Small herbivores and abiotic heterogeneity promote trait variation of a saltmarsh plant in local communities

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Intraspecific trait variation (ITV) enables plants to respond to global changes. However, causes for ITV, especially from biotic components such as herbivory, are not well understood. We explored whether small vertebrate herbivores (hares and geese) impact ITV of a dominant clonal grass (*Elytrigia atherica*) in local communities. Moreover, we looked at the relative importance of their direct (e.g. selective grazing) and indirect effects (altering genotypic richness/diversity and abiotic environment) on ITV. We used exclosures at two successional stages in a Dutch saltmarsh, where the early stage had higher grazing pressure than the intermediate one . We measured key functional traits of *E. atherica* including height, aboveground biomass, flowering (flower or not), specific leaf area, and leaf dry matter content in the local communities (1 m ×1m plots) inside and outside the exclosures. We also determined genotypic richness and diversity using molecular markers. We further measured abiotic variations in topography and clay thickness (a proxy for soil total nitrogen). Structural equation models revealed that small herbivores significantly promoted ITV in height and flowering at the early stage, while they marginally promoted ITV in height at the intermediate stage. Moreover, the direct effects of herbivores played a major role in promoting ITV. Small herbivores decreased genotypic diversity at the intermediate stage, but genotypic richness and diversity did not impact ITV. Small herbivores did not alter topographic variation and variation in clay thickness, but these variations increased ITV in all traits at the early stage. Small herbivores may not only impact trait means as studies have shown but also ITV in plants.

1   **Small herbivores and abiotic heterogeneity promote trait variation of a saltmarsh plant in**

2   **local communities**

3   **running title:** small herbivores and trait variation

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10   **Abstract**

11   Intraspecific trait variation (ITV) enables plants to respond to global changes. However, causes

12   for ITV, especially from biotic components such as herbivory, are not well understood. We

13   explored whether small vertebrate herbivores (hares and geese) impact ITV of a dominant clonal

14   grass (*Elytrigia atherica*) in local communities. Moreover, we looked at the relative importance

15   of their direct (e.g. selective grazing) and indirect effects (altering genotypic richness/diversity

16   and abiotic environment) on ITV. We used exclosures at two successional stages in a Dutch

17   saltmarsh, where the early stage had higher grazing pressure than the intermediate one. We

18   measured key functional traits of *E. atherica* including height, aboveground biomass, flowering

19   (flower or not), specific leaf area, and leaf dry matter content in the local communities (1 m ×1m

20   plots) inside and outside the exclosures. We also determined genotypic richness and diversity

21   using molecular markers. We further measured abiotic variations in topography and clay

22   thickness (a proxy for soil total nitrogen). Structural equation models revealed that small

23   herbivores significantly promoted ITV in height and flowering at the early stage, while they

24   marginally promoted ITV in height at the intermediate stage. Moreover, the direct effects of

25   herbivores played a major role in promoting ITV. Small herbivores decreased genotypic

26   diversity at the intermediate stage, but genotypic richness and diversity did not impact ITV.

27   Small herbivores did not alter topographic variation and variation in clay thickness, but these

28   variations increased ITV in all traits at the early stage. Small herbivores may not only impact

29   trait means in plants as studies have shown but also their ITV.

30   **Keywords:** trait variation; plant-herbivore interaction; grazing; abiotic; genetic diversity;

31   saltmarsh

32 **Introduction**

33   Studies show that intraspecific trait variation (i.e. variability in traits of conspecific individuals;

34   hereafter ITV) can enable plant species to respond to global changes (Westerband, Funk &

35   Barton, 2021), impact community composition and structure (Whitlock, Grime & Burke, 2010),

36   and govern ecosystem processes (Boege & Dirzo, 2004; Crutsinger et al., 2006; Crutsinger,

37   Souza & Sanders, 2008; Lecerf & Chauvet, 2008). The importance of ITV has been increasingly

38   realized over the years (Violle et al., 2012; Siefert et al., 2015; Funk et al., 2017; Westneat et al.,

39   2019; Westerband, Funk & Barton, 2021), therefore, exploring the causes for ITV is of prime

40   importance, particularly under the current rapid global changes (Westerband, Funk & Barton,

41   2021). Several components including genotypic richness/diversity and phenotypic plasticity

42   triggered by biotic and abiotic environments can drive ITV (Valladares, Gianoli & Gomez,

43   2007). However, biotic components, particularly herbivory, received much less attention than

44   genotypic richness/diversity and abiotic environments (Valladares, Gianoli & Gomez, 2007).

45

46   Whilst herbivores are one of the major drivers for plant trait differentiation globally (Díaz et al.,

47   2007; He & Silliman, 2016), the majority of the studies that document the effects of herbivores

48   on plant traits focus on trait means without considering ITV (e.g. Bullock et al., 2001; Louault et

49   al., 2005; Kahmen & Poschlod, 2008). Limited studies suggest that herbivores may also impact

50   ITV (e.g. Jessen et al., 2020). However, these studies mainly focus on large herbivores. Large

51   and small herbivores are relative. Here we refer to small herbivores as vertebrate herbivores with

52   body mass range from 1 to 10 kg such as hares and geese. Small herbivores sometimes can have

53   stronger impacts on plant communities than large ones especially when their abundance is high

54   (Olofsson et al., 2004). Additionally, small herbivores are usually more selective with forage

55   plants (Olff & Ritchie, 1998). Therefore, small herbivores may also impact ITV, however, we

56   currently lack empirical evidence.

57

58   Small herbivores may impact ITV via multiple non-exclusive processes. First, small herbivores

59   can promote ITV through selective grazing. Studies show that large herbivores generally

60   consume tall plants, thus promoting short plants (Díaz et al., 2007; Evju et al., 2009). This is

61   probably because short plants, which are usually young, are generally more nutritious (e.g.

62   higher protein and lower lignin and cellulose) (Augustine & McNaughton, 1998). Similarly,

63   small herbivores are more likely to consume young (short) plants over old (tall) ones, but some

64   young (short) plants may escape from grazing via association with unpalatable species (Van Der

65   Wal et al., 2000; Kuijper & Bakker, 2008). Thus, small herbivores may promote variation in

66   plant height. Second, small herbivores may impact ITV indirectly through altering genotypic

67   richness and diversity. Increased genotypic richness and diversity can increase ITV (Evans et al.,

68   2016). Studies looking at the effects of large herbivores on genotypic diversity show that large

69   herbivores can increase genotypic diversity by promoting seed transportation (Rico & Wagner,

70   2016) or decrease it by promoting clonal growth (Kleijn & Steinger, 2002). Small herbivores can

71   have similar effects to large ones on many processes such as seed dispersal (Bakker & Olff,

72   2003). Therefore, small herbivores can either increase or decrease genotypic richness and

73   diversity. Third, small herbivores may promote ITV indirectly through altering abiotic

74   heterogeneity (e.g. topographic variation and variation in soil nutrients) via grazing the

75   vegetation at particular spatial scales, trampling, and localized deposition of droppings (Adler,

76   Raff & Lauenroth, 2001). Abiotic heterogeneity usually promotes ITV (Westerband, Funk &

77 Barton, 2021). However, whether small herbivores impact ITV via these processes and the

78 relative importance of them in shaping ITV remains underexplored.

79

80 In the eastern part of the saltmarsh of the island of Schiermonnikoog, hares and geese are the

81 abundant herbivores while large herbivores are absent (Kuijper & Bakker, 2005; Schrama et al.,

82 2015; Chen et al., 2019). A well-calibrated successional gradient is present here (Olff et al.,

83 1997). Hares and geese are more abundant at early successional stages but their abundance

84 decreases at intermediate and late successional stages due to less preferred late successional

85 plants such as *Elytrigia atherica* (synonym *Elymus athericus*) increase in dominance (Kuijper &

86 Bakker, 2005; Schrama et al., 2015; Chen et al., 2019). Although adult plants of *E. atherica* are

87 less preferred, hares and geese considerably graze on its seedling/young plants (Kuijper, Nijhoff

88 & Bakker, 2004; Fokkema et al., 2016). However, short seedlings/young plants may escape from

89 grazing by associating with non-preferred plants such as *Artemisia maritima*. *Elytrigia atherica*

90 mainly reproduces clonally, but sexual reproduction may occur occasionally via windows of

91 opportunity, thus genotypic richness is high in this grass (Bockelmann et al., 2003; Chen, 2020).

92 Hares and geese play a less important role than tidal water in seed dispersal (Chang et al., 2005),

93 therefore, it is unlikely that hares and geese can increase genotypic richness and diversity by

94 dispersing seeds. Instead, hares and geese may decrease genotypic richness and diversity by

95 promoting clonal spread of *E. atherica* (Van Der Graaf, Stahl & Bakker, 2005). Hares and geese

96 may increase topographic variation via trampling (Wijnen, Wal & Bakker, 1999; Elschot et al.,

97 2015), they may also impact sediment accumulation (measured by clay thickness) by trampling

98 and altering vegetation structure (Boorman, Garbutt & Barratt, 1998). Clay thickness is highly

99 correlated with soil total Nitrogen (Olff et al., 1997). Topographic variation and variation in clay

100   thickness are important factors for the growth and expansion of *E. atherica* in this system (Olff

101   et al., 1997; Nolte et al., 2019). Taken together, hares and geese may increase ITV of *E. atherica*

102   through selective grazing and promoting topographic variation and variation in clay thickness but

103   may decrease ITV by decreasing genotypic richness and diversity.

104

105   To test these hypotheses, we used long-term (22-year) exclosures at the early and intermediate

106   successional stages, where abiotic conditions are relatively similar, but grazing pressure differs

107   markedly (Kuijper & Bakker, 2005; Chen et al., 2019). As ITV is usually strongly driven by

108   processes operating at small spatial scales (Westerband, Funk & Barton, 2021), we, therefore,

109   looked at ITV in local communities (1 m ×1m plots). Specifically, we measured key functional

110   traits of this dominant grass including height, aboveground biomass, flowering (flower or not),

111   specific leaf area, and leaf dry matter content within local communities inside and outside the

112   exclosures. Using structural equation models, we test whether hares and geese impact ITV of *E.*

113   *atherica*, and the relative importance of their direct effects (e.g. selective grazing) and indirect

114   effects (via altering genotypic richness/diversity and abiotic variables) on ITV. We expect that

115   the effects of hares and geese on ITV would be more apparent at the early successional stage

116   (relative to the intermediate one) where grazing pressure was higher.

117

118   **Materials and methods**

119   *Study site*

120   A natural successional gradient is present in the back-barrier saltmarsh of the island of

121   Schiermonnikoog (53°30' N, 6°10' E), the Netherlands. Because this island expands eastward,

122   thus the eastern part of the island is younger relative to the western part (Olff et al., 1997). The

123   western part of the saltmarsh is enclosed for cattle grazing, while the eastern part of it is grazed

124   by wild small herbivores such as spring staging geese, year-round present hares and rabbits.

125   Hares and geese are the most abundant herbivores (Van de Koppel et al., 1996; Van Der Wal,

126   Kunst & Drent, 1998; Van Der Wal et al., 2000; Kuijper & Bakker, 2005; Schrama et al., 2015).

127   Rabbits are very rare. Kuijper and Bakker (2005) found that biomass removed by rabbits in 2000

128   was 6.33% and 0.16% of the total biomass removed by small herbivores at the early and

129   intermediate stage, respectively.

130

131   We used long-term hare and goose exclosures that were initiated in 1994 (details in Chen et al.,

132   2019). We selected exclosures located at the early and intermediate successional stages, which

133   are approximately 2.5 km apart (Fig. 1A). Vegetation succession has undergone around 30 and

134   60 years at the early and intermediate stage, respectively. Age of the vegetation succession was

135   counted from the year of vegetation establishment to 2016. The year of vegetation establishment

136   was determined by checking aerial photographs (Olff et al., 1997). Grazing pressure from hares

137   and geese was much higher at the early stage than the intermediate stage. Grazing pressure was

138   calculated using year-round dropping count in 2000 and 2016 (see Table S1 for more details;

139   Kuijper & Bakker, 2005; Chen et al., 2019; Chen et al., 2019a).

140

141   Hare and goose exclosures (one per each stage) were located in a similar elevation (early stage:

142   1.42 ± 0.004; intermediate stage:1.44 ± 0.004; mean $\pm$ 1se; N=24; m+NAP; Normal Amsterdam

143   Water Level). Exclosures (8 m $\times$ 12 m and 6 m $\times$ 8 m at the early and intermediate stages) were

144   made by chicken mesh (mesh width 25 mm) supported by wooden posts every 3.5 m to exclude

145   hare grazing inside the exclosures. Exclosures were around 1 m in height, ropes were suspended

146   on top of the wooden posts to stop geese flying into the exclosures. At the beginning of the

147   exclosure experiment (1995), *E. atherica* rarely occurred (< 2.5 %; percent cover) inside and

148   outside the exclosures at these two stages. Vegetation composition differed in the grazed areas

149   and inside the exclosures in 2016 (Fig.1; also see Table S2 for species composition for the three

150   most abundant species). Specifically, *Artemisia maritima* was the most dominant plant in the

151   grazed area at these two stages, while *E. atherica* was the most dominant plant inside the

152   exclosures.

153

154   *Experimental design*

155   We marked an area, ca. 6 m $\times$ 8 m, which corresponds to the smaller size of the exclosures (at

156   the intermediate successional stage), inside and outside the exclosure for both stages in June

157   2017. The distance between the area and the exclosure measured ca. 80 m. We randomly set up 7

158   plots (1m $\times$ 1m) inside these two grazed areas and exclosures (Fig. 1). The distance between

159   these plots ranged from 0.5 m to 9 m. We divided each plot into 25 grids (0.2 m $\times$ 0.2 m). Within

160   each grid, we collected one individual stem of *E. atherica* (with roots), usually in the middle of

161   the grid (Fig. 1). *Elytrigia atherica* did not occur everywhere, particularly not in the grazed area,

162   thus sample size per plot varied from 9 to 25.

163

164   *Trait measurement*

165   We measured traits for individual stems of *E. atherica* in the field. We sampled individual stems

166   without obvious grazing marks. We cut individual stems at the ground level and measured

167   maximum height (cm) from the base to the highest point. We measured the maximum leaf width

168   and length of the first fully grown leaf, usually the third leaf from the top. We also recorded

169   whether individual stems flowered or not. After these measurements, each stem was stored in a

170   paper bag, sealed, labeled, and then put in a self-sealing plastic bag to reduce water loss in

171   tissues. At the end of each day, samples were brought to the lab, we measured fresh biomass of

172   the individual stems and fully-grown leaves. We also measured dried biomass (g) after oven-dry

173   (70 °C) to constant mass (ca. 3 days). Specific leaf area (mm^2 mg^{-1}) was calculated as (leaf width

174   ×leaf length) / dried leaf biomass. Leaf dry matter content (mg g^{-1}) was calculated as dried leaf

175   biomass / fresh leaf biomass. Note that leaf dry matter content was only measured in 3, 5, 4, and

176   4 plots in the grazed and ungrazed treatment at the early and intermediate stage, respectively.

177   This is because we needed to bring all samples and measure the fresh weight of each leaf sample

178   and the whole plants in the field station. We also need to do this quickly, otherwise, water will be

179   lost in the plant tissues, which may bias estimation of fresh weight. Due to lack of manpower, we

180   measured fresh leaf weight for around half of the randomly selected plots. Also, specific leaf

181   area and leaf dry matter content was not measured in the standard way (Pérez-Harguindeguy et

182   al., 2016), and caution should be taken when comparing our data with other studies. We

183   measured leaf width and length because they are easier to measure in the field. A similar method

184   was also used in a previous study in this system (Veeneklaas et al., 2011). Because we measured

185   leaf width and leaf length for all plots in this way, thus our results are not very likely to be biased

186   by the method used here.

187

188   *Genotyping and genotype richness and diversity*

189   We used oven-dried leaf samples (ca. 2 mg per individual stem) for DNA extraction. We first

190   shredded leaf samples into smaller pieces using tissuelyser. We then extracted DNA from each

191   leaf sample using the CTAB method (Doyle & Doyle, 1987) and stored DNA samples at -20 °C

192   before PCR. We amplified DNA using PCR with fluorescence-labeled primers. We used five

193   microsatellite markers (ECGA89, WMS6, WMS44, WMS2, and ECGA89) originally designed

194   for the other Poaceae species, *Elymus caninus* (Sun, Salomon & Bothmer, 1998) and *Triticum*

195   *aestivum* (Röder et al., 1998). These five markers were used for genotyping *E. atherica* in this

196   system (Bockelmann et al., 2003; Chen 2020). The PCR products from primer ECGA89, WMS6,

197   and WMS44 were pooled together, while the PCR products from WMS2 and ECGA89 were

198   pooled together. Additionally, 1 µL rROX was added in each sample as the internal size standard

199   (Gene ScanTM–350 ROXTM, Applied Biosystem). We visualized the pooled PCR products

200   using the 3730 DNA analyzer and scored the microsatellite peak patterns (height > 100)

201   manually using GeneMapper. In total, we successfully genotyped 579 individual stems of *E.*

202   *atherica*, but 2 were excluded for further analyses as some trait data measured in the field for

203   these samples were missing.

204

205   *Abiotic variables*

206 We measured clay thickness and topographic variation for each plot (3 replicates). We measured

207 clay thickness using a 2 cm Ø soil corer with tick marks as a proxy for soil total nitrogen (Olff et

208 al., 1997). Previous studies show that clay thickness is strongly positively correlated with soil

209 total nitrogen in this system (e.g. Olff et al., 1997), and is used as a proxy of soil fertility (e.g.

210 Schrama et al., 2017). We measured topography using Trimble R8 (precision for elevation ca. 1

211 cm).

212

213 **Data analysis**

214

215 *Calculating trait means, ITV, topographic variation, and variation in clay thickness*

216 We calculated means for each trait by averaging trait values over 9 -25 individual stems in each

217 plot. We calculated ITV in each trait each plot as the standard deviation /mean. Topographic

218 variation and variation in clay thickness were calculated as standard deviation /mean (over 3

219 samples) of elevation and clay thickness, respectively.

220

221 *Calculating genotypic richness, genotypic diversity, and genetic differentiation*

222 To calculate genotype richness and diversity per plot, we first calculated pairwise genetic

223 distance using Dice dissimilarity from the R package ade4 (Dray & Dufour, 2015) based on the

224 presence/absence matrix of 42 allele bands from those five markers. We then assigned genotypes

225 based on dice dissimilarity, using the function "assignClones" from the R package polysat

226 (Lindsay, Clark & Clark, 2018). We calculated genotypic richness as the number of unique

227 genotypes detected divided by the number of individual stems genotyped for each plot.

228 Genotypic diversity — taking into account the abundance of different genotypes—was

229   calculated using the function "genotypeDiversity" with the index of "Shannon" from the package

230   polysat (Lindsay, Clark & Clark, 2018). Because small herbivores may select for some particular

231   genotypes that are more resistant or tolerant to herbivore grazing (Kotanen & Bergelson, 2000),

232   which may also impact traits. We explored genetic differentiation using principal coordinates

233   analysis (PCoA) from the R package ade4 (Dray & Dufour, 2015). We looked at genetic

234   differentiation at the treatment level, that is, grazed and ungrazed across plots at each stage,

235   result can be found in Fig. S1.

236

237   *Effects of small herbivores on trait means and ITV*

238   Although our focus is on ITV, to compare whether the effects of small herbivores on ITV are

239   stronger than trait means, we also looked at the effects of small herbivores on trait means. We

240   use analysis of variance, function "lm", to look at the effects of small herbivores on means and

241   ITV of each trait at the early and intermediate successional stage, separately. We checked

242   residuals for each model, in all cases, models fitted the data reasonably well.

243

244   *Direct and indirect effects of small herbivores on ITV*

245   We estimated the direct, indirect effects through altering genotypic richness and diversity,

246   indirect effects through altering abiotic variables, and total effects (sum of direct and indirect

247   effects) of small herbivores on ITV. We estimated these effects in each trait at each successional

248   stage based on the standardized path coefficients using structural equation models from the R

249   package "lavaan" (Rosseel, 2012). Grazing (0: ungrazed, 1: grazed), genotypic richness,

250   genotypic diversity, topographic variation, and variation in clay thickness were included in the

251   models. Note that genotypic diversity was not included for ITV at the early stage, as it was

252   significantly correlated with genotypic richness (variance inflation factor > 4). See online

253   supporting text for an example of a structural equation model for ITV in height at the

254   intermediate stage and calculation of the direct and indirect effects. Note that leaf dry matter

255   content was not evaluated in structural equation models due to limited sample size. Effects are

256   significant when $p{\leq}0.05$, while marginally significant when $p{>}0.05$ and $p{<}0.1$. Data analysis was

257   performed in R3.5.3 (R Core Team 2020).

258

259   **Results**

260   *Effects of small herbivores on trait means and ITV*

261   At the early successional stage, small herbivores significantly decreased means in height,

262   aboveground biomass, and flowering of *E. atherica*, but had no effects on means in specific leaf

263   area and leaf dry matter content. Also, small herbivores significantly increased ITV in flowering

264   and marginally increased ITV in height, but had no effects on ITV in other traits. At the

265   intermediate successional stage, small herbivores significantly decreased means in all traits

266   except for leaf dry matter content. However, small herbivores had no effects on ITV in all traits

267   (Fig. 2; see Table S3 for test statistics).

268

269   *Direct and indirect effects of small herbivores on ITV*

270   The structural equation model reveals that overall (summing up the direct and indirect effects),

271   small herbivores tended to promoted ITV in height and flowering, but not in other traits at the

272   early successional stage. Overall, small herbivores tended to promoted ITV in height, but not in

273   other traits at the intermediate stage. Moreover, these positive overall effects of small herbivores

274   on ITV in height and flowering were mainly attributable to their direct effects. Although the

275   overall effects of small herbivores on ITV in aboveground biomass and specific leaf area were

276   not significant, small herbivores also directly significantly impacted ITV in these two traits at the

277   early successional stage (Fig 3; see Table 1 for standardized path coefficient estimated from

278   SEM for the direct, indirect, and total effects of small herbivores on ITV).

279

280   At the early stage, small herbivores did not have significant effects on genotypic richness and

281   diversity, but genotypic richness decreased ITV in flowering. At the intermediate stage, small

282   herbivores did not have significant effects on genotypic richness, but significantly reduced

283   genotypic diversity. However, genotypic richness and diversity did not impact ITV in any traits

284   (Fig. 3).

285

286   Small herbivores did not impact topographic variation and variation in clay thickness at both

287   stages. However, these variations significantly increased ITV in all traits at the early stage except

288   that the effects of topographic variation on ITV in flowering were not significant, their effects

289   were less apparent at the intermediate stage (Fig. 3).

290

291   **Discussion**

292

293   In this study, using long-term exclosures, in combination with field observations and genetic

294   analyses, we explored ITV of a dominant clonal grass (*Elytrigia atherica*) and the direct and

295   indirect effects of small herbivores on ITV. We found that, at the early successional stage, small

296   herbivores promoted ITV in height and flowering of this grass. At the intermediate stage, small

297   herbivores marginally promoted ITV in height. Moreover, small herbivores promoted ITV

298   mainly through direct effects (e.g. selective grazing) but not through altering genotypic

299   richness/diversity or topographic variation and variation in clay thickness.

300

301   *Effects of small herbivores on trait means and ITV*

302   Our results suggest that small herbivores can impact both means and variations in functional

303   traits of *E. atherica*, but their effects differed in different traits. At the early stage, we found that

304   small herbivores strongly impacted means in three traits measured (height, aboveground

305   biomass, and flowering) and they strongly promoted ITV in two traits (height and flowering). At

306   the intermediate stage, small herbivores impacted means in four traits (height, aboveground

307   biomass, flowering, and specific leaf area) and they marginally promoted ITV in one trait

308   (height; Fig. 2). This suggests that trait means and variations may be driven by different

309   processes. Moreover, we only observed ITV in height and flowering may be because these two

310   traits have higher plasticity than other traits, and traits with a higher level of plasticity also show

311   higher ITV (Givnish, 2002). Further, the effects of small herbivores on trait means (relative to

312   ITV) were less dependent on grazing pressure. This may be due to that the preferred plant, *F.*

313   *rubra*, was more abundant in the grazed area at the early successional stage (ca. 3 times relative

314   to that of the intermediate successional stage). Thus, small herbivores (although more abundant)

315   may graze more on *F. rubra* rather than *E. atherica*. At the intermediate stage, the abundance of

316   *F. rubra* deceased, while the abundance of *E. atherica* increased in the grazed area (ca. 5 times

317   relative to that of the early successional stage), thus small herbivores (less abundant) may also

318   substantially grazed on less preferred *E. atherica*. Therefore, overall, the effects of small

319   herbivores on trait means were similar at these two stages. Meanwhile, non-preferred species (*A.*

320   *maritima*) was more abundant in the grazed area at the early successional stage (ca. 2 times

321   relative to that of the intermediate successional stage; see Table S2 for more detailed percent

322   cover of these species), which may facilitate young/short *E. atherica* escape from herbivore

323   grazing. Therefore, ITV was more apparent at the early successional stage than the intermediate

324   one. Previous studies in this system have shown that hares and geese are the important drivers

325   for plant community composition and structure along this successional gradient (Olff et al.,

326   1997; Kuijper & Bakker, 2005; Chen et al., 2019). Here, extending the current knowledge, we

327   show that small herbivores may also impact trait means and variations, which in turn may impact

328   community composition and structure (Whitlock, Grime & Burke, 2010).

329

330   *Direct and indirect effects of small herbivores on ITV*

331   As we hypothesized, the direct effects of small herbivores through selective grazing increased

332   ITV. This is not only true for height and flowering but also for aboveground biomass and

333   specific leaf area, despite the overall effects of small herbivores on aboveground biomass and

334   specific leaf area were not significant. As explained in the previous paragraph, the direct effects

335   of small herbivores may be mediated by community composition especially the proportion of

336   preferred and non-preferred plants. Herz et al. (2017) found that local neighborhood diversity

337   can explain a large amount of ITV in German meadows and pastures, possibly through increased

338   plant-plant interactions (e.g. competition). In this study, plant diversity was higher in the grazed

339   than the ungrazed plots at both successional stages after 22-year grazing (Table S2). Thus, higher

340   plant diversity may also contribute to increased ITV in height under grazing at both successional

341   stages. Future studies/experiments looking at the effects of herbivores on ITV in plant

342   communities with the same composition are needed to fully separate the effects of selective

343   grazing from the effects of neighboring plants.

344

345   We found that small herbivores did not impact genotype richness and diversity of *E. atherica* at

346   the early successional stage but decreased genotypic diversity at the intermediate stage. A

347   previous study in the west part of this saltmarsh found that cattle grazing does not impact

348   genotype richness and diversity of this grass, but cattle grazing alters its morphological traits in

349   the field such as decreasing height and leaf width (Veeneklaas et al., 2011). Here, we found that

350   genotypic richness and diversity generally had no significant effects on ITV. Possibly because

351   microsatellite markers are selectively neutral, thus they do not control gene expression for the

352   traits measured here. A low correlation between variation in morphological traits and DNA

353   markers was also reported before (Kolliker et al., 1998). Additionally, we found genetic

354   differentiation of *E. atherica* in the grazed and ungrazed areas at the early successional stage, but

355   not at the intermediate successional stage (Fig. S1), however, more data (presumably from more

356   exclosures) are needed to consolidate this result. Thus, the observed trait changes (at both

357   successional stages) may not be associated with genetic changes in *E. atherica*. Taken together,

358   plasticity induced by herbivore grazing may play a major role in trait changes (both in means and

359   variations).

360

361   In contrast, we found no effects of small herbivores on topographic variation and variation in

362   clay thickness within plots. These abiotic variations may be primarily induced by flooding and

363   inundation, which may overrule the effects of small herbivores in salt marshes. However,

364   variations in these two abiotic variables had substantial positive impacts on ITV particularly at

365   the early stage. Abiotic environment may be more heterogenous at the early stage because this

366   stage has more frequent sediment deposition than the intermediate stage (Schrama, Berg & Olff,

367   2012). A previous study conducted in the western part of this saltmarsh also found that

368   topographic variation at the small spatial scales (0.1 m^2 and 10 m^2) is positively correlated with

369   species richness and cattle grazing additionally increased species richness (Ruifrok et al., 2014).

370   Thus, abiotic variations, even at very small spatial scales, may play an important role in

371   promoting ITV and altering other plant community properties.

372

373   *Long-term exclosures*

374   Although we used 22-year old herbivore exclosures, our data cannot answer whether duration of

375   herbivore grazing impacts ITV, as we only collected data for one year. To our knowledge, no

376   studies have compared the effects of short- and long-term grazing on ITV. Didiano et al. (2014)

377   found that tolerance to rabbit grazing decreased as the age of the exclosures increased in *F.*

378   *rubra*, the most abundant plant in Silwood Park, England. Therefore, grazing duration may also

379   impact ITV.

380

381   **Conclusion**

382   Our results yield substantial insight into (1) small herbivores may not only impact means but also

383   ITV in some key functional traits of a dominant grass (*E.atherica*); (2) small herbivores

384   impacted ITV mainly through plasticity induced by selective grazing but not through altering

385   genotypic richness/diversity and abiotic variations. However, topographic variation and variation

386   in clay thickness may contribute to ITV. Small herbivore populations are changing rapidly due to

387   human actions. For instance, populations of European brown hares have declined dramatically

388   due to land-use changes (Smith, Jennings & Harris, 2005), while populations of geese are rapidly

389   increasing globally (Menu, Gauthier & Reed, 2002). These changes in small herbivore

390   populations could thus impact their effects on ITV, which may have consequences for saltmarsh

391   plants to respond to global changes.

392

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394

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397   **Availability of data and material (data transparency)**: Data can be found

398   https://figshare.com/s/d7e2cee03c89cf0a9d6a. Date will be made public once the manuscript is

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400   **Authors' contributions**: QC conceived and designed the experiments, performed the

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403

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413

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598

Figure 1

Sampling location and scheme

Location for the 22-year exclosures at the early and intermediate successional stage in the saltmarsh of the island of Schiermonnikoog. Pictures of the exclosures at the early and intermediate successional stages are shown. Sampling plots (1× 1 m) within an exclosure and sampling scheme for individuals of *Elytrigia atherica* within a 1× 1 m plot are shown. We followed the same sampling scheme for each plot. *Elytrigia atherica* did not always occur within each grid, thus sample size varied for each plot. Sampling plots and scheme were similar outside the exclosures (in the grazed area) at both successional stages. Number of hares and geese indicate the abundance of small herbivores such that the early successional stage had higher grazing pressure (indicated by two hares and two geese) relative to the intermediate stage (indicated by one hare and one goose). Note, only the map of the saltmarsh, but not exclosures and sampling plots, is projected according to its actual size

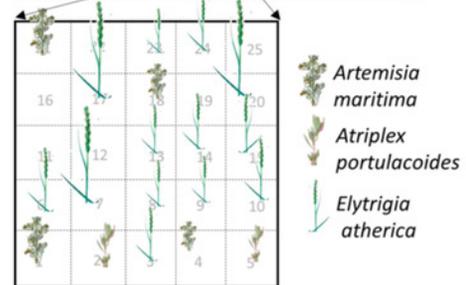
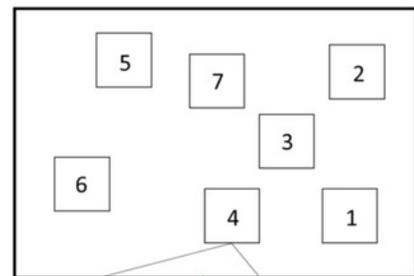



Figure 2

Means and variations in functional traits of individual stems of *Elytrigia atherica* in the grazed and ungrazed plots at the early and intermediate stages.

Traits include height (cm), aboveground biomass (g), flower frequency, and specific leaf area (cm^2 g^{-1}), leaf dry matter content (g g^{-1}). Dots are the means, error bars show 1 se. Asterisks indicate significant levels: * P<0.1; ** P<0.05; *** P<0.001. See Table S3 for test statistics.

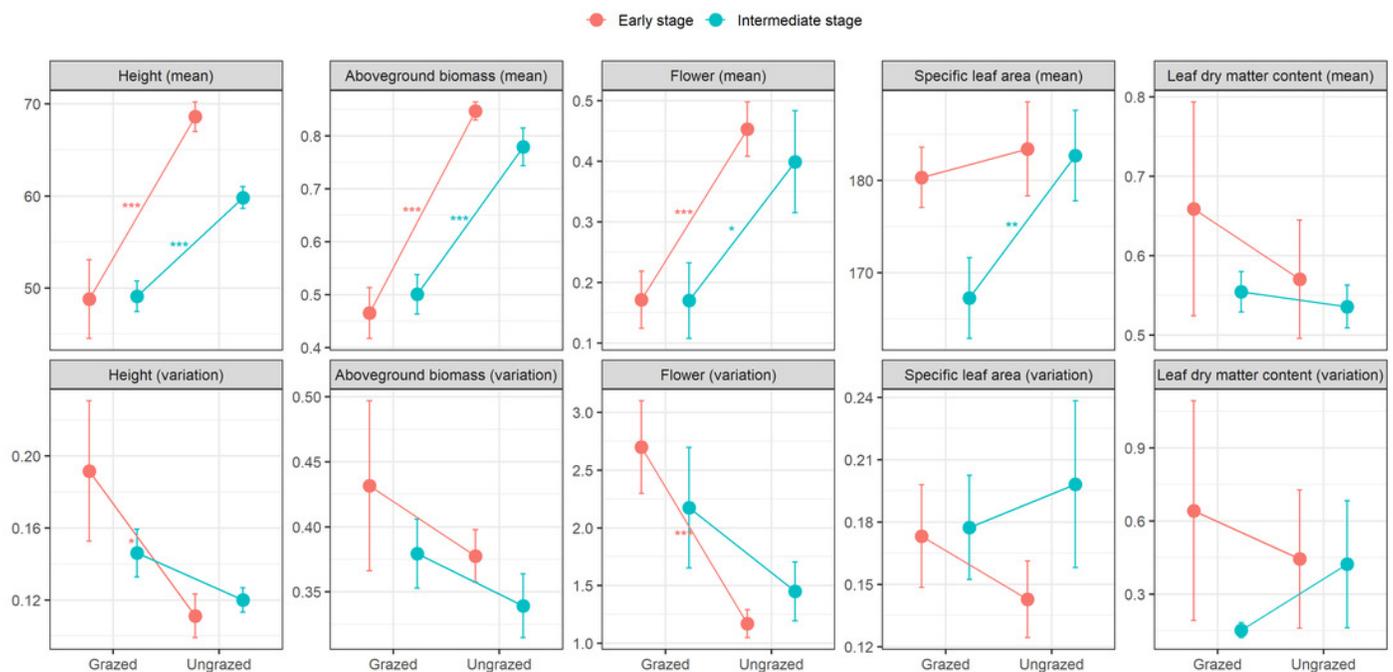
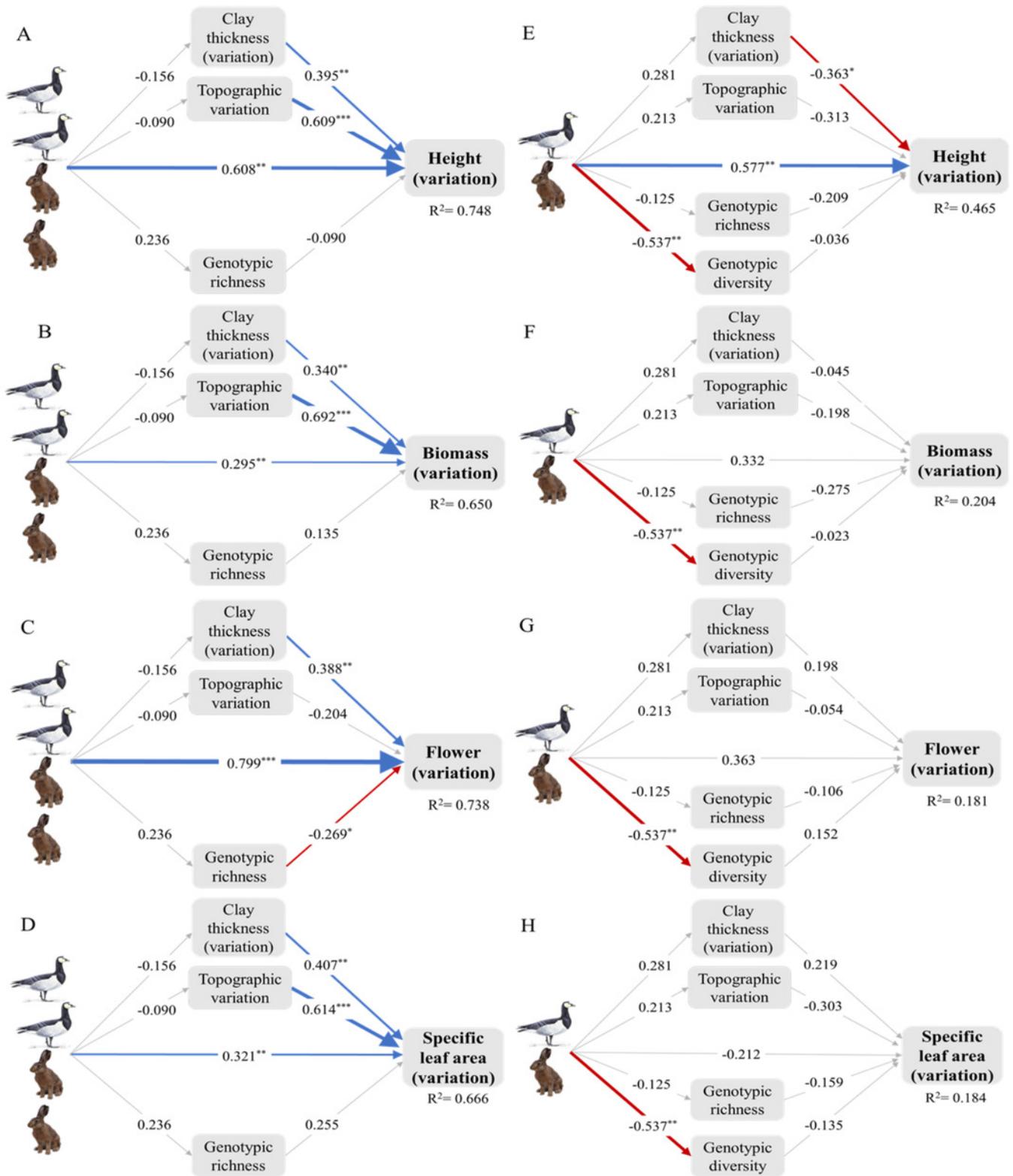



Figure 3

Intraspecific trait variation (ITV) of the dominant grass *Elytrigia atherica* and the direct and indirect effects of small herbivores on ITV in local communities at the early (A-D) and intermediate stage (E-G).

The direct effects, indirect effects through genotypes, indirect effects through abiotic variations, and total effects of small herbivores on ITV in each trait are summarized in Table 1. Model fit the data well (for all models at the early successional stage: $\chi^2 = 4.409$, df=3, N=14, $p>0.05$; for all models at the intermediate stage: $\chi^2 = 6.559$, df=6, N=14, $p>0.05$). Variance explained for clay thickness (variation), topographic variation, and genotypic richness for models at the early successional stage are 0.024, 0.008, and 0.056, respectively. Variance explained for clay thickness (variation), topographic variation, genotypic richness, and genotype diversity for models at the intermediate successional stage are 0.079, 0.045, 0.016, and 0.289, respectively. Number of hares and geese indicate the abundance of small herbivores such that the early successional stage had higher grazing pressure (indicated by two hares and two geese) relative to the intermediate stage (indicated by one hare and one goose). Boxes are measured variables. Arrows denote unidirectional relationships among variables. Blue arrows are significant positive relationships, red arrows are significant negative relationships, and grey arrows show non-significant relationships. The width of the arrows indicates the strength of the pathways. The values on the arrows denote standardized path coefficients. Asterisks indicate significant paths: * P<0.1; ** P<0.05; *** P<0.001.

Direct and indirect effects of hares and geese on intraspecific trait variation (ITV) of *Elytrigia atherica* at the early and intermediate successional stages.

See online supporting text for an example of how these data were calculated and how significance was determined using lavaan SEM. Asterisks indicate significant effects: * P<0.1; ** P<0.05; *** P<0.001.

Successional stages	Traits	Direct effects	Indirect effects through genotypes	Indirect effects through abiotic variations	Total effects
Early	Height	0.608***	-0.021	-0.117	0.47**
	Biomass	0.295**	0.032	-0.116	0.211
	Specific leaf area	0.321**	0.06	-0.119	0.262
	Flowering	0.799***	-0.063	-0.042	0.694***
Intermediate	Height	0.577**	0.045	-0.169	0.454*
	Biomass	0.332	0.022	-0.055	0.299
	Specific leaf area	-0.212	0.092	-0.003	-0.122
	Flowering	0.363	-0.068	0.044	0.339