

Herbivore preference drives plant community composition

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

Key words: biotic factors; generalist herbivores; growth–defense trade-off; growth-rate hypothesis; herbivore performance; herbivore preference; leaf traits; plant coexistence; plant resistance.

INTRODUCTION

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

Among important biotic interactions, herbivores are commonly invoked to explain the coexistence of plant species in a community, because they alter competitive interactions between species (Pacala and Crawley 1992, Chesson 2000). Herbivores can promote coexistence if their consumption depends on the frequency of plants and thereby impairs abundant species more than less abundant ones (negative frequency dependence, Janzen-Connell hypothesis), which is seen as a stabilizing mechanism. Theory suggests that stabilizing mecha-

nisms are mainly driven by specialist herbivores that hold down the density of their host plant (e.g., Chesson 2000). However, also generalist herbivores can have stabilizing effects on plant communities when they switch host or food plant species and consume disproportionately whichever species is most abundant (Murdoch 1969, Chase et al. 2002). Other theories on how herbivores can promote coexistence require a trade-off between the vigorous growth of plants and their defense against consumers, assuming that defense is costly and constrains investment in other important traits (Coley et al. 1985, Herms and Mattson 1992, Viola et al. 2010, Kempel et al. 2011, Lind et al. 2013). If the plants growing most vigorously in a community are also the least defended ones, herbivores promote coexistence by selectively feeding on more vigorously growing and hence less defended plant species (Pacala and Crawley 1992, Carson and Root 1999), thereby reducing average fitness differences between species, which is considered as an equalizing mechanism (Chesson 2000).

In spite of the importance of plant defense, it is notoriously difficult to assess. Plants evolved a variety of defense strategies to cope with their enemies. Such

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

The balancing role of herbivores has been demonstrated several times, especially in grassland communities, using herbivore exclusion experiments. Results from these experiments often found a shift in plant community composition and a loss of diversity due to competitive exclusion when herbivores are excluded (Brown and Gange 1992, Carson and Root 1999, Allan et al. 2010, Stein et al. 2010). Such experimental manipulations can provide strong evidence that herbivores are critical for plant community composition and diversity. If the most abundant plant species also benefit most from herbivore exclusion such experiments can demonstrate a stabilizing effect of herbivores on plant communities. However, they cannot demonstrate an equalizing effect of herbivores, since they neither demonstrate costs of defense nor a mechanistic link to a growth-defense trade-off, which requires species-specific information on plant defense values: if defense is costly and trading off with vigorous growth, then the least defended species should suffer most from herbivores, thus benefit most from their exclusion. Accordingly, highly defended species should decrease in cover if herbivores are excluded, since they lose their fitness advantage over less defended species and suffer from interspecific competition (Fig. 1a). Indeed, it has been shown that the exclusion of vertebrate herbivores resulted in an increase of plant species that were

preferred by the grazers (Diaz 2000, Bråthen and Oksanen 2001). Similarly, the abundance of plant species in communities allowed to assemble from seeds was correlated with herbivore preference, assessed in feeding trials (Burt-Smith et al. 2003). However, whether the shift in composition of an entire plant community in response to invertebrate herbivore exclusion can be explained by differences in herbivore performance or preference among plant species has rarely been tested (but see Schädler et al. 2003), largely due to the difficulty of assessing the ability to defend against herbivores for many different plant species.

Here, we used existing data from a five-year-long herbivore-exclusion experiment that was replicated in 14 Central German grasslands sites (total study area 114 km², distances between neighboring sites from 120 m to 6.5 km), where aboveground and belowground herbivores were excluded with pesticides (Stein et al. 2010). Aboveground herbivore exclusion resulted in a shift in the community composition (Fig. 1a) and above- and belowground herbivore removal in a reduction of plant diversity (Stein et al. 2010; aboveground herbivore removal alone did not decrease diversity). Among the 14 sites, 28 plant species were common enough to estimate species-specific responses to aboveground herbivore exclusion. In the greenhouse, we performed multi-species feeding experiments with generalist herbivores to assess species-specific values of plant defense. Specifically, we performed a no-choice feeding experiment with caterpillars of the generalist herbivore *Spodoptera littoralis* to assess herbivore performance (growth) on each of the 28 plant species. Moreover, we assessed the feeding preferences of the generalist caterpillar and the generalist slug species *Arion vulgaris* in a series of pairwise choice tests. Because of their extreme polyphagy, both herbivores are commonly used to integratively measure plant resistance against generalist herbivores (van Zandt 2007, Kempel et al. 2011). In a so-called “plant tournament” we created a ranking of the most to the least preferred plant species. We focused on generalist herbivores because, in assessing 28 different plant species, it is impossible to gather comparable data on performance and preference of specialist herbivores. Additionally, we assessed several leaf traits (chlorophyll content, leaf thickness, and specific leaf area) which are known to affect the palatability of plant species. Specifically, we addressed the following questions: (1) Is the variation in plant defense against generalist herbivores measured in feeding trials related to the response of plants to herbivore exclusion in the field? If yes, which attributes of plant defense (performance, preference or leaf traits) can explain the shift in the plant community? (2) Are herbivore preference and performance related to each other and do different generalist herbivores respond to plant defense in a similar way?

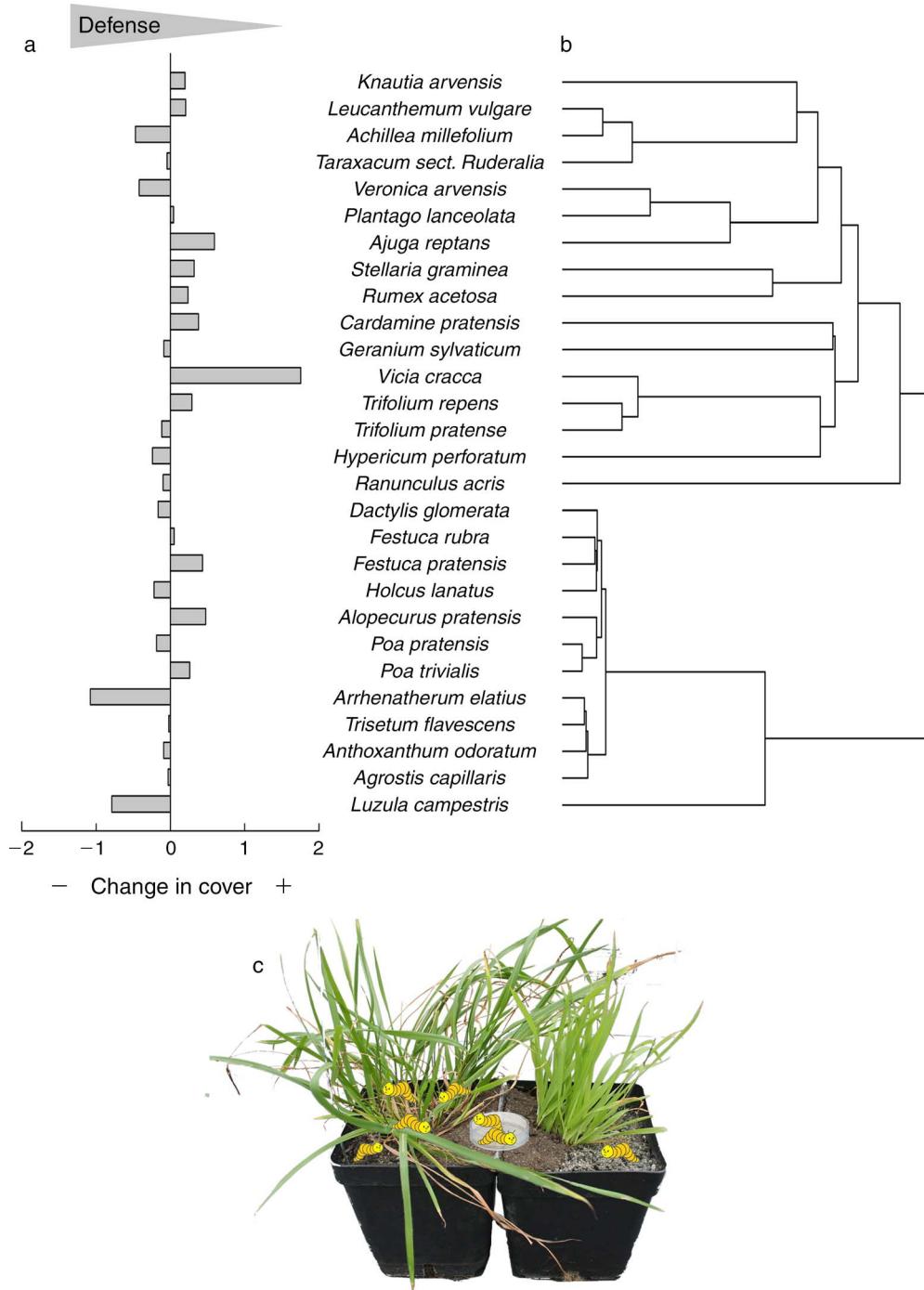


FIG. 1. (a) Change in cover ($\ln R_{\text{cover}}$) in response to herbivore exclusion in the field of the 28 plant species. Species that increased in cover (+) due to herbivore exclusion in the field are hypothesized to be less defended than species that decreased in cover (-). Cover is measured as log response ratio ($\ln R$) of species grown in herbivore-exclusion plots relative to control plots. (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 has four goals, plant on the right has one goal). Following the rules of association football, we awarded three points 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., plant on the left has three points, plant on the right has zero points).

MATERIAL AND METHODS

Response of plant species to herbivore exclusion in the field

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

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

Cultivation of plant species to assess indicators of plant defense

For 28 plant species of the field experiment (Fig. 1a, b), we assessed several indicators of plant defense in independent greenhouse experiments, namely herbivore performance, herbivore preference, and several leaf traits related to palatability. In both herbivore experiments in the greenhouse, we used entire adult plants instead of, e.g., leaf discs, to allow herbivores to feed on all plant parts, and because detaching leaves might

change plant chemistry and inhibit induced resistance responses in plants (Karban and Baldwin 1997). Further, the preference of herbivores might be affected by other components of a plant than just the ones of a single leaf, such as architecture or scent.

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

Assessment of herbivore performance using bioassays.—To assess herbivore performance, we used caterpillars of the generalist herbivore *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), which is known to feed on a wide range of plant species (Brown and Dewhurst 1975). Although *Spodoptera littoralis* does not naturally occur on the investigated grassland sites, the species is an adequate model organism for assessing plant resistance against generalist herbivores (van Zandt 2007, Kempel et al. 2011). Caterpillars were hatched from eggs (Syngenta, Stein, Switzerland) and reared on artificial diet before they entered the experiments.

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

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

For each herbivore species, we performed a series of pairwise choice tests, which we called a “plant tournament,” where herbivores could choose between two different plant species. We connected the pots of two plant species with tape and a sand ramp, allowing herbivores to walk easily from one plant to the other (Fig. 1c). Thus, all plant cues (olfactorial, visual, mechanical, and taste) could influence herbivore choice. Pairs of plants were kept at the same greenhouse conditions as described in *Assessment of herbivore performance using bioassays*. At the beginning of each choice test, we placed seven naïve third- to fourth-instar caterpillars or five naïve adult slugs, respectively, on a petri dish in the middle between the two pots and enclosed both pots together with nylon gauze ($24 \times 12 \times 70$ cm). Herbivores were then allowed to choose their preferred plant species (Fig. 1c). After 24 hours, we counted the number of herbivores on each of the two plant species, whereas herbivores that stayed in the middle between the plants, and thus did not make a choice, were not counted. To estimate the degree of preferences, we followed the rules of association football (FIFA 2014), and 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 an equal number of caterpillars or slugs or differed only in one individual, one point was awarded to each plant (a draw), thereby minimizing chance results. In addition to the points, we summed up the number of caterpillars or slugs per plant species, which we called goals in analogy to association football, as an alternative measure of preference. Because points and goals weighted the relative preference slightly differently, we present results from both analyses.

A perfect preference ranking of all plant species would require all 28 plant species to play against each other, resulting in a prohibitively large number of 378 tests ($n(n - 1)/2 = 378$). To reduce the number of tests, we allocated species to groups and conducted two rounds of round-robin tournaments. In the first round, we randomly assigned the 28 species into seven groups of four species each and tested all combinations between pairs of species within groups. After this first round, we ranked the species within each group based on points. In the second round the species were randomly distributed into four new groups of seven species each. Each new group had to contain one species from each group of the first round, and no more than two species of a given rank recorded from the first round to create equally powerful groups (see Appendix A: section A1 and Fig. A1). Then we tested again all combinations between pairs of species within groups and calculated the overall ranking of species by summing the points, respectively the goals, of all tests per species of both rounds. Thereby each species was tested against nine other species (three and six in the first and second round, respectively), resulting in a total of 126 tests. From these data we

obtained preference rankings for plant species according to goals and points, for both caterpillars and slugs. We assume that highly preferred plant species are poorly defended and vice versa.

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

Statistical analysis

We tested the relationships between caterpillar performance from the bioassays, caterpillar and slug preference from the plant tournaments, leaf characteristics (specific leaf area, chlorophyll content, and leaf thickness), and the change in plant cover due to herbivore exclusion by pesticide in the field experiment, using Pearson’s correlation. To test whether herbivores affected abundant species more than less-abundant species (indicating frequency dependence), we also tested whether the change in plant cover due to herbivore exclusion was related to the absolute cover of plant species in control plots using a randomization test that accounts for spurious correlation. To assess whether the grasslands were dominated by highly or less-defended plant species, we tested whether the absolute cover of the plant species on control and pesticide plots in the field was correlated with herbivore performance, preference, and leaf characteristics from the greenhouse experiments. Because more closely related plant species are likely to be phenotypically more similar than others, we included phylogenetic relationships for all our analyses. First, we constructed a phylogenetic tree of all 28 plant species based on a dated phylogeny of the European flora (Durka and Michalski 2012) and tested for phylogenetic signals for each of our measured variables using K statistics on a random walk model of phenotypic evolution (Blomberg et al. 2003). Second, we calculated phylogenetic independent contrasts (PICs) for all variables. Because variables were more or less phylogenetically constrained, we performed a phylogenetic regression (PGLS) to calculate the strength of the phylogenetic signal in the residual variation (Grafen’s ρ) and used this parameter to compute a specific tree with adjusted branch length, for each of our variables. We then used these variable-specific trees to calculate the PICs (see Appendix B: section B1).

We also performed correlations without considering phylogeny, which yielded qualitatively similar results as

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).

Response variable	Performance		Preference		Leaf characteristics			Phylogenetic signal	
	Caterpillars	Caterpillar		Slug		SLA	Leaf thickness	Chlorophyll content	K
		Goals	Points	Goals	Points				
Caterpillar performance								0.56**	
Caterpillar preference, goals	0.42*							0.36	
Caterpillar preference, points	0.45*	0.91***						0.40*	
Slug preference, goals	0.42*	0.71***	0.64***					0.27	
Slug preference, points	0.39*	0.68***	0.61***	0.96***				0.26	
Specific leaf area, SLA	-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		0.62**	
Chlorophyll content	0.02	0.34	0.33	0.42*	0.36	-0.03	0.26	0.24	
Change in cover	-0.059	0.4*	0.27	0.46*	0.46*	-0.02	0.16	0.11	
Absolute cover control	-0.29	-0.12	-0.19	-0.31	-0.39*	-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

Notes: 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. Significant values are highlighted in boldface type.

* $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$.

the correlations using PICs (see Appendix C: Table C1). We performed all analysis in R using the package ape (R Development Core Team 2010).

RESULTS

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

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

The most abundant plant species in the field did not benefit most from herbivore exclusion (no relationship of change in plant cover in response to herbivore exclusion with absolute plant cover in control plots, $r = -0.11$ not significant in randomization test accounting for spurious correlation), suggesting that frequency-dependent stabilizing effects of herbivores in these grasslands were not very strong. The most abundant plant species in the field tended to be the least preferred by the bioassay herbivores, especially of slugs (Table 1, see Appendix C: Fig. C1). However, after herbivore exclusion, the negative plant-abundance–herbivore-preference relationship diminished (relationship of absolute plant cover with slug preference (measured as points) in control plots, $r = -0.39$, $P = 0.047$; in pesticide plots, $r = -0.31$, $P = 0.120$), although not significantly, suggesting that dominance may possibly have shifted toward less-defended species.

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

DISCUSSION

Indicators of plant defense in relation to plant cover changes in the field.—Our combination of a field exclusion experiment with plant defense experiments in the greenhouse showed that the plant species decreasing in cover in response to herbivore exclusion in the field were the ones less preferred by generalist herbivores, and

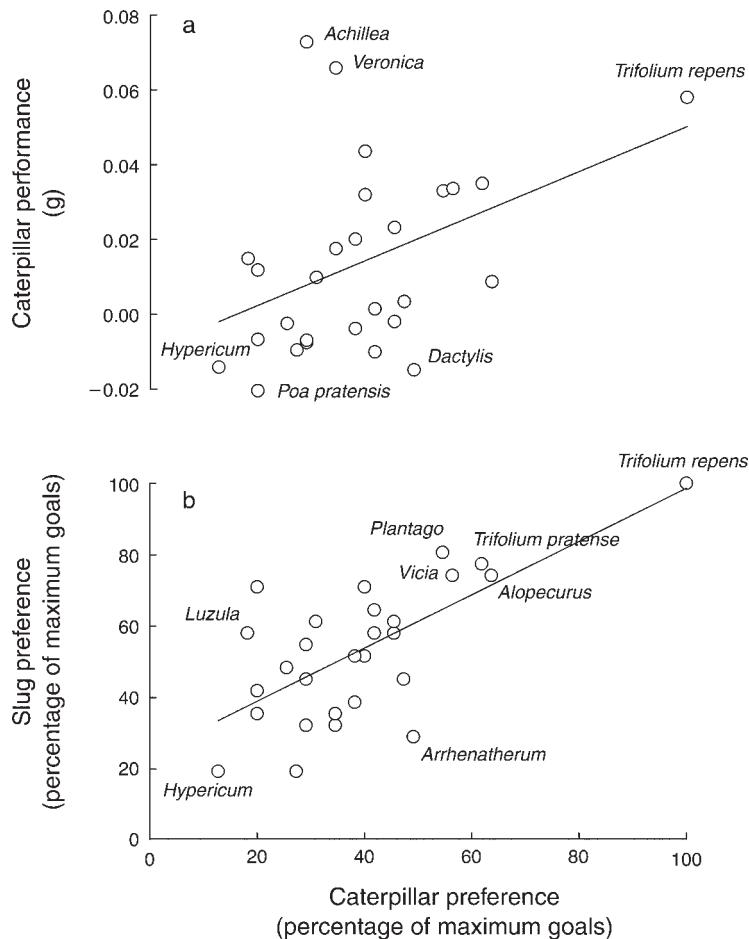


FIG. 2. Relationship between (a) the performance (growth, measured as final caterpillar biomass adjusted for initial biomass in the bioassay experiment) and the preference (plant tournament) of the caterpillar *Spodoptera littoralis* and (b) the preference of the caterpillars and the slug *Arion vulgaris*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

thus better defended. Plant defense against herbivores measured as herbivore preference therefore comes at the expense of weaker growth under competitive conditions, a strong indication for the presence of a growth–defense trade-off in the grassland communities (Fig. 1a). Interestingly, only herbivore preference, but not herbivore performance or any of the measured leaf traits, was related to plant species' change in cover in response to herbivore exclusion in the field. This suggests that plant defenses will only be effective if herbivores are sensitive to plant traits that provide resistance and avoid defended plants. From a plant's point of view, reducing herbivore performance through “antibiotic” effects is not necessarily favored by selection unless herbivores avoid these plants and make decisions. Preference, i.e., herbivore behavior, which is influenced by a whole congregation of factors such as nutritional value, plant defensive structures or compounds, risk of predation or parasitism, etc., might therefore be key to the effects of consumers in structuring plant communities. The fact that traits affecting preference are acting in concert

might explain why it is so difficult to identify single plant traits that capture variation in herbivory (Pearse and Hipp 2009, Carmona et al. 2011). The importance of herbivore behavior has been pointed out repeatedly (Adler and Grunbaum 1999, Karban 2011), but has received little recognition, although information on herbivore choice might help us to better understand the costs and benefits of defense (a defense that reduces herbivore performance might not be beneficial to a plant if herbivores feed longer and hence damage a plant more in order to complete development). A simple growth–defense trade-off might therefore not adequately reflect opposing selection pressures occurring in nature. A plant–growth–herbivore–preference trade-off seems to be much more realistic. Our data shows that herbivore preference is related to the vigorous growth of plant species in grasslands. This indirectly demonstrates that by selectively feeding, generalist herbivores can change dominance hierarchy among plant species and shift plant community structure toward less-preferred plant species.

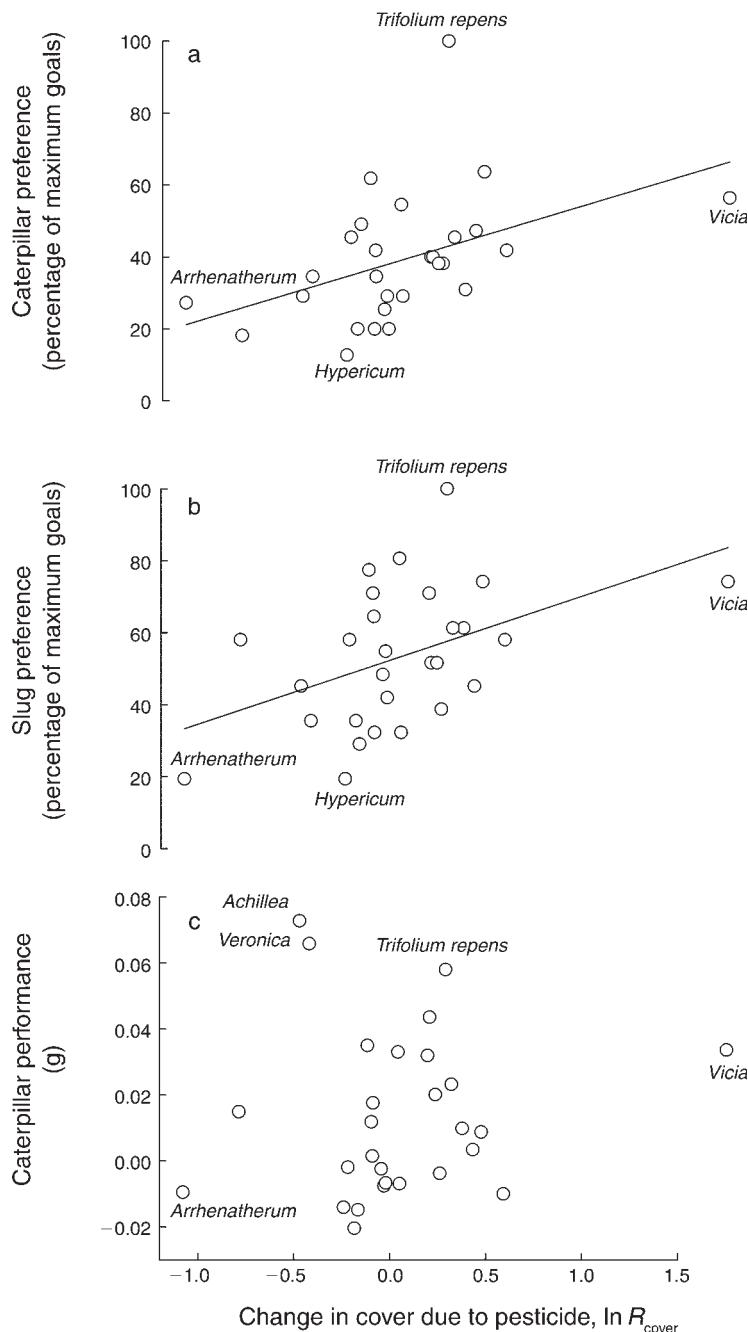


FIG. 3. Relationship between the change in cover in response to herbivore exclusion in the field of the 28 plant species and (a) caterpillar preference, (b) slug preference, and (c) caterpillar performance (growth, measured as final caterpillar biomass adjusted for initial biomass in the bioassay experiment) assessed in independent greenhouse experiments. Depicted are raw data points, and significant relationships indicated by a fitted line for visualization. Only some species are labeled for clarity.

Although many studies have experimentally manipulated abiotic or biotic limiting factors (e.g., through nitrogen addition or herbivore exclusion) to identify filters driving the assembly and composition of plant communities and to search for trade-offs between plant strategies (Viola et al. 2010, Lind et al. 2013), only few have provided insight into the underlying ecological

mechanisms (HilleRisLambers et al. 2012). Schädler et al. (2003) related the response of 13 herbaceous plant species to invertebrate herbivore exclusion with plant palatability based on herbivore performance of a generalist slug and the generalist house cricket, but found no relationship between the palatability of plants and their cover change due to herbivore exclusion. This

is either because a growth–defense trade-off might play a minor role in their study system, a successional field, in comparison to grasslands or, as suggested by our data, because they measured palatability, which is based on herbivore performance, rather than preference. Harpole and Tilman (2006) assessed species-specific indices for competitive ability for nitrogen and could show that nitrogen addition in an old field led to an increase of poor competitors at the expense of strong competitors for nitrogen. With our assessment of species-specific values of plant defense measured as herbivore preference of entire plant communities we could show that invertebrate herbivore exclusion in the field led to an increase of highly preferred (and likely poorly defended) species at the expense of less preferred (and likely strongly defended) species, which adds novel information on the ecological mechanism of how biotic limiting factors affect the composition of plant communities.

Generalist and specialist herbivores can both have stabilizing and equalizing effects on plant communities. Generalist herbivores are suggested to mainly equalize fitness between plant species by selective feeding and inflicting greater damage on vigorously growing but poorly defended plant species, but they can also stabilize plant communities if they feed preferentially on whatever is the most abundant plant species in a community (Murdoch 1969, Chase et al. 2002). Specialist herbivores are suggested to mainly stabilize plant communities via frequency-dependent predation, creating a rare plant species advantage, but they can theoretically also equalize fitness differences between species if they specialize predominantly on fast growing but poorly defended plant species (Chesson 2000, Chase et al. 2002). The frequency-dependent effects (stabilizing mechanisms) are essential for coexistence and have been mainly attributed to specialist herbivores. Therefore, ecologists have assigned specialist insect herbivores a stronger role in promoting plant diversity and coexistence than generalist insect herbivores (Pacala and Crawley 1992, Carson and Root 1999, Allan and Crawley 2011). We did not find a frequency-dependent effect of herbivores in the field experiment, i.e., the most abundant plant species did not benefit most from herbivore exclusion, suggesting that stabilizing effects of herbivores via frequency-dependent consumption, were rather low in our grasslands. Instead, herbivores influenced the composition of plant communities: the change in plant cover might have been mainly driven by selective feeding of herbivores and potentially a trade-off between the vigorous growth of plants and herbivore preference. The fact that generalist herbivores, in particular grasshoppers, were dominating on the 14 grassland sites that our study is based on (for detailed information on the amount of generalist and specialist herbivores, see Unsicker et al. [2006]) suggests that generalist rather than specialist herbivores were mainly responsible for the compositional shift of plants in

these grasslands. In line with our data, Bagchi et al. (2014) also found no evidence for stabilizing density-dependent effects of insect herbivores on plants in a tropical forest, but effects on plant species composition. This together with our findings therefore emphasize the importance of equalizing effects of invertebrate aboveground herbivores on plant communities, and shed new light on our mechanistic understanding of grassland ecosystems.

So far, the relative importance of generalist and specialist aboveground invertebrate herbivores for the composition of plant communities is not known because of the difficulty to manipulate specifically the density of either of two groups. Therefore it remains speculative whether the equalizing effect of herbivores in our study was caused by generalists preferring poorly defended plant species or by specialists that have predominantly specialized on fast growing but poorly defended plant species. Whether the impact of specialist herbivores differs between more or less abundant plant species or whether variation in plant defense against generalist herbivores is related with different loads of specialist herbivores (Novotny and Basset 2005), remains unclear. Similarly, we lack knowledge on feeding preferences of belowground herbivores (but see Schallhart et al. 2012), although they did affect plant community diversity and composition in our field experiments (e.g., Stein et al. 2010). Lower mobility belowground might generally impede selective feeding, leading to more frequency-dependent herbivory. Thus, identifying the different roles of generalist and specialist as well as of above- and belowground herbivores for the assembly and composition of plant communities and the maintenance of plant diversity therefore is a major challenge for future research.

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

Herbivore performance and preference.—In insects, the preference and the performance of herbivores are hypothesized to be tightly linked (Gripengberg et al. 2010). Although this pattern, also known as the “mother-knows-best-principle” (Jaenike 1978), mainly refers to oviposition preferences of female insects and the performance of their offspring, our results show that, also within the life of an individual herbivore, generalist caterpillars, when given a choice, prefer those plant species on which they perform best. While the positive preference–performance relationship may be of little surprise, the considerable amount of scatter is remarkable. *Achillea millefolium*, for example, the plant species on which caterpillars gained most mass, was hardly preferred (rank 8 out of 28) by the herbivores. Similarly, feeding on the second-most preferred species, *Alopecurus pratensis*, caterpillars gained only little mass. Thus, although food preference seems to be linked to herbivore performance, other factors, such as either chemical feeding deterrents that do not directly affect herbivore performance (Dicke 2009), or the suitability of plants as a protective structure from natural enemies (Björkman et al. 1997) or competitors (Wise and Weinberg 2002), might affect a herbivore’s preference and hence fine tune performance–preference relationships.

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

Conclusions

We show that variation in plant defense against herbivory that do affect herbivore preference may drive the composition of plant communities. Generalist herbivores reduce the performance of less-defended plant species, thereby indirectly boosting the more highly defended plant species. That more preferred plant species were those that increased in cover after herbivores were excluded is a strong indication for a trade-off between plant growth and herbivore preference. Such a growth–preference trade-off might much better reflect opposing selection pressures in nature than

the usually described growth-defense trade-off, as it better takes into account the costs and benefit of defenses for plants. Our results indicate that generalist herbivores seem to equalize fitness between plant species by selectively feeding on more preferred (ergo less defended), but more competitive (vigorously growing), species. Interestingly, different generalist herbivores perceive plant resistance similarly—an essential prerequisite if the differential abilities of species to defend themselves, at least against generalist herbivores, are expected to translate into changes in natural communities. Therefore, our approach of assessing a “species-specific value of plant defense” against generalist herbivores measured as herbivore preference for an entire plant community provides novel information on ecological mechanisms that is required to understand how biotic limiting factors affect the assembly of plant communities.

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SUPPLEMENTAL MATERIAL

Ecological Archives

Appendices A–C are available online: <http://dx.doi.org/10.1890/14-2125.1.sm>