Global Ecology and Biogeography

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Latitudinal variation in plant defense against herbivory in a marine foundation species does not follow a linear pattern: the importance of resource availability

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Manuscripts

1 **Title:** Latitudinal variation in plant defense against herbivory in a marine foundation

2 species does not follow a linear pattern: the importance of resource availability

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BIOSKETCH

We are a group of researchers of the Zostera Experimental Network (http://zenscience.org/) interested in conducting coordinated research in beds of eelgrass (Zostera marina) throughout the northern hemisphere to tackle big questions about how biodiversity, climate change, and natural variability across the globe influence

51   ecosystem structure and functioning.

52

1   **ABSTRACT**

2   *Aim*

3   Studies on latitudinal patterns in plant defense have traditionally overlooked the potential

4   effect that resource availability may have in shaping plant defense. Likewise, latitudinal

5   patterns of tolerance traits have rarely been studied, yet they can be a critical component

6   of plant defense. Therefore, the aim of our study was to examine latitudinal variation in

7   the production of tolerance and resistance traits against herbivory along a latitudinal

8   range and a natural gradient of resource availability from upwelling conditions.

9   *Location*

10   Canada, North America and Mexico

11   *Time period*

12   Summer months of 2015

13   *Major taxa used*

14   The seagrass *Zostera marina*

15   *Methods*

16   We conducted experiments simulating macroherbivore (e.g., bird, fish) damage at 10 sites

17   across the Eastern Pacific coast (Canada-Mexico) and Quebec and analyzed several

18   seagrass traits related to resistance and tolerance strategies against herbivory. In addition,

19   we examined the effects of potential seagrass changes in defense strategies by performing

20   a series of feeding experiments with mesoherbivores in a subset of sites.

21   *Results*

22   We found that eelgrass resistance defenses did not follow a linear latitudinal pattern but

23   rather followed a bell-shaped curve which correlated with bottom-up control. In sites with

24 higher nutrient availability, plants allocated resources to tolerance strategies and had

25 lower resistance traits. Furthermore, seagrasses did not respond linearly to increased

26 herbivory pressure; while they tolerated moderate levels of herbivory, they underwent a

27 significant reduction in tolerance and resistance under high herbivory levels, which also

28 made them more susceptible to consumers in feeding experiments.

29 *Main conclusions*

30 Our results highlight the importance that nutrient availability has in shaping latitudinal

31 patterns of plant defense against herbivory and show how these defenses may not respond

32 linearly to increased herbivory pressure in seagrasses.

33 **Keywords:** Seagrass, plant-herbivore interactions, latitudinal gradient, herbivory,

34 resource availability, Limited Resource Model, phenolic compounds, *Zostera marina*,

35 nutrients, upwelling.

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3   **MAIN TEXT**
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8   species does not follow a linear pattern: the importance of resource availability
9
10   **Running title:** Latitudinal patterns of seagrass defense
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16   **INTRODUCTION**
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18
19   Biotic interactions such as herbivory strongly influence plant fitness and
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21   distribution patterns (Wisz et al., 2013) and consequently plants have evolved diverse
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23   defense strategies to tolerate and resist herbivory (Núñez-Farfan, Fornoni, & Valverde,
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25   2007; Strauss & Agrawal, 1999). Tolerance strategies mitigate the negative effects of
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27   herbivory to plant fitness, for example, by compensating the biomass lost through
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37   interactions (i.e., biotic interactions hypothesis, BIH), and thus herbivory, is hypothesized
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39   to follow a latitudinal gradient with higher herbivory towards lower latitudes (Schemske,
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41   Mittelbach, Cornell, Sobel, & Roy, 2009). Such phenomenon has led to the proposal that
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43   plants are better defended towards lower latitudes (i.e. the Latitudinal-Herbivory-
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45   Defense-Hypothesis; LHDH; Coley & Aide, 1991). For instance, salt marsh plant
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47   palatability decreases towards lower latitudes as a result of lower leaf nitrogen content,
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51   Pennings, Siska, & Bertness, 2001; Siska, Pennings, Buck, & Hanisak, 2002). In the
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53   marine environment, algae collected from tropical sites have higher chemical defenses
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Line numbers in left margin of text (37–61):

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42   **INTRODUCTION**

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45   defense strategies to tolerate and resist herbivory (Núñez-Farfan, Fornoni, & Valverde,

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59   Pennings, Siska, & Bertness, 2001; Siska, Pennings, Buck, & Hanisak, 2002). In the

60   marine environment, algae collected from tropical sites have higher chemical defenses

61   (phenolic compounds), and thus lower palatability, when compared to temperate sites

62   (Bolser & Hay, 1996; Demko et al., 2017).

63   However, both the BIH and the LHDH remain topics of controversy (Anstett,

64   Nunes, Baskett, & Kotanen, 2016; Hillebrand, 2004; A. Moles, 2013) and recent reviews

65   have found weak support for the LHDH both in terrestrial and marine environments.

66   There is conflicting evidence in relation to plant chemical defenses (e.g., plants have

67   more chemical defenses in temperate regions; Moles, Bonser, Poore, Wallis, & Foley,

68   2011), and contradictory results in feeding patterns (Moles et al., 2011; Poore et al., 2012;

69   Zhang, Zhang, Ma, & Shefferson, 2016). Importantly, these reviews, as well as many of

70   the works therein, do not consider important aspects that can drive plant-herbivore

71   interactions when examining these relationships besides chemical defense; such as

72   nutritional quality, tolerance strategies, or environmental resources. In fact, other

73   mechanisms (e.g., changes in cost of defense due to biogeography; Kooyers et al., 2017)

74   may be explaining the latitudinal variability observed in plant defense. It has been

75   pointed out that the solution to this controversy requires new investigations that

76   encompass broad latitudinal geographic areas and that use consistent standardized

77   methods among sites. Also, studies need to consider more types of defense traits (Anstett,

78   Nunes, et al., 2016; Schemske et al., 2009) including defense induction (Anstett, Chen, &

79   Johnson, 2016) and nutritional quality (Moles et al., 2011), and incorporate the use of

80   feeding preference experiments to understand the mechanisms of interaction.

81   Most studies exploring latitudinal patterns in plant defense have focused on

82   herbivore damage and resistance strategies, while latitudinal patterns in tolerance

83   strategies have been largely unexplored (Anstett, Nunes, et al., 2016), yet they are a

84   critical element of plant defense strategies (Núñez-Farfan et al., 2007; Strauss &

85   Agrawal, 1999). The few existing tolerance studies have only measured compensatory

86   growth, and have yielded inconsistent results likely due to biogeographical effects such as

87   similar regrowth responses driven by different selective pressures (e.g., leaf loss by

88   freezing at high latitudes, and herbivore damage at low latitudes; Wieski & Pennings,

89   2014; Woods, Hastings, Turley, Heard, & Agrawal, 2012). Importantly, plants have

90   several other tolerance traits against herbivory (e.g., belowground reserves) whose

91   variations have not been examined in a latitudinal context.

92   The availability of resources greatly influences the production of defensive traits,

93   since resources allocated to defense can trade off with growth and reproduction (Strauss,

94   Rudgers, Lau, & Irwin, 2002). The Resource Availability Hypothesis (RAH; Coley,

95   Bryant, & Chapin, 1985) postulates that plants in resource-rich environments invest in

96   induction of secondary metabolites (produced in response to herbivory) instead of

97   investing in constitutive resistance (produced regardless of the risk of attack; Agrawal &

98   Karban, 1999). However, recent works assessing the predictions of RAH for intraspecific

99   variations in plant defense (intraspecific-RAH), propose the opposite; i.e. that plant

100   populations from resource-rich environments have higher constitutive resistance, which is

101   mediated by the higher herbivory pressure often found in these environments.

102   Accordingly, in low-resource environments, inducibility of resistance traits should be

103   more effective and would trade-off with constitutive resistance (Hahn & Maron, 2016).

104   Regarding tolerance strategies, resources have also been postulated to drive their

105   production. For instance, the Limiting Resource Model (LRM) proposes that plants are

106   more tolerant to herbivory when limiting resources are available and if herbivore damage

107   does not actually hamper the acquisition or use of those resources (Wise & Abrahamson,

108   2007).

109   Since environmental resources (e.g., light, nutrients) change with latitude,

110   latitudinal patterns of both resistance and tolerance defense strategies are also likely to be

111   influenced by resource availability in addition to herbivore pressure or damage.

112 Furthermore, the level of herbivore pressure (i.e., duration and/or intensity) can also shift

113 plant defense responses. While some traits may be more prevalent under high herbivory

114 rates (e.g., induction of secondary metabolites; Dostálek, Rokaya, Maršík, & Rezek,

115 2016), others are induced under moderate pressure (e.g., compensatory growth; Vergés,

116 Pérez, Alcoverro, & Romero, 2008). Understanding how resource availability and

117 herbivory may interact to modify plant defense traits in a latitudinal context is highly

118 relevant, especially in marine environments, given that eutrophication is usually a

119 concern in coastal areas and upwelling events are predicted to increase their intensity due

120 to climate change (García-Reyes et al., 2015; Xiu, Chai, Curchitser, & Castruccio, 2018).

121 However, to our knowledge no studies have analyzed these interactions in a wide

122 latitudinal range.

123 Beyond environmental factors, the suite of defense traits that plants exhibit

124 against herbivory involve different traits that are heritable and influenced by genetic

125 constraints that modulate the expression of these traits (e.g., genetic variation; Andrew,

126 Wallis, Harwood, Henson, & Foley, 2007; O'Reilly-Wapstra, McArthur, & Potts, 2002).

127 In fact, reduction of genotypic richness or increased inbreeding reduce plant resistance to

128 herbivory (Du, Winsor, Smith, DeNicco, & Stephenson, 2008; Moreira, Abdala-Roberts,

129 Parra-Tabla, & Mooney, 2014). Therefore, it is important to consider genetic variations in

130 influencing patterns of plant defense, especially in wide spatial range investigations such

131 as latitudinal studies.

132 Plant-herbivore interactions can have profound effects in ecosystems, especially

133 when involving foundation species, due to direct (e.g., loss of feeding resources) and

134 indirect (e.g., loss of refuge) impacts on associated species as well as on ecosystem

135 properties (e.g., nutrient cycling; Silliman et al., 2013). As foundation species, seagrasses

136 create critical coastal ecosystems and contribute to many ecosystem services (e.g., carbon

137   burial, reduction of coastal erosion; Nordlund, Koch, Barbier, & Creed, 2016). Seagrasses

138   are chemically defended (Zidorn, 2016) and studies have shown that some seagrass traits

139   such as below and aboveground biomass vary with latitude (Clausen, Krause-Jensen,

140   Olesen, & Marbà, 2014; Ruesink et al., 2018; Soissons et al., 2018), yet, to our

141   knowledge, no works have studied latitudinal variations in defenses against herbivory in

142   seagrasses. Importantly, given their critical role as foundation species, variations in the

143   ecological roles of seagrasses are expected in concert with variations in plant traits and

144   interactions with herbivores.

145   The aim of this study was to investigate the applicability of the resource

146   hypotheses (RAHs, LRM) in explaining latitudinal patterns of plant tolerance and

147   resistance traits against herbivory and the potential inductive responses to different

148   herbivory rates. Integrating these elements is necessary in wide latitudinal studies

149   because plants are exposed to gradients of herbivory and resource availability, and thus,

150   their allocation of resources to defense is likely constrained by the interaction between

151   these two factors. We predicted that marine plants from regions with more resources

152   available (e.g. upwelling sites) would allocate more resources to tolerance (following

153   LRM), having higher (following intraspecific-RAH) constitutive resistance and lower

154   inducibility of secondary metabolites under grazing pressure. Consequently, we expected

155   that herbivores would modify their feeding behavior in response to changes in plant traits.

156   We tested these predictions by performing a simulated herbivory experiment with the

157   seagrass *Zostera marina*. *Z. marina* is a dominant foundation species in estuaries and

158   coastal areas across the northern hemisphere and is consumed by a wide variety of

159   grazers including birds and small invertebrates (e.g. amphipods, isopods) which can

160   strongly influence eelgrass abundance and distribution (Kollars et al., 2017; Reynolds,

161   Carr, & Boyer, 2012; Tomas et al., 2011). Although, to our knowledge, there are no

162 comprehensive studies on the levels of herbivory along the sites of this study, numerous

163 herbivores that feed on seagrass such as isopods, amphipods or waterfowl are present in

164 the study sites (Personal observation; Duffy, Macdonald, Rhode, & Parker, 2013;

165 Hayduk, Hacker, Henderson, & Tomas, 2019; Kollars et al., 2017; Reynolds et al., 2012).

166 While the studies that have explored patterns in herbivory pressure in seagrasses do not

167 find a consistent latitudinal tendency (Vergés et al., 2018), temperate seagrasses are

168 expected to suffer increasingly higher herbivory damage due to the poleward expansion

169 of tropical herbivores (Hyndes et al., 2016; Vergés et al., 2014).

170   We replicated the simulated herbivory experiment on several sites across the US

171 west coast, Mexico and Quebec to 1) examine the influence of latitude (and associated

172 changes in some environmental factors) on plant defense strategies against herbivory and

173 to 2) assess the effects of latitude (and associated changes in environmental factors) and

174 herbivory on these strategies and their trade-offs. We followed this manipulative

175 experiments with feeding choice experiments to 3) investigate how herbivore-driven

176 changes in plant traits affect seagrass palatability to other herbivores within the

177 community.

178

179 **MATERIALS AND METHODS**

180   *Experimental design*

181   We performed simulated herbivory experiments at ten study sites (nine of them

182 along the eastern coast of the Pacific Ocean) ranging approximately 20 degrees in latitude

183 (52ºN–32°N), from Calvert Island in Canada to Ensenada in Mexico (Fig. 1, S1 and

184 Table S1). Along this latitudinal range, mean coastal sea surface temperature (SST)

185 decreases from south to north (Fig. 1) while the mean nutrient (i.e., NO_3) concentration

186 exhibits a maximum off the coast of California where coastal upwelling is most intense

187   (Fig, 1b., S2). This upwelling strongly influences the nutrient availability and community

188   structure of coastal reefs (Menge & Menge, 2013) and estuaries in the US west coast

189   (Hayduk et al., 2019; Hessing-Lewis & Hacker, 2013). Indeed, leaf N of seagrasses in

190   study sites exhibits a significant positive correlation with NO_3 values. (Fig. S3). Further

191   descriptions about the abiotic factors of the study sites are provided in the Supplementary

192   material (Study site description S1).

193   The experiment started the first week of May 2015 and was maintained for a total

194   of 10-11 weeks in order to capture plant chemical and size changes. A total of fifteen 4m^2

195   experimental plots, five per treatment, were randomly distributed in shallow subtidal

196   (0.5-1 m at low tide) homogeneous monospecific meadows *Z. marina* (low wave

197   exposure and low natural herbivory) avoiding the edges of meadows or patchy areas.

198   Plots were separated by 3 m and the treatments were randomly assigned to each plot.

199   The experimental treatments consisted of three simulated herbivory levels; control

200   (i.e., natural low levels), moderate (i.e., removal of 40% of maximum leaf length), and

201   high (i.e., removal of 80% of maximum leaf length), with maximum leaf length being

202   quantified initially as the average maximum leaf length of 5 plants measured per plot.

203   Herbivory simulation was performed by leaf clipping, a standard procedure for

204   simulating herbivory from macroherbivores such as fish or birds in seagrasses (Holzer &

205   McGlathery, 2016; Sanmartí, Saiz, Llagostera, Pérez, & Romero, 2014; Tomas,

206   Martinez-Crego, Hernan, & Santos, 2015; Valentine, Blythe, Madhavan, & Sherman,

207   2004; Vergés et al., 2008) that produces similar effects to natural grazing in seagrasses

208   (Fourqurean, Manuel, Coates, Kenworthy, & Smith, 2010; Holzer & McGlathery, 2016).

209   All the seagrass shoots in the plot and 20 cm outside the plot were clipped every two

210   weeks throughout the experimental period. At the end of the experiment, five to ten

211   shoots were collected from the experimental plots to perform analyses of tolerance and

212 resistance traits (see below and Table S2).

Plant tolerance traits

214   Leaf relative growth rate and shoot size are tolerance traits related to compensatory

215 growth response (Tiffin, 2000; Vergés et al., 2008; Table S2). Leaf relative growth rate

216 (RGR; day^{-1}) was measured in five to ten shoots per plot by hole punching leaves two

217 weeks before the end of the experiment following the method of Zieman (1974) and

218 dividing the growth area by the total leaf area of the shoot and the number of days

219 elapsed since punching. Leaf width, maximum leaf length, number of leaves, total leaf

220 area, leaf biomass, sheath area (proxy of shoot size and unaffected by clipping; Ruesink

221 et al., 2018), rhizome biomass, and number of rhizome internodes were measured at the

222 end of the experiment.

223   Nitrogen, carbon and sucrose of the three newest formed leaves (hereafter young

224 leaves) and of rhizomes as well as belowground (i.e. rhizome) starch content were

225 analyzed from pooled plant material collected at the end of the experiment. All these

226 chemical traits are considered tolerance traits (Table S2) since belowground resources

227 can be reallocated to reproduction or regrowth after herbivory (Stowe, Marquis,

228 Hochwender, & Simms, 2000; Tiffin, 2000). N and C in all tissues were analyzed using a

229 Carlo-Erba CNH elemental analyzer. Sucrose and starch were analyzed with the

230 Anthrone assay. A more extensive explanation for the categorization of tolerance traits,

231 and detailed methods for chemical analyses of plant traits are described in the

232 Supplementary material (Methodology S1, Table S2).

Plant resistance traits

234   Specific phenolic compounds, nitrogen, carbon, and fiber content in leaves are

235 considered resistance traits (Table S2) due to their effects in the palatability or

236 digestibility of the plant and therefore herbivore preference (Hernán, Castejón, Terrados,

237 & Tomas, 2019; Sieg & Kubanek, 2013). These traits were analyzed in young leaves

238 from pooled plant material collected at the end of the experiment. Specific phenolic

239 compounds were analyzed with ultra-performance liquid chromatography-tandem mass-

240 spectrometry as in Hernán et al., (2017) with modifications (see Methodology S1). We

241 identified 7 phenolic compounds; Rosmarinic acid (RA), and 6 flavonoids (i.e., apigenin-

242 7-sulfate, APS; diosmetin-7-sulfate, DS; luteolin-7-sulfate, LUTS; luteolin-7-O-β-

243 glucoside, LUTG; luteolin-7,3'-disulfate, LUTS2; luteolin-7-O-β-(6''-malonyl)

244 glucoside, LUTMG). Specific leaf area (SLA= leaf area/ leaf weight; cm^2/g), which is

245 used to evaluate leaf toughness (with high SLA indicating low toughness; Paul et al.,

246 2012), was quantified in five to ten shoots per plot at the end of the experiment. A

247 detailed methodology for all chemical analyses of plant traits can be found in the

248 Supplementary material (Methodology S1).

249 ### *Genetic metrics*

250 Allelic richness and genotypic diversity (clonality) may follow a linear or

251 unimodal latitudinal gradient, reflecting phylogeographic history in which range edges

252 show low diversity and an overall attenuation from south to north. These variables were

253 assessed to be used as covariates to control for genetic variance. Genetic metrics (allelic

254 richness and genotypic richness) were assessed on 20 shoots per site (maintaining 2 m

255 between shoots) with 24 microsatellite loci. Details about the methodology for genetic

256 metrics of eelgrass can be found in the Supplementary material (Methodology S2).

257 ### *Herbivore feeding assays*

258 To examine the effect of simulated herbivory-driven changes on feeding behavior

259 of other herbivores within the community, we conducted a series of three-choice feeding

260 experiments in which we measured tissue consumption (i.e., tissue consumed from one

261 treatment relative to the total tissue consumed). The experiments were performed with an

262 invertebrate grazer commonly found in *Z. marina* seagrass beds along the Central and

263 North Pacific coast, the isopod *Pentidotea resecata*. The isopods were collected from two

264 sites (Coos Bay and Yaquina Bay) and were offered fresh eelgrass leaves without

265 epiphytes collected from all three simulated herbivory treatments of their own sites.

266 Additionally, in order to examine if the feeding patterns observed were driven by

267 mechanical-structural or chemical traits, we performed three-choice feeding experiments

268 with agar-based artificial food (Siska et al., 2002; Tomas et al., 2015). The experiments

269 consisted of 10-15 replicates and ended when approximately 50% of initial material was

270 consumed. Further details about the methodology of the feeding assays are provided in

271 the Supplementary material (Methodology S3).

272   ***Statistical analyses***

273   We calculated one mean value of the response variables measured per plot having

274 thus 5 replicates per treatment and site. We performed a Principal Component Analysis

275 (PCA) with all the 31 plant response variables measured or calculated from the variables

276 measured (e.g., rhizome biomass per internode= rhizome biomass/number of internodes;

277 SLA; RGR) in order to visualize possible patterns of plant defense strategies across

278 clipping treatments and sites (Fig. 2). We tested our predictions (effects of latitude,

279 resource availability, and simulated herbivory treatments, and their interactions) while

280 controlling for potential covariation introduced by the genetic traits using univariate

281 General Linear Models (GLMs). Prior to performing the GLMs, we explored the

282 correlation among our response variables to reduce the number of GLMs to fit (Fig. S3)

283 and selected 15 response variables which are also the more relevant as defense traits (i.e.,

284 leaf RGR, number of leaves, sheath area, rhizome biomass per internode, leaf nitrogen,

285   carbon and sucrose content, rhizome nitrogen, carbon, sucrose and starch content, SLA,

286   leaf fiber content, total flavonoids and specific phenolic compounds) to test our

287   hypotheses. We also explored the correlation between potential explanatory variables

288   (genetic covariates, SST, latitude and NO3) to avoid co-linearity in the model fitting and

289   discarded SST due to its high negative correlation with NO3 and latitude (R^2=-0.7 for

290   both; Fig. S3). Consequently, NO3 and latitude were use as explanatory variables and

291   genotypic richness as a covariate. We reduced the full GLM of each response variable

292   using a stepwise selection procedure to find the model with the lowest Akaike

293   information criterion (AIC) value to avoid type I errors (Table S3). The coefficients,

294   standard errors, and p-values of fixed effects in all minimum adequate models were

295   estimated via iteratively reweighted least squares using the glm function of the core R-

296   package (R Core Team, 2007). Standardized generalized variance-inflation factors were

297   also calculated as an index of multicollinearity. All models were fitted considering a

298   Gaussian (i.e., normal) distribution of errors. Hence, the distribution of the residuals

299   (errors) was checked for normality and response variables were transformed when the

300   distribution of the residuals deviated from normality.

301   For the results of the feeding assays, we tested whether the level of simulated

302   herbivory affected the amount of eelgrass biomass consumed by *P. resecata* using

303   multivariate Hotelling's T^2 test without transformation of the variables. Post hoc analyses

304   were performed with Tukey multiple comparisons of means. The detailed methodology

305   for all statistical analyses is described in the Supplementary material (Methodology S4).

306

RESULTS

308   ***Latitudinal variation of defense traits***

309   We found that both tolerance and resistance traits (except number of leaves and

310   rhizome biomass per internode) varied with latitude.

311   The PCA (Fig 2) differentiates two groups in axis 1 (PC1); the sites at the

312   extremes of the *Z. marina* distribution range, and the sites at intermediate latitudes where

313   the shoots reach larger sizes with higher growth rates and belowground resources (PC1,

314   Fig. 2). The results of the analyses on tolerance and size traits show that leaf sucrose,

315   sheath area and correlated morphometric traits (Fig. S3) peaked at mid-latitudes (Fig. 3

316   and Fig.S4). Carbon-based belowground resources (i.e., rhizome starch, sucrose and C)

317   increased with increasing latitude (Table 1, Fig. 3), whereas belowground N and leaf

318   relative growth rate were lower towards higher latitudes (Table 1, Fig. 3). Genotypic

319   richness statistically influenced all tolerance traits, except rhizome C, but there were no

320   overall consistent patterns with latitude (Table 1).

321   The PCA also shows a separation between mid-latitude and extreme-range

322   populations in relation to resistance traits. In particular, marginal populations seem to

323   invest in mechanical and phenolic defenses (Fig. 2 and Fig. 4). SLA increased with

324   increasing latitude, while leaf fiber, leaf C, and total flavonoids (driven by the most

325   abundant compound; DS) exhibited their minimum values at mid-latitudes (Fig. 4 and

326   Fig. S5). However, some less abundant flavonoids and RA, exhibited the opposite pattern

327   (Fig. S5, Table S4). On the other hand, leaf N content peaked at mid-latitudes, decreasing

328   more towards higher latitudes (Table 1, Fig. 4).

329   ***Resource availability and defense traits***

330   Sheath area as well as rhizome C, N, sucrose and biomass (and correlated traits,

331   Fig S3, S6 and Fig. S7) increased with nitrate availability, while number of leaves and

332   rhizome starch decreased (Table 1, Fig. 5). Leaf N, phenolic compounds and flavonoids

14

333   increased with higher nitrate availability whereas, leaf fiber content exhibited the

334   opposite pattern (Table 1, Table S4, Fig. 6 Fig. S6 and Fig. S7)

335   *Latitudinal patterns of defense traits in response to herbivory*

336   Plant traits did not differ between the control and the moderate herbivory

337   treatments, and only the high herbivory treatment had effects on defense traits in *Z.*

338   *marina*, which affected most of the tolerance traits. For instance, plants exhibited a higher

339   relative growth rate under high simulated herbivory, which was further enhanced under

340   high nutrient availability (Table 1, Fig. 5). Number of leaves, rhizome biomass per

341   internode, and sucrose content of both leaves and rhizomes were lower under the high

342   herbivory treatments. In addition, rhizome C and sheath area exhibited only a significant

343   decrease in high herbivory treatments at high and mid latitudes, respectively (Table 1,

344   Fig. 3). The only traits related to resistance that increased significantly under the high

345   herbivory treatment were SLA and leaf N, being the increase in leaf N stronger towards

346   higher latitudes (Table 1, Fig. 4).

347   *Herbivore responses to simulated herbivory-driven changes in plant palatability*

348   Isopods from both Coos Bay and Yaquina Bay consumed significantly higher

349   amounts of fresh leaf biomass and agar-base food from the high herbivory treatment

350   when compared to the control treatment in the three-choice experiments (Fig. 7).

351

352   **DISCUSSION**

353   This work shows that most eelgrass defense traits against herbivory follow a bell-

354   shaped distribution, which suggests that latitudinal differences in defense traits of *Z.*

355   *marina* are strongly driven by bottom-up forces, supporting the Resource Availability

356   Hypothesis (RAH). Tolerance traits (6 of the 9 traits) were strongly associated with high

357   resource availability (i.e., upwelling) as expected by the limiting resource model (LRM).

358   On the other hand, we found higher investment in resistance traits towards both extremes

359   of the distribution of eelgrass (and not only at lower latitudes), likely influenced by lower

360   resources at these extremes in comparison to mid-latitudes (as proposed by the RAH and

361   contrary to the intraspecific-RAH). Furthermore, none of the *Z. marina* traits analyzed

362   were significantly affected by moderate (i.e. 40% loss) herbivore damage, while high

363   herbivory induced compensatory growth, decreased resistance, and increased palatability,

364   which further enhanced susceptibility to herbivory by other grazers within the

365   community.

366   Most tolerance traits were higher in the sites with higher nutrient availability,

367   coincident with the region of strong upwelling (Schwing & Mendelssohn, 1997).

368   Upwelling brings nutrient-rich waters which also have higher $p\mathrm{CO}_2$ (Feely, Sabine,

369   Hernandez-Ayon, Ianson, & Hales, 2008), thus providing resources that generally limit

370   seagrass productivity (Beer, Bjork, Hellblom, & Axelsson, 2002; Lee, Park, & Kim,

371   2007). In accordance with the LRM, we observed higher belowground resources and

372   aboveground growth rates with higher nutrient availability under herbivory. At mid

373   latitudes, higher resource availability appears to translate into higher aboveground

374   (Ruesink et al., 2018) and belowground per capita biomass. Consequently, populations in

375   high resource availability environments are likely to be better prepared to tolerate

376   herbivory or other disturbances that can remove aboveground biomass (e.g. harsher

377   hydrodynamic conditions) having more resources to invest for regrowth. Our study also

378   indicates that summertime leaf relative growth rate decreases with increasing latitude,

379   perhaps due to lower temperature in northern sites, which has been suggested as an

380   important factor controlling annual seagrass productivity (Clausen et al., 2014). In

381   addition, towards the northern sites, carbon mainly accumulates into carbohydrate

382   reserves in belowground tissues rather than being invested in plant growth, probably as

383   an adaptation to overwintering (Clausen et al., 2014; Robertson & Mann, 1984; Soissons

384   et al., 2018). Indeed, the non-linearity of the relationship of some of the variables with

385   nutrient availability is likely due to this adaptation, leading to lower values of some

386   variables (e.g., number of leaves) at intermediate levels of nitrate concentrations, which

387   correspond to high latitude sites. Moreover, genotypic richness appears to influence most

388   seagrass size traits, which may be related to previous findings of higher eelgrass biomass

389   in more genotypically diverse sites (Hughes & Stachowicz, 2004; Reusch, Ehlers,

390   Hämmerli, & Worm, 2005). It has been suggested that higher genetic diversity increases

391   positive interactions such as resource partitioning, improving performance in more

392   genotypically diverse populations (Hughes, Inouye, Johnson, Underwood, & Vellend,

393   2008).

394   The production of resistance traits also appears to be strongly influenced by

395   available resources. As predicted by the RAH, plants from the sites with high nutrient

396   availability tended to have higher nitrogen and lower fiber contents in leaves, and thus

397   lower constitutive resistance. Indeed, experimental evidence shows that increases in

398   nutrient availability usually lead to higher nutrient content (Hernán et al., 2019; Tomas et

399   al., 2015) and lower fiber production in plant tissues (Goecker, Heck, & Valentine, 2005;

400   Hernán et al., 2019). On the other hand, the pattern that we detected of lower resistance in

401   high-resource areas does not follow the predictions of the intraspecific-RAH, which

402   predicts that resistance is mediated (and higher) by the higher herbivory suffered at

403   resource-rich sites. In fact, some plant species inhabiting high resource environments do

404   not undergo high herbivory rates and thus, the predictions of the intraspecific-RAH may

405   not always be applicable (Lamarre et al., 2012). Such lower investment in structural

406   defenses and higher nutritional quality typically enhance plant susceptibility to herbivory

407   (e.g., Goecker et al., 2005; Hernán et al., 2019; Lucas, 2000; Tomas et al., 2011), and

408   thus would likely make mid-latitude plants more susceptible to consumers.

409   Furthermore, according to LHDH, we would expect a negative linear relationship

410   between the concentrations of phenolic compounds and latitude (Anstett, Nunes, et al.,

411   2016; Rasmann & Agrawal, 2011), and we would also expect an induction in the

412   production of chemical defenses in response to herbivory. Yet, we did not observe either

413   of those phenomena. In fact, in our system, the production of chemical defenses appears

414   to be driven by resource availability rather than herbivory pressure. We found that total

415   flavonoid compounds and total phenolic compounds were slightly higher in higher

416   nutrient availability conditions. Nevertheless, specific compounds exhibited contrasting

417   responses. The most abundant flavonoid (DS) was lower at mid latitudes (Fig. S5) while

418   other less abundant flavonoids (LUTG, LUTMG, LUTS), which have been related to UV

419   protection (LUTG, LUTMG; Mierziak et al., 2014), exhibited the opposite pattern (Fig.

420   S5), increasing with higher nutrient availability (Fig. S7). Likewise, Rosmarinic acid

421   (RA) was one of the most abundant phenolic compounds found in this study. RA has

422   been found to have antibacterial activity (Guan, Saha, & Weinberger, 2019) and has been

423   suggested to play a role in pathogen and herbivore protection (Khan et al., 2019;

424   Simmonds, Stevenson, & Hanson, 2019). This compound exhibited higher concentrations

425   at mid latitudes (Fig. S5) and, in fact, previous studies have found a strong positive

426   correlation among RA and leaf N in seagrasses (Ravn et al., 1994). The idiosyncratic

427   responses in the content of specific phenolic compounds may affect the capacity of plants

428   to defend against pathogens, herbivores and UV-radiation. However, further studies need

429   to be performed in order to actually determine the specific function of many of these

430   particular compounds in seagrasses. On the other hand, genotypic richness seemed to

431   influence some chemical resistance traits such as leaf N, total flavonoids and half of the

432 phenolic compounds identified. Genetic identity influences the production of total

433 phenolic compounds in *Z. marina* (Tomas et al., 2011), and intraspecific (genetically-

434 driven) differences in production of secondary metabolites have major ecosystem effects

435 on *Populus*-based ecosystems (see reviews by Schweitzer et al., 2008; Whitham et al.,

436 2006) highlighting the potential role that intraspecific genetic variations may have in

437 driving chemical composition in eelgrass and consequently influencing herbivory and

438 other important ecological processes (e.g. decomposition, nutrient cycling; Schweitzer et

439 al., 2008; Whitham et al., 2006).

440 The only resistance trait that followed the predictions of the LHDH (i.e. more

441 investment in defense at lower latitudes) was specific leaf area (SLA), with southern

442 populations having tougher leaves (i.e., lower SLA). While higher SLA could potentially

443 be a response to higher grazing impact at the southern sites, it may also be related to

444 higher temperature stress that plants suffer during low tides in southern sites (Ramírez-

445 Valiente, Valladares, Sánchez-Gómez, Delgado, & Aranda, 2014). On the other hand,

446 higher SLA at northern sites may be a consequence of the need to increase plant

447 photosynthetic capacity (and thus leaf area) due to light limitation (Enriquez & Sand-

448 Jensen, 2003).

449 Regarding impacts of simulated herbivory, *Z. marina* plants appear to be able to

450 tolerate moderate levels of herbivory throughout the latitudinal range examined, as those

451 plants had similar traits to control plants, even though they were regularly being denuded

452 of 40% in leaf length. Thus, plants under moderate treatments were able to compensate

453 for leaf loss, and this may have been achieved by increasing photosynthetic activity of the

454 remaining leaf tissue (Tiffin, 2000). On the other hand, high leaf denudation rates did

455 cause negative effects on plants. For example, *Z. marina* exhibited a compensatory

456 growth response, which has been linked to a reduction in belowground resources

457   (Sanmartí et al., 2014), and which was also observed in our study, with an important

458   reduction in rhizome biomass per internode, and sucrose content. Importantly, such

459   reduction was attenuated at mid and high-latitudes, likely as a result of higher resource

460   availability (LRM; Wise & Abrahamson, 2007). Furthermore, rhizomes suffered a

461   stronger reduction of carbon reserves towards northern latitudes, which may be driven by

462   a limitation in carbon uptake often observed at high latitudes as a result of light limitation

463   (Reich & Oleksyn, 2004). In addition, given that at these northern sites leaves had higher

464   nitrogen content (see below), lower C reserves may also be due to the use of carbon for

465   nitrogen assimilation (Touchette & Burkholder, 2000).

466   Interestingly, plants under intense herbivory did not increase their resistance

467   against consumption. On the contrary, we observed that high herbivory rates made leaves

468   more palatable (with decreased toughness and higher nitrogen content), which may have

469   resulted in part from a stimulation of nitrogen uptake due to defoliation under high

470   herbivory (Jaramillo & Detling, 1988; Valentine et al., 2004). The increase of nitrogen

471   pools under intense herbivory was particularly stronger at the northern sites, and this may

472   be a consequence of a lower use of nitrogen for growth (Kerkhoff, Enquist, Elser, &

473   Fagan, 2005; Reich & Oleksyn, 2004). The changes that we observed in SLA and

474   nutrients under high herbivory would likely make these plants even more attractive to

475   grazers. In fact, herbivores are known to perform "cultivation" or "gardening" grazing

476   whereby they maintain certain species or plant tissues that optimize their foraging

477   (Bjorndal, 1985; Preen, 1995), and we indeed observed that this herbivory-driven

478   decrease in resistance made plants consistently more susceptible to consumption by

479   isopods. Consequently, negative impacts of macroherbivores such as fish or waterfowl on

480   eelgrass may be further enhanced by facilitating susceptibility to other consumers within

481   the community. Indeed, isopods from both populations tended to prefer the most clipped

482   leaves, which were also the more nutritious (i.e., high leaf nitrogen content), more tender

483   (i.e., higher SLA) and which had not exhibited any induction of phenolic compounds or

484   other resistance traits. The fact that isopods exhibited the same preferences when we

485   performed the agar-base feeding experiment suggests that nutritional quality and possibly

486   structural traits are driving the feeding behavior of this herbivore (Tomas et al., 2011,

487   2015).

488   Our study highlights the importance that resource availability has in shaping

489   latitudinal patterns of plant defense strategies against herbivory in a dominant foundation

490   species. Furthermore, our results also indicate that while eelgrass populations are highly

491   tolerant to moderate levels of herbivory, they are not adapted to high herbivory.

492   Importantly, tolerance to high herbivory was enhanced in the sites with high resource

493   availability. Although there are no comprehensive studies on patterns of herbivory

494   pressure on *Z. marina*, this adaptation to moderate herbivory could be primarily driven by

495   consumption from waterfowl (Kollars et al., 2017; Rivers & Short, 2007). Importantly,

496   warming-driven range expansion of tropical herbivores, such as fish or sirenians, into

497   temperate areas is already occurring in many regions (e.g., Mediterranean Sea, Gulf of

498   Mexico, Australian coast), strongly enhancing consumption pressure on benthic

499   macrophytes (Hyndes et al., 2016; Vergés et al., 2016, 2014). The establishment of these

500   new species will increase herbivory pressure to these systems, particularly at their lower

501   edge of their distribution. Moreover, migration patterns of waterfowl species are being

502   altered with climate change and degradation of habitats (Ward et al., 2005) and may shift

503   spatial patterns of grazing pressure. These new scenarios of grazing could have strong

504   detrimental effects on temperate seagrasses, not only by directly reducing plant standing

505   stock, but also by reducing resistance traits and facilitating herbivory by other grazers in

506   the community. Such processes could be especially problematic under conditions of high

507 resource availability, because while more resources may initially enable to better tolerate

508 herbivory, they also make plant tissues less resistant to herbivores.

509

510 **REFERENCES**

511 Agrawal, A. A., & Karban, R. (1999). Why induced defenses may be favored over

512 constitutive strategies in plants. In R. Tollrian & C. D. Harwell (Eds.), *The ecology*

513 *and evolution of inducible defenses* (pp. 45–61). Princeton: Princeton University

514 Press. https://doi.org/10.1159/000331745

515 Andrew, R. L., Wallis, I. R., Harwood, C. E., Henson, M., & Foley, W. J. (2007).

516 Heritable variation in the foliar secondary metabolite sideroxylonal in Eucalyptus

517 confers cross-resistance to herbivores. *Oecologia*, *153*, 891–901.

518 https://doi.org/10.1007/s00442-007-0784-1

519 Anstett, D. N., Chen, W., & Johnson, M. T. J. (2016). Latitudinal gradients in induced

520 and constitutive resistance against herbivores. *Journal of Chemical Ecology*, *42*(8),

521 772–781. https://doi.org/10.1007/s10886-016-0735-6

522 Anstett, D. N., Nunes, K. A., Baskett, C., & Kotanen, P. M. (2016). Sources of

523 controversy surrounding latitudinal patterns in herbivory and defense. *Trends in*

524 *Ecology and Evolution*, *31*(10), 789–802. https://doi.org/10.1016/j.tree.2016.07.011

525 Beer, S., Bjork, M., Hellblom, F., & Axelsson, L. (2002). Inorganic carbon utilisation in

526 marine angiosperms (seagrasses). *Functional Plant Biology*, *29*, 349–354.

527 Bjorndal, K. A. (1985). Nutritional ecology of sea turtles. *Copeia*, *3*, 736–751.

528 https://doi.org/10.2307/1444767

529 Bolser, R. C., & Hay, M. E. (1996). Are tropical plants better defended? Palatability and

530 defenses of temperate vs. tropical seaweeds. *Ecology*, *77*(8), 2269–2286.

531 Clausen, K. K., Krause-Jensen, D., Olesen, B., & Marbà, N. (2014). Seasonality of

532　　eelgrass biomass across gradients in temperature and latitude. *Marine Ecology*

533　　*Progress Series*, *506*, 71–85. https://doi.org/10.3354/meps10800

534　Coley, P. D., & Aide, T. M. (1991). Comparison of herbivory and plant defenses in

535　　temperate and tropical broad-leaved forests. In P. W. Price, T. M. Lewinsohm, G.

536　　W. Fernandes, & W. W. Benson (Eds.), *Plant-Animal Interactions : Evolutionary*

537　　*Ecology in Tropical and Temperate Regions* (pp. 25–49). New York: Wiley.

538　Coley, P. D., Bryant, J. P., & Chapin, F. S. (1985). Resource availability and plant

539　　antiherbivore defense. *Science*, *230*(4728), 895–899.

540　Demko, A. M., Amsler, C. D., Hay, M. E., Long, J. D., McClintock, J. B., Paul, V. J., &

541　　Sotka, E. E. (2017). Declines in plant palatability from polar to tropical latitudes

542　　depend on herbivore and plant identity. *Ecology*, *98*(9), 2312–2321.

543　　https://doi.org/10.1002/ecy.1918

544　Dostálek, T., Rokaya, M. B., Maršík, P., & Rezek, J. (2016). Trade-off among different

545　　anti-herbivore defence strategies along an altitudinal gradient. *AoB Plants*, *8*.

546　　https://doi.org/10.1093/aobpla/plw026

547　Du, D., Winsor, J. A., Smith, M., DeNicco, A., & Stephenson, A. G. (2008). Resistance

548　　and tolerance to herbivory changes with inbreeding and ontogeny in a wild gourd

549　　(Cucurbitaceae). *American Journal of Botany*, *95*(1), 84–92.

550　　https://doi.org/10.3732/ajb.95.1.84

551　Duffy, J. E., Macdonald, K. S., Rhode, J. M., & Parker, J. (2013). Grazer diversity,

552　　functional redundancy and productivity in seagrass beds: an experimental test.

553　　*Ecology*, *82*(9), 2417–2434.

554　Enriquez, S., & Sand-Jensen, K. (2003). Variation in light absorption properties of

555　　*Mentha aquatica* L. as a function of leaf form: Implications for plant growth.

556　　*International Journal of Plant Sciences*, *164*(1), 125–136.

557   https://doi.org/10.1086/344759

558 Feely, R. A., Sabine, C. L., Hernandez-Ayon, J. M., Ianson, D., & Hales, B. (2008).

559   Evidence for upwelling of corrosive "acidified" water onto the continental shelf.

560   *Science*, *320*(5882), 1490–1492. https://doi.org/10.1126/science.1155676

561 Fourqurean, J., Manuel, S., Coates, K., Kenworthy, W., & Smith, S. (2010). Effects of

562   excluding sea turtle herbivores from a seagrass bed: Overgrazing may have led to

563   loss of seagrass meadows in Bermuda. *Marine Ecology Progress Series*, *419*, 223–

564   232. https://doi.org/10.3354/meps08853

565 Fritz, R. S., & Simms, E. L. (1992). *Plant Resistance to Herbivores and Pathogens,*

566   *ecology, evolution, and genetics*. (R. S. Fritz & E. L. Simms, Eds.), *Univ. of*

567   *Chicago Press*. Chicago: Univ. of Chicago Press.

568 García-Reyes, M., Sydeman, W. J., Schoeman, D. S., Rykaczewski, R. R., Black, B. A.,

569   Smit, A. J., … Lin, P. (2015). Under Pressure : Climate Change , Upwelling , and

570   Eastern Boundary Upwelling Ecosystems. *Frontiers in Marine Science*, *2*(109), 1–

571   10. https://doi.org/10.3389/fmars.2015.00109

572 Goecker, M. E., Heck, K. L., & Valentine, J. F. (2005). Effects of nitrogen concentrations

573   in turtlegrass *Thalassia testudinum* on consumption by the bucktooth parrotfish

574   *Sparisoma radians* . *Marine Ecology Progress Series*, *286*, 239–248.

575   https://doi.org/10.3354/meps286239

576 Guan, C., Saha, M., & Weinberger, F. (2019). Chemical Defence of a Seagrass against

577   Microfoulers and Its Seasonal Dynamics. *Applied Sciences*, *9*(1258).

578   https://doi.org/10.3390/app9061258

579 Hahn, P. G., & Maron, J. L. (2016). A Framework for Predicting Intraspecific Variation

580   in Plant Defense. *Trends in Ecology and Evolution*, *31*(8), 646–656.

581   https://doi.org/10.1016/j.tree.2016.05.007

582   Hayduk, J. L., Hacker, S. D., Henderson, J. S., & Tomas, F. (2019). Evidence for

583   regional-scale controls on eelgrass (*Zostera marina*) and mesograzer community

584   structure in upwelling-in fl uenced estuaries. *Limnology and Oceanography*, *64*,

585   1120–1134. https://doi.org/10.1002/lno.11102

586   Hernán, G., Castejón, I., Terrados, J., & Tomas, F. (2019). Herbivory and resource

587   availability shift plant defense and herbivore feeding choice in a seagrass system.

588   *Oecologia*, *189*(3), 719–732. https://doi.org/10.1007/s00442-019-04364-6

589   Hernán, G., Ortega, M. J., Gándara, A. M., Castejón, I., Terrados, J., & Tomas, F. (2017).

590   Future warmer seas: Increased stress and susceptibility to grazing in seedlings of a

591   marine habitat-forming species. *Global Change Biology*, (January), 1–14.

592   https://doi.org/10.1111/gcb.13768

593   Hessing-Lewis, M. L., & Hacker, S. D. (2013). Upwelling-influence , macroalgal blooms

594   , and seagrass production; temporal trends from latitudinal and local scales in

595   northeast Pacific estuaries. *Limnology and Oceanography*, *58*(3), 1103–1112.

596   https://doi.org/10.4319/lo.2013.58.3.1103

597   Hillebrand, H. (2004). On the generality of the latitudinal diversity gradient. *American

598   Naturalist*, *163*(2), 192–211. https://doi.org/10.1086/381004

599   Ho, C. K., & Pennings, S. C. (2013). Preference and performance in plant-herbivore

600   interactions across latitude-A study in U.S. Atlantic salt marshes. *PLoS ONE*, *8*(3),

601   1–11. https://doi.org/10.1371/journal.pone.0059829

602   Holzer, K. K., & McGlathery, K. J. (2016). Cultivation grazing response in seagrass may

603   depend on phosphorus availability. *Marine Biology*, *163*(4), 1–11.

604   https://doi.org/10.1007/s00227-016-2855-5

605   Hughes, A. R., Inouye, B. D., Johnson, M. T. J., Underwood, N., & Vellend, M. (2008).

606   Ecological consequences of genetic diversity. *Ecology Letters*, *11*(6), 609–623.

607   https://doi.org/10.1111/j.1461-0248.2008.01179.x

608   Hughes, A. R., & Stachowicz, J. J. (2004). Genetic diversity enhances the resistance of a

609   seagrass ecosystem to disturbance. *Proceedings of the National Academy of*

610   *Sciences*, *101*(24), 8998–9002.

611   Hyndes, G. A., Heck, K. L., Vergés, A., Harvey, E. S., Kendrick, G. A., Lavery, P. S., …

612   Wilson, S. (2016). Accelerating tropicalization and the transformation of temperate

613   seagrass meadows. *BioScience*, *66*(11), 938–948.

614   https://doi.org/10.1093/biosci/biw111

615   Jaramillo, V., & Detling, J. K. (1988). Grazing history, defoliation, and competition:

616   effects on shortgrass production and nitrogen accumulation. *Ecology*, *69*(5), 1599–

617   1608.

618   Kerkhoff, A. J., Enquist, B. J., Elser, J. J., & Fagan, W. F. (2005). Plant allometry,

619   stoichiometry and the temperature-dependence of primary productivity. *Global*

620   *Ecology and Biogeography*, *14*, 585–598. https://doi.org/10.1111/j.1466-

621   822x.2005.00187.x

622   Khan, S., Nji, C., Taning, T., Bonneure, E., Id, S. M., Id, G. S., … Asif, M. (2019).

623   Bioactivity-guided isolation of rosmarinic acid as the principle bioactive compound

624   from the butanol extract of Isodon rugosus against the pea aphid , Acyrthosiphon

625   pisum. *PLoS ONE*, *14*(6), 1–14.

626   Kollars, N. M., Henry, A. K., Whalen, M. A., Boyer, K. E., Cusson, M., Eklöf, J. S., …

627   Ruesink, J. L. (2017). Meta-Analysis of Reciprocal Linkages between Temperate

628   Seagrasses and Waterfowl with Implications for Conservation. *Frontiers in Plant*

629   *Science*, *8*(December), 1–14. https://doi.org/10.3389/fpls.2017.02119

630   Kooyers, N. J., Blackman, B. K., & Holeski, L. M. (2017). Optimal defense theory

631   explains deviations from latitudinal herbivory defense hypothesis. *Ecology*, *98*(4),

632   1036–1048. https://doi.org/10.1002/ecy.1731

633   Lamarre, G. P. A., Baraloto, C., Fortunel, C., Dávila, N., Mesones, I., Grandez-Rios, J.,

634   … Fine, P. V. A. (2012). Herbivory , growth rates , and habitat specialization in

635   tropical tree lineages : implications for Amazonian beta-diversity. *Ecology*, *93*(8),

636   195–210. Retrieved from http://www.esajournals.org/doi/full/10.1890/11-0397.1

637   Lee, K.-S., Park, S. R., & Kim, Y. K. (2007). Effects of irradiance, temperature, and

638   nutrients on growth dynamics of seagrasses: A review. *Journal of Experimental*

639   *Marine Biology and Ecology*, *350*(1–2), 144–175.

640   https://doi.org/10.1016/j.jembe.2007.06.016

641   Lucas, P. (2000). Mechanical defences to herbivory. *Annals of Botany*, *86*(5), 913–920.

642   https://doi.org/10.1006/anbo.2000.1261

643   Menge, B. A., & Menge, D. N. L. (2013). Dynamics of coastal meta-ecosystems: the

644   intermittent upwelling hypothesis and a test in rocky intertidal regions. *Ecological*

645   *Monographs*, *83*(3), 283–310.

646   Mierziak, J., Kostyn, K., & Kulma, A. (2014). Flavonoids as important molecules of

647   plant interactions with the environment. *Molecules*, *19*, 16240–16265.

648   https://doi.org/10.3390/molecules191016240

649   Moles, A. (2013). Dogmatic is problematic: Interpreting evidence for latitudinal gradients

650   in herbivory and defense. *Ideas in Ecology and Evolution*, *6*(1), 1–4.

651   https://doi.org/10.4033/iee.2013.6.1.c

652   Moles, A. T., Bonser, S. P., Poore, A. G. B., Wallis, I. R., & Foley, W. J. (2011).

653   Assessing the evidence for latitudinal gradients in plant defence and herbivory.

654   *Functional Ecology*, *25*(2), 380–388. https://doi.org/10.1111/j.1365-

655   2435.2010.01814.x

656   Moreira, X., Abdala-Roberts, L., Parra-Tabla, V., & Mooney, K. A. (2014). Positive

657   effects of plant genotypic and species diversity on anti-herbivore defenses in a

658   tropical tree species. *PLoS ONE*, *9*(8). https://doi.org/10.1371/journal.pone.0105438

659   Nordlund, L. M., Koch, E. W., Barbier, E. B., & Creed, J. C. (2016). Seagrass ecosystem

660   services and their variability across genera and geographical regions. *PLoS ONE*,

661   *11*(10), 1–23. https://doi.org/10.1371/journal.pone.0163091

662   Núñez-Farfan, J., Fornoni, J., & Valverde, P. (2007). The evolution of resistance and

663   tolerance to herbivores. *Annual Review of Ecology Evolution and Systematics*, *38*,

664   541–566. https://doi.org/doi:10.1146/annurev.ecolsys.38.091206.095822

665   O'Reilly-Wapstra, J. M., McArthur, C., & Potts, B. M. (2002). Genetic variation in

666   resistance of Eucalyptus globulus to Marsupial browsers. *International Association*

667   *for Ecology*, *130*(2), 289–296. https://doi.org/10.1007/S004420100797

668   Paul, G. S., Montagnini, F., Berlyn, G. P., Craven, D. J., van Breugel, M., & Hall, J. S.

669   (2012). Foliar herbivory and leaf traits of five native tree species in a young

670   plantation of Central Panama. *New Forests*, *43*(1), 69–87.

671   https://doi.org/10.1007/s11056-011-9267-7

672   Pennings, S. C., Siska, E. L., & Bertness, M. D. (2001). Latitudinal differences in plant

673   palatability in Atlantic coast salt marshes. *Ecology*, *82*(5), 1344–1359.

674   https://doi.org/10.1890/0012-9658(2001)082[1344:LDIPPI]2.0.CO;2

675   Poore, A. G. B., Campbell, A. H., Coleman, R. A., Edgar, G. J., Jormalainen, V.,

676   Reynolds, P. L., … Emmett Duffy, J. (2012). Global patterns in the impact of

677   marine herbivores on benthic primary producers. *Ecology Letters*, *15*(8), 912–922.

678   https://doi.org/10.1111/j.1461-0248.2012.01804.x

679   Preen, A. (1995). Impacts of dugong foraging on seagrass habitats: observational and

680   experimental evidence for cultivation grazing. *Marine Ecology Progress Series*, *124*,

681   201–213. Retrieved from http://www.int-res.com/articles/meps/124/m124p201.pdf

682   R Core Team. (2007). R: a language and environment for statistical Computing. Viena,

683   Austria: R Foundation for Statistical Computing.

684   Ramírez-Valiente, J. A., Valladares, F., Sánchez-Gómez, D., Delgado, A., & Aranda, I.

685   (2014). Population variation and natural selection on leaf traits in cork oak

686   throughout its distribution range. *Acta Oecologica*, *58*, 49–56.

687   https://doi.org/10.1016/j.actao.2014.04.004

688   Rasmann, S., & Agrawal, A. A. (2011). Latitudinal patterns in plant defense: Evolution

689   of cardenolides, their toxicity and induction following herbivory. *Ecology Letters*,

690   *14*(5), 476–483. https://doi.org/10.1111/j.1461-0248.2011.01609.x

691   Ravn, H., Pedersen, M. F., Borum, J., Andary, C., Anthoni, U., Christophen, C., &

692   Nielsen, P. H. (1994). Seasonal variation and distribution of two phenolic

693   compounds, rosmarinic acid and caffeic acid, in leaves and roots-rhizomes of

694   eelgrass (*Zostera marina* L.). *Ophelia*, *40*(1), 51–61.

695   Reich, P. B., & Oleksyn, J. (2004). Global patterns of plant leaf N and P in relation to

696   temperature and latitude. *Proceedings of the National Academy of Sciences*, *101*(30),

697   11001–11006.

698   Reusch, T. B. H., Ehlers, A., Hämmerli, A., & Worm, B. (2005). Ecosystem recovery

699   after climatic extremes enhanced by genotypic diversity. *Proceedings of the*

700   *National Academy of Sciences*, *102*(8), 2826–2831.

701   Reynolds, L. K., Carr, L. A., & Boyer, K. E. (2012). A non-native amphipod consumes

702   eelgrass inflorescences in San Francisco Bay. *Marine Ecology Progress Series*,

703   *451*(April), 107–118. https://doi.org/10.3354/meps09569

704   Rivers, D. O., & Short, F. T. (2007). Effect of grazing by Canada geese *Branta*

705   *canadensis* on an intertidal eelgrass *Zostera marina* meadow. *Marine Ecology*

706   *Progress Series*, *333*(Ganter 2000), 271–279. https://doi.org/10.3354/meps333271

707   Robertson, A. I., & Mann, K. H. (1984). Disturbance by ice and life-history adaptations

708   of the seagrass *Zostera marina*. *Marine Biology*, *80*(2), 131–141.

709   https://doi.org/10.1007/BF02180180

710   Rosenthal, J. P., & Kotanen, P. M. (1994). Terrestrial plant tolerance to herbivory. *Trends*

711   *in Ecology & Evolution*, *9*(4), 145–148. https://doi.org/10.1016/0169-

712   5347(94)90180-5

713   Ruesink, J. L., Stachowicz, J. J., Reynolds, P. L., Boström, C., Cusson, M., Douglass, J.,

714   … Duffy, J. E. (2018). Form–function relationships in a marine foundation species

715   depend on scale: a shoot to global perspective from a distributed ecological

716   experiment. *Oikos*, *127*(3), 364–374. https://doi.org/10.1111/oik.04270

717   Sanmartí, N., Saiz, L., Llagostera, I., Pérez, M., & Romero, J. (2014). Tolerance

718   responses to simulated herbivory in the seagrass *Cymodocea nodosa*. *Marine*

719   *Ecology Progress Series*, *517*, 159–169. https://doi.org/10.3354/meps11084

720   Schemske, D. W., Mittelbach, G. G., Cornell, H. V, Sobel, J. M., & Roy, K. (2009). Is

721   there a latitudinal gradient in the importance of biotic interactions? *Annual Review*

722   *of Ecology Evolution and Systematics*, *40*(2009), 245–269.

723   https://doi.org/10.1146/annurev.ecolsys.39.110707.173430

724   Schweitzer, J. A., Madritch, M. D., Bailey, J. K., Leroy, C. J., Fischer, D. G., Rehill, B.

725   J., … Whitham, T. G. (2008). From Genes to Ecosystems : The Genetic Basis of

726   Condensed Tannins and Their Role in Nutrient Regulation in a Populus Model

727   System. *Ecosystems*, *11*, 1005–1020. https://doi.org/10.1007/s10021-008-9173-9

728   Schwing, F. B., & Mendelssohn, R. (1997). Increased coastal upwelling in the California

729   Current System, *102*, 3421–3438.

730   Sieg, R. D., & Kubanek, J. (2013). Chemical Ecology of Marine Angiosperms :

731   Opportunities at the Interface of Marine and Terrestrial Systems. *Journal of*

732   *Chemical Ecology*, *39*, 687–711. https://doi.org/10.1007/s10886-013-0297-9

733   Silliman, B. R., Mccoy, M. W., Angelini, C., Holt, R. D., Griffin, J. N., & Koppel, J. Van

734   De. (2013). Consumer fronts, global change, and runaway collapse in ecosystems.

735   *Annual Review of Ecology, Evolution, and Systematics*, *44*, 503–538.

736   https://doi.org/10.1146/annurev-ecolsys-110512-135753

737   Simmonds, M. S., Stevenson, P. C., & Hanson, F. E. (2019). Rosmarinic acid in Canna

738   generalis activates the medial deterrent chemosensory neurone and deters feeding in

739   the tobacco hornworm Manduca sexta. *Physiological Entomology*, *44*, 140–147.

740   https://doi.org/10.1111/phen.12284

741   Siska, E. L., Pennings, S. C., Buck, T. ., & Hanisak, M. D. (2002). Latitudinal variation

742   in palatability of salt-marsh plants: which traits are responsible? *Ecology*, *83*(12),

743   3369–3381.

744   Soissons, L. M., Haanstra, E. P., van Katwijk, M. M., Asmus, R., Auby, I., Barillé, L., …

745   Bouma, T. J. (2018). Latitudinal Patterns in European Seagrass Carbon Reserves:

746   Influence of Seasonal Fluctuations versus Short-Term Stress and Disturbance

747   Events. *Frontiers in Plant Science*, *9*(February), 88.

748   https://doi.org/10.3389/fpls.2018.00088

749   Stowe, K. A., Marquis, R. J., Hochwender, C. G., & Simms, E. L. (2000). The

750   evolutionary ecology of tolerance to consumer damage. *Annual Review of Ecology

751   and Systematics*, *31*(2000), 565–595. Retrieved from

752   http://www.jstor.org/stable/221743

753   Strauss, S. Y., & Agrawal, A. A. (1999). The ecology and evolution of plant tolerance to

754   herbivory. *Trends in Ecology and Evolution*, *14*(5), 179–185.

755   https://doi.org/10.1016/S0169-5347(98)01576-6

756   Strauss, S. Y., Rudgers, J. A., Lau, J. A., & Irwin, R. E. (2002). Direct and ecological

757 costs of resistance to herbivory. *Trends in Ecology and Evolution*, *17*(6), 278–285.

758 https://doi.org/10.1016/S0169-5347(02)02483-7

759 Tiffin, P. (2000). Mechanisms of tolerance to herbivore damage: What do we know?

760 *Evolutionary Ecology*, *14*(4–6), 523–536. https://doi.org/10.1023/A:1010881317261

761 Tomas, F., Abbott, J. M., Steinberg, C., Balk, M., Williams, S. L., & Stachowicz, J. J.

762 (2011). Plant genotype and nitrogen loading influence seagrass productivity,

763 biochemistry, and plant-herbivore interactions. *Ecology*, *92*(9), 1807–1817.

764 Tomas, F., Martinez-Crego, B., Hernan, G., & Santos, R. (2015). Responses of seagrass

765 to anthropogenic and natural disturbances do not equally translate to its consumers.

766 *Global Change Biology*, *21*(11), 1–10. https://doi.org/10.1111/gcb.13024

767 Touchette, B. W., & Burkholder, J. A. M. (2000). Overview of the physiological ecology

768 of carbon metabolism in seagrasses. *Journal of Experimental Marine Biology and*

769 *Ecology*, *250*(1–2), 169–205. https://doi.org/10.1016/S0022-0981(00)00196-9

770 Valentine, J. F., Blythe, E. F., Madhavan, S., & Sherman, T. D. (2004). Effects of

771 simulated herbivory on nitrogen enzyme levels, assimilation and allocation in

772 *Thalassia testudinum*. *Aquatic Botany*, *79*(3), 235–255.

773 https://doi.org/10.1016/j.aquabot.2004.04.001

774 Vergés, A., Doropoulos, C., Czarnik, R., McMahon, K., Llonch, N., & G. B. Poore, A.

775 (2018). Latitudinal variation in seagrass herbivory: Global patterns and explanatory

776 mechanisms. *Global Ecology and Biogeography*, (July 2017), 1068–1079.

777 https://doi.org/10.1111/geb.12767

778 Vergés, A., Doropoulos, C., Malcolm, H. A., Skye, M., Garcia-Pizá, M., Marzinelli, E.

779 M., … Steinberg, P. D. (2016). Long-term empirical evidence of ocean warming

780 leading to tropicalization of fish communities, increased herbivory, and loss of kelp.

781 *Proceedings of the National Academy of Sciences*, *113*(48), 13791–13796.

782   https://doi.org/10.1073/pnas.1610725113

783 Vergés, A., Pérez, M., Alcoverro, T., & Romero, J. (2008). Compensation and resistance

784   to herbivory in seagrasses: induced responses to simulated consumption by fish.

785   *Oecologia*, *155*(4), 751–760. https://doi.org/10.1007/s00442-007-0943-4

786 Vergés, A., Steinberg, P. D. ., Hay, M. E., Poore, A. G. B., Campbell, A. H., Ballesteros,

787   E., … Wilson, S. K. (2014). The tropicalization of temperate marine ecosystems:

788   climate-mediated changes in herbivory and community phase shifts. *Proceedings of*

789   *The Royal Society B*, *281*(1789), 1–10. https://doi.org/10.1098/rspb.2014.0846

790 Ward, D. H., Reed, A., Sedinger, J. S., BLack, J. M., Derksen, D. V., & Castelli, P. M.

791   (2005). North American Brant: Effects of changes in habitat and climate on

792   population dynamics. *Global Change Biology*, *11*(6), 869–880.

793   https://doi.org/10.1111/j.1365-2486.2005.00942.x

794 Whitham, T. G. W., Bailey, J. K., Schweitzer, J. A., Shuster, S. M., Bangert, R. K.,

795   LeRoy, C. J., … Wooley, S. C. (2006). A framework for community and ecosystem

796   genetics : from genes to ecosystems. *Nature Review Genetics*, *7*(7), 510–523.

797   https://doi.org/10.1038/nrg1877

798 Wieski, K., & Pennings, S. (2014). Latitudinal variation in resistance and tolerance to

799   herbivory of a salt marsh shrub. *Ecography*, *37*(8), 763–769.

800   https://doi.org/10.1111/ecog.00498

801 Wise, M. J., & Abrahamson, W. G. (2007). Effects of resource availability on tolerance

802   of herbivory: a review and assessment of three opposing models. *The American*

803   *Naturalist*, *169*(4), 443–454. https://doi.org/10.1086/512044

804 Wisz, M. S., Pottier, J., Kissling, W. D., Pellissier, L., Lenoir, J., Damgaard, C. F., …

805   Svenning, J. C. (2013). The role of biotic interactions in shaping distributions and

806   realised assemblages of species: Implications for species distribution modelling.

807   *Biological Reviews*, *88*(1), 15–30. https://doi.org/10.1111/j.1469-

808   185X.2012.00235.x

809   Woods, E. C., Hastings, A. P., Turley, N. E., Heard, S. B., & Agrawal, A. A. (2012).

810   Adaptive geographical clines in the growth and defense of a native plant. *Ecological*

811   *Monographs*, *82*(2), 149–168. https://doi.org/10.1890/11-1446.1

812   Xiu, P., Chai, F., Curchitser, E. N., & Castruccio, F. S. (2018). Future changes in coastal

813   upwelling ecosystems with global warming : The case of the California Current

814   System. *Scientific Reports*, (October 2017), 1–9. https://doi.org/10.1038/s41598-

815   018-21247-7

816   Zhang, S., Zhang, Y., Ma, K., & Shefferson, R. (2016). Latitudinal variation in herbivory:

817   hemispheric asymmetries and the role of climatic drivers. *Journal of Ecology*,

818   *104*(4), 1089–1095. https://doi.org/10.1111/1365-2745.12588

819   Zidorn, C. (2016). Secondary metabolites of seagrasses (Alismatales and

820   Potamogetonales; Alismatidae): Chemical diversity, bioactivity, and ecological

821   function. *Phytochemistry*, *124*, 5–28.

822   https://doi.org/10.1016/j.phytochem.2016.02.004

823   Zieman, J. (1974). Methods for the study of the growth and production of Turtlegrass,

824   *Thalassia Testudinum* Konig. *Aquaculture*, *4*, 139–143.

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827 **DATA AVAILABILITY**

828   All data supporting the results in the paper are publicly available at Figshare data

829 repository 10.6084/m9.figshare.13048871.

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Table 1: Results of GLM on defense traits. Intercept (i) and slopes of latitude (β_{lat}), herbivory simulation moderate (β_H) and high level (β_{HH}), nitrate (β_{NO3}), their interactions (marked with asterisc), and genotypic richness ($\beta_{G.R.}$). Sqrt, Ln arcsin and logit indicate square root, napierian logarithm, arcsine and logit transformation respectively. SLA indicates specific leaf area; TF Total flavonoids; TPC total phenolic compounds; Est, estimate; SE, standard error and P, p-value (n=5 per treatment and site).

		i	β_{lat}	β_H	β_{HH}	β_{NO3}	$\beta_{G.R.}$	β_{lat*H}	β_{lat*HH}	β_{NO3*H}	β_{NO3*HH}
Sqrt (Relative Growth Rate (d-1))	Est.	0.39	-0.003	0.002	0.013	-0.003	-0.099			0.007	0.011
	SE	0.033	<0.001	0.012	0.012	0.004	0.026			0.005	0.05
	P	**<0.001**	**<0.001**	0.892	0.284	0.45	**<0.001**			0.149	**0.027**
Number of leaves	Est.	2.825		0.106	-0.396	-0.239	3.117				
	SE	0.469		0.14	0.139	0.039	0.5				
	P	**<0.001**		0.452	0.06	**<0.001**	**<0.001**				
Sheath area(cm2)	Est.	22.102	0.757	4.214	12.944	2.324	-45.043	-0.148	-0.489		
	SE	8.28	0.159	9.423	9.423	0.386	5.008	0.225	0.225		
	P	**0.009**	**<0.001**	0.655	0.172	**<0.001**	**<0.001**	0.512	**0.031**		
Log (Rhizome biomass (grFW/internode))	Est.	0.665		-0.078	-0.671	0.387	-3.487				
	SE	0.512		0.153	0.153	0.042	0.546				
	P	0.196		0.61	**<0.001**	**<0.001**	**<0.001**				
Leaf sucrose (%)	Est.	5.276	0.318	-0.483	-1.855		-11.55				
	SE	3.966	0.058	0.878	0.878		3.155				
	P	0.186	**<0.001**	0.583	**0.037**		**<0.001**				
Logit (Rhizome starch(%))	Est.	0.216	0.03			-0.8					
	SE	0.33	0.008			0.032					
	P	0.514	**<0.001**			**0.016**					
arcsine (Rhizome sucrose (%))	Est.	-0.027	0.002	0.001	-0.004	0.006	-0.011				
	SE	0.009	<0.001	0.002	0.002	0.006	0.007				
	P	**0.003**	**<0.001**	0.537	**0.025**	**<0.001**	0.135				
Logit Rhizome C (%)	Est.	-1.674	0.018	0.217	0.248	0.029	0.14	-0.006	-0.007		
	SE	0.125	0.002	0.142	0.142	0.006	0.076	0.003	0.003		
	P	**<0.001**	**<0.001**	0.13	0.084	**<0.001**	0.066	0.105	**0.037**		
Rhizome N (%)	Est.	2.829	-0.027			0.044	-0.835				
	SE	0.309	0.005			0.019	0.247				
	P	**<0.001**	**<0.001**			**0.023**	**<0.001**				
SLA(cm2/g)	Est.	-419.589	23.442	23.189	178.176						
	SE	176.321	4.111	62.413	62.413						
	P	**0.019**	**<0.001**	0.711	**0.005**						
Logit (Fibre (%))	Est.	-0.157	-0.034			-0.148	1.326				
	SE	0.25	0.004			0.015	0.2				
	P	0.532	**<0.001**			**<0.001**	**<0.001**				
Logit (LeafC (%))	Est.	-0.929	0.005				0.097				
	SE	0.076	0.001				0.061				
	P	**<0.001**	**<0.001**				0.112				
Logit (Leaf N (%))	Est.	-1.231	-0.007	-0.045	-0.116	0.286	-0.231	0.001	0.005	-0.001	-0.016
	SE	0.081	0.002	0.092	0.092	0.07	0.049	0.002	0.002	0.009	0.009
	P	**<0.001**	**<0.001**	0.629	0.21	**<0.001**	**<0.001**	0.533	**0.02**	0.876	0.081
Sqrt (TF (mg/gDW))	Est.	3.613	-0.045			0.078	1.81				
	SE	0.802	0.012			0.05	0.643				
	P	**<0.001**	**<0.001**			0.118	**0.006**				
Sqrt (TPC (mg/gDW))	Est.	3.007	0.027			0.143					
	SE	0.646	0.015			0.064					
	P	**<0.001**	0.077			**0.027**					

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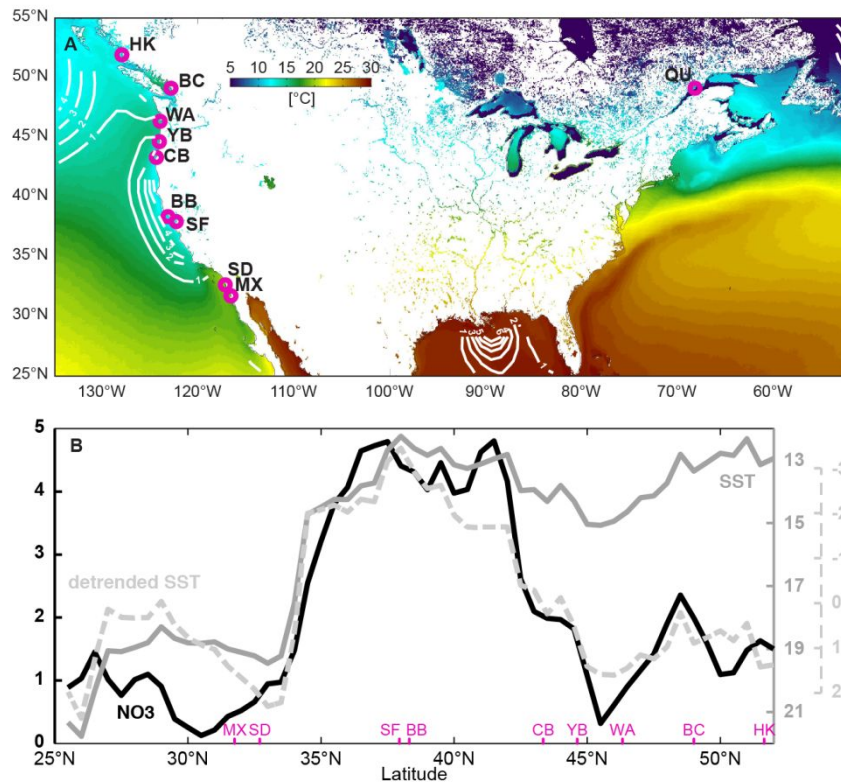48
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FIGURES

Figure 1. (a) Mean May-June-July satellite SST derived from the 1982-2008 AVHRR Pathfinder Project data set. White contours indicate mean May-June-July NO_3 (μmol/L) from the 1878-2012 World Ocean Atlas data set. The locations of the study sites; Calvert (Hakai, Canada; HK), Baie St. Ludger (Quebec, Canada; QU), Tsawwaseen (British Columbia, Canada; BC), Willapa (Washington, United States; WA), Yaquina Bay (Oregon, US; YB), Coos Bay (Oregon, US; CB), West side Regional Park (Bodega Bay, California, US; BB), Point Molate (San Francisco, California, US; SF), San Diego Bay (San Diego, California, US; SD) and Punta Banda Estuary (Ensenada, Baja California, Mexico; MX), are indicated with magenta circles. (b) Comparison of the May-June-July NO_3 (μmol/L; black), with the May June July SST (ºC; grey) and the SST anomaly (SST without latitudinal gradient; dashed light grey) along the Pacific coast. Note the reverse axis for the SST and the SST anomaly (i.e., detrended SST).
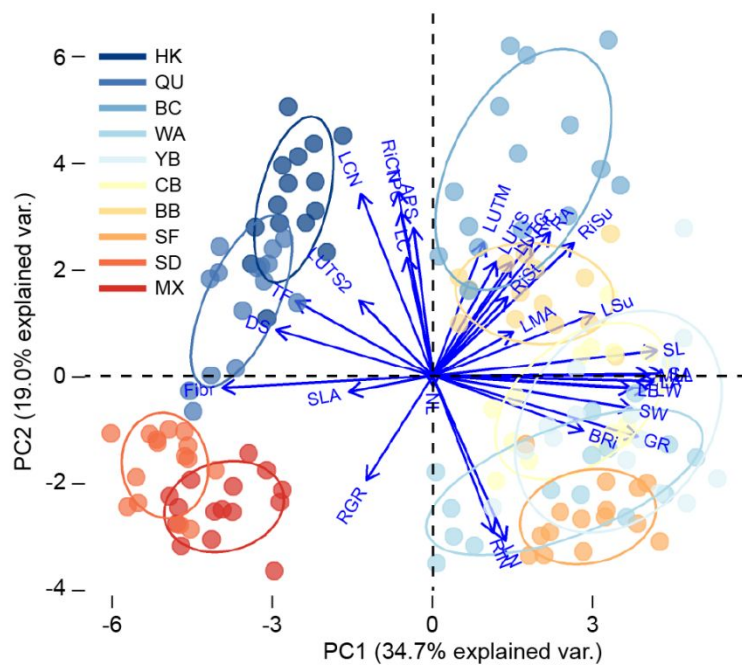
Figure 2. Principal Component Analysis. Arrows represent the loading of the variables. Dots represent the score of each replicate on PC1 and PC2. Circles group the replicates within each site and sites are coded as in Figure 1. Leaf relative growth rate (RGR), number of leaves (NL), leaf width (LW), leaf biomass (LB), leaf area (LA), maximum leaf length (MLL), sheath area (SA), leaf mass area (LMA), sheath length (SL), sheath width (SW), biomass of rhizome per internode (BRi), leaf sucrose content (LSu), rhizome starch content (RiSt), rhizome sucrose content (RiSu), rhizome nitrogen content (RiN), rhizome carbon content (RiC), rhizome C/N (RiCN), specific leaf area (SLA), leaf nitrogen content (LN), leaf carbon content (LC), leaf C/N (LCN), and leaf fiber (Fiber), total flavonoids (TF), total phenolic compounds (TPC), flavonoids (LUTG, LUTS2 ,LUTMG, LUTS, APS, DS) and rosmarinic acid (RA).
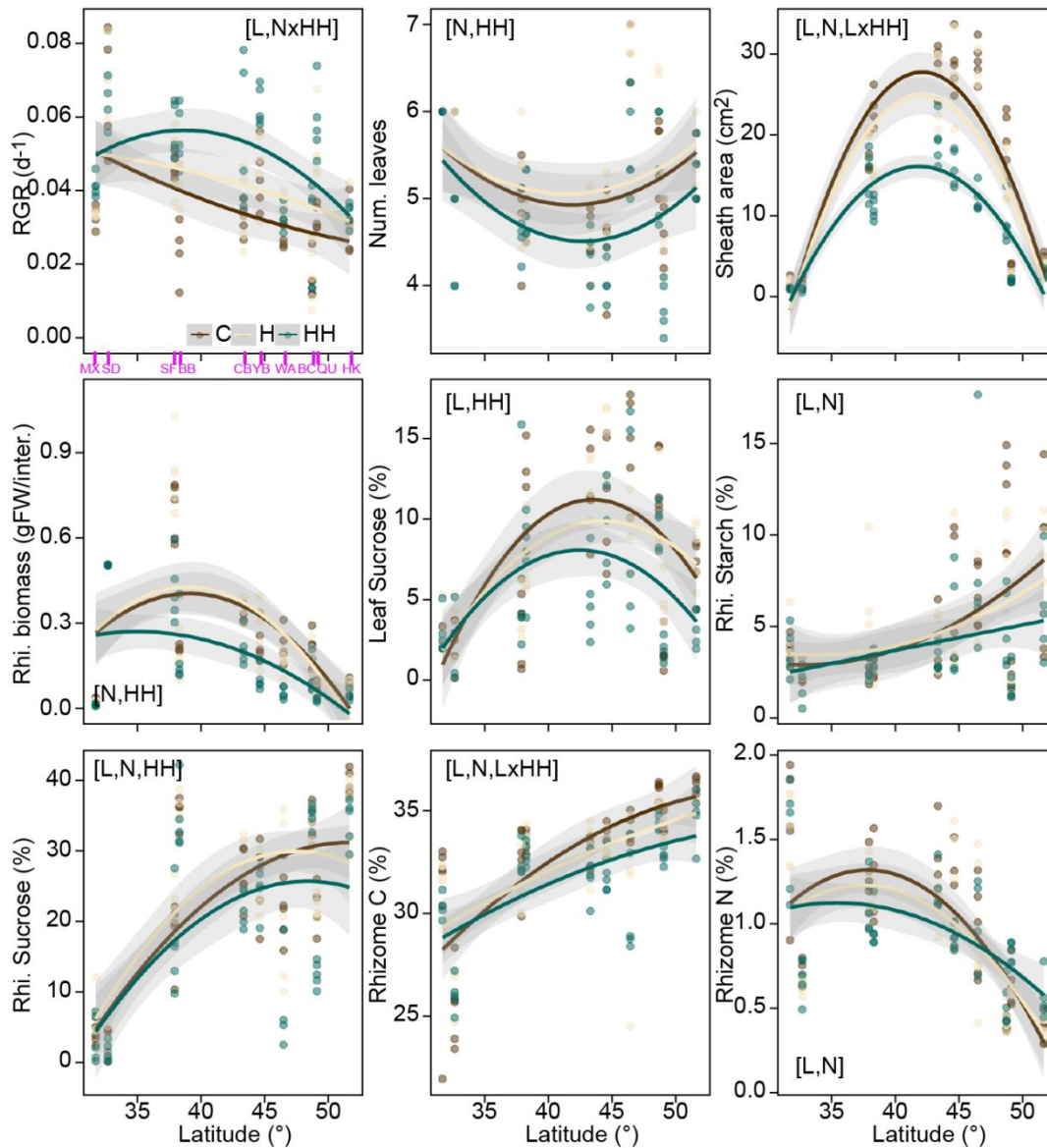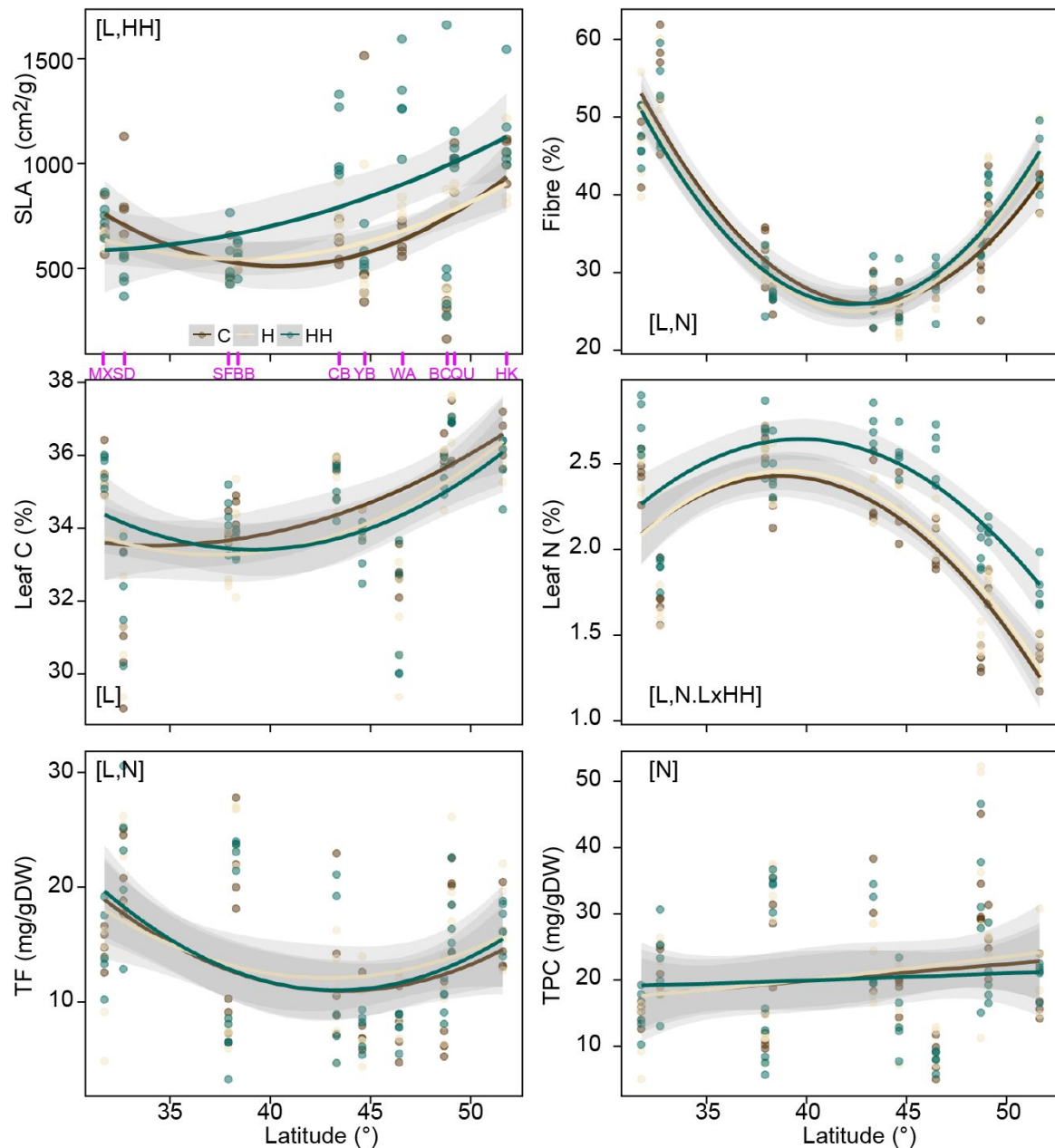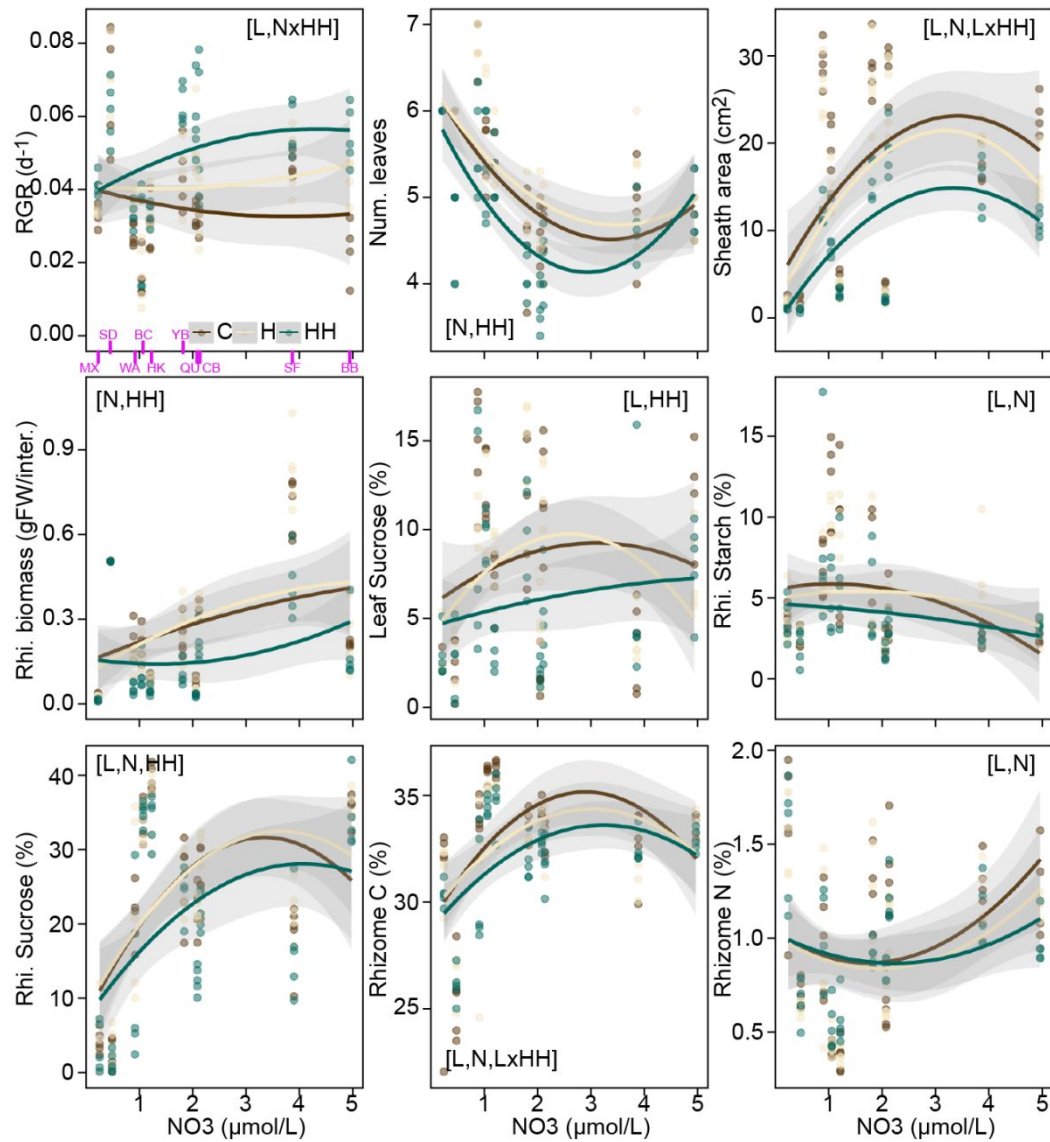
Figure 3. Distribution of tolerance traits along the latitudinal gradient. Grey bands indicate 95% confidence boundaries for the control (C; dark brown), moderate (H; beige) and high (HH; green) herbivory treatment fitted curves (general linear models). Letters indicate statistical differences due to latitude (L), nitrate (N), high herbivore treatment (HH), and interactions. Rhi= rhizome, RGR= Relative growth rate. The first panel shows in magenta the latitude of the study sites (n=5 per treatment and site).

Figure 4. Distribution of resistance traits along the latitudinal gradient. Grey bands indicate 95% confidence boundaries for the control (C; dark brown), moderate (H; beige) and high (HH; green) herbivory treatment fitted curves (general linear models). Letters indicate statistical differences due to latitude (L), nitrate (N), high herbivore treatment (HH), and interactions. SLA= specific leaf area, TF=Total flavonoids, TPC= total phenolic compounds. The first panel shows in magenta the latitude of the study sites (n=5 per treatment and site).

Figure 5. Distribution of tolerance traits along the nitrate availability gradient. Grey bands indicate 95% confidence boundaries for the control (C; dark brown), moderate (H; beige) and high (HH; green) herbivory treatment fitted curves (general linear models). Letters indicate statistical differences due to latitude (L), nitrate (N) high herbivore treatment (HH), and interactions. The first panel shows in magenta the latitude of the study sites (n=5 per treatment and site).
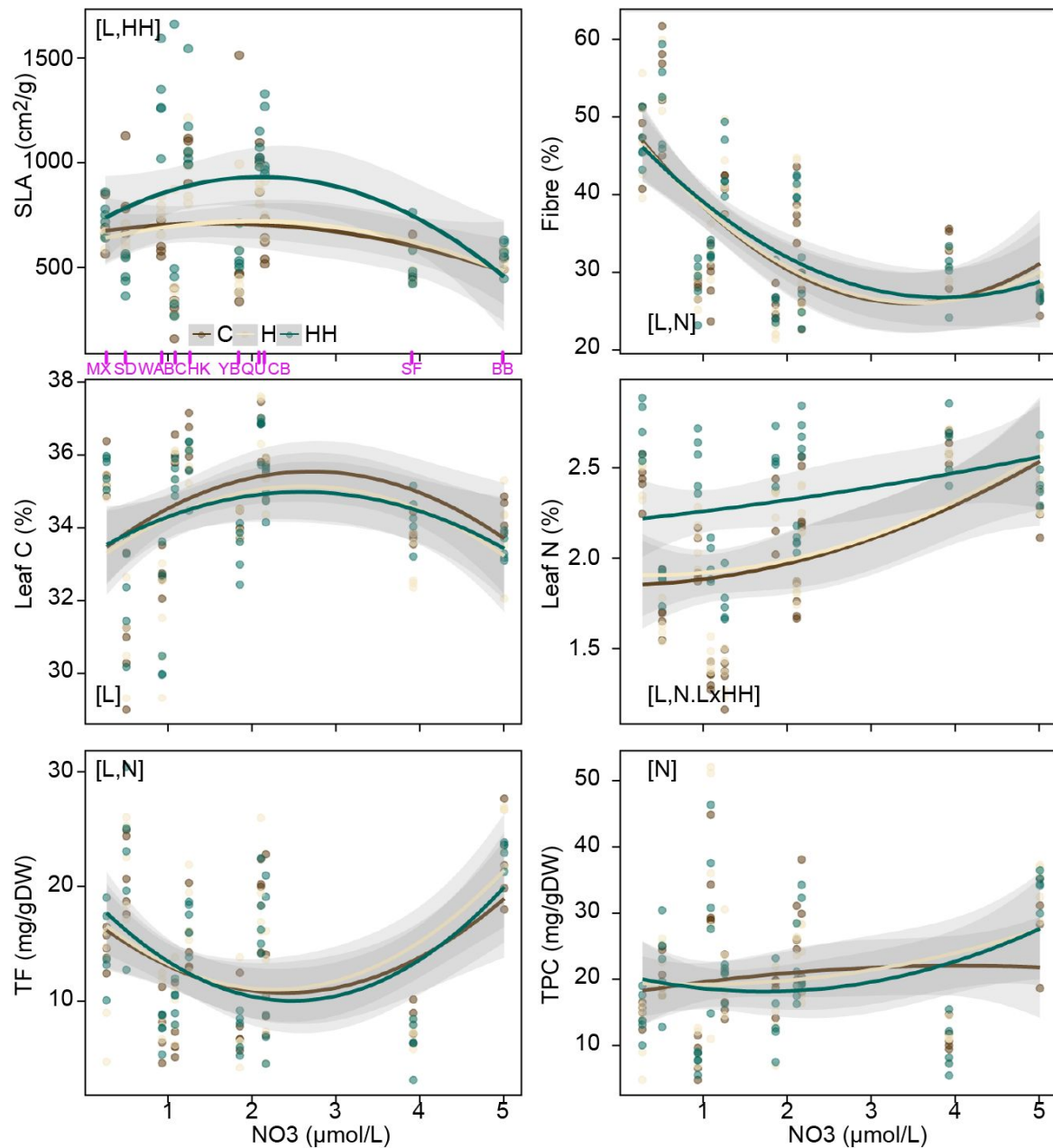
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Figure 6. Distribution of resistance traits along the nitrate availability gradient. Grey bands indicate 95% confidence boundaries for the control (C; dark brown), moderate (H; beige) and high (HH; green) herbivory treatment fitted curves (general linear models). Letters indicate statistical differences due to latitude (L), nitrate (N), high herbivore treatment (HH), and interactions (X). SLA= specific leaf area, TF=Total flavonoids, TPC= total phenolic compounds. The first panel shows in magenta the latitude of the study sites (n=5 per treatment and site).

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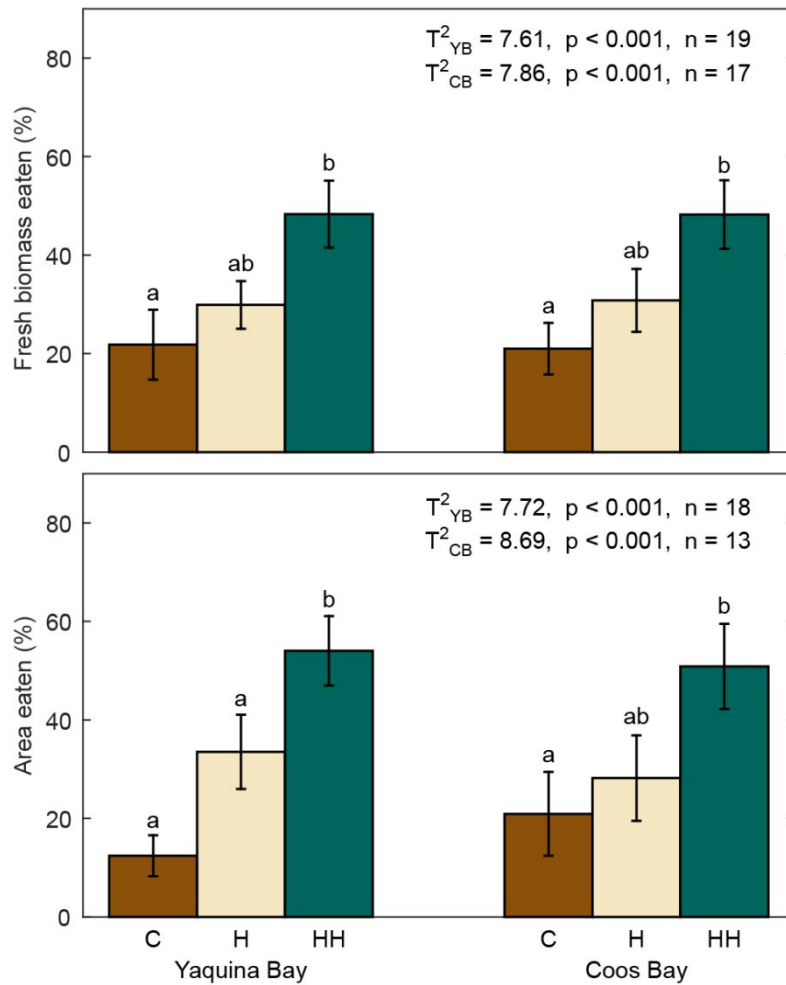
Figure 7. Mean percentage of (A) fresh biomass and (B) agar-based reconstructed food of plant material from different experimental treatments eaten by isopods collected in Yaquina Bay and Coos Bay and fed seagrass from their respective bays. Error bars indicate standard error, and different letters indicate statistically significant differences across treatments (Tukey). Hotelling test results (T^2) from samples collected in Yaquina Bay (T^2_{YB}) and Coos Bay (T^2_{CB}), p-value (p), and number of replicates (n) shown.

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SUPPLEMENTARY MATERIAL

Latitudinal variation in plant defense against herbivory in a marine foundation species does not follow a linear pattern: the importance of resource availability

Study site description S1.

The abiotic factors of the study sites located in the Pacific coast (Table S1) are affected by the variability of the California Current System (CCS). The CCS extends from the North Pacific Current (~50°N) to Baja California (~25°N), where the nearshore equatorward winds drive the upwelling of cold nutrient-rich deep waters. The CCS is therefore associated with an increase of dissolved nitrate concentration where coastal upwelling occurs (e.g., up to 34 µmol/L at Yaquina Bay, Oregon; Sigleo *et al.* 2005) and a lower surface temperature (SST; Fig. 1.b). The annual cycle of coastal wind stress displays two distinct regimes (Fig. S1, S2). In the southern sites (San Diego and Ensenada), the annual cycle features a weak upwelling season in early spring (Schwing and Mendelssohn 1997). In contrast, in the central sites, the greatest upwelling season, which is more intense than further south, occurs from April-July (Fig., S1, S2). Additionally, alongshore variations of upwelling are also driven by the interaction of the coastal current and the coastline/bathymetric features. This upwelling influences the nutrient availability and community structure of coastal reefs (Menge et al. 2003; Barth et al. 2007; Menge and Menge 2013) and estuaries in the US west coast, including estuaries in this study (Brown and Ozretich 2009; Hessing-Lewis and Hacker 2013; Hayduk et al. 2019). More information about ecological community traits and biological seagrass traits from the sites sampled in this work can be found in Duffy et al. (2013, 2015) and Ruesink et al. (2018).

Sea surface temperature data was extracted from the 1982-2008 AVHRR Pathfinder Project data set with a 4 km resolution. Nitrate concentrations were extracted from the NOAA World Ocean Atlas database that compiles *in situ* observations at a 1°Latitude.

Methodology S1. *Plant chemical traits*

Specific phenolic compounds, nitrogen, carbon, sucrose, and fiber content of young leaves (the three newest leaves formed) and nitrogen, carbon, sucrose and starch content of rhizomes were analyzed from samples collected at the end of the experiment. Pooled plant material of five to ten shoots was analyzed per plot. Plants were cleaned of epiphytes, ultrafrozen (-80ºC), freeze-dried, and ground to a fine powder to determine the concentration of chemical traits in young leaves and rhizomes. Carbon and nitrogen content in young leaves and rhizomes were analyzed using a CNH elemental analyzer (EA1108, Carlo-Erba, Italy). Non-structural carbohydrates in young leaves (sucrose), and rhizomes (sucrose and starch) were measured using methodology described by Invers *et al.*, (2004) with spectrophotometer (U-2900, Hitachi High Technologies, TX USA). Neutral detergent fiber content (NDF), referred as 'fiber content', was measured as in Hernán *et al.* (2017).

Phenolic compounds were extracted from powdered leaf samples (0.1-0.2 g) with MeOH:H_2O (3mL x 3) under maceration followed by vacuum evaporation. The organic extracts obtained were suspended in H_2O to 100 ppm. Separation and identification of compounds was performed as in Hernán *et al.* (2017) with the following modifications: single components were identified by their UV, mass spectra, retention times, accurate molecular masses and comparison with standards (purity of the compounds determined by NMR, ≥95%) with the MassLynx 4.1ChromaLynx Application Manager software (Waters). The reference compounds used for quantification were: rosmarinic acid (RA), apigenin-7-sulfate (APS), diosmetin-7-sulfate (DS), luteolin-7-O-β-glucoside (LUTG), luteolin-7-sulfate (LUTS). For those compounds for which no standard was available, a reference compound was selected based on the principle of structure-related target analyte/standard (chemical structure and functional group). Hence, the calibration curves of luteolin-7-sulfate (LUTS) and luteolin-7-O-β-glucoside (LUTG) were used for luteolin-7,3'-disulfate (LUTS2) and luteolin-7-O-β-(6''-malonyl) glucoside (LUTMG), respectively. The sum of LUTS, LUTS2, LUTG, LUTMG, DS and APS was calculated as the total Flavonoids (TF) content (mg/gDW). The sum of flavonoids, and RA was calculated as the total phenolic compounds (TPC, mg/gDW).

Methodology S2. *Genetic metrics*

Leaf samples were stored in silica gel for later DNA extraction and genotyping following Jahnke et al. (2018). Twenty-four microsatellite loci were use in the analysis: the original set of eight from Reusch *et al.* (1999), used in numerous genetic surveys including Duffy *et al.* (2015); and 16 new loci (Jahnke et al. 2018). The number of unique genotypes (genets) present among the ramets sampled at a site was distinguished with GenClone 2.0 (Arnaud-Haond and Belkhir 2007), considering as identical only those genets with non-significant probabilities of identity by chance (Psex (FIS)). Genotypic richness, R (number of unique genets, G–1 divided by the number of sampled ramets, N–1) was also calculated with GenClone 2.0. Following removal of duplicate genotypes, genotypic diversity, allelic diversity and the inbreeding coefficient, were calculated in Genetix 4.05 (Belkhir et al. 2001). Allelic diversity A (number of alleles per locus) was corrected for the minimum number of genets identified among all sites (Â (n = 7 genets)). Details of DNA extraction, microsatellite amplification, genotyping and subsequent data analysis can be found in (Jahnke et al. 2018).

Methodology S3. *Herbivore feeding assays*

To examine the effect of herbivory-driven changes on herbivore feeding behavior we conducted a series of three-choice feeding experiments with isopods (*Pentidotea resecata*) of similar size (CB = 27.55 ± 0.6mm length, YB = 26.88 ± 0.79mm; One-way ANOVA: F1/78=0.466, p=0.497) from two sites, (i.e. Coos Bay and Yaquina Bay), maintained at 15ºC (Mean summer SST at those sites). *P. resecata* is an invertebrate grazer commonly found in *Z. marina* seagrass beds along the Central and North Pacific coast. Isopods were acclimated for 24h prior to the start of the experiments in food deprivation, being kept in individual containers (500cm^3 plastic cups with two parallel windows and the top covered with a 1.5 mm mesh) inside tanks with aerated seawater under controlled light conditions (12/12h).

We performed two (one for each isopod population) three-choice experiments with 20 replicates in which we offered leaves without epiphytes from the control, moderate and high herbivory treatments. To avoid confounding factors such as within tissue differences in structural (Enríquez 2005) or chemical traits (McKey 1979; Cronin and Hay 1996), the tissue offered was clipped from the second leaf avoiding basal and apical tissue, and the length of all the pieces offered was similar (ca. between 2 and 4 cm depending on the trial). In order to measure any potential changes in leaf tissue biomass not related to grazing (e.g. increase or reduction of wet weight due to gain or loss of water content), control cups without herbivores were used to correct for changes in weight. Leaf weight at the end of the experiment was corrected with the controls prior to the statistical analyses.

Additionally, in order to examine if the feeding patterns observed were driven by mechanical-structural or chemical traits, we performed the equivalent three-choice feeding experiment but using agar-base artificial food (Siska et al. 2002; Vergés et al. 2007b; Prado and Heck 2011; Tomas et al. 2015). One gram of agar was added to 25 mL of boiling distilled water, the mix was chilled while stirring until reaching approximately 60ºC (to avoid sample degradation by temperature). Then, two grams of pulverized leaf seagrass (young leaves without apical and basal parts) were added to the mix. The mixture was then poured onto a thin plastic window screen and flattened. Once the agar mix was solid, it was cut into rectangles of 1 x 3.5 cm and offered to the herbivores following the same procedure as with fresh leaf tissue. Consumption was measured by counting the number of mesh squares that were cleared of food and calculated as percentage of area eaten. The experiment consisted of 20 (for YB) and 15 (for CB) replicates. In order to measure any potential changes in the agar rectangles not related to grazing, control cups without herbivores were used to correct for changes in area. Since we found no changes in area in these controls, those data were not used. All the experiments ended

when approximately 50% of the initially offered material was consumed. Following the procedures of previous feeding behavior experiments in seaweeds and seagrasses (e.g., Bolser et al., 1998; Vergés et al., 2007; Tomas et al., 2015), replicates where all the offered material was consumed or remained intact were not considered in the statistical analyses. Consumption was expressed as plant material consumed (fresh biomass or agar-based area) from one treatment to the total consumed of all treatments.

Methodology S4. *Statistical analyses*

We performed a Principal Component Analysis (PCA) with all the 33 response variables measured in order to visualize the data and possible patterns of plant defense strategies across clipping treatments and sites (Fig. S4). Variables were centered and scaled to have unit variance before PCA analysis (van den Berg et al. 2006). We tested our predictions (main effects of latitude, resource availability and herbivory treatments, and their interaction) while controlling for potential covariation introduced by the genetic traits using univariate General Linear Models (GLMs). Before fitting the GLMs to our response variables, we first explored i) the correlation between latitude and co-variates to avoid co-linearity in the model fitting and ii) correlation among our response variables. We performed pairwise correlations between each of the predictor variables (mean May-June-July SST, NO_3, latitude, genotypic richness, allelic richness and inbreeding) and among all the response variables using Pearson correlation coefficients (Fig. S3). We identified a strong negative correlation between latitude and SST, and SST with NO_3, while genotypic richness and allelic richness were latitude-independent but positively correlated between them. We therefore discarded SST as explanatory variable and included genotypic richness into the analysis as a control variable. Based on the results of the correlation analysis, we selected 15 response variables (i.e., relative leaf growth rate, number of leaves, sheath area, rhizome biomass per internode, leaf nitrogen, carbon and sucrose content, rhizome nitrogen, carbon, sucrose and starch content, SLA, leaf fiber content, total flavonoids and specific phenolic compounds) to test our hypotheses.

The main goal of our work was to statistically test the interactive effect between latitude and herbivory treatment (i.e., control, moderate and high herbivory) and the effect of latitude in defense traits while controlling for genetic co-variates (i.e., genotypic richness) using the GLM. In addition, the main effect of latitude and treatment were also interpreted when there was no significant effect of the interaction. Since resource availability also changes with latitude, we added nitrate concentration as an explanatory variable effect in our model. We reduced the full GLM of each response variable (with all explanatory variables and interactions) using a stepwise selection procedure to find the minimum adequate model, that is, the one with the lowest Akaike information criterion (AIC) value (Table S1). This is a widely use model-building procedure to avoid type I errors or false positives. The coefficients, standard errors, and p-values of explanatory variables in all minimum adequate models were estimated through the iteratively reweighted least squares using the glm function of the R-package (R Core Team 2007). Multicollinearity among the explanatory variables (Latitude, treatment, NO3 and genotypic richness) was verified by the generalized variance inflation factors (GVIF, Fox and Weisberg

2011). We calculated the standardized generalised variance inflation factor (SGVIF= GVIF^ [1/2*df]). Values of GVIF higher than 10 (SGVIF$_{d.f.=1}$= 3.16, SGVIF$_{d.f.=2}$= 1.78) in some cases, or more generally 4 (SGVIF$_{d.f.=1}$= 2, SGVIF$_{d.f.=2}$= 1.41) could indicate collinearity. However, higher values are expected for interaction terms (Hair et al. 1995; O'Brien 2007).

All models were fitted considering a Gaussian (i.e., normal) distribution of errors. Hence, the distribution of the residuals (errors) was checked for normality and an adequate natural logarithm- (ln) or square root- (sqrt) transformation was applied to response variables when the distribution of the residuals strongly deviated from normality. Proportions were transformed when residuals were not normal with logit (ln[p/(1-p)]; ln=naeperian logarithm, p=variable(%)/100) and arcsine (arcsine(sqrt (p)); sqrt=square root) transformations as recommended by Warton et al. (2011).

The analyses of the feeding experiments were performed using Hotelling tests as in Prince, LeBlanc & Maciá (2004) which is a widely used test for multiple choice feeding preference analysis. Variables were not transformed and Post hoc analyses were performed with Tukey multiple comparisons of means.

Table S1. Location, latitude and longitude of the study sites

Label	Site	Location	Latitude (°N)	Longitude (°W)
MX	Mexico	Punta Banda Estuary	31.753	116.626
SD	San Diego	Coronado	32.701	117.173
SF	San Francisco	Point Molate	37.945	122.416
BB	Bodega Bay	West Side Regional Park	38.319	123.047
CB	Oregon	Coos bay	43.346	124.338
YB	Oregon	Yaquina Bay	44.624	124.004
WA	Washington	Willapa	46.328	124.026
BC	Canada (British Columbia south coast)	Tsawwassen	49.008	123.093
QU	Canada (Quebec)	Baie St. Ludger	49.087	68.320
HK	Canada (Hakai, BC central coast)	Calvert	51.647	128.119

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Table S2. Traits related to plant defense strategies against herbivory

Defense strategy	Trait	Reason	References
RESISTANCE	SLA	Inversely correlated with leaf toughness, which is a good determinant of herbivory. Toughness affects penetration of plant tissues and increases breaking strength. Even though it is considered a physical characteristic, leaf toughness is achieved by chemical compounds, such as fibers (i.e. lignins, cellulose), produced and accumulated in the plants at different levels (molecules, cell walls).	(e.g., Coley 1983; Howlett et al. 2001)
	Fibers	Increasing toughness and being a C-based compound, it is inversely correlated to leaf nutritional quality. Moreover, some components that confer toughness such as lignin can bind to nutrients such as proteins, hindering their absorption. Seagrasses with higher fiber content are less preferred by herbivores (e.g., fish, sea urchins) in choice experiments.	(e.g., Hagerman & Butler 1991; Mariani & Alcoverro 1999; de los Santos et al. 2012; Hernán et al. 2017)
	Leaf N content	Herbivores need to fulfill a determined requirement of nitrogen. However, plants have low N contents since in most ecosystems there is a limited availability of N. Thus, increases in N can improve the palatability and nutritional quality of plants, and herbivore preference.	(e.g.,Mattson 1980; Valentine & Heck 2001; De Bruyn et al. 2002; Hemmi & Jormalainen 2002; Güsewell 2004; Prado et al. 2010)
	Secondary metabolites	Secondary metabolites are considered to play an important role in defense against herbivores through their activity as disruptive chemicals making tissues difficult to digest or unpalatable (e.g., tannins), as protective chemicals making tissues toxic (e.g., alkaloids), and as modifiers of feeding behavior (e.g., volatile compounds).	(e.g., Rosenthal & Berenbaum 1992; Bennett & Wallsgrove 1994; Nykänen & Koricheva 2004; Lambers et al. 2008)
TOLERANCE	Growth rate, shoot biomass or number of leaves	Many experiments report increases in leaf growth as a compensatory response after leaf loss by herbivory in terrestrial plants and marine macrophytes.	(e.g.,McNaughton 1983; Hawkes and Sullivan 2001; Moran and Bjorndal 2005; van Staalduinen and Anten 2005; Vergés et al. 2008; Sanmartí et al. 2014)
	Leaf sucrose	One of the main products of photosynthesis and thus, indicative of photosynthetic activity. Increasing photosynthetic activity is a well-studied tolerance mechanism.	(e.g.,Strauss & Agrawal 1999; Tiffin 2000; Lunn 2008)
	Belowground reserves and biomass	Belowground reserves (e.g. sucrose, starch) and reallocation of those resources to reproduction or biomass regrowth after herbivory is commonly cited and it is the most studied mechanism.	(reviewed by Stowe et al. 2000; Tiffin 2000; Orians et al. 2011)

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Supplementary References

Arnaud-Haond S, Belkhir K (2007) GENCLONE: A computer program to analyse genotypic data, test for clonality and describe spatial clonal organization. Mol Ecol Notes 7:15–17. doi: 10.1111/j.1471-8286.2006.01522.x

Barth JA, Menge BA, Lubchenco J, et al (2007) Delayed upwelling alters nearshore coastal ocean ecosystems in the northern California current. Proc Natl Acad Sci 104:3719–3724

Belkhir K, Borsa P, Goudet J, et al (2001) GENETIX 4.05, logiciel sous WindowsTM pour la genetique des populations. Laboratoire Genome, Populations, Interactions

Bennett BYRN, Wallsgrove RM (1994) Secondary metabolites in plant defence mechanisms. New Phytol 72:617–633

Bolser RC, Hay ME, Lindquist N (1998) Chemical defenses of freshwater macrophytes against crayfish herbivory. J Chem Ecol 24:1639–16. J Chem Ecol che 24:1639–1658

Brown CA, Ozretich RJ (2009) Coupling Between the Coastal Ocean and Yaquina Bay, Oregon: Importance of Oceanic Inputs Relative to Other Nitrogen Sources. Estuaries and Coasts 32:219–237. doi: 10.1007/s12237-008-9128-6

Coley PD (1983) Herbivory and defensive characteristics of tree species in lowland tropical forest. Ecol Monogr 53:209–234

Cronin G, Hay ME (1996) Within-plant variation in seaweed palatability and chemical defenses: optimal defense theory versus the growth-differentiation balance hypothesis. Oecologia 105:361–368. doi: 10.1007/BF00328739

De Bruyn L, Scheirs J, Verhagen R (2002) Nutrient stress, host plant quality and herbivore performance of a leaf-mining fly on grass. Oecologia 130:594–599. doi: 10.1007/s00442-001-0840-1

de los Santos CB, Brun FG, Onoda Y, et al (2012) Leaf-fracture properties correlated with nutritional traits in nine Australian seagrass species: implications for susceptibility to herbivory. Mar Ecol Prog Ser 458:89–102. doi: 10.3354/meps09757

Duffy JE, Macdonald KS, Rhode JM, Parker J (2013) Grazer diversity, functional redundancy and productivity in seagrass beds: an experimental test. Ecology 82:2417–2434

Duffy JE, Reynolds PL, Boström C, et al (2015) Biodiversity mediates top-down control in eelgrass ecosystems: A global comparative-experimental approach. Ecol Lett 18:696–705. doi: 10.1111/ele.12448

Enríquez S (2005) Light absorption efficiency and the package effect in the leaves of the seagrass *Thalassia testudinum*. Mar Ecol Prog Ser 289:141–150. doi: 10.3354/meps289141

Güsewell S (2004) N:P ratios in terrestrial plants: Variation and functional significance. New Phytol 164:243–266. doi: 10.1111/j.1469-8137.2004.01192.x

Hagerman A, Butler L (1991) Tannins and lignins. In: Rosenthal GA, Berenbaum MR (eds) Herbivores: Their Interactions with Secondary Plant Metabolites, 2nd edn. Academic Press, pp 355–388

Hair JFJ, Anderson RE, Tatham RL, Black WC (1995) Multivariate Data Analysis, 3rd edn. MACMILLAN, New York

Hawkes C V., Sullivan JJ (2001) The impact of herbivory on plants in different resource conditions: a meta-analysis. Ecology 82:2045–2058. doi: 10.1890/0012-9658(2001)082[2045:TIOHOP]2.0.CO;2

Hayduk JL, Hacker SD, Henderson JS, Tomas F (2019) Evidence for regional-scale controls on eelgrass (*Zostera marina*) and mesograzer community structure in upwelling-in fl uenced estuaries. Limnol Oceanogr 64:1120–1134. doi: 10.1002/lno.11102

Hemmi A, Jormalainen V (2002) Nutrient enhancement increases performance of a marine herbivore via quality of its food alga. Ecology 83:1052–1064. doi: 10.1890/0012-9658(2002)083[1052:NEIPOA]2.0.CO;2

Hernán G, Ortega MJ, Gándara AM, et al (2017) Future warmer seas: Increased stress and susceptibility to grazing in seedlings of a marine habitat-forming species. Glob Chang Biol 1–14. doi: 10.1111/gcb.13768

Hessing-Lewis ML, Hacker SD (2013) Upwelling-influence , macroalgal blooms , and seagrass production; temporal trends from latitudinal and local scales in northeast Pacific estuaries. Limnol Oceanogr 58:1103–1112. doi: 10.4319/lo.2013.58.3.1103

Howlett BG, Clarke AR, Madden JL (2001) The influence of leaf age on the oviposition preference of *Chrysophtharta bimaculata* (Olivier) and the establishment of neonates. Agric For Entomol 3:121–127. doi: 10.1046/j.1461-9563.2001.00096.x

Invers O, Kraemer GP, Pérez M, Romero J (2004) Effects of nitrogen addition on nitrogen metabolism and carbon reserves in the temperate seagrass *Posidonia oceanica* . J Exp Mar Bio Ecol 303:97–114. doi: 10.1016/j.jembe.2003.11.005

Jahnke M, Jonsson PR, Moksnes PO, et al (2018) Seascape genetics and biophysical connectivity modelling support conservation of the seagrass Zostera marina in the Skagerrak–Kattegat region of the eastern North Sea. Evol Appl 11:645–661. doi: 10.1111/eva.12589

Lambers H, Stuart Chapin III F, Pons TL (2008) Plant Physiological Ecology, Second. Springer, New York

Lunn EJ (2008) Sucrose metabolism. In: Encyclopedia of life sciences. John Wiley & Sons, Chichester

Mariani S, Alcoverro T (1999) A multiple-choice feeding-preference experiment utilising seagrasses with a natural population of herbivorous fishes. Mar Ecol Prog Ser 189:295–299. doi: 10.3354/meps189295

Mattson WJJ (1980) Herbivory in relation to plant nitrogen content. Annu Rev Ecol Syst 11:119–61. doi: 10.1146/annurev.es.11.110180.001003

McKey D (1979) The distribution of secondary compounds within plants. In: Rosenthal GA, Janzen DH (eds) Herbivores: Their Interaction with Secondary Plant Metabolites. Academic Press, New York, pp 55–133

McNaughton SJ (1983) Compensatory plant growth as a response to herbivory. Oikos 40:329–336. doi: 10.2307/3544305

Menge BA, Lubchenco J, Bracken MES, et al (2003) Coastal oceanography sets the pace of rocky intertidal community dynamics. Proc Natl Acad Sci 100:12229–12234

Menge BA, Menge DNL (2013) Dynamics of coastal meta-ecosystems: the intermittent upwelling hypothesis and a test in rocky intertidal regions. Ecol Monogr 83:283–310

Moran KL, Bjorndal KA (2005) Simulated green turtle grazing affects structure and productivity of seagrass pastures. Mar Ecol Prog Ser 305:235–247. doi: 10.3354/meps305235

Nykänen H, Koricheva J (2004) Damage-induced changes in woody plants and their effects on insect herbivore performance : a meta-analysis. Oikos 104:247–268

O'Brien RM (2007) A caution regarding rules of thumb for variance inflation factors. Qual Quant 41:673–690. doi: 10.1007/s11135-006-9018-6

Orians CM, Thorn A, Gómez S (2011) Herbivore-induced resource sequestration in plants: Why bother? Oecologia 167:1–9. doi: 10.1007/s00442-011-1968-2

Prado P, Heck K (2011) Seagrass selection by omnivorous and herbivorous consumers: determining factors. Mar Ecol Prog Ser 429:45–55. doi: 10.3354/meps09076

Prado P, Romero J, Alcoverro T (2010) Nutrient status, plant availability and seasonal forcing mediate fish herbivory in temperate seagrass beds. Mar Ecol Prog Ser 409:229–239. doi: 10.3354/meps08585

Prince JS, LeBlanc WG, Maciá S (2004) Design and analysis of multiple choice feeding preference data. Oecologia 138:1–4. doi: 10.1007/s00442-003-1413-2

R Core Team (2007) R: a language and environment for statistical Computing

Reusch TBH, Boström C, Stam WT, Olsen JL (1999) An ancient eelgrass clone in the Baltic. Mar Ecol Prog Ser 183:301–304. doi: 10.3354/meps183301

Rosenthal GA, Berenbaum MR (1992) Herbivores: their interactions with secondary metabolites, 2nd edn. Academic Press, New York

Ruesink JL, Stachowicz JJ, Reynolds PL, et al (2018) Form–function relationships in a marine foundation species depend on scale: a shoot to global perspective from a distributed ecological experiment. Oikos 127:364–374. doi: 10.1111/oik.04270

Sanmartí N, Saiz L, Llagostera I, et al (2014) Tolerance responses to simulated herbivory in the seagrass *Cymodocea nodosa*. Mar Ecol Prog Ser 517:159–169. doi: 10.3354/meps11084

Schwing FB, Mendelssohn R (1997) Increased coastal upwelling in the California Current System. 102:3421–3438

Sigleo AC, Mordy CW, Stabeno P, Frick WE (2005) Nitrate variability along the Oregon coast : Estuarine-coastal exchange. Estuar Coast Shelf Sci 64:211–222. doi: 10.1016/j.ecss.2005.02.018

Siska EL, Pennings SC, Buck T., Hanisak MD (2002) Latitudinal variation in palatability of salt-marsh plants: which traits are responsible? Ecology 83:3369–3381

Stowe KA, Marquis RJ, Hochwender CG, et al (2000) The evolutionary ecology of tolerance to consumer damage. Annu Rev Ecol Syst 31:565–595

Strauss SY, Agrawal AA (1999) The ecology and evolution of plant tolerance to herbivory. Trends Ecol Evol 14:179–185. doi: 10.1016/S0169-5347(98)01576-6

Tiffin P (2000) Mechanisms of tolerance to herbivore damage: What do we know? Evol Ecol 14:523–536. doi: 10.1023/A:1010881317261

Tomas F, Martinez-Crego B, Hernan G, Santos R (2015) Responses of seagrass to anthropogenic and natural disturbances do not equally translate to its consumers. Glob Chang Biol 21:1–10. doi: 10.1111/gcb.13024

Valentine JF, Heck KL (2001) The role of leaf nitrogen content in determining turtlegrass (*Thalassia testudinum*) grazing by a generalized herbivore in the northeastern Gulf of Mexico. J Exp Mar Bio Ecol 258:65–86

van den Berg RA, Hoefsloot HCJ, Westerhuis JA, et al (2006) Centering, scaling, and transformations: improving the biological information content of metabolomics data. BMC Genomics 7:142. doi: 10.1186/1471-2164-7-142

van Staalduinen M a, Anten NPR (2005) Differences in the compensatory growth of two co-occurring grass species in relation to water availability. Oecologia 146:190–199. doi: 10.1007/s00442-005-0225-y

Vergés A, Becerro M, Alcoverro T, Romero J (2007a) Experimental evidence of chemical deterrence against multiple herbivores in the seagrass *Posidonia oceanica*. Mar Ecol Prog Ser 343:107–114. doi: 10.3354/meps06885

Vergés A, Becerro MA, Alcoverro T, Romero J (2007b) Variation in multiple traits of vegetative and reproductive seagrass tissues influences plant-herbivore interactions. Oecologia 151:675–86. doi: 10.1007/s00442-006-0606-x

Vergés A, Pérez M, Alcoverro T, Romero J (2008) Compensation and resistance to herbivory in seagrasses: induced responses to simulated consumption by fish. Oecologia 155:751–60. doi: 10.1007/s00442-007-0943-4

Warton, David I, Hui, Francis KC (2011) The arcsine is asinine: the analysis of proportions in ecology. Ecology 92:3–10

1 **Table S3.** Akaike information criterion (AIC), adjusted R2 of the models, and standardized generalized variance-inflation factors (SGVIF) for

2 explanatory variables of the selected model. Models considering effects of latitude (L), nutrient availability (NO3), herbivory treatment (H) and

3 genotypic richness (G) and their interactions (marked with asterisk) are shown.

Trait	Model	AIC	Adjusted R2	SGVIF$_{Lat}$	SGVIF$_{Treat}$	SGVIF$_{NO3}$	SGVIF$_{G.R.}$	SGVIF Lat*Treat	SGVIF$_{NO3*Treat}$
Sqrt (Relative Growth Rate (d-1))	L*H+NO3*H+G	-516.41							
	L+H+NO3+G+L*H	-514.41	0.36	1.005	1.648	1.792	1.006		1.920
Number of leaves	L*H+NO3*H+G	279.29							
	L+H+NO3+G+L*H	277.29							
	L+H+NO3+G	276.38							
	H+NO3+G	275.25	0.41		1.000	1.001	1.001		
Sheath area(cm2)	L*H+NO3*H+G	898.16							
	L+H+NO3+G+L*H	894.36	0.61	1.738	6.777	1.002	1.005	6.846	
Leaf sucrose (%)	L*H+NO3*H+G	773.37							
	L+H+NO3+G+L*H	769.83							
	L+H+NO3+G	768.23							
	L+H+G	767.97	0.29	1.004	1.000		1.004		
Ln(Rhizome biomass (grFW/internode))	L*H+NO3*H+G	367.85							
	L+H+NO3+G+L*H	364.25							
	L+H+NO3+G	361.66	0.36	1.005	1.000	1.002	1.005		
Logit (Rhizome starch(%))	L*H+NO3*H+G	235.77							
	L+H+NO3+G+H*NO3	232.71							
	L+H+NO3+G	230.71							
	L+H+NO3	228.75							
	L+NO3	228.44	0.33	1.001		1.001			
arcsine(Rhizome sucrose (%))	L*H+NO3*H+G	-862.45							
	L+H+NO3+G+L*H	-865.80							
	L+H+NO3+G	-869.13	0.71	1.005	1.000	1.002	1.005		

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Trait	Model	AIC	Adjusted R2	SGVIF$_{Lat}$	SGVIF$_{Treat}$	SGVIF$_{NO3}$	SGVIF$_{G.R.}$	SGVIF$_{Lat*Treat}$	SGVIF$_{NO3*Treat}$
Logit (Rhizome C (%))	L*H+NO3*H+G	-2225.92							
	L+H+NO3+G+L*H	-229.64	0.53	1.738	6.777	1.002	1.005	6.846	
Rhizome N (%)	L*H+NO3*H+G	92.98							
	L+H+NO3+G+L*H	90.00							
	L+H+NO3+G	87.48							
	L+NO3+G	84.36	0.27	1.010		1.004	1.011		
SLA (cm2/g)	L*H+NO3*H+G	904.19							
	L+H+NO3+G+L*H	900.48	0.59	1.000	1.000				
Logit (Fiber (%))	L*H+NO3*H+G	37.64							
	L+H+NO3+G+L*H	33.74							
	L+H+NO3+G	32.07							
	L+NO3+G	28.21	0.65	1.010		1.004	1.011		
Logit (Leaf C (%))	L*H+NO3*H+G	-279.85							
	L+H+NO3+G+L*H	-283.67							
	L+H+NO3+G	-285.81							
	L+NO3+G	-288.91							
	L+G	-290.47	0.32	1.009			1.009		
Logit(Leaf N (%))	L*H+NO3*H+G	-346.14	0.49	1.739	6.859	1.793	1.005	6.851	1.922
Sqrt (TF (mg/gDW))	L*H+NO3*H+G	351.31							
	L+H+NO3+G+L*H	347.71							
	L+H+NO3+G	344.39							
	L+NO3+G	340.49	0.17	1.010		1.004	1.011		
Sqrt (TPC (mg/gDW))	L*H+NO3*H+G	420.15							
	L+H+NO3+G+L*H	416.78							
	L+H+NO3+G	413.55							
	L+NO3+G	409.79							
	L+NO3	408.45	0.15	1.001		1.001			

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Table S4: Results of GLM on Specific phenolic compounds. Intercept (i) and slopes of latitude (β_{lat}), herbivory simulation moderate (β_H) and high level (β_{HH}), nitrate (β_{NO3}), their interactions (marked with asterisc), and genotypic richness ($\beta_{G.R.}$). Sqrt, and logit indicate square root and logit transformation, respectively. RA indicates Rosmarinic acid, DS diosmetin-7-sulfate, LUTS luteolin-7-sulfate, LUTS2 luteolin-7,3'-disulfate, LUTG luteolin-7-O-β-glucoside and LUTMG luteolin-7-O-β-(6''-malonyl) glucoside

|		i	β_{lat}	β_H	β_{HH}	β_{NO3}	$\beta_{G.R.}$	β_{lat*H}	β_{NO3*H}	β_{NO3*HH}	Adjusted R2
Sqrt (RA (g/gDW))	Est.	-1.648	0.126			0.325	-2.278				0.4
	SE	1.203	0.018			0.074	0.964				
	P	0.173	**<0.001**			**<0.001**	**0.020**				
Sqrt (DS (mg/g DW))	Est.	4.562	-0.049			-0.198					0.16
	SE	0.623	0.015			0.062					
	P	**<0.001**	**0.001**			**0.002**					
sqrt (LUTS (mg/g DW))	Est.	0.258	0.010			0.220					0.35
	SE	0.282	0.007			0.028					
	P	0.361	0.121			**<0.001**					
LUTS2 (mg/g DW)	Est.	2.966	-0.144	-3.655	-4.156	0.781	8.256	0.089			0.53
	SE	1.996	0.038	2.271	2.271	0.093	1.207	0.054			
	P	0.140	**<0.001**	0.110	0.070	**<0.001**	**<0.001**	0.100			
Logit (LUTG (mg/g DW))	Est.	-409.598	8.507			31.091	-36.875				0.14
	SE	161.377	3.351			12.427	14.925				
	P	**0.019**	**0.019**			**0.021**	**0.022**				
Sqrt (LUTMG (mg/g DW))	Est.	-0.049	<0.001			0.002	0.020				0.262
	SE	0.017	<0.001			0.001	0.013				
	P	**0.004**	**<0.001**			**0.020**	0.124				
Sqrt (APS (mg/g DW))	Est.	-0.158	0.005	0.026	-0.033	0.022	0.276		0.003	0.023	0.4
	SE	0.079	0.001	0.028	0.28	0.062	0.062		0.012	0.012	
	P	0.047	**<0.001**	0.363	0.248	**<0.001**	**<0.001**		0.784	0.057	

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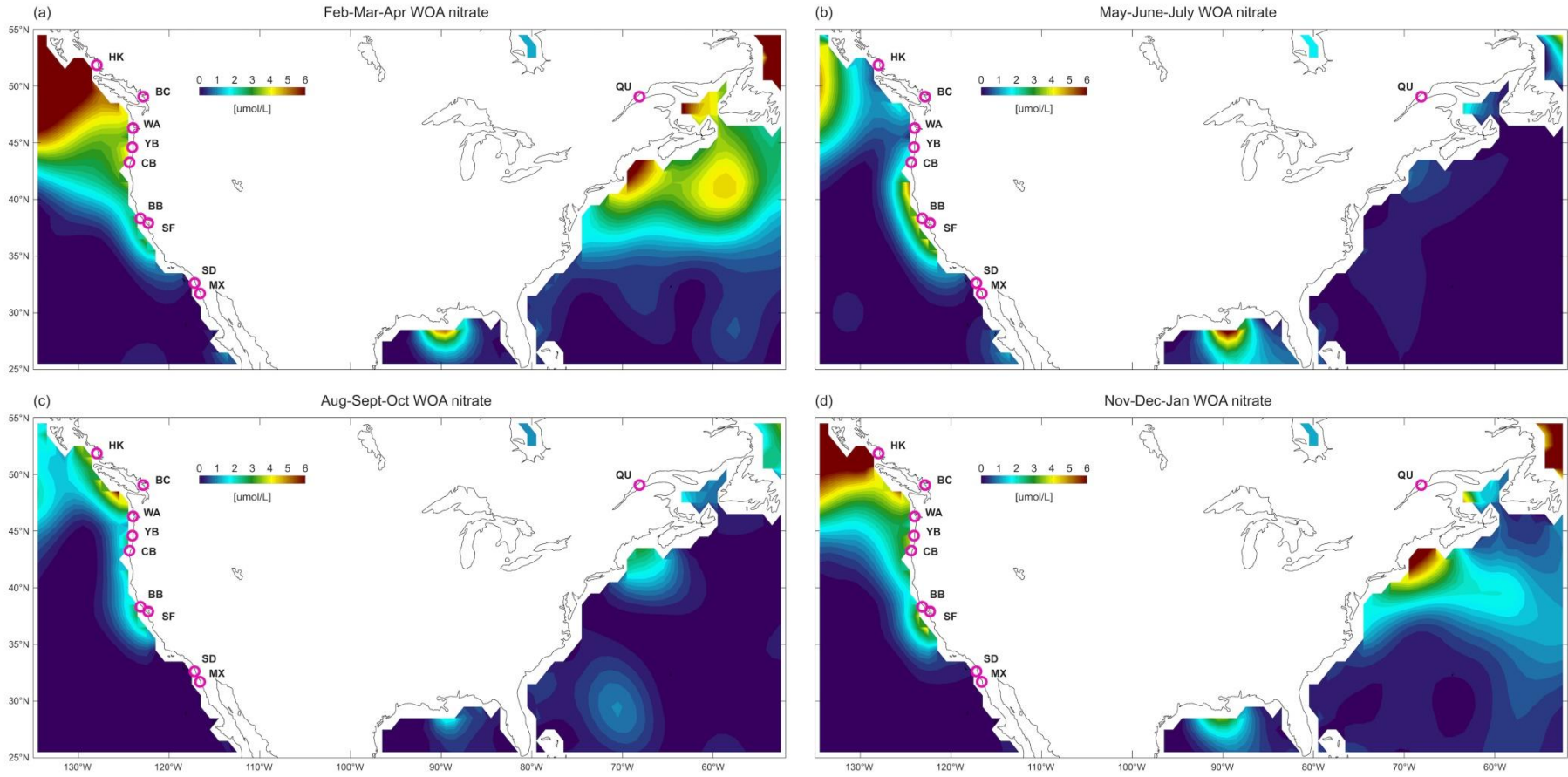
FIGURES

Figure S1. Mean (a) February- March- April, (b) May-June-July, (c) August-September-October, (d) November-December-January nitrate concentration (μmol/L) from the 1878-2012 World Ocean Atlas data set. Location of study sites is marked with a magenta circle. See Table S1 for coding of the sites.
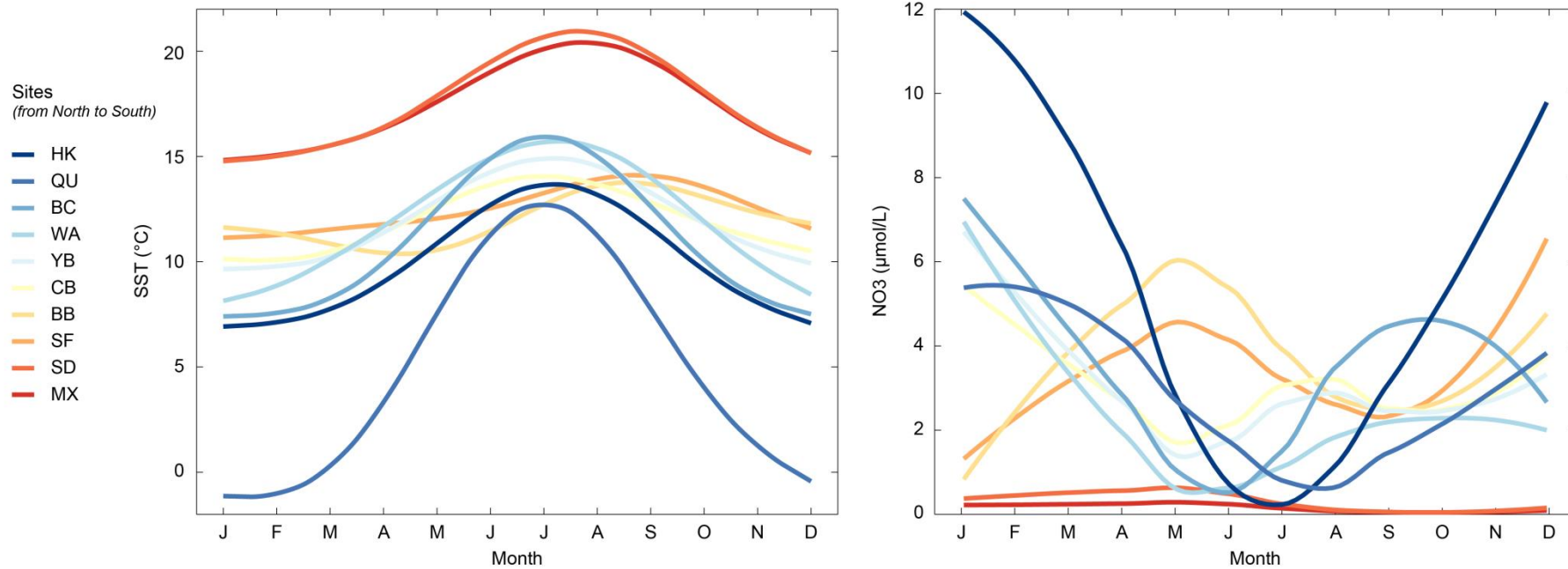
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Figure S2. Mean sea surface temperature (SST; ºC) on the left and mean nitrate concentration (µmol/L) on the right of the study sites from the 1878-2012 World Ocean Atlas data set. MX, Mexico; SD, San Diego (California); SF, San Francisco (California); BB, Bodega Bay (California); CB, Coos Bay (Oregon); YB Yaquina Bay (Oregon); WA, Washington; BC (British Columbia south coast) Canada; QU Quebec (Canada); HK, Hakai, (BC central coast, Canada).
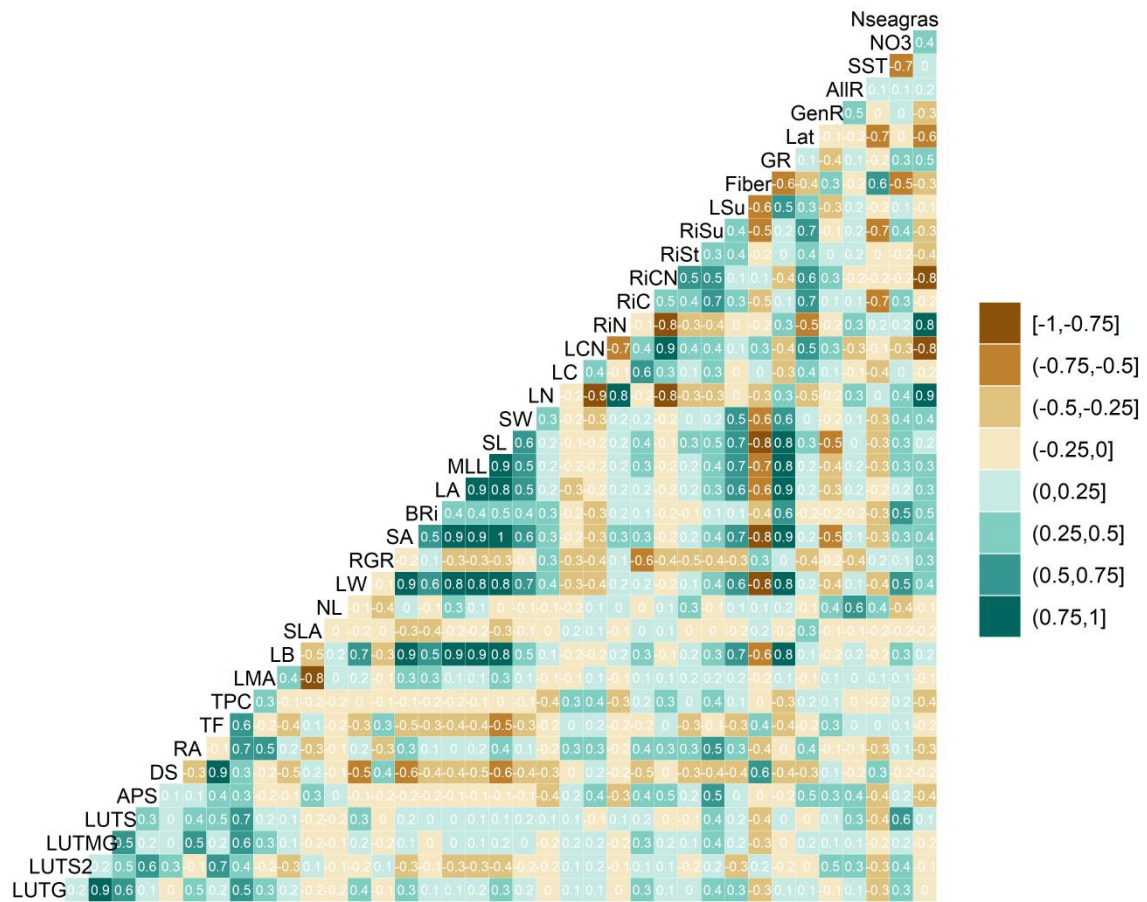
18

Figure S3. Pairwise correlation matrix of Flavonoids (LUTG, LUTS2, LUTMG, LUTS, APS, DS), Rosmarinic acid (RA), Total flavonoids (TF), total phenolic compounds (TPC), leaf mass area (LMA), leaf biomass (LB), specific leaf area (SLA), number of leaves (NL), leaf width (LW), relative leaf growth rate (RGR), sheath area (SA), biomass of rhizome per internode (BRi), leaf area (LA), maximum leaf length (MLL), sheath length (SL), sheath width (SW), leaf nitrogen content (LN), leaf carbon content (LC), leaf C/N (LCN), rhizome nitrogen content (RiN), rhizome carbon content (RiC), rhizome C/N (RiCN), rhizome starch content (RiSt), rhizome sucrose content (RiSu), leaf sucrose content (LSu), leaf fiber (Fiber), latitude(Lat), genotypic richness (GenR), allelic richness (AllR), inbreeding (InB) and mean May-June-July sea surface temperature (SST), nitrate (NO3) and leaf nitrogen of ambient seagrass (Nseagrass). Numbers indicate Pearson correlation coefficients.
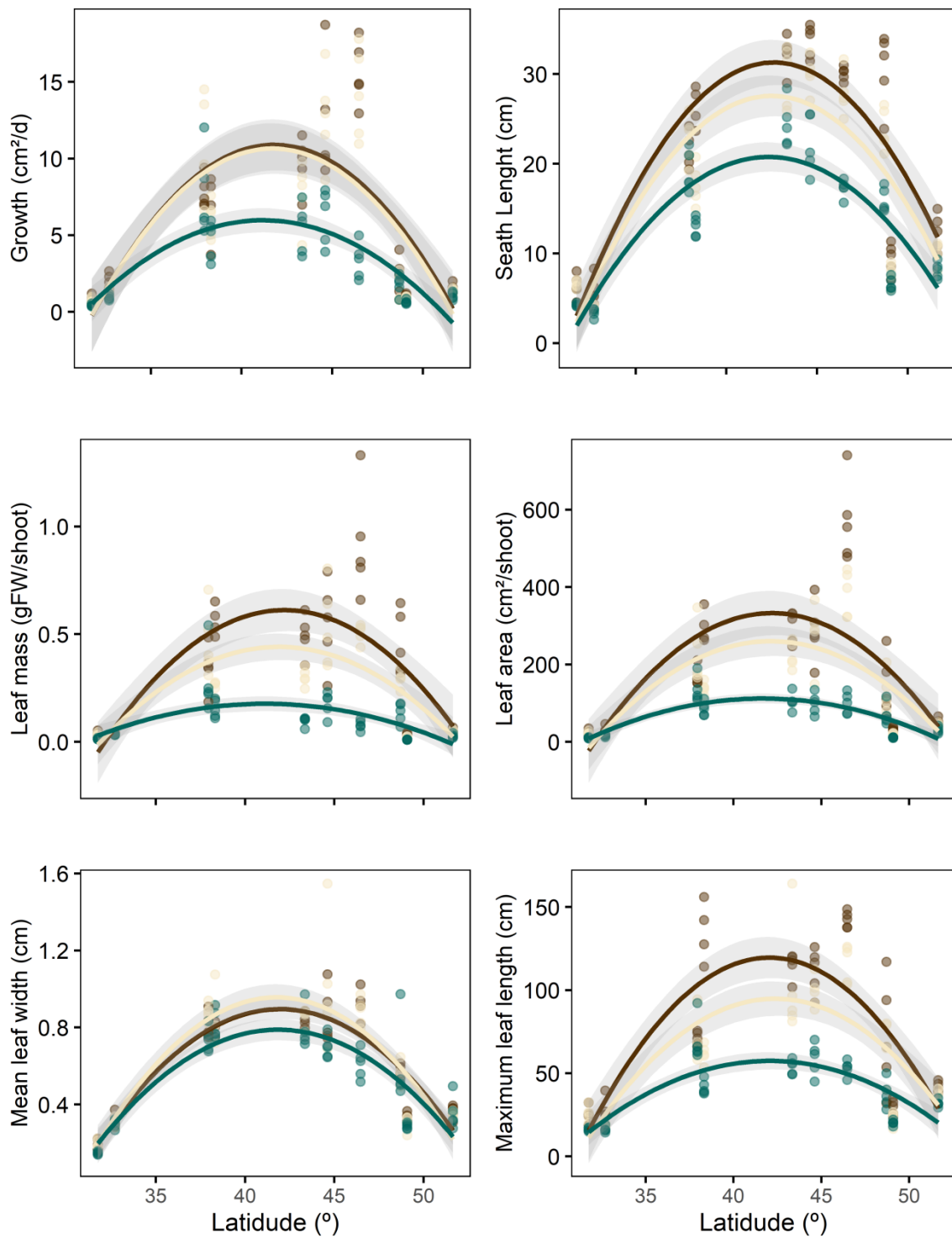
Figure S4. Distribution of seagrass size traits along the latitudinal gradient. Grey bands indicate 95% confidence boundaries for the control (dark brown), moderate (beige) and high (green) herbivory treatment fitted curves (glm).
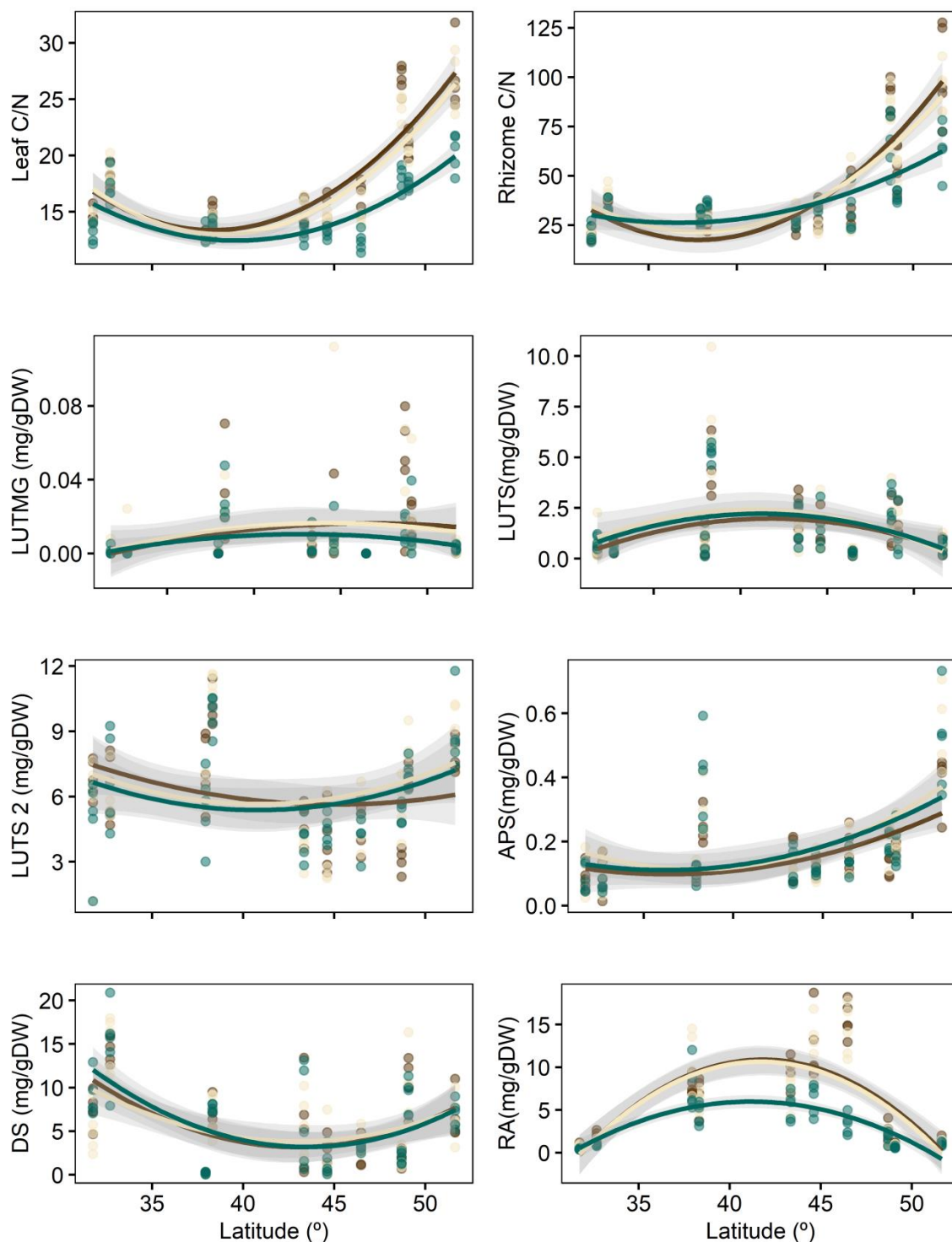
Figure S5. Distribution of seagrass chemical traits along the latitudinal gradient. Grey bands indicate 95% confidence boundaries for the control (dark brown), moderate (beige) and high (green) herbivory treatment fitted curves (glm). APS indicates Apigenin-7-sulfate, DS diosmetin-7-sulfate, LUTS luteolin-7-sulfate, LUTG luteolin-7-O-β-glucoside, LUTS2 luteolin-7,3'-disulfate, LUTMG luteolin-7-O-β-(6''-malonyl) glucoside, and RA Rosmarinic acid.
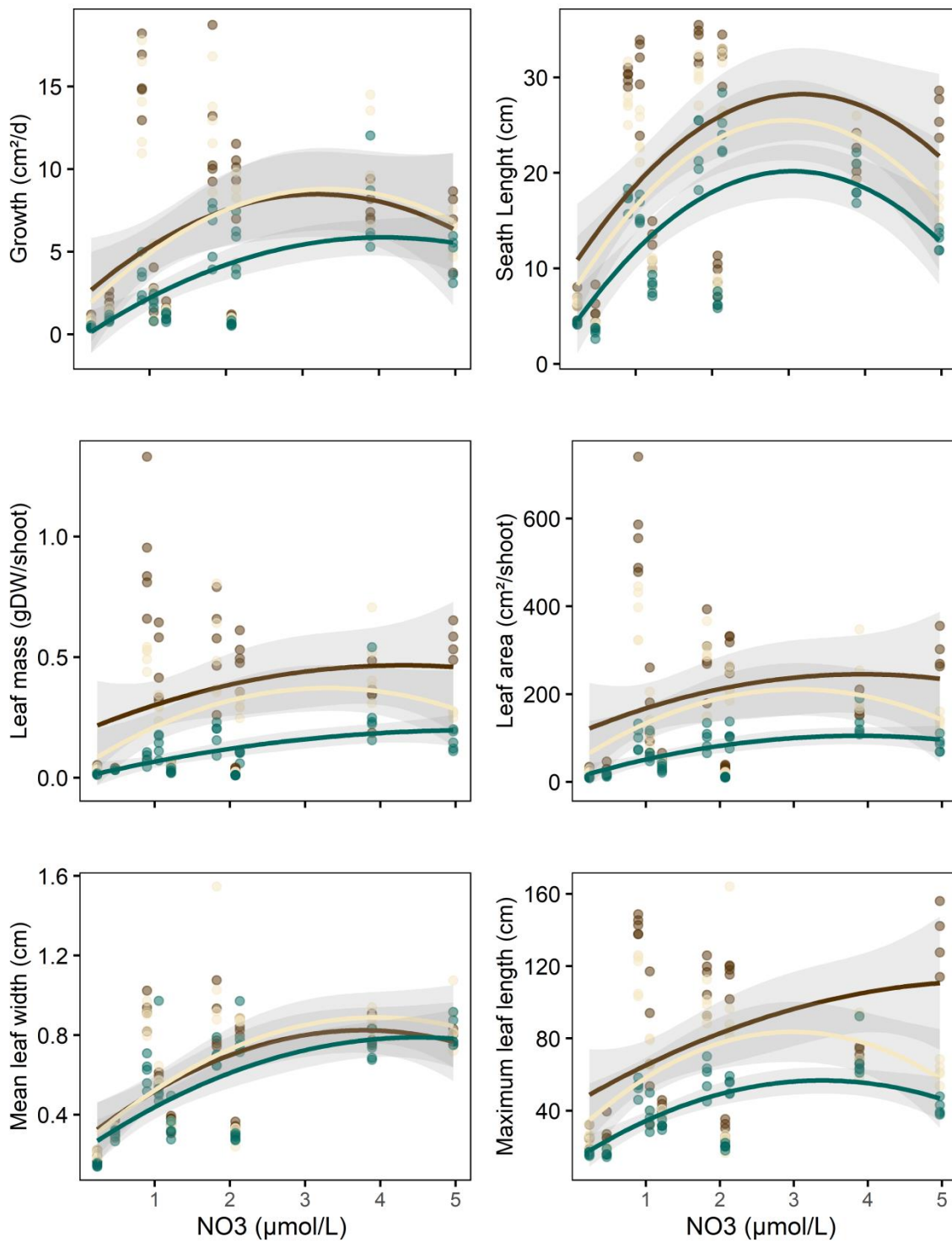
Figure S6. Distribution of seagrass size traits along the nitrate availability gradient. Grey bands indicate 95% confidence boundaries for the control (dark brown), moderate (beige) and high (green) herbivory treatment fitted curves (glm).
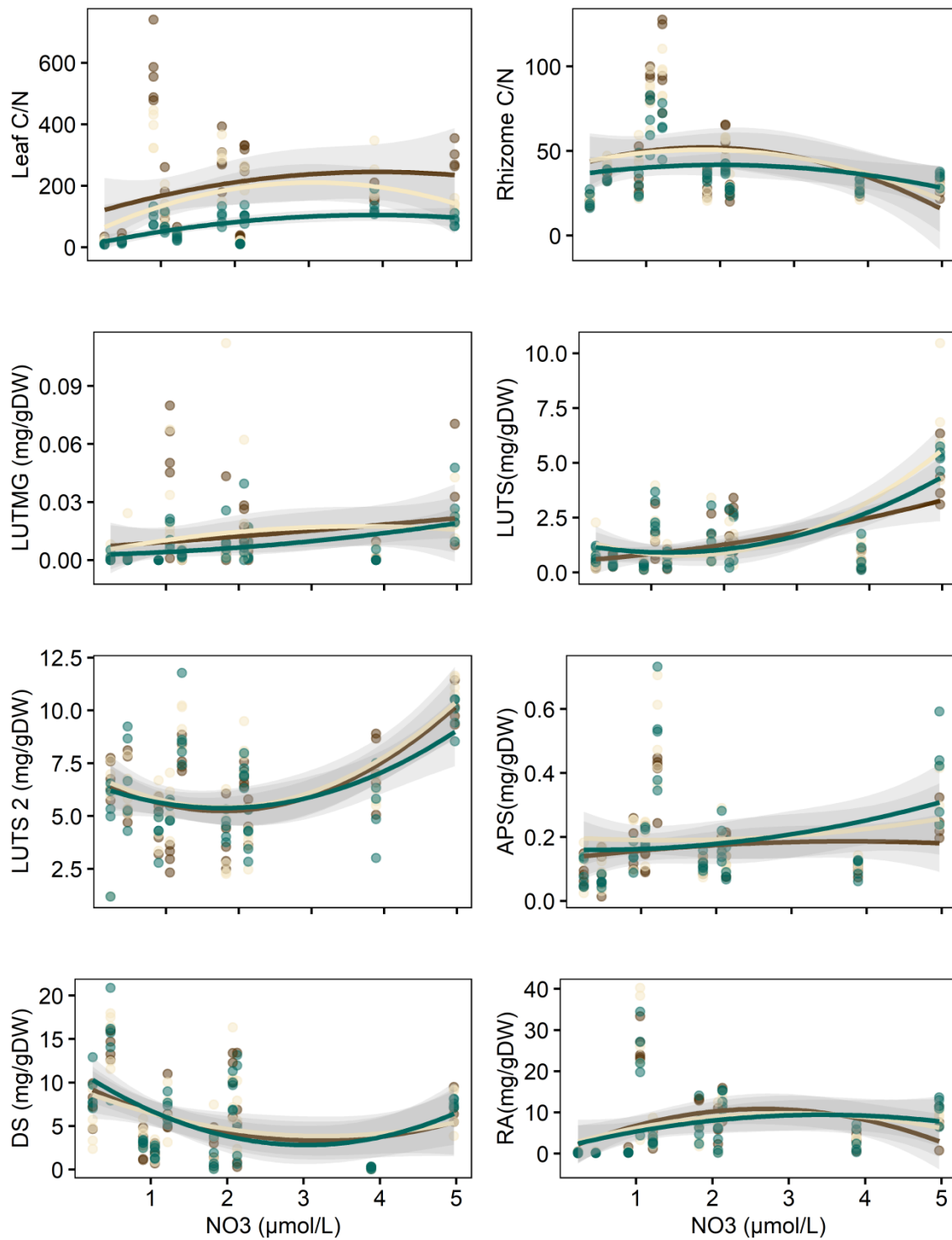
Figure S7. Distribution of seagrass chemical traits along the nitrate availability gradient. Grey bands indicate 95% confidence boundaries for the control (dark brown), moderate (beige) and high (green) herbivory treatment fitted curves (glm). APS indicates Apigenin-7-sulfate, DS diosmetin-7-sulfate, LUTS luteolin-7-sulfate, LUTG luteolin-7-O-β-glucoside, LUTS2 luteolin-7,3'-disulfate, LUTMG luteolin-7-O-β-(6''-malonyl) glucoside, and RA Rosmarinic acid.