1   Running Head：Effects of nutrient enrichment and alien snails on alien and native plants

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3   **Opposite effects of nutrient enrichment and herbivory by an alien snail on growth of an**

4   **invasive macrophyte and native macrophytes**

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18   **Abstract**

19   Human-mediated introduction of plant and animal species into biogeographic ranges where they

20   did not occur before has been so pervasive globally that many ecosystems are now co-invaded by

21   multiple alien plant and animal species. Although empirical evidence of invaders modifying

22   recipient ecosystems to the benefit of other aliens is accumulating, these interactions remain

23   underexplored and underrepresented in heuristic models of invasion success. Many freshwater

24   ecosystems are co-invaded by aquatic macrophytes and mollusks and at the same time

25   experience nutrient enrichment from various sources. However, studies are lacking that test how

26   nutrient enrichment and co-invasion by alien herbivores and plant species can interactively affect

27   native plant communities in aquatic habitats. To test such effects, we performed a freshwater

28   mesocosm experiment in which we grew a synthetic native macrophyte community of three

29   species under two levels of nutrient enrichment (enrichment vs. no-enrichment) treatment and

30   fully crossed with two levels of competition from an invasive macrophyte *Myriophyllum*

31   *aquaticum* (competition vs. no-competition), and two levels of herbivory by an invasive snail

32   *Pomacea canaliculata* (herbivory vs. no-herbivory) treatments. Results show that herbivory by

33   the invasive snail enhanced above-ground biomass yield of the invasive macrophyte. Moreover,

34   the invasive herbivore preferentially fed on biomass of the native macrophytes over that of the

35   invasive macrophyte. However, nutrient enrichment reduced above-ground biomass yield of the

36   invasive macrophyte. Our results suggest that eutrophication of aquatic habitats that are already

37   invaded by *M. aquaticum* may slow down invasive spread of the invasive macrophyte. However,

38   herbivory by the invasive snail *P. canaliculata* may enhance invasive spread of *M. aquaticum* in

39   the same habitats. Broadly, our study underscores the significance of considering several factors

40   and their interaction when assessing the impact of invasive species, especially considering that

41   many habitats experience co-invasion by plants and herbivores and simultaneously undergo

42   varous other disturbances including nutrient enrichment.

43

44   **Key words**: Biological invasion, alien species, non-native plants, herbivory, shallow lake,

45   invasion debt, early establishment, invasional-meltdown.

46

INTRODUCTION

47

48 Human-mediated introduction of plant and animal species into biogeographic ranges where they

49 did not occur before has been so pervasive globally that many ecosystems are now co-invaded by

50 multiple alien plant and animal species (Dawson et al. 2017). The invasive alien species could

51 interact with native species as well as with each other (Green et al. 2011). Invasive species often

52 have negative interactions with native species through herbivory, parasitism, predation, and

53 competition, which often impacts negatively on abundance and diversity of native plant and

54 animal species and disrupt ecosystem processes (Mack et al. 2000, Wikelski et al. 2004, Parker

55 et al. 2006, Oduor et al. 2010, Vilà et al. 2011, Ricciardi et al. 2013, Schirmel et al. 2016,

56 Stephens et al. 2019), although there are instances where invasive species can facilitate native

57 species diversity (Callaway et al. 2000, Oduor et al. 2018). However, far fewer studies have

58 investigated effects of invader-invader interactions on attributes of recipient communities and

59 ecosystem processes (O'Loughlin and Green 2017, Ricciardi et al. 2021).

60 Complex invader-invader interactions can lead to a wide range of outcomes for native

61 communities and ecosystem processes (Grosholz 2005). Negative invader-invader interactions

62 through competition and/or predation can minimize joint effects of invasive species on native

63 biota (Ross et al. 2004). In other cases, invasive species do not have net effect on each other

64 (Cope and Winterbourn 2004). Invasive species can also facilitate each other's establishment,

65 dominance, spread and ecological impacts, in accordance with the invasional meltdown

66 hypothesis (Simberloff and Von Holle 1999, Ricciardi 2001, Best and Arcese 2009). Although

67 empirical evidence of invaders modifying recipient ecosystems to the benefit of other aliens is

68 accumulating, these interactions remain underexplored and underrepresented in heuristic models

69 of invasion success (O'Loughlin and Green 2017). The complex interactions among invasive

70 species warrant experimental research to identify how invasive species interact and their

71 consequences for native communities and ecosystems (Johnson et al. 2009). Such studies may be

72 particularly informative in aquatic ecosystems where experimental approaches to understanding

73 the multi-scale effects of invasive species have historically been underrepresented compared to

74 terrestrial ecosystems (Johnson et al. 2009, Havel et al. 2015). For most aquatic invaders, our

75 knowledge regarding specific ecological impacts remains limited particularly at scales extending

76 beyond the population level (Stephens et al. 2019). When ecological changes are associated with

77 an aquatic invader, the direct and indirect mechanisms responsible are often unknown or

78 confounded by other forms of environmental change, precluding identification of the invader's

79 role in observed shifts (Stephens et al. 2019). This situation may severely undermine our ability

80 to forecast how future increases or decreases in invader abundances are likely to influence

81 ecosystem conditions (Strayer et al. 2006).

82   It has been suggested that herbivory reinforces competition between plant species, which in

83 turn diminishes the chance of coexistence among species by favoring species that are better

84 competitors (Gurevitch et al. 2000). However, in spite of this prediction, in invasion ecology, the

85 interactive effects of herbivory and competition on plant communities have been rarely assessed

86 (Suwa and Louda 2012, Li et al. 2014, Zhang et al. 2018) as studies have typically assessed

87 single biotic mechanisms (Santamaría et al. 2021). This hinders accurate estimation of the

88 relative significance of competition and herbivory in structuring plant communities in invaded

89 habitats, particularly in aquatic ecosystems that remain understudied (Santamaría et al. 2021).

90   Many freshwater ecosystems are co-invaded by aquatic macrophytes and mollusks

91 (Tricarico et al. 2016) and at the same time experience nutrient enrichment from various sources

92 including agricultural activities in watersheds (Havel et al. 2015). However, few studies have

93   investigated whether effects of the multiple invaders are additive, synergistic, or antagonistic

94   (Havel et al. 2015). Observations from field surveys that were conducted mostly in terrestrial

95   ecosystems have found that invasive plant species generally thrive in nutrient-rich habitats (Funk

96   and Vitousek 2007, Buckley and Catford 2016). Factorial experiments have also found that

97   nutrient enrichment favours growth performance of invasive plant species over native plants

98   (González et al. 2010, Liu et al. 2017). These findings generally support the suggestion that

99   nutrient enrichment may promote plant invasion (Davis et al. 2000, Dawson et al. 2012). Theory

100   predicts that herbivores that share evolutionary history with particular host plants will have a

101   lower feeding preference for those plants than for plants with which they have not co-evolved

102   (Colautti et al. 2004, Parker et al. 2006). This prediction is premised on the idea that host plants

103   that do not share evolutionary history with herbivores have not evolved strong defences against

104   the herbivores. Indeed, a previous synthesis of 11 studies found support for the prediction (Oduor

105   et al. 2010). However, studies are lacking that test how nutrient enrichment and co-invasion by

106   alien herbivores and alien plant species can interactively affect native plant communities in

107   aquatic habitats.

108   Some aquatic habitats in China have been co-invaded by the apple snail *Pomacea*

109   *canaliculata* and a macrophyte *Myriophyllum aquaticum* (Qiu and Kwong 2009), which are both

110   native to South America (Hayes et al. 2008, Gillard et al. 2017), and co-occur in large areas of

111   their native region (for details see the GBIF database; www.gbif.org). *Pomacea canaliculata* is

112   one of the 100 malignant invasive species in the world (Lowe et al. 2000), which has become

113   established in many tropical East Asian countries (Hayes et al. 2008). It often feeds on

114   submerged and emergent macrophytes (Qiu and Kwong 2009) and causes economic losses and

115   alters wetland ecosystems in its introduced region (Carlsson et al. 2004, Hayes et al. 2008). On

116 the other hand, the macrophyte *M. aquaticum* was initially introduced as a wetland ornamental

117 plant and sewage treatment plant (Cui et al. 2021), but spread quickly and occupied large

118 wetlands and shallow water areas later (i.e. become invasive; Wang et al. 2016). Separate studies

119 have shown that nutrient enrichment can enhance growth of the invasive macrophyte (Xie et al.

120 2010, Shen et al. 2019, Zhang et al. 2021). Studies have also shown that the invasive snail

121 exhibits feeding preference for plants with lower defences (Qiu and Kwong 2009). Furthermore,

122 the invasive plants often have higher resistance to generalist herbivore over native plants due to

123 their novel allelochemicals (Schaffner et al. 2011, Qi et al. 2020). Therefore, the aquatic habitats

124 offer a good system to test the combined effects of nutrient enrichment and co-invasion by alien

125 herbivores and alien plant species on native plant communities.

126 Using a mesocosm experiment, we experimentally investigated the individual and combined

127 effects of nutrient enrichment and co-invasion by the invasive herbivore *P. canaliculata* and the

128 invasive plant *M. aquaticum* on biomass yield of a synthetic community of three native

129 macrophyte species. Specifically, we tested the following predictions: (1) Nutrient enrichment

130 enhances growth of the invasive macrophyte more than that of native macrophytes; (2) the

131 invasive herbivore consumes more biomass of the native macrophyte than that of the invasive

132 macrophyte because the invasive macrophyte has with a stronger defence against the herbivore,

133 while the native macrophytes are evolutionarily naïve to the herbivore; (3) Preferential feeding

134 by the invasive herbivore on competitor native macrophytes confers growth advantage to the

135 invasive macrophyte.

136 **MATERIAL AND METHODS**

137 *Study location and species*

138 The study was conducted in a mesocosm of the Dongting Lake Wetland Ecosystem Observation

139 and Research Station (29.30°N, 150.74°E) of the Chinese Academy of Sciences in Yueyang,

140 China. The local climate is subtropical. The mesocosm comprises a fixed steel structure, with a

141 transparent acrylic plate placed on the top. Light and temperature conditions in the mesocosm are

142 similar to that of the ambient conditions. The roof of the mesocosm structure is covered with a

143 waterproof nylon bag to keep out rain water. The mesocosm was subdivided into 128 individual

144 ponds that each measured 1 m^3.

145   We constructed a three-species native macrophyte community using perennial herbaceous

146 species that commonly occur in the local wetland habitat *Vallisneria natans*, *Hydrilla verticillata*,

147 and *Myriophyllum spicatum*. To simulate natural invasion of the native macrophyte community,

148 we introduced *M. aquaticum* to the submerged native community. We procured seedlings of all

149 the four macrophyte species from an aquatic plant-producing company (Guangzhou Beishanshui

150 Ecological Technology Co., Ltd, Ezhou, China). The seedlings were kept in pond water until use

151 in the experimental set up described below. On 5th July 2020, we collected individuals of the

152 apple snail from a pond around the research station. The snails were maintained in pond water

153 with similar conditions as the experimental mesocosm for two weeks where they were fed daily

154 with leaf tissues of the four macrophyte species that were used in the current study. Water in the

155 pond that harbored the snails was changed daily.

156 *Experimental design*

157 On 3rd June 2020, we added soil into each of the 128 ponds to a depth of 15 cm. The soil was

158 obtained from the experimental station and was sifted to remove stones and plant and animal

159   debris. We used terrestrial topsoil instead of lake bottom silt to avoid the risk of undesired

160   macrophytes emerging from an aquatic plant seed bank. Immediately after adding the soil, we

161   added 150 L of groundwater to each pond. Two days later, we transplanted into the ponds

162   similar-sized stem cuttings of all the experimental plant species except for *V. natans* that was

163   propagated with seedlings. To facilitate transplanting, we placed a metallic framework (100 cm ×

164   100 cm) of 36 grids (each grid measured 15 cm × 15 cm) in the center of each pond and then

165   randomly placed 12 individual stem cuttings or seedlings of each native species in 12 grids to

166   create a three-species native macrophyte community per pond.

167   Using the 128 ponds that all planted with native macrophyte community, we employed a

168   fully crossed factorial experiment with two levels of competition from an invasive macrophyte

169   *Myriophyllum aquaticum* (competition vs. no-competition), two levels of nutrient enrichment

170   (enrichment vs. no-enrichment) treatment, and two levels of herbivory by an invasive snail

171   *Pomacea canaliculata* (herbivory vs. no-herbivory) treatments. To simulate invasion of an

172   established native macrophyte community by the invasive macrophyte *M. aquaticum*, we

173   introduced 12 individuals of *M. aquaticum* haphazardly into half of the 128 ponds (i.e. 64 ponds;

174   **Fig.1**) on 5th June 2020. After transplanting all plants, we removed the metal frame from the

175   ponds.

176   On 28th June 2020, we added additional 450 L of water to attain a water depth of 45 cm,

177   and immediately thereafter imposed a nutrient enrichment treatment. We added 4.5 g of Peters

178   Professional® water-soluble fertilizer (Total Nitrogen - 20%; Available Phosphate - 20%;

179   Soluble Potash - 20%; Magnesium - 0.05%; Boron - 0.0125%; Copper - 0.0125%; Iron - 0.05%;

180   Manganese - 0.025%; Molybdenum - 0.005%; Zinc - 0.025%) to a half of the ponds (i.e., 64

181   ponds). The fertilizer was dissolved in five liters of water and then gradually released into the

182 pond to ensure its even distribution across the pond. As a control for nutrient enrichment, we

183 added five liters of water to the other 64 ponds. On 13th July, 2020, we introduced six

184 similar-sized (diameter: 2.5 to 3.5 cm) individuals of the apple snail into each pond for a half the

185 ponds. We let only the introduced snails to feed; hence, we cleared the pond of snail eggs every

186 three days for the two-week period when herbivory treatment was imposed to prevent a new

187 generation of snails from feeding on the macrophytes. During the first week, three dead snails

188 from three ponds were replaced.

189 On 2nd August, 2020, we ended the experiment when some individuals of *M. spicatum*

190 started to flower. We removed all the apple snails and then harvested aboveground biomass of

191 the experimental macrophytes separately for each pond. Immediately after harvest, the plants

192 were wiped dry with absorbent paper and dried to a constant biomass in an oven at 65 ^{o}C for 72

193 hours. Biomass was harvested from 125 ponds as three ponds were infested by an insect that

194 consumed all the aboveground biomass, and hence were omitted from a statistical analysis.

195 Weight of the dry biomass was then taken to the nearest 0.1g.

196 To test whether the invasive snail *P. canaliculata* had a lower feeding preference for the

197 invasive macrophytes than the native macrophytes, we set up a feeding bioassay on 7th July,

198 2020. Ten grams of above-ground parts (including leaves and stems) of each macrophyte species

199 was placed in a conical flask that had been filled with 1L water. Then two individuals of *P.*

200 *canaliculata* of the same size (diameter: 2.5cm) were added into each flask. The flask was

201 covered with a gauze on the top part to prevent escape of the snails and ingress of any unwanted

202 herbivore. As a control, we set up flasks with the same amount of water and above-ground

203 biomass of the individual macrophyte species but without the snail being introduced. Each

204 treatment and control was replicated three times. All flasks were kept in a room at a constant

205   temperature (27 ^{o}C) for 24h after which the experiment was stopped. Immediately at the end of

206   the feeding bioassay, we removed all the snails and dried the plant biomass at 65 ^{o}C for 72 hours

207   and took measurements of the individual biomass samples per flaks per herbivory treatment.

208   The biomass records were used in the statistical tests described below.

209   ***Statistical analysis***

210   To test the main and interactive effects of herbivory by the invasive snail *P. canaliculata*,

211   competition by the invasive macrophyte *M. aquaticum*, and nutrient enrichment on growth

212   performance of the native macrophyte community, we performed a three-way ANOVA.

213   Aboveground biomass of the native macrophyte community in each pond was specified as

214   dependent variable. The independent variables included main and all possible three-way, and

215   two-way interactive effects of nutrient enrichment (enrichment vs. no-enrichment), competition

216   from *M. aquaticum* (competition vs. no-competition), and herbivory by *P. canaliculata*

217   (herbivory vs. no-herbivory). The above-ground biomass data were log-transformed to assure

218   normality of residuals and homogeneity of variance.

219   To test the main and interactive effects of herbivory by the invasive snail *P. canaliculata* and

220   nutrient enrichment on absolute and relative growth performance of the invasive macrophyte, we

221   conducted a two-way ANOVA using a subset of data from ponds that were invaded by *M.*

222   *aquaticum*. The absolute above-ground biomass of *M. aquaticum* and the proportional

223   above-ground biomass of *M. aquaticum* relative to the whole community above-ground biomass

224   (i.e. an indicator of relative growth performance of *M. aquaticum*) were specified as dependent

225   variables. The independent variables included main and two-way interactive effects of nutrient

226   enrichment and herbivory by the invasive snail. To assure normality of residuals and

227   homogeneity of variance, the absolute above-ground biomass and the relative above-ground

228   biomass of the invasive macrophyte were log-transformed.

229   We also performed a two-way ANOVA for the feeding bioassay experiment to test the main

230   and interactive effects of herbivory by the invasive snail and plant species identity on the

231   biomass of above-ground parts. In the ANOVA, amount of biomass of each of the four

232   individual macrophyte species was treated as a dependent variable, while herbivory treatment

233   (herbivory *vs* no-herbivory) and species identity (*M. aquaticum*, *V. natans*, *H. verticillata* and *M.*

234   *spicatum*) were specified as an independent variable. Then, we did a post-hoc analysis with

235   Tukey's Test to test whether the invasive snail had a lower preference for the invasive

236   macrophyte than for the native macrophytes. All analyses were performed in R 4.0.3 (R Core

237   Team 2020).

238

239 **RESULTS**

240 Nutrient enrichment increased the above-ground biomass of the native macrophyte community

241 (**Fig. 2; Table S1**). However, herbivory by the invasive snail significantly decreased the

242 above-ground biomass of the native macrophyte community (**Fig. 2; Table S1**). The negative

243 effect of herbivory by the snail was stronger when the macrophyte community was grown under

244 nutrient-enrichment treatment than in the absence of nutrient enrichment (**Fig. 2**; significant N ×

245 H effects in **Table S1**).

246   Herbivory by the invasive snail tended to decrease above-ground biomass of the invasive

247 macrophyte (**Fig. 3a;** marginal significant effect [$p = 0.083$] in **Table S2**). However, herbivory

248 by the invasive snail significantly increased proportional biomass of the invasive macrophyte in

249 the invaded community (**Fig. 3b; Table S2**). Although nutrient enrichment did not affect the

250 absolute above-ground biomass of the invasive macrophyte, nutrient enrichment decreased the

251 proportional above-ground biomass of the invader (**Fig. 3b; Table S2**).

252   In the feeding assay, herbivory by the invasive snail reduced significantly biomass of two of

253 the native macrophytes *H. verticillata* and *V. natans* (**Fig. 4; Table S3**). However, biomass of a

254 native macrophyte *M. spicatum* and the invader *M. aquaticum* was not reduced significantly by

255 herbivory (**Fig. 4**).

256 **DISCUSSION**

257 In line with the invasional meltdown hypothesis, we found that herbivory by the invasive snail

258 may enhance the dominance of the invasive macrophyte in the invaded communities. On the

259 other hand, our study does not support the prediction that nutrient enrichment may enhance

260 invasion by the invasive macrophyte, although invasive plant species have been shown to thrive

261 in nutrient-rich habitats in many studies (Funk and Vitousek 2007, Seabloom et al. 2015). These

262 results generally underscore the significance of considering several factors and their interaction

263 when assessing the impact of invasive species, especially considering that many habitats

264 experience co-invasion by plants and herbivores (Green et al. 2011, Dawson et al. 2017) and

265 simultaneously undergo varous other disturbances including human-induced nutrient enrichment

266 (Bobbink et al. 2010).

267 Alien herbivores can facilitate invasion by alien plant species through preferential feeding

268 on herbivory-intolerant native plants in the invaded communities (Hobbs 2001, Parker et al. 2006,

269 Oduor et al. 2010). Indeed our results show that the invasive snail consumed more biomass of

270 two of the three native macrophytes than that of the co-introduced invader *M. aquaticum*. As the

271 native macrophyte species are evolutionarily naïve to the herbivore, it is likely that the native

272 macrophytes have not evolved strong defences against the invasive snail. On the other hand,

273 because the invasive macrophyte   is native to the same biogeographic region as the invasive

274 snail, it is likely that the invasive macrophyte has evolved strong defence against the herbivore,

275 and hence is less palatable to the invasive snail than native macrophytes. In fact, a previous

276 feeding assay with the invasive snail *P. canaliculata* and ten aquatic macrophyte species that

277 included *M. aquaticum* found that the invasive snail had low survivorship and growth rate, and

278 did not reproduce when fed on the invasive macrophyte *M. aquaticum* (Qiu and Kwong 2009). In

279   the previous study, macrophytes with high nutritional contents and low chemical defences (i.e.,

280   low phenolic content) were more palatable to the invasive snail than macrophytes with low

281   nutritional content and high chemical defences (Qiu and Kwong 2009). Future studies may test

282   whether the three native macrophytes that were studied presently differ in nutritional content and

283   chemical defences from the invasive macrophyte.

284   Herbivory can enhance growth performance of alien plants over native plants by inducing

285   compensatory growth and enhanced competitive ability of alien plants (Best and Arcese 2009).

286   Given that invasive plant species often grow faster than native species (van Kleunen et al. 2010),

287   greater compensatory growth might be expected for the former. Plants can defend against

288   herbivory through two strategies: resistance (i.e., plant traits that minimize damage from

289   herbivores, e.g. defence compounds and leaf toughness) and tolerance (i.e., plant traits that

290   enable a plant to maintain fitness after damage has occurred, e.g., increased photosynthetic and

291   growth rates) (Strauss and Agrawal 1999, Stowe et al. 2000). Resistance and tolerance are not

292   necessarily mutually exclusive defence strategies as plant species often deploy both. For instance,

293   herbivory by two folivores (*Lema daturaphila* and *Epitrix parvula*) selected for plant *Datura*

294   *stramonium* genotypes with intermediate resistance and high tolerance (Carmona and Fornoni

295   2013). Separately, the plant *Senecio jacobaea* exhibited both greater resistance to and tolerance

296   of herbivory in the introduced range than in the native range (Stastny et al. 2005), while native

297   populations of the invasive grass *Phragmites australis* did not display tolerance-resistance

298   tradeoff (Croy et al. 2020). A positive association between tolerance and induced chemical

299   defence was demonstrated in plant *Arabidopsis thaliana* (Mesa et al. 2017). Therefore, our

300   finding that the invasive macrophyte experienced a lower herbivory by the invasive snail than

301   native macrophytes likely because that the invasive macrophyte has high resistance.

302   Mutualism between invaders is posited to initiate invasional meltdown by generating

303   reciprocal, positive population-level responses that amplify invader-specific impacts. These

304   impacts then facilitate further invasions and accelerate the overall rate of invasion (Parker et al.

305   1999). Nevertheless, few studies have demonstrated positive population-level effects between

306   invaders that amplify their impacts (Havel et al. 2015, Braga et al. 2018). Therefore, future

307   studies may investigate population-level effects of any positive interactions between *M.*

308   *aquaticum* and *P. canaliculata*.

309   Many empirical studies suggest that nutrient enrichment will promote alien plant invasion

310   (Davis et al. 2000, Leishman and Thomson 2005, González et al. 2010, Dawson et al. 2012,

311   Seabloom et al. 2015, Liu et al. 2017). However, in the present study, nutrient enrichment

312   reduced the proportional above-ground biomass of the invasive macrophyte. It remains unclear

313   why our results contradict those of several other studies. A plausible explanation for the current

314   finding may be that because the current test native macrophyte species are also invasive

315   elsewhere in the world, they have similarly high or higher growth response to nutrient

316   enrichment than *M. aquaticum*. The native *H. verticillata* is a naturalized species in much of Asia

317   that has invaded aquatic habitats in the United States (Zhu et al. 2017), Brazil (Sousa 2011) and

318   South Africa (Coetzee et al. 2009). Similarly, *M. spicatum* is also listed as an invasive plant in

319   the United States (Moody and Les 2007) and Egypt (Ali and Soltan 2006), while *H. verticillata*

320   is highly competitive and often dominates the invasive areas and local large plant communities

321   (Sousa et al. 2009, Hofstra et al. 2010). Although previous studies found that nutrient enrichment

322   enhanced growth of the invader *M. aquaticum* (Xie et al. 2010, Shen et al. 2019, Zhang et al.

323   2021), the effect of nutrient enrichment on competitive ability of *M. aquaticum* against native

324   macrophytes was not investigated. Therefore, it is likely that these native macrophytes have

325   similar or higher competitive ability than *M. aquaticum* under nutrient enrichment.

326   In conclusion, our study suggests that nutrient enrichment in freshwater lakes may slow

327   down invasion by the invasive macrophyte, while the inasive herbivore may enhance dominance

328   of the invasive macrophyte in the invaded community. These results broadly support the idea

329   that co-invasion by alien species that come from the same biogeographic region and possibly

330   share evolutionary history can be detrimental to recipient native communities. To date, most

331   documented cases of facilitation of one invader by another invader are from terrestrial habitats

332   (Braga et al. 2018), and our study aims to help fill the knowledge gap of positive interactions

333   among invasive species in aquatic habitats. Identification of interaction networks among invasive

334   species offers opportunities beyond a single-species approach for managing invasive species in

335   multiply invaded systems (Bull and Courchamp 2009). In the present context, dissolution of

336   positive interactions between the invasive snail *P. canaliculata* and the invasive macrophyte *M.*

337   *aquaticum* may mitigate negative impacts of the invasive macrophyte on native macrophyte

338   communities.

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AUTHOR CONTRIBUTIONS

YL conceived the idea and designed the experiment. YY, FL and YX performed the experiment. YY and YL analyzed the data. YY, YL and AMO wrote the first draft of the manuscript, with further inputs from FL and YX.

DATA ACCESSIBILITY

Should the manuscript be accepted, the data supporting the results will be archived in Dryad and the data DOI will be included at the end of the article.

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538 **FIGURES**

539
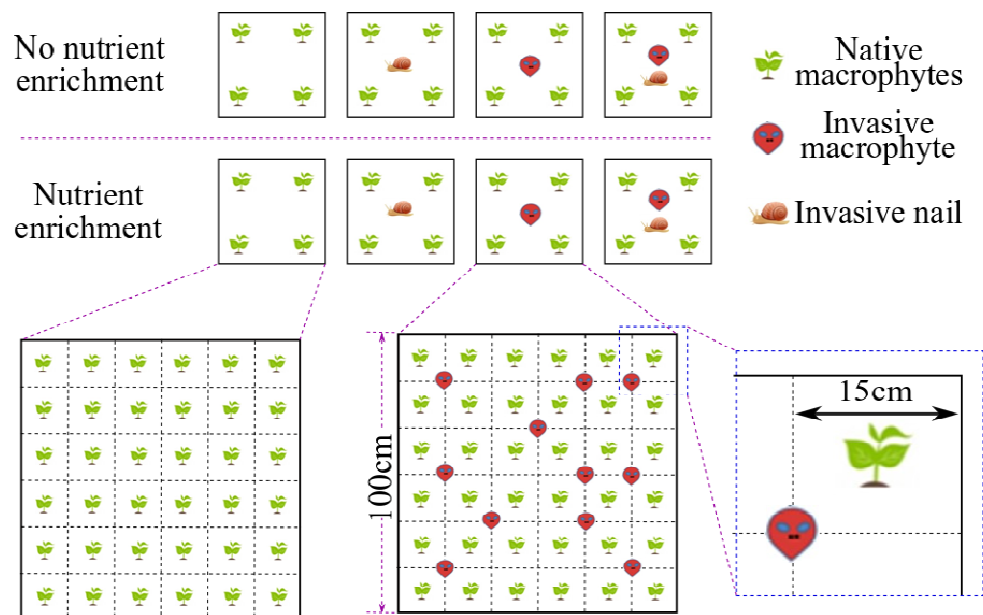
Figure 1. A schematic of an experimental set up to test the main and interactive effects of co-invasion by an alien snail *Pomacea canaliculata* and an alien macrophyte *Myriophyllum aquaticum* and nutrient enrichment on growth performance of a three-species community of native macrophytes (*Vallisneria natans, Hydrilla verticillata,* and *Myriophyllum spicatum*) and the invader *M. aquaticum.* Each grid represents a 1m^3 pond; a total of 128 ponds were used in the experiment. Grids with a blue outline represent ponds with no-nutrient enrichment treatment, while grids with a red outline represent ponds with a nutrient-enrichment treatment. Six similar-sized individuals of *P. canaliculata* were introduced into each pond for a total of 64 ponds, while the other 64 ponds served as a control (no-herbivory). Nutrient-enrichment treatment was applied to 64 ponds (32 with herbivory and 32 with no-herbivory), while another 64 ponds served as a control and did not receive nutrient enrichment (32 with herbivory and 32 with no-herbivory).
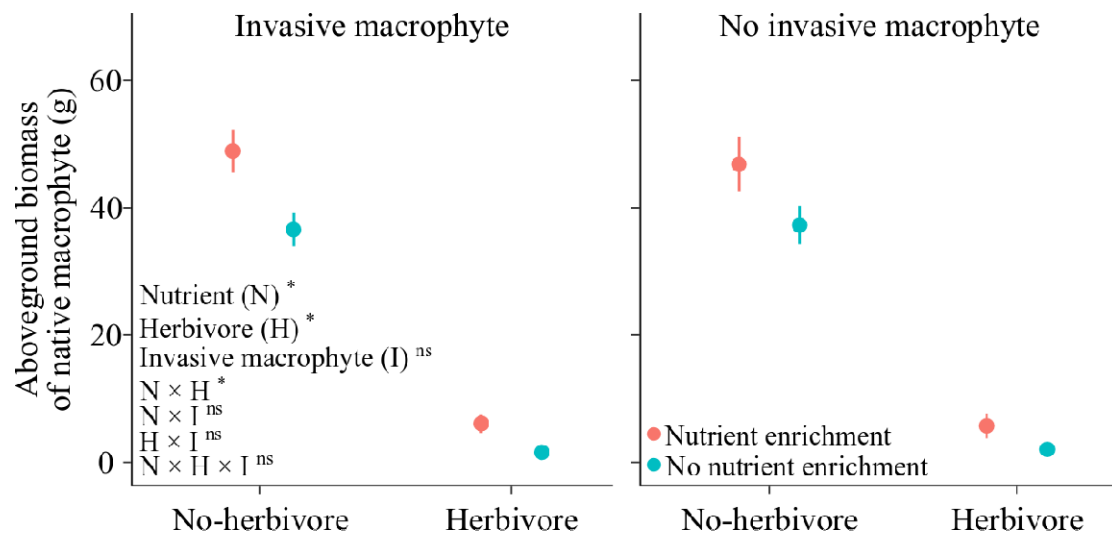
Figure 2. Mean ($\pm$ 1 SE) above-ground biomass of a three-species native macrophyte community. The panels show the effects of invasion by an aquatic macrophyte *Myriophyllum aquaticum* under two levels of nutrient enrichment (enrichment vs. no-enrichment) and herbivory (herbivory vs. no-herbivory) by an invasive snail *Pomacea canaliculata*. The asterisks indicate level of statistical significance: * denotes $p < 0.05$, while [ns] denotes $p > 0.05$.
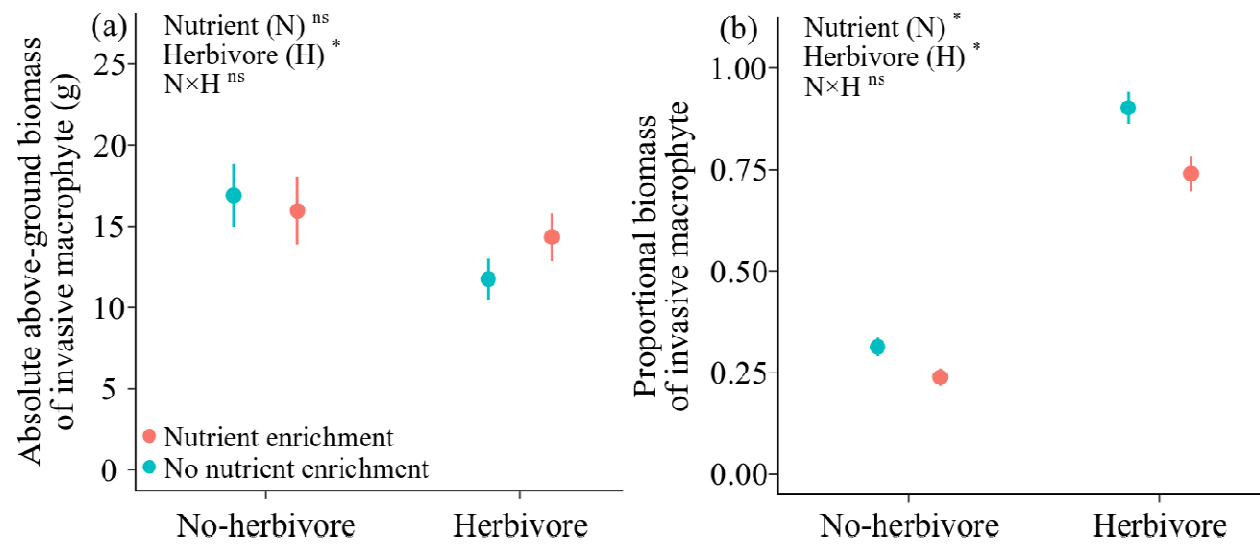
Figure 3. Mean (± 1 SE) absolute (**a**) and proportional (**b**) above-ground biomass of an invasive macrophyte *Myriophyllum aquaticum* when grown in a three-species native aquatic macrophyte community (*Vallisneria natans, Hydrilla verticillata,* and *Myriophyllum spicatum*). The asterisks indicate level of statistical significance: * denotes $p < 0.05$, while [ns] denotes $p > 0.05$.

563
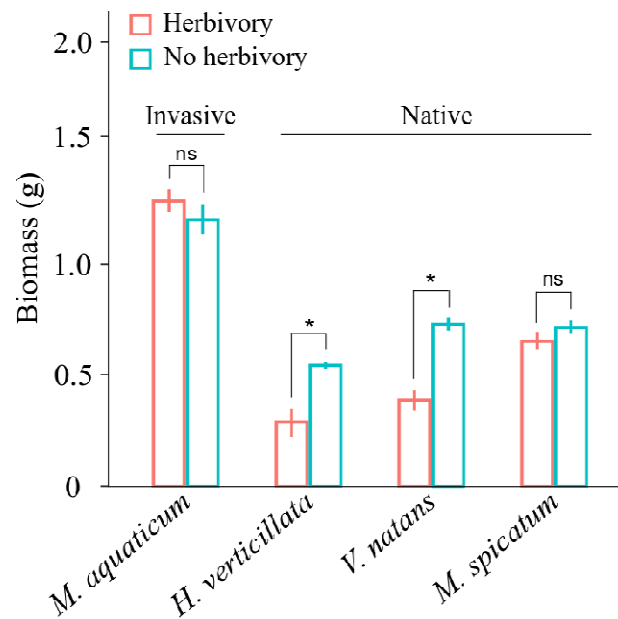
Figure 4. Mean (± 1 SE) biomass of three native aquatic macrophytes (*Vallisneria natans,*

Hydrilla verticillata, and *Myriophyllum spicatum*) and an invasive macrophyte *Myriophyllum*

aquaticum that were subjected to herbivory or not (control) by an invasive aquatic snail *Pomacea*

canaliculata. Symbols above bars (*: $p < 0.05$; [ns]: $p > 0.05$) show levels of differences between

feeding and no feeding treatment based on the post-hoc analysis with Tukey's Test.

569

570 **Supporting information**

571 **Table S1** Results of a three-way ANOVA that tested for the effects of nutrient enrichment

572 (enrichment vs. no-enrichment), herbivory by an invasive snail *Pomacea canaliculata* (herbivory

573 vs. no-herbivory), and presence of an invasive macrophyte *Myriophyllum aquaticum* (present vs.

574 absent) and their interaction on above-ground biomass of a community three native aquatic

575 macrophytes (*Vallisneria natans*, *Hydrilla verticillata*, and *Myriophyllum spicatum*). Significant

576 effects ($P < 0.05$) are shown in bold font.

Factor	Df	F	p
Nutrient enrichment (N)	1	17.03	**<0.001**
Herbivory (H)	1	89.18	**<0.001**
Plant invasion (I)	1	0.46	0.139
N × H	1	10.34	**0.011**
N × I	1	1.97	0.419
H × I	1	0.37	0.703
N × H × I	1	1.53	0.141
Residuals	Df	Mean Squares	
	120	24.7	

577

578 **Table S2**. Results of two-way ANOVAs that tested the effects of nutrient enrichment (enrichment

579 vs. no-enrichment), herbivory by an invasive snail *Pomacea canaliculata* (herbivory vs. no-

580 herbivory), and interaction between them on absolute above-ground biomass and proportional

581 biomass of an invasive macrophyte *Myriophyllum aquaticum* when grown together with a

582 community of three native macrophyte species (*Vallisneria natans, Hydrilla verticillata,* and

583 *Myriophyllum spicatum*). Significant effects ($P < 0.05$) are shown in bold font.

Factor	Df	Absolute above-ground F	p	Df	Proportional above-ground biomass F	p
Nutrient enrichment (N)	1	0.25	0.616	1	12.66	**0.001**
Herbiovry (H)	1	3.12	0.083	1	212.06	**<0.001**
N × H	1	1.92	0.171	1	0.27	0.603
Residuals	Df	Mean Squares		Df	Mean Squares	
	57	0.090		57	0.205	

584

585   **Table S3.** Results of a two-way ANOVA that tested for the effect of herbivory by an invasive

586   alien snail *Pomacea canaliculata* on above-ground biomass of an invasive alien macrophyte

587   *Myriophyllum aquaticum* and three native macrophyte species *Vallisneria natans, Hydrilla*

588   *verticillata,* and *Myriophyllum spicatum*. Species identity of the four macrophytes and herbivory

589   by the snail were treated as independent variables.

Factors	Df	F	*p*
Species	1	53.70	**<0.001**
Feeding	1	25.29	**<0.001**
Species × Feeding	1	8.18	**0.002**
Residuals	Df	Mean Squares	
	16	0.027	

590