Examination of nitrogen accumulation rate in a *Lemna minor* aquatic system

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Abstract

Duckweeds (Lemnaceae) are small, free-floating aquatic macrophytes that can recover nutrients from water while producing biomass suitable for valorization. This study examined biomass production and nitrogen–phosphorus dynamics during a 75-day cultivation of *Lemna minor* in 30 Petri-dish batch reactors, each containing 45 mL of modified Hoagland solution. Frond number, dry biomass, nitrogen species, phosphate, total organic carbon, and biomass TKN were monitored using destructive time-course sampling. Frond number increased from 30 to 243, with a maximum of 251 on day 69, while dry biomass increased from 2.5 to 40.2 mg and accelerated markedly after approximately day 29. Biomass protein content ranged from 28.5% to 38.8% during days 18–75. By day 75, nitrate-N mass had decreased by 79.1% and phosphate concentration by 88.1%; the maximum phosphate reduction was 97.4% on day 56. Total measured N in the combined liquid and biomass pools varied from 45.6% below to 8.0% above the initial inventory and ended 8.23% lower. These mass-balance deviations are compatible with unmeasured N transformations, including denitrification

and biological N fixation, but do not directly demonstrate either process. The substantial nutrient removal and production of protein-rich biomass support further evaluation of *L. minor* systems for tertiary wastewater treatment and for feed or biorefinery applications.

Keywords: *Lemna minor*, nitrogen mass balance, nutrient recovery, biomass protein, acclimation

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1.Introduction

Lemna minor, a small floating macrophyte, thrives in nutrient-rich freshwater environments such as lakes and ponds. Belonging to the family *Lemnaceae*, it is one of the smallest flowering plants and propagates vegetatively without a seed stage. The plant is distributed worldwide, except in deserts and permanently frozen regions. Optimal growth occurs in tropical and temperate zones. Its biomass is composed of up to 93% moisture, with dry weight containing up to 45% protein and 30% carbohydrates. The plant is also known for high phosphorus accumulation [1] [2]. Under favorable conditions, *L. minor* exhibits rapid productivity, with an intrinsic growth rate of 0.29 per day [3]. *L. minor* is widely studied for its ability to absorb large quantities of nutrients [4], making it an effective tool for wastewater remediation [5]. It has been used to lower chemical oxygen demand (COD), biological oxygen demand (BOD), nitrates, phosphates, and total suspended solids (TSS) in various water treatment systems [6], [7]. Additionally, its ability to bioaccumulate heavy metals makes it suitable for phytoremediation [8], [9]. It has also been used as a test species to assess the toxicity of engineered nanoparticles [10] and other contaminants released into the

environment [11]. Contaminants absorbed into its biomass can be removed via harvesting.

In phytoremediation systems, plant growth rate directly influences the total biomass available for contaminant uptake and stabilization. As noted by Cunningham and Ow [12] the effectiveness of a plant for remediation is a function of both its accumulation capacity and its overall biomass production, which is tied to growth rate. Faster-growing species can therefore accumulate more tissue over time, potentially increasing removal efficiency, provided that nutrient availability does not become limiting [13].

Among essential nutrients, nitrogen plays a central role in supporting vegetative growth, as it is a key component of chlorophyll, amino acids, and nucleic acids and overall crop productivity [14] [15]. However, nitrogen uptake is not always proportional to growth; imbalances can occur depending on nitrogen form [16], concentration [17] and plant physiological status [18].

Based on contemporary research, the nitrogen cycle is a paradigm of biochemical complexity governed by an intricate network of biotic and abiotic transformations [19] [20] [21]. The nitrogen fluxes do not follow a simple circular pathway. Instead, they transition through a complex web involving multiple oxidation states that are mostly mediated by microbial activities [22], sensitive to environmental variables such as temperature, pH [23] and available O_2 [24]. Nitrogen availability influences the processes of growth and metabolism of *L. minor*, but since its forms and pools are not static, a mass balance approach is required to accurately track uptake and transformation through the system [25]. Studies using the acetylene reduction assay estimate that up to 15–20% of the nitrogen required by *Lemna minor* can be acquired

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through Biological Nitrogen Fixation (BNF). Nitrogen-fixing microorganisms associated with duckweed include diazotrophic bacteria such as *Klebsiella* [26], *Exiguobacterium* [27], *Pseudomonas* spp. [28], *Ensifer* spp. [29], and *Azotobacter vinelandii* [30]. These bacteria colonize the plant's roots and fronds, forming symbiotic relationships that enhance nitrogen assimilation [31].

While the role of plants in remediation has been widely studied, less attention has been given to the interplay between growth rate, nitrogen uptake, and nitrogen mass balance. Understanding this relationship is important because nitrogen availability can directly influence biomass production and therefore remediation efficiency, while also being subject to transformations and losses that complicate interpretation. This study aims to quantify the phytoremediation capabilities of *L. minor* as well as the nitrogen – phosphorus kinetics during the plant's growth by evaluating the plant biomass produced in a long-term batch system and assessing the process's efficacy. A mass balance approach was adopted to track nitrogen fluxes through the *L. minor* and growth medium system and to accurately interpret growth and removal efficiency since nitrogen pools are inherently dynamic, subject to uptake, transformation and losses. By doing so, we seek to provide a more integrated assessment of the plant's potential for phytoremediation and to identify key factors influencing its performance.

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2. Materials and methods

2.1 Materials

Lemna minor plants were collected from the Benaki Phytopathological Institute and maintained before the experiment as stock cultures in a controlled artificial growth medium.

This medium consisted of a modified Hoagland solution [32], prepared with chemicals purchased from Merck and Sigma-Aldrich, Germany. Stock cultures were stored and cultivated in 0.75 L plastic tanks in an incubator at 20–25°C under 1200 lux with a 10-h light/14-hours dark photoperiod. The growth medium was replenished weekly. These conditions applied only to pre-experiment stock maintenance.

2.2 Cultivation of *Lemna minor*

For time-resolved monitoring under batch conditions, L. minor was cultivated in 30 Petri-dish reactors (Figure 1; 9 cm diameter).

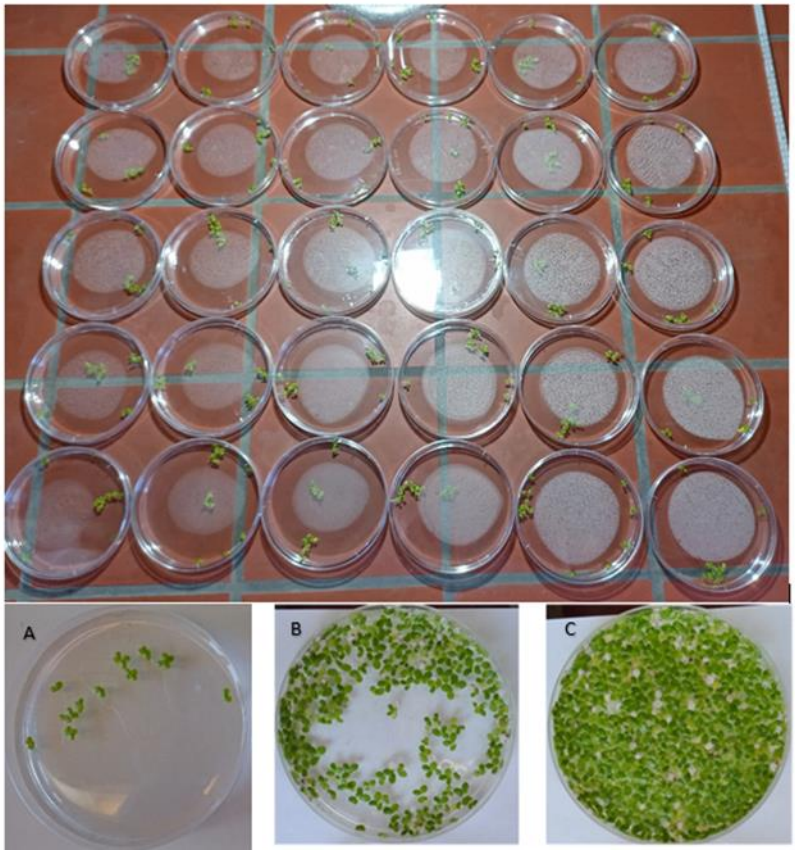


Figure 1. Cultivation of *Lemna minor* in petri dishes, (A) day 3, (B) day 48, and (C) day 75).

Each reactor contained 45 mL of the modified Hoagland nutrient solution and 30 *L. minor* fronds. During the 75-day experiment, the reactors were maintained at 20 ± 3°C

for their experimental conditions with a 14-hour photoperiod provided by a daylight lamp emitting 1900 LUX intensity. These experimental conditions differed from the stock-maintenance regime described in Section 2.1. Table 1 summarizes the nutrient composition of the modified Hoagland solution.

Table 1. Composition of modified Hoagland solution

Modified Hoagland Composition	Concentration (mg/L)
$MgSO_4$ $7H_2O$	246.0
$Ca(NO_3)_2$ $4H_2O$	543.0
KH_2PO_4	68.0
KNO_3	253.0
H_3BO_3	1.43
$MnCl_2$ $4H_2O$	0.90
$ZnSO_4$ $7H_2O$	0.11
Na_2MoO_4 $2H_2O$	0.045
$CuSO_4$ $5H_2O$	0.045
$FeCl_3$ $6H_2O$	0.50
EDTA	0.50

The cultivation experiment lasted 75 days, spanning late autumn to winter (November to late January). The petri dishes were opened every 3 days to permit atmospheric exposure. Every 3-5 days, up to two Petri dishes were destructively sampled for analysis of the plant biomass and nutrient solution. Samples were filtered through 0.7 µm glass fiber filters to separate the liquid and solid phases. The liquid phase was stored at 4°C until analysis, whereas plant biomass was dried sequentially at 70°C and then at 105°C subsequent analyses.

2.3 Analysis of samples

Liquid samples were analysed for nitrate-N (NO_3^--N), ammonium-N (NH_4^+-N), nitrite-N (NO_2 −N), total Kjeldahl nitrogen (TKN), phosphate-P (PO_4^{3-}-P), and total

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organic Carbon (TOC) in the liquid samples. Nitrite-N was below the detection limit in all samples. Standard water and wastewater testing protocols were followed.

2.3.1 Ammonium determination

Ammonium-N was measured using the Nessler method (Standard Test Method D1426), with a HANNA HI83303 Multiparameter Photometer for Aquaculture. One mL of liquid sample was used, and results were quantified over the range of 0–100 mg N/L.

2.3.2 Nitrate determination

Nitrate-N was determined using the ultraviolet spectrophotometric screening method. Absorbance was measured at 220 nm, with a corrective reading at 275 nm to account for interference from dissolved organic matter. The analysis was performed using a Hitachi U-2000 double-beam UV-visible spectrophotometer and required 50 mL of sample. Nitrate-N was quantified over the range of 0–7 mg N/L, with appropriate dilutions as needed.

2.3.3 Phosphates determination

Phosphate-P was measured using the ascorbic acid method with a NANOCOLOR 500D spectrophotometer (Macherey-Nagel). Twenty milliliters of liquid sample were analysed over the range of 0–6.5 mg P/L, with dilution as required.

2.3.4 Total Kjeldahl Nitrogen (TKN)

TKN, representing the sum of organic and ammonium nitrogen, was determined by Kjeldahl digestion. Twenty-five millilitres of sample were heated with concentrated sulfuric acid (H_2SO_4) and a catalyst mixture (K_2SO_4, $CuSO_4$, TiO_2), followed by alkalization with 32% sodium hydroxide. Released ammonia was collected in 2% boric

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acid (H_3BO_3) solution during distillation and quantified by titration with 0.02 sulfuric acid using a bromocresol green/methyl red indicator. Analyses were performed using a KjelFlex K-360, SpeedDigester K-425, and Scrubber K-415 (Büchi). For the nitrogen inventory, dissolved organic N was calculated as TKN minus measured ammonium-N to avoid double counting.

2.3.5 Total Organic Carbon (TOC)

TOC in liquid samples was determined by catalytic combustion oxidation. Samples were oxidized at 680°C in a platinum catalyst-filled combustion tube, and the resulting CO_2 was quantified using an infrared gas analyser. A Shimadzu TOC-L analyser was used.

2.3.6 Analysis of plant biomass

Plant biomass growth was monitored by measuring frond number and fresh and dry biomass. Biomass was separated using 0.7-µm glass-fiber filters (Macherey-Nagel), dried at 70°C for 48 h, and then dried at 105°C. Protein content was estimated from biomass TKN using a conversion factor of 6.25. Initial frond number was 30 per reactor, was recorder at each destructive sampling point.

2.4 Nitrogen inventory and mass-balance assessment

A deterministic nitrogen inventory was constructed to quantify changes in total measured nitrogen within the defined system boundary, comprising plant biomass, growth medium, and the associated microbial community. The measured liquid and biomass pools were combined according to Eq. (1). Because atmospheric N_2 incorporation and gaseous nitrogen products were not measured directly, the calculation does not independently quantify biological nitrogen fixation or

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denitrification. Positive and negative deviations are therefore reported as apparent net gains or losses and are used only to formulate mechanistic hypotheses.

$$N_i = N_{i,solution} + N_{i,biomass} \text{ (Eq. 1)}$$

$N_{i,solution}$ represents the total measured nitrogen remaining in the growth medium, calculated as the sum of nitrate-N, nitrite-N, ammonium-N and dissolved organic N. $N_{i,biomass}$ represents biomass TKN. All terms were expressed as absolute nitrogen mass (mg N per reactor).

Since the Petri dishes remained closed for almost the entire experimental period and were located indoors, atmospheric nitrogen deposition was considered negligible. Based on typical indoor concentrations of the most common nitrogenous components included in the air (NH_3, NO_2, HNO_3) and their respective deposition velocities [33] [34], the maximum potential deposition during the brief opening periods was estimated at less than 0.2 µg N (<0.003 µg N/day) which is well below the analytical detection limit and negligible compared to the observed nitrogen fluxes [33]. Likewise, ammonia volatilization was assumed to be negligible. According to Zimmo et al. [35], while factors like pH, temperature and mixing conditions influence ammonia transfer, gaseous losses primarily depend on the concentration of ammonia in the liquid phase. Since ammonia concentrations were undetectable or very low throughout the experiments, volatilization losses were considered insignificant. Accordingly, atmospheric deposition and ammonia volatilization were treated as minor relative to the observed inventory deviations; however, this assumption does not identify the remaining unmeasured nitrogen fluxes.

2.5 Kinetic modelling and uncertainty treatment

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The logistic models for dry biomass and biomass TKN were fitted to the mean time-course data by nonlinear least-squares regression. Goodness of fit was evaluated using the coefficient of determination (R^2) and root mean square error (RMSE). Sampling was destructive. Two independently cultivated Petri-dish reactors were analysed at most time points; at selected time points and for some analytes, only one valid reactor measurement was available. Results are presented as mean ± standard deviation when n = 2 and as an individual value when n = 1; where shown, error bars represent standard deviation. Given the limited replication, no inferential statistical tests were applied, and process-level interpretations are presented as hypotheses.

3. Results and discussion

3.1 *Lemna minor* biomass growth

The growth of *Lemna minor* was monitored to evaluate biomass production over 75 days of cultivation under the specified growth conditions. Table 2 illustrates the progression of plant biomass.

Table 2: Frond number and fresh and dry biomass during the 75-day cultivation experiment.

Day	Number of Fronds*	Fronds Standard deviation	Plants - Fresh Weight (mg)*	Fresh Weight Standard deviation (mg)	Plants - Dry Weight (mg)*	Dry Weight Standard deviation (mg)
0	30.0	N/A	24.30	N/A	2.50	N/A
5	37.5	2.121	23.55	5.869	1.65	0.354
8	39.5	2.121	29.55	2.616	1.70	0.283
13	49.5	3.536	42.00	4.667	2.25	0.212
18	53.5	0.707	40.20	4.525	2.50	0.141
21	53.5	0.707	32.05	7.707	2.40	0
25	54.5	0.707	47.15	3.889	3.05	0.212
29	68.0	2.828	68.75	6.152	3.65	0.636

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33	87.5	0.707	105.05	34.860	4.60	0.141
40	105.5	4.950	114.70	1.131	6.30	0.990
48	139.5	13.435	155.20	12.162	9.10	0.849
53	140.5	7.778	197.50	43.841	10.35	1.061
56	173.0	1.414	239.65	6.859	11.80	N/A
60	224.5	14.849	356.75	61.872	18.95	0.212
64	239.0	N/A	313.00	N/A	20.03	N/A
69	251.0	N/A	471.90	N/A	33.70	N/A
75	243.0	N/A	458.50	N/A	40.20	N/A

*Means are reported where duplicate reactor measurements were available; individual values are reported when only one valid measurement was available.

Lemna minor showed a prolonged initial slow-growth period followed by marked biomass acceleration after approximately day 29. Frond number increased from 30 on day 0 to a maximum of 251 on day 69 and then declined slightly to 243 on day 75. Dry biomass increased from 2.5 to 40.2 mg and continued to rise through day 75. The early slow-growth period was longer than commonly reported under optimal conditions near 25°C [36] [37], likely reflecting the lower experimental temperature range of 17–23°C.

Because dry biomass did not reach a plateau or decline within the observation period, a complete sigmoid growth cycle was not captured. Equation (2) should therefore be interpreted as an empirical logistic fit to the measured trajectory. The fit gave $R^2 = 0.987$ and RMSE = 1.29 mg, and its inflection point at 73.95 days occurred near the end of cultivation, consistent with continued biomass acceleration rather than a stationary or death phase. The modelled maximum accumulation rate was 1.77 mg/day; extrapolation beyond day 75 is not warranted. The mean moisture content of fresh biomass was 93.68% ± 1.44%.

$$W_{DB}(t) = 2.284 + \frac{73.93}{1+e^{-0.096(t-73.953)}} \text{ (Eq.2)}$$

The experimental dry-biomass data and logistic fit are shown in Figure 2. Error bars denote standard deviation where n = 2; no error bar is shown where n = 1.

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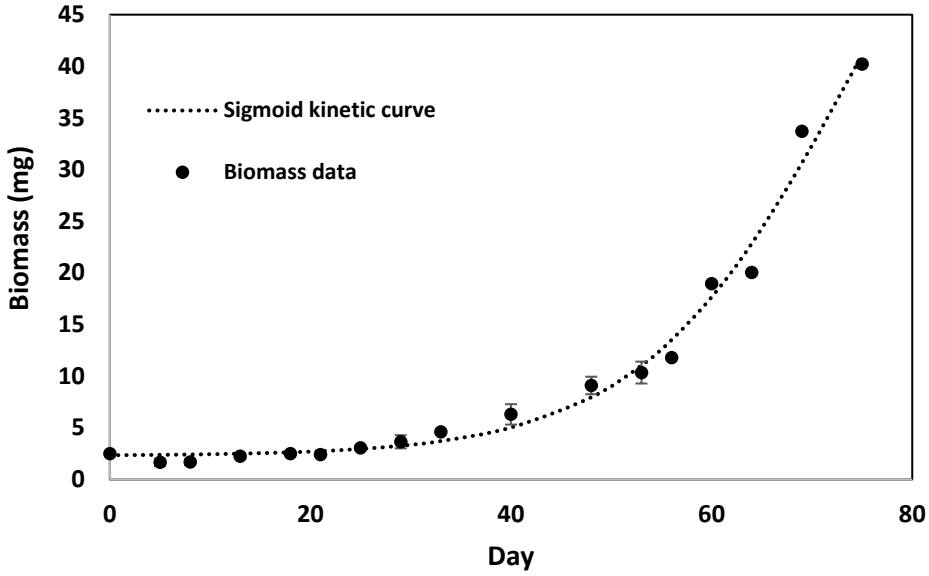


Figure 2. Dry-biomass time course and logistic fit. Error bars denote standard deviation where n = 2; no error bar is shown where n = 1.

3.2 Nutrient dynamics in plant biomass and the liquid phase

During the cultivation period, significant changes were observed in the nutrient concentrations of the liquid phase, reflecting the uptake and assimilation of nutrients by *Lemna minor*.

3.2.1 Nitrogen dynamics

Biomass TKN increased by 619%, from 0.340 mg N initially to 2.322 mg N on day 75. After an early decrease, nitrogen accumulation rose markedly from day 29 onward. The logistic model in Eq. (3) gave R^2 = 0.985 and RMSE = 0.076 mg N. Because the measured trajectory did not include a clear upper plateau and replication was limited, the model is used as an empirical description rather than evidence for a specific mechanism.

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$$N_{TKN}(t) = 0.224 + \frac{2.713}{1+e^{-0.152(t-66.695)}} \text{ (Eq.3)}$$

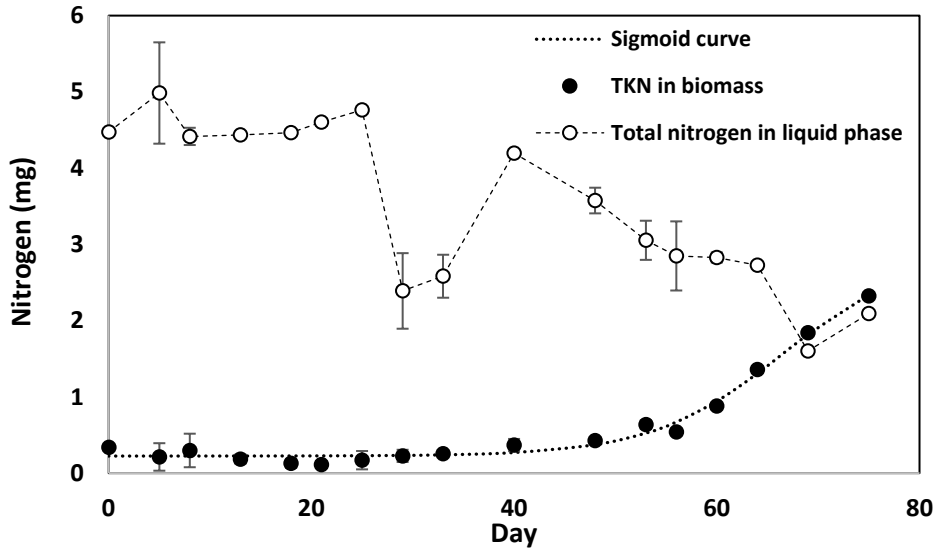


Figure 3. Nitrogen mass in plant biomass and the liquid phase, with the logistic fit for biomass TKN. Error bars denote standard deviation where n = 2; no error bar is shown where n = 1.

Protein content in the dry plant biomass ranged from 28.5% to 38.8% during days 18-75, with an average of 33.7% (Figure 4).

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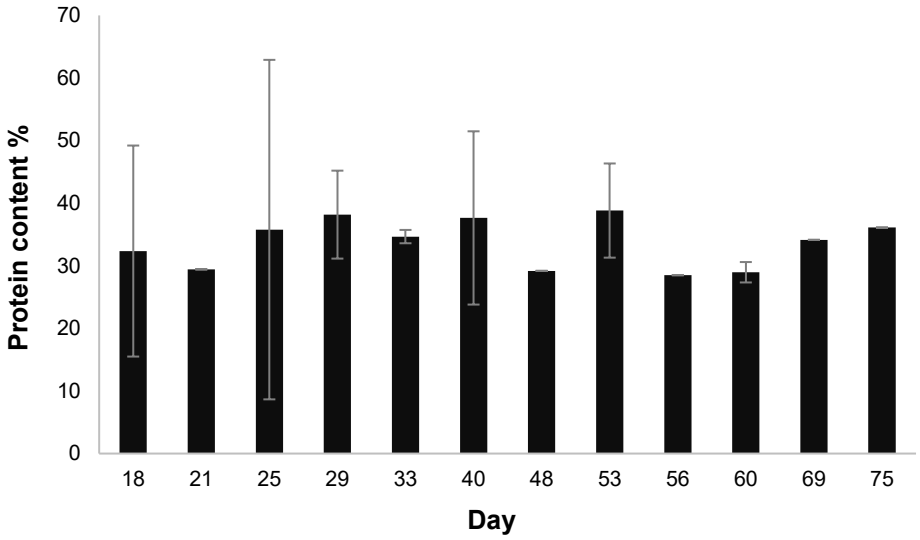


Figure 4. Protein content of plant biomass. Error bars denote standard deviation where n = 2; no error bar is shown where n = 1.

The total measured nitrogen mass in the liquid phase decreased from 4.472 to 2.094 mg N per reactor (53.2%) between days 0 and 75. Nitrate-N, the predominant initial nitrogen pool in the nutrient solution, showed a net decrease of 79.1% by day 75 (Table 3).

Table 3. Measured nitrogen inventory of the 45-mL reactors. All values are absolute masses (mg N per reactor).

Day	Nitrate-N (mg N)		Dissolved Organic-N (mg N)		Ammonium-N (mg N)		TN in Sample (mg N)	
	Average	st. deviation	Average	st. deviation	Average	st. deviation	Average	st. deviation
0	4.472	N/A	0	N/A	0	N/A	4.812	N/A
5	4.565	0.070	0.421	0.595	0	0	5.198	0.484
8	4.416	0.113	0	0	0	0	4.714	0.107
13	4.406	0.043	0.030	0.042	0	0	4.620	0.062
18	4.467	0.015	0	0	0	0	4.594	0.075
21	4.560	0.013	0.043	0.060	0	0	4.748	N/A
25	4.602	0.087	0.162	0.113	0	0	4.934	0.146

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29	2.059	0.194	0.331	0.301	0	0	2.616	0.416
33	2.243	0.013	0.264	0.282	0.076	0.014	2.837	0.282
40	3.691	0.200	0.506	0.169	0	0	4.565	0.049
48	3.428	0.377	0.1475	0.209	0	0	4	0.129
53	3.054	0.257	0	0	0	0	3.690	0.315
56	2.849	0.453	0	0	0	0	3.707	N/A
60	2.069	0.163	0.544	0.050	0.216	0.179	3.706	0.0277
64	2.309	N/A	0.241	N/A	0.178	N/A	4.087	N/A
69	1.286	N/A	0.316	N/A	0	N/A	3.443	N/A
75	0.935	N/A	0.674	N/A	0.485	N/A	4.416	N/A

Values are means where duplicate measurements were available and individual values where n = 1. Total measured system N includes nitrate-N, nitrite-N (below detection), ammonium-N, dissolved organic N, and biomass TKN.

Nitrite-N remained below detection throughout the experiment. Ammonium-N was detected intermittently from day 33 onward and reached 0.485 mg N per reactor (10.77 mg N/L) on day 75. Between days 0 and 75, biomass TKN increased by 1.982 mg N while total measured liquid-phase nitrogen decreased by 2.379 mg N. This complementary trend is compatible with plant uptake but does not imply a one-to-one transfer because other measured and unmeasured pools may have contributed. On day 75, dissolved organic N was 0.674 mg N per reactor (14.98 mg N/L), the highest value measured for this pool.

Total measured nitrogen in the combined liquid and biomass pools varied from 45.6% below to 8.0% above the initial inventory. Consecutive sampling points differed by −47.0% to +65.3%. These deviations describe non-closure of the measured inventory and may reflect a combination of analytical and sampling uncertainty, unmeasured microbial pools, and nitrogen exchange with the atmosphere. They cannot, by themselves, identify biological nitrogen fixation or denitrification.

During the early cultivation period, total measured nitrogen remained relatively stable. Between days 25 and 29, however, nitrate-N mass fell from 4.602 to 2.059 mg

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N while biomass TKN increased only from 0.170 to 0.227 mg N. The disproportionate nitrate decrease is compatible with transfer to an unmeasured or gaseous pool. Denitrification is one plausible explanation because anoxic micro-zones can occur in aerated systems [38] and some bacteria express denitrification enzymes in the presence of oxygen [39], but denitrification was not measured directly. Analytical uncertainty and temporary storage in unmeasured microbial biomass cannot be excluded. Ammonia volatilization is unlikely to explain the magnitude of the apparent loss because ammonium concentrations remained low.

After day 29, liquid-phase nitrate-N increased again by day 40, coinciding with a decline in phosphate and an increase in biomass nitrogen. Internal recycling through decomposition and mineralization, followed by nitrification of released ammonium, is a plausible explanation. Uptake by algae or other phosphorus-scavenging microorganisms may also have contributed, but microbial and algal biomass were not measured; these interpretations therefore remain tentative.

Between days 56 and 64, total measured system nitrogen increased by 0.700 mg N while liquid-phase nitrogen declined and biomass TKN rose from 0.538 to 1.360 mg N. This apparent gain is compatible with an unmeasured nitrogen input such as biological nitrogen fixation, but it could also arise from sampling or analytical variability or from unmeasured pools. The concurrent ammonium signal does not confirm N_2 fixation. Over the full 75 days, total measured system nitrogen declined by 8.23%. Without direct gas or isotope measurements, this net change cannot be partitioned among fixation, denitrification, or other nitrogen sources and sinks.

Overall, the data demonstrate redistribution of nitrogen between the liquid and plant pools, with temporary apparent gains and losses in the measured inventory. These

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patterns generate hypotheses but do not establish specific gaseous nitrogen processes. Confirmation would require direct approaches such as an acetylene-reduction assay or $^{15}N_2$ tracing for nitrogen fixation, together with N_2O/N_2 flux measurements for denitrification and greater biological replication.

3.2.2 Phosphorus dynamics and N-P interactions

Nitrogen and phosphorus dynamics were partly coupled through biological uptake, but their timing differed. Between days 21 and 29, nitrate-N mass fell from 4.56 to 2.06 mg N with only a small increase in biomass nitrogen, whereas phosphate declined modestly. From days 29 to 40, nitrate-N recovered while phosphate concentration decreased from 16.43 to 6.26 mg P/L. Internal recycling and nitrification, together with uptake by algae or other microorganisms, are plausible explanations, but these pools were not measured. Between days 53 and 64, total measured system nitrogen showed an apparent gain of approximately 0.7 mg N while phosphate remained low. Thus, the temporary decoupling of nitrogen and phosphorus is compatible with several plant and microbial processes, including denitrification and possible nitrogen fixation, but does not confirm a specific pathway.

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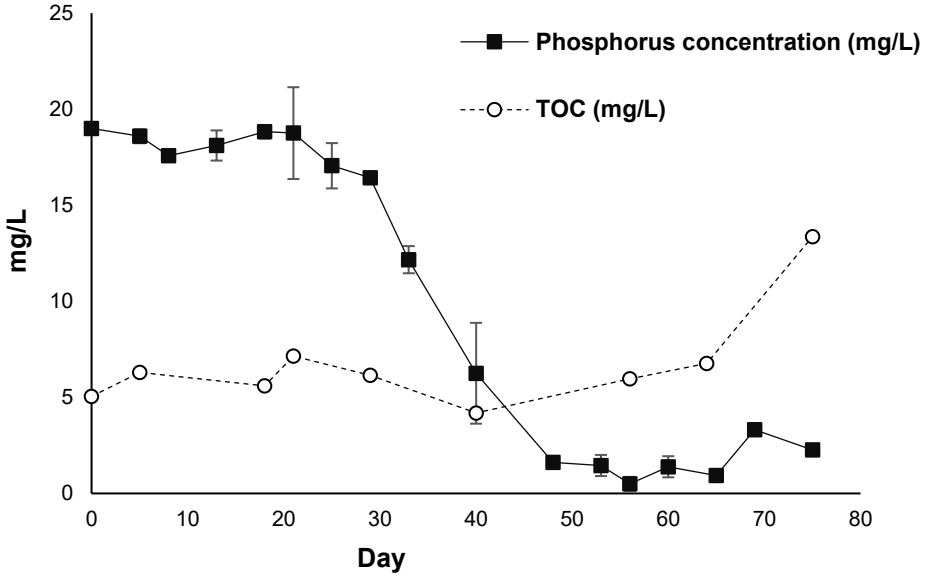


Figure 5. Phosphorus and TOC concentrations in the liquid phase. Error bars denote standard deviation where n = 2; no error bar is shown where n = 1.

3.2.3 TOC Dynamics

The TOC levels in the liquid phase ranged between 4.188 and 7.140 mg/L throughout most of the cultivation period but nearly doubled to 13.366 mg/L during the final days (Figure 5). Increase in extracellular carbon excretion has been observed during the stationary phase of bacterial growth [40]. Nutrient limitation has been linked to increased carbon excretion by phytoplankton as an energy conservation mechanism [41]. Moreover, limited phosphorus conditions in diatoms have been proposed to cause excretion of excess carbon from diatom cells in the form of extracellular polysaccharides [42].

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4. Conclusions

Under the present cultivation conditions, *L. minor* dry biomass increased from 2.5 to 40.2 mg per reactor and frond number increased from 30 to 243 over 75 days, reaching a maximum of 251 on day 69. Biomass protein content during days 18–75 averaged 33.7% and ranged from 28.5% to 38.8%. Nitrate-N mass decreased by 79.1%, whereas phosphate concentration was 88.1% lower on day 75 and reached a maximum reduction of 97.4% on day 56. Total measured system nitrogen varied between −45.6% and +8.0% relative to the initial inventory and ended 8.23% lower. These inventory deviations are compatible with unmeasured nitrogen transformations but do not directly demonstrate biological nitrogen fixation or denitrification. Direct gas or isotope measurements and greater replication are required to resolve the responsible pathways. Despite these limitations, the substantial nutrient removal and generation of protein-rich biomass support further evaluation of *L. minor* for tertiary wastewater treatment and biomass valorization, including feed or biorefinery applications, subject to wastewater-specific safety and quality assessment.

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Declarations

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Author Contributions Statement: The presented idea was conceived by Apostolos Vlyssides. The project and experiments were planned and supervised by Apostolos Vlyssides and Anestis Vlysidis. Ioanna Goudeli and Vaya Kati contributed to material preparation. The experiments, data collection and processing and original draft composition of the manuscript were carried out by Ioanna Goudeli. Anestis Vlysidis contributed to the funding acquisition and resources. All authors reviewed the results, contributed to editing and approved the final version of the manuscript.

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Figure Captions

Fig. 1 Cultivation of Lemna minor in petri dishes, (A) day 3, (B) day 48, and (C) day 75.

Fig. 2 Dry-biomass time course and logistic fit. Error bars denote standard deviation where n = 2; no error bar is shown where n = 1.

Fig. 3 Nitrogen mass in plant biomass and the liquid phase, with the logistic fit for biomass TKN. Error bars denote standard deviation where n = 2; no error bar is shown where n = 1.

Fig. 4 Protein content of plant biomass. Error bars denote standard deviation where n = 2; no error bar is shown where n = 1.

Fig. 5 Phosphorus and TOC concentrations in the liquid phase. Error bars denote standard deviation where n = 2; no error bar is shown where n = 1.

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