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Acidification increases efficiency of *Lemna minor* N and P recovery from diluted cattle slurry

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ABSTRACT

Livestock slurry is high in N and P and as such is a valuable fertiliser, however, poorly timed or overapplication can result in the eutrophication of surface water. As a result, the agricultural industry is subject to increasingly stringent slurry management regulations, leading to interest in novel methods of recovering nutrients from wastewater. This study investigated the potential for using *Lemna minor* to recover N and P from slurry over a five-week trial, assessing whether previously reported increased growth on acidified wastewater translates into greater nutrient removal. It was hypothesised that acidifying diluted slurry with H_2SO_4 would lead to greater (i) reductions in total ammonia nitrogen (TAN) and PO_4^{3-} concentrations and (ii) cumulative N and P uptake by *L. minor*, relative to unamended diluted slurry (control). Consistent with previous studies, *L. minor* growth was significantly higher in the acidified treatment relative to the control, with greater reductions in PO_4^{3-} concentrations also observed in the acidified treatment. Contrary to the first hypothesis, the same was not observed for TAN, where final concentrations were lowest in the control despite poor growth on this medium. Consistent with the second hypothesis, greater cumulative *L. minor* N uptake over the five-week trial was observed from the acidified treatment, with *L. minor* uptake accounting for 94.8 % of the reduction in inorganic N concentration, relative to 7.5 % in the control. Cumulative P recovery from the acidified treatment was also significantly higher than in the control, with *L. minor* uptake accounting for 99.5 % of the reduction in the inorganic P concentration of the acidified diluted slurry, relative to only 18 % in the control. Our findings have important implications for the operation of duckweed growing systems in practice. We show that lowering pH enabled efficient PO_4^{3-} recovery, to the point where its concentrations may have been limiting to growth. Lowering pH also increased the proportion of N and P removal directly attributable to *L. minor* uptake, increasing the efficiency of the nutrient recovery process whilst also minimising the amount of N lost via other pathways such as gaseous emissions.

1. Introduction

The intensification of livestock farming in response to a growing demand for meat and dairy products has resulted in farms generating more wastewater (Sońta et al., 2020). The wastewater, commonly referred to as slurry, contains high concentrations of N and P, and as such, is seen as a resource in agriculture due to its fertiliser value (Johnson and Dawson, 2005). However, over-application or poorly-timed application of slurry onto land poses a severe pollution risk to surface and groundwater (Dungait et al., 2012; Johnson and Dawson, 2005; Lloyd et al., 2016). Whilst P loss from agriculture is low relative to N due to its precipitation and adsorption in soils (Dungait et al., 2012; Johnson and Dawson, 2005), P pollution is considered to be the major cause of eutrophic conditions as it is often the limiting nutrient in aquatic habitats (Diaz et al., 1994; Johnson and Dawson, 2005). Intensive livestock farming has been identified as a major source of P introduction to streams (Jarvie et al., 2010), mostly due to point-source pollution attributable to manure or slurry application, particularly on steeper gradients or when application is followed by high rainfall (Johnson and Dawson, 2005; Lloyd et al., 2016; Withers and Hodgkinson, 2009).

Due to the risks posed to water quality, slurry management on farms is subject to increasingly stringent regulations in Europe (Lloyd et al., 2016). For example, recent legislation in Wales includes restrictions on slurry application rates and the time of year spreading is permitted, with farms required to have sufficient storage capacity for all slurry produced during the closed period between October and January (Welsh Government, 2021). Storage infrastructure is costly, with many farms

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requiring substantial investment to meet regulations without reducing production.

The financial implications of such regulations, along with increasing awareness of the environmental impacts of nutrient losses, has led to interest in alternative methods of recovering nutrients from slurry, such as ammonia stripping, air scrubbing, membrane filtration and struvite crystallisation (Fattah et al., 2022; Shi et al., 2022). Whilst these methods efficiently recover nutrients from wastewaters, examples of their implementation in agriculture remain rare due to the substantial investment and technical expertise required for their operation and maintenance (Shi et al., 2022).

There is therefore a need to develop low-tech, cheaper alternative methods of recovering nutrients that can be easily implemented on farms. Phytoremediation may be one such alternative, where plants or algae are used to uptake N and P from wastewater (Hu et al., 2020; Shi et al., 2022). The use of duckweed, a family of floating aquatic plants, has received particular recent interest (Devlamynck et al., 2021a; Sońta et al., 2020). This is partly due to duckweed's rapid growth rates (Ziegler et al., 2015), whilst there is also interest in using the harvested biomass as a sustainable livestock feed (Sońta et al., 2019), given its high crude protein content (Stadtlander et al., 2022) and favourable amino acid profile (Appenroth et al., 2017; Rusoff et al., 1980).

Duckweed effectively removes both N and P from growth media, with recovery rates comparable to the aforementioned physico-chemical processes. For example, Dinh et al. (2020), observed an 84 % reduction in both the total N and P (TN and TP) concentrations of diluted pig slurry after nine days of *Lemna minor* growth. Direct uptake by duckweed only represents a proportion of the total reductions in wastewater nutrient concentrations (Dinh et al., 2020), with N and P also lost via other pathways. These include gaseous N loss via ammonia volatilisation and denitrification (Zimmo et al., 2004), assimilation of N and P by coexisting algae and microorganisms (Körner and Vermaat, 1998; Zimmo et al., 2004), and the precipitation, adsorption and sedimentation of P and organic N forms (Dinh et al., 2020; Körner and Vermaat, 1998). The relative contribution of duckweed to reducing N and P concentrations is dependent on growth rates; Zimmo et al. (2004) observed that whilst duckweed uptake accounted for 30 % of TN removal from diluted sewage over a four month period, this decreased to 10 % during colder winter months. Therefore, by optimising growth rates, the relative contribution of duckweed to nutrient removal can be increased. Improving efficiency in this way increases the amount of N and P available for reuse as duckweed biomass, and also reduces the proportion of N lost as gaseous emissions, important given the environmental impacts of NH_3 deposition (Galloway et al., 2008) and greenhouse gas potential of N_2O (Sims et al., 2013).

Acidifying wastewater may improve the efficiency of duckweed-based nutrient recovery. Duckweed can maintain high growth rates in higher total ammonia nitrogen (TAN) concentrations where the pH of the solutions has been lowered (Caicedo et al., 2000; Körner et al., 2001), and a recent laboratory-scale experiment showed these findings also extend to increasing concentrations of agricultural wastewater (Jones et al., 2023). The impact of solution pH on duckweed growth has been attributed to its influence on the NH_4^+:NH_3 equilibrium. At a pH of 6, 99.95 % of the TAN is in the ionised (NH_4^+) form, with the proportion of free ammonia (NH_3) increasing by one order of magnitude for every 1 unit increase in pH (Goopy et al., 2004; Warren, 1962). NH_3 is toxic to living organisms at relatively low concentrations as it crosses membranes into cells more readily than the less toxic ionised form (Körner et al., 2001). Given that slurry pH is often >8, acidification may increase growth rates, and in turn, duckweed nutrient uptake from the medium.

Slurry acidification also influences the bioavailability of P (Li et al., 2020). In solution, P primarily occurs as PO_4^{3-} and its solubility is determined by precipitation-dissolution and sorption-desorption reactions (Holtan et al., 1988). pH exerts a strong influence on these processes in freshwater (Diaz et al., 1994) and livestock manure (Chapuis-Lardy et al., 2004), with PO_4^{3-} availability decreasing above a pH of 8 due to precipitation as Ca-P (Diaz et al., 1994) and adsorption onto mineral surfaces (Li et al., 2020). As such, duckweed growing on slurry can only access a proportion of the total P (Chapuis-Lardy et al., 2004; Li et al., 2020). Acidifying slurry results in desorption and dissolution of precipitated P, thus increasing the plant available PO_4^{3-} concentration (Li et al., 2020), and in turn, potentially leading to greater duckweed growth and P recovery.

Whilst previous work has demonstrated the positive effect of acidifying slurry on duckweed growth under laboratory conditions (Jones et al., 2023) as well as on the speciation and bioavailability of slurry N (Kavanagh et al., 2019) and P (Li et al., 2020), the implications of lowering pH for nutrient recovery using duckweed has not been investigated. This study investigated the nutrient dynamics in outdoor duckweed growing systems, with the aim of improving the efficiency of N and P uptake from diluted slurry by lowering pH. Two hypotheses were tested: firstly, that higher growth rates in acidified media observed in previous laboratory experiments would lead to greater decreases in inorganic N and P concentrations in acidified diluted slurry, relative to unamended diluted slurry (control). Secondly, that acidification increases *L. minor* N and P recovery, due to the aforementioned effect of lowering pH on growth via NH_3 toxicity and increased P bioavailability.

2. Materials and methods

2.1. Experimental design

The experiment was undertaken outdoors out at the Aberystwyth University Botany Gardens (52°25′06″N 4°03′54″W), beginning on September 6 2022 and running for a period of five weeks. Duckweed growing tanks were prepared by removing the tops of 1 m^3 intermediate bulk containers (IBCs). The IBCs were covered with a 4 mm transparent acrylic sheet secured to the metal cages with U-bolts to prevent rain from entering the tanks whilst still allowing air flow. The sides of the IBCs were covered with black parcel wrap up to the water level to prevent light penetration into the water column from the sides, thus limiting submerged algal growth. Dataloggers (Ystumtec, Wales, UK), as described by Newnes et al. (2021), were used to record air and water temperature during the experiment (Fig. 1). The air temperature sensor was at a height of 5 cm above the duckweed, whereas the water temperature sensor was at a depth of 5 cm. Mean, minimum, and maximum solar radiation values of 119, 0, and 1156 W m^{-2}, respectively, were recorded during the experimental period at the Gogerddan weather station (52°25′55″ N 04°01′14 W).

Raw cattle slurry was collected from a farm in north-west Wales, UK. The slurry storage tank received daily inputs of manure, urine, and leachate from a farmyard manure heap, and was thoroughly mixed prior to sample collection to ensure homogeneity. Preliminary analysis was conducted to assess the dilution rate required in the experiment. This involved analysing a representative sub-sample for its NH_4^+ concentration (1154.5 mg L^{-1}) using ion exchange chromatography and pH (8.71) with a benchtop meter (more detailed information of the methods provided in Section 2.2. Chemical Analysis), two variables that are known to strongly influence duckweed growth in wastewater (Körner et al., 2001). Data for the concentrations of other ions relevant to wastewater treatment and duckweed growth are presented in Table 1.

The experiment had two treatments, each with four replicates. The first treatment was the control, where the tanks were filled to 250 L with 1:20 diluted slurry, lowering the TAN concentration (Table 1) to a level previously shown to be tolerated by *L. minor* when grown on agricultural wastewater (Jones et al., 2023). In the second treatment, the pH of 1:20 diluted slurry was lowered to 6.96 ± 0.11 (standard error) by adding 375 mL of 1 M H_2SO_4 at the start of the experiment (Jones et al., 2023). The pH of the acidified treatment increased over the course of the experiment, therefore further additions of 1 M H_2SO_4 were made at the end of weeks 1 (12.5 mL), 2 (6.25 mL), and 3 (50 mL) to return the pH to 7, with each tank gently mixed to ensure the even dispersal of the acid.

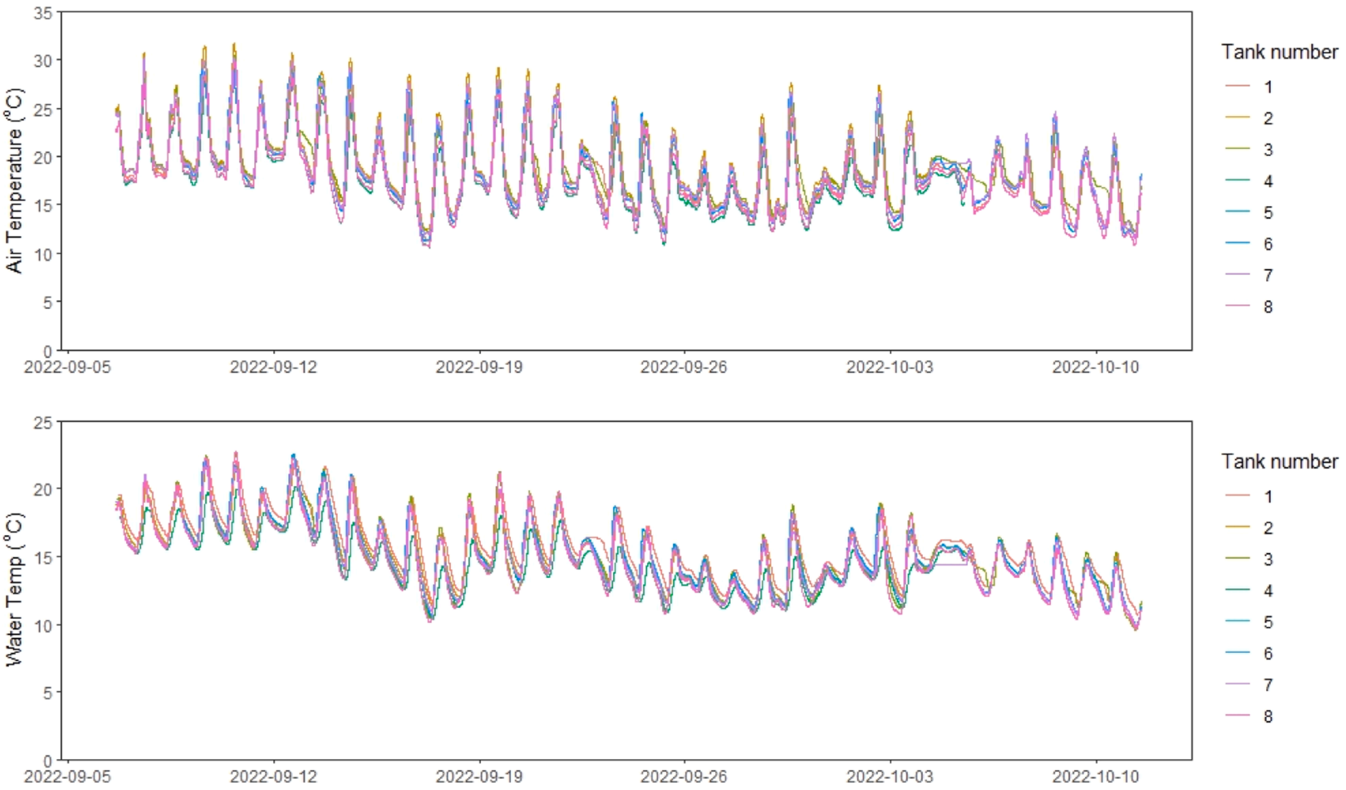

Fig. 1. Air and water temperature (°C) within each of the eight tanks over the five-week duration of the experiment.

Table 1
Concentrations of various ions in the undiluted raw slurry collected for use in the study, and the diluted control and acidified slurry used in the experiment. Concentrations of unionised NH_3, expressed in brackets, were calculated using the equations from Emerson et al. (1975). All data are expressed as mg L^{-1}, except for pH data.

	Concentration (mg L^{-1})		
	Raw slurry	Diluted control slurry	Diluted acidified slurry
TAN (NH_3)	1154.5 (232.5)	57.44 (10.74)	58.76 (0.26)
NO_2^-	5.9	0.23	0.27
NO_3^-	72.6	20.62	16.83
PO_4^{3-}	102.7	8.17	10.17
K^+	11,826.5	611.22	590.12
SO_4^{3-}	76.5	19.80	422.12
Ca^{2+}	233.8	26.19	26.28
Mg^{2+}	257.4	17.05	17.88
Na^+	2016.4	127.53	120.99
Cl^-	787.0	533.29	515.80
pH	8.71	8.67	6.96

To replace water lost through evapotranspiration, all tanks were topped up to 250 L with tap water at the end of weeks 2 and 4.

Lemna minor L. (Blarney strain, number 5500 in the Rutgers stock) was cultured for three weeks in adjacent IBCs on the N medium described by Appenroth and Horn (1996). At the start of the experiment, each tank was inoculated with 500 g fresh weight of *L. minor*. The media was sampled at three depths corresponding to the root-zone, middle and bottom of the water column (2 cm, 12.5 cm, and 25 cm), using a 50 mL syringe attached to 2 mm internal diameter silicone tubing. The media samples collected on day 0 were subsequently repeated weekly throughout the experiment. After sampling the media during each week, the *L. minor* biomass in each tank was collected and suspended on high-density polyethylene netting above the tank to drain for 15 min. Once drained, the fresh weight was recorded to calculate the weekly relative growth rate (RGR) using the following equation (Hunt, 1978):

$$RGR = \frac{\ln\left(\frac{FW_2}{FW_1}\right)}{T}$$

where ln is the natural logarithm, FW_1 is the initial *L. minor* fresh weight, FW_2 the fresh weight at the end of the experiment, and T the duration of the experiment in days. Each tank was then re-inoculated with 500 g of the harvested *L. minor*, or as close to this figure as possible if biomass was lost during a particular week. A representative sub-sample of *L. minor* biomass was collected from all tanks during each week and was stored at –80 °C prior to chemical analysis.

2.2. Chemical analyses

The raw slurry sample collected was transported to the laboratory for analysis, which involved measurement of pH (Hydrus 500, Fisherbrand, UK) and electrical conductivity (EC) (SevenMulti, Mettler Toledo, USA). A sub-sample of the raw slurry was filtered through 0.22 μm membrane syringe filters (Fisherbrand, UK) before ion-exchange chromatography analysis (Metrosep C4 250/4.0 and A Supp 5 250/4.0 columns, Metrohm, Switzerland) for a range of anions and cations relevant to agricultural wastewater pollution and plant growth. The media samples collected weekly over the duration of both experiments were also analysed for pH, EC, and ion concentrations as described above. Using the media TAN, pH and temperature data, the proportion of TAN as NH_3 was calculated using the two following equations from Emerson et al. (1975):

$$\mathrm{p}Ka = \frac{0.09108 + 2729.92}{(273.15 + T)}$$

$$NH_3 \quad (\%) = \frac{100}{1 + 10^{(\mathrm{p}Ka - \mathrm{pH})}}$$

where T = temperature (°C). The *L. minor* samples collected were freeze-dried, with dry matter content calculated gravimetrically. The total carbon and nitrogen contents of freeze-dried material were measured using the Dumas method with a Vario MAX cube elemental analyser (Elementar, Germany), whilst total P, K, S, Mg and Ca was measured by inductively coupled plasma optical emission spectroscopy (ICP-OES) (Varian, USA) following nitric acid digestion (Newnes et al., 2021).

2.3. Statistical analyses

All statistical analyses were conducted in R (Version 4.1.0.). Prior to analysis, data were explored using the protocol described by Zuur et al. (2010) to ensure the data met model assumptions. To test for differences in *L. minor* RGR grown on control and acidified diluted slurry over time, linear mixed effects models were used with the tanks included as a random effect to account for repeated measurements. To account for the potential influence of temperature on growth, mean weekly water temperature was included as a covariate. TAN and PO_4^{3-} concentration data, as well as cumulative N and P uptake data, were square root transformed to avoid violation of the homogeneity of variance assumption prior to analysis using linear mixed effects models with tanks as a random effect. Plots of model residuals v fitted values were used to verify the transformation had improved homogeneity of variance.

3. Results

3.1. Lemna minor growth

L. minor RGR over the course of the experiment was higher in the acidified treatment, relative to the control ($P = 0.001$) (Fig. 2). Growth was poor in the control treatment during the first week, with only 99 g FW m^{-2} produced relative to 382 g FW m^{-2} in the acidified treatment. During the following weeks, symptoms of toxicity including chlorosis and mortality were observed in the control treatment, resulting in negative RGR values during all weeks except for the fourth. Conversely, positive growth rates were maintained throughout the experiment in the acidified treatment. Time also had a significant effect on *L. minor* RGR ($P < 0.001$), with growth rates generally declining over the course of the trial (Fig. 2). A significant interaction between treatment and time was observed ($P < 0.001$), reflecting the fact that growth in the acidified treatment decreased each week, whereas in the control growth remained low for the duration of the experiment. Mean water temperature within each tank was included as a covariate in the model, however, this had no significant effect on RGR ($P = 0.933$).

3.2. Changes in nutrient concentrations over the course of the experiment

Contrary to the first hypothesis, TAN concentrations over the duration of the experiment were significantly lower in the control tanks relative to the acidified treatment ($P = 0.007$; Fig. 3), despite higher growth rates in the acidified treatment (Fig. 2). TAN concentrations also significantly varied over time ($P < 0.001$), whilst an interaction was observed between time and treatment ($P < 0.001$), reflecting the higher rate of depletion in the control treatment. Over the five-week duration of the experiment, the slurry TAN was reduced by 87 % in the control tanks, and by 57 % in the acidified treatment. The TAN concentration did not significantly vary with depth ($P = 0.226$). Statistical analysis of NO_3^- concentration data was not conducted as the concentrations rapidly reduced during the first week (85 % reduction in both control and acidified slurry treatments), resulting in low concentrations for the remainder of the experimental period that were occasionally below the detection limits of the analytical method used. Similarly, NO_2^- concentrations remained low throughout the experiment and were therefore not statistically analysed.

Unlike the TAN concentrations and consistent with the first hypothesis, PO_4^{3-} concentrations were significantly lower in the acidified treatment relative to the control ($P = 0.001$; Fig. 3). PO_4^{3-} concentrations also significantly varied with depth ($P = 0.004$), with concentrations at the root zone level tending to be higher than at the other two lower sampling depths. Significant variation over time was observed ($P < 0.001$), whilst there was a significant interaction between treatment and time ($P < 0.001$), reflecting the faster rate of depletion in the acidified treatment. After five weeks, PO_4^{3-} concentrations were 60 % and 93 % lower in the control and acidified treatments, respectively.

3.3. N and P recovery by Lemna minor

To assess the direct contribution of *L. minor* in lowering slurry nutrient concentrations, the *L. minor* samples collected during the experiment were analysed for their elemental composition (Table 2). Using these data, along with the weekly yield and dry matter content

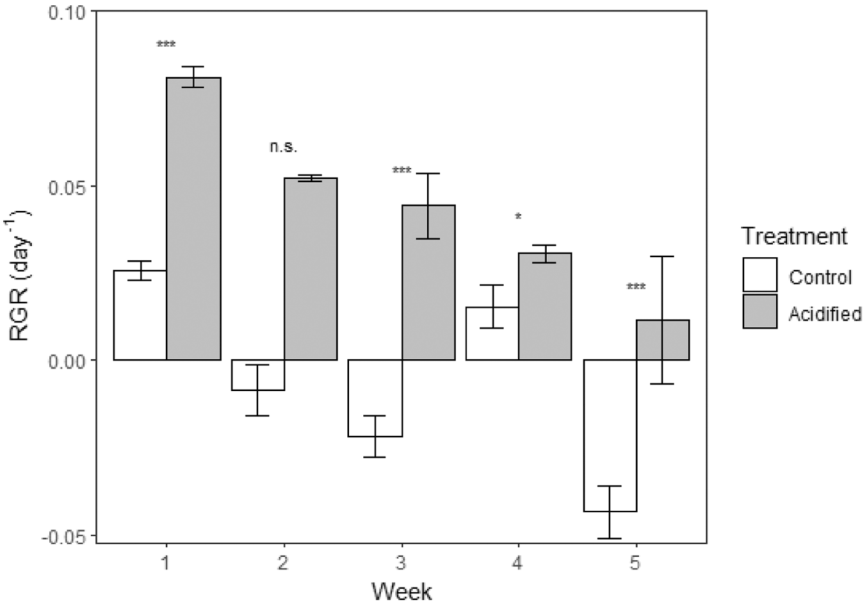

Fig. 2. *L. minor* relative growth rates (RGR) when grown in the control (initial pH of 8.67) and acidified (initial pH of 6.96) diluted cattle slurry over a five-week period. Error bars represent the standard error of the mean.

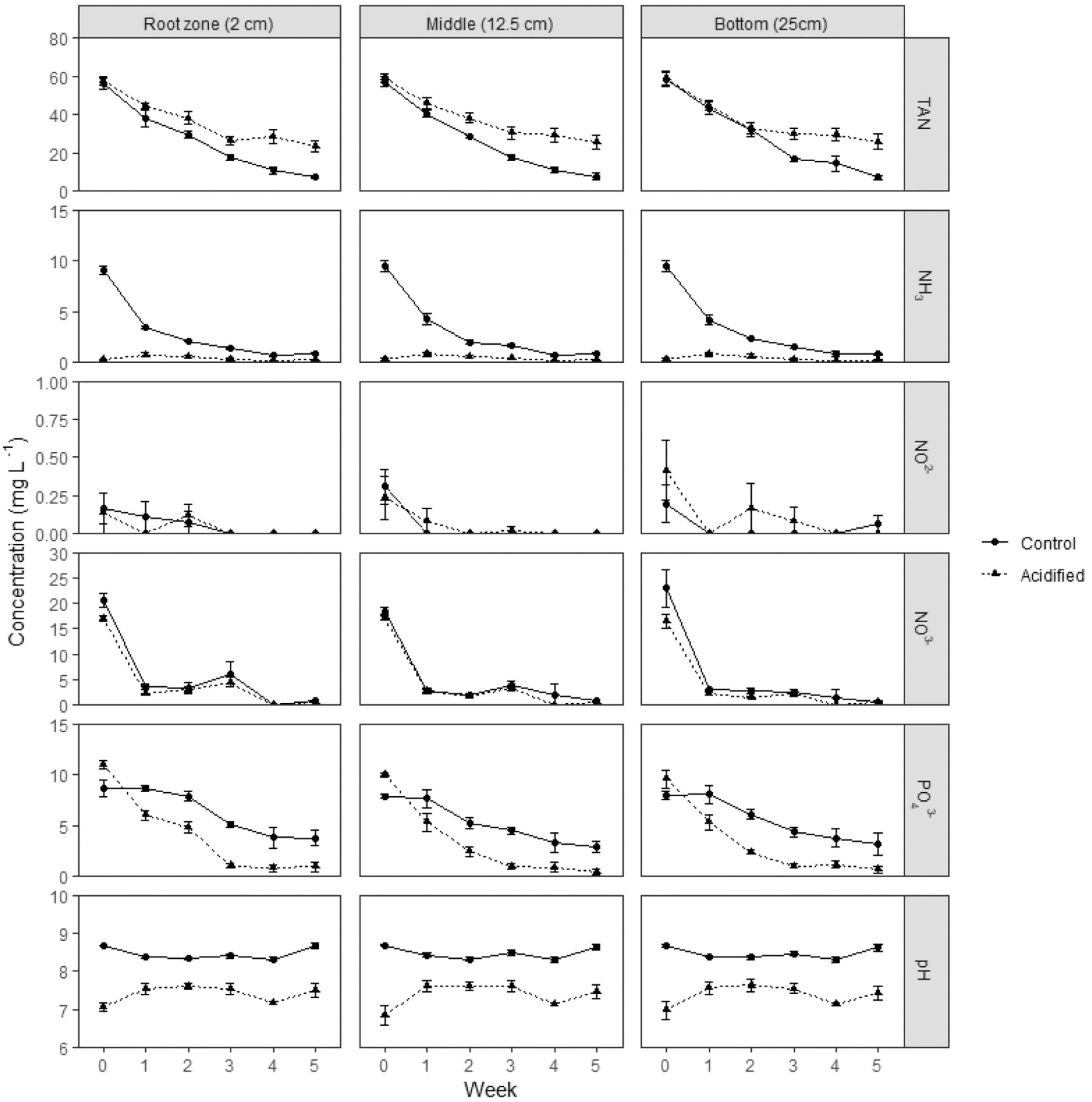

Fig. 3. Mean concentrations of TAN, NH_3, NO^{2-}, NO^{3-}, PO_4^{3-}, and the pH (± standard error) of the control and acidified diluted slurry at three different depths during the experiment.

Table 2
Total content of C, N, P, K, and S of the *L. minor* samples collected during each week of the experiment (± standard error). The first row contains data for a homogenised sub-sample of the *L. minor* plants used to inoculate the tanks at the start of the experiment before the treatments were applied.

Week	Treatment	Total content (mg g^{-1} DM)					C:N ratio	Crude protein (%)
		C	N	P	K	S		
0	Initial *L. minor*	464.8	64.3	11.0	23.4	4.3	7.23	40.18
1	Control	475.7 ± 2.8	69.9 ± 3.1	6.1 ± 0.1	13.7 ± 1.2	3.5 ± 0.1	6.84 ± 0.25	43.68 ± 1.92
1	Acidified	478.8 ± 5.1	70.5 ± 1.6	8.2 ± 0.1	18.3 ± 1.4	5.5 ± 0.1	6.79 ± 0.09	44.09 ± 0.98
2	Control	497.6 ± 3.4	67.6 ± 1.3	5.3 ± 0.5	13.8 ± 1.0	3.2 ± 0.3	7.36 ± 0.10	42.28 ± 0.83
2	Acidified	487.8 ± 3.0	65.8 ± 2.3	6.9 ± 0.3	14.6 ± 0.8	5.3 ± 0.2	7.44 ± 0.26	41.14 ± 1.43
3	Control	514.2 ± 9.4	63.5 ± 2.0	6.0 ± 0.4	13.8 ± 1.4	3.7 ± 0.2	8.14 ± 0.38	39.66 ± 1.24
3	Acidified	495.7 ± 4.7	68.2 ± 2.8	7.2 ± 0.3	16.4 ± 1.6	6.4 ± 0.1	7.30 ± 0.27	42.65 ± 1.72
4	Control	508.7 ± 4.2	63.5 ± 2.3	5.9 ± 0.4	14.4 ± 0.4	3.6 ± 0.2	8.04 ± 0.28	39.67 ± 1.43
4	Acidified	484.6 ± 1.1	64.7 ± 3.1	5.9 ± 0.7	16.6 ± 1.5	6.2 ± 0.2	7.55 ± 0.38	40.43 ± 1.91
5	Control	513.7 ± 3.6	65.1 ± 0.9	5.2 ± 0.4	11.3 ± 1.2	3.1 ± 0.1	7.89 ± 0.16	40.71 ± 0.57
5	Acidified	491.9 ± 4.6	64.1 ± 3.0	4.9 ± 0.8	16.3 ± 1.8	6.2 ± 0.1	7.72 ± 0.31	40.05 ± 1.85

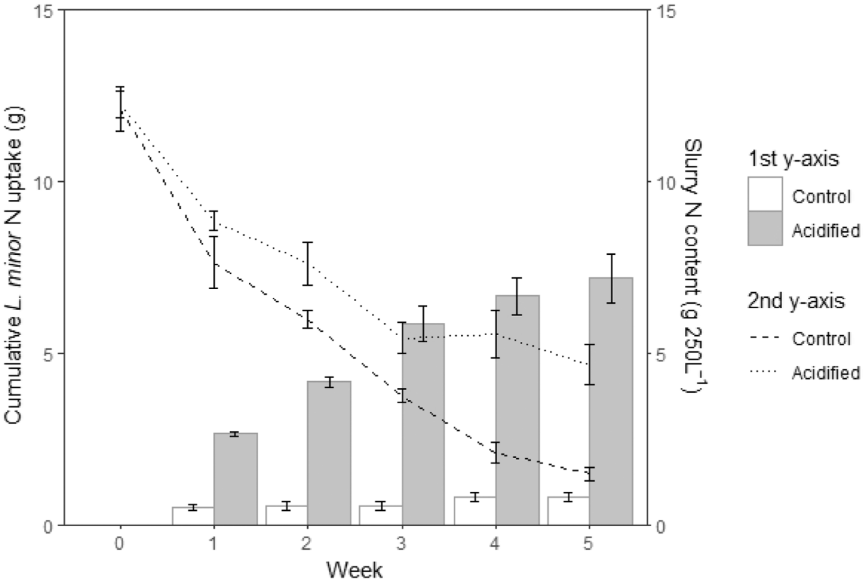

Fig. 4. Cumulative *L. minor* N uptake ($\pm$ standard error) over the duration of the experiment from control and acidified treatments (plotted on the primary y-axis). The dissolved N concentrations of the diluted slurries ($\pm$ standard error), calculated from the weekly TAN, NO_2^- and NO_3^- concentration data, are plotted on the secondary y-axis.

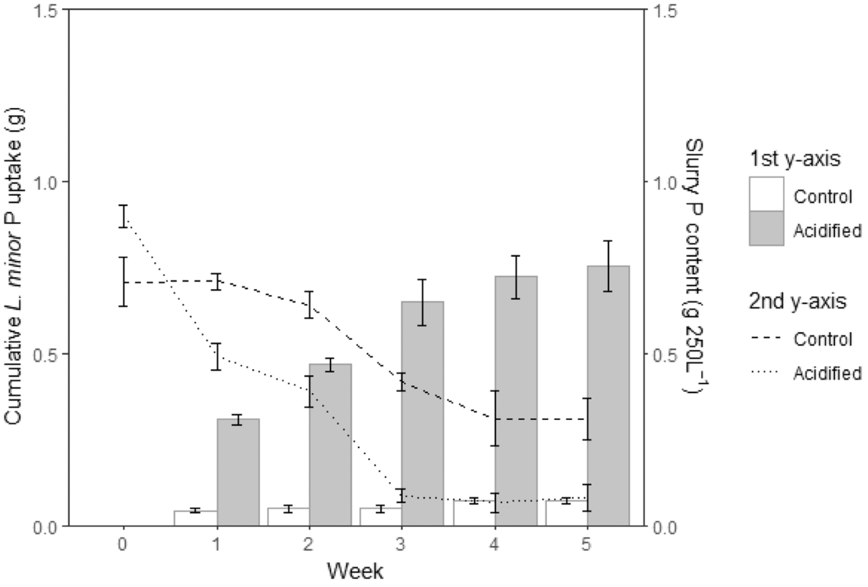

Fig. 5. Cumulative *L. minor* P uptake ($\pm$ standard error) over the duration of the experiment from control and acidified diluted slurry treatments (plotted on the primary y-axis). The dissolved P concentration of the slurry ($\pm$ standard error), calculated from the weekly PO_4^{3-} concentration data, is plotted on the secondary y-axis.

data, the total N and P removed by *L. minor* over the course of the experiment was estimated (Figs. 4 and 5). As previously reported, *L. minor* showed poor growth in the control treatment with only two weeks where positive growth was observed. This resulted in significantly higher cumulative *L. minor* N uptake in the acidified treatment ($P < 0.001$), with a total of 7.17 g recovered from the acidified diluted slurry over the course of the trial, relative to only 0.80 g recovered from the control during the same period. This corresponds to uptake rates from the control and acidified treatments of 23 mg m^{-2} d^{-1} and 205 mg m^{-2} d^{-1} over the five-week period, respectively. Significant temporal variation in cumulative N recovery was observed ($P < 0.001$) in addition to a significant interaction between time and treatment ($P < 0.001$), reflecting the fact that the differences between sampling timepoints were greatest in the acidified treatment, and that cumulative N uptake generally remained low throughout the experiment in the control (Fig. 4).

The total inorganic N concentrations of the control and acidified treatments at the start of the experiment, calculated from the TAN, NO_2^-, and NO_3^- data, were 48.43 mg N L^{-1} and 48.87 mg N L^{-1}, respectively. By the end of the five-week experiment, these concentrations had reduced to 5.92 mg N L^{-1} in the control and 18.64 mg N L^{-1} in the acidified treatments. Based on these reductions in inorganic N concentrations, it was estimated that over five weeks a total of 10.63 g and 7.56 g of N was lost from the diluted slurry of the control and acidified treatments, respectively. Using the *L. minor* N content data reported above, it was estimated that direct uptake accounted for 7.5 % of the N removed from the control, and 94.8 % of the N removed from the acidified treatment.

Similar estimates were observed for P removal (Fig. 5); cumulative *L. minor* uptake was significantly lower in the control relative to the acidified treatment ($P < 0.001$), with a total of 71 mg and 754 mg of P removed from the diluted slurries, respectively, corresponding to uptake rates of 2 mg m^{-2} d^{-1} and 22 mg m^{-2} d^{-1} over the five-week period. As with N uptake, significant temporal variation was also observed ($P < 0.001$) in addition to an interaction between time and treatment ($P < 0.001$), reflecting the fact that cumulative P uptake increased over time in the acidified treatment but remained low throughout the experiment in the control (Fig. 5). Using the PO_4^{3-} concentration data of Fig. 3, it was calculated that the control and acidified diluted slurry at the start of the experiment contained 2.66 mg P L^{-1} and 3.32 mg P L^{-1}, respectively, which was reduced to 1.08 mg L^{-1} and 0.29 mg L^{-1} within five weeks (Fig. 3). Based on the above estimates of cumulative P uptake, *L. minor* was therefore responsible for an estimated 18 % and 99.5 % of the reductions in dissolved inorganic P concentrations presented in Fig. 5.

The N and P content data of the *L. minor* biomass samples collected, expressed as mg g^{-1} dry matter (DM), were analysed with mixed effect models. Whilst N removal from acidified diluted slurry via *L. minor* uptake was significantly higher than in the control, as mentioned above, the N content of the harvested biomass did not vary between treatments ($P = 0.851$; Table 2). *L. minor* N content significantly varied with sampling week ($P < 0.001$), decreasing over time in both treatments and reflecting the decreasing N concentrations of the diluted slurry. A significant interaction was also observed between treatment and sampling week ($P = 0.039$), indicating that the rate of decreasing N content varied between treatments. Pairwise analysis was used to compare the N content of *L. minor* grown in the control and acidified treatments within the same week, however, no significant differences were observed for any of the weeks ($P > 0.05$).

Similar analysis for the P content (mg g^{-1} DM) of the *L. minor* biomass samples found a significant effect of treatment ($P = 0.026$) along with sampling week ($P < 0.001$), with *L. minor* P content generally higher in the acidified treatment and decreasing over time (Table 2). A significant interaction between treatment and sampling week was also detected ($P < 0.001$), indicating that the rate of decreasing *L. minor* P content also varied depending on the growth medium. Pairwise comparisons were used to investigate this interaction. At the end of the first and second weeks, *L. minor* P content was higher in the acidified treatment, relative to the control ($P = 0.002$ and $P = 0.007$, respectively). The differences between treatments in P content diminished as the experiment progressed, with no significant difference observed at end of weeks 3 ($P = 0.055$), 4 ($P = 0.990$), or 5 ($P = 0.632$).

4. Discussion

This study investigated the impact of lowering the pH of diluted slurry on *L. minor* uptake of N and P over the course of a five-week experiment. Acidifying wastewater has previously been shown under laboratory conditions to lead to higher duckweed growth (Jones et al., 2023), therefore, it was hypothesised that more N and P would also be removed from acidified diluted slurry, relative to unamended diluted slurry (control). This proved to be true for P, with the mean PO_4^{3-} concentration of acidified diluted slurry reduced by 93 % to <1 mg L^{-1} by the end of the experiment, compared to 60 % in the control. In contrast, the greatest reduction in N concentrations was seen in the control. However, *L. minor* growth data and N content analysis of the harvested biomass showed that plant uptake accounted for only 7.5 % of the reduction in the N concentration of control diluted slurry, relative to 94.9 % in the acidified treatment. Therefore, most of the N lost from the control treatment was removed via other pathways, rather than *L. minor* uptake.

Without acidification, *L. minor* growth was poor, and negative growth rates were observed during three of the five weeks measured. This was most likely due to the relatively high concentrations of NH_3, with concentrations during the first week in excess of 8 mg L^{-1}, which caused *L. minor* mortality in Körner et al. (2001). Growth was higher in the acidified treatment, again indicating the importance of the impact of pH on the NH_4^+:NH_3 equilibrium (Körner et al., 2001) and reflecting the results of a previous laboratory-scale study using anaerobically digested cattle slurry (Jones et al., 2023). Growth rates in the acidified treatment decreased over time. As temperature is known to cause temporal variation in duckweed productivity (Devlamynck et al., 2021b), air and water temperature within each tank were monitored during the experiment. Both air and water temperature showed a slight decrease over the course of the experiment (Fig. 1), from weekly means of 22.0 °C and 18.2 °C during the first week to 17.3 °C and 14.0 °C during the fifth week, respectively. Whilst these temperatures are below the optimal range of 20–25 °C for *L. minor* growth (Paterson et al., 2020), neither water nor air temperature had significant effects on *L. minor* RGR when included as covariates in a mixed effects model, indicating that they were unlikely to be the main factors behind the declining growth rates in the acidified treatment.

Nutrient concentrations in the acidified treatment also decreased over the course of the experiment. Dissolved inorganic N was mainly in the form of TAN, the concentrations of which decreased by 57 % during the experiment. However, N depletion is unlikely to have been a limiting factor in this study, as the final TAN concentration (25.13 mg L^{-1}) remained above 19 mg L^{-1}, reported by Stadtlander et al. (2022) to be optimal for the growth of two duckweed spp. (*Spirodela polyrhiza* and *Landoltia punctata*). The PO_4^{3-} concentration in the acidified treatment became considerably more depleted, being reduced to around 1 mg PO_4^{3-} L^{-1} by the third week and remaining low for the remainder of the experiment. This reduction in the PO_4^{3-} concentration is most likely explained by the high growth rates and P recovery in the acidified treatment, and is reflected in the P content of the *L. minor* biomass (Table 2). The plants used to inoculate the tanks at the beginning of the experiment had a P content of 1.1 % DM after being cultured on the N-medium described by Appenroth et al. (1996). Within five weeks, the P content of the acidified treatment *L. minor* had reduced to 0.49 %, which is within the critical internal P content range of 0.46–0.65 % identified by Huebert and Shay (1991) as being limiting to *Lemna trisulca* growth. This indicates that P availability may have limited growth by the latter part of the experiment, explaining the reduced yields over time. Acidifying slurry is known to increase P solubility (Li et al., 2020), potentially explaining the higher *L. minor* P content in the acidified treatment relative to the control during the first two weeks. This difference in *L. minor* P content was not seen from week 3 onwards, indicating that the PO_4^{3-} concentration of the acidified media may be beginning to limit growth at this point.

The varying growth rates in the control and acidified treatments are reflected in the *L. minor* nutrient uptake data, as N and P recovery using duckweed relies on the growth of additional biomass (Körner and Vermaat, 1998). Poor growth in the control resulted in *L. minor* recovering an average of 23 mg N m^{-2} d^{-1} and 2 mg P m^{-2} d^{-1} over the 5-week trial, around ten times lower than the 205 mg N m^{-2} d^{-1} and 22 mg P m^{-2} d^{-1} recovered from the acidified treatment over the same period. The decreasing yields in the acidified treatment are also reflected in the nutrient uptake rates, with removal highest during the first week (377 mg N m^{-2} d^{-1} and 44 mg P m^{-2} d^{-1}) and lowest during the final week (73 mg N m^{-2} d^{-1} and 5 mg P m^{-2} d^{-1}). The high rates observed during the first week are close to the 327 mg N m^{-2} d^{-1} and 67 mg P m^{-2} d^{-1} reported by Devlamynck et al. (2021b), where *L. minor* was grown on a similar pilot-scale system consisting of a series of three connected IBCs receiving a continuous supply of pig slurry. Whilst comparison between studies is difficult given the number of variables that influence duckweed performance (Körner and Vermaat, 1998), the lower nutrient removal rates observed by the end of the current study may again be a result of P depletion, whereas the continuous supply of nutrients fed into the system used by Devlamynck et al. (2021b) maintained higher rates of growth and therefore nutrient uptake.

Despite the lack of growth in the control treatment, dissolved N concentrations still decreased over time, indicating that N was also being lost in other ways in addition to plant uptake. N can be removed from duckweed wastewater treatment systems through algal and microbial uptake, in addition to sedimentation of organic matter and microorganisms (Körner and Vermaat, 1998; Peng et al., 2007). However, sedimentation and assimilation by organisms other than *L. minor* are unlikely to have been the main difference between treatments in the current study, as if this was the case, we would also have expected higher rates of P removal from the control treatment, which was not observed. Gaseous emissions can represent substantial N losses from duckweed ponds (Körner and Vermaat, 1998; Peng et al., 2007; Vermaat and Hanif, 1998). For example, N can be lost through the nitrification of TAN and the subsequent denitrification of NO_3^- (Sims et al., 2013; Zhao et al., 2022; Zimmo et al., 2003), its contribution comparable to that of duckweed itself in some cases (Körner and Vermaat, 1998). Measured NO_3^- concentrations decreased rapidly within one week in both the control and acidified treatments, remaining low for the remainder of the experiment, suggestive of high rates of denitrification as well as uptake by *L. minor*. Throughout the experiment, both treatments had pH values within or close to the optimal range for denitrification of 7–8 (Knowles, 1982), potentially explaining the rapid decreases in NO_3^- concentrations observed.

The pH of a solution also influences the NH_4^+:NH_3 ratio (Warren, 1962), as can be seen from the higher pH and NH_3 concentration data of the control treatment (Fig. 3). Solution pH and NH_3 volatilisation are positively correlated, with slurry acidification proven to reduce NH_3 emissions from slurry stores (Husted et al., 1991; Kavanagh et al., 2019) and following field application (Stevens et al., 1989). Whilst NH_3 volatilisation was not directly measured in this study, the lower NH_3 concentration and lower rate of decreasing TAN concentrations in the acidified diluted slurry, relative to the higher pH control, suggests it may have contributed to the difference between treatments in N removal that was not accounted for by *L. minor* uptake. Previous work by Zimmo et al. (2003) support this suggestion, who modelled NH_3 volatilisation from a duckweed ponds system treating domestic wastewater, estimating that whilst NH_3 volatilisation accounted for less than 3 % of total N loss with a pH range of 7.7–8.0, this increases to 21 % at a pH of 9.0. This has important implications for the implementation of duckweed growing systems on farms, given the current concern over NH_3 emissions from agriculture (Misselbrook et al., 2016).

Unlike N, P is not lost from wastewater as gaseous emissions, with duckweed uptake the main pathway for its removal (Vermaat and Hanif, 1998). It was estimated that *L. minor* accounted for 18 % of the reduction in the soluble P concentration of the control diluted slurry and 99.5 % in the acidified treatment. The higher P recovery from the acidified treatment most likely resulted from the previously discussed differences in growth between treatments. Other than *L. minor* uptake, PO_4^{3-} concentrations may also have been influenced by adsorption and precipitation reactions (Li et al., 2020). These processes impact upon the solubility of inorganic P and are influenced by changes in pH, particularly in media with high Ca concentrations (Diaz et al., 1994; Picot et al., 1991) such as livestock slurry (Chapuis-Lardy et al., 2004). Li et al. (2020) found lowering cattle slurry pH from 7.4 to 5.5 caused desorption of mineral surface adsorbed P and dissolved precipitated Ca-P, resulting in a higher proportion of the TP being water soluble. In the current study, Ca^{2+} concentrations remained relatively stable throughout the experiment at around 25 mg L^{-1} in both treatments (Table 1). This indicates that the effect of Ca-P precipitation/dissolution was limited, possibly due to the lower pH reported by Li et al. (2020) and the lower Ca^{2+} concentrations of our slurry post-dilution (Diaz et al., 1994), although adsorption may still have removed PO_4^{3-} from solution in the control treatment. Assimilation by algae and microorganisms can also contribute to P removal (Körner and Vermaat, 1998); however, as discussed above, this was unlikely to have been the main cause of the differences in N and P uptake between treatments.

The efficient P recovery observed has important implications in practice given that P is recognised as the main cause of eutrophication in aquatic habitats, with concentrations as low as 20 μg P L^{-1} known to accelerate algal growth (Johnson and Dawson, 2005). Our findings highlight the viability of using duckweed to recover P from wastewater, minimising losses to the environment and thus mitigating the associated risks to aquatic habitats. Additionally, we show that the contribution of *L. minor* uptake to P removal can be substantially increased by lowering pH, thus increasing the amount of P recovered as biomass. This was also the case for N, particularly important given current concerns over gaseous losses of NH_3 and N_2O from agriculture (Misselbrook et al., 2016). Finally, our findings show that the beneficial impacts of lowering wastewater pH on duckweed growth previously observed in laboratory-scale studies (Caicedo et al., 2000; Jones et al., 2023) are transferable to a larger outdoor scale that was more representative of a system that could be employed in practice.

5. Conclusions

For the first time, this study investigated the impact of lowering pH on the efficiency of N and P recovery from diluted slurry using duckweed. *L. minor* growth was significantly higher in the acidified treatment, confirming the results of previous laboratory studies on a scale relevant to industry. Whilst higher growth was observed in the acidified treatment, this did not translate into greater reductions in diluted slurry N concentrations, with the final N concentration lowest in the control treatment. In contrast, P removal was higher in the acidified treatment, mostly due to direct uptake by *L. minor*. Growth rates in the acidified treatment steadily declined during the experiment which was attributed to P depletion.

Declaration of Competing Interest

The 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.

Data Availability

Data will be made available on request.

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