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Impact of temperature and nutrients on carbon:nutrient tissue stoichiometry of submerged aquatic plants: an experiment and meta-analysis

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13 **Abstract**

14 Human activity is currently changing our environment rapidly, with predicted temperature
15 increases of 1-5°C over the coming century and increased nitrogen and phosphorus inputs in
16 aquatic ecosystems. In the shallow parts of these ecosystems, submerged aquatic plants
17 enhance water clarity by resource competition with phytoplankton, provide habitat and serve
18 as a food source for other organisms. The carbon:nutrient stoichiometry of submerged aquatic
19 plants can be affected by changes in both temperature and nutrient availability. We
20 hypothesized that elevated temperature leads to higher carbon:nutrient ratios through
21 enhanced nutrient-use efficiency, while nutrient addition leads to lower carbon:nutrient ratios
22 by the luxurious uptake of nutrients.

23

24 We addressed these hypotheses with an experimental and a meta-analytical approach. We
25 performed a full-factorial microcosm experiment with the freshwater plant *Elodea nuttallii*
26 grown at 10, 15, 20 and 25 °C on sediment consisting of pond soil/sand mixtures with 100,
27 50, 25 and 12.5% pond soil. To address the effect of climatic warming and nutrient addition
28 on the carbon:nutrient stoichiometry of submerged aquatic plants in general, we performed a
29 meta-analysis on experimental studies that elevated temperature and/or added nutrients
30 (nitrogen and phosphorus).

31

32 In the microcosm experiment, C:N ratios of *Elodea nuttallii* decreased with increasing
33 temperature*,* and this effect was most pronounced at intermediate nutrient availability.
34 Furthermore, higher nutrient availability led to decreased aboveground C:P ratios. In the
35 meta-analysis, nutrient addition led to a 25, 22 and 16% reduction in aboveground C:N and
36 C:P ratios and belowground C:N ratios, accompanied with increased N content. No consistent
37 effect of elevated temperature on plant stoichiometry could be observed, as very few studies
38 were found on this topic and contrasting results were reported.

39

40 We conclude that while nutrient addition consistently leads to decreased carbon:nutrient
41 ratios, elevated temperature does not change submerged aquatic plant carbon:nutrient
42 stoichiometry in a consistent manner. This effect is rather dependent on nutrient availability
43 and may be species-specific. As changes in the carbon:nutrient stoichiometry of submerged
44 aquatic plants can impact the transfer of energy to higher trophic levels, these results suggest
45 that eutrophication may enhance plant consumption and decomposition, which could in turn
46 have consequences for carbon sequestration.

47

48 Keywords: submerged aquatic plant, meta-analysis, microcosm experiment, *Elodea nuttallii*,
49 eutrophication, global warming, carbon:nutrient stoichiometry, growth rate

Introduction

Human activity has led to rapid environmental changes on our planet (Vitousek et al. 1997, Steffen et al. 2015). Water temperatures in marine and freshwater systems have increased over the last decades and are expected to increase further over the course of the century (Mooij et al. 2008, Adrian et al. 2009, IPCC 2014). Furthermore, agriculture and industrialization have a strong impact on nutrient cycles (Carpenter et al. 1998, Tilman et al. 2001) and are major sources of nitrogen and phosphorus input in freshwater and marine ecosystems. Changes in temperature and nutrient availability can have consequences for the abundance of submerged aquatic plants that occur in the shallow parts of aquatic ecosystems (Bornette and Puijalon 2011).

Changes in plant abundances and growth rates can have consequences for their nutrient demand and uptake and as such can influence their carbon:nutrient stoichiometry (Sterner and Elser 2002). Alterations in internal stoichiometry in turn can have consequences for ecosystem functioning, as lower carbon:nutrient ratios can make aquatic plants more palatable to herbivores (Dorenbosch and Bakker 2011), resulting in higher herbivory rates and stimulated top-down control (Olsen and Valiela 2010, Bakker and Nolet 2014) and leading to lowered carbon stocks in the form of plant biomass (Heithaus et al. 2014, van Altena et al. 2016).

However, contrasting hypotheses exist on how temperature and nutrient availability may affect carbon:nutrient ratios in aquatic plants. Elevated temperature can lead to an increase in plant biomass and a biomass dilution effect, where increased growth rates are accompanied by reduced tissue content (per unit of biomass) of a particular element (Taylor et al. 1991, Vermaat and Hootsmans 1994). In terrestrial plant and phytoplankton research, this effect is referred to as enhanced nutrient-use efficiency (An et al. 2005, De Senerpont Domis et al. 2014). According to this hypothesis, elevated temperature would lead to reduced N and P content in aquatic plants and a subsequent increase in carbon:nutrient ratios. Alternatively, higher temperatures can increase the rate of cellular processes, but do not necessarily lead to an unbalanced nutrient uptake, provided that enough nutrients are available in the environment, and therefore would not result in changes in carbon:nutrient ratios.

Similarly, nutrient addition can positively affect the nutritional quality of aquatic plants (e.g. lower carbon:nutrient ratios (Burkholder et al. (2007), Bakker and Nolet (2014)) as they may take up relatively more nutrients compared to carbon. This fertilization effect was demonstrated for terrestrial plants in a recent meta-analysis (Sardans et al. 2012). Furthermore, the combined effect of elevated temperature and nutrient addition may be antagonistic under the hypotheses of enhanced nutrient-use efficiency and luxurious uptake, as the former would be expected to increase carbon:nutrient ratios, while the latter would decrease carbon:nutrient ratios.

Here, we aim to quantify the effects of temperature and nutrient addition on the carbon:nutrient stoichiometry of submerged aquatic angiosperms. We hypothesized that (1) both elevated temperature and nutrient addition lead to elevated enhanced growth rates of submerged angiosperms, (2) if the biomass-dilution effect applies, elevated temperature will lead to higher carbon:nutrient ratios, whereas (3) nutrient addition is expected to lead to decreased carbon:nutrient ratios. These hypothesized changes in carbon:nutrient ratios are

96   expected to be driven by changes in nutrient contents as opposed to carbon (4). Furthermore,
97   we hypothesized that elevated temperature and nutrient addition are antagonists in their
98   combined effect on carbon:nutrient ratios (5).

99

100 We tested these hypotheses using two complementary approaches. First, we performed a full-
101 factorial experiment on the effects of temperature and sediment nutrient content (and their
102 interaction) on the growth and carbon:nutrient stoichiometry of the submerged freshwater
103 angiosperm *Elodea nuttallii. E. nuttallii* is native to North America, but has become common
104 throughout the northern hemisphere in the 1900s (Cook and Urmi-König 1985).
105 Subsequently, a meta-analytic approach was used to address the effect of elevated temperature
106 and nutrient addition on submerged angiosperms in general. We performed a meta-analysis
107 using experimental studies that simulated temperature rise and/or increased nutrient (nitrogen
108 and phosphorus) input and documented the effects on plant growth and carbon:nutrient
109 stoichiometry. In this analysis, we included both marine and freshwater plants. Whereas the
110 responses of aquatic plants to environmental change in marine and freshwater systems are
111 mostly discussed independently, we expected that responses in growth and carbon:nutrient
112 stoichiometry similarly apply to both submerged marine and freshwater angiosperms alike.

113

114 **Material and Methods**

115 **Elodea laboratory experiment**

116 **Experimental set-up**

117 To test the effect of temperature and sediment nutrient content on *Elodea nuttallii*, a full-
118 factorial microcosm experiment was set up. Shoots of *E. nuttallii* were collected from a small
119 pond on the grounds of The Netherlands Institute of Ecology (NIOO-KNAW), Wageningen,
120 The Netherlands (51°59'15.0"N; 5°40'14.8"E) on 07-09-2015. After collection, the plants
121 were rinsed and acclimatised at room temperature for two days prior to the start of the
122 experiment.

123

124 The experiment was carried out in 4 L plastic microcosms ($14 \times 14 \times 21$ cm), which contained
125 1.1 L of sediment and 2.7 L of water. Nutrient treatments were achieved by mixing artificial
126 pond sediment (20% organic matter, Velda, Enschede, The Netherlands) with sand and
127 consisted of 12.5, 25, 50 and 100% (v/v) of pond sediment (n=5), covered with a one
128 centimetre layer of sand. The artificial pond sediment contained 31 ± 1.8, 0.80 ± 0.048 and
129 0.11 ± 0.0084 % (mean ± SE) C, N and P respectively. One shoot fragment of *E. nuttallii* of ±
130 5.5 cm (C:N = 19 ± 1.4 , C:P = 435 ± 72, n=5) was placed in the middle of each microcosm
131 and the microcosm was topped off with nutrient-poor tap water (3.5 ± 0.5 (mean ± SE) µM
132 DIN and undetectable levels of DIP). The microcosms were placed in four aquaria, which
133 served as temperature-regulated water baths. The temperature treatments were 10, 15, 20 and
134 25°C, which were obtained by a computer-controlled (Specview 32/859, SpecView Ltd.,
135 Uckfield, UK) custom-made climate control system. These temperatures are within the range
136 of natural temperatures *E. nuttallii* would encounter, as water temperatures in the Netherlands
137 vary seasonally between 4 and 23 °C (van Dam 2009). Light (14:10 hours light:dark) was
138 provided by two 28W TL5 HE lamps (Philips, Eindhoven, The Netherlands), hung above the
139 aquaria with an average light intensity at the water surface of 30 µmol s^{-1} m^{-2}. To ensure equal
140 light conditions between treatments, position of the microcosms in the water bath was

randomized once a week and evaporation losses were compensated by additions of demi-water. To prevent excessive periphyton and phytoplankton growth during the experiment, one periphyton-grazing snail (*Planorbarius corneus*) and one filtering mussel (*Dreissena polymorpha*) were put in each microcosm. In pilot tests, *P. corneus* did not feed on *E. nuttallii* (Peiyu Zhang, personal observation) and no grazing on the plants was observed during the experiment. The snails were retrieved from the same pond as the plants, and the mussels were collected from the Nether Rhine, Wageningen, the Netherlands (51°57'12.9"N 5°39'48.2"E). In case either snail or mussel died, another one was added.

Harvest

After 58 days the experiment was terminated. From the middle of each microcosm, water samples were taken to determine dissolved nutrient concentrations, filtered over prewashed GF/F filters (Whatman, Maidstone, U.K.) and stored at -20°C until further analysis. Samples for pore water nutrients were taken in each microcosm through a 10 cm Rhizon SMS (Rhizosphere, Wageningen, the Netherlands) and stored at -20°C until further analysis. Plants were cut at the sediment level, and the above- and belowground biomass was harvested and rinsed with demi-water. All plant materials (above- and belowground) were dried at 60°C until constant dry mass and weighed. During the harvest, basic parameters were measured that describe the growing conditions (pH, alkalinity and seston chlorophyll-a). Methods and results of these measurements can be found in supplementary material S1.

Chemical analysis

Plant material was grinded to a fine powder on a microfine grinder (MF 10 basic, IKA-werke, Staufen, Germany) or in test tube with a 1/8" ball bearing (Weldtite, Lincolnshire, UK) on a Tissuelyser II (QIAGEN, Germantown, USA). For nitrogen (N) and carbon (C) content, 0.2-2 mg dry mass was analyzed on a NC analyser (FLASH 2000 NC elemental analyser, Brechbueler Incorporated, Interscience B.V., Breda, The Netherlands). For phosphorus (P) content, 1-4 mg dry mass was combusted in a Pyrex glass tube at 550°C for 30 minutes. Subsequently, 5 mL of persulfate (2.5%) was added and samples were autoclaved for 30 minutes at 121°C. Digested P (as PO_4^{3-}) was measured on a QuAAtro39 Auto-Analyzer (SEAL Analytical Ltd**.,** Southampton, U.K.). Concentrations of dissolved nutrients (PO_4^{3-}, NO_2^-, NO_3^- and NH_4^+) of thawed pore water-samples were determined on a QuAAtro39 Auto-Analyzer (SEAL Analytical Ltd**.,** Southampton, U.K.).

Calculations and statistics

Plant specific growth rate (SGR) was calculated with the following formula:

$$SGR = \frac{\ln(DWt) - \ln(DW0)}{t}$$

Where DW_t is the plant aboveground dry weight at the end of the experiment, DW_0 the dry weight at the beginning of the experiment (determined by multiplying the initial wet weight with the plants wet weight/dry weight ratio) and t the experimental duration (=58 days).

Data on aboveground and belowground parameters (specific growth rate, above- and belowground biomass, carbon:nutrient stoichiometry and elemental contents) and dissolved nutrient concentrations (DIN and DIP) in the water column and the pore water were tested for

184 effects of temperature, nutrients and their interaction with generalized linear models (function
185 *glm* from stats package). Visual examination of the data distribution (function *hist*) led to the
186 use of a gamma distribution. Post-hoc tests within treatment levels were carried out using
187 Tukey contrasts (function glht from multcomp package (Hothorn et al. 2008)), with P-values
188 corrected for multiple comparison as described by Benjamini and Hochberg (1995).
189
190 **Meta-analysis**

191 **Systematic literature review and data collection**

192 A systematic literature review was carried out in Web of Science based on the guidelines
193 described by the Collaboration for Environmental Evidence (2013). The search term
194 ("submer?ed macrophyte*" OR "aquatic plant" OR isoetid OR macrophyte* OR "aquatic
195 weed" OR seagrass*) AND (stoichiometr* OR "*chemical composition" OR "nutritional
196 quality" OR "nutrient composition" OR "elemental composition" OR "nutrient content" OR
197 "nutrient ratio*" OR C:N OR C:P OR N:P OR "plant nutrient concentration*") AND
198 (warming OR eutrophication OR temperature* or enrichment or fertilis* or "nutrient
199 availability") on 01-11-2016 gave 414 hits. Further selection based on abstracts, graphs and
200 tables led to 47 papers that contained information on temperature and/or nutrient effects on
201 elemental composition of submerged angiosperms. Data originating from light limited
202 conditions (as indicated in the paper itself) were excluded from analysis, as well as studies
203 without reported standard errors or deviations, and studies with limited ($n<2$) or non-reported
204 sample size. From the selected papers, data on C:N and C:P ratios were extracted with use of
205 Plotdigitizer and Engauge and converted to molar ratios when necessary. In addition, C, N
206 and P contents, growth rates (above- or belowground), habitat (marine or freshwater), which
207 part of the plant was analyzed (above- or belowground) and sample size were extracted when
208 reported. If the described methodology indicated possible additional results that were not
209 reported, corresponding authors were contacted to retrieve those data. If experiments reported
210 several measurements over time, only the final measurement was extracted. If papers
211 contained multiple experiments, on the same or on different species, these were extracted as
212 being separate studies.

213

214 **Data selection**

215 Control and elevated treatments were defined for both temperature and nutrient addition and
216 for each experiment separately. The lowest water temperature reported was defined as the
217 control temperature treatment and 3-6 °C above that temperature (equivalent to RCP scenario
218 8.5 from IPCC (2014)) was defined as the elevated temperature treatment. For the nutrient
219 addition studies, those studies that manipulated both nitrogen and phosphorus simultaneously
220 were selected. The lowest nutrient condition reported was defined as the control treatment and
221 the highest as the elevated treatment. The data was then split up into above- and belowground
222 plant responses, as different parts of plants were expected to respond differently (Bloom et al.
223 1985). These selection criteria led to a total of 50 studies on nutrient addition spread over 26
224 papers (of which 50 and 11 on above- and belowground responses respectively) and 3 studies
225 on temperature (only on aboveground responses) originating from 3 papers. Temperature
226 studies were all conducted in mesocosms, whereas nutrient studies included in situ
227 fertilization experiments (38), mesocosm experiments (9) and laboratory experiments (3). Of
228 the nutrient addition studies, 9 studies tested a range of nutrient concentrations of which the
229 highest and lowest were selected, while the majority (41) specifically looked at the addition of
230 nitrogen and phosphorus to the system relative to a control level. An overview of the dataset

231 selection can be found in Fig. S2 and an overview of the selected papers in supplementary
232 material S3.

233

234 **Response factors and statistics**

235 Delta response ratios and their variances were calculated for each separate study according to
236 Lajeunesse (2015):

237 $$RR\Delta = Ln\left(\frac{Xtreatment}{Xcontrol}\right) + \frac{1}{2}\left[\frac{(SDtreatment)^2}{Ntreatment*Xtreatment^2} - \frac{(SDcontrol)^2}{Ncontrol*Xcontrol^2}\right]$$

238 $$var(RR\Delta) = \frac{(SDtreatment)^2}{Ntreatment*Xtreatment^2} + \frac{(SDcontrol)^2}{Ncontrol*Xcontrol^2}$$

239 $$+ \frac{1}{2}\left[\frac{(SDtreatment)^4}{Ntreatment^2*Xtreatment^4} + \frac{SDcontrol^4}{Ncontrol^2*Xcontrol^4}\right]$$

240 Where X denotes mean of the fixed factor of interest (C:N and C:P ratio, growth rate (μ) and
241 C, N and P contents), SD the standard deviation of that mean and N the sample size.

242 All statistics were carried out in R (R Core Team 2015). To test whether response ratios
243 deviated from zero, mixed effect models were fitted to the response ratios and their variances
244 with the function *rma.mv* (package metafor; Viechtbauer (2010)), incorporating reference and
245 species as random effects. To test whether freshwater and marine systems differed in response
246 ratio, separate models were compared for significant differences between the two habitat
247 types (by adding habitat as a moderator to the function *rma.mv*).

248

Results

Elodea experiment

Biomass responses

Temperature affected the specific growth rate and above- and belowground biomass of *E. nuttallii* (Table 1). As indicated by the interaction term, temperature only affected specific growth rate at intermediate sediment nutrient content (e.g. 25%), with optimal growth at 15 and 20°C ($P<0.05$, Tukey post-hoc comparison; Fig. S4). Similarly, aboveground biomass was highest at these temperatures, irrespective of nutrient treatment ($P<0.05$; Fig. 1a). Belowground biomass of *E. nuttallii* was affected by temperature, and this effect interacted with nutrient treatment (Fig. 1b, Table 1). The effects of temperature on belowground biomass seemed strongest in the lowest nutrient treatments (e.g. 12.5%), where biomass tended to increase 3-fold between 15 and 20°C but these effects were not significant in post-hoc tests ($P=0.09$).

Sediment nutrient content affected the specific growth rate, above- and belowground biomass of *Elodea nuttallii* (Table 1). However, no significant differences between nutrient treatments could be observed for specific growth rate (Fig. S4), nor for aboveground biomass (Fig. 1a) in the post-hoc comparisons. Belowground biomass decreased with increasing sediment nutrient content, and this effect interacted with temperature treatment (Table 1; Fig. 1b). Belowground biomass tended to decrease 6-fold over the entire range of nutrient treatments at 25°C, but this effect was not significant ($P=0.08$).

Carbon:nutrient stoichiometry

Temperature negatively affected aboveground C:N ratios (Table 1, Fig. 2a), which was most visible at intermediate sediment nutrient content (25%). In this treatment, aboveground C:N ratios decreased moderately but significantly between 10 and 25°C ($P<0.001$, Tukey post-hoc comparison). No effects of temperature on aboveground C:P ratios were observed, nor on belowground C:N and C:P ratios (Table 1, Fig. 2b-d).

Sediment nutrient content affected aboveground C:P ratio, while no effect on aboveground C:N ratios was observed (Table 1). Aboveground C:P ratios of *E. nuttallii* were negatively affected by increasing sediment nutrient content (Fig. 2b, Table 1). This effect was most visible at 15°C, where the C:P ratio significantly decreased 4-fold the entire range of nutrient treatments ($P<0.001$, Tukey post-hoc comparison). Belowground C:N and C:P ratios were affected by nutrient treatment, and the effect on C:N interacted with temperature (Fig. 2c). Belowground C:N ratios significantly increased between 25 and 100% nutrient treatments at 20°C, while belowground C:P ratios increased 4-fold between those nutrient treatments at the same temperature ($P<0.01$; Fig. 2d).

Elemental contents

Accompanied by the changes in aboveground C:N ratio, temperature seemed to affect aboveground N content (Table 1). However, no differences between any of the temperature treatments could be detected in post-hoc comparisons (Fig. S5b). Belowground carbon

292 content was affected by temperature, and halved between 10 and 25°C in the lowest sediment
293 nutrient treatments (12.5 and 25%; $P<0.05$). No effects of temperature on aboveground C and
294 P content were observed, nor on belowground N and P contents (Table 1).

295

296 Sediment nutrient content affected aboveground P content, with a 3-fold increase over the
297 entire range of nutrient treatments (P<0.05; Fig. S5c). No effect on aboveground C or N
298 content was observed for nutrient content. Belowground C content significantly increased
299 14% over the entire range of nutrient treatments at 25°C ($P<0.01$; Fig. S5d), while
300 belowground N and P content were not affected by nutrient treatment (Table 1).

301

Abiotic conditions

303 Temperature affected dissolved nutrient concentrations in the pore water, and this effect
304 interacted with nutrient treatment (Table 1). Temperature effects on pore water DIN
305 concentrations were strongest at intermediate sediment nutrient content (50%), where values
306 significantly doubled from 10 to 15°C, and decreased at higher temperatures ($P<0.01$, Tukey
307 post-hoc comparison; Fig. 3a). Pore water DIP concentrations were significantly higher at
308 15°C than other temperature treatments in the highest nutrient treatment ($P<0.01$; Fig. 3b).
309 This response was less pronounced in other nutrient treatments. Similarly to temperature,
310 sediment nutrient content affected DIN and DIP concentrations in the pore water. Pore water
311 DIN and DIP increased 7- and 16-fold, respectively, from the 12.5 to 50% nutrient treatment
312 irrespective of temperature ($P<0.01$; Fig. 3a-b).

313

Meta-analysis

Effects of elevated temperature on carbon:nutrient stoichiometry

316 No significant effects of elevated temperature were observed on aboveground C:N and C:P
317 ratios (Fig. 4), nor on aboveground C, N and P contents (Fig. S4a) or belowground N and P
318 contents (Fig. S4b). Sample sizes were too low to analyze effects of elevated temperature on
319 aboveground growth rates (n=0), on belowground C:N and C:P ratios and C content (n=1) or
320 on potential differences between marine and freshwater ecosystems (n=1 and 2 respectively).

321

Effects of nutrient addition on carbon:nutrient stoichiometry

323 Nutrient addition significantly decreased aboveground carbon:nutrient ratios, with 24.7% and
324 21.9% for C:N and C:P ratios, respectively (Fig. 5a). This decrease in aboveground
325 carbon:nutrient ratios was accompanied by a 23.5% increase in aboveground N content, while
326 P content tended to increase as well (with 20.6%, $P=0.06$), whereas C content remained
327 unaffected (Fig. S6c). Furthermore, aboveground growth rates tended to increase 83.1% with
328 nutrient addition, but this effect was not significant ($P=0.08$, Fig. 5a). Similar to aboveground
329 responses, belowground C:N ratio also declined 15.6% with nutrient addition, while no effect
330 on belowground C:P ratios was observed (Fig. 5b). This decline in C:N ratio was
331 accompanied by an 18.2% increase in belowground N content (Fig. S6d).

332

9

Aboveground carbon:nutrient stoichiometry of marine and freshwater plants responded qualitatively similar to nutrient addition, though the number of studies in the latter group was far lower (Fig. 6). Quantitatively, responses in C:N and C:P were stronger for freshwater compared to marine plants ($P<0.001$). Sample sizes were too low to analyze differences in aboveground growth rates between freshwater and marine plants (n=0 for freshwater plants).

Discussion

To address the impacts of temperature and nutrient availability on the growth and carbon:nutrient stoichiometry of aquatic plants, we performed a microcosm experiment and a meta-analysis. In line with our first hypothesis, elevated temperatures led to higher growth rates and standing stock biomass of the freshwater plant *Elodea nuttallii*, with an optimal growth at 15°C. In contrast to the biomass-dilution effect (second hypothesis), aboveground C:N ratios were negatively affected by temperature, and this effect interacted with nutrient treatment. Aboveground C:P ratios of E*. nuttallii* were lower with higher sediment nutrient content, in line with our third hypothesis. The observed decrease in aboveground C:P ratio coincided with an increase in P content, confirming our fourth hypothesis. However, in contrast to our third and fourth hypotheses, belowground C:N and C:P ratios as well as belowground C content increased with higher sediment nutrient content.

In the meta-analysis, elevated temperature did not lead to enhanced growth rates or increased carbon:nutrient ratios of submerged aquatic plants in general, in contrast to our first and second hypotheses. However, it should be noted that overall sample sizes were very low (n=3), which may (partly) explain the lack of effect. In line with our first hypothesis, nutrient (e.g. nitrogen and phosphorus) addition tended to increase plant growth rates, though this effect was not significant. Nutrient addition led to decreased C:N and C:P ratios and increased N content, in agreement with the third and fourth hypotheses. The carbon:nutrient ratio declined in both marine and freshwater plants upon nutrient addition, although the absolute level of the response was stronger in freshwater systems.

Effects of temperature on plant carbon:nutrient stoichiometry

Aboveground C:N ratio of *E. nuttallii* decreased moderately with increasing temperatures in our experiment. This is in direct contrast with the hypotheses of enhanced nutrient-use efficiency with elevated temperatures (2), which would lead to increased carbon:nutrient ratios (as is observed for other aquatic plants such as *Zostera marina* (Kaldy 2014)). The decrease in C:N ratios was most pronounced between 10 and 25 °C, even though the aboveground biomass did not differ between those temperatures. Thus, temperature does not seem to indirectly affect C:N ratios through changes in biomass. Accompanied by the decreased C:N ratios in our *Elodea* experiment with higher temperatures, N contents tended to be higher as well, but this effect was not significant. Increased N content over similar temperature range has been documented for *E. canadensis* (Ventura et al. 2008) and *Ruppia drepanensis* (Santamaria and Hootsmans 1998) and could indicate resource allocation to nitrogen-rich compounds such as chlorophyll-a (Santamaria and Hootsmans 1998). Furthermore, elevated temperature can increase nitrogen availability in the sediment pore water through enhanced nitrogen mobilization (Alsterberg et al. 2012), thereby indirectly leading to higher nitrogen availability for plant growth. In our experiment, the temperature

378 treatments with highest aboveground biomass of *E. nuttallii* (e.g. 15 and 20°C) varied
379 considerably in their pore water nitrogen availability, indicating that those are not directly
380 related. Furthermore, as the temperature effect on C:N ratios was most pronounced at
381 intermediate sediment nutrient content (as indicated by the temperature × nutrient interaction
382 term), these results indicate that stoichiometric responses of plants to changes in temperature
383 may be directly and indirectly altered by nutrient availability.

384

385 In our meta-analysis, we observed no overall effect of an 3-6°C elevated temperature on
386 carbon:nutrient ratios of submerged aquatic plants, which contradicts findings in other groups
387 of primary producers, such as phytoplankton (Toseland et al. 2013, De Senerpont Domis et al.
388 2014) and terrestrial plants (An et al. 2005). The number of studies in our analysis was rather
389 low (n=3) and included a positive (Zhang et al. 2016), negative (Ventura et al. 2008) and
390 neutral (Touchette et al. 2003) response. The different directions of responses indicate that
391 effects of temperature on the carbon:nutrient stoichiometry of aquatic plants are not
392 necessarily linked to the temperature increments they are exposed to. Thus, it may indicate
393 species-specific responses or possibly even a phylogenetic relationship considering the similar
394 response of *E. nuttallii* in our experiment and *E. canadensis* (Ventura et al. 2008), which both
395 have an optimal growth temperature of around 15°C (Olesen and Madsen 2000). However,
396 due to the limited sample size of each species (n=1), we currently cannot distinguish between
397 species-specific and study-specific responses (such as experimental set-up and environmental
398 conditions) of carbon:nutrient stoichiometry in our analysis.

399

Effects of nutrient addition on carbon:nutrient tissue stoichiometry

401 Aboveground C:P ratios decreased about 4-fold with increasing sediment nutrient content in
402 the *Elodea* experiment, confirming our third hypothesis. However, in contrast to this
403 hypothesis, belowground C:P ratios of *E. nuttallii* and carbon content rather increased with
404 sediment nutrient availability. Higher belowground carbon content can indicate thicker cell
405 walls and thicker roots. Possibly, with sufficient nutrient availability, *Elodea* may shift from
406 investment in root structures for nutrient uptake to thicker roots for anchorage in the sediment
407 (Sand-Jensen and Madsen 1991). In the meta-analysis, nutrient addition led to a 25% and 22%
408 decrease in aboveground C:N and C:P ratios of submerged aquatic plants, consistent with the
409 results from the *Elodea* experiment. While some variability in response can be detected at the
410 species level, responses are consistently either absent or negative (Fig. S6). Similar to the
411 aboveground responses, nutrient addition led to a 16% decrease in belowground C:N ratios.
412 These decreases in above- and belowground carbon:nutrient ratios were accompanied by
413 increased tissue N and P contents and demonstrate the flexibility in carbon:nutrient
414 stoichiometry of aquatic plants under fluctuating nutrient availability (Sardans et al. 2012).

415

416 Increased plant nutrient content as observed in our meta-analysis and *Elodea* experiment may
417 have resulted from excess or luxurious uptake of nutrients (Millard 1988), as terrestrial plants
418 can store excess P in cell vacuoles (Bieleski 1973) and N in specialized storage organs (Aerts
419 and Chapin 2000). Similar to our results, meta-analytic studies on terrestrial plants observed
420 elevated foliar N and P contents in response to nutrient addition (Yuan and Chen 2015) and a
421 decrease in C:N in photosynthetic tissues to N addition (Sardans et al. 2012). Combined with
422 our results, this indicates that these effects are not ecosystem specific, but can be seen as a
423 general qualitative response of primary producers to nutrient addition.

424

Our analysis indeed indicated qualitatively similar responses to nutrient addition in both marine and freshwater submerged plants, though the responses were stronger in the latter group. Sample sizes for freshwater plants were far lower than for marine plants, highlighting the potential for freshwater research to learn from physiological studies on marine plants. Mean C:N ratios of freshwater plants are lower than marine plants (Bakker et al. 2016) and could result from higher levels of fertilization as nutrient levels in freshwater are generally considered higher than in marine systems (Smith et al. 1999). However, as these ecosystems differ greatly in retention time, sediment characteristics and osmotic stress from salinity (Short et al. 2016), caution must be taken when interpreting these differences.

Possible implications for carbon cycling and food-web dynamics

Changes in plant carbon:nutrient stoichiometry in aquatic systems can have consequences for carbon cycling. In our meta-analysis, nutrient addition tended to increase plant growth rates, with positive (Murray et al. 1992, Udy et al. 1999, Peralta et al. 2003) and neutral (Erftemeijer et al. 1994, Holzer and McGlathery 2016) responses reported. Thus, carbon sequestration in the form of plant standing stock biomass can be enhanced by nutrient addition (Armitage and Fourqurean 2016). Furthermore, changes in carbon:nutrient stoichiometry can have consequences for the energy transfer to higher trophic levels as elevated nutrient content in aquatic plants can lead to increased herbivore grazing rates (Bakker and Nolet 2014) and subsequent reduction in standing-stock biomass (van Altena et al. 2016). This may counteract positive effects of fertilization on plant growth rates and carbon sequestration. Furthermore, eutrophic conditions can enhance plant litter quality (Emsens et al. 2016) and plants with lower carbon:nutrient ratios decompose faster than those with higher ratios (Wang et al. 2017), indicating an accelerated release of sequestered carbon and nutrients. We therefore hypothesize that eutrophication can affect carbon stocks in submerged aquatic vegetation, through changes in their nutritional quality (e.g. reduced carbon:nutrient stoichiometry) and subsequent effects on grazing and decomposition. Given the current knowledge about the effects of temperature on carbon:nutrient stoichiometry of aquatic plants presented in this study, we cannot draw any general conclusions on the effect of global warming on aquatic carbon cycling. However, our results suggest species-specific responses, which indicate that given the community composition in an ecosystem, effects may be substantial. Our current analysis focuses on individual plant responses and their stoichiometric flexibility. On a community level, interspecific variability can drive changes in C:N:P stoichiometry (Frost and Hicks 2012), with consequences for community composition under elevated nutrient availability and temperature. For instance, elevated temperature can shift aquatic plant community composition towards floating vegetation (Netten et al. 2010), while nutrient addition can lead to a decline in overall plant abundance at the expense of algae (Scheffer et al. 1993, Short and Neckles 1999). Therefore, hypotheses on an ecosystem level should also take these changes into account.

Conclusion

We conclude that nutrient (e.g. nitrogen and phosphorus) addition decreases carbon:nutrient stoichiometry in submerged aquatic plants, while no consistent effects of elevated temperature on these ratios were observed. The latter could be an effect of low sample size or could indicate species-specific responses in carbon:nutrient stoichiometry to global warming, which

470 is an interesting avenue for future research. Furthermore, our experiment shows that the
471 impact of temperature on aquatic plant stoichiometry depends on the availability of nutrients
472 for plant growth, which is seldom taken into account. The impact of temperature may thus be
473 modified by nutrient availability. The observed decline in carbon:nutrient stoichiometry of
474 aquatic plants in response to nutrient addition can stimulate the further energy transfer to
475 herbivores and decomposers, leading to reduced carbon stocks. With ongoing global
476 warming, the knowledge gap of temperature effects on carbon:nutrient stoichiometry of
477 submerged aquatic plants is in urgent need for further investigation.

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Figure legends

Fig. 1. Above- (A) and belowground (B) biomass of *Elodea nuttallii* grown at different temperatures and sediment nutrient content. Temperature treatments include 10 (■), 15 (●), 20 (▲) and 25 (♦) °C. Dots represent means and error bars standard error of the mean (n=5). Capital and lower case letters indicate post-hoc differences between temperature and nutrient treatments, respectively.

Fig. 2. Above- (A-B) and belowground (C-D) carbon:nutrient stoichiometry of *Elodea nuttallii* to sediment nutrient content, with (A,C) C:N and (B,D) C:P ratios. Temperature treatments include 10 (■), 15 (●), 20 (▲) and 25 (♦) °C. Dots represent means and error bars standard error of the mean. Capital and lower case letters indicate post-hoc differences between temperature and nutrient treatments, respectively.

Fig. 3. Dissolved nutrient concentrations in the pore water in response to sediment nutrient content, with dissolved inorganic nitrogen (DIN) (A) and dissolved inorganic phosphorus (DIP) (B) at the end of the experiment. Temperature treatments include 10 (■), 15 (●), 20 (▲) and 25 (♦) °C. Dots represent means and error bars standard error of the mean. Capital and lower case letters indicate post-hoc differences between temperature and nutrient treatments, respectively.

Fig. 4. Natural-log response ratios of aboveground carbon:nutrient stoichiometry and plant growth rates (μ) to 3-6 degrees elevated temperature from the meta-analysis on submerged aquatic plants. Values represent means, error bars 95% confidence intervals and sample size is indicated between brackets. No response ratios were significantly different from zero. N.A. indicates that data were not available.

Fig. 5. Natural-log response ratios of carbon:nutrient stoichiometry and plant growth rates (μ) to nutrient (nitrogen and phosphorus) addition in (A) above- and (B) belowground biomass on submerged aquatic plants. Values represent means, error bars 95% confidence intervals, sample size is indicated between brackets and response ratios significantly different from zero are indicated as follows: ***:P<0.001, **:P<0.01, *:P<0.05 and ⋅:P<0.10. N.A. indicates that data were not available.

Fig. 6. Natural-log response ratios of aboveground carbon:nutrient stoichiometry and growth rates (μ) to nutrient (nitrogen and phosphorus) addition in freshwater (open circles) and marine (closed circles) submerged aquatic plants. Values represent means, error bars 95% confidence intervals, sample size is indicated between brackets and significance levels are indicated as follows: ***:P<0.001, **:P<0.01 , *:P<0.05 and ⋅:P<0.10. N.A. indicates that data were not available.

677 **Tables**

678 Table 1: Summary of generalized linear model analysis of the *Elodea* experiment, describing
679 the effect of temperature treatment, nutrient treatment and their interaction on the biomass,
680 carbon:nutrient stoichiometry and elemental contents of *Elodea nuttallii* and nutrient
681 concentrations. Significant results are indicated in bold, with ***:P<0.001, **:P<0.01 and
682 *:P<0.05.

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	Unit	Chi-square values		
		Temperature	Nutrients	Temperature × Nutrients
Biomass variables				
Specific growth rate	day^{-1}	**16.7*****	**8.8***	7.3
Aboveground biomass	mg DW	**62.4*****	**17.7*****	13.1
Belowground biomass	mg DW	**10.8***	**18.6*****	**20.8***
Carbon:nutrient stoichiometry				
Aboveground C:N	molar	**43.6*****	3.5	**22.5****
C:P	molar	7.7	**45.6*****	8.2
Belowground C:N	molar	4.7	**8.8***	**21.8****
C:P	molar	6.0	**12.0****	7.5
Elemental contents				
Aboveground C	mol g DW^{-1}	5.1	0.7	1.3
N	mol g DW^{-1}	**15.5****	1.4	3.1
P	mol g DW^{-1}	7.6	**83.4*****	**45.7*****
Belowground C	mol g DW^{-1}	**9.4***	**35.2*****	9.8
N	mol g DW^{-1}	7.4	0.9	14.3
P	mol g DW^{-1}	6.1	1.2	**17.3***
Nutrient concentrations				
Pore water DIN	μM	**42.1*****	**163.9*****	**19.8***
Pore water DIP	μM	**47.9*****	**317.6*****	**29.3*****

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