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Sustainable hydroponic systems: Effects of nutrient and substrate variations on plant growth and rhizosphere bacterial morphological diversity

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ABSTRACT

This study investigated the effects of nutrient concentrations (0, 0.8, 1.6, 2.4, and 3.2 dS m^{-1}) and substrate types (volcanic sand, rice husk charcoal, and a 1:1 mixture) on *Limnocharis flava* growth and rhizosphere bacterial morphological diversity in a hydroponic system. A factorial experiment was arranged in a randomized complete block design (RCBD) with three replications. Low to moderate nutrient levels (0.8–1.6 dS·m^{-1}) enhanced the leaf area ratio (LAR), relative growth rate (RGR), net assimilation rate (NAR), and harvest index (HI), while rice husk charcoal and the mixed substrate supported greater plant growth and root volume. Bacterial density increased under low nutrient concentrations but declined at higher levels. A total of 138 bacterial isolates were obtained and classified into 23 morphotypes based on macroscopic traits. Diversity analysis showed Shannon–Wiener and Simpson index values ranging from 0.2–1.6 and 0.2–0.75, respectively, indicating low to moderate diversity. Higher diversity was associated with nutrient concentrations of 0–1.6 dS·m^{-1}, whereas elevated levels promoted dominance. Substrate type did not significantly affect bacterial density or pH but strongly influenced root volume, with volcanic sand and mixed substrates providing better aeration and porosity. These findings demonstrate that nutrient substrate interactions regulate both plant morpho-physiological traits and rhizosphere bacterial structure, offering a basis for sustainable hydroponics and potential biofertilizer applications.

Keywords: sustainable, hydroponic, substrate, nutrient, bacterial, morphological diversity.

INTRODUCTION

Global population growth and increasing pressure on natural resources have driven the development of more efficient and environmentally friendly agricultural systems. In this context, hydroponic systems are gaining popularity as a sustainable alternative to conventional agriculture. Hydroponics enables precise control of water and nutrient use while reducing ecological impacts on the environment (Tuxun et al., 2025). The long-term sustainability of hydroponic systems, however, depends not only on crop productivity but also on the ability of these systems to sustain microbial ecology in the root zone, an aspect that is often overlooked.

The concentration of the nutrient solution and the type of substrate are key factors in hydroponic cultivation, as both determine overall plant growth performance. Nutrient solutions directly influence plant metabolism, photosynthesis, and biomass accumulation (Guevara et al., 2020), whereas substrates not only provide mechanical support but also regulate aeration, water retention capacity, and nutrient ion distribution. Thus, while nutrient

solutions control the physiological processes underlying plant growth, substrates ensure structural stability and optimal root environment (Dhandapani et al., 2025; Swain et al., 2021).

Beyond plant growth aspects, variations in nutrient concentration and substrate type can also shape unique rhizosphere microenvironments in hydroponic systems. Although hydroponics is generally regarded as a soilless and relatively sterile cultivation method, hydroponic media can still harbor diverse microbial communities, particularly rhizosphere bacteria that actively interact with plant roots (Stegelmeier et al., 2022). Rhizosphere bacterial communities are highly responsive to changes in nutrient regimes and substrate composition, which may alter microbial abundance, diversity, and ecological interactions among taxa (Nisar et al., 2024; Vogelmann et al., 2025). Recent studies have shown that hydroponic systems can enrich beneficial bacterial taxa such as *Rhodanobacter*, *Chujalbacter*, and *Thermomonas*, which support plant growth and enhance nutrient use efficiency (Alkaabi et al., 2025; Chowdhury and Samarakoon, 2024; Spencer et al., 2024).

Although rhizosphere bacteria are well recognized for their roles in soil-based systems, their functions in hydroponic cultivation remain underexplored, particularly regarding how nutrient concentrations and substrate types influence microbial community structures and, indirectly, plant performance (Vlasselaer, 2024). Optimizing these two factors is therefore essential not only for maximizing plant growth but also for enhancing microbial ecology within hydroponic systems (Herna et al., 2025). Understanding these interactions can inform pro-ecological hydroponic practices and contribute to sustainable ecological engineering solutions that minimize resource consumption and mitigate anthropogenic impacts (Rajendran et al., 2024).

To date, studies on the relationships between environmental factors in hydroponics and rhizosphere bacterial communities remain limited. Most investigations have focused on plant performance, with microbial aspects seldom considered in parallel (Banboukian et al., 2025). Yet microbial diversity and abundance play critical roles in nutrient availability, pathogen suppression, and overall system resilience. Therefore, integrative evaluations that encompass both plant growth and microbial indicators are essential to fully understand hydroponic sustainability.

This study aims to analyze the effects of nutrient solution concentration and substrate variation on plant growth and rhizosphere bacterial morphological diversity in hydroponic systems. The findings are expected to provide a scientific foundation for the design of sustainable hydroponic systems that not only prioritize crop productivity but also incorporate rhizosphere bacteria as integral components of an engineered ecosystem. Preliminary observations on bacterial morphological diversity in hydroponics offer baseline insights into the influence of nutrient and substrate conditions on plant–microbe interactions, a topic that remains underexplored. Consequently, this research may be regarded as an initial step toward the development of pro-ecological hydroponic models aligned with the principles of ecological engineering, emphasizing resource efficiency, environmental friendliness, and long-term sustainability.

MATERIALS AND METHODS

Study area

The study was conducted from August to December 2024 at the hydroponic greenhouse and Soil Biology and Biotechnology Laboratory, Faculty of Agriculture, Universitas Sebelas Maret, Central Java, Indonesia (altitude 131 m above sea level; coordinates 7°33′41.8″ S and 110°51′32.36″ E). The greenhouse temperature ranged from 26–41 °C, with an average of 31–32 °C, while relative humidity varied from 45% to 90%, with a mean of 68–70%.

Procedure

Floating raft hydroponic system setup

A hydroponic floating raft system without aeration was assembled using 45 plastic boxes. Each box was covered with a perforated Styrofoam sheet, where holes were spaced 20 × 20 cm apart, allowing six planting positions per box. The experiment was arranged in three replications, with each replication consisting of 15 boxes. Substrates were provided according to the treatment, namely volcanic sand, rice husk charcoal, or a 1:1 volumetric mixture of both, layered to a depth of 15 cm. Nutrient solution was added until the liquid level stood about 2 cm above the substrate surface. The system was deliberately maintained without aeration to replicate the naturally flooded habitat where *L. flava* typically grows.

Transplanting of L. flava

Seedlings of *L. flava* were collected from Semarang Regency. The seedlings selected for transplanting were approximately 15 cm in height and had three fully developed leaves. They were transplanted by inserting each seedling into the pre-formed holes on the Styrofoam cover of the hydroponic box.

Nutrient solution and substrate material preparation

The nutrient formulation contains 180 ppm NO_3^-, 37 ppm NH_4^+, 66 ppm P, 286 ppm K, 154 ppm Ca, 66 ppm Mg, and 122 ppm S, supplemented with 40 g of Vitaflex™ micronutrient mix per 5 L concentrated stock. Two types of stock solutions were prepared: Stock Solution A (nitrate, ammonium, potassium, calcium, and micronutrients, but excluding phosphate and sulfate salts) and Stock Solution B (containing magnesium, sulfate, and phosphate salts). Working solutions were diluted with water to reach the desired electrical conductivity levels (0, 0.8, 1.6, 2.4, and 3.2 dS m^{-1}). Electrical conductivity (EC) was regularly measured with a calibrated meter (Hanna HI 98301). Volcanic sand with a particle size of 3–5 mm was collected from volcanic deposits, while rice husk charcoal was obtained through pyrolysis at 400 °C under restricted oxygen conditions. The mixed substrate was prepared in a 1:1 volume ratio of sand and husk charcoal.

Crop management and harvesting

Pest and disease management was performed manually, without pesticides, by handpicking insects or discarding infected plants. Destructive sampling was carried out at 21 and 28 days after transplanting (DAT) by carefully uprooting plants from the planting holes. Harvested samples were immediately placed into labeled containers and transported for subsequent measurements and analysis.

Experimental design and treatments

A factorial experiment was arranged in a Randomized complete block design (RCBD) with three replications. Factor A was the nutrient solution concentration, consisting of five levels (0, 0.8, 1.6, 2.4, 3.2 dS m^{-1}), while Factor B was the substrate type, including volcanic sand, rice husk charcoal, and a 1:1 (v/v) mixture of both. The interaction of these factors resulted in 15 treatment combinations, each replicated three times, for a total of 45 experimental units. The treatments are shown in Table 1.

Sample preparation and analysis

Plant growth measurement

Root volume was measured using the water displacement method, and nutrient solution pH was determined with an pH meter (Hanna

Table 1. Experimental treatments combining nutrient solution concentrations and substrate types in the hydroponic cultivation of *L. flava*

Code	Nutrient concentration (dS m^{-1})	Substrate type	Combination
A	0	Volcanic sand	0.0 dS m^{-1} × volcanic sand
B	0	Rice husk charcoal	0.0 dS m^{-1} × rice husk charcoal
C	0	Mixed substrate	0.0 dS m^{-1} × mixed substrate
D	0.8	Volcanic sand	0.8 dS m^{-1} × volcanic sand
E	0.8	Rice husk charcoal	0.8 dS m^{-1} × rice husk charcoal
F	0.8	Mixed substrate	0.8 dS m^{-1} × mixed substrate
G	1.6	Volcanic sand	1.6 dS m^{-1} × volcanic sand
H	1.6	Rice husk charcoal	1.6 dS m^{-1} × rice husk charcoal
I	1.6	Mixed substrate	1.6 dS m^{-1} × mixed substrate
J	2.4	Volcanic sand	2.4 dS m^{-1} × volcanic sand
K	2.4	Rice husk charcoal	2.4 dS m^{-1} × rice husk charcoal
L	2.4	Mixed substrate	2.4 dS m^{-1} × mixed substrate
M	3.2	Volcanic sand	3.2 dS m^{-1} × volcanic sand
N	3.2	Rice husk charcoal	3.2 dS m^{-1} × rice husk charcoal
O	3.2	Mixed substrate	3.2 dS m^{-1} × mixed substrate

HI98107). Destructive sampling of plants was performed at 21 and 28 day after transplanting (DAT) to measure growth parameters, including leaf area ratio (LAR), leaf area duration (LAD), relative growth rate (RGR), net assimilation rate (NAR), and harvest index (HI). The parameters were calculated using the following formulae:

$$\text{LAR } (\text{cm}^2.\ \text{g}^{-1}) = = \frac{leaf\ area}{total\ dry\ weight} \quad (1)$$

$$\text{LAD } (\text{cm}^2.\text{d}^{-1}) = \frac{(LA1+LA2)X(T2-T1)}{2} \quad (2)$$

where: $LA1$, $LA2$ – leaf area at the first and second observations, repectively; $T1$, $T2$ – time of the first and second observations (day).

$$\text{RGR } (\text{g.d}^{-1}) = \frac{lnW2-lnW1}{T2-T1} \quad (3)$$

where: $W1$, $W2$ – total dry weight of plants at times $T1$ and $T2$ respectively.

$$\text{NAR } (\text{g.m}^{2\text{-}1}.\text{day}^{-1}) = \frac{W2-W1}{L2-L1} \times \frac{lnL2-lnL1}{t2-t1} \quad (4)$$

where: $W1$, $W2$ = total plant dry weight at $t1$ and $t2$; $L1$, $L2$ – leaf area at $t1$ and $t2$; $T1$, $t2$ – time intervals for measurement.

$$\text{HI } (\%) = \frac{ekonomis\ yield}{biological\ yield} \times 100 \quad (5)$$

(Banerjee et al., 2012)

Isolation and total density of bacteria from L. flava rizosfer

Rhizosphere bacteria were isolated from the substrate surrounding *L. flava* roots cultivated in the hydroponic system. Substrate samples (10 g) were suspended in 90 mL of physiological saline solution and homogenized using a shaker. Serial dilutions up to 10^{-9} were prepared and vortexed for uniformity. Dilutions of 10^{-3}, 10^{-4}, and 10^{-5} were selected for bacterial density determination and isolation. Aliquots of 0.1 mL were spread onto nutrient agar (NA) plates using the spread plate method (Pujiati et al., 2025), with each dilution replicated twice. Plates were incubated at room temperature for five days until colonies developed. The total bacterial population in the rhizosphere was quantified using the Total Plate Count (TPC) method. The total bacterial population was expressed as CFU per gram of rhizosphere sample using the following equation:

$$Total\ bacterial\ population\ (\text{CFU g}^{-1}) = \frac{N \times D}{W} \quad (6)$$

where: N is the number of colonies, D is the dilution factor, and W is the sample weight (g).

Colony morphological characteristics

Colony morphology was characterized macroscopically by direct observation on NA plates. Observed features included color, diameter, colony shape, colony edge, elevation, and opacity (Linda et al., 2023; Masi et al., 2021).

Dendogram

Morphological data from colony characterization, including color, diameter, colony shape, colony edge, elevation, and opacity, were converted into numerical scores (Table 2). The scoring data were subjected to cluster analysis to evaluate morphological similarities among isolates. A dendrogram

Table 2. Morphological colony characters and scoring scheme used for dendogram

Color	Score	Diameter	Score	Colony shape	Score	Colony edge	Score	Elevasi	Score	Opacity	Score
White	1	0–1	1	Circular	1	Entire	1	Effuse	1	Transparent	1
Milky white	2	>1–2	2	Amoeboid	2	Erose	2	Law convex	2	Transculent	2
Cream	3	>3–4	3	Iregular	3	Crenate	3	Raised	3	Opaque	3
Yellow	4	>4–5	4	Curled	4	Undulate	4	Convex	4	Smooth	4
Orange	5	>5–6	5	Filamentous	5	Lobate	5	Conver papillate	5	Finely granular	5
Brick red	6	>6–7	6	Rhizoid	6	Ciliate	6	Convex rugose	6	Coarsely granular	6
Pink	7	>7–8	7	Myceloid	7	Fimbriate	7	Raised with concave bevelfed edge	7	Wavy enteriaced	7
Red	8	>8–9	8	Toruloid	8	Lacerate	8	Umbonate	8	Filamentous	8
				Spindle	9	Ramose	9	Pulvinate	9	Arborescent	9

was constructed using the single linkage method with interval rescale distance to visualize the relationships among the bacterial isolates.

Diversity and dominance index

The diversity and dominance of the 138 selected isolates were calculated using the Shannon–Wiener Index (H') and the Simpson Index (D). The formulas used were as follows:

$$H' = \sum_{i=1}^{S} p_i . \ln (p_i) \quad (7)$$

where: S – total number of species (or morphotypes) observed; p_i – proportion of individuals belonging to the i-th species, calculated as n_i/N, where n_i is the number of individuals of species i, and N is the total number of individuals in the community; ln – natural logarithm.

$$D = \sum_{i=1}^{S} {p_i}^2 \quad (8)$$

where: p_i – proportion of individuals belonging to the i-th species; S – total number of species (or morphotypes).

The values of the Shannon–Wiener Index (H') and Simpson Index (D) were presented as bar graphs.

Data analysis

The experimental data were subjected to analysis of variance (ANOVA) following a factorial arrangement in a randomized complete block design (RCBD) using SPSS version 26. When a significant F-value was obtained, mean separation was conducted using Duncan's multiple range test (DMRT) at a 5% probability level. Pearson's correlation analysis was employed to determine the relationships among bacterial density, nutrient solution pH, and root volume, and the results were illustrated through a heatmap in R. Additionally, a dendrogram was generated in R to group bacterial isolates based on morphological similarity.

RESULTS AND DISCUSSION

Results

Plant growth

The analysis of plant growth traits (leaf area ratio (LAR), leaf area duration (LAD), relative growth rate (RGR), net asimilation rate (NAR) and harvest Indeks (HI)) of *L. flava* at 28 day after transplanting (DAT) revealed significant effects of nutrient solution concentration and substrate type, while no interaction between the two factors (Table 3).

The highest LAR was recorded at 0.8 dS m^{-1} (0.070 ± 0.02 a), statistically comparable with 1.6 dS m^{-1} (0.067 ± 0.02 a) and 2.4 dS m^{-1} (0.055 ± 0.01 ab). In contrast, the lowest values LAR exhibited clear differences among treatments were obtained at 3.2 dS m^{-1} (0.039 ± 0.02 c) and 0 dS m^{-1} (0.042 ± 0.01 b). These results clearly demonstrate that both nutrient excess and deficiency impose substantial constraints on leaf expansion relative to biomass accumulation.

LAD measurements further confirmed the positive influence of moderate nutrient concentrations. LAD reached its peak at 0.8 dS m^{-1} (6.40 ± 0.62 a), followed by 1.6 dS m^{-1} (5.34 ± 0.57 b). In contrast, the control remained minimal (1.08 ± 0.09 e), while excessive nutrient input at 3.2 dS m^{-1} reduced LAD to 3.22 ± 0.31. Such outcomes confirm that moderate nutrient availability prolongs the persistence of photosynthetically active leaves, whereas extremes in nutrient supply curtail their functional lifespan. RGR at 28 DAT showed a similar tendency, with the highest value observed at 0.8 dS m^{-1} (0.11 ± 0.02 a), which was statistically comparable with 1.6 dS m^{-1} (0.07 ± 0.02 ab) and 3.2 dS m^{-1} (0.09 ± 0.01 ab). The control exhibited the lowest RGR (0.06 ± 0.01 b). This pattern implies that moderate nutrient enrichment accelerates biomass accumulation more effectively than either nutrient deprivation or excess supply.

NAR displayed an intriguing response across treatments. The highest NAR values were recorded at 0.8 dS m^{-1} (5.99 ± 0.17 a), 1.6 dS m^{-1} (5.62 ± 1.80 a), 2.4 dS m^{-1} (5.55 ± 1.41 a), and 3.2 dS m^{-1} (6.90 ± 0.51 a), whereas the control maintained a much lower value (2.70 ± 0.97 b). This outcome illustrates that beyond a certain threshold, nutrient addition does not proportionally increase NAR, reflecting a physiological plateau in carbon assimilation. HI significantly increased with nutrient addition, reaching the maximum at 3.2 dS m^{-1} (76.69 ± 3.28 a), comparable with 1.6 dS m^{-1} (75.54 ± 1.88 a). The control treatment produced the lowest HI (69.46 ± 1.61 b). The data reveal that nutrient supplementation not only enhances total biomass but also improves biomass allocation efficiency toward economically valuable yield.

Substrate analysis indicated that rice husk consistently produced higher values for LAR

Table 3. Plant growth parameters of *L. flava* under different substrate and nutrient concentrations at 28 days after transplanting (DAT)

Treatment	LAR 28 DAT	LAD 28 DAT	RGR 28 DAT	NAR 28 DAT	HI 28 DAT
Concentration (dS m^{-1})					
0	0.042 ± 0.01 b	1.08 ± 0.09 e	0.06 ± 0.01 b	2.70 ± 0.97 b	69.46 ± 1.61 b
0.8	0.070 ± 0.02 a	6.40 ± 0.62 a	0.11 ± 0.02 a	5.99 ± 0.17 a	72.59 ± 6.26 ab
1.6	0.067 ± 0.02 a	5.34 ± 0.57 b	0.07 ± 0.02 ab	5.62 ± 1.80 a	75.54± 1.88 a
2.4	0.055 ± 0.01 ab	3.97 ± 0.57 c	0.07± 0.02 b	5.55 ± 1.41 a	71.97 ± 4.87 ab
3.2	0.039 ± 0.02 c	3.22 ± 0.31 d	0.09± 0.01 ab	6.90 ± 0.51 a	76.69 ± 3.28 a
Substrate					
Volcanic sand	0.035 ± 0.01 b	3.88 ± 1.69 b	0.07 ± 0.01 b	4.72 ± 1.23	75.53 ± 4.62
Husk charcoal	0.077 ± 0.01 a	4.12 ± 1.66 ab	0.09 ± 0.03 a	5.86 ± 2.33	72.12 ± 2.88
Volcanic sand+ husk charcoal (1:1)	0.052 ± 0.01 b	4.44 ± 2.09 a	0.08 ± 0.01 ab	5.48 ± 1.70	72.10 ± 5.10
Interaction	-	-	-	-	-

Note: numbers followed by the same letter indicate there are no significant differences based on ANOVA and DMRT at the α level of 5%.

(0.077 ± 0.01 a), NAR (5.86 ± 2.33 a), and HI (72.12 ± 2.88 a), while volcanic sand showed the lowest performance in most parameters. The mixed substrate (volcanic sand + rice husk) yielded intermediate but stable responses, particularly for LAD (4.44 ± 2.09 a). This finding emphasizes that substrate selection plays a crucial role in optimizing plant growth performance, with rice husk proving to be the most favorable medium under hydroponic conditions.

Bacterial density, nutrient solution pH, and root volume

Observations on bacterial density, nutrient solution pH, and root volume of *L. flava* were conducted across all treatments (Table 4). Bacterial density reflects the population size within a sample and is commonly used to assess microbial activity under specific conditions, which in this study corresponds to the rhizosphere of *L. flava* cultivated under varying nutrient solution concentrations and substrate types. Higher bacterial density values indicate larger populations, which may be beneficial if the bacteria present possess plant growth-promoting capabilities.

The results demonstrated that increasing nutrient concentrations significantly affected the rhizosphere bacterial density of *L. flava* grown in a hydroponic system, whereas substrate type and the interaction between substrate and nutrient concentration did not show significant effects. Bacterial density decreased with increasing

Table 4. Bacterial density, nutrient solution pH, and root volume of *L. flava* under different substrate and nutrient concentrations

Treatment	Bacterial density (log CFU/g)	Nutrient solution pH	Root volume (cm^3)
Concentration (dS m^{-1})			
0	6.24 ± 0.11 a	7.50 ± 0.08 a	3.55 ± 0.50 c
0.8	6.01 ± 0.02 b	6.60 ± 0.13 b	14.00 ± 1.52 a
1.6	6.02 ± 0.03 b	5.70 ± 0.08 c	12.22 ± 2.67 a
2.4	5.94 ± 0.01 cd	5.80 ± 0.05 c	12.00 ± 2.21 a
3.2	5.89 ± 0.02 d	5.70 ± 0.00 c	9.11 ± 2.00 b
Substrate			
Volcanic sand	6.05 ± 0.14 a	6.20 ± 0.80 a	8.06 ± 2.49 b
Husk charcoal	6.06 ± 0.18 a	6.20 ± 0.74 a	11.80 ± 4.01 a
Volcanic sand+ husk charcoal (1:1)	6.01 ± 0.10 a	6.20 ± 0.83 a	10.66 ± 3.28 a
I Interaction	-	-	-

Note: numbers followed by the same letter indicate there are no significant differences based on ANOVA and DMRT at the α level of 5%.

nutrient concentration. The highest bacterial density (6.24 ± 0.11 log CFU/g) was observed at 0 dS m^{-1} nutrient concentration. Nutrient concentrations of 0.8 dS m^{-1} and 1.6 dS m^{-1} resulted in bacterial densities of 6.01 ± 0.02 log CFU/g and 6.02 ± 0.03 log CFU/g, respectively, which were higher than those recorded at 2.4 dS m^{-1} and 3.2 dS m^{-1}, which only reached 5.94 ± 0.01 log CFU/g and 5.89 ± 0.02 log CFU/g, respectively.

Increasing nutrient concentrations resulted in a decrease in nutrient solution pH, whereas substrate type and the interaction between substrate and nutrient concentration did not cause significant differences in pH. The acidity of the nutrient solution decreased with increasing nutrient concentration. The highest pH value (7.50 ± 0.08, neutral) was observed at 0 dS m^{-1} nutrient concentration. A nutrient concentration of 0.8 dS m^{-1} yielded a pH of 6.60 ± 0.13, which was higher than the values recorded at 1.6 dS m^{-1}, 2.4 dS m^{-1}, and 3.2 dS m^{-1}, which were 5.70 ± 0.08, 5.80 ± 0.05, and 5.70 ± 0.00, respectively. The results also indicated that nutrient deficiency led to minimal root volume, measured at 3.55 cm^3. At nutrient concentrations ranging from 0.8 to 2.4 dS m^{-1}, the root volume of *L. flava* was highest, ranging from 12 to 14 cm^3 compared to other treatments. However, at the highest nutrient concentration (3.2 dS m^{-1}), root volume declined to 9.11 cm^3. Regarding substrate effects, volcanic sand and the mixture of volcanic sand + rice husk charcoal (1:1) produced higher root volumes than rice husk charcoal alone.

Bacterial morphology, diversity, and dominance

Microbial isolation yielded a total of 138 colonies. These colonies were subsequently examined macroscopically on Petri dishes to assess their morphological characteristics, including colony color, diameter, shape, margin, elevation, and internal structure. Based on these morphological

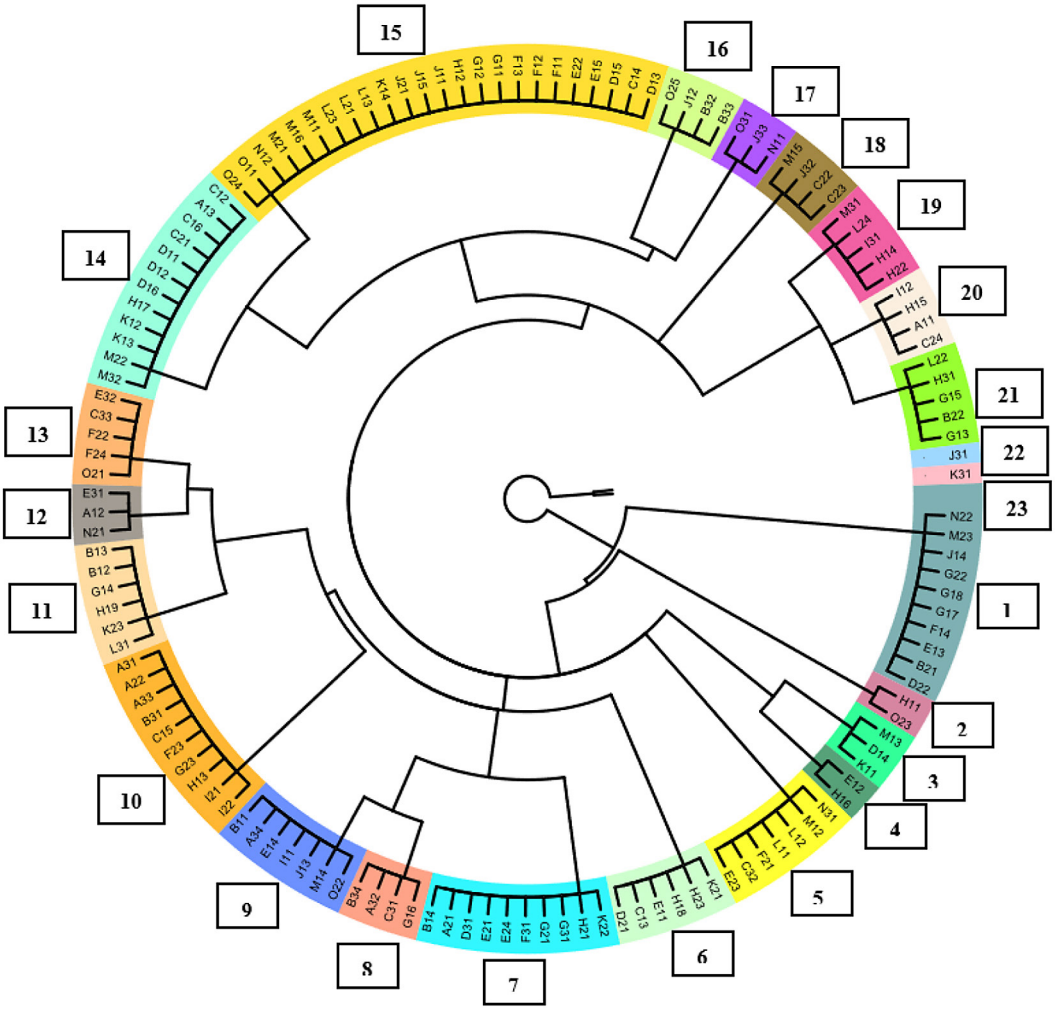

Figure 1. Cluster analysis of rhizosphere bacterial isolates derived from morphological characteristics

traits, colonies were grouped accordingly. The grouping results indicated the presence of 23 distinct bacterial morphological groups (Figure 1).

The observation of rhizosphere bacterial morphological diversity revealed several variations in colony color, diameter, shape, margin, elevation, and internal structure (Table 5). Colony colors included milky white, cream, yellow, orange, pink, brick red, and red. Isolates with yellow, orange, pink, brick red, and red pigmentation demonstrated the ability to produce pigments. Colony diameters ranged from 0.55 to 1.70 mm, with circular forms being the most dominant. Other colony shapes observed included irregular, myceloid, and spindle forms. Five distinct colony margins were identified: undulate, fimbriate, entire, erose, and lobate. Elevation types varied considerably, encompassing umbonate, low convex, convex rugose, convex papillate, raised, and effuse. Internal structures also exhibited wide variation, including finely granular, opaque, filamentous, wavy entrapped, translucent, coarsely granular, arborescent, and smooth. No isolates shared completely identical combinations of margin, elevation, and internal structure.

Diversity and dominance indices of rhizosphere bacteria were evaluated using the Shannon–Wiener Index (H') and Simpson Index (D). The results of these analyses for *L. flava* cultivated under different nutrient solution concentrations and substrate types indicated that bacterial diversity fell within the low to moderate categories (Figure 2). The Simpson Index across all treatments reflected moderate to low levels of dominance by specific taxa. Treatments D, F, G, H, I, J, K, L, N, and O exhibited Simpson Index values categorized as moderate, whereas treatments A, B, C, E, and M showed values categorized as low.

Discussion

Plant growth

The results demonstrate that moderate nutrient concentrations (0.8–1.6 dS m^{-1}) create optimal conditions for leaf development and growth in *L. flava*. The peak of LAR and LAD at these levels indicates enhanced assimilate allocation to leaf tissues, thereby increasing the photosynthetic surface area per unit biomass. Similar responses have been reported in hydroponically grown leafy vegetables, where moderate electrical conductivity values of 1.0–1.5 dS m^{-1} promoted maximum leaf expansion and dry matter accumulation. Spinach grown under EC 1.2–1.5 dS m^{-1} exhibited significantly higher leaf area and shoot biomass compared with lower or higher EC treatments, suggesting an optimal balance between nutrient availability and osmotic stress (Dewir et al., 2022). Likewise, Yang et al. (2024) reported that lettuce exposed to 1.2–1.5 dS m^{-1} achieved superior vegetative growth and assimilate distribution to leaf tissues, reinforcing the notion that moderate EC levels enhance the efficiency

Table 5. Morphological characteristics of rhizosphere bacterial isolates from *L. flava* cultivated under different substrate and nutrient concentrations

Code	Nutrient concentration (dS m^{-1})	Substrate type	Combination
A	0	Volcanic sand	0.0 dS m^{-1} × volcanic sand
B	0	Rice husk charcoal	0.0 dS m^{-1} × rice husk charcoal
C	0	Mixed substrate	0.0 dS m^{-1} × mixed substrate
D	0.8	Volcanic sand	0.8 dS m^{-1} × volcanic sand
E	0.8	Rice husk charcoal	0.8 dS m^{-1} × rice husk charcoal
F	0.8	Mixed substrate	0.8 dS m^{-1} × mixed substrate
G	1.6	Volcanic sand	1.6 dS m^{-1} × volcanic sand
H	1.6	Rice husk charcoal	1.6 dS m^{-1} × rice husk charcoal
I	1.6	Mixed substrate	1.6 dS m^{-1} × mixed substrate
J	2.4	Volcanic sand	2.4 dS m^{-1} × volcanic sand
K	2.4	Rice husk charcoal	2.4 dS m^{-1} × rice husk charcoal
L	2.4	Mixed substrate	2.4 dS m^{-1} × mixed substrate
M	3.2	Volcanic sand	3.2 dS m^{-1} × volcanic sand
N	3.2	Rice husk charcoal	3.2 dS m^{-1} × rice husk charcoal
O	3.2	Mixed substrate	3.2 dS m^{-1} × mixed substrate

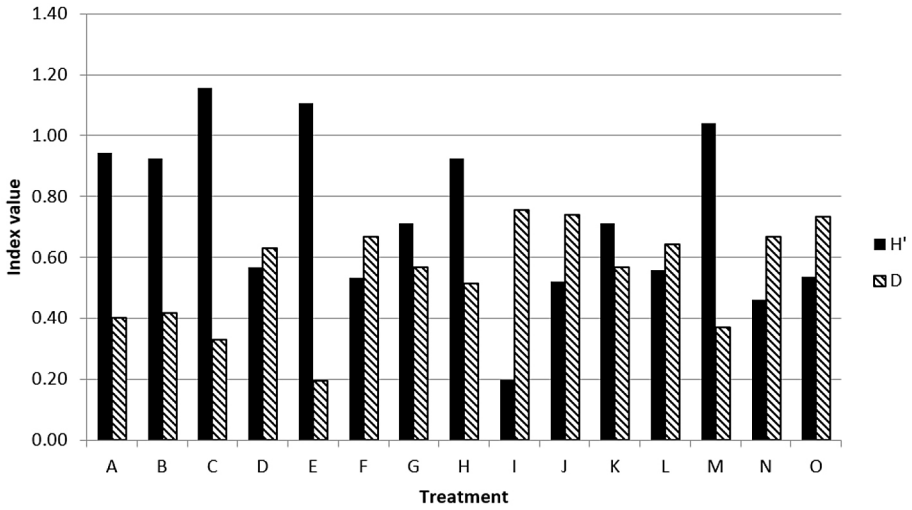

Figure 2. Bacterial diversity (Shannon–Wiener index) and dominance (Simpson index) of rhizosphere communities of *L. flava* under different nutrient concentrations and substrates. A = 0 dS m^{-1} × volcanic sand, B = 0 dS m^{-1} × rice husk charcoal, C = 0 dS m^{-1} × mixed substrate, D = 0.8 dS m^{-1} × volcanic sand, E = 0.8 dS m^{-1} × rice husk charcoal, F = 0.8 dS m^{-1} × mixed substrate, G = 1.6 dS m^{-1} × volcanic sand, H = 1.6 dS m^{-1} × rice husk charcoal, I = 1.6 dS m^{-1} × mixed substrate, J = 2.4 dS m^{-1} × volcanic sand, K = 2.4 dS m^{-1} × rice husk charcoal, L = 2.4 dS m^{-1} × mixed substrate, M = 3.2 dS m^{-1} × volcanic sand, N = 3.2 dS m^{-1} × rice husk charcoal, and O = 3.2 dS m^{-1} × mixed substrate

of resource allocation to economically important organs. This pattern suggests the existence of an optimal nutrient concentration window that maximizes leaf expansion relative to total biomass. In contrast, both nutrient deficiency and excess constrain leaf enlargement, either due to inadequate nutrient availability or ionic toxicity. Excessive nutrient concentration (3.2 dS m^{-1}) markedly reduced LAR and LAD, likely as a consequence of osmotic stress inhibiting cellular expansion. A similar outcome was reported in hydroponic Lactuca sativa, where elevated nutrient concentrations suppressed leaf area expansion despite slight improvements in certain leaf-level physiological traits (Kappel et al., 2021).

The observed decline in LAR and LAD with increasing nutrient concentrations, accompanied by stable or even relatively high NAR values at the highest nutrient concentration, indicates a physiological compensation strategy. Under ion-rich conditions that limit leaf expansion, plants tend to optimize photosynthetic performance per unit area through increased chlorophyll content, improved light use efficiency, and activation of key photosynthetic enzymes. Thus, even with reduced total leaf area, carbon assimilation capacity per unit area remains preserved. This phenomenon is supported by,He et al. (2024) who reported that elevated nutrient concentrations in hydroponically grown cucumber triggered the upregulation of photosynthetic gene expression, thereby sustaining carbon assimilation efficiency despite reductions in leaf expansion. Similarly, Ikiz et al. (2024) demonstrated that lettuce subjected to high salinity stress exhibited smaller leaf areas due to osmotic constraints but maintained photosynthetic efficiency per unit area through enhanced chlorophyll concentration and improved stomatal conductance. Together, these findings highlight the adaptive strategies plants employ to balance structural limitations with functional efficiency under elevated EC conditions.

Furthermore, the stability of NAR at higher nutrient concentrations, which remains statistically comparable to moderate levels (0.8–2.4 dS m^{-1}), suggests the presence of a physiological plateau in assimilation capacity. In other words, once plants reach their optimal nutrient threshold, additional nutrient supply does not further enhance their carbon assimilation ability. Barros et al. (2024) demonstrated that NAR primarily reflects physiological efficiency rather than leaf expansion, which explains why NAR remains stable even when LAR and LAD decrease under nutrient-rich conditions. Excessive nutrient supply in leafy vegetables did not increase biomass production but instead maintained assimilation efficiency at a constant level, supporting the concept of an upper limit in nutrient-use efficiency. These findings suggest that the hydroponic

cultivation of *L. flava* should prioritize moderate nutrient levels to ensure efficient resource utilization while avoiding unnecessary nutrient inputs that increase costs and environmental burdens without improving crop productivity.

The highest RGR observed at moderate nutrient concentrations (0.8 dS m^{-1}) reinforces the notion that neither excessively low nor excessively high nutrient availability supports optimal growth. At this concentration, nutrient uptake, leaf area expansion, and biomass production were maximized, indicating that plants were able to maintain balanced physiological processes. Soufi et al. (2023) similarly demonstrated that hydroponic systems operated under elevated EC levels experienced reduced water uptake and nutrient absorption, directly limiting biomass accumulation and growth efficiency. These findings highlight that biomass accumulation rate is not solely dependent on nutrient supply, but also on the plant's capacity to sustain physiological homeostasis under specific EC conditions, thereby emphasizing the importance of maintaining nutrient concentrations within a moderate range.

In contrast, the higher harvest index (HI) observed at elevated nutrient concentration levels (1.6–3.2 dS m^{-1}) indicates a strategic shift in biomass allocation toward economically valuable organs, even though the overall growth rate (RGR) was not maximized. This pattern reflects a trade-off between total biomass accumulation and allocation efficiency, with plants prioritizing carbon partitioning into harvestable tissues rather than sustaining structural growth (LAR or LAD). Rajaseger et al. (2023) reported that excessive nutrient availability often promotes greater allocation of assimilates into reproductive or harvestable organs, even when vegetative expansion slows, highlighting an adaptive reallocation strategy to safeguard yield. Practically, this suggests that in cultivation systems targeting economic yield, such as leaves or fruits, maintaining nutrient concentrations slightly above the RGR optimum may represent an efficient agronomic approach.

Moderate nutrient concentrations (0.8–1.6 dS m^{-1}) create optimal conditions for *L. flava* by balancing growth and physiological efficiency, resulting in maximal leaf expansion, biomass production, and effective carbon assimilation. Excessive nutrients (>1.6 dS m^{-1}) limit leaf enlargement through osmotic stress but maintain photosynthetic performance per unit area, while also favoring allocation to harvestable organs, increasing the harvest index. These results highlight the importance of managing nutrient supply to enhance productivity and resource-use efficiency in hydroponic cultivation without incurring unnecessary environmental or economic costs.

Bacterial density, nutrient solution pH, and root volume

Bacterial density was strongly influenced by nutrient solution concentration. Higher bacterial densities were observed under low nutrient concentrations. This increase may be attributed to several factors, one of which is root stress caused by nutrient deficiency. Such stress often enhances root exudation, which serves as the primary energy source for rhizosphere bacteria. Consequently, under nutrient-poor conditions, bacterial populations increase due to greater availability of root exudates in the rhizosphere (Camli-Saunders and Villouta, 2025; Ma et al., 2021). Bai et al. (2022) also reported that in low-fertility soils, bacterial and fungal populations in the rhizosphere increased by 205–254%. Similarly, Ma et al. (2022) demonstrated that nutrient-deficient soils stimulate plants to release more primary metabolites, including root exudates, as an adaptive response to stress conditions. The presence of rhizosphere bacteria is therefore highly dependent on a balanced microenvironment. Although low nutrient availability favors microbial proliferation, this condition must be considered carefully, as insufficient nutrients may negatively affect plant growth.

In contrast, bacterial density declined with increasing nutrient concentrations. At high nutrient levels, ion accumulation creates osmotic stress (Ding et al., 2022). Elevated ion concentrations and osmotic pressure are unfavorable for microbial survival, as they disrupt protein and membrane structures, thereby reducing microbial metabolic efficiency and replication (Zhang et al., 2024).

In addition to influencing bacterial density, increasing nutrient concentrations from 0 to 3.2 dS m^{-1} also decreased nutrient solution pH, from 7.5 to 5.7. *L. flava*, as a leafy vegetable, utilizes NH_4^+ in addition to NO_3^- as a nitrogen source. The use of NH_4^+ is typically higher in leafy vegetable nutrient formulations than in fruiting vegetables. Greater NH_4^+ input lowers pH due to the release of H^+ ions into the nutrient solution. Zhu et al. (2021) confirmed that higher NH_4^+ concentrations increase H^+ release, resulting in lower pH values. However, in this study, the pH decline remained within the tolerance range of *L. flava*, as indicated by root volume

data (Table 4). Root volume differences were primarily influenced by nutrient concentrations.

Root volume increased with rising nutrient concentrations up to a certain threshold, beyond which further increases caused toxicity and reduced root development. Similarly, nutrient deficiency also suppressed root volume due to limited resource availability. Root volume is a critical parameter reflecting a plant's capacity to absorb water and nutrients from its environment. Larger root volumes provide greater surface area for efficient uptake. Excessive nutrient concentrations, however, may induce osmotic stress, inhibiting root development. (Sakamoto and Suzuki (2020) reported a similar trend in sweet potato, where nutrient concentrations up to EC 2.6 dS increased storage root fresh weight compared with EC 0.8 dS m^{-1} and EC 1.4 dS m^{-1}, while higher concentrations inhibited plant growth. Enhanced root volume in response to increased nutrient concentrations is closely linked to nutrient availability in the growth medium. Adequate nutrient supply promotes optimal root development, whereas nutrient scarcity alters root architecture, favoring elongation to explore for nutrients (López-Bucio et al., 2003).

In this study, substrate type had no significant effect on bacterial density or nutrient solution pH but significantly influenced root volume. Volcanic sand and a mixture of volcanic sand + rice husk charcoal (1:1) produced greater root volumes than rice husk charcoal alone. This effect is attributed to the higher porosity and aeration of volcanic sand-based substrates, which promote root development.

Correlation between bacterial density, nutrient solution pH, and root volume

Bacterial density was found to be closely associated with the acidity of the growth medium. Correlation analysis revealed a very strong positive relationship between bacterial density and the pH of the nutrient solution. This indicates that higher pH values were associated with greater bacterial density, whereas lower pH values corresponded with reduced bacterial density. Conversely, a strong negative correlation was observed between nutrient solution pH and root volume (Figure 3). As the pH increased, root volume tended to decrease, while lower pH levels promoted greater root volume. Notably, bacterial density showed no correlation with root volume.

The increase in bacterial density with rising pH and its decline with decreasing pH are consistent with earlier findings, where neutral soils supported higher bacterial richness compared to acidic soils (Wei et al., 2025). Plant growth-promoting rhizobacteria (PGPR), such as *Azotobacter* and *Azospirillum*, are more active and effective under neutral to slightly alkaline conditions (Artyszak and Gozdowski, 2020). Although

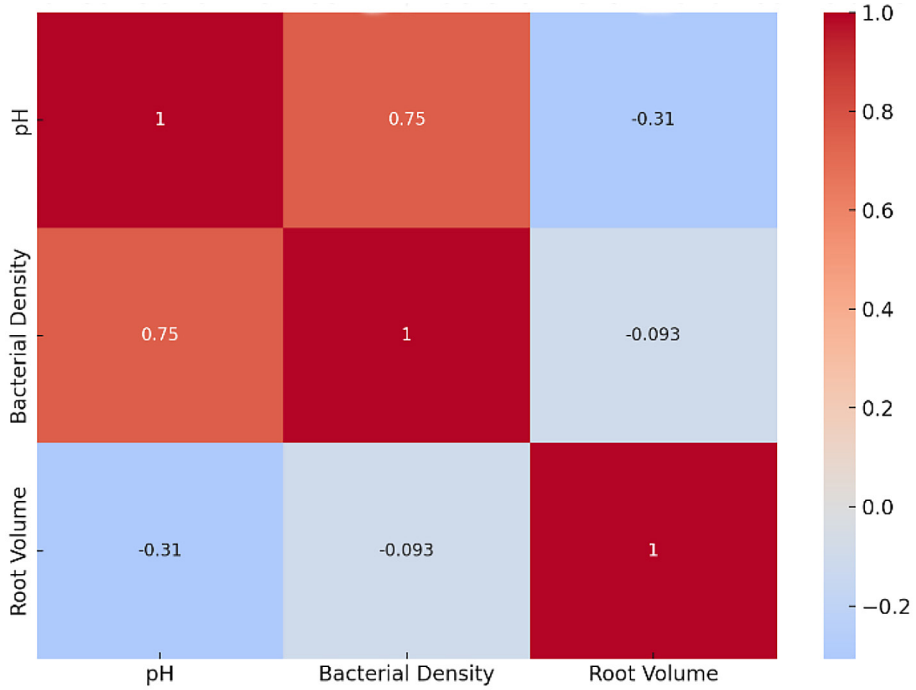

Figure 3. Heatmap of correlation between pH, bacterial density, and root volume

low pH does not completely inhibit bacterial growth, it may affect the growth rate and duration. For instance, *Bacillus cereus* and *Pseudomonas aeruginosa* showed optimal growth at pH 5–6 (6×10^8 CFU/ml), with exponential phases lasting 48 h and 24 h, respectively, while *Escherichia coli* exhibited optimal growth at pH 5 (4×10^{10} CFU/ml), maintaining an exponential phase for 24 h (Razmi et al., 2023).

Despite variations in pH affecting total bacterial counts (TPC), the impact is species-specific, as each bacterium exhibits distinct tolerance to acidic conditions. Even minor shifts in soil pH can significantly alter microbial composition (Li et al., 2023). Soil acidity also influences the diversity, structure, interactions, and functions of rhizosphere bacterial communities. Rhizosphere bacterial functions and structures are more tightly coupled in acidic soils, with crop yield reductions potentially linked to diminished microbial functionality (Wan et al., 2020). Anzalone et al. (2022) reported that tomato plants cultivated in soil had greater rhizosphere bacterial diversity compared to those grown hydroponically in cocopeat substrates. Similarly, Sherpa et al. (2021) highlighted that soil acidity and available phosphorus were the strongest factors shaping Proteobacteria distribution in the rhizosphere. Cordero et al. (2020) further demonstrated that the relative abundance of specific bacterial groups in the rhizosphere correlated with soil pH, silt content, and organic matter levels.

The strong negative correlation between nutrient solution pH and root volume (Figure 3) indicates that elevated pH reduces root volume, while lower pH enhances root growth. Supporting this, Kaiwen et al. (2020) found that *Medicago sativa* grown at high pH (pH 9) exhibited severe root structural damage, whereas plants cultivated at neutral pH (pH 7) showed no such impairment. Extreme reductions in nutrient solution pH can also disrupt plant growth, particularly root development (Gillespie et al., 2021). Generally, the optimal pH range for plant growth, including root development, is between 5.5 and 6.5. Deviations above or below this range may reduce nutrient availability, induce physiological stress in roots, and disturb rhizosphere microbial balance (Balliu et al., 2024).

Bacterial morphology, diversity, and dominance

Macroscopic characterization of bacterial colonies is an essential preliminary step for identifying and classifying bacterial taxa. The macroscopic traits observed included colony color, diameter, colony shape, colony edge, elevation, and opacity (Sheikh et al., 2024). The 23 morphological groups identified in this study exhibited colony colors ranging from milky white, cream, yellow, orange, pink, brick red, to red. Isolates with yellow, orange, pink, brick red, and red pigmentation are likely to produce secondary metabolites in the form of pigments. These bacterial pigments serve diverse ecological and functional roles. For instance, *Chryseobacterium* species produce pigments such as carotenoids, ranging from yellow to reddish-purple, which may enhance plant growth by mitigating environmental stress. Pigmented bacteria are also recognized for their potential in green biotechnology as natural pesticides and bioremediation agents (Orlandi et al., 2022). The red pigment of *Bacillus subtilis*, identified as pulcherrimin, functions as an antimicrobial compound against yeasts, microscopic fungi, and postharvest pathogens (Salo and Novero, 2020). Similarly, *Serratia* species produce the red pigment prodigiosin, which has demonstrated antimicrobial and biocontrol potential (Soenens and Imperial, 2020).

Other macroscopic traits observed included colony diameter, shape, margin, elevation, and internal structure, all of which exhibited substantial variability. No isolates shared identical combinations of margin, elevation, and internal structure. Such morphological variation is influenced by bacterial strain differences as well as environmental conditions, including incubation time, population density, culture media composition, and growth methods (Sousa et al., 2013).

Biodiversity indices are critical tools for quantifying the diversity of organisms in an ecosystem. They represent not only the richness of species present but also the evenness of their distribution within a community. Diversity indices increase both with greater species richness and with higher distributional evenness (Omayio and Mzungu, 2019). Among the most widely applied indices are the Shannon-Wiener Index and the Simpson Index (Sharashy, 2022). Both of which estimate richness, abundance, and dominance within a microbial community.

According to Ulfah et al. (2019), Shannon diversity values ($H' \leq 1$ indicate low diversity, while $1 < H' \leq 3$ represent moderate diversity. In this study, treatments C, E, and M fell within the moderate category. The observed pattern

indicated that treatments with lower nutrient solution concentrations (0-1.6 dS m^{-1}), regardless of substrate type, tended to display relatively higher Shannon-Wiener index values compared with treatments at higher nutrient concentrations. This suggests that reduced nutrient input promotes rhizosphere bacterial diversity. Supporting this, Mejia et al. (2025) reported that reducing fertilizer input by 50% in hydroponic systems, combined with soil-derived inoculum, resulted in higher rhizosphere bacterial diversity and biomass compared to full fertilization (100%).

The Simpson Index provides a measure of dominance within a community. Dominance values of $0.75 < D \leq 1.0$ indicate high dominance, $0.5 < D < 0.75$ indicate moderate dominance, and $0 < D < 0.5$ indicate low dominance (Ulfah et al., 2019). In the present study, no single genus exhibited absolute dominance across treatments. The general pattern observed was that higher bacterial diversity corresponded to lower dominance, whereas lower diversity was associated with higher dominance. Moreover, the use of different substrates did not reveal a consistent trend in either the Shannon-Wiener or Simpson indices.

CONCLUSIONS

Hydroponic cultivation of *L. flava* showed that moderate nutrient concentrations (0.8–1.6 dS m^{-1}) resulted in optimal plant growth, as indicated by higher LAR, RGR, NAR, and HI values compared to other treatments. Rice husk charcoal and a mixed medium of volcanic sand and rice husk charcoal produced higher LAR, LAD, RGR, and HI values than volcanic sand.

Of the 138 bacterial isolates, 23 distinct morphologies were identified, with Shannon–Wiener values ranging from 0.2–1.6 (low to moderate) and Simpson index values from 0.2–0.75 (low to moderate dominance). The highest bacterial density and diversity occurred at low to moderate nutrient concentrations combined with porous substrates (volcanic sand and mixed media). Bacterial density correlated strongly with nutrient pH, while root volume negatively correlated with pH.

Overall, a combination of moderate nutrient concentrations (0.8–1.6 dS m^{-1}) and mixed substrates (volcanic sand + rice husk charcoal) is recommended, as it simultaneously maximizes plant growth and maintains rhizosphere bacterial diversity, thus providing an integrated ecological basis for sustainable hydroponic engineering.

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