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Pedersen, Morten Foldager; Lørup Johnsen, Kathrine

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Nutrient (N and P) dynamics of the invasive macroalga *Gracilaria vermiculophylla:* Nutrient uptake kinetics and nutrient release through decomposition

Morten F. Pedersen* and Kathrine L. Johnsen

Department of Science and Environment (DSE)

Roskilde University, Box 260, DK-4000 Roskilde, Denmark.

* Corresponding author: Morten Foldager Pedersen, email: mfp@ruc.dk, phone: +45 46742088.

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Abstract

The invasive alga *Gracilaria vermiculophylla* was introduced to Europe two decades ago and has since become dominant in many shallow estuaries. *G. vermiculophylla* is a relatively fast-growing alga that thrives well at low nutrient availability in summer, suggesting that it uses nutrients efficiently, which might give it a competitive advantage over many native species. We studied therefore the nutrient dynamics of *G. vermiculophylla* and compared it to those of a range of native macroalgal species. Nutrient uptake rates (NH_4^+, NO_3^- and PO_4^{3-}) were compared to growth related requirements and we found that *G. vermiculophylla* needs relatively high nutrient concentrations to sustain fast and non-limited growth. This compares to the nutrient dynamics of many fast-growing algae and we found thus no indication that *G. vermiculophylla* should have any particularly advantage relative to other, sympatric species. The nutrient storage capacity of *G. vermiculophylla* was, in contrast, relatively large and comparable to that of more slow-growing algae, which, when combined with the low nutrient uptake experienced in summer, could explain how *G. vermiculophylla* can sustain non-nutrient limited growth through most of the growth season. The biomass of *G. vermiculophylla* can be massive and estimates showed that gross nutrient uptake could exceed the amount of nutrients received from land. The turnover of *Gracilaria* biomass is however fast and nutrients bound in the resulting detritus are quickly mineralized during decomposition, which is especially important during late summer when water temperatures are high. Invasion and subsequent dominance by *G. vermiculophylla* may thus affect local nutrient cycling significantly.

Key words: Nitrate, ammonium, phosphate, uptake kinetics, mineralization, seaweed.

Introduction

The red alga *Gracilaria vermiculophylla* originates from NE Asia (Tseng and Xia 1999), but was introduced to North America and Europa with imported oysters intended for aquaculture in the 1990's (Mollet et al. 1998). *G. vermiculophylla* is invasive (Nyberg et al. 2009) and has spread along the Atlantic coast of Europe and is now found from Venice lagoon in the Mediterranean to southern Norway in Scandinavia (Thomsen et al. 2007; Sfriso et al. 2012). *G. vermiculophylla* was first observed in Danish waters in 2003 and is now common in many estuaries of the western Baltic Sea (Thomsen et al. 2007; Weinberger et al. 2008). *G. vermiculophylla* is mostly abundant in sheltered, soft-bottom areas where it may become dominant and sometimes replaces previously common seaweeds such as *Ulva* sp. (Nejrup and Pedersen 2010) or *Fucus vesiculosus* (Weinberger et al. 2008). *G. vermiculophylla* is considered an ecosystem engineer (Wallentinus and Nyberg 2007; Byers et al. 2012) and where abundant it may not only affect the composition of the algal assemblage, but also affect local biogeochemistry, nutrient cycling including the transfer of nutrients between abiotic and biotic components (Tyler and McGlathery 2006; Hardison et al. 2010; Gulbransen & McGlathery 2013) and alter trophic relations in the food web (Wallentinus and Nyberg 2007).

Gracilaria vermiculophylla has a number of traits that are typical for invasive algae; it is relatively fast-growing (22% d^{-1}; Raikar et al. 2001), recruits from both spores and fragments (Rueness 2005; Thomsen et al. 2007; Nyberg et al. 2009), it is tolerant to desiccation and extreme levels of light, temperature and salinity (Yokoya et al. 1999; Raikar et al. 2001; Nejrup and Pedersen 2012; Nejrup et al. 2013). Recent studies have further shown that *G. vermiculophylla* is avoided by many native herbivores within its invaded range (Thomsen and McGlathery 2007; Weinberger et al. 2008; Nejrup et al. 2012; Hammann et al. 2013) due to inducible chemical defenses (Nylund et al. 2011; Hammann et al. 2016).

Low nutrient availability (permanently or periodically) restricts growth of fast-

74 growing species more than that of more slow-growing species because the latter are better
75 adapted to cope with low nutrient availability (Pedersen and Borum 1996; Pedersen and
76 Borum 1997; Pedersen et al. 2010) and nutrient richness may therefore affect species
77 composition of algal assemblages (Bokn et al. 2003; Karez et al. 2004; Kraufvelin et al. 2006;
78 Kraufvelin et al. 2010). *G. vermiculophylla* is relatively fast growing and should therefore
79 have high nutrient demands per unit biomass and time and be susceptible to nutrient
80 limitation during late spring and summer where insolation and water temperature is high
81 enough to support rapid growth, but where the availability of nutrients is low. Nejrup and
82 Pedersen (2010) studied seasonal variations in biomass and growth of *G. vermiculophylla* in
83 two Danish estuaries with low nutrient availability in summer, but were unable to detect any
84 significant increase in growth following experimental nutrient enrichment. This result
85 indicates that *G. vermiculophylla* has the capacity to acquire dissolved nutrients efficiently
86 even when these are present at low concentrations or, that it is able to sustain growth in
87 summer by using internal nutrient reserves obtained during winter and early spring where
88 nutrient availability is high. Both strategies are comparable to those of more slow-growing
89 species that can sustain near maximum growth rates during extended periods of low nutrient
90 availability (Pedersen and Borum 1996; Pedersen and Borum 1997; Pedersen et al. 2010).
91 Fast growth combined with an efficient uptake capacity (relative to its demands)
92 and/or high nutrient storage capacity may leave *G. vermiculophylla* competitively superior
93 under low nutrient availability, which could add to explain its recent success in European and
94 north American estuarine waters. Few studies have investigated the nutrient dynamics of *G.*
95 *vermiculophylla* and most of these have only assessed one or a few aspects of the nutrient
96 dynamics, i.e. either uptake kinetics (Tyler et al. 2005; Tyler and McGlathery 2006; Abreu et
97 al. 2011) or the role of *in situ* nutrient limitation (e.g. Thomsen and McGlathery 2007; Nejrup
98 and Pedersen 2010). No studies have yet evaluated the full set of dynamics including uptake

kinetics, requirements for growth and the role of stored nutrients.

Seaweeds may acquire and temporarily immobilize a large proportion of the nutrients received from land, especially in shallow estuaries and coastal lagoons where algal biomass can be substantial (Tyler and McGlathery 2003; Pedersen et al. 2004). Nutrients incorporated into macroalgal biomass become temporarily unavailable for other primary producers until they are released through grazing or decomposition. The turnover rate of algal biomass and, hence, the release of nutrients, differs systematically among seaweeds with different life strategies; slow-growing macroalgae tend generally to be grazed less and decompose more slowly than fast-growing species (e.g. Buchsbaum et al. 1991; Enriquez et al. 1993; Banta et al. 2004; Conover et al. 2016). Algal assemblages dominated by fast-growing and bloom-forming macroalgae do therefore have a faster and more variable turnover of nutrients than those dominated by slow-growing, perennial macrophytes (Duarte and Cebrián 1996; Banta et al. 2004) and release of nutrients from dense populations of opportunistic seaweeds may periodically exceed land-derived inputs and the efflux of nutrients from sediments (Tyler et al. 2003). Grazing on *G. vermiculophylla* is insignificant (e.g. Weinberger et al. 2008; Nejrup and Pedersen 2010) so most of the nutrients contained in the biomass must consequently be released through decomposition. Invasion and subsequent dominance by *G. vermiculophylla* may thus potentially influence nutrient dynamics at the ecosystem level if the decomposition rate of *G. vermiculophylla* differs markedly from the species it has replaced.

The aim of this study was 2-fold. The major aim was to evaluate all aspects of the nutrient dynamics of *G. vermiculophylla* and to compare it to those of other common, indigenous algal species to assess whether *G. vermiculophylla* uses nutrients more efficiently than the algae it is potentially competing with. We wanted next to study the release of major nutrients (N and P) from decomposing *G. vermiculophylla* and compare it to that of other common, indigenous algal species to assess how *G. vermiculophylla* may affect the turn over

of nutrients in estuarine systems once it has become dominant. We measured nutrient uptake kinetics for ammonium (NH_4^+), nitrate (NO_3^-) and phosphate (PO_4^{3-}) and compared those to growth related N and P requirements, the latter being determined from culturing algae under a range of nutrient concentrations. These data were combined and used to model how growth relates to nutrient availability and additionally, used to estimate the storage capacity for N and P in *G. vermiculophylla*. We conducted finally a series of decomposition experiments to evaluate how fast N and P bound in biomass was released from dead and decaying *G. vermiculophylla* under different temperature regimes

Methods

Gracilaria vermiculophylla was collected at Fyns Hoved, Denmark (55° 36.9' N, 10° 36.7' E) in October 2013. The algae were cleaned and transported to the laboratory where they were kept in 80 L storage tanks until being used in the experiments. The storage tanks and the experimental chambers used for the uptake and growth experiments were kept at constant temperature (15°C) and salinity 25 (PSU) and were illuminated by lamps equipped with halogen spots (OSRAM 12V, 35W) providing a light intensity of ca. 90 µmol photons m^{-2} s^{-1} PAR in a 16:8 hr light:dark cycle. The chosen temperature and salinity is optimal for *G. vermiculophylla* (Nejrup and Pedersen 2012) while the light intensity is sufficient to saturate growth of this species (ca. 60 µmol photons m^{-2} s^{-1}: Nejrup et al. 2013). The initial N and P content in the algae were 1.7 % DW and 0.19 % DW, respectively.

Nutrient uptake kinetics. Uptake kinetics for NH_4^+, NO_3^- and PO_4^{3-} were determined in three separate experiments using a combination of the multiple-flask and the depletion methods as described by Harrison et al. (1989) and Pedersen (1994). Eight PVC-beakers (Vol = 1.6 L) were filled with a known volume (1.0-1.4 L) of seawater (salinity 25) with different added concentrations of the nutrient species in question (NO_3^- range: 15-76 µM; NH_4^+ range: 1-76

149 μM; PO_4^{3-} range: 1-14 μM). Each beaker was bubbled with air to ensure circulation and
150 reduce the thickness of boundary layers. Measurements of nutrient uptake were initiated by
151 adding an algal sample (5-10 g FW) to each beaker where it was attached to a PVC-net to
152 keep it submerged during the incubation. Three replicate water samples (each 5.0 mL) were
153 taken from each beaker during the first hour of the incubation (at time = 0, 15, 30, 45 and 60
154 minutes) and then subsequently at every 30 min for the remaining part of the experiment.
155 Water samples were immediately frozen at -20°C for later analysis of nutrients. The
156 incubations lasted for 120 – 300 minutes and all algae were subsequently harvested and dried
157 to constant dry weight (DW) at 85°C and weighted. Concentrations of NO_3^- in the water
158 samples were analyzed using a Lachat (QuickChem FIA+ 8000 Series) autoanalyzer while
159 concentrations of NH_4^+ and PO_4^{3-} were analyzed manually; the concentration of NH_4^+ was
160 analyzed using the salicylate-hypochlorite method (Bower and Holm-Hansen 1980) while that
161 of PO_4^{3-} was analyzed spectrophotometrically following Strickland and Parsons (1968).
162 Biomass specific nutrient uptake rates (V) were estimated from changes in substrate
163 concentration (S) over the course of the experiment:

164 $$V = \frac{(S_0 \times Vol_0) - (S_T \times Vol_T)}{t \times B} \quad \text{(Eq. 1)}$$

165 where V is the uptake rate (in μmol g^{-1} DW h^{-1}), S_0 and Vol_0 are the substrate concentration (in
166 μM) and volume (in L) at the beginning of a time interval, while S_T and Vol_T are the substrate
167 concentration and volume at the end of a time interval. t is the time elapsed between two
168 successive samplings and B is the DW biomass. Uptake rates determined on algae from
169 different beakers, but obtained during identical time intervals, were plotted against the mean
170 substrate concentrations obtained in each beaker during that specific time interval. The
171 Michaelis-Menten function (Eq. 2) was fitted to data by least square non-linear regression
172 using SYSTAT v. 13:

173 $$V = \frac{V_{max} \times S}{K_m + S} \quad \text{(Eq. 2)}$$

where V is the uptake rate (in μmol g^{-1} DW h^{-1}), V_{max} is the maximum uptake rate, K_m is the half-saturation constant (in μM) and S is the substrate concentration (in μM). Uptake rates of NH_4^+ and PO_4^{3-} were initially enhanced when the algae were exposed to nutrients (figure 1) while no such transiently enhanced uptake was observed in the case of NO_3^-. Parameter estimates of V_{max} and K_m (table 1) were therefore represented by 2 sets of uptake kinetics: (1) one representing transiently enhanced uptake rates (i.e. surge uptake), measured over the initial 30 minutes after exposure to nutrients and, (2) one representing rates obtained after 90 minutes in the case of NH_4^+ and later than 30 minutes after exposure in the case of NO_3^- and PO_4^{3-} (hereafter called assimilation).

Nutrient requirements. Algae were first pre-conditioned for 4-6 weeks in 10 aquaria (volume = 20 L) receiving different quantities of dissolved N or P, to obtain specimens with different tissue nutrient concentrations. Nutrients were added from stock-solutions of NH_4NO_3 and KH_2PO_3. Algae receiving different levels of NH_4NO_3 received KH_2PO_3 in excess while algae receiving different levels of KH_2PO_3 received NH_4NO_3 in excess to ensure that only one nutrient was limiting at the time. The aquaria were exposed to low light (40 μmol photons m^{-2} s^{-1} PAR in a16:8 hr light:dark cycle) using shade screens to ensure slow growth, which eased the accumulation of nutrients in the tissues. Tissue N-concentrations in algae intended for the N-growth experiment ranged from 1.0 to 3.7% N of DW at the end of the pre-condition period while the P-content in these algae averaged 0.23 % of DW. Final tissue P-concentrations in algae intended for the P-growth experiment ranged from 0.04 to 0.27 % of DW while the N-content in these algae averaged 2.4 % of DW .

Growth measurements were subsequently conducted in PVC-beakers (volume = 1.6L), which were filled with GF/C-filtered seawater (salinity 25) that had been stripped for inorganic nutrients by letting *Ulva lactuca* grow in it for 2-3 days prior to use. The beakers were placed under saturating light (90 μmol m^{-2} s^{-1} PAR) and each beaker was bubbled with

199 atmospheric air to create circulation and ensure exchange of O_2 and CO_2. The initial FW
200 biomass was determined on all pre-conditioned specimens prior to the growth measurements
201 and the DW:FW ratio (and initial tissue N or P concentrations) was determined on a number
202 of sacrificed subsamples after drying them to constant weight at 85°C. The algae were left to
203 grow for 5-7 days after which they were harvested, dried to constant weight and analyzed for
204 final tissue N or P content. Growth rates were estimated from changes in biomass over time
205 assuming exponential growth:

206 $$\mu = \frac{\ln B_T - ln B_0}{t} \quad \text{Eq. 3}$$

207 where μ is the relative growth rate (d^{-1}), B_0 and B_T are the initial and final DW biomass and t
208 is the incubation time. Growth rates were plotted against tissue nutrient concentrations and
209 the Droop equation was fitted to data using least square, non-linear regression (SYSTAT v.
210 13):

211 $$\mu = \mu_{max} \times \left(1 - \frac{Q_S}{Q}\right) \quad \text{Eq. 4}$$

212 where μ is the relative growth rate (d^{-1}), μ_{max} is the maximum growth rate, Q_S is the
213 subsistence cell quota (in % of DW), i.e. the lowest tissue nutrient concentration that allows
214 growth and Q is the average tissue N or P concentration (i.e. = (Q_{Init} + Q_{Final})/2). The critical
215 cell quota (Q_C) was defined as the tissue nutrient concentration above which growth is not
216 limited by lack of nutrients. The Droop function is a continuous function so we arbitrarily
217 determined Q_C as the tissue nutrient concentration where the corresponding growth rate
218 equaled 67% of the estimated μ_{max} (Pedersen and Borum 1996).
219 **Modeling substrate dependent growth.** The kinetics of substrate dependent growth were
220 estimated by combining data for substrate dependent uptake kinetics and tissue nutrient
221 dependent growth. Substrate dependent growth of macroalgae is often described by Monod
222 kinetics (e.g. Rosenberg et al. 1984) with parameters μ^*_{max} and $K_{\mu,}$ which can be estimated

223 from equations 5 and 6 assuming that nutrient uptake and use of nutrients for growth is in
224 steady state (Turpin 1988):

225 $$\mu^*_{max} = \frac{(\mu_{max} \times V_{max})}{[(\mu_{max} \times Q_S) + V_{max}]} \quad \text{Eq. 5}$$

226 $$K_\mu = \frac{(K_m \times \mu_{max} \times Q_{min})}{[(\mu_{max} \times Q_S) + V_{max}]} \quad \text{Eq. 6}$$

227 where V_{max} and K_m are the maximum uptake rate (in μ mol g^{-1} DW h^{-1}) and the Michaelis-
228 Menten constant (in μM), respectively, obtained from the nutrient uptake experiments (Eq. 2)
229 while μ_{max} and Q_S are the maximum growth rate (d^{-1}) and subsistence cell quota (in % DW)
230 obtained from the growth experiments (Eq. 4).
231 **Storage capacity.** The amount of N or P being stored in excess of what is needed to obtain
232 maximum growth was estimated as the difference between the critical quota (Q_C) and the
233 highest observed quota (Q_{max}) in algae from the growth experiment. The excess amount of
234 cellular N or P, respectively, can support growth at maximum rates until Q_C is reached after
235 which growth ceases as the cell quota approaches Q_S. We defined storage capacity as the
236 number of days ($T_{Storage}$) that this pool of N or P could support growth (at maximum and
237 reduced rates) without additional acquisition of N or P from the medium. The storage capacity
238 was estimated from a numerical solution of equation 4 for Q. We used a time step of 0.1 day
239 for each iteration and started out with an initial cell quota equal to the observed Q_{max}. A new
240 cell quota was estimated for each time step using the estimated growth rate from the previous
241 time step. The storage capacity that could support non-nutrient limited growth was then
242 defined as the time passing until the cell quota reached Q_C while the storage capacity that
243 could support growth at reduced rates was defined as the time it took to reduce the quota from
244 Q_C to Q_S. Potential time lags to mobilize nutrient reserves were not accounted for.
245 **Decomposition and mineralization.** Decomposition of *G. vermiculophylla* and
246 mineralization of N and P bound in its tissue was studied using litterbags. 105 algal samples

247 (each 0.6 – 2.5 g FW) were cleaned and their initial FW biomass determined. Fifteen samples
248 were initially sacrificed and dried for determination of the initial DW:FW ratio. The
249 remaining ninety samples were placed in separate litterbags with a mesh size of 1 mm, which
250 were distributed equally among nine aquaria. Each aquarium had a volume of 45 L and was
251 filled with ca. 20 L of sediment from the sampling site and 20 L of seawater (salinity 25). The
252 aquaria were kept dark in 3 climate chambers with temperatures 5, 15 and 25°C, respectively,
253 thus simulating a typical winter, spring/fall or summer situation. The water was bubbled with
254 atmospheric air to keep it aerated and exchanged with freshly collected seawater monthly.
255 The litterbags with live algae were covered by ca. 1 cm of sediment in an attempt to simulate
256 slight burial as observed in the field. Three litterbags (one per replicate aquarium at each
257 temperature) were retrieved periodically, and the algal remains were rinsed for sand and mud,
258 dried to constant DW at 85°C, weighed and stored for later analysis of tissue nutrients (C, N
259 and P). Decomposition rates were estimated by fitting a multiple-G model (Eq.; Westrich and
260 Berner 1984) to the data (i.e. remaining biomass *vs.* time). This model assumes that detritus
261 may be made up by several fractions, each decomposing at a specific rate (k_i) plus a refractory
262 fraction (G_R) that does not decompose within the time-scale studied. If only one actively
263 decomposing pool of matter and a refractory pool can be deduced from data, the model
264 simplifies to:

265 $$B_T = B_0\, e^{-kt} + G_R \qquad \text{Eq. 7}$$

266 where B_T and B_0 are the final and initial biomass (measured in units of C, N or P), k is the
267 decay rate, t is the number of days elapsed since initiating the experiment and G_R is the size of
268 the residual (i.e. non-reactive) fraction of the detritus.
269 **Tissue nutrient analyses.** Tissue concentrations of C and N were determined on dried and
270 ground samples using an EA 1110 CHNS elemental analyze (CE Instruments). Tissue P was
271 determined on dried and ground algae after oxidation with boiling H_2SO_4 followed by

spectrophotometric analysis (Strickland and Parsons 1968).

Results

Nutrient uptake kinetics. Uptake rates of NH_4^+, NO_3^- and PO_4^{3-} increased with increasing substrate concentration and saturated at high concentrations (figure 1) why uptake kinetics were described by the Michaelis-Menten function (R^2-values ranged from 0.629 to 0.930; all p-values <0.001). The maximum surge uptake rate of NH_4^+ was 2-fold higher than the maximum assimilation rate, while the half-saturation constant for uptake (K_m) during surge uptake was 3-fold higher than that for assimilation. These differences resulted in a higher affinity (α) for NH_4^+ at low substrate concentrations during assimilation than during surge uptake. The initial maximum uptake rate for NO_3^- was not different from that observed later in the experiment (i.e. no surge uptake), but it was 3 to 4-fold lower than both V_{max}^{Surge} and V_{max}^{Ass} for NH_4^+. K_m for NO_3^- averaged 9 µM and the affinity at low substrate concentration ca. 1.7 µM. Initial V_{max} and K_m for PO_4^{3-} were ca. 2-fold higher than for P-uptake occurring after 30 minutes whereas the affinity for P at low PO_4^{3-} concentrations (α) was approximately the same for surge uptake and assimilation.

Growth rate and nutrient requirements. Growth rate increased as a function of N or P quotas (figure 2; R^2 for N = 0.876, p<0.001; R^2 for P = 0.876, p<0.001). Estimated maximum growth rate at infinite N or P quota varied slightly between the two experiments (0.088 – 0.105 d^{-1}), but did not differ significantly according to the 95% CL's (table 2). The N subsistence quota (Q_S) was 0.71% of DW while the critical N quota (Q_C) was 2.14% of DW, resulting in a N-requirement of 169 µmol N g^{-1} DW d^{-1} at maximum growth rate. The subsistence quota for P was 0.05% of DW while the critical P quota was 0.14% of DW, resulting in a P-demand of 4.1 µmol P g^{-1} DW d^{-1}.

296 **Substrate dependent growth.** Substrate dependent growth was modeled from data on uptake
297 kinetics and quota dependent growth and was represented by the derived Monod parameters
298 (table 3). Estimated maximum growth rate based on uptake of NH_4^+ (0.098 d^{-1}) or NO_3^- (0.091
299 d^{-1}) as the N source did not differ much. In contrast, the half-saturation constant for growth
300 (Kμ) based on NO_3^- was 2-fold larger than that for growth on NH_4^+ indicating a higher affinity
301 for NH_4^+. Substrate concentrations needed to saturate growth (S_{Sat}) ranged from 5 μM for NH_4^+
302 to 10 μM for NO_3^-. Estimated maximum growth rate based on uptake of PO_4^{3-} (0.084 d^{-1}) was
303 slightly lower than for NH_4^+ or NO_3^- and K_μ was 0.12 μM PO_4^{3-}. The substrate concentration
304 needed to support maximum growth rate was ca. 1 μM PO_4^{3-}.
305 **Nutrient storage capacity.** The amount of N stored in excess of the critical quota (i.e. Q_{max} -
306 Q_C) attained 14.0 mg N g^{-1} DW, while the internal quantity of N that could support sustained
307 growth at reduced rates (i.e. $Q_C - Q_S$) was 14.3 mg N g^{-1} DW (table 2). These internal stocks
308 of N could support continual growth at maximum or reduced rates for a total of 33 days (6.5
309 days at maximum rate and 26.6 days at reduced rates) without acquisition of dissolved N from
310 the surroundings (table 4). The amount of P in excess of the critical limit was 1.3 mg P g^{-1}
311 DW and the amount of P that could support sustained growth at reduced rates was 1.0 mg P
312 g^{-1} DW (table 2). The internal stocks of P were could support growth for a total period of 43
313 days (10.5 days at maximum rate and 32.5 days at reduced rates; table 4) without having to
314 acquire dissolved P from the medium.
315 **Decomposition and mineralization.** Decomposition of *G. vermiculophylla* followed a simple
316 1-G model without a significant refractory pool (i.e. a simple exponential decline; figure 3,
317 table 5; R^2 ranged from 0.774 to 0.908, all p-values <0.001) since this model provided better
318 fits (higher R^2-values) than multiple G-models with or without a refractory pool. All biomass
319 disappeared within 120 days in the 15 and 25°C treatments, while about 30% of the original
320 biomass was left in the 5°C treatment at the end of the experiment. Decay rates based on loss

of C biomass ranged from 0.013 to 0.065 d^{-1} depending on temperature and increased almost 5-fold across the temperature range from 5 to 25°C. Temperature had the same effect on loss of tissue-bound N and P. Decomposition rates ranged from 0.016 to 0.079 d^{-1} when expressed in units of N and were marginally higher than when expressed in units of C. Decay rates expressed in units of P ranged from 0.023 to 0.1 d^{-1} depending on temperature and were substantially higher than those expressed in units of C or N, indicating that P was mineralized faster than C and N during decomposition.

Discussion

The balance between uptake capacity and nutrient requirements and, thus, the risk of suffering nutrient limitation during periods of low nutrient availability is related to the maximum growth rate of algae. Fast-growing algae have a large relative surface area (i.e. high SA:V ratio) and a high capacity for nutrient uptake (Wallentinus 1984; Hein et al. 1995), but also large nutrient requirements per unit biomass and time dictated by their fast growth (Pedersen and Borum 1997; Pedersen et al. 2010). In contrast, slow-growing species have lower uptake capacities and lower requirements, but uptake rate and nutrient demands are better scaled at low nutrient availability in these algae (Pedersen and Borum 1997; Pedersen et al. 2010), why slow-growing algae generally can grow at near maximum rates when concentrations of dissolved inorganic N (DIN) and P (DIP) in the water are low.

Our findings failed to support the hypothesis presented by Nejrup and Pedersen (2010); *G. vermiculophylla* did not have an exceptionally high affinity for inorganic nutrients relative to its requirements for growth. Nutrient demands are largely determined by apparent growth rate and critical cell quotas. *G. vermiculophylla* attained maximum growth rates between 0.09 and 0.11 d^{-1}, which is comparable to those reported from other studies (Yokoya et al. 1999; Raikar et al. 2001; Nejrup and Pedersen 2010; Nejrup et al. 2013), but lower than

346 for truly fast-growing, sheet-like and filamentous species and higher than for most fucoids
347 and Laminarians (see table 6 for a comparison with algae indigenous to Scandinavian waters).
348 *G. vermiculophylla* can thus be ranked as an algal species with an intermediate growth rate.
349 The experimentally determined critical N quota of *G. vermiculophylla* is rather high
350 and comparable to those of fast-growing species such as *U. lactuca* and *C. virgatum* (table 6).
351 Red algae have generally higher N quotas than green and brown algae due to the prevalence
352 of protein rich pigments in red algae (Hurd et al. 2014). The critical P quota of *G.*
353 *vermiculophylla* was, in contrast, low and corresponded to those of more slow-growing
354 species such as *F. vesiculosus* and *A. nodosum* (table 6). The observed maximum growth rates
355 and critical N and P quotas resulted in maximum N and P requirements corresponding to ca.
356 169 and 4 μmol N or P g^{-1} DW d^{-1}, respectively, which is lower than for truly fast-growing
357 algae such as *U. lactuca* and *C. virgatum*, but higher than those for more slow-growing
358 species (Pedersen and Borum 1997; Pedersen et al. 2010).
359 High nutrient requirements do not necessarily represent a problem if the affinity for these
360 nutrients is high enough. The obtained nutrient uptake kinetics of *G. vermiculophylla*
361 corresponded to those found by other authors (e.g. Tyler et al. 2005; Abreu et al. 2011) and
362 show that maximum uptake rates for dissolved inorganic N and P by far exceed the
363 requirements even when *G. vermiculophylla* is growing at maximum rate. Such uptake rates
364 can, however, only be obtained at relatively high and often ecologically irrelevant substrate
365 concentrations. The main question was therefore whether the capacity of *G. vermiculophylla*
366 to acquire nutrients is better scaled to growth related nutrient demands than in native algae
367 that it potentially competes with. This is best evaluated by comparing species-specific
368 substrate dependent growth and its related parameters across species. The half-saturation
369 constants (K_{μ}) for growth and the substrate concentrations needed to saturate growth (S_{SAT})
370 on either NH_4^+ or NO_3^- tended to be higher than those of more slow-growing algae and

371 comparable to those of faster growing algae showing that *G. vermiculophylla* requires
372 relatively high DIN concentrations to saturate growth while the opposite is true for DIP. The
373 half-saturation constant (K_μ) for growth on PO_4^{3-} and the concentration of DIP needed to
374 saturate growth were low and comparable to those of more slow-growing algae such as *Fucus*
375 *vesiculosus* and *F. serratus*, which could give *G. vermiculophylla* and advantage under low P
376 availability. P-limitation is however rare in most temperate estuaries with terrigenous
377 sediments, where N-limitation is more common (e.g. Howarth and Marino 2006). Nejrup and
378 Pedersen (2010) showed that the tissue N:P ratio in *G. vermiculophylla* from Holckenhavn
379 Fjord and Fyns Hoved reached 40-45 for a short period in early spring, thus inferring P-
380 limitation. The absolute P-content in these algae was however relatively high (>0.2 % of DW)
381 and experimental fertilization did not stimulate growth. Slow growth in early spring seems
382 thus to be dictated by low water temperature and insolation rather than by P-limitation and
383 low P-requirements do therefore not seem to represent a clear advantage for *G.*
384 *vermiculophylla*. In summary, we found no clear evidence that the nutrient kinetics of *G.*
385 *vermiculophylla* result in higher nutrient use efficiency than in comparable, sympatric
386 indigenous algal species and therefore, no indication that *G. vermiculophylla* should have a
387 specific advantage relative to native species under nutrient replete conditions.
388 Nutrient storage plays an essential role for seaweeds in areas where nutrient
389 availability undergoes large seasonal variations. Little is know about the forms in which
390 excess P is stored in macroalgae, but N is mainly stored as proteins and amino acids while
391 inorganic N and N bound in pigments may constitute smaller and less important N reserves
392 (Bird et al. 1982; McGlathery et al. 1996). Although some amino acids play essential roles for
393 osmoregulation (e.g. proline; Kirst 1989) and photoprotection (e.g. mycosporine-like amino
394 acids; Karsten et al. 2000) there is a strong correlation between total N content and the pool
395 sizes of proteins, amino acids, inorganic N and pigment bound N, which shows that all these

pools take part in N storage and can be mobilized when necessary (Bird et al. 1982; McGlathery et al. 1996).

The amount of N and P stored in excess of that needed to support the maximum growth rate in *G. vermiculophylla* was ca. 14 mg N g^{-1} DW and 1.3 mg P g^{-1} DW, respectively. The observed N-reserve was somewhat less than for a range of indigenous algal species (Pedersen and Borum 1996; Pedersen et al. 2010), which suggests that the algae used in our experiments may not have been saturated with nutrients. The storage capacity for nutrients (defined as the time where growth could be sustained without additional nutrient uptake from the medium) was ca. 33 days for N and 43 days for P, respectively. The storage capacity for N was larger for a number of fast-growing indigenous algae (*U. lactuca, C. virgatum, Cladophora* sp. and *Chaetomorpha linum*: range from 9.6 to 17.5 days), but smaller than for slow-growing *F. vesiculosus* (45.9 days; Pedersen and Borum 1996).

The N and P-reserves obtained through winter and spring can obviously not sustain maximum growth of *G. vermiculophylla* throughout extended periods of low nutrient availability (i.e. mid May to mid September). However, even though *in situ* nutrient concentrations in Holckenhavn Fjord and Fyns Hoved were low (Nejrup and Pedersen 2010), they were not low enough to prevent nutrient uptake from the water completely. The observed concentrations of DIN ranged between 1 and 3 μM from mid May to mid September and should be high enough to cover about 50% of the N-demand for maximum growth, meaning that the N reserves could last for about twice as long as estimated assuming no uptake (i.e. ca. 2 months). Equally important, average *in situ* growth rates in Holckenhavn Fjord during the growing season were some what lower than those attained in this study (ca. 0.05 d^{-1}, range 0.032 – 0.065 d^{-1}; Nejrup and Pedersen 2010), meaning that 'dilution' of the internal nutrient reserves would occur more slowly and the stores last longer than predicted when assuming growth at maximum attainable rates. In other words, the combined effect of continuous

421 nutrient uptake (albeit at reduced rates) and slower growth (i.e. lower nutrient demands per
422 unit biomass and time) increases the storage capacity of the algae by a factor of ca. 4, i.e. to
423 130-160 days, which should be enough to sustain non-nutrient limited growth during most of
424 the major growth season. This conclusion is supported by the fact that Nejrup and Pedersen
425 (2010) were unable to stimulate *in situ* growth significantly by experimental nutrient
426 enrichment and by the fact that the N content in algae from the control treatment in
427 Holckenhavn Fjord and Fyns Hoved remained close to ca. 2.5% of DW (i.e. above the critical
428 N content) throughout summer. Relatively fast and non-nutrient limited growth by *G.*
429 *vermiculophylla* during summer can thus be explained by the use of reserve N (and P) rather
430 than by an extraordinarily high affinity for inorganic nutrients.
431 The large biomass of *G. vermiculophylla* found in some estuaries, e.g. >1000 g DW
432 m^{-2} in some samples from Hog Island Bay, Virginia, USA (Thomsen et al. 2006), 464 g DW
433 m^{-2} on average in Holckenhavn Fjord, Denmark (Nejrup and Pedersen 2010) and 6-700 g DW
434 m^{-2} in parts of Venice lagoon, Italy (Sfriso et al. 2012) suggests that large amounts of DIN
435 and DIP are assimilated and become bound in living or dead biomass and, thus, that *G.*
436 *vermiculophylla* has the potential to affect nutrient cycling significantly in such systems (e.g.
437 Tyler et al. 2003; Tyler and McGlathery 2006). Like for other bloom-forming algae, the
438 biomass of *G. vermiculophylla* might undergo large and quick temporal variations where
439 algae suffer high mortality and the biomass is turned into detritus that decomposes under
440 release of nutrients. Whether detritus originating from *G. vermiculophylla* serves as a
441 temporary sink for nutrients during summer or functions as an internal source of nutrients
442 within the system depends on: 1) the turn-over of biomass and, thus, the production of detritus
443 and, 2) the rate at which that detritus decays and the bound nutrients become mineralized.
444 We have no direct estimates of the biomass turnover of *G. vermiculophylla*, but data
445 on seasonal changes in biomass and *in situ* growth rates from Holckenhavn Fjord, from

446 Nejrup and Pedersen (2010) show that the potential production of *G. vermiculophylla* may
447 reach ca. 5200 g DW m^{-2} during the growing season (from March to October) corresponding
448 to a net incorporation of ca. 78 g N and ca. 10 g P m^{-2} (assuming a mean N and P content of
449 1.5% of DW and 0.2% of DW, respectively). The average load of total nitrogen (TN) and
450 total phosphorus (TP) per unit of estuarine area across 47 Danish estuaries is ca. 20 g TN m^{-2}
451 y^{-1} and 0.8 g TP m^{-2} y^{-1} (Conley et al. 2000) so *G. vermiculophylla* may have a substantial
452 effect on nutrient cycling in Holckenhavn Fjord. The biomass of *G. vermiculophylla* changed
453 little over the season (ranging from 464 g DW m^{-2} in March to 176 g DW m^{-2} in October), so
454 biomass losses and, hence, the production of detritus must have been in the same order of
455 magnitude as the production of biomass (estimated to ca. 5400 g DW m^{-2} in 7 months) since
456 grazing on *G. vermiculophylla* is insignificant (Nejrup and Pedersen 2010). Large amounts of
457 nutrient are thus being acquired and bound into living and dead algal biomass and will
458 become released when the detritus decomposes. Decomposition rates of *G. vermiculophylla*
459 ranged from 0.013 to 0.065 d^{-1} depending on temperature and were markedly lower than those
460 reported by Conover et al. (2016). Conover et al. (2016) used dead tissues killed by freezing
461 which were placed on top of the sediment (i.e. under aerobic conditions) whereas we used live
462 material that was slightly covered by sediment (i.e. partly hypoxic conditions). Freezing may
463 break the cell membrane and wall and cause a rapid loss of soluble compounds from the
464 detritus and, thus, speed up decomposition. The use of live material may, in contrast, delay
465 initial decay and the initial loss of biomass may be due to respiration rather than decay. Dark
466 respiration rate in *G. vermiculophylla* corresponds, however, to a much smaller biomass loss
467 than that observed in our decomposition study (e.g. <0.003 d^{-1}; Nejrup et al. 2013) suggesting
468 that the biomass losses we observed were caused by decomposition mainly.
469 Decomposition of *G. vermiculophylla* was strongly temperature dependent;
470 decomposition was 4-5 fold faster at 25°C than at 5°C, corresponding to a half-time of about

471 10 days under late summer conditions suggesting that nutrients bound in detritus generated
472 during summer and early autumn are released almost completely within the time-scale of
473 weeks whereas it takes much longer during winter and early spring. An increasing algal
474 biomass in early spring and subsequent production of detritus during early summer may thus
475 immobilize bio-available nutrients whereas the opposite will be true in late summer and early
476 autumn where the production of detritus increases and dead *G. vermiculophylla* decomposes
477 fast and, thus, will act as a major source of nutrients. Nutrients released from living and dead
478 *G. vermiculophylla* may not only be recycled back to live and nutrient deplete *Gracilaria* in
479 the upper layers of the mats, but may also be taken up by other primary producers including
480 phytoplankton and microphytobenthos or diffuse into the sediment where it can take part in
481 biogeochemical processes (Hardison et al. 2010; Gulbransen and McGlathery 2013).
482 Decomposition rate for *G. vermiculophylla* at 15°C were obtained under the same
483 conditions as rates for other estuarine macroalgae reported by Banta et al. (2004) and
484 Pedersen et al. (2005), which allows for a direct comparison with these data. Detritus from *G.*
485 *vermiculophylla* decomposed completely within 120 days at 15°C. The decay rate (0.032 d^{-1})
486 was lower than those of *Ulva lactuca* and *Ceramium virgatum* (0.038 - 0.040 d^{-1}) and similar
487 to that of *Fucus vesiculosus* (0.028 d^{-1}; Banta et al. 2004). *F. vesiculosus* contains however
488 refractory compounds, which will leave ca. 7% of the initial biomass after 340 days (Banta et
489 al. 2004). Detritus from *G. vermiculophylla* decomposed markedly faster than that from
490 *Sargassum muticum* (0.016 d^{-1}) and *Halidrys siliquosa* (0.019 d^{-1}), respectively (Pedersen et
491 al. 2005). The release of nutrients through decomposition and, thus, the potential impact of *G.*
492 *vermiculophylla* on local nutrient cycling depends therefore partly on which species it has
493 succeeded. Nutrient cycling may be slowed down when fast-growing species like *Ulva* sp.
494 and *Ceramium* sp. are replaced by *Gracilaria* because the former are grazed more and their
495 detritus decomposes faster than for *G. vermiculophylla*. Dominance by *G. vermiculophylla*

will thus tend cause a slower turnover of nutrients and the biomass of living and dead *Gracilaria* may act as a temporary sink for nutrients. The opposite is expected to be the case if *G. vermiculophylla* succeeds slow-growing species such as *Fucus* sp. *Halidrys siliquosa* etc., which are less susceptible to grazing and produces detritus that decomposes slowly and/or incompletely. Here dominance of *G. vermiculophylla* will tend to speed up the turnover of nutrients due to its relatively fast and complete decomposition.

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657 **Figure legends**

658 **Fig. 1** *Gracilaria vermiculophylla.* Uptake rates (V) of ammonium, nitrate and phosphate as a
659 function of substrate concentration (S). Surge uptale (●) was measured over the initial 30
660 minutes after exposure to nutrients, while assimilation rates (○) were measured later than 30
661 minutes after exposure. Lines represent the best fits of the Michalis-Menten function to the
662 data

663

664 **Fig. 2** *Gracilaria vermiculophylla.* Relationship between relative growth rate (μ) and N
665 quota (figure A) or P quota (figure B) as determined in laboratory experiments. Lines
666 represent the best fits of the Droop equation to the data

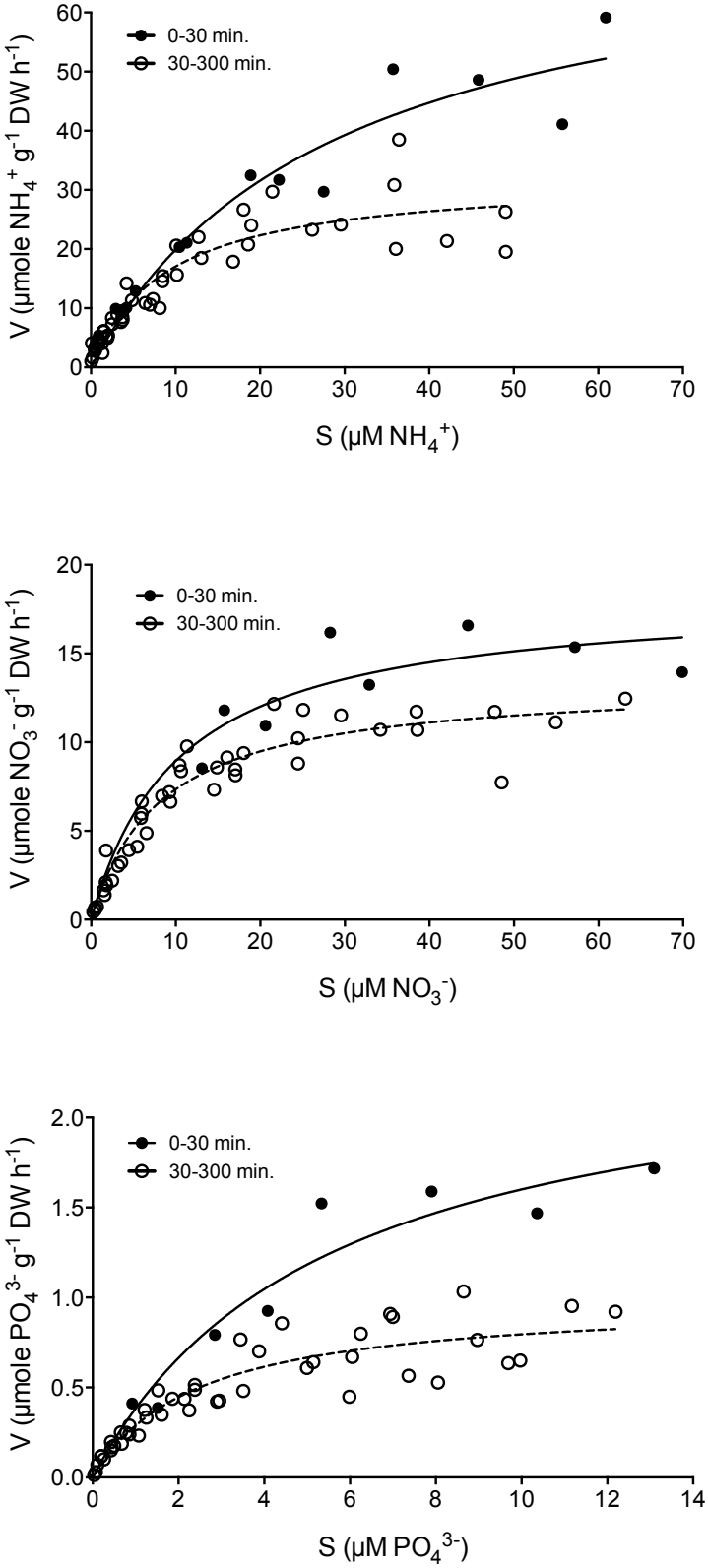
667

668 **Fig. 3** *Gracilaria vermiculophylla.* Changes in C, N and P biomass during decomposition of
669 dead *Gracilaria vermiculophylla* at three different temperatures. A: 5°C, B: 15°C and C:
670 25°C. Lines represent the best fits to an exponential function. Values are means ±95%
671 confidence limits (n=3)

672

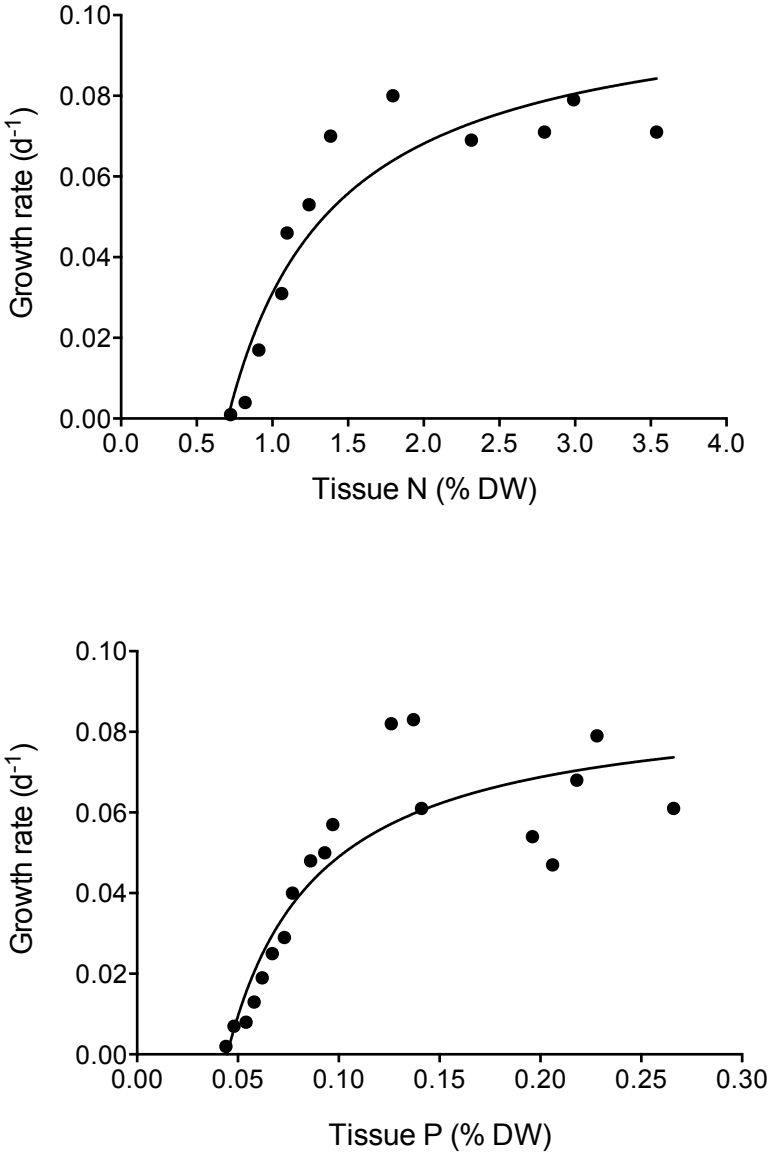
673

Figure 1.


0-30 min.
30-300 min.
V (µmole NH4+ g-1 DW h-1)
S (µM NH4+)
V (µmole NO3- g-1 DW h-1)
S (µM NO3-)
V (µmole PO43- g-1 DW h-1)
S (µM PO43-)

674

675 **Figure 2.**



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678 **Figure 3.**

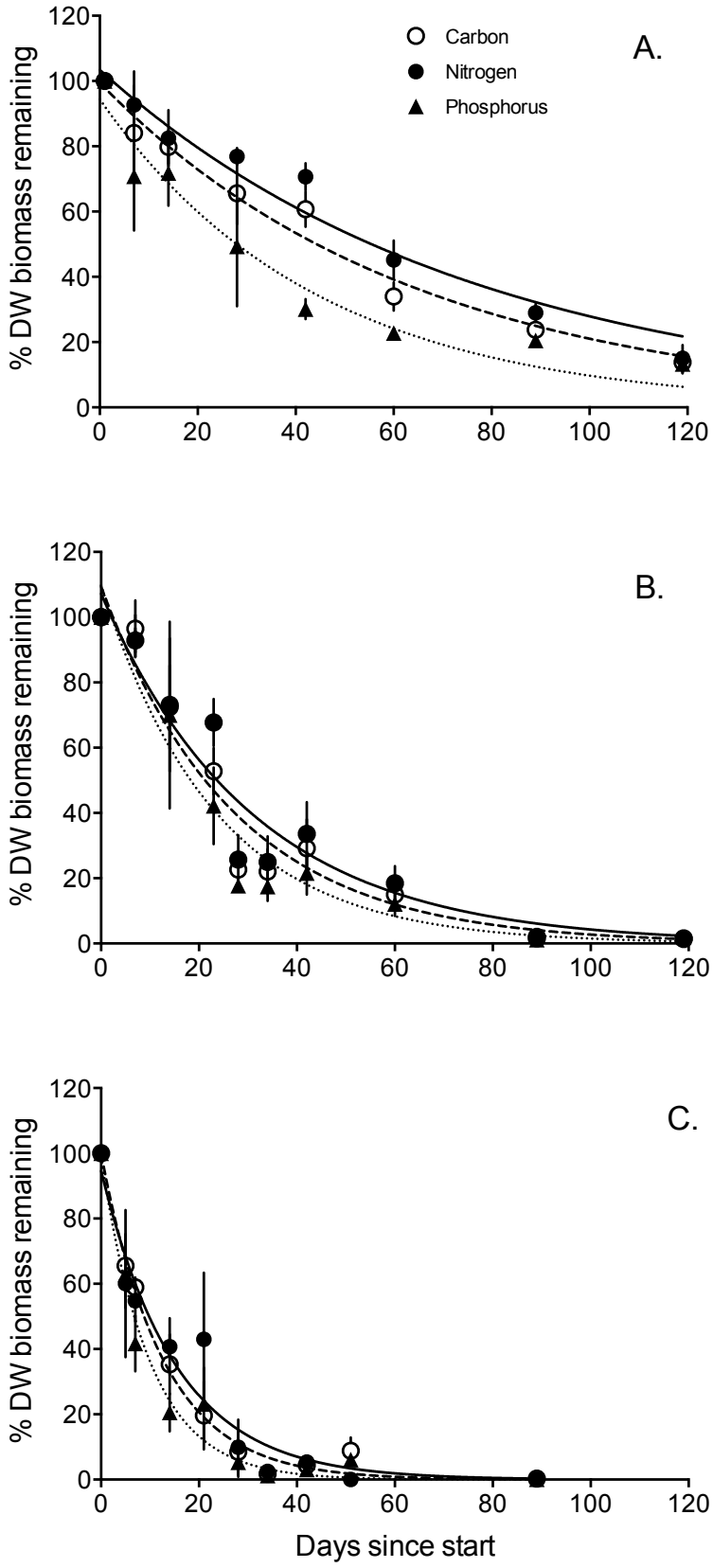

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Table 1. Nutrient (NH_4^+, NO_3^- and PO_4^{3-}) uptake kinetics. Parameters V_{max} (µmol N or P g^{-1} DW h^{-1}), K_m (µM) and affinity for uptake at low substrate concentrations (V_{max}/K_m; Healy 1980) are given for both initial surge uptake measured over the first 30 minutes of exposure to nutrients and for uptake and assimilation measured later than 30 minutes after exposure to nutrients. Numbers are means ±95% confidence limits.

	Surge uptake (0-30 min.)			Assimilation (30-300 min.)		
	V_{max} (µmol g^{-1} DW h^{-1})	K_m (µM)	V_{max}/K_m	V_{max} (µmol g^{-1} DW h^{-1})	K_m (µM)	V_{max}/K_m
NH_4^+	76.8 ±24.5	28.7 ±19.7	2.68	32.3 ±4.4	8.9 ±3.2	3.63
NO_3^-	18.3 ±5.6	10.4 ±12.4	1.76	13.4 ±1.3	8.3 ±2.3	1.61
PO_4^{3-}	2.47 ±1.01	5.5 ±5.1	0.45	1.0 ±0.2	2.4 ±1.0	0.42

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Table 2. Parameter estimates of maximum growth rate (μ_{max}) and subsistance cell quota (Q_S) as determined from fitting the Droop function to data (i.e. growth rates *vs.* cell quota). Estimated means ±95% confidence limits. Critical cell quotas (Q_C) were estimated as the quota where growth rate equalled 66.7% of μ_{max} while maximum cell quotas (Q_{max}) correspond to the highest quotas obtained in the experiment.

	μ_{max} (d^{-1})	Q_S (% of DW)	Q_C (% of DW)	Q_{max} (% of DW)	Requirement (µmol g^{-1} DW d^{-1})
Nitrogen	0.105 ±0.016	0.706 ±0.098	2.14	3.54	169.2
Phosphorus	0.088 ±0.013	0.045 ±0.007	0.14	0.27	4.1

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Table 3. Modelled substrate dependent growth kinetics. Monod parameters (μ^*_{max} and K_μ) and the substrate concentration at which 0.9 of the maximum growth rate is obtained (S_{sat}).

	μ^*_{max} (d^{-1})	K_μ (μM)	μ_{max}/K_μ	S_{sat} (μM)
Ammonium	0.098	0.57	0.17	5.1
Nitrate	0.091	1.17	0.08	10.4
Phosphorus	0.084	0.12	0.70	1.03

Table 4. Storage capacity ($T_{Storage}$) for N and P, respectively, in *Gracilaria vermiculophylla*.

	$T_{Storage}$ at max. growth rate (days)	$T_{Storage}$ at reduced growth rate (days)	$T_{Storage}$ total (days)
Storage capacity for N	6.5	26.5	33.0
Storage capacity for P	10.5	32.5	43.0

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Table 5. Parameter estimates (Y_0: intercept with Y-axis; k: decay rate and, $T_{0.5}$: half time) for decomposition of C biomass and mineralization of tissue bound N and P in *Gracilaria vermiculophylla*. Estimated means ± 95% confidence limits. Decomposition experiments were conducted at 5°C (A), 15°C (B) and 25°C (C), respectively.

	Y_0 (%)	k (d^{-1})	$T_{0.5}$ (days)	R^2
C at 5°C	103.4 ± 3.4	0.013 ± 0.002	52.9 ± 8.9	0.895
C at 15°C	107.2 ± 14.0	0.032 ± 0.007	21.5 ± 4.1	0.857
C at 25°C	94.6 ± 17.3	0.065 ± 0.023	10.7 ± 2.8	0.774
N at 5°C	99.5 ± 9.5	0.016 ± 0.004	44.6 ±7.9	0.877
N at 15°C	109.5 ±15.1	0.037 ± 0.009	18.8 ± 3.8	0.842
N at 25°C	99.9 ± 9.8	0.079 ± 0.015	12.7 ± 5.3	0.926
P at 5°C	94.4 ± 14.5	0.023 ± 0.008	30.5 ± 7.8	0.780
P at 15°C	109.8 ± 17.8	0.043 ± 0.012	16.2 ± 3.7	0.805
P at 25°C	98.8 ± 11.2	0.100 ± 0.022	6.9 ± 1.2	0.908

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Table 6. Maximum relative growth rate (μ_{max}), critical N and P quotas (N_C *and* P_C), half-saturation constants for growth (K_μ) and saturating substrate concentrations (S_{sat}) for *Gracilaria vermiculophylla* and eleven common algal species native to Scandinavian waters.

Species		Critical quota		NH_4^+		NO_3^-		PO_4^{3-}	
	μ_{max}	N_C	P_C	K_μ	S_{sat}	K_μ	S_{sat}	K_μ	S_{sat}
	(d^{-1})	(%DW)		(µM)		(µM)		(µM)	
Ulva lactuca [1,2,3]	0.196 - 0.452	2.17	0.20	0.75	6.71	1.45	13.02	0.27	2.39
Ceramium virgatum [1,2,3]	0.136 - 0.299	3.10	0.44	0.63	5.65	-	-	0.22	1.94
Cladophora sp. [1,2]	0.188 – 0.208	2.05	-	0.36	3.28	0.83	7.45	-	-
Chaetomorpha linum [1,2]	0.139 – 0.142	1.15	-	0.11	1.00	0.13	1.18	-	-
Gracilaria vermiculophylla	0.084 – 0.098	2.14	0.14	0.57	5.10	1.17	10.40	0.12	1.03
Chordaria flagelliformis [4]	0.091 – 0.126			0.31					
Codium fragile [1,2]	0.074 – 0.083	1.58	-	0.25	2.22	0.57	5.11	-	-
Fucus disticus [4]	0.067 – 0.081			0.24					
Fucus vesiculosus [1,2,3]	0.038 – 0.040	1.71	0.12	0.29	2.66	0.81	7.32	0.09	0.78
Fucus serratus [3]	0.040	-	0.22	-	-	-	-	0.16	0.88
Ascophyllum nodosum [3]	0.014	-	0.15	-	-	-	-	0.06	0.35
Laminaria digitata [3]	0.006	-	0.22	-	-	-	-	0.03	0.15

Data sources:
[1)] *Pedersen and Borum 1996*
[2)] *Pedersen and Borum 1997*
[3)] *Pedersen et al. 2010*
[4)] *Rosenberg et al. 1984*

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