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1 **Running Head:** Invertebrate herbivory in European grasslands

2

3 **Impact of invertebrate herbivory in grasslands depends on plant species diversity**

4

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16 **ABSTRACT**

17 Invertebrate herbivores are ubiquitous in most terrestrial ecosystems and theory predicts that
18 their impact on plant community biomass should depend on diversity and productivity of the
19 associated plant communities. To elucidate general patterns in the relationship between
20 invertebrate herbivory, plant diversity, and productivity we carried out a long-term herbivore
21 exclusion experiment at multiple grassland sites in a mountainous landscape of central Germany.
22 Over a period of five years, we used above-and belowground insecticides as well as a
23 molluscicide to manipulate invertebrate herbivory at 14 grassland sites, covering a wide range of
24 plant species diversity (13 – 38 species m⁻²) and aboveground plant productivity (272 - 1125 g m⁻²
25 year⁻¹), where plant species richness and productivity of the sites were not significantly
26 correlated.

27 Herbivore exclusion had significant effects on the plant communities: it decreased plant
28 species richness and evenness, and it altered plant community composition. In particular,
29 exclusion of belowground herbivores promoted grasses at the expense of herbs. In contrast to our
30 expectation, herbivore effects on plant community biomass were not influenced by productivity.
31 However, effect size of invertebrate herbivores was negatively correlated with plant diversity of
32 the grasslands: the effect of herbivory on biomass tended to be negative at sites of high diversity
33 and positive at sites of low diversity. In general, the effects of aboveground herbivores were
34 relatively small as compared to belowground herbivores which were important drivers of plant
35 community composition. Our study is the first to show that variation in the effects of invertebrate
36 herbivory on plant communities across a landscape is significantly influenced by plant species
37 richness.

38

39 **Keywords:** biodiversity, ecosystem functioning, plant functional groups, long term insect
40 exclusion, invertebrate herbivores, semi-natural grasslands, primary productivity

41 **INTRODUCTION**

42 Herbivorous invertebrates outweigh vertebrates as much as 10-fold in temperate terrestrial
43 ecosystems (Pimentel and Andow 1984). Nevertheless, their role in determining plant
44 community dynamics is generally considered to be less important when compared to vertebrates
45 (Crawley 1989). The most immediate effect of invertebrate herbivores is the consumption of
46 plant tissue (Crawley 1983, Karban and Baldwin 1997, Crawley 1997). Additionally,
47 invertebrate herbivores may influence plant species composition and diversity by selective
48 feeding on dominant species and thereby, releasing subordinate species from competition
49 (Crawley 1989, Pacala and Crawley 1992, Carson and Root 1999, Long et al. 2003). These
50 effects, however, are not consistent across different ecosystems (Hillebrand et al. 2007), or
51 across different herbivore guilds. For example, plant diversity in early successional plant
52 communities was found to be enhanced by aboveground (Brown and Gange 1992, Carson and
53 Root 2000), and reduced by belowground herbivores (Brown and Gange 1989a, Brown and
54 Gange 1992). Environmental factors and plant community characteristics are considered to be
55 further sources of variation in the effects of herbivores on plant diversity (Olf and Ritchie 1998).

56 Ecosystem level effects of herbivores, e.g. on plant community biomass, are suggested to
57 decrease with increasing plant diversity via either a reduced abundance of specialist herbivores
58 or an increased abundance of natural enemies (Pimentel 1961, Root 1973). However, results
59 from previous studies have been ambiguous (Andow 1991, Jactel et al. 2005, Jactel and
60 Brockerhoff 2007). A few studies have confirmed the predicted decrease in herbivore abundance
61 or damage in more diverse plant communities (Mulder et al. 1999, Unsicker et al. 2006, Jactel
62 and Brockerhoff 2007), but others have found a positive or negative relationship depending on
63 the type of herbivore studied (Koricheva et al. 2000, Otway et al. 2005). Yet others have found

64 no such relationship suggesting that particular plant functional groups may be more important
65 than species richness *per se* (Scherber *et al.* 2006). Furthermore, ecosystem level effects of
66 herbivores are suggested to be affected by primary productivity (Fretwell 1977, Oksanen *et al.*
67 1981, Fretwell 1987, Oksanen 1990) with the strongest herbivore impact expected at
68 intermediate levels of productivity (“hypothesis of exploitation ecosystems”, also known as the
69 Fretwell-Oksanen model). At low primary productivity, plant biomass may not suffice to sustain
70 appreciable populations of herbivores (bottom-up control of herbivores), whereas at high levels
71 of productivity top-down control of herbivores by predators is expected. Even though this model
72 was originally devised for vertebrate herbivores (Oksanen *et al.* 1981, Oksanen 1990), it has
73 been suggested to also apply to invertebrates (Oksanen and Oksanen 2000, Schädler *et al.* 2003).

74 Although a number of invertebrate exclusion experiments have been conducted to date, it
75 is still difficult to draw general conclusions about how productivity and plant diversity of
76 terrestrial ecosystems influence herbivory. There are several reasons for this. First, most studies
77 have investigated the effects of plant diversity and productivity on herbivory separately, and
78 those that have considered both factors simultaneously (e.g., Siemann 1998) did not focus on
79 herbivore effects on the plant community. Second, many studies have been carried out in
80 agricultural or forest systems where the diversity gradient was low, often ranging from just one
81 species (monoculture) to two (intercropping), raising the question of the generality of the results
82 (Andow 1991). Third, the effects of invertebrates on plant communities can often only be
83 detected after years (e.g., Cain *et al.* 1991, Long *et al.* 2003). What is needed therefore are long-
84 term studies in natural ecosystems that simultaneously investigate productivity and plant
85 diversity effects on the influence that invertebrate herbivores exert on a plant community.

86 In our study, we investigated the effects of above- and belowground invertebrate
87 herbivory on plant species diversity and aboveground plant community biomass along natural
88 gradients of plant species richness and productivity. This enabled us to explore whether variation
89 in herbivory among sites can be explained by these two community characteristics. Using 14
90 extensively managed hay meadows in one region of central Germany, we were able to minimize
91 the impact of confounding factors such as site management. Over a period of five years, using a
92 factorial design, we applied pesticides to exclude mollusks and insects from experimental plots
93 both above- and belowground, to test the following hypotheses:

94 (1) Invertebrate herbivores generally decrease aboveground biomass.

95 (2) Invertebrate herbivores increase species diversity of plant communities.

96 (3) The effect of herbivory on aboveground biomass decreases with increasing plant diversity
97 and is strongest at intermediate levels of primary productivity.

98

99 **METHODS**

100 *Study sites*

101 The study was conducted in the Frankenwald and Thüringer Schiefergebirge (11°00'-11°37'E
102 and 50°21'-50°34'N), a plateau-like mountain range at the border between Bavaria and
103 Thuringia in central Germany with a elevation ranging from 500 to 870 m a.s.l. The bedrock
104 material in the area consists mainly of schist and greywacke and produces a carbonate-free,
105 nutrient-poor soil. Mean annual air temperature is 5°C to 7°C, and mean annual precipitation
106 ranges from 840 to 1200 mm with a slight summer maximum (Hiekel et al. 2004). Before human
107 settlement, montane spruce-fir-beech forests formed the natural vegetation. Since human
108 settlement in medieval times much of the forest has been converted into an agricultural landscape
109 with a large proportion of montane hay meadows (Hundt 1964).

110 Based on a survey of more than 70 grassland sites in 2001 (Kahmen et al. 2005, Perner et
111 al. 2005), we selected a subset of 14 montane hay meadows (phytosociologically classified as
112 Geranio-Trisetetum, Knapp ex Oberd. 1975) that vary in plant species diversity but are similar
113 with respect to orographic conditions and management regime. The size of the total study area
114 was 114 km². The distance between two neighboring sites varied between 120 m and 6.5 km.
115 Plant species richness, as measured in June 2002, ranged from 13 to 38 vascular plants m⁻² across
116 sites. Mean standing aboveground biomass, also measured in June 2002, ranged from 230 to
117 591 g m⁻² (dry biomass). Prior to the onset of the study, all study sites had been managed
118 extensively for at least ten years by cutting for hay, with two cuts per year around the end of June
119 and at the beginning of September, and no grazing and no fertilizer application. During the
120 course of the study, this management regime was continued.

121 In 2004, a total of 21,624 insects were caught by sweep-netting in nine of the 14 study
122 sites (150 sweep net counts per site) on six dates throughout the growing season. The most
123 abundant herbivore taxa were Aphidina (16.1%), Coleoptera (10.2%), Auchenorrhyncha (8.4%),
124 Orthoptera (3.3%) and Lepidoptera (1.5%) (Unsicker *et al.* 2006). Mean damage levels measured
125 in the same year as percentage of leaf area loss were below 6% (Unsicker *et al.* 2006) which is in
126 line with other studies in grasslands (e.g., Scherber *et al.* 2006).

127

128 *Manipulation of invertebrate herbivory*

129 At each of the 14 grassland sites, we established permanent plots separated by 1 m guard strips.
130 Pesticides were applied to the plots to exclude aboveground herbivores (insects and mollusks,
131 *aboveground treatment*, plot size: 5x5 m), belowground herbivores (insects only, *belowground*
132 *treatment*, plot size: 2x2.5 m) or both above- and belowground herbivores (*combined treatment*,
133 plot size: 2x2.5 m). Treatments started in June 2002, after the first mowing of the grasslands in
134 that year, and continued through to the end of 2007. To exclude aboveground herbivores we
135 sprayed Dimethoate (Perfekthion, BASF, Germany), a broad spectrum quasi-systemic foliar
136 insecticide (Tomlin 2006). Diluted in water, it was applied in biweekly intervals at the
137 recommended rate of 0.15 g of active ingredient m⁻². The aboveground treatment also included
138 the application of molluscicide pellets (metaldehyde, 0.6 g of active ingredient m⁻²) every two or
139 four weeks. When rainfall intensity in the study area was low, monthly application of
140 molluscicide was deemed sufficient. The belowground treatment consisted of the application of
141 Chlorpyrifos, a contact soil pesticide without systemic effect (Tomlin 2006). It was applied
142 monthly at a concentration of 0.9 g m⁻² of the active compound. From June 2002 to October
143 2004, we applied a granular form of the belowground pesticide (Hortex, Scotts Celaflor,

144 Germany). After 2004, we could only purchase a form of Chlorpyrifos (Dow AgroSciences
145 GmbH, Germany) that had to be dissolved in water and sprayed onto the soil surface of the plots
146 (0.05 L m^{-2}). The combined treatment consisted of an application of the molluscicide, the foliar
147 and the soil pesticide. Two control treatments were established: an untreated *control* (plot size:
148 $5 \times 5 \text{ m}$) and a *water control* (plot size: $2 \times 2.5 \text{ m}$). The water control received the same amount of
149 water as used for application of the foliar pesticide (0.04 L m^{-2}). Due to logistic reasons and
150 shortage of space for the experimental plots in the respective grasslands, we were neither able to
151 set up $5 \times 5 \text{ m}$ plots for each of the treatments nor to set up replications within the study sites.
152 Treatments were always applied on the same day at all sites from May to October in each year,
153 resulting in a total number of 9 to 11 aboveground and 4 to 5 belowground pesticide applications
154 per year depending on the length of the growing season in each particular year. All pesticides
155 used in this study are commonly applied in agriculture and they have been successfully
156 employed in other experimental grassland studies (Brown and Gange 1989b, Carson and Root
157 2000).

158

159 *Measurement of plant diversity and productivity*

160 In June 2002, pre-treatment plant diversity and productivity were determined at each site.
161 Measurement of treatment effects started one year after the first pesticide applications. In June
162 2003, four $1 \times 1 \text{ m}$ subplots separated by 1 m were permanently marked in the center of plots of
163 the aboveground treatment as well as the control, and due to the smaller size of the plots, only
164 one $1 \times 1 \text{ m}$ subplot was marked in the center of the belowground treatment, the combined
165 treatment and the water control within each grassland site. In late June of every year, from 2003
166 until 2006, at peak standing biomass and directly before the first mowing, all vascular plants in

167 these eleven subplots per site were identified to species level. Percent cover m^{-2} of each species
168 was visually estimated using a modified Londo scale (Londo 1976). For the aboveground
169 treatment and the control, species richness as well as plant cover were determined for each
170 subplot, and then averaged across subplots in the respective treatment plot. To ensure that these
171 calculations did not affect our results we repeated the statistical analyses described below
172 including only one randomly chosen subplot for each of these treatments per study site. Since
173 the results did not differ qualitatively, all analyses presented in this paper refer to the calculations
174 described above.

175 We used plant species cover to calculate evenness values for each treatment separately,
176 based on the Shannon index:

$$177 \quad HE' = \frac{-\sum (p_i)(\ln p_i)}{\ln S},$$

178 where p_i is the proportion of total abundance of the i th species and S is the number of
179 species (Magurran 1988). Cover data were also collected for plant functional groups. Following
180 the classification of herbs by Klotz *et al.* (2002), we classified all plants into one of five
181 functional groups: grasses, legumes, rosette forming herbs, hemirosette herbs and erosulate herbs
182 (Appendix 1).

183 Aboveground plant community biomass was harvested twice a year (late June and
184 September), according to the existing mowing regime of the grasslands, from 2002-2006.
185 Immediately before mowing of the entire sites, biomass in each plot was sampled in 20 x 50 cm
186 squares (0.10 m^2) for each plot by cutting the vegetation 2 cm above the ground. Four samples
187 were taken from each plot in June 2002 (to measure pre-treatment productivity). From 2003

188 onwards, eight samples were taken in the aboveground treatment and the control and four in the
189 other plots. After drying the biomass samples at 60°C to 70°C for at least 48 h to achieve weight
190 constancy, aboveground dry biomass was determined in g m^{-2} . Aboveground plant community
191 biomass was used (1) to quantify the effects of herbivores on the plant community from 2003 to
192 2006 (henceforth ‘aboveground biomass’) but also (2) as an estimate of site productivity before
193 the start of the experiment in 2002 (henceforth ‘productivity’) which in turn might affect
194 herbivory.

195

196 *Data analysis*

197 The water control treatment was not included in the analyses, as there were no significant
198 differences between this treatment and the untreated control treatment with respect to the number
199 of plant species (control: 19.97 ± 0.20 species m^{-2} , water control: 19.79 ± 0.15 species m^{-2}),
200 evenness (control: 0.71 ± 0.01 , water control: 0.70 ± 0.01) and productivity (control: $445.93 \pm$
201 21.70 g dry mass m^{-2} , water control: 432.60 ± 33.10 g dry mass m^{-2} ; means averaged over four
202 years \pm SE, $P > 0.05$ in each case). This suggests that the amount of water used for pesticide
203 application (0.04 L m^{-2}) was too small to have any effects in itself.

204 We analyzed the effects of 5-years of invertebrate herbivory suppression on aboveground
205 biomass, plant species richness, evenness and plant functional group cover using two-factorial
206 repeated-measures ANOVAs, with aboveground treatment and belowground treatment as
207 factors. Site was included in the model as blocking factor. The fact that we excluded the water
208 control from the analyses and that we had no replicates within study sites resulted in a total
209 sample size of 56 ($2 \times 2 \times 14$) per year. To account for repeated measurements, we applied a

210 multivariate approach using MANOVA as implemented in JMP (version 5.1.2, SAS Institute
211 Inc., Cary, NC, USA, 1989-2005) and results are not dependent on the order in which the
212 independent variables are listed. Degrees of freedom were adjusted with Greenhouse–Geisser
213 Epsilon to accommodate for a potential lack of variance–covariance matrix compound
214 symmetry. Biomass data were log-transformed prior to analysis to achieve normality and
215 homoscedasticity. To account for multiple comparisons within each hypothesis, we applied the
216 Benjamini-Hochberg method (Verhoeven et al. 2005) separately to the analyses of the diversity
217 measurements (plant species richness, evenness) and to these of the cover of plant functional
218 groups.

219 To analyze whether invertebrate herbivory was dependent on plant diversity and
220 productivity of the study sites we calculated for each site a log response ratio of aboveground
221 biomass:

222

$$223 \quad (1) \quad \ln RR_B = \ln(B_{\text{combined}}/B_{\text{control}}),$$

224

225 where B_{combined} and B_{control} represent biomass of the plant community in the combined and
226 the control treatment, respectively. Positive values of $\ln RR_B$ indicate that pesticide application
227 increased community biomass whereas negative values of $\ln RR_B$ indicate a decrease in biomass
228 due to pesticide applications.

229 The $\ln RR_B$ was calculated for each site and year, and regressed against pre-treatment
230 plant species richness and pre-treatment productivity using a similar repeated measures approach

231 as described above. We used the pre-treatment data as explanatory variables to avoid a spurious
232 regression resulting from the same productivity in the dependent and the independent variables
233 (for a detailed discussion see Jackson 1997, Coupe and Cahill 2003). To test for a unimodal
234 relationship between herbivore impact and productivity as predicted by the exploitation
235 ecosystem hypothesis, we explicitly included a quadratic term. After analyzing their effects on
236 $\ln RR_B$ separately, we simultaneously included plant diversity, productivity and its quadratic
237 term in the model to explore whether each of them has still an effect after correcting for the other
238 variable.

239 To evaluate general treatment effects on individual plant species, we calculated the log
240 response ratio (Hedges et al. 1999) for each plant species i as

241

$$242 \quad (2) \quad \ln RR_C(i) = \ln(C_{\text{combined}}(i)/C_{\text{control}}(i)),$$

243

244 where $C_{\text{combined}}(i)$ is the cover of plant species i in the combined treatment and $C_{\text{control}}(i)$ is
245 its cover in the control treatment, both averaged across all study sites and years. When a species
246 was absent from a study site over all years, this was treated as a missing value. A positive $\ln RR_C$
247 indicates that the cover of a plant species did increase in response to pesticide application
248 whereas a negative $\ln RR_C$ indicates the opposite. Only plant species recorded in more than 20%
249 of the study sites (i.e. the 37 most frequent plant species) were included in this analysis.

250 To analyze whether species' responses to pesticide application were related to their
251 abundances, we calculated an ANCOVA on $\ln RR_C$ values using abundance data of individual

252 species as covariate and site as a factor, and including the site x abundance interaction to test for
253 homogeneity of slopes. Abundance data per study site were averaged across all study years to
254 avoid too many zero values for which $\ln RR_C$ are not defined. Species abundances were taken
255 from the pre-treatment census in 2002 to avoid that the same abundance data were used to
256 calculate the $\ln RR_C$ values. Rare plant species, i.e. species with a mean cover of $< 3\%$
257 determined during the pre-treatment census in 2002, were not included in this analysis.

258

259 **RESULTS**

260 The response of aboveground biomass to the pesticide treatments was similar in both June and
261 September, but aboveground biomass was always higher in June than in September. We
262 therefore only present results based on the annual sums. There were no significant differences in
263 productivity between plots designated for the different treatments before the long term treatments
264 started (2002 harvest, data not shown). Plant species richness and productivity of the grassland
265 sites were not correlated in any year. When analyzing the untreated control plots of all sites,
266 productivity tended to decrease with increasing species richness, but the correlation was not
267 significant (2002-2006, Pearson's r : $-0.38 < r < -0.23$, $0.20 < P < 0.43$).

268

269 *Effects of herbivore exclusion on aboveground biomass*

270 Aboveground biomass was not influenced consistently by either the aboveground treatment or
271 the belowground treatment (Tab. 1). There was a highly significant interaction between the
272 belowground treatment and time, indicating that the effect of belowground herbivores depended
273 on conditions of the particular year. This is, for instance, reflected by the negative effect of soil
274 pesticide application on aboveground biomass in 2003 and its positive effect in 2004, while there
275 were no significant effects in 2005 (Fig. 1). Across all years and sites, the average effect of
276 pesticide application on aboveground biomass was close to zero (mean $\ln RR_B = -0.01$) which is
277 in line with the results of the repeated measures ANOVA (Tab. 1). However, as individual
278 grasslands responded quite differently, ranging from negative to positive responses (-0.25 to
279 0.32) we used a repeated measures analysis to investigate whether this variation in responses
280 could be explained by the diversity and productivity of the grasslands.

281 Analysing the effects of diversity and productivity on herbivore impact separately,
282 revealed that the response of aboveground biomass to pesticide application was significantly
283 positively correlated with plant species richness ($F_{1,11} = 10.59, P < 0.007$), i.e. invertebrate
284 herbivory had a positive effect on biomass in low diversity grasslands and a negative effect in
285 more diverse sites (Fig. 2). Furthermore, the response to pesticides was significantly negatively
286 correlated with site productivity ($F_{1,11} = 6.75, P < 0.025$), i.e. herbivory had a negative effect on
287 plant community biomass in sites with generally low productivity and a positive effect in sites
288 with high productivity. We found no evidence for a unimodal relationship between herbivore
289 impact and productivity. However, the significant effect of site productivity vanished when
290 included together with plant diversity in the model (Tab. 2), which points to collinearity between
291 these two variables. The conclusion is that the response of plant community biomass to
292 invertebrate herbivore exclusion was depended on plant species richness but not on site
293 productivity (Fig. 2, Tab. 2).

294

295 *Effects of herbivore exclusion on plant diversity and plant species composition*

296 There were consistent and significant effects of belowground herbivore exclusion on plant
297 species richness. Exclusion of belowground herbivory significantly decreased plant species
298 richness by 1.44 ± 0.15 (mean \pm SE) species m^{-2} , averaged across all years and study sites (Tab.
299 1, Fig. 3a). On the other hand, the aboveground treatment did not significantly affect species
300 richness, and we found no interaction between the two treatments (Tab. 1, Fig. 3a). Evenness
301 was significantly reduced by the combined treatment, but responses of evenness to the
302 aboveground or belowground treatments were more variable (Fig. 3b). The fact that the effect of

303 the combined treatment was much higher than the sum of the effects of individual treatments on
304 their own indicates that the two pesticides acted synergistically rather than additively which is
305 also expressed by the significant interaction term (Tab. 1). Time-treatment interactions were not
306 significant (Tab. 1).

307 The treatments significantly influenced plant species composition, measured as change in
308 cover of the different plant functional groups. In particular the exclusion of belowground
309 herbivores caused a significant increase in the cover of grasses by $31.25 \pm 2.63\%$ (mean
310 averaged over all study sites and years \pm SE) and a significant decrease in the cover of erosulate
311 herbs by $3.14 \pm 0.68\%$ (mean averaged over all study sites and years \pm SE) whereas the cover of
312 rosette and hemirosette herbs as well as of legumes was not affected (Tab. 1, Fig. 4, Appendix 1
313 for plant functional groups). By contrast, the aboveground treatment had no effect on cover of
314 any functional group, and there was no interaction between the belowground and aboveground
315 treatments.

316 Because evenness was most strongly affected by the combined treatment (Fig. 3b), we
317 analyzed the response of individual plant species for this treatment only. The analysis of the log
318 response ratios ($\ln RR_C$) of the 37 most frequent plant species revealed that, averaged across all
319 study sites and years, two species were significantly positively affected and seven species were
320 significantly negatively affected by the combined treatment (Fig. 5). The two positively affected
321 species are the grass *Festuca rubra* and the legume *Vicia cracca*. Four of the seven negatively
322 affected species are hemirosette herbs. This result seems to contradict the results from the
323 repeated measure ANOVAs, which revealed no significant response of the functional group of
324 hemirosette forbs (Tab. 1). Log response ratios of individual plant species to pesticide
325 application increased significantly with their abundance ($F_{1, 189} = 3.98$, $P < 0.05$). Although

326 average species responses differed between the study sites ($F_{13, 189} = 3.68$, $P < 0.001$), dominant
327 species showed a consistently stronger response than less abundant species in all study sites
328 (interaction site x abundance not significant: $F_{13, 189} = 1.52$, $P = 0.12$).

329

330 **DISCUSSION**

331 Over a period of five years, we excluded above- and belowground herbivores from 14
332 extensively managed grasslands along existing gradients of plant diversity and productivity in
333 central Germany. Our first hypothesis, that invertebrate herbivores generally decrease
334 aboveground biomass, was not confirmed (Tab. 1, Fig. 1). In accordance with our second
335 hypothesis, invertebrate herbivores had a positive effect on plant species diversity as pesticide
336 application reduced both plant species richness and evenness (Tab. 1, Fig. 3). This particularly
337 applied to the exclusion of belowground herbivores which shifted plant functional composition
338 towards grasses at the expense of erosulate herbs. Our third hypothesis, that the effect of
339 herbivory on aboveground biomass decreases with increasing plant diversity and is strongest at
340 intermediate levels of primary productivity was also not confirmed. In contrast to our
341 expectation, productivity did not have a significant impact on the effect of herbivory. The impact
342 of plant species richness was surprising: the effect of herbivore exclusion on plant community
343 biomass was positively correlated with plant species richness, with negative effects at sites of
344 low diversity and positive at sites of high plant diversity. To our knowledge such a strong
345 connection of plant species richness to the effects of invertebrate herbivores on plant
346 communities has never been shown before. In the following, we will discuss experimental
347 restrictions of our study, explore possible mechanisms behind our findings and outline their
348 implications for biodiversity-ecosystem functioning research.

349 First, we would like to emphasize that invertebrate exclusion experiments are likely to
350 underestimate the real effects of herbivory, as the use of pesticides under field conditions usually
351 does not result in a complete exclusion of invertebrates (see Coupe & Cahill 2003) - even when,
352 as in our study, they are applied frequently at high dosages just below the maximum

353 recommended dosage. In our experiment, pesticides were effective in reducing insect density.
354 Our sites were mown twice a year, consequently there was no dense litter layer present
355 preventing pesticides from penetrating the soil. Extraction of soil invertebrates, carried out
356 accordingly to McFadyen (1962) and Kempson et al. (1963), revealed that the belowground
357 treatment was effective at least to a depth of 10 cm: we found that soil pesticide application
358 reduced abundance of soil invertebrates by 46% (excluding Collembola, Wilcoxon signed-rank
359 test, $P = 0.036$) and also caused a decrease in the overall number of invertebrate taxa found in the
360 soil by 47% (Wilcoxon signed-rank test, $P = 0.016$, Stein et al., unpublished data). These data
361 highlight the above mentioned fact that the application of pesticides does not result in a complete
362 exclusion of herbivores. Instead, by applying pesticides one rather compares the effect of an
363 intact herbivore community with that of a resistant subcommunity. A general weakness of using
364 pesticides are potential side effects, such as physiological effects on plant growth. However,
365 greenhouse experiments on a large number of plant species have shown that direct effects of the
366 pesticides used in our study on plant performance are negligible (Hector *et al.* 2004) or
367 nonexistent (Schädler *et al.* 2004b, Auge, unpublished data). A further complication of pesticide
368 use is that all insects and not just herbivores are killed by these pesticides, possibly masking the
369 potential role of herbivores: when herbivores are normally controlled by parasitoids and
370 predators, pesticide use will result in no changes for the plant community (Siemann et al. 2004).
371 Finally, invertebrate decomposers and plant nutrient availability may also be affected by
372 pesticide use (Siemann *et al.* 2004, Schädler *et al.* 2004a, Fountain *et al.* 2007). In our study, the
373 changes observed in the cover of plant functional groups could have been the result of a
374 fertilization effect due to the killing of belowground insects. In this case, however, we would
375 have expected a simultaneous increase in aboveground biomass, which was not observed. In

376 addition, such a fertilization effect would have been expected to be strongest in the first year of
377 application and much lower in subsequent years, when the insecticide treatment would have
378 killed eggs and small larvae early in their development. Even though we did not detect negative
379 effects of pesticides on the density of Collembola (Stein et al., unpublished data), there might be
380 effects on the dominance structure of the decomposer community as it has been shown in an old
381 field (Endlweber *et al.* 2006). Despite potential side effects, and even with the appropriate
382 caution in interpreting chains of causation, a number of previous studies have demonstrated that
383 the use of pesticides is a valuable tool for studying herbivore effects in terrestrial ecosystems
384 (Siemann et al. 2004). Furthermore, pesticides are so far the only way to selectively reduce
385 abundance of belowground insects, and hence insect herbivory, under natural conditions. Based
386 on the arguments above, we feel certain that our results are, at least for the most part, reflecting
387 the effects of invertebrate herbivores rather than possible side effects of pesticides.

388 Belowground invertebrates significantly affected plant diversity and functional group
389 composition of the investigated 14 grasslands. In response to the exclusion of belowground
390 invertebrates plant species richness and evenness decreased. This is in line with the conceptual
391 framework proposed by Hillebrand et al. (2007) who suggested that in communities where
392 productivity and dominance are high herbivores have a positive effect on plant species richness
393 and evenness. The dominant plant species in our sites, the grasses, became more dominant when
394 belowground herbivores were excluded and erosulate herbs declined. However, an analysis of
395 the response of the most abundant plant species revealed that several hemirosette herbs were also
396 significantly affected (Fig. 5). Thus, the definition of functional groups based on one single
397 attribute – in our case growth form – is obviously not sufficient to predict plant species response
398 to invertebrate herbivory (Petchey 2004). One potential mechanism underlying the positive effect

399 of the soil insecticide on grasses could be that belowground herbivores may generally be less
400 specialized than aboveground herbivores (Crawley 1983, Lal 2006 but see also) (Blossey and
401 Hunt-Joshi 2003), and feed as a consequence on the roots that are most abundant. Since grasses
402 typically have more extensive root systems than herbs (e.g., Kutschera 1960), they are also more
403 exposed to belowground herbivory and would consequently benefit most from the exclusion of
404 belowground herbivory. While many grass species increased in cover, several grasses such as
405 *Holcus lanatus* or *Arrhenaterum elatius* did not increase, suggesting at least some specificity in
406 the herbivore effect. In contrast to our study, previous investigations have found a promotion of
407 herbs in response to belowground herbivore exclusion (Brown and Gange 1989b, Brown and
408 Gange 1992). However, those studies were conducted in early successional plant communities in
409 which herbs represented the most abundant group, which is consistent with our hypothesis that
410 belowground herbivores are affecting the most abundant species in a community. In accordance
411 with this hypothesis, we found a consistently positive relationship between response to herbivory
412 and abundance of individual plant species across our study sites. There is a clear need for further
413 studies distinguishing whether the effect of belowground herbivores is driven by their preference
414 for particular plant species or by plant abundance.

415 Effects of invertebrate herbivory on aboveground biomass were small and inconsistent
416 when averaged across the 14 sites. Our findings support a recent meta-analysis (Schädler et al.
417 2003) that also demonstrated high variability in effect size when insects and/or molluscs were
418 excluded in field experiments. Interestingly, however, in our experiment, 42% of the variance in
419 the aboveground biomass response to invertebrate herbivory could be attributed to plant species
420 richness in the study sites. Remarkably, invertebrate herbivory tended to have a positive effect on
421 biomass in low diversity grasslands and a negative effect in more diverse sites, thus excluding

422 invertebrates using pesticides led to an increase in aboveground biomass only in the more diverse
423 sites (Fig. 2). Such a pattern was not reported in a recent meta-analysis of insect effects on
424 primary productivity in different herbaceous communities (Coupe and Cahill 2003). Using
425 experimental grassland, Mulder et al. (1999) also found that more diverse grasslands gained
426 more biomass when herbivores were excluded but there was no indication of a negative effect of
427 pesticide use on biomass use in low-diversity mixtures. In a different grassland biodiversity
428 experiment, Scherber et al. (2006) found no increase in herbivore damage of individual plants
429 across a gradient of 1 to 60 plant species. A previous study carried out in the same grasslands
430 that were used in the present study showed that damage caused by aboveground feeding
431 invertebrate herbivores, measured as percent leaf area removed, did decrease with increasing
432 plant species richness (Unsicker *et al.* 2006). However, the same study indicated that herbivory
433 was mainly due to generalist rather than specialist herbivores, with aphids being the most
434 abundant group. These and other results show that (1) the mechanisms that are hypothesized to
435 lead to a decrease in herbivore impact with increasing plant diversity (Pimentel 1961, Root 1973)
436 do not appear to apply universally and need to be studied in more detail, (2) herbivory as
437 measured as leaf area loss or related quantities does not necessarily reflect herbivore impact at
438 the community level as plants may compensate for damage and differ in their response to it, (3)
439 insect abundance data or herbivore load may likewise be only poor predictors of herbivore effect
440 at the plant community level. Unfortunately, in order to understand how plant diversity
441 influences herbivore effects on the plant community it appears to be necessary to measure
442 simultaneously the response of both generalist and specialist herbivores (and their natural
443 enemies) to plant species richness, the resulting impact on individual plant species, and the
444 consequences at the community level. Such comprehensive studies have not been performed and

445 our study also falls short of measuring all of these parameters. Studying herbivore effects on
446 plant-plant competition in detail in differently diverse plant communities may be very rewarding
447 as our study indicates that even an increase in community biomass under herbivory is possible
448 when species richness is low.

449 The effect of invertebrate herbivores on aboveground biomass was not related to the
450 productivity of the plant communities. In contrast to previous studies (Fraser and Grime 1997,
451 Fraser 1998) our results are not consistent with the exploitation ecosystem hypothesis (Fretwell
452 1977, Oksanen *et al.* 1981, Fretwell 1987, Oksanen 1990). Instead, they support the suggestion
453 that this model does not apply to invertebrates (Schädler *et al.* 2003). For large mammalian
454 herbivores, Chase *et al.* (2000) have shown a decline in the effect size of herbivores on
455 aboveground biomass with increasing productivity of the plant communities. However, our study
456 suggests that effect size of invertebrate herbivores is controlled by plant diversity rather than
457 productivity.

458 Our results emphasize the fact that invertebrate herbivores affect ecosystem functioning
459 (Weisser and Siemann 2004). However, in the studied grasslands, these effects varied with
460 specific ecosystem characteristics (plant diversity), and depended on the type of herbivory (i.e.
461 aboveground versus belowground herbivory). At our study sites, the effects of aboveground
462 herbivores were generally small in comparison to those of belowground herbivores. In
463 combination, above- and belowground herbivory seemed to affect plant diversity only in terms of
464 evenness synergistically rather than additively. This is in line with the results of previous studies
465 in early successional plant communities where observed effects were additive (Brown and Gange
466 1989b). Our results indicate that belowground herbivores can be important drivers in semi-
467 natural grasslands. Since the main effect of invertebrate herbivory might be on root biomass (but

468 see McNaughton et al. 1998), future studies should in particular examine belowground primary
469 productivity.

470

471 In conclusion, our results show that invertebrate herbivores can affect ecosystem
472 processes under field conditions, and that effect sizes can depend on species diversity of the plant
473 community. Furthermore, our results indicate that the influence of herbivores on a plant
474 community does not necessarily become evident in aboveground biomass. Instead, plant
475 community composition measured as the contribution of plant functional groups to the
476 community, was more strongly affected by our treatments than total aboveground biomass.
477 Future studies should therefore include more measures of ecosystem functioning, and not just
478 aboveground biomass. Finally, our results emphasize the importance of longer-term studies.
479 While the effect of the combined treatment on evenness was already visible within one year of
480 study, the effects on productivity were quite variable so that the conclusions would have been
481 different had our study stopped after only one or two years (Figs. 2, 4). Because effects can be
482 subtle and may be influenced by interannual variation in temperature and rainfall patterns, short-
483 term studies may fail to elucidate such effects.

484

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493 laws.

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629 **TABLES**

630 **Table 1.** Results of the repeated measures ANOVA (F-values) of the effect of pesticide treatments (above = aboveground pesticide, below =
 631 belowground pesticide) on aboveground biomass, diversity (number of species and Shannon evenness), and on cover of plant functional groups.
 632 Pesticides were applied during four years on 14 grassland sites. Significance levels are denoted with: * P < 0.05; ** P < 0.01; *** P < 0.001 after
 633 adjusting the degrees of freedom by Greenhouse–Geisser Epsilon (error degrees of freedom are fewer for biomass because of missing values) and
 634 after accounting for multiple comparisons with the Benjamini-Hochberg method (for each hypothesis).

Effects	d.f.	Above- ground biomass	Plant species richness	Evenness	Cover grasses	Cover erosulate herbs	Cover hemirosette herbs	Cover rosette herbs	Cover legumes
Between subjects									
Site	13, 39 (36)	53.25***	28.87***	8.97***	7.98***	13.61***	10.62***	3.59**	4.15***
Above	1, 39 (36)	2.61	0.71	6.64*	1.12	0.29	0.07	1.44	0.23
Below	1, 39 (36)	0.38	4.17*	10.47**	27.77***	13.51**	0.55	0.15	1.93

Above*below	1, 39 (36)	1.12	0.57	6.58*	2.73	0.01	0.49	0.32	0.45
Within subjects									
Time	3, 117 (108)	26.66***	1.43	18.20***	7.92***	0.76	5.74**	2.49	1.65
Time x site	39, 117 (108)	2.57**	1.62*	2.45***	4.36***	2.87***	3.90***	2.40***	2.10**
Time x above	3, 117 (108)	2.46	0.54	1.04	1.65	1.68	0.26	0.59	2.11
Time x below	3, 117 (108)	20.83***	0.28	1.29	1.73	2.29	2.87	1.68	1.14
Time x above x below	3, 117 (108)	0.87	0.03	0.95	2.23	0.26	0.31	1.23	0.40
Greenhouse-Geisser epsilon		0.92	0.71	0.88	0.89	0.75	0.72	0.87	0.76

635

636

637 **Table 2.** Results of repeated measures analysis (F-values) for the log response ratio of
 638 aboveground biomass responding to the combined application of above- and belowground
 639 pesticides (LnRR_B) depending on the diversity and productivity of the study sites. Pesticides
 640 were applied over four years across 14 grassland sites in Thuringia and Bavaria, Germany.
 641 Significance levels are given after adjusting the degrees of freedom by Greenhouse–Geisser
 642 Epsilon.

Effects	d.f.	F	P
Between subject			
Number of plant species	1, 8	7.03	0.029
Productivity	1, 8	0.73	0.42
Productivity ^{^2}	1, 8	1.11	0.32
Number of plant species x productivity	1,8	1.86	0.21
Within subjects			
Time	3, 24	0.50	0.63
Time x number of plant species	3, 24	0.14	0.88
Time x productivity	3, 24	0.41	0.68
Time x productivity ^{^2}	3, 24	0.41	0.68
Time x number of plant species x productivity	3, 24	0.93	0.42
Greenhouse-Geisser epsilon		0.72	

643

644 **FIGURE LEGENDS**

645 **Fig. 1.** Aboveground biomass in untreated plots (“control”) and in plots treated with
646 aboveground pesticide and molluscicide (“above”), belowground pesticide (“below”), and in a
647 combination of above- and belowground pesticides (“combined”). Means (\pm SE) were
648 calculated using 14 different grassland sites.

649

650 **Fig. 2.** Response of aboveground biomass ($\ln RR_B$) of the 14 grassland sites to application of
651 above- and belowground pesticides (“combined” treatment) is positively correlated with plant
652 diversity ($R^2 = 0.38$, $P < 0.05$). A positive $\ln RR_B$ indicates that aboveground biomass
653 increases due to pesticide application whereas a negative $\ln RR_B$ indicates the opposite. The
654 dotted line marks the base line of $\ln RR_B = 0$. For reasons of illustration, curve is fitted using
655 simple regression ($\ln RR_B$ values for each site are averaged over 4 years).

656

657 **Fig. 3.** (a) Plant species richness and (b) Shannon evenness in untreated plots (“control“), and
658 in plots treated with aboveground pesticide and molluscicide (“above”), belowground
659 pesticide (“below”), and in a combination of above- and belowground pesticides
660 (“combined”). Means (\pm SE) were calculated using 14 different grassland sites.

661

662 **Fig. 4.** Cover of plant functional groups in untreated plots (“control”) and in plots treated with
663 aboveground pesticide and molluscicide (“above”), belowground pesticide (“below”), and in a
664 combination of above- and belowground pesticides (“combined”). Means (\pm SE) were
665 calculated using 14 different grassland sites.

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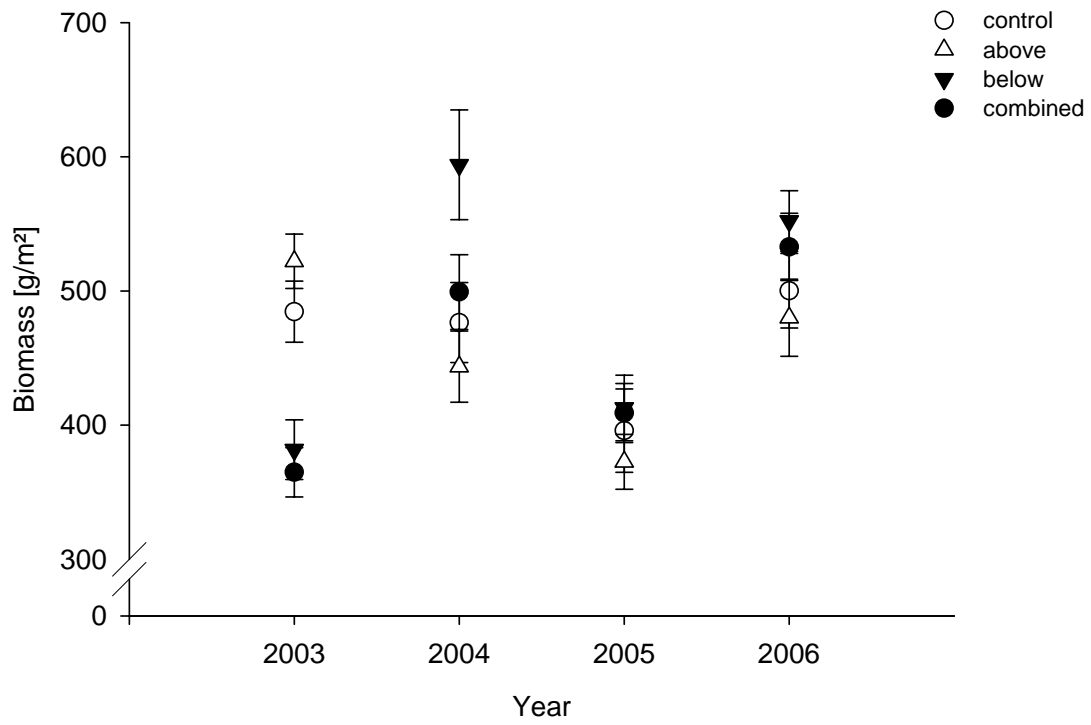
667 **Fig. 5.** The influence of above- and belowground pesticide application on the cover of plant
668 species. Bars represent the relative response of species cover (\pm 95% confidence interval) to
669 the “combined” pesticide treatment relative to the control (averaged over 14 study sites and 4
670 years). A positive $\ln RR_C$ indicates that the cover of a plant species increases due to pesticide
671 application whereas a negative $\ln RR_C$ indicates a negative effect of pesticide application on
672 the plant species. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; (*) $P < 0.1$.

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674

675 **FIGURES**

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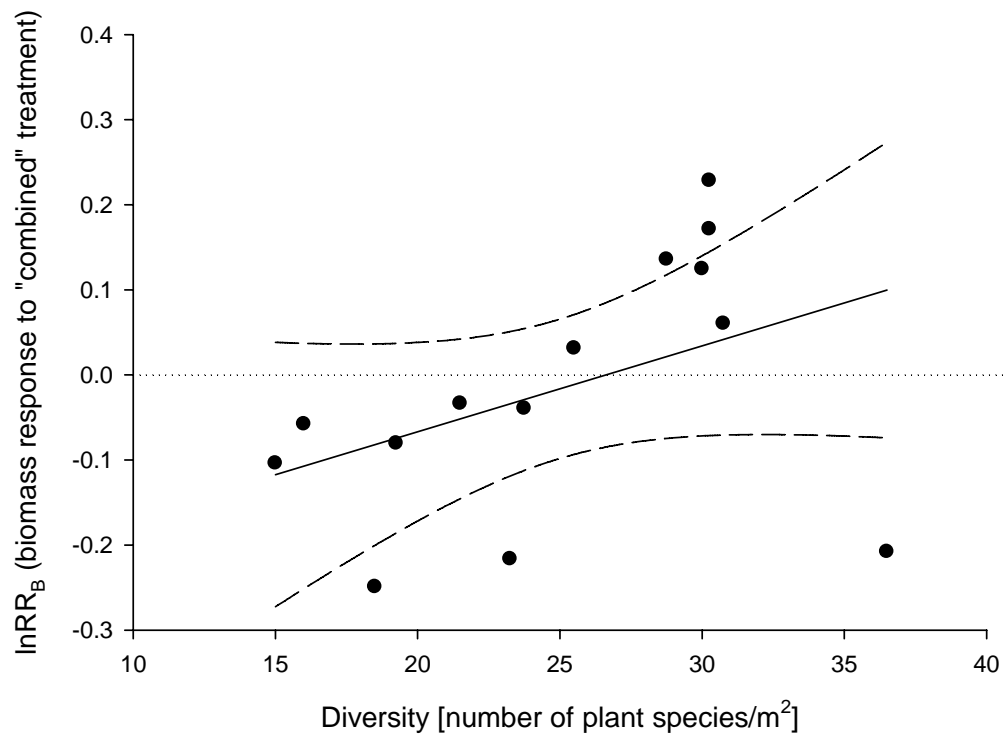


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678 **Fig. 1.**

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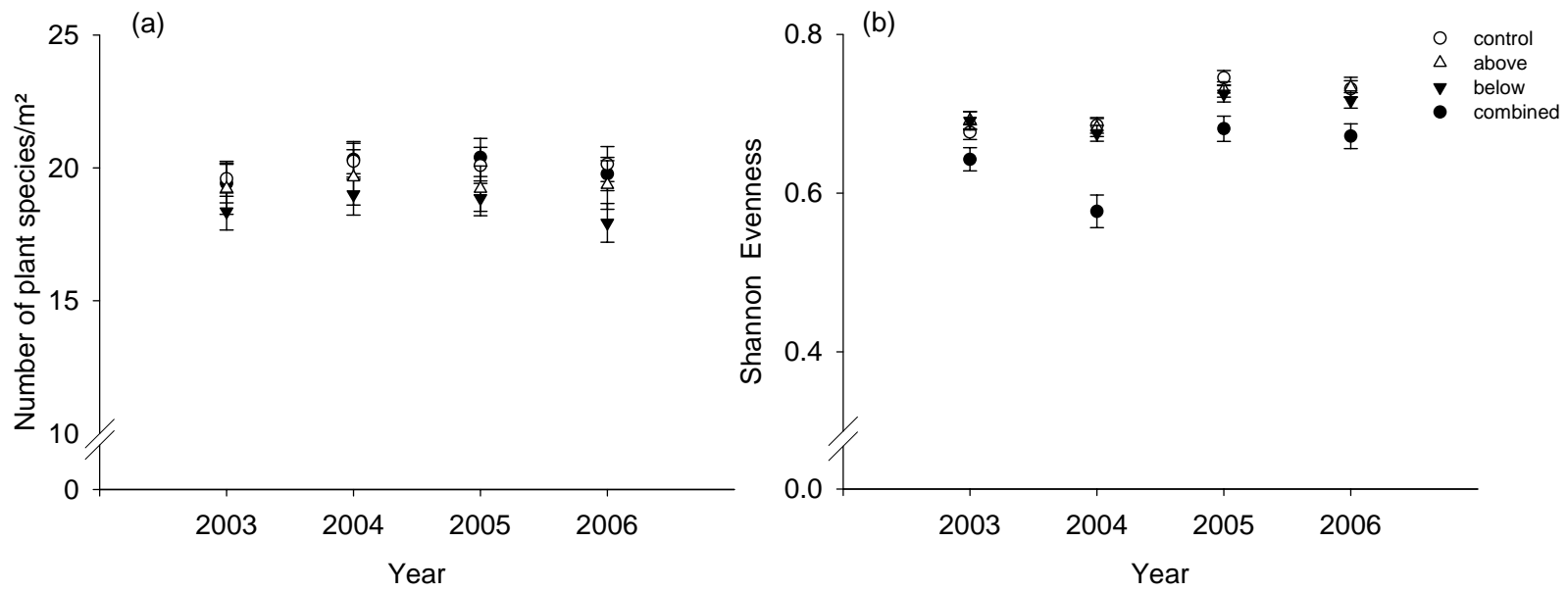
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683 **Fig. 2.**

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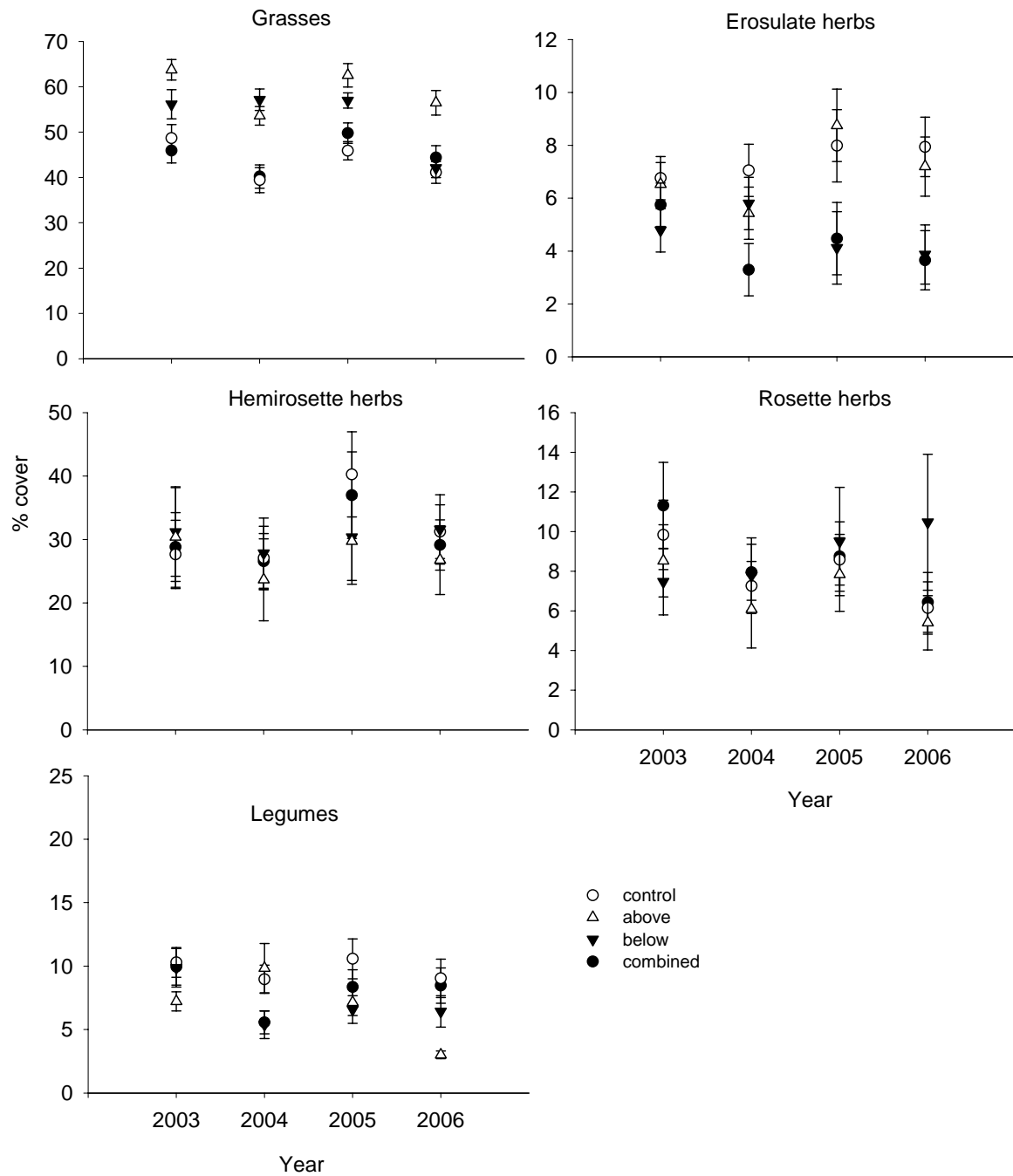
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687 **Fig. 3.**

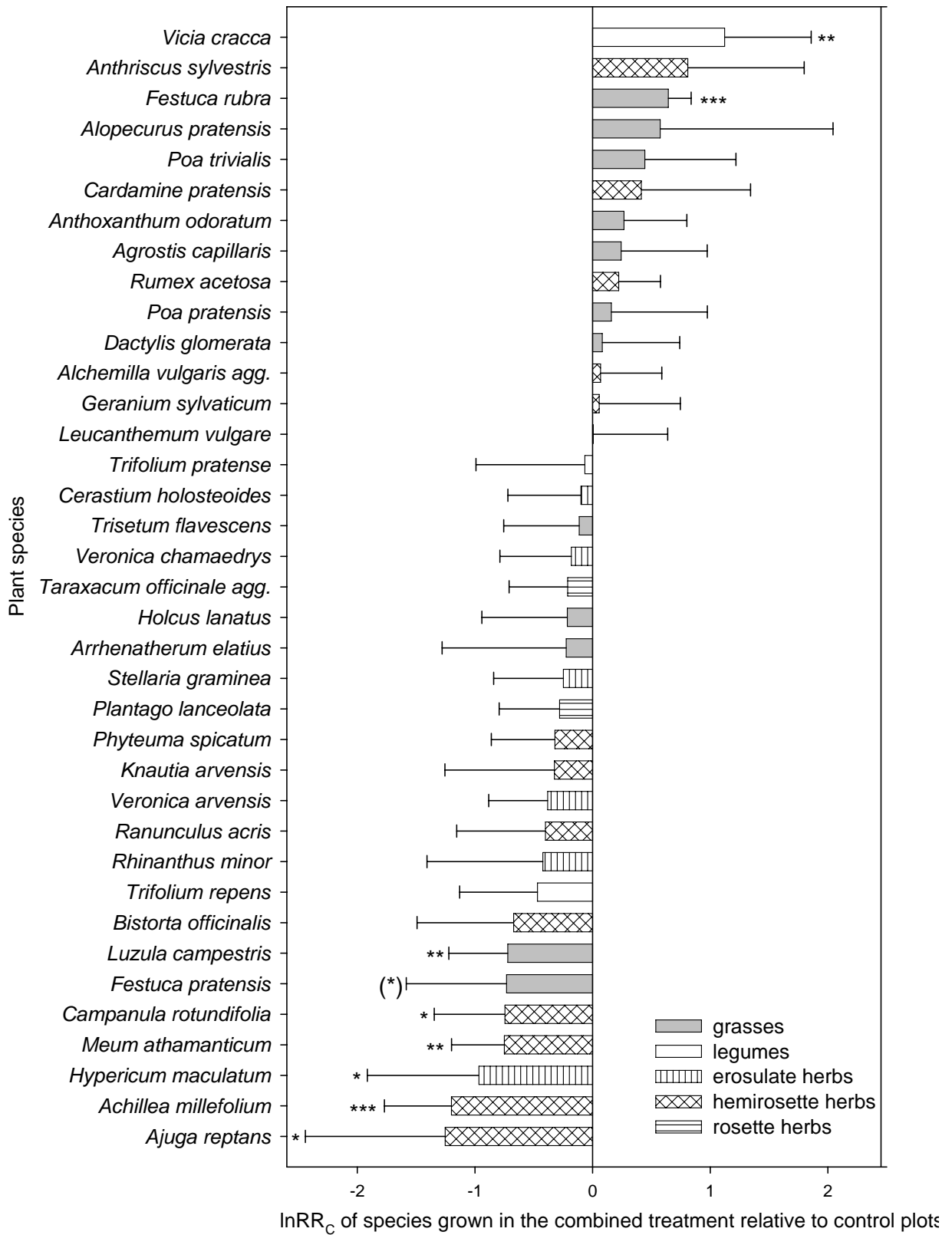
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691 **Fig. 4.**

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693

694 **Fig. 5.**

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