

**Little evidence for release from herbivores as a driver of plant invasiveness from a multi-species herbivore-removal experiment.**

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Abstract:	<p>Enemy release is frequently posed as a main driver of invasiveness of alien species. However, an experimental multi-species test examining performance and herbivory of invasive alien, non-invasive alien and native plant species in the presence and absence of natural enemies is lacking. In a common garden experiment in Switzerland, we manipulated exposure of seven alien invasive, eight alien non-invasive and fourteen native species from six taxonomic groups to natural enemies (invertebrate herbivores), by applying a pesticide treatment under two different nutrient levels. We assessed biomass production, herbivore damage and the major herbivore taxa on plants. Across all species, plants gained significantly greater biomass under pesticide treatment. However, invasive, non-invasive and native species did not differ in their biomass response to pesticide treatment at either nutrient level. The proportion of leaves damaged on invasive species was significantly lower compared to native species, but not when compared to non-invasive species. However, the difference was lost when plant size was accounted for. There were no differences between invasive, non-invasive and native species in herbivore abundance. Our study offers little support for herbivore release as a driver of plant invasiveness, but suggests that future enemy release studies should account for differences in plant size among species.</p>

1 **Little** evidence for release from herbivores as a driver of plant invasiveness from a  
2 multi-species herbivore-removal experiment.

3

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24 Running title: Experimental enemy release of plants

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27 experimental multi-species test examining performance and herbivory of invasive alien, non-  
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37 non-invasive species. However, the difference was lost when plant size was accounted for. There  
38 were no differences between invasive, non-invasive and native species in herbivore abundance.  
39 Our study offers little support for herbivore release as a driver of plant invasiveness, but suggests  
40 that future enemy release studies should account for differences in plant size among species.

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43 Key-words: exotic, herbaceous plants, natural enemies, naturalisation, phytophagous insects,  
44 resource availability.

45

46

## 47 Introduction

48 The enemy release hypothesis (ERH) is one of the most widely invoked mechanisms used to  
49 explain why some alien plant species become invasive in their introduced ranges (see reviews of  
50 (Colautti et al. 2004, Liu and Stiling 2006, Chun et al. 2010). Plant species introduced to a new  
51 range may escape natural enemies, such as pathogens and herbivores (Keane and Crawley 2002).  
52 **The absence of natural enemies** may increase plant performance compared to the native range,  
53 and also relative to native plant species in the new range as the latter should still suffer from their  
54 full suite of natural enemies (Colautti et al. 2004). Under the ERH, alien plant species that are  
55 **sufficiently released from enemy damage** to increase performance and fitness, may greatly  
56 increase their populations and become invasive. In contrast, aliens that undergo less release from  
57 enemy damage do not have the same performance advantage, and are less likely to outcompete  
58 native species and become invasive (Keane and Crawley 2002). Thus, for the ERH to be a  
59 general rule, only invasive alien, but not non-invasive alien species should benefit through a  
60 reduction in enemy damage, which results in greater performance, relative to native species.

61 A number of studies have compared damage from herbivory on alien and native plant  
62 species in the introduced range (Agrawal and Kotanen 2003, Colautti et al. 2004, Agrawal et al.  
63 2005, Chun et al. 2010, Funk and Throop 2010). Others have compared the level of damage by  
64 natural enemies on native, non-invasive alien and invasive alien species (Liu et al. 2007, Parker  
65 and Gilbert 2007), or investigated the relationship between viral/fungal pathogen release and  
66 invasiveness of alien plant species (Mitchell and Power 2003, van Kleunen and Fischer 2009).  
67 These approaches test the ERH partially, in that reduced attack and damage of invasive alien  
68 species does not necessarily result in increased plant performance relative to native species, or  
69 non-invasive aliens. To fully test the ERH, plant performance in the absence and presence of

70 enemies has to be assessed, which only few studies did so far (Parker and Gilbert 2007, Chun et  
71 al. 2010).

72 Further, the identity of the herbivores may determine the outcome of the ERH (Cripps et  
73 al. 2006, Ando et al. 2010, Alba et al. 2012), as it assumes release from specialist herbivores  
74 (Keane and Crawley 2002, Mitchell et al. 2006). However, many alien species occur in urban  
75 environments (Pyšek 1998), where it is likely that most herbivores are generalists (Niemelä et al.  
76 2011). Whether release from generalist herbivores also contributes to plant invasion remains  
77 open: while some studies show that also generalist herbivores avoid alien and alien invasive  
78 plant species (Jogesh et al. 2008, Tallamy et al. 2010, Schaffner et al. 2011), others did not find  
79 this pattern (Parker and Hay 2005).

80 Plants growing under higher nutrient levels are likely to be more susceptible to herbivory  
81 due to greater tissue nutrient content (Mattson 1980, Butler et al. 2012). Therefore, plant species  
82 that grow in high-nutrient environments and benefit most from increased nutrient availability  
83 may suffer greater levels of herbivory than species in low nutrient environments (Coley et al.  
84 1985, Dostal et al. 2013, Lind et al. 2013). The resource-enemy release hypothesis (Blumenthal,  
85 2005, 2006) states that alien plants from nutrient-rich environments will benefit more from  
86 enemy release. A prediction made by this hypothesis is that if invasive alien species already  
87 benefit from enemy release in terms of performance, they may do so to a greater extent under  
88 higher nutrient levels compared to more susceptible natives.

89 We tested the ERH by manipulating exposure of native, alien invasive and alien non-  
90 invasive herbaceous plant species in Switzerland to invertebrate herbivores. If invasive species  
91 already benefit from natural enemy release, they should benefit less strongly from  
92 experimentally reduced herbivore exposure compared to native and non-invasive alien species.

93 We recorded 1) the abundance and identity of major invertebrate herbivores, which we  
94 subsequently classified according to their feeding preferences as generalists or specialists; 2) leaf  
95 damage, and 3) biomass in response to enemy exclusion. For biomass, we tested the effect of  
96 nutrient availability on the level of enemy release experienced by the plants by exposing plants  
97 to low and high nutrient levels. Specifically our hypotheses were:

98 1) herbivore damage and herbivore abundance should be lower in invasive alien than  
99 non-invasive alien and native species;

100 2) Invasive species should show no or little decrease in herbivore damage and abundance  
101 when treated with pesticide, while non-invasive and native species should exhibit  
102 significantly lower herbivore damage/abundance when treated with pesticide;

103 3) Plant performance (biomass) of invasive species should show no or little increase  
104 when treated with pesticide, while non-invasive and native species should show a larger  
105 increase in biomass.

106 4) We also expect the differences between native and non-invasive species, and invasive  
107 species in pesticide effects to be more pronounced with the addition of nutrients.

108

## 109 **Materials and Methods**

### 110 *Study species*

111 Seeds of 29 species were collected in 2008 and 2009 from plants in wild populations throughout  
112 Switzerland (Table 1). These 29 species included fourteen native, eight non-invasive alien and  
113 seven invasive alien species within Switzerland, and represent six taxonomic confamilial groups

114 (Table 1). Species belonging to the Plantaginaceae, Phrymaceae and Scrophulariaceae, which  
115 until recently all belonged to the Scrophulariaceae, were considered one group, and the  
116 Asteraceae species were split into two groups (*Bidens* genus and non-*Bidens* species). Except for  
117 five native species, all other species occur in nitrophilous plant communities (Landolt et al. 2010;  
118 see Table S1). For the non-*Bidens* Asteraceae group, the native *Solidago virgaurea* was used to  
119 assess herbivory damage, while the natives *Gnaphalium luteoalbum* and *Artemisia vulgaris* were  
120 used to assess biomass responses to pesticide and nutrient addition treatments (see below), due to  
121 limited numbers of plants available. Thus, for native non-*Bidens* Asteraceae species, herbivory  
122 damage and biomass responses to pesticide and nutrient treatments are not directly comparable.  
123 Expert opinion from the National Data and Information Centre of the Swiss Flora, and the  
124 associated Info Flora website (<http://www.infoflora.ch/de/flora/art-abfragen.html>) were used in  
125 order to assign species status as native, alien invasive or alien non-invasive, with invasive  
126 species generally being more widespread in Switzerland according to Atlas records (Table S1).

127

### 128 *Experiment set-up*

129 Seeds were planted as individual seed families collected from mother plants, in a 1:1  
130 mixture of seedling compost and sharp sand in the third week of April 2010 in a greenhouse.  
131 After germination, individual seedlings were transplanted to separated compartments within  
132 trays, filled with a 1:1 mixture of alluvial soil and sharp sand. These seedlings were then grown  
133 for a further 4-5 weeks until the end of June 2010.

134 We set up a common-garden experiment in a 1000 m<sup>2</sup> field in Bern, Switzerland,  
135 surrounded by short grassland and gardens (i.e. the type of habitat where many plant invasions

136 have started). Eight experimental blocks were set up (in a four by two configuration), and each  
137 block was split into two halves (Fig. 1). Plants in each block were represented by one seed  
138 family per species, to **minimise the contribution of genetic differences to variation among plants**  
139 **in different treatments within blocks**. One half of each block was later randomly assigned to the  
140 herbivore exclusion treatment (see below), **and the block-halves were separated by 2 m**. Each  
141 block-half contained seven sub-blocks (Fig. 1), and sub-blocks were paired according to their  
142 position across the block-halves. One pair of sub-blocks was randomly assigned a taxonomic  
143 group per block (Fig. 1; the non-*Bidens* Asteraceae group was split into two plots, giving seven  
144 instead of six sub-blocks in total, see Table 1). Two plants per species per sub-block were  
145 planted individually in 3-L pots, with the same soil as the seedling trays. Pots within each sub-  
146 block had either no nutrient addition or nutrient addition **applied once prior to planting** (12 g of  
147 slow-release NPK fertiliser pellets; Osmocote™ Exact Standard, N:P:K 16:9:12 + 2MgO + trace  
148 elements). The soil had a nitrogen content of 1.79 g kg<sup>-1</sup> of dry weight substrate (5.83 g N pot<sup>-1</sup>),  
149 and nutrient addition resulted in a 33% increase in nitrogen concentration. These plants were  
150 used for assessment of plant performance in response to herbivore exclusion and nutrient  
151 addition (hereafter referred to as the performance set). **An additional plant per species in each**  
152 **taxonomic group per sub-block was planted in a 3-L pot containing the same soil as other plants**  
153 **and without nutrient addition**. These plants were subsequently used to record rates of herbivore  
154 damage and invertebrate herbivores (hereafter referred to as the herbivory set). The total  
155 numbers of performance plants without nutrient addition and with nutrient addition, and plants  
156 used for measuring herbivory per sub-block, are indicated in Fig. 1. Pot positions within each  
157 sub-block were randomised.

158 **In summary, 11 native, 8 non-invasive alien and 7 invasive alien species were used to**  
159 **assess herbivory damage (Table 1), with initially 8 plants per pesticide treatment per species**



160 (giving 416 plants in total). For measuring plant biomass in response to pesticide and nutrient  
161 addition treatments, there were also 8 plants per species for each pesticide/nutrient treatment  
162 combination (giving a total of 896 plants). During the experiment, some plants died, and others  
163 were lost due to complete consumption by molluscs before the enemy exclusion experimental  
164 treatment started. Thus, 200 complete pairs of pesticide/non-pesticide treated plants remained of  
165 those for measuring herbivory (paired according to block, 400 in total), and 382 pairs remained  
166 for measuring nutrient/pesticide effects on biomass (764 in total). Table S1 shows final sample  
167 sizes (numbers of plants) per species per treatment.

168 Two weeks after planting (to allow plants to overcome transplant stress), one block-half  
169 per block was randomly assigned to the herbivore-exclusion treatment. A belowground pesticide  
170 was applied in pellet form (Cortilan®, Maag/Syngenta Agro AG, 1.5 % Chlorpyrifos) once to  
171 each pot individually, at a rate of 5 g per m<sup>2</sup> (0.16 g per pot). An above-ground pesticide  
172 (Perfekthion®, Maag/Syngenta Agro AG, 500 g per L dimethoate) was applied as a fine mist  
173 spray with a concentration of 1 ml per L of water, and was repeated two weeks later at the  
174 beginning of August, and in the last week of August. Spray treatments were applied on calm,  
175 non-windy days. As a control, a fine mist spray of water was applied to the plants in the non-  
176 pesticide treatment. To contain the application of pesticide and procedural controls to the target  
177 plants, the treatments were carefully applied at a constant rate to each plant individually at close  
178 range, and not above the plants using a pump-action canister. Anti-mollusc pellets (Mioplant®  
179 Schneckenkörner, Migros AG, active ingredient: Metaldehyde) were applied liberally on the  
180 ground surrounding the pots in the enemy exclusion block-halves at the end of July and in the  
181 third week of August. Whilst it is unlikely that all herbivores were excluded by the pesticide  
182 treatment, the abundance of invertebrate herbivores on plants was reduced compared with plants  
183 with no pesticide applied (see results). To encourage colonisation by invertebrate herbivores,

184 grassland was allowed to grow in strips separating and bordering the blocks, with care taken to  
185 avoid shading of the experimental plants. The plants were grown for 12 weeks until 22<sup>nd</sup>  
186 September 2010, giving a period of 10 weeks for the herbivore exclusion treatment.

187

### 188 *Herbivory and performance measurements*

189 On the herbivory set of plants, we collected herbivores once every two weeks, for a total of six  
190 times throughout the experimental period between 9 am and 4 pm (6 - 9 am for molluscs), under  
191 sunny and calm weather conditions. Most insects were sampled through removal. For aphids,  
192 however, we counted the number of individuals on the plants, and we removed only a few  
193 individuals for identification. This was done to avoid artificially decreasing aphid abundance  
194 through harvesting over time. Molluscs and aphids were identified to species level, while other  
195 invertebrates were identified to family or order level. Aphids, molluscs, orthopterans and  
196 thysanopterans were the most abundant herbivore groups, and thus further analysed. On a species  
197 level, aphids and molluscs were classified as generalists or specialists according to host plants  
198 cited in Lampel and Meier (2007) for aphids, and in Frömring (1954) and Boschi (2011) for  
199 molluscs. Species were judged to be generalist if they were known to feed on host plants from  $\geq$   
200 two families/recorded as strongly polyphagous. The abundance of aphids on each plant was  
201 summed across all survey times. Damage sustained by the herbivory set of plants was recorded  
202 as the proportion of leaves on plants that were damaged, at the end of the experiment. Leaf  
203 chewing, gall formation and leaf mining were all considered forms of damage, but only leaf  
204 chewing was present. For plants with few leaves, all leaves were surveyed for signs of damage.  
205 For branched plants with many leaves, one branch per plant was randomly chosen and surveyed.  
206 Mean damage and aphid abundance values are given in Table S2.

207 For the performance set of plants, we harvested aboveground and belowground biomass  
208 of surviving plants after 12 weeks, and dried it at 80-85 °C for at least 72 hours prior to  
209 weighing. After 12 weeks, we also harvested, dried and weighed the aboveground biomass of the  
210 herbivory set of plants, for use as a covariate in subsequent analyses.

211

## 212 *Analysis*

213 Mixed effects models were used for all analyses, with species nested in taxonomic group, and  
214 block added as random effects throughout. An identity variance structure modelling different  
215 variances per species was included in all linear mixed effects models using the 'varIdent'  
216 function in the R package 'nlme' (plus an additional identity variance structure according to  
217 nutrient treatment for biomass models) to meet error normality and homoscedasticity  
218 assumptions. For non-pesticide treated plants from the herbivory set, we analysed aphid  
219 abundance ( $\ln(x+1)$  transformed) with a linear mixed model, and the proportion of leaves  
220 damaged and the presence of other herbivores using binomial generalized linear mixed models.  
221 In each case, species status was a fixed effect. We also re-analysed the proportion of leaves  
222 damaged with aboveground biomass (square-root transformed) and the number of leaves per  
223 plant (natural-log transformed) as covariates, to account for differences in plant size (both  
224 centred to the mean and scaled to one standard deviation, to allow estimates of differences  
225 among invasive, non-invasive and native species to be calculated for the average-sized plant).  
226 The three Poaceae species had no plants with damaged leaves and were excluded from analyses  
227 of leaves damaged to avoid zero-inflation.

228 As measures of experimental enemy release, we calculated the difference in the  
229 proportion of leaves damaged and the difference in aphid abundance between pairs of plants of a  
230 species treated and not treated with pesticide for each block (giving up to eight values per  
231 species, one per block). Linear mixed models were used to analyse these two variables, with  
232  $\ln(x+22)$  transformation for the difference in aphid abundance, because the most negative  
233 difference between paired plants was -21 (21 aphids fewer on the non-pesticide plant than the  
234 pesticide-treated plant). Species status was a fixed effect. The difference in proportion of leaves  
235 damaged was reanalysed with average biomass (natural-log transformed) and the difference in  
236 the number of leaves between plants in each pesticide/non-pesticide pair added as covariates  
237 (centred and scaled). In addition, in order to assess whether or not biomass actually correlated  
238 with proportion of leaves damaged, we analysed the aboveground biomass (square-root  
239 transformed) as a function of the proportion of leaves damaged for non-pesticide and pesticide-  
240 treated plants separately. In these analyses, the intercept and slope were allowed to vary  
241 according to taxonomic group and species nested within taxonomic group. A fixed variance  
242 structure (variance increasing with increasing biomass) was used to account for variance  
243 heterogeneity.

244 To quantify the effect of experimental enemy release on plant performance, we analysed  
245 total biomass (square-root transformed) of the performance set of plants using a linear mixed  
246 effects model. Only data points representing complete pairs were used, where both the 'enemy-  
247 excluded' and 'enemy-exposed' plants were present and surviving in a block per nutrient  
248 treatment. Species status, nutrient treatment and pesticide treatment were fixed effects. We used  
249 likelihood ratio tests ( $\chi^2$  values) to assess the significance of interactions and main effects of these  
250 three factors throughout, and in order to obtain a minimum adequate model explaining plant  
251 performance. All analyses were conducted in R version 3.0.2 (R Core Team 2013). We used the

252 function ‘lme’ in the package ‘nlme’ (Pinheiro et al. 2013) for linear mixed effects models, and  
253 the function ‘glmer’ in the package ‘lme4’ (Bates et al. 2013), for generalised linear mixed  
254 effects models.

255

## 256 **Results**

### 257 *Herbivore damage*

258 In the treatment without pesticides, 20% of leaves on native species (n=10) were damaged on  
259 average, compared to 13% and 7 % on invasive alien species (n= 6) and non-invasive alien  
260 species (n=7), respectively; the proportion of leaves damaged on invasive species was  
261 significantly lower compared to native species, but not to non-invasive species (Figure 2a; Table  
262 2). However, the significant difference was lost when plant biomass and total number of leaves  
263 per plant were accounted for (**Likelihood ratio test**:  $\chi^2=2.37$ , df=2, p=0.306; Table 2). There was  
264 a significant reduction in proportion of leaves damaged as both biomass ( $\chi^2=16.10$ , df=1,  
265 p<0.001) and the number of leaves increased ( $\chi^2=19.34$ , df=1, p<0.001; Table 2).

266 The proportion of leaves damaged on non-pesticide-treated plants was significantly  
267 greater than on pesticide-treated plants across all species, on average (mean increase in  
268 proportion of leaves damaged on non-pesticide treated plants= +0.041, SE= 0.008, t=5.238,  
269 p<0.001). The difference in proportion of leaves damaged between treatments was significantly  
270 smaller for invasive species (+0.0095 ±0.026%) compared to non-invasive alien species (+0.077  
271 ±0.021%;), but only marginally compared to native species (+ 0.056 ±0.026%; Figure 2b, Table  
272 2). When plant-size covariates were included, differences according to status were no longer  
273 significant ( $\chi^2=2.119$ , df=2, p=0.347; Figure 1b, Table 2). The difference in proportion of leaves

274 damaged between non-pesticide plants and pesticide-treated plants decreased significantly with  
275 increasing average biomass ( $\chi^2=7.01$ ,  $df=1$ ,  $p=0.008$ ) and difference in the number of leaves for  
276 each plant pair ( $\chi^2=9.16$ ,  $df=1$ ,  $p=0.003$ ; Table 2).

277 Among non-pesticide-treated plants, aboveground biomass of plants used to survey  
278 herbivory decreased significantly with increasing proportion of leaves damaged (Table S3; Fig  
279 S1). In contrast, aboveground biomass was not significantly related to proportion of leaves  
280 damaged on pesticide-treated plants (Table S3; Fig. S1). In both cases, models with slopes and  
281 intercepts varying according to taxonomic group and species explained significantly more  
282 variation than random intercept models (non-pesticide plants  $\chi^2=40.06$ ,  $df=1$ ,  $p<0.001$ ; pesticide  
283 plants  $\chi^2=34.71$ ,  $df=1$ ,  $p<0.001$ ). This indicated that the effect of proportion of leaves damaged  
284 on biomass was variable among species (see Table S4 for intercepts and slopes per species).

285

### 286 *Herbivore abundance*

287 All eight species of aphid found on the plants were considered generalists (Table S5);  
288 *Aphis frangulae* (Kaltenbach), *Aphis fabae* (Scopoli), *Aulocorhthum solani* (Kaltenbach),  
289 *Macrosiphum euphorbiae* (Thomas), *Myzus persicae* (Sulzer), *Rhopalosiphum nymphaeae* (L.),  
290 *R. padi* (L.) and *Sitobion avenae* (F.). According to Wittenberg et al. (2006), *Myzus persicae* and  
291 *Macrosiphum euphorbiae* are alien to Switzerland. On average, 21 aphids were found per plant  
292 on native plant species ( $n=11$ ) without pesticide treatment, compared with 24 and 21 for invasive  
293 ( $n=7$ ) and non-invasive ( $n=8$ ) species, respectively; these differences were not significant (Table  
294 3). There were significantly more aphids on non-pesticide treated plants than on pesticide-treated  
295 plants, across all species (mean difference in aphid abundance= +22.34, 95% CI= 7.63 – 44.37).

296 However, the difference in aphid abundance between treatments was similar for invasive, non-  
297 invasive and native plants (Table 3).

298 Four species of mollusc were found; *Arion vulgaris* (Moquin-Tandon), *Deroceras*  
299 *reticulatum* (Mueller), *Succinea putris* (L.) and *Xerolenta obvia* (Menke). Wittenberg et al.  
300 (2006) list *Arion vulgaris* as alien to Switzerland. Molluscs and orthopterans were no more likely  
301 to be present on native species, than on invasive or non-invasive alien species, while  
302 thysanopterans were marginally (but not significantly) less likely to occur on native than on  
303 invasive species (Table S6).

#### 305 *Effect of pesticide and nutrient treatments on biomass*

306 There was an overall significant effect of pesticide treatment ( $\chi^2=14.55$ ,  $df=1$ ,  $p<0.001$ ),  
307 with significantly more biomass on average for plants treated with pesticide than compared to  
308 plants not treated with pesticide (mean difference in biomass= 1.092 g, 95% CI= 0.210 – 1.974).  
309 Responses of individual species were variable (Fig. S2). Invasive, non-invasive and native  
310 species did not significantly differ from one another in their responses to pesticide treatment (2-  
311 way interaction: ( $\chi^2=2.97$ ,  $df=2$ ,  $p=0.227$ ; Fig. 3a). The effects of pesticide treatment on biomass  
312 also did not significantly differ according to nutrient treatment (2-way interaction: ( $\chi^2=0.88$ ,  
313  $df=1$ ,  $p=0.348$ ). Species of different status responded differently to nutrient addition (2-way  
314 interaction:  $\chi^2=29.257$ ,  $df=2$ ,  $p<0.001$ ), with a greater relative increase in biomass for native  
315 (n=13) and invasive (n=7) species compared to non-invasive species (n=8), and native compared  
316 to invasive species (Figure 3b; Table 4). The invasive species increased their biomass from 25.44  
317 g to 48.54 g with nutrient addition, compared to 14.57 and 36.34 g for native species, and 17.82

318 and 28.92 g for non-invasive species without and with nutrient addition, respectively. There was  
319 no significant three-way interaction between pesticide treatment, nutrient treatment and species  
320 status affecting plant biomass ( $\chi^2=0.69$ ,  $df=2$ ,  $p=0.708$ ).

321

## 322 Discussion

323 Evidence for the enemy release hypothesis (ERH) involving herbivores would require a  
324 smaller response to experimental herbivore exclusion for invasive alien species than for native  
325 and non-invasive alien species (Keane and Crawley 2002). In our study, invasive species  
326 suffered less foliar herbivory than native, but not than non-invasive species, and there was a  
327 positive effect of pesticide treatment in terms of reduced herbivory for native and non-invasive  
328 species, but not for invasive species, which would partially support the ERH. However, the  
329 differences between invasive and other species were lost after accounting for plant size,  
330 indicating that differences in enemy attack may be confounded by plant size (i.e. larger plants  
331 have a tendency to suffer herbivory on a smaller proportion of their leaves). The change in  
332 response for invasive but not native/non-invasive species to pesticide treatment when accounting  
333 for size might reflect the influence of larger invasive species, such as *Bidens frondosa*, suffering  
334 less herbivory due to their size. Unfortunately, tests of the ERH involving herbivores rarely  
335 consider the effects of variation in plant size among compared species on enemy attack, and the  
336 apparent partial support for the ERH from our results indicate that it should be taken into account  
337 in such studies. In addition, there were no differences among invasive, non-invasive and native  
338 species in herbivore presence and abundance, or in their biomass responses to pesticide  
339 treatment. This is similar to a recent review of the few studies comparing herbivore abundance  
340 and damage in introduced and native ranges of invasive species, showing that plants in the



341 introduced range suffer less herbivory overall, but the magnitude of difference was small (Liu  
342 and Stiling 2006). When using a phylogenetically controlled multi-species approach the enemy  
343 release hypothesis involving invertebrate herbivores does not appear to be a general mechanism  
344 explaining the invasiveness of plant species. We acknowledge, however, that release from other  
345 guilds of natural enemies, such as fungal pathogens and seed predators could potentially still  
346 play a role in the success of the invasive species considered here, although evidence for release  
347 from these types of enemies in general is not conclusive (Parker and Gilbert 2007, van Kleunen  
348 and Fischer 2009, Hill and Kotanen 2011).

349 Measures of herbivory and enemy damage commonly used to test the ERH may not  
350 always translate into plant performance effects. Even though Parker & Gilbert (2007) found that  
351 herbivore damage and pathogen infection were lower on alien compared to native plant species  
352 in North America, this did not translate into greater survival of alien compared to native species,  
353 or of invasive alien compared to non-invasive alien species. Also, a recent study on *Artemisia*  
354 *ambrosiifolia*, in its native range, found that enemy exclusion reduced damage on adult leaves,  
355 but did not result in increased growth or reproduction (MacDonald and Kotanen 2010).  
356 Moreover, a meta-analysis of enemy-release studies that manipulated the presence of natural  
357 enemies found no consistent differences in plant-performance responses to enemy exclusion  
358 between invasive species and native comparators (Chun et al. 2010). The lack of clear evidence  
359 for reduced plant damage resulting in greater plant performance may be a consequence of  
360 differing abilities among plant species to tolerate herbivory. We found that for non-pesticide  
361 treated plants, those suffering herbivory on more leaves had a lower biomass overall, but there  
362 was a significant amount of variation in the relationship according to species. Understanding  
363 how herbivory (and natural enemy impacts in general) actually relate to plant performance is  
364 essential if we want to adequately assess the relevance of enemy release to plant invasions. Other

365 effects of defoliation on plant performance can include reduced over-winter survival and  
366 reproduction (Rose et al. 2009). Nonetheless, our results and others mentioned suggest it is  
367 unlikely that release from herbivory alone can increase growth performance of invasive  
368 compared to native plants.

369 Nutrient addition did not affect the differences in total biomass between pesticide and  
370 non-pesticide treated plants, and also did not affect the differences among native, non-invasive  
371 and invasive species. This result appears to suggest that while plants obviously increased  
372 biomass with nutrient addition, overall, the absolute difference in biomass with enemy exclusion  
373 did not vary greatly, which runs counter to the prediction of the resource-enemy release  
374 hypothesis (Blumenthal, 2006). As we only assessed herbivore damage and herbivores on a  
375 subset of plants that did not include a nutrient-addition treatment, we were not able to verify that  
376 the number of leaves damaged by herbivores or the abundance of herbivores found on nutrient-  
377 treated plants remained the same as on non-nutrient treated plants. Notwithstanding this, while  
378 invasive species are able to increase biomass more than non-invasive alien species under nutrient  
379 addition (in line with theory on fluctuating resources; Davis et al. 2000), our results suggest that  
380 nutrient levels may not mediate enemy release differences between invasive and non-invasive  
381 alien species in terms of performance. Moreover, native species increased biomass significantly  
382 more than invasive species in response to nutrient addition. This could be due to, in part, the  
383 inclusion of common, fast-growing native species, which may perform at least as well as  
384 invasive aliens under higher nutrient levels (Dawson et al. 2012). However, invasive alien species  
385 were also already ~ 1.7 times larger on average than native species without nutrient addition,  
386 which may have limited their potential to increase biomass with nutrient addition.

387 A potential limitation of our study (and garden experiments in general), could be that  
388 plants were not exposed to the whole suite of potential herbivores of the species used, especially  
389 those that would occur belowground. Thus, whether the ERH **in relation to invertebrate**  
390 **herbivores** explains the invasion success of a plant species might not only depend on the species  
391 involved but also the herbivore community present. **We only found species of the two most**  
392 **abundant herbivore groups known to feed on multiple genera and plant families (Table S5). If**  
393 **present, specialist species of the herbivore groups not identified to species level (because they**  
394 **were mostly still larvae) may have played a minor role. However, the meaning of generalism**  
395 **versus specialism is not clearly dichotomous; even among species considered ‘generalist’, there**  
396 **may be some preference shown for certain food plants, and this could be mediated by the choice**  
397 **of plant species available in the community. Notwithstanding this, if invertebrate herbivores**  
398 **show preferences, then such preferences did not result in enemy release differences of invasive**  
399 **compared to native and non-invasive species. This may not be surprising if preferences are**  
400 **expressed at higher (e.g. familial) taxonomic levels; differences in herbivory may be greater**  
401 **among families than according to species status within families. A further potential caveat is that**  
402 **the pesticides used in the experiment were organophosphates. We cannot rule out entirely the**  
403 **possibility that additional phosphorus from the pesticide treatment could have increased plant**  
404 **biomass; however we estimate that only ~ 4 mg of P in total was added to plants receiving**  
405 **pesticide treatment. The soil used was a relatively nutrient-rich agricultural soil, and we therefore**  
406 **think a confounding fertilisation effect of pesticide use is unlikely. However, we cannot rule out**  
407 **other potential non-target effects of pesticides, such as impacts on soil microbiota.**

408

409

## 410 **Conclusions**

411 To the best of our knowledge, this is the first multi-species experiment that assessed  
412 herbivore loads and herbivore damage as well as performance with and without herbivore  
413 suppression of invasive alien, non-invasive alien and native species. We found equivocal support  
414 for enemy release involving invertebrate herbivores as a mechanism explaining invasion success  
415 of alien plant species in our study. The species of herbivores identified were considered  
416 generalists, and plant size was an important variable explaining variation in herbivory in our  
417 multi-species approach. In addition, our study suggests that increased resource availability may  
418 not necessarily increase the extent to which species benefit from enemy release. The degree of  
419 herbivore release experienced by alien plant species under varying resource availability would be  
420 better considered relative to plant size, and under a plant community context with manipulation  
421 of different herbivore guilds.

422

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428 Madalin Parepa and Yvonne Zuercher for help in preparing seedlings, setting up and harvesting  
429 the experiment.

430

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432

433 **Appendices**

434 **Table S1** Sample sizes of pesticide effects per species per nutrient treatment, and per response  
435 variable in the study.

436 **Table S2** Mean (and standard error) abundance of aphids and proportion of leaves damaged per  
437 plant per species, treated either without or with pesticide in the experiment.

438 **Table S3** Parameter estimates (and standard errors in parentheses) for linear mixed models of  
439 aboveground biomass (square root transformed) in relation to proportion of leaves damaged, for  
440 the herbivory set of plants

441 **Table S4** Modelled intercept and slope estimates per species (as random effects), from linear  
442 mixed models assessing the relationship between aboveground biomass and proportion of leaves  
443 damaged for plants without and with pesticide treatment.

444 **Table S5** Information on host plant families, genera and species, distribution and native status of  
445 aphid and mollusc species identified in the study.

446 **Table S6** Parameter estimates (and standard errors in parentheses) for binomial generalised  
447 linear mixed models of mollusc, orthopteran and thysanopteran presence on non-pesticide treated  
448 plants.

449 **Figure S1** Relationship between aboveground biomass and proportion of leaves damaged on  
450 plants without and with pesticide treatment, showing overall fitted relationship (thick line) and  
451 individual fitted lines for the 23 species.

452 **Figure S2** Mean total biomass (square-root scale) of plants with pesticide treatment (circles) and  
453 without pesticide treatment (squares), without nutrient (unfilled symbols) and with nutrient  
454 addition (filled symbols). Error bars represent  $\pm 1$  SE.

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585

586 Figure 1. Schematic plan of the experimental set-up. Each of the eight blocks was split in half,  
587 and one half was randomly assigned the pesticide application treatment. Each half-block  
588 contained seven sub-blocks, with one of the seven taxonomic groups randomly assigned to each  
589 of them. Each sub-block contained plants belonging to the designated taxonomic group. The  
590 numbers shown in each sub-block represent the total number of plants (across species) per sub-  
591 block without nutrients added/with nutrients added/used for assessing herbivory levels. As one  
592 plant per treatment per species is in each sub-block, the numbers also represent the number of  
593 species, which varies among the taxonomic groups.

594

595 Figure 2. a) Mean proportion of leaves damaged on pesticide-treated plants and b) mean  
596 differences in proportion of leaves damaged on pesticide versus non-pesticide treated plants, for  
597 native, invasive alien and non-invasive alien plant species. Error bars represent  $\pm 1$  standard  
598 error. In both a) and b), means are shown from models excluding and including plant-size  
599 covariates. The dashed line in b) signifies zero difference in proportion of leaves damaged  
600 between non-pesticide and pesticide-treated plants.

601

602 Figure 3. a) Mean difference in square-root transformed total biomass between pesticide and  
603 non-pesticide treated plants, for native, invasive alien and non-invasive alien species without (-)  
604 and with (+) nutrient addition. The dashed line signifies zero difference in biomass. b) Mean  
605 difference in square-root transformed total biomass between nutrient-treated and non-nutrient  
606 treated plants, for native, invasive alien and non-invasive alien species (averaged across  
607 pesticide-treatments). Error bars in a) and b) represent  $\pm 1$  standard error. For reference, numbers

608 above the x axis represent mean biomass without pesticide treatment in a), and without nutrient  
609 addition in b).

610

For Review Only

611 Table 1. The 29 species used in this study, their status, and the corresponding code per species  
 612 shown in the figures. Numbers in parentheses for Asteraceae species are **sub-block** groupings for  
 613 this family. \*Species used in leaf damage analyses. † Species used in analysis of herbivore  
 614 presence/abundance. The Phrymaceae, Plantaginaceae and Scrophulariaceae species were  
 615 considered as one taxonomic group

616

Species	Family	Status
<i>Bidens radiata</i> *†	Asteraceae ( <i>Bidens</i> )	Native
<i>Bidens tripartita</i> *†	Asteraceae ( <i>Bidens</i> )	Native
<i>Bidens bipinnata</i> *†	Asteraceae ( <i>Bidens</i> )	Alien non-invasive
<i>Bidens frondosa</i> *†	Asteraceae ( <i>Bidens</i> )	Alien invasive
<i>Artemisia borealis</i> *†	Asteraceae (1)	Native
<i>Artemisia vulgaris</i>	Asteraceae (1)	Native
<i>Cirsium montanum</i> *†	Asteraceae (2)	Native
<i>Gnaphalium luteo-album</i>	Asteraceae (1)	Native
<i>Inula helvetica</i>	Asteraceae (2)	Native
<i>Solidago virgaurea</i> *†	Asteraceae (2)	Native
<i>Aster lanceolatus</i> *†	Asteraceae (2)	Alien non-invasive
<i>Rudbeckia hirta</i> *†	Asteraceae (1)	Alien non-invasive
<i>Conyza canadensis</i> *†	Asteraceae (2)	Alien invasive
<i>Senecio inaequidens</i> *†	Asteraceae (1)	Alien invasive
<i>Solidago canadensis</i> *†	Asteraceae (2)	Alien invasive
<i>Oenanthe lachenalii</i> *†	Apiaceae	Native
<i>Eryngium giganteum</i> *†	Apiaceae	Alien non-invasive
<i>Heracleum mantegazzianum</i> *†	Apiaceae	Alien invasive
<i>Veronica anagallis-aquatica</i> *†	Plantaginaceae	Native
<i>Veronica peregrina</i> *†	Plantaginaceae	Alien non-invasive
<i>Veronica persica</i> *†	Plantaginaceae	Alien invasive
<i>Brachypodium sylvaticum</i> †	Poaceae	Native
<i>Eleusine indica</i> †	Poaceae	Alien non-invasive
<i>Panicum capillare</i> †	Poaceae	Alien invasive
<i>Rumex maritimus</i> *†	Polygonaceae	Native
<i>Rumex obtusifolius</i> *†	Polygonaceae	Native
<i>Persicaria orientalis</i> *†	Polygonaceae	Alien non-invasive
<i>Scrophularia nodosa</i> *†	Scrophulariaceae	Native
<i>Mimulus guttatus</i> *†	Phrymaceae	Alien non-invasive

617 Table 2. Parameter estimates (and standard errors in parentheses) from models of  
 618 differences in proportion of leaves damaged between pesticide and non-pesticide treated  
 619 plants, and the proportion of leaves damaged on non-pesticide treated plants only.  
 620 Models were analysed with and without covariates of plant size, and with species status  
 621 as fixed effects. Values given for random effects are standard deviations. Significant  
 622 estimates ( $P < 0.05$ ) are shown in bold.

Variable	Proportion of leaves damaged on non-pesticide plants	
	Excluding covariates	Including covariates
<i>sqrt (biomass)</i>		<b>-0.628 (0.141)</b>
<i>ln (number of leaves)</i>		<b>-0.492 (0.104)</b>
<i>Intercept</i>	<b>-2.623 (0.432)</b>	<b>-2.198 (0.399)</b>
<i>Non-invasive</i>	0.738 (0.476)	0.419 (0.472)
<i>Native</i>	<b>1.240 (0.443)</b>	0.707 (0.440)
Random effects		
<i>Block</i>	0.445	0.382
<i>Family</i>	0.168	0.267
<i>Species</i>	0.592	0.760
	Difference in proportion of leaves damaged	
	Excluding covariates	Including covariates
<i>ln (biomass)</i>		<b>-0.030 (0.012)</b>
<i>Difference in number of leaves</i>		<b>-0.021 (0.001)</b>
<i>Intercept</i>	0.001 (0.026)	<b>0.036 (0.016)</b>
<i>Non-invasive</i>	<b>0.077 (0.021)</b>	0.023 (0.026)
<i>Native</i>	0.056 (0.026)	0.038 (0.021)
Random effects		
<i>Block</i>	$2.97 \times 10^{-9}$	$4.97 \times 10^{-5}$
<i>Family</i>	0.033	0.014
<i>Species</i>	$2.24 \times 10^{-6}$	$3.096 \times 10^{-8}$
<i>Residual</i>	0.201	0.176

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630 Table 3. Parameter estimates (and standard errors in parentheses) from models of differences in  
 631 aphid abundance between pesticide and non-pesticide treated plants, and the number of aphids  
 632 on non-pesticide treated plants only. Fixed effects included species status. Values given for  
 633 random effects are standard deviations. Significant estimates ( $P < 0.05$ ) are shown in bold.

Variable	Aphid abundance on non-pesticide plant [ $\ln(x+1)$ transformed]	Difference in Aphid abundance [ $\ln(x+22)$ -transformed]
<i>Intercept</i>	<b>3.820 (0.227)</b>	<b>3.129 (0.618)</b>
<i>Native</i>	-0.054 (0.138)	0.341 (0.696)
<i>Non-invasive</i>	0.002 (0.155)	0.463 (0.754)
Random effects		
<i>Block</i>	$6.596 \times 10^{-6}$	$8.099 \times 10^{-6}$
<i>Family</i>	0.511	0.586
<i>Species</i>	0.209	1.252
<i>Residual</i>	1.562	1.170

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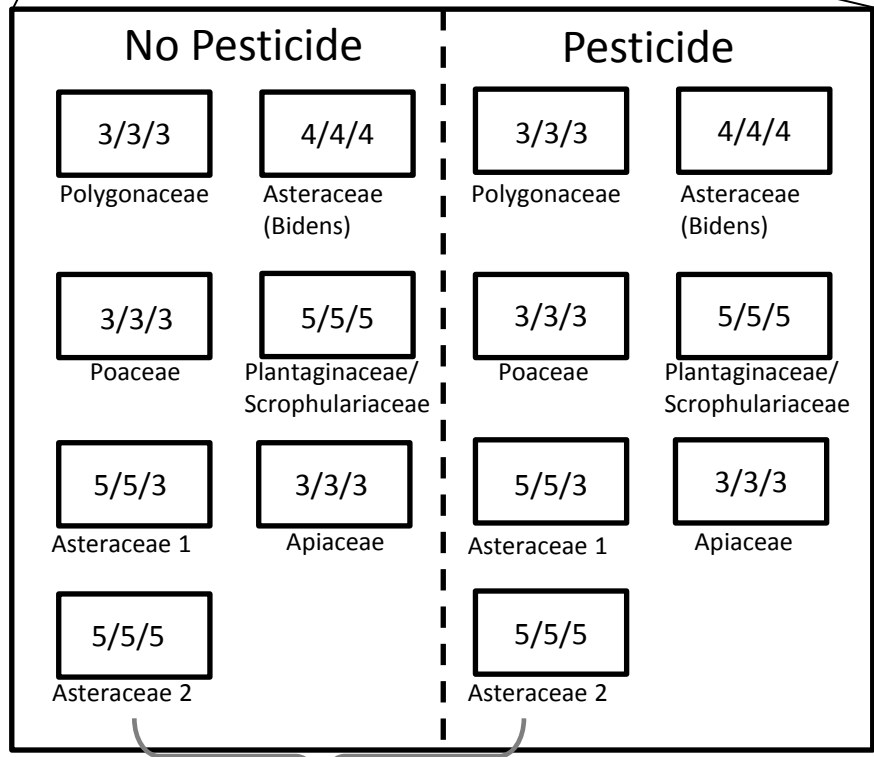
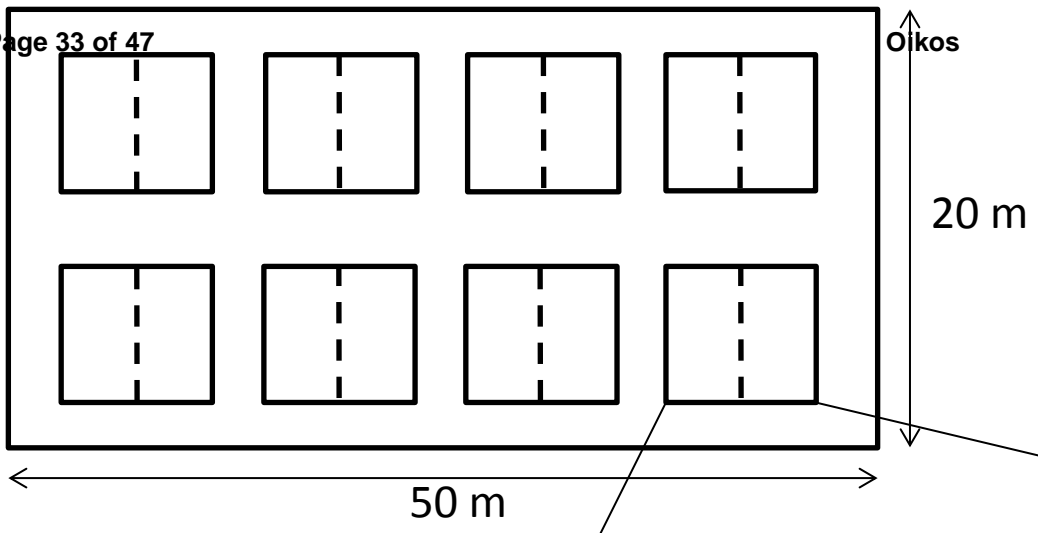
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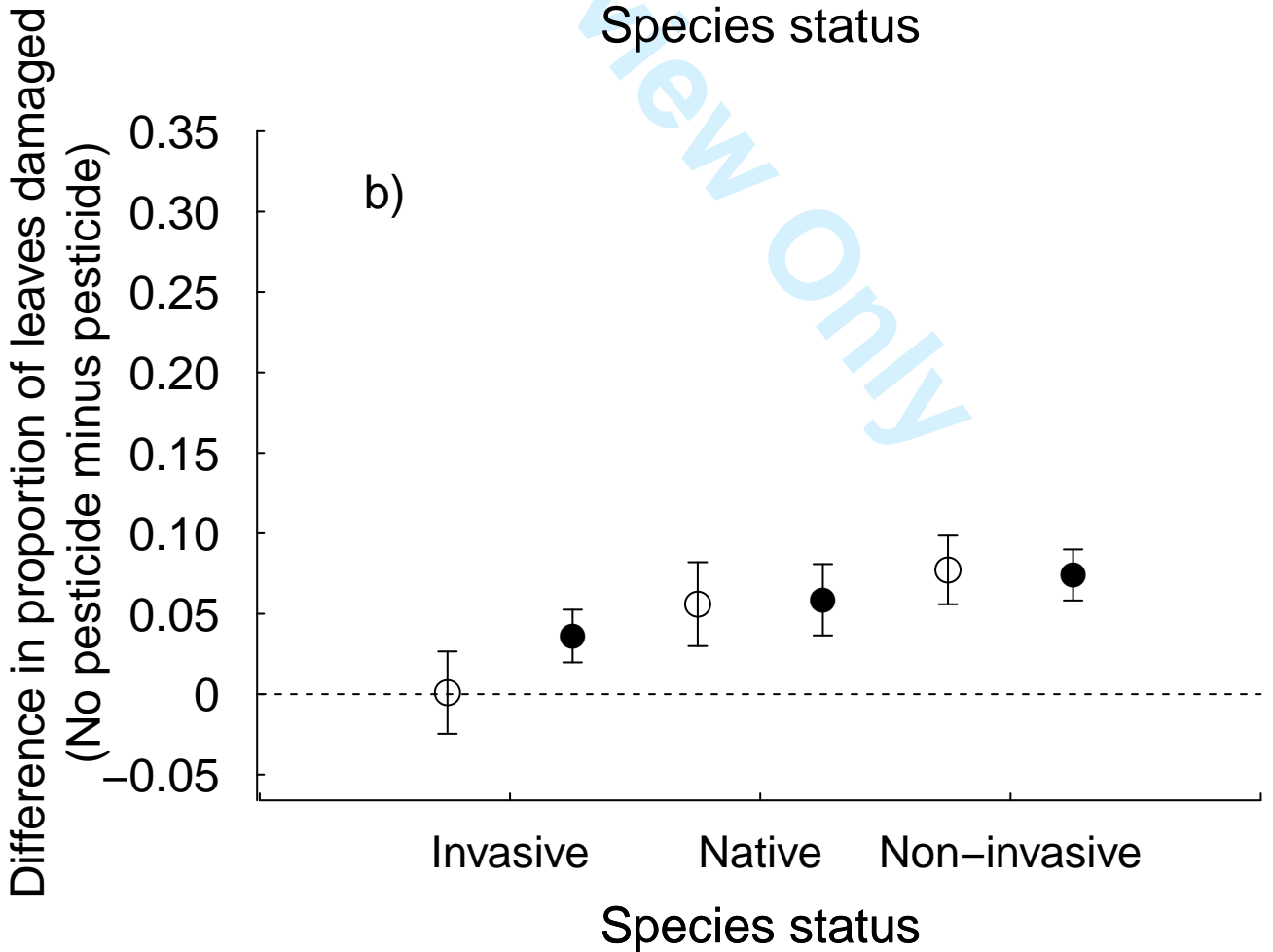
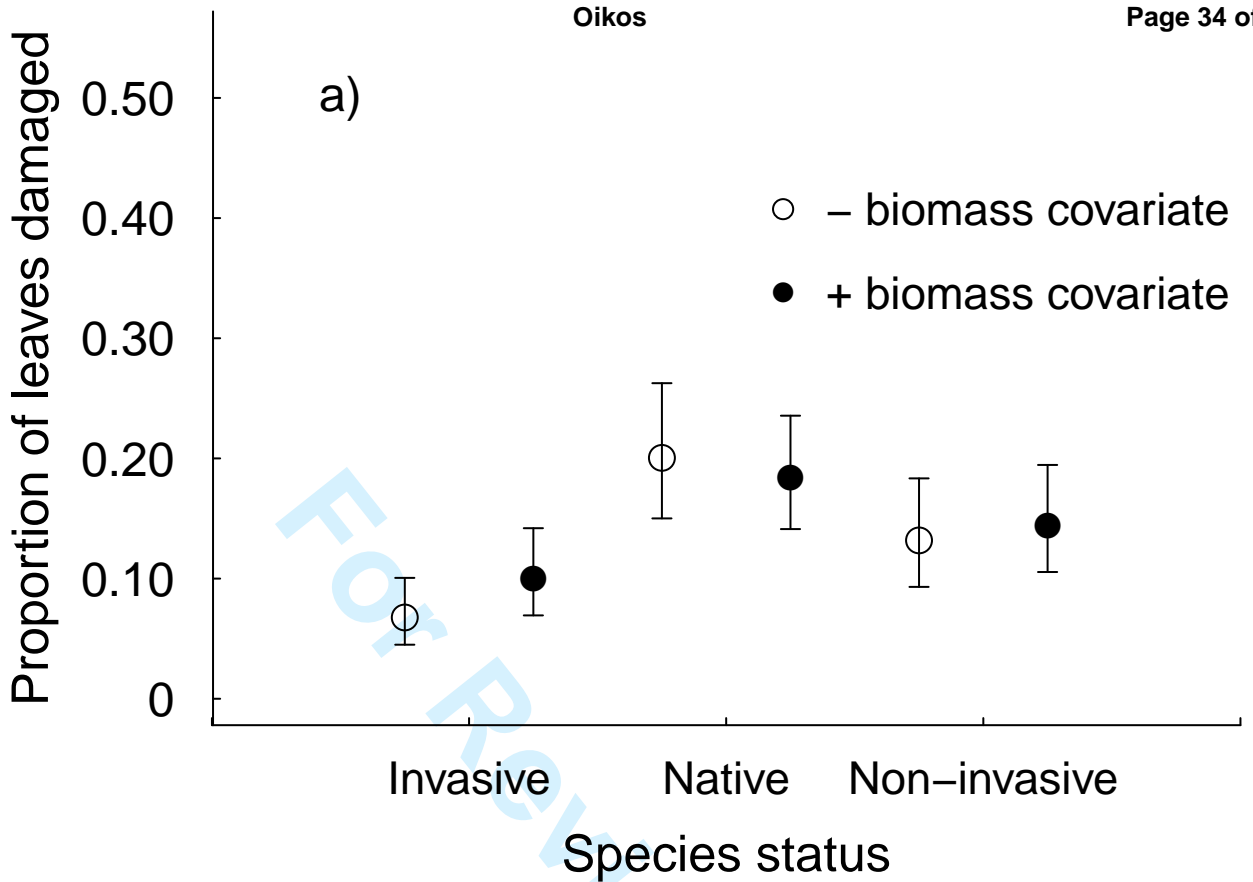
643 Table 4. Parameter estimates (and standard errors in parentheses) from full and minimum models  
 644 explaining total biomass of native, non-invasive and invasive plant species, under pesticide and  
 645 non-pesticide treatments, and with or without nutrient addition. Values given for random effects  
 646 are standard deviations. Significant estimates ( $P < 0.05$ ) are shown in bold.

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Variable	Model	
	Full	Minimum adequate
Intercept	<b>5.479 (0.965)</b>	<b>5.412 (0.963)</b>
Native	-1.757 (0.918)	-1.722 (0.912)
Non-invasive	-1.183 (1.010)	-1.106 (1.004)
Nutrient addition	<b>1.871 (0.158)</b>	<b>1.911 (0.111)</b>
Pesticide absent	<b>-0.364 (0.157)</b>	<b>-0.231 (0.059)</b>
Native : Nutrient addition	0.385 (0.209)	<b>0.352 (0.147)</b>
Non-invasive : Nutrient addition	<b>-0.839 (0.211)</b>	<b>-0.758 (0.150)</b>
Native : Pesticide absent	0.068 (0.207)	
Non-invasive : Pesticide absent	0.152 (0.209)	
Nutrient addition : Pesticide absent	0.075 (0.223)	
Native : Nutrient addition : Pesticide absent	-0.058 (0.295)	
Non-invasive : Nutrient addition : Pesticide absent	0.165 (0.298)	
Random effects		
Block	$1.727 \times 10^{-17}$	0.0008
Family	1.372	1.372
Species	1.884	1.884
Residual	0.911	0.914



Plants paired according to species and treatment



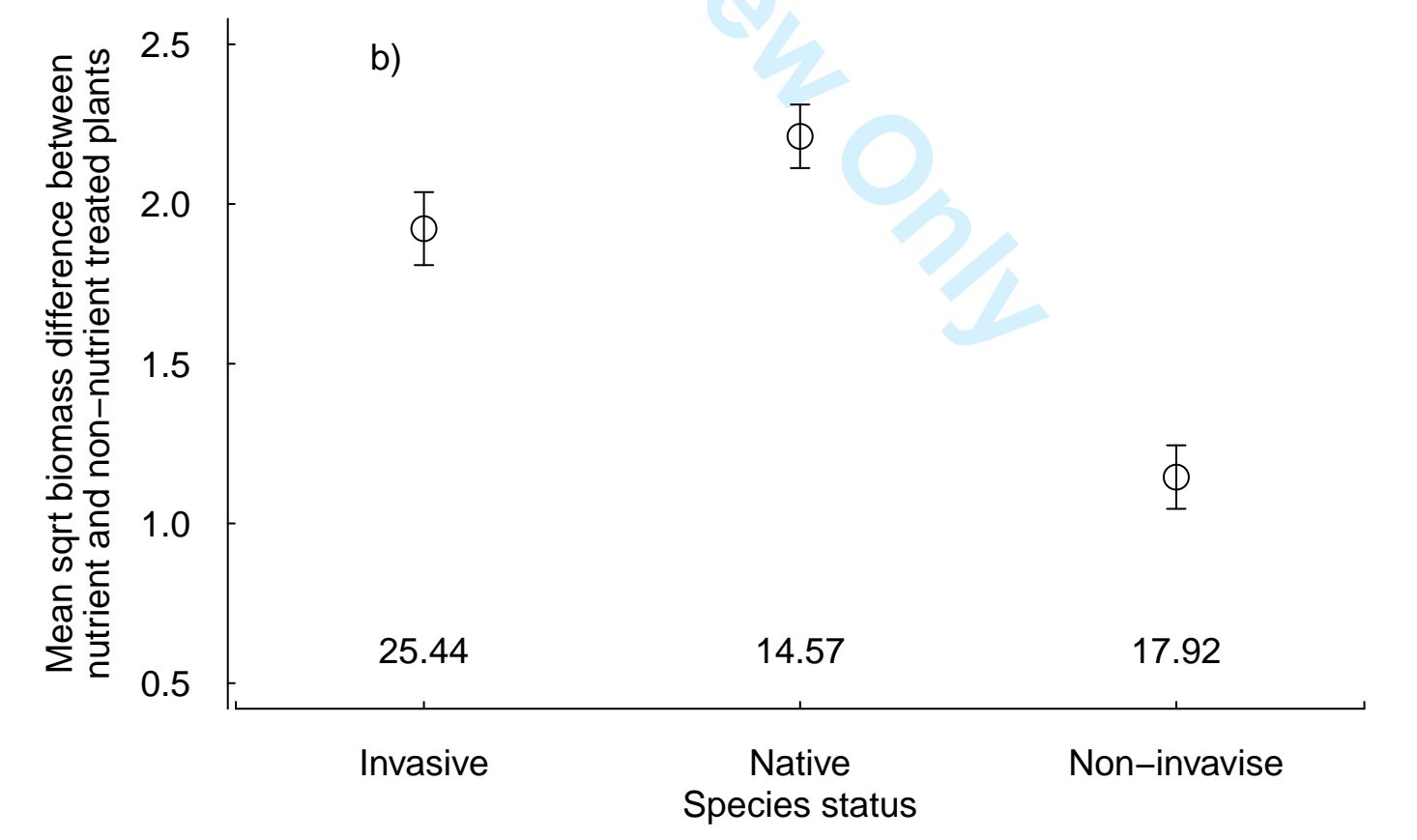
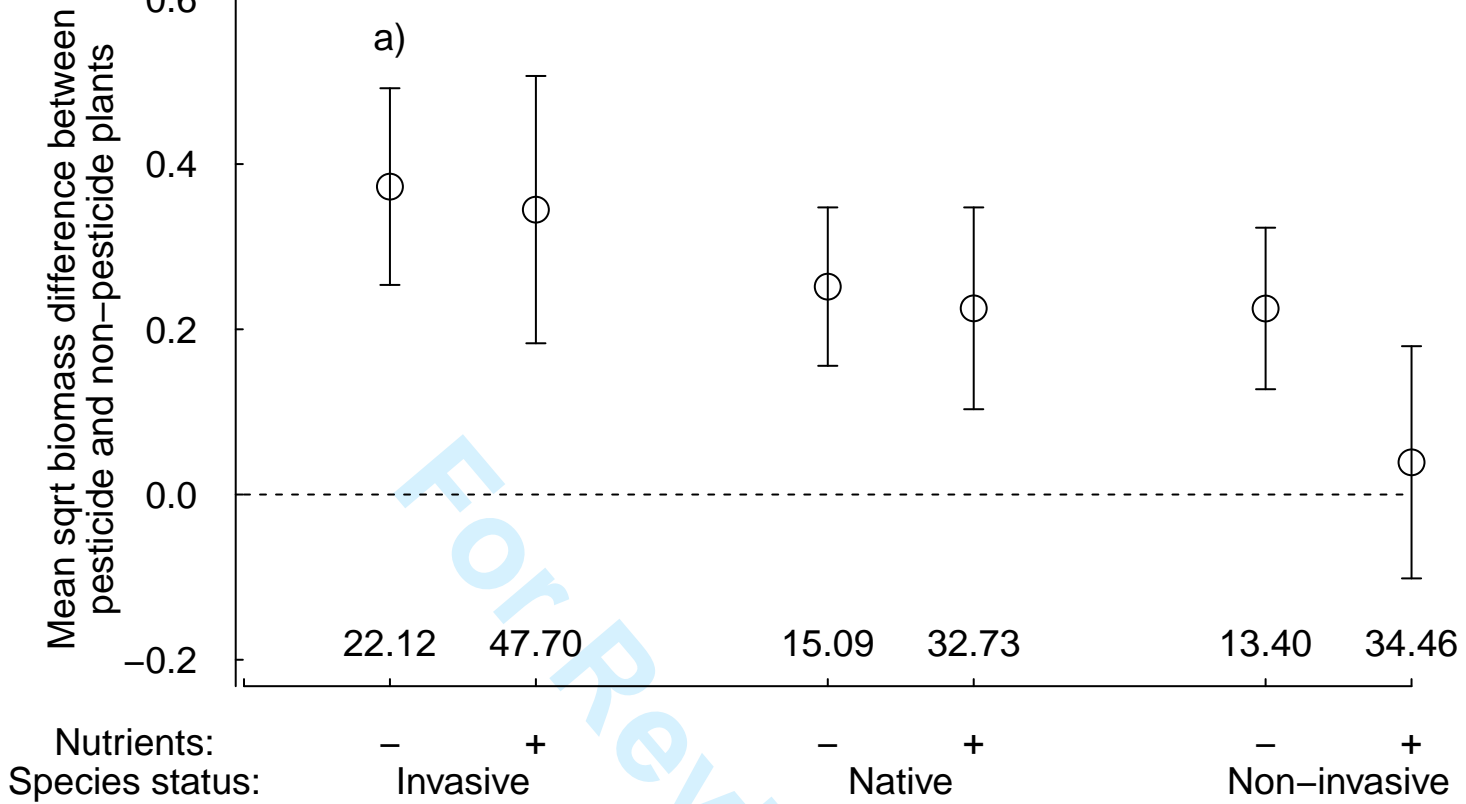


Table S1. The 29 species used in this study, their status, the number of 5km x 5km grid cells in Switzerland occupied, and first date alien species were recorded as naturalised in Central Europe (Klotz et al. 2002). Numbers in parentheses for Asteraceae species are sub-subplot groupings for this family. ‡From Hegi (1954). Habitat codes follow Landolt et al (2010): 1- Water bodies, banks and ditches; 2- Eutrophic terrestrial vegetation; 3- Outcrops, screes, sandy/gravel habitats; 4- Water sources and streams; 5- Mires; 6- Grasslands and meadows; 7-Dwarf shrub and tall herb communities; 8- Shrubland.

Species	Status	Characteristics			Biomass		Herbivory		
		Number of grid cells	Habitats	Year of establishment	No Nutrient	Nutrient	Leaves damaged	Difference in leaves damaged	Invertebrates
<i>Artemisia borealis</i>	Native	6	3,7		3	4	8	8	8
<i>Artemisia vulgaris</i>	Native	253	2,7		8	8	—	—	—
<i>Aster lanceolatus</i>	Non-invasive	17	2,7	1850	8	7	7	7	8
<i>Bidens bipinnata</i>	Non-invasive	4	2,3,7	1754 ‡	8	8	7	7	8
<i>Bidens frondosa</i>	Invasive	45	1,2,9	1736	8	8	7	7	8
<i>Bidens radiata</i>	Native	3	1,2		3	3	3	3	5
<i>Bidens tripartita</i>	Native	57	1,2		7	6	6	6	8
<i>Brachypodium sylvaticum</i>	Native	494	7,9		7	8	—	—	8
<i>Cirsium montanum</i>	Native	1	5,7,8,9		6	4	4	4	7
<i>Conyza canadensis</i>	Invasive	140	2	1646-1880	7	7	8	8	8
<i>Eleusine indica</i>	Non-invasive	21	2	1900	8	8	—	—	8
<i>Eryngium giganteum</i>	Non-invasive	4	2	Unknown	6	6	8	8	8
<i>Gnaphalium luteo-album</i>	Native	29	1,2		7	7	—	—	—
<i>Heracleum mantegazzianum</i>	Invasive	420	2,7	1890	8	8	8	8	8
<i>Inula helvetica</i>	Native	40	2,5,7,9		6	6	—	—	—
<i>Mimulus guttatus</i>	Non-invasive	23	1,2,4	1824	8	8	8	8	8
<i>Oenanthe lachenalii</i>	Native	8	1,6		6	5	8	8	8
<i>Panicum capillare</i>	Invasive	136	2	1867	8	8	—	—	8
<i>Persicaria orientalis</i>	Non-invasive	4	2,7	Unknown	4	4	4	4	4
<i>Rudbeckia hirta</i>	Non-invasive	27	2,7	1860	5	5	5	5	8
<i>Rumex maritimus</i>	Native	1	2		8	8	8	8	8
<i>Rumex obtusifolius</i>	Native	602	2,6		8	8	8	8	8
<i>Scrophularia nodosa</i>	Native	358	2,9		8	7	8	8	8
<i>Senecio inaequidens</i>	Invasive	121	2,3	1889	8	7	8	8	8
<i>Solidago canadensis</i>	Invasive	491	1,2	1736	8	8	7	7	8
<i>Solidago virgaurea</i>	Native	207	7,9		—	—	8	8	8
<i>Veronica anagallis-aquatica</i>	Native	131	1,2,4		8	8	8	8	8
<i>Veronica peregrina</i>	Non-invasive	57	2	1760	8	7	8	8	8
<i>Veronica persica</i>	Invasive	458	2	1805	7	7	8	8	8

Table S2. Mean (and standard error) abundance of aphids and proportion of leaves damaged per plant per species, treated either without or with pesticide in the experiment. \*- Species treated as 'Scrophulariaceae' taxonomic group; however *Mimulus guttatus* is in the Phrymaceae, and *Veronica* spp. are in the Plantaginaceae.

Species	Family	Status	Aphid abundance without pesticide	Aphid abundance with pesticide	Proportion of leaves damaged without pesticide	Proportion of leaves damaged with pesticide
<i>Artemisia borealis</i>	Asteraceae	Native	14.00 (5.73)	1.88 (0.95)	0.180 (0.040)	0.065 (0.059)
<i>Aster lanceolatus</i>	Asteraceae	Non-invasive	31.43 (8.75)	9.29 (3.00)	0.280 (0.051)	0.125 (0.061)
<i>Bidens bipinnata</i>	Asteraceae (Bidens)	Non-invasive	61.71 (14.71)	18.43 (3.82)	0.053 (0.012)	0.012 (0.029)
<i>Bidens frondosa</i>	Asteraceae (Bidens)	Invasive	1.29 (0.29)	1.71 (0.29)	0.024 (0.048)	0.070 (0.024)
<i>Bidens radiata</i>	Asteraceae (Bidens)	Native	178.00 (57.46)	60.33 (16.05)	0.178 (0.058)	0.106 (0.178)
<i>Bidens tripartita</i>	Asteraceae (Bidens)	Native	2.33 (0.21)	2.00 (0.37)	0.015 (0.032)	0.046 (0.015)
<i>Brachypodium sylvaticum</i>	Poaceae	Native	1.63 (0.84)	0.38 (0.26)	0.000 (0.000)	0.000 (0.000)
<i>Cirsium montanum</i>	Asteraceae	Native	73.25 (28.30)	7.75 (3.52)	0.544 (0.096)	0.302 (0.096)
<i>Conyza canadensis</i>	Asteraceae	Invasive	43.13 (15.17)	4.50 (1.27)	0.062 (0.013)	0.027 (0.023)
<i>Eleusine indica</i>	Poaceae	Non-invasive	6.50 (0.82)	0.75 (0.41)	0.000 (0.000)	0.000 (0.000)
<i>Eryngium giganteum</i>	Apiaceae	Non-invasive	17.50 (7.64)	1.88 (0.95)	0.155 (0.055)	0.102 (0.080)
<i>Heracleum mantegazzianum</i>	Apiaceae	Invasive	116.38 (15.10)	41.63 (13.13)	0.213 (0.055)	0.106 (0.088)
<i>Mimulus guttatus</i>	Scrophulariaceae*	Non-invasive	75.00 (12.89)	20.75 (8.42)	0.189 (0.036)	0.124 (0.059)
<i>Oenanthelachenalii</i>	Apiaceae	Native	42.75 (15.85)	6.50 (2.11)	0.344 (0.066)	0.147 (0.119)
<i>Panicum capillare</i>	Poaceae	Invasive	4.00 (0.80)	1.25 (0.41)	0.000 (0.000)	0.000 (0.000)
<i>Polygonum orientale</i>	Polygonaceae	Non-invasive	80.75 (31.47)	33.50 (12.98)	0.346 (0.08)	0.159 (0.028)
<i>Rudbeckia hirta</i>	Asteraceae	Non-invasive	36.40 (16.77)	6.40 (5.42)	0.212 (0.031)	0.118 (0.059)
<i>Rumex maritimus</i>	Polygonaceae	Native	228.50 (82.89)	66.75 (19.49)	0.202 (0.059)	0.235 (0.050)
<i>Rumex obtusifolius</i>	Polygonaceae	Native	216.88 (30.90)	67.75 (10.12)	0.257 (0.063)	0.153 (0.087)
<i>Scrophularia nodosa</i>	Scrophulariaceae*	Native	51.13 (12.53)	14.75 (3.26)	0.108 (0.050)	0.063 (0.037)
<i>Senecio inaequidens</i>	Asteraceae	Invasive	11.88 (5.34)	3.13 (1.32)	0.063 (0.028)	0.034 (0.035)
<i>Solidago canadensis</i>	Asteraceae	Invasive	26.57 (8.78)	1.57 (0.87)	0.057 (0.011)	0.017 (0.015)
<i>Solidago virgaurea</i>	Asteraceae	Native	1.88 (1.60)	0.38 (0.18)	0.418 (0.033)	0.081 (0.112)
<i>Veronica anagallis-aquatica</i>	Scrophulariaceae*	Native	91.50 (19.77)	7.63 (1.08)	0.249 (0.089)	0.184 (0.045)
<i>Veronica peregrina</i>	Scrophulariaceae*	Non-invasive	85.38 (10.40)	14.25 (3.80)	0.063 (0.008)	0.011 (0.022)
<i>Veronica persica</i>	Scrophulariaceae*	Invasive	182.50 (64.06)	38.50 (19.18)	0.193 (0.060)	0.141 (0.068)

Table S3. Parameter estimates (and standard errors in parentheses) for linear mixed models of aboveground biomass (square root transformed) in relation to proportion of leaves damaged, for the herbivory set of plants that were either a) not treated with pesticide, or b) had pesticide treatment. Significant estimates ( $P < 0.05$ ) are shown. Values for random effects represent standard deviations.

	<b>Variable</b>	<b>a) without pesticide</b>	<b>b) with pesticide</b>
<b>Fixed Effects</b>	<i>Intercept</i>	<b>2.760 (0.449)</b>	<b>2.930 (0.499)</b>
	<i>Proportion of leaves damaged</i>	<b>-0.892 (0.294)</b>	0.450 (0.998)
<b>Random Intercepts</b>	<i>Family</i>	0.868	0.966
	<i>Species</i>	1.000	1.126
<b>Random Slopes</b>	<i>Family</i>	0.209	1.906
	<i>Species</i>	0.825	1.319
	<i>Residual</i>	0.291	0.365

Table S4. Modelled intercept and slope estimates per species (as random effects), from linear mixed models assessing the relationship between aboveground biomass and proportion of leaves damaged for plants without and with pesticide treatment. Note that the intercepts and slopes were calculated from taxonomic group and species-level random effects.

Family	Species	Without pesticide		With pesticide	
		Intercept	Slope	Intercept	Slope
Apiaceae	<i>Eryngium giganteum</i>	0.609	-0.143	0.898	-0.801
Apiaceae	<i>Heracleum mantegazzianum</i>	1.660	-0.451	3.071	-3.572
Apiaceae	<i>Oenanthe lachenalii</i>	2.690	-2.588	4.377	2.588
Asteraceae	<i>Artemisia borealis</i>	1.660	-1.439	6.339	4.143
Asteraceae	<i>Aster lanceolatus</i>	3.266	-1.504	3.218	1.931
Asteraceae	<i>Bidens bipinnata</i>	2.732	-1.341	3.485	2.509
Asteraceae	<i>Bidens frondosa</i>	4.033	-1.713	1.811	-0.992
Asteraceae	<i>Bidens radiata</i>	1.440	-0.858	2.156	-2.140
Asteraceae	<i>Bidens tripartita</i>	1.881	-1.010	0.692	-0.657
Asteraceae	<i>Cirsium montanum</i>	3.643	-0.540	1.932	-0.021
Asteraceae	<i>Conyza canadensis</i>	3.758	-0.825	3.371	-0.046
Asteraceae	<i>Rudbeckia hirta</i>	3.076	0.087	2.522	-1.517
Asteraceae	<i>Senecio inaequidens</i>	4.585	-1.236	5.815	2.459
Asteraceae	<i>Solidago canadensis</i>	3.658	-0.550	1.820	-1.753
Asteraceae	<i>Solidago virgaurea</i>	2.691	-0.083	3.551	1.584
Polygonaceae	<i>Polygonum orientale</i>	4.990	-0.992	1.727	1.699
Polygonaceae	<i>Rumex maritimus</i>	3.360	-0.528	2.967	0.264
Polygonaceae	<i>Rumex obtusifolius</i>	1.710	-0.019	3.069	-1.425
Scrophulariaceae	<i>Mimulus guttatus</i>	3.207	-0.989	2.025	-1.299
Scrophulariaceae	<i>Scrophularia nodosa</i>	2.772	-1.022	0.999	-1.044
Scrophulariaceae	<i>Veronica anagallis-aquatica</i>	3.173	-1.086	3.206	0.323
Scrophulariaceae	<i>Veronica peregrina</i>	1.268	-1.021	1.620	0.050
Scrophulariaceae	<i>Veronica persica</i>	2.443	-0.468	2.642	0.020



Table S5. The identified aphid and mollusc species in the study, with details of main host plant species, other families, genera and species used as host plants, other food types, presence in Switzerland, distribution and alien status in Switzerland. Sources are as follows- 1: Lampel, G. & Meier, W. (2007) Hemiptera Sternorrhyncha - Aphidina. Centre Suisse de Cartographie de la Faune, Neuchâtel. 2: Boschi, C. (2011) Die Schneckenfauna der Schweiz, Haupt Verlag. 3: Frömming, E. (1954) Biologie der mitteleuropäischen Landgastropoden. Duncker & Humblot, Berlin. 4: Turner, H. (1998) Atlas der Mollusken der Schweiz und Liechtensteins. Centre Suisse de Cartographie de la Faune, Neuchâtel. 5: Wittenberg, R. & Schweiz Bundesamt Für Umwelt. (2006) Invasive alien species in Switzerland an inventory of alien species and their threat to biodiversity and economy in Switzerland. Federal Office for the Environment FOEN, Bern.

Species	Main host <sup>1</sup>	Other host families <sup>1</sup>	Host genera <sup>1</sup>	Host species <sup>1,2,3</sup>	Records in Switzerland <sup>1,4</sup>	Distribution <sup>1,4</sup>	Alien? <sup>5</sup>
<i>Aphis frangulae</i> (Kaltenbach)	<i>Frangula alnus</i>	Brassicaceae, Lamiaceae, Onagraceae, Convolvulaceae, Lythraceae, Plantaginaceae, Rhamnaceae, Solanaceae	several, for example <i>Capsella</i> , <i>Epilobium</i> , <i>Galeopsis</i> , <i>Ipomaea</i> , <i>Lamium</i> , <i>Lysimachia</i> , <i>Veronica</i>	<i>Solanum tuberosum</i>	13	palearctic, N-American	
<i>Aulocorthum solani</i> (Kaltenbach)		Extremely polyphagous, but no grasses; certain strains mainly on <i>Solanum tuberosum</i> ; Asteraceae, Euphorbiaceae, Rosaceae		<i>Cichorium endivia</i> , <i>Cirsium acaule</i> , <i>Euphorbia exigua</i> , <i>E.stricta</i> , <i>Potentilla grandiflora</i> , <i>Sanguisorba officinalis</i>	16	cosmopolitan, probably originally European	
<i>Aphis fabae</i> (Scopoli)	<i>Euonymus europaea</i>	Celastraceae, Fabaceae, Chenopodiaceae		<i>Vicia faba</i> , <i>Phaseolus spp.</i> , <i>Chenopodium spp.</i> , <i>Beta vulgaris</i>	7	Eurasian, N-American	
<i>Macrosiphum euphorbiae</i>		Extremely polyphagous, important families are Asteraceae and Solanaceae		<i>Solanum tuberosum</i> , <i>Beta vulgaris</i> ,	28	cosmopolitan, nearctic origin	yes, origin N-

(Thomas)				<i>Lactuca sativa</i>			America
<i>Rhopalosiphum nymphaeae</i> (L.)	<i>Prunus sp.</i>	Acoraceae, Alismataceae, Butomaceae, Araceae, Callitrichaceae, Hydrocharitaceae, Juncaceae, Menyanthaceae, Haloragaceae, Nymphaeaceae, Poaceae, Polygonaceae, Potamogetonaceae, Ranunculaceae, Cyperaceae, Sparganiaceae, Typhaceae, Lythraceae	several, <i>Acorus</i> , <i>Alisma</i> , <i>Butomus</i> , <i>Calla</i> , <i>Callitriche</i> , <i>Echinodorus</i> , <i>Elodea</i> , <i>Glyceria</i> , <i>Hippuris</i> , <i>Hydrocharis</i> , <i>Juncus</i> , <i>Lemna</i> , <i>Menyanthes</i> , <i>Myriophyllum</i> , <i>Nelumbo</i> , <i>Nuphar</i> , <i>Nymphaea</i> , <i>Nymphoides</i> , <i>Pistia</i> , <i>Polygonum</i> , <i>Potamogeton</i> , <i>Ranunculus</i> , <i>Sagittaria</i> , <i>Schoenoplectus</i> , <i>Scirpus</i> , <i>Sparganium</i> , <i>Stratiotes</i> , <i>Trapa</i> , <i>Typha</i> , <i>Wedelis</i> , etc.		12	cosmopolitan	
<i>Myzus persicae</i> (Sulzer)	<i>Prunus sp.</i>	In CH: Amaranthaceae, Apiaceae, Asteraceae, Boraginaceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, Convolvulaceae, Cucurbitaceae, Malvaceae, Orobanchaceae, Poaceae, Polygonaceae, Rosaceae, Rubiaceae, Scrophulariaceae, Solanaceae, Tamaricaceae, Violaceae			29	cosmopolitan	yes, probably from Asia
<i>R. padi</i> (L.)	<i>Prunus sp.</i>	Rosaceae. Also possible on: Cyperaceae, Juncaceae, Typhaceae, Iridaceae, Poaceae, Brassicaceae	several Poaceae, including <i>Agropyron</i> ,	<i>Capsella bursa-pastoris</i>	28	cosmopolitan	

			<i>Agrostis, Arrhenatherum, Avena, Bromus, Calamagrostis, Cynosurus, Dactylis, Deschampsia, Festuca, Glyceria, Helictotrichon, Holcus, Hordelymus, Hordeum, Melica, Phalaris, Phleum, Poa, Triticum, Zea, etc.</i>			
<i>Sitobion avenae</i> (F.)		several, but often on Poaceae, Amaranthaceae, Asparagaceae	<i>Amaranthus, Asparagus, Avena, Calamagrostis, Glyceria, Hordeum, Lagurus, Lolium, Molinia, Phalaris, Poa, Secale, Setaria, Triticum, Zea</i>	36	cosmopolitan, probably origin westpalaeartic	
<i>Arion vulgaris</i> (Moquin-Tandon)		higher plants but also faeces and carcasses			abundant, pest	yes, origin unknown
<i>Deroceras reticulatum</i> (Mueller)		higher plants, prefers fresh plants; eats also fungi and carcasses			abundant, pest	
<i>Succinea putris</i> (L.)		higher plants, in particular herbs; algae			abundant	
<i>Xerolenta obvia</i>		higher plants, several herbs			abundant	

(Menke)							
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Table S6. Parameter estimates (and standard errors in parentheses) for binomial generalised linear mixed models of mollusc, orthopteran and thysanopteran presence on non-pesticide treated plants. Results are shown for models including all species (n= 26), and only the nitrophilous species (n=21). Values for random effects represent standard deviations. Significant estimates ( $P < 0.05$ ) are shown in bold.

	<b>Variable</b>	<b>Mollusc</b>	<b>Orthopteran</b>	<b>Thysanopteran</b>
<b>Fixed Effects</b>	<i>Intercept</i>	<b>-1.782 (0.556)</b>	<b>-1.744 (0.590)</b>	-0.255 (0.794)
	<i>Native</i>	0.217 (0.647)	-0.797 (0.620)	-1.226 (0.697)
	<i>Non-invasive</i>	0.291 (0.688)	-0.505 (0.627)	-0.474 (0.741)
<b>Random effects</b>	<i>Block</i>	0.615	0.311	<0.0001
	<i>Family</i>	0.018	0.943	1.495
	<i>Species</i>	0.915	0.401	0.976

**References:**

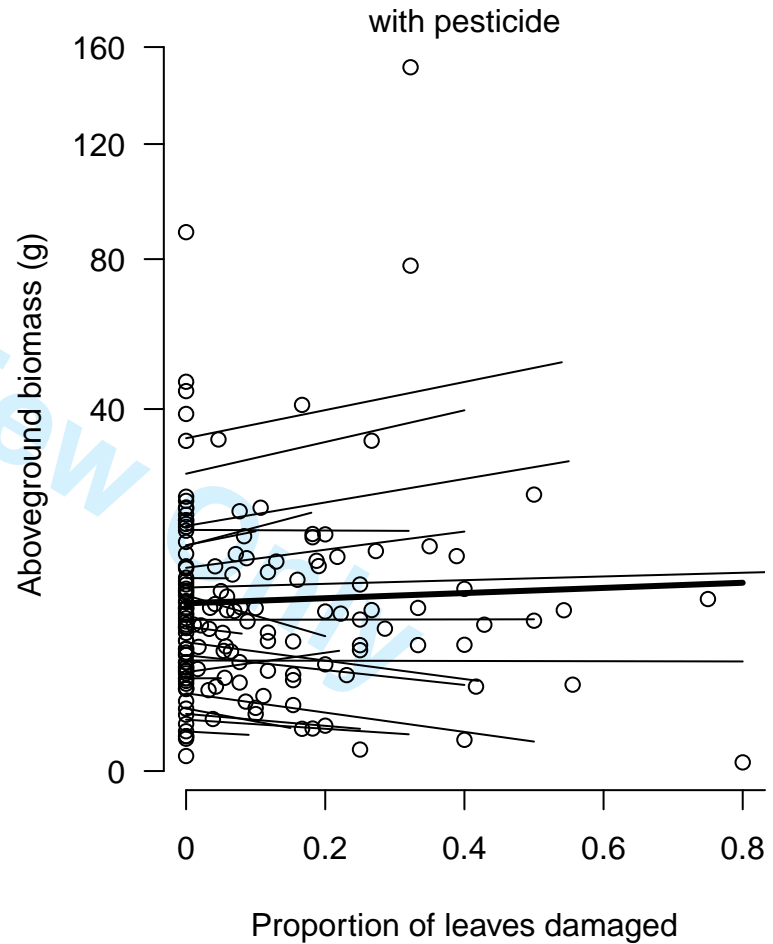
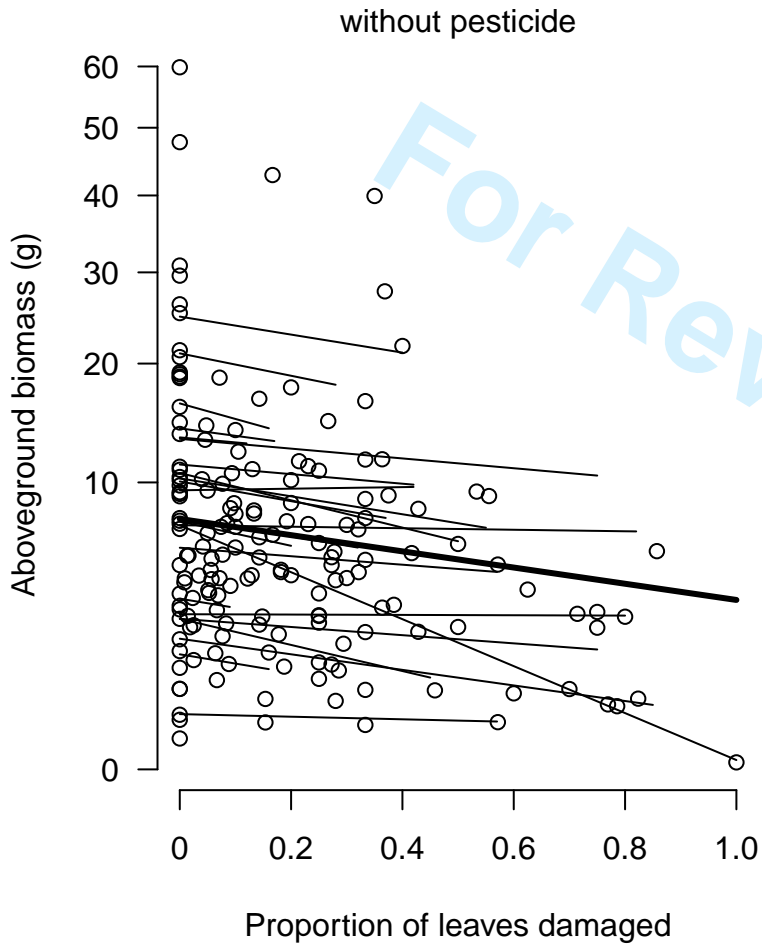
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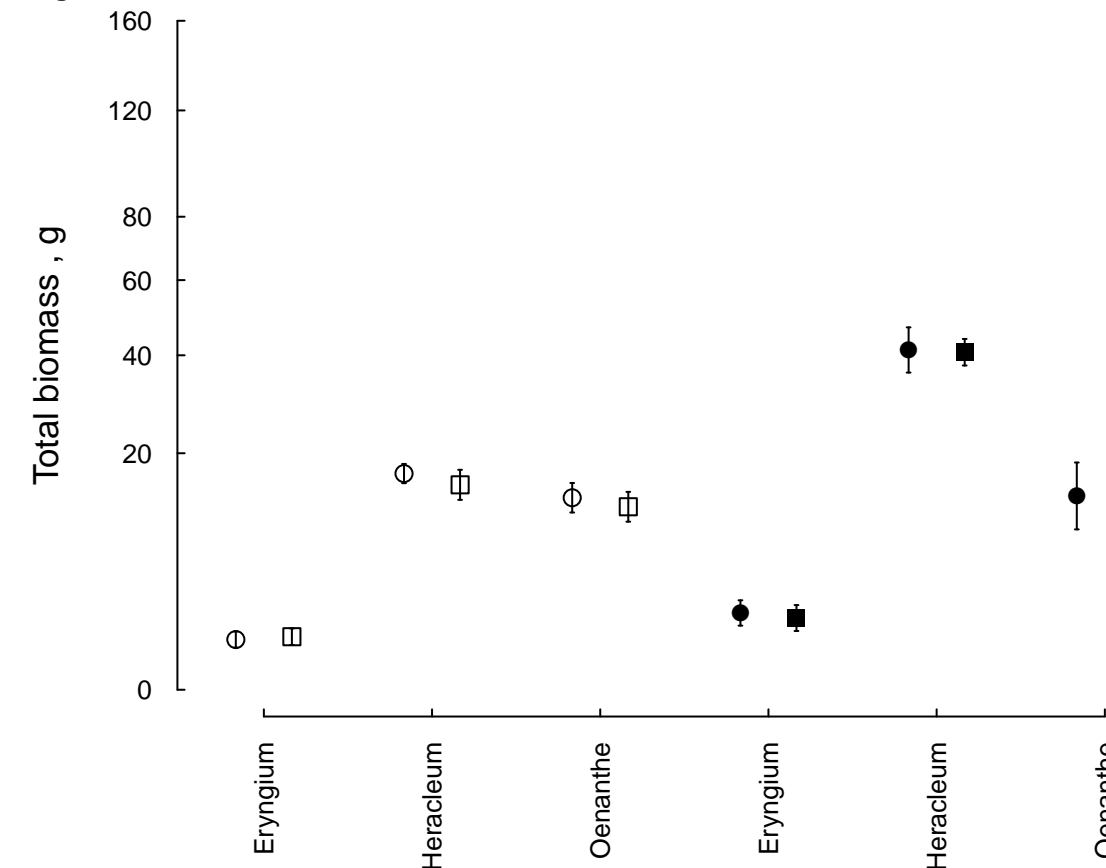
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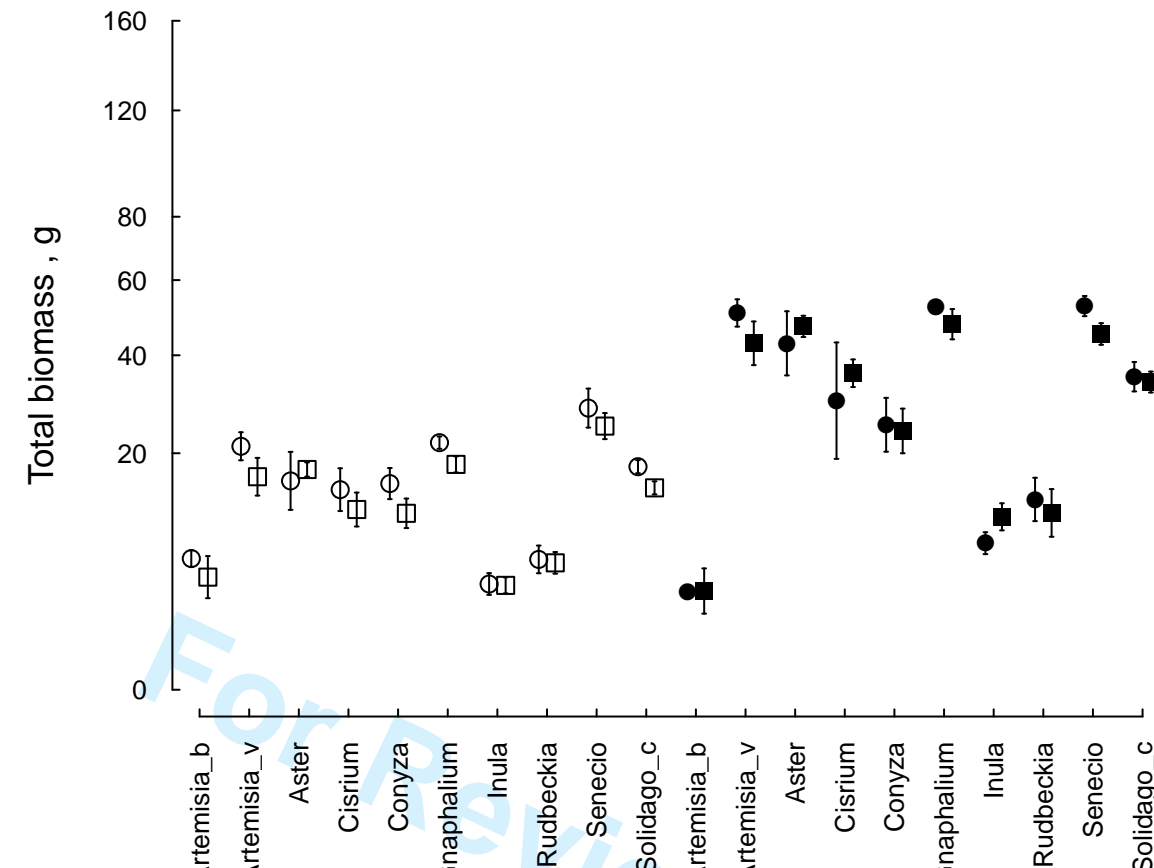
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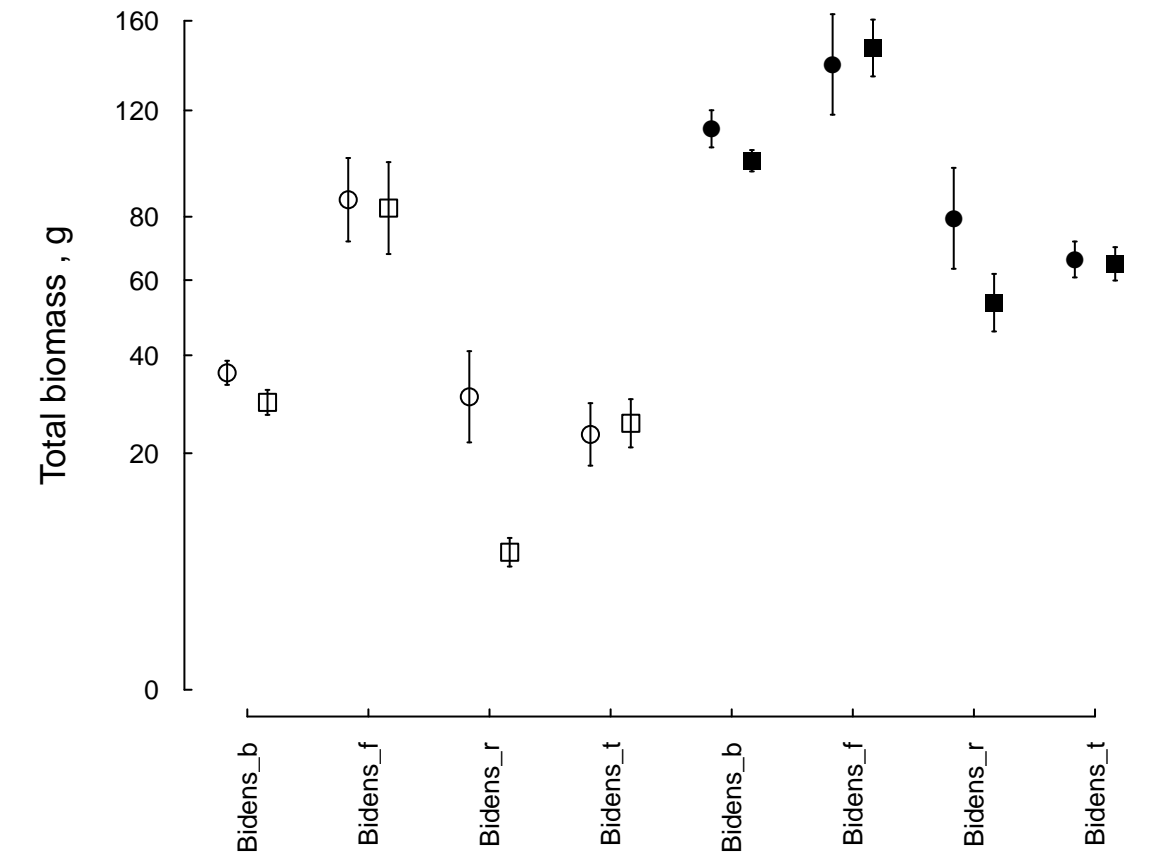
### Apiaceae



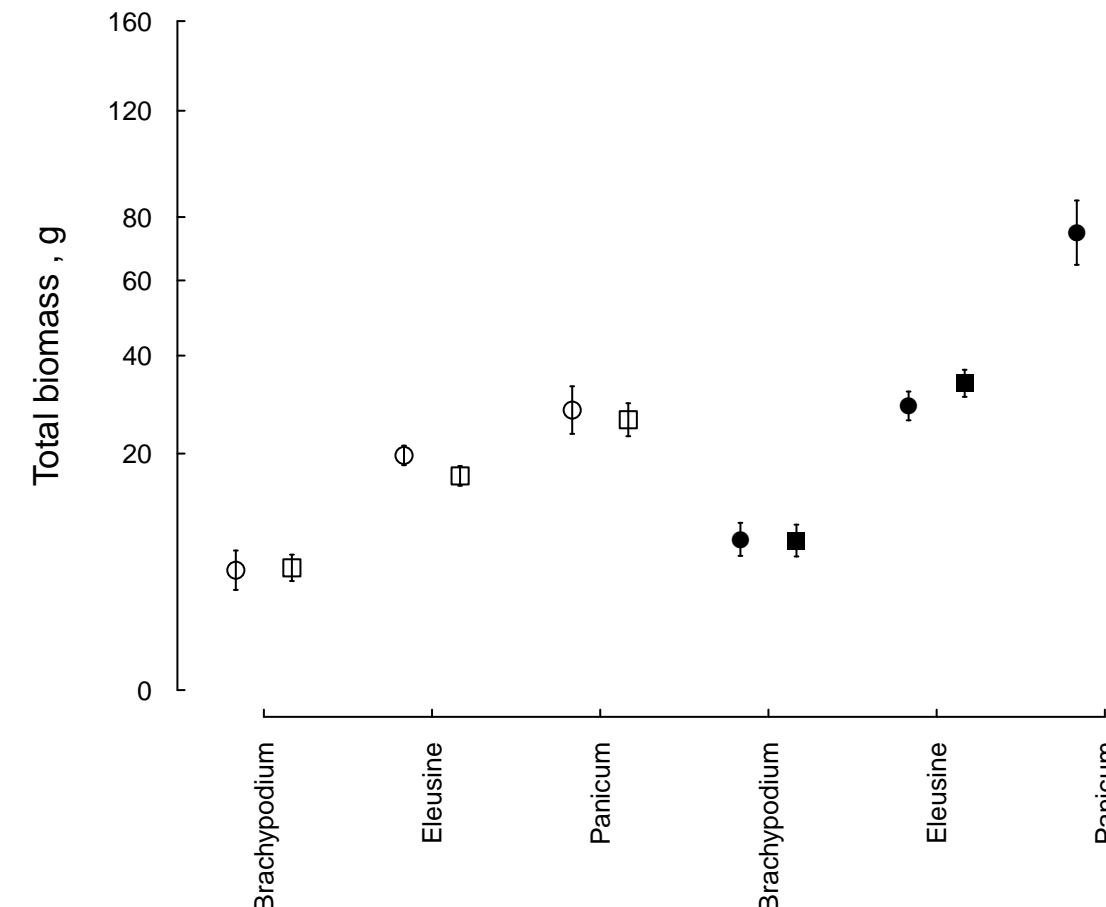
### Asteraceae



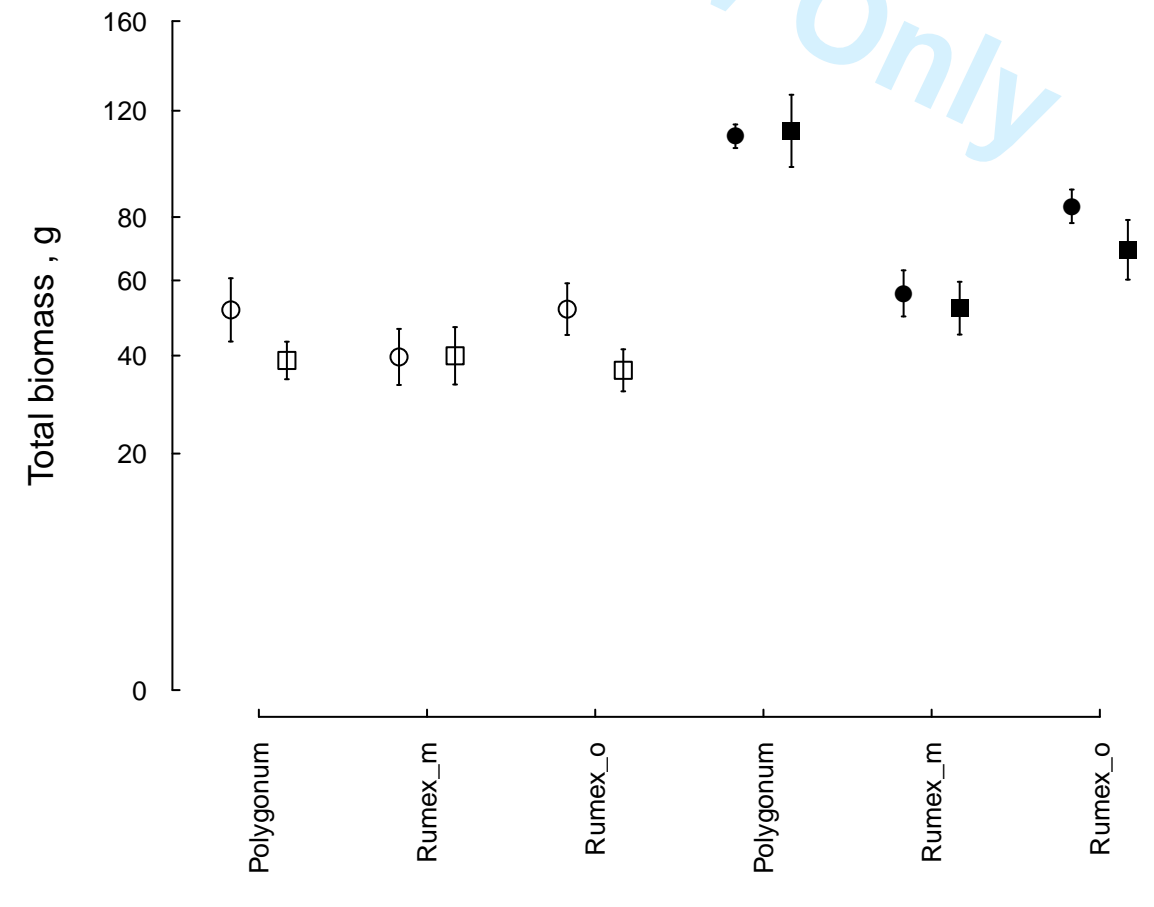
### Bidens



### Poaceae



### Polygonaceae



### Scroph./Plant./Phrym.

