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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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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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45

#### 46 BIOSKETCH

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

51 ecosystem structure and functioning.

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2       **ABSTRACT**  
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5       **Aim**  
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8       Studies on latitudinal patterns in plant defense have traditionally overlooked the potential  
9       effect that resource availability may have in shaping plant defense. Likewise, latitudinal  
10      patterns of tolerance traits have rarely been studied, yet they can be a critical component  
11      of plant defense. Therefore, the aim of our study was to examine latitudinal variation in  
12      the production of tolerance and resistance traits against herbivory along a latitudinal  
13      range and a natural gradient of resource availability from upwelling conditions.  
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16       **Location**  
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19       Canada, North America and Mexico  
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22       **Time period**  
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25       Summer months of 2015  
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28       **Major taxa used**  
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31       The seagrass *Zostera marina*  
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34       **Methods**  
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37       We conducted experiments simulating macroherbivore (e.g., bird, fish) damage at 10 sites  
38       across the Eastern Pacific coast (Canada-Mexico) and Quebec and analyzed several  
39       seagrass traits related to resistance and tolerance strategies against herbivory. In addition,  
40       we examined the effects of potential seagrass changes in defense strategies by performing  
41       a series of feeding experiments with mesoherbivores in a subset of sites.  
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44       **Results**  
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47       We found that eelgrass resistance defenses did not follow a linear latitudinal pattern but  
48       rather followed a bell-shaped curve which correlated with bottom-up control. In sites with  
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3     24 higher nutrient availability, plants allocated resources to tolerance strategies and had  
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5     25 lower resistance traits. Furthermore, seagrasses did not respond linearly to increased  
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7     26 herbivory pressure; while they tolerated moderate levels of herbivory, they underwent a  
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9     27 significant reduction in tolerance and resistance under high herbivory levels, which also  
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11     28 made them more susceptible to consumers in feeding experiments.  
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15     29 **Main conclusions**  
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18     30 Our results highlight the importance that nutrient availability has in shaping latitudinal  
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20     31 patterns of plant defense against herbivory and show how these defenses may not respond  
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22     32 linearly to increased herbivory pressure in seagrasses.  
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25     33 **Keywords:** Seagrass, plant-herbivore interactions, latitudinal gradient, herbivory,  
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27     34 resource availability, Limited Resource Model, phenolic compounds, *Zostera marina*,  
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29     35 nutrients, upwelling.  
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3       **37           MAIN TEXT**  
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6       **38     Title:** Latitudinal variation in plant defense against herbivory in a marine foundation  
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8       **39     species does not follow a linear pattern: the importance of resource availability**  
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10      **40     Running title:** Latitudinal patterns of seagrass defense  
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15      **42           INTRODUCTION**  
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18      Biotic interactions such as herbivory strongly influence plant fitness and  
19 distribution patterns (Wisz et al., 2013) and consequently plants have evolved diverse  
20 defense strategies to tolerate and resist herbivory (Núñez-Farfán, Fornoni, & Valverde,  
21 2007; Strauss & Agrawal, 1999). Tolerance strategies mitigate the negative effects of  
22 herbivory to plant fitness, for example, by compensating the biomass lost through  
23 herbivory with compensatory growth or by accumulating resources belowground to  
24 invest in regrowth (Rosenthal & Kotanen, 1994; Tiffin, 2000). Resistance strategies  
25 reduce the feeding preference or performance of herbivores by, for instance, reducing  
26 plant palatability or nutritional quality (Fritz & Simms, 1992). The strength of trophic  
27 interactions (i.e., biotic interactions hypothesis, BIH), and thus herbivory, is hypothesized  
28 to follow a latitudinal gradient with higher herbivory towards lower latitudes (Schemske,  
29 Mittelbach, Cornell, Sobel, & Roy, 2009). Such phenomenon has led to the proposal that  
30 plants are better defended towards lower latitudes (i.e. the Latitudinal-Herbivory-  
31 Defense-Hypothesis; LHDH; Coley & Aide, 1991). For instance, salt marsh plant  
32 palatability decreases towards lower latitudes as a result of lower leaf nitrogen content,  
33 higher toughness, or higher content of secondary compounds (Ho & Pennings, 2013;  
34 Pennings, Siska, & Bertness, 2001; Siska, Pennings, Buck, & Hanisak, 2002). In the  
35 marine environment, algae collected from tropical sites have higher chemical defenses  
36 (phenolic compounds), and thus lower palatability, when compared to temperate sites  
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3 62 (Bolser & Hay, 1996; Demko et al., 2017).  
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6 63 However, both the BIH and the LHDH remain topics of controversy (Anstett,  
7 Nunes, Baskett, & Kotanen, 2016; Hillebrand, 2004; A. Moles, 2013) and recent reviews  
8 have found weak support for the LHDH both in terrestrial and marine environments.  
9  
10 66 There is conflicting evidence in relation to plant chemical defenses (e.g., plants have  
11 more chemical defenses in temperate regions; Moles, Bonser, Poore, Wallis, & Foley,  
12 67 2011), and contradictory results in feeding patterns (Moles et al., 2011; Poore et al., 2012;  
13 68 Zhang, Zhang, Ma, & Shefferson, 2016). Importantly, these reviews, as well as many of  
14 69 the works therein, do not consider important aspects that can drive plant-herbivore  
15 70 interactions when examining these relationships besides chemical defense; such as  
16 71 nutritional quality, tolerance strategies, or environmental resources. In fact, other  
17 72 mechanisms (e.g., changes in cost of defense due to biogeography; Kooyers et al., 2017)  
18 73 may be explaining the latitudinal variability observed in plant defense. It has been  
19 74 pointed out that the solution to this controversy requires new investigations that  
20 75 encompass broad latitudinal geographic areas and that use consistent standardized  
21 76 methods among sites. Also, studies need to consider more types of defense traits (Anstett,  
22 77 Nunes, et al., 2016; Schemske et al., 2009) including defense induction (Anstett, Chen, &  
23 78 Johnson, 2016) and nutritional quality (Moles et al., 2011), and incorporate the use of  
24 79 feeding preference experiments to understand the mechanisms of interaction.  
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27 81 Most studies exploring latitudinal patterns in plant defense have focused on  
28 herbivore damage and resistance strategies, while latitudinal patterns in tolerance  
29 strategies have been largely unexplored (Anstett, Nunes, et al., 2016), yet they are a  
30 critical element of plant defense strategies (Núñez-Farfán et al., 2007; Strauss &  
31 84 Agrawal, 1999). The few existing tolerance studies have only measured compensatory  
32 85 growth, and have yielded inconsistent results likely due to biogeographical effects such as  
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3     87 similar regrowth responses driven by different selective pressures (e.g., leaf loss by  
4         88 freezing at high latitudes, and herbivore damage at low latitudes; Wieski & Pennings,  
5         89 2014; Woods, Hastings, Turley, Heard, & Agrawal, 2012). Importantly, plants have  
6         90 several other tolerance traits against herbivory (e.g., belowground reserves) whose  
7         91 variations have not been examined in a latitudinal context.  
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15     92              The availability of resources greatly influences the production of defensive traits,  
16         93 since resources allocated to defense can trade off with growth and reproduction (Strauss,  
17         94 Rudgers, Lau, & Irwin, 2002). The Resource Availability Hypothesis (RAH; Coley,  
18         95 Bryant, & Chapin, 1985) postulates that plants in resource-rich environments invest in  
19         96 induction of secondary metabolites (produced in response to herbivory) instead of  
20         97 investing in constitutive resistance (produced regardless of the risk of attack; Agrawal &  
21         98 Karban, 1999). However, recent works assessing the predictions of RAH for intraspecific  
22         99 variations in plant defense (intraspecific-RAH), propose the opposite; i.e. that plant  
23         100 populations from resource-rich environments have higher constitutive resistance, which is  
24         101 mediated by the higher herbivory pressure often found in these environments.  
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26     102 Accordingly, in low-resource environments, inducibility of resistance traits should be  
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28     103 more effective and would trade-off with constitutive resistance (Hahn & Maron, 2016).  
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30     104 Regarding tolerance strategies, resources have also been postulated to drive their  
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32     105 production. For instance, the Limiting Resource Model (LRM) proposes that plants are  
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34     106 more tolerant to herbivory when limiting resources are available and if herbivore damage  
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36     107 does not actually hamper the acquisition or use of those resources (Wise & Abrahamson,  
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38     108 2007).

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41     109              Since environmental resources (e.g., light, nutrients) change with latitude,  
42         110 latitudinal patterns of both resistance and tolerance defense strategies are also likely to be  
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44         111 influenced by resource availability in addition to herbivore pressure or damage.

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3 112 Furthermore, the level of herbivore pressure (i.e., duration and/or intensity) can also shift  
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5 113 plant defense responses. While some traits may be more prevalent under high herbivory  
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7 114 rates (e.g., induction of secondary metabolites; Dostálek, Rokaya, Maršík, & Rezek,  
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9 115 2016), others are induced under moderate pressure (e.g., compensatory growth; Vergés,  
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11 116 Pérez, Alcoverro, & Romero, 2008). Understanding how resource availability and  
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13 117 herbivory may interact to modify plant defense traits in a latitudinal context is highly  
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15 118 relevant, especially in marine environments, given that eutrophication is usually a  
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17 119 concern in coastal areas and upwelling events are predicted to increase their intensity due  
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19 120 to climate change (García-Reyes et al., 2015; Xiu, Chai, Curchitser, & Castruccio, 2018).  
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21 121 However, to our knowledge no studies have analyzed these interactions in a wide  
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23 122 latitudinal range.

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26 123 Beyond environmental factors, the suite of defense traits that plants exhibit  
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28 124 against herbivory involve different traits that are heritable and influenced by genetic  
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30 125 constraints that modulate the expression of these traits (e.g., genetic variation; Andrew,  
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32 126 Wallis, Harwood, Henson, & Foley, 2007; O'Reilly-Wapstra, McArthur, & Potts, 2002).  
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34 127 In fact, reduction of genotypic richness or increased inbreeding reduce plant resistance to  
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36 128 herbivory (Du, Winsor, Smith, DeNicco, & Stephenson, 2008; Moreira, Abdala-Roberts,  
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38 129 Parra-Tabla, & Mooney, 2014). Therefore, it is important to consider genetic variations in  
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40 130 influencing patterns of plant defense, especially in wide spatial range investigations such  
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42 131 as latitudinal studies.

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45 132 Plant-herbivore interactions can have profound effects in ecosystems, especially  
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47 133 when involving foundation species, due to direct (e.g., loss of feeding resources) and  
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49 134 indirect (e.g., loss of refuge) impacts on associated species as well as on ecosystem  
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51 135 properties (e.g., nutrient cycling; Silliman et al., 2013). As foundation species, seagrasses  
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53 136 create critical coastal ecosystems and contribute to many ecosystem services (e.g., carbon

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3     137 burial, reduction of coastal erosion; Nordlund, Koch, Barbier, & Creed, 2016). Seagrasses  
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5     138 are chemically defended (Zidorn, 2016) and studies have shown that some seagrass traits  
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7     139 such as below and aboveground biomass vary with latitude (Clausen, Krause-Jensen,  
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9     140 Olesen, & Marbà, 2014; Ruesink et al., 2018; Soissons et al., 2018), yet, to our  
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11     141 knowledge, no works have studied latitudinal variations in defenses against herbivory in  
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13     142 seagrasses. Importantly, given their critical role as foundation species, variations in the  
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15     143 ecological roles of seagrasses are expected in concert with variations in plant traits and  
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17     144 interactions with herbivores.  
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22     145 The aim of this study was to investigate the applicability of the resource  
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24     146 hypotheses (RAHs, LRM) in explaining latitudinal patterns of plant tolerance and  
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26     147 resistance traits against herbivory and the potential inductive responses to different  
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28     148 herbivory rates. Integrating these elements is necessary in wide latitudinal studies  
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30     149 because plants are exposed to gradients of herbivory and resource availability, and thus,  
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32     150 their allocation of resources to defense is likely constrained by the interaction between  
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34     151 these two factors. We predicted that marine plants from regions with more resources  
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36     152 available (e.g. upwelling sites) would allocate more resources to tolerance (following  
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38     153 LRM), having higher (following intraspecific-RAH) constitutive resistance and lower  
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40     154 inducibility of secondary metabolites under grazing pressure. Consequently, we expected  
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42     155 that herbivores would modify their feeding behavior in response to changes in plant traits.  
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45     156 We tested these predictions by performing a simulated herbivory experiment with the  
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47     157 seagrass *Zostera marina*. *Z. marina* is a dominant foundation species in estuaries and  
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49     158 coastal areas across the northern hemisphere and is consumed by a wide variety of  
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51     159 grazers including birds and small invertebrates (e.g. amphipods, isopods) which can  
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53     160 strongly influence eelgrass abundance and distribution (Kollars et al., 2017; Reynolds,  
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55     161 Carr, & Boyer, 2012; Tomas et al., 2011). Although, to our knowledge, there are no  
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3 162 comprehensive studies on the levels of herbivory along the sites of this study, numerous  
4 163 herbivores that feed on seagrass such as isopods, amphipods or waterfowl are present in  
5 164 the study sites (Personal observation; Duffy, Macdonald, Rhode, & Parker, 2013;  
6 165 Hayduk, Hacker, Henderson, & Tomas, 2019; Kollars et al., 2017; Reynolds et al., 2012).  
7 166 While the studies that have explored patterns in herbivory pressure in seagrasses do not  
8 167 find a consistent latitudinal tendency (Vergés et al., 2018), temperate seagrasses are  
9 168 expected to suffer increasingly higher herbivory damage due to the poleward expansion  
10 169 of tropical herbivores (Hyndes et al., 2016; Vergés et al., 2014).  
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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 ( $52^{\circ}\text{N}$ – $32^{\circ}\text{N}$ ), 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.,  $\text{NO}_3$ ) concentration  
186 exhibits a maximum off the coast of California where coastal upwelling is most intense

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3 187 (Fig. 1b., S2). This upwelling strongly influences the nutrient availability and community  
4 188 structure of coastal reefs (Menge & Menge, 2013) and estuaries in the US west coast  
5 189 (Hayduk et al., 2019; Hessing-Lewis & Hacker, 2013). Indeed, leaf N of seagrasses in  
6 190 study sites exhibits a significant positive correlation with NO<sub>3</sub> values. (Fig. S3). Further  
7 191 descriptions about the abiotic factors of the study sites are provided in the Supplementary  
8 192 material (Study site description S1).  
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17 193 The experiment started the first week of May 2015 and was maintained for a total  
18 194 of 10-11 weeks in order to capture plant chemical and size changes. A total of fifteen 4m<sup>2</sup>  
19 195 experimental plots, five per treatment, were randomly distributed in shallow subtidal  
20 196 (0.5-1 m at low tide) homogeneous monospecific meadows *Z. marina* (low wave  
21 197 exposure and low natural herbivory) avoiding the edges of meadows or patchy areas.  
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29 198 Plots were separated by 3 m and the treatments were randomly assigned to each plot.  
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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; Sammartí, 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

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3 212 resistance traits (see below and Table S2).  
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6 213 ***Plant tolerance traits***  
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8 214 Leaf relative growth rate and shoot size are tolerance traits related to compensatory  
9 215 growth response (Tiffin, 2000; Vergés et al., 2008; Table S2). Leaf relative growth rate  
10 216 ( $\text{RGR}; \text{ day}^{-1}$ ) was measured in five to ten shoots per plot by hole punching leaves two  
11 217 weeks before the end of the experiment following the method of Zieman (1974) and  
12 218 dividing the growth area by the total leaf area of the shoot and the number of days  
13 219 elapsed since punching. Leaf width, maximum leaf length, number of leaves, total leaf  
14 220 area, leaf biomass, sheath area (proxy of shoot size and unaffected by clipping; Ruesink  
15 221 et al., 2018), rhizome biomass, and number of rhizome internodes were measured at the  
16 222 end of the experiment.  
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19 223 Nitrogen, carbon and sucrose of the three newest formed leaves (hereafter young  
20 224 leaves) and of rhizomes as well as belowground (i.e. rhizome) starch content were  
21 225 analyzed from pooled plant material collected at the end of the experiment. All these  
22 226 chemical traits are considered tolerance traits (Table S2) since belowground resources  
23 227 can be reallocated to reproduction or regrowth after herbivory (Stowe, Marquis,  
24 228 Hochwender, & Simms, 2000; Tiffin, 2000). N and C in all tissues were analyzed using a  
25 229 Carlo-Erba CNH elemental analyzer. Sucrose and starch were analyzed with the  
26 230 Anthrone assay. A more extensive explanation for the categorization of tolerance traits,  
27 231 and detailed methods for chemical analyses of plant traits are described in the  
28 232 Supplementary material (Methodology S1, Table S2).  
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31 233 ***Plant resistance traits***  
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33 234 Specific phenolic compounds, nitrogen, carbon, and fiber content in leaves are  
34 235 considered resistance traits (Table S2) due to their effects in the palatability or  
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3     236 digestibility of the plant and therefore herbivore preference (Hernán, Castejón, Terrados,  
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5     237 & Tomas, 2019; Sieg & Kubanek, 2013). These traits were analyzed in young leaves  
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7     238 from pooled plant material collected at the end of the experiment. Specific phenolic  
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9     239 compounds were analyzed with ultra-performance liquid chromatography-tandem mass-  
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11     240 spectrometry as in Hernán et al., (2017) with modifications (see Methodology S1). We  
12  
13     241 identified 7 phenolic compounds; Rosmarinic acid (RA), and 6 flavonoids (i.e., apigenin-  
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15     242 7-sulfate, APS; diosmetin-7-sulfate, DS; luteolin-7-sulfate, LUTS; luteolin-7-O- $\beta$ -  
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17     243 glucoside, LUTG; luteolin-7,3'-disulfate, LUTS2; luteolin-7-O- $\beta$ -(6''-malonyl)  
18  
19     244 glucoside, LUTMG). Specific leaf area (SLA= leaf area/ leaf weight; cm<sup>2</sup>/g), which is  
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21     245 used to evaluate leaf toughness (with high SLA indicating low toughness; Paul et al.,  
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23     246 2012), was quantified in five to ten shoots per plot at the end of the experiment. A  
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25     247 detailed methodology for all chemical analyses of plant traits can be found in the  
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27     248 Supplementary material (Methodology S1).

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33     249         ***Genetic metrics***  
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36     250         Allelic richness and genotypic diversity (clonality) may follow a linear or  
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38     251 unimodal latitudinal gradient, reflecting phylogeographic history in which range edges  
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40     252 show low diversity and an overall attenuation from south to north. These variables were  
41  
42     253 assessed to be used as covariates to control for genetic variance. Genetic metrics (allelic  
43  
44     254 richness and genotypic richness) were assessed on 20 shoots per site (maintaining 2 m  
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46     255 between shoots) with 24 microsatellite loci. Details about the methodology for genetic  
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48     256 metrics of eelgrass can be found in the Supplementary material (Methodology S2).

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53     257         ***Herbivore feeding assays***  
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56     258         To examine the effect of simulated herbivory-driven changes on feeding behavior  
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58     259 of other herbivores within the community, we conducted a series of three-choice feeding

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3 experiments in which we measured tissue consumption (i.e., tissue consumed from one  
4 treatment relative to the total tissue consumed). The experiments were performed with an  
5 invertebrate grazer commonly found in *Z. marina* seagrass beds along the Central and  
6 North Pacific coast, the isopod *Pentidotea resecata*. The isopods were collected from two  
7 sites (Coos Bay and Yaquina Bay) and were offered fresh eelgrass leaves without  
8 epiphytes collected from all three simulated herbivory treatments of their own sites.  
9 Additionally, in order to examine if the feeding patterns observed were driven by  
10 mechanical-structural or chemical traits, we performed three-choice feeding experiments  
11 with agar-based artificial food (Siska et al., 2002; Tomas et al., 2015). The experiments  
12 consisted of 10-15 replicates and ended when approximately 50% of initial material was  
13 consumed. Further details about the methodology of the feeding assays are provided in  
14 the Supplementary material (Methodology S3).

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19 **Statistical analyses**

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21 We calculated one mean value of the response variables measured per plot having  
22 thus 5 replicates per treatment and site. We performed a Principal Component Analysis  
23 (PCA) with all the 31 plant response variables measured or calculated from the variables  
24 measured (e.g., rhizome biomass per internode= rhizome biomass/number of internodes;  
25 SLA; RGR) in order to visualize possible patterns of plant defense strategies across  
26 clipping treatments and sites (Fig. 2). We tested our predictions (effects of latitude,  
27 resource availability, and simulated herbivory treatments, and their interactions) while  
28 controlling for potential covariation introduced by the genetic traits using univariate  
29 General Linear Models (GLMs). Prior to performing the GLMs, we explored the  
30 correlation among our response variables to reduce the number of GLMs to fit (Fig. S3)  
31 and selected 15 response variables which are also the more relevant as defense traits (i.e.,  
32 leaf RGR, number of leaves, sheath area, rhizome biomass per internode, leaf nitrogen,  
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3     285 carbon and sucrose content, rhizome nitrogen, carbon, sucrose and starch content, SLA,  
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5     286 leaf fiber content, total flavonoids and specific phenolic compounds) to test our  
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7     287 hypotheses. We also explored the correlation between potential explanatory variables  
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9     288 (genetic covariates, SST, latitude and NO<sub>3</sub>) to avoid co-linearity in the model fitting and  
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11     289 discarded SST due to its high negative correlation with NO<sub>3</sub> and latitude ( $R^2=-0.7$  for  
12  
13 both; Fig. S3). Consequently, NO<sub>3</sub> and latitude were used as explanatory variables and  
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15 genotypic richness as a covariate. We reduced the full GLM of each response variable  
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17 using a stepwise selection procedure to find the model with the lowest Akaike  
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19 information criterion (AIC) value to avoid type I errors (Table S3). The coefficients,  
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21 standard errors, and p-values of fixed effects in all minimum adequate models were  
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23 estimated via iteratively reweighted least squares using the glm function of the core R-  
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25 package (R Core Team, 2007). Standardized generalized variance-inflation factors were  
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27 also calculated as an index of multicollinearity. All models were fitted considering a  
28  
29 Gaussian (i.e., normal) distribution of errors. Hence, the distribution of the residuals  
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31 (errors) was checked for normality and response variables were transformed when the  
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33 distribution of the residuals deviated from normality.  
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40     301 For the results of the feeding assays, we tested whether the level of simulated  
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42 herbivory affected the amount of eelgrass biomass consumed by *P. resecata* using  
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44 multivariate Hotelling's T<sup>2</sup> test without transformation of the variables. Post hoc analyses  
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46 were performed with Tukey multiple comparisons of means. The detailed methodology  
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48 for all statistical analyses is described in the Supplementary material (Methodology S4).  
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55     307 **RESULTS**  
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58     308 ***Latitudinal variation of defense traits***  
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3 309 We found that both tolerance and resistance traits (except number of leaves and  
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5 310 rhizome biomass per internode) varied with latitude.  
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8 311 The PCA (Fig. 2) differentiates two groups in axis 1 (PC1); the sites at the  
9 extremes of the *Z. marina* distribution range, and the sites at intermediate latitudes where  
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11 312 the shoots reach larger sizes with higher growth rates and belowground resources (PC1,  
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13 313 Fig. 2). The results of the analyses on tolerance and size traits show that leaf sucrose,  
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15 314 sheath area and correlated morphometric traits (Fig. S3) peaked at mid-latitudes (Fig. 3  
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17 315 and Fig. S4). Carbon-based belowground resources (i.e., rhizome starch, sucrose and C)  
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19 316 increased with increasing latitude (Table 1, Fig. 3), whereas belowground N and leaf  
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21 317 relative growth rate were lower towards higher latitudes (Table 1, Fig. 3). Genotypic  
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23 318 richness statistically influenced all tolerance traits, except rhizome C, but there were no  
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25 319 overall consistent patterns with latitude (Table 1).  
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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).

### 50 329 ***Resource availability and defense traits***

53 330 Sheath area as well as rhizome C, N, sucrose and biomass (and correlated traits,  
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55 331 Fig. S3, S6 and Fig. S7) increased with nitrate availability, while number of leaves and  
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57 332 rhizome starch decreased (Table 1, Fig. 5). Leaf N, phenolic compounds and flavonoids

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3     333 increased with higher nitrate availability whereas, leaf fiber content exhibited the  
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5     334 opposite pattern (Table 1, Table S4, Fig. 6 Fig. S6 and Fig. S7)  
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8     335           *Latitudinal patterns of defense traits in response to herbivory*  
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11     336           Plant traits did not differ between the control and the moderate herbivory  
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13     337 treatments, and only the high herbivory treatment had effects on defense traits in *Z.*  
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15     338 *marina*, which affected most of the tolerance traits. For instance, plants exhibited a higher  
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17     339 relative growth rate under high simulated herbivory, which was further enhanced under  
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19     340 high nutrient availability (Table 1, Fig. 5). Number of leaves, rhizome biomass per  
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21 internode, and sucrose content of both leaves and rhizomes were lower under the high  
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23 herbivory treatments. In addition, rhizome C and sheath area exhibited only a significant  
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25 decrease in high herbivory treatments at high and mid latitudes, respectively (Table 1,  
26  
27 Fig. 3). The only traits related to resistance that increased significantly under the high  
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29 herbivory treatment were SLA and leaf N, being the increase in leaf N stronger towards  
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31 higher latitudes (Table 1, Fig. 4).  
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37     347           *Herbivore responses to simulated herbivory-driven changes in plant palatability*  
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40     348           Isopods from both Coos Bay and Yaquina Bay consumed significantly higher  
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42 amounts of fresh leaf biomass and agar-base food from the high herbivory treatment  
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44     350 when compared to the control treatment in the three-choice experiments (Fig. 7).  
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50     352           **DISCUSSION**  
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53     353           This work shows that most eelgrass defense traits against herbivory follow a bell-  
54 shaped distribution, which suggests that latitudinal differences in defense traits of *Z.*  
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56 *marina* are strongly driven by bottom-up forces, supporting the Resource Availability  
57 Hypothesis (RAH). Tolerance traits (6 of the 9 traits) were strongly associated with high  
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3 357 resource availability (i.e., upwelling) as expected by the limiting resource model (LRM).  
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5 358 On the other hand, we found higher investment in resistance traits towards both extremes  
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7 359 of the distribution of eelgrass (and not only at lower latitudes), likely influenced by lower  
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9 resources at these extremes in comparison to mid-latitudes (as proposed by the RAH and  
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11 360 contrary to the intraspecific-RAH). Furthermore, none of the *Z. marina* traits analyzed  
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13 361 were significantly affected by moderate (i.e. 40% loss) herbivore damage, while high  
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15 362 herbivory induced compensatory growth, decreased resistance, and increased palatability,  
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17 363 which further enhanced susceptibility to herbivory by other grazers within the  
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19 364 community.  
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24 366 Most tolerance traits were higher in the sites with higher nutrient availability,  
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26 367 coincident with the region of strong upwelling (Schwing & Mendelssohn, 1997).  
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28 368 Upwelling brings nutrient-rich waters which also have higher  $p\text{CO}_2$  (Feely, Sabine,  
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30 369 Hernandez-Ayon, Ianson, & Hales, 2008), thus providing resources that generally limit  
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32 370 seagrass productivity (Beer, Bjork, Hellblom, & Axelsson, 2002; Lee, Park, & Kim,  
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34 371 2007). In accordance with the LRM, we observed higher belowground resources and  
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36 372 aboveground growth rates with higher nutrient availability under herbivory. At mid  
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38 373 latitudes, higher resource availability appears to translate into higher aboveground  
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40 374 (Ruesink et al., 2018) and belowground per capita biomass. Consequently, populations in  
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42 375 high resource availability environments are likely to be better prepared to tolerate  
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44 376 herbivory or other disturbances that can remove aboveground biomass (e.g. harsher  
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46 377 hydrodynamic conditions) having more resources to invest for regrowth. Our study also  
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48 378 indicates that summertime leaf relative growth rate decreases with increasing latitude,  
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50 379 perhaps due to lower temperature in northern sites, which has been suggested as an  
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52 380 important factor controlling annual seagrass productivity (Clausen et al., 2014). In  
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54 381 addition, towards the northern sites, carbon mainly accumulates into carbohydrate  
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3       382 reserves in belowground tissues rather than being invested in plant growth, probably as  
4       383 an adaptation to overwintering (Clausen et al., 2014; Robertson & Mann, 1984; Soissons  
5       384 et al., 2018). Indeed, the non-linearity of the relationship of some of the variables with  
6       385 nutrient availability is likely due to this adaptation, leading to lower values of some  
7       386 variables (e.g., number of leaves) at intermediate levels of nitrate concentrations, which  
8       387 correspond to high latitude sites. Moreover, genotypic richness appears to influence most  
9       388 seagrass size traits, which may be related to previous findings of higher eelgrass biomass  
10      389 in more genotypically diverse sites (Hughes & Stachowicz, 2004; Reusch, Ehlers,  
11      390 Hämmelerli, & Worm, 2005). It has been suggested that higher genetic diversity increases  
12      391 positive interactions such as resource partitioning, improving performance in more  
13      392 genotypically diverse populations (Hughes, Inouye, Johnson, Underwood, & Vellend,  
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31       394 The production of resistance traits also appears to be strongly influenced by  
32       395 available resources. As predicted by the RAH, plants from the sites with high nutrient  
33       396 availability tended to have higher nitrogen and lower fiber contents in leaves, and thus  
34       397 lower constitutive resistance. Indeed, experimental evidence shows that increases in  
35       398 nutrient availability usually lead to higher nutrient content (Hernán et al., 2019; Tomas et  
36       399 al., 2015) and lower fiber production in plant tissues (Goecker, Heck, & Valentine, 2005;  
37       400 Hernán et al., 2019). On the other hand, the pattern that we detected of lower resistance in  
38       401 high-resource areas does not follow the predictions of the intraspecific-RAH, which  
39       402 predicts that resistance is mediated (and higher) by the higher herbivory suffered at  
40       403 resource-rich sites. In fact, some plant species inhabiting high resource environments do  
41       404 not undergo high herbivory rates and thus, the predictions of the intraspecific-RAH may  
42       405 not always be applicable (Lamarre et al., 2012). Such lower investment in structural  
43       406 defenses and higher nutritional quality typically enhance plant susceptibility to herbivory

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3 407 (e.g., Goecker et al., 2005; Hernán et al., 2019; Lucas, 2000; Tomas et al., 2011), and  
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5 408 thus would likely make mid-latitude plants more susceptible to consumers.  
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8 409 Furthermore, according to LHDH, we would expect a negative linear relationship  
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10 410 between the concentrations of phenolic compounds and latitude (Anstett, Nunes, et al.,  
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12 411 2016; Rasmann & Agrawal, 2011), and we would also expect an induction in the  
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14 412 production of chemical defenses in response to herbivory. Yet, we did not observe either  
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16 413 of those phenomena. In fact, in our system, the production of chemical defenses appears  
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18 414 to be driven by resource availability rather than herbivory pressure. We found that total  
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20 415 flavonoid compounds and total phenolic compounds were slightly higher in higher  
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22 416 nutrient availability conditions. Nevertheless, specific compounds exhibited contrasting  
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24 417 responses. The most abundant flavonoid (DS) was lower at mid latitudes (Fig. S5) while  
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26 418 other less abundant flavonoids (LUTG, LUTMG, LUTS), which have been related to UV  
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28 419 protection (LUTG, LUTMG; Mierziak et al., 2014), exhibited the opposite pattern (Fig.  
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30 420 S5), increasing with higher nutrient availability (Fig. S7). Likewise, Rosmarinic acid  
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32 421 (RA) was one of the most abundant phenolic compounds found in this study. RA has  
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34 422 been found to have antibacterial activity (Guan, Saha, & Weinberger, 2019) and has been  
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36 423 suggested to play a role in pathogen and herbivore protection (Khan et al., 2019;  
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38 424 Simmonds, Stevenson, & Hanson, 2019). This compound exhibited higher concentrations  
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40 425 at mid latitudes (Fig. S5) and, in fact, previous studies have found a strong positive  
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42 426 correlation among RA and leaf N in seagrasses (Ravn et al., 1994). The idiosyncratic  
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44 427 responses in the content of specific phenolic compounds may affect the capacity of plants  
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46 428 to defend against pathogens, herbivores and UV-radiation. However, further studies need  
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48 429 to be performed in order to actually determine the specific function of many of these  
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50 430 particular compounds in seagrasses. On the other hand, genotypic richness seemed to  
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52 431 influence some chemical resistance traits such as leaf N, total flavonoids and half of the  
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3     432 phenolic compounds identified. Genetic identity influences the production of total  
4 phenolic compounds in *Z. marina* (Tomas et al., 2011), and intraspecific (genetically-  
5 driven) differences in production of secondary metabolites have major ecosystem effects  
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7     433 on *Populus*-based ecosystems (see reviews by Schweitzer et al., 2008; Whitham et al.,  
8  
9     434 2006) highlighting the potential role that intraspecific genetic variations may have in  
10 driving chemical composition in eelgrass and consequently influencing herbivory and  
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12     435 other important ecological processes (e.g. decomposition, nutrient cycling; Schweitzer et  
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14     436 al., 2008; Whitham et al., 2006).  
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22     440 The only resistance trait that followed the predictions of the LHDH (i.e. more  
23 investment in defense at lower latitudes) was specific leaf area (SLA), with southern  
24 populations having tougher leaves (i.e., lower SLA). While higher SLA could potentially  
25 be a response to higher grazing impact at the southern sites, it may also be related to  
26 higher temperature stress that plants suffer during low tides in southern sites (Ramírez-  
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28     441 Valiente, Valladares, Sánchez-Gómez, Delgado, & Aranda, 2014). On the other hand,  
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30     442 higher SLA at northern sites may be a consequence of the need to increase plant  
31 photosynthetic capacity (and thus leaf area) due to light limitation (Enriquez & Sand-  
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33     443 Jensen, 2003).  
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449     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

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3 457 (Sanmartí et al., 2014), and which was also observed in our study, with an important  
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5 reduction in rhizome biomass per internode, and sucrose content. Importantly, such  
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7 reduction was attenuated at mid and high-latitudes, likely as a result of higher resource  
8 availability (LRM; Wise & Abrahamson, 2007). Furthermore, rhizomes suffered a  
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10 stronger reduction of carbon reserves towards northern latitudes, which may be driven by  
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12 a limitation in carbon uptake often observed at high latitudes as a result of light limitation  
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14 (Reich & Oleksyn, 2004). In addition, given that at these northern sites leaves had higher  
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16 nitrogen content (see below), lower C reserves may also be due to the use of carbon for  
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18 nitrogen assimilation (Touchette & Burkholder, 2000).  
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24 466 Interestingly, plants under intense herbivory did not increase their resistance  
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26 against consumption. On the contrary, we observed that high herbivory rates made leaves  
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28 more palatable (with decreased toughness and higher nitrogen content), which may have  
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30 resulted in part from a stimulation of nitrogen uptake due to defoliation under high  
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32 herbivory (Jaramillo & Detling, 1988; Valentine et al., 2004). The increase of nitrogen  
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34 pools under intense herbivory was particularly stronger at the northern sites, and this may  
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36 be a consequence of a lower use of nitrogen for growth (Kerkhoff, Enquist, Elser, &  
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38 Fagan, 2005; Reich & Oleksyn, 2004). The changes that we observed in SLA and  
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40 nutrients under high herbivory would likely make these plants even more attractive to  
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42 grazers. In fact, herbivores are known to perform “cultivation” or “gardening” grazing  
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44 whereby they maintain certain species or plant tissues that optimize their foraging  
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46 (Bjorndal, 1985; Preen, 1995), and we indeed observed that this herbivory-driven  
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48 decrease in resistance made plants consistently more susceptible to consumption by  
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50 isopods. Consequently, negative impacts of macroherbivores such as fish or waterfowl on  
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52 eelgrass may be further enhanced by facilitating susceptibility to other consumers within  
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54 the community. Indeed, isopods from both populations tended to prefer the most clipped  
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3 482 leaves, which were also the more nutritious (i.e., high leaf nitrogen content), more tender  
4 483 (i.e., higher SLA) and which had not exhibited any induction of phenolic compounds or  
5 other resistance traits. The fact that isopods exhibited the same preferences when we  
6 performed the agar-base feeding experiment suggests that nutritional quality and possibly  
7 structural traits are driving the feeding behavior of this herbivore (Tomas et al., 2011,  
8 486 2015).  
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17 488 Our study highlights the importance that resource availability has in shaping  
18 latitudinal patterns of plant defense strategies against herbivory in a dominant foundation  
19 species. Furthermore, our results also indicate that while eelgrass populations are highly  
20 tolerant to moderate levels of herbivory, they are not adapted to high herbivory.  
21 491 Importantly, tolerance to high herbivory was enhanced in the sites with high resource  
22 availability. Although there are no comprehensive studies on patterns of herbivory  
23 pressure on *Z. marina*, this adaptation to moderate herbivory could be primarily driven by  
24 494 consumption from waterfowl (Kollars et al., 2017; Rivers & Short, 2007). Importantly,  
25 495 warming-driven range expansion of tropical herbivores, such as fish or sirenians, into  
26 496 temperate areas is already occurring in many regions (e.g., Mediterranean Sea, Gulf of  
27 497 Mexico, Australian coast), strongly enhancing consumption pressure on benthic  
28 498 macrophytes (Hyndes et al., 2016; Vergés et al., 2016, 2014). The establishment of these  
29 500 new species will increase herbivory pressure to these systems, particularly at their lower  
30 501 edge of their distribution. Moreover, migration patterns of waterfowl species are being  
31 502 altered with climate change and degradation of habitats (Ward et al., 2005) and may shift  
32 503 spatial patterns of grazing pressure. These new scenarios of grazing could have strong  
33 504 detrimental effects on temperate seagrasses, not only by directly reducing plant standing  
34 505 stock, but also by reducing resistance traits and facilitating herbivory by other grazers in  
35 506 the community. Such processes could be especially problematic under conditions of high  
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3 507 resource availability, because while more resources may initially enable to better tolerate  
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5 508 herbivory, they also make plant tissues less resistant to herbivores.  
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3     **DATA AVAILABILITY**  
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5         All data supporting the results in the paper are publicly available at Figshare data  
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7         repository 10.6084/m9.figshare.13048871.  
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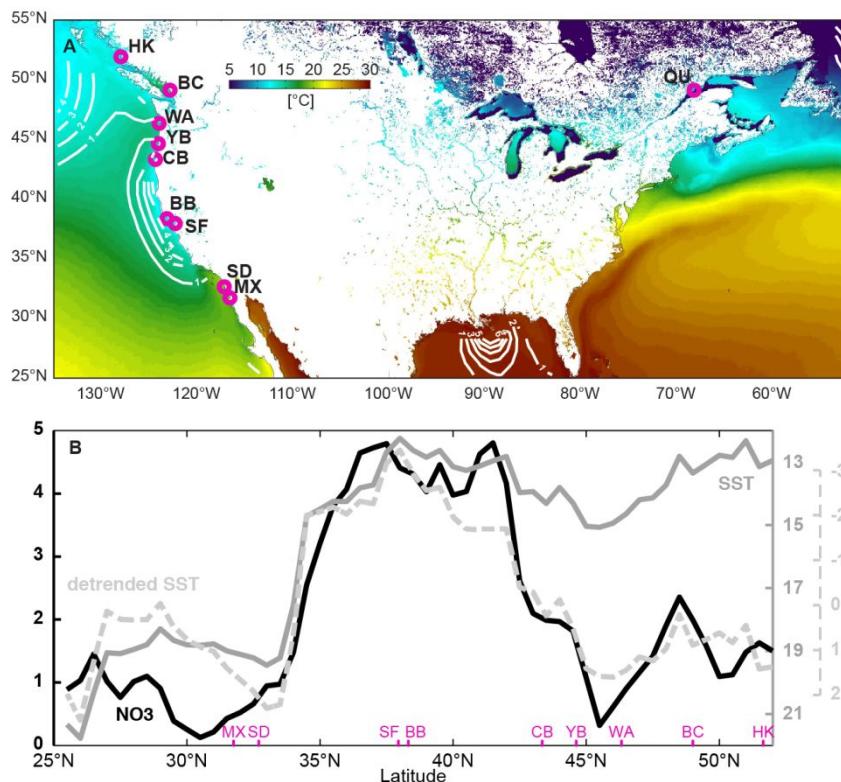
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**Table 1:** Results of GLM on defense traits. Intercept (i) and slopes of latitude ( $\beta_{\text{lat}}$ ), herbivory simulation moderate ( $\beta_H$ ) and high level ( $\beta_{HH}$ ), nitrate ( $\beta_{\text{NO}_3}$ ), their interactions (marked with asterisc), and genotypic richness ( $\beta_{\text{G.R.}}$ ). 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).

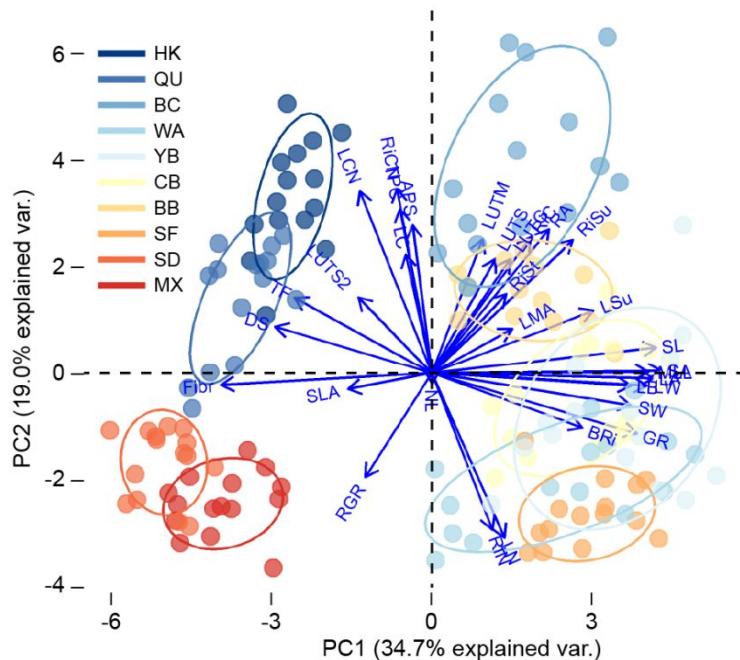
		i	$\beta_{\text{lat}}$	$\beta_H$	$\beta_{HH}$	$\beta_{\text{NO}_3}$	$\beta_{\text{G.R.}}$	$\beta_{\text{lat}^*H}$	$\beta_{\text{lat}^*HH}$	$\beta_{\text{NO}_3^*H}$	$\beta_{\text{NO}_3^*HH}$
<b>Sqrt (Relative Growth Rate (d-1))</b>	Est.	0.39	-0.003	0.002	0.013	-0.003	-0.099			0.007	0.011
	SE	0.033	<0.001	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	<b>0.027</b>
<b>Number of leaves</b>	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				
<b>Sheath area(cm<sup>2</sup>)</b>	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	<b>0.009</b>	<0.001	0.655	0.172	<0.001	<0.001	0.512	<b>0.031</b>		
<b>Log (Rhizome biomass (grFW/internode))</b>	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				
<b>Leaf sucrose (%)</b>	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	<b>0.037</b>		<0.001				
<b>Logit (Rhizome starch(%))</b>	Est.	0.216	0.03		-0.8						
	SE	0.33	0.008		0.032						
	P	0.514	<0.001		<b>0.016</b>						
<b>arcsine (Rhizome sucrose (%))</b>	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	<b>0.003</b>	<0.001	0.537	<b>0.025</b>	<0.001	0.135				
<b>Logit Rhizome C (%)</b>	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	<b>0.037</b>		
<b>Rhizome N (%)</b>	Est.	2.829	-0.027		0.044	-0.835					
	SE	0.309	0.005		0.019	0.247					
	P	<0.001	<0.001		<b>0.023</b>	<0.001					
<b>SLA(cm<sup>2</sup>/g)</b>	Est.	-419.589	23.442	23.189	178.176						
	SE	176.321	4.111	62.413	62.413						
	P	<b>0.019</b>	<0.001	0.711	<b>0.005</b>						
<b>Logit (Fibre (%))</b>	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					
<b>Logit (LeafC (%))</b>	Est.	-0.929	0.005		0.097						
	SE	0.076	0.001		0.061						
	P	<0.001	<0.001		0.112						
<b>Logit (Leaf N (%))</b>	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	<b>0.02</b>	0.876	0.081
<b>Sqrt (TF (mg/gDW))</b>	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	<b>0.006</b>					
<b>Sqrt (TPC (mg/gDW))</b>	Est.	3.007	0.027		0.143						
	SE	0.646	0.015		0.064						
	P	<0.001	0.077		<b>0.027</b>						

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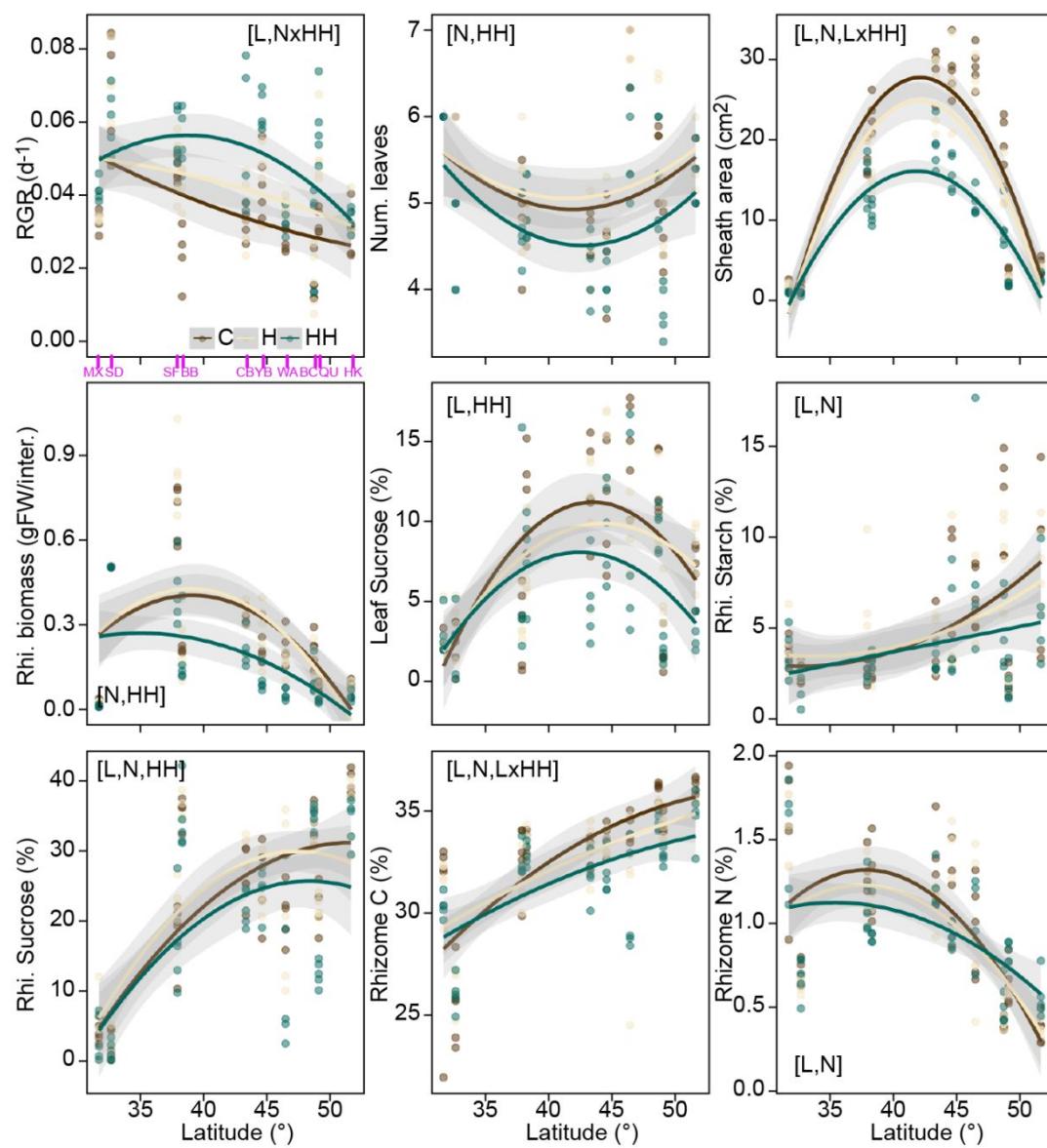
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**FIGURES**

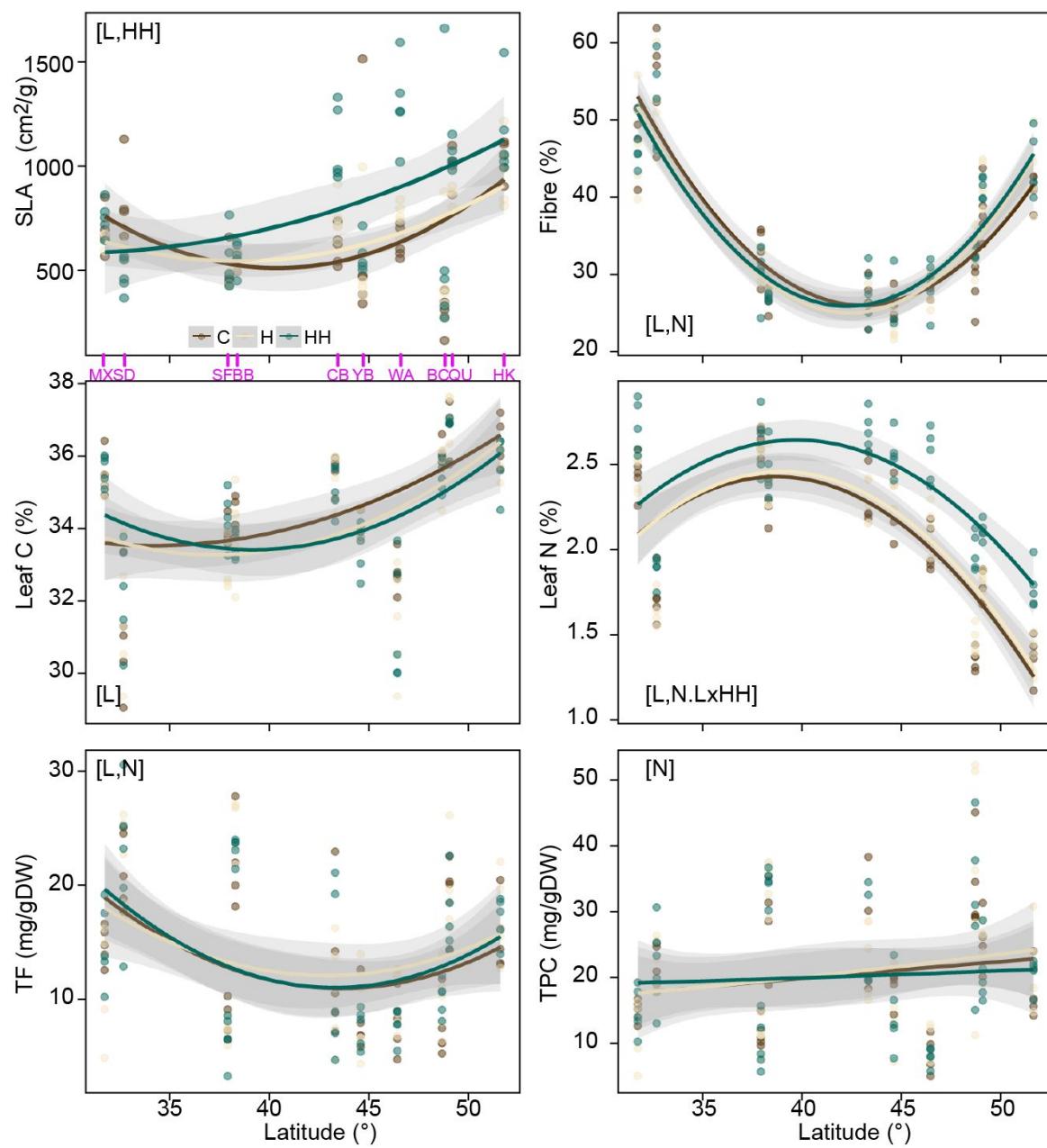
**Figure 1.** (a) Mean May-June-July satellite SST derived from the 1982-2008 AVHRR Pathfinder Project data set. White contours indicate mean May-June-July  $\text{NO}_3$  ( $\mu\text{mol/L}$ ) from the 1878-2012 World Ocean Atlas data set. The locations of the study sites; Calvert (Hakai, Canada; HK), 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  $\text{NO}_3$  ( $\mu\text{mol/L}$ ; black), with the May June July SST ( $^{\circ}\text{C}$ ; 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).



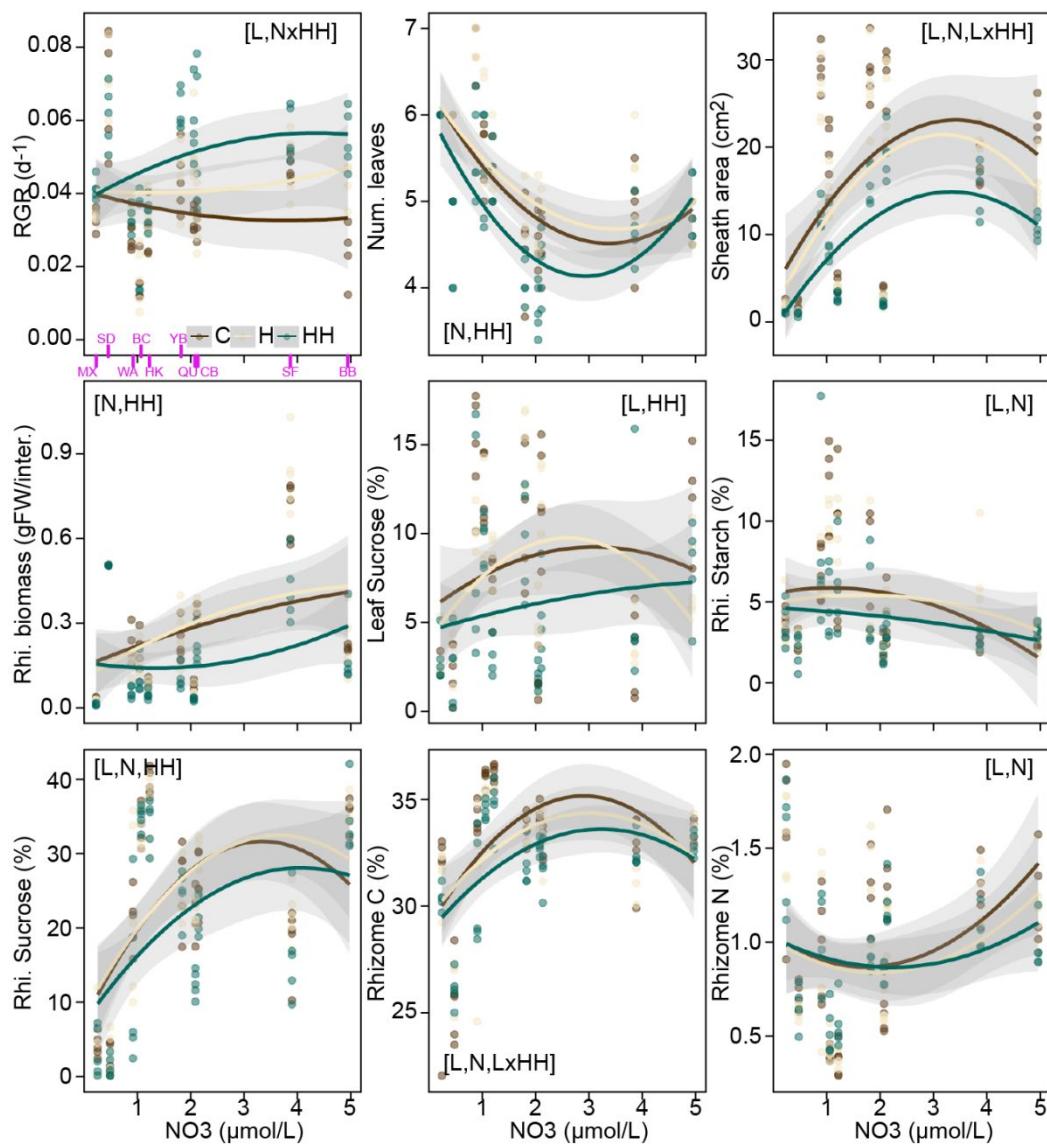
**Figure 2.** Principal Component Analysis. Arrows represent the loading of the variables. Dots represent the score of each replicate on PC1 and PC2. Circles group the replicates within each site and sites are coded as in Figure 1. Leaf relative growth rate (RGR), number of leaves (NL), leaf width (LW), 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).



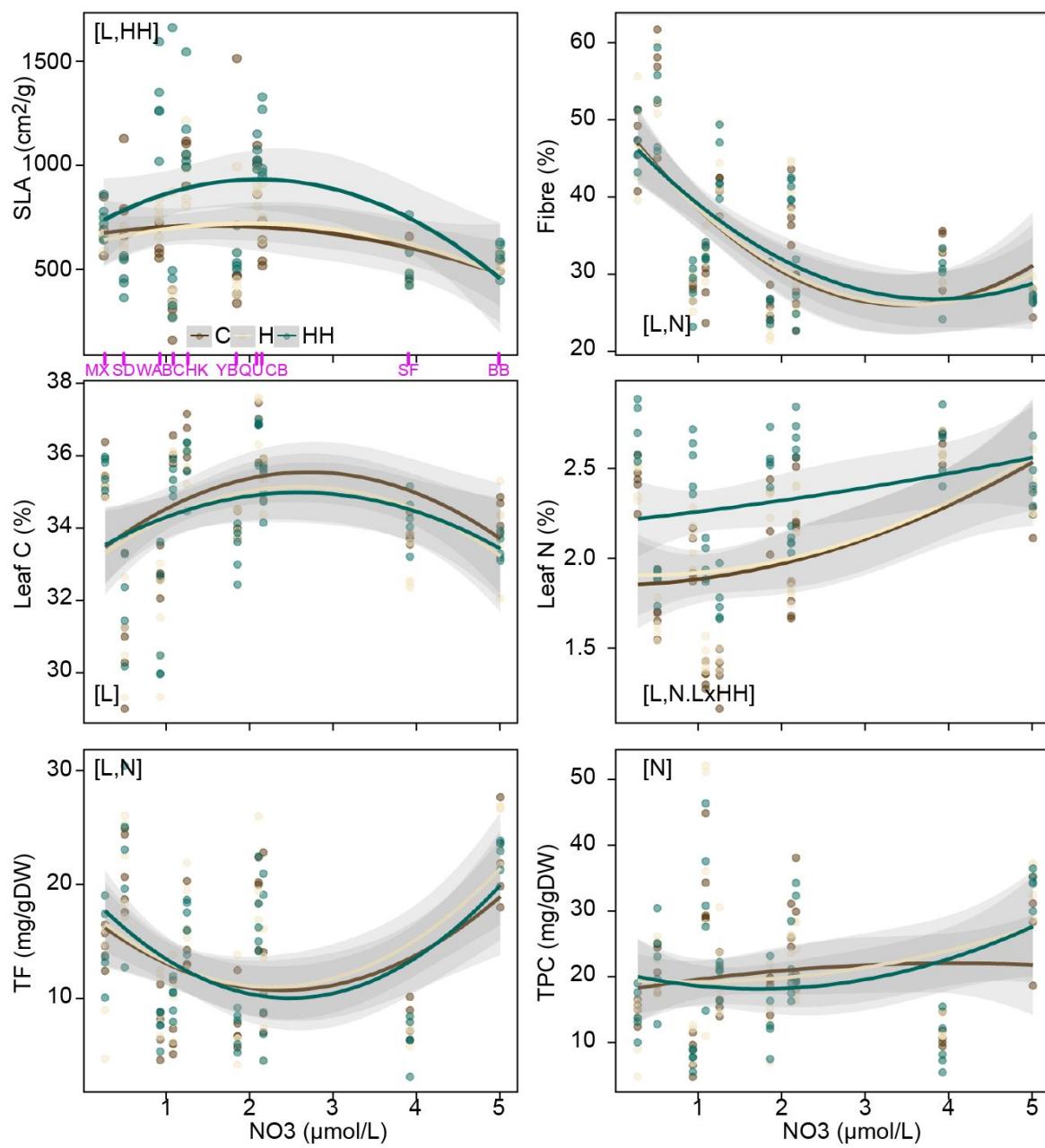
**Figure 3.** Distribution of tolerance traits along the latitudinal gradient. Grey bands indicate 95% confidence boundaries for the control (C; dark brown), moderate (H; beige) and high (HH; green) herbivory treatment fitted curves (general linear models). Letters indicate statistical differences due 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).



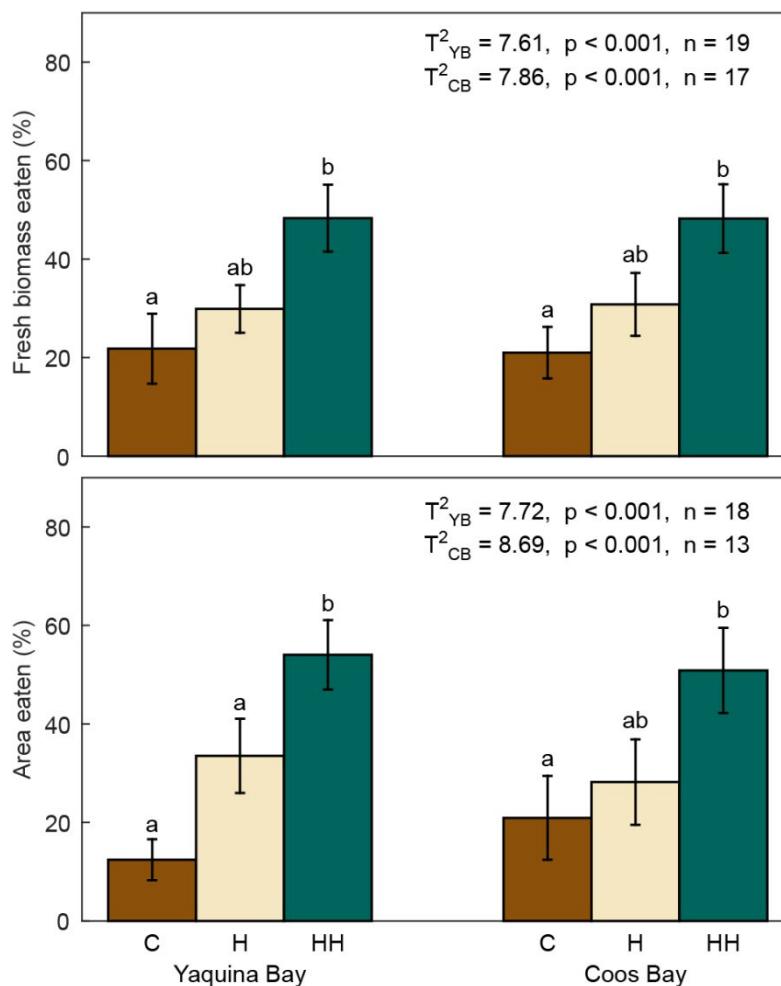
**Figure 4.** Distribution of resistance traits along the latitudinal gradient. Grey bands indicate 95% confidence boundaries for the control (C; dark brown), moderate (H; beige) and high (HH; green) herbivory treatment fitted curves (general linear models). Letters indicate statistical differences due 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).



**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).



**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.

**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 Mendelsohn 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.

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2     **Methodology S1. Plant chemical traits**  
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5     Specific phenolic compounds, nitrogen, carbon, sucrose, and fiber content of young  
6     leaves (the three newest leaves formed) and nitrogen, carbon, sucrose and starch content of  
7     rhizomes were analyzed from samples collected at the end of the experiment. Pooled plant  
8     material of five to ten shoots was analyzed per plot. Plants were cleaned of epiphytes, ultrafrozen  
9     (-80°C), freeze-dried, and ground to a fine powder to determine the concentration of chemical  
10    traits in young leaves and rhizomes. Carbon and nitrogen content in young leaves and rhizomes  
11    were analyzed using a CNH elemental analyzer (EA1108, Carlo-Erba, Italy). Non-structural  
12    carbohydrates in young leaves (sucrose), and rhizomes (sucrose and starch) were measured using  
13    methodology described by Invers *et al.*, (2004) with spectrophotometer (U-2900, Hitachi High  
14    Technologies, TX USA). Neutral detergent fiber content (NDF), referred as ‘fiber content’, was  
15    measured as in Hernán *et al.* (2017).

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17     Phenolic compounds were extracted from powdered leaf samples (0.1-0.2 g) with  
18     MeOH:H<sub>2</sub>O (3mL x 3) under maceration followed by vacuum evaporation. The organic extracts  
19     obtained were suspended in H<sub>2</sub>O to 100 ppm. Separation and identification of compounds was  
20     performed as in Hernán *et al.* (2017) with the following modifications: single components were  
21     identified by their UV, mass spectra, retention times, accurate molecular masses and comparison  
22     with standards (purity of the compounds determined by NMR, ≥95%) with the MassLynx  
23     4.1ChromaLynx Application Manager software (Waters). The reference compounds used for  
24     quantification were: rosmarinic acid (RA), apigenin-7-sulfate (APS), diosmetin-7-sulfate (DS),  
25     luteolin-7-O-β-glucoside (LUTG), luteolin-7-sulfate (LUTS). For those compounds for which no  
26     standard was available, a reference compound was selected based on the principle of structure-  
27     related target analyte/standard (chemical structure and functional group). Hence, the calibration  
28     curves of luteolin-7-sulfate (LUTS) and luteolin-7-O-β-glucoside (LUTG) were used for  
29     luteolin-7,3'-disulfate (LUTS2) and luteolin-7-O-β-(6''-malonyl) glucoside (LUTMG),  
30     respectively. The sum of LUTS, LUTS2, LUTG, LUTMG, DS and APS was calculated as the  
31     total Flavonoids (TF) content (mg/gDW). The sum of flavonoids, and RA was calculated as the  
32     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 used 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 ( $P_{sex}$  (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 ( $\hat{A}$  ( $n = 7$  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 15°C (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<sup>3</sup> 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 60°C (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<sub>3</sub>, 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<sub>3</sub>, 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 used 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, NO<sub>3</sub> and genotypic richness) was verified by the generalized variance inflation factors (GVIF, Fox and Weisberg

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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

**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 <i>et al.</i> 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 <i>et al.</i> 2012; Hernán <i>et al.</i> 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 <i>et al.</i> 2002; Hemmi & Jormalainen 2002; Güsewell 2004; Prado <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 2008; Sanmartí <i>et al.</i> 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 <i>et al.</i> 2000; Tiffin 2000; Orians <i>et al.</i> 2011)

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1      1 **Table S3.** Akaike information criterion (AIC), adjusted R<sup>2</sup> 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 (NO<sub>3</sub>), herbivory treatment (H) and  
 3 genotypic richness (G) and their interactions (marked with asterisk) are shown.  
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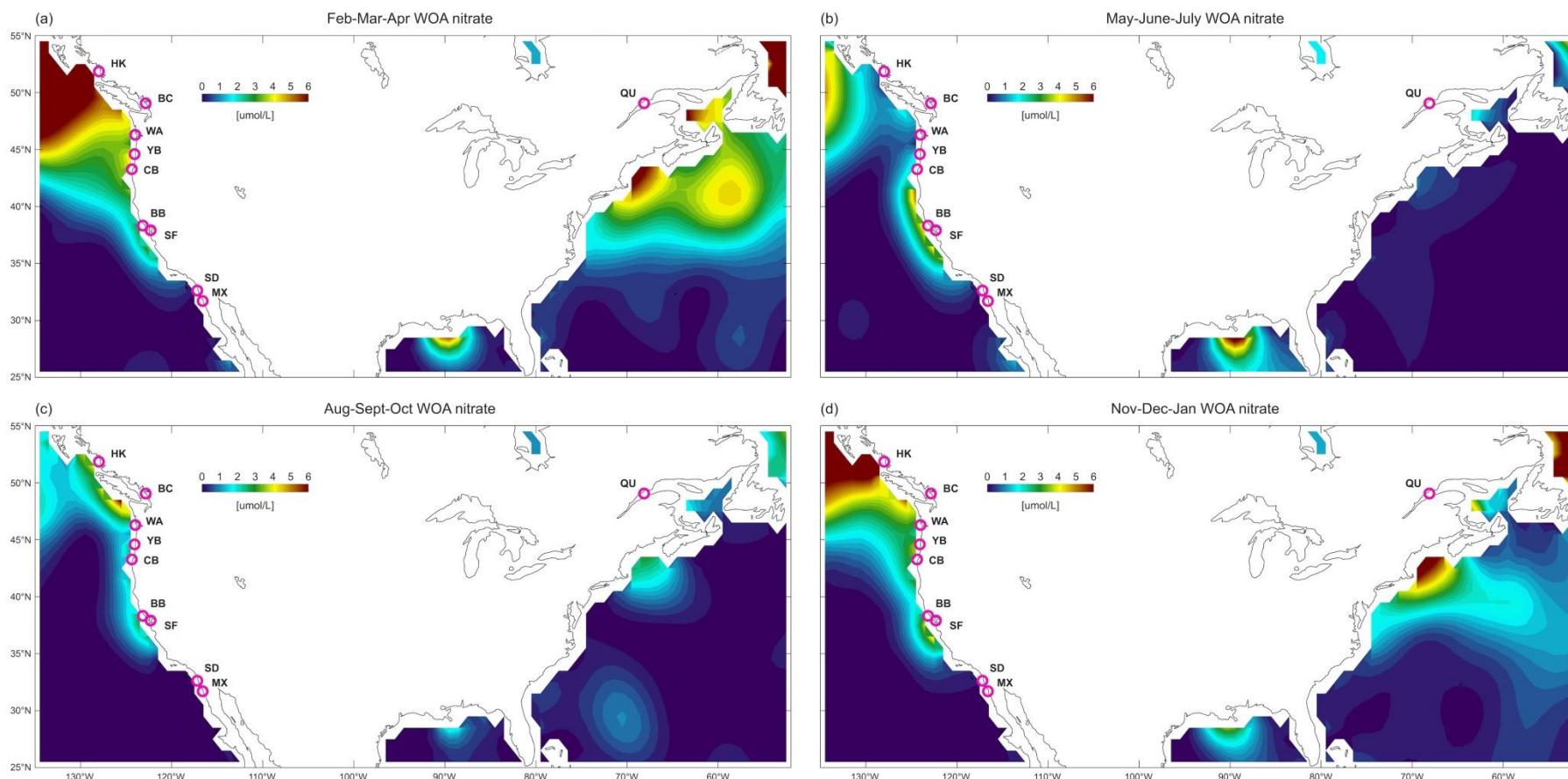
Trait	Model	AIC	Adjusted R <sup>2</sup>	SGVIF <sub>Lat</sub>	SGVIF <sub>Treat</sub>	SGVIF <sub>NO<sub>3</sub></sub>	SGVIF <sub>G.R.</sub>	SGVIF <sub>Lat*Treat</sub>	SGVIF <sub>NO<sub>3</sub>*Treat</sub>
<b>Sqrt (Relative Growth Rate (d-1))</b>	L*H+NO <sub>3</sub> *H+G	-516.41							
	L+H+NO <sub>3</sub> +G+L*H	-514.41	0.36	1.005	1.648	1.792	1.006		1.920
<b>Number of leaves</b>	L*H+NO <sub>3</sub> *H+G	279.29							
	L+H+NO <sub>3</sub> +G+L*H	277.29							
	L+H+NO <sub>3</sub> +G	276.38							
	H+NO <sub>3</sub> +G	275.25	0.41		1.000	1.001	1.001		
<b>Sheath area(cm<sup>2</sup>)</b>	L*H+NO <sub>3</sub> *H+G	898.16							
	L+H+NO <sub>3</sub> +G+L*H	894.36	0.61	1.738	6.777	1.002	1.005	6.846	
<b>Leaf sucrose (%)</b>	L*H+NO <sub>3</sub> *H+G	773.37							
	L+H+NO <sub>3</sub> +G+L*H	769.83							
	L+H+NO <sub>3</sub> +G	768.23							
	L+H+G	767.97	0.29	1.004	1.000		1.004		
<b>Ln(Rhizome biomass (grFW/internode))</b>	L*H+NO <sub>3</sub> *H+G	367.85							
	L+H+NO <sub>3</sub> +G+L*H	364.25							
	L+H+NO <sub>3</sub> +G	361.66	0.36	1.005	1.000	1.002	1.005		
<b>Logit (Rhizome starch(%))</b>	L*H+NO <sub>3</sub> *H+G	235.77							
	L+H+NO <sub>3</sub> +G+H*NO <sub>3</sub>	232.71							
	L+H+NO <sub>3</sub> +G	230.71							
	L+H+NO <sub>3</sub>	228.75							
<b>arcsine(Rhizome sucrose (%))</b>	L+NO <sub>3</sub>	228.44	0.33	1.001		1.001			
	L*H+NO <sub>3</sub> *H+G	-862.45							
	L+H+NO <sub>3</sub> +G+L*H	-865.80							
	L+H+NO <sub>3</sub> +G	-869.13	0.71	1.005	1.000	1.002	1.005		

	Trait	Model	AIC	Adjusted R <sub>2</sub>	SGVIF <sub>Lat</sub>	SGVIF <sub>Treat</sub>	SGVIF <sub>NO3</sub>	SGVIF <sub>G.R.</sub>	SGVIF <sub>Lat*Treat</sub>	SGVIF <sub>NO3*Treat</sub>
4 5 6	<b>Logit (Rhizome C (%) )</b>	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	
7 8 9 10	<b>Rhizome N (%)</b>	L*H+NO3*H+G	92.98							
		L+H+NO3+G+L*H	90.00							
		L+H+NO3+G	87.48							
11 12 13 14	<b>SLA (cm<sup>2</sup>/g)</b>	L+NO3+G	84.36	0.27	1.010		1.004	1.011		
		L*H+NO3*H+G	904.19							
		L+H+NO3+G+L*H	900.48	0.59	1.000	1.000				
15 16 17 18	<b>Logit (Fiber (%) )</b>	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		
20 21 22 23 24	<b>Logit (Leaf C (%) )</b>	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			
26 27	<b>Logit(Leaf N (%) )</b>	L*H+NO3*H+G	-346.14	0.49	1.739	6.859	1.793	1.005	6.851	1.922
		L*H+NO3*H+G	351.31							
29 30 31	<b>Sqrt (TF (mg/gDW))</b>	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		
33 34 35 36 37	<b>Sqrt (TPC (mg/gDW))</b>	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			

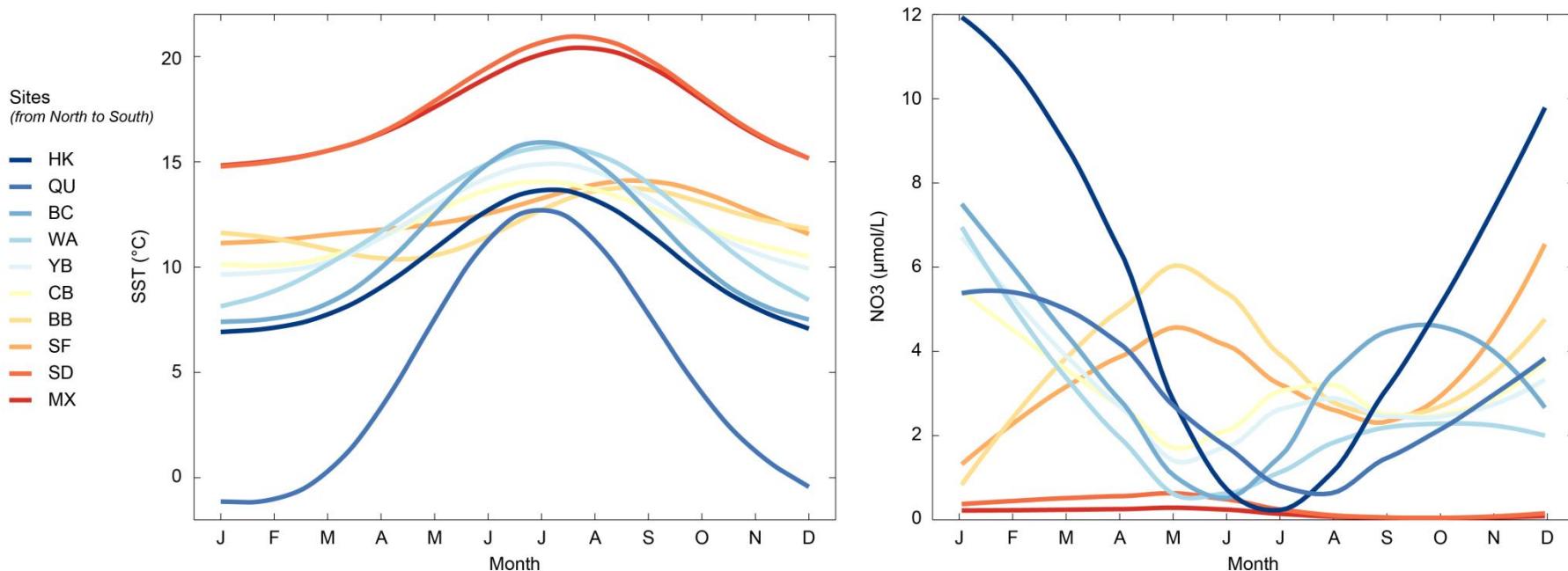
**Table S4:** Results of GLM on Specific phenolic compounds. Intercept ( $i$ ) and slopes of latitude ( $\beta_{lat}$ ), herbivory simulation moderate ( $\beta_H$ ) and high level ( $\beta_{HH}$ ), nitrate ( $\beta_{NO_3}$ ), their interactions (marked with asterisc), and genotypic richness ( $\beta_{G.R.}$ ). 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- $\beta$ -glucoside and LUTMG luteolin-7-O- $\beta$ -(6"-malonyl) glucoside

		$i$	$\beta_{lat}$	$\beta_H$	$\beta_{HH}$	$\beta_{NO_3}$	$\beta_{G.R.}$	$\beta_{lat*H}$	$\beta_{NO_3*H}$	$\beta_{NO_3*HH}$	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	<b>&lt;0.001</b>			<b>&lt;0.001</b>	<b>0.020</b>				
Sqrt (DS (mg/g DW))	Est.	4.562	-0.049			-0.198					0.16
	SE	0.623	0.015			0.062					
	P	<b>&lt;0.001</b>	<b>0.001</b>			<b>0.002</b>					
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			<b>&lt;0.001</b>					
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	<b>0.140</b>	<b>&lt;0.001</b>	0.110	0.070	<b>&lt;0.001</b>	<b>&lt;0.001</b>	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	<b>0.019</b>	<b>0.019</b>			<b>0.021</b>	<b>0.022</b>				
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	<b>0.004</b>	<b>&lt;0.001</b>			<b>0.020</b>	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	<b>&lt;0.001</b>	0.363	0.248	<b>&lt;0.001</b>	<b>&lt;0.001</b>		0.784	0.057	

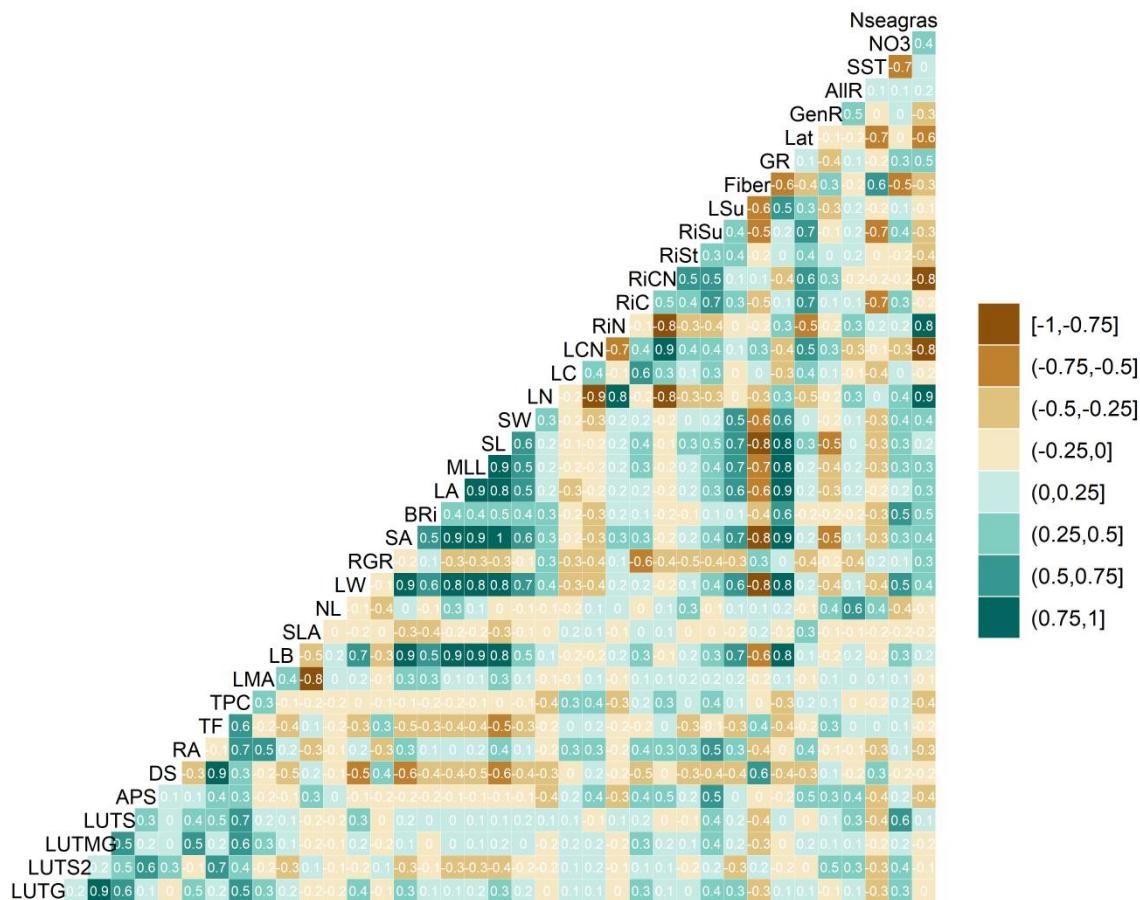
1  
2 **FIGURES**  
3



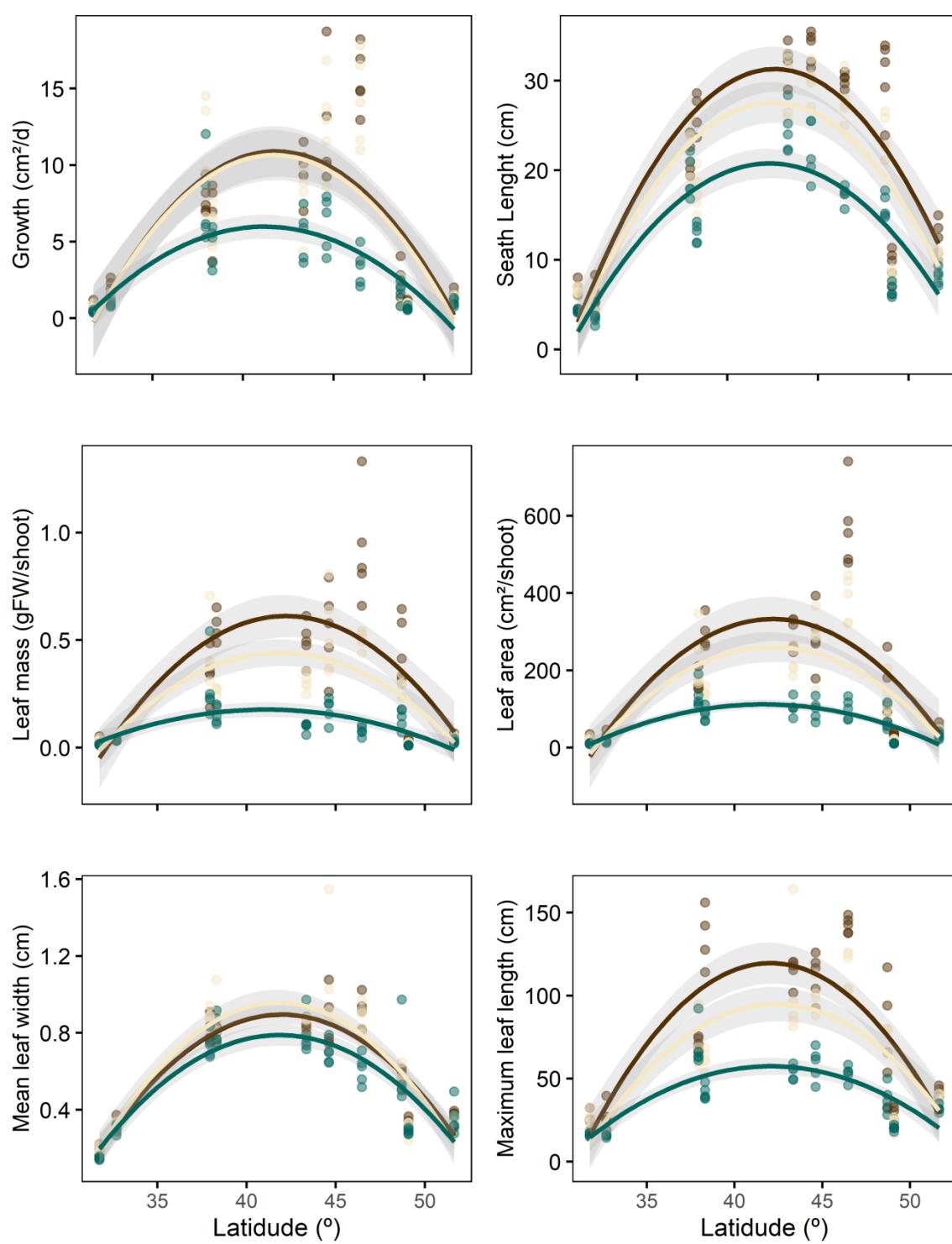
33 **Figure S1.** Mean (a) February- March- April, (b) May-June-July, (c) August-September-October, (d) November-December-January nitrate  
34 concentration ( $\mu\text{mol/L}$ ) from the 1878-2012 World Ocean Atlas data set. Location of study sites is marked with a magenta circle. See Table S1  
35 for coding of the sites.  
36  
37



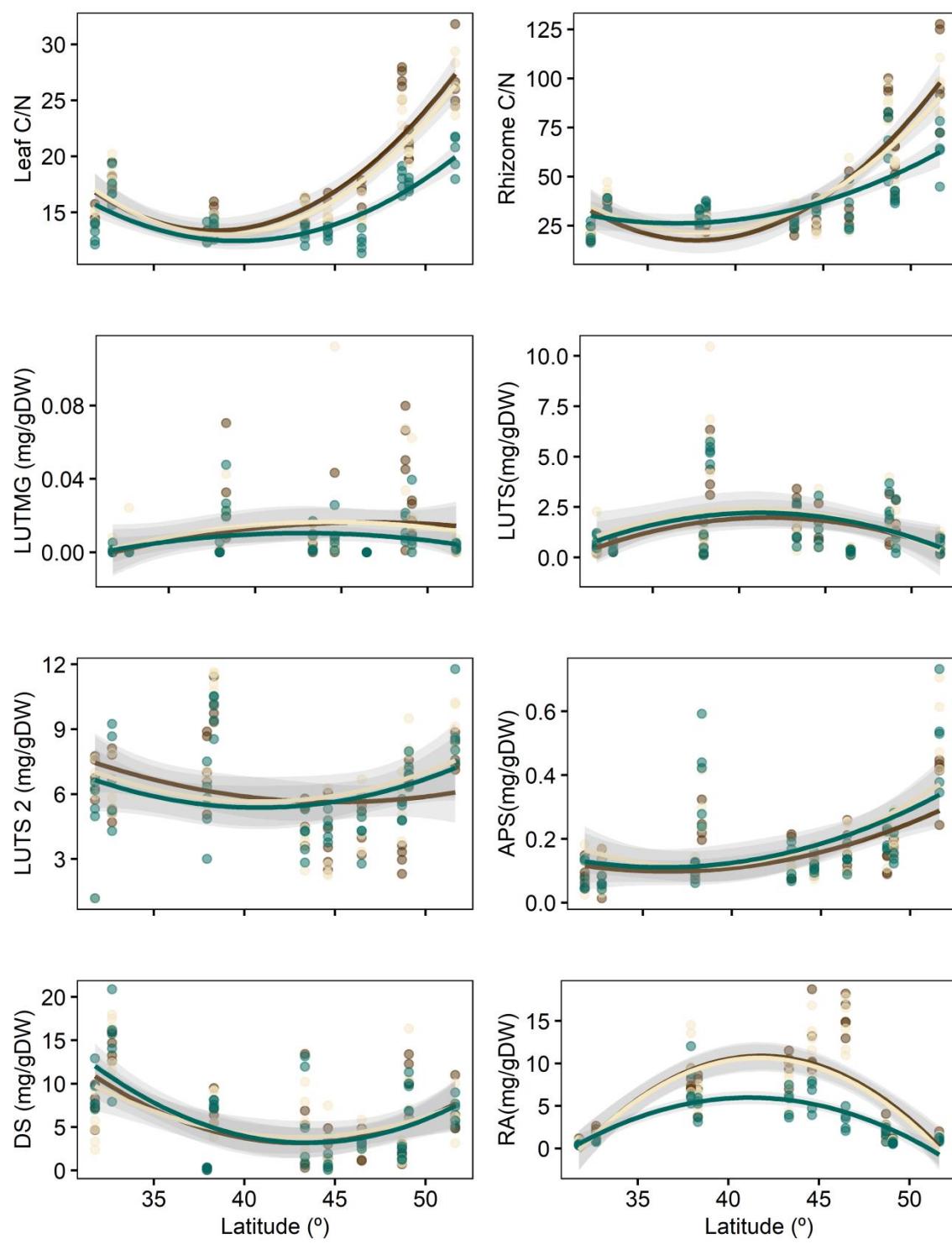
**Figure S2.** Mean sea surface temperature (SST; °C) on the left and mean nitrate concentration (μmol/L) on the right of the study sites from the 1878-2012 World Ocean Atlas data set. MX, Mexico; SD, San Diego (California); SF, San Francisco (California); BB, Bodega Bay (California); CB, Coos Bay (Oregon); YB Yaquina Bay (Oregon); WA, Washington; BC (British Columbia south coast) Canada; QU Quebec (Canada); HK, Hakai, (BC central coast, Canada).



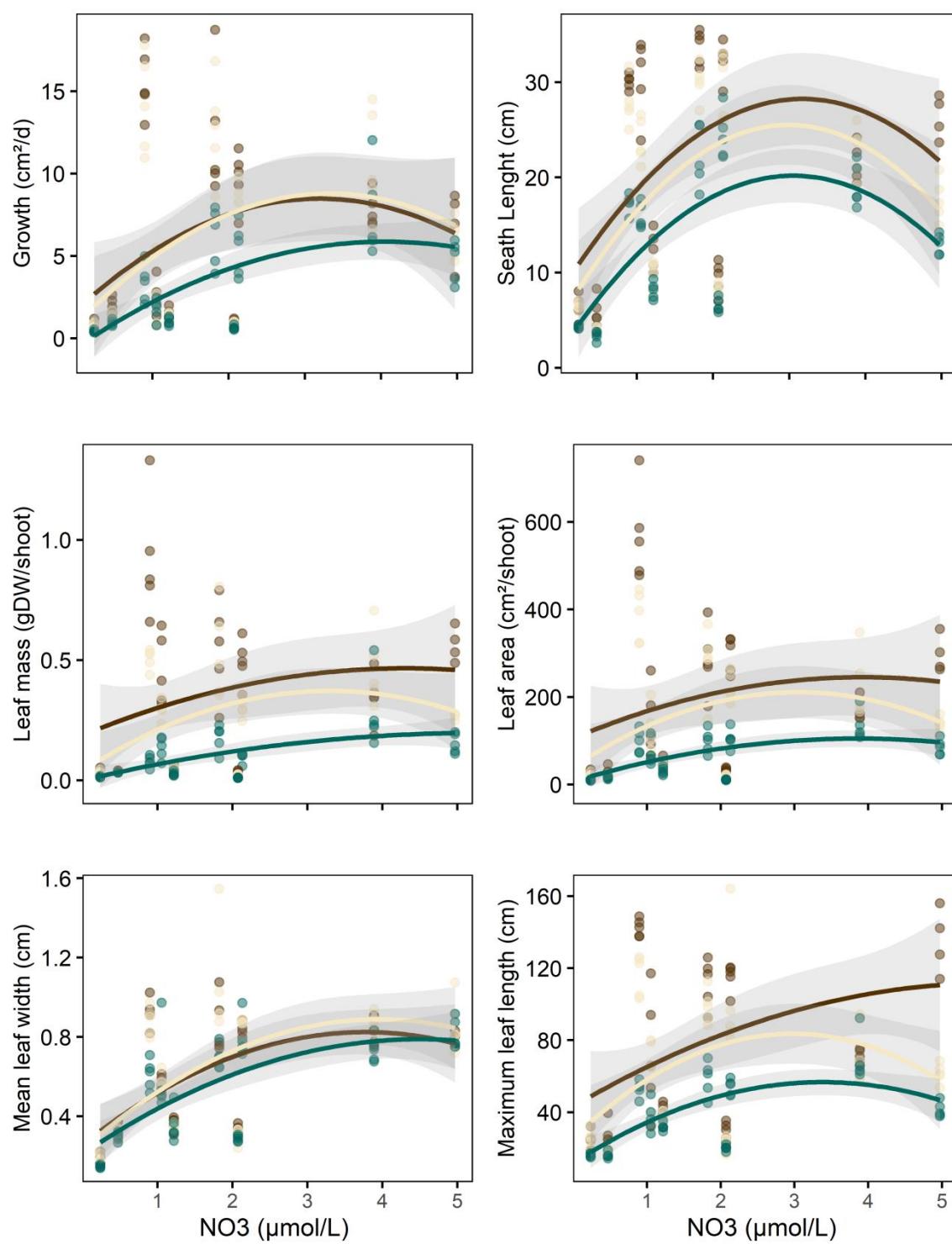
**Figure S3.** Pairwise correlation matrix of Flavonoids (LUTG, LUTS2, LUTMG, LUTS, APS, DS), Rosmarinic acid (RA), Total flavonoids (TF), total phenolic compounds (TPC), leaf mass area (LMA), leaf biomass (LB), specific leaf area (SLA), number of leaves (NL), leaf width (LW), relative leaf growth rate (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.



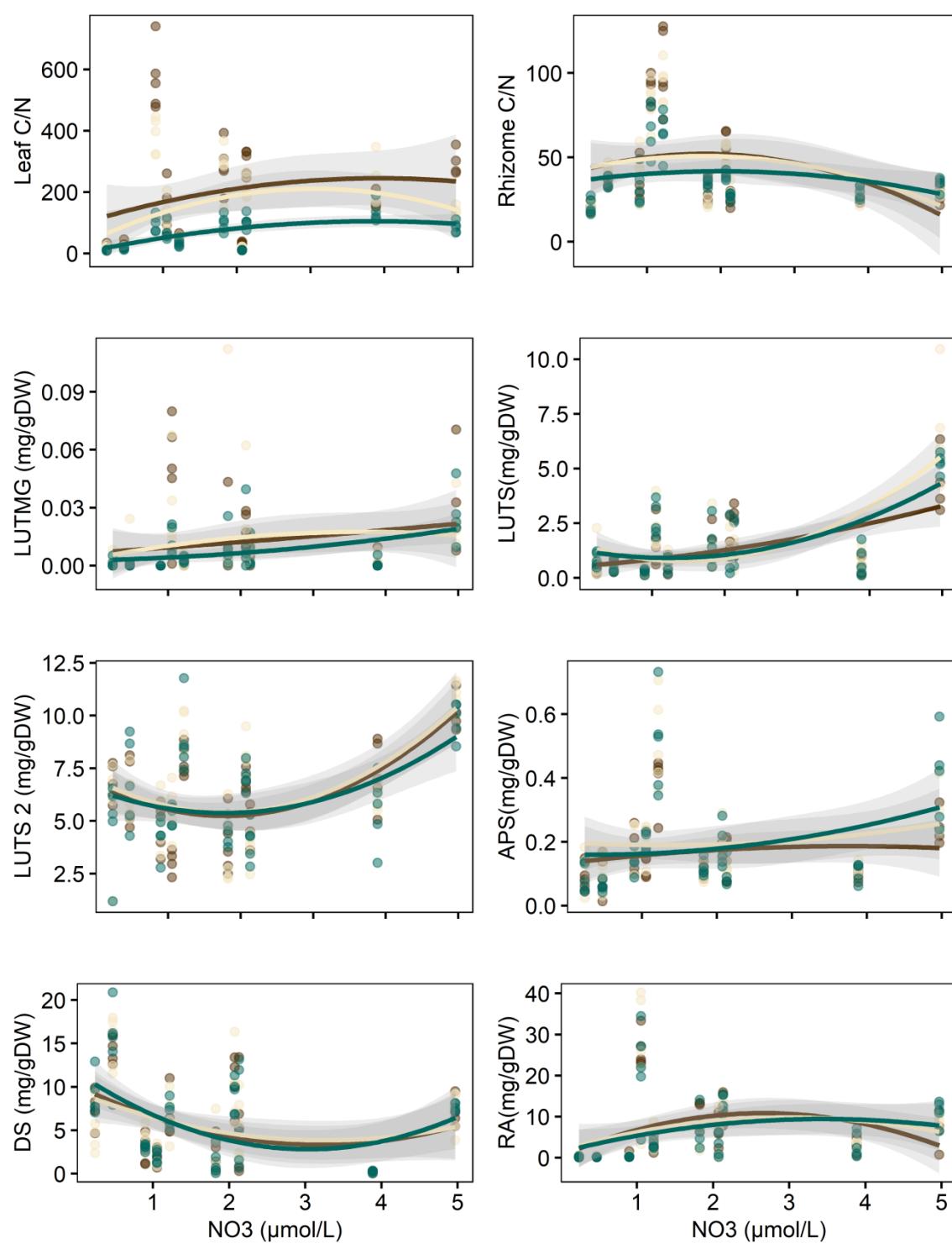
**Figure S4.** Distribution of seagrass size traits along the latitudinal gradient. Grey bands indicate 95% confidence boundaries for the control (dark brown), moderate (beige) and high (green) herbivory treatment fitted curves (glm).



**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- $\beta$ -glucoside, LUTS2 luteolin-7,3'-disulfate, LUTMG luteolin-7-O- $\beta$ -(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.