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Growth Dynamics, Harvest Performance, and Biomass Partitioning of Lettuce Cultivars Under Contrasting Hydroponic Configurations: Implications for Sustainable Protected Horticulture

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Abstract: Hydroponic production can contribute to resource-efficient protected horticulture, although cultivar responses may vary among cultivation configurations. This study characterized pre-harvest growth, harvest morphology, fresh and dry biomass, and biomass partitioning of three lettuce cultivars—White Boston, Waldmann's Green, and Black Rose—grown in pyramidal nutrient film technique (NFT), floating raft (FR), and semihydroponic (SH) configurations. The experiment included 36 units and 192 plants. Nondestructive measurements were performed at 30 and 60 days after sowing, followed by destructive harvest measurements at 60 days. Temporal changes and cultivar responses were evaluated separately within each configuration because hydraulically independent units differed among configurations; between-configuration patterns were interpreted descriptively. Most temporal changes were similar among cultivars. At harvest, cultivar-dependent responses were configuration-specific and involved morphology, leaf fresh weight, total dry matter content, and biomass allocation. Total fresh and dry weight did not differ significantly among cultivars within any configuration. Nevertheless, FR–White Boston showed the highest numerical total fresh weight (189.96 ± 48.90 g plant^{-1}), whereas FR–Black Rose showed the greatest leaf fresh-weight proportion (81.0%). Waldmann's Green showed comparatively high total dry matter content, including 5.18% in FR, where cultivar differences were statistically supported. The first two principal components explained 56.9% of the total variance and revealed overlapping multivariate patterns among configuration × cultivar combinations. Overall, cultivar performance depended on the trait and cultivation configuration: White Boston was associated with higher numerical fresh biomass, Black Rose with greater leaf biomass allocation, and Waldmann's Green with comparatively higher dry matter content. These findings provide an agronomic basis for cultivar selection in protected hydroponic production.

Keywords: Controlled-environment agriculture; Biomass partitioning; Cultivar response; Hydroponic systems; Lettuce; Sustainable protected horticulture; Crop management

1. Introduction

Global horticultural production is under increasing pressure from soil degradation, water scarcity, limited availability of productive land, and the need to intensify production under environmentally sustainable frameworks (FAO, 2021). In this context, controlled-environment agriculture and hydroponic systems have gained particular relevance as alternatives capable of partially decoupling crop production from soil constraints while potentially improving water- and nutrient-use efficiency. Hydroponics comprises soilless cultivation systems in which plant roots receive water, oxygen, and essential mineral nutrients through a managed nutrient solution, either directly or through an inert substrate (Savvas & Gruda, 2018). Within organic agriculture and sustainable food system research, hydroponic and soilless production can be discussed in terms of their potential contribution to resource-efficient protected horticulture, particularly where production systems aim to reduce pressure on soil and water

resources. Controlled and recirculating hydroponic systems are relevant in this context when they are evaluated according to sustainability criteria such as water conservation, nutrient-use efficiency, reduction of nutrient losses, and the potential incorporation of bio-based nutrient strategies. Therefore, comparative studies of system architecture and cultivar response can provide useful evidence for designing more efficient protected horticulture systems and for identifying management components that may support the transition toward more sustainable and organic-oriented production models (Chowdhury et al., 2024; Fathidarehnijeh et al., 2024; Maucieri et al., 2018).

Lettuce (*Lactuca sativa* L.) is one of the most representative crops for this type of evaluation, given that its short growth cycle makes it suitable for high-turnover production under controlled conditions (Sandoya et al., 2021). Lettuce is highly responsive to changes in nutrient-solution composition, which has motivated numerous studies assessing the effects of different formulations on key agronomic parameters such as biomass, leaf number, and marketable yield (Ricardo Morales & Pertierra Lazo, 2020). In addition, variation in macro- and micronutrient concentrations within the rhizosphere generates significant differential responses in vegetative growth, including plant height, stem diameter, and leaf area, which confirms lettuce as a suitable model species not only for mineral nutrition studies but also for evaluating how hydroponic system design regulates oxygen, water, and nutrient availability in the root zone and, consequently, final crop performance (Chowdhury et al., 2024; Lopez Vicente, 2010).

Among the hydroponic systems used for lettuce production, three contrasting configurations are particularly relevant: nutrient film technique (NFT), floating raft (FR) or deep-water culture, and substrate-based systems. NFT supplies a shallow recirculating film of nutrient solution along cultivation channels, FR systems maintain roots in a larger aerated solution volume, and substrate-based configurations provide mechanical support while allowing intermittent nutrient delivery and drainage (Sandoya et al., 2021; Savvas & Gruda, 2018). System architecture, solution flow, root-zone volume, oxygenation, and plant support can modify growth, nutrient uptake, water consumption, and crop quality; therefore, the available evidence does not support the universal superiority of a single hydroponic configuration (Abbas et al., 2024; Chowdhury et al., 2024).

Nutrient solution formulation and management constitute determining factors, since the appropriate choice of cultivar in combination with macro- and micronutrient concentrations is critical for optimizing biomass accumulation, plant height, and fresh weight. In addition, the genotype-by-environment interaction (G × E) can generate significant differential responses among cultivars under varying environmental conditions, reinforcing the need to assess the performance of different genotypes under controlled production environments (Tobar-Tosse et al., 2022). Indeed, recent literature recognizes lettuce as one of the most extensively studied species in hydroponics and protected agriculture, because of its usefulness for comparing production and management strategies (Sapkota et al., 2019).

In addition to system-related variation, cultivar identity can substantially influence crop response. In lettuce, cultivars may differ in leaf expansion, leaf number, chlorophyll content, root length, and the accumulation of fresh and dry biomass, even when grown under similar environmental conditions (Leiva Espinoza et al., 2018; Sapkota et al., 2019). Sapkota et al. (2019) demonstrated that hydroponic productivity varies as a function of the cultivar × nutrient-solution composition combination. Consequently, evaluating hydroponic systems without considering cultivar identity may lead to incomplete conclusions regarding treatment performance and limit the transferability of recommendations across production conditions (Leiva Espinoza et al., 2018; Sapkota et al., 2019).

From this perspective, assessing only fresh weight at harvest is insufficient to interpret lettuce agronomic performance in soilless systems. Pre-harvest growth, leaf number, leaf expansion, and dry matter accumulation provide a more robust view of crop adaptation to each production environment (Fraile-Robayo et al., 2017). Likewise, evaluating shoot and root fresh and dry biomass together with the root:shoot ratio helps determine whether treatment differences reflect changes in organ allocation, tissue water content, or structural biomass accumulation (Germer et al., 2023; Li et al., 2018).

Previous studies have evaluated cultivar × nutrient-solution combinations within individual hydroponic systems (Sapkota et al., 2019), nutrient dynamics in recirculating NFT installations (Vought et al., 2024), and lettuce performance under contrasting hydroponic system designs (Chowdhury et al., 2024; Yang et al., 2024). However, integrated evaluations of multiple commercial cultivars under contrasting system architectures and the same protected environment, incorporating both nondestructive growth assessments and destructive biomass partitioning, remain limited. Therefore, comprehensive evaluations integrating system type, cultivar response, pre-harvest growth, and biomass allocation are needed to support more precise agronomic decisions (Fraile-Robayo et al., 2017).

Within this framework, this study aimed to characterize pre-harvest growth, harvest morphology, fresh and dry biomass accumulation, and biomass partitioning of three lettuce cultivars grown under three contrasting hydroponic configurations in a protected environment. Cultivar responses and temporal changes were evaluated separately within each configuration, whereas patterns among configurations were interpreted descriptively because the number and structure of hydraulically independent units were not equivalent. We expected cultivar performance to vary according to the evaluated trait and cultivation configuration, with differences expressed more clearly in plant morphology, dry matter content, and biomass allocation than in total biomass production. From

the perspective of sustainable protected horticulture and organic-oriented agriculture, this study provides an agronomic basis for future assessments incorporating direct indicators of resource-use efficiency and alternative nutrient-management strategies. More broadly, evidence supporting the development of horticultural systems with the potential for more efficient water and nutrient management is consistent with Sustainable Development Goals 2, 6, and 12, which are oriented toward food security, sustainable water management, and responsible production, respectively (United Nations, 2015).

2. Methodology

2.1 Study Site and Experimental Conditions

The study was conducted at the Donoso Agricultural Experimental Station of the National Institute of Agrarian Innovation (INIA), located at approximately 11°31′18″ S and 77°14′07″ W, at 180–188 m above sea level, where the hydroponic systems were installed inside the shade house facilities of the Hydroponics Program. Nutrient solutions were prepared and plant samples were dried in the Legume Laboratory, whereas germination tests were carried out in the Seed Laboratory. The use of a protected environment reduced variability associated with external climatic factors and allowed more uniform crop management, a condition widely recommended in comparative hydroponic studies with lettuce (Chowdhury et al., 2024).

During the experimental period, mean air temperature and relative humidity inside the shade house were 21.48 ± 0.78 °C and 78.85 ± 3.75%, respectively, thereby providing a relatively homogeneous growth environment for comparisons among hydroponic systems and cultivars.

2.2 Plant Material and Seedling Establishment

Three commercial cultivars of lettuce were evaluated: ‘White Boston’ (V1), ‘Waldmann’s Green’ (V2), and ‘Black Rose’ (V3). For seedling production, washed and disinfected river sand was used as the substrate and placed in 40 × 30 cm polypropylene trays. Seeds were sown in continuous rows, with 500 seeds per cultivar per tray. Irrigation was applied daily and increased to twice per day during periods of higher temperature. The use of uniform and vigorous seedlings is essential to minimize initial bias in comparative hydroponic system trials, particularly in short-cycle crops such as lettuce (Sapkota et al., 2019).

2.3 Germination Test

Initial seed physiological quality was assessed through germination tests conducted in accordance with the International Rules for Seed Testing issued by the International Seed Testing Association (ISTA, 2025). For each cultivar, four replicates of 100 seeds were placed on filter paper moistened with distilled water in Petri dishes. Seeds were incubated in a germination chamber (Incucell Ecoline, MMM Group, Munich, Germany) at 20 °C for 7 days, and normal seedlings, abnormal seedlings, and ungerminated seeds were recorded.

2.4 Hydroponic Systems Evaluated

Three contrasting hydroponic configurations were evaluated: a pyramidal NFT system, an FR system, and an SH system. The NFT system comprised two pyramidal modules, each equipped with 10 cultivation channels made of 4-inch polyvinyl chloride (PVC) pipes. Plants were spaced 20 cm apart, and each module included channels with a 0.5% slope, a 100-L reservoir, a 0.5-hp electric pump, and an independent recirculation circuit. The FR system comprised 12 geomembrane-lined floating-raft boxes (approximately 0.8 m long × 1.0 m wide), each containing approximately 100–110 L of nutrient solution, supporting plants on expanded-polystyrene sheets at 20-cm spacing, and operating with its own aeration pump. The SH system used cultivation sleeves (20 cm wide × 20 cm high × 80 cm long) filled with disinfected gravel and supplied through arrow-type drip emitters connected to a common reservoir, pump, and recirculation network. Dimensions, plant spacing, nutrient formulation, and crop-management procedures were standardized as far as permitted by the physical architecture of each configuration.

2.5 Nutrient Solution Management

Two concentrated nutrient solutions formulated by the Hydroponics and Mineral Nutrition Research Center (CIHNM) of the Universidad Nacional Agraria La Molina were prepared. Solution A supplied macronutrients and included ammonium nitrate, potassium nitrate, and calcium triple superphosphate, whereas Solution B supplied magnesium sulfate, iron chelate, manganese sulfate, zinc sulfate, boric acid, copper sulfate, and ammonium molybdate.

Seedlings were established in washed and disinfected river sand from 0 to 15 days after sowing (DAS). At 15

DAS, they were transferred to an aerated floating-root nursery box, where they remained for a further 15-day acclimatization and early-growth period. This nursery box was used exclusively for seedling acclimatization and did not constitute an experimental hydroponic treatment. During this stage, the working solution was prepared using 2.5 mL L^{-1} of Solution A and 1.0 mL L^{-1} of Solution B, and pH was maintained between 5.5 and 6.5.

At 30 DAS, uniform seedlings were transferred to the definitive NFT, FR, and SH experimental units, where they remained for 30 days until harvest at 60 DAS. For this definitive experimental phase, the working solution was prepared using 4.0 mL L^{-1} of Solution A and 2.0 mL L^{-1} of Solution B. Electrical conductivity was maintained between 1.5 and 1.8 dS m^{-1}, and pH was maintained between 4.5 and 5.5.

During the definitive 30-day phase, reservoir water was replenished five to six times, at intervals of approximately 5–7 days, depending on crop consumption and the remaining operating volume. The nutrient solution was not completely replaced, and no complete nutrient-solution renewal was performed during this period. Water was added to restore the required volume, while electrical conductivity and pH were monitored to verify that they remained within the established ranges. The NFT modules and FR boxes operated according to the independent hydraulic structure described in Sections 2.4 and 2.7, whereas all SH analytical units shared the common irrigation network.

Nutrient-solution pH and electrical conductivity were monitored every 3 days at the hydraulic-unit level using a portable multiparameter meter (Hanna Instruments, Combo series, model HI98129). Instrument calibration was performed using pH 4.01 and 7.01 buffer solutions and a 1413 µS cm^{-1} conductivity standard. The measurements were used to verify that the nutrient solution remained within the physicochemical target ranges established for the definitive cultivation phase.

The nutrient solution was mineral-based and corresponded to the institutional CIHNM formulation. Compost-derived extracts, leachates, or other organically derived and circular nutrient sources were not evaluated. Therefore, all three configurations were managed under the same mineral-nutrition formulation and physicochemical target ranges during the definitive experimental phase.

2.6 Transplanting, Crop Management, and Evaluated Variables

Before seedling transfer, all materials intended to come into direct contact with the root system were disinfected by immersion in 1% sodium hypochlorite for 20 min and subsequently rinsed with clean water to minimize contamination risks and avoid phytotoxic residues. At 15 DAS, seedlings were carefully removed from the sand substrate, their roots were washed to remove adhering particles, and the plants were positioned in support sponges at collar level before transfer to the aerated floating-root nursery box. Seedlings remained in this acclimatization unit until 30 DAS.

At 30 DAS, uniform seedlings were selected and transferred to the definitive NFT, FR, and SH experimental units. The comparative phase therefore extended from 30 to 60 DAS. D2 was recorded at 30 DAS and served as the baseline assessment at the beginning of the definitive cultivation phase. D3 was recorded at 60 DAS, after 30 days of cultivation in the definitive hydroponic configurations. The D3 assessment was conducted nondestructively and in situ, with the plants maintained in their cultivation position, immediately before plant removal and destructive harvesting on the same day.

The nondestructive assessments conducted at D2 and D3 comprised in situ plant height, leaf number, leaf length, leaf width, and leaf chlorophyll content (Table 1). D1 corresponded to emergence and early seedling establishment and was excluded from the comparative analyses. Accordingly, the comparative pre-harvest dataset was restricted to D2 and D3. Nutrient-solution pH and electrical conductivity were monitored separately at the hydraulic-unit level, as described in Section 2.5.

Leaf chlorophyll content was measured every 2 days at 12:00 h using a SPAD-502Plus chlorophyll meter (Konica Minolta, Japan). For each plant, one leaf located in the middle portion of the canopy was selected, and five consecutive readings were obtained from the middle region of the leaf blade. The instrument's internal averaging function was used to calculate a single Soil Plant Analysis Development (SPAD) value per plant. Plant-level values were subsequently averaged within each analytical cultivation unit. Only the measurements corresponding to D2 and D3 were included in the comparative statistical analyses.

Immediately after the nondestructive D3 assessment, plants were removed from the cultivation units and subjected to destructive harvest evaluation. Root length after plant removal, harvest plant height, total leaf number at harvest, leaf length at harvest, and leaf width at harvest were determined according to the harvest-specific measurement protocol. Although several D3 and harvest variables had similar names, they were recorded under different operational conditions and therefore represented complementary rather than directly interchangeable measurements.

Following the morphological evaluation, plants were separated into leaves, roots, and stems. Leaf, root, and stem fresh weights were measured immediately after organ separation using a Nimbus NBL 4602 series precision balance (Adam Equipment, UK; capacity: 4600 g; readability: 0.01 g). The separated plant organs were subsequently dried according to the laboratory drying protocol, and the same balance was used to determine leaf,

root, and stem dry weights.

All derived variables were calculated separately for each analytical cultivation unit before treatment summaries and statistical analyses were obtained, using the following equations:

$$\text{Total fresh weight} = \text{Leaf fresh weight} + \text{Root fresh weight} + \text{Stem fresh weight} \quad (1)$$

$$\text{Total dry weight} = \text{leaf dry weight} + \text{root dry weight} + \text{stem dry weight} \quad (2)$$

$$\text{Total dry matter content (\%)} = \frac{\text{Total dry weight}}{\text{Total fresh weight}} \times 100 \quad (3)$$

$$\text{Root:shoot fresh-weight ratio} = \frac{\text{Root fresh weight}}{\text{Leaf fresh weight} + \text{Stem fresh weight}} \quad (4)$$

$$\text{Leaf fresh-weight proportion} = \frac{\text{Leaf fresh weight}}{\text{Total fresh weight}} \quad (5)$$

These variables jointly characterized marketable fresh biomass, structural dry-matter accumulation, and biomass allocation under the evaluated hydroponic configurations.

Table 1. Evaluated variables, measurement units, and timing of assessment in lettuce grown under three hydroponic configurations

Analytical Block	Evaluated Variable	Unit	Timing of Assessment
Nondestructive plant assessments	Plant height (in situ)	cm	D2 and D3
	Leaf number (in situ)	count (n)	D2 and D3
	Leaf length (in situ)	cm	D2 and D3
	Leaf width (in situ)	cm	D2 and D3
	Leaf chlorophyll content	SPAD units	Every 2 days; D2 and D3 used in comparative analyses
Nutrient-solution monitoring	pH	pH units	Every 3 days at the hydraulic-unit level
	Electrical conductivity	dS m^{-1}	Every 3 days at the hydraulic-unit level
Destructive harvest assessment	Root length	cm	Harvest
	Plant height	cm	Harvest
	Leaf number	count (n)	Harvest
	Leaf length	cm	Harvest
	Leaf width	cm	Harvest
	Leaf fresh weight	g plant^{-1}	Harvest
	Root fresh weight	g plant^{-1}	Harvest
	Stem fresh weight	g plant^{-1}	Harvest
	Leaf dry weight	g plant^{-1}	Harvest
	Root dry weight	g plant^{-1}	Harvest
	Stem dry weight	g plant^{-1}	Harvest
Harvest-derived variables	Total dry weight	g plant^{-1}	Calculated at harvest
	Total fresh weight	g plant^{-1}	Calculated at harvest
	Total dry matter content	%	Calculated at harvest
	Root:shoot fresh-weight ratio	dimensionless	Calculated at harvest
	Leaf fresh-weight proportion	dimensionless	Calculated at harvest

Note: D1 corresponded to emergence and early seedling establishment and was excluded from the comparative statistical analyses. D2 and D3 were conducted at 30 and 60 days after sowing, respectively. D2 represented the baseline assessment at the beginning of the definitive 30-day cultivation phase, whereas D3 was conducted nondestructively and in situ at the end of this phase, with plants maintained in their cultivation position. Destructive harvest measurements were obtained immediately afterward following plant removal. Variables with similar names at D3 and harvest were measured under different operational conditions and should therefore be interpreted as complementary rather than directly equivalent measurements. Leaf chlorophyll content was monitored every 2 days, although only the D2 and D3 values were included in the comparative analyses. Nutrient-solution pH and electrical conductivity were monitored every 3 days at the hydraulic-unit level and were not treated as independent analytical-unit observations. Total fresh weight was calculated as the sum of leaf, root, and stem fresh weights. SPAD = Soil Plant Analysis Development.

2.7 Experimental Design and Unit Structure

The study comprised three hydroponic configurations—pyramidal NFT, FR, and SH—and three lettuce cultivars

under a 3 × 3 treatment structure. Because the configurations differed in their physical and hydraulic architecture, hydraulic units were distinguished from analytical cultivation units. Hydraulic units corresponded to reservoirs and circulation or aeration systems operating independently, whereas analytical cultivation units corresponded to spatially defined groups of plants whose measurements were averaged before statistical analysis. Individual plants were treated as subsamples rather than as independent replicates.

The FR configuration consisted of 12 hydraulically independent floating-raft boxes, each containing approximately 100–110 L of nutrient solution and equipped with its own aeration pump. Four boxes were assigned to each cultivar, and measurements from five plants within each box were averaged. Each box therefore represented both a hydraulic unit and an analytical cultivation unit, yielding four independent FR units per cultivar.

The NFT configuration consisted of two hydraulically independent modules, each connected to its own 100-L reservoir, pump, and recirculation circuit. All three cultivars were represented in both modules, and four analytical cultivation units per cultivar were distributed across the two modules, with two analytical units per cultivar in each module. Measurements from five plants were averaged within each analytical unit. Because multiple analytical units shared the reservoir and recirculation circuit of the same module, these units were treated as nested within module rather than as four hydraulically independent replicates of the NFT configuration.

The SH configuration consisted of cultivation sleeves filled with disinfected gravel and supplied through a common drip-irrigation network connected to a single reservoir and pump. Four spatial analytical units were defined per cultivar, and each analytical mean was calculated from six sampled plants. Although these units represented spatially distinct groups, they shared the same nutrient solution and hydraulic circuit and were therefore interpreted as analytical units within one SH installation rather than as hydraulically independent system replicates.

Overall, the dataset contained 36 analytical cultivation units, corresponding to four units for each configuration × cultivar combination, and comprised 192 plants: 60 in NFT, 60 in FR, and 72 in SH. Cultivars and analytical-unit positions were assigned within each configuration using restricted randomization because the existing infrastructure prevented complete randomization of the configuration factor. Plant spacing, nutrient-solution formulation, and crop-management practices were standardized as far as permitted by the architecture of each configuration.

Because the number and structure of hydraulically independent units were not equivalent among NFT, FR, and SH, the configuration factor was not treated as a fully replicated experimental effect. Inferential analyses focused on cultivar responses and temporal changes within each configuration, whereas contrasts among configurations were interpreted descriptively as installation-specific patterns under the evaluated experimental conditions.

2.8 Data Processing and Statistical Analysis

Analyses were conducted using analytical cultivation-unit means, with individual plants treated as subsamples. D1 was excluded because it corresponded to emergence and early seedling establishment. D2 and D3 represented the baseline and end-of-phase assessments at 30 and 60 DAS, respectively, and harvest measurements were obtained immediately after D3.

Because hydraulic replication was not equivalent among the three configurations, analytical units sharing a reservoir or circulation network were not treated as independent replicates of the configuration factor. Inferential analyses were therefore conducted separately within FR, NFT, and SH. Differences among configurations were summarized descriptively and interpreted as conditional on the specific installations evaluated.

For each nondestructive growth trait, D2 and D3 values were summarized by configuration and cultivar. Cultivar-dependent temporal responses during the definitive experimental phase were evaluated using the change score $\Delta = D3 - D2$ calculated for each analytical unit. Within FR and SH, change scores were analyzed using linear models with cultivar as a fixed effect. Within NFT, cultivar and module were included as fixed effects, with module serving as a blocking factor. This change-score formulation was used because only two repeated assessments were available and several preliminary random-intercept models produced singular fits.

As a sensitivity analysis, overall cultivar effects on change scores were evaluated using 999 label permutations. NFT permutations were restricted within module to preserve the blocking structure. When an overall cultivar effect was detected, pairwise permutation comparisons were adjusted using the Holm procedure. The same permutation framework was applied to harvest variables as a robustness check when residual assumptions were uncertain.

Harvest morphology, fresh biomass, dry biomass, and allocation traits were summarized using the mean, standard deviation, standard error, minimum, maximum, and coefficient of variation for each configuration × cultivar combination. Cultivar effects were tested separately within each configuration using analytical-unit means. FR and SH were analyzed using one-way analysis of variance with cultivar as a fixed factor. NFT was analyzed using linear models containing cultivar and module as fixed effects. When the overall cultivar effect was significant and model assumptions were adequate, pairwise comparisons of estimated marginal means were conducted using Tukey adjustment (Lenth & Piaskowski, 2024).

Residual normality was evaluated using the Shapiro–Wilk test and quantile–quantile plots, whereas

homogeneity of variances was assessed using Levene's test and residual-versus-fitted plots (Shapiro & Wilk, 1965). Parametric results were interpreted together with the permutation sensitivity analyses, particularly for variables showing assumption alerts or disagreement between analytical approaches.

Principal component analysis (PCA) was conducted using centered and scaled harvest variables calculated at the analytical-unit level. Total fresh weight was excluded from the PCA because it was exactly equal to the sum of leaf, root, and stem fresh weights and would therefore introduce an exact linear dependency. Pearson correlation coefficients were calculated between five nondestructive traits measured at D3 plant height, leaf number, leaf length, leaf width, and chlorophyll content and harvest traits across the 36 analytical cultivation units. Electrical conductivity was excluded from this analysis because it was measured at the hydraulic-unit level, hydraulic replication differed among configurations, and no D3 conductivity values were available for the SH installation. To account for the number of pairwise tests, *p*-values were adjusted using the Benjamini–Hochberg false-discovery-rate procedure (Benjamini & Hochberg, 1995). Correlations were interpreted as exploratory associations rather than as evidence of causality or validated prediction. Data handling was performed using dplyr and tidyr, graphical visualizations were produced using ggplot2 (Wickham, 2016) and ggrepel, and all statistical analyses were conducted in R (R Core Team, 2024).

3. Results

3.1 Pre-Harvest Performance

Table 2 summarizes the analytical-unit means recorded during the nondestructive in situ assessments at baseline (D2, 30 DAS) and on the harvest day (D3, 60 DAS). Because D2 coincided with the beginning of the definitive 30-day experimental phase, differences among hydroponic configurations at D2 represent baseline variation and were not interpreted as configuration effects. Across the observed values, FR combinations frequently showed high chlorophyll and morphological measurements, whereas NFT–Black Rose displayed high leaf length and leaf width. These cross-configuration patterns are descriptive because hydraulic replication differed among the evaluated installations. The D3 values characterize plants in their cultivation position and are not directly interchangeable with similarly named measurements obtained after plant removal at harvest.

Table 2. Pre-harvest performance of three lettuce cultivars within the evaluated hydroponic configurations

System	Cultivar	D2 (Baseline)					D3 (Harvest Day)				
		Chlorophyll (SPAD)	Leaf Length (cm)	Leaf Number (n)	Leaf Width (cm)	Plant Height (cm)	Chlorophyll (SPAD)	Leaf Length (cm)	Leaf Number (n)	Leaf Width (cm)	Plant Height (cm)
NFT	White Boston	24.61 ± 0.56	19.65 ± 1.67	13.35 ± 0.96	10.10 ± 1.31	11.76 ± 2.47	25.61 ± 1.60	19.88 ± 1.61	13.30 ± 0.93	11.96 ± 1.75	22.86 ± 3.41
	Waldmann's Green	28.39 ± 2.21	19.38 ± 0.64	9.05 ± 0.30	11.30 ± 0.66	11.31 ± 0.67	27.73 ± 1.04	20.28 ± 1.51	10.35 ± 0.38	13.57 ± 1.53	22.62 ± 1.91
	Black Rose	27.75 ± 3.04	22.98 ± 0.78	12.85 ± 0.50	12.96 ± 0.89	11.07 ± 1.17	27.52 ± 3.60	23.62 ± 2.32	14.05 ± 1.33	15.34 ± 1.22	22.30 ± 1.36
FR	White Boston	27.07 ± 1.55	23.82 ± 1.44	15.30 ± 1.09	12.78 ± 0.30	15.33 ± 1.07	29.21 ± 0.85	23.55 ± 1.22	15.80 ± 1.23	13.86 ± 0.42	24.40 ± 1.72
	Waldmann's Green	30.61 ± 1.82	21.39 ± 0.68	10.15 ± 0.50	13.66 ± 0.51	13.11 ± 0.47	29.26 ± 1.01	19.64 ± 1.37	10.40 ± 0.63	14.48 ± 1.37	21.20 ± 2.33
	Black Rose	28.12 ± 1.43	24.95 ± 0.57	13.50 ± 0.35	13.14 ± 0.66	13.11 ± 0.97	27.68 ± 1.45	22.65 ± 2.84	14.35 ± 1.43	13.25 ± 0.82	19.50 ± 2.18
SH	White Boston	22.35 ± 2.39	17.15 ± 0.81	10.67 ± 0.56	8.75 ± 0.50	7.85 ± 0.32	23.89 ± 0.67	19.20 ± 1.41	13.63 ± 1.03	12.31 ± 1.36	20.20 ± 2.23
	Waldmann's Green	24.70 ± 0.50	17.90 ± 1.12	8.74 ± 3.41	11.05 ± 2.41	10.22 ± 4.81	25.72 ± 0.74	18.70 ± 1.21	9.54 ± 0.90	15.05 ± 1.54	21.44 ± 1.19
	Black Rose	22.77 ± 1.85	17.27 ± 0.78	10.33 ± 0.56	9.34 ± 0.32	7.57 ± 0.39	24.32 ± 1.99	17.35 ± 0.92	13.08 ± 0.93	12.63 ± 0.34	16.46 ± 2.09

Note: NFT = nutrient film technique; FR = floating raft; SH = semihydroponic. SPAD = Soil Plant Analysis Development. Values are analytical cultivation-unit means ± standard deviation (n = 4 units per configuration × cultivar combination). D2 and D3 were conducted nondestructively and in situ at 30 and 60 days after sowing, respectively, with plants maintained in their cultivation position. D2 represented the baseline assessment at the beginning of the definitive 30-day experimental phase, and D3 was completed immediately before plant removal and destructive harvest sampling. Temporal responses were evaluated using D3 − D2 change scores separately within each hydroponic configuration. An overall cultivar effect on the change in chlorophyll content was detected within FR; however, no pairwise cultivar comparison remained significant after adjustment for multiple comparisons. Therefore, letter-based groupings are not presented. D3 values should not be interpreted as directly equivalent to similarly named destructive harvest measurements. Comparisons among hydroponic configurations are descriptive.

At D3, FR–White Boston and FR–Waldmann's Green recorded the highest numerical chlorophyll values (29.21 ± 0.85 and 29.26 ± 1.01 SPAD, respectively). FR–White Boston also recorded the greatest numerical plant height (24.40 ± 1.72 cm) and leaf number (15.80 ± 1.23) within FR, whereas NFT–Black Rose showed the greatest

numerical leaf length across the observed combinations (23.62 ± 2.32 cm). These values describe the evaluated installations and should not be interpreted as inferential comparisons among hydroponic configurations.

Change-score analysis showed that cultivar-dependent temporal responses were generally limited. The only significant overall cultivar effect on D3 − D2 was detected for chlorophyll content within FR ($F(2, 9) = 4.39$, $p = 0.047$; permutation $p = 0.037$). White Boston increased by 2.14 ± 1.70 SPAD, whereas Waldmann's Green and Black Rose changed by −1.35 ± 2.26 and −0.43 ± 0.97 SPAD, respectively. No individual pairwise comparison remained significant after Holm adjustment, so this finding was interpreted at the overall cultivar-effect level. For plant height, leaf number, leaf length, and leaf width, cultivar effects on change scores were not significant within FR, NFT, or SH (parametric $p = 0.117$–0.994; permutation $p = 0.114$–0.990; Appendix Table A1).

Figure 1 illustrates the within-installation trajectories from D2 to D3. Plant height increased in every configuration × cultivar combination, whereas changes in leaf number and leaf width were generally positive but more moderate. Leaf length showed small increases or decreases depending on the combination, and chlorophyll content remained comparatively stable except for the divergent FR responses described above.

Taken together, the descriptive trajectories and change-score tests indicate that most cultivars followed broadly similar growth changes during the definitive phase within each configuration. The figure is therefore used to visualize temporal patterns rather than to infer a general hydroponic-configuration effect.

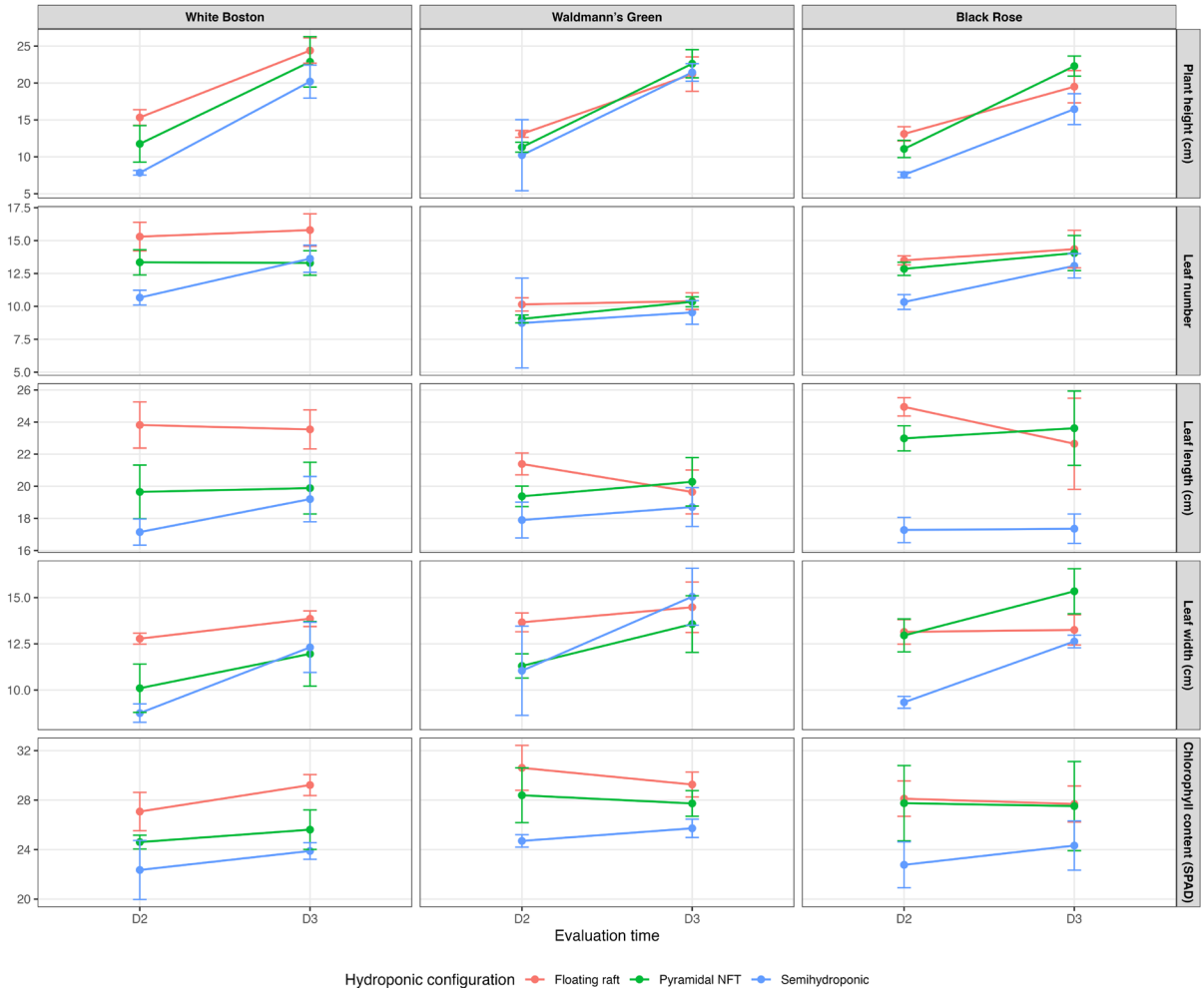

Figure 1. Nondestructive in situ variation in plant height, leaf number, leaf length, leaf width, and chlorophyll content from the baseline assessment at 30 days after sowing (D2) to the harvest-day assessment at 60 days after sowing (D3) in three lettuce cultivars grown under contrasting hydroponic configurations

Note: V1 = White Boston; V2 = Waldmann's Green; V3 = Black Rose; FR = floating raft; NFT = nutrient film technique; SH = semihydroponic; SPAD = Soil Plant Analysis Development. D2 and D3 measurements were obtained nondestructively with plants maintained in their cultivation position. Points represent analytical cultivation-unit means, and error bars indicate standard deviations for each configuration × cultivar combination. Comparisons among configurations are descriptive.

3.2 Harvest Performance and Configuration-Specific Cultivar Responses

Harvest responses were configuration-specific (Table 3). Harvest morphology was recorded after plants had

been removed from the cultivation units and therefore represents a destructive assessment that is complementary to, but not directly interchangeable with, the nondestructive in situ D3 measurements. Within FR, overall cultivar effects were supported for root length, plant height, leaf number, leaf length, and leaf width (parametric $p < 0.001$–0.013; permutation $p = 0.003$–0.013). White Boston recorded the greatest numerical root length (34.23 ± 5.83 cm), plant height (28.90 ± 1.43 cm), leaf number (25.35 ± 4.98), and leaf length (23.84 ± 1.61 cm), whereas Waldmann's Green recorded the greatest leaf width (17.02 ± 0.90 cm).

Table 3. Harvest morphology, fresh and dry biomass, and key allocation traits of three lettuce cultivars grown under three hydroponic configurations

System	Cultivar	Root Length at Harvest (cm)	Plant Height at Harvest (cm)	Leaf Number at Harvest (n)	Leaf Length at Harvest (cm)	Leaf Width at Harvest (cm)	Total Fresh Weight (g plant⁻¹)	Leaf Fresh Weight (g plant⁻¹)	Total Dry Weight (g plant⁻¹)	Total Dry Matter Content (%)	Root:Shoot Fresh-Weight Ratio (dimensionless)
NFT	White Boston	23.27 ± 1.38	25.49 ± 3.30	18.76 ± 1.92	21.45 ± 2.31	13.24 ± 1.35	129.38 ± 19.17	88.66 ± 11.62	6.66 ± 1.44	5.11 ± 0.50	0.30 ± 0.06
	Waldmann's Green	22.18 ± 1.14	26.29 ± 1.55	12.90 ± 1.10	20.77 ± 1.97	16.82 ± 1.50	135.26 ± 19.34	89.84 ± 10.70	6.23 ± 1.13	4.60 ± 0.43	0.30 ± 0.10
	Black Rose	23.77 ± 0.64	24.18 ± 1.34	21.05 ± 0.67	22.57 ± 2.19	17.14 ± 0.82	168.94 ± 22.96	123.19 ± 18.12	6.73 ± 1.10	3.99 ± 0.47	0.24 ± 0.03
FR	White Boston	34.23 ± 5.83	28.89 ± 1.43	25.35 ± 4.98	23.84 ± 1.61	14.86 ± 0.90	189.96 ± 48.90	142.53 ± 33.6	7.80 ± 1.47	4.19 ± 0.46	0.17 ± 0.07
	Waldmann's Green	21.52 ± 2.02	23.54 ± 2.01	15.80 ± 2.61	19.64 ± 1.36	17.02 ± 0.90	126.34 ± 29.84	94.07 ± 24.56	6.36 ± 0.56	5.18 ± 0.86	0.14 ± 0.02
	Black Rose	31.58 ± 2.17	22.19 ± 0.61	20.55 ± 2.25	20.83 ± 0.88	16.44 ± 0.66	170.55 ± 25.53	138.17 ± 20.52	6.09 ± 0.66	3.59 ± 0.21	0.11 ± 0.01
SH	White Boston	17.47 ± 0.65	29.04 ± 1.37	21.92 ± 3.07	22.37 ± 1.53	15.35 ± 0.87	146.36 ± 15.55	115.36 ± 12.37	7.31 ± 0.36	5.03 ± 0.5	0.07 ± 0.01
	Waldmann's Green	17.27 ± 1.19	28.88 ± 0.77	15.21 ± 1.34	21.51 ± 0.39	19.23 ± 0.86	140.75 ± 6.15	108.04 ± 6.77	7.33 ± 0.27	5.21 ± 0.31	0.09 ± 0.05
	Black Rose	19.38 ± 3.00	23.72 ± 0.72	22.94 ± 2.77	18.69 ± 0.90	16.22 ± 1.10	147.24 ± 17.83	117.04 ± 16.27	7.27 ± 1.73	4.90 ± 0.63	0.08 ± 0.01

Note: NFT = nutrient film technique; FR = floating raft; SH = semihydroponic. Values are analytical cultivation-unit means ± standard deviation ($n = 4$ units per configuration × cultivar combination). Morphological harvest variables were measured after plant removal using the destructive harvest protocol; therefore, they are not directly interchangeable with similarly named nondestructive in situ variables measured at D3. Cultivar effects were tested separately within FR, NFT, and SH; full parametric and permutation results are presented in Appendix Table A2. Letter-based comparisons are not displayed because the primary interpretation emphasizes overall cultivar effects and because sensitivity pairwise tests were conservative at $n = 4$. Comparisons among configurations are descriptive owing to unequal hydraulic replication.

Within NFT, cultivar effects were supported for leaf number ($p < 0.001$; permutation $p = 0.001$) and leaf width ($p = 0.003$; permutation $p = 0.008$), with Black Rose recording the greatest numerical values for both traits (21.05 ± 0.67 leaves and 17.14 ± 0.82 cm). Within the evaluated SH installation, cultivar effects were supported for plant height, leaf number, leaf length, and leaf width (parametric $p \leq 0.004$; permutation $p = 0.003$–0.006). White Boston and Waldmann's Green had the two highest numerical means for plant height, whereas the highest numerical means for leaf number, leaf length, and leaf width were observed in Black Rose, White Boston, and Waldmann's Green, respectively.

Total fresh weight did not differ significantly among cultivars within FR, NFT, or SH (parametric $p = 0.087$, 0.065, and 0.785; permutation $p = 0.083$, 0.084, and 0.797, respectively). FR–White Boston nevertheless recorded the numerically highest total fresh weight (189.96 ± 48.90 g plant⁻¹), followed by FR–Black Rose (170.55 ± 25.53 g plant⁻¹) and NFT–Black Rose (168.94 ± 22.96 g plant⁻¹). Total dry weight also showed no cultivar effect within any configuration (parametric $p = 0.075$–0.997; permutation $p = 0.060$–0.998). Leaf fresh weight differed among cultivars only within NFT ($p = 0.017$; permutation $p = 0.018$), whereas total dry matter content differed within FR and NFT ($p = 0.011$ and 0.037; permutation $p = 0.013$ and 0.024, respectively). Complete results are provided in Appendix Table A2.

Figure 2 shows the distributions of leaf fresh weight, root length, and total fresh weight. FR–White Boston and FR–Black Rose displayed high leaf fresh weight and root length, whereas NFT–Black Rose showed high leaf fresh weight and the highest total fresh weight within NFT. The comparatively broad spread of FR–White Boston total fresh weight reflects substantial variation among its four independent FR boxes.

Although FR–White Boston recorded the largest overall numerical mean for total fresh weight, the absence of a significant cultivar effect for this variable within each configuration means that the ranking should be interpreted descriptively. Likewise, the figure does not support inferential ranking among FR, NFT, and SH because the configurations were not represented by equivalent numbers of independent hydraulic units.

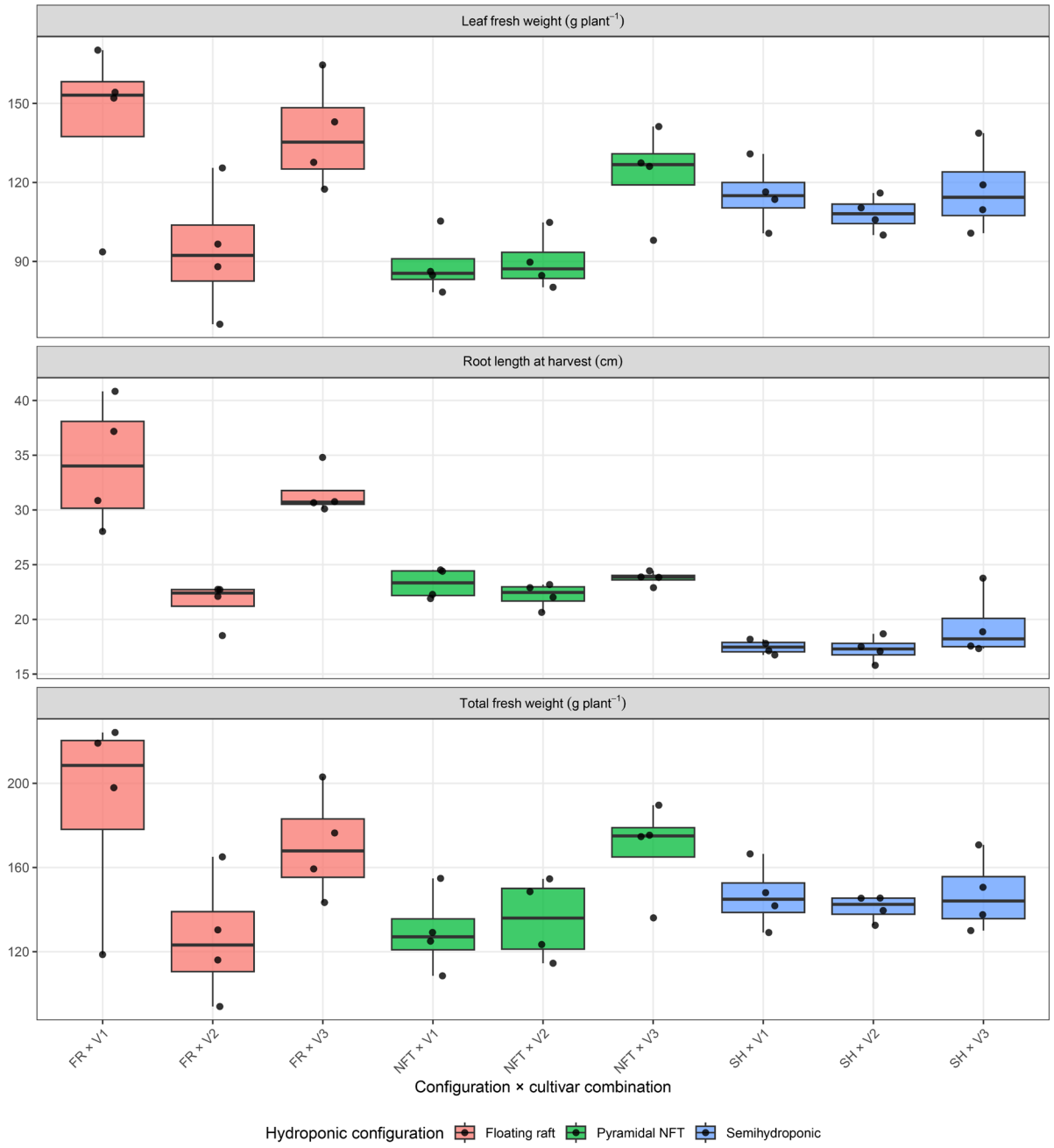

Figure 2. Variation in leaf fresh weight, root length at harvest, and total fresh weight among configuration × cultivar combinations

Note: V1 = White Boston; V2 = Waldmann's Green; V3 = Black Rose; NFT = nutrient film technique; FR = floating raft; SH = semihydroponic. Boxplots represent analytical cultivation-unit means, and points indicate individual analytical units. Comparisons among configurations are descriptive.

3.3 Biomass Partitioning and Dry Matter Accumulation

Figure 3 presents the fresh-biomass partitioning among leaves, roots, and stems. Leaves represented the dominant fresh-biomass component in every configuration × cultivar combination, accounting for 66.4–81.0% of total fresh weight. FR–White Boston recorded the numerically highest total fresh weight (189.96 ± 48.90 g plant^{-1}), followed by FR–Black Rose (170.55 ± 25.53 g plant^{-1}) and NFT–Black Rose (168.94 ± 22.96 g plant^{-1}). The lowest numerical totals occurred in FR–Waldmann's Green (126.34 ± 29.84 g plant^{-1}) and NFT–White Boston (129.38 ± 19.17 g plant^{-1}). These rankings are descriptive because hydraulic replication was not equivalent among configurations.

Organ allocation also differed descriptively among the evaluated installations. The root fraction represented 19.0–23.2% of total fresh weight in NFT, compared with 9.8–15.1% in FR and 6.9–8.1% in SH. Conversely, stem

contribution was relatively greater in SH (13.4–15.2%) than in most FR and NFT combinations. Within each configuration, cultivar effects on total dry weight were not significant in FR (parametric $p = 0.075$; permutation $p = 0.060$), NFT ($p = 0.847$; permutation $p = 0.824$), or SH ($p = 0.997$; permutation $p = 0.998$). Total dry weight consequently varied within a comparatively narrow range despite the larger numerical differences observed for fresh biomass, supporting the interpretation that hydration and organ allocation contributed substantially to the observed fresh-weight patterns.

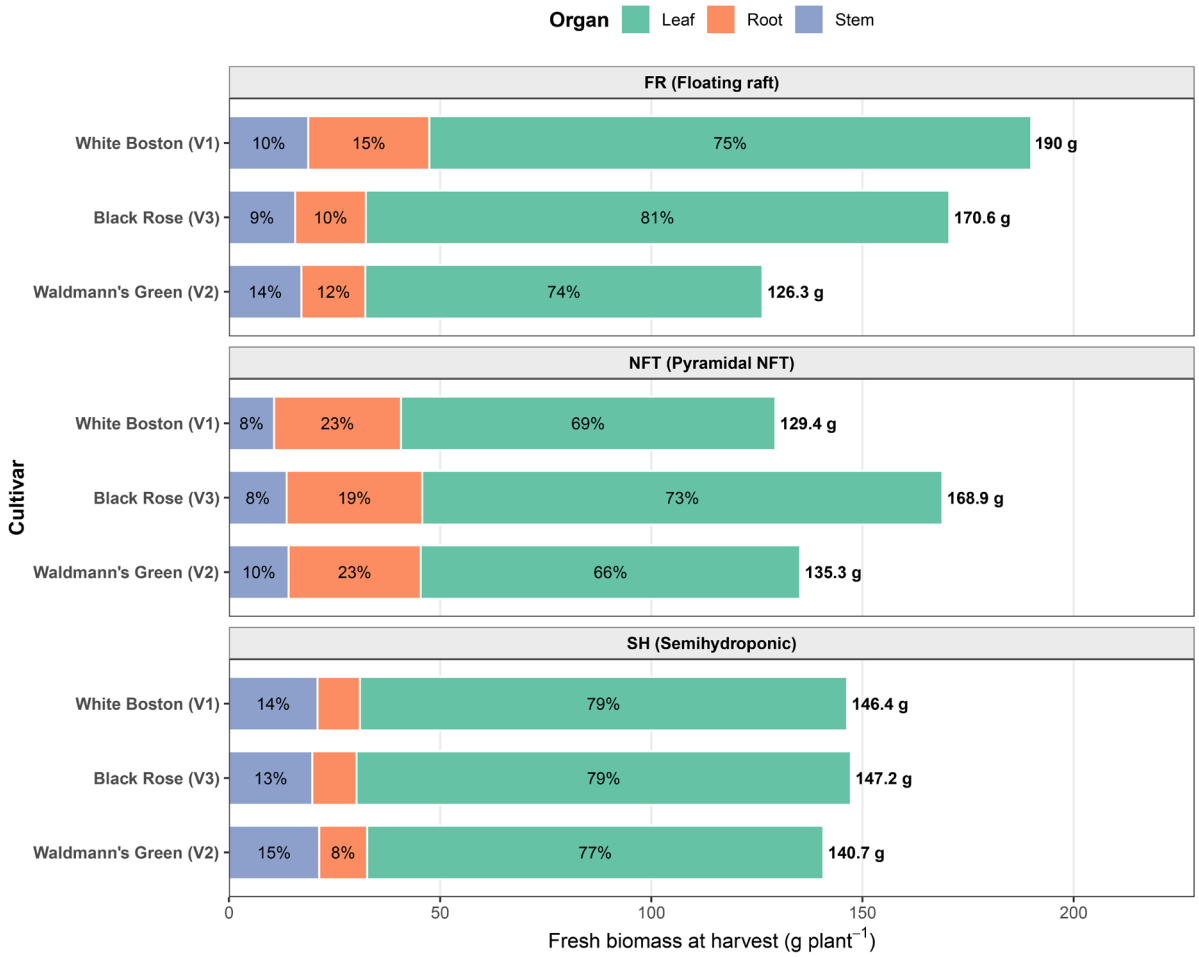

Figure 3. Fresh-biomass partitioning among leaves, roots, and stems across configuration × cultivar combinations

Note: V1 = White Boston; V2 = Waldmann's Green; V3 = Black Rose; NFT = nutrient film technique; FR = floating raft; SH = semihydroponic. Horizontal stacked bars represent mean organ fresh biomass at harvest. Internal labels show the percentage contribution of each organ to total fresh weight, and values at the ends of the bars show total fresh weight. Comparisons among hydroponic configurations are descriptive.

Figure 4 shows configuration-specific patterns in leaf fresh-weight proportion, root:shoot fresh-weight ratio, and total dry matter content. Within FR, cultivar effects were supported for total dry matter content (parametric $p = 0.011$; permutation $p = 0.013$) and leaf fresh-weight proportion ($p = 0.026$; permutation $p = 0.028$). The numerical mean for total dry matter content was highest in Waldmann's Green (5.18 ± 0.86%), followed by White Boston (4.19 ± 0.46%) and Black Rose (3.59 ± 0.21%). The numerical mean for leaf fresh-weight proportion was highest in Black Rose (0.810 ± 0.007), followed by White Boston (0.756 ± 0.046) and Waldmann's Green (0.741 ± 0.026).

Within NFT, cultivar effects were likewise detected for total dry matter content (parametric $p = 0.037$; permutation $p = 0.024$) and leaf fresh-weight proportion ($p = 0.042$; permutation $p = 0.036$). White Boston recorded the largest numerical mean for total dry matter content (5.11 ± 0.50%), compared with 4.60 ± 0.43% for Waldmann's Green and 3.99 ± 0.47% for Black Rose. A different pattern was observed for leaf fresh-weight proportion, for which Black Rose had the largest numerical mean (0.728 ± 0.012), followed numerically by White Boston (0.687 ± 0.027) and Waldmann's Green (0.667 ± 0.043). No cultivar effect on these variables was detected within SH (parametric and permutation $p \geq 0.339$). Root:shoot fresh-weight ratio did not differ among cultivars in the parametric analyses of any configuration. A permutation-based signal within FR ($p = 0.045$) was not supported

by the corresponding parametric model ($p = 0.125$) and was therefore interpreted cautiously. Across configurations, the higher root:shoot ratios in NFT and the comparatively high leaf proportions in FR and SH remain descriptive installation-level patterns.

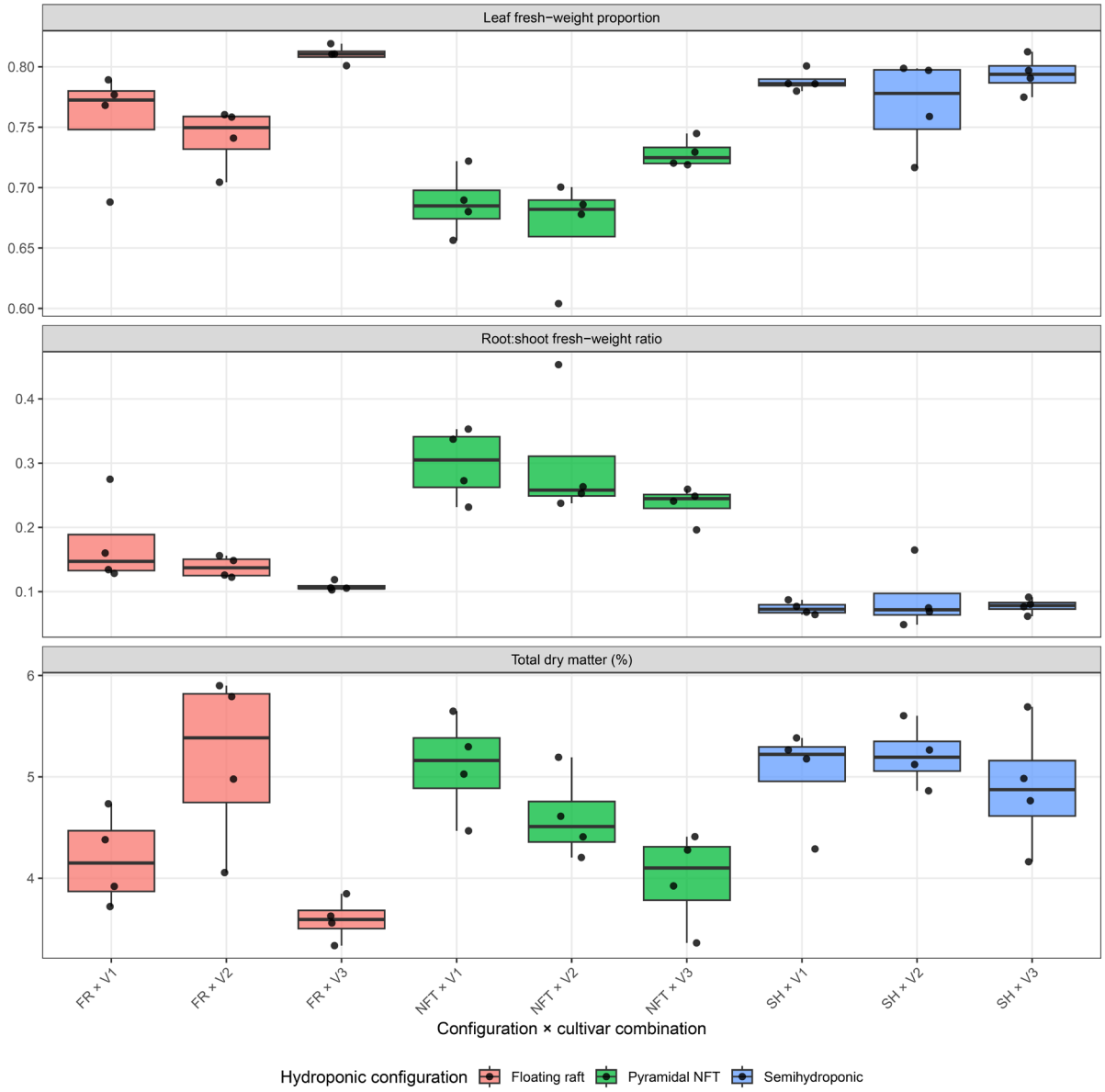

Figure 4. Variation in leaf fresh-weight proportion, root:shoot fresh-weight ratio, and total dry matter content among configuration × cultivar combinations

Note: V1 = White Boston; V2 = Waldmann's Green; V3 = Black Rose; NFT = nutrient film technique; FR = floating raft; SH = semihydroponic. Boxplots show analytical cultivation-unit means, and points represent individual analytical units. Overall cultivar effects were tested separately within each hydroponic configuration. Numerical differences among cultivars are descriptive because adjusted pairwise comparisons are not presented. Comparisons among configurations are also descriptive.

3.4 Multivariate Relationships Between Pre-Harvest and Harvest Traits

3.4.1 Principal component analysis of harvest traits

The first two principal components explained 56.9% of the standardized variation in the selected harvest traits, with PC1 accounting for 31.3% and PC2 for 25.5% (Figure 5). Total fresh weight was excluded from the PCA because it was exactly equal to the sum of leaf, root, and stem fresh weights and would therefore duplicate the same biomass information. The PCA was used as an exploratory visualization of multivariate tendencies rather than as an inferential test of separation among hydroponic configurations.

Positive PC1 loadings were strongest for leaf fresh-weight proportion, stem fresh weight, leaf fresh weight, and leaf number, whereas root:shoot fresh-weight ratio and root fresh weight loaded negatively on this axis. PC2 was

positively associated mainly with total dry matter content and leaf width and negatively associated with root length, root fresh weight, leaf fresh weight, and leaf number. This structure indicates that the first two axes primarily represented contrasts between leaf- and stem-oriented fresh-biomass allocation, root investment, and dry-matter concentration.

NFT analytical units occurred predominantly on the negative side of PC1, whereas SH units were more frequently located at positive PC1 values and often at positive PC2 values. FR units showed the broadest dispersion and extended across positive and negative sectors of both axes. Nevertheless, the configuration ellipses overlapped substantially, and cultivar symbols did not form discrete groups. These patterns therefore describe the evaluated installations and do not demonstrate definitive multivariate discrimination among configurations or cultivars.

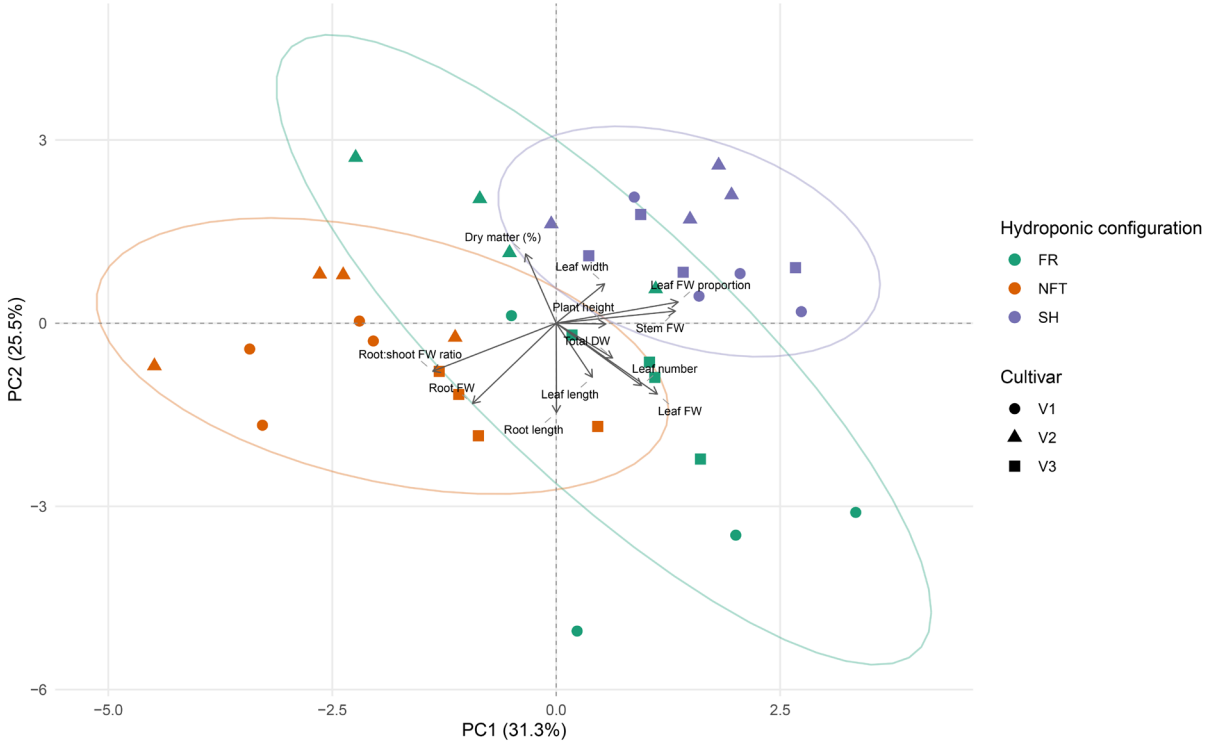

Figure 5. Principal component analysis (PCA) biplot of harvest morphology, organ-level fresh biomass, dry biomass, and biomass-allocation traits

Note: V1 = White Boston; V2 = Waldmann's Green; V3 = Black Rose; NFT = nutrient film technique; FR = floating raft; SH = semihydroponic. Points represent analytical cultivation units, colors indicate hydroponic configurations, and symbols indicate lettuce cultivars. Ellipses show the dispersion of analytical units within each evaluated configuration. Total fresh weight was excluded because it was the exact sum of leaf, root, and stem fresh weights. PC1 and PC2 explained 31.3% and 25.5% of the total variance, respectively. The PCA is exploratory and does not represent a fully replicated inferential comparison among configurations.

3.4.2 Correlations between harvest-day nondestructive measurements and harvest traits

Figure 6 summarizes exploratory Pearson correlations between five nondestructive traits measured in situ at D3 and destructive harvest traits across the 36 analytical cultivation units. After Benjamini–Hochberg adjustment for 60 pairwise tests, 12 correlations remained significant. The strongest association occurred between leaf number at D3 and total leaf number at harvest ($r = 0.79$, adjusted $p < 0.001$).

Leaf length at D3 was positively associated with root length at harvest ($r = 0.65$, adjusted $p = 0.001$) and leaf length at harvest ($r = 0.57$, adjusted $p = 0.003$), and negatively associated with total dry matter content ($r = −0.62$, adjusted $p = 0.001$). Leaf number at D3 was positively associated with root length ($r = 0.58$, adjusted $p = 0.003$) and leaf fresh weight ($r = 0.54$, adjusted $p = 0.006$), and negatively associated with leaf width at harvest ($r = −0.52$, adjusted $p = 0.008$) and total dry matter content ($r = −0.45$, adjusted $p = 0.030$).

Additional significant associations occurred between leaf width at D3 and leaf width at harvest ($r = 0.54$, adjusted $p = 0.006$), between plant height at D3 and both leaf length ($r = 0.52$, adjusted $p = 0.008$) and plant height at harvest ($r = 0.45$, adjusted $p = 0.030$), and between chlorophyll content at D3 and root length at harvest ($r = 0.47$, adjusted $p = 0.023$). Because the analysis pooled observations from the three evaluated configurations, these coefficients represent exploratory associations and may partly reflect installation-specific differences.

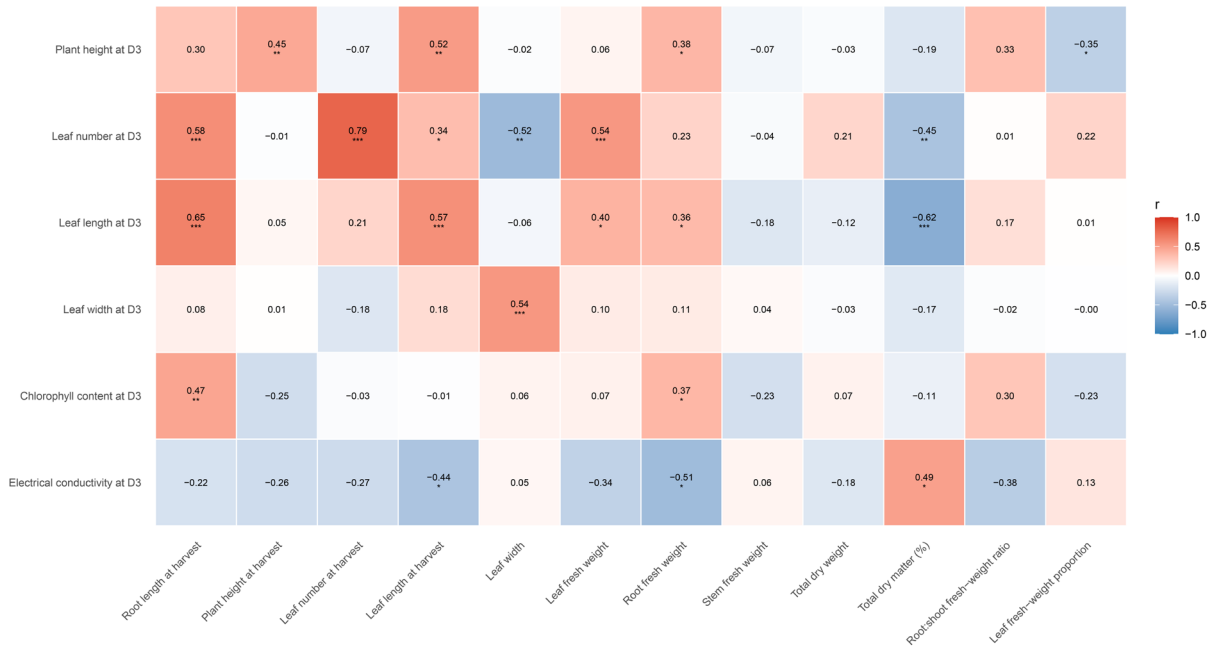

Figure 6. Exploratory Pearson correlations between nondestructive in situ traits measured at D3 (60 days after sowing) and destructive harvest traits in lettuce grown under three hydroponic configurations

Note: Cells show Pearson correlation coefficients (*r*) calculated across 36 analytical cultivation units. D3 traits were measured nondestructively while plants remained in their cultivation positions, whereas harvest traits were recorded after plant removal using a destructive protocol. Asterisks denote significance based on Benjamini–Hochberg-adjusted *p*-values (* adjusted $p < 0.05$, ** adjusted $p < 0.01$, and *** adjusted $p < 0.001$). Electrical conductivity was excluded because it was measured at the hydraulic-unit level and D3 values were unavailable for the semihydroponic (SH) installation. Positive and negative correlations are shown in red and blue, respectively. These correlations represent exploratory associations and should not be interpreted as causal or predictive relationships. D3 = third nondestructive assessment.

4. Discussion

4.1 Configuration-Specific Patterns in Pre-Harvest Growth and Harvest Morphology

Most cultivar differences in temporal response were modest during the definitive 30-day phase. Changes from D2 to D3 in plant height, leaf number, leaf length, and leaf width did not differ significantly among cultivars within FR, NFT, or SH. The only overall cultivar effect on a D3 − D2 change score was detected for chlorophyll content within FR, although no individual pairwise contrast remained significant after adjustment for multiple comparisons. Nevertheless, the descriptive profiles and harvest morphology indicate that the three evaluated installations created contrasting root-zone environments. FR maintained roots in continuously aerated solution, NFT exposed roots to a recirculating nutrient film, and SH combined gravel support with intermittent drip delivery and drainage. These physical differences can influence oxygen availability, hydraulic continuity, root-zone temperature, nutrient transport, and plant water status, all of which may affect leaf expansion, root elongation, and fresh-biomass accumulation.

This interpretation is consistent with reports that hydroponic architecture and operating conditions modify lettuce growth, nutrient uptake, phytochemical composition, and water consumption (Yang et al., 2024), and that system performance depends on the interaction between root-zone design, nutrient delivery, and crop-management conditions (Chowdhury et al., 2024). Within NFT, oxygen enrichment and flow management can alter root development and crop performance (Nitu et al., 2024), while controlled modification of root-zone temperature has also been shown to affect lettuce productivity, nutrient uptake, and metabolite accumulation (Hayashi et al., 2024). However, dissolved oxygen, root-zone temperature, and flow characteristics were not measured directly in the present trial. Consequently, these mechanisms should be regarded as biologically plausible explanations for the observed installation-specific patterns, not as experimentally demonstrated causes or as evidence that one hydroponic configuration was universally superior.

4.2 Cultivar Responses Within Hydroponic Configurations

Cultivar responses were clearly trait dependent within the evaluated configurations. In FR, cultivar effects were supported for several harvest morphological traits, total dry matter content, and leaf fresh-weight proportion.

Within NFT, cultivar effects were detected for leaf number, leaf width, leaf fresh weight, total dry matter content, and leaf fresh-weight proportion, whereas within SH the significant responses were concentrated mainly in harvest morphology rather than biomass. In contrast, total fresh weight did not differ significantly among cultivars within FR, NFT, or SH. Thus, FR–White Boston, FR–Black Rose, and NFT–Black Rose should be described as combinations with high numerical fresh-biomass values under the evaluated conditions, rather than as statistically superior treatments.

The dependence of lettuce performance on cultivar identity is well documented. Sapkota et al. (2019) showed that hydroponic productivity changes with cultivar × nutrient-solution combinations, while Leiva Espinoza et al. (2018) reported marked variation among lettuce cultivars grown in recirculating NFT. Genotype evaluations across environments likewise demonstrate that lettuce cultivars differ in their stability and adaptation (Tobar-Tosse et al., 2022). Treatment-related differences in growth, fresh biomass, physiology, and nutrient uptake have also been reported under contrasting nutrient sources and operational conditions (Ahmed et al., 2021; Nitu et al., 2024). The present results therefore support cultivar-specific management, but they do not provide a fully replicated test of a general configuration × cultivar interaction because hydraulic replication differed among FR, NFT, and SH. Recommendations should consequently be framed according to cultivar, target trait, and the particular installation evaluated.

4.3 Fresh Yield, Biomass Partitioning, and Dry Matter Patterns

FR–White Boston recorded the highest numerical total fresh weight, followed by FR–Black Rose and NFT–Black Rose. Across all combinations, leaves represented 66.4–81.0% of total fresh biomass, confirming that the marketable foliar fraction dominated plant fresh mass. NFT displayed a comparatively larger root fraction, whereas SH showed a relatively greater stem contribution. These patterns indicate that similar total fresh weights can arise from different organ-allocation strategies.

Fresh mass alone, however, does not distinguish tissue expansion caused by water accumulation from structural biomass formation. Total dry weight did not differ among cultivars within any configuration, despite the larger numerical variation in total fresh weight. Cultivar effects on total dry matter content and leaf fresh-weight proportion were detected within FR and NFT, but not within SH. This combination of results suggests that differences in hydration and organ allocation contributed substantially to the observed fresh-biomass patterns. Similar studies have emphasized the importance of evaluating root and shoot fractions, dry matter, and water-related traits together when interpreting lettuce performance in hydroponic or soilless systems (Germer et al., 2023; Li et al., 2018). Variation among cultivars in fresh and dry biomass has also been reported under NFT conditions (Leiva Espinoza et al., 2018).

From a production perspective, high leaf fresh-weight proportion can be advantageous because leaves constitute the commercial product, whereas a higher dry matter percentage may indicate less water-diluted tissue. Neither trait alone is sufficient to define agronomic superiority, because commercial quality also depends on texture, nutrient composition, appearance, and postharvest behavior, which were not measured here. A robust assessment of sustainable protected horticulture should therefore integrate marketable fresh yield with dry matter, organ allocation, water and nutrient use, and crop quality. This broader interpretation is consistent with recent work emphasizing that hydroponic performance depends on both crop output and management efficiency rather than yield alone (Chowdhury et al., 2024; Fathidarehnijeh et al., 2024; Wang et al., 2023).

4.4 Interpretation of Exploratory Multivariate and Correlation Patterns

The PCA summarized complementary dimensions of harvest performance rather than identifying a single axis of superiority. PC1 and PC2 jointly explained 56.9% of the standardized variation and contrasted leaf- and stem-oriented fresh-biomass allocation with root investment and dry matter concentration. Total fresh weight was excluded because it was exactly equal to the sum of leaf, root, and stem fresh weights; retaining it would have duplicated the same biomass information and introduced an exact linear dependency. The substantial overlap among configuration ellipses and the absence of discrete cultivar clusters further indicate that the ordination should be interpreted as an exploratory representation of tendencies within the evaluated installations, not as evidence of statistically distinct multivariate groups.

The pooled Pearson correlations identified a limited set of associations that remained significant after controlling the false-discovery rate. The strongest relationships involved leaf number and leaf length measured nondestructively at D3, which were associated with selected morphological, fresh-biomass, and dry-matter traits at harvest. However, because observations from FR, NFT, and SH were pooled, part of these coefficients may reflect configuration-level contrasts rather than relationships operating consistently within every installation. Moreover, D3 and harvest traits were recorded using complementary nondestructive and destructive protocols. The correlations should therefore be interpreted as exploratory associations rather than as evidence of measurement agreement, causal effects, or operational prediction equations.

Even with these limitations, the results illustrate the potential value of nondestructive measurements close to harvest for crop monitoring. Previous work has shown that repeated measurements of growth and physiological status can help characterize lettuce development under recirculating hydroponics (Fraile-Robayo et al., 2017), while dedicated sensing approaches have been developed to quantify water circulation and plant mass dynamics nondestructively in controlled hydroponic environments (Huang et al., 2024). Translating the present correlations into decision-support tools would require independent calibration and validation across crop cycles, cultivars, and hydraulically replicated installations, together with direct monitoring of root-zone and nutrient-solution conditions.

4.5 Limitations, Practical Implications, and Future Research

The principal limitation of this study was the unequal hydraulic replication of the three configurations. FR was represented by 12 independently aerated boxes, NFT by two independent recirculation modules containing nested analytical cultivation units, and SH by one shared reservoir and irrigation network. Consequently, the four analytical units per configuration × cultivar combination were not equivalent independent replicates of the configuration factor. The trial was also conducted during a single crop cycle, and the definitive comparison lasted 30 days. These features restrict generalization beyond the specific installations and environmental conditions evaluated. Additional limitations include the absence of direct measurements of dissolved oxygen, root-zone temperature, nutrient uptake, complete water and nutrient balances, product quality, and postharvest performance.

Within this scope, the study still provides useful installation-specific evidence for cultivar management in protected lettuce production. The results indicate that no single configuration produced the most favorable response for every cultivar and trait. Growers should therefore align configuration choice with the intended product and management goal, such as high foliar fresh mass, a larger root fraction, greater dry matter concentration, or architectural constraints. For protected horticulture in arid coastal environments, these agronomic outcomes must ultimately be considered together with water productivity, nutrient-use efficiency, energy demand, labor requirements, and economic performance. Recent comparative research similarly emphasizes that the sustainability of hydroponic production cannot be inferred from yield alone (Chowdhury et al., 2024; Fathidarehnijeh et al., 2024; Wang et al., 2023).

Future experiments should include multiple independent hydraulic installations for each configuration, randomized cultivar positions within each installation, and repeated crop cycles or seasons. Direct measurements of dissolved oxygen, flow rate, root-zone temperature, evapotranspiration, reservoir replenishment, nutrient uptake, and nutrient losses would help link system architecture to plant response. Commercial quality, sensory attributes, nitrate content, and shelf life should also be evaluated. Finally, mineral nutrient solutions should be compared with well-characterized organic or circular nutrient sources, including treated waste-derived solutions, compost or vermicompost extracts, and biodigester effluents, while controlling for nutrient availability and microbial safety. Such work would allow the configuration- and cultivar-specific patterns observed here to be tested under more broadly replicated and resource-aware production scenarios (Ahmed et al., 2021; Germer et al., 2023; Hayashi et al., 2024).

5. Conclusions

In relation to the study objective, the results characterized cultivar-specific patterns of pre-harvest growth, harvest morphology, fresh and dry biomass, and biomass partitioning under the evaluated pyramidal NFT, FR, and SH configurations. Temporal responses from the baseline assessment at 30 DAS (D2) to the harvest-day assessment at 60 DAS (D3) were generally similar among cultivars within each configuration. The only overall cultivar effect detected for a D3 − D2 change corresponded to chlorophyll content in FR, although no individual pairwise comparison remained significant after adjustment for multiple comparisons. At harvest, cultivar effects were evident for selected morphological and biomass-allocation traits within each configuration, whereas total fresh weight and total dry weight did not differ significantly among cultivars. FR–White Boston recorded the numerically highest total fresh weight (189.96 ± 48.90 g plant^{-1}), but this value should not be interpreted as statistical superiority.

Taken together, the findings indicate that no evaluated configuration was consistently superior across all cultivars and response variables. Fresh-biomass patterns were associated with differences in foliar development, organ allocation, and likely tissue water content rather than with corresponding increases in total structural dry biomass. Consequently, configuration choice should be aligned with cultivar identity and the intended production criterion, such as foliar fresh mass, root development, dry matter concentration, or spatial and operational constraints. Because hydraulic replication was unequal among FR, NFT, and SH, comparisons among configurations remain descriptive and installation-specific. Likewise, the PCA and pooled correlations should be interpreted as exploratory evidence of multivariate organization and trait association rather than as definitive discrimination, prediction, or causation.

The relevance of these results to sustainable and organic-oriented protected horticulture lies in providing a

controlled agronomic baseline for improving the integration of cultivar selection, system architecture, and resource management. Because all configurations were managed using the same mineral nutrient formulation, the present trial does not demonstrate organic hydroponic production. Rather, it establishes an experimental reference for future studies that compare standardized mineral nutrition with safe and well-characterized organic or circular nutrient sources. Further research should incorporate independently replicated hydraulic installations, multiple crop cycles, and direct measurements of water productivity, nutrient-use efficiency, energy demand, crop quality, postharvest performance, and microbial safety. Under this framework, hydroponic and soilless systems may be evaluated more rigorously for their potential contribution to resource-efficient production and to the progressive development of organic-oriented and circular horticultural strategies.

Author Contributions

Conceptualization, K.O.-Q., L.O.-R., M.P.-M., and P.C.-I.; methodology, L.O.-R., P.C.-I., and S.Y.C.-V.; software, K.O.-Q. and C.A.-L.; validation, C.A.-L., S.Y.C.-V., and K.O.-Q.; formal analysis, P.C.-I., M.P.-M., and L.O.-R.; investigation, K.O.-Q.; resources, S.Y.C.-V. and C.A.-L.; data curation, P.C.-I., L.O.-R., and K.O.-Q.; writing—original draft preparation, K.O.-Q.; writing—review and editing, K.O.-Q. and P.C.-I.; visualization, P.C.-I. and K.O.-Q.; supervision, S.Y.C.-V. and C.A.-L.; project administration, M.P.-M. and C.A.-L.; funding acquisition, M.P.-M. and C.A.-L. All authors have read and agreed to the published version of the manuscript.

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Data Availability

The data used to support the research findings are available from the corresponding author upon request.

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Conflicts of Interest

The authors declare no conflicts of interest.

Declaration on the Use of Generative AI and AI-assisted Technologies

The authors declare that generative AI tools were used solely to improve the language, clarity, and stylistic consistency of the manuscript. The authors remain fully responsible for the accuracy, originality, integrity, and final content of the work.

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Appendix

Table A1. Cultivar effects on D3 − D2 change scores for pre-harvest traits within each hydroponic configuration

Configuration	Variable	Parametric Test Statistic	Parametric *p*-Value	Permutation *p*-Value
NFT	Chlorophyll content	$F(2, 8) = 0.79$	0.486	0.444
	Leaf length	$F(2, 8) = 0.28$	0.763	0.734
	Leaf number	$F(2, 8) = 2.84$	0.117	0.114
	Leaf width	$F(2, 8) = 0.15$	0.867	0.868
	Plant height	$F(2, 8) = 0.01$	0.994	0.990
FR	Chlorophyll content	$F(2, 9) = 4.39$	0.047	0.037
	Leaf length	$F(2, 9) = 0.77$	0.490	0.522
	Leaf number	$F(2, 9) = 0.14$	0.867	0.852
	Leaf width	$F(2, 9) = 0.69$	0.525	0.541
	Plant height	$F(2, 9) = 1.28$	0.325	0.332
SH	Chlorophyll content	$F(2, 9) = 0.08$	0.921	0.918
	Leaf length	$F(2, 9) = 1.72$	0.232	0.241
	Leaf number	$F(2, 9) = 1.41$	0.293	0.243
	Leaf width	$F(2, 9) = 0.24$	0.789	0.797
	Plant height	$F(2, 9) = 1.59$	0.257	0.226

Note: NFT = nutrient film technique; FR = floating raft; SH = semihydroponic. Tests evaluate the overall cultivar effect on the analytical-unit change score Δ = D3 − D2. FR and SH were analyzed with cultivar as a fixed factor; NFT models also included module as a fixed blocking factor. Permutation *p*-values are based on 999 label permutations, restricted within NFT module. Only FR chlorophyll content showed a significant overall cultivar effect; no pairwise contrast remained significant after Holm adjustment.

Table A2. Overall cultivar effects on harvest morphology, biomass, and allocation traits within each hydroponic configuration

Configuration	Variable	Parametric Test Statistic	Parametric *p*-Value	Permutation *p*-Value
NFT	Root length at harvest	$F(2, 8) = 2.07$	0.189	0.134
	Plant height at harvest	$F(2, 8) = 0.87$	0.456	0.458
	Leaf number at harvest	$F(2, 8) = 36.47$	<0.001	0.001
	Leaf length at harvest	$F(2, 8) = 0.65$	0.548	0.493
	Leaf width at harvest	$F(2, 8) = 13.19$	0.003	0.008
	Total fresh weight	$F(2, 8) = 3.92$	0.065	0.084
	Leaf fresh weight	$F(2, 8) = 7.09$	0.017	0.018
	Root fresh weight	$F(2, 8) = 0.06$	0.944	0.975
	Stem fresh weight	$F(2, 8) = 3.08$	0.102	0.104
	Leaf dry weight	$F(2, 8) = 0.28$	0.765	0.696
	Root dry weight	$F(2, 8) = 0.77$	0.494	0.417
	Stem dry weight	$F(2, 8) = 0.69$	0.527	0.529
	Total dry weight	$F(2, 8) = 0.17$	0.847	0.824
	Total dry matter content	$F(2, 8) = 5.14$	0.037	0.024
	Root:shoot fresh-weight ratio	$F(2, 8) = 1.07$	0.387	0.443
	Leaf fresh-weight proportion	$F(2, 8) = 4.82$	0.042	0.036
FR	Root length at harvest	$F(2, 9) = 12.61$	0.002	0.003
	Plant height at harvest	$F(2, 9) = 23.31$	<0.001	0.005
	Leaf number at harvest	$F(2, 9) = 7.46$	0.012	0.013
	Leaf length at harvest	$F(2, 9) = 10.79$	0.004	0.003
	Leaf width at harvest	$F(2, 9) = 7.25$	0.013	0.007
	Total fresh weight	$F(2, 9) = 3.24$	0.087	0.083
	Leaf fresh weight	$F(2, 9) = 4.01$	0.057	0.057
	Root fresh weight	$F(2, 9) = 2.90$	0.107	0.047
	Stem fresh weight	$F(2, 9) = 0.64$	0.547	0.557
	Leaf dry weight	$F(2, 9) = 2.37$	0.149	0.137
	Root dry weight	$F(2, 9) = 3.66$	0.069	0.039
	Stem dry weight	$F(2, 9) = 1.05$	0.390	0.351
	Total dry weight	$F(2, 9) = 3.51$	0.075	0.060
	Total dry matter content	$F(2, 9) = 7.85$	0.011	0.013
	Root:shoot fresh-weight ratio	$F(2, 9) = 2.64$	0.125	0.045
	Leaf fresh-weight proportion	$F(2, 9) = 5.66$	0.026	0.028

SH	Root length at harvest	$F(2, 9) = 1.51$	0.272	0.215
	Plant height at harvest	$F(2, 9) = 36.77$	<0.001	0.005
	Leaf number at harvest	$F(2, 9) = 11.20$	0.004	0.006
	Leaf length at harvest	$F(2, 9) = 13.53$	0.002	0.005
	Leaf width at harvest	$F(2, 9) = 18.41$	<0.001	0.003
	Total fresh weight	$F(2, 9) = 0.25$	0.785	0.797
	Leaf fresh weight	$F(2, 9) = 0.59$	0.573	0.576
	Root fresh weight	$F(2, 9) = 0.14$	0.871	0.930
	Stem fresh weight	$F(2, 9) = 0.36$	0.707	0.720
	Leaf dry weight	$F(2, 9) = 0.00$	0.999	0.997
	Root dry weight	$F(2, 9) = 0.03$	0.967	0.969
	Stem dry weight	$F(2, 9) = 0.53$	0.604	0.568
	Total dry weight	$F(2, 9) = 0.00$	0.997	0.998
	Total dry matter content	$F(2, 9) = 0.40$	0.682	0.717
	Root:shoot fresh-weight ratio	$F(2, 9) = 0.26$	0.777	0.931
	Leaf fresh-weight proportion	$F(2, 9) = 1.22$	0.339	0.350

Note: NFT = nutrient film technique; FR = floating raft; SH = semihydroponic. Overall cultivar effects were tested separately within each hydroponic configuration using analytical cultivation-unit means. One-way linear models were used for FR and SH, while NFT models included module as a fixed blocking factor. Permutation *p*-values were based on 999 label permutations, restricted within module for NFT. Parametric and permutation results should be interpreted jointly, particularly where residual diagnostics indicated potential assumption violations. The tests assess overall cultivar effects only and do not establish significant pairwise differences among cultivars. Comparisons among configurations are descriptive.