

Original Article

Comparative assessment of growth performance and water nutrient of duckweed (*Lemna minor*) cultured with and without fertilizer in an industrial-scale raceway setting

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Abstract: Duckweed (*Lemna minor*) has gained increasing attention as a sustainable biomass resource because of its rapid growth and high nutrient-recovery potential. This study investigated the effects of fertilizer application on the growth performance, productivity, and water-nutrient dynamics of *L. minor* cultured in an industrial-scale raceway system. Two enclosed raceway tanks (29×9×0.5 m) were established under fertilized and non-fertilized conditions and monitored over seven independent production cycles. The experiment was conducted over seven independent production cycles. Growth performance was assessed using final biomass, biomass yield increase, doubling time, relative growth rate (RGR), and productivity, while water quality parameters were analyzed at the end of each culture cycle. Fertilized raceways produced significantly higher final biomass (77.21±26.90 kg), biomass yield increase (451.49±192.13%), RGR (23.62±5.18% day⁻¹), and productivity (9.03±3.84 kg day⁻¹) compared with non-fertilized raceways (P<0.05). Fertilizer application also significantly influenced nitrate, nitrite, total Kjeldahl nitrogen, total nitrogen, chemical oxygen demand, pH, and dissolved oxygen concentrations. Lower nitrite and total nitrogen levels in fertilized raceways indicated efficient nutrient assimilation by duckweed. These findings suggest that fertilizer supplementation substantially enhances biomass production and nutrient utilization efficiency, supporting the development of sustainable, large-scale commercial raceway duckweed cultivation systems for biomass generation and nutrient recovery.

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Introduction

The rapid proliferation of duckweed, particularly *Lemna minor*, as a high-protein bioresource has intensified interest in developing larger-scale production for sustainable biomass production (Petersen et al., 2022; Coughlan et al., 2023). Previous studies have primarily focused on duckweed production in small-scale laboratory settings, earthen ponds, or tank-based culture systems, where controlled environmental conditions are easier to maintain (Chakrabarti et al., 2018; Ullah et al., 2021). Although these studies demonstrated *L. minor*'s strong potential for rapid biomass accumulation and nutrient recovery, information on its production performance in large-scale commercial raceway systems remains

limited.

Most studies mainly evaluated the effects of manipulated nitrogen and phosphorus concentrations on biomass production and nutrient composition in pond or tank culture systems (Devlamynck et al., 2021). However, the cultivation dynamics in large commercial-scale raceway systems may differ substantially. Consequently, directly applying findings from laboratory or small-scale systems to industrial raceway production may not fully reflect commercial cultivation performance. Furthermore, the effects of fertilizer application on long-term biomass productivity and nutrient uptake efficiency in continuous large-scale raceway production remain poorly understood.

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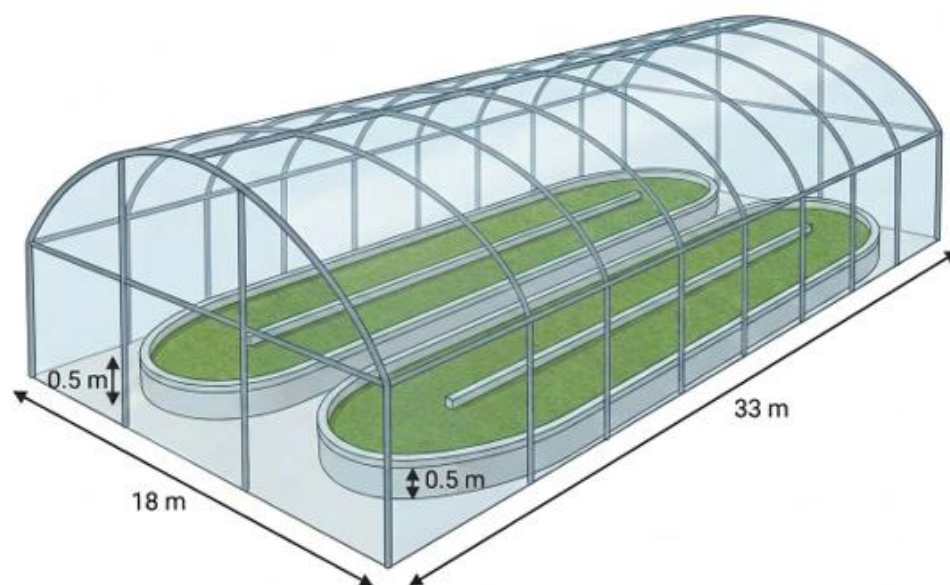


Figure 1. Industrial-scale *Lemna minor* raceway systems under (A) non-fertilized and (B) fertilized conditions.

Therefore, this study compared the growth performance, biomass productivity, and water and nutrient composition of duckweed grown under fertilized and non-fertilized conditions in an industrial-scale raceway system. The results will provide important insights into the feasibility of reducing fertilizer dependency while maintaining satisfactory biomass production and nutritional quality. This information is essential for establishing scalable, economically viable duckweed farming systems that support sustainable bioresource production and the development of a circular bioeconomy (O'Mahoney et al., 2022; Maissour et al., 2023). The findings can also provide valuable insights into environmentally sustainable biomass cultivation strategies that support circular resource utilization and improved water and nutrient management.

Materials and Methods

Experimental raceway system: The experiment was conducted using 2 units of enclosed industrial-scale raceway systems designed for large-scale *L. minor* cultivation (Figs. 1, 2). The raceway measured 29 m in length, 9 m in width, and 0.5 m in depth, and was covered with a greenhouse-like enclosure to minimize environmental fluctuations and external contamination. Because the production system was

commercial-scale, experimental replication within each treatment was not feasible. Each treatment consisted of a single raceway containing approximately 73 tonnes of water, making it impractical to establish multiple independent replicate units due to infrastructure, space, and operational constraints. Instead, the study was conducted over seven consecutive production cycles under the same management and environmental conditions. Each production cycle was treated as an independent temporal replicate, allowing evaluation of the consistency and repeatability of treatment effects over time. This approach is commonly adopted in commercial aquaculture and agricultural production studies where true spatial replication is constrained by the large scale of the production system. We applied a commercial organic hybrid fertilizer (QL Eco-Green, Malaysia) at 4 kg per raceway once during the experimental period. The fertilizer contained a balanced nitrogen–phosphorus–potassium (NPK) formulation of 5–5–5.

Growth performance: At the end of the 7-day culture period, the total biomass of *L. minor* in each tank was harvested and weighed to determine the final biomass (Anjur et al., 2024). Growth performance was evaluated based on biomass yield increase (%), doubling time (DT), relative growth rate (RGR), and

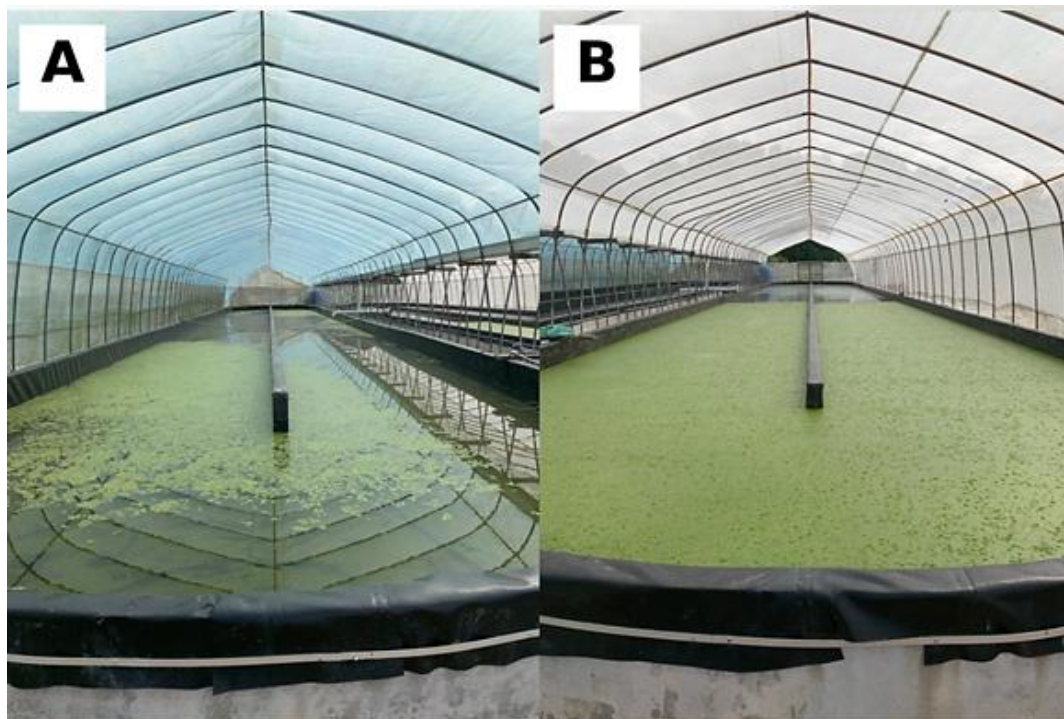


Figure 2. Industrial-scale *Lemna minor* raceway systems under (A) non-fertilized and (B) fertilized conditions.

productivity per day and per gram (Ziegler et al., 2015). The following are the equations used for each analysis:

Biomass yield increase (%) = ((Final biomass – Initial biomass) / Initial biomass) * 100

Doubling time (DT) = $t \times \ln(2) / \ln(W_t) - \ln(W_0)$

Relative growth rate (RGR) = $\ln(W^t) - \ln(W_0) / t$

Productivity per day ($g\ day^{-1}$) = (Final biomass – Initial biomass) / t

Productivity per day ($g\ day^{-1}$) = (Final biomass – Initial biomass) / (Surface area x t)

Water nutrient analysis: At the end of the experiment, we collected water samples from both raceway tanks for nutrient and water quality analyses. The samples were analyzed for nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_3), orthophosphate (PO_4^{3-}), chemical oxygen demand (COD), biological oxygen demand (BOD), and total nitrogen (TN), with all parameters expressed in mg/L. Nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_3), and orthophosphate (PO_4^{3-}) were analyzed using standard colorimetric methods. Chemical oxygen demand (COD) was determined according to HACH Method 8000, while five-day biochemical oxygen demand (BOD_5) was determined according to APHA Standard Methods

5210 B, 4500-O G. Total nitrogen (TN) and Total Kjeldahl Nitrogen (TKN) were analyzed using the persulfate digestion method followed by spectrophotometric analysis according to the APHA Standard Methods 4500-Norg. All results were expressed in mg/L.

Statistical analysis: We compared the growth performance and nutrient composition of *L. minor* grown in large commercial-scale raceways with and without fertilizer. Data were collected over seven culture rounds for both treatments. We performed descriptive statistics and tests for normality and homogeneity of variance, then tested the hypothesis using the Wilcoxon Signed-Rank Test. We also calculated Spearman rank-order correlations for both groups.

Results

Growth performance: The two treatments differed significantly in growth performance indicators (Tables 1, 2). When fertilized, the average final biomass was 77.2126.90 kg, whilst the no-fertilizer tank biomass was 17.72 ± 2.93 Kg. In the fertilized group, the biomass yield increased by 451.49192.13%, whereas in the non-fertilized group,

Table 1. Growth performance and biomass productivity of *Lemna minor* cultured in commercial-scale raceway systems under fertilized and non-fertilized conditions. Values are presented as mean±standard deviation. Different superscript letters within the same variable indicate significant differences between treatments ($P<0.05$).

Parameter	Group	Mean±SD
Final Biomass (kg)	Without Fertilizer	17.72±2.93 ^a
	With Fertilizer	77.21±26.90 ^b
Biomass Yield Increase (%)	Without Fertilizer	26.58±20.93 ^a
	With Fertilizer	451.49±192.13 ^a
Doubling Time (days)	Without Fertilizer	20.41±11.13 ^a
	With Fertilizer	1.67±0.28 ^b
Relative Growth Rate (% per day)	Without Fertilizer	3.21±2.29 ^a
	With Fertilizer	23.62±5.18 ^b

Table 2. Productivity of *Lemna minor* cultured in commercial-scale raceway systems under fertilized and non-fertilized conditions. Values are presented as mean±standard deviation. Different superscript letters within the same variable indicate significant differences between treatments ($P<0.05$).

Parameter	Group	Mean±SD	Increase (%)
Productivity (kg/day)	Without Fertilizer	0.53±0.42 ^a	+1604
	With Fertilizer	9.03±3.84 ^b	
Productivity (g/m ² /day)	Without Fertilizer	2.04±1.60 ^a	+1596
	With Fertilizer	34.60±14.72 ^a	

it increased by only 26.58±20.93%. Without fertilizer, *L. minor* required 20.41 days to double biomass, while with fertilizer, the doubling time was 1.67 days. With fertilizer, the relative growth rate was 23.62±5.18% per day, and without fertilizer, 3.21±2.29% per day. The results showed that fertilizer affects *L. minor* growth, with biomass yield increasing sharply from 28.57 to 479.71%. Doubling time was reduced to 1.56 days, indicating that duckweed grew rapidly with fertilizer use. Relative growth rate improved from 3.59% per day to 25.11% per day. The productivity indicators also significantly increased.

In terms of productivity, *L. minor* cultured under fertilized conditions achieved 9.03±3.84 kg/day, approximately 16-fold higher than the non-fertilized treatment (0.53±0.42 kg/day). Similarly, productivity increased significantly from 2.04±1.60 g/m²/day in the non-fertilized raceway to 34.60±14.72 g/m²/day in the fertilized raceway (Table 2). These findings demonstrate that nutrient supplementation substantially accelerates duckweed growth and biomass accumulation, leading to markedly higher production efficiency under commercial-scale raceway conditions.

Water nutrients: The study revealed significant differences between fertilized and non-fertilized

raceway systems for nitrate ($P=0.018$), nitrite ($P=0.017$), COD ($P=0.018$), TKN ($P=0.018$), total nitrogen ($P=0.018$), pH ($P=0.018$), and dissolved oxygen ($P=0.043$) (Table 3). Fertilized raceways exhibited significantly higher nitrate, COD, TKN, pH, and dissolved oxygen concentrations than non-fertilized raceways, reflecting increased nutrient availability and biological activity following fertilizer application. Conversely, nitrite and total nitrogen concentrations were significantly lower in the fertilized treatment, suggesting active nutrient uptake and assimilation by duckweed. No significant differences were observed in ammonia ($P=0.144$), orthophosphate ($P=0.340$), and BOD ($P=0.207$) between treatments. Overall, fertilizer application significantly influenced nutrient dynamics and water quality, supporting enhanced duckweed growth and nutrient utilization efficiency in the industrial-scale raceway system.

Discussions

Fertilizer, which typically contains nitrogen- and phosphorus-rich elements, is critical for optimizing duckweed biomass yield and nutritional composition, particularly for photosynthesis, protein synthesis, and cell division (Okwuosa et al., 2021). Specifically,

Table 3. Water quality and nutrient composition of industrial-scale raceway systems for *Lemna minor* cultured with and without fertilizer application. Values are presented as mean±standard deviation. Different superscript letters within the same parameter indicate significant differences between treatments ($P<0.05$).

Nutrient	Group	Mean±SD
Nitrate (mg/L)	Without Fertilizer	1.42±0.22 ^a
	With Fertilizer	1.93±0.23 ^b
Nitrite (mg/L)	Without Fertilizer	3.80±0.56 ^a
	With Fertilizer	2.07±0.59 ^b
Ammonia (mg/L)	Without Fertilizer	0.39±0.26 ^a
	With Fertilizer	0.59±0.30 ^a
Orthophosphate (mg/L)	Without Fertilizer	0.31±0.23 ^a
	With Fertilizer	0.35±0.10 ^a
COD (mg/L)	Without Fertilizer	32.33±9.74 ^a
	With Fertilizer	50.14±7.11 ^b
BOD ₅ (mg/L)	Without Fertilizer	8.61±1.56 ^a
	With Fertilizer	7.37±1.27 ^a
Total Kjeldahl Nitrogen (mg/L)	Without Fertilizer	3.06±0.47 ^a
	With Fertilizer	6.49±0.11 ^b
Total nitrogen (mg/L)	Without Fertilizer	37.19±1.05 ^a
	With Fertilizer	16.67±1.06 ^b
pH	Without Fertilizer	1.30±0.17 ^a
	With Fertilizer	2.34±0.09 ^b
Dissolved oxygen (mg/L)	Without Fertilizer	2.16±1.23 ^a
	With Fertilizer	5.67±1.13 ^b

providing optimal ammonia concentrations is essential for maintaining high crude protein content, while adequate phosphorus availability is a primary driver of rapid duckweed expansion (Chakrabarti et al., 2018). Biomass yield in the fertilized treatment was more than 17 times higher than in the non-fertilized treatment. This pronounced increase suggests that nutrient limitation was a major constraint on growth in unfertilized raceways. Duckweed is known for its rapid nutrient uptake capacity (Pulido et al., 2024), and adequate nutrient availability (Ullah et al., 2021) enables efficient conversion of inorganic nutrients into plant biomass, resulting in accelerated growth and higher yields. Fertilizer application also significantly reduced the biomass doubling time from 20.41 days in the non-fertilized treatment to only 1.67 days. This rapid doubling rate reflects the exceptional growth potential of *L. minor* under nutrient-rich conditions and falls within the range reported for highly productive duckweed cultivation systems (Hong et al., 2024). Shorter doubling times are desirable in commercial production because they allow more frequent harvesting cycles and greater profitable annual biomass output.

Similarly, RGR increased significantly from

3.21±2.29% day⁻¹ without fertilizer to 23.62±5.18% day⁻¹ with fertilizer, indicating substantial gains in overall productivity. The improved RGR indicates enhanced physiological performance, likely due to increased nutrient assimilation, chlorophyll synthesis, and photosynthetic efficiency. Fertilizer-derived nutrients support new frond production and accelerate vegetative propagation, the primary mode of reproduction in duckweed (Hong et al., 2024).

The significant increase in productivity observed in the fertilized raceways further highlights the importance of nutrient management in commercial-scale duckweed cultivation. This enhanced productivity shows that fertilization not only increases standing biomass but also improves biomass generation per unit area and time, making the system more economically viable. These findings support previous reports (Ullah et al., 2021; Anjur et al., 2024) that optimized fertilization strategies are essential to maximize duckweed biomass production and ensure sustainable commercial-scale cultivation.

The water quality and nutrient findings indicate that fertilizer application significantly impacted nutrient dynamics and physicochemical conditions within the commercial-scale *L. minor* raceway system.

Fertilized raceways recorded significantly higher concentrations of nitrate, COD, TKN, pH, and DO than the non-fertilized treatment, showing that nutrient supplementation increased the availability of essential resources for duckweed growth (Jurga et al., 2026).

The elevated nitrate and TKN concentrations likely resulted from the direct addition of nitrogen-rich fertilizer, which provided an immediately available nutrient source and stimulated rapid biomass production. Similar findings have been reported in duckweed cultivation systems, where increased nutrient inputs promote greater plant productivity and nutrient assimilation (Petersen et al., 2022; Coughlan et al., 2023). The significantly higher COD values in the fertilized treatment may reflect increased organic matter accumulation due to enhanced plant growth and microbial activity. Greater duckweed biomass production increases root exudates, organic particles, and microbial metabolism, which collectively increase oxygen demand for chemical oxidation. Nevertheless, the elevated COD levels did not appear to adversely affect duckweed performance, as reflected in the substantially higher biomass yields and growth rates observed in the fertilized raceways.

Interestingly, despite the higher nutrient inputs, nitrite and total nitrogen concentrations were significantly lower in the fertilized treatment. This finding suggests efficient nutrient removal and assimilation by *L. minor*, which is well known for rapidly taking up dissolved nitrogen compounds (Pasos-Panqueva et al., 2024). The vigorous growth in fertilized raceways likely accelerated nitrogen assimilation into plant tissues, thereby reducing the accumulation of intermediate nitrogen forms such as nitrite and lowering residual total nitrogen concentrations in the water column. This result highlights duckweed's dual role as both a biomass-producing crop and an effective phytoremediation agent that removes excess nutrients from aquatic environments (Chen et al., 2018).

The increase in pH and dissolved oxygen in fertilized raceways further indicates enhanced photosynthetic activity. Under nutrient-rich

conditions, duckweed grows rapidly and increases carbon fixation, consuming dissolved carbon dioxide and shifting the carbonate equilibrium toward higher pH values. At the same time, intensified photosynthesis releases oxygen into the water, resulting in significantly higher dissolved oxygen concentrations. Elevated DO generally indicates a healthy and productive aquatic system and can further support beneficial microbial processes involved in nutrient cycling (Cheng and Stomp, 2009; Gupta and Prakash, 2013).

In contrast, ammonia, orthophosphate, and BOD did not differ significantly between treatments. The absence of significant differences in ammonia may indicate rapid nitrification and plant uptake, preventing ammonia accumulation despite fertilizer addition. Similarly, orthophosphate concentrations remained relatively stable, suggesting duckweed efficiently utilized phosphorus, which it requires in large amounts for cell division and biomass development. The non-significant differences in BOD indicate that fertilizer application did not substantially increase the system's biodegradable organic load, suggesting the raceways remained environmentally stable despite enhanced nutrient inputs (Azab and Soror, 2020).

Conclusion

This study concludes that fertilizer application significantly improved nutrient availability and enhanced nutrient utilization by *L. minor*. The reduction in nitrite and total nitrogen, alongside increased biomass production, suggests efficient nutrient recovery and conversion into plant biomass. Therefore, fertilized raceway systems not only promote excellent duckweed growth but also improve nutrient removal efficiency, highlighting their potential as a sustainable approach for large-scale biomass production and water quality management.

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