

1 This is the AUTHORS' ACCEPTED MANUSCRIPT, for the published article, please see:

2 <https://doi.org/10.1016/j.jembe.2018.07.005>

3 Full citation: ANGOVE, C., NORKKO, A. and GUSTAFSSON, C., 2018. Assessing the
4 efficiencies and challenges for nutrient uptake by aquatic plants. *Journal of Experimental*
5 *Marine Biology and Ecology*, **507**, pp. 23-30.

6

7 Assessing the efficiencies and challenges for nutrient uptake by aquatic plants

8

9 Charlotte Angove, Alf Norkko, Camilla Gustafsson

10

11 Charlotte Angove (corresponding author), Tvärminne Zoological Station, University of

12 Helsinki, J.A. Palménin tie 260, 10900 Hanko, Finland

13 charlotte.angove@helsinki.fi, phone: +358-406433609

14 Alf Norkko, Tvärminne Zoological Station, University of Helsinki, J.A. Palménin tie 260,

15 10900 Hanko, Finland

16 Baltic Sea Centre, Stockholm University, SE-106 91, Stockholm, Sweden

17 Camilla Gustafsson, Tvärminne Zoological Station, University of Helsinki, FIN-10900

18 Hanko, Finland

19

20

21 Abstract

22 Aquatic plant meadows are valuable components to the ‘coastal filter’ and it is important to
23 understand the processes that drive their ability to cycle nutrients. However, at present, the
24 field-based evidence for understanding the drivers of nutrient uptake by plants is lacking.
25 This study aimed to investigate how well individual shoots of aquatic plants could meet their
26 nitrogen demands using the sediment nutrient pool (porewater ammonium) and to explore
27 which traits helped to facilitate such uptake. Several species were investigated in shallow,
28 submerged (2-4 m) mixed-species communities in the northern Baltic Sea using incubation
29 experiments with enriched ammonium. After a 3.5 h incubation time, individuals were
30 collected and analysed for nitrogen (% DW) and ^{15}N (at-%) concentrations. Uptake by plants
31 was calculated per unit nitrogen in response to the ^{15}N -labelled source and to overall nitrogen
32 availability. Background porewater ammonium availability was highly variable between
33 individual plants. Species identity did not significantly affect uptake metrics and the effect of
34 ambient porewater availability was weak. As biomass increased there were significant
35 logarithmic declines in the 95th quantiles of nutrient uptake rates, ambient porewater nutrient
36 availability and aboveground nitrogen tissue concentrations (% DW). Such findings
37 suggested that uptake rates of plants were significantly demand-driven and the nutrient
38 conditions of the porewater were significantly driven by the demands of the plant. Findings
39 parameterised the unfulfilled potential for some aquatic plants to cycle nutrients more
40 efficiently and highlighted the potential importance of access to new nutrient sources as a
41 way of enhancing nutrient cycling by aquatic plants. Plant traits and community properties
42 such as the activity of infauna could facilitate such an access and are likely important for
43 nutrient uptake.

44

45 Keywords: Nitrogen, functional traits, seagrass, Baltic Sea, sediment, *Zostera marina*,
46 nutrient cycling, nutrient enrichment.

47 Running page head: Nitrogen uptake by aquatic plants

48 Introduction

49 Aquatic plant meadows are valuable for cycling nutrients in coastal zones (McGlathery et al.,
50 2007). They absorb dissolved nutrients and store them as biomass, which is then available for
51 further processes such as burial or grazing by consumers, and it ultimately slows the re-
52 release of inorganic nutrients into the surrounding environment (McGlathery et al., 2007).
53 Understanding uptake processes is particularly important in the Baltic Sea because many of
54 its ecosystems are threatened by eutrophication (see Andersen et al., 2009; Gustafsson et al.,
55 2012) which has been accelerated by an increase in nutrient concentrations from
56 anthropogenic nutrient loading to coastal zones (Gustafsson et al., 2012). It is hence
57 imperative to investigate how well aquatic plants can access and utilise nutrient pools in the
58 surrounding environment and understand what enhances their ability to cycle nutrients.

59 Aquatic plants absorb dissolved organic and inorganic nutrients from the water
60 column and, mainly, the sediment porewater (Erfteimeijer and Middelburg, 1995; Touchette
61 and Burkholder, 2000). Nutrient availability in the sediment is increased by processes such as
62 excretion by fauna (Peterson and Heck, 2001; Peterson and Heck, 1999) as well as the
63 decomposition of organic matter (Kenworthy and Thayer, 1984), including vegetation and
64 fauna, phytodetritus (Evrard et al., 2005) and other particles entrained from the water column
65 (Kennedy et al., 2010). Such factors are highly spatiotemporally variable (e.g. Hemminga,
66 1998; Wirachwong and Holmer, 2010; Fourqurean et al., 1992). Nutrients can be depleted to
67 the extent that aquatic plant growth is nutrient-limited (Perez et al., 1991; Ferdie and
68 Fourqurean, 2004; Armitage et al., 2011); which is not always the case (as observed by Olsen

69 and Valiela, 2010; Mutchler and Hoffman, 2017; Campbell and Fourqurean, 2014), but
70 nutrient availability can drive spatial differences in plant patch development (Furman et al.,
71 2017). Such a large-scale response to nutrient availability is owing to the responsive foraging
72 and growth strategies by roots and clonal ramets to nutrient availability (Furman et al., 2017;
73 Kembel et al., 2008; Campbell et al., 1991; de Kroon and Mommer, 2006). Thus, nutrient
74 variability is highly influential to the development of aquatic plant meadows and their ability
75 to cycle nutrients.

76 If an individual plant had a greater capacity to meet its nutrient demands using the
77 resources that were available (e.g. Comas and Eissenstat, 2004), it would cycle nutrients
78 more efficiently in the place that it grows, be more robust to temporal change in nutrient
79 availability and in turn the meadow structure would be more robust to changes in nutrient
80 availability. The growth of the root network of a plant is coordinated by its hormonal cues in
81 response to nutrient demands. In terrestrial environments, the growth responses and root
82 architectural strategies to meet nutrient demands change between species (Kembel et al.,
83 2008; Campbell et al., 1991). These strategies can enhance uptake to different levels of
84 success depending on the environment. For instance, having a higher root length per unit root
85 mass (Specific Root Length, SRL) reduces the distance that nutrients must diffuse to reach
86 the roots, and this benefits plants in nutrient poor environments because it catalyses nutrient
87 supply (Aerts, 1999). However, in nutrient-rich zones the nutrient supply is no longer the
88 limiting factor; instead it is the rate at which plants can absorb the available nutrients and so
89 their physiological nutrient uptake kinetics become more important for enhancing nutrient
90 uptake (Aerts, 1999). In the context of aquatic plant communities, we do not know which
91 strategies are valuable for uptake and thus, what the nutrient microenvironment is like for an
92 individual plant. While the physiology of different aquatic plant species can vary (e.g.
93 Gustafsson and Norkko, 2016), coarser-scale morphological variations which affect uptake

94 rates are still yet to be explored. For instance, seagrasses and other aquatic plants often
95 develop a network of ramets which can share nutrients (Marbà et al., 2002); a strategy which
96 enhances nutrient uptake in terrestrial environments with heterogeneous nutrient availability
97 (Roiloa and Hutchings, 2013). Yet for any single shoot in natural conditions, we do not
98 understand which conditions are most important for uptake success. For seagrass, root traits
99 are likely to represent extremely poor nutrient conditions because the spatiotemporal
100 variability of nutrients can be so influential on meadow growth (Furman et al., 2017) that the
101 variability of traits which enhance uptake kinetics are likely to be redundant compared to the
102 ability for plants to increase their reach to access new nutrient sources. In this case, growing
103 longer roots would likely be the most important attribute for nutrient uptake rather than the
104 amount of absorptive surfaces (i.e. Root biomass). However, without evidence we do not
105 know whether nutrient uptake by each seagrass shoot is dictated by the abundance of
106 nutrients available to be absorbed, whether plants benefit from investing more in extracting
107 nutrients from their immediate environment (e.g. High SRL) or the ability for roots to access
108 new nutrient pools (e.g. Maximum root length) is the most important trait in the natural
109 environment.

110 As nitrogen is hypothesised to be the main nutrient which limits growth at higher
111 latitudes (Hemminga, 1998; Short, 1987), and sediment porewater ammonium the main
112 nitrogen source, porewater ammonium was the focal nutrient of this study. Our aim was to
113 quantify nitrogen uptake rates by several aquatic plant species from the sediment porewater
114 environment. We hypothesised that species would have significantly different nutrient uptake
115 rates, and these differences would be owing to the variation in morphological root traits
116 between species. It would be highly difficult to conduct such an investigation in typical
117 seagrass meadows because they are often monocultures (Hemminga and Duarte, 2000),
118 which means that there would not likely be enough variability in uptake strategies to find

119 insightful trends. In the northern Baltic Sea, the brackish-water conditions allow for a unique
120 assembly of marine, estuarine and limnic species to coincide within the same meadow
121 (Kautsky, 1988; Gustafsson and Norkko, 2016), therefore these communities were ideal for
122 capturing a variety of uptake strategies facilitated by interspecific differences, while
123 environmental conditions in the experiment remained relatively consistent. In this
124 experiment, we examined how efficiently individual shoots could fulfil their nitrogen demand
125 in response to a nutrient enrichment, and by comparing uptake rates between several species,
126 we explored which uptake strategies were most beneficial to uptake rates. It is important to
127 investigate which strategies enhance uptake in aquatic plants because it allows us to identify
128 which mechanisms might otherwise be limiting nutrient uptake, thus nutrient cycling, in
129 aquatic plant communities in the northern Baltic Sea.

130 Methods

131 Study site and vegetation

132 The experiment was conducted in the shallow, submerged (2-4 m) mixed species vegetative
133 communities around Tvärminne Zoological Station, Finland. Tvärminne is situated on the
134 Hanko Peninsula of the Finnish archipelago, Baltic Sea (59° 50' 400" N, 23° 14' 56" E
135 WGS84). The species measured both have a limnic and marine origin; the freshwater species
136 *Ceratophyllum demersum*, *Myriophyllum spicatum*, *Stuckenia pectinata*, *Potamogeton*
137 *perfoliatus* and *Zannichellia major*, and the marine/brackish species *Zostera marina* and
138 *Ruppia cirrhosa*. We incubated independent ramets of a clone to prevent translocation during
139 incubation. Individuals with a single shoot were selected, however for small species with a
140 trailing growth (*R. cirrhosa* and *Z. major*), incubating multiple shoots was unavoidable. To
141 prevent multiple species from being in the same incubation, we focussed on sparse stands.
142 While sparse stands may not facilitate optimal conditions for aquatic plants, there is also less

143 competition for nutrients and it is possible to investigate the nutrient uptake drivers of a
144 single plant shoot without the influence of other shoots around. All incubations were at least
145 1 m apart from each other and individuals did not have noticeable grazer marks nor
146 biofouling. Species-averaged canopy heights can range from 10-70 cm in this area and season
147 (Gustafsson and Norkko, 2016), but individuals in this study did not exceed 20 cm in height.

148 The sediment of the study site had approximately 0.5-0.8 % OM content and 1.2-7.3
149 % silt fraction (<0.063 mm) (Gustafsson and Norkko, 2018).

150 Field work

151 Short term nutrient enrichment incubations were conducted *in situ* using SCUBA (Figure 1).
152 ¹⁵N - enriched ammonium sulfate solution (40 ml, 47 μM, 99 at-%) was used as an
153 immediate-release sediment fertiliser followed by incubations (Figure 1). 36 incubations were
154 conducted on 4 days, 9 incubations on each day, during the late growth season (August-
155 September) 2015 and 10 further incubations were conducted in September 2016 (Table 2). A
156 minimum of 3 replicates of each species were incubated on each day and a further 3
157 specimens were collected intact from the surrounding meadow as ambient samples to obtain
158 the background ¹⁵N in plant tissue. Not all incubations were successful due to insufficient
159 root or belowground biomass available for the analysis, but this remained unknown until
160 harvest (see Table 2). Rooting depths can vary between species (approx. 5-20 cm, pers. obs.)
161 and within species (length can change by a magnitude of 10, Gustafsson and Norkko, 2018),
162 which meant that selecting a standardised depth for enriching the sediment porewater and for
163 measuring the ambient porewater nutrient availability to plants required due consideration.
164 For instance, injecting the liquid fertiliser too shallow into the sediment would likely lead to
165 an immediate re-release of the majority of fertiliser into the water column. Likewise, too deep
166 an enrichment and the fertiliser would be out of reach of roots for some of the individuals.

167 The sediment porewater was enriched at 7-8cm deep; approximately half way down the depth
168 of the core and close to the maximum lengths that the roots of aquatic plants in the study area
169 typically reached (Gustafsson and Norkko, unpubl.). At this depth, it was predicted that the
170 nutrients would reach the plants by diffusion and they would not be immediately diffused into
171 the water column (Cheng et al., 2014, Chowdhury and Bakri, 2006; Clavero et al., 2000). The
172 plants absorbed a fraction of the enriched source, meaning that there was enriched solution
173 available for the plants throughout the experiment (Figure 1). Porewater samplers collected
174 depth-integrated water samples in the upper 10 cm of sediment (see Gustafsson and Boström,
175 2011; Figure 1) to provide a best estimate of nutrient availability to plants in the region where
176 roots were mostly found (pers. obs.) before the enrichment occurred. This data was used to
177 estimate background porewater nutrient availability within the area which varied widely
178 (Table 1).

179 Water column samples were periodically collected (13/08/15; 10/09/15; 11/09/15;
180 02/09/16) to estimate ammonium, nitrate, nitrite and phosphate availability to the
181 aboveground parts of the aquatic plants (Table 1).

182 Uptake metric calculations

183 The increase in nitrogen availability induced by the experimental enrichment was calculated
184 as the percentage of nitrogen added compared to ambient NH_4^+ concentrations, in moles, for
185 each individual. Uptake by plants was calculated per unit nitrogen in order to detect the
186 small-scale changes in nitrogen attributed to the short incubation time. The ability for plants
187 to respond to the specific nutrient enrichment (response rates, $\mu\text{gN gN}^{-1} \text{h}^{-1}$, hereafter RR)
188 was determined using the equation:

$$189 \quad {}^{15}\text{N} (\text{enriched plant}) - {}^{15}\text{N} (\text{ambient plant})$$

190 Where the ^{15}N of an ambient plant represented an average of 3 replicates of each
191 species. Uptake rates which considered additional nutrient uptake by the ambient porewater
192 nutrient pool (uptake rates, $\mu\text{gN gN}^{-1} \text{h}^{-1}$, hereafter UR) were calculated using mass-balanced
193 isotope mixing equations (Collos, 1987) as described by Lepoint et al. (2002). The important
194 difference between uptake response rate (RR) and uptake rate (UR) is that RR is a
195 measurement of uptake of the enriched source, but UR incorporates ambient porewater
196 ammonium measurements to estimate total ammonium uptake during the incubation. To
197 avoid confusion, both units of uptake rate are named uptake metrics when they are being
198 referred to collectively.

199 Statistical analysis

200 Analyses were performed using the statistical software R (version 3.02.2, R Core Team
201 2015). For all analyses, the relevant assumptions relating to heteroscedasticity and the
202 distribution of residuals were checked and in order to meet the criteria, some of the variables
203 (RR, UR, porewater NH_4^+ , total biomass, root biomass) were logarithmically transformed.

204 The effect of total biomass on uptake metrics, NH_4^+ and leaf tissue nitrogen
205 concentrations were analysed using 95th quantile regression. Quantile regression, such as 95th
206 quantile analysis, is an effective technique to explore the maximum (or factor ceiling) effect
207 that a factor has on a dependent variable (Thomson et al., 1996; Thrush et al., 2003). 95th
208 quantiles were calculated for 50mg biomass bins, which were then analysed using linear
209 regression. Thus, statistical outputs represent trends of the boundary lines for the 95th
210 quantiles. One individual was removed from biomass-specific analyses because of its
211 inordinately high biomass compared to the rest of the samples. The 2016 data was pooled
212 with 2015 data for quantile regression analyses, but not for analyses which required species

213 averages because only two species were incubated in 2016 and their pooled values may have
214 led to misrepresentative differences between species.

215 A multiple-regression style General Linear Model (GLM) was used to analyse the
216 effect of species identity, ambient porewater ammonium availability (log-transformed,
217 hereafter NH_4^+) and their main effects on uptake metrics. For species comparisons, 3
218 replicates of each species were randomly selected from 2015 data for equal group sizes,
219 however *Ceratophyllum demersum* was discounted because it did not have true roots and
220 another species (*Zannichellia major*) was removed due to replicate loss.

221 Morphological characteristics of the experimental plants were not measured because it
222 was important to process plant samples as quickly as possible after harvesting in order to
223 prevent ^{15}N -loss from samples through leaching. Instead, the 95th quantile regressions were
224 used to infer which traits were likely to be important to enhance uptake rates, and pre-existing
225 data was compared to uptake rates as a supportive tool for highlighting opportunities for
226 future study. Species-averaged root traits were derived from a plant survey in the same study
227 area at the same time of year, the previous year (Gustafsson and Norkko, 2018). These traits
228 represented relative species differences in root traits for individuals growing in the study area
229 (Table 3); from up to 5 quadrats per species, wherein up to 10 of the longest roots were
230 measured. While rooting depth can vary between individuals, by using maximum root length
231 the data represented the length that the roots of each species could typically extend to within
232 the study area. Then, individual effects of NH_4^+ and root biomass on uptake metrics were
233 analysed using linear regression. Species-averaged root traits (Mean Max Root Length,
234 MMRL; Specific Root Length, SRL) were tested against uptake metrics with rank regression
235 using up to three randomly selected uptake rates for each species. *Z. major*, which had been
236 previously discounted from the species comparison, was included in the trait analyses

237 because it did not risk violation of the test assumptions and its presence did not change the
238 significant outcome of the tests.

239 Results

240 Porewater nutrient availability

241 Background porewater ammonium availability was highly variable within the replicates of
242 individual plants (Table 1), which meant that when plants were experimentally enriched with
243 identical doses of fertiliser the effect on porewater nutrient availability varied widely
244 (approximately 2-74 % NH_4^+ enrichment, based on 20% sediment porosity measured at the
245 site) and the starting nutrient conditions were not consistent between individuals. Such
246 porewater variability had a significant, yet weak effect on uptake metrics (Table 4). Uptake
247 response rates (RR) and uptake rates (UR) exhibited large variation too; ranging from -1.31
248 and $-30.15 \mu\text{gN gN}^{-1} \text{h}^{-1}$ respectively, where uptake of the enriched source did not exceed the
249 natural variability of ^{15}N , to 109.43 and $1032.25 \mu\text{gN gN}^{-1} \text{h}^{-1}$. It was highly unlikely that the
250 nutrient enrichment produced toxic effects for the plants because the enrichment was minor
251 in comparison to the variability of ambient nutrients in the sediment porewater.

252 Biomass and nutrient cycling

253 There were no trends between root biomass and uptake metrics (Table 4), however total
254 biomass was significantly linked to the upper limits of all the nutrient cycling metrics that
255 were measured in this study. For instance, there were significant logarithmic declines in the
256 95th percentiles of uptake metrics (UR & RR) as biomass increased (Figure 2A & B, Table 5),
257 and larger individuals were more likely to be limited in their overall uptake rate (UR) rather
258 than their capacity to respond to the experimental enrichment (RR, Figure 2A & B, Table 5).
259 Similarly, the sediment porewater surrounding larger plants was significantly less likely to be
260 NH_4^+ replete (Figure 2C, Table 5) and leaf tissue nitrogen concentrations of larger plants did

261 not reach the same maximum concentrations as for smaller plants; a trend which was not
262 singly controlled by species identity (Figure 2D).

263 Root traits and uptake rates

264 Species did not have significantly different uptake rates (UR) nor response rates (RR) ($t_{17,14} =$
265 $.654, p > .05$; $t_{17,14} = .765, p > .05$, respectively), nor did species identity interact with
266 porewater ammonium availability to produce significantly different URs and RRs ($t_{17,14} = -$
267 $.998, p > .05$; $t_{17,14} = -.881, p > .05$). Supportive analyses which refer to an external trait
268 dataset suggest that while there may not have been species differences, species traits could
269 provide insights into the drivers of uptake rates. For instance, species capable of growing
270 longer roots (Mean Max Root Length, MMRL) were significantly more adept at responding
271 to the experimental nutrient pulse by rank (RR; Figure 3A, Table 4) but MMRL was not
272 significantly beneficial for the rank order of overall uptake rates (UR; Figure 3B, Table 4).
273 There were no significant relationships between Specific Root Lengths (SRL) and the ranks
274 of uptake metrics (UR & RR, Table 4).

275 Discussion

276 This study has provided novel insights into the short-term dynamics of nutrient uptake by
277 aquatic plants, not only for plants in the northern Baltic Sea but also for vascular plants in other
278 submerged aquatic ecosystems. Overall, results showed that the nutrient demands of the plants
279 significantly affected uptake rates, nutrient storage in plant tissue and the nutrient availability
280 in the sediment porewater (Figure 2, Figure 4). Therefore, short-term nutrient uptake in these
281 aquatic plant meadows was likely to be largely driven by the presence and characteristics of
282 the aquatic plants, more so than by environmental nutrient availability. For example, as plants
283 increased in size, their ability to cycle nutrients to their full potential was significantly hindered
284 and the nutrient availability in the sediment porewater was likewise depleted (Figure 2). This
285 suggests that access to new nutrient sources is likely to benefit nutrient uptake and nutrient
286 cycling by plants. Such conclusions are likewise suggested by supportive analyses which
287 identify a potential link between maximum root lengths and uptake rates (Table 4) . These
288 insights are informative and beneficial because they described the challenges for nutrient
289 uptake experienced by a single aquatic plant shoot, and findings parameterise the unfulfilled
290 potential for some aquatic plants to cycle nutrients more efficiently.

291 The biomass paradox

292 Unlike other characteristics of a plant, such as root length and thickness, biomass does not
293 only represent the capacity for plants to acquire nitrogen (e.g. Tessier and Raynal, 2003) but
294 it also represents their nitrogen demand (Poorter and Nagel, 2000). As an individual increases
295 in size, the amount of nitrogen it needs to maintain the same nutrient concentrations in its
296 more abundant tissues becomes exponentially higher (Figure 2, Blackman, 1919). While the
297 overall nitrogen uptake rates of the plants in this study were likely to be higher as plants
298 increased in biomass (hereafter ‘larger plants’) their capacity to meet their increased demand

299 was significantly limited (Figure 2A & B). Thus, the increased capacity to absorb nitrogen
300 that a larger biomass might provide, such as by having more absorptive surfaces (Blackman,
301 1919) , was outweighed by the increased demand from having larger standing nitrogen stocks
302 (Figure 2). Also, larger plants were less likely to store the same standing nitrogen stocks
303 (%DW) as smaller plants (Figure 2D), suggesting that larger plants may not be absorbing
304 nitrogen to their full potential. This is a new insight into the efficiency of nutrient cycling by
305 aquatic plants. In addition, the porewater nitrogen was more likely to be depleted in the
306 vicinity of larger plants (Figure 2C). All trends were highly variable for plants with a smaller
307 biomass, which made factor ceiling analysis a highly valuable tool. Its results suggest that
308 firstly, demand was represented more strongly than supply in biomass-uptake relationships
309 (Figure 2A & B), secondly the larger plants did not store N (%DW) standing stocks as high
310 as smaller plants, which was not necessarily related to species (Figure 2D) and lastly an
311 individual plant can deplete porewater NH_4^+ (Figure 2C). Due to the aforementioned
312 observations, the evidence suggests that the larger plants were nitrogen deprived. Such
313 nitrogen deprivation may not have limited growth because plants are capable of luxury
314 nutrient uptake (e.g. Göransson and Eldhuset, 1991) and the threshold N (%DW)
315 concentration above which further uptake becomes luxurious is unknown. Nevertheless,
316 larger plants were likely to not have been cycling nitrogen to their fullest potential and even
317 larger plants that may be growing naturally at higher densities than the selection criteria of
318 this study are probably more N-deprived. Thus, nitrogen is likely to be a competitive resource
319 in aquatic plant meadows of the north Baltic Sea, and interpretations of species interactions
320 during nitrogen uptake should be re-evaluated.

321 Aquatic plants are subject to a nutrient compromise in the water column (Fourqurean
322 et al., 1992); that with increasing nutrient availability in the water column, the light
323 availability decreases as responsive phytoplankton blooms attenuate the light which is

324 transmitted to the plants from the surface. But results from this study exhibited an additional
325 nitrogen compromise for plants that might not be expected in a eutrophicated sea (Gustafsson
326 et al., 2012). Ammonium concentrations in the sediment porewater were lower than the
327 global median for aquatic plants meadows (Hemminga, 1998). Trends showed that overall,
328 the more investment in biomass the less capable plants were at meeting their nutrient
329 demands (Figure 2D), which has been previously unobserved in the field. These trends
330 between aquatic plant biomass and tissue nitrogen concentrations represented not only the
331 ability for plants to absorb their nutrients from the sediment, but also the water column, and
332 the trade-off between investing in belowground and aboveground biomass for uptake is also
333 accounted for. Therefore, these interpretations apply to the capacity for a whole plant to meet
334 its nutrient demands. It is noteworthy that this experiment was conducted at the peak for plant
335 biomass, but not for production, and so if the experiment was conducted at the peak of
336 productivity the biomass-uptake trends observed could become even more accentuated.
337 Overall, these findings show that larger aquatic plants are not cycling nutrients to their full
338 potential. Thus, they could potentially become more efficient at their role in the coastal
339 nutrient filter if circumstances allowed it. Strategies, such as morphological root traits, could
340 thus be highly valuable for helping plants to cycle nutrients more effectively. For instance,
341 depending on the extent of nutrient variability, the ability for plants to absorb nutrients in the
342 vicinity (i.e. Root biomass, Specific Root Length) or to access new nutrient pools (i.e.
343 Maximum root length) could be highly valuable for enhancing nutrient uptake.

344 Root traits and uptake rates

345 In general, an increase in ambient nutrient availability leads to an accelerating increase in the
346 rate of nutrient uptake by an individual, typically described by a hyperbolic trend (Caassen
347 and Barber, 1976). However, such a trend was not detected in this experiment. Porewater
348 estimates may not have been representative of the relative availability of nutrients in the

349 depletion zones, or an interaction with other factors that were more influential drivers of
350 uptake rate (e.g. Plant traits, plant biomass, Figure 4) could have convoluted the conventional
351 porewater nutrient-uptake trend. This was likewise the case for root biomass (Table 4),
352 because root biomass represents a dynamic equilibrium between environmental nutrient
353 availability and plant nutrient demands, making it highly unlikely that a direct relationship
354 could be observed between root biomass and any plant nutrient parameters (e.g. Ruffel et al.,
355 2011; Figure 4). Given that the uptake rates, plant stores and sediment supply of nutrients
356 were largely influenced by the plant biomass in this study, the nutrient dynamics were likely
357 to be largely plant-driven. These findings suggest that access to new nutrient sources would
358 enhance uptake rates and plant stores. Similarly, when uptake rates were compared to pre-
359 existing trait data, species with the potential to grow longer roots appeared to be more
360 equipped to respond to the nutrient source (Figure 2). This seemed less beneficial when the
361 effect of porewater availability was also taken into account to estimate overall uptake rates
362 (Figure 2). Thus, the plants would potentially benefit from longer roots to respond to the
363 nutrient enrichment, but because longer roots cannot guarantee a proportional increase in
364 access to more nutrients it does not necessarily mean that the overall uptake rate would be
365 faster. Within the zone from which plants can deplete nutrients, it is unlikely that properties
366 that increase the absorptive properties of the roots would be as helpful as accessing new
367 zones with nutrient sources. Otherwise, one would expect that porewater nutrient availability
368 or root biomass would have had stronger roles in the different uptake rates of individuals of
369 this study (Figure 2; Figure 4). Instead, the plant biomass (i.e. Demand) had the prevailing
370 influence (Figure 2). Supporting this result, specific root length was not significantly linked
371 to the rank order of uptake rates in the supportive analyses. It is unlikely that variability of
372 further physiological strategies for absorption would significantly affect uptake rates.
373 Therefore, findings imply that even for a single aquatic plant shoot, the variability in access

374 to a nutrient source is more important than variability for plants to retrieve the nitrogen once
375 it had been accessed. Other traits which might enhance access to nutrient sources include
376 expanding the spatial reach for nutrients than seeking nutrients within a smaller zone, based
377 on terrestrial examples (see Kembel et al., 2008; Campbell et al., 1991 and references
378 within), which could include growing multiple ramets because labour is divided between
379 ramets, simultaneously increasing the reach (Roiloa and Hutchings, 2013).

380 Conclusion

381 To our knowledge, this is the first study to measure the nitrogen uptake of a variety of
382 different aquatic species in relatively similar environmental conditions in the field.
383 Furthermore, the influence of different plant characteristics was surveyed in a natural
384 environment while other factors remained relatively similar. Results show a previously
385 unidentified, extreme spatial variability of sediment porewater ammonium availability within
386 an aquatic plant meadow, and its variability is reflected by the uptake strategies, challenges
387 and successes for even a single aquatic plant shoot. Overall, our results suggest that the larger
388 plants could perform better at their role as nutrient filters in the coastal zone, and increasing
389 access to new nutrient sources could help plants to uptake and store nutrients more
390 effectively. Thus, larger plants have the potential to cycle nutrients more effectively, and this
391 could potentially be facilitated by increasing the access to new nutrient pools which would
392 benefit from nutrient cycling by aquatic plants. These findings fill a knowledge gap because
393 such conditions for aquatic plants in their natural settings were previously unknown. Future
394 studies which investigate the mechanisms that increase root access to new nutrient sources
395 could help to understand how nutrient cycling by aquatic plants might be enhanced. For
396 example, more diverse aquatic meadows may enhance resource partitioning (Gustafsson and
397 Boström, 2011) and thus nutrients may be cycled more effectively, and nutrient cycling by
398 fauna can increase plant growth (Peterson and Heck, 2001; Peterson and Heck, 1999). To

399 follow from this study, there is an opportunity to investigate how community composition
400 (Gustafsson and Boström, 2011), the activity of infauna (e.g. Villnäs et al., 2018) and other
401 factors increase the connectivity of aquatic plant nutrient depletion zones to areas which
402 would benefit from their role as nutrient filters. Ultimately, such studies may help to develop
403 effective conservation strategies to mitigate eutrophication by providing the most effective
404 solutions to enhance the role of aquatic plant meadows as nutrient filters.

405 Acknowledgements

406 We would like to thank Hanna Halonen; research technician, and volunteers Mari
407 Vanharanta, Ella Pippingsköld and Pauliina Saarman for assistance in the field. Thanks also
408 to Iván Rodil and Anna Villnäs for their analytical and literary advice during the preparation
409 of the manuscript. This work was funded by the Walter and Andrée de Nottbeck Foundation
410 (CA), Academy of Finland (CG, grant number 295443; AN, grant number 294853) and
411 Svenska kulturfonden (CG) and conducted at Tvärminne Zoological Station, University of
412 Helsinki.

413

414 Literature cited

415 Aerts, R., 1999. Interspecific competition in natural plant communities: Mechanisms, trade-
416 offs and plant-soil feedbacks. *Journal of Experimental Botany*, **50**(330), pp. 29-37.

417 Andersen, J.H., Laamamen, M., Aigars, J., Axe, P., Blomqvist, M., Carstensen, J., Claussen,
418 U., Josefson, A.B., Flemming-Lehtinen, V. and Järvinen, M., 2009. Eutrophication in the
419 Baltic Sea - An integrated thematic assessment of the effects of nutrient enrichment in the
420 Baltic Sea region.

421 Armitage, A.R., Frankovich, T.A. and Fourqurean, J.W., 2011. Long-term effects of adding
422 nutrients to an oligotrophic coastal environment. *Ecosystems*, **14**(3), pp. 430-444.

423 Blackman, V., 1919. The compound interest law and plant growth. *Annals of Botany*,
424 **33**(131), pp. 353-360.

- 425 Caassen, N. and Barber, S., 1976. Simulation model for nutrient uptake from soil by a
426 growing plant root system. *Agronomy Journal*, **68**(6), pp. 961-964.
- 427 Campbell, B., Grime, J. and Mackey, J., 1991. A trade-off between scale and precision in
428 resource foraging. *Oecologia*, **87**(4), pp. 532-538.
- 429 Campbell, J.E. and Fourqurean, J.W., 2014. Ocean acidification outweighs nutrient effects in
430 structuring seagrass epiphyte communities. *Journal of Ecology*, **102**(3), pp. 730-737.
- 431 Cheng, X., Zeng, Y., Guo, Z. and Zhu, L., 2014. Diffusion of nitrogen and phosphorus across
432 the sediment-water interface and in seawater at aquaculture areas of Daya Bay, China.
433 *International Journal of Environmental Research and Public Health*, **11**(2), pp. 1557-1572.
- 434 Chowdhury, M. and Bakri, D.A., 2006. Diffusive nutrient flux at the sediment—water
435 interface in Suma Park Reservoir, Australia. *Hydrological Sciences Journal*, **51**(1), pp. 144-
436 156.
- 437 Clavero, V., Izquierdo, J., Fernandez, J. and Niell, F., 2000. Seasonal fluxes of phosphate and
438 ammonium across the sediment-water interface in a shallow small estuary (Palmones River,
439 southern Spain). *Marine Ecology Progress Series*, pp. 51-60.
- 440 Collos, Y., 1987. Calculations of ¹⁵N uptake rates by phytoplankton assimilating one or
441 several nitrogen sources. *International Journal of Radiation Applications and
442 Instrumentation. Part A. Applied Radiation and Isotopes*, **38**(4), pp. 275-282.
- 443 Comas, L. and Eissenstat, D., 2004. Linking fine root traits to maximum potential growth rate
444 among 11 mature temperate tree species. *Functional Ecology*, **18**(3), pp. 388-397.
- 445 De Kroon, H. and Mommer, L., 2006. Root foraging theory put to the test. *Trends in Ecology
446 & Evolution*, **21**(3), pp. 113-116.
- 447 Erftemeijer, P.L. and Middelburg, J.J., 1995. Mass balance constraints on nutrient cycling in
448 tropical seagrass beds. *Aquatic Botany*, **50**(1), pp. 21-36.
- 449 Evrard, V., Kiswara, W., Bouma, T.J. and Middelburg, J.J., 2005. Nutrient dynamics of
450 seagrass ecosystems: ¹⁵N evidence for the importance of particulate organic matter and root
451 systems. *Marine Ecology Progress Series*, **295**, pp. 49-55.
- 452 Ferdie, M. and Fourqurean, J.W., 2004. Responses of seagrass communities to fertilization
453 along a gradient of relative availability of nitrogen and phosphorus in a carbonate
454 environment. *Limnology and Oceanography*, **49**(6), pp. 2082-2094.
- 455 Fourqurean, J.W., Zieman, J.C. and Powell, G.V.N., 1992. Relationships between Porewater
456 Nutrients and Seagrasses in a Subtropical Carbonate Environment. *Marine Biology*, **114**(1),
457 pp. 57-65.
- 458 Furman, B.T., Jackson, L.J. and Peterson, B.J., 2017. Edaphic resource foraging by *Zostera
459 marina* (Linnaeus) patches. *Journal of Experimental Marine Biology and Ecology*, **486**, pp.
460 214-221.

- 461 Göransson, A. and Eldhuset, T.D., 1991. Effects of aluminium on growth and nutrient uptake
462 of small *Picea abies* and *Pinus sylvestris* plants. *Trees-Structure and Function*, **5**(3), pp. 136-
463 142.
- 464 Gustafsson, B.G., Schenk, F., Blenckner, T., Eilola, K., Meier, H.M., Müller-Karulis, B.,
465 Neumann, T., Ruoho-airola, T., Savchuk, O.P. and Zorita, E., 2012. Reconstructing the
466 development of Baltic Sea eutrophication 1850–2006. *Ambio*, **41**(6), pp. 534-548.
- 467 Gustafsson, C. and Norkko, A., 2018. Quantifying the importance of functional traits for
468 primary production in aquatic plant communities. *Journal of Ecology*, **00**, pp. 1-13.
- 469 Gustafsson, C. and Norkko, A., 2016. Not all plants are the same: Exploring metabolism and
470 nitrogen fluxes in a benthic community composed of different aquatic plant species.
471 *Limnology and Oceanography*, **61**(5), pp. 1787-1799.
- 472 Gustafsson, C. and Boström, C., 2011. Biodiversity influences ecosystem functioning in
473 aquatic angiosperm communities. *Oikos*, **120**(7), pp. 1037-1046.
- 474 Hemminga, M.A., 1998. The root/rhizome system of seagrasses: an asset and a burden.
475 *Journal of Sea Research*, **39**(3-4), pp. 183-196.
- 476 Hemminga, M.A. and Duarte, C.M., 2000. *Seagrass Ecology*. Cambridge University Press.
- 477 Kautsky, L., 1988. Life strategies of aquatic soft bottom macrophytes. *Oikos*, pp. 126-135.
- 478 Kembel, S.W., De Kroon, H., Cahill JR, J.F. and Mommer, L., 2008. Improving the scale and
479 precision of hypotheses to explain root foraging ability. *Annals of Botany*, **101**(9), pp. 1295-
480 1301.
- 481 Kennedy, H., Beggins, J., Duarte, C.M., Fourqurean, J.W., Holmer, M., Marbà, N. and
482 Middelburg, J.J., 2010. Seagrass sediments as a global carbon sink: Isotopic constraints.
483 *Global Biogeochemical Cycles*, **24**, pp. GB4026-GB4026.
- 484 Kenworthy, J.W. and Thayer, G.W., 1984. Production and decomposition of the roots and
485 rhizomes of seagrasses, *Zostera marina* and *Thalassia testudinum*, in temperate and
486 subtropical marine ecosystems. *Bulletin of Marine Science*, **35**(3), pp. 364-379.
- 487 Lepoint, G., Millet, S., Dauby, P., Gobert, S. and Bouquegneau, J., 2002. Annual nitrogen
488 budget of the seagrass *Posidonia oceanica* as determined by in situ uptake experiments.
489 *Marine Ecology Progress Series*, **237**, pp. 87-96.
- 490 Marbà, N., Hemminga, M.A., Mateo, M.A., Duarte, C.M., Mass, Y.E., Terrados, J. and
491 Gacia, E., 2002. Carbon and nitrogen translocation between seagrass ramets. *Marine Ecology
492 Progress Series*, **226**, pp. 287-300.
- 493 McGlathery, K.J., Sundbäck, K. and Anderson, I.C., 2007. Eutrophication in shallow coastal
494 bays and lagoons: the role of plants in the coastal filter. *Marine Ecology Progress Series*,
495 **348**, pp. 1-18.

- 496 Mutchler, T. and Hoffman, D.K., 2017. Response of seagrass (*Thalassia testudinum*) metrics
497 to short-term nutrient enrichment and grazing manipulations. *Journal of Experimental*
498 *Marine Biology and Ecology*, **486**, pp. 105-113.
- 499 Olsen, Y.S. and Valiela, I., 2010. Effect of Sediment Nutrient Enrichment and Grazing on
500 Turtle Grass *Thalassia testudinum* in Jobos Bay, Puerto Rico. *Estuaries and Coasts*, **33**(3),
501 pp. 769-783.
- 502 Pérez, M., Romero, J., Duarte, C.M. and Sand-Jensen, K., 1991. Phosphorus limitation of
503 *Cymodocea nodosa* growth. *Marine Biology*, **109**(1), pp. 129-133.
- 504 Peterson, B.J. and Heck JR, K.L., 2001. An experimental test of the mechanism by which
505 suspension feeding bivalves elevate seagrass productivity. *Marine Ecology Progress Series*,
506 **218**, pp. 115-125.
- 507 Peterson, B.J. and Heck, K.L., 1999. The potential for suspension feeding bivalves to
508 increase seagrass productivity. *Journal of Experimental Marine Biology and Ecology*, **240**(1),
509 pp. 37-52.
- 510 Poorter, H. and Remkes, C., 1990. Leaf-Area Ratio and Net Assimilation Rate of 24 Wild-
511 Species Differing in Relative Growth-Rate. *Oecologia*, **83**(4), pp. 553-559.
- 512 Roiloa, S.R. and Hutchings, M.J., 2013. The effects of physiological integration on biomass
513 partitioning in plant modules: an experimental study with the stoloniferous herb *Glechoma*
514 *hederacea*. *Plant Ecology*, **214**(4), pp. 521-530.
- 515 Ruffel, S., Krouk, G., Ristova, D., Shasha, D., Birnbaum, K.D. and Coruzzi, G.M., 2011.
516 Nitrogen economics of root foraging: transitive closure of the nitrate-cytokinin relay and
517 distinct systemic signaling for N supply vs. demand. *Proceedings of the National Academy of*
518 *Sciences of the United States of America*, **108**(45), pp. 18524-18529.
- 519 Short, F.T., 1987. Effects of Sediment Nutrients on Seagrasses - Literature-Review and
520 Mesocosm Experiment. *Aquatic Botany*, **27**(1), pp. 41-57.
- 521 Tessier, J.T. and Raynal, D.J., 2003. Use of nitrogen to phosphorus ratios in plant tissue as an
522 indicator of nutrient limitation and nitrogen saturation. *Journal of Applied Ecology*, **40**(3), pp.
523 523-534.
- 524 Thomson, J.D., Weiblen, G., Thomson, B.A., Alfaro, S. and Legendre, P., 1996. Untangling
525 multiple factors in spatial distributions: lilies, gophers, and rocks. *Ecology*, **77**(6), pp. 1698-
526 1715.
- 527 Thrush, S.F., Hewitt, J.E., Norkko, A., Nicholls, P.E., Funnell, G.A. and Ellis, J.I., 2003.
528 Habitat change in estuaries: predicting broad-scale responses of intertidal macrofauna to
529 sediment mud content. *Marine Ecology Progress Series*, **263**, pp. 101-112.
- 530 Touchette, B.W. and Burkholder, J.M., 2000. Review of nitrogen and phosphorus metabolism
531 in seagrasses. *Journal of Experimental Marine Biology and Ecology*, **250**(1-2), pp. 133-167.

532 Villnäs, A., Hewitt, J., Snickars, M., Westerbom, M. and Norkko, A., 2018. Template for
 533 using biological trait groupings when exploring large-scale variation in seafloor
 534 multifunctionality. *Ecological Applications*, 28(1), pp. 78-94.

535 Wirachwong, P. and Holmer, M., 2010. Nutrient dynamics in 3 morphological different
 536 tropical seagrasses and their sediments. *Aquatic Botany*, **93**(3), pp. 170-178.

537

538 Tables

539

Table 1. Overall mean, standard deviation (SD) and frequency (n) of ammonium (NH₄⁺), nitrate and nitrite (NO₂⁻ + NO₃⁻) and phosphate (PO₄³⁻) concentrations (µg L⁻¹) in the sediment porewater next to individual plants and the water column.

	Mean	SD	N
Porewater			
NH ₄ ⁺	21.63	13.12	58
NO ₂ ⁻ + NO ₃ ⁻	1.98	2.75	11
PO ₄ ³⁻	3.44	2.76	11
Water column			
NH ₄ ⁺	0.10	0.03	6
NO ₂ ⁻ + NO ₃ ⁻	0.14		2
PO ₄ ³⁻	0.13	0.04	3

540

Table 2. Details of enrichment events including dates, which species were enriched (Treatment), number of successful replicates for each species (originally 3 replicates per species per day in 2015. No replicates lost in 2016), average temperature (°C) and photosynthetically active radiation (PAR; mol m⁻² h⁻¹; HOBO Pendant® Temperature/Light Data Logger 64 K, Onset, USA, light data converted to PAR as described by Gustafsson, Norkko 2016) during each incubations.

Enrichment	Date	Treatment	Successful replicates	Temp. °C	PAR (mol m ⁻² h ⁻¹)
1	13.08.15	Cerdem, Rupcir, Zanmaj	3, 3, 2	16	1.2
2	26.08.15	Potper, Zosmar	3, 3	17	1.2
3	27.08.15	Myrspi, Stupec	2, 1	18	0.9
4	10.09.15	Myrspi, Potper	1, 1	19	0.7
5	11.09.15	Myrspi, Potper, Stupec	3, 1, 3	19	0.6
6	02.09.16	Myrspi, Potper	7, 3	16	1.1

541

Table 3. Mean Max Root Length (mm) and Specific Root Length means (\pm Standard Error, SE) of study species in the same area of the experiment. Data source Gustafsson & Norkko (2018).

Species	MMRL (mm)	SRL
<i>Myriophyllum spicatum</i>	9.8 \pm 1.3	5.7 \pm 1.6
<i>Potamogeton perfoliatus</i>	7.4 \pm 0.4	14.0 \pm 1.2
<i>Ruppia cirrhosa</i>	8.7 \pm 1.2	11.6 \pm 1.3
<i>Stuckenia pectinata</i>	6.4 \pm 1.2	19.5 \pm 2.0
<i>Zannichellia major</i>	7.5 \pm 1.4	11.2 \pm 0.6
<i>Zostera marina</i>	6.4 \pm 0.8	8.5 \pm 0.7

542

Table 4. Regression statistics for aquatic plant uptake rate (UR, $\mu\text{gN gN}^{-1} \text{h}^{-1}$) and uptake response rate (RR, $\mu\text{g}^{15}\text{N gN}^{-1} \text{h}^{-1}$), in response to changes in porewater ammonium availability (NH_4^+ , μM) and root biomass (mg). Rank regression statistics for UR and RR with changes in species-averaged Mean Max Root Length (MMRL, mm) and species-averaged specific root length (SRL).

	Coefficient	Intercept	Regression statistics			
			r^2	n	F	P
Porewater [NH_4^+]						
UR	.9112	3.5734	.2902	30	11.45	.002
RR	.2556	2.3424	.0694	30	2.088	.160
Root biomass (mg)						
UR	-.007912	6.185049	.02568	17	.3954	.539
RR	-.002668	2.204714	.002517	23	.05299	.820
	Coefficient	Intercept	Rank regression statistics			
			n	Overall Wald test	P	
Mean max root length						
UR	262.63	-1570.02	6	3.5724		.053
RR	4.0781	-21.4567	6	4.2730		.034
Specific root length						
UR	-.69579	18.2358	6	1.3207		.296
RR	-29.799	657.346	6	0.6716		.526

543

Table 5. 95th quantile regression statistics for aquatic plant uptake rate ($\ln(\text{UR})$, $\mu\text{gN gN}^{-1} \text{h}^{-1}$), uptake response rate ($\ln(\text{RR})$, $\mu\text{g}^{15}\text{N gN}^{-1} \text{h}^{-1}$), aboveground nitrogen concentration (AG N, %DW) and porewater ammonium availability (NH_4^+ , μM) when compared to total biomass ($\ln(\text{biomass})$, mg) of an individual plant.

	Coefficient	Intercept	95 th quantile regression statistics			
			r^2	N	F	P
Total biomass						
UR	-2.1609	18.0999	0.774	12	38.67	<.0001
RR	-1.5419	11.1518	0.7212	12	29.46	<.0001
AG N (%DW)	-0.8825	7.3764	0.7969	15	55.92	<.0001
Porewater NH_4^+	-12.380	93.398	0.5943	12	17.11	.002

544

545 Figure legends

546 Figure 1. Experimental design of an incubation for a single aquatic plant. Multiple incubations (min.
547 batch of 9) were conducted simultaneously within an incubation day.

548 Figure 2. Total plant biomass ($\ln(\text{biomass})$, mg dry weight (DWT)) and, A: Uptake rates ($\ln(\text{UR})$,
549 $\mu\text{gN gN}^{-1} \text{h}^{-1}$), B: Uptake response rates ($\ln(\text{RR})$, $\mu\text{gN gN}^{-1} \text{h}^{-1}$), C: Porewater ammonium (NH_4^+ ,
550 μM) and D: Aboveground nitrogen concentration (AG N, percentage dry weight; %DW). 'D' displays
551 data for enriched and non-enriched plants. Dotted lines denote 95th percentiles. 95th percentiles were
552 calculated before the data was logarithmically transformed; two values are missing from A and B due
553 to disproportionately negative values.

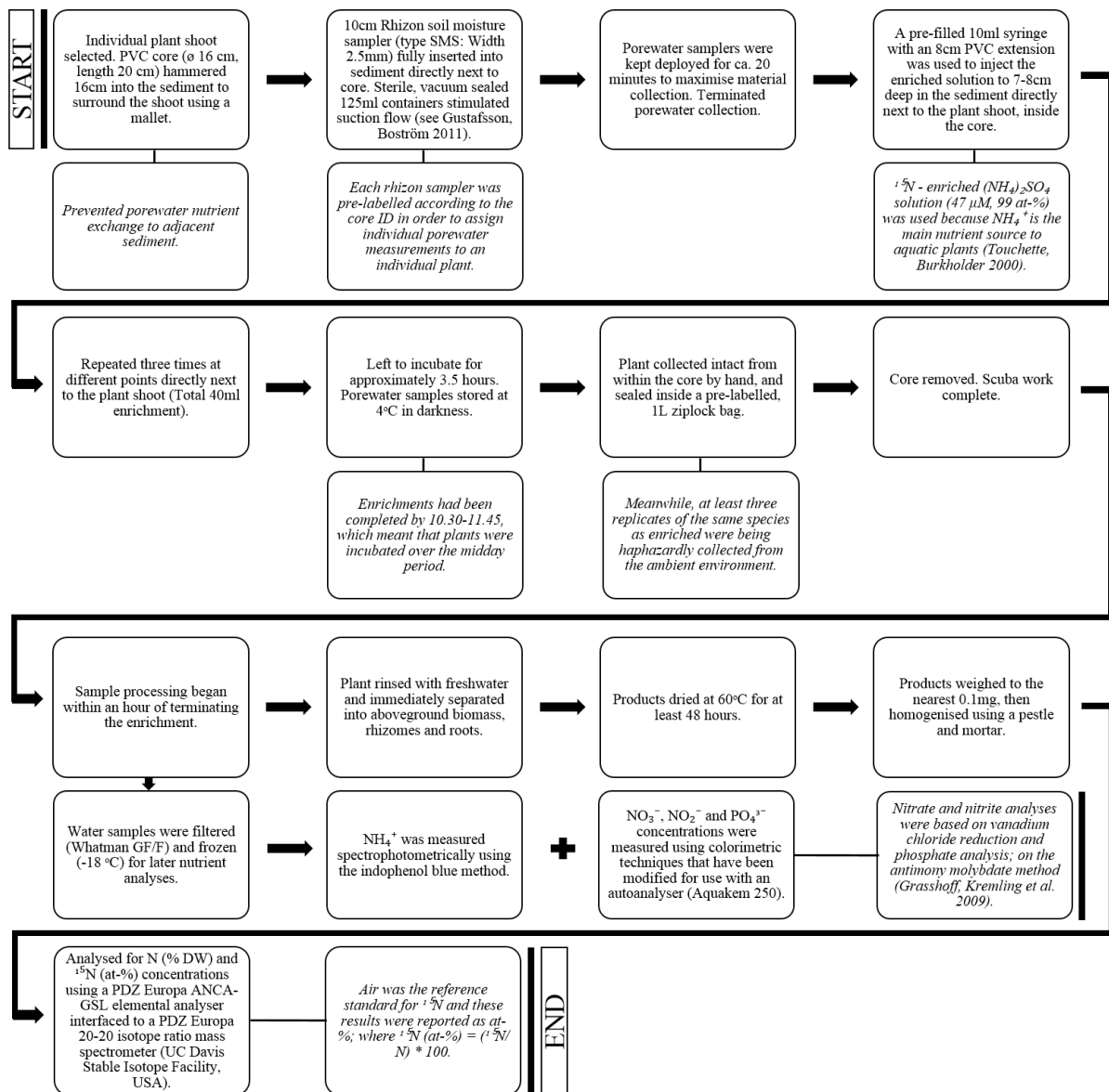
554

555 Figure 3. Species-averaged mean max root lengths (mm) and (A) uptake response rates ($\ln(\text{RR}+10)$),
556 (B) species-averaged uptake rates ($\ln(\text{UR}+100)$), ($\mu\text{gNgN}^{-1}\text{h}^{-1}$).

557

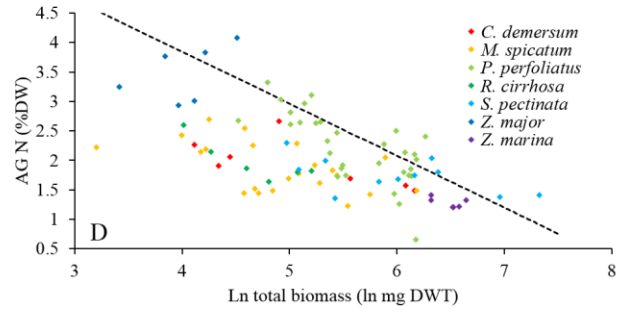
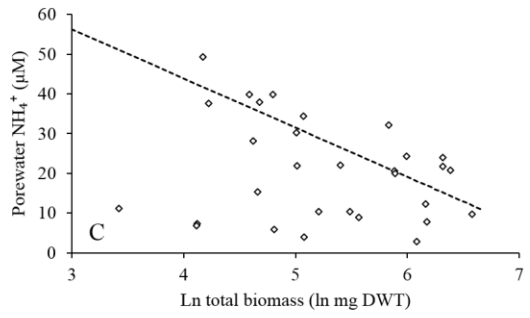
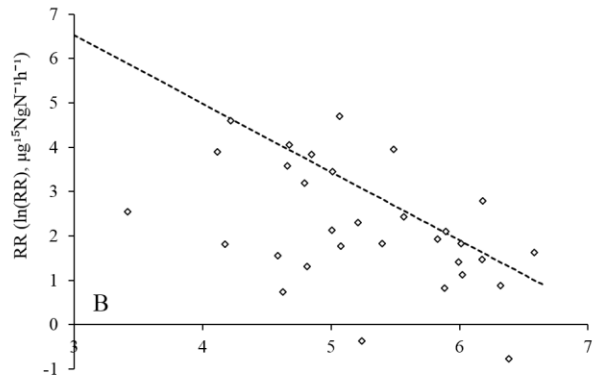
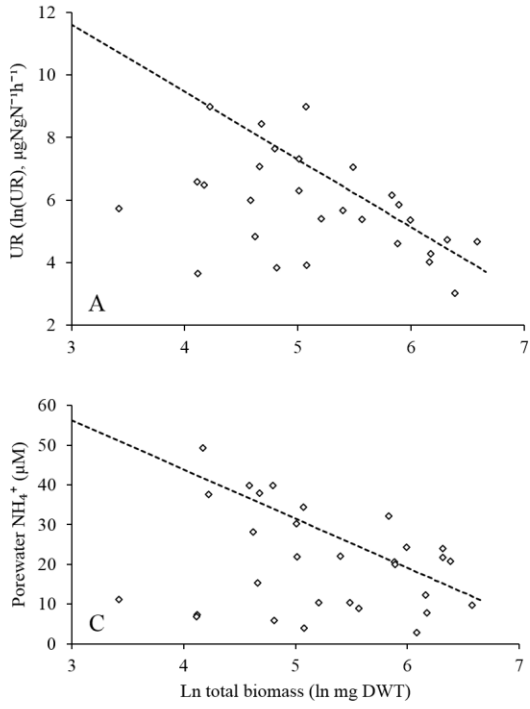
558 Figure 4. Conceptual diagram of the trade-offs occurring in nutrient uptake for aquatic plants in the
559 northern Baltic Sea, based on current evidence from this study and published literature. Solid lines:

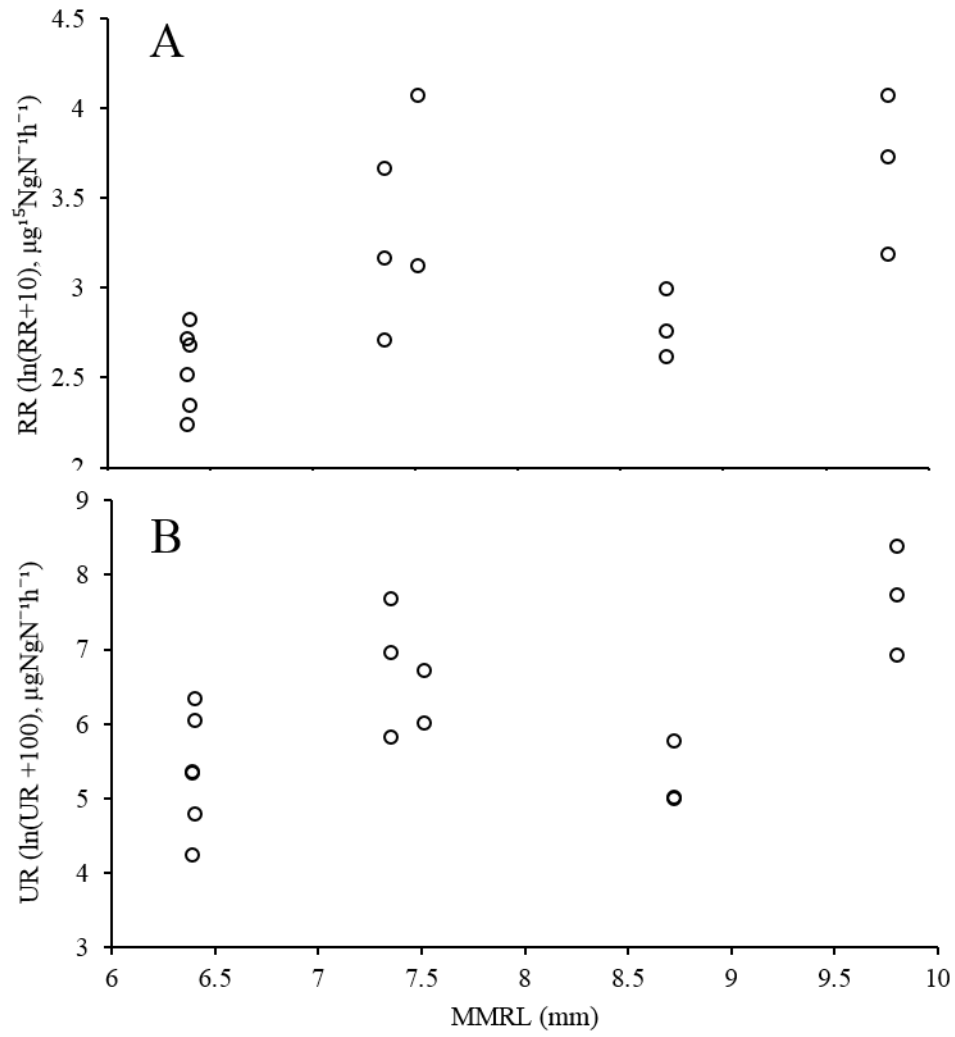
560 Evidence-based trends considering interspecific and intraspecific trends. Dashed line: May not apply
 561 to a single shoot of a plant that has multiple ramets (Roiloa, Hutchings 2013). Reverse trend for plants
 562 with multiple ramets. ^a= Marba et al. 2002, while other components to the model were inferred from
 563 the results of this study.



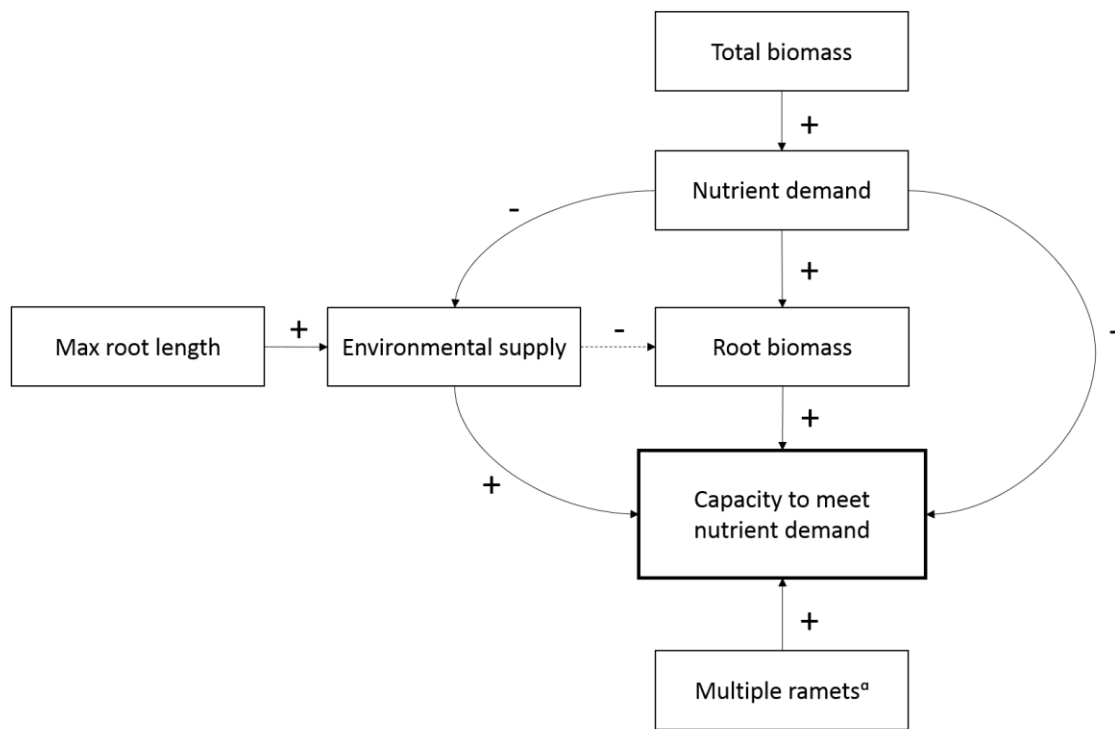
564

565





567



568