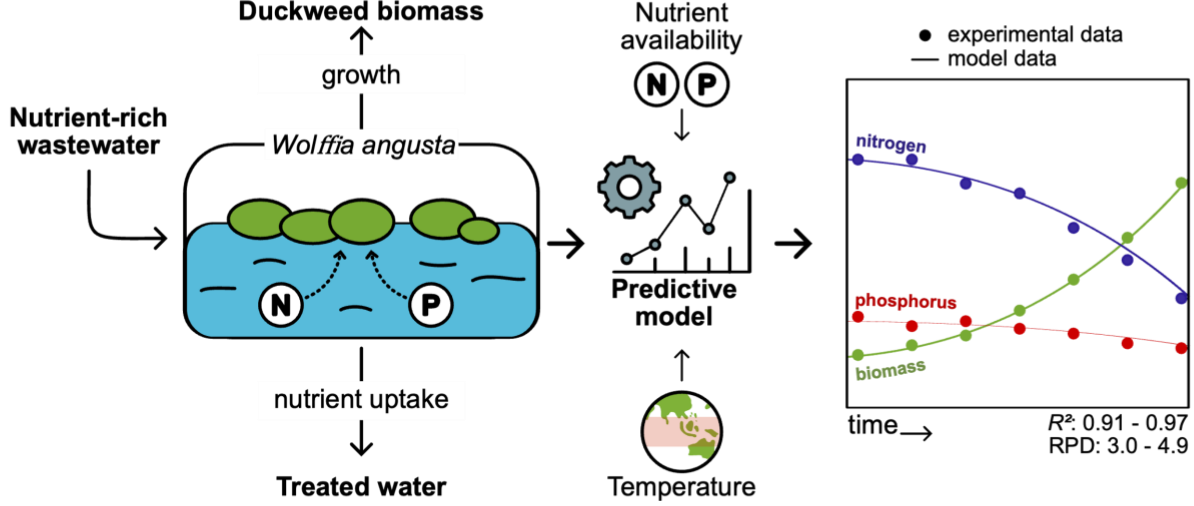
Decoding *Wolffia*: A Predictive Model for Duckweed Growth and Nutrient Uptake for Resource Recovery from Wastewater in Tropical Countries

Johan Pasos-Panqueva [1,2,3], Anie Yulistyorini[4], Alison Baker [2] and Miller Alonso Camargo-Valero [1,4]

[1] BioResource Systems Research Group, School of Civil Engineering, University of Leeds, Leeds LS2 9JT, UK
[2] School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK
[3] Departamento de Ingeniería Química, Universidad Nacional de Colombia, Campus La Nubia, Manizales, 170003, Colombia
[4] Environmental Engineering Study Program, Department of Civil Engineering and Planning, Faculty of Engineering, Universitas Negeri Malang, Malang 65145, Indonesia

Corresponding authors:
Miller Alonso Camargo-Valero (M.A.Camargo-Valero@leeds.ac.uk)
Alison Baker (A.Baker@leeds.ac.uk)

Graphical Abstract



Highlights

- A first kinetic model for *Wolffia*'s growth and nutrient uptake was developed
- The model integrates temperature and nutrient dynamics into duckweed growth
- The model accounts for in-planta nutrients reserves contributing to growth under low nutrient conditions
- The model accurately predicts *Wolffia* biomass and nutrient removal even under tropical conditions

Keywords
Duckweed, Growth kinetics, Nutrient uptake, Wastewater treatment, *Wolffia angusta*

Abstract

Duckweed-based wastewater treatment systems offer immense potential for sustainable water pollution control, yet predictive tools to optimise their operation under real-world conditions are lacking. In this work, we developed and validated an empirical kinetic model capable of accurately predicting biomass productivity and nutrient uptake in *Wolffia angusta* cultures across variable environmental conditions. Our model integrates the combined effects of temperature and nutrient availability, capturing dynamic responses and internal nutrient reserves. This model was used to assess the performance of duckweed ponds to improve the quality of effluents from anaerobic baffled reactors, typically used for domestic wastewater treatment in Indonesia. Experimental cultivation under controlled conditions (20, 25 and 30°C; varying phosphorus and nitrogen supplies, including nutrient limiting scenarios) revealed that temperature predominantly drives biomass growth, while nutrient availability more strongly governs biological nutrient uptake and removal efficiency. Optimal biomass production was observed between 25 - 30°C, though specific nitrogen uptake rates declined at higher temperatures, indicating metabolic constraints. Nutrient removal is mainly biomass-driven rather than resulting from enhanced metabolic rates, which benefits nutrient recovery and reuse. Model calibration markedly improved predictive accuracy ($R^2 > 0.9$), even under nutrient limiting conditions. This work advances the mechanistic understanding of *Wolffia*'s growth and nutrient uptake, and delivers a practical, transferable modelling framework to enhance nutrient control in decentralised wastewater treatment systems, like anaerobic baffled reactors, particularly relevant for tropical regions facing nutrient pollution. This approach represents a significant step towards integrating nature-based biotechnologies into circular, sustainable water management strategies.

1. Introduction

The increasing demand for sustainable and decentralised wastewater treatment has intensified the search for nature-based systems that not only remove pollutants but also enable resource recovery. In regions where the discharge of nutrients to water bodies is the major factor contributing to water pollution, the challenge of removing excess nitrogen and phosphorus remains urgent, particularly in the tropics (Ahmad et al., 2022; Liu et al., 2024). If left untreated, nutrient-rich effluents can lead to eutrophication, hypoxia, degrading ecosystems, diminishing water quality, and threatening biodiversity (Tom et al., 2021). While conventional nutrient control technologies are effective at large centralised wastewater treatment works, they are often expensive, energy-intensive and dependent on complex infrastructure that do not promote nutrient recovery and reuse, making them less suitable for small and decentralised wastewater treatment works in low-resource or rural contexts (Chatla et al., 2020; Tom et al., 2021).

On average, high-income countries treat approximately 70% of their domestic and industrial wastewater, compared to 38%, 28%, and 8% in upper-middle-income, lower-middle-income, and low-income countries, respectively (Slocombe et al., 2020). In high-income countries such as the United Kingdom (UK), phosphorus removal is primarily achieved through chemical precipitation at small wastewater treatment works, resulting in the formation of ferric and aluminium phosphate precipitates, and through enhanced biological phosphorus removal using phosphorus-accumulating organisms (PAOs) at large works (>100,000 population equivalent - P.E.) (Manyumba et al., 2009). This approach, driven mainly by large, centralised facilities, has reduced annual river's total reactive phosphorus flux from 71 kt P year^{-1} in the year 2000 to 10 kt P year^{-1} (Worrall et al., 2016). However, compliance with the UK Water Framework Directive now requires many small works (<5,000 P.E.) to achieve very low effluent phosphorus concentrations, in some cases below 0.5 mg PO_4-P L^{-1}, to prevent eutrophication in sensitive catchments. For nitrogen control, the focus has been on removing ammoniacal nitrogen due to its toxicity to fish, primarily through biological nitrification (oxidation of ammonium to nitrate) coupled with denitrification at large works to meet total nitrogen removal targets. Consequently, ammoniacal nitrogen flux in UK rivers has declined significantly, but nitrate flux has increased, reaching approximately 676 kt N year^{-1} (69% of total annual nitrogen flux) (Worrall et al., 2009). Data from England confirm that effluents from wastewater treatment works substantially increase nutrient concentrations in surface waters, with phosphate and nitrate increasing from upstream averages of 0.37 mg PO_4-P L^{-1} and 4.05 mg NO_3-N L^{-1} to downstream values of 0.62 mg PO_4-P L^{-1} and 6.54 mg NO_3-N L^{-1} (Yang et al., 2025).

In low- and middle-income countries, onsite sanitation and decentralised systems remain the predominant solutions for faecal sludge and wastewater management (WHO and UNICEF, 2025). For example, in Indonesia, only 0.8% of the population has access to sewer-based sanitation, while 86.6% rely on septic systems (WHO and UNICEF, 2025). These include conventional anaerobic baffled reactors (ABRs) and hybrid designs incorporating aerated chambers to facilitate ammonium removal via nitrification (Lau and Trzcinski, 2024). Such systems have been widely implemented under the Sanitation by Communities (SANIMAS) program, a community-led initiative launched in 2002. However, Yulistyorini et al. (2019) reported that most ABR systems discharge effluents of poor quality, failing to meet regulatory standards set by the Ministry of Environment and Forestry of Indonesia (Regulation No. P.68/2016), and contribute marginally to nutrient control targets.

Technological advancements for achieving ammoniacal nitrogen and phosphorus discharge limits have largely focused on economies of scale and intensive processes suited to large, centralised wastewater treatment facilities. These approaches have limited applicability to small and decentralised systems in both high and low- and middle-income countries, where existing infrastructure must be upgraded to meet nitrate and phosphorus removal targets. Such improvements are essential to mitigate eutrophication in receiving waters and to promote nutrient recovery and reuse.

In that sense, the use of floating aquatic plants such as duckweeds (DW) has emerged as a promising alternative to conventional nutrient control in wastewater treatment systems due to their rapid growth, low operation and maintenance requirements, and exceptional nutrient uptake capacity (Körner et al., 2003; Ziegler et al., 2023). In nature, these plants thrive in shallow water bodies, capturing nutrients directly from the water column while producing valuable biomass. Among nature-based technologies for wastewater treatment, duckweed pond systems stand out for their simplicity, scalability and potential for integration into decentralised treatment setups (Coughlan et al., 2022; Sha et al., 2024). Moreover, duckweed ponds can serve as polishing stages to conventional wastewater treatment plants, effectively removing residual nitrogen and phosphorus and improving effluent quality before discharge or reuse (Chiemchaisri et al., 2025).

Over the past decades, research on such duckweed systems has expanded rapidly, exploring the effects of environmental and biological factors on growth and nutrient removal. Biotic factors, such as species variability and acclimation (Körner & Vermaat, 1998; Paolacci et al., 2021; Sembada & Faizal, 2019; Ziegler et al., 2015); cultivation density (Demirezen et al., 2007; Driever et al., 2005; Monette et al., 2006; Njambuya et al., 2011); and the role of root-associated microbiota (Ishizawa et al., 2017; Ma et al., 2023; Yamakawa et al., 2018), have been well documented. Similarly, extensive work has evaluated abiotic parameters, including temperature, light, salinity and nutrient concentrations, and their impact on duckweed growth (Bal Krishna & Polprasert, 2008; Calicioglu et al., 2021; Iqbal et al., 2019; Lasfar et al., 2007; McCann, 2016a; Nesan et al., 2020; Peng et al., 2007; Yin et al., 2015). While valuable, much of this work has focused on individual variables in isolation, which limits the use results in real set-ups. On the other hand, very few studies have integrated research findings into optimised duckweed culture conditions using pre-treated effluent characteristics (Pasos-Panqueva et al., 2024) or translated them into predictive tools or practical models that can be easily used for the design and operation of a wastewater treatment system (Calicioglu et al., 2021; Korner et al., 2003). A critical gap remains in understanding how multiple environmental conditions interact to influence duckweed performance, especially in dynamic wastewater environments.

Within the duckweed family, *Wolffia* species offer exceptional potential for wastewater treatment and beyond. Native to many tropical and subtropical regions (Kew Royal Botanic Gardens, 2025), *Wolffia* thrives under warm conditions and is well-suited for deployment in regions most affected by nutrient pollution. It is also the world’s smallest flowering plant and one of the fastest growing, with an ability to double its biomass in under 48 hours under optimal conditions (Sree et al., 2015). Beyond its phytoremediation capacity, *Wolffia* also serves as a rich source of plant-based protein, positioning it as a promising organism for integrated systems that couple wastewater treatment and nutrient recovery with biomass valorisation for food or feed production. Its nutritional profile, rich in essential amino acids, vitamins (including B12), and antioxidants, has already led to its use as a food in parts of Southeast Asia (Appenroth et al., 2018; On-Nom et al., 2023), and more recently to its approval as a novel food in the European Union (Regulation 2025/153). These unique traits make *Wolffia* an ideal organism for developing sustainable nature-based wastewater treatment solutions in tropical regions.

However, despite its potential, the ecological and operational performance of duckweed ponds using *Wolffia* under variable environmental conditions, such as temperature and nutrient availability, remains poorly understood (Pasos-Panqueva et al., 2024). There is a lack of predictive tools that effectively capture its growth dynamics and nutrient uptake capacity, which limits its wider use in wastewater treatment systems. *Wolffia* pond systems have strong potential to polish pre-treated wastewater effluents and meet nutrient control targets, particularly in tropical countries where weather conditions are most favourable all year around.

This study seeks to bridge this gap by investigating the growth and nutrient uptake behaviour of *Wolffia angusta*. We developed and validated a simple yet effective predictive model that reflects the key dynamics of biomass production and nutrient uptake and removal under simulated tropical climate conditions. The model incorporates temperature and nutrient availability within a simplified kinetic framework. Accounting for critical factors, such as the role of internal (*in-planta*) nutrient reserves, it enhances prediction accuracy, even when external nutrient levels are low. In addition to experimental validation, the model was applied to evaluate the performance of a *Wolffia*-based system for polishing effluents from hybrid (aerated) anaerobic baffled reactors under realistic operational conditions in Indonesia. Ultimately, this work provides both a practical tool for the design and management of *Wolffia*-based wastewater treatment systems, and a robust framework for model development that can be extended to other duckweed species and weather conditions.

2. Materials and Methods

2.1. Plant material

A clone of the duckweed species *Wolffia angusta* (strain 8878), native to Southeast Asia and originally isolated from Malaysia, was obtained from the Landolt Duckweed Culture Collection (ETH Zurich, Switzerland). This clone was maintained under sterile (axenic) conditions in the lab at the University of Leeds. Stock cultures were kept in plastic containers containing Hoagland's growth solution in a Sanyo MLR-351H growth chamber at a constant temperature of 20°C, with an 8-hour light cycle and a light intensity of 150 μmol m^{-2} s^{-1}. The growth solution was replaced every two weeks, and any dead plants on the surface or bottom of the containers were removed monthly.

2.2. Culture medium

Hoagland's growth media (HM) was selected for this study because it contains nitrate as the sole nitrogen source, allowing for a more accurate representation of the targeted nitrogen species typically found in effluents from small and decentralised wastewater treatment systems, where nitrification processes often convert ammonium into nitrate. HM was prepared from six autoclaved stock solutions containing all the required macro- and micro-nutrients for optimal duckweed growth, as described by

Paterson et al. (2020). The final concentrations of nutrients in media are as follows: 543 mM $Ca(NO_3)_2*4H_2O$, 1 mM $MgSO_4*7H_2O$, 0.5 mM KH_2PO_4, 2.5 mM KNO_3, 23.1 µM H_3BO_3, 0.19 µM $NaMoO_4*2H_2O$, 4.6 µM $MnSO_4*5H_2O$, 0.13 µM $ZnSO_4*7H_2O$, 0.18 µM $CuSO_4*5H_2O$, 35.8 µM $FeCl_3*6H_2O$, 103 µM EDTA.

For all experiments, unless stated otherwise, the initial pH of the culture medium was adjusted to 7 using 1M NaOH, without the addition of any buffer. In experiments involving varying inorganic phosphorus levels, the volume of the KH_2PO_4 stock solution was adjusted to achieve the desired phosphorus concentration. Any resultant potassium deficiency was compensated by adding 1M KCl to maintain nutrient balance. Similarly, when modifying the total nitrogen concentration in the medium, the volumes of the $Ca(NO_3)_2$ and KNO_3 stock solutions were proportionally adjusted. Any imbalances in potassium and calcium levels due to these adjustments were corrected by adding 1M KCl and 1M $CaCl_2$, respectively.

2.3. Experimental setup

The first part of the experimental setup involved pre-cultivation. Healthy plants from the stock culture were selected, rinsed with deionised water, and pre-cultivated under specific conditions required for each experiment. Pre-cultures were maintained for a week in 1-litre plastic containers with Hoagland's media (HM), replenished every two days. Before each experiment, the pre-cultivated fronds were thoroughly washed with deionised water and placed in a separate container with phosphorus-deficient HM. These fronds underwent phosphorus starvation for at least five days to maximise phosphorus uptake response during the experiments (Paterson et al, 2020). Potassium deficiency was corrected by adding KCl.

Batch experiments were conducted in a Fitotron® growth chamber (model SG066 CHX-F) using 150 mL plastic pots (6.25 cm diameter) containing 100 mL sterile growth solution. All experiments were performed under non-sterile conditions. Light was provided by fluorescent tubes at 150 µmol photons m^{-2} s^{-1}, in 12-hour light cycles. Experimental pots were placed in a cardboard box to prevent lateral light incidence and algae proliferation. Experiments were conducted in triplicate using 100 mg initial fresh duckweed mass per pot.

2.3.1. Temperature-dependent growth experiments

W. angusta was cultivated at three different temperatures (20, 25 and 30°C) using HM with no nutrient limitations (100 mg NO_3-N L^{-1} and 15 mg PO_4-P L^{-1}). Pre-culture containers were maintained at each set temperature to acclimate the plants. Experiments lasted eight days, with water samples taken every second day for nutrient characterisation, together with top-view photographs for calculating biomass growth using changes in surface area coverage. Experimental pots were replenished daily with HM lacking nitrogen and phosphorus to mitigate evapotranspiration. The optimum temperature for nutrient uptake was used for the following nutrient experiments.

2.3.2. Phosphorus and nitrogen uptake and removal experiments

Nutrient-dependent growth kinetics were measured independently for phosphorus and nitrogen in short-term batch cultures by varying initial nutrient concentrations. At the start of each experiment series, healthy plants were inoculated into plastic pots containing 100 mL of modified culture medium – i.e., HM with set N and P concentrations. Phosphate experiments were conducted at 0.2, 2.0, 4.0, 6.0 and 8.0 mg PO_4-P L^{-1}, while nitrogen experiments were carried out at 0.5, 5.0, 10.0, 15.0 and

20.0 mg NO_3-N L^{-1}. For phosphorus experiments, the initial nitrogen concentration was kept constant at 100 mg NO_3-N L^{-1}, and for nitrogen experiments, the initial phosphorus concentration was kept constant at 15 mg PO_4-P L^{-1}, to ensure single-nutrient limitation. The medium was supplemented with KCl and $CaCl_2$ as needed to keep a similar ionic strength across all experiments. Experimental pots were topped up daily with Hoagland's medium lacking either phosphorus or nitrogen to counter evapotranspiration and to keep single-nutrient limitation conditions. Experiments were conducted at 25°C for eight days, with samples taken every two days.

2.3.3. Model validation experiments

To validate the kinetic model and constants, *W. angusta* was cultivated under varying temperature and nutrient conditions. Temperature ranged from 20 to 30°C, and phosphorus and nitrogen concentrations were within levels typically found in effluents from anaerobic baffled reactors (0.2–4.0 mg P L^{-1}, 0.5–5 mg N L^{-1}) (Yulistyorini et al., 2019; Lau and Trzcinski, 2024). Plants were grown in 150 mL plastic pots with an initial inoculum of 100 mg fresh biomass. Light was provided at 150 µmol photons m^{-2} s^{-1} in 12-hour light cycles. Over five days, biomass growth and nutrient removal from the medium were monitored.

2.4. Analytical methods

2.4.1. Duckweed growth monitoring

The growth of *Wolffia angusta* was assessed by measuring changes in the total frond area over time using image analysis. Overhead photographs of the cultivation pots were taken at a fixed height and analysed using Fiji (ImageJ, version 1.54g; Java 1.8.0_345, 64-bit), a free and open-source image processing software.

2.4.2. Analysis of nutrients in media

Water samples were collected and filtered using 0.45 µm syringe filters (Fisher Scientific, UK). Samples were diluted with distilled water when necessary. Phosphate concentrations were determined using the standard vanadate-molybdate colorimetric method (Holman, 1943). Nitrate concentrations were measured using Hach® test kits (NO_3-N, LCK339), which have a detection range of 0.23 to 13.5 mg NO_3-N L^{-1}.

2.4.3. Data analysis

All the data were analysed using analysis of variance (ANOVA) followed by Tukey's post-hoc test to determine statistically significant differences at $p < 0.05$. Model fitting and statistical analysis were conducted using Minitab software.

2.5. Model development and accuracy assessment

Building on experimental data, we developed and validated a kinetic model to describe the interconnected dynamics of duckweed growth and nutrient removal. The model combines classical first-order Monod kinetics with temperature-dependent functions and yield coefficients that are adjusted according to nutrient availability. Although internal nutrient reserves were not directly modelled, their influence was indirectly captured in the calibrated model. Experiments were allowed to continue even after the depletion of external nutrients, reflecting Duckweed's known ability to utilise *in-planta* phosphorus and nitrogen reserves (Paterson et al., 2020).

The effects of light intensity and photoperiod, though recognised as influential factors in duckweed growth and nutrient uptake, were excluded from the model. This decision aligns well with the model's intended application in tropical regions, where day length is relatively consistent all year-round at about 12 hours and ambient light levels are typically in excess of the minimum required to support optimal growth – i.e., sunlight intensity in the tropics ranges from 460 to 6,000 μmol photons m^{-2} s^{-1} during the day (Handara et al., 2016). Under these conditions, light variations are less likely to limit growth or significantly impact nutrient dynamics compared to temperature and nutrient availability.

Model output prediction intervals were calculated at the 95% confidence level, using the standard error of predicted values derived from the linear regression model based on the *t*-distribution of the data. Model performance, both pre- and post-calibration, was assessed using three key accuracy metrics: the coefficient of determination (R^2), root mean square error (*RMSE*), and residual predictive deviation (*RPD*). A well-performing model is typically characterised by a high R^2 value (>0.9), a low *RMSE*, and an *RPD* exceeding 2.5.

3. Results and Discussion

3.1. Temperature-dependent biomass growth and nutrient removal

Duckweed growth, expressed as the relative increase in total frond area with respect to the cultivation pot area (fronds cover percentage), at three different temperatures, is presented in Figure 1A. The results indicate that duckweed grew at all tested temperatures without a lag phase, thanks to pre-culture conditions. However, significant differences in growth rates were observed across the tested temperatures. At 20°C, plants exhibited the slowest growth, achieving only 45% surface coverage by the end of the 8-day experiment. In contrast, plants cultivated at 25 and 30°C displayed substantially higher growth, reaching full surface coverage by days 7 and 6, respectively. Once plants covered 100% of the surface, fronds began overlapping, making further growth quantification via image analysis impossible. The relative growth rate (*RGR*) of duckweed was determined by fitting an exponential growth model to data collected before plants reached full surface area coverage. A statistically significant effect of temperature on *RGR* was found (Table 1). Specifically, increasing the temperature within the tested range enhanced the growth rate of *Wolffia angusta*, with *RGR* values rising from 0.197 day^{-1} at 20°C to 0.427 day^{-1} at 30°C.

Nutrient removal from the growth medium was closely linked to temperature as well. Figure 1B illustrates phosphorus depletion, which occurred more rapidly as the temperature increased. By the end of the experiment, phosphorus was nearly undetectable in media from pots maintained at 25 and 30°C, while approximately 37% of the initial phosphorus supply remained in pots at 20°C. Nitrogen removal followed a similar trend (Figure 1C), with final nitrogen removal efficiencies of 29%, 52%, and 67% at 20, 25, and 30°C, respectively.

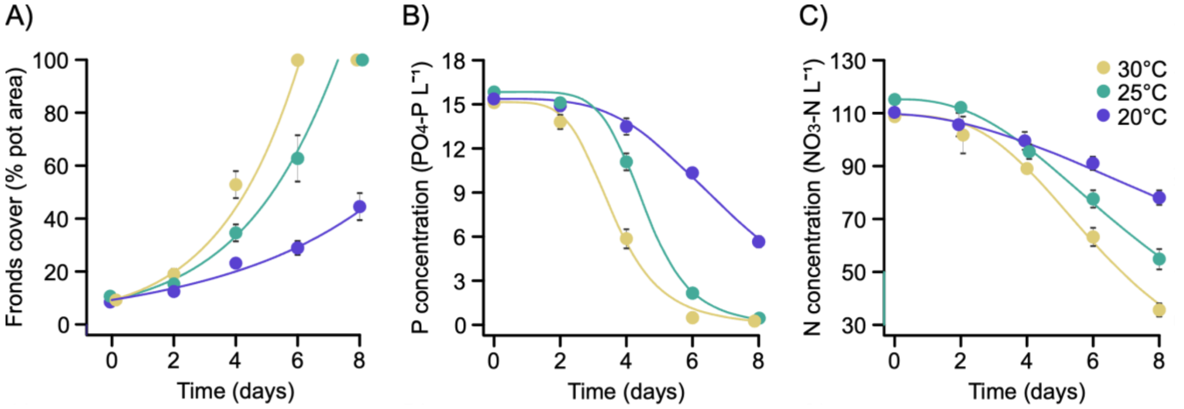


Figure 1. Influence of temperature on *Wolffia angusta* growth and nutrient removal. (A) Increase in duckweed fronds cover. (B) Remaining orthophosphate concentration in media, expressed as mg PO_4-P L^{-1}. (C) Remaining nitrate concentration, expressed as mg NO_3-N L^{-1}. The plants were grown at 20, 25, and 30°C in triplicates in standard Hoagland's media over 8 days. Error bars correspond to the standard deviation.

Table 1. Temperature-dependent rates of growth and nutrient removal in duckweed. The table summarises the relative growth rate and nutrient removal rates and specific nutrient uptake (phosphorus and nitrogen) of *Wolffia angusta* at three different temperatures. Values are presented as means with standard errors in parentheses. Statistical significance between mean values ($p < 0.05$, ANOVA + Tukey HSD test) at tested temperatures is indicated by different letters (a, b and c).

Temperature	20°C	25°C	30°C
Relative growth rate, d^{-1}	0.197 (0.014) ^{a}	0.337 (0.010) ^{b}	0.427 (0.018) ^{c}
Max P removal rate, mg P L^{-1} d^{-1}	2.08 (0.25) ^{a}	5.65 (0.57) ^{b}	5.11 (0.88) ^{b}
Specific P uptake, g P m^{-2} DW	0.74 (0.04) ^{a}	0.79 (0.06) ^{a}	0.75 (0.06) ^{a}
Max N removal rate, mg N L^{-1} d^{-1}	5.92 (1.16) ^{a}	9.21 (1.16) ^{b}	13.29 (1.98) ^{c}
Specific N uptake, g P m^{-2} DW	2.62 (0.33) ^{a}	2.18 (0.20) ^{b}	1.63 (0.20) ^{c}

Analysis of the maximum nutrient removal rates revealed two distinct trends (Table 1). For phosphorus, the maximum phosphorus removal rate (PPR_{max}) was lowest at 20°C (2.08 mg P L^{-1} d^{-1}) and increased by a factor of 2.7 at 25°C, reaching a plateau. Temperatures above 25°C did not further enhance the phosphorus removal rate. In contrast, the maximum nitrogen removal rate (NRR_{max}) showed a positive correlation with increasing temperature, rising from 5.92 mg N L^{-1} d^{-1} at 20°C to 13.29 mg N L^{-1} d^{-1} at 30°C. Regarding specific nutrient uptake, temperature changes did not significantly affect specific phosphorus uptake, which remained consistent at an average of 0.77g of P per m^2 of duckweed cover across all tested temperatures. However, temperature appeared to have an inverse effect on specific nitrogen uptake, decreasing from 2.62g of N per m^2 of duckweed cover at 20°C to 1.63 g N m^{-2} at 30°C (Table 1).

Our results corroborate previous studies indicating that duckweed growth and nutrient removal are temperature-dependent processes. For instance, Wedge and Burris (1982) reported an optimal photosynthetic temperature range of 30–35°C for duckweeds, aligning with our observation that *W. angusta* exhibited maximum relative growth rate at 30°C. Similarly, Lasfar et al. (2007) suggested an optimal growth temperature range of 23–28°C, reinforcing the idea that duckweed growth efficiency declines when temperatures deviate from this range. The observed disparity in optimal temperature conditions among different duckweed species and isolates underscores the necessity for species-specific studies to refine environmental conditions for maximal growth and nutrient removal (Pasos-Panqueva et al., 2024; Ziegler et al., 2015), particularly for isolates within the *Wolffia* genus.

Results from Table 1 indicate that the primary factor driving increased nutrient removal at elevated temperatures is greater biomass accumulation, rather than enhanced metabolic activity. This is further supported by the observation that while *RGR* improves with rising temperatures, specific P uptake remains unaffected, and specific N uptake is altered. The low Q10 temperature coefficient for nitrogen uptake (0.62) suggests a decline in metabolic processes related to nitrogen assimilation as temperatures rise (Mundim et al., 2020). This decline could be due to morphological changes, altered transport protein activity, or temperature-driven shifts in cellular mechanisms, as observed in terrestrial plant species (Mishra et al., 2023; Pregitzer & King, 2005). In *Spirodela polyrhiza*, the reduction in specific nitrogen uptake at higher temperatures is likely due to decreased protein synthesis, with resources being redirected towards the production of heat shock proteins that require less nitrogen (Shang et al., 2023). Meanwhile, stable phosphorus uptake is linked to activated metabolic pathways for phosphorus and carbon storage, which require significant ATP and extracellular phosphorus uptake (Shang et al., 2023).

3.2. Impact of nutrient supply on *Wolffia angusta* growth and nutrient removal

Two separate experiments were conducted to investigate how phosphorus and nitrogen supplies affect both nutrient removal and the growth of *Wolffia angusta*. In each experiment, the concentration of only one nutrient was varied, while the other nutrient was replenished daily. This approach ensured that any limitations in duckweed growth were due solely to the nutrient being tested. In the phosphorus experiment, media concentrations ranged from 0.2 to 8 mg PO_4-P L^{-1}. Phosphorus in media reached its minimum value more rapidly when the initial supply was lower (Figure 2A).

Over an 8-day period, complete removal of phosphorus was achieved only in the pots with an initial supply of 0.2 mg P L^{-1}; in all other treatments, removal ranged between 96% and 98%. *W. angusta* exhibited robust growth at all phosphorus concentrations except at 0.2 mg PO_4-P L^{-1} (Figure 2B). Under this low-phosphorus condition, duckweed fronds began to bleach and settle at the bottom of the pots from day 6 onwards. By the end of the experiment, duckweed in these pots covered only 36% of the water surface, compared with complete coverage in the other treatments. Apart from the lowest phosphorus treatment, the *RGR* of duckweed was not influenced by the initial phosphorus concentration ($p < 0.05$, ANOVA with Tukey HSD test), maintaining an average value of 0.35 day^{-1}; at 0.2 mg PO_4-P L^{-1}, the *RGR* decreased by 26%.

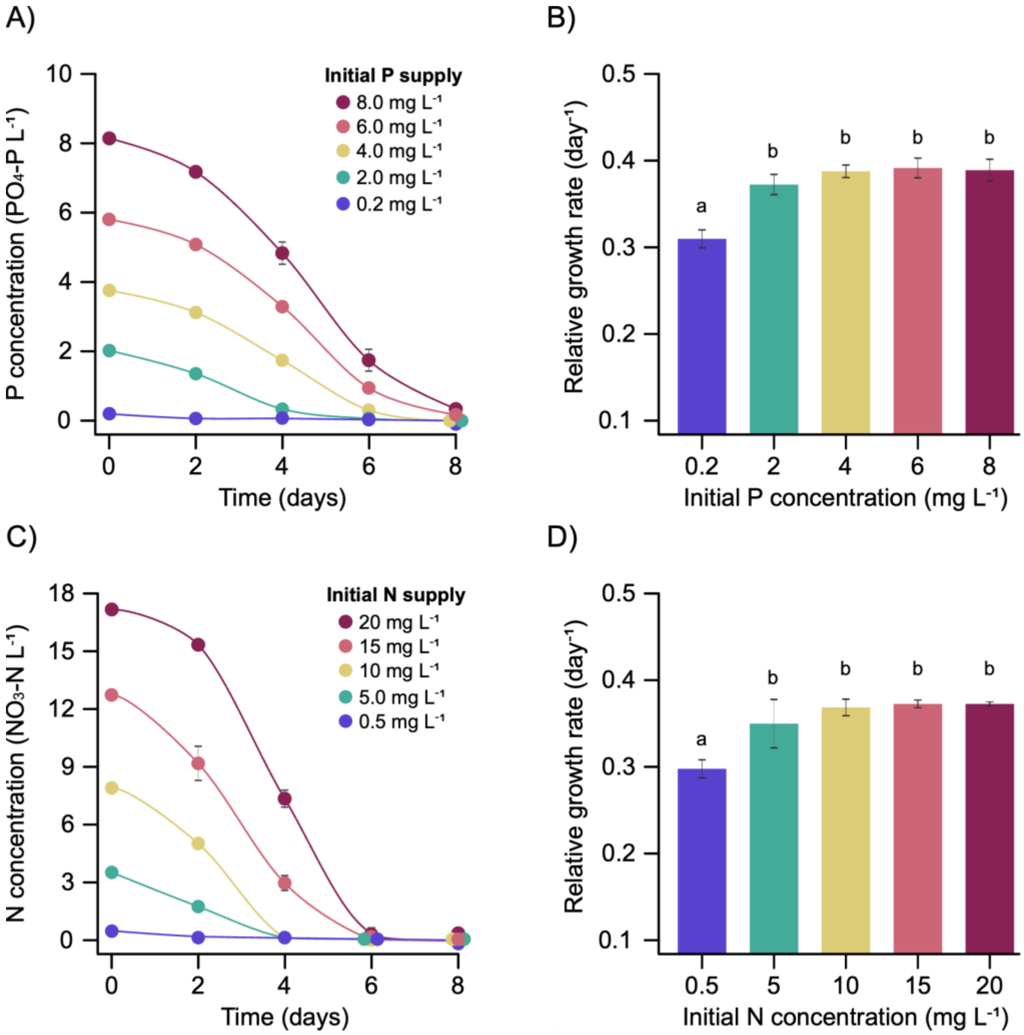


Figure 2. Nutrient removal and growth response of *Wolffia angusta* under different phosphorus and nitrogen supplies. (A) Change in orthophosphate concentration, expressed as mg PO_4-P L^{-1}, and (B) relative growth rate of plants cultivated at 25°C with varying initial phosphorus concentrations while maintaining a constant nitrogen concentration of 100 mg NO_3-N L^{-1}. (C) Change in nitrate concentration, expressed as mg NO_3-N L^{-1}, and (D) relative growth rate of plants cultivated at 25°C with varying initial nitrogen concentrations while keeping phosphorus concentration constant at 15 mg PO_4-P L^{-1}. Error bars correspond to the standard error of three experimental replicates. Bars with the same letter (a or b) are not statistically different from each other (p < 0.05, ANOVA + Tukey HSD test).

Similarly, the nitrogen experiment involved initial nitrate concentrations ranging from 0.5 to 20 mg NO_3-N L^{-1}. In all cases, duckweed removed the available nitrogen by the end of the experiment (Figure 2C). When the initial nitrogen concentration was below 15 mg NO_3-N L^{-1}, complete removal was achieved by day 4; at higher concentrations, full removal occurred by day 6. With respect to growth, a low initial nitrogen concentration (0.5 mg NO_3-N L^{-1}) resulted in many fronds settling at the bottom from day 4, yielding a final frond cover of only 24%. At 5 mg NO_3-N L^{-1}, fronds began to settle from day 6, although the final cover increased to 43%. At higher nitrogen concentrations, the duckweed completely covered the pot surfaces. The RGR showed a similar pattern to that observed in the phosphorus experiment. At 0.5 mg NO_3-N L^{-1}, the *RGR* was 0.25 day^{-1}, but at higher nitrogen concentrations the *RGR* increased and stabilised at an average of 0.34 day^{-1} (Figure 2D, $p < 0.05$, ANOVA with Tukey HSD test).

In parallel with these growth responses, nutrient removal rates showed clear dependencies on the initial nutrient supply (Table 2). The maximum phosphorus removal rate increased from 0.73 to 1.85 mg PO_4-P L^{-1} d^{-1} as the initial phosphorus concentration rose from 2 to 8 mg PO_4-P L^{-1}. For nitrogen, the maximum removal rate was 1.70 mg NO_3-N L^{-1} d^{-1} at the lowest supply of nitrogen, peaking at 6.85 mg NO_3-N L^{-1} d^{-1} at the highest. Moreover, the specific uptake of both nutrients was directly correlated with their initial concentrations. A 4-fold rise in the initial supply of phosphorus and nitrogen resulted in increases of 79% and 68% in their respective specific uptakes.

The observed trends in nutrient removal are consistent with earlier reports for *Landoltia punctata*, where the highest phosphorus and nitrogen removal rates occurred at the highest initial nutrient supplies (Cheng et al., 2002). However, *Wolffia angusta* showed lower removal rates, likely due to species-specific physiological differences, the use of lower nutrient concentrations, and the choice of nitrate (instead of ammonium) as the nitrogen source. Similar species-specific responses have been documented (Bergmann et al., 2000; McCann, 2016b; Ziegler et al., 2015), underscoring that nutrient removal efficiency depends on both the inherent traits of the species and the chemical form of the nutrients. Notably, removal rates in *W. angusta* increased steadily with each increment in nutrient supply, indicating that saturation of nutrient uptake was not reached (Pasos-Panqueva et al., 2024).

Table 2. Nutrient removal and uptake rates of duckweed at different nutrient concentrations. The table presents the nutrient removal and specific uptake rates of phosphorus and nitrogen by *Wolffia angusta* at varying initial concentrations of these nutrients in the growth medium. Duckweed was cultivated under controlled conditions at a constant temperature of 25°C and a daily light integral of 6.5 mol m^{-2}. Values are reported as means with standard errors in parentheses (n = 3). Statistical significance (p < 0.05, ANOVA + Tukey HSD test) between temperatures is indicated by different letters (a, b and c).

P supply (mg P L^{-1})	**2.0**	**4.0**	**6.0**	**8.0**
Max P removal rate mg P L^{-1} d^{-1}	0.73 (0.03) ^{a}	0.94 (0.19) $^{a, b}$	1.44 (0.27) $^{b, c}$	1.85 (0.33) ^{c}
Specific P uptake g P m^{-2} DW	0.19 (0.02) ^{a}	0.25 (0.01) $^{a, b}$	0.30 (0.02) $^{b, c}$	0.34 (0.04) ^{c}
Initial N:P (w:w)	50:1	25:1	17:1	13:1
N supply (mg N L^{-1})	**5.0**	**10**	**15**	**20**
Max N removal rate mg N L^{-1} d^{-1}	1.70 (0.33) ^{a}	4.67 (0.65) ^{b}	4.59 (0.75) $^{b, c}$	6.85 (0.33) ^{d}
Specific N uptake g P m^{-2} DW	0.81 (0.21) ^{a}	1.00 (0.06) $^{a, b}$	1.20 (0.09) $^{b, c}$	1.36 (0.10) ^{c}
Initial N:P (w:w)	0.3:1	0.7:1	1.0:1	1.3:1

Growth responses further illustrate these dynamics (Figure 2B and 2D). *Wolffia angusta* growth was adversely affected only when the initial phosphorus concentration dropped below 2 mg P L^{-1}, a threshold similar to that observed in *Lemna minor* at 27°C (Lasfar et al., 2007). The ability to thrive at low phosphorus levels depends on species-specific traits, the plant's internal phosphorus reserves, and prior environmental acclimation (Chaiprapat et al., 2005; Paterson et al., 2020). Even at minimal phosphorus, sustained growth suggests that *W. angusta* mobilises internal reserves to support its metabolism – i.e., in this case, due to pre-culture conditions.

For nitrogen, growth limitation became evident when nitrate levels fell below 10 mg N L^{-1}, aligning with previous findings in *Lemna minor* (Lasfar et al., 2007). This greater sensitivity to nitrogen likely reflects the higher nitrogen content in dry duckweed biomass (N:P = 15:1) and its essential role in protein synthesis, chlorophyll production and reproduction (Marschner, 2011). Moreover, although plant growth was not significantly affected by changes in nutrient concentrations above 0.2 mg P L^{-1} and 0.5 mg N L^{-1}, nutrient removal rates continued to increase. This pattern suggests that nutrient availability beyond a minimum threshold enhances biological uptake per unit biomass until nutrient transport systems reach saturation.

Research indicates that duckweeds reallocate nitrogen to support biomass growth and protein synthesis (Körner & Vermaat, 1998) and accumulate phosphorus as a safeguard against future shortages (Gérard & Triest, 2014). These insights are particularly relevant for wastewater treatment systems, especially those handling domestic wastewater, where phosphorus is often low and nitrogen is predominantly present as ammonium in raw influents (or after anaerobic pre-treatment), or as nitrate after aerobic pre-treatment (Di Capua et al., 2022). Recognising the threshold concentrations limiting duckweed growth can inform operational strategies, such as optimising hydraulic residence times and adjusting biomass harvesting schedules, to maintain robust duckweed growth and enhance nutrient removal and uptake efficiency.

3.3. Determination of batch kinetics coefficients

For the formulation of the batch kinetics, several assumptions were made: the cultivation pots are small enough that there is no vertical stratification of nutrient concentrations; duckweed growth is directly related to the concentrations of nitrogen and phosphorus in the medium as water and other nutrients are replenished during the test; no interference from other microorganisms occurs (sterile conditions); temperature remains constant over the course of the experiments; light is not a limiting factor (daily light integral between 5 - 20 mol m^{-2}; Pasos-Panqueva et al., 2024); water evaporation is negligible; environmental CO_2 is the sole carbon source; and there are no mass transfer limitations affecting nutrient diffusion and transport. Under nutrient-rich conditions, the biomass growth rate can be described by an exponential growth model.

(1) $$\boldsymbol{r_X = \frac{dX}{dt} = \mu * X}$$

Here, r_X (or dX/dt), represents the plant growth rate (expressed as the increase in the pot's surface coverage in cm^2 cm^{-2} d^{-1}); μ is the relative growth rate (d^{-1}), and X denotes the plants surface cover in comparison with the full surface area at any given time (cm^2 cm^{-2}).

To capture the influence of external nutrient concentrations on growth, the Monod kinetic model (Monod, 1949) is applied:

(2) $$\boldsymbol{\mu = \mu_{max} * \frac{P}{K_P+P} * \frac{N}{K_N+N}}$$

In Equation 2, μ_{max} is the maximum relative growth rate, while P and N are the phosphorus (mg PO_4-P L^{-1}) and nitrogen (mg NO_3-N L^{-1}) concentrations in the medium, respectively. The constants K_P and K_N represent the substrate half-saturation constants – i.e., the nutrient concentrations at which the growth rate is half of μ_{max}.

Under conditions of nutrient excess and constant light, μ reaches μ_{max}, which can be further described as a function of temperature using an Arrhenius-type equation:

(3) $$\boldsymbol{\mu_{max} = A_1 * exp^{\left(\frac{-E_1}{R*T}\right)}}$$

Here, A_1 is a constant (day^{-1}), E_1 is the activation energy (J mol^{-1}), *R* is the universal gas constant (J mol^{-1} K^{-1}), and *T* is the absolute temperature (K).

The removal of phosphorus and nitrogen from the medium is directly mediated by plant growth. Therefore, the rates of substrate utilization are given by:

(4) $$\boldsymbol{r_P = \frac{r_X}{Y_{XP}}}$$

(5) $$\boldsymbol{r_N = \frac{r_X}{Y_{XN}}}$$

In these equations, r_P and r_N (mg substrate L^{-1} d^{-1}) are the rates of phosphorus and nitrogen removal, and Y_{XP} and Y_{XN} (cm^2 per mg substrate) are the biomass yield coefficients for each nutrient. The inverses of these coefficients represent the specific uptake rates.

It is important to note that the biomass yield for each nutrient is not constant during treatment but varies with the residual nutrient concentration in the cultivation medium (as previously shown in Table 2). This variation was modelled using a logistic function to describe yields at high nutrient concentrations (denoted as ${Y_{XP}}^{min}$ and ${Y_{XN}}^{min}$) and the maximum yields observed in the absence of external nutrient supply (${Y_{XP}}^{max}$ and ${Y_{XN}}^{max}$). The constants K_P' and K_N' determine the rate of change of the yield. Additionally, a modified Arrhenius equation was used to adjust these parameters for temperature changes relative to values obtained at 25°C:

(6) $$\boldsymbol{Y_{XP} = \frac{K_P' * {Y_{XP}}^{max} + P}{K_P' + (1/{Y_{XP}}^{min}) * P}}$$

(7) $$\boldsymbol{Y_{XN} = \frac{K_N' * {Y_{XN}}^{max} + N}{K_N' + (1/{Y_{XN}}^{min}) * N}}$$

(8) $$\boldsymbol{Value_T = Value_{25°C} * \theta^{(T-25)}}$$

The kinetic parameters presented in Equations 1-8 were determined from experimental data, and the compiled values are summarized in Table 3. The maximum relative growth rate of *Wolffia angusta* at 25°C aligns with values reported for other *Wolffia* species (Ziegler et al., 2015). Although half-saturation values for phosphorus and nitrogen in *W. angusta* have not been previously reported, the estimated K_P and K_N values are significantly higher than those reported for *Lemna minor* (1.89 µg P L^{-1}, 0.02 mg N L^{-1}) and *Spirodela polyrhiza* (0.87 mg P L^{-1}, 0.02 mg N L^{-1}) (Kufel et al., 2012). These elevated half-saturation constants indicate that *W. angusta* requires higher nutrient concentrations to reach half of its maximum growth rate, which explain similar growth rate results obtained from this work for a wider range of N:P ratios (0.7:1 < N:P < 25:1). Consequently, while it may grow robustly under nutrient-rich conditions, it could be less competitive in nutrient-poor environments compared to other duckweed species.

Table 3. Kinetic constants for the growth model of *Wolffia angusta*

Parameter	Description	Value (SD)	Unit
${\mu_{max}}^{25°C}$	Maximum RGR at 25°C	0.30 (0.01)	day^{-1}
$Ln(A_1)$	N-log Arrhenius pre-exponential factor	18.59 (3.90)	day^{-1}
E_1	Growth activation energy	48.97 (9.68)	kJ mol^{-1}
K_P	Half saturation constant for phosphorus	0.048 (0.012)	mg P L^{-1}
K_N	Half saturation constant for nitrogen	0.244 (0.030)	mg N L^{-1}
${Y_{XP}}_{25°C}^{max}$	Maximum biomass-to-phosphorus yield at 25°C	113.6 (37.1)	cm^2 mg^{-1}
${Y_{XP}}_{25°C}^{min}$	Minimum biomass-to-phosphorus yield at 25°C	18.9 (2.52)	cm^2 mg^{-1}
${K'_P}_{25°C}$	Biomass-to-phosphorus yield rate constant	0.055 (0.014)	mg^2 L^{-1} cm^{-2}
${Y_{XN}}_{25°C}^{max}$	Maximum biomass-to-nitrogen yield at 25°C	24.7 (6.6)	cm^2 mg^{-1}
${Y_{XN}}_{25°C}^{min}$	Minimum biomass-to-nitrogen yield at 25°C	4.94 (0.48)	cm^2 mg^{-1}
${K'_P}_{25°C}$	Biomass-to-phosphorus yield rate constant	0.566 (0.356)	mg^2 L^{-1} cm^{-2}
θ	Temperature correction factor	1.054 (0.012)	-

3.4. Batch model validation and prediction

In a batch duckweed cultivation system, biomass accumulation and nutrient removal are dynamically interlinked and hence, our model couples an exponential growth formulation (1) with substrate utilization (4 and (5), to capture this relationship. In axenic cultures, nutrient uptake is directly proportional to biomass growth (Paterson et al., 2020), meaning that as the duckweed surface coverage increases, phosphorus and nitrogen are removed from the medium in fixed proportions defined by their yield coefficients. Furthermore, by incorporating temperature effects on the maximum growth rate via an Arrhenius-type adjustment (3), the model becomes adaptable to environmental fluctuations.

The model was calibrated using experimental data from duckweed grown under simulated tropical conditions with varying temperature, (see Section 2.3.3) where phosphorus and nitrogen supplies were varied within ranges typically found in nutrient-rich wastewaters (Figure).

Experiments that maintained sufficient nutrient availability throughout, such as those at 20°C with 2 mg PO_4-P L^{-1} and 10 mg NO_3-N L^{-1}, were better reproduced by the model ($R^2 > 0.787$), whereas experiments in which one or both nutrients were absent or completely depleted (e.g., at 30°C) over the course of the experiment resulted in poorer model performance. These discrepancies can be attributed to the limitations of the traditional Monod kinetic model, which only accounts for external nutrient concentrations and neglects the role of internal nutrient reserves. In reality, duckweed can mobilise internal stores of phosphorus and nitrogen to sustain growth under nutrient scarcity (Paterson et al., 2020). Consequently, the original model assumed that plant growth would cease once an external nutrient was depleted (i.e., N and P in the media), leading to underestimations of biomass accumulation.

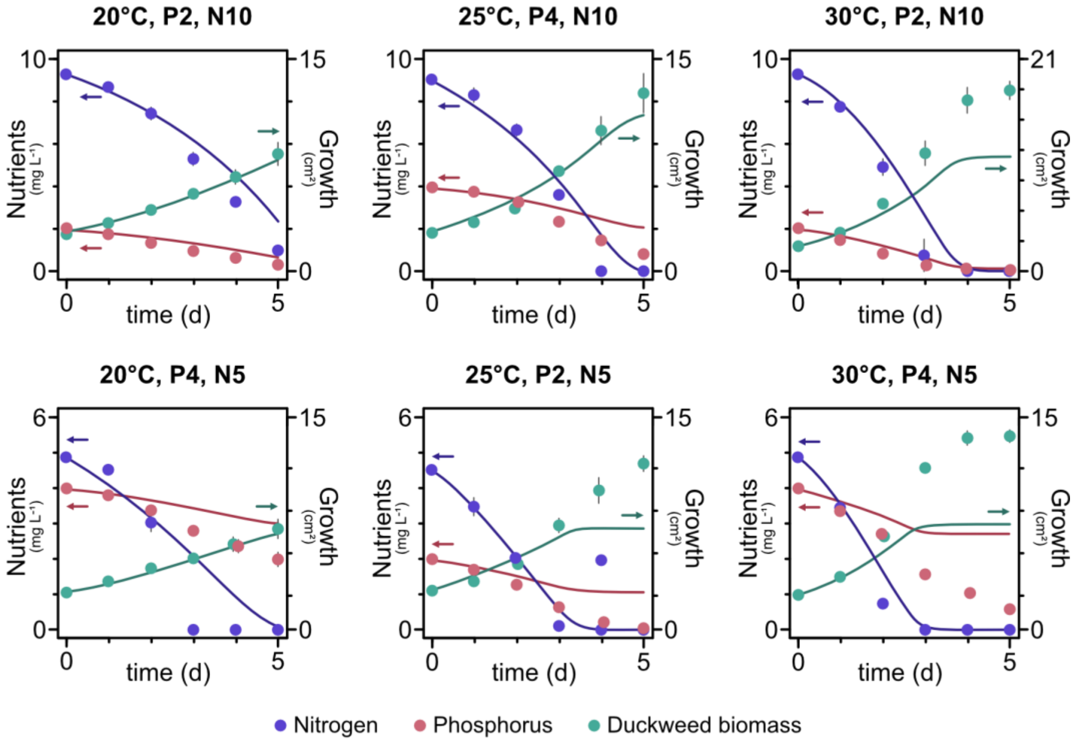


Figure 3. Experimental and modelled outcomes for the cultivation of *W. angusta* at varying culture conditions. Dots represent experimental data while continuous lines predict values from the kinetic model. Data for nitrogen and phosphorus concentration should be read against the left axis while duckweed growth data (expressed as increased in total fronds area) should be read against the right axis, as marked by the arrows. Culture conditions for each experiment are shown at the top of each graph. The initial concentration of phosphorus (P) and nitrogen (N) is represented as the number next to the letters, in mg PO$_4$-P L^{-1} and mg NO$_3$-N L^{-1}, respectively**.**

To overcome this shortcoming, the kinetic model was modified to accommodate a finite growth rate under nutrient-depleted conditions by adopting a logistic growth formulation (9). This modification introduces two new parameters: a specific growth rate (μ_i), which takes on distinct values (μ_{P_0}, μ_{N_0}, or μ_{P_0,N_0}) depending on whether phosphorus, nitrogen, or both are absent in media, and a carrying capacity (L_i) that represents the maximum duckweed coverage achievable under nutrient limitation. In parallel, the expression for the relative growth rate of *Wolffia angusta* was adjusted ()

to integrate the effects of internal nutrient reserves, thereby refining the model's representation of plant growth in the absence of external nutrients.

$$(9)\qquad \boldsymbol{r_X = \frac{dX}{dt} = \begin{cases} \mu * X, & [P],[N] > 0 \\ \mu_i * X * \left(\frac{L_i - X}{L_i}\right), & [P],[N] = 0 \end{cases}}$$

$$(10)\qquad \boldsymbol{\mu_i} = \begin{cases} \mathbf{\mu_1 = \mu_{max} * \frac{P}{K_P + P} * \frac{N}{K_N + N}} \\ \mathbf{\mu_2 = \mu_{P_0} * \frac{K_P}{K_P + P} * \frac{N}{K_N + N}} \\ \mathbf{\mu_3 = \mu_{N_0} * \frac{K_N}{K_N + N} * \frac{P}{K_P + P}} \\ \mathbf{\mu_4 = \mu_{P_0, N_0} * \frac{K_P}{K_P + P} * \frac{K_N}{K_N + N}} \end{cases}$$

Prior to calibration, the model exhibited variable predictive ability, particularly under conditions characterised by poor nutrient concentrations in the medium. This is evident in the lower *R²* values and wider spread of data points around the 1:1 line in the corresponding correlation plots. For instance, in the pre-calibration plots (Figure , left column), *R²* values were noticeably moderate to low, suggesting only a modest proportion of the variance in observed values was explained by the model. In addition, *RMSE* values were relatively high, indicating greater discrepancies between predicted and observed values. The *RPD* values, which offer insight into the practical utility of the model (with values below 1.5 typically indicating poor predictions), were also unsatisfactory before calibration.

Following calibration (Figure , right column), a substantial improvement in model performance is evident across all conditions. *R²* values increased significantly, in some cases approaching or exceeding 0.9, which reflects a much stronger agreement between observed and predicted values. The *RMSE* decreased notably across calibrated conditions, demonstrating a reduction in prediction errors.

Correspondingly, *RPD* values improved, often exceeding the threshold of 2.0, suggesting the model's predictions became reliable and suitable for analytical purposes. Particularly under conditions with low nutrient concentrations, the calibration procedure had a marked impact. The improvement in prediction is highlighted by the narrowing of the 95% confidence intervals and the tighter clustering of data points along the ideal 1:1 line, reducing bias and enhancing model robustness.

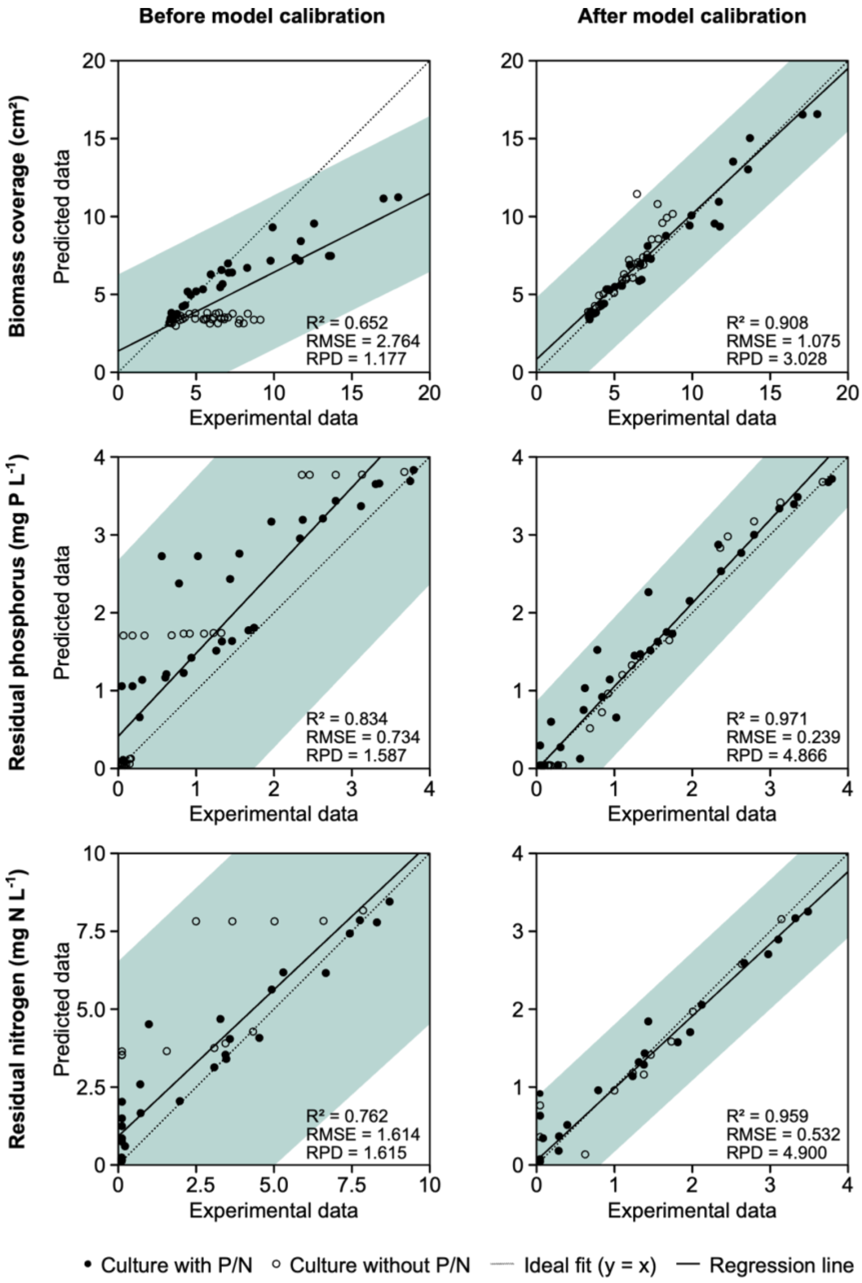


Figure 3. Correlation plots between experimental and predicted data from uncalibrated and calibrated kinetic batch models. The figure shows six scatterplots comparing observed and predicted under different modelling conditions, illustrating model performance before (left column) and after calibration (right column). Shaded areas represent the 95% confidence intervals around the predicted values, indicating the uncertainty of model estimates. Three statistical metrics, the coefficient of determination (*R^2*), the root mean square error (RMSE), and the ratio of performance to deviation (RPD), are reported within each plot to quantitatively assess model accuracy. Data points represent experimental results from cultures with high (solid dots) and low (hollow dots) nutrient availability.

3.5. Model application: Design of duckweed-based polishing systems

Using the calibrated kinetic model, we simulated the performance of a *Wolffia*-based polishing system treating the effluent from a hybrid anaerobic baffled reactor (HyABR) system, which are commonly used in decentralised urban wastewater management in Indonesia (World Bank/WSP, 2013; Yulistyorini et al., 2019). The simulations assumed a freshwater consumption of 120 L person^{-1} day^{-1}, with 80% returned as wastewater (Utami et al., 2024). HyABR effluent was modelled with nutrient concentrations of 20 mg N L^{-1} and 5 mg P L^{-1} (Yulistyorini et al., 2019) and assuming that aeration chambers achieve full nitrification. The number of duckweed polishing ponds arranged in series was varied from 1 to 12 under simulated tropical conditions (25°C, 12-hour photoperiod, and non-limiting light intensity). Initial duckweed surface coverage in each pond was set at 50% and allowed to increase until full (100%) coverage was reached, after which sufficient biomass was harvested to restore coverage to 50%.

Simulations set to meet the regulatory phosphorus discharge limit of 1 mg P L^{-1} in the final effluent are presented in Figure 5. The results indicate that increasing the number of duckweed ponds in series reduces both the total hydraulic retention time (HRT) and the treatment area per person necessary to meet the phosphorus target (Figures 5A and 5B). However, this also results in lower duckweed biomass productivity (Figure 5C). Specifically, a single polishing pond requires an HRT of 52.5 days and a treatment area of 10.1 m^2 per person, producing 1.448 tonnes of fresh duckweed biomass per day. Increasing the number of ponds from 1 to 12 leads to a 28.8% reduction in total HRT, area, and biomass productivity, but results in a 94.1% reduction in these values per pond.

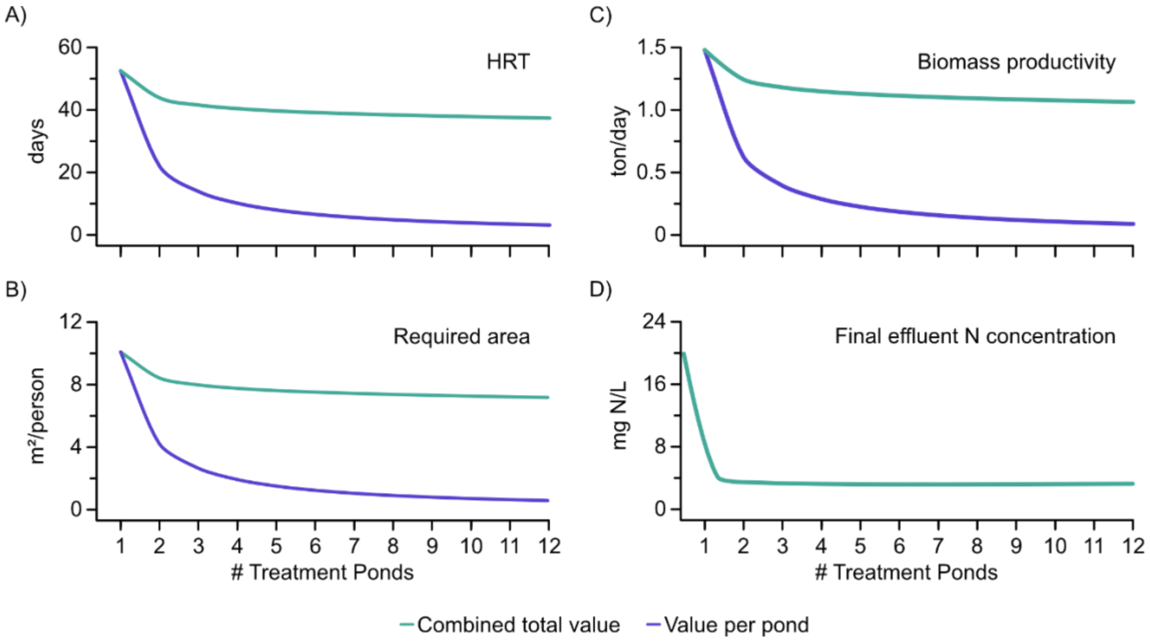


Figure 5. Simulated performance of a duckweed-based polishing system treating hybrid anaerobic baffled reactor (HyABR) effluent under tropical conditions. System performance was evaluated based on (A) hydraulic retention time (HRT), (B) treatment area required per person, (C) fresh duckweed biomass productivity, and (D) concentration of nitrogen in the final effluent, across systems with varying numbers of ponds. Results are shown as both per-pond values and total values for the entire treatment system. Simulations assumed typical tropical conditions (25°C, 12-hour photoperiod, and sufficient light intensity), for a population of 2,000 people using 120 L person^{-1} day^{-1} of water. Wastewater return factor was set at 0.8. Post-ABR effluent concentrations were assumed to be 20 mg N L^{-1} and 5 mg P L^{-1}. Duckweed coverage in each pond was initially set at 50% and was reset to the same value whenever the plants covered the entire pond surface. In all cases, the simulation aimed to achieve a final effluent phosphorus concentration of 1 mg P L^{-1}.

In all simulated cases, nitrogen removal exceeded 80%, with final concentrations below 4 mg N L^{-1} (Figure 5D), in compliant with current regulatory thresholds in Indonesia (< 10mg N L^{-1}, Ministry of Environment and Forestry of the Republic of Indonesia, Regulation No. P.68/2016), and sensitive areas in the UK (< 10mg N L^{-1} and minimum nitrogen removal between 70-80% of influent load (Environment Agency, 2019)).

Based on the results, a polishing system consisting of two to three ponds in series appears to be optimal. This configuration is sufficient to comply with both phosphorus and nitrogen discharge standards, while effectively complementing the HyABR treatment for communities of up to 2,000 inhabitants. The addition of further ponds does not provide significant benefits, as it neither reduces the required surface area nor increases biomass production.

Beyond treatment performance, this configuration also offers advantages from a resource recovery perspective. At this scale, and based on the reported composition of the duckweed biomass (28% protein dry weight, 12.1 g P kg^{-1} dry weight; Appenroth et al., 2018), the harvested *W. angusta* biomass could enable the recovery of approximately 0.44 tonnes of phosphorus and 1.65 tonnes of nitrogen per year. This highlights the role of polishing ponds not only in improving effluent quality but also in supporting future circular economy strategies in urban wastewater treatment.

4. Conclusion

This study presents a robust kinetic model that accurately predicts duckweed biomass productivity and nutrient depletion under varying tropical conditions, including nutrient-limited scenarios. The model offers a practical tool for optimising decentralised, nature-based wastewater treatment systems using *Wolffia angusta*, particularly in tropical and subtropical regions impacted by nutrient-rich discharges. Our findings reveal temperature as a key driver of biomass growth and nutrient removal, with optimal performance between 25 and 30°C. While higher temperatures boost biomass, nutrient uptake rates remain stable, indicating growth governs nutrient removal. Nutrient availability strongly influenced uptake patterns, revealing three distinct responses: growth limitation at low levels, increased uptake efficiency at moderate N:P ratios, and inhibition under nutrient-saturated conditions. Batch systems performed best at high nutrient concentrations, maximising both growth and nutrient recovery. Beyond controlled experiments, the model was applied to simulate a duckweed-based polishing stage for hybrid anaerobic baffled reactor (HyABR) effluents, a common decentralised treatment approach in Indonesia. The results support the feasibility of using duckweed systems to meet discharge standards while enabling nutrient recovery, reinforcing their potential role in sustainable urban wastewater management. Future work should explore internal nutrient reserves, microbial interactions, and system resilience under dynamic environmental conditions.

Declaration of Competing Interest

All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Johan Pasos-Panqueva: Conceptualisation, Experimental design, Experimental work, Data curation and processing, Model development and validation, Writing – original draft, review and editing. Anie Yulistyorini: Writing – review and editing. Alison Baker: Conceptualisation, Experimental design, Data curation, Supervision, Resources, Writing – review and editing. Miller Alonso Camargo-Valero Conceptualisation, Experimental design, Data curation, Supervision, Resources, Funding acquisition, Writing – review and editing.

Data access statement

All data underlying the results are available as part of the article and no additional source data are required.

Acknowledgements

This work was funded by the School of Civil Engineering, University of Leeds, United Kingdom through a PhD scholarship granted to Dr Pasos-Panqueva; the UKRI funded GCRF Water Security and Sustainable Development Hub project (ES/S008179/1); and the Colombia’s Ministry of Science, Technology and Innovation (MINCIENCIAS), Colombia for international doctoral scholarship number 860–2019 (contract no.106124).

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