



Research article

Growth response to nitrate enrichment helps facilitate success of an alien *Potamogeton* in New Zealand streams

Louis Johansen Skovsholt^{a,b,*}, Tenna Riis^c, Fleur Matheson^b, Ian Hawes^a^a University of Waikato, School of Science, New Zealand^b NIWA, New Zealand^c Aarhus University, Department of Biology, Denmark

ARTICLE INFO

Keywords:

Freshwater
Macrophyte
Aquatic plant
Eutrophication
Nutrients
Invasive
Traits
Potamogeton crispus

ABSTRACT

Motivated by stream ecosystem degradation by eutrophication, we mimicked slow flowing low-land stream conditions with a novel experimental setup to further our understanding of aquatic plant responses to increases in nitrate and light. We conducted a mesocosm growth experiment of two species from the genus *Potamogeton*: *P. crispus* (alien) and *P. ochreatus* (native), grown at four nitrate and four light levels. We hypothesised that (i) internal nutrient status of the plants would scale with water column nutrient concentration, and that (ii) plant performance would reflect the nutrient status of the plant. Furthermore, we hypothesised that (iii) a low irradiance level would negate the effects of an increased nitrate level. In relation to (ii) we hypothesised that (iv) the traits of the alien species would enable it to outperform the native species where both the availability of light and nutrient resources was high. Internal tissue N content was broadly similar in the two higher (>250 $\mu\text{g NO}_3^- \text{L}^{-1}$) and the two lower nutrient treatments (<20 $\mu\text{g NO}_3^- \text{L}^{-1}$) in both species and plants were therefore collapsed into high and low N-groups. High-N individuals had higher growth rates than low-N ones regardless of species or light treatment and plants had reduced growth rates at the lowest light treatment, however this response was less evident for *P. crispus*. The highest growth rate was found at the high-N individuals of *P. crispus* at the highest light treatment, and correspondingly, in this treatment this species exhibited an increase in branching degree and lateral spread from the low-N plants. As *P. crispus* spreads by fragmentation, our results show it to be a highly effective competitor in anthropogenically impacted areas compared to its native counterpart. Our study exemplifies how light can influence eutrophication responses of plants and how both need to be accounted for in management decisions.

1. Introduction

Aquatic submerged macrophytes support many important services in stream ecosystems. They provide food and habitat for invertebrates and fish [1–4], alter the physico-chemical environment [5] and affect nutrient cycling [6]. However, increased anthropogenic influences have impacted stream macrophyte communities and the services they support [7–9]. Of particular relevance to macrophytes has been the global increase in fertilizer use, which has led to widespread eutrophication across ecosystems [10] and excessive biomass production [11,12], also termed ‘nuisance growth’ [13,14].

* Corresponding author. University of Waikato, School of Science, New Zealand.

E-mail address: ljs@bio.au.dk (L.J. Skovsholt).<https://doi.org/10.1016/j.heliyon.2023.e15528>

Received 26 September 2022; Received in revised form 27 March 2023; Accepted 12 April 2023

Available online 17 April 2023

2405-8440/© 2023 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Nuisance biomass accrual by macrophytes is dependent on the complex interplay of multiple environmental and biotic factors, and is most frequently occurring in lowland reaches, where water velocity is sufficiently low to reduce scouring [15] and light is not limiting macrophyte growth [7]. When these conditions are met, in-stream biomass scales with nutrient levels [16–23]. When these conditions are not met, no direct link between macrophyte biomass and stream nutrient concentrations is observed [24–27], making simple relationships unlikely and underpins the importance of disentangling the specific effects of eutrophication [16].

There are many factors that potentially confound relationships between water nutrient status and plant biomass. Firstly, spot measurements of water column nutrient concentrations do not integrate potential temporal variations in nutrient levels that the plants have been exposed to prior to the date of sampling. This is particularly problematic when trying to correlate water column nutrient concentrations with biomass *in situ* [20]. Most submerged macrophytes can also take up nutrients from the sediment as well as the water column via root uptake [28] or have nutrients stored in their tissue from times when nutrient availability was higher [i.e. luxury uptake; [29]]. Thus, studies of plant-nutrient relations should consider these issues in experimental design. For example, measurement of tissue nutrients is a more direct indicator of the supply available for growth than external concentrations [30–33].

Where occurring, extreme macrophyte proliferation can clog waterways and lead to diverse ecological alterations, including anoxia, altered pH, loss of plant species diversity [12,34–36] and subsequently loss of biodiversity in other trophic levels [1,37,38]. Consequently, there is an interest from managers to limit nuisance growths and controlling incoming nutrient loads to streams to prevent prolific macrophyte growth is seen as a tractable option. However, our knowledge on how substantial nutrient reduction needs to be to reverse eutrophication effects and prevent nuisance growth is still lacking [but see Ref. [12]].

Biotic factors, including intrinsic potential of macrophytes, also add complexity to nutrient-plant responses. Large scale studies of lowland streams in Europe suggest that, when plants are no longer competing for nutrients, traits related to enhancing either light interception or light utilization are dominant in submerged macrophyte communities in eutrophic streams [12,39]. Ellenberg et al. [40] introduced Ellenberg Indicator Values (EIVs) to vegetation science to classify plant responses to environmental variables. Baattrup-Pedersen et al. [39] applied the EIV concept to aquatic macrophytes in lowland streams and reported traits of successful taxa in response to eutrophication to include apical and multi-apical growth meristems as strategies for canopy formation at the water surface for better light capture, but also that plants with low EIV(Light) trait scores can be promoted, as these systems are often highly productive and shade-dominated [39]. These traits are commonly found in invasive nuisance species and as such these species often dominate stream sections where light and nutrient levels are high [7]. In New Zealand, one such species is *Potamogeton crispus* [41], which is also known as a problematic species in the US [42]. However, only few experimental investigations of how the interaction of different levels of light and nutrients affect the performance of aquatic macrophytes exist, although these could shed light on how these abiotic factors facilitate certain species in streams.

To further our understanding of species' response to nutrient increases and how this response is affected by variations in incoming light, we conducted a mesocosm growth experiment of two species occurring in New Zealand from the genus *Potamogeton*: *P. crispus* and *P. ochreatus*. The species were specifically chosen such that we might elucidate how the focal factors and potential interaction of these influenced the response of a native and an alien invasive species with otherwise similar habits of growth. The study used a novel mesocosm setup to mimic slow flowing lowland river conditions, as this is where these two macrophytes often proliferate. The setup consisted of four large (~10 m) flume channels with continuous inflow of purified water dosed with macro- and micronutrients and dissolved inorganic carbon at controlled concentrations. By using clean, low-nutrient, sand as a rooting medium, and providing P and other essential growth elements at non-limiting concentrations in the water column, the effects of N could be isolated. We employed a four-by-four factorial design with four nitrate and four irradiance levels. We used nitrate as a dissolved inorganic N (DIN) source, although ammonium (NH_4^+) is taken up by plants with lower energetic costs, as nitrate (NO_3^-) is the dominant form of DIN in streams, and the one most readily leached from agricultural soils [43]. We hypothesised that (i) internal nutrient status of the plants would scale with water column nutrient concentration and that (ii) plant performance would reflect the nutrient status of the plant. Furthermore, we hypothesised that (iii) a low irradiance level would negate the effects of an increased nitrate level. In relation to (ii) we hypothesised that (iv) the traits of the alien species would enable it to outperform the native species where both the availability of light and nutrient resources was high. A suite of relevant plant traits was measured to test the above hypotheses.

2. Methods

2.1. *Potamogeton crispus* and *P. Ochreatus*

Were collected from a lowland stream near Morrinsville, New Zealand (37°42'18.59 S; 175°33'4.15 E). *P. crispus* is a naturalised, widely distributed alien species in Aotearoa New Zealand (Figure SI-1), considered native to Eurasia, Africa and Australia [44]. It is regarded as having a ruderal (R) strategy [sensu Grimes [45]]; characterised by fast growth rates and a high disturbance tolerance (i.e. high stem flexibility and root:shoot ratio). *P. ochreatus* is a native species, however not endemic [46], and is also found nation-wide (Figure SI-1), as well as in Australia and south-east Asia. *P. ochreatus* is classified as a competitive-ruderal (CR) strategist [sensu Grimes; 45] and thus shares characteristics of *P. crispus* but is less resistant or resilient to disturbance. At low disturbance and high resource availability both species show traits of high stature, dense canopy formation and high morphological plasticity [45].

2.2. Mesocosms

The experimental setup consisted of four flume mesocosms, each 10 m long and 0.55 m wide, with a water level of 0.35 m and a constant inflow of water at the top (1.5 L min^{-1}) giving a water residence time of ca. 0.8 d. A pump at the lower end of each flume

recirculated water to the top end, to increase water velocity, homogenize water column elemental concentrations and prevent stagnation (Fig. 1). By preventing water stagnation in the high light treatment, this reduced phytoplankton build-up that could affect light climate [e.g. Ref. [47]]. Water from a groundwater bore was purified using a reverse osmosis system (Ecosoft MO10000) with subsequent addition of micro- and macronutrients to create a growth solution excluding nitrogen, bicarbonate and potassium [cf [48]]. Bicarbonate and potassium, to complete the growth medium, were added to the water via peristaltic pumps immediately before entering the flumes, as well as different amounts of NO_3^- targeting four different N concentrations across the flumes: control ($0 \mu\text{g N L}^{-1}$), low ($50 \mu\text{g N L}^{-1}$), medium ($500 \mu\text{g N L}^{-1}$) and high ($5000 \mu\text{g N L}^{-1}$). The target P content (as sodium hydrogen phosphate) of the growth medium was $100 \mu\text{g P L}^{-1}$ and was considered to be in excess in all nitrogen treatments. The flow rates of all pumps were checked at least every three days.

Water samples were taken to verify nutrient concentrations in each treatment. Both top and bottom of the flumes were sampled to test for potential gradients due to nutrient uptake by the plants. Each flume was covered by shade cloth in four different densities, yielding four light treatments. By measuring incoming irradiation (I) (LI-COR LI-200 sensor) above and below the cloth, the irradiance of each was calculated ($I_{\text{below}} \cdot I_{\text{above}}^{-1} \times 100$) as 70%, 44%, 32%, 8% of incident. This is very close to the nominated percentages, and for ease of reading we maintain the naming scheme of 70%, 50%, 30%, and 10% light treatments in which 70% is highest and 10% is lowest. Mean daily incoming irradiance for the duration of the experiment was $96.8 \pm 29.5 \text{ mol photons m}^{-2} \text{ d}^{-1}$ (mean \pm s.d.), thus the light treatments yielded on average 67.7, 44.5, 30.9, and 7.8 $\text{mol photons m}^{-2} \text{ d}^{-1}$ (Figure SI-2).

2.3. Plants

Plants used in experiments comprised 15 cm apical shoots cut from healthy donor plants, potted in 700 ml plastic pots $\frac{3}{4}$ filled with nutrient poor sand [Particulate carbon, particulate nitrogen, and TRP contents were $0.03 \pm 0.006\%$, $0.02 \pm 0.001\%$ and $0.01 \pm 0.001\%$, respectively; [49]]. Cuttings were set to acclimate for at least 3 weeks in their respective nutrient treatments under 50% shade cloth, to ensure internal nitrogen was in equilibrium with the external medium and stored N could not affect growth. At experiment start, 15 cm apical shoots taken from recovered plants were transplanted to new pots and 5 replicates of each species were assigned to each of the 16 treatments. From each nutrient treatment, 5 shoots of each species were taken for mean initial biomass (DW_{start}) and internal C and N measurements (20 in total per species). The plants were grown under experimental conditions for ~ 10 weeks.

2.4. Estimation of photosynthetic parameters

Immediately prior to harvest, we used pulse amplitude modulated (PAM) fluorometry (MONI-DA/S, Heinz Walz GmbH, Germany) to create photosynthesis-irradiance (PI) curves of dark acclimated leaves. PAM utilizes the fluorescence of chlorophyll excited by absorption of photons to estimate the saturation state of photosystem II (PSII) by comparing fluorescence yield under ambient light, when some reaction centres are closed and some open, with that under saturating light (when all reaction centres are closed). Quantum yield of PSII is estimated as $Y = (F_m' - F)/F_m'$, where F is the fluorescence at ambient light and F_m' is fluorescence under saturating light sufficient to close all the reaction centres [50]. The quantum yield of PSII is subsequently used to estimate electron transport rate (ETR) (see below).

The 3rd leaf from the apex was taken from three individuals of each treatment for measurement. If the 3rd leaf was too small to produce viable readings the 4th leaf was taken instead. Leaves were fixed in measuring clips and left in the dark for 30 min before measuring yield at 12 actinic light intensities of increasing magnitude. Subsequently, light absorption factor (AF) of the leaves was determined as the proportion of incident irradiance absorbed by each leaf by measuring irradiance reaching the LiCOR Li200 sensor from a fixed light source (white LED flashlight, similar to the white LED of the PAM instrument) with and without the leaves/top covering the light sensor [50]. Leaves were then frozen for chl. *an* analysis (see below).

ETR of the plants was calculated as $ETR = Y \cdot PAR \cdot AF \cdot 0.5$ where Y is the yield, PAR is the light intensity emitted by the PAM-sensor and AF is the absorption factor of the leaf. ETR was then plotted by the PAR to make the PI-curves. For easier literature comparison we present photosynthesis as mol oxygen produced rather than electrons transferred, we assumed a conversion factor of 0.25 oxygen evolved per electron transferred [50]. To obtain the maximum photosynthetic rate P_{max} and saturation irradiance I_c from the curves, a hyperbolic tangent function $y = P_{\text{max}} \tanh(\alpha x P_{\text{max}}^{-1})$ was fitted to each replicate, and I_c was calculated as $I_c = \alpha P_{\text{max}}^{-1}$ [51]. The parameters P_{max} , I_c and the yield at $PAR_i = 0$ were all chl. *A* corrected.

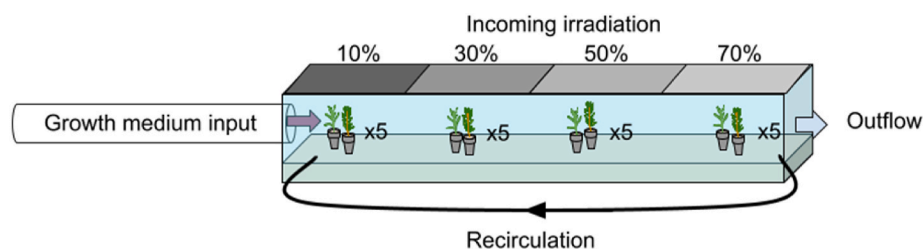


Fig. 1. Schematic representation of the mesocosm flume experimental setup. See text for details.

2.5. Harvest

At harvest, for each plant, the length of the main stem and each branch was measured, and biomass was separated into above and below ground fractions, then dried for 48 h at 60 °C to obtain dry weight (DW). Internal tissue C and N concentrations were obtained from ~2 mg subsamples of finely ground dried biomass, by flash combustion followed by separation of the gaseous products and subsequent mass spectroscopy (Vario EL cube, Elementar, Germany; <0.1% absolute precision). Ratios of tissue C and N are given by weight. Water samples were analyzed for DIN (NH_4^+ , NO_3^-) and dissolved reactive phosphorus (DRP) on a Lachat flow injection analyser (Quikchem® 8500, Hach, USA), with a detection limit of 1 $\mu\text{g L}^{-1}$. Trace elements were analyzed at Hills laboratories in Hamilton, NZ (see supplementary information for details).

Chlorophyll and carotenoid content of the leaves from PAM measurements was determined by UV-VIS spectroscopy of ethanol extracts. Plant material was submerged in liquid nitrogen and left to lyophilise in a freeze drier after which ~5 mg (when possible) was weighed and transferred to a test tube and rehydrated with 0.1 ml Milli-Q water. Some leaves were too small for accurate analysis and thus three leaves of a treatment were pooled together as one replicate. Ethanol (4 ml) was added, and samples were left to extract overnight, in the dark at 4 °C. Absorbance was subsequently measured at 470, 648 and 664 nm on a UV-1800 spectrophotometer (Shimadzu). An additional measurement was taken at 750 nm to be able to correct for impurities. Where a measurable absorbance was measured at 750 nm this was subtracted from that at all other wavelengths.

From the lengths and weights, branching degree (number of branches · main stem length⁻¹), lateral spread (total length · main stem length⁻¹), density (DW · total length⁻¹), root to shoot ratio and relative growth rate (RGR) were calculated. RGR was calculated [cf [52]] as

$$RGR (d^{-1}) = \frac{\ln(DW_{end}) - \ln(DW_{start})}{time} \quad (1)$$

From the UV-VIS spectroscopic absorbances, chlorophyll *a* (C_a), *b* (C_b), total (C_{a+b}) and total carotenoids (C_{x+c} ; all in mg g^{-1} dw) were calculated as follows [53]:

$$C_a = (13.36A_{664} - 5.19A_{648}) \bullet 4.1 \bullet DW^{-1} \quad (2)$$

$$C_b = (27.43A_{648} - 8.12A_{664}) \bullet 4.1 \bullet DW^{-1} \quad (3)$$

$$C_{a+b} = (5.24A_{664} + 22.24A_{648}) \bullet 4.1 \bullet DW^{-1} \quad (4)$$

$$C_{x+c} = (1000A_{470} - 2.13C_a - 97.64C_b) \bullet 4.1 \bullet DW^{-1} \bullet 209^{-1} \quad (5)$$

Where A_{648} , A_{664} and A_{470} is absorbance at 648, 664 and 470 nm respectively and 4.1 is the volume of liquid added to the sample.

2.6. Data analysis

To investigate differences of internal nitrogen (%N), carbon (%C) content and C:N ratio, amongst nutrient treatments, species, and light, we performed Wilcoxon tests between species grouped by nutrient and light treatments, as well as Kruskal-Wallis tests between light and nutrient treatments, grouped by species and nutrient and species and light respectively. Following the Kruskal-Wallis tests, we conducted post hoc pairwise Wilcoxon tests on individual group pairings of light and nutrient treatments. Non-parametric statistics were used as data failed tests of normality and heteroskedasticity.

To evaluate effects of nutrient and light on response parameters, two-way ANOVA tests were attempted, but all parameters failed either or both of Shapiro-Wilk test of normality ($p < 0.05$) or Levene's test of variance homogeneity ($p < 0.05$), even after log transformation of data. Thus, non-parametric Wilcoxon rank-sum tests were employed to ascertain differences between species within light and N groups and between N groups within light treatments and species. Kruskal-Wallis tests were carried out on light treatments within N group and species, with post hoc pairwise Wilcoxon tests (with Bonferroni-Holm p adjustment method) between specific light levels.

Table 1

Resulting nutrient concentrations of the water samples taken at top end (upstream) and lower end (downstream) of the mesocosm flumes ($n = 1$).

		DRP $\mu\text{g L}^{-1}$	NH_4^+ $\mu\text{g L}^{-1}$	NO_3^- $\mu\text{g L}^{-1}$	DIN $\mu\text{g L}^{-1}$	DIN:DRP
Control	Upstream	52	20	1	21	0.4 : 1
	Downstream	57	1	<1	<2	<0.04 : 1
Low	Upstream	63	8	21	29	0.5 : 1
	Downstream	71	7	17	24	0.3 : 1
Medium	Upstream	49	23	264	287	5.9 : 1
	Downstream	54	62	252	314	5.8 : 1
High	Upstream	60	15	5470	5485	91 : 1
	Downstream	49	20	5580	5600	114 : 1

3. Results

3.1. Nutrient concentrations in water and plants

Dissolved reactive phosphorous concentrations were similar amongst flumes, whereas a clear distinction was evident between DIN concentrations of the four nutrient treatments (Table 1). Differences between inflow and outflow were negligible except for the control flume which had more ammonium (NH_4^+) in the upstream part. DIN:DRP ratios ranged from <0.04:1 in the control flume to as much as 114:1 in the high DIN flume.

Both species within light treatments showed significant differences in tissue %N content amongst nutrient treatments, whereas in only two instances difference in tissue %C were significant (Kruskal-Wallis rank-sum test $p < 0.05$; Table 2, Fig. 2). Similarly, both species mostly differed in %N content amongst the light treatments and less so for %C content (Table 3, Fig. 2). C:N ratios tended to follow %N, given the greater between-treatment variation in %N than %C. As the number of post hoc Wilcoxon tests between % N, % C, and C:N ratios, within light and nutrient treatments are very high (288 tests in total), the results of the individual tests are omitted here but in summary the results showed two consistent patterns: 1) Within nutrient treatments, there were more significant differences found between control or low and medium or high nutrient treatments than between control and low, and medium and high. Out of 24 comparisons, control differed from low only 6 times, whereas control differed from medium and high 15 and 19 times, respectively, and low differed from medium and high 14 and 15 times, respectively. Medium and high differed in only 3 out of 24 comparisons. 2) Within light treatments, there were more differences between the 10% light treatment and any other light treatment than amongst the 70, 50, and 30% light treatments. Out of 72 comparisons involving the 10% light treatment 27 of these were significantly different, while of the 72 comparisons involving the other treatments only 6 were significantly different.

Thus, internal tissue N content was broadly similar in the two higher and the two lower nutrient treatments in both species (Fig. 2a). Consequently, in terms of internal N status, the four N treatments collapsed into high (High and Medium) and low (Low and Control) N-groups (Fig. 2c). For further analyses, we therefore combined high and medium N treatments, and low and control N treatments as high-N and low-N, respectively, as well as individuals from the high-N and low-N treatments will be referred to as high-N individuals and low-N individuals, respectively.

The only noteworthy difference between species was that *P. crispus* incorporated more N in the 70% and 50% light treatment than *P. ochreatus*, but only at 70% light did it lead to a difference in C:N ratio (Supplementary Table 3). No obvious differences were seen in the C content of the two plants. Although *P. ochreatus* tended to have slightly higher C content than *P. crispus* (Fig. 2b), this did not give rise to differences in C:N ratio (Fig. 2c).

3.2. Growth and morphology

High-N individuals had higher RGR than low-N ones regardless of species or light treatment save for two instances (Table 4), although *P. crispus* at 70% light was close to the 0.05 mark ($p = 0.08$). Generally, *P. crispus* had higher variation than *P. ochreatus* meaning that some individuals achieved much higher RGR's than *P. ochreatus* at high-N, the highest (0.06 d^{-1}) found under high

Table 2

Results of Kruskal-Wallis tests of internal tissue carbon and nitrogen content difference amongst nutrient treatments for each of the two *Potamogeton* species within light treatments. $P < 0.05$ denoted by **, $P < 0.01$ by ***.

Species	Light treatment	Response parameter	n	Kruskal-Wallis rank-sum statistic	P
<i>P. crispus</i>	70%	% N	20	14.3	0.002 **
<i>P. crispus</i>	50%	% N	20	16.1	0.001 **
<i>P. crispus</i>	30%	% N	20	14.8	0.002 **
<i>P. crispus</i>	10%	% N	18	10.8	0.013 *
<i>P. ochreatus</i>	70%	% N	20	14.7	0.002 **
<i>P. ochreatus</i>	50%	% N	20	16.2	0.001 **
<i>P. ochreatus</i>	30%	% N	20	16.1	0.001 **
<i>P. ochreatus</i>	10%	% N	18	13.4	0.004 **
<i>P. crispus</i>	70%	% C	20	6.9	0.075
<i>P. crispus</i>	50%	% C	20	9.2	0.027 *
<i>P. crispus</i>	30%	% C	20	3.4	0.34
<i>P. crispus</i>	10%	% C	18	1.7	0.64
<i>P. ochreatus</i>	70%	% C	20	14.4	0.002 **
<i>P. ochreatus</i>	50%	% C	20	6.4	0.093
<i>P. ochreatus</i>	30%	% C	20	4.3	0.23
<i>P. ochreatus</i>	10%	% C	18	6.2	0.10
<i>P. crispus</i>	70%	C:N ratio	20	14.6	0.002 **
<i>P. crispus</i>	50%	C:N ratio	20	16.1	0.001 **
<i>P. crispus</i>	30%	C:N ratio	20	14.9	0.002 **
<i>P. crispus</i>	10%	C:N ratio	18	11.0	0.012 *
<i>P. ochreatus</i>	70%	C:N ratio	20	15.6	0.001 **
<i>P. ochreatus</i>	50%	C:N ratio	20	16.4	0.001 **
<i>P. ochreatus</i>	30%	C:N ratio	20	16.2	0.001 **
<i>P. ochreatus</i>	10%	C:N ratio	18	13.4	0.004 **

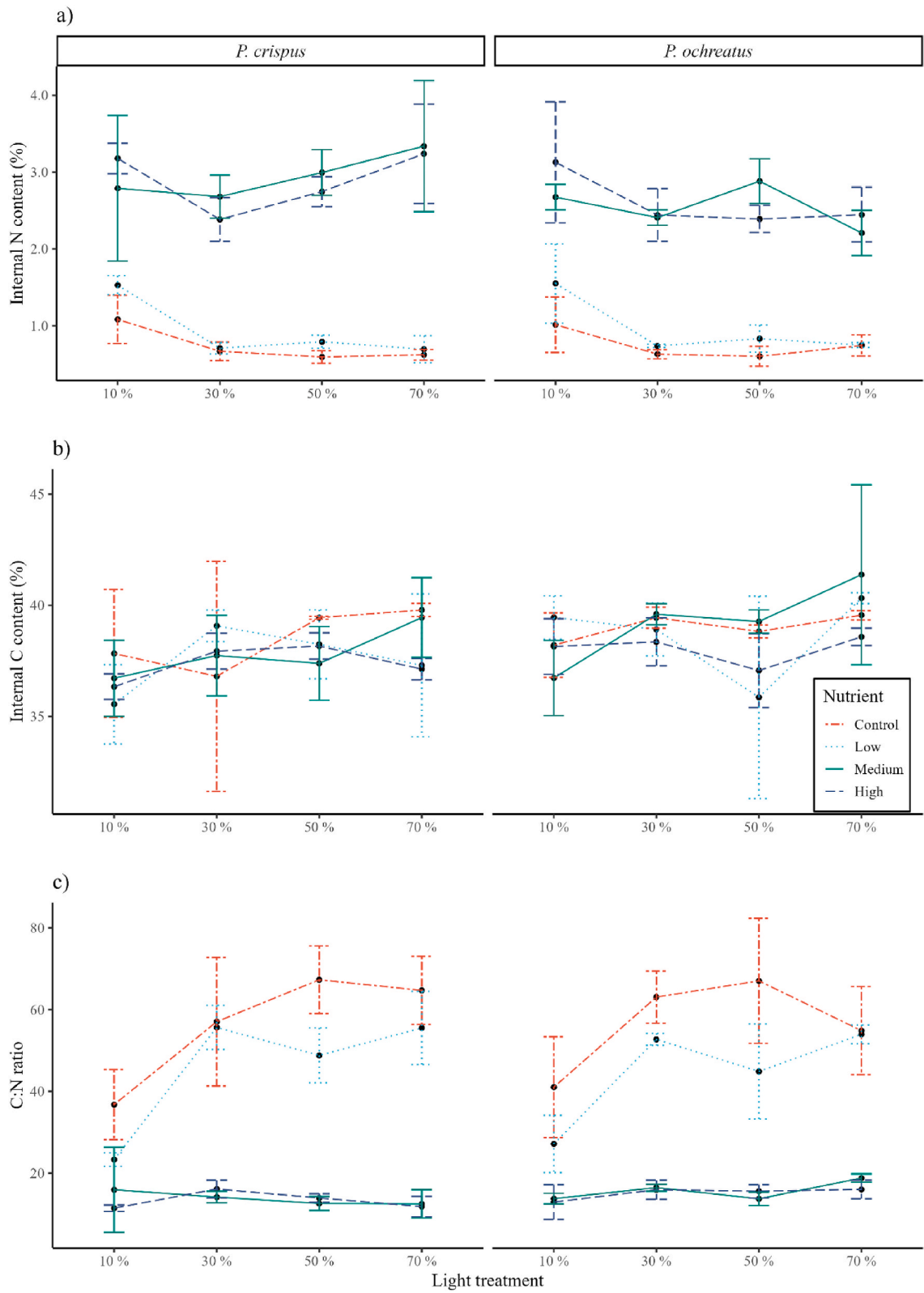


Fig. 2. Internal tissue % nitrogen (N), % carbon (C), and C:N ratio of *Potamogeton crispus* and *P. ochreatus* grown at four nutrient and light levels. Lines connect mean values between light treatments and errorbars denote standard deviation.

Table 3

Results of Kruskal-Wallis tests of internal tissue carbon and nitrogen content difference amongst light treatments for each of the two *Potamogeton* species within nutrient treatments. P < 0.05 denoted by ‘*’, P < 0.01 by ‘**’.

Species	Nutrient treatment	Response parameter	n	Kruskal-Wallis rank-sum statistic	P
<i>P. crispus</i>	Control	% N	19	9.4	0.02 *
<i>P. crispus</i>	Low	% N	19	10.8	0.01 *
<i>P. crispus</i>	Medium	% N	20	3.8	0.29
<i>P. crispus</i>	High	% N	20	11.0	0.01 *
<i>P. ochreatus</i>	Control	% N	20	8.0	0.05 *
<i>P. ochreatus</i>	Low	% N	19	9.9	0.02 *
<i>P. ochreatus</i>	Medium	% N	20	11.5	0.009 **
<i>P. ochreatus</i>	High	% N	19	2.6	0.46
<i>P. crispus</i>	Control	% C	19	4.7	0.20
<i>P. crispus</i>	Low	% C	19	6.2	0.10
<i>P. crispus</i>	Medium	% C	20	4.5	0.22
<i>P. crispus</i>	High	% C	20	13.3	0.004 **
<i>P. ochreatus</i>	Control	% C	20	8.9	0.03 *
<i>P. ochreatus</i>	Low	% C	19	7.7	0.05
<i>P. ochreatus</i>	Medium	% C	20	12.0	0.007 **
<i>P. ochreatus</i>	High	% C	19	2.5	0.47
<i>P. crispus</i>	Control	C:N ratio	19	9.4	0.02 *
<i>P. crispus</i>	Low	C:N ratio	19	10.4	0.02 *
<i>P. crispus</i>	Medium	C:N ratio	20	3.3	0.35
<i>P. crispus</i>	High	C:N ratio	20	11.3	0.01 *
<i>P. ochreatus</i>	Control	C:N ratio	20	8.9	0.03 *
<i>P. ochreatus</i>	Low	C:N ratio	19	8.8	0.03 *
<i>P. ochreatus</i>	Medium	C:N ratio	20	15.3	0.002 **
<i>P. ochreatus</i>	High	C:N ratio	19	2.8	0.43

irradiance (70%), while many individuals at low-N had negative RGR's regardless of light treatment. However, a significant difference between species was only found between means of the low-N individuals at the lowest irradiance (10% - $W = 14$, $p = 0.04$; Table 4), which was the only treatment where *P. ochreatus* individuals had negative RGR's. Both species showed a similar response trend to changes in light, by having reduced RGR's at the lowest light treatment (10%), but for the high-N plants, the significant difference was for *P. ochreatus*, while for low-N plants it was for *P. crispus* (Table 4).

P. crispus showed a significant difference between high-N and low-N in branching degree and lateral spread at the highest light treatment only (Table 5). Generally, *P. crispus* responded to increased N content with main shoot length increases (Table 5), while *P. ochreatus* responded with increases in branching degree and lateral spread (Table 5). Both species responded to increase in N content by increased root extension (Table 5) and increase in below-ground/above-ground biomass ratio (Table 5). For all traits but the below-ground/above-ground biomass ratio, no difference between high-N and low-N plants were found at the 10% light treatment. For high-N *P. crispus* plants decreasing irradiance intensity tended to decrease branching degree and lateral spread and high-N *P. ochreatus* plants had the lowest main shoot length at 10% light (Table 5). *P. ochreatus* achieved longer shoot lengths than *P. crispus* at low-N, and higher densities overall (Table 5), while *P. crispus* had generally higher branching degree and lateral spread than *P. ochreatus*, regardless of light treatment and N group (Table 5).

Table 4

RGR mean (\pm SD) values of High-N and Low-N groups of *Potamogeton crispus* and *P. ochreatus* at four different light treatments. Difference between N groups for each species are reported below. Difference between species within N group and light treatment are shown as bold text on the highest scoring species if significant (Wilcoxon rank sum test: W stat < 10, $p < 0.05$). Differences of the means between light treatments within species and N-group are shown as alphabetical operators after each mean value (i.e. ^{abc}), as tested by pairwise Wilcoxon tests.

Parameter	N-group	Species	Light treatment			
			70%	50%	30%	10%
RGR (d^{-1})	High-N	<i>P. crispus</i>	0.021 ^a	0.019 ^a	0.025 ^a	0.006 ^a
		<i>P. ochreatus</i>	± 0.018	± 0.011	± 0.013	± 0.011
		<i>P. ochreatus</i>	0.018 ^a	0.021 ^a	0.017 ^a	0.005 ^b
		<i>P. ochreatus</i>	± 0.006	± 0.006	± 0.007	± 0.002
	Low-N	<i>P. crispus</i>	0.003 ^a	0.008 ^a	0.005 ^{ab}	-0.006 ^b
		<i>P. ochreatus</i>	± 0.009	± 0.004	± 0.011	± 0.005
		<i>P. ochreatus</i>	0.006 ^{ab}	0.009 ^a	0.008 ^a	0.000^b
		<i>P. ochreatus</i>	± 0.004	± 0.004	± 0.004	± 0.006
Difference between N groups	<i>P. crispus</i>	$W = 74$	$W = 84$	$W = 65$	$W = 89$	
	<i>P. ochreatus</i>	$W = 98$	$W = 88$	$W = 93$	$W = 53$	

Table 5

Mean (+ SD) values for growth responses of High-N and Low-N groups of *Potamogeton crispus* and *P. ochreatus* at four different light treatments. Difference between N groups for each species are reported below each parameter. Difference between species within N group and light treatment are shown as bold text on the highest scoring species if significant (Wilcoxon rank sum test: W stat <10, p < 0.05). Differences of the means between light treatments within species and N-group are shown as alphabetical operators after each mean value (i.e. ^{abc}), as tested by pairwise Wilcoxon tests.

Parameter	N-group	Species	% Incoming irradiation			
			70%	50%	30%	10%
Branching degree (cm ⁻¹)	High-N	<i>P. crispus</i>	1.3^a	0.8 ^b	1.1 ^{ab}	0.4 ^c
		<i>P. ochreatus</i>	±0.5	±0.4	±1.1	±0.2
	Low-N	<i>P. crispus</i>	0.6 ^{ab}	1.0 ^a	0.4 ^{ab}	0.2 ^b
		<i>P. ochreatus</i>	±0.6	±0.8	±0.5	±0.1
		<i>P. crispus</i>	0.6^a	1.1^a	0.7^a	0.4^a
		<i>P. ochreatus</i>	±0.2	±0.9	±0.3	±0.2
Difference between N groups	<i>P. crispus</i>	0.2 ^a	0.4 ^a	0.1 ^a	0.1 ^a	
	<i>P. ochreatus</i>	±0.1	±0.4	±0.0	±0.1	
Lateral spread (cm cm ⁻¹)	High-N	<i>P. crispus</i>	W = 94	W = 94	W = 47	W = 66
		<i>P. ochreatus</i>	W = 81	W = 77	W = 67	W = 48
	Low-N	<i>P. crispus</i>	6.6^a	3.9 ^b	4.3 ^b	2.5 ^c
		<i>P. ochreatus</i>	±2.6	±1.3	±1.5	±1.3
		<i>P. crispus</i>	3.2 ^{ab}	4.8 ^a	2.8 ^{ab}	1.8 ^b
		<i>P. ochreatus</i>	±2.2	±2.7	±2.3	±0.6
Difference between N groups	<i>P. crispus</i>	3.1^a	4.2 ^a	3.5 ^{ab}	2.1 ^a	
	<i>P. ochreatus</i>	±1.0	±2.7	±1.3	±0.7	
Main shoot length (cm ⁻¹)	High-N	<i>P. crispus</i>	1.6 ^a	2.5 ^a	1.4 ^{ab}	1.8 ^a
		<i>P. ochreatus</i>	±0.5	±1.5	±0.4	±0.9
	Low-N	<i>P. crispus</i>	W = 96	W = 96	W = 51	W = 63
		<i>P. ochreatus</i>	W = 71	W = 81	W = 79	W = 39
		<i>P. crispus</i>	11.4 ^a	14.9 ^a	20.1 ^a	9.1 ^a
		<i>P. ochreatus</i>	±6.7	±11.7	±12.3	±4.6
Difference between N groups	<i>P. crispus</i>	13.0 ^a	11.6 ^a	14.0 ^a	9.4 ^b	
	<i>P. ochreatus</i>	±3.3	±1.8	±3.4	±1.6	
Root length (cm)	High-N	<i>P. crispus</i>	7.5 ^a	6.1 ^a	7.2 ^a	7.0 ^a
		<i>P. ochreatus</i>	±1.9	±2.0	±2.4	±3.0
	Low-N	<i>P. crispus</i>	10.6^a	9.7^a	11.8^a	10.1^a
		<i>P. ochreatus</i>	±1.9	±3.5	±1.0	±1.8
		<i>P. crispus</i>	W = 72	W = 72	W = 82	W = 88
		<i>P. ochreatus</i>	W = 74	W = 67	W = 74	W = 25
Difference between N groups	<i>P. crispus</i>	9.6 ^a	10.6 ^a	13.3 ^a	5.6 ^a	
	<i>P. ochreatus</i>	±4.4	±5.8	±7.4	±4.1	
Density (mg cm ⁻¹)	High-N	<i>P. crispus</i>	7.7 ^{ab}	13.9 ^a	9.6 ^{ab}	5.6 ^b
		<i>P. ochreatus</i>	±6.6	±6.1	±6.6	±4.5
	Low-N	<i>P. crispus</i>	4.1	4.8	3.6	3.4
		<i>P. ochreatus</i>	±2.5	±3.6	±3.4	±3.4
		<i>P. crispus</i>	4.3	4.7	4.1	6.2
		<i>P. ochreatus</i>	±3.0	±3.1	±4.5	±4.3
Difference between N groups	<i>P. crispus</i>	W = 88	W = 88	W = 76	W = 91	
	<i>P. ochreatus</i>	W = 64	W = 88	W = 77	W = 39	
Below-/aboveground biomass ratio	High-N	<i>P. crispus</i>	0.003 ^a	0.002 ^a	0.002 ^a	0.003 ^a
		<i>P. ochreatus</i>	±0.002	±0.001	±0.001	±0.002
	Low-N	<i>P. crispus</i>	0.004^a	0.004^a	0.004^a	0.004^a
		<i>P. ochreatus</i>	±0.001	±0.001	±0.001	±0.001
		<i>P. crispus</i>	0.003 ^a	0.003 ^a	0.003 ^a	0.003 ^a
		<i>P. ochreatus</i>	±0.001	±0.001	±0.001	±0.005
Difference between N groups	<i>P. crispus</i>	0.004^a	0.004 ^a	0.005^a	0.003^a	
	<i>P. ochreatus</i>	±0.002	±0.001	±0.001	±0.001	
Below-/aboveground biomass ratio	High-N	<i>P. crispus</i>	W = 45	W = 45	W = 20	W = 49
		<i>P. ochreatus</i>	W = 43	W = 46	W = 38	W = 52
	Low-N	<i>P. crispus</i>	0.63 ^a	0.22 ^a	0.36 ^a	0.20 ^a
		<i>P. ochreatus</i>	±0.57	±0.09	±0.55	±0.23
		<i>P. crispus</i>	0.23 ^a	0.21 ^a	0.18 ^a	0.27 ^a
		<i>P. ochreatus</i>	±0.26	±0.10	±0.16	±0.28
Difference between N groups	<i>P. crispus</i>	0.07 ^a	0.05 ^a	0.01 ^a	0.01 ^a	
	<i>P. ochreatus</i>	±0.09	±0.06	±0.02	±0.01	
Difference between N groups	<i>P. crispus</i>	0.05 ^a	0.06 ^a	0.02 ^a	0.11 ^a	
	<i>P. ochreatus</i>	±0.06	±0.08	±0.02	±0.19	
Difference between N groups	<i>P. crispus</i>	W = 84	W = 84	W = 76	W = 80	
	<i>P. ochreatus</i>	W = 53	W = 79	W = 49	W = 53	

3.3. Photosynthetic parameters

No clear patterns emerged from the photosynthetic parameters, although in all cases of statistical difference, high-N plants had the higher trait score (Table 6). Two trends can be noticed, however; 1) high-N plants of both species tended to have higher saturation irradiance (E_k) than low-N plants, and for *P. ochreatus* at 70 and 30% light, this was reflected in a trend of a lower quantum yield (Φ); 2) high-N plants had higher maximum photosynthetic rate (P_{max}) at 10% light. Significant differences between species were only found for low-N individuals at 50% light, where *P. ochreatus* had higher P_{max} and E_k , and *P. crispus* had higher yield at PAR = 0 (Table 6).

Significant differences between light treatments were found for low-N individuals of *P. ochreatus*, that had lower P_{max} and higher Yield at PAR = 0 at 10% light compared to all other shade treatments (Table 6). *P. ochreatus* also showed difference between E_k at 70% and 10% light for high-N plants (Table 6). Kruskal-Wallis test results showed differences amongst light treatments in low-N *P. crispus* plants for E_k and Yield at PAR = 0 ($P = 0.02$), but none of the pairwise Wilcoxon tests were significant (Table 6).

As pigment samples had to be pooled across replicates to obtain sufficient for analysis, robust statistical comparisons were not possible. However it was evident that the content chlorophyll-a, chlorophyll-b and total carotenoids increased with N content for all light treatments in both species (Figure SI-3). The range of content of each pigment showed no difference between species.

4. Discussion

We tested differences between growth rates, morphological traits, and photosynthetic performance parameters of two species of

Table 6

Mean (+ SD) values of photosynthetic parameters from High-N and Low-N groups of *Potamogeton crispus* and *P. ochreatus* grown at four different light treatments. Difference between N groups for each species are reported below each parameter. Difference between species within N group and light treatment are shown as bold text on the highest scoring species if significant (Wilcoxon rank sum test: W stat <10, $p < 0.05$). Differences of the means between light treatments within species and N-group are shown as alphabetical operators after each mean value (i.e. ^{abc}), as tested by pairwise Wilcoxon tests.

Parameter	N-group	Species	% Incoming irradiation				
			70%	50%	30%	10%	
Quantum yield Φ	High-N	<i>P. crispus</i>	0.013 ^a ±0.008	0.017 ^a ±0.013	0.013 ^a ±0.009	0.019 ^a ±0.015	
		<i>P. ochreatus</i>	0.015 ^a ±0.010	0.014 ^a ±0.010	0.007 ^a ±0.007	0.014 ^b ±0.010	
	Low-N	<i>P. crispus</i>	0.017 ^a ±0.000	0.009 ^a ±0.007	0.009 ^a ±0.010	0.012 ^a ±0.007	
		<i>P. ochreatus</i>	0.028 ^a ±0.018	0.012 ^a ±0.006	0.015 ^a ±0.007	0.006 ^a ±0.005	
	Difference between N groups		<i>P. crispus</i>	W = 3	W = 18	W = 16	W = 25
			<i>P. ochreatus</i>	W = 8	W = 17	W = 7	W = 28
E_k (mmol photons $m^{-2}s^{-1}$)	High-N	<i>P. crispus</i>	51 ^a ±37	62 ^a ±43	66 ^a ±22	34 ^a ±13	
		<i>P. ochreatus</i>	88 ^a ±19	53 ^{ab} ±27	66 ^{ab} ±15	42 ^b ±14	
	Low-N	<i>P. crispus</i>	29 ^a ±0	13 ^a ±3	49 ^a ±27	20 ^a ±3	
		<i>P. ochreatus</i>	33 ^a ±8	41 ^a ±23	47 ^a ±30	18 ^a ±7	
	Difference between N groups		<i>P. crispus</i>	W = 4	W = 24	W = 16	W = 32
			<i>P. ochreatus</i>	W = 24	W = 19	W = 20	W = 35
P_{max} (mmol O ₂ $m^{-2}s^{-1}$)	High-N	<i>P. crispus</i>	0.6 ^a ±0.4	1.0 ^a ±1.0	0.8 ^a ±0.5	0.6 ^a ±0.5	
		<i>P. ochreatus</i>	1.2 ^a ±0.8	0.7 ^a ±0.5	0.4 ^a ±0.3	0.6 ^b ±0.5	
	Low-N	<i>P. crispus</i>	0.5 ^a ±0.0	0.1 ^a ±0.1	0.6 ^a ±0.9	0.2 ^a ±0.1	
		<i>P. ochreatus</i>	0.8 ^a ±0.4	0.4 ^a ±0.3	0.8 ^a ±0.7	0.1 ^b ±0.1	
	Difference between N groups		<i>P. crispus</i>	W = 3	W = 22	W = 14	W = 26
			<i>P. ochreatus</i>	W = 16	W = 22	W = 9	W = 32
Yield (PS II) at PAR = 0	High-N	<i>P. crispus</i>	0.6 ^a ±0.1	0.6 ^a ±0.1	0.7 ^a ±0.1	0.6 ^a ±0.1	
		<i>P. ochreatus</i>	0.6 ^a ±0.1	0.7 ^a ±0.0	0.5 ^a ±0.2	0.6 ^b ±0.1	
	Low-N	<i>P. crispus</i>	0.7 ^a ±0.0	0.7 ^a ±0.0	0.5 ^a ±0.1	0.6 ^a ±0.0	
		<i>P. ochreatus</i>	0.5 ^a ±0.0	0.5 ^a ±0.1	0.5 ^a ±0.0	0.6 ^b ±0.0	
	Difference between N groups		<i>P. crispus</i>	W = 5	W = 10	W = 24	W = 15
			<i>P. ochreatus</i>	W = 19	W = 30	W = 24	W = 15

Potamogeton, the naturalised *P. crispus* and the native *P. ochreatus*, grown under four N concentrations then split into two N-groups based on internal N content in combination with four irradiances.

4.1. Factors controlling rate of growth - interplay of light and nutrients

Our hypotheses were partly supported; internal N content increased with N treatment, but this did not directly correspond to external concentration; the four nitrogen treatments appeared to collapse to two. Except at the lowest irradiance, the two lowest N treatments in both species yielded % N contents of less than 1%, while at all irradiances the two highest N treatments yielded similar N contents between 2 and 3%. The internal nutrient content of a plant is widely recognised as an indicator of its growth potential, as this represents the nutrients available for production of biomass; below certain %N thresholds, the growth of the plant will be limited, or the plant will senesce [54]. These thresholds can vary between species [55], but a mean N content for 95% maximum growth and 95% maximum quantum yield for aquatic macrophytes is reported as 1.82% N and 1.14% N, respectively [see 31]. Minimum macrophyte content of N to permit growth is reported to be 0.6% [54] and in general, N contents of <1% are rare in natural freshwater macrophytes [e.g. 31]. Internal tissue concentrations above 3% is considered high for *P. crispus* [42] though Cavalli et al. [32] reports concentrations as high as 4.7% N. Furthermore, N limitation thresholds can be approximated by plotting N content against C:N ratios, and estimating when % N starts changing more so than the C:N ratio [56]. Using this approach, Cavalli et al. [32] suggests a limitation threshold of <2% for tissue N which corresponds reasonably well with our data when we plot our C:N ratios against % N (Fig. 3a) and look at the correlation between RGR and % N overall (Fig. 3b).

Large scale surveys have found that *in situ* freshwater angiosperms normally contain between 2 and 3% tissue N [33], and Demars and Edwards [31] confirmed this, citing a median N content of 2.64% in a dataset of 268 samples across 765 sites. Higher N content (and consequently lower C:N ratio) in plants grown at low light is probably due to lower nutrient demand from growth. Similar effects of other abiotic factors influencing N limitation have been found by Madsen et al. [57], who finds that low inorganic carbon concentrations lower the growth rates of two aquatic macrophytes and consequently allows an increase in their N content due to lower demand. Adding to the complexity of nutrient dynamics is the fact that the ability to maintain a high N content during growth will vary between species as they inherently vary in nutrient uptake rates, and that these rates differ for NO_3^- and NH_4^+ [52]. Nevertheless, we have effectively created eutrophic and oligotrophic environments, which yield the high-N and low-N groups, and plants that are at

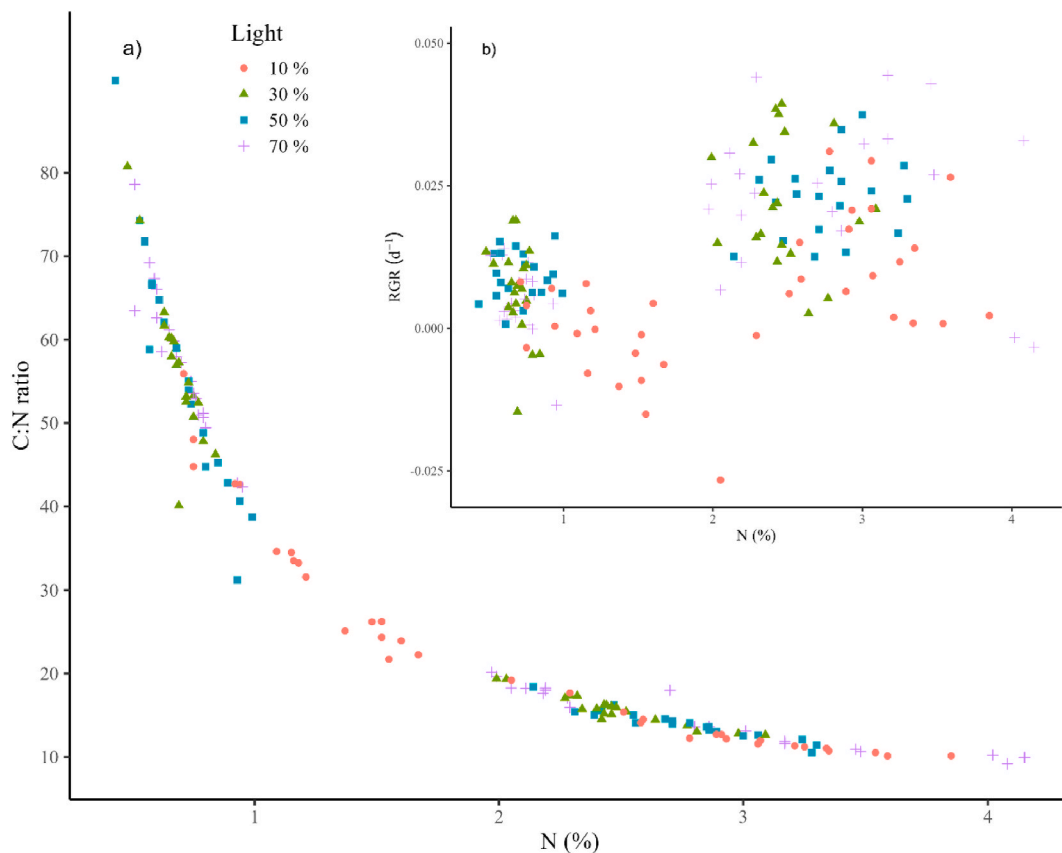


Fig. 3. a) % N content of all plants from the experiment plotted against C:N ratio for threshold analysis [56]. The inflexion point of the curve indicates onset of N saturation. And b) the relationship between % N content and RGR - a clear threshold can be witnessed at the 2% N mark.

growth saturating and extreme growth limiting nitrogen content. At water column concentrations of more than $\sim 290 \mu\text{g NO}_3^- \text{L}^{-1}$ internal N content of our plants did not scale with higher water column N and similarly, plants from $\sim 30 \mu\text{g NO}_3^- \text{L}^{-1}$ achieved minimum N content as well as those with no N supply (Fig. 2).

There was an obvious effect of nutrient content on the response parameters of the plants, in support of our second hypothesis; plant traits were generally higher for high-N plants, as were the contents of pigments. Still, we expected a larger difference between the high-N and low-N plants, particularly at the higher irradiances. We have no good explanation as to why so many of the *P. crispus* high-N plants did not attain better RGR's. Manolaki et al. [52] finds for *P. crispus* a mean RGR of 0.06 day^{-1} under non-limiting light and nutrient conditions, suggesting an inherent ability of this species to grow even faster than what was attained here. Not much research has been done on growth of *P. ochreatus*, but Cenzato and Ganf [58] finds maximum RGR of *P. ochreatus* grown in eutrophic sediment to be around 0.02 d^{-1} , comparable to our results. Both species are classified as high N loving species [45] and as such are well adapted to be able to utilize a high resource availability for growth as we see in our experiment. Indeed, pigment content for both species were high compared to previously reported values for similar plants [59–61], suggesting ample capability to upscale the photosynthetic apparatus when resource supply is high. Thus, even though evidently, our plants have not been able to perform to their maximum potential (see supplementary info for details), from the increased growth rates we can see that ecological issues like nuisance biomass will most likely occur when available N concentrations are higher, even when light is quite reduced. We find it interesting that the largest main shoot length difference was found between N groups for *P. crispus*, while *P. ochreatus* differed more in branching degree and lateral spread, when main shoot length is classified as a C-strategist trait, while the others are R-strategist traits [45] and thus reversed cf. the species Grime's classifications. Possibly, this is an evolutionary strategy of the plants to allocate relatively more resources into traits that fall within their Grime's classification category when resources are scarce and when resources are plenty, there is a surplus to allocate to additional traits. We had anticipated the low-N plants to increase root length more than the high-N plants, to explore the sediment as a nutrient source. A possible explanation for longer roots at high N is flow induced root biomass allocation [62].

Our third hypothesis was mostly supported, as we see no difference between N groups for most parameters at the lowest irradiance and most of the differences between irradiance levels was between 10% light and the other levels. Still, we did not expect a difference in RGR between low-N and high-N plants at this irradiance level (*P. crispus* Table 4). Although certainly light limited when compared to the other irradiance levels, 10% light high-N plants were still able to grow. Compensation irradiance points for the plants are reported as $152 \pm 23 \text{ lux}$ ($\sim 2.5 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) at 15°C for *P. crispus* [63] and $37 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ [58] for *P. ochreatus* (daily average), both of which are lower than what the plants are exposed to in our experiment. We note however, that the compensation point irradiance of the *P. ochreatus* is the ecological, while for *P. crispus* it is the instantaneous and the ecological is expected to be higher [64]. Furthermore, the conversion from lux to mol photons depends on the type of light source used and this is not stated in the experiment [63], thus the conversion made here can only ever be approximate. Thus, even at these low irradiances both species can maintain positive growth rates, but evidently shading is a constraining factor. Low irradiance has recently been shown to be important in restricting the presence and growth of invasive species [7] and can influence the plants ability to cope with adverse conditions [65]. The results of our study further exemplify the importance of the light environment and highlights the need for this to be considered when investigating the effects of nutrients. Successful strategies of plants in eutrophic streams are either to grow to the water surface for better light capture or better light utilization for tolerance of out shading [39]. As neither of the species exhibited signs of acclimation to shading at the lowest irradiance, i.e. increased quantum yield (α^B) and lower P_{max} , we see no evidence that either of the tested species were opting for the light utilization strategy although we did not test other parameters that respond to irradiance such as reduced specific leaf area [66].

We see some evidence in support of our fourth hypothesis, partly due to *P. crispus* having higher maximum RGR's and thus have the potential to outgrow *P. ochreatus*. Moreover, *P. crispus* had increases in branching degree and lateral spread only in the high light treatment when the N content was high. Furthermore, when the light was only at an intermediate level, the increase in lateral spread and branching degree was not significantly different between N groups but was different for main shoot length. Thus, although growth rates are similar, when resources are high, as we would expect to see in streams in an anthropogenic setting, we see a response of extending laterally with many branches rather than vertically, which is beneficial for dispersal (i.e. increases likelihood of fragmentation), as *P. crispus* spreads by vegetative formation in New Zealand [67]. This response is virtually absent in *P. ochreatus* which generally show the same responses to increases in N content in all but the lowest irradiance treatment. Certainly, the species that suffered the most under low light and low nutrient levels was *P. crispus*, showing the importance of ample resource supply for this species.

4.2. Implications for thresholds and management perspective

Eutrophication of stream ecosystems have consequences for macrophyte communities that then propagate through the higher trophic levels and adversely affect ecosystem services. In this study we present results showing how low $\text{NO}_3\text{-N}$ concentrations in the water column can significantly reduce internal N content and growth rates of plants and how this can be seen across a wide range of irradiance but was less evident below 10% incident. It is important to acknowledge that the mesocosm environment does not mirror a natural setting to perfection. In our case, the plants were exposed to a continuous, stable supply of nutrients and flow rate was kept at a constant low level, creating a constant flux of nutrients from the water to the plants. In a natural setting, precipitation events will likely play an important, pulsed role in supplying N to stream plants, by washing out N from soils and diminishing boundary layer thickness. Especially for oligotrophic streams, this will, at least temporarily, provide a boost of N that can be stored in the plants and help maintain growth even though consequent stream conditions are yet again oligotrophic. In addition, internal N content of the pre

experiment plants were mostly above the 1.82% threshold for growth as determined by Demars and Edwards [31] and thus it must be assumed that this stored N has in part been available to support growth during the experiment. N uptake rates vary little between *Potamogeton* species [52], thus utilization might be the key differentiator. Further studies are required in the field of temporarily elevated N concentrations and the legacy effect they can have on plant communities.

Despite these experimental limitations, our hypotheses were able to be tested and were partly supported. Most importantly, plants grown under higher nitrate conditions sustained high % N, had higher growth rates, and higher trait scores than plants grown under lower nitrate conditions, suggesting eutrophication thresholds for nitrate-N lie somewhere between the concentrations of the low ($47 \mu\text{g N L}^{-1}$) and medium ($290 \mu\text{g N L}^{-1}$) nutrient treatments in our experiment. The current guideline trigger value for nitrate concentration in lowland streams in New Zealand is $444 \mu\text{g N L}^{-1}$, a concentration which would offer little protection from macrophyte proliferation. Mean concentrations of nitrate-N in lowland streams draining pastoral and urban areas in New Zealand are above these levels (1102 and $705 \mu\text{g N L}^{-1}$ respectively), while that of streams draining natural catchments is $78 \mu\text{g N L}^{-1}$ [68]. Pastoral and urban streams also had much lower ecological health scores [69], consistent with eutrophication loss of ecosystem values. There is thus little expectation that growth of existing populations of either species in lowland rivers of Aotearoa New Zealand, of which only 5% of those in Larned et al. [68] were in natural catchments, are nutrient limited.

Under conditions prevailing in the few lowland streams of Aotearoa New Zealand with minimal anthropogenic change to their catchments, water nitrate concentrations may be sufficiently low to limit the rate of growth of the two *Potamogeton* spp investigated here. In addition, lowland forest provides shading and under the combination of low nitrogen supply with a high degree of shading the native *P. ochreatus* did exhibit a higher RGR than the alien *P. crispus*. It was generally better and maintaining positive growth rates under combined nutrient and light limiting conditions. The suggestion in our results is that there is potential for the alien *P. crispus* to out-grow the native *P. ochreatus* in more eutrophic, brightly lit systems and informs the “phenotypic convergence – phenotypic divergence” paradigm in invasive species theory [70]. Our example suggests that the non-native species may be successful because it is pre-adapted to better exploit conditions prevailing in newly modified catchments, specifically increased resource supply, than pre-existing native organisms.

Our results provide some insight into setting limits to manage macrophyte proliferation in streams of Aotearoa New Zealand. They suggest that reducing resource supply could be an effective management tool to both reverse the advantage from *P. crispus* back to *P. ochreatus*, and also reduce growth rates, as could the restoration of riparian shade. However, a substantial reduction, up to tenfold in many cases [68] in nitrate may be needed that would require significant changes to lowland catchment land use. Such socioeconomic challenges of lowland stream restoration are persistent, and a recent review of forty years of effort in the Netherlands concludes “biophysical objectives and restoration measures, a monitoring deficiency and restoration plans neglecting large scale catchment wide effects hampered the success of ecological stream restoration. It is therefore recommended to improve the monitoring programmes accompanying restoration projects by applying a proper design, matching the relevant spatiotemporal dimensions for the ecosystem under study” [71]. Our results support the need for large-scale catchment consideration if the underlying causes of eutrophication, macrophyte proliferation and species spread are to be addressed.

Author contribution statement

Louis Skovsholt: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Tenna Riis: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Fleur Matheson; Ian Hawes: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

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.

Acknowledgements

We would like to thank NIWA and the University of Waikato for giving access to laboratory facilities and materials. Additionally, the authors acknowledge the invaluable contributions of laboratory staff and technicians to the chemical analyses used in this study. Lastly, a special thanks goes to Daniel Clements for assisting in plant collection and Ngapera Keegan for assisting in harvesting and measuring the plants.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2023.e15528>.

References

- [1] K.J. Collier, P.D. Champion, G.F. Croker, Patch- and reach-scale dynamics of a macrophyte-invertebrate system in a New Zealand lowland stream, *Hydrobiologia* 392 (2) (1999) 89–97, <https://doi.org/10.1023/A:1003653717805>.
- [2] A. Giorgi, C. Feijóo, G. Tell, Primary producers in a Pampean stream: temporal variation and structuring role, *Biodivers. Conserv.* 14 (7) (2005) 1699–1718, <https://doi.org/10.1007/s10531-004-0694-z>.
- [3] R.L. Paice, J.M. Chambers, B.J. Robson, Native submerged macrophyte distribution in seasonally-flowing, south-western Australian streams in relation to stream condition, *Aquat. Sci.* 79 (1) (2017) 171–185, <https://doi.org/10.1007/s00027-016-0488-x>.
- [4] B.A. Quirino, N. Carniatto, S.M. Thomaz, M.H.F. Aleixo, R. Fugí, The amphibian macrophyte *Polygonum punctatum* as a temporary habitat and feeding ground for fish, *Aquat. Ecol.* 53 (3) (2019) 441–452, <https://doi.org/10.1007/s10452-019-09700-9>.
- [5] K. Sand-Jensen, Influence of submerged macrophytes on sediment composition and near-bed flow in lowland streams, *Freshw. Biol.* 39 (4) (1998) 663–679, <https://doi.org/10.1046/j.1365-2427.1998.00316.x>.
- [6] T. Riis, J.L. Tank, A.J. Reisinger, A. Aubenau, K.R. Roche, P.S. Levi, A. Baattrup-Pedersen, A.B. Alnoee, D. Bolster, Riverine macrophytes control seasonal nutrient uptake via both physical and biological pathways, *Freshw. Biol.* 65 (2) (2019) 178–192, <https://doi.org/10.1111/fwb.13412>.
- [7] T.L. Mouton, F.E. Matheson, F. Stephenson, P.D. Champion, S. Wadhwa, M.P. Hamer, A. Catlin, T. Riis, Environmental filtering of native and non-native stream macrophyte assemblages by habitat disturbances in an agricultural landscape, *Sci. Total Environ.* 659 (2019) 1370–1381, <https://doi.org/10.1016/j.scitotenv.2018.12.277>.
- [8] K. Sand-Jensen, T. Riis, O. Vestergaard, S.E. Larsen, Macrophyte decline in Danish lakes and streams over the past 100 years, *J. Ecol.* 88 (6) (2000) 1030–1040, <https://doi.org/10.1046/j.1365-2745.2000.00519.x>.
- [9] K. Steffen, T. Becker, W. Herr, C. Leuschner, Diversity loss in the macrophyte vegetation of northwest German streams and rivers between the 1950s and 2010, *Hydrobiologia* 713 (1) (2013) 1–17, <https://doi.org/10.1007/s10750-013-1472-2>.
- [10] L. Vilmin, J.M. Mogollon, A.H.W. Beusen, A.F. Bouwman, Forms and subannual variability of nitrogen and phosphorus loading to global river networks over the 20th century, *Global Planet. Change* 163 (2018) 67–85, <https://doi.org/10.1016/j.gloplacha.2018.02.007>.
- [11] J. Hilton, M. O'Hare, M.J. Bowes, J.L. Jones, How green is my river? A new paradigm of eutrophication in rivers, *Sci. Total Environ.* 365 (1–3) (2006) 66–83, <https://doi.org/10.1016/j.scitotenv.2006.02.055>.
- [12] M.T. O'Hare, A. Baattrup-Pedersen, I. Baumgarte, A. Freeman, I.D.M. Gunn, A.N. Lazar, R. Sinclair, A.J. Wade, M.J. Bowes, Responses of aquatic plants to eutrophication in rivers: a revised conceptual model, *Front. Plant Sci.* 9 (2018) 451, <https://doi.org/10.3389/fpls.2018.00451>.
- [13] S.M. Haslam, *River Plants; the Macrophytic Vegetation of Watercourses*, 1978.
- [14] F.E. Matheson, J. Quinn, C. Hickey, *Review of the New Zealand Instream Plant and Nutrient Guidelines and Development of an Extended Decision Making Framework: Phases 1 and 2 Final Report*, NIWA, 2012.
- [15] T. Riis, B.J.F. Biggs, Hydrologic and hydraulic control of macrophyte establishment and performance in streams, *Limnol. Oceanogr.* 48 (4) (2003) 1488–1497, <https://doi.org/10.4319/lo.2003.48.4.1488>.
- [16] A. Baattrup-Pedersen, E. Gothe, T. Riis, M.T. O'Hare, Functional trait composition of aquatic plants can serve to disentangle multiple interacting stressors in lowland streams, *Sci. Total Environ.* 543 (Pt A) (2016) 230–238, <https://doi.org/10.1016/j.scitotenv.2015.11.027>.
- [17] G.M. Carr, S.A.E. Bod, H.C. Duthie, W.D. Taylor, Macrophyte biomass and water quality in Ontario rivers, *J. North Am. Benthol. Soc.* 22 (2) (2003) 182–193, <https://doi.org/10.2307/1467991>.
- [18] C. Garbey, G. Thiebaut, S. Muller, Morphological plasticity of a spreading aquatic macrophyte, *Ranunculus peltatus*, in response to environmental variables, *Plant Ecol.* 173 (1) (2004) 125–137, <https://doi.org/10.1023/B:VEGE.0000026336.44576.f6>.
- [19] S.I. Heaney, R.H. Foy, G.J.A. Kennedy, W.W. Crozier, W.C.K. O'Connor, Impacts of agriculture on aquatic systems: lessons learnt and new unknowns in Northern Ireland, *Mar. Freshw. Res.* 52 (1) (2001) 151–163, <https://doi.org/10.1071/Mf00055>.
- [20] C.A. Mebane, N.S. Simon, T.R. Maret, Linking nutrient enrichment and streamflow to macrophytes in agricultural streams, *Hydrobiologia* 722 (1) (2014) 143–158, <https://doi.org/10.1007/s10750-013-1693-4>.
- [21] M.T. O'Hare, R.T. Clarke, M.J. Bowes, C. Cailes, P. Henville, N. Bissett, C. McGahey, M. Neal, Eutrophication impacts on a river macrophyte, *Aquat. Bot.* 92 (3) (2010) 173–178, <https://doi.org/10.1016/j.aquabot.2009.11.001>.
- [22] T. Riis, C. Lambertini, B. Olesen, J.S. Clayton, H. Brix, B.K. Sorrell, Invasion strategies in clonal aquatic plants: are phenotypic differences caused by phenotypic plasticity or local adaptation? *Ann. Bot.* 106 (5) (2010) 813–822, <https://doi.org/10.1093/aob/mcq176>.
- [23] A. Sosiak, Long-term response of periphyton and macrophytes to reduced municipal nutrient loading to the Bow River (Alberta, Canada), *Can. J. Fish. Aquat. Sci.* 59 (6) (2002) 987–1001, <https://doi.org/10.1139/F02-071>.
- [24] D.E. Canfield, M.V. Hoyer, Influence of nutrient enrichment and light availability on the abundance of aquatic macrophytes in Florida streams, *Can. J. Fish. Aquat. Sci.* 45 (1988) 1467–1472.
- [25] U. Kern-Hansen, F.H. Dawson, The standing crop of aquatic plants in lowland streams in Denmark and the interrelationships of nutrients in plant, sediment, and water, in: *Proc. EWRS 5th Symp. on Aquatic Weeds*, 1978.
- [26] F.A. Kouwe, Phosphorous removal in a sewage treatment plant and plant biomass in an effluent receiving lowland stream, *Hydrol. Bull.* 17 (2) (1983) 143–156.
- [27] D. Levesque, C. Hudon, P.M.A. James, P. Legendre, Environmental factors structuring benthic primary producers at different spatial scales in the St. Lawrence River (Canada), *Aquat. Sci.* 79 (2) (2016) 345–356, <https://doi.org/10.1007/s00027-016-0501-4>.
- [28] T.V. Madsen, N. Cedergreen, Sources of nutrients to rooted submerged macrophytes growing in a nutrient-rich stream, *Freshw. Biol.* 47 (2) (2002) 283–291, <https://doi.org/10.1046/j.1365-2427.2002.00802.x>.
- [29] P. Millard, The accumulation and storage of nitrogen by herbaceous plants, *Plant Cell Environ.* 11 (1) (1988) 1–8, <https://doi.org/10.1111/j.1365-3040.1988.tb01769.x>.
- [30] S. Gusewell, M. Koerselman, Variation in nitrogen and phosphorus concentrations of wetland plants, *Perspect. Plant Ecol. Evol. Systemat.* 5 (1) (2002) 37–61, <https://doi.org/10.1078/1433-8319-0000022>.
- [31] B.O.L. Demars, A.C. Edwards, Tissue nutrient concentrations in freshwater aquatic macrophytes: high inter-taxon differences and low phenotypic response to nutrient supply, *Freshw. Biol.* 52 (11) (2007) 2073–2086, <https://doi.org/10.1111/j.1365-2427.2007.01817.x>.
- [32] G. Cavalli, A. Baattrup-Pedersen, T. Riis, Nutrient availability and nutrient use efficiency in plants growing in the transition zone between land and water, *Plant Biol.* 18 (2) (2016) 301–306, <https://doi.org/10.1111/plb.12397>.
- [33] C.M. Duarte, Nutrient concentration of aquatic plants - patterns across species, *Limnol. Oceanogr.* 37 (4) (1992) 882–889, <https://doi.org/10.4319/lo.1992.37.4.0882>.
- [34] P. Lacoul, B. Freedman, Environmental influences on aquatic plants in freshwater ecosystems, *Environ. Rev.* 14 (2) (2006) 89–136, <https://doi.org/10.1139/A06-001>.
- [35] G. Bornette, S. Puijalon, Response of aquatic plants to abiotic factors: a review, *Aquat. Sci.* 73 (1) (2010) 1–14, <https://doi.org/10.1007/s00027-010-0162-7>.
- [36] R. Hrivnak, J. Kochjarova, H. Otahelova, P. Palove-Balang, M. Slezak, P. Slezak, Environmental drivers of macrophyte species richness in artificial and natural aquatic water bodies – comparative approach from two central European regions, *Annales de Limnologie - Int. J. Limnol.* 50 (4) (2014) 269–278, <https://doi.org/10.1051/limn/2014020>.
- [37] N.F. Caraco, J.J. Cole, Contrasting impacts of a native and alien macrophyte on dissolved oxygen in a large river, *Ecol. Appl.* 12 (5) (2002) 1496–1509, <https://doi.org/10.2307/3099987>.
- [38] A. Baczyk, M. Wagner, T. Okruszko, M. Grygoruk, Influence of technical maintenance measures on ecological status of agricultural lowland rivers - systematic review and implications for river management, *Sci. Total Environ.* 627 (2018) 189–199, <https://doi.org/10.1016/j.scitotenv.2018.01.235>.
- [39] A. Baattrup-Pedersen, E. Gothe, S.E. Larsen, M. O'Hare, S. Birk, T. Riis, N. Friberg, Plant trait characteristics vary with size and eutrophication in European lowland streams, *J. Appl. Ecol.* 52 (6) (2015) 1617–1628, <https://doi.org/10.1111/1365-2664.12509>.

- [40] H. Ellenberg, U. Arndt, R. Bretthauer, B. Ruthsatz, L. Steubing, *Biological Monitoring. Signals from the Environment*, 1991.
- [41] P. Champion, D. Rowe, B. Smith, R. Wells, C. Kilroy, M. de Winton, *Freshwater Pests of New Zealand*, NIWA, Wellington, 2013.
- [42] B.R. Bolduan, G.C. Van Eeckhout, H.W. Quade, J.E. Gannon, *Potamogeton crispus*— the other invader, *Lake Reservoir Manag.* 10 (2) (1994) 113–125, <https://doi.org/10.1080/07438149409354182>.
- [43] B. Kronvang, E. Jeppesen, D.J. Conley, M. Sondergaard, S.E. Larsen, N.B. Ovesen, J. Carstensen, Nutrient pressures and ecological responses to nutrient loading reductions in Danish streams, lakes and coastal waters, *J. Hydrol.* 304 (1–4) (2005) 274–288, <https://doi.org/10.1016/j.jhydrol.2004.07.035>.
- [44] Champion, P. and D. Hofstra. *Potamogeton crispus* Fact Sheet (continuously updated). New Zealand Plant Conservation Network. [Web page] [cited 2021 October]; Available from: <https://www.nzpcn.org.nz/flora/species/potamogeton-crispus/>.
- [45] R. Riis, B.J.F. Biggs, Distribution of macrophytes in New Zealand streams and lakes in relation to disturbance frequency and resource supply - a synthesis and conceptual model, *N. Z. J. Mar. Freshw. Res.* 35 (2) (2001) 255–267, <https://doi.org/10.1080/00288330.2001.9516996>.
- [46] de Lange, P.J. *Potamogeton ochreatus* Fact Sheet (content continuously updated). New Zealand Plant Conservation Network. [cited 2021 October]; Available from: <https://www.nzpcn.org.nz/flora/species/potamogeton-ochreatus/>.
- [47] T. Barker, K.O.C. Hatton, L. Connor, B. Moss, Effects of nitrate load on submerged plant biomass and species richness: results of a mesocosm experiment, *Fundamental and Applied Limnology* 173 (2) (2008) 89–100, <https://doi.org/10.1127/1863-9135/2008/0173-0089>.
- [48] R. Michael Smart, J.W. Barko, Laboratory culture of submersed freshwater macrophytes on natural sediments, *Aquat. Bot.* 21 (3) (1985) 251–263, [https://doi.org/10.1016/0304-3770\(85\)90053-1](https://doi.org/10.1016/0304-3770(85)90053-1).
- [49] C. Ellawala Kankanamge, F.E. Matheson, T. Riis, Shading constrains the growth of invasive submerged macrophytes in streams, *Aquat. Bot.* 158 (2019), <https://doi.org/10.1016/j.aquabot.2019.06.004>.
- [50] S. Beer, M. Bjork, Measuring rates of photosynthesis of two tropical seagrasses by pulse amplitude modulated (PAM) fluorometry, *Aquat. Bot.* 66 (2000) 69–76.
- [51] A.D. Jassby, T. Platt, Mathematical formulation of the relationship between photosynthesis and light for phytoplankton, *Limnol. Oceanogr.* 21 (4) (1976) 540–547.
- [52] P. Manolaki, M.B. Mouridsen, E. Nielsen, A. Olesen, S.M. Jensen, T.L. Lauridsen, A. Baatrup-Pedersen, B.K. Sorrell, T. Riis, A comparison of nutrient uptake efficiency and growth rate between different macrophyte growth forms, *J. Environ. Manag.* 274 (2020), 111181, <https://doi.org/10.1016/j.jenvman.2020.111181>.
- [53] H.K. Lichtenthaler, Chlorophylls and carotenoids - pigments of photosynthetic biomembranes, *Methods Enzymol.* 148 (1987) 350–382.
- [54] G.E. Hutchinson, *A Treatise on Limnology: Limnological Botany*, vol. 3, Wiley-Interscience, 1975.
- [55] G.C. Gerloff, *Nutritional Ecology of Nuisance Aquatic Plants*, National Environmental Research Center, Office of Research and Development, 1975.
- [56] C.M. Duarte, Seagrass nutrient content, *Mar. Ecol. Prog. Ser.* 67 (2) (1990) 201–207, <https://doi.org/10.3354/meps067201>.
- [57] T.V. Madsen, P. Hahn, J. Johansen, Effects of inorganic carbon supply on the nitrogen requirement of two submerged macrophytes, *Elodea canadensis* and *Callitriche cophocarpa*, *Aquat. Bot.* 62 (2) (1998) 95–106, [https://doi.org/10.1016/S0304-3770\(98\)00087-4](https://doi.org/10.1016/S0304-3770(98)00087-4).
- [58] D. Cenzato, G. Ganf, A comparison of growth responses between two species of *Potamogeton* with contrasting canopy architecture, *Aquat. Bot.* 70 (1) (2001) 53–66, [https://doi.org/10.1016/S0304-3770\(00\)00143-1](https://doi.org/10.1016/S0304-3770(00)00143-1).
- [59] W. Huang, S. Han, Q. Zhou, W. Li, W. Xing, Assessing interactions between environmental factors and aquatic toxicity: influences of dissolved CO₂ and light on Cd toxicity in the aquatic macrophyte *Potamogeton crispus*, *Aquat. Toxicol.* 212 (2019) 247–258, <https://doi.org/10.1016/j.aquatox.2019.05.005>.
- [60] S. Jana, M.A. Choudhuri, Changes occurring during aging and senescence in a submerged aquatic angiosperm (*Potamogeton-Pectinatus*), *Physiol. Plantarum* 55 (3) (1982) 356–360, <https://doi.org/10.1111/j.1399-3054.1982.tb00305.x>.
- [61] S.Q. Su, Y.M. Zhou, J.G. Qin, W.Z. Yao, Z.H. Ma, Optimization of the method for chlorophyll extraction in aquatic plants, *J. Freshw. Ecol.* 25 (4) (2010) 531–538, <https://doi.org/10.1080/02705060.2010.9664402>.
- [62] S. Puijalon, J.P. Lena, G. Bornette, Interactive effects of nutrient and mechanical stresses on plant morphology, *Ann. Bot.* 100 (6) (2007) 1297–1305, <https://doi.org/10.1093/aob/mcm226>.
- [63] H. Zhou, J. Ren, X. Cai, The concept and measurement of diurnal light compensation point of submerged macrophyte, *Acta Sci. Circumstantiae* 17 (1997) 256–258.
- [64] T.J. Givnish, Adaptation to sun and shade - a whole-plant perspective, *Aust. J. Plant Physiol.* 15 (1–2) (1988) 63–92, <https://doi.org/10.1071/Pp9880063>.
- [65] T. Cao, P. Xie, L.Y. Ni, M. Zhang, J. Xu, Carbon and nitrogen metabolism of an eutrophication tolerant macrophyte, *Potamogeton crispus*, under NH₄⁺ stress and low light availability, *Environ. Exp. Bot.* 66 (1) (2009) 74–78, <https://doi.org/10.1016/j.envexpbot.2008.10.004>.
- [66] L. Wang, X. Wang, X. Han, Y. Gao, B. Liu, X. Zhang, G. Wang, *Potamogeton crispus* responses to varying water depth in morphological plasticity and physiological traits, *Environ. Sci. Pollut. Res. Int.* 28 (4) (2021) 4253–4261, <https://doi.org/10.1007/s11356-020-10806-z>.
- [67] D.E. Hofstra, K.D. Adam, J.S. Clayton, Isozyme variation in new-zealand populations of *Myriophyllum* and *Potamogeton* species, *Aquat. Bot.* 52 (1–2) (1995) 121–131, [https://doi.org/10.1016/0304-3770\(95\)00494-K](https://doi.org/10.1016/0304-3770(95)00494-K).
- [68] S.T. Larned, T. Snelder, M.J. Unwin, G.B. McBride, Water quality in New Zealand rivers: current state and trends, *N. Z. J. Mar. Freshw. Res.* 50 (3) (2016) 389–417, <https://doi.org/10.1080/00288330.2016.1150309>.
- [69] S.T. Larned, J. Moores, J. Gadd, B. Baillie, M. Schallenberg, Evidence for the effects of land use on freshwater ecosystems in New Zealand, *N. Z. J. Mar. Freshw. Res.* 54 (3) (2019) 551–591, <https://doi.org/10.1080/00288330.2019.1695634>.
- [70] A. Ordonez, I.J. Wright, H. Olff, Functional differences between native and alien species: a global-scale comparison, *Funct. Ecol.* 24 (6) (2010) 1353–1361, <https://doi.org/10.1111/j.1365-2435.2010.01739.x>.
- [71] P.C. Dos Reis Oliveira, H.G. van der Geest, M.H.S. Kraak, J.J. Westveer, R.C.M. Verdonshot, P.F.M. Verdonshot, Over forty years of lowland stream restoration: lessons learned? *J. Environ. Manag.* 264 (2020), 110417 <https://doi.org/10.1016/j.jenvman.2020.110417>.