Environmental Science and Pollution Research (2021) 28:16394–16407
https://doi.org/10.1007/s11356-020-11792-y

RESEARCH ARTICLE

Light intensity alters the phytoremediation potential of *Lemna minor*

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Received: 26 June 2020 / Accepted: 22 November 2020 / Published online: 2 January 2021

Abstract

Lemnaceae, i.e. duckweed species, are attractive for phytoremediation of wastewaters, primarily due to their rapid growth, high nutrient uptake rates, tolerance to a broad range of growing conditions and ability to expeditiously assimilate a variety of pollutants. Light is essential for plant growth, and therefore, phytoremediation. Nevertheless, the effect of light intensity remains poorly understood in relation to phytoremediation, a knowledge gap that impedes the development of indoor, fully controlled, stacked remediation systems. In the present study, the effect of light intensity (10–850 μmol m^{-2} s^{-1}) on the phytoremediation potential of *Lemna minor* was assessed. Plants were grown on either an optimal growth medium (half-strength Hutner's) or synthetic dairy processing wastewater, using stationary axenic (100 mL) or re-circulating non-sterile (11.7 L) systems. The relative growth rate (RGR) of *L. minor* grown on half-strength Hutner's increased proportionally with increasing light intensity. In contrast, the RGR of *L. minor* grown on synthetic dairy wastewater did not increase with light over an intensity range from 50 to 850 μmol m^{-2} s^{-1}. On synthetic dairy wastewater, total nitrogen and total phosphorous removal also remained unchanged between 50 and 850 μmol m^{-2} s^{-1}, although *L. minor* protein content (% fresh weight) increased from 1.5 to 2% at higher light intensities. Similar results were obtained with the larger re-circulating system. The results demonstrate interactive effects of light intensity and wastewater composition on growth and phytoremediation potential of *L. minor*. The data imply that light intensities above 50 μmol m^{-2} s^{-1} may not necessarily confer benefits in duckweed wastewater remediation, and this informs engineering of stacked, indoor remediation systems.

Keywords Duckweed · Lemna · Phytoremediation · Wastewater · Dairy processing · Light · Nutrient removal · Protein

Introduction

Food security, including the availability of clean water, is increasingly threatened by rapid human population growth, climatic change and pollution (Porter et al. 2014; Caine et al. 2019; Hamann 2020). As such, there is an urgent requirement to develop more sustainable food production and processing methods that can deliver high nutritional value, whilst reducing both the consumption of finite resources and the generation of waste (Caine et al. 2019; Rufí-Salís et al. 2020). In recent years, through the efficient use of resources and waste minimisation, an economic model known as the circular economy has sought to enhance food security, environmental protection and the socioeconomic benefits of food production systems worldwide (Morseletto 2020; Rufí-Salís et al. 2020). In principle, the circular economy is reliant on long-term value retention, reduced use of primary resources and closed-loop production systems, whereby waste materials are recovered and transformed into new resources (Morseletto 2020).

The dairy industry is a major economic component of the global agricultural sector, and has experienced enormous growth in recent decades as the demand for milk products continues to increase worldwide (Sheng et al. 2020). However, the conversion of milk into an array of dairy products results in the generation of large volumes of dairy

Responsible Editor: Elena Maestri

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processing wastewater, primarily through cleaning, sanitisation, heating and cooling activities (Chokshi et al. 2016). Typically, a dairy processing facility can process between 200 and 550 million litres of milk a year (Baskaran et al. 2003; Gösta 2015), and it is estimated that between 0.2 and 10 L of wastewater are produced for every litre of milk processed (Baskaran et al. 2003; Gösta 2015; Wang and Serventi 2019). This variation in wastewater production reflects the manufacturing of a wide range of different dairy products, as well as the operational parameters of individual processing plants. Dairy processing wastewater generally contains a broad range of organic and inorganic components, and is customarily rich in nitrogen and phosphorus compounds (Demirel and Yenigun 2004; Carvalho et al. 2013; Tikariha and Sahu 2014).

Although dairy processing plants employ a number of physicochemical and biological treatments to remediate wastewater (Wang and Serventi 2019), phytoremediation technologies have begun to emerge as alternatives for low-cost, eco-friendly and sustainable purification of tertiary, secondary and even primary dairy effluents (Lutterbeck et al. 2017; Akansha et al. 2020). Lemnaceae are a group of floating aquatic plants that are considered particularly suitable for wastewater remediation due to their rapid growth (Ziegler et al. 2015), high nutrient uptake rate (Zhao et al. 2014), relative ease of harvesting (Landolt and Kandeler 1987) and tolerance of a wide range of growing conditions, including high ammonia levels (Landolt and Kandeler 1987; Caicedo et al. 2000). Lemnaceae biomass can be used as a biofuel, fertiliser or feed (Ahmad et al. 1990; Cheng and Stomp 2009). In particular, there is significant interest in using duckweed as an animal feed (Anderson et al. 2011; Stadtlander et al. 2019), and even for human consumption (Appenroth et al. 2017), due to its high protein content and favourable amino acid profile (Cheng and Stomp 2009; Anderson et al. 2011). Accordingly, the integration of Lemnaceae phytoremediation technology into wastewater purification regimes could enhance the sustainability of dairy production plants, whilst adding value to the production chain (Adhikari et al. 2015).

Duckweed-driven phytoremediation has been employed to remove a variety of pollutants including excess macronutrients, such as nitrogen and phosphorous-containing compounds like ammonia, nitrate and phosphate (Körner et al. 2003; Cheng and Stomp 2009). The removal of these macronutrients is linked to the growth rate of the duckweed (Cheng et al. 2002), and a faster growth rate is considered to translate into greater nutrient uptake. Accordingly, to increase the phytoremediation capacity of duckweed-based systems, operational parameters should be designed to maximise duckweed growth, in relation to the nutrient composition of wastewaters (Caicedo et al. 2000), light intensity (Paolacci et al. 2018), temperature (Wedge and Burris 1982), plant density (Driever et al. 2005; Kufel et al. 2018) and photoperiod (Yin et al. 2015). Interactive effects among some of these parameters have previously been identified (Ögren et al. 1984). The interactive effects of light and medium composition are, however, less well understood. In particular, given that nutrient deficiencies and/or surpluses can have negative effects on plant growth and health (Morales et al. 2008; Nagajyoti et al. 2010; Paolacci et al. 2016; Walsh et al. 2020), an improved understanding of potential interactive effects between wastewater composition, light and plant growth is required.

As a primary source of energy, light is a key determinant of plant growth (Paolacci et al. 2018). Duckweed growth typically increases with increasing light intensity up to a saturation point, beyond which growth is no longer accelerated by light and may even decrease due to photo-inhibitory damage (Wedge and Burris 1982; Landolt and Kandeler 1987). Light intensity curves have been measured for various species of Lemnaceae (Wedge and Burris 1982; Landolt and Kandeler 1987; Paolacci et al. 2018). For example, light has been noted as a limiting factor for *L. minor* growth up to an intensity of 400 μmol m^{-2} s^{-1} (Paolacci et al. 2018). Thus, it appears that moderately high light intensities of at least 400 μmol m^{-2} s^{-1} would be required for optimisation of duckweed-based phytoremediation systems, although this will depend on possible interactive effects between medium composition and light intensity. In addition, uncertainties surrounding optimal light intensity can also impact the financial viability of large-scale indoor phytoremediation systems due to mismatch in the provision of costly lamps and their associated energy demand (including cooling systems) (Gupta and Jatothu 2013; Poulet et al. 2014). Accordingly, to advance indoor, high-output duckweed remediation, there is a need to examine specific context dependencies between media types and light intensity.

Aside from possible interactive effects between wastewater composition and light intensity, an important consideration in dairy wastewater phytoremediation is increasing the scale beyond small and highly controlled laboratory conditions. For example, a large-scale phytoremediation system may experience water currents and algal or microbial growth, unlike stationary axenic systems. In particular, excessive algal and microbial growth can compete with duckweed for the acquisition of nutrients in non-sterile systems (Körner and Vermaat 1998; Roijackers et al. 2004), and this can reduce the health, nutritional value and phytoremediation capacity of cultivated duckweed. However, the introduction of a moderate current to a phytoremediation system can decrease nutrient depletion zones and increase nutrient availability at the plant surface, as has been noted for other species (Parker 1981). The combination of these distinct influences makes upscaling an important component of the development of remediation approaches.

Overall, whilst effective remediation of dairy processing wastewater with *L. minor* has been demonstrated (e.g. Walsh et al. (2020)), possible interactive effects concerning

wastewater composition and other growth parameters on duckweed health and phytoremediation capacity remain unknown. Therefore, in the present study, we assessed the interactive effects between light intensity and growing medium on key *L. minor* phytoremediation parameters, using either an optimal laboratory growing medium (i.e. half-strength Hutner's medium) or a standardised synthetic dairy wastewater, in both an axenic stationary and a non-sterile re-circulating system. We hypothesise that duckweed phytoremediation capacity will increase with greater light intensity until a plateau is reached. Results will inform the design and operational parameters of indoor duckweed-based phytoremediation systems for dairy processing wastewaters.

Materials and methods

Stock cultivation

The duckweed strain used in this study was *Lemna minor* L.—Blarney strain, number 5500 in the Rutgers Duckweed Stock Cooperative database (Lahive et al. 2012; Van Hoeck et al. 2015). A sterile stock of *L. minor* was cultivated on half-strength Hutner's medium (Hutner 1953) under an average light intensity of 50 μmol m^{-2} s^{-1} PAR (photosynthetically active radiation) within a controlled growth room (22 °C, 14:10 h light:dark photoperiod). Prior to experiments, *L. minor* plants were acclimated for 7 days to experimental light conditions whilst grown on either synthetic dairy wastewater or half-strength Hutner's medium.

Experimental design

Synthetic dairy wastewater

The composition of synthetic wastewater mimics real dairy processing wastewater (Table S1, Online Resource 1; Tarpey 2016). A breakdown of the elemental and compound composition of this synthetic wastewater is detailed in Walsh et al. (2020). Synthetic dairy wastewater is naturally around pH 8.0 but was reduced to 4.5–5.0 using 1 M H_2SO_4, to encourage optimal duckweed growth. Furthermore, additional calcium was added to maintain a favourable Ca:Mg ratio at 1:1.6 (mM) as detailed in Walsh et al. (2020).

Stationary remediation system

As an initial assessment, plants were grown for 5 days (days 0–5) on 100 mL of synthetic wastewater under ten different light intensities (10–850 μmol m^{-2} s^{-1}; Table S2, Online Resource 1) in Magenta vessels (GA-7, 7.7-cm length × 7.7-cm width × 9.7-cm height, surface area 42.25 cm^2 with 100 mL of liquid). Following this, in a second experiment, a reduced range of three light intensities were selected (50, 200 and 850 μmol m^{-2} s^{-1}) for a comparative assessment of duckweed grown on either 100 mL of synthetic dairy wastewater or half-strength Hutner's medium in Magenta vessels for 5 days (days 0–5). The length of the experiment was determined by the need to achieve measurable increases in plant biomass and decreases in media nutrients, without achieving overcrowding and nutrient depletion. Eight replicates were conducted in total with the number of replicates for each measured parameter detailed in figure legends (Figs. 1, 2, 3, 4 and 5). Both experiments were conducted under controlled laboratory conditions (18 °C, 16:8 h light:dark photoperiod). Different light intensities were generated by placing plants at different distances from LED-based lamps (AP67 R-series, Valoya, Finland). Starting biomass was eight colonies, averaging 25–30 fronds, per replicate. Colonies were taken at random from plants acclimated for 7 days to experimental light and media conditions. Throughout the experiment, there was some water loss due to evaporation, but deionised water was added to maintain media volume at 100 mL.

Re-circulating remediation system

Lemna minor was grown on synthetic wastewater (Table S1, Online Resource 1) in a re-circulating tank system at three different light conditions (100, 300 and 900 μmol m^{-2} s^{-1} PAR, 18–21 °C, 16:8 h light:dark photoperiod). In this re-circulating system, wastewater was pumped from a lower sump tank (at an average rate of 125 L per hour) to an upper duckweed tank and then it drained back down to the sump tank. The total re-circulating synthetic wastewater volume contained in both tanks was 11.7 L. Experiments were conducted over 3 days ($n = 8$). The length of the experiment was determined by the need to achieve measurable increases in plant biomass and decreases in media nutrients, without achieving overcrowding and nutrient depletion. Initially, tanks were seeded with duckweed to achieve a plant density of 60% surface cover (i.e. 360-cm^2 out of 600-cm^2 surface area was plant covered). To determine how much excess material was to be removed to maintain 60% coverage, surface coverage was monitored using the image analysis software EasyLeafArea (Easlon and Bloom 2014).

Measured parameters

Growth in stationary remediation system

At the start of the experiment (day 0), starting biomass was determined by measuring the biomass of 'representative' colonies. At the end of the experiment (day 5), fresh biomass was again measured. Fresh plant biomass was measured after removing excess water with tissue. The relative growth rate

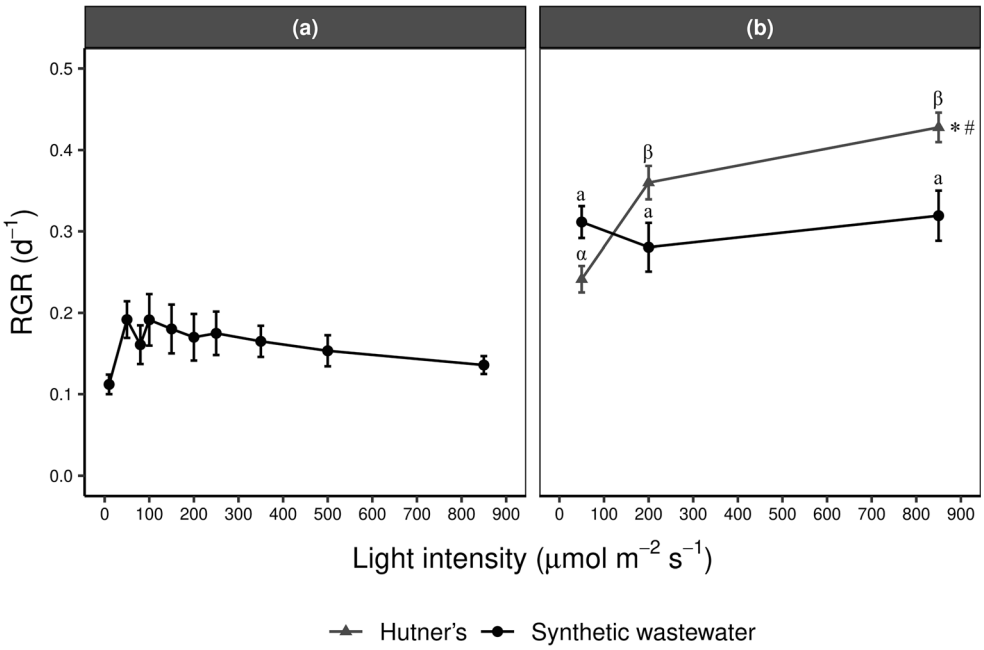

Fig. 1 Mean (± SE) fresh biomass RGR (day^{-1}) of (a) *Lemna minor* grown on synthetic wastewater under ten light intensities ($n = 8$), and (b) *L. minor* grown under three different light intensities on synthetic wastewater or half-strength Hutner's medium ($n = 6$). An asterisk (*) denotes an effect of media for $P < 0.05$, whilst a hash symbol (#) denotes an effect of light intensity for $P < 0.01$, as per the two-way ANOVA (see Table 1). Points that do not share the same letter significantly differ from one another, as per the Tukey post hoc test, $P < 0.05$ (see Table S6, Online Resource 1)

(RGR) was calculated based on measurements of fresh biomass using the formula (Connolly and Wayne 1996):

$$\mathrm{RGR} = \frac{ln\frac{W_2}{W_1}}{\Delta T} \quad (1)$$

where ln is the natural log, W_1 is the starting biomass, W_2 is the final biomass and ΔT is the length of the experiment.

Growth in re-circulating remediation system

Lemna minor cover was maintained at 60% (or 360 cm^2) of the surface area of the tank. A proxy of RGR was calculated as per above. The biomass of excess, harvested plants was used to estimate the final fresh biomass (W_2). The initial fresh biomass (W_1) was calculated from the initial surface cover, by using the biomass per square centimetre, which was determined for each replicate tank.

Chlorophyll *a* fluorometry

Chlorophyll *a* fluorescence was measured using a pulse amplitude modulated fluorometer (WALZ Imaging fluorometer, Effeltrich, Germany). Chlorophyll fluorescence measurements were taken of plants on the initial (day 0) and final days (day 5) of the stationary experiment, in which plants were grown on either half-strength Hutner's or synthetic wastewater under three different light intensities (50, 200 and 850 µmol m^{-2} s^{-1}). Day 0 measurements were not included in the final analysis but showed the baseline fluorescence characteristics in each medium and under each light intensity. Chlorophyll fluorescence measurements were not taken in the re-circulating system as the purpose of these experiments was limited to the study of upscaling. Immediately before a measurement, plants were dark-adapted for 15 min. For each replicate, three random colonies were selected for measurements. These three measurements were averaged together and treated as a single replicate. The chlorophyll fluorescence analysis procedure was as follows: first, a low-intensity modulated measuring light was turned on to measure F_0 on the dark-adapted plant, and secondly a saturating pulse of light (2700 µmol m^{-2} s^{-1}) was applied to obtain the maximum fluorescence F_m. Subsequently, actinic light (photosynthetically active light of 186 µmol m^{-2} s^{-1}) was applied to the plants and at 20-s intervals saturating pulses were applied to measure F_m', the maximum fluorescence under light-adapted conditions. F_t is the value of fluorescence immediately before the first saturating pulse is applied, i.e. the steady-state value of fluorescence. F_v/F_m, the maximum quantum efficiency of photosystem II (PSII), Y(II), the quantum efficiency of PSII under steady-state light conditions, and NPQ, the non-photochemical quenching, were calculated according to Maxwell and Johnson (2000) using Eqs. (2), (3) and (4).

$$F_v/F_m = (F_m - F_0)/F_m \quad (2)$$

$$\mathrm{Y(II)} = \left(F_m' - F_t\right)/F_m' \quad (3)$$

$$\mathrm{NPQ} = \left(F_m - F_m'\right)/F_m' \quad (4)$$

Two further quenching parameters were calculated using Eqs. (5) and (6): the yield of non-regulated energy dissipation, Y(NO), and the yield of regulated energy dissipation, Y(NPQ) (Kramer et al. 2004).

$$Y(NO) = \frac{1}{\left(NPQ + 1 + qL\left(\frac{F_m}{F_0 - 1}\right)\right)} \quad (5)$$

$$Y(NPQ) = 1 - Y(II) - Y(NO) \quad (6)$$

Analysis of total nitrogen and total phosphorous in the stationary remediation system

Samples of synthetic wastewater and half-strength Hutner's medium were taken on the initial and final days of the 5-day experiments in order to quantify total nitrogen (TN) and total phosphorous (TP) using a Hach machine (DR3900). For TN, Hach test LCK138 was used. Firstly, the sample was digested with peroxo-disulphate for 1 h at 100 °C causing inorganically and organically bonded nitrogen to oxidise to nitrate (Koroleff digestion). The resulting oxidised nitrate was then analysed photometrically in a reaction with 2,6-dimethylphenol. For TP, Hach test LCK348 was used. Firstly, the wastewater sample was digested using the persulphate digestion method for 1 h at 100 °C. The resulting solution was then analysed photometrically through the ascorbic acid/phosphomolybdenum blue method.

Analysis of total nitrogen and total phosphorous analysis in the re-circulating remediation system

Samples of wastewater were taken for TN and TP analysis on the initial and final days of the 3-day experiment. These samples were sent to an external lab for analysis (Aquatic Services Unit, Environmental Research Institute, University College Cork, Ireland). For TN, the unfiltered sample was digested with potassium persulfate and boric acid in alkaline conditions in an autoclave at 120 °C for 30 min. The resulting total oxidised nitrogen was analysed by automated cadmium reduction method using Lachat Quikchem 8000 by Zellweger Analytics, Inc. Milwaukee, USA (Grasshoff et al. 2009). For TP, the unfiltered sample was digested with ammonium persulfate in acidic conditions in an autoclave at 120 °C for 30 min. The resulting phosphate was analysed manually using the Murphy and Riley Method (Rice et al. 2005).

Protein analysis

Lemna minor biomass samples were taken on the final day of each experiment and stored in a − 20 °C freezer. Protein was extracted from stored samples using 50 mM potassium phosphate buffer, pH 7, containing 0.1 mM EDTA and 0.1 mM polyvinylpyrrolidone (PVP). For protein measurements, between 50 and 80 mg of plant sample was weighed out, then homogenised in cold potassium phosphate buffer (1 mL of buffer to 80 mg of plant sample). The homogenised sample was centrifuged at 16000 rpm for 30 min at 4 °C (Balen et al. 2011). The supernatant was used for soluble protein analysis using the Bradford method with bovine serum albumin as a standard (Bradford 1976). Following this, 5 μL of sample was added to 1 mL of Bradford reagent in a cuvette and left for 5 min in dark conditions, then this sample was measured at 595 nm using a spectrophotometer (Shimadzu UV-160A).

Data analysis

Statistical analyses were conducted using R software (R Core Team (2019), R 3.4.3). Numbers of independent replicates are stated in figure legends. One-way and two-way ANOVAs were used to analyse differences in RGR, TN and TP removal rates, and protein content between treatments. A post hoc Tukey test was used for pairwise comparisons of treatment groups. For heteroscedastic datasets, a Welch's ANOVA was used. A significant result indicates a P value that is less than 0.05 ($P < 0.05$).

Results

Stationary remediation system

RGR for *L. minor* grown on synthetic wastewater or half-strength Hutner's medium

When grown on synthetic wastewater under a range of ten light intensities, *L. minor* kept under 50 μmol m^{-2} s^{-1} showed a higher RGR (day^{-1}) compared to plants kept under 10 μmol m^{-2} s^{-1} PAR (Fig. 1(a)). However, above 50 μmol m^{-2} s^{-1}, the RGR plateaued and gradually decreased as light intensity increased (Welch's ANOVA: $P = 0.1$; Fig. 1(a)).

Table 1 Summary of two-way ANOVA test for the effects of light intensity and media on *Lemna minor* RGR (day^{-1}), TN and TP removal rate (mg N/P m^{-2} day^{-1}) and protein content (%)

Measurement	Term	df	*F*-statistic	*P* value
RGR	Light intensity	2	8.759	0.001
	Media	1	4.297	0.047
	Light intensity × media	2	8.502	0.001
TN	Light intensity	2	1.945	0.160
	Media	1	5.447	0.027
	Light intensity × media	2	0.405	0.671
TP	Light intensity	2	0.113	0.894
	Media	1	7.665	0.010
	Light intensity × media	2	0.153	0.858
Protein	Light intensity	2	17.260	0.00001
	Media	1	0.000	0.997
	Light intensity × media	2	2.066	0.144

RGR for *L. minor* grown on either of two media, synthetic wastewater or half-strength Hutner's, displayed different trends in response to increasing light intensity (Fig. 1(b)). Both light (two-way ANOVA: $P = 0.001$; Table 1) and media (two-way ANOVA: $P = 0.047$; Table 1) affected *L. minor* RGR. When grown on half-strength Hutner's, *L. minor* RGR increased with increasing light intensity, rising from 0.24 day^{-1} at 50 μmol m^{-2} s^{-1} to 0.43 day^{-1} at 850 μmol m^{-2} s^{-1} (post hoc Tukey: $P < 0.001$; Fig. 1(b); Table S6, Online Resource 1). For *L. minor* grown on synthetic wastewater, the mean RGR values remained close to 0.3 day^{-1} at all light intensities (post hoc Tukey: $P > 0.05$; Fig. 1(b); Table S6, Online Resource 1). An interactive effect was found between light intensity and media (two-way ANOVA: $P = 0.001$; Table 1), which indicates that light and media had a combined effect on *L. minor* RGR.

Chlorophyll *a* fluorescence of *L. minor* grown on synthetic wastewater or half-strength Hutner's medium

Chlorophyll fluorescence measurements were taken for *L. minor* grown on either synthetic wastewater or half-strength Hutner's medium under three different light intensities on the final day of the experiment. The maximum quantum efficiency of photosystem II (F_v/F_m) decreased for *L. minor* on both media as the light intensity increased (two-way ANOVA: $P < 0.001$; Fig. 2(a); Table 2). F_v/F_m for *L. minor* grown on synthetic wastewater decreased from 0.78 at 50 μmol m^{-2} s^{-1} to 0.67 at 850 μmol m^{-2} s^{-1} (post hoc Tukey: $P = 0.003$; Fig. 2(a); Table S7, Online Resource 1). The post hoc tests did not reveal any significant decrease for plants on half-strength Hutner's.

At 50 μmol m^{-2} s^{-1}, the mean Y(II), the quantum efficiency of PSII under steady-state light conditions, was almost equal irrespective of the medium. As light intensity increased, however, differences in Y(II) between *L. minor* grown on synthetic wastewater or half-strength Hutner's were observed (two-way ANOVA: $P = 0.01$; Fig. 2(b); Table 2). As light intensity increased to 200 μmol m^{-2} s^{-1}, the Y(II) of *L. minor* grown on half-strength Hutner's increased more than the Y(II) of *L. minor* grown on synthetic wastewater. At 850 μmol m^{-2} s^{-1}, Y(II) decreased irrespective of medium, but this decrease was much stronger for *L. minor* grown on synthetic wastewater. In addition, an interactive effect on Y(II) between light intensity and media was observed (two-way ANOVA: $P = 0.052$; Table 2).

Y(NPQ), the yield of regulated energy dissipation, for *L. minor* grown under 50 μmol m^{-2} s^{-1} was similar irrespective of medium. At higher light intensities, Y(NPQ) values diverged depending on the medium (two-way ANOVA: $P = 0.015$; Fig. 2(c); Table 2). As light intensity increased to 200 and 850 μmol m^{-2} s^{-1}, *L. minor* grown on half-strength Hutner's showed a lower Y(NPQ) value than that of *L. minor* grown on synthetic wastewater (Fig. 2(c)). The Y(NO), the yield of non-regulated energy dissipation, of *L. minor* was mostly steady across all light intensities and media conditions (two-way ANOVA: $P > 0.05$; Fig. 2(d); Table 2).

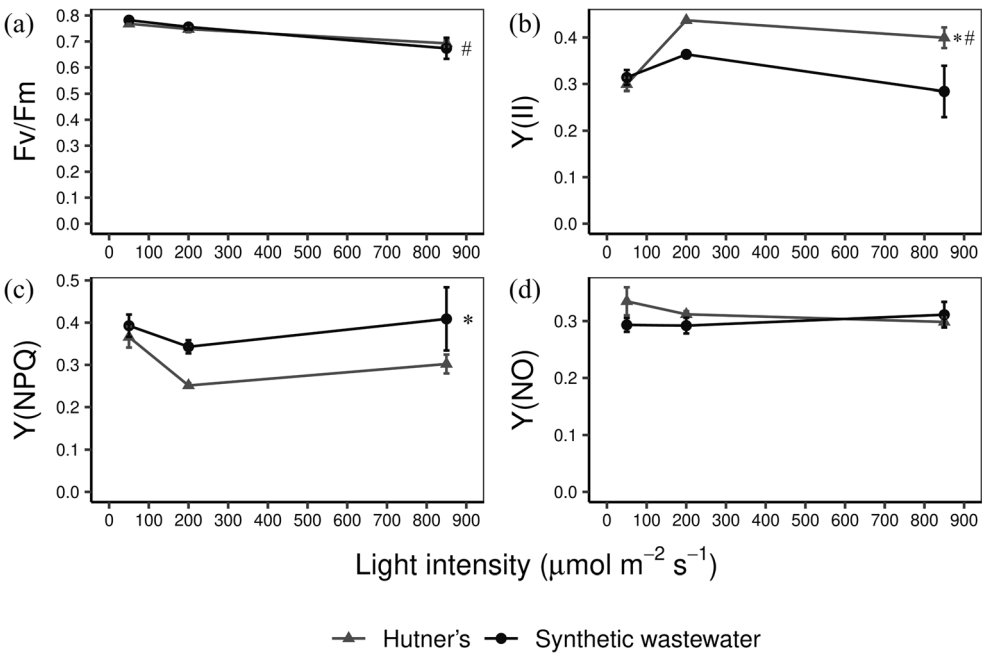

Fig. 2 Mean (± SE) values of (a) F_v/F_m, (b) Y(II), (c) Y(NPQ) and (d) Y(NO) for *Lemna minor* grown under three different light intensities on synthetic wastewater or half-strength Hutner's medium ($n = 6$). An asterisk (*) denotes an effect of media for $P < 0.05$, whilst a hash symbol (#) denotes an effect of light intensity for $P < 0.01$, as per the two-way ANOVA (see Table 2)

Table 2 Summary of two-way ANOVA tests for the effects of light intensity and media on *Lemna minor* F_v/F_m, Y(II), Y(NPQ) and Y(NO)

Measurement	Term	df	*F*-statistic	*P* value
F_v/F_m	Light intensity	2	13.241	0.00007
	Media	1	0.004	0.952
	Light intensity × media	2	0.454	0.639
Y(II)	Light intensity	2	6.647	0.004
	Media	1	7.475	0.010
	Light intensity × media	2	3.270	0.052
Y(NPQ)	Light intensity	2	2.800	0.077
	Media	1	6.596	0.015
	Light intensity × media	2	0.704	0.503
Y(NO)	Light intensity	2	0.306	0.739
	Media	1	1.528	0.226
	Light intensity × media	2	1.439	0.253

Total nitrogen removal for *L. minor* grown on synthetic wastewater or half-strength Hutner's medium

Removal of TN from synthetic wastewater by *L. minor* was measured under ten different light intensities (Table S3, Online Resource 1), and then used to calculate the mean daily TN removal rates for the duration of the experiment, i.e. milligrams of N removed per initial m^2 of *L. minor* per day (mg N m^{-2} day^{-1}: days 0–5). No particular pattern or trend of TN removal was found as a function of light intensity (ANOVA: $P = 0.688$; Fig. 3(a)). Nevertheless, mean TN removal rates were variable but standard errors were substantial.

Removal of TN from synthetic wastewater or half-strength Hutner's medium by *L. minor* was measured under three different light intensities (Table S4, Online Resource 1), and then used to calculate TN removal rates (mg N m^{-2} day^{-1}). Overall, *L. minor* grown on synthetic wastewater had higher TN removal rates than *L. minor* grown on half-strength Hutner's (two-way ANOVA: $P = 0.027$; Fig. 3(b); Table 1). For *L. minor* grown on half-strength Hutner's, the mean TN removal rate increased with increasing light intensity, whereas for *L. minor* grown on synthetic wastewater, the mean TN removal rate increased only at the highest light intensity, 850 μmol m^{-2} s^{-1}, but at all light intensities substantial standard errors were observed (Fig. 3(b)). A post hoc Tukey test of TN removal rate did not show significant difference between the light intensity treatments (Table S6, Online Resource 1). Further analysis showed that plants grown on half-strength Hutner's displayed a strong linear correlation between growth rate (RGR) and TN removal rate (Fig. 3(c)), whereas plants grown on synthetic wastewater had similar growth rates with no particular association with TN removal rate.

Total phosphorous removal for *L. minor* grown on synthetic wastewater or half-strength Hutner's medium

Removal of TP from synthetic wastewater by *L. minor* was measured under ten different light intensities (Table S3,

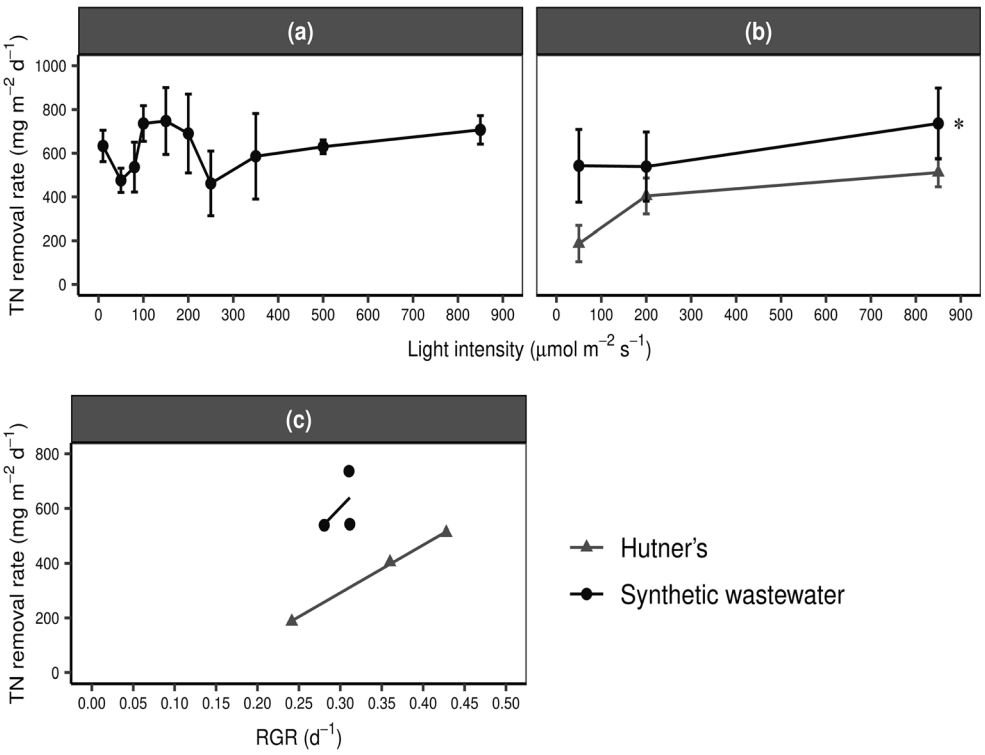

Fig. 3 Mean (± SE) values for (a) TN removal rate (mg N m^{-2} day^{-1}) from synthetic wastewater under ten light intensities ($n = 3$), (b) TN removal rate (mg N m^{-2} day^{-1}) under three different light intensities from synthetic wastewater or half-strength Hutner's medium ($n = 6$) and (c) mean TN removal rate (mg N m^{-2} day^{-1}) against RGR for synthetic wastewater and half-strength Hutner's medium, with linear fit lines ($n = 3$). An asterisk (*) denotes an effect of media for $P < 0.05$, as per the two-way ANOVA (see Table 1)

Online Resource 1), and then used to calculate the mean daily TP removal rates for the duration of the experiment, i.e. milligrams of P removed per initial square meters of *L. minor* per day (mg P m^{-2} day^{-1}: days 0–5). As observed for TN, no light dependency of TP removal was discerned (ANOVA: $P = 0.75$; Fig. 4(a)).

Removal of TP from synthetic wastewater or half-strength Hutner's medium by *L. minor* was measured under three different light intensities (Table S4, Online Resource 1), and then used to calculate TP removal rates (mg P m^{-2} day^{-1}). The TP removal rate differed between *L. minor* grown on synthetic wastewater and *L. minor* grown on half-strength Hutner's (two-way ANOVA: $P = 0.01$; Fig. 4(b); Table 1). However, light intensity did not exert a strong effect on TP removal rate (two-way ANOVA: $P = 0.894$; Fig. 4(b); Table 1). The mean TP removal rate for plants grown on half-strength Hutner's increased moderately from 50 to 200 μmol m^{-2} s^{-1} but then reduced at 850 μmol m^{-2} s^{-1} (Fig. 4(b)). The mean TP removal for plants grown on synthetic wastewater medium increased moderately with increasing light intensity (Fig. 4(b)). A post hoc Tukey test did not identify significant differences between light intensity treatments (Table S6, Online Resource 1).

Protein content of *L. minor* grown on synthetic wastewater or half-strength Hutner's medium

When grown on synthetic wastewater under a range of ten light intensities, *L. minor* protein content (% protein of fresh biomass) increased with increasing light intensity (ANOVA: $P < 0.001$; Fig. 5(a)). A post hoc Tukey test showed significant differences between plant protein content at 10 and 50 μmol m^{-2} s^{-1} and that at 350, 500 and 850 μmol m^{-2} s^{-1} ($P < 0.01$ and $P < 0.05$, respectively; Table S8, Online Resource 1).

Lemna minor protein content (% protein of fresh biomass) for plants grown on either half-strength Hutner's medium or synthetic wastewater increased with increasing light intensity (two-way ANOVA: $P < 0.001$; Fig. 5(b); Table 1). For *L. minor* grown on half-strength Hutner's, significant differences were found between protein content at 50 and 200 μmol m^{-2} s^{-1}, and that at 850 μmol m^{-2} s^{-1} (post hoc Tukey, 50–850: $P < 0.001$, 200–850: $P = 0.003$; Table S6, Online Resource 1). For *L. minor* grown on synthetic wastewater, a borderline significant P value of $P = 0.055$ was found (as per post hoc Tukey, Table S6, Online Resource 1), comparing 50 and 850 μmol m^{-2} s^{-1}.

Re-circulating remediation system

The mean RGR of *L. minor* grown in re-circulating tanks under three different light intensities increased with increasing light intensity, from 0.21 to 0.27 day^{-1} (ANOVA: $P = 0.475$; Fig. 6a). TN and TP removal from synthetic wastewater in re-circulating tanks was measured over the course of the experiment (Table S5, Online Resource 1). TN and TP removal rates (mg N/P m^{-2} day^{-1}) were calculated from the change in concentration between start and end date (days 0–3). The mean TN removal rate increased with light intensity but exhibited substantial standard errors at 300 and 900 μmol m^{-2} s^{-1} (ANOVA: $P = 0.479$; Fig. 6b). The TP removal rate was similar at 100 and 300 μmol m^{-2} s^{-1} but increased at 900 μmol m^{-2} s^{-1} (ANOVA: $P = 0.008$; Fig. 6c). Significant differences were

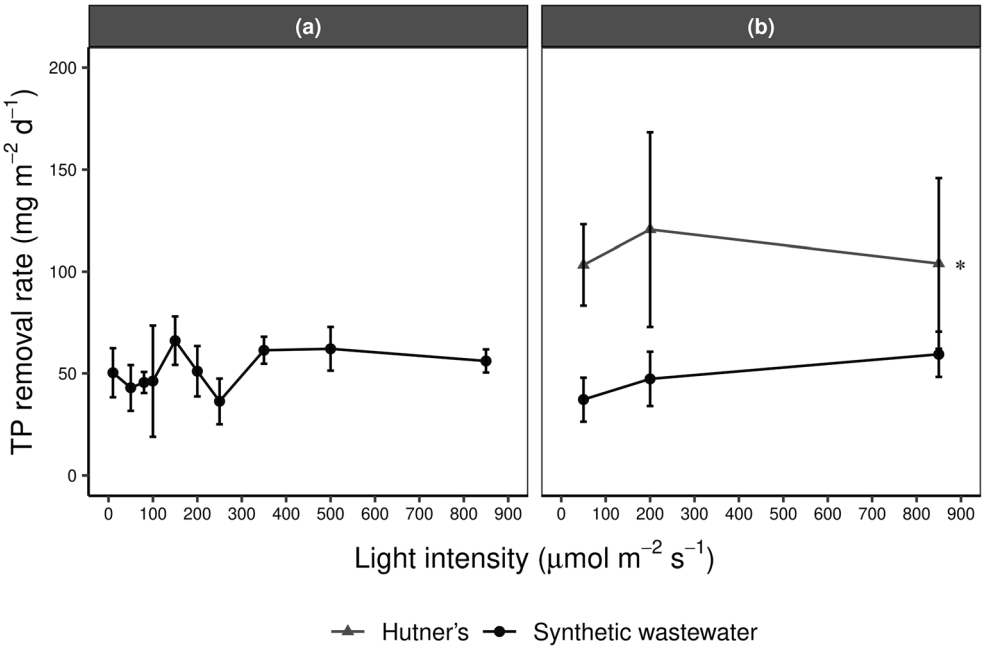

Fig. 4 Mean (± SE) values for (a) TP removal rate (mg P m^{-2} day^{-1}) from synthetic wastewater under ten light intensities ($n = 4$, except at 100 and 150 μmol m^{-2} s^{-1} where $n = 3$), and (b) TP removal rate (mg P m^{-2} day^{-1}) under three different light intensities from synthetic wastewater or half-strength Hutner's medium ($n = 6$). An asterisk (*) denotes an effect of media for $P < 0.05$, as per the two-way ANOVA (see Table 1)

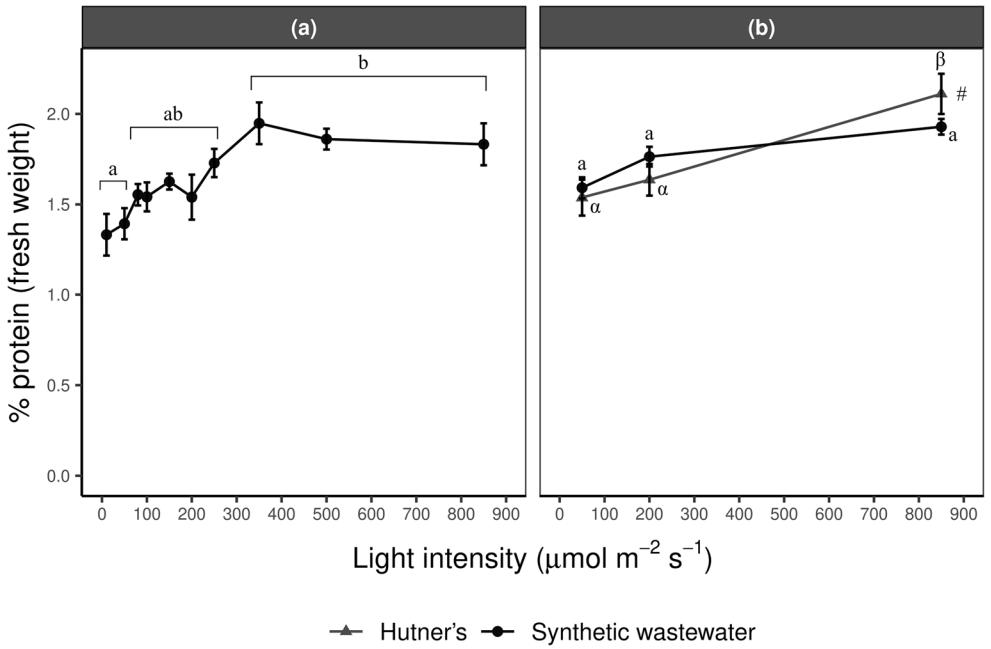

Fig. 5 Mean (± SE) values for (a) *L. minor* protein content (% protein of fresh *Lemna minor* biomass) under ten light intensities ($n = 6$), and (b) *L. minor* protein content (% protein of fresh *L. minor* biomass) under three different light intensities on synthetic wastewater or half-strength Hutner's medium ($n = 6$). Based on a typical dry biomass content of 4%, the protein content on a dry weight basis is on average 33–50% and 38–53% for plants grown on half-strength Hutner's or synthetic wastewater, respectively. A hash symbol (#) denotes an effect of light intensity for $P < 0.001$, as per the two-way ANOVA (see Table 1). Points that do not share the same letter, significantly differ from one another for $P < 0.05$, as per the Tukey post hoc test (see Tables S6 and S8, Online Resource 1)

found between the TP removal rate at 100 and 300 µmol m^{-2} s^{-1} compared to that at 900 µmol m^{-2} s^{-1} (post hoc Tukey: $P = 0.01$, $P = 0.03$, respectively; Fig. 6c). *L. minor* protein concentration (% protein of fresh duckweed biomass) increased moderately with increasing light intensity (ANOVA: $P = 0.294$; Fig. 6d).

Discussion

In general, growth rates documented in the present study for *Lemna minor* 'Blarney', cultivated on synthetic dairy processing wastewater, are slightly lower or similar to those found in the literature for duckweed grown on optimised media (Ziegler et al. 2015; Paolacci et al. 2016, 2018), but similar or greater than those for duckweed grown on wastewater (Caicedo et al. 2000; Iatrou et al. 2015). *L. minor* grown on half-strength Hutner's medium displayed comparable growth rates to those found in the literature (Ziegler et al. 2015). However, there was a major difference in the way *L. minor* responded to light when grown on either synthetic wastewater or half-strength Hutner's. Typically, duckweed growth rates increase until light is saturating, at which point growth rates plateau. The growth saturation point for Lemnaceae has been found to range between 250 and 750 µmol m^{-2} s^{-1}, depending on species and clone (Landolt and Kandeler 1987). Specifically, the saturation point for *L. minor* has been identified as between 400 and 600 µmol m^{-2} s^{-1} (Wedge and Burris 1982; Paolacci et al. 2018). In this context, the light response curve for *L. minor* grown on synthetic wastewater is unusual in that it already shows growth saturation at 50 µmol m^{-2} s^{-1}. The same pattern was found in the scaled-up 11.7-L re-circulating tank system in which *L. minor* reached growth saturation at around 100 µmol m^{-2} s^{-1}.

To explore why the growth of *L. minor* on synthetic wastewater did not increase with increasing light intensities, photosynthetic efficiency was quantified. Measurements of *L. minor* Y(II), the quantum yield of PSII (Murchie and Lawson 2013) and Y(NPQ), the proportion of energy being quenched by non-photochemical processes such as heat dissipation (Kramer et al. 2004), diverged at light intensities above 50 µmol m^{-2} s^{-1} between the two media. *L. minor* grown on synthetic wastewater was less capable of using additional light energy, as shown by lower Y(II) values (Genty et al. 1989), and dissipated increasing amounts of radiation energy through the xanthophyll cycle (Horton et al. 1996), as shown by higher values for Y(NPQ) (Klughammer and Schreiber 2008). Such regulated energy dissipation does not necessarily mean photoinhibitory damage has occurred, and this is seen in the similar values for F_v/F_m, the maximum quantum yield of photosystem II (Murchie and Lawson 2013), and Y(NO), the proportion of light energy being dissipated in a non-regulated manner (Kramer et al. 2004), that were observed for *L. minor* grown in both media. An increase in Y(NO) would have been

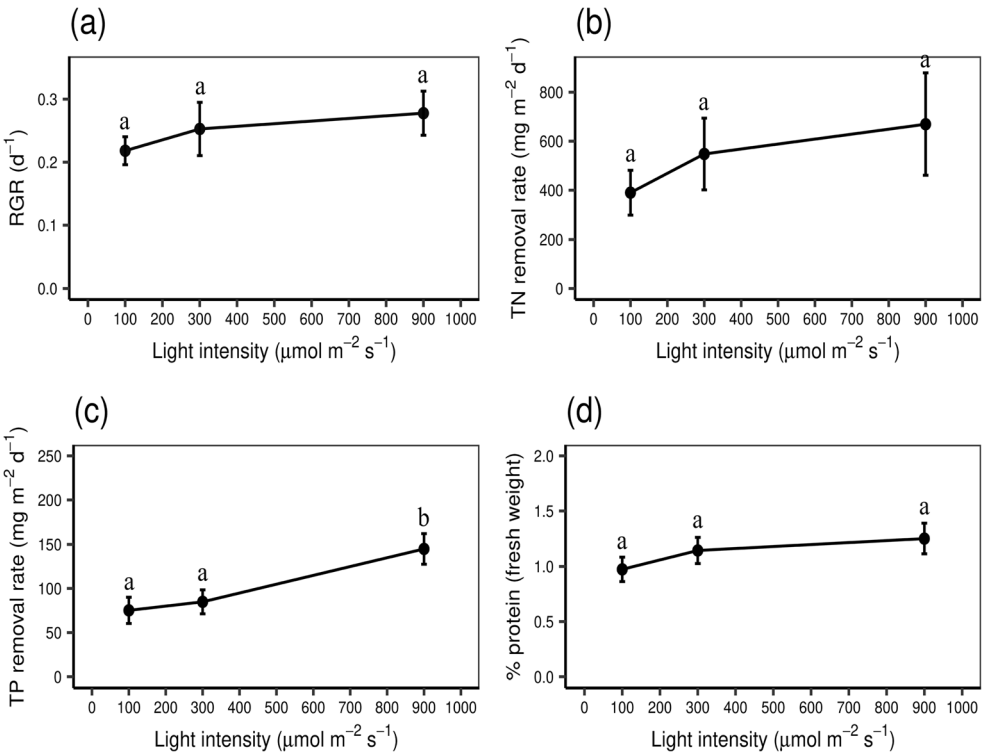

Fig. 6 Mean (± SE) for *Lemna minor* (**a**) RGR, (**b**) TN removal rate (mg N m^{-2} day^{-1}), (**c**) TP removal (mg P m^{-2} day^{-1}), and (**d**) protein content (% protein of fresh weight) grown on synthetic wastewater under three different light intensities in re-circulating tanks ($n = 8$). Based on a typical dry biomass content of 4%, the protein content on a dry weight basis is on average 24–30%. Points that do not share the same letter significantly differ from one another for $P < 0.05$, as per the Tukey post hoc test

indicative of a plant struggling to cope with excess radiation due to photochemical damage or damage to its light-protective mechanisms (Klughammer and Schreiber 2008). Similar F_v/F_m values indicate that PSII is not directly negatively affected in either growth medium. Rather, photosynthesis in *L. minor* growing in synthetic wastewater is likely to be disrupted at a point beyond PSII (Kanazawa and Kramer 2002; Vredenberg 2018).

A previous study, Walsh et al. (2020), which documented the concentration of elemental components in synthetic wastewater and half-strength Hutner's medium revealed some elements that, due to their concentration, may potentially impede ATP generation or the Calvin cycle. In particular, copper, which is present in higher amounts in synthetic wastewater than in half-strength Hutner's (5 and 0.12 µM, respectively), can inhibit phosphorylation by ATP synthase (Uribe and Stark 1982; Maksymiec 1998). A consequence of impeding ATP generation is the build-up of a proton gradient across the thylakoid lumen (Kramer et al. 2003). In turn, this gradient causes non-photochemical quenching, Y(NPQ), to be increased through the xanthophyll cycle (Horton et al. 1996; Li et al. 2002), as was observed in this study. Moreover, magnesium, which is present at much lower concentrations in synthetic wastewater than in half-strength Hutner's (0.2 and 3 mM, respectively), can adversely affect ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity if present in deficient amounts (Farhat et al. 2016), whilst manganese, which can negatively affect carbon assimilation when present in excess amounts (Li et al. 2010), is present in a higher concentration in synthetic wastewater than in half-strength Hutner's (37 and 0.591 µM, respectively). Therefore, we argue that through subtle disruptions in plant physiology, the cultivation of *L. minor* on synthetic dairy wastewater resulted in increased energy dissipation, inhibition of photosynthetic yield and a lack of growth acceleration at higher light intensities. It can be concluded that synthetic dairy wastewater can support the cultivation of *L. minor* under low light intensities but is less suitable under higher light intensities due to interactive effects between light and media composition.

The detected removal rates of TN from synthetic wastewater are in line with those documented for duckweed within the literature, which range from 500 to 2100 mg N m^{-2} day^{-1} (Körner and Vermaat 1998; Cheng et al. 2002; Mohedano et al. 2012). Removal rates vary depending on the Lemnaceae species or clone used (Zhao et al. 2014), the types of nitrogen source available (Fang et al. 2007), the type of wastewater being remediated (Toyama et al. 2018) and the type of system used (e.g. outdoor or indoor) (Cheng and Stomp 2009). In addition, luxury uptake of nitrogen has been reported for some plant species (Lipson et al. 1996), and this would distort the relationship between growth and nitrogen uptake. However, to our knowledge, no records of luxury nitrogen uptake by duckweed species have been reported. In the present study, nitrogen uptake was closely linked with *L. minor* RGR for each specific medium. Although half-strength Hutner's medium contains approximately 20% more available nitrogen than synthetic

dairy wastewater (see Table S1, Online Resource 1; Hutner 1953), greater nitrogen removal by *L. minor* occurred for plants grown on synthetic wastewater, despite these plants having lower growth rates. The relatively high TN removal rates from synthetic wastewater are likely due to the type of nitrogen available. Synthetic wastewater contains ammonia and urea as its nitrogen sources, whereas in Hutner's medium nitrate is the sole nitrogen source (Hutner 1953). It has previously been shown that ammonia is more readily taken up than nitrate by *L. minor* (Feller and Erismann 1971; Landolt and Kandeler 1987). Furthermore, *L. minor* shows a preference for ammonia over nitrate when both nutrients are available (Feller and Erismann 1971; Porath and Pollock 1982). Thus, the relationship between RGR and TN removal is further modified by the available form of nitrogen.

Lemna minor has been shown to take up more phosphorus than it requires when this is available in high concentrations; consequently, such luxury uptake can distort the relationship between RGR and TP uptake (Chaiprapat et al. 2005). Whilst the TP removal rates observed in this study are in the lower portion of the published range (i.e. 20–590 mg P m^{-2} day^{-1}) (Körner and Vermaat 1998; Cheng et al. 2002; Mohedano et al. 2012), the TP removal rate for *L. minor* grown on half-strength Hutner's was double the rate for *L. minor* grown on synthetic wastewater. As half-strength Hutner's medium contained substantially more phosphorus than synthetic dairy wastewater (93 mg L^{-1} and 10.9 mg L^{-1}, respectively; Table S1, Online Resource 1; Hutner 1953), luxury phosphorus uptake by plants grown in half-strength Hutner's may have occurred.

Typically, duckweed protein content can vary between 10 and 40% of dry duckweed biomass (Landolt and Kandeler 1987; Bergmann et al. 2000). The protein content found in this study is presented as milligrams of protein per milligram of fresh *L. minor* biomass, which was used to calculate protein per dry *L. minor* biomass for a comparison with literature sources (a direct measurement of dry weight would be worthwhile for future studies). Based on a *L. minor* dry biomass content of 4% (Landolt and Kandeler 1987; Appenroth et al. 2017), the average protein content for *L. minor* grown on half-strength Hutner's was 44% and on synthetic wastewater 42%. The protein content of *L. minor* increased with greater light intensity, for plants grown on both synthetic wastewater and half-strength Hutner's. Previous studies have documented that higher light intensities lead to greater allocation of total nitrogen from non-protein nitrogen-containing components to soluble protein in C3 plants such as duckweed (Evans 1989; Evans and Seemann 1989). It is thought that this process may be associated with the increased production of Rubisco, a soluble protein, at higher light intensities (Evans 1989). The increase in TN removal with increasing light intensity, as seen for *L. minor* grown on half-strength Hutner's, does not increase the proportion of soluble protein content in these plants any more than was observed for *L. minor* grown on synthetic wastewater. This greater TN uptake may instead have increased the overall nitrogen content of *L. minor* grown on half-strength Hutner's (Landolt and Kandeler 1987; Evans and Seemann 1989).

In general, *L. minor* grown on synthetic wastewater in re-circulating tanks had similar growth rates, TN and TP removal rates, and protein content, as *L. minor* grown on synthetic wastewater in stationary conditions. The protein content for *L. minor* grown on synthetic wastewater in re-circulating tanks was generally lower, but exhibited the same trend under different light intensity conditions as documented for plants grown in stationary conditions. The lower protein content detected for *L. minor* grown in re-circulating systems may reflect an unknown effect linked to upscaling and this should be considered for future research. The effects of light intensity on the measured parameters were mostly similar to the stationary system with the exception of TN and TP removal at the highest light intensities. The presence of algae and microbes in re-circulating tanks may have contributed to a modest increase in the mean TN removal at higher light intensities without an associated increase in *L. minor* RGR (Zhao et al. 2015). The presence of microbiota can lead to the loss of nitrogen from a wastewater treatment system through the bacterial nitrification-denitrification process (Thakur and Medhi 2019). Furthermore, the presence of algae and a microbial biofilm has also been reported to contribute to the removal of nitrogen and phosphorous in a duckweed-based system (Körner and Vermaat 1998). Algae in particular can compete with duckweed for nutrients (Roijackers et al. 2004), potentially decreasing duckweed-mediated uptake of nitrogen and phosphorus but increasing nutrient uptake overall. The relatively high density of duckweed in the system (60%) may have negated a strong effect of algal competitors (Roijackers et al. 2004). The volume of wastewater to *L. minor* biomass, which is greater in re-circulating tanks, around 1.6 L to 1 g compared to 0.5 L to 1 g in stationary experiments, may have been a factor in the response of TP removal rate to different light intensities. The greater availability of phosphate may saturate luxury uptake and restore the relationship between growth and phosphorous removal (Paolacci et al. 2016). As duckweeds naturally come from still or slow-moving waters (Landolt 1986), water movement and currents, such as those introduced by the re-circulating system, can have a negative impact on *L. minor* growth (Iqbal 1999). However, the high density of duckweed seemed to have a positive effect by stabilising

the duckweed and reducing the impact of moving water. Overall, no adverse effects on *L. minor* growth were noted in the re-circulating system. Despite the noted differences, it can be argued that simple stationary, sterile systems can meaningfully inform on phytoremediation potential prior to investment in larger and more complex re-circulating systems.

Conclusion

Lemna minor has been shown to grow on and remediate a synthetic wastewater which mirrors real dairy processing wastewater. Attempts to accelerate this process by using higher light intensities showed that, remarkably, *L. minor* growth rates and TN and TP removal rates did not differ significantly across a wide range of light intensities. However, when compared to *L. minor* grown on half-strength Hutner's medium, a media-dependent effect of light intensity was detected. These findings will inform the design and operational parameters of indoor duckweed-driven phytoremediation systems, as a lower light intensity could reduce costs and energy consumption. Yet, the use of higher light intensities can also result in higher *L. minor* protein content, which may represent a supplementary source of income, improving the financial viability of the phytoremediation process. As such, an evaluation between the cost of higher light intensity and the potential financial gain of additional protein content will need to be considered.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-020-11792-y.

Acknowledgments This project was funded under the EPA Research Programme 2014–2020 (grant 2016-W-LS-11). MAKJ also acknowledges support by WoB.

Authors' contributions ÉW, HK and MAKJ contributed to the study conception and design. Material preparation, data collection and analysis were performed by ÉW, HK and SOB. The first draft of the manuscript was written by ÉW and all authors contributed to reviewing and editing the manuscript.

Funding This project was funded under the EPA Research Programme 2014–2020 (grant 2016-W-LS-11). The EPA Research Programme is a Government of Ireland initiative funded by the Department of Communications, Climate Action and Environment. It is administered by the Environmental Protection Agency, which has the statutory function of coordinating and promoting environmental research.

Data availability The datasets used in the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Ethical approval Not applicable

Competing interests The authors declare that they have no conflict of interest.

Consent to participate Not applicable

Consent to publish Not applicable

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