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1 **Herbivore preference drives plant community composition**

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23

24 Running head: Herbivore preference and plant community composition

25

26 **Abstract**

27 Herbivores are important drivers of plant species coexistence and community assembly.  
 28 However, detailed mechanistic information on how herbivores affect dominance hierarchies  
 29 between plant species is scarce. Here, we used data of a multi-site herbivore exclusion  
 30 experiment in grasslands to assess changes in the cover of 28 plant species in response to  
 31 aboveground pesticide application. Moreover, we assessed species-specific values of plant  
 32 defense of these 28 species measured as the performance of a generalist caterpillar, and the  
 33 preference of the caterpillar and a slug species in no-choice and choice feeding experiments,  
 34 respectively. We show that more preferred species in the feeding experiments were those that  
 35 increased in cover after herbivore exclusion in the field, whereas less preferred ones  
 36 decreased. Herbivore performance and several measured leaf traits were not related to the  
 37 change in plant cover in the field in response to herbivore removal. Additionally, the  
 38 generalist slug and the generalist caterpillar preferred and disliked the same plant species,  
 39 indicating that they perceive the balance between defense and nutritional value similarly. We  
 40 conclude that the growth-defense tradeoff in grassland species acts via the preference of  
 41 herbivores and that among-species variation in plant growth and preference to herbivores  
 42 drives plant community composition.

43

44

45 Key words: herbivore performance, herbivore preference, growth-defense tradeoff, growth-  
 46 rate hypothesis, leaf traits, generalist herbivores, plant resistance, plant coexistence, biotic  
 47 factors

48

49 **Introduction**

50 Environmental conditions and biotic interactions with other organisms have been suggested  
 51 to drive the distribution and abundance of plant species (Soberon 2007). While environmental  
 52 constraints have been studied intensively (Thuiller et al. 2004), the importance of biotic  
 53 interactions for the assemblage of plant communities and coexistence of species, and their  
 54 underlying mechanisms, are much less understood (Chesson 2000, HilleRisLambers et al.  
 55 2012).

56         Among important biotic interactions, herbivores are commonly invoked to explain the  
 57 coexistence of plant species in a community, because they alter competitive interactions  
 58 between species (Pacala and Crawley 1992, Chesson 2000). Herbivores can promote  
 59 coexistence if their consumption depends on the frequency of plants and thereby impairs  
 60 abundant species more than less abundant ones (negative frequency dependence, Janzen-  
 61 Connell hypothesis), which is seen as a stabilizing mechanisms. Theory suggests that  
 62 stabilizing mechanisms are mainly driven by specialist herbivores that hold down the density  
 63 of their host plant (e.g. Chesson 2000). However, also generalist herbivores can have  
 64 stabilizing effects on plant communities when they switch host or food plant species and  
 65 consume disproportionately whichever species is most abundant (Murdoch 1969, Chase et al.  
 66 2002). Other theories on how herbivores can promote coexistence require a tradeoff between  
 67 the vigorous growth of plants and their defense against consumers, assuming that defense is  
 68 costly and constrains investment in other important traits (Coley et al. 1985, Herms and  
 69 Mattson 1992, Viola et al. 2010, Kempel et al. 2011, Lind et al. 2013). If the plants growing  
 70 most vigorously in a community are also the least defended ones, herbivores promote  
 71 coexistence by selectively feeding on more vigorously growing and hence less defended plant  
 72 species (Pacala and Crawley 1992, Carson and Root 1999), thereby reducing average fitness

73 differences between species, which is considered as an equalizing mechanism (Chesson  
 74 2000).

75         In spite of the importance of plant defense, it is notoriously difficult to assess. Plants  
 76 evolved a variety of defense strategies to cope with their enemies. Such defenses can be  
 77 mechanical, chemical, or indirect, they can be constitutive, i.e. independent from herbivore  
 78 attack, or induced after damage (Karban and Baldwin 1997, Walling 2000). Most of those  
 79 defenses reduce the performance or the preference of herbivores, collectively called plant  
 80 resistance (Karban and Baldwin 1997), and hence decrease the amount of consumed plant  
 81 tissue. The variety of plant defense strategies (Walling 2000) combined with the vast amount  
 82 of herbivore species differing in host specificity (Ali and Agrawal 2012) and feeding  
 83 strategies (Strong et al. 1984) represents a major challenge for assessing a “species-specific  
 84 value of plant defense”. Such a value must incorporate both the performance of herbivores  
 85 (which is related to herbivore fitness, and tested in no-choice experiments) as well as their  
 86 preference (which is related to feeding behavior, and tested in choice or cafeteria  
 87 experiments), because both may contribute to a plant’s defense. A “species specific value of  
 88 plant defense” would be necessary to better understand the ecological mechanisms  
 89 underlying the balancing role of herbivores in plant communities. A promising attempt is the  
 90 use of herbivores as an evaluation of plant defense (or of the combined effects of many traits  
 91 acting in concert that provide resistance to plants, such as nutritional value and defense)  
 92 across many species. At least for generalist herbivores, it might be possible to tell whether a  
 93 plant species is more or less defended than another if one screens herbivore performance  
 94 using no-choice experiments together with the preference of herbivores using choice  
 95 experiments to all plant species of a community.

96         The balancing role of herbivores has been demonstrated several times, especially in  
 97 grassland communities, using herbivore exclusion experiments. Results from these

98 experiments often found a shift in plant community composition and a loss of diversity due to  
 99 competitive exclusion when herbivores are excluded (Brown and Gange 1992, Carson and  
 100 Root 1999, Allan et al. 2010, Stein et al. 2010). Such experimental manipulations can provide  
 101 strong evidence that herbivores are critical for plant community composition and diversity. If  
 102 the most abundant plant species also benefit most from herbivore exclusion such experiments  
 103 can demonstrate a stabilizing effect of herbivores on plant communities. However, they  
 104 cannot demonstrate an equalizing effect of herbivores, since they neither demonstrate costs  
 105 of defense nor a mechanistic link to a growth-defense tradeoff which requires species-specific  
 106 information on plant defense values: if defense is costly and trading off with vigorous  
 107 growth, then the least defended species should suffer most from herbivores, thus benefit most  
 108 from their exclusion. Accordingly, highly defended species should decrease in cover if  
 109 herbivores are excluded, since they lose their fitness advantage over less defended species  
 110 and suffer from interspecific competition (Fig. 1a). Indeed, it has been shown that the  
 111 exclusion of vertebrate herbivores resulted in an increase of plant species that were preferred  
 112 by the grazers (Diaz 2000, Bråthen and Oksanen 2001). Similarly, the abundance of plant  
 113 species in communities allowed to assemble from seeds was correlated with herbivore  
 114 preference, assessed in feeding trials (Burt-Smith et al. 2003). However, whether the shift in  
 115 composition of an entire plant community in response to invertebrate herbivore exclusion can  
 116 be explained by differences in herbivore performance or preference among plant species has  
 117 rarely been tested (but see Schädler et al. 2003), largely due to the difficulty of assessing the  
 118 ability to defend against herbivores for many different plant species.

119 Here, we used existing data from a five-year long herbivore exclusion experiment that  
 120 was replicated in 14 Central German grasslands sites (total study area 114 km<sup>2</sup>, distances  
 121 between neighboring sites from 120 m to 6.5 km), where aboveground and belowground  
 122 herbivores were excluded with pesticides (Stein et al. 2010). Aboveground herbivore

123 exclusion resulted in a shift in the community composition (Fig. 1a) and above- and  
 124 belowground herbivore removal in a reduction of plant diversity (Stein et al. 2010,  
 125 aboveground herbivore removal alone did not decrease diversity). Among the 14 sites, 28  
 126 plant species were common enough to estimate species-specific responses to aboveground  
 127 herbivore exclusion. In the greenhouse, we performed multi-species feeding experiments with  
 128 generalist herbivores to assess species-specific values of plant defense. Specifically, we  
 129 performed a no-choice feeding experiment with caterpillars of the generalist herbivore  
 130 *Spodoptera littoralis* to assess herbivore performance (growth) on each of the 28 plant  
 131 species. Moreover, we assessed the feeding preferences of the generalist caterpillar and the  
 132 generalist slug species *Arion vulgaris* in a series of pairwise choice tests. Because of their  
 133 extreme polyphagy, both herbivores are commonly used to integratively measure plant  
 134 resistance against generalist herbivores (van Zandt 2007, Kempel et al. 2011). In a so-called  
 135 “plant tournament” we created a ranking of the most to the least preferred plant species. We  
 136 focused on generalist herbivores because, assessing 28 different plant species, it is impossible  
 137 to gather comparable data on performance and preference of specialist herbivores.  
 138 Additionally, we assessed several leaf traits (chlorophyll content, leaf thickness and specific  
 139 leaf area) which are known to affect the palatability of plant species. Specifically, we  
 140 addressed the following questions: i) Is the variation in plant defense against generalist  
 141 herbivores measured in feeding trials related to the response of plants to herbivore exclusion  
 142 in the field? And if yes, which attributes of plant defense (performance, preference or leaf  
 143 traits) can explain the shift in the plant community? ii) Are herbivore preference and  
 144 performance related to each other and do different generalist herbivores respond to plant  
 145 defense in a similar way?

146

147 **Material and methods**

148 ***Response of plant species to herbivore exclusion in the field***

149 To assess the response of plant species to herbivore exclusion in the field we used existing  
 150 data from a large herbivore exclusion experiment performed in 14 grassland sites in Central  
 151 Germany collected from Stein et al. (2010). Over five years (2002-2006) one of two 5x5m  
 152 plots in each site was regularly treated with pesticide (Dimethoate, Perfekthion, BASF,  
 153 Ludwigshafen, Germany; and molluscicide pellets: metylaldehyde, 0.6 g of active ingredient  
 154 per square meter) to reduce aboveground invertebrate herbivores (arthropods and molluscs),  
 155 whereas the other plot served as a control (for details on the herbivore exclusion experiment  
 156 see Stein et al. 2010, for details on the herbivore community in these grasslands see Unsicker  
 157 et al. 2006). The application of pesticides did not result in a complete exclusion of herbivores,  
 158 but reduced aboveground herbivory significantly (Unsicker, *personal observation*), however  
 159 we use the term herbivore exclusion hereafter. In each plot four 1 x 1 m subplots were  
 160 permanently marked. From 2003 to 2006 the percent cover of all vascular plants per subplot  
 161 was visually estimated twice during growing season and averaged across subplots in the  
 162 respective treatment plot. From these data we could calculate the response to the pesticide  
 163 treatment for 37 plant species that occurred on pesticide and control plots of at least three  
 164 study sites, however we only used 28 plant species for the greenhouse experiments as for  
 165 some species we did not obtain seeds or seeds did not germinate in sufficient numbers (Fig.  
 166 1a,b). In the exclusion experiment by Stein et al. (2010) also belowground herbivores were  
 167 excluded. However, here we only used data on the response of plants to aboveground  
 168 herbivore exclusion.

169 To evaluate the change in cover due to the pesticide treatment of each plant species, we  
 170 calculated the log response ratio  $\ln R$  (Hedges et al. 1999) as the logarithm of the cover in the  
 171 pesticide treatment divided by its cover in the control, averaged across all study sites and  
 172 years. Thus, a positive  $\ln R_{\text{cover}}$  indicates an increase in plant cover of a species in response to



173 pesticide application. In addition, absolute cover of a plant species in control and pesticide  
 174 plots was calculated as averaged cover across all study sites and years.

175

176 ***Cultivation of plant species to assess indicators of plant defense***

177 For 28 plant species of the field experiment (Fig. 1a, b), we assessed several indicators of  
 178 plant defense in independent greenhouse experiments, namely herbivore performance,  
 179 herbivore preference and several leaf traits related to palatability. In both herbivore  
 180 experiments in the greenhouse we used entire adult plants instead of e.g. leaf discs, to allow  
 181 herbivores to feed on all plant parts, and because detaching leaves might change plant  
 182 chemistry and inhibit induced resistance responses in plants (Karban and Baldwin 1997).  
 183 Further, the preference of herbivores might be affected by other components of a plant than  
 184 just the ones of a single leaf, such as architecture or scent.

185 In spring 2011, 14 seedlings of each of the 28 species were individually planted to 1.4 liter  
 186 pots filled with a nutrient poor mixture of washed sand and humus (ratio 9:1) and placed  
 187 outside in a common garden (Muri, near Bern, Switzerland) where they grew until the  
 188 experiments started in October 2011. All plants were watered when needed and were exposed  
 189 to natural levels of herbivory. Seeds of the species were obtained from a commercial supplier  
 190 of seeds of wild plants (Rieger-Hoffmann, Blaufelden-Raboldshausen, Germany).

191

192 **Assessment of herbivore performance using bioassays.** To assess herbivore performance,  
 193 we used caterpillars of the generalist herbivore *Spodoptera littoralis* (Boisduval)  
 194 (Lepidoptera: Noctuidae), which is known to feed on a wide range of plant species (Brown  
 195 and Dewhurst 1975). Although *Spodoptera littoralis* does not naturally occur on the

196 investigated grassland sites, the species is an adequate model organism for assessing plant  
 197 resistance against generalist herbivores (van Zandt 2007, Kempel et al. 2011). Caterpillars  
 198 were hatched from eggs (Syngenta, Stein, Switzerland) and reared on artificial diet before  
 199 they entered the experiments.

200 To assess the performance of *Spodoptera littoralis* on all 28 plant species, in October 2011  
 201 we transferred all adult plants to a greenhouse (14 °C, to 30 °C, a constant day length of 14 h,  
 202 and additional light) and individually bagged five plants per species with nylon gauze (12 cm  
 203 × 12 cm × 70 cm) which we randomly assigned to five blocks in the greenhouse. We added  
 204 two naïve caterpillars to each plant, and allowed them to feed for five days. To quantify  
 205 herbivore performance we assessed the increase in biomass of the caterpillars per plant by  
 206 recording mean caterpillar fresh mass before and after feeding. Using block and initial  
 207 caterpillar mass as covariates we used this adjusted caterpillar growth as a measure of  
 208 herbivore performance.

209 **Assessment of herbivore preference in a “plant tournament”.** We assessed the variation  
 210 in herbivore preference for the 28 grassland plant species using the generalist caterpillar  
 211 *Spodoptera littoralis*, and the generalist slug *Arion vulgaris* Moquin-Tandon (syn. *Arion*  
 212 *lusitanicus* Mabille; Arionidae). *Arion vulgaris* is widespread throughout Europe and  
 213 occupies a broad range of habitat types, including grasslands. Similar to *Spodoptera littoralis*,  
 214 *Arion vulgaris* is known to feed on a variety of plant species and is often used in bioassays  
 215 (Dirzo 1980, Pérez-Harguindeguy et al. 2003). Individuals of *Arion vulgaris* were collected  
 216 in the wild (Bremgartenwald, Bern, Switzerland) and kept in the lab for several weeks before  
 217 they entered the experiments.

218 For each herbivore species, we performed a series of pairwise choice-tests, which we called a  
 219 “plant tournament”, where herbivores could choose between two different plant species. We

220 connected the pots of two plant species with tape and a sand ramp, allowing herbivores to  
 221 walk easily from one plant to the other (Fig. 1c). Thus, all plant cues (olfactorial, visual,  
 222 mechanical and taste) could influence herbivore choice. Pairs of plants were kept at the same  
 223 greenhouse conditions as described above. At the beginning of each choice test, we placed  
 224 seven naïve third to fourth instar caterpillars or five naïve adult slugs, respectively, on a petri-  
 225 dish in the middle between the two pots and enclosed both pots together with nylon gauze (24  
 226 cm × 12 cm × 70 cm). Herbivores were then allowed to choose their preferred plant species  
 227 (Fig. 1c). After 24 hours, we counted the number of herbivores on each of the two plant  
 228 species, whereas herbivores that stayed in the middle between the plants, and thus did not  
 229 make a choice, were not counted. To estimate the degree of preferences we followed the rules  
 230 of association football (FIFA 2014), and awarded three points (a win) to the plant species  
 231 attracting two or more caterpillars or slugs more than the other plant, which received zero  
 232 points (a loss). When both plants attracted equal number of caterpillars or slugs or differed  
 233 only in one individual, one point was awarded to each plant (a draw), thereby minimizing  
 234 chance results. In addition to the points, we summed up the number of caterpillars or slugs  
 235 per plant species, which we called goals in analogy to association football, as an alternative  
 236 measure of preference. Because points and goals weighted the relative preference slightly  
 237 differently, we present results from both analyses.

238 A perfect preference ranking of all plant species would require all 28 plant species to play  
 239 against each other, resulting in a prohibitively large number of 378 tests ( $n(n-1)/2 = 378$ ). To  
 240 reduce the number of tests, we allocated species to groups and conducted two rounds of  
 241 round-robin tournaments. In the first round, we randomly assigned the 28 species into seven  
 242 groups of four species each and tested all combinations between pairs of species within  
 243 groups. After this first round, we ranked the species within each group based on points. In the  
 244 second round the species were randomly distributed into four new groups of seven species

245 each. Each new group had to contain one species from each group of the first round, and no  
 246 more than two species of a given rank recorded from the first round to create equally  
 247 powerful groups (see Appendix A, Text A1 and Fig. A1). Then we tested again all  
 248 combinations between pairs of species within groups and calculated the overall ranking of  
 249 species by summing the points, respectively the goals, of all tests per species of both rounds.  
 250 Thereby each species was tested against nine other species (three and six in the first and  
 251 second round, respectively), resulting in a total of 126 tests. From these data we obtained  
 252 preference rankings for plant species according to goals and points, for both caterpillars and  
 253 slugs. We assume that highly preferred plant species are poorly defended and vice versa.

254

255 **Leaf characteristics.** We measured several leaf traits, including leaf greenness as a proxy for  
 256 chlorophyll content, leaf thickness and specific leaf area. High chlorophyll content, low leaf  
 257 thickness and high specific leaf area are all suggested to increase plant palatability, and thus  
 258 to reduce plant resistance (Coley and Barone 1996, Poorter et al. 2004, Schuldt et al. 2012).  
 259 We assessed leaf greenness using a portable chlorophyll meter (SPAD-501) and leaf  
 260 thickness with a caliper on three randomly assigned leaves from each of five plants per  
 261 species used in the herbivore preference experiment and extracted values for specific leaf  
 262 area from a trait database (LEDA, (Kleyer et al. 2008).

263

264 *Statistical analysis*

265 We tested the relationships between caterpillar performance from the bioassays, caterpillar  
 266 and slug preference from the plant tournaments, leaf characteristics (specific leaf area,  
 267 chlorophyll content and leaf thickness) and the change in plant cover due to herbivore  
 268 exclusion by pesticide in the field experiment, using Pearson's correlation. To test whether  
 269 herbivores affected abundant species more than less abundant species (indicating frequency-

270 dependence) we also tested whether the change in plant cover due to herbivore exclusion was  
 271 related to the absolute cover of plant species in control plots using a randomization test that  
 272 accounts for spurious correlation. To assess whether the grasslands were dominated by highly  
 273 or less defended plant species, we tested whether the absolute cover of the plant species on  
 274 control and pesticide plots in the field was correlated with herbivore performance, preference  
 275 and leaf characteristics from the greenhouse experiments. Because more closely related plant  
 276 species are likely to be phenotypically more similar than others we included phylogenetic  
 277 relationships for all our analyses. First, we constructed a phylogenetic tree of all 28 plant  
 278 species based on a dated phylogeny of the European flora (Durka and Michalski 2012) and  
 279 tested for phylogenetic signals for each of our measured variables using K statistics on a  
 280 random walk model of phenotypic evolution (Blomberg et al. 2003). Second, we calculated  
 281 phylogenetic independent contrasts (PICs) for all variables. Because variables were more or  
 282 less phylogenetically constrained, we performed a phylogenetic regression (PGLS) to  
 283 calculate the strength of the phylogenetic signal in the residual variation (Grafen's rho) and  
 284 used this parameter to compute a specific tree with adjusted branch length, for each of our  
 285 variables. We then used these variable-specific trees to calculate the PICs (see Appendix B,  
 286 Text B1).

287 We also performed correlations without considering phylogeny, which yielded qualitatively  
 288 similar results as the correlations using PICs (see Appendix C, Table C1). We performed all  
 289 analysis in R using the package ape (R Development Core Team 2010).

290

## 291 **Results**

292 **Herbivore performance and preference.** Caterpillars preferred those plant species on which  
 293 they performed best (Table 1, Fig. 2a). Both herbivores, caterpillars and slugs, preferred the

294 same plant species (Fig. 2b, preference measured by points and by goals were highly  
 295 correlated for both caterpillars and slugs, Table 1). Accordingly, *Trifolium repens* was the  
 296 most preferred whereas *Hypericum perforatum* the least preferred plant species by both  
 297 herbivores. Moreover, when we excluded *Trifolium repens* from the analysis to test for  
 298 robustness, this turned out not to change the positive relationship between caterpillar and slug  
 299 preference ( $r = 0.56$ ,  $P = 0.002$  for goals,  $r = 0.44$ ,  $P = 0.02$  for points), indicating that  
 300 herbivores as different as insects and mollusks perceive plant defense in a similar way.

301 **Herbivore performance and preference in relation to plant cover changes and absolute**  
 302 **cover in the field.** Plant species that increased in cover in response to herbivore exclusion in  
 303 the field were also more preferred, and thus poorer defended, by both caterpillars and slugs in  
 304 the plant tournaments (Fig. 3a, b, Table 1; when we excluded *Vicia cracca* from the analysis  
 305 to test for robustness, both relationships also remained significant:  $r = 0.44$ ,  $P = 0.022$  for  
 306 caterpillar preference;  $r = 0.43$ ,  $P = 0.024$  for slug preference measured as goals). In contrast,  
 307 no relationship was found between caterpillar performance and plant cover changes in the  
 308 field (Fig. 3c, Table 1). This suggests that less defended species, measured as herbivore  
 309 preference rather than performance, can increase in cover, while better defended species (i.e.  
 310 less preferred species) lose their advantage over less defended species when herbivores are  
 311 absent, indicating a growth-defense tradeoff.

312 The most abundance plant species in the field did not benefit most from herbivore exclusion  
 313 (no relationship of change in plant cover in response to herbivore exclusion with absolute  
 314 plant cover in control plots:  $r = -0.11$  not significant in randomization test accounting for  
 315 spurious correlation), suggesting that frequency-dependent stabilizing effects of herbivores in  
 316 these grasslands were not very strong. The most abundant plant species in the field tended to  
 317 be the least preferred by the bioassay herbivores, especially of slugs (Table 1, see Appendix  
 318 C, Fig. C1). However, after herbivore exclusion, the negative plant abundance-herbivore

319 preference relationship diminished (relationship of absolute plant cover with slug preference  
 320 (measured as points) in control plots:  $r = -0.39$ ,  $P = 0.047$ ; in pesticide plots:  $r = -0.31$ ,  $P =$   
 321  $0.120$ ), although not significantly – suggesting that dominance may possibly have shifted  
 322 towards less defended species.

323 **Leaf traits in relation to herbivore performance, herbivore preference and cover**  
 324 **changes in the field.** Generally, the measured leaf characteristics neither were correlated  
 325 with herbivore performance nor with preference. Only chlorophyll content was positively  
 326 related with preference. In addition, all leaf characteristics that we measured were not related  
 327 to the change in cover in response to herbivore exclusion (Table 1). This indicates that  
 328 herbivore preference, and not herbivore performance or the measured leaf characteristics, is  
 329 the most relevant indicator of plant defenses affecting community composition.

330

## 331 **Discussion**

332 *Indicators of plant defense in relation to plant cover changes in the field.* Our combination  
 333 of a field exclusion experiment with plant defense experiments in the greenhouse showed that  
 334 the plant species decreasing in cover in response to herbivore exclusion in the field were the  
 335 ones less preferred by generalist herbivores, and thus better defended. Plant defense against  
 336 herbivores measured as herbivore preference therefore comes at the expense of weaker  
 337 growth under competitive conditions – a strong indication for the presence of a growth-  
 338 defense tradeoff in the grassland communities (Fig. 1a). Interestingly, only herbivore  
 339 preference, but not herbivore performance or any of the measured leaf traits, was related to  
 340 plant species' change in cover in response to herbivore exclusion in the field. This suggests  
 341 that plant defenses will only be effective if herbivores are sensitive to plant traits that provide  
 342 resistance and avoid defended plants. From a plant's point of view, reducing herbivore

343 performance through “antibiotic” effects is not necessarily favored by selection unless  
 344 herbivores avoid these plants and make decisions. Preference, i.e. herbivore behavior, which  
 345 is influenced by a whole congregation of factors such as nutritional value, plant defensive  
 346 structures or compounds, risk of predation or parasitism etc., might therefore be key to the  
 347 effects of consumers in structuring plant communities. The fact that traits affecting  
 348 preference are acting in concert might explain why it is so difficult to identify single plant  
 349 traits that capture variation in herbivory (Pearse & Hipp 2009, Carmona et al. 2011). The  
 350 importance of herbivore behavior has been pointed out repeatedly (Adler & Grunbaum 1999,  
 351 Karban 2011), but has received little recognition, although information on herbivore choice  
 352 might help us to better understand the costs and benefits of defense (a defense that reduces  
 353 herbivore performance might not be beneficial to a plant if herbivores feed longer and hence  
 354 damage a plant more in order to complete development). A simple growth-defense tradeoff  
 355 might therefore not adequately reflect opposing selection pressures occurring in nature. A  
 356 plant growth - herbivore preference tradeoff seems to be much more realistic. Our data shows  
 357 that herbivore preference is related to the vigorous growth of plant species in grasslands. This  
 358 indirectly demonstrates that by selectively feeding, generalist herbivores can change  
 359 dominance hierarchy among plant species and shift plant community structure towards less  
 360 preferred plant species.

361         Although many studies have experimentally manipulated abiotic or biotic limiting  
 362 factors (e.g. through nitrogen addition or herbivore exclusion) to identify filters driving the  
 363 assembly and composition of plant communities and to search for tradeoffs between plant  
 364 strategies (Viola et al. 2010, Lind et al. 2013), only few have provided insight into the  
 365 underlying ecological mechanisms (HilleRisLambers et al. 2012). Schädler et al. (2003)  
 366 related the response of 13 herbaceous plant species to invertebrate herbivore exclusion with  
 367 plant palatability based on herbivore performance of a generalist slug and the generalist



368 house cricket, but found no relationship between the palatability of plants and their cover  
 369 change due to herbivore exclusion. This is either because a growth-defense tradeoff might  
 370 play a minor role in their study system, a successional field, in comparison to grasslands or,  
 371 as suggested by our data, because they measured palatability which is based on herbivore  
 372 performance, rather than preference. Harpole and Tilman (2006) assessed species-specific  
 373 indices for competitive ability for nitrogen and could show that nitrogen addition in an old  
 374 field led to an increase of poor competitors at the expense of strong competitors for nitrogen.  
 375 With our assessment of species-specific values of plant defense measured as herbivore  
 376 preference of entire plant communities we could show that invertebrate herbivore exclusion  
 377 in the field led to an increase of highly preferred (and likely poorly defended) species at the  
 378 expense of less preferred (and likely strongly defended) species, which adds novel  
 379 information on the ecological mechanism of how biotic limiting factors affect the  
 380 composition of plant communities.

381         Generalist and specialist herbivores can both have stabilizing and equalizing effects  
 382 on plant communities. Generalist herbivores are suggested to mainly equalize fitness between  
 383 plant species by selective feeding and inflicting greater damage on vigorously growing but  
 384 poorly defended plant species, but they can also stabilize plant communities if they feed  
 385 preferentially on whatever is the most abundant plant species in a community (Chase et al.  
 386 2002, Murdoch et al. 1969). Specialist herbivores are suggested to mainly stabilize plant  
 387 communities via frequency-dependent predation, creating a rare plant species advantage, but  
 388 they can theoretically also equalize fitness differences between species if they specialize  
 389 predominantly on fast growing but poorly defended plant species (Chesson 2000, Chase et al.  
 390 2002). The frequency-dependent effects (stabilizing mechanisms) are essential for  
 391 coexistence and have been mainly attributed to specialist herbivores. Therefore, ecologists  
 392 have assigned specialist insect herbivores a stronger role in promoting plant diversity and

393 coexistence than generalist insect herbivores (Carson & Root 1999, Pacala & Crawley 1992,  
 394 Allan & Crawley 2011). We did not find a frequency-dependent effect of herbivores in the  
 395 field experiment, i.e. the most abundant plant species did not benefit most from herbivore  
 396 exclusion, suggesting that stabilizing effects of herbivores via frequency-dependent  
 397 consumption, were rather low in our grasslands. Instead, herbivores influenced the  
 398 composition of plant communities: the change in plant cover might have been mainly driven  
 399 by selective feeding of herbivores and potentially a trade-off between the vigorous growth of  
 400 plants and herbivore preference. The fact that generalist herbivores, in particular  
 401 grasshoppers, were dominating on the 14 grassland sites that our study is based on (for  
 402 detailed information on the amount of generalist and specialist herbivores see Unsicker et al.  
 403 2006) suggests that generalist rather than specialist herbivores were mainly responsible for  
 404 the compositional shift of plants in these grasslands. In line with our data, Bagchi et al.  
 405 (2014) also found no evidence for stabilizing density-dependent effects of insect herbivores  
 406 on plants in a tropical forest, but effects on plant species composition. This together with our  
 407 findings therefore emphasize the importance of equalizing effects of invertebrate  
 408 aboveground herbivores on plant communities, and shed new light on our mechanistic  
 409 understanding of grassland ecosystems.

410         So far, the relative importance of generalist and specialist aboveground invertebrate  
 411 herbivores for the composition of plant communities is not known because of the difficulty to  
 412 manipulate specifically the density of either of two groups. Therefore it remains speculative  
 413 whether the equalizing effect of herbivores in our study was caused by generalists preferring  
 414 poorly defended plant species or by specialists that have predominantly specialized on fast  
 415 growing but poorly defended plant species. Whether the impact of specialist herbivores  
 416 differs between more or less abundant plant species or whether variation in plant defense  
 417 against generalist herbivores is related with different loads of specialist herbivores (Novotny

418 and Basset 2005), remains unclear. Similarly, we lack knowledge on feeding preferences of  
 419 belowground herbivores (but see Schallhart et al. 2012), although they did affect plant  
 420 community diversity and composition in our field experiments (e.g. Stein et al. 2010). Lower  
 421 mobility belowground might generally impede selective feeding, leading to more frequency-  
 422 dependent herbivory. Thus, identifying the different roles of generalist and specialist as well  
 423 as of above- and belowground herbivores for the assembly and composition of plant  
 424 communities and the maintenance of plant diversity therefore is a major challenge for future  
 425 research.

426 Whether plant communities are dominated by good competitors that are vulnerable to  
 427 herbivores, or by poor competitors that are highly defended, depends on the overall herbivore  
 428 pressure at a given site (Holt and Lawton 1994). In our study, the most abundant plant species  
 429 tended to be least preferred by the bioassay herbivores, suggesting that herbivore pressure in  
 430 the grassland sites is rather high (see Appendix C, Fig. C1). This was mainly driven by the  
 431 most abundant species *Festuca rubra*, which is among the least preferred plant species,  
 432 especially by slugs. However, after five years of herbivore exclusion, the negative  
 433 relationship between plant abundance and herbivore preference diminished (see Appendix C,  
 434 Fig. C1) – suggesting that dominance might shift away from highly defended species –  
 435 however, although in the expected direction, this change in slope was far from being  
 436 significant (no significant abundance  $\times$  pesticide treatment interaction). Therefore, while  
 437 aboveground herbivores are likely to select for defended plant species and to co-control the  
 438 abundance of plant species in a community, it might take longer than five years of herbivore  
 439 exclusion to be reversed (Allan and Crawley 2011).

440 ***Herbivore performance and preference.*** In insects, the preference and the performance of  
 441 herbivores are hypothesized to be tightly linked (Gripengberg et al. 2010). Although this  
 442 pattern, also known as the “mother-knows-best-principle” (Jaenike 1978), mainly refers to

443 oviposition preferences of female insects and the performance of their offspring, our results  
 444 show that, also within the life of an individual herbivore, generalist caterpillars, when given a  
 445 choice, prefer those plant species on which they perform best. While the positive preference-  
 446 performance relationship may be of little surprise, the considerable amount of scatter is  
 447 remarkable. *Achillea millefolium*, for example, the plant species on which caterpillars gained  
 448 most weight, was hardly preferred (rank 8 out of 28) by the herbivores. Similarly, feeding on  
 449 the second-most preferred species, *Alopecurus pratensis*, caterpillars gained only little  
 450 weight. Thus, although food preference seems to be linked to herbivore performance, other  
 451 factors, such as either chemical feeding deterrents that do not directly affect herbivore  
 452 performance (Dicke 2009), or the suitability of plants as a protective structure from natural  
 453 enemies (Björkman et al. 1997) or competitors (Wise and Weinberg 2002), might affect a  
 454 herbivore's preference and hence fine-tune performance-preference relationships.

455         Although herbivores are diverse and cover a variety of feeding strategies, generalist  
 456 herbivores are suggested to respond similarly to components of plant leaf quality, such as  
 457 chemical defense, physical defense and nutritive quality (Herms and Mattson 1992,  
 458 Pérez-Harguindeguy et al. 2003). Accordingly, in our experiment the preference of the two  
 459 herbivore species to the 28 plant species was highly correlated. Both showed higher  
 460 preferences for legumes, supporting the view of a strong influence of a plant's nutritive value  
 461 on herbivore preference (Dirzo 1980, Pérez-Harguindeguy et al. 2003, Loranger et al. 2012).  
 462 Similarly, both herbivores disliked *Hypericum perforatum*, whose chemical arsenal is known  
 463 to be toxic to phytophagous insects (Maron et al. 2004). Thus, generalist herbivores as  
 464 different as mollusks and lepidopteran larvae seem to perceive the interplay of several factors  
 465 such as defensive compounds and nutritive value of plants in a similar way.

466

467 **Conclusions**

468 We show that variation in plant defense against herbivory that do affect herbivore preference  
 469 may drive the composition of plant communities. Generalist herbivores reduce the  
 470 performance of less defended plant species, thereby indirectly boosting the more highly  
 471 defended plant species. That more preferred plant species were those that increased in cover  
 472 after herbivores were excluded is a strong indication for a tradeoff between plant growth and  
 473 herbivore preference. Such a growth-preference tradeoff might much better reflect opposing  
 474 selection pressures in nature than the usually described growth-defense tradeoff, as it better  
 475 takes into account the costs and benefit of defenses for plants. Our results indicate that  
 476 generalist herbivores seem to equalize fitness between plant species by selectively feeding on  
 477 more preferred (ergo less defended), but more competitive (vigorously growing), species.  
 478 Interestingly, different generalist herbivores perceive plant resistance similarly - an essential  
 479 prerequisite if the differential abilities of species to defend themselves, at least against  
 480 generalist herbivores, are expected to translate into changes in natural communities.  
 481 Therefore, our approach of assessing a “species-specific value of plant defense” against  
 482 generalist herbivores measured as herbivore preference for an entire plant community  
 483 provides novel information on ecological mechanisms which is required to understand how  
 484 biotic limiting factors affect the assembly of plant communities.

485

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492

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636 **Supplemental material**

637 **Appendix A:** Detailed description of the assessment of herbivore preference in a “plant  
638 tournament” (Text A1) and mode of the plant tournament (Figure A1).

639 **Appendix B:** Manual to compute phylogenetic independent contrasts for variables differing  
640 in the strength of phylogenetic signal, using the statistical software R.

641 **Appendix C:** Additional results on the relationship between the mean absolute cover of the  
642 plant species in the grassland sites and the preference of the slug *Arion vulgaris* (Fig. C1),  
643 and raw correlations between plant species traits assessed in the greenhouse and field data on  
644 plant cover (Table C1).

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**Table 1:** Correlations between herbivore performance, herbivore preference and leaf characteristics, and the change in cover in response to pesticide and the absolute cover in control and pesticide plots from the field experiment (Stein et al. 2010). Shown are Pearson’s correlation coefficients using phylogenetic independent contrasts (with adjusted trees for each variable), and K statistics as a measure of a phylogenetic signal. Significance levels are denoted with \*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001 and significant values presented in bold.

	Performance		Preference				Leaf characteristics			Phylogenetic signal
	Caterpillars		Caterpillar		Slug		SLA	Leaf	Chlorophyll	K
	goals	points	goals	points	goals	points	thickness	content		
Performance caterpillar									<b>0.56</b> **	
Preference caterpillar (goals)	<b>0.42</b> *								0.36	
Preference caterpillar (points)	<b>0.45</b> *	<b>0.91</b> ***							<b>0.40</b> *	
Preference slugs (goals)	<b>0.42</b> *	<b>0.71</b> ***	<b>0.64</b> ***						0.27	
Preference slugs (points)	<b>0.39</b> *	<b>0.68</b> ***	<b>0.61</b> ***	<b>0.96</b> ***					0.26	
Specific leaf area	-0.15	0.32	0.25	-0.02	-0.01				0.17	
Leaf thickness	-0.29	-0.33	-0.36	0.1	0.06	-0.18			<b>0.62</b> **	
Chlorophyll content	0.02	0.34	0.33	<b>0.42</b> *	0.36	-0.03	0.26		0.24	
Change in cover	-0.059	<b>0.4</b> *	0.27	<b>0.46</b> *	<b>0.46</b> *	-0.02	0.16	0.11	0.23	
Absolute cover control	-0.29	-0.12	-0.19	-0.31	<b>-0.39</b> *	-0.17	-0.10	-0.04	0.16	
Absolute cover pesticide	-0.24	-0.48	-0.11	-0.22	-0.31	-0.18	-0.060	-0.02	0.17	

**Figure 1** a) Change in cover in response to herbivore exclusion in the field of the 28 plant species. Species that increased in cover due to herbivore exclusion in the field are hypothesized to be less defended than species that decreased in cover. b) Phylogenetic tree of the studied plant species according to Durka and Michalski (2012). c) Schematic picture of a pairwise choice-test for caterpillars. In a test, seven caterpillars of the generalist *Spodoptera littoralis* (or five individuals of the generalist slug *Arion vulgaris*, not shown) were placed between two plant species. Herbivores were allowed to explore the playing field and feed on their preferred plant species. After 24 hours, herbivores on each plant were counted as goals, herbivores that stayed close to the half-way line and thus did not make a choice, were not counted (e.g. plant on the left = 4 goals, plant on the right = 1 goal). Following the rules of association football we awarded three points (a win) to the plant species attracting two or more caterpillars or slugs more than the other plant, which received zero points (a loss). When both plants attracted equal number of caterpillars or slugs or differed only in one individual, one point was awarded to each plant (a draw) (e.g. plants on the left = 3 points, plant on the right = 0 points).

**Figure 2** Relationship between a) the performance (measured as final caterpillar biomass [g] adjusted for initial biomass in the bioassay experiment) and the preference (“plant tournament”) of the caterpillar *Spodoptera littoralis*, and b) the preference of the caterpillars and the slug *Arion vulgaris* (“plant tournaments”) for the 28 plant species. Depicted are raw data points and significant relationships indicated by a fitted line for visualization.

**Figure 3** Relationship between the change in cover in response to herbivore exclusion in the field of the 28 plant species and a) caterpillar preference, b) slug preference and c) caterpillar

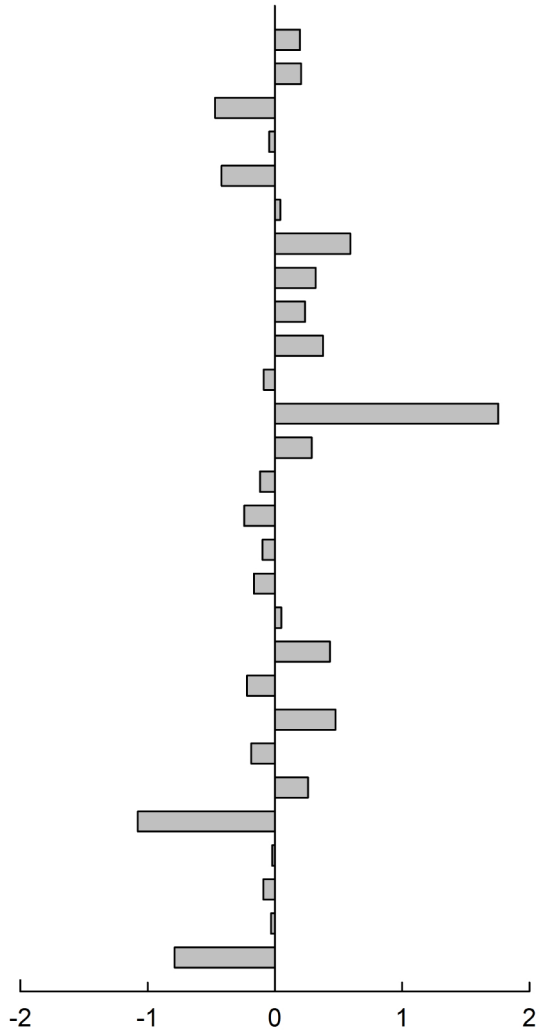
performance (measured as final caterpillar biomass [g] adjusted for initial biomass in the bioassay experiment) assessed in independent greenhouse experiments. Depicted are raw data points, and significant relationships indicated by a fitted line for visualization.

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a)

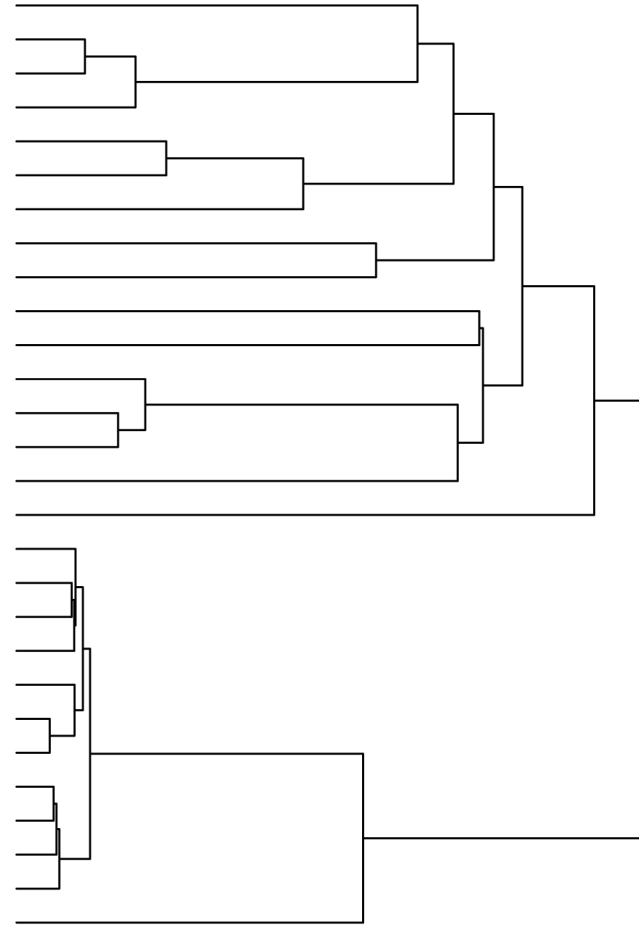
Defence?



- Change in cover +

( $\ln R_{\text{cover}}$  of species grown in herbivore exclusion plots relative to control plots)

b)



c)





