Ocean warming increases the nitrogen demand and the uptake of organic nitrogen of the globally distributed seagrass Zostera marina

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25   Running head: Shifts in *Zostera marina* N uptake with temperature

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29

30   Abstract

31

32   1. The impact of global warming on the metabolic state of a species may be examined

33   by either measuring physiological rates across a latitudinal gradient or by assessing

34   short-term responses under experimentally controlled temperature regimes. The

35   combination of the two approaches is seldom used but it provides valuable information

36   on an organism's responses to temperature at broader temporal and spatial scales while

37   allowing the isolation of temperature effects from other environmental variables.

38   2. Here we used both approaches to assess the warming effects on the total acquisition

39   of dissolved inorganic nitrogen (DIN; nitrate, ammonium) and organic N (DON; amino

40   acids, peptides) by the globally widespread seagrass *Zostera marina*. DIN and DON

41   uptake rates were measured in plants from three sites covering the species latitudinal

42   distribution in Europe (Iceland, United Kingdom and Portugal). The responses of DIN

43   and DON uptake rates of plants from the middle latitude (UK) to a latitudinal range of

44   temperatures (8, 12 and 17 ºC) were also measured. We further examined the microbial

45   uptake of DON along the latitudinal distribution and whether temperature is the main

46   driver of that uptake.

47   3. Our results showed that warming greatly increased the total N uptake by *Z. marina*

48   and also the relative contribution of DON to total N acquisition. The microbial uptake

49   of DON increased towards warmer latitudes, and temperature was the main driver of

50   these observations.

51   4. Ocean warming will increase the nitrogen demand of *Z. marina* and this demand may

52   be met by an increasing uptake of organic nitrogen forms. This indicates that *Z. marina*,

53   and probably other seagrass species, can be winners under global change as nitrogen

54 uptake capacity will not limit growth driven by increased photosynthetic assimilation of

55 CO_2.

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60 Introduction

61

62 Global warming (IPCC, 2018) is affecting the metabolic rates of organisms and the

63 distribution of species, shaping the structure and functioning of marine ecosystems and

64 their trajectories (Walther et al., 2002; Poloczanska et al., 2013). The potential impacts

65 of rising temperatures on organisms and ecosystems are often investigated by short-term

66 experimental temperature manipulation (see Shaver et al., 2000, Rustad et al., 2001 and

67 Aronson & McNulty, 2009 for a review of methods). Controlled temperature

68 experimentation allows separation of temperature effects from other confounding

69 environmental factors that covary with temperature in natural conditions. However, this

70 approach may fail to accurately predict both the magnitude and the direction of species

71 responses due to the transient nature (short-duration) of the experiments and the step

72 increases in experimental temperatures, which are often unrealistic (Rustad, 2008).

73 Testing a species response across a wide latitudinal range is an alternative to address the

74 effects of temperature on organisms because sites along the gradients integrate the

75 geographical climate variation over large temporal scales, thus providing empirical-

76 based predictions of consequences of temperature changes at broader temporal and

77 spatial scales (Fukami & Wardle, 2005; De Frenne, 2013). In the present study, we

78 combined latitudinal gradient analysis with laboratory responses to temperature

79   manipulation to address how climate warming may affect seagrasses' nutrient

80   acquisition, a key physiological process driving production.

81   Seagrasses represent one of the most heterogeneous landscape structures of shallow-

82   water marine ecosystems in the world, however, it is predicted that they will be highly

83   impacted by climate change (Bostrom et al., 2006; Chefaoui et al., 2018). Warming may

84   affect seagrasses directly by altering nutrient uptake rates (e.g. nitrogen (N) and

85   phosphorus (P)), or indirectly via a potential acceleration of N mineralisation that may

86   increase seagrass productivity by increasing inorganic nutrient availability in nutrient-

87   limited environments. This process has been well described in terrestrial ecosystems

88   (Rustad et al., 2001; Bai et al., 2013) but is poorly understood in seagrass communities

89   (Duarte et al., 2018).

90   Seagrasses can take up both dissolved inorganic nitrogen (DIN; nitrate, ammonium) and

91   dissolved organic nitrogen (DON; amino acids, peptides), with a general preference for

92   the uptake of ammonium (NH_4^+) (Alexandre et al., 2011 and references therein), but it

93   is not known how this preference may shift with temperature. In fact, there is little

94   information on whether, or how, seagrass nutrient uptake will respond to global

95   warming (Moore & Short, 2006). A recent study showed that nitrate (NO_3^-) uptake by

96   *Zostera marina* increased by 50% under warming, however, this was undertaken under

97   an unrealistic warming scenario (10 to 18/25 ℃) and exposure to a high nitrate

98   concentration (100 µM; Kaldy, 2014). In terrestrial habitats, it is expected that increased

99   soil temperature will increase plant uptake of N and P in species from warm habitats

100 more than in those from colder environments (BassiriRad, 2000), but warming is also

101 expected to increase plant N and P uptake in arctic species (Jonasson et al., 1999). The

102 few available data also indicate that increased soil temperature elicits a differential

103 effect on the uptake of ammonium versus nitrate, i.e. the ratio of ammonium to nitrate

104   uptake will consistently decrease with increasing temperature in various plant species

105   (BassiriRad, 2000). Potential future changes in the availability of the different N forms

106   in the sediment and water column alongside changes in seagrass N preference under

107   global warming might shift seagrasses' competitive interactions with other species.

108   N availability is one of the major factors limiting primary productivity of seagrasses,

109   particularly in oligotrophic environments where nutrient concentrations are very low

110   (e.g. Agawin et al., 1996; Alcoverro et al., 1997). Seagrasses are well adapted to these

111   conditions, being highly efficient at taking up ephemeral pulses of dissolved inorganic

112   nitrogen (DIN) (Alexandre et al., 2017). On the other hand, the uptake of dissolved

113   organic nitrogen (DON) by seagrasses has been largely overlooked, even though this

114   represents a large component of the total dissolved N pool in coastal waters and is a

115   precursor to DIN formation (Sharp, 1983, 2002; Bronk et al., 2007). In fact, seagrasses

116   can take up N from both simple (amino acids and urea) and complex (peptides) organic

117   substrates at ecologically relevant rates (Vonk et al., 2008; Van Engeland et al., 2011;

118   Alexandre et al., 2015), suggesting that DON may be an important N source for these

119   plants.

120   *Z. marina* is the most abundant seagrass species in the northern hemisphere spreading

121   over a wide latitudinal range, from warm subtropical to arctic regions (Cabello-Pasini et

122   al., 2003; Green & Short 2003). The use of DON by this species was studied at the

123   southern limit of its geographical distribution range, in Ria Formosa lagoon, south

124   Portugal (Alexandre et al., 2015). In this coastal lagoon, DON forms a significant pool

125   of bioavailable N in the water column and in sediments (~ 60 % of total soluble N) and

126   the uptake of DON (as amino acids and peptides) by *Z. marina* represented a significant

127   fraction (~ 30 %) of the total N taken up by the species. However, it is not known how

128   widespread is the use of DON by *Z. marina* and how it may vary along the species'

129 latitudinal range. Latitude may be relevant as the availability of DON is expected to

130 increase at higher and colder latitudes as DIN regeneration from the mineralisation of

131 organic matter decreases due to decreasing rates of microbial N mineralisation (Chapin

132 et al., 1993; Mozdzer et al., 2014). This trend has been reported in coastal saltmarshes

133 colonized by *Spartina alterniflora* along a latitudinal distribution range (30 - 44 ºN)

134 (Mozdzer et al., 2014). In boreal forests, the low mineralisation rates result in one order

135 of magnitude higher concentrations of DON than those of DIN (Näsholm et al., 2009).

136 Further, in arctic ecosystems the ratios of primary production to N mineralisation are

137 high and consequently arctic vascular plants use amino acid and peptidic N to avoid N

138 limitation derived from low DIN regeneration (Hill et al., 2011). It is also energetically

139 more favourable to take up DON in comparison to DIN as the compounds taken up can

140 be directly used in cell metabolism.

141 This study assesses how global warming may affect the N uptake rates by the globally

142 widespread seagrass *Z. marina*. Specically we tested the following questions: 1) What is

143 the availability of DON relative to DIN in *Z. marina* meadows along a latitudinal

144 gradient?, 2) What is the latitudinal variation of the uptake rates of DIN and DON by *Z.*

145 *marina* plant parts (leaves and roots)?, 3) What is the latitudinal variation of the

146 contribution of DIN and DON to the total N budget of *Z. marina*?, 4) What is the

147 latitudinal variation of the microbial uptake of DON and 5) Is temperature the main

148 driver of the latitudinal variations? Laboratory experiments will allow to assess if the

149 latitudinal observations are mainly driven by temperature and consequently what will be

150 the response of *Z. marina* meadows N cycling to global warming.

151

152 Methods

153

154   Sampling sites

155   The latitudinal variation of *Zostera marina* uptake rates were assessed in the summer of

156   2014, when seagrass productivity is highest, in plants collected from meadows located

157   along the species latitudinal distribution range in Europe: a high-latitude meadow

158   located in Alftafjordur, Iceland (64°59'N; 22°36'W), a mid-latitude meadow located in

159   Porthdinllaen, United Kingdom (52°56'N, 4°33'W), and a lower-latitude meadow

160   located in Ria Formosa lagoon, Portugal (36°58'N, 8°02'W) (see Fig. S1 in Supporting

161   Information). Iceland (high-latitude) and Portugal (lower-latitude) represent the

162   northern and southernmost limits of the species distribution, respectively, and United

163   Kingdom (mid-latitude) an intermediate point. The seagrass uptake rates along the

164   latitudinal gradient was assessed by measuring uptake rates of each meadow at the local

165   mean annual temperature. These were combined with temperature manipulation

166   experiments with plants collected at the intermediate site within the distribution (UK),

167   to isolate the temperature effects from other environmental variables. Plant biomass and

168   N availability at each site were used to calculate the contribution of DIN and DON to

169   the total N budget of *Z. marina*.

170   Samples (n = 5) of seawater (20 mL each) and sediment porewater were collected

171   during low tide (approximate water height of 20 cm) at each meadow to determine the

172   concentration of inorganic (ammonium and nitrate) and organic nitrogen (free amino

173   acids and peptides), as well as total soluble nitrogen (TN). Seawater samples were

174   filtered (Whatman cellulose acetate filters, 0.45 μm pore size) and stored at -20 ℃ until

175   analysis. A total of four sediment cores (3 cm diameter, 5 cm depth) were pooled for

176   each porewater sample (~20 mL) to reduce the effect of nutrient heterogeneity. The four

177   pooled cores were randomly collected within the meadow at an approximate distance of

178   10 cm, and the different groups of pooled cores were collected at a distance of at least 2

179 m. The sediment samples were centrifuged (2300 g, 15 min at 4 ℃) and the

180 supernatants were filtered (Whatman cellulose acetate filters, 0.45 µm pore size) and

181 frozen until analysis.

182 Shoot density (no. shoots m^{-2}) and areal biomass (g dry weight m^{-2}) were also

183 determined for each site by counting the number of shoots and weighing the dried plants

184 (48 h at 60 ℃) collected within a sampling quadrat (20 cm x 20 cm, n = 3). The

185 quadrats were haphazardly tossed inside the meadow, ensuring distances higher than 20

186 cm between different tossings.

187

188 <u>Plant collection and acclimation</u>

189 *Z. marina* plants were collected during low tide from the meadows and cleaned of

190 adhering sediment, avoiding damage of the root hairs, and epiphytes removed from the

191 leaves by gentle hand scraping. The plants were transported in seawater collected at the

192 sampling site to the laboratory, where they were acclimated for 3 days at local summer

193 conditions of light and mean annual temperatures (Marine and Freshwater Research

194 Institute; climate-data.org; Global Sea Temperature database). In Iceland, plants were

195 acclimated at 8 ℃ with a 20:4 h light:dark cycle; in the UK, plants were acclimated at

196 12 ℃ with a 18:6 h light:dark cycle and in Portugal at 17 ℃ with a 16:8 h light:dark

197 cycle.

198

199 <u>*Z. marina* nitrogen uptake experiments</u>

200 Uptake rates of inorganic (ammonium and nitrate) and organic (alanine and trialanine)

201 N were determined by incubating whole plants fully immersed in 300 mL of N-free

202 artificial seawater (0.2 µm filtered water, salinity of 35, pH 8.2) containing either

203 $^{15}NH_4Cl$, $^{15}KNO_3$ or $^{13}C_3H_7{}^{15}NO_2$ (L-alanine) at concentrations of 5, 25, 50 and 100 µM

204   or $^{13}C_9H_{17}^{15}N_3O_4$ (L-trialanine) at concentrations of 5, 15 and 50 µM (atom % = 98,

205   alanine obtained from Cambridge Isotope Laboratories; trialanine obtained from CK

206   Gas Products, Hook, UK). Above and belowground biomass of incubated tissues are

207   given in Table S1. Alanine was selected because it is one of the amino acids with higher

208   rates of occurrence in the proteins of all types of organisms and therefore is important in

209   N cycling. Trialanine was chosen because of its ecological significance as it represents a

210   common structure that is released by protein hydrolysis. All experiments were

211   performed at constant light ($\approx$ 100 µmol quanta m^{-2} s^{-1}) for 0.5 h and at each site-

212   specific acclimation temperature. Incubations with the four N sources at the different

213   nutrient concentrations were done simultaneously whereas replicates (n = 3) were done

214   sequentially to overcome feasibility constrains derived form the setup of a large number

215   of incubations chambers necessary to test all treatments simultaneously (N sources and

216   N concentrations, n = 16 chambers).

217   At the end of uptake experiments, the leaves were immediately separated from the

218   rhizomes and roots. Tissues were rinsed with deionised water to remove adhering salt

219   and isotopic labels before being dried (60 ºC, 48 h), and reduced to a fine powder for

220   analysis of the atom % of ^{15}N and total N content.

221

222   <u>Microbial DON uptake experiments</u>

223   At each meadow, seawater samples and sediment cores of 3 cm diameter and 5 cm

224   depth were collected (n = 3), placed in 50 mL polypropylene centrifuge tubes, sealed

225   immediately and transported on ice to the laboratory. Sediment samples were

226   acclimated for three days to the site-specific temperature (8, 12 and 17º C in Iceland,

227   UK and Portugal, respectively). Sediment sub-samples of 1 g of fresh weight were then

228   placed in 1.5 mL polypropylene tubes. The water content of natural sediments (~ 0.5

229   mL g^{-1} fresh weight) was previously determined by drying at 105 ºC for 24 h and used

230   to calculate the amount of seawater needed to add to the sediment in order to obtain a

231   final incubation volume of 1 mL after sediment centrifugation. Artificial seawater with

232   a salinity equal to the natural seawater of each site was purged with N_2 to remove

233   oxygen and added to the sediment tubes to reach the final incubation volume of 1 mL of

234   water content in each tube. Microbial uptake rates of amino acids and peptides were

235   determined by adding ^{14}C-labelled alanine or trialanine solutions (20 µL; 2.8 kBq mL^{-1}

236   seawater or g^{-1} soil; American Radiolabeled Chemicals, St Louis, MO, USA) to the

237   seawater and sediment sub-samples at a range of concentrations (5, 25, 50 and 100 µM

238   for alanine and 5, 15 and 50 µM for trialanine). Samples from Iceland, UK and Portugal

239   were incubated respectively at 8, 12 and 17 ºC for 0.5 h under vigorous shaking. After

240   incubation, samples were centrifuged (18 000 g, 10 min) and the supernatant recovered.

241   Subsequently, 0.2 mL of HCl (1 M) was added to release any $H^{14}CO_3^-$ trapped in

242   solution (Brailsford et al., 2019) and the ^{14}C activity in the supernatant (0.3 mL)

243   determined on a Wallac 1404 liquid scintillation counter with automatic quench

244   correction (Wallac EG&G, Milton Keynes, UK) after addition of 4 mL of HiSafe3

245   liquid scintillation cocktail (Perkin Elmer Corp., Waltham, MA). Unfortunately, it was

246   not possible to measure the microbial uptake of alanine and trialanine in the water in the

247   absence of sediment as the ^{14}C activity measured at the end of the experiment was never

248   lower than the initial ^{14}C activity, even when the incubation time was extended (i.e. no

249   measureable uptake).

250

251   <u>Effects of temperature on N uptake</u>

252   To evaluate the effect of seawater temperature on the uptake rates of *Z. marina* (DIN

253   and DON) and microbes (DON), plants and sediment cores (3 cm diameter, 5 cm depth)

254   were collected from the mid-latitude site (UK) and were incubated at the latitudinal

255   range of temperatures of 8, 12 and 17 ℃. The rationale for this was that mid-latitude

256   plants are within the range of mean annual temperatures (8, 12 and 17 ℃) of the three

257   latitudes studied, i.e. the minimum (8 ℃) and maximum (17 ℃) temperatures to which

258   mid-latitude plants are exposed coincide with the mean annual temperature of plants

259   from the high-latitude site in Iceland (8 ℃) and from the lower-latitude site in Portugal

260   (17 ℃). Plants and sediments were acclimated for three days to the experimental

261   conditions of light (∼ 100 µmol quanta m^{-2} s^{-1}) and temperature under a 18:6 h

262   light:dark cycle. The uptake rates of inorganic (ammonium and nitrate) and organic

263   (alanine and trialanine) nitrogen by *Z. marina* and the microbial uptake of DON were

264   determined as described above. The average leaf biomass per incubated shoot was 0.24

265   g dry weight, whereas the average belowground biomass was 0.14 g dry weight.

266

267   <u>Sample analysis</u>

268   Concentrations of dissolved inorganic nitrogen (DIN) were determined colorimetrically

269   in a loop-flow analyzer (µMAC-1000 Systea, Agnani, Italy). Samples for determination

270   of total free amino acids and amino acids bound in short peptides were passed through a

271   1 kDa ultrafiltration membrane (Millipore, Billerica, Massachussets, USA). Amino acid

272   N was determined fluorometrically before and after hydrolysis in 6 M HCl at 105 °C for

273   16 h under N_2 using glycine as a standard (Jones et al., 2002). Glycine was used because

274   it is the most predominant amino acid and its relative fluorescent intensity is similar to

275   that of other dominant amino acids (Parsons et al. 1984). Peptide N was determined as

276   the difference between the free and total amino acid pools, determined before and after

277   hydrolysis, respectively. Total nitrogen (TN) was determined after combustion to

278   nitrogen monoxide by chemiluminescence using a TOC-V-TN analyser (Shimadzu,

279   Kyoto, Japan). TN content and atom % ^{15}N of dried tissues were determined using a

280   PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope

281   ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Precision of the $\delta\,^{15}$N analysis was

282   higher than 0.03 ‰. Leaf and root ^{15}N background level was measured on five replicate

283   samples.

284

285   <u>Data analysis</u>

286   The N uptake rates of Z. *marina* were expressed in µmol g^{-1} dry weight h^{-1}. ^{15}N

287   enrichment (%) of tissues after incubation was calculated by subtracting the post-

288   incubation ^{15}N levels from the initial background levels, multiplied by the total N

289   content (g) in the tissue and divided by its dry weight (g dry weight). Uptake rates were

290   plotted against substrate concentration (µM) and the uptake kinetic parameters were

291   derived using the Michaelis-Menten kinetic model

292     $V = (V_{max} \times S) / (K_m + S)$

293   where V is uptake rate (µmol g^{-1} dry weight h^{-1}), V_{max} is maximum uptake rate (µmol g^{-1}

294   DW h^{-1}), S is substrate concentration (µM) and K_m is the half-saturation constant (µM).

295   Data not displaying saturation kinetics were fitted to a linear regression model ($V =$

296   uptake rate, S = substrate concentration).

297   Whole-plant nitrogen budgets per ground area (µmol m^{-2} h^{-1}) of Z. *marina* along the

298   latitudinal gradient and at each experimental temperature were estimated by multiplying

299   the N uptake rates (µmol g^{-1} dry weight h^{-1}) of the plants at the ambient nutrient

300   concentrations (µM) measured in each site by the areal biomass (g^{-1} dry weight m^{-2}).

301   The DIN and DON contribution to the total N acquisition by the leaves and roots were

302   calculated by dividing the sum of the acquisition of ammonium and nitrate, or amino

303   acids and peptides, of each plant part by the total plant N acquisition.

304   Microbial DON uptake rates were expressed in nmol mL^{-1} h^{-1}. ^{14}C microbial uptake of

305   alanine and trialanine was calculated based on the percentage of ^{14}C-labelled solution

306   taken up by the microbes, obtained from the proportion of initial ^{14}C activity remaining

307   in solution after incubation.

308   Differences in ambient concentrations between study sites were tested for each N source

309   separately using one-way analysis of variance (ANOVA). The effects of latitude /

310   nutrient concentration and temperature / nutrient concentration on N uptake rates by *Z.*

311   *marina* were tested using two-way ANOVAs for each N source and plant part. All other

312   differences in *Z. marina* uptake rates were tested using the non-parametric Kruskal-

313   Wallis test because the assumption of data normality was not met even after data

314   transformation.

315   The effects of latitude / trialanine concentration and of temperature / alanine

316   concentration on microbial uptake rates were tested using two-way ANOVA. The

317   effects of latitude / alanine concentration and of temperature / trialanine concentration

318   on microbial uptake rates were tested with the non-parametric Kruskal-Wallis test

319   because the ANOVA assumptions of data normality and equal variance were not met

320   even after data transformation. Data were square root transformed when normality was

321   not verified. All tests were performed at a level of significance of $p < 0.05$.

322

323   Results

324

325   <u>Concentration of inorganic and organic N</u>

326   The concentrations of the different forms of soluble N were generally much higher in

327   the sediment porewater than in the water column at all sites (Table 1). The nutrient

328   concentrations in the water column did not vary significantly among sites except for

329   ammonium and amino acids, which were higher in Portugal and in the UK, respectively.

330   The concentration of ammonium in the sediment porewater was not significantly

331   different among the study sites, whereas the concentration of all other N sources varied

332   significantly but with no consistent latitudinal pattern.

333   Amino acids and peptides, a fraction of the dissolved organic nitrogen (DON) pool,

334   constituted an important component of the total nitrogen available in both the sediment

335   and water column of the studied sites, representing 53%, 29% and 43% of the total

336   nitrogen in the sediment and 52%, 13% and 41% in the water, respectively in Iceland,

337   UK and Portugal. No latitudinal consistency was found.

338

339   <u>Plant density and biomass</u>

340   Shoot density in Iceland (1483 $\pm$ 379 shoots m^{-2}) was much higher than in the UK (533

341    $\pm$ 113 shoots m^{-2}) and Portugal (408 $\pm$ 52 shoots m^{-2}) but the aboveground biomass was

342   lower at the northern limit of the species distribution (44 $\pm$ 11 g dry weight m^{-2})

343   compared to the UK (185 $\pm$ 12 g dry weight m^{-2}) and Portugal (130 $\pm$ 9 g DW m^{-2}) due

344   to the smaller size of the leaves (leaf length: 15 $\pm$ 4 cm in Iceland, 37 $\pm$ 10 cm in the UK

345   and 32 $\pm$ 9 cm in Portugal). Below-ground biomass was conservative: in Iceland it was

346   55 $\pm$ 5 g dry weight m^{-2}, in the UK it was 56 $\pm$ 12 g dry weight m^{-2} and in Portugal it

347   was 55 $\pm$ 5 g dry weight m^{-2}. *Z. marina* from Iceland showed higher concentrations of

348   nitrogen in the leaf tissues (2.35 $\pm$ 0.2 % dry weight) compared to plants from the UK

349   and Portugal (1.65 $\pm$ 0.4 and 1.83 $\pm$ 0.2 % DW, respectively).

350

351   <u>N uptake by *Zostera marina*</u>

352   Overall, the DIN and DON uptake rates by *Z. marina* was higher towards the equator,

353   and reduced in the poleward direction, particularly at higher substrate concentrations

354 (Fig. 1, Table S2). *Z. marina* growing at the southern limit of its distribution (Portugal)

355 showed significantly higher uptake rates compared to plants growing further north

356 except for the uptake of ammonium through the roots, which was similar between

357 latitudes at all nutrient concentrations (Fig. 1e, Table S2). Differences in the uptake

358 rates between latitudes at the lowest N concentration (5 µM) were only detected for

359 organic N sources (alanine and trialanine). Maximum nitrogen (inorganic and alanine)

360 uptake rates (V_{max}) consistently increased with decreasing latitude, with a 2 to 4-fold

361 decrease from Portugal to Iceland (Table S3). The latitudinal effects on nutrient affinity

362 (α) and on the half-saturation constant (K_m) were not as clear. The increase of V_{max} with

363 decreasing latitude is mostly caused by temperature, as indicated by the temperature

364 experiment, where V_{max} increased significantly with temperature (Table S4). The results

365 of the two-way analysis of variance examining the effects of temperature (T) and

366 nutrient concentration (N) on the nitrogen uptake rates of *Zostera marina* support that

367 the latitudinal pattern observed is caused by temperature as higher temperatures resulted

368 in higher N uptake (Fig. 2, Table S5).

369

370 <u>Whole-plant N budget</u>

371 The leaf, root and whole-plant N budgets of *Z. marina* increased with decreasing

372 latitude both for DIN and DON (Fig. 3a, Table S6). Root uptake was more important for

373 *Z. marina* nitrogen budget than leaf uptake as it contributed 93%, 72% and 70% to the

374 whole-plant budget, respectively in Iceland, UK and Portugal (Fig. 3b, Table S6). These

375 values also show that root contribution decreases with decreasing latitude. Overall, the

376 uptake of DON by *Z. marina* contributed about one third to the whole-plant nitrogen

377 budget, showing that this N form is ecologically relevant throughout the latitudinal

378 gradient. Ammonium represented the largest contributor to the total DIN budget of the

379 species at all sites, particularly via root uptake from the sediment (66% in Iceland, 44%

380 in UK and 41% in Portugal, Table S6). The contribution of amino acids and peptides

381 via root uptake (26 % in Iceland, 27% in UK and 24% in Portugal) was smaller

382 compared to ammonium but much higher than that of nitrate (2, 0.5 and 5%,

383 respectively).

384 The temperature experiments showed a general pattern of increasing DIN and DON

385 budgets towards warmer sites, except for the DIN and DON of leaves (Fig. 3c, Table

386 S7). The relative contributions of DIN and DON to the whole-plant budgets did not

387 vary with latitude, but the relative contribution of DON to the whole plant N budget

388 increased with temperature, as opposed to DIN (Fig. 3d, Table S7).

389

390 <u>Microbial DON uptake</u>

391 The sediment microbial uptake of alanine did not change significantly among sites (H =

392 4.491, p = 0.106), as opposed to the uptake of trialanine that increased with decreasing

393 latitude (Fig. 4a and b). Despite the lack of a significant latitudinal variation of alanine,

394 the temperature experiments showed significant effects on the microbial uptake rates of

395 alanine at the highest nutrient concentration (100 µM) (F = 20.46, P < 0.001) (Fig. 4c).

396 The microbial uptake of trialanine increased significantly from 8 ℃ to 12 ℃ and 17 ℃

397 (H = 6.423, P = 0.04) (Fig. 4d).

398

399 Discussion

400

401 Our study showed that the uptake rates of inorganic and organic N by both leaves and

402 roots and the total meadow uptake under local conditions (N budget) of the globally

403 widespread seagrass *Zostera marina* increased from northern to southern sites. The

404   hypothesis that temperature is the main driver of this latitudinal trend was supported by

405   the temperature experiments.

406

407   <u>N uptake rates and N budget of *Z. marina*</u>

408   For all N sources, the uptake rates and the uptake kinetics of *Z. marina* increased with

409   temperature as reported for the uptake rates of ammonium and glycine by the Arctic

410   saltmarsh grass *Pucinellia phryganodes* (Henry & Jefferies, 2003). Kaldy (2014) is, to

411   our knowledge, the only other report that has experimentally tested the effects of

412   temperature on the acquisition of nitrogen by *Z. marina*. This study showed a 50 %

413   increase in nitrate uptake as the temperature increased from 10 to 18/25 ºC in plants

414   exposed to elevated nitrate concentrations (100 µM). Indirect indications of temperature

415   effects on the acquisition of nitrogen by seagrasses have been presented in studies

416   evaluating the seasonal variation of N uptake rates (Lee & Dunton, 1999; Hasegawa et

417   al., 2005) or the influence of season and temperature on the activity of enzymes

418   involved in the nitrogen metabolism, such as glutamine synthetase (Kraemer &

419   Mazzella, 1999) and nitrate reductase (Alexandre et al., 2004). In these studies, the

420   uptake rates and the enzymatic activities generally increased with increasing

421   temperature. Despite the greater amount of bioavailable DON relative to DIN in all

422   studied sites, the uptake of DIN represented the largest fraction of the total N budget of

423   *Z. marina* across its latitudinal distribution. This was due to the high uptake of

424   ammonium (54 - 68 % of the total N uptake), which is the preferential nitrogen source

425   of *Z. marina* (Short & McRoy, 1984; Hemminga et al., 1994; Alexandre et al., 2015).

426   Even though the rate of nitrogen uptake through the leaves was generally higher than

427   through the roots at similar nutrient concentrations, the overall contribution of root

428   uptake to the total N budget of *Z. marina* (70 - 94 %) was much higher than that of

429   leaves (7 - 30 %) because the concentrations of the different nitrogen sources in the

430   sediment porewater were several-fold higher than in the water column. This relative

431   contribution of the different plant parts contrasts with other reports showing an equal

432   contribution of leaves and roots to the total annual N acquisition by *Z. marina* (Pedersen

433   & Borum, 1993; Lee & Dunton, 1999), but their calculations accounted only for

434   inorganic nitrogen. Amino acids and oligopeptides are a relevant source of nitrogen for

435   *Z. marina*, particularly through root uptake. The average contribution of amino acids

436   (12 %) and peptides (14 %) via root uptake exceeded that of nitrate (2 %) by several-

437   fold at the extremes of the latitudinal gradient. This result supports the relevant role of

438   organic nitrogen as a complementary N source for *Z. marina*, previously reported by

439   Alexandre et al. (2015).

440   In contrast to our initial hypothesis, the pool of bioavailable DON did not increase with

441   latitude driven by lower microbial N mineralisation and this was not reflected in higher

442   DON uptake by *Z. marina*, as observed for arctic and antarctic vascular plants (Chapin

443   et al., 1993, Hill et al., 2011). Both the uptake of DON by *Z. marina* and the DON

444   contribution to the whole-plant N budget increased towards lower latitude and this trend

445   was driven by higher temperature. At high latitude, the lower contribution of DON to

446   the total N budget of *Z. marina* is determined by the plants' lower DON uptake rates

447   rather than by a lower availability of DON sources.

448

449   <u>Microbial DON uptake</u>

450   The microbial uptake of DON in *Z. marina* sediments increased towards southern

451   latitudes and this was driven by temperature. DON uptake is ecologically relevant as

452   amino acids and peptides accounted for a significant pool of bioavailable nitrogen in *Z.*

453   *marina* meadows, particularly in the sediments (30 to 50 % of the total N). The

454   observed latitudinal pattern contrasts with that reported by Mozdzer et al. (2014), who

455   found significant increases in sediment DON availability with increasing latitude in

456   *Spartina alterniflora* saltmarshes, which coincided with a significant decrease in

457   microbial DON uptake.

458   To the best of our knowledge, this was the first report of the latitudinal effects of

459   temperature on the DON uptake of both micobes and seagrasses, which is relevant to

460   understand their competition for these nitrogen sources. Dissolved organic nitrogen

461   serves both as a direct source of nitrogen for *Z. marina* and as a mineralisation substrate

462   for its associated microbial community, and both of their uptake rates increase with

463   temperature. Seagrasses in general and *Z. marina* in particular may have a competitive

464   advantage over microbes for DON uptake because they also acquire nutrients through

465   the leaves from the water column, where microbial uptake of alanine and trialanine was

466   very low, according to the lower ^{14}C uptake when compared to the sediment.

467   The northern distribution limits of *Z. marina* are warming twice as fast as the rest of the

468   globe (IPCC, 2018) creating the conditions for its expansion towards the pole. This

469   warming trend will increase the nitrogen demand of *Z. marina* that may be met by an

470   increasing uptake of organic nitrogen forms. The competition with microbes for this

471   nutrient source is also expected to increase as warming will also enhance the microbial

472   uptake of DON. Our results highlight that *Z. marina*, and probably other seagrass

473   species, can be winners under global change conditions of increased temperature and

474   CO_2. Many reports have shown that higher CO_2 levels will increase the photosynthetic

475   production of seagrasses (Jiang et al., 2010, Alexandre et al., 2012, Ow et al., 2015),

476   which may result in higher growth that will not be limited by the plants' nitrogen uptake

477   capacity.

478

479   Acknowledgements

480

490

491   Authors contribution

492

493   A.A. and R.S. conceived and designed the study. A.A., R.Q. and P.W.H. carried out the

494   experimental work. A.A., P.W.H., D.J. and R.S. analysed the data. A.A. wrote the

495   manuscript. All authors contributed critically to the drafts and gave final approval for

496   publication.

497

498   Data availability statement

499

500   Data are available from the Mendeley Data under doi: 10.17632/r4m88s8m8w.1

501   (Alexandre and Santos 2020 Zm N uptake latitude and temperature).

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716

717 SUPPORTING INFORMATION

718 Additional supporting information may be found in the online version of this article.

719

720 Table S1. Average aboveground and belowground biomass of *Zostera marina* incubated

721 in each site along the latitudinal distribution gradient.

722 Table S2. Nitrogen uptake kinetic parameters of *Zostera marina* along the species'

723 latitudinal distribution gradient.

724 Table S3. Results of the two-way ANOVA examining the effects of latitude and nutrient

725 concentration on the nitrogen uptake rates of *Zostera marina*.

726   Table S4. Nitrogen uptake kinetic parameters of *Zostera marina* at three different

727   temperatures.

728   Table S5. Results of the two-way ANOVA examining the effects of temperature and

729   nutrient concentration on the nitrogen uptake rates of *Zostera marina*.

730   Table S6. Whole-plant nitrogen budget of *Zostera marina* along the species latitudinal

731   distribution range in Europe.

732   Table S7. Whole-plant nitrogen budget of *Zostera marina* at three different

733   temperatures.

734   Figure S1. Aspect of the *Zostera marina* meadows in Iceland, United Kingdom and

735   Portugal.

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743   Table 1. Concentration (µM) of the different nitrogen sources (ammonium, nitrate, free

744   amino acids, peptides and total nitrogen) in the water column and sediment porewater in

745   the study sites (Iceland, United Kingdom and Portugal). Values are mean ± S.D. (n = 5).

746   Levels of significance of the statistical tests are indicated as (*) $P < 0.05$, (**) $P < 0.01$,

747   (***) $P < 0.001$, (ns) not significant. Different letters indicate significant differences.

748   Peptide values in italic are not reliable as they are higher than total nitrogen and higher

749   than sediment values (see text in Supporting Information).

750

	Iceland	**United Kingdom**	**Portugal**	**Test statistic**
Water column				
Ammonium	0.18 ± 0.19^a	0.77 ± 0.36^a	1.35 ± 1.06^b	$H = 7.322^*$
Nitrate	0.43 ± 0.68	0.14 ± 0.06	3.12 ± 1.89	$H = 4.842^{ns}$
Amino acids	0.29 ± 0.10^a	0.67 ± 0.30^b	0.29 ± 0.11^a	$F = 6.483^*$
Peptides (estimated)[§]	2.50 ± 1.83	1.03 ± 0.49	4.82 ± 4.04	$H = 5.700^{ns}$
Total nitrogen	5.33 ± 3.90	12.86 ± 6.14	12.36 ± 10.35	$F = 2.784^{ns}$
Sediment				
Ammonium	20.87 ± 8.49	20.03 ± 8.98	30.17 ± 6.75	$F = 1.769^{ns}$
Nitrate	0.66 ± 0.55^a	0.54 ± 0.16^a	15.08 ± 5.09^b	$F = 26.201^{***}$
Amino acids	7.83 ± 4.25^a	62.25 ± 20.06^b	6.72 ± 2.12^a	$H = 7.385^*$
Peptides	42.23 ± 16.43^{ab}	17.70 ± 13.33^a	50.59 ± 12.59^b	$F = 5.081^*$
Total nitrogen	93.93 ± 29.35^a	278.73 ± 102.57^b	134.72 ± 33.49^{ab}	$H = 9.118^{***}$

751 [§]Concentrations estimated from the percentage of peptides relative to the total soluble

752 nitrogen in the sediment porewater (see text).

753

754   Figure 1. Uptake rates (µmol g^{-1} dry weight h^{-1}) of the different nitrogen sources by

755   leaves and roots of *Zostera marina* as a function of concentration (µM) along the

756   species latitudinal distribution gradient (Iceland, United Kingdom and Portugal):

757   ammonium (a), nitrate (b), alanine (c) and trialanine (d) by the leaves; ammonium (e),

758   nitrate (f), alanine (g) and trialanine (h) by the roots. Values are mean ± S.D. (n = 3).

759   DW = dry weight.

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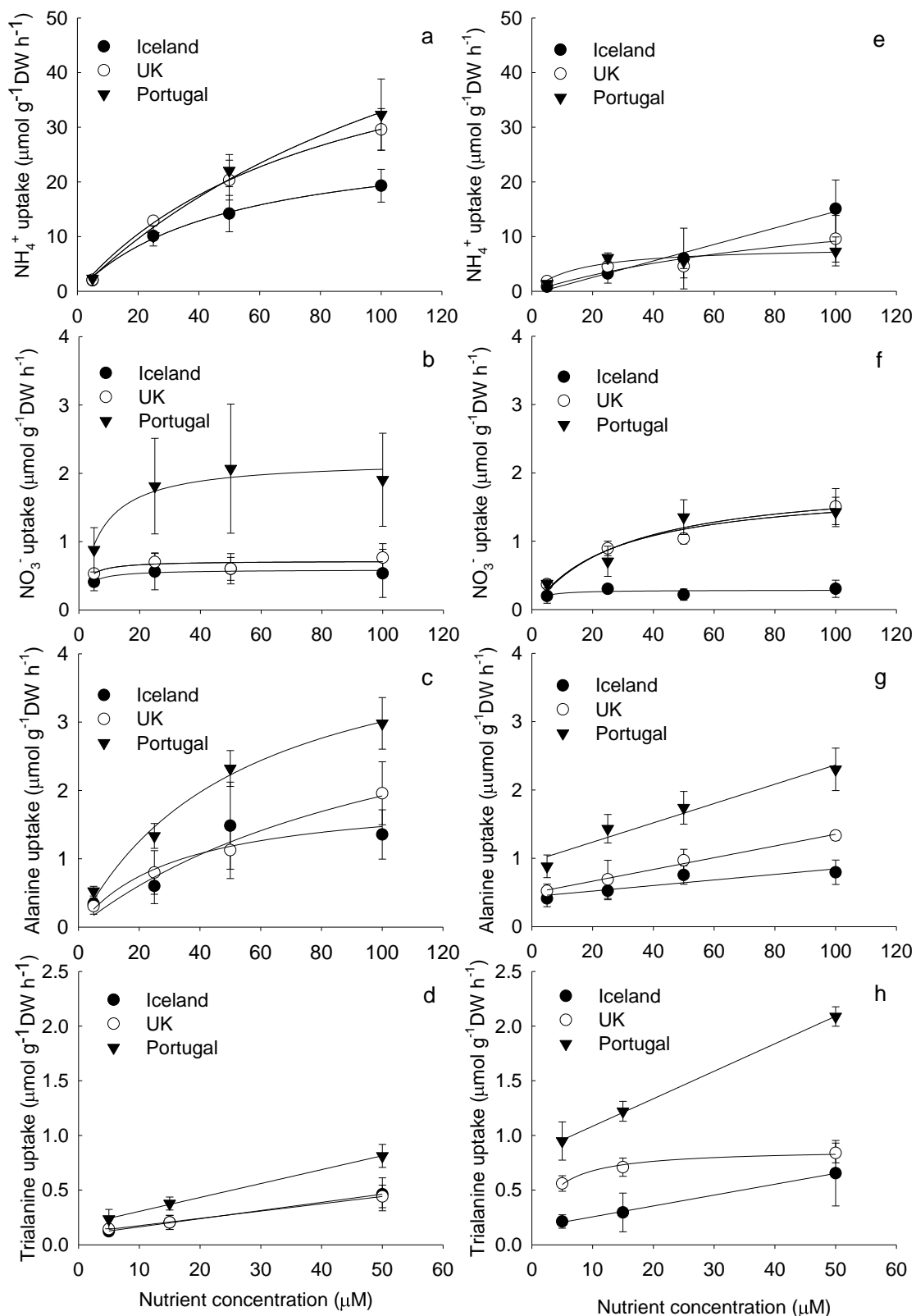
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768   Figure 2. Uptake rates (μmol g^{-1} dry weight h^{-1}) of the different nitrogen sources by

769   leaves and roots of *Zostera marina* as a function of concentration (μM) at three

770   incubation temperatures (8, 12 and 17 ℃): ammonium (a), nitrate (b), alanine (c) and

771   trialanine (d) by the leaves; ammonium (e), nitrate (f), alanine (g) and trialanine (h) by

772   the roots. Values are mean ± S.D. (n = 3). DW = dry weight.
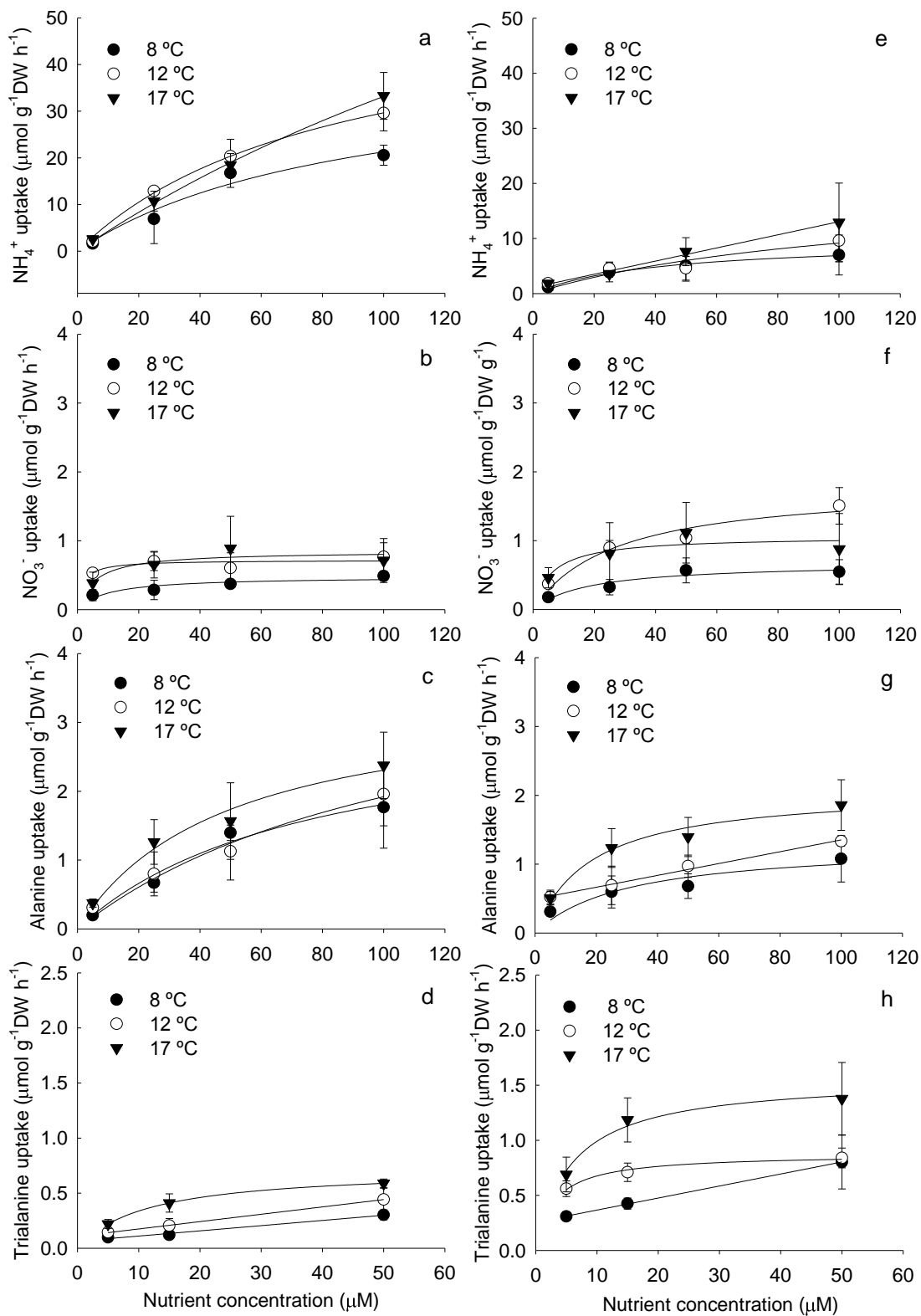
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779 Figure 3. Whole-plant nitrogen budget of *Zostera marina* along the species latitudinal

780 distribution range in Europe (Iceland, United Kingdom and Portugal) as µmol m^{-2} h^{-1} (a)

781 and % contribution (b), and the experimental effects of temperature on the whole-plant

782 nitrogen budget as µmol m^{-2} h^{-1} (c) and % contribution (d).

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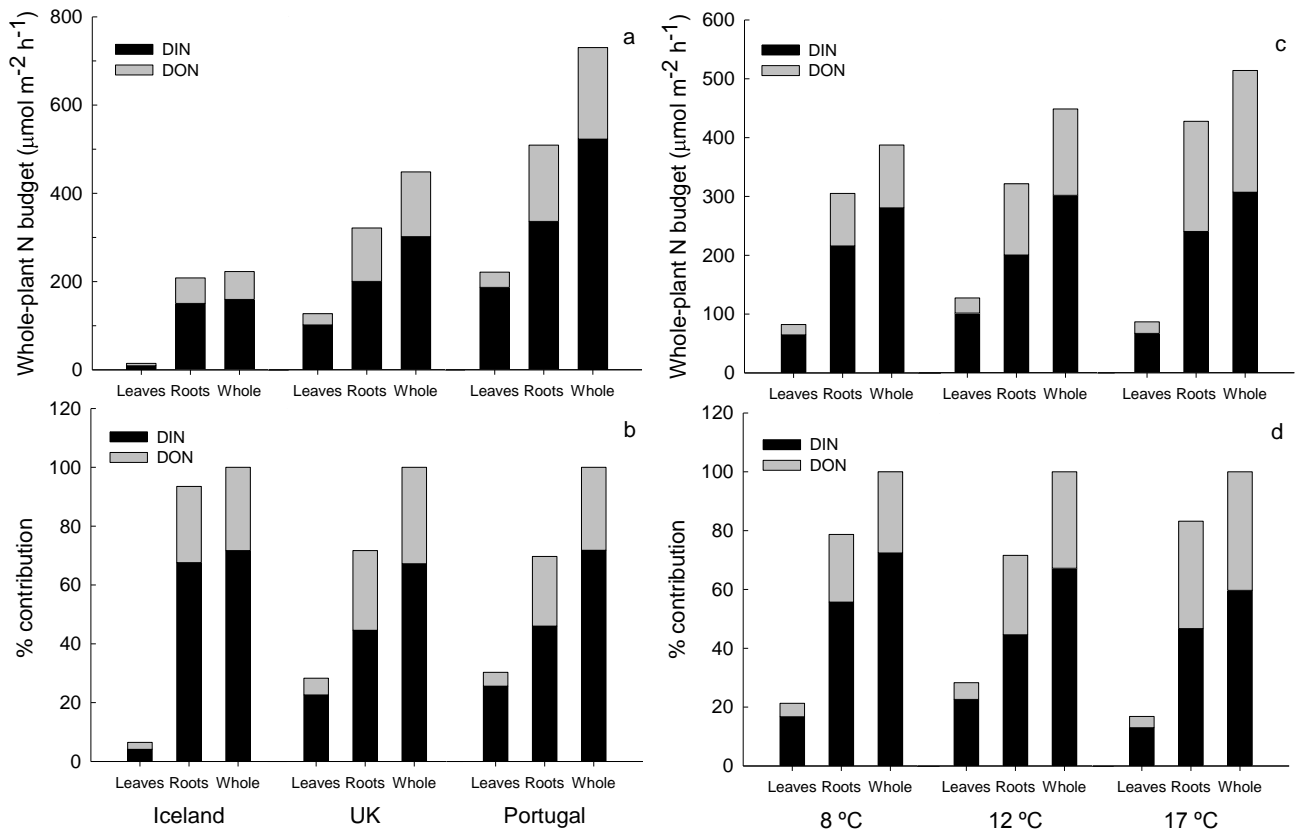
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798 Figure 4. Microbial uptake (nmol mL^{-1} h^{-1}) of alanine (a) and trialanine (b) as a function

799 of substrate concentration (µM) in *Zostera marina* meadows at three sites (Iceland,

800 United Kingdom and Portugal) along the species latitudinal distribution gradient, and

801 the experimental effects of temperature on the microbial uptake of alanine (c) and

802 trialanine (d) . Values are mean ± SD (n = 3). Significant differences are indicated by

803 different letters. (P < 0.05).

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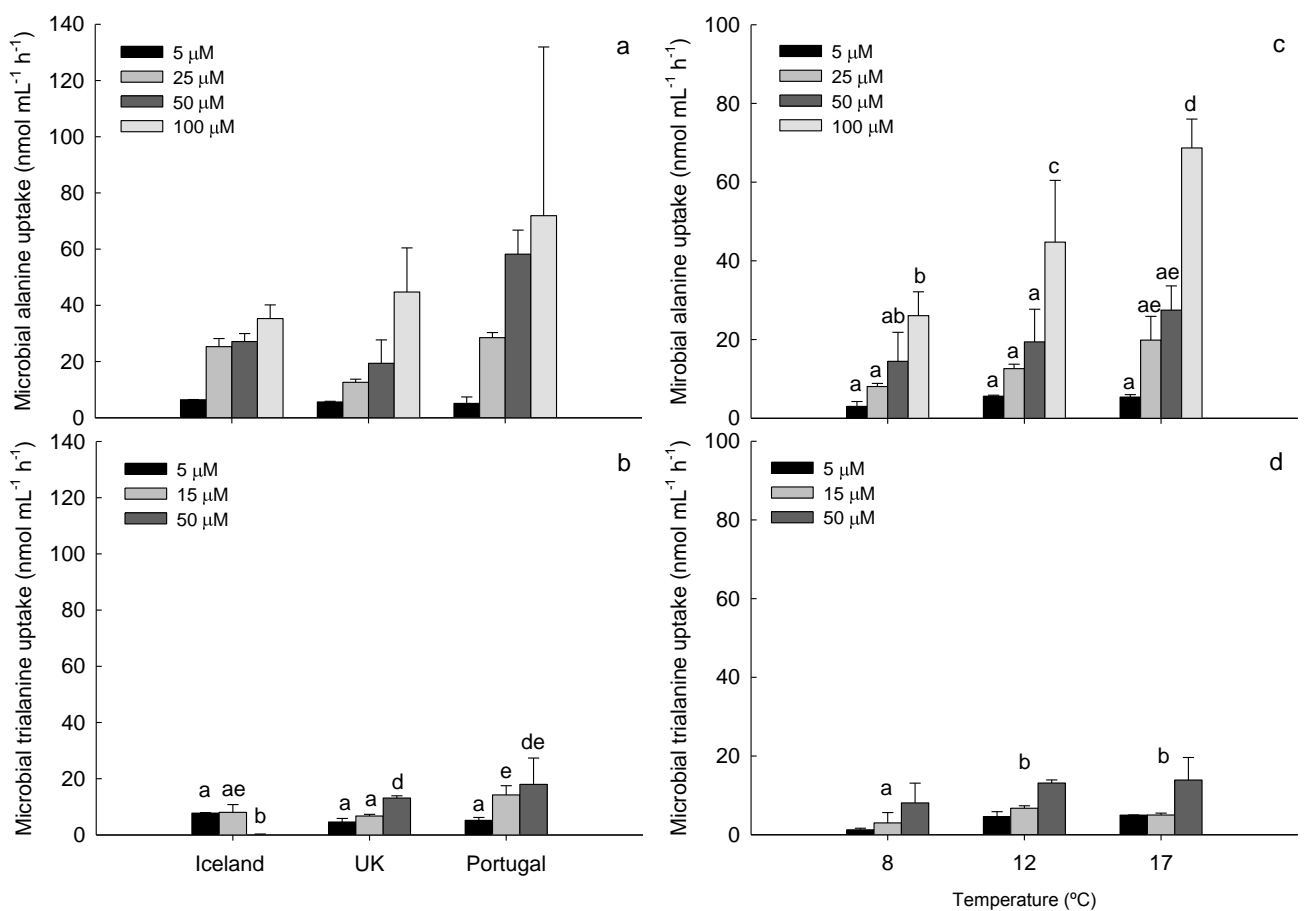
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811   SUPPORTING INFORMATION

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813   Supplementary Methods

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815   *Z. marina* nitrogen uptake experiments

816   During incubations, plant shoots were loose in the incubation chambers and naturally

817   gained a relatively vertical alignment, with their roots pointing towards the bottom of

818   the chamber and the leaves to the surface. The media were constantly stirred during

819   incubation to decrease the thickness of the boundary layer at the leaf/root surface and to

820   ensure a homogeneous distribution of the isotopic labels and that leaf surfaces received

821   similar amounts of photosynthetic active radiation. The authors acknowledge that, in

822   this experiment, belowground plant parts were incubated in oxygenated conditions,

823   which is in contrast with the anoxic natural environment. However, preliminary

824   experiments showed no effect of rhizosphere oxygenation on the ammonium uptake

825   rates of leaves and roots, as was also reported for the sister species *Z. noltei* (Alexandre

826   et al., 2010; Alexandre et al., 2011).

827   Because no significant internal translocation of incorporated ^{15}N between leaves and

828   roots is expected within the chosen experimental incubation time (Vonk et al., 2008;

829   Alexandre et al., 2011), we assumed that the ^{15}N content determined in leaf and root

830   tissues after incubation represents only the amount of N that was taken up by the

831   respective plant parts. The removal of a substantial part of the microbial community

832   from the incubation media (artificial seawater filtered through 0.2 µm) and the short

833   incubation time (0.5 h) restricted exogenous DON mineralisation during the incubations

834   (Sharkey, 2007; Bauwe et al., 2010). Therefore based on these many previous studies,

835   we assume that most, if not all, of the amino acids and peptides were taken up by the

836   leaves and roots of *Z. marina* without prior cleavage.

837

838   <u>Sample analysis</u>

839   The concentration of ammonium was determined using the hypochlorite/indophenol

840   method (detection and quantification limits = 0.03 and 0.07 μM), whereas nitrate was

841   determined by the cadmium reduction method (detection and quantification limits =

842   0.06 and 0.11 μM). Amino acid N was determined according to Jones et al. (2002).

843   Briefly, 20 µL of sample or standard and 200 µL of working reagent (5 mL of OPAME

844   concentrate + 200 mL of borate buffer 0.02 M pH 9.5) were combined and read after 1

845   min on a LS-5B Fluorometer (Perkin Elmer Corp., Boston, MA) with an excitation

846   wavelength of 340 nm and an emission wavelength of 450 nm. The OPAME reagent

847   was obtained by dissolving 50 mg of *o*-phthaldialdehyde in 5 mL of methanol and

848   adding 100 µL of β-mercaptoethanol.

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850   <u>Supplementary Results</u>

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852   <u>Concentration of inorganic and organic N</u>

853   The concentration of peptides in the water column was higher than in the sediment, and

854   exceeded the total N by 3 to 10-fold, an incongruity that was not observed in the

855   sediment porewater (Table 1). We concluded that these values are not reliable,

856   suggesting an unresolved technical issue (e.g. contamination or unknown intereference).

857   To overcome this, water column peptide values were estimated from the percentage of

858   peptides relative to the total soluble nitrogen observed in the sediment porewater (50 %

859   in Iceland, 8 % in the UK and 40 % in Portugal).

860

861 <u>N uptake by *Zostera marina*</u>

862 The uptake of dissolved inorganic nitrogen (DIN) by *Z. marina* generally displayed

863 saturation kinetics whereas the uptake of DON was in most cases best described by a

864 linear model, indicating that saturation was not reached within the range of nutrient

865 concentrations used in the experiment (Fig. 1, Table S2).

866 The uptake of trialanine from the sediments increased only slightly with nutrient

867 concentration (F = 4.083, p = 0.035) except for trialanine in Iceland, where values at 50

868 µM (0.12 nmol mL^{-1} h^{-1}) were much smaller than at 5 µM and 15 µM (7.75 and 8.05

869 nmol mL^{-1} h^{-1}, respectively) (Fig. 4).

870

871 Supplementary Tables

872

873 Table S1. Average aboveground (leaves) and belowground (rhizomes + roots) biomass

874 (g dry weight) of *Zostera marina* incubated in the high-, mid- and lower-latitude sites

875 along the latitudinal distribution gradient.

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	High-latitude	Mid-latitude	Lower-latitude
Aboveground	0.05	0.24	0.29
Belowground	0.03	0.13	0.22

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886　Table S2. Uptake kinetic parameters of nitrogen uptake by *Zostera marina* plant parts at
887　the three sites (Iceland, United Kingdom and Portugal) along the species' latitudinal
888　distribution gradient. V_{max} = maximum uptake rate (µmol N g^{-1} dry weight h^{-1}); K_m =
889　half-saturation constant (µM); and α = affinity constant (V_{max}/K_m). The coefficient of
890　determination (r^2), level of significance (P) and the standard error of the estimate
891　(values in brackets) are given. Data not displaying saturation kinetics were fitted with a
892　linear regression model (V = uptake rate; S = substrate concentration).
893

	V_{max}	K_m	α	r^2	P
Ammonium (leaves)					
Iceland	28.91 (2.30)	49.92 (8.73)	0.58	0.996	0.002
United Kingdom	54.63 (5.18)	84.24 (14.42)	0.65	0.997	0.001
Portugal	82.08 (24.48)	150.62 (67.20)	0.55	0.990	0.005
Nitrate (leaves)					
Iceland	0.59 (0.03)	2.05 (0.86)	0.29	0.83	0.087
United Kingdom	0.72 (0.06)	1.75 (1.27)	0.41	0.60	0.224
Portugal	2.19 (0.16)	6.56 (2.30)	0.33	0.94	0.029
Alanine (leaves)					
Iceland	1.95 (0.78)	32.60 (34.34)	0.06	0.82	0.096
United Kingdom	4.19 (1.50)	118.18 (67.95)	0.04	0.97	0.014
Portugal	4.62 (0.63)	53.68 (15.66)	0.09	0.99	0.006
Trialanine (leaves)					
Iceland	$V = 0.089+0.007\ S$	-	-	1.00	0.012
United Kingdom	$V = 0.108+0.007\ S$	-	-	1.00	0.012
Portugal	$V = 0.178+0.013\ S$	-	-	0.99	0.016
	V_{max}	K_m	α	r^2	P
Ammonium (roots)					
Iceland	$V = -0.486+0.151\ S$	-	-	0.99	0.007
United Kingdom	19.39 (14.37)	111.61 (135.66)	0.17	0.87	0.069
Portugal	8.26 (1.58)	15.39 (10.65)	0.54	0.89	0.054
Nitrate (roots)					
Iceland	0.29 (0.04)	2.02 (2.19)	0.14	0.43	0.346
United Kingdom	1.81 (0.30)	27.00 (2.70)	0.07	0.95	0.027
Portugal	1.93 (0.45)	30.37 (19.23)	0.06	0.92	0.042
Alanine (roots)					
Iceland	$V = 0.439+0.004\ S$	-	-	0.82	0.093
United Kingdom	$V = 0.493+0.009\ S$	-	-	0.99	0.004
Portugal	$V = 0.954+0.014\ S$	-	-	0.96	0.022
Trialanine (roots)					
Iceland	$V = 0.156+0.010\ S$	-	-	0.99	0.023
United Kingdom	0.88 (0.03)	2.96 (0.56)	0.30	0.98	0.09
Portugal	$V = 0.832+0.025\ S$	-	-	1.00	0.011

894 Table S3. Results of the two-way ANOVA examining the effects of latitude (L) and

895 nutrient concentration (N) on the nitrogen uptake rates of *Zostera marina*. Test statistic

896 (F) and levels of significance are indicated as (*) $P < 0.05$, (**) $P < 0.01$, (***) $P <$

897 0.001, (^{ns}) not significant.

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	Latitude (L)	Nutrient concentration (N)	L x N
Leaves			
Ammonium	$F = 11.87^{***}$	$F = 118.2^{***}$	$F = 3.73^{**}$
Nitrate	$F = 24.48^{***}$	$F = 2.45^{ns}$	$F = 1.15^{ns}$
Alanine	$F = 22.51^{***}$	$F = 45.70^{***}$	$F = 3.16^{*}$
Trialanine	$F = 18.31^{***}$	$F = 55.05^{***}$	$F = 2.49^{ns}$
Roots			
Ammonium	$F = 0.02^{ns}$	$F = 24.33^{***}$	$F = 2.12^{ns}$
Nitrate	$F = 101.73^{**}$	$F = 38.47^{***}$	$F = 7.86^{***}$
Alanine	$F = 84.83^{***}$	$F = 35.18^{***}$	$F = 3.96^{**}$
Trialanine	$F = 118.12^{***}$	$F = 43.46^{***}$	$F = 8.45^{***}$

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918 Table S4. Uptake kinetic parameters of nitrogen uptake by *Zostera marina* plant parts at
919 three different temperatures (8, 12 and 17 °C). V_{max} = maximum uptake rate (μmol N g^{-1}
920 dry weight h^{-1}); K_m = half-saturation constant (μM); and α = affinity constant (V_{max}/K_m).
921 The coefficient of determination (r^2), level of significance (P) and the standard error of
922 the estimate (values in brackets) are given. Data not displaying saturation kinetics were
923 fitted with a linear regression model (V = uptake rate; S = substrate concentration).
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	V_{max}	K_m	α	r^2	P
Ammonium (leaves)					
8 °C	40.48 (16.53)	90.20 (64.87)	0.45	0.96	0.021
12 °C	54.63 (5.18)	84.24 (14.42)	0.65	0.99	0.001
17 °C	130.76 (25.89)	294.41 (74.16)	0.44	0.99	0.001
Nitrate (leaves)					
8 °C	0.48 (0.08)	9.96 (7.12)	0.05	0.79	0.113
12 °C	0.72 (0.06)	1.75 (1.27)	0.41	0.60	0.224
17 °C	0.85 (0.11)	5.77 (3.77)	0.15	0.80	0.105
Alanine (leaves)					
8 °C	3.20 (0.81)	76.95 (36.09)	0.04	0.98	0.011
12 °C	4.19 (1.50)	118.18 (67.95)	0.04	0.97	0.014
17 °C	3.43 (0.59)	48.69 (18.49)	0.07	0.98	0.012
Trialanine (leaves)					
8 °C	$V = 0.063 + 0.005\,S$	-	-	0.99	0.073
12 °C	$V = 0.108 + 0.007\,S$	-	-	1.00	0.012
17 °C	0.72 (0.00)	11.44 (0.03)	0.06	1.00	0.001
	V_{max}	K_m	α	r^2	P
Ammonium (roots)					
8 °C	9.66 (0.81)	40.38 (8.15)	0.24	0.99	0.004
12 °C	19.39 (14.37)	111.61 (135.66)	0.17	0.87	0.069
17 °C	$V = 1.113 + 0.119\,S$	-	-	0.99	0.004
Nitrate (roots)					
8 °C	0.68 (0.13)	18.20 (11.77)	0.04	0.89	0.055
12 °C	1.81 (0.30)	27.00 (2.70)	0.07	0.95	0.027
17 °C	1.06 (0.14)	6.11 (4.20)	0.17	0.79	0.110
Alanine (roots)					
8 °C	1.30 (0.34)	29.78 (21.59)	0.04	0.87	0.066
12 °C	$V = 0.493 + 0.009\,S$	-	-	0.99	0.004
17 °C	2.09 (0.22)	18.26 (6.32)	0.12	0.97	0.017
Trialanine (roots)					
8 °C	$V = 0.257 + 0.011\,S$	-	-	1.00	0.010
12 °C	0.88 (0.03)	2.96 (0.56)	0.3	0.98	0.089
17 °C	1.57 (0.10)	5.82 (1.42)	0.27	0.98	0.090

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926 Table S5. Results of the two-way ANOVA examining the effects of temperature (T) and

927 nutrient concentration (N) on the nitrogen uptake rates of *Zostera marina*. Test statistic

928 (F) and levels of significance are indicated as (*) $P < 0.05$, (**) $P < 0.01$, (***) $P <$

929 0.001, (^{ns}) not significant.

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	Temperature (T)	Nutrient concentration (N)	T x N
Leaves			
Ammonium	$F = 10.36^{***}$	$F = 124.00^{***}$	$F = 2.82^{*}$
Nitrate	$F = 9.26^{**}$	$F = 3.21^{*}$	$F = 0.76^{ns}$
Alanine	$F = 4.25^{*}$	$F = 37.84^{***}$	$F = 0.55^{ns}$
Trialanine	$F = 38.70^{***}$	$F = 61.99^{***}$	$F = 2.44^{ns}$
Roots			
Ammonium	$F = 12.30^{***}$	$F = 45.36^{***}$	$F = 12.23^{***}$
Nitrate	$F = 6.27^{**}$	$F = 4.37^{*}$	$F = 0.96^{ns}$
Alanine	$F = 20.03^{***}$	$F = 28.42^{***}$	$F = 1.40^{ns}$
Trialanine	$F = 26.91^{***}$	$F = 18.84^{***}$	$F = 1.80^{ns}$

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950 Table S6. Whole-plant nitrogen budget (μmol m^{-2} h^{-1}) of *Zostera marina* along the

951 species latitudinal distribution range in Europe, calculated using the nitrogen uptake

952 rates (μmol g^{-1} dry weight h^{-1}), areal biomass (g^{-1} dry weight m^{-2}) and ambient nutrient

953 concentrations (μM) determined at each site (Iceland, United Kingdom and Portugal).

954 Values in brackets show the percentage contribution of each nitrogen source to the

955 plant's total N acquisition. DIN = dissolved inorganic nitrogen, DON = dissolved

956 organic nitrogen.

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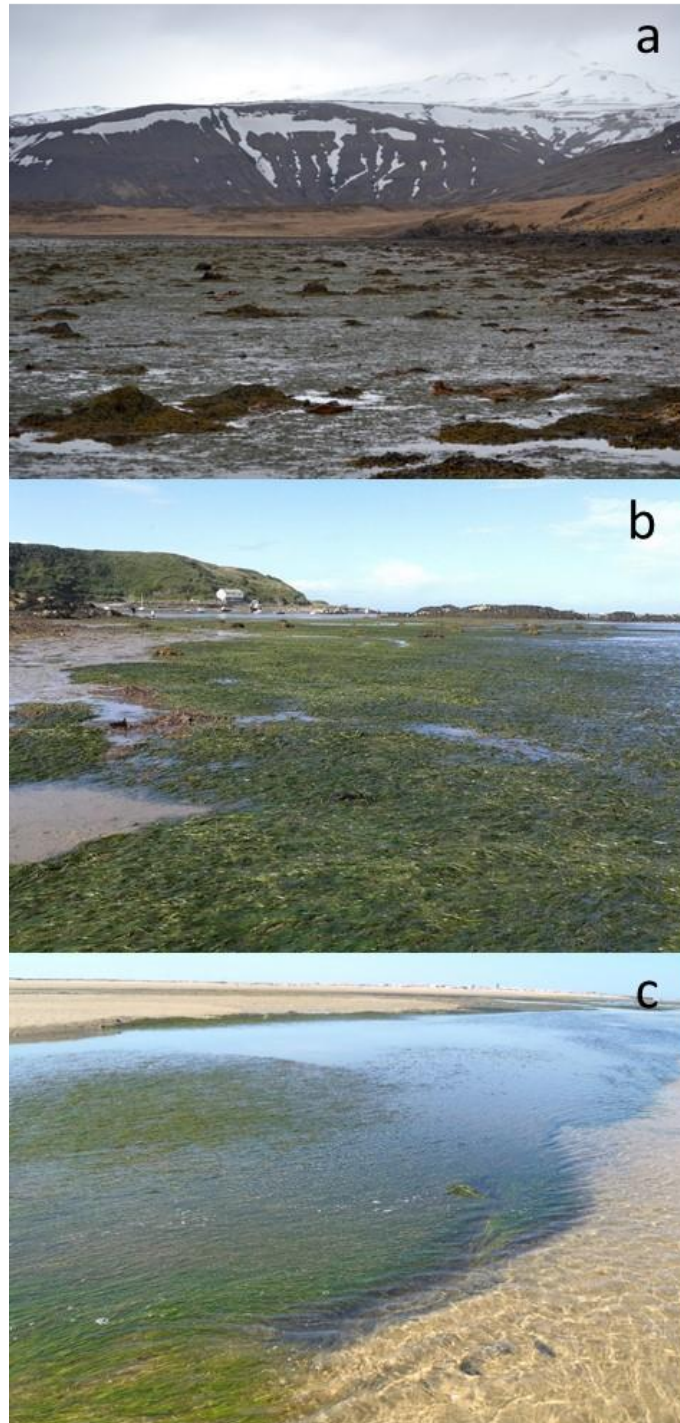|	Iceland	United Kingdom	Portugal
Leaves			
Ammonium	4.6 (2.1 %)	91.6 (20.4 %)	94.8 (13 %)
Nitrate	4.5 (2.0 %)	9.9 (2.2 %)	91.8 (12.6 %)
Amino acids	0.8 (0.3 %)	4.4 (1.0 %)	3.2 (0.4 %)
Peptides	4.7 (2.1 %)	21.3 (4.8 %)	31.3 (4.3 %)
DIN	9.1 (4.1 %)	101.4 (22.6 %)	186.6 (25.6 %)
DON	5.4 (2.4 %)	25.7 (5.7 %)	34.5 (4.7 %)
Roots			
Ammonium	146.6 (65.8 %)	197.7 (44.1 %)	300.8 (41.2 %)
Nitrate	3.9 (1.8 %)	2.4 (0.5 %)	35.2 (4.8 %)
Amino acids	25.9 (11.6 %)	70.6 (15.7 %)	57.6 (7.9 %)
Peptides	31.8 (14.3%)	50.5 (11.3 %)	115.3 (15.8 %)
DIN	150.5 (67.6 %)	200.1 (44.6 %)	336.1 (46.0 %)
DON	57.7 (25.9 %)	121.1 (27.1 %)	173.0 (23.7 %)
Whole-plant			
Total DIN	159.6 (71.7 %)	301.5 (67.2 %)	522.7 (71.8 %)
Total DON	63.1 (28.3 %)	146.8 (32.8 %)	207.5 (28.2 %)

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971 Table S7. Whole-plant nitrogen budget (μmol m^{-2} h^{-1}) of *Zostera marina* at three

972 different temperatures (8, 12 and 17 ºC), calculated using the nitrogen uptake rates

973 (μmol g^{-1} dry weight h^{-1}) obtained at each temperature, areal biomass (g^{-1} dry weight m^{-}

974 ^{2}) and ambient nutrient concentrations (μM) at the intermediate site (United Kingdom).

975 Values in brackets show the percentage contribution of each nitrogen source to the

976 plant's total N acquisition. DIN = dissolved inorganic nitrogen, DON = dissolved

977 organic nitrogen.

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	8 ºC	12 ºC	17 ºC
Leaves			
Ammonium	63.4 (16.4 %)	91.6 (20.4 %)	63.1 (12.3 %)
Nitrate	1.2 (0.3 %)	9.9 (2.2 %)	3.7 (0.7 %)
Amino acids	5.1 (1.3 %)	4.4 (1.0 %)	8.6 (1.7 %)
Peptides	12.6 (3.3 %)	21.3 (4.8 %)	11.0 (2.1 %)
DIN	64.6 (16.7 %)	101.4 (22.6 %)	66.8 (13.0 %)
DON	17.7 (4.6 %)	25.7 (5.7 %)	19.6 (3.8 %)
Roots			
Ammonium	214.6 (55.4 %)	197.7 (44.1 %)	243.3 (45.6 %)
Nitrate	1.3 (0.3 %)	2.4 (0.5 %)	5.7 (1.1 %)
Amino acids	58.9 (15.2 %)	70.6 (15.7 %)	108.3 (21.1 %)
Peptides	30.3 (7.8 %)	50.5 (11.3 %)	79.2 (15.4 %)
DIN	215.9 (55.7 %)	200.1 (44.6 %)	240.0 (46.7 %)
DON	89.2 (23.0 %)	121.1 (27.0 %)	187.4 (36.5 %)
Whole-plant			
Total DIN	280.5 (72.4 %)	301.5 (67.2 %)	306.8 (59.7 %)
Total DON	106.9 (27.6 %)	146.8 (32.8 %)	207.0 (40.3 %)

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992   Figure S1. Aspect of the *Zostera marina* meadows in (a) Iceland (high-latitude), (b)

993   United Kingdom (mid-latitude), and (c) Portugal (lower-latitude) during summer at low

994   tide.

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