

1 **Uptake of nitrogen from compound pools by the seagrass *Zostera noltii***

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14 **ABSTRACT**

15 In nature, seagrasses are confronted with a compound pool of low concentrations inorganic
16 and organic nitrogen-containing substances of varying bioavailability. Nevertheless, the
17 majority of research on nitrogen acquisition by seagrasses has been largely limited to studies
18 addressing a single nitrogen substrate at a time. Using a combination of one of ^{15}N -labelled
19 substrates and one ^{14}N -labelled background substrate, we investigated how the rate of
20 nitrogen uptake by the seagrass *Zostera noltii* varies with nitrogen background. Leaf and root
21 mediated uptake were studied separately for different combinations of inorganic (ammonium,
22 nitrate) and organic substrates (urea, glycine). Ammonium uptake rates were higher than for
23 the other substrates. However, substrate uptake was not dependent on the background
24 nutrient. Similar patterns and uptake rates were found for above- and belowground plant
25 parts. The dependence of uptake rate on substrate type, combined with an independence of
26 nutrient background is explained as difference in uptake capacity, rather than substrate
27 preference. For the dual labeled (^{15}N and ^{13}C) urea and glycine, strong relationships existed
28 between nitrogen and carbon uptake, but with deviations from expectations under complete
29 uptake of the molecules. Overall, this study indicates that at realistically low ambient
30 concentrations, seagrasses can simultaneously use inorganic and organic sources for their
31 nitrogen needs, and do not distinguish between substrates. In other words, they take up
32 whatever is available.

33 **Keywords:** Nitrogen uptake; seagrass; *Zostera noltii*; isotope label; inorganic nitrogen;
34 organic nitrogen

35

36 1. Introduction

37 Like all plants, seagrasses need nitrogen (N) to maintain their high productivity. However,
38 unlike many terrestrial plants, the resorption of N from the senescent leaves is very limited
39 and a lot of N is lost due to the high leaf detachment (Romero et al., 2006; Stapel and
40 Hemminga, 1997). This makes seagrasses strongly dependent on external nutrient sources
41 (Short and McRoy, 1984) from the sediment and water column (Short and McRoy, 1984;
42 Stapel et al., 1996; Touchette and Burkholder, 2000).

43 Nitrogen is available to seagrasses as a mixture of compounds, of which some are expected
44 to be more immediately useful to them than others. Usually, affinities for ammonium are
45 higher than for nitrate in kinetic uptake experiments (e.g. Hasegawa et al. 2005; Alexandre et
46 al., 2010), which is generally attributed to additional costs associated with nitrate reduction
47 (Turpin, 1991). If this increased affinity for ammonium is inherent to the organism, and exists
48 without external stimulus (and literature shows at least that this property is very common in
49 seagrasses in general (Touchette and Burkholder, 2000), and in *Zostera noltii* in particular;
50 (Alexandre et al. 2010)), it could be called a ‘constitutive preference’. In addition, nutrient-
51 nutrient interactions have been reported, where nitrate uptake is down-regulated under
52 increasing ammonium availability (Alexandre et al. 2010). In their uptake experiments,
53 Alexandre and co-workers (2010) could also demonstrate an up-regulation of ammonium
54 uptake by *Zostera noltii* under increased nitrate concentrations, which they attributed to a
55 signaling function of nitrate in the ammonium metabolism. The latter mechanism could be
56 addressed as an ‘induced preference’, where ammonium uptake is stimulated by an external
57 factor.

58 Whereas for a long time nitrogen research has solely focused on dissolved inorganic
59 nitrogen (DIN) uptake by seagrasses (e.g. Cornelisen and Thomas, 2004; Stapel et al., 2001),
60 recent studies suggest that seagrasses are also able to use dissolved organic matter as a
61 nitrogen source. This enables them to shortcut N cycling (Barron et al., 2006; Evrard et al.,
62 2006; Vonk et al., 2008). Similar to terrestrial plants (Harrison et al., 2007), seagrasses exhibit
63 distinct uptake rates for different organic nitrogen substrate, that seem to be related to the
64 substrate's bioavailability, molecular complexity and/or chemical stability of the molecules
65 (Vonk et al., 2008; Van Engeland 2011, 2013). For instance, urea is a very simple molecule
66 that provides two amine groups per molecule. Amino acids with chemically very stable
67 phenyl-groups may be less prone to breakdown and uptake. It is currently not clear if any
68 nutrient-nutrient interactions exist in the uptake dynamics of organic nitrogen.

69 In oligotrophic coastal systems (usually in tropical regions), effective use of nitrogen
70 sources are vital to maintain a high productivity, whereas in eutrophic areas (usually in
71 temperate regions) nitrogen overloading may occur (e.g., Touchette and Burkholder, 2007).
72 Recently, it was discovered that dissolved organic nitrogen pools in coastal waters are
73 relatively high and not refractory, even in oligotrophic systems (Bronk et al., 2007). In
74 oligotrophic systems, the availability of additional nitrogen sources may help to explain the
75 high productivity of seagrass systems. In eutrophic systems, the availability of additional
76 nitrogen sources may form an additional threat. Therefore, we aim to (1) quantify uptake rates
77 of each of the dominant nitrogen sources, i.e., inorganic and organic nitrogen, and (2) detect
78 whether the availability on one of these sources affects uptake rates of the other sources. We
79 studied this in a temperate seagrass species, *Zostera noltii*, as this species usually occurs in
80 meso- or eutrophic situations (e.g. Wadden Sea, Cadiz Bay e.g. Dolch et al., 2013; Brun et al.,
81 2003), but can also be found in oligotrophic lagoons (Honkoop et al., 2008) We tested this in

82 the lower range of nutrient concentrations as observed in Cadiz Bay in summer (Van
83 Engeland et al., 2013)

84 Using stable isotope labelling, we investigated uptake by the temperate seagrass, *Zostera*
85 *noltii* Horneman, of ^{15}N nitrogen from different inorganic (ammonium, nitrate) and organic
86 substrates (urea and glycine) as a function of the presence of one of the other substrates as
87 background (^{14}N). By adding fairly low concentrations, we focused on nutrient interactions in
88 uptake at nutritional conditions that are realistic for the source population of the studied plants
89 (Cadiz Bay, Spain). Dual labeling (^{13}C and ^{15}N) was used to track potential dissolved organic
90 carbon uptake.

91

92 **2. Materials and methods**

93

94 2.1. Biological material and experimental setup

95 Shoots of *Zostera noltii* Horneman were collected from an intertidal meadow of Cadiz Bay
96 (Southern Spain, 36°29'19.79"N; 6°15'53.05"E), brought to the lab in a cool box, wrapped in
97 moist paper, and then boxed in an ice-chest to be transported to the Netherlands. The plants
98 arrived after two days and were immediately put in a tank with 2 μm filtered water from
99 Oosterschelde (south-west Netherlands) under controlled temperature (19°C) and light (278
100 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) conditions. Inorganic nitrogen concentration in the tank were as in
101 Oosterschelde ($\text{NH}_4^+ = 4.7\mu\text{mol N}$, $\text{NO}_x^- = 1.17\mu\text{mol N}$, $\text{DON} = 20.1 \mu\text{mol N}$). After an
102 acclimation period of two full days, plants were cut into single complete shoots (with leaves,
103 rhizomes and roots) and gently cleaned from epiphytes with a razor blade to minimize
104 microbial degradation by *e.g.* free living bacteria, exo-enzymes etcetera, Van Engeland et al.,

105 2011). This enabled us to focus on the ability of the seagrass itself to process or use nitrogen
106 forms, rather than facilitation by better equipped micro-organisms. Six days after harvest in
107 Cadiz bay, the plants were incubated in a climate-controlled room (temperature 19°C and
108 lights 254 mol photons m⁻² s⁻¹) in 250 ml plastic cups. Plants (2 - 3 shoots) were left intact
109 with their belowground and aboveground parts submersed in separate cups (Van Engeland et
110 al., 2011) (Fig. 1). As the plants would protrude out of the water, the cups were filled almost
111 until the edge to prevent desiccation, while exchange of water between cups was prevented.
112 We used artificial seawater (constituents from Merck and Sigma-Aldrich) that we manually
113 prepared to exclude unintended nutrient addition (modified F2 medium containing only the
114 major constituents, without the nitrogen salts; see for instance De Brouwer et al., 2005), and
115 to minimize interference of microorganisms (e.g. competition for nutrients, remineralisation).
116 In this setup, either the aboveground or the belowground tissue received a nutrient treatment,
117 and were incubated for approximately 3 hours under continuous bubbling to prevent local
118 depletion and the built-up of concentration gradients. For logistic reasons the labelling of the
119 aboveground and belowground tissues were performed on consecutive days.

120 At the start of the experiment, the plants received a combination of one heavy isotope
121 labelled nitrogen substrate (¹⁵N 99% pure ¹⁵N, Cambridge Isotope Laboratories) at a
122 concentration of 1 μM, and one background substrate in the light isotope form (¹⁴N) at 1 μM
123 (both added with a pipet). The substrates were ammonium, nitrate, urea, and glycine
124 (Cambridge Isotope Laboratories). Urea and glycine (amino acid) also contained isotope
125 labelled carbon (¹³C, universally labelled 99%, Cambridge Isotope Laboratories) to track
126 potential carbon uptake. The different substrate combinations are given in table 1. These
127 nutrient concentrations are similar to those found in the water column of Cadiz bay (Van
128 Engeland et al., 2013) and for ammonium and nitrate in the range commonly found in the

129 water column of seagrass ecosystems (0 – 8 μM and 0 – 3.2 μM , respectively; Touchette and
 130 Burkholder, 2000). Control treatments were performed where only the substrate was added
 131 without background. Each nutrient treatment was replicated 5 times. Since the cups were
 132 relatively small, there may have been substrate depletion during the experiment. However,
 133 because the objective of this study is to determine the uptake capacity of nitrogen from a pool
 134 of nitrogen, rather than quantifying their uptake kinetics, this was not a problem.

135 After the incubation, plants were rinsed and cleaned with artificial seawater containing
 136 only the nutrient background, and dabbed with paper tissues. Aboveground and belowground
 137 parts were separated and immediately stored in glass vials at $-20\text{ }^{\circ}\text{C}$. Later they were freeze-
 138 dried for 48 hours. Dried samples were weighed and ground to a homogenous powder for
 139 further analysis.

140

141 2.2. Sample and data treatment

142 Dried samples were analysed for their nitrogen and carbon content, and nitrogen and
 143 carbon isotope composition using Thermo EA 1112 elemental analyzer coupled to a Thermo
 144 Delta V Advantage isotope ratio mass spectrometer with a ConFlo II interface (EA-IRMS).
 145 Specific ^{15}N uptake rates ($V_{15\text{N}}$; expressed $\mu\text{mol N g DW}^{-1} \text{ h}^{-1}$; DW = dry weight) were
 146 calculated as :

$$147 \quad V_{15\text{N}} = \left((AF_{sa} - AF_{bg}) * F_N \right) / (M_N * time),$$

148 where AF_{sa} and AF_{bg} are the ^{15}N fraction in the sample and the natural isotope fraction in the
 149 plant tissue, respectively. F_N is the nitrogen fraction in the sample's dryweight (gN gDW^{-1}),
 150 M_N is the molar mass of nitrogen (14 gmol^{-1}) and time is the length of the incubation period

151 (hours). These specific ^{15}N uptake rates were converted to specific N uptake rates using the
 152 ^{15}N fraction in the substrate ($F_{\text{substr}}^{15\text{N}}$):

$$V_N = V_{15\text{N}} / F_{\text{substr}}^{15}$$

153 This fraction was 1 for all substrate-background combinations, except those where the labeled
 154 substrate (^{15}N) and the non-labeled background (^{14}N) were the same ($F_{\text{substr}}^{15\text{N}} = 1/2$). Total N
 155 uptake (ρ_N ; $\mu\text{mol N}$) after incubation was calculated for individual treatment as:

$$\rho_N = V_N * \text{time} * DW_{\text{sa}}$$

156 where DW_{sa} is the sample's dryweight (g).

157 Depletion was calculated as the percentage of available substrate (N_{added}) that was taken up:

$$\text{Depletion} = (\rho_N / N_{\text{added}}) \times 100$$

158 Note that in the treatment where the substrate (^{15}N) was also added as background (^{14}N), this
 159 background was also taken into account. Similar formulas were used for the carbon uptake
 160 rates from the organic molecules.

161

162 2.3. Statistical analysis

163 Treatment and background effects were tested through variance analysis (ANOVA). When
 164 needed, asymmetry in distribution per group was compensated by log-transformations.

165 Regression analysis (ordinary least square) was performed to compare carbon and nitrogen
 166 uptake from the organic substrates.

167

168 3. Results

169

170 3.1. Seagrass DIN and DON uptake independency on nutrient background

171 Our results showed different uptake rates for different substrates (Fig. 2) with similar
172 patterns for above- and belowground tissues. Variance analyses per substrate, indicated
173 systematically higher uptake rates in aboveground than in belowground tissues (always $p <$
174 0.01), except for the labelled glycine addition. In the leaf-mediated substrate uptake, only
175 substrate type exhibited a significant effect (ANOVA; $F_{3, 80} = 75$, $p < 0.001$), but the
176 background type did not (ANOVA; $F_{4, 80} = 75$, $p > 0.05$). Tukey HSD tests indicated
177 differences between all labelled substrates ($p < 0.01$), except between nitrate and urea. Root-
178 mediated uptake rates were significantly affected by both the substrate type and the
179 background type, but the latter effect was very weak (ANOVA; $F_{3, 80} = 28$, $p < 0.001$;
180 ANOVA; $F_{4, 80} = 2$, $p < 0.05$; respectively). Ammonium uptake rates were higher than for the
181 other substrates (Tukey, always $p < 0.5$), and a significant difference existed between nitrate
182 and urea uptake rates (Tukey, $p < 0.05$). From these analyses it is clear that substrate uptake
183 showed no clear dependence on the presence and type of a background substrate (Fig. 2).

184 Considering the small volumes and low (but realistic) concentrations used, it is imperative
185 that we investigated the potential for depletion. Substrate depletion was significantly affected
186 by the tissue type and labelled substrate, but not by the background substrate (Tab. 2). The
187 amounts of ammonium taken up represent a considerable fraction of the added amounts (Fig.
188 3), indicating a strong potential for depletion-related underestimation of the corresponding
189 uptake rates. This is supported by the similar degrees of depletion in ammonium, with and
190 without ammonium background (i.e. doubling of the ammonium concentration “visible” to
191 the plant). The fraction taken up for the other substrates were far less (Fig. 3). Hence,
192 depletion-related under-estimation of the uptake rates are not likely for these substrates.

193

194 3.2. Carbon versus nitrogen uptake from organic N-sources

195 For the dual-labelled glycine and urea, a strong linear relationship existed between carbon
196 and nitrogen uptake (Linear regression per substrate; only the slope coefficients were
197 significant $p < 0.05$; Fig. 4). If the organic molecules would be taken up intact, one could
198 expect that the total uptake of carbon and nitrogen by the plants occurred in proportions
199 dictated by the C:N ratio or the substrates. This hypothetical uptake is in figure 4 indicated by
200 the lines. The observed C:N ratios of this uptake were clearly lower than expected from the
201 C:N ratios in the substrates (lines in Fig. 4), indicating preferential nitrogen uptake over
202 carbon uptake. However, C:N ratio of uptake was stronger for the more carbon-rich glycine
203 than for urea (R^2 values of 99% and 88%, from the respective regression analyses).

204

205 4. Discussion

206

207 In nature, nitrogen is available to marine macrophytes as a mixture of inorganic and
208 organic molecules. In coastal and estuarine areas the dissolved organic nitrogen constitutes
209 13-18% of the nitrogen pool (except dissolved N_2 ; Berman and Bronk, 2003) of which
210 substantial parts can be non-refractory (Bronk et al., 2007). It is currently established that
211 dissolved organic matter also serves as an effective source of nitrogen to marine macrophytes
212 (Van Engeland et al., 2011; Vonk et al., 2008). Our study supports these findings and
213 demonstrates organic nitrogen uptake by *Zostera noltii* under conditions of a strongly reduced
214 microbial community (epiphyte removal and artificial seawater). Our ammonium uptake rates
215 are slightly higher than those reported by Morris et al. (2008) for *Zostera noltii* shoots from
216 the same source population under low current conditions (data not shown). Our uptake rates

217 for the aboveground tissue are also in the same range as those reported by Van Engeland et al.
218 (2011) for the same substrates, but somewhat higher for the belowground tissue. Variability
219 between substrates also resembles those reported by Van Engeland et al. (2013) for the same
220 seagrass species and by Vonk et al. (2008) for tropical species. These similarities with
221 literature show that our data are of good quality. In addition, our study takes research in
222 organic nitrogen uptake by marine macrophytes one step further by considering the role of
223 organic substrates in nutrient-nutrient interactions in nitrogen uptake by a temperate seagrass
224 species.

225

226 4.1. Seagrass DIN and DON uptake independency on nutrient background

227 With regard to the inorganic nitrogen substrates, our results agree with earlier studies that
228 show higher uptake rates for ammonium than for nitrate (Alexandre et al., 2010; Touchette
229 and Burkholder, 2000; Van Engeland et al., 2011) and organic N-sources (Vonk et al., 2008;
230 Van Engeland et al., 2011; 2013). This effectively results in a ‘constitutive preference’ for
231 ammonium over the other substrates. If all substrates are supplied in the same concentrations
232 (like in this study), ammonium is taken up in higher amounts than the others. As the presence
233 of a background substrate did not affect the uptake rates of the labelled substrate in any of the
234 treatments (Fig. 2; comparison within panels), no down- or up-regulation was observed that
235 favored one nitrogen source over the others (i.e. an induced preference). This contrasts with
236 the findings of (Alexandre et al., 2010), who showed an inhibition effect of ammonium on
237 nitrate uptake, and stimulated ammonium uptake under higher nitrate concentrations.

238 Considering the low (but close to ambient) nitrogen concentrations applied in our study, we
239 may have not reached certain threshold concentrations to induce inhibition or stimulation of
240 substrate uptake. It is likely that, at these low concentrations *Zostera noltii* is “programmed”

241 to take up whatever nutrients it can find. Clearly, our experiment was conducted in nutritional
242 conditions characteristic of the quasi-linear part of the Michaelis-Menten curve for uptake of
243 nitrogen sources.

244 Under nutritionally poor conditions, other seagrass species also seem to take up nutrients
245 from whatever source is available. *Posidonia oceanica* in Revellata Bay (Corsica) seems to
246 take up inorganic nitrogen according to the available water column concentrations (Lepoint et
247 al., 2002). However, although the same applies to *Phyllospadix iwatensis*, this species still
248 exhibits a preference for ammonium as revealed by its uptake affinities (Hasegawa et al.,
249 2005). Inorganic nitrogen concentrations in the latter study varied so much that they simply
250 drowned out the difference in affinities. This shows the value of kinetic studies in unravelling
251 nutrient preference mechanisms. To summarize, *Zostera noltii* exhibits a constitutive and
252 induced preference for ammonium under higher nutritional conditions (Alexandre et al.,
253 2010), but only a constitutive preference at lower nutritional conditions (this study). Apart
254 from that, the eventual contribution of different sources in the overall nitrogen acquisition
255 may further depend on the relative concentrations of the different sources.

256 Due to the strong depletion in the labelled ammonium additions, the true ammonium
257 uptake rates may have been underestimated, although they were roughly similar to those
258 reported by Morris et al. (2008) for *Zostera noltii* from the same source population under low
259 current conditions. Underestimating uptake rates due to depletion would imply that a potential
260 down- or up-regulation of ammonium uptake could remain undetected. However, since the
261 up-regulation, demonstrated by Alexandre et al., (2010) was more pronounced at substrate
262 concentrations of 25 μM than at 5 μM , we consider such an effect at concentrations of 1 μM
263 would not likely to occur.

264 The organic nitrogen substances in our study did not have any effect on the uptake of any
265 nitrogen source, nor were their uptake rates influenced by the presence of another substrate.
266 Considering that the pattern in uptake rates for the aboveground tissue was similar to that
267 found by Van Engeland et al. (2011), it probably reflects a ‘constitutive preferential’ order
268 from ammonium as most preferred, to urea, nitrate and glycine as least preferred (note
269 however that the differences with glycine were not statistically significant in our study).
270 Whether an inducible preference mechanism exists in *Zostera noltii* involving organic
271 nitrogen substances remains an open question. Note however, that amino acids concentrations
272 of 1 μM are really at (or beyond) the upper limit of the observed range for seagrass
273 ecosystems (e.g. Hansen et al., 2000). This implies that the chance of not detecting an existing
274 role for amino acids in the down-regulation of the uptake of some nitrogen source is much
275 smaller than the chance that such a role actually exists.

276

277 4.2. Carbon versus nitrogen uptake from organic N-sources

278 A strong relationship existed between nitrogen and carbon uptake from organic molecules
279 in *Zostera noltii*. However, the uptake C:N ratios were lower than expected from the
280 respective molecule C:N ratios, indicating occurrence of carbon loss. Several reasons can be
281 put forward for this partial decoupling: 1) remineralisation outside the plant with subsequent
282 uptake of the products (NH_4^+ and dissolved inorganic carbon), 2) uptake of the entire
283 molecule with subsequent loss of a part of the carbon, or 3) remineralisation outside plant by
284 epiphytic bacteria (in the boundary layer) with transport of the products influenced by
285 boundary layer effects (i.e. coupling through limited physical transport after external
286 remineralisation).

287 The fact that the uptake C:N ratio is stronger for the more carbon-rich glycine (C:N=2)
288 than for urea (C:N=0.5) seems to support remineralisation (see Harrison et al., 2007; von
289 Felten et al., 2008), considering that coupled uptake implies a specific uptake mechanism
290 which is most likely not directly dependent on the molecule's C:N ratio. However,
291 explanation 1 is problematic in the sense that the produced DIC would still enter a large
292 background pool (micromolar versus millimolar concentrations). In contrast, explanation 3
293 does not suffer from this problem as remineralisation within the boundary layer would cause
294 less dilution losses of labelled DIC in the unlabelled DIC background pool. Present study
295 does however not provide an affirmative answer to the mechanisms causing constant uptake
296 C:N ratios that deviate from theoretical expectations.

297

298 4.3. Summarising conclusion

299 Overall, this study suggests that at low ambient concentrations, *Zostera noltii* exhibits a
300 'constitutive preference' for ammonium over other (in)organic nitrogen sources, in-line with
301 findings from kinetic studies. However, contrary to the demonstrated ammonium-nitrate
302 interaction in nitrogen uptake by *Zostera noltii* at higher nitrogen concentrations, no similar
303 regulation seems to exist in lower ambient concentrations, indicating that in low-nutrient
304 environments *Zostera noltii* takes whatever (in)organic nutrients are available.

305

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312 **LIST OF REFERENCES**

- 313 Alexandre, A., Silva, J., Santos, R., 2010. Inorganic nitrogen uptake and related enzymatic
314 activity in the seagrass *Zostera noltii*. *Marine Ecology-an Evolutionary Perspective*
315 31(4), 539-545.
- 316 Alexandre, A., Silva, J., Bouma, T.J., Santos, R., 2011. Inorganic nitrogen uptake kinetics and
317 whole-plant nitrogen budget in the seagrass *Zostera noltii*. *J. Exp.Mar. Biol. Ecol.*
318 401(1-2), 7-12.
- 319 Barron, C., Middelburg, J.J., Duarte, C.M., 2006. Phytoplankton trapped within seagrass
320 (*Posidonia oceanica*) sediments are a nitrogen source : An in situ isotope labelling
321 experiment. *Limnol. Oceanogr.* 51, 1648-1653.
- 322 Berman, T., Bronk, D.A., 2003. Dissolved organic nitrogen: a dynamic participant in aquatic
323 ecosystems. *Aquat. Microb. Ecol.* 31, 279-305.
- 324 Bronk, D.A., See, J.H., Bradley, P., Killberg, L.. 2007. DON as a source of bioavailable
325 nitrogen for phytoplankton. *Biogeosciences* 4, 283-296.
- 326 Brun, F. G., Vergara, J. J., Navarro, G., Hernández, I., & Pérez-Lloréns, J. L., 2003. Effect of
327 shading by *Ulva rigida* canopies on growth and carbon balance of the seagrass *Zostera*
328 *noltii*. *Mar. Ecol. Prog. Ser.* 265, 85-96.
- 329 Cornelisen, C.D., Thomas, F.I.M., 2004. Ammonium and nitrate uptake by leaves of the
330 seagrass *Thalassia testudinum*: impact of hydrodynamic regime and epiphyte cover on
331 uptake rates. *Journal of Mar.Syst.* 49(1-4), 177-194.
- 332 De Brouwer, J.F.C., Wolfstein, K., Ruddy, G.K., Jones, T.E.R., Stal, L.J., 2005. Biogenic
333 stabilization of intertidal sediments: the importance of extracellular polymeric
334 substances produced by benthic diatoms. *Microb. Ecol* 49(4), 501-512.

- 335 Dolch T., Buschbaum C., Reise K., 2013. Persisting intertidal seagrass beds in the northern
336 Wadden Sea since the 1930s. *J. of Sea Research* 82: 134-141 2013
- 337 Duarte, C.M., 1990. Seagrass nutrient content. *Mar. Ecol. Prog. Ser.* 67(2), 201-207.
- 338 Evrard, V., Kiswara, W., Bouma, T.J, Middelburg, J.J. 2005. Nutrient dynamics of seagrass
339 systems : ^{15}N evidence for the importance of particulate organic matter and root
340 systems. *Mar. Ecol. Prog. Ser.* 295: 49-55.
- 341 Honkoop, P.J.C., Berghuis, E.M., Holthuijsen, S., Lavaleye, M.S.S., Piersma, T., 2008.
342 Molluscan assemblages of seagrass-covered and bare intertidal flats on the Banc
343 d'Arguin, Mauritania, in relation to characteristics of sediment and organic
344 matter. *J. Sea Res.* 60:255-263
- 345 Hansen, J.W., Udy, J.W., Perry, C.J., Dennison, W.C., Lomstein, B.A., 2000. Effect of the
346 seagrass *Zostera capricorni* on sediment microbial processes. *Mar. Ecol. Prog. Ser.*
347 199, 83–96
- 348 Harrison, K.A., Bol, R., Bardgett, R.D., 2007. Preferences for different nitrogen forms by
349 coexisting plant species and soil microbes. *Ecology* 88(4), 989-999.
- 350 Hasegawa, N., Iizumi, H., Mukai, H., 2005. Nitrogen dynamics of the surfgrass *Phyllospadix*
351 *iwatensis*. *Mar. Ecol. Prog. Ser.* 293, 59-68.
- 352 Lepoint, G., Millet, S., Dauby, P., Gobert, S., Bouquegneau, J.M., 2002. An annual nitrogen
353 budget of the seagrass *Posidonia oceanica* as determined by in situ uptake
354 experiments. *Mar. Ecol. Prog. Ser.* 237,87–96.
- 355 Morris, E.P., Peralta, G., Brun, F.G., van Duren, L., Bouma, T.J., Pérez-Lloréns., J.L., 2008.
356 Interactions between hydrodynamics and seagrass canopy structure: spatially explicit
357 effects on ammonium uptake rates. *Limnol. Oceanog.* 53: 1531-1539.

- 358 Romero, J., Lee, K.S., Pérez, M., Mateo, M.A., Alcoverro, T., 2006. Nutrient dynamics in
359 seagrass ecosystems. In: Larkum, A.W.D., Orth, R.J., Duarte, C.M. (Eds.), Seagrasses:
360 biology, ecology and conservation. Springer, the Netherlands, pp. 227-254.
- 361 Short, F.T., McRoy, C.P., 1984. Nitrogen uptake by leaves and roots of the seagrass *Zostera*
362 *marina* L. Bot. Mar. 27(12), 547-555.
- 363 Stapel, J., Aarts, T.L., van Duynhoven, B.H.M., de Groot, J.D., van den Hoogen, P.H.W.,
364 Hemminga, M.A., 1996. Nutrient uptake by leaves and roots of the seagrass *Thalassia*
365 *hemprichii* in the Spermonde Archipelago, Indonesia. Mar. Ecol. Prog. Ser. 134(1-3),
366 195-206.
- 367 Stapel, J., Hemminga, M.A., 1997. Nutrient resorption from seagrass leaves. Mar. Biol.
368 128(2), 197-206.
- 369 Stapel, J., Hemminga, M.A., Bogert, C.G., Maas, Y.E.M., 2001. Nitrogen (N-15) retention in
370 small *Thalassia hemprichii* seagrass plots in an offshore meadow in South Sulawesi,
371 Indonesia. Limnol. Oceanogr. 46(1), 24-37.
- 372 Touchette, B.W., Burkholder, J.M., 2000. Review of nitrogen and phosphorus metabolism in
373 seagrasses. J. Exp. Mar. Biol. Ecol. 250(1-2), 133-167.
- 374 Turpin, D.A., 1991. Effects of inorganic N availability on algal photosynthesis and carbon
375 metabolism. J. Phycol. 27, 14-20.
- 376 Van Engeland, T., Bouma, T.J., Morris, E.P., Brun, F.G., Peralta, G., Lara, M., Hendriks, I.E.,
377 Soetaert, K., Middelburg, J.J., 2011. Potential uptake of dissolved organic matter by
378 seagrasses and macroalgae. Mar. Ecol. Prog. Ser. 427, 71-81.
- 379 Van Engeland, T., Bouma, T.J., Morris, E.P., Brun, F.G., Peralta, G., Lara, M., Hendriks, I.E.,
380 van Rijswijk, P., Veuger, B., Soetaert, K., Middelburg, J.J., 2013. Dissolved organic
381 matter uptake in a temperate seagrass ecosystem. Mar. Ecol. Prog. Ser. 478, 87-100.

382 von Felten, S., Buchmann, N., Scherer-Lorenzen, M., 2008. Preferences for different nitrogen
383 forms by coexisting plant species and soil microbes: Comment. *Ecology* 89(3), 878-
384 879.

385 Vonk, J.A., Middelburg, J.J., Stapel, J., Bouma, T.J., 2008. Dissolved organic nitrogen uptake
386 by seagrasses. *Limnol. Oceanogr.* 53(2), 542-548.

387

388 Figure captions

389 Figure 1. Experimental setup with two cups containing the aboveground and belowground
390 parts of intact *Zostera noltii* plants. Bubbling was used to stir the water in order to prevent
391 concentration gradients from developing during the incubation.

392 Figure 2. Boxplots of the ^{15}N specific uptake rates for the different labelled substrates
393 (grouped in separate graphs) in different backgrounds of nitrogen containing substances
394 (horizontal axis) in leaves (upper panels) and roots (lower panels). Both the labelled (^{15}N) and
395 background (^{14}N) were added in final concentration of $1\mu\text{M}$. The small lines, boxes, whiskers
396 and dots indicate median, interquartile range (IQR), $1.5\times\text{IQR}$ and outliers (deviation from
397 median larger than $1.5 \times \text{IQR}$). The thick horizontal lines and grey zones indicate the mean
398 and (\pm) standard deviation of the uptake rate for the ^{15}N substrate in a background of the same
399 substances in ^{14}N form. NB = No Background (indicating no ^{14}N -nutrient added).

400 Figure 3. Total N uptake as a percentage of the added substrate N in leaves (upper panels) and
401 roots (lower panels). The small lines, boxes, whiskers and dots indicate median, interquartile
402 range (IQR), $1.5\times\text{IQR}$ and outliers (deviation from median larger than $1.5\times\text{IQR}$). Background
403 N is not taken into account unless the background was the same n species as the substrate.

404 Figure 4. Total uptake of substrate C versus substrate N for the two organic substrates. The
405 theoretically expected relationship between C and N uptake, derived from the substrate C:N
406 ratios are for urea and glycine shown by the dotted and dashed line, respectively. These
407 calculations assumed absence of fractionation. Root and leaf-mediated uptake are for both
408 substrates indicated with different symbols (*cf.* legend in figure). Equations carbon uptake (C)
409 as function of nitrogen uptake (N) are given for the theoretical lines (normal font) and the

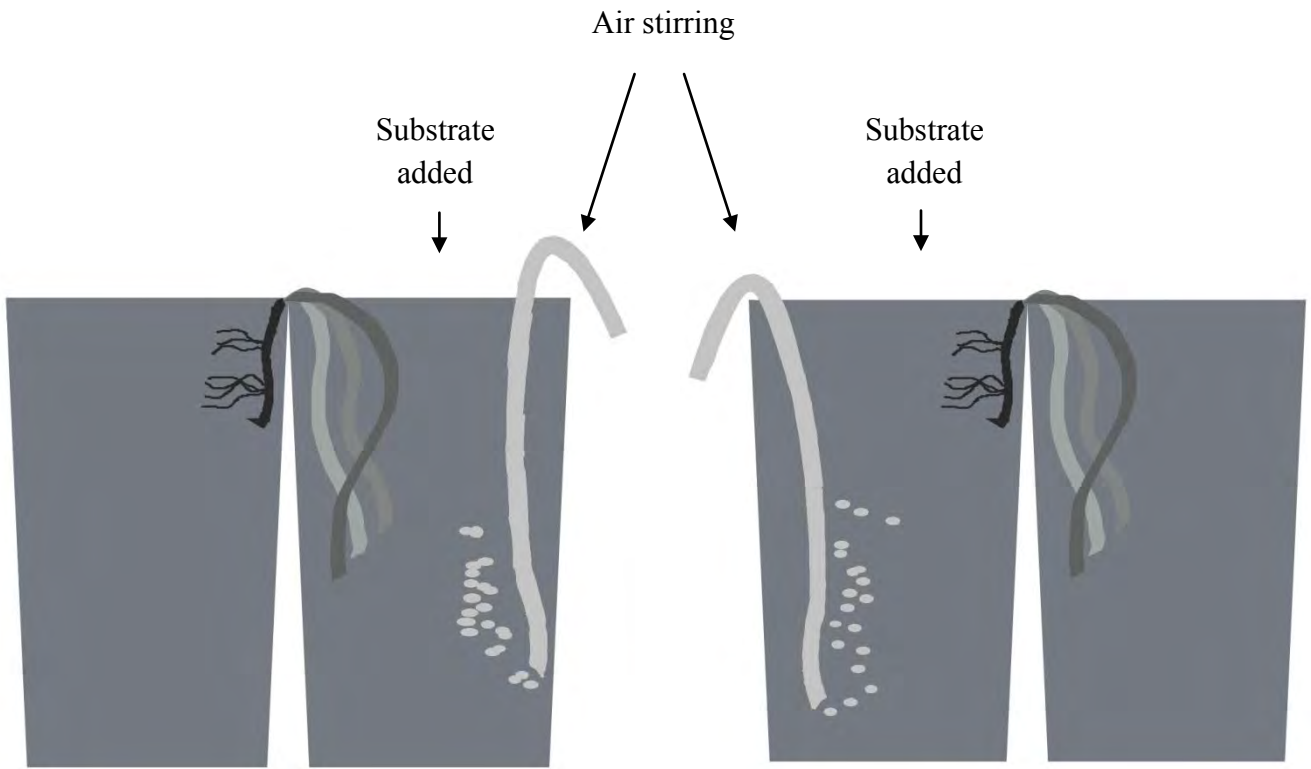
410 empirical data (bold font). In the latter case, only the slope coefficients were significant in the
411 linear regression (*cf.* text).

Table 1. Experimental design showing concentrations of non-labelled-background and labelled-substrate applied to both above and belowground tissues. Values should be interpreted as “background substrate concentration – labelled substrate concentration” in micromolar.

| Background concentration (non-labelled) (μM) | Substrate concentration (isotope labelled) (μM) | | | |
|---|--|-----------------|-------|---------|
| | NH_4^+ | NO_3^- | Urea | Glycine |
| None (control) | 0 - 1 | 0 - 1 | 0 - 1 | 0 - 1 |
| NH_4^+ | 1 - 1 | 1 - 1 | 1 - 1 | 1 - 1 |
| NO_3^- | 1 - 1 | 1 - 1 | 1 - 1 | 1 - 1 |
| Urea | 1 - 1 | 1 - 1 | 1 - 1 | 1 - 1 |
| Glycine | 1 - 1 | 1 - 1 | 1 - 1 | 1 - 1 |

Table 2. Analysis of variance (ANOVA) table for the substrate depletion, indicating the degrees of freedom of the F statistic (df), the value of the F statistic, and the corresponding probability value (p).

| Factor | df | F | p |
|----------------|---------|------|---------|
| Intercept | 1, 160 | 1633 | < 0.001 |
| Label (L) | 3, 160 | 70 | < 0.001 |
| Tissue (T) | 1, 160 | 62 | < 0.001 |
| Background (B) | 4, 160 | 0.5 | 0.7 |
| L x T | 3, 160 | 5.8 | < 0.001 |
| L x B | 12, 160 | 1.4 | 0.2 |
| T x B | 4, 160 | 1 | 0.4 |
| L x T x B | 12, 160 | 0.5 | 0.9 |



Aboveground incubation

Belowground incubation

