

Effects of Different Watering Regimes on Culturable Bacterial Communities in the Rhizosphere of *Terminalia ivorensis* Seedlings in Nursery Systems

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ABSTRACT

Water availability influences seedling growth and rhizosphere bacterial communities in nursery systems. This study evaluated the effects of watering frequency on growth, leaf development, biomass characteristics, and cultivable rhizosphere bacterial populations of *Terminalia ivorensis* seedlings during a 42-day nursery experiment. Twelve seedlings were assigned to daily watering and watering every four days, with six seedlings per treatment. Height, collar diameter, leaf characteristics, leaf biomass, moisture content, dry matter, and bacterial abundance were monitored at seven-day intervals, while isolates were provisionally characterized using cultural, morphological, and biochemical methods. Initial measurements were comparable, with seedling height of 42.50 ± 7.04 cm and 44.00 ± 7.45 cm, respectively, and identical leaf numbers of 314.50 ± 62.49 . By day 42, daily watering produced greater height (61.00 ± 2.86 cm), collar diameter (12.50 ± 1.51 mm), and leaf number (554.50 ± 99.00) than four-day watering (54.50 ± 13.02 cm, 8.00 ± 0.76 mm, and 411.50 ± 1.00 , respectively). Daily watering also increased leaf length, width, individual leaf area, and total leaf area. Fresh and dry leaf biomass, moisture, and dry matter did not differ significantly. Bacterial abundance increased to 1.61×10^7 CFU/g (7.21 Log_{10} CFU/g) under daily watering but declined to 8.50×10^3 CFU/g (3.93 Log_{10} CFU/g) under four-day watering. *Bacillus* spp. predominated among recovered isolates, although bacterial genus distribution did not differ significantly between treatments. Principal Coordinates Analysis demonstrated separation between watering regimes (94.52% of variation). Findings indicate that consistent water availability promotes vegetative development and maintains higher rhizosphere bacterial abundance during nursery establishment.

Keywords: *Terminalia ivorensis*, Watering Frequency, Rhizosphere Bacteria, Seedling Growth, Bacterial Abundance.

Introduction

Water availability is one of the most important environmental factors regulating seedling establishment, growth and survival in nursery systems. Adequate water is required for nutrient transport, photosynthesis, cell expansion and maintenance of cellular turgidity. However, insufficient or excessive watering can negatively affect plant development by altering soil moisture, aeration, nutrient availability and microbial activity (Chieb and Gachomo, 2023). These effects are particularly important within the rhizosphere, the narrow zone of soil directly influenced by plant roots, where roots, microorganisms and nutrients interact continuously. Bandopadhyay et al. (2024) reported that drought produced distinct changes in active rhizosphere bacterial communities, demonstrating that moisture conditions can influence microorganisms through changes in plant physiology.

This relationship is especially important in nursery systems, where irrigation can be controlled and differences in watering frequency can create moisture environments. Such conditions provide an opportunity to examine bacterial responses under uniform conditions. *Terminalia ivorensis* A. Chev., commonly known as “Idigbo”, is an economically valuable tropical timber species native to West and Central Africa. The species is valued for its wood (Bamigboye et al., 2026). Successful establishment of *T. ivorensis* depends on producing healthy and vigorous seedlings. Although previous studies have examined watering frequency in relation to germination and early growth of *T. ivorensis*, little attention has been given to its rhizosphere bacterial communities under different moisture conditions.

The rhizosphere is a biologically active environment containing diverse bacterial populations. Rhizosphere bacteria contribute to organic matter decomposition, nutrient mineralization, and nitrogen transformation.

Rhizosphere bacteria also contribute to phosphorus solubilization and production of compounds that stimulate plant growth. Plant roots also influence microbial populations through root exudates. These substances provide resources for microorganisms and can influence bacterial establishment (Chen & Liu, 2024).

Consequently, factors that change root activity or soil moisture may modify rhizosphere bacterial communities. Huang et al. (2024) observed that drought stress altered rhizosphere bacterial community structure in cassava and indicated that particular bacterial groups were associated with plant responses to water limitation.

Watering regime may therefore function as an important ecological factor controlling bacterial community development around seedlings. Frequent watering can maintain higher soil moisture and support microbial activity, whereas excessive water application may reduce soil aeration and alter bacterial community structure (Li et al. 2024). Conversely, prolonged intervals between watering can decrease microbial activity and alter nutrient and root-exudate availability. Benmrid et al. (2024) reported that drought-tolerant plant growth-promoting rhizobacteria can support plants under drought through mechanisms involving nutrient acquisition, phytohormonal regulation, root development and stress tolerance.

Moisture fluctuations may also affect microbial communities beyond water limitation. Repeated drying and rewatering under different nursery irrigation schedules can influence microbial abundance, community composition and interactions. Rehman et al. (2024) reported that rehydration following extreme drought produced substantial changes in rhizosphere microbial communities and network characteristics. Such findings suggest that watering regimes should be considered as factors shaping the biological environment around roots.

Despite increasing interest in plant-microbiome interactions under water stress, information on how controlled watering regimes shape rhizosphere bacterial communities of tropical timber seedlings remains limited. This gap is particularly relevant to *T. ivorensis* because nursery establishment supports plantation development, restoration and timber production. Understanding bacterial responses to irrigation may provide useful insight into biological processes supporting seedling growth and adaptation.

Therefore, this study investigates the effects of different watering regimes on the rhizosphere bacterial community of *T. ivorensis* seedlings in nursery systems. The study seeks to determine how differences in water application influence bacterial abundance and community characteristics associated with developing roots. The findings are expected to contribute to improved nursery water-management practices and a better understanding of plant-microbe interactions during early *T. ivorensis* establishment. Integrating irrigation management with knowledge of rhizosphere bacterial communities may ultimately support production of vigorous *T. ivorensis* seedlings capable of successful establishment under variable environmental conditions.

Materials and Methods

Study Area

The study was conducted in a nursery facility within Igbinedion University community, Okada, Ovia North-East Local Government Area, Edo State, Nigeria. It is located approximately at 6°43'49"N and 5°23'45"E and has a tropical rainforest climate. Annual rainfall is about 1,783 mm, while mean annual temperature is approximately 25.9°C (Agbonaye and Izinyon, 2025). Rainfall occurs mainly from April to October, supporting seedling growth.

Experimental Design

The experiment followed a completely randomized design (CRD). After establishment, uniform seedlings were randomly assigned to two watering regimes: daily watering and watering once every four days. Each watering event supplied 500 mL of water, measured with a graduated cylinder. Pot positions were periodically rearranged to minimize differences associated with light, temperature and other nursery micro-environmental conditions.

Seed Collection and Seedling Propagation

Mature *T. ivorensis* seeds were obtained from Okada, Edo State. Damaged, diseased and abnormal seeds were discarded, while eighty-four healthy seeds were retained. Seeds were cleaned, surface-disinfected in 1% sodium hypochlorite for 2–3 minutes, and rinsed with sterile distilled water. They were sown at approximately 2 cm depth in polypots containing homogenized topsoil, river sand and compost at a 3:1:1 ratio.

Seedlings were maintained under uniform moisture, shade, temperature and humidity and protected from pests following Salami et al. (2023). Uniform seedlings were subsequently selected for the watering experiment. No chemical fertilizer or antimicrobial treatment was applied.

Measurement of Seedling Growth

Plant height was measured from the soil surface to the apical growing point using a graduated ruler (Chisa et al., 2025). Collar diameter was measured at the stem-soil junction with a digital vernier caliper (Musa et al., 2025). Fully expanded leaves were counted directly. Leaf area was estimated using the maximum length $\times$ maximum width method (Yan et al., 2024). Leaves were placed flat without folding or overlapping. Maximum length was measured from the leaf base to the tip, and maximum width across the widest part of the blade. Leaf area (LA) was calculated as:

$$LA = L \times W \times K \quad \dots\dots\dots (1)$$

where LA is leaf area (cm²), L is maximum leaf length (cm), W is maximum leaf width (cm), and K is the correction factor. An adopted correction factor of 0.75 was applied.

Mean leaf area was calculated by dividing the summed leaf areas by the number of leaves measured and expressed as cm² per leaf, while total leaf area per seedling was calculated using the equation:

$$TLA = \sum_{i=1}^n LA_i \quad \dots\dots\dots (2)$$

Where TLA is total leaf area (cm²/plant) and LA_i is the area of the *i*th leaf.

Leaf Biomass Determination

Fresh leaves were used to determine biomass as previously described by (Berruto and Demirel 2024). Leaves were detached with clean scissors, inspected for adhering debris and blotted with clean paper towels. Fresh weight was measured using a calibrated analytical balance. Samples were transferred into labelled crucibles and dried in a hot-air oven at 70°C for 24 hours. Drying and weighing continued at 6–12-hour intervals until constant weight, defined by two successive measurements differing by less than 0.01 g. The final value represented dry leaf biomass. Moisture content was calculated as:

$$\text{Moisture content (\%)} = [(W_f - W_d) / W_f] \times 100 \quad \dots\dots\dots (3)$$

Where W_f is fresh sample weight (g) and W_d is dry sample weight (g).

Dry matter was calculated as:

$$\text{Dry matter (\%)} = (W_d / W_f) \times 100 \quad \dots\dots\dots (4)$$

Moisture content plus dry matter equalled 100%.

Total dry leaf biomass was estimated from the fresh-to-dry weight relationship. Mean biomass was calculated for each seedling and treatment. Values were expressed as grams of fresh or dry biomass per seedling. Dry biomass was used for statistical comparisons because it minimizes variation associated with tissue water content.

Collection of Rhizosphere Soil

Rhizosphere sampling followed Edwards et al. 2018. At the end of the experiment, seedlings were carefully removed from their pots. Loosely attached soil was removed gently, while soil closely adhering to roots was regarded as rhizosphere soil. Ten grams was aseptically collected from each seedling with a sterile spatula and transferred into sterile labelled containers. Samples were processed for microbiological analyses within four hours.

Enumeration of Cultivable Rhizosphere Bacteria

Cultivable bacteria were enumerated by serial dilution and spread plating following the methods of Jiang et al. (2023). Ten grams of rhizosphere soil was transferred into 90 mL sterile physiological saline and homogenized thoroughly. Ten-fold serial dilutions were prepared, and dilutions were inoculated onto nutrient agar. Approximately 0.1 mL was spread evenly over each agar surface using sterile spreaders. Plates were incubated aerobically, and countable plates were used for enumeration. Results were expressed as colony-forming units per gram (CFU/g). Bacterial abundance was determined as colony-forming units per gram of rhizosphere soil (CFU/g) and expressed as Log₁₀ CFU/g for comparative analysis.

Isolation and Characterization of Bacterial Isolates

Distinct colonies differing in colour, size, shape, elevation, margin and surface characteristics were selected. Colonies were repeatedly subcultured on fresh agar until pure cultures were obtained.

Pure isolates were characterized and identified using conventional methods (Krieg and Holt, 1984; Cheesbrough, 2006), including Gram staining, cell morphology and appropriate biochemical tests.

Statistical Analysis

Data from seedling growth and bacterial counts were entered into Microsoft Excel and analyzed using Number Cruncher Statistical Software (NCSS), version 12. Differences among watering treatments in seedling growth and cultivable bacterial abundance were evaluated using one-way analysis of variance (ANOVA). Statistical significance was accepted at $P < 0.05$.

Results

Table 1 presents the initial growth characteristics of *T. ivorensis* seedlings that were assessed in six seedlings under each of two watering regimes: daily watering and watering every four days. Measurements of seedling height, collar diameter, and number of leaves were obtained at the beginning of the experiment to establish baseline morphological characteristics before evaluating the effects of watering frequency on subsequent plant growth and rhizosphere bacterial communities. Seedlings subjected to daily watering had an initial mean height of 42.50 ± 7.04 cm, whereas those watered every four days recorded a slightly higher mean height of 44.00 ± 7.45 cm. This represented a mean difference of 1.50 cm, equivalent to approximately 3.5% greater height in the four-day watering group. However, initial collar diameter differed only marginally between treatments, with mean values of 8.50 ± 0.58 mm for daily watering and 8.40 ± 0.62 mm for four-day watering. The difference was only 0.10 mm.

Similarly, both treatments recorded an identical mean initial leaf number of 314.50 ± 62.49 leaves, indicating no numerical difference in foliar development at baseline. Independent-samples *t*-tests were performed at a significance level of $\alpha = 0.05$ to determine whether the observed baseline differences were statistically significant. Initial seedling height did not differ significantly between treatments ($t = -0.395$, $df = 10$, $p = 0.701$). Although the four-day treatment had a slightly higher mean height, the difference was small relative to within-group variability, with a Cohen's *d* of approximately 0.21, indicating a small effect. Collar diameter also showed no significant difference ($t = 0.322$, $df = 10$,

$p = 0.754$), with a Cohen's *d* of approximately 0.17, representing a very small effect. Initial leaf number was identical between treatments, producing $t = 0.000$, $df = 10$, and $p = 1.000$. Overall, the results indicate that seedlings were broadly comparable in height, collar diameter, and leaf number before the experimental watering regimes commenced.

Table 1: Initial growth characteristics of *Terminalia ivorensis* seedlings

Parameter	Treatment (n = 6)	
	Daily watering	Watering every 4 days
Initial height (cm)	42.50 ± 7.04	44.00 ± 7.45
Initial collar diameter (mm)	8.50 ± 0.58	8.40 ± 0.62
Initial number of leaves	314.50 ± 62.49	314.50 ± 62.49

The effect of watering frequency on growth characteristics of *T. ivorensis* seedlings is as presented in Table 2; under daily watering and watering every four days, using six seedlings per treatment. Seedling height, collar diameter, and number of leaves were monitored at seven-day intervals. Seedling height generally increased throughout the experiment following an initial decline at day 7. Daily-watered seedlings increased from 42.50 cm initially to 61.00 cm at day 42, representing a 43.5% net increase. Seedlings watered every four days increased from 44.00 to 54.50 cm, equivalent to 23.9%. Although daily watering produced greater numerical height gains, differences between treatments were not statistically significant at any observation period ($p > 0.05$), partly because of variability within treatments.

Collar diameter showed a clearer response to watering frequency. Both treatments began at approximately 8.50 mm, followed by an initial decline at day 7. Thereafter, collar diameter increased progressively, reaching 12.50 mm under daily watering and 8.00 mm under four-day watering by day 42. Differences became statistically significant from day 21 ($p = 0.035$), increased at day 28 ($p = 0.004$), and were highly significant at days 35 and 42 ($p < 0.001$). These findings demonstrate a stronger influence of watering frequency on stem thickening than on height growth. Leaf number also fluctuated initially before increasing under both treatments.

Daily watering produced an increase from 314.50 leaves at day 0 to 554.50 at day 42, whereas four-day watering increased from 314.50 to 411.50 leaves. The respective net increases were 76.3% and 30.8%. Significant differences occurred at days 7, 35, and 42. Daily watering promoted greater final values for height, collar diameter, and leaf production.

However, statistically significant effects were observed primarily for collar diameter and leaf number, indicating that watering frequency exerted a stronger demonstrable influence on structural development and foliage production than on seedling height during the experimental period.

Table 2: Effect of Watering Frequency on selected growth characteristics of *Terminalia ivorensis* seedlings during the 42-day experiment

Days of experiment	Height(cm)		Collar diameter(cm)		Number of leaves	
	Daily Watering	Watering every4 days	Daily Watering	Watering every4 days	Daily Watering	Watering every4 days
	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6
0	42.50 ± 7.04	44.00 ± 7.45	8.50 ± 0.58	8.50 ± 0.62	314.50 ± 62.49	314.50 ± 62.49
7	33.00 ± 1.62	35.50 ± 8.06	6.00 ± 0.60	5.50 ± 0.60	294.00 ± 49.85	229.00 ± 0.00
14	38.50 ± 1.86	40.00 ± 8.89	6.50 ± 0.50	6.00 ± 0.65	282.50 ± 49.85	248.00 ± 2.00
21	44.50 ± 2.12	43.00 ± 9.90	7.50 ± 0.71	6.50 ± 0.71	339.00 ± 99.00	267.00 ± 2.83
28	50.00 ± 2.44	47.00 ± 10.91	8.50 ± 0.75	7.00 ± 0.66	377.00 ± 99.00	286.00 ± 0.00
35	55.50 ± 2.65	50.50 ± 12.01	11.00 ± 1.41	7.50 ± 0.71	510.00 ± 49.85	305.50 ± 1.00
42	61.00 ± 2.86	54.50 ± 13.02	12.50 ± 1.51	8.00 ± 0.76	554.50 ± 99.00	411.50 ± 1.00

Table 3 presents the effects of daily watering and watering every four days on leaf development of *T. ivorensis* seedlings. Five characteristics were assessed at seven-day intervals: mean leaf length, maximum leaf width, individual leaf area, number of leaves per seedling, and total leaf area per seedling. Six seedlings were evaluated under each watering regime. At day 0, seedlings in both treatments had comparable leaf characteristics, with no statistically significant differences. Mean leaf length, maximum width, individual leaf area, leaf number, and total leaf area were similar between treatments, indicating comparable baseline conditions. During the first week, daily watering began producing modestly greater values for most characteristics. Although leaf number declined under both regimes, the decline was greater under four-day watering. Significant differences were detected in leaf number and total leaf area at day 7. By day 14, leaf dimensions and total leaf area had increased in both treatments. Daily-watered seedlings generally recorded higher values, and total leaf area was significantly greater than that of seedlings watered every four days. This trend continued at day 21, when significant differences emerged in individual leaf area and total leaf area. The results indicate that more frequent water availability increasingly supported leaf expansion and overall foliage development.

At day 28, the divergence between treatments became more pronounced. Daily watering produced significantly greater maximum leaf width, individual leaf area, and total leaf area, although differences in leaf length and leaf number remained statistically non-significant.

By day 35, all five measured leaf characteristics differed significantly between treatments. Daily watering resulted in greater leaf dimensions, individual leaf area, leaf number, and total leaf area, demonstrating an increasingly strong response to watering frequency. At day 42, daily watering produced the highest values for every measured characteristic. Mean leaf length, maximum width, individual leaf area, leaf number, and total leaf area were significantly greater under daily watering than under four-day watering. Compared with the four-day regime, daily watering produced approximately 17.4% greater leaf length, 37.5% greater maximum width, 61.4% greater individual leaf area, 34.7% more leaves, and 47.4% greater total leaf area. Overall, the findings demonstrate progressive divergence in leaf development between watering regimes throughout the experiment. While differences were limited during the early stages, daily watering increasingly enhanced leaf expansion, leaf production, and total foliage accumulation.

Table 3: Leaf Characteristics of *Terminalia ivorensis* Seedlings during the 42-Day Experiment

Days	Mean length (cm)		Mean maximum width (cm)		Mean leaf area (cm ² /leaf)		Number of leaves/seedling		Total leaf area	
	Daily watering	Watering every 4 days	Daily watering	Watering every 4 days	Daily watering	Watering every 4 days	Daily watering	Watering every 4 days	Daily watering	Watering every 4 days
	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6
0	8.50 ± 0.72	8.60 ± 0.76	3.20 ± 0.28	3.20 ± 0.30	20.40 ± 2.84	20.64 ± 2.96	314.50 ± 62.49	314.50 ± 62.49	6,415.80 ± 1,124.30	6,491.28 ± 1,156.20
7	8.80 ± 0.69	8.70 ± 0.74	3.35 ± 0.27	3.15 ± 0.29	22.11 ± 2.71	20.55 ± 2.83	294.00 ± 49.85	229.00 ± 0.00	6,500.34 ± 1,035.40	4,706.81 ± 614.80
14	9.70 ± 0.73	9.30 ± 0.79	3.70 ± 0.29	3.40 ± 0.31	26.92 ± 3.05	23.71 ± 3.12	282.50 ± 49.85	248.00 ± 2.00	7,604.19 ± 1,105.60	5,881.32 ± 782.10
21	10.60 ± 0.81	9.90 ± 0.85	4.05 ± 0.32	3.60 ± 0.33	32.20 ± 3.28	26.73 ± 3.31	339.00 ± 99.00	267.00 ± 2.83	10,914.95 ± 1,683.40	7,136.91 ± 876.40
28	11.50 ± 0.91	10.50 ± 0.92	4.45 ± 0.35	3.75 ± 0.35	38.38 ± 3.62	29.53 ± 3.48	377.00 ± 99.00	286.00 ± 0.00	14,469.73 ± 2,095.60	8,445.94 ± 995.30
35	12.60 ± 1.04	11.10 ± 0.98	4.95 ± 0.39	3.90 ± 0.36	46.78 ± 3.94	32.47 ± 3.61	510.00 ± 49.85	305.50 ± 1.00	23,856.52 ± 2,793.50	9,918.82 ± 1,104.20
42	13.50 ± 1.18	11.50 ± 1.02	5.50 ± 0.43	4.00 ± 0.37	55.69 ± 4.26	34.50 ± 3.74	554.50 ± 99.00	411.50 ± 1.00	30,878.72 ± 1,421.48	14,196.75 ± 1,975.30

Table 4: Leaf Biomass and Moisture Characteristics of Seedlings under Different Watering Frequencies

Days of Experiment	Fresh leaf biomass (g/seedling)		Dry leaf biomass (g/seedling)		Moisture content (%)		Dry matter (%)	
	Daily watering	Watering every 4 days	Daily watering	Watering every 4 days	Daily watering	Watering every 4 days	Daily watering	Watering every 4 days
	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6
0	0.96 ± 0.18	0.95 ± 0.17	0.30 ± 0.06	0.30 ± 0.05	68.75 ± 3.86	68.42 ± 4.12	31.25 ± 3.86	31.58 ± 4.12
7	0.88 ± 0.17	0.81 ± 0.15	0.28 ± 0.05	0.27 ± 0.04	68.18 ± 4.25	66.67 ± 5.08	31.82 ± 4.25	33.33 ± 5.08
14	0.94 ± 0.19	0.86 ± 0.17	0.30 ± 0.06	0.29 ± 0.05	68.09 ± 4.38	66.28 ± 5.42	31.91 ± 4.38	33.72 ± 5.42
21	1.05 ± 0.22	0.94 ± 0.18	0.34 ± 0.07	0.31 ± 0.05	67.62 ± 4.17	67.02 ± 5.91	32.38 ± 4.17	32.98 ± 5.91
28	1.16 ± 0.25	1.02 ± 0.19	0.37 ± 0.08	0.34 ± 0.06	68.10 ± 4.05	66.67 ± 6.13	31.90 ± 4.05	33.33 ± 6.13
35	1.29 ± 0.32	1.17 ± 0.22	0.41 ± 0.09	0.39 ± 0.06	68.22 ± 4.17	66.67 ± 6.87	31.78 ± 4.17	33.33 ± 6.87
42	1.41 ± 0.41	1.36 ± 0.25	0.44 ± 0.11	0.45 ± 0.05	68.28 ± 4.23	66.32 ± 7.80	31.72 ± 5.84	33.69 ± 2.80

The statistically significant differences observed from days 7 through 42, particularly for total leaf area, indicate that watering frequency substantially influenced vegetative development. Thus, daily watering was associated with superior leaf growth and greater canopy development in *T. ivorensis* seedlings during the 42-day nursery period.

The effects of daily watering and watering every four days on leaf biomass, moisture content and dry matter of *T. ivorensis* seedlings are shown in Table 4. The findings revealed progressive biomass accumulation under both watering regimes, although differences between treatments were not statistically significant. At the beginning of the experiment, fresh leaf biomass was comparable between daily-watered seedlings (0.96 ± 0.18 g/seedling) and seedlings watered every four days (0.95 ± 0.17 g/seedling). Both treatments experienced slight reductions during the first week, followed by gradual increases throughout the remaining experimental period. By day 42, fresh biomass reached 1.41 ± 0.41 g/seedling under daily watering and 1.36 ± 0.25 g/seedling under four-day watering, representing increases of 46.9% and 43.2%, respectively. Although daily watering generally supported slightly greater fresh biomass, the differences remained statistically insignificant. Dry leaf biomass followed a similar developmental pattern, declining slightly during the initial week before increasing steadily. At day 42, seedlings watered daily recorded 0.44 ± 0.11 g/seedling, compared with 0.45 ± 0.05 g/seedling under four-day watering.

These closely comparable values indicated that both treatments supported similar dry biomass accumulation despite differences in watering frequency. Leaf moisture content remained relatively stable throughout the experiment, ranging between 67.62% and 68.75% under daily watering and between 66.28% and 68.42% under four-day watering. At day 42, moisture content was $68.28 \pm 4.23\%$ and $66.32 \pm 7.80\%$, respectively. Corresponding dry matter percentages were $31.72 \pm 15.84\%$ and $33.69 \pm 2.80\%$. These findings suggest that seedlings maintained relatively consistent leaf hydration and dry matter proportions under both treatments. Welch's independent-samples t-tests revealed no statistically significant differences between watering regimes for any measured parameter throughout the experiment ($p > 0.05$). At day 42, the probability values for fresh biomass, dry biomass, moisture content and dry matter were 0.805, 0.845, 0.604 and 0.776, respectively.

Table 5 presents cultivable heterotrophic bacterial populations in the rhizosphere soil of *T. ivorensis* seedlings subjected to daily watering and watering at four-day intervals over a 42-day experimental period. At the beginning of the experiment, bacterial populations were similar between treatments. Daily-watered seedlings recorded $5.88 \times 10^5 \pm 5.30 \times 10^4$ CFU/g ($5.77 \text{ Log}_{10} \text{ CFU/g}$), while the four-day treatment recorded $6.25 \times 10^5 \pm 3.54 \times 10^4$ CFU/g ($5.80 \text{ Log}_{10} \text{ CFU/g}$). This similarity indicated comparable initial microbial conditions before the watering treatments were established.

Table 5: Cultivable bacterial population in rhizosphere soil during the experiment

Days of experiment	Cultivable bacterial population in rhizosphere soil during the experiment			
	Daily watering		Watering every 4 days	
	CFU/g	Log ₁₀ CFU/g	CFU/g	Log ₁₀ CFU/g
	n = 6	n = 6	n = 6	n = 6
0	587500 ± 53033	5.77	625000 ± 35355	5.80
7	825000 ± 106066	5.92	27125 ± 1237	4.43
14	887500 ± 53033	5.95	23500 ± 1061	4.37
21	1037500 ± 229810	6.02	26500 ± 6717	4.42
28	1112500 ± 88388	6.05	14125 ± 2298	4.15
35	1325000 ± 106066	6.12	12000 ± 2475	4.08
42	16125000 ± 1237437	7.21	8500 ± 1061	3.93

Following treatment initiation, contrasting bacterial population patterns emerged. Under daily watering, bacterial abundance increased gradually from 5.92 Log₁₀ CFU/g on day 7 to 6.12 Log₁₀ CFU/g on day 35. A marked increase occurred by day 42, when the population reached $1.61 \times 10^7 \pm 1.24 \times 10^6$ CFU/g, equivalent to 7.21 Log₁₀ CFU/g. This represented approximately a 27-fold increase relative to the initial population. In contrast, four-day watering produced a substantial and progressive decline in bacterial abundance. The population decreased from 5.80 Log₁₀ CFU/g at day 0 to 4.43 Log₁₀ CFU/g at day 7 and continued declining throughout the experiment. By day 42, only $8.50 \times 10^3 \pm 1.06 \times 10^3$ CFU/g (3.93 Log₁₀ CFU/g) remained. Consequently, the bacterial population under daily watering was approximately 1,897 times greater than that under four-day watering at the end of the experiment. Welch's independent-samples *t*-test showed no significant difference between treatments at day 0 (*p* = 0.185).

However, significant differences emerged from day 7 and persisted through day 42, with all subsequent comparisons showing *p* < 0.001. These results demonstrate clear statistical separation between watering treatments following the establishment of the experimental conditions.

Fifteen isolates were examined at each sampling point and provisionally identified using cultural appearance, cellular morphology, Gram staining and biochemical characteristics of cultivable bacterial isolates recovered from the rhizosphere soil of *T. ivorensis* seedlings exposed to daily watering and watering at four-day intervals revealed the presence of *Bacillus* spp., *Pseudomonas* spp., *Enterobacter* spp., *Micrococcus* spp., *Staphylococcus* spp., and *Serratia* spp. The percentage frequency of occurrence of the bacterial isolates in the rhizosphere is as presented in Figure 1.

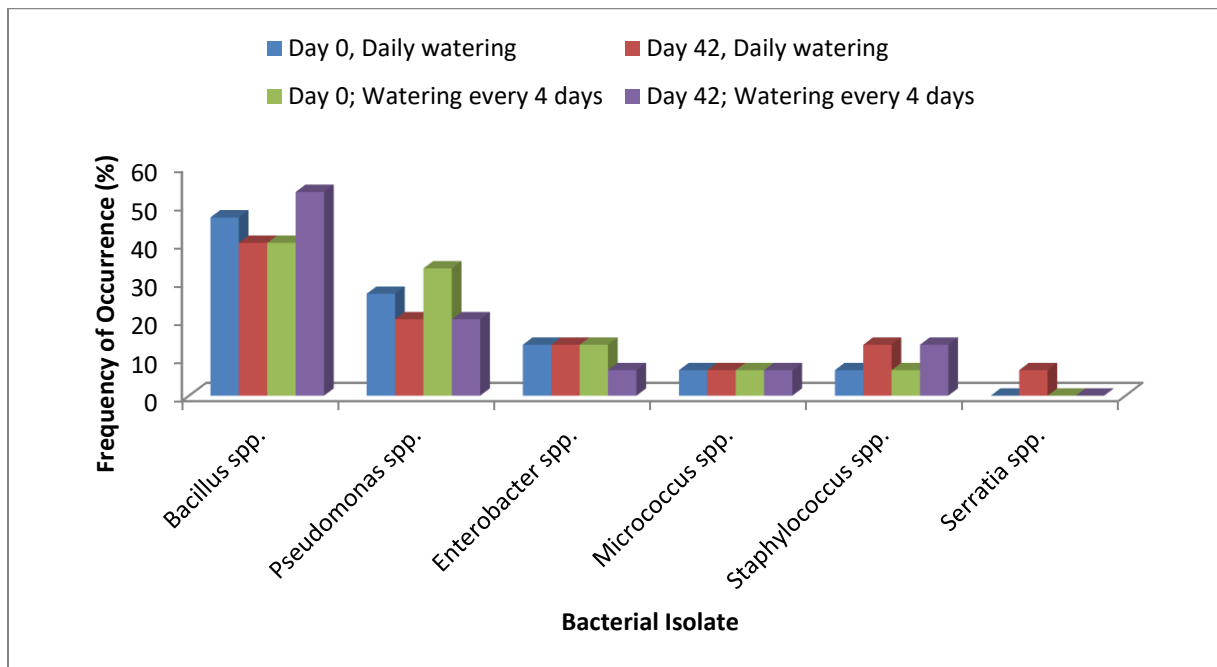


Figure 1: Frequency (%) of occurrence of the bacterial isolates in the rhizosphere soil of *T. ivorensis* seedlings

At the beginning of daily watering, *Bacillus* spp. were dominant, comprising 46.67% of isolates, followed by *Pseudomonas* spp. at 26.67%. *Enterobacter* spp. represented 13.33%, while *Micrococcus* and *Staphylococcus* each accounted for 6.67%. After 42 days, *Bacillus* remained predominant at 40.00%, while *Pseudomonas* declined to 20.00%.

Enterobacter remained at 13.33%, *Staphylococcus* increased to 13.33%, and *Serratia* appeared at 6.67%. Under four-day watering, the initial distribution was similarly dominated by *Bacillus* spp. at 40.00%, followed by *Pseudomonas* spp. at 33.33%. *Enterobacter* accounted for 13.33%, and *Micrococcus* and *Staphylococcus* each represented 6.67%.

By day 42, *Bacillus* increased to 53.33%, whereas *Pseudomonas* decreased to 20.00% and *Enterobacter* to 6.67%. *Staphylococcus* remained at 13.33%, while *Micrococcus* represented 6.67%; *Serratia* was not recovered.

Pearson chi-square analysis showed no significant difference in bacterial distribution between watering regimes at day 0 ($\chi^2 = 0.188$, $df = 4$, $p = 0.996$) or day 42 ($\chi^2 = 1.619$, $df = 5$, $p = 0.899$). Overall, *Bacillus* spp. consistently predominated, while other genera occurred less frequently.

Although numerical shifts were observed, watering frequency did not significantly alter the categorical distribution of recovered bacterial genera.

Figure 2 shows the heatmap describing the shared growth characteristics of *T. ivorensis* at Day 42. The heatmap compares the relative direction of each measured characteristic between the two watering regimes using standardized values ranging from -1 to +1.

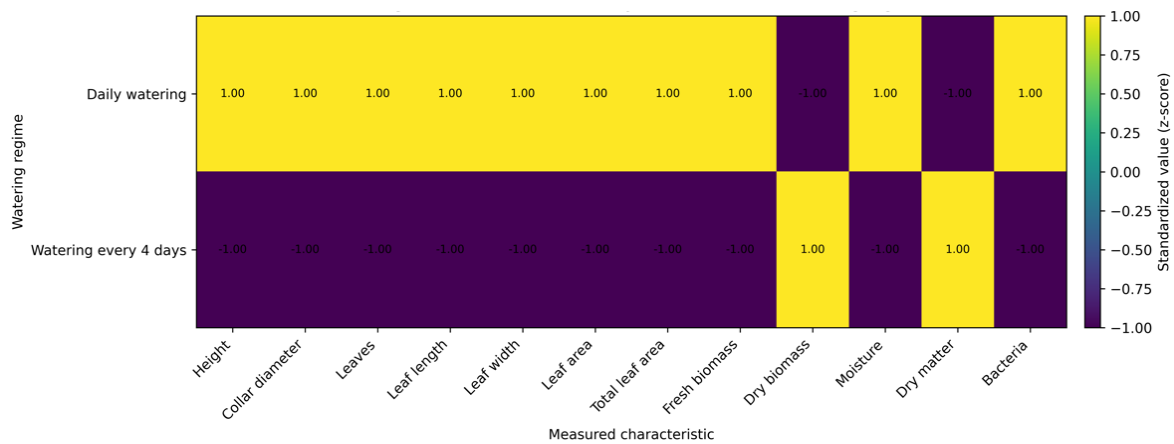


Figure 2: Shared growth characteristics at day 42 under the two watering regimes

The heatmap revealed distinct patterns in the growth, leaf, biomass, moisture, and rhizosphere bacterial characteristics of *Terminalia ivorensis* seedlings subjected to daily watering (0 to +1) and watering every four days (0 to -1). Daily watering was associated with higher standardized values for most morphological and microbial characteristics, particularly collar diameter, leaf development, total leaf area, fresh biomass, moisture content, and cultivable bacterial abundance (0 to +1).

In contrast, dry leaf biomass and dry matter showed slightly higher values under four-day watering (0 to +1), although these differences were not statistically significant. Welch's *t*-tests confirmed significant treatment differences for collar diameter, leaf number, leaf length, leaf width, individual leaf area, total leaf area, and bacterial population.

Seedling height, fresh and dry biomass, moisture content, and dry matter did not differ significantly.

Overall, the findings indicate that regular watering generally promoted vegetative development and substantially increased cultivable rhizosphere bacterial abundance, while intermittent watering produced limited differences in biomass-related characteristics at day 42.

Multivariate response of *Terminalia ivorensis* seedlings to watering regime: Principal Coordinates Analysis (PCoA) shown in Figure 3 provided an integrated view of changes in the growth, leaf, biomass, and cultivable rhizosphere bacterial characteristics of *Terminalia ivorensis* seedlings under daily watering and watering every four days.

The first two axes explained 94.52% of the total variation, with axis 1 accounting for 70.59% and axis 2 for 23.93%. Both treatments started from relatively similar positions at day 0, indicating comparable initial characteristics.

However, their trajectories progressively diverged throughout the 42-day experiment. Daily-watered seedlings moved toward increasingly negative values on axis 1, whereas seedlings watered every four days shifted mainly toward positive values on axis 2. The greatest separation occurred at days 35 and 42, indicating substantial treatment-associated changes in the combined characteristics.

Univariate analyses supported this pattern, with significant differences observed in collar diameter, leaf number, leaf dimensions, total leaf area, and bacterial abundance. Thus, watering frequency progressively influenced the overall multivariate response of seedlings.

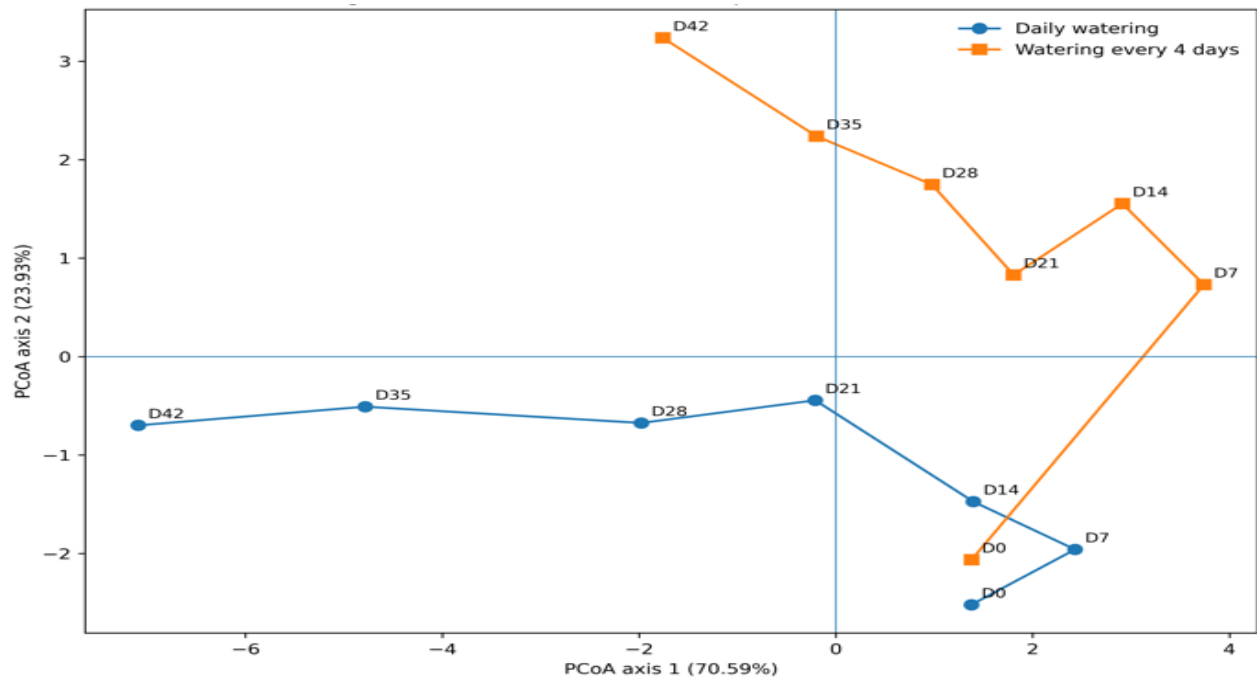


Figure 3: PCoA of growth, leaf, biomass, and rhizosphere bacterial characteristics

Discussion

Terminalia ivorensis seedlings began the experiment in broadly comparable condition under daily watering and watering every four days. Initial height, collar diameter and leaf number did not differ significantly, providing a useful baseline for evaluating later plant and microbial responses.

Bandopadhyay et al. (2024) demonstrated that drought can alter active rhizosphere bacterial communities through interacting plant-mediated and environmental processes. Williams et al. (2022) likewise associated increasing drought severity with changes in root-exudate concentration and composition, while Cheng et al. (2024) showed that plant nutritional and signalling status can alter exudates and bacterial community assembly.

Over 42 days, watering frequency increasingly influenced seedling development. Daily watering produced greater collar diameter and leaf development, with collar diameter becoming significantly different from day 21 and reaching 12.50 ± 1.51 mm under daily watering compared with 8.00 ± 0.76 mm under four-day watering at day 42. Yadav et al. (2025) and Yadav et al. (2024) described water limitation as involving coordinated changes in roots, stomatal regulation, nutrient acquisition, hormones and plant-microbe interactions.

Leaf development followed a similar trajectory: daily watering generally promoted greater leaf length, width, individual area, leaf number and total canopy area. At day 42, individual leaf area was 55.69 ± 4.26 cm² under daily watering and 34.50 ± 3.74 cm² under four-day watering.

Vadez et al. (2024) emphasized coordinated adjustments in growth, water acquisition and resource-use strategies during drought. Because soil-water and plant-water potentials were not measured, the four-day treatment is more appropriately described as reduced or intermittent watering rather than severe drought.

Leaf biomass increased under both treatments. Fresh biomass reached 1.41 ± 0.41 g/seedling with daily watering and 1.36 ± 0.25 g/seedling with four-day watering, while dry biomass, moisture content and dry matter showed no significant treatment differences. The relatively stable leaf moisture suggests that seedlings maintained tissue hydration despite contrasting irrigation frequencies. Olaoye and Oyun (2019) similarly reported that watering frequency affected early growth of indigenous tree species, including *T. ivorensis*. Thus, the biomass findings indicate physiological adjustment rather than severe deterioration.

The clearest treatment response occurred in cultivable heterotrophic bacteria. Initial populations were similar, at 5.77 and 5.80 Log₁₀ CFU/g for daily and four-day watering, respectively. By day 42, daily watering increased abundance to 7.21 Log₁₀ CFU/g, whereas four-day watering reduced it to 3.93 Log₁₀ CFU/g. Differences were nonsignificant initially but became highly significant from day 7 onward. Bandopadhyay et al. (2024) and Liu et al. (2025) support the importance of soil-moisture availability and temporal stability in regulating rhizosphere microbial communities. Regular moisture can facilitate microbial metabolism, nutrient diffusion and substrate movement, whereas drying can restrict activity and impose osmotic stress. Plant-mediated processes may have contributed to the bacterial response. Shafi and Shahid (2025) emphasized that root exudates influence microbial recruitment, nutrient cycling and community assembly. Fu et al. (2024) similarly described drought-induced changes in root exudates as important mediators of carbon transfer and plant–soil–microbe interactions. Bhattacharyya et al. (2025) demonstrated that water-stressed wheat root exudates containing compounds such as choline and glycine betaine supported osmotic-stress tolerance in *Pseudomonas* and enhanced biofilm-associated protection. Zhang et al. (2025) further reported drought-related changes in rhizosphere microbial strategies in *Syringaoblata*.

These findings provide plausible mechanisms for contrasting bacterial responses, although root exudates were not measured.

The culture-dependent bacterial assessment recovered six presumptive genera: *Bacillus*, *Pseudomonas*, *Enterobacter*, *Micrococcus*, *Staphylococcus* and *Serratia*. *Bacillus* dominated across treatments, representing 40.00–53.33% of isolates and becoming proportionally more frequent under four-day watering. Its persistence is biologically plausible because many *Bacillus* species form endospores and tolerate environmental stress. Ren et al. (2025) reported that *Bacillus subtilis* could improve soil water retention, rhizosphere microbial structure and plant water-use efficiency under drought, while Buqori et al. (2025) demonstrated that drought-tolerant *Bacillus* could improve plant growth and physiological performance. Liu et al. (2025) also identified *Bacillus* among potentially useful plant-growth-promoting rhizobacteria. *Pseudomonas* was the second most frequent group and persisted under both regimes, although its proportion declined under four-day watering. Bhattacharyya et al. (2025) showed that *Pseudomonas* can use plant-derived compatible solutes and biofilms to improve survival during osmotic stress. *Enterobacter* occurred less frequently and declined under four-day watering, while *Micrococcus* remained consistently low. *Staphylococcus* increased numerically, and *Serratia* was detected only at day 42 under daily watering.

Pearson chi-square analysis found no significant difference in bacterial genus distribution between treatments at either day 0 or day 42. Therefore, watering frequency appeared to influence total cultivable bacterial abundance more strongly than the broad proportional distribution of recovered genera. The increase in *Bacillus* under four-day watering was not statistically significant and cannot by itself establish selective enrichment. Culture-based enumeration also measures only organisms capable of growing under the selected laboratory conditions; reduced counts may therefore reflect reduced culturability as well as lower abundance. Overall, the findings support an integrated pathway in which watering frequency modifies soil moisture, plant growth, root physiology and exudation, which together influence the culturable rhizosphere bacterial community.

Daily watering maintained substantially higher cultivable bacterial abundance, whereas four-day watering produced progressive suppression

The study showed that watering frequency influenced several aspects of growth and rhizosphere bacterial abundance in *T. ivorensis* seedlings. Daily watering promoted greater collar diameter, leaf number, leaf length, width, individual leaf area, and total leaf area, with significant treatment differences becoming evident by day 42. In contrast, seedling height, fresh and dry leaf biomass, moisture content, and dry matter remained statistically similar, suggesting that seedlings retained capacity for physiological adjustment under intermittent watering. The strongest response occurred in the cultivable rhizosphere bacterial population, which increased markedly under daily watering but declined substantially with four-day watering.

Recent studies indicate that water availability can affect rhizosphere microorganisms through changes in soil moisture, root exudation, nutrient cycling, and plant–microbe interactions (Bhattacharyya et al., 2025). Although several bacterial genera were recovered, watering did not significantly alter their overall distribution. Overall, regular watering supported vegetative development and maintained substantially greater cultivable bacterial abundance than intermittent watering during nursery establishment.

The PCoA demonstrated that watering frequency progressively influenced the combined growth, leaf, biomass, and cultivable rhizosphere bacterial characteristics of *T. ivorensis* seedlings. The first two axes explained 94.52% of the total variation, with axis 1 contributing 70.59% and axis 2 contributing 23.93%. Both treatments were initially similar at day 0 but followed increasingly distinct trajectories as the experiment progressed.

Daily watering was associated with greater collar diameter, leaf development, total leaf area, and cultivable bacterial abundance, whereas four-day watering produced comparatively lower values for these characteristics. Biomass-related variables remained relatively stable between treatments, indicating that multivariate separation was driven mainly by strongly responsive morphological and bacterial characteristics.

The observed pattern is consistent with evidence that water availability affects plant growth, root activity, microbial processes, and plant–microbe interactions (Chattaraj et al., 2025; Yadav et al., 2025). Overall, the PCoA indicates progressive multivariate differentiation associated with watering frequency.

Conclusion

The study demonstrated that watering frequency influenced the growth performance, leaf development, and cultivable rhizosphere bacterial population of *Terminalia ivorensis* seedlings during the 42-day nursery experiment. The two treatment groups were comparable at baseline, confirming that subsequent differences were associated with the watering regimes. Daily watering produced greater increases in seedling height, collar diameter, and leaf production, with the strongest effects observed in collar diameter and foliage development. Most notably, cultivable bacterial abundance increased under daily watering but declined under four-day watering. *Bacillus* spp. remained predominant, although genus distribution was not significantly affected. Principal Coordinates Analysis (PCoA) supports the overall influence of watering frequency on plant–rhizosphere responses. These findings indicate that consistent water availability promotes vegetative development and maintains higher cultivable rhizosphere bacterial abundance during nursery establishment.

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