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nutrient uptake kinetics and nutrient release through decomposition

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1	Nutrient (N and P) dynamics of the invasive macroalga Gracilaria
2	vermiculophylla: Nutrient uptake kinetics and nutrient release through
3	decomposition
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26 Abstract

27 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-28 29 growing alga that thrives well at low nutrient availability in summer, suggesting that it uses 30 nutrients efficiently, which might give it a competitive advantage over many native species. 31 We studied therefore the nutrient dynamics of G. vermiculophylla and compared it to those of a range of native macroalgal species. Nutrient uptake rates $(NH_4^+, NO_3^- \text{ and } PO_4^{3-})$ were 32 33 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 34 35 to the nutrient dynamics of many fast-growing algae and we found thus no indication that G. 36 *vermiculophylla* should have any particularly advantage relative to other, sympatric species. 37 The nutrient storage capacity of G. vermiculophylla was, in contrast, relatively large and 38 comparable to that of more slow-growing algae, which, when combined with the low nutrient 39 uptake experienced in summer, could explain how G. vermiculophylla can sustain non-40 nutrient limited growth through most of the growth season. The biomass of G. 41 vermiculophylla can be massive and estimates showed that gross nutrient uptake could exceed 42 the amount of nutrients received from land. The turnover of Gracilaria biomass is however 43 fast and nutrients bound in the resulting detritus are quickly mineralized during 44 decomposition, which is especially important during late summer when water temperatures 45 are high. Invasion and subsequent dominance by G. vermiculophylla may thus affect local 46 nutrient cycling significantly.

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

49 Introduction

50 The red alga Gracilaria vermiculophylla originates from NE Asia (Tseng and Xia 1999), but 51 was introduced to North America and Europa with imported oysters intended for aquaculture 52 in the 1990's (Mollet et al. 1998). G. vermiculophylla is invasive (Nyberg et al. 2009) and has 53 spread along the Atlantic coast of Europe and is now found from Venice lagoon in the 54 Mediterranean to southern Norway in Scandinavia (Thomsen et al. 2007; Sfriso et al. 2012). 55 G. vermiculophylla was first observed in Danish waters in 2003 and is now common in many 56 estuaries of the western Baltic Sea (Thomsen et al. 2007; Weinberger et al. 2008). G. 57 vermiculophylla is mostly abundant in sheltered, soft-bottom areas where it may become 58 dominant and sometimes replaces previously common seaweeds such as Ulva sp. (Nejrup and 59 Pedersen 2010) or Fucus vesiculosus (Weinberger et al. 2008). G. vermiculophylla is 60 considered an ecosystem engineer (Wallentinus and Nyberg 2007; Byers et al. 2012) and 61 where abundant it may not only affect the composition of the algal assemblage, but also affect 62 local biogeochemistry, nutrient cycling including the transfer of nutrients between abiotic and 63 biotic components (Tyler and McGlathery 2006; Hardison et al. 2010; Gulbransen & 64 McGlathery 2013) and alter trophic relations in the food web (Wallentinus and Nyberg 2007). 65 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 66 fragments (Rueness 2005; Thomsen et al. 2007; Nyberg et al. 2009), it is tolerant to 67 desiccation and extreme levels of light, temperature and salinity (Yokoya et al. 1999; Raikar 68 69 et al. 2001; Nejrup and Pedersen 2012; Nejrup et al. 2013). Recent studies have further shown 70 that G. vermiculophylla is avoided by many native herbivores within its invaded range 71 (Thomsen and McGlathery 2007; Weinberger et al. 2008; Nejrup et al. 2012; Hammann et al. 72 2013) due to inducible chemical defenses (Nylund et al. 2011; Hammann et al. 2016). 73 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. Neirup 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 and Pedersen 2010). No studies have yet evaluated the full set of dynamics including uptake 98

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

100 Seaweeds may acquire and temporarily immobilize a large proportion of the 101 nutrients received from land, especially in shallow estuaries and coastal lagoons where algal 102 biomass can be substantial (Tyler and McGlathery 2003; Pedersen et al. 2004). Nutrients 103 incorporated into macroalgal biomass become temporarily unavailable for other primary 104 producers until they are released through grazing or decomposition. The turnover rate of algal 105 biomass and, hence, the release of nutrients, differs systematically among seaweeds with 106 different life strategies; slow-growing macroalgae tend generally to be grazed less and 107 decompose more slowly than fast-growing species (e.g. Buchsbaum et al. 1991; Enriquez et al. 108 1993; Banta et al. 2004; Conover et al. 2016). Algal assemblages dominated by fast-growing 109 and bloom-forming macroalgae do therefore have a faster and more variable turnover of 110 nutrients than those dominated by slow-growing, perennial macrophytes (Duarte and Cebrián 111 1996; Banta et al. 2004) and release of nutrients from dense populations of opportunistic 112 seaweeds may periodically exceed land-derived inputs and the efflux of nutrients from 113 sediments (Tyler et al. 2003). Grazing on G. vermiculophylla is insignificant (e.g. Weinberger 114 et al. 2008; Nejrup and Pedersen 2010) so most of the nutrients contained in the biomass must 115 consequently be released through decomposition. Invasion and subsequent dominance by G. 116 *vermiculophylla* may thus potentially influence nutrient dynamics at the ecosystem level if the 117 decomposition rate of G. vermiculophylla differs markedly from the species it has replaced. 118 The aim of this study was 2-fold. The major aim was to evaluate all aspects of the 119 nutrient dynamics of G. vermiculophylla and to compare it to those of other common, 120 indigenous algal species to assess whether G. vermiculophylla uses nutrients more efficiently 121 than the algae it is potentially competing with. We wanted next to study the release of major 122 nutrients (N and P) from decomposing G. vermiculophylla and compare it to that of other

123 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 124 kinetics for ammonium (NH_4^+) , nitrate (NO_3^-) and phosphate (PO_4^{3-}) and compared those to 125 126 growth related N and P requirements, the latter being determined from culturing algae under a 127 range of nutrient concentrations. These data were combined and used to model how growth 128 relates to nutrient availability and additionally, used to estimate the storage capacity for N and 129 P in G. vermiculophylla. We conducted finally a series of decomposition experiments to 130 evaluate how fast N and P bound in biomass was released from dead and decaying G. 131 vermiculophylla under different temperature regimes

132

133 Methods

Gracilaria vermiculophylla was collected at Fyns Hoved, Denmark (55° 36.9' N, 10° 36.7' E) 134 in October 2013. The algae were cleaned and transported to the laboratory where they were 135 136 kept in 80 L storage tanks until being used in the experiments. The storage tanks and the 137 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 138 139 halogen spots (OSRAM 12V, 35W) providing a light intensity of ca. 90 µmol photons m⁻² s⁻¹ 140 PAR in a 16:8 hr light:dark cycle. The chosen temperature and salinity is optimal for G. 141 vermiculophylla (Nejrup and Pedersen 2012) while the light intensity is sufficient to saturate 142 growth of this species (ca. 60 μ mol 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. 143 Nutrient uptake kinetics. Uptake kinetics for NH_4^+ , NO_3^- and PO_4^{3-} were determined in three 144 145 separate experiments using a combination of the multiple-flask and the depletion methods as 146 described by Harrison et al. (1989) and Pedersen (1994). Eight PVC-beakers (Vol = 1.6 L) 147 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 (NO₃⁻range: 15-76 μ M; NH⁺₄ range: 1-76 148

 μ M; PO₄³⁻ range: 1-14 μ M). Each beaker was bubbled with air to ensure circulation and 149 150 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 60154 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 to constant dry weight (DW) at 85°C and weighted. Concentrations of NO_3^- in the water 157 158 samples were analyzed using a Lachat (QuickChem FIA+ 8000 Series) autoanalyzer while concentrations of NH_4^+ and PO_4^{3-} were analyzed manually; the concentration of NH_4^+ was 159 160 analyzed using the salicylate-hypochlorite method (Bower and Holm-Hansen 1980) while that of PO₄³⁻ was analyzed spectrophotometrically following Strickland and Parsons (1968). 161

Biomass specific nutrient uptake rates (V) were estimated from changes in substrateconcentration (S) over the course of the experiment:

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

where V is the uptake rate (in μ mol g⁻¹ DW h⁻¹), S₀ and Vol₀ are the substrate concentration (in 165 166 μ M) 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
$$V = \frac{V_{max} \times S}{K_m + S} \quad (Eq. 2)$$

where V is the uptake rate (in μ mol g⁻¹ DW h⁻¹), V_{max} is the maximum uptake rate, K_m is the 174 175 half-saturation constant (in μ M) and S is the substrate concentration (in μ M). Uptake rates of NH_4^+ and PO_4^{3-} were initially enhanced when the algae were exposed to nutrients (figure 1) 176 while no such transiently enhanced uptake was observed in the case of NO₃. Parameter 177 estimates of V_{max} and K_m (table 1) were therefore represented by 2 sets of uptake kinetics: (1) 178 179 one representing transiently enhanced uptake rates (i.e. surge uptake), measured over the 180 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 181 182 PO_4^{3-} (hereafter called assimilation).

183 **Nutrient requirements.** Algae were first pre-conditioned for 4-6 weeks in 10 aquaria

184 (volume = 20 L) receiving different quantities of dissolved N or P, to obtain specimens with

185 different tissue nutrient concentrations. Nutrients were added from stock-solutions of

186 NH₄NO₃ and KH₂PO₃. Algae receiving different levels of NH₄NO₃ received KH₂PO₃ in

187 excess while algae receiving different levels of KH₂PO₃ received NH₄NO₃ in excess to ensure

that only one nutrient was limiting at the time. The aquaria were exposed to low light (40

189 μ mol photons m⁻² s⁻¹ PAR in a16:8 hr light:dark cycle) using shade screens to ensure slow

algae intended for the N-growth experiment ranged from 1.0 to 3.7% N of DW at the end of

growth, which eased the accumulation of nutrients in the tissues. Tissue N-concentrations in

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

194 0.27 % of DW while the N-content in these algae averaged 2.4 % of DW .

190

195 Growth measurements were subsequently conducted in PVC-beakers (volume = 196 1.6L), which were filled with GF/C-filtered seawater (salinity 25) that had been stripped for 197 inorganic nutrients by letting *Ulva lactuca* grow in it for 2-3 days prior to use. The beakers 198 were placed under saturating light (90 μ mol m⁻² s⁻¹ PAR) and each beaker was bubbled with

199 atmospheric air to create circulation and ensure exchange of O₂ and CO₂. The initial FW200 biomass was determined on all pre-conditioned specimens prior to the growth measurements201 and the DW:FW ratio (and initial tissue N or P concentrations) was determined on a number202 of sacrificed subsamples after drying them to constant weight at 85°C. The algae were left to203 grow for 5-7 days after which they were harvested, dried to constant weight and analyzed for204 final tissue N or P content. Growth rates were estimated from changes in biomass over time205 assuming exponential growth:

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

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

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

where μ is the relative growth rate (d⁻¹), μ_{max} is the maximum growth rate, Q_S is the subsistence cell quota (in % of DW), i.e. the lowest tissue nutrient concentration that allows growth and Q is the average tissue N or P concentration (i.e. = $(Q_{Init} + Q_{Final})/2$). The critical cell quota (Q_C) was defined as the tissue nutrient concentration above which growth is not limited by lack of nutrients. The Droop function is a continuous function so we arbitrarily determined Q_C as the tissue nutrient concentration where the corresponding growth rate 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_{μ} , which can be estimated from equations 5 and 6 assuming that nutrient uptake and use of nutrients for growth is insteady state (Turpin 1988):

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

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

where V_{max} and K_m are the maximum uptake rate (in μ mol g⁻¹ DW h⁻¹) and the Michaelis-Menten constant (in μ M), respectively, obtained from the nutrient uptake experiments (Eq. 2) while μ_{max} and Q_S are the maximum growth rate (d⁻¹) and subsistence cell quota (in % DW) 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 cellular N or P, respectively, can support growth at maximum rates until Q_C is reached after 234 235 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 $B_T = B_0 e^{-kt} + G_R$ Eq. 7

where B_T and B_0 are the final and initial biomass (measured in units of C, N or P), *k* is the decay rate, *t* is the number of days elapsed since initiating the experiment and G_R is the size of the residual (i.e. non-reactive) fraction of the detritus.

Tissue nutrient analyses. Tissue concentrations of C and N were determined on dried and
ground samples using an EA 1110 CHNS elemental analyze (CE Instruments). Tissue P was
determined on dried and ground algae after oxidation with boiling H₂SO₄ followed by

272 spectrophotometric analysis (Strickland and Parsons 1968).

273

274 Results

Nutrient uptake kinetics. Uptake rates of NH_4^+ , NO_3^- and PO_4^{3-} increased with increasing 275 276 substrate concentration and saturated at high concentrations (figure 1) why uptake kinetics were described by the Michaelis-Menten function (R²-values ranged from 0.629 to 0.930; all 277 278 p-values < 0.001). The maximum surge uptake rate of NH₄⁺ was 2-fold higher than the maximum assimilation rate, while the half-saturation constant for uptake (K_m) during surge 279 280 uptake was 3-fold higher than that for assimilation. These differences resulted in a higher affinity (α) for NH₄⁺ at low substrate concentrations during assimilation than during surge 281 uptake. The initial maximum uptake rate for NO_3^- was not different from that observed later in 282 the experiment (i.e. no surge uptake), but it was 3 to 4-fold lower than both V_{max}^{Surge} and V_{max}^{Ass} 283 for $NH_4^+.$ K_m for NO_3^- averaged 9 μM and the affinity at low substrate concentration ca. 1.7 284 μ M. Initial V_{max} and K_m for PO₄³⁻ were ca. 2-fold higher than for P-uptake occurring after 30 285 minutes whereas the affinity for P at low PO_4^{3-} concentrations (α) was approximately the 286 287 same for surge uptake and assimilation.

288 Growth rate and nutrient requirements. Growth rate increased as a function of N or P

289 quotas (figure 2; R^2 for N = 0.876, p<0.001; R^2 for P = 0.876, p<0.001). Estimated maximum

290 growth rate at infinite N or P quota varied slightly between the two experiments (0.088 –

291 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,
- 293 resulting in a N-requirement of 169 μ mol 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,
- 295 resulting in a P-demand of 4.1 μ mol 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 (table 3). Estimated maximum growth rate based on uptake of NH_4^+ (0.098 d⁻¹) or NO_3^- (0.091 298 d⁻¹) as the N source did not differ much. In contrast, the half-saturation constant for growth 299 $(K\mu)$ based on NO₃⁻ was 2-fold larger than that for growth on NH₄⁺ indicating a higher affinity 300 for NH₄⁺. Substrate concentrations needed to saturate growth (S_{Sat}) ranged from 5 μ M for NH₄⁺ 301 to 10 μ M for NO₃⁻. Estimated maximum growth rate based on uptake of PO₄³⁻ (0.084 d⁻¹) was 302 slightly lower than for NH₄⁺ or NO₃⁻ and K_{μ} was 0.12 μ M PO₄³⁻. The substrate concentration 303 needed to support maximum growth rate was ca. 1 μ M PO₄³⁻. 304

Nutrient storage capacity. The amount of N stored in excess of the critical quota (i.e. Q_{max} -305 Q_C) attained 14.0 mg N g⁻¹ DW, while the internal quantity of N that could support sustained 306 growth at reduced rates (i.e. $Q_C - Q_S$) was 14.3 mg N g⁻¹ DW (table 2). These internal stocks 307 308 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 the surroundings (table 4). The amount of P in excess of the critical limit was 1.3 mg P g^{-1} 310 311 DW and the amount of P that could support sustained growth at reduced rates was 1.0 mg P g^{-1} DW (table 2). The internal stocks of P were could support growth for a total period of 43 312 313 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.

Decomposition and mineralization. Decomposition of *G. vermiculophylla* followed a simple 1-G model without a significant refractory pool (i.e. a simple exponential decline; figure 3, table 5; R^2 ranged from 0.774 to 0.908, all p-values <0.001) since this model provided better fits (higher R^2 -values) than multiple G-models with or without a refractory pool. All biomass disappeared within 120 days in the 15 and 25°C treatments, while about 30% of the original 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.

328

329 Discussion

330 The balance between uptake capacity and nutrient requirements and, thus, the risk of suffering 331 nutrient limitation during periods of low nutrient availability is related to the maximum 332 growth rate of algae. Fast-growing algae have a large relative surface area (i.e. high SA:V 333 ratio) and a high capacity for nutrient uptake (Wallentinus 1984; Hein et al. 1995), but also 334 large nutrient requirements per unit biomass and time dictated by their fast growth (Pedersen 335 and Borum 1997; Pedersen et al. 2010). In contrast, slow-growing species have lower uptake 336 capacities and lower requirements, but uptake rate and nutrient demands are better scaled at 337 low nutrient availability in these algae (Pedersen and Borum 1997; Pedersen et al. 2010), why 338 slow-growing algae generally can grow at near maximum rates when concentrations of 339 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

for truly fast-growing, sheet-like and filamentous species and higher than for most fucoids
and Laminarians (see table 6 for a comparison with algae indigenous to Scandinavian waters). *G. vermiculophylla* can thus be ranked as an algal species with an intermediate growth rate.

The experimentally determined critical N quota of *G. vermiculophylla* is rather high and comparable to those of fast-growing species such as *U. lactuca* and *C. virgatum* (table 6).

Red algae have generally higher N quotas than green and brown algae due to the prevalence

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

and critical N and P quotas resulted in maximum N and P requirements corresponding to ca.

356 169 and 4 μ mol N or P g⁻¹ DW d⁻¹, respectively, which is lower than for truly fast-growing

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}) on either NH⁺₄ or NO⁻₃ tended to be higher than those of more slow-growing algae and 370

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 half-saturation constant (K_{μ}) for growth on PO₄³⁻ and the concentration of DIP needed to 373 374 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

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

397 McGlathery et al. 1996).

398 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, 399 400 respectively. The observed N-reserve was somewhat less than for a range of indigenous algal 401 species (Pedersen and Borum 1996; Pedersen et al. 2010), which suggests that the algae used 402 in our experiments may not have been saturated with nutrients. The storage capacity for 403 nutrients (defined as the time where growth could be sustained without additional nutrient 404 uptake from the medium) was ca. 33 days for N and 43 days for P, respectively. The storage 405 capacity for N was larger for a number of fast-growing indigenous algae (U. lactuca, C. 406 virgatum, Cladophora sp. and Chaetomorpha linum: range from 9.6 to 17.5 days), but smaller 407 than for slow-growing F. vesiculosus (45.9 days; Pedersen and Borum 1996). 408 The N and P-reserves obtained through winter and spring can obviously not sustain 409 maximum growth of G. vermiculophylla throughout extended periods of low nutrient

410 availability (i.e. mid May to mid September). However, even though *in situ* nutrient

411 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

413 concentrations of DIN ranged between 1 and 3 μ M from mid May to mid September and

414 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.

416 2 months). Equally important, average *in situ* growth rates in Holckenhavn Fjord during the

417 growing season were some what lower than those attained in this study (ca. $0.05 d^{-1}$, range

418 $0.032 - 0.065 d^{-1}$; Nejrup and Pedersen 2010), meaning that 'dilution' of the internal nutrient

419 reserves would occur more slowly and the stores last longer than predicted when assuming

420 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 m⁻² on average in Holckenhavn Fjord, Denmark (Nejrup and Pedersen 2010) and 6-700 g DW 433 m⁻² in parts of Venice lagoon, Italy (Sfriso et al. 2012) suggests that large amounts of DIN 434 435 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 reach ca. 5200 g DW m⁻² during the growing season (from March to October) corresponding 447 to a net incorporation of ca. 78 g N and ca. 10 g P m⁻² (assuming a mean N and P content of 448 449 1.5% of DW and 0.2% of DW, respectively). The average load of total nitrogen (TN) and total phosphorus (TP) per unit of estuarine area across 47 Danish estuaries is ca. 20 g TN m⁻² 450 y^{-1} and 0.8 g TP m⁻² y⁻¹ (Conley et al. 2000) so G. vermiculophylla may have a substantial 451 452 effect on nutrient cycling in Holckenhavn Fjord. The biomass of G. vermiculophylla changed little over the season (ranging from 464 g DW m⁻² in March to 176 g DW m⁻² in October), so 453 454 biomass losses and, hence, the production of detritus must have been in the same order of magnitude as the production of biomass (estimated to ca. 5400 g DW m^{-2} in 7 months) since 455 456 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 ranged from 0.013 to 0.065 d^{-1} depending on temperature and were markedly lower than those 459 460 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 than that observed in our decomposition study (e.g. <0.003 d⁻¹; Nejrup et al. 2013) suggesting 467 468 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. *vermiculophylla* decomposed completely within 120 days at 15°C. The decay rate (0.032 d^{-1}) 485 was lower than those of Ulva lactuca and Ceramium virgatum (0.038 - 0.040 d⁻¹) and similar 486 to that of Fucus vesiculosus (0.028 d⁻¹; Banta et al. 2004). F. vesiculosus contains however 487 488 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 Sargassum muticum (0.016 d⁻¹) and Halidrys siliquosa (0.019 d⁻¹), respectively (Pedersen et 490 491 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

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

- **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 (\bigcirc) were measured later than 30
- 661 minutes after exposure. Lines represent the best fits of the Michalis-Menten function to the

662 data

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- **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
- represent the best fits of the Droop equation to the data

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- 668 Fig. 3 *Gracilaria vermiculophylla*. Changes in C, N and P biomass during decomposition of
- 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 $\pm 95\%$
- 671 confidence limits (n=3)

Figure 1.



Figure 2.



Figure 3.



Table 1. Nutrient (NH₄⁺, NO₃⁻ and PO₄³⁻) 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 upt	ake (0-30 min.	.)	Assimilation (30-300 min.)			
	V_{max} K_m V_{max}/K_m			V _{max}	K _m	V_{max}/K_m	
	$(\mu mol g^{-1} DW h^{-1})$	(µM)		$(\mu mol g^{-1} DW h^{-1})$	(µM)		
$\mathrm{NH_4}^+$	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	
PO4 ³⁻	2.47 ± 1.01	5.5 ±5.1	0.45	1.0 ± 0.2	2.4 ± 1.0	0.42	

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}	Qs	Q _C	Q _{max}	Requirement
	(d^{-1})	(% of DW)	(% of DW)	(% of DW)	$(\mu mol g^{-1} DW d^{-1})$
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

	$\begin{array}{c} \mu^*_{max} \\ (d^{-1}) \end{array}$	Κ _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 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}).

Table 4. Storage capa	city $(T_{Storage})$ for N and	l P, respectively, in	Gracilaria vermiculophylla.
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		T _{Storage} at max.	T _{Storage} at reduced	T _{Storage} total
		growth rate	growth rate	
		(days)	(days)	(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 \pm 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

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

Species		Crit que	ical ota	N	H_4^+	N	03	PC) ₄ ³⁻
	μ_{max}	N _C	P _C	K_{μ}	\mathbf{S}_{sat}	K_{μ}	\mathbf{S}_{sat}	K_{μ}	\mathbf{S}_{sat}
	(d^{-1})	(%[OW)	(μ	M)	(μ	.M)	(μ	M)
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
<i>Ceramium</i> <i>virgatum</i> ^{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 flagelliformis⁴	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
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Data sources:

Pedersen and Borum 1996
 Pedersen and Borum 1997
 Pedersen et al. 2010

⁴⁾ Rosenberg et al. 1984