

# Diversity and beyond: plant functional identity determines herbivore performance

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## Summary

1. Recent biodiversity studies have addressed various community-level effects of biodiversity change, but the number of studies on specific biotic interactions is still rather limited. An open question in the context of plant–insect–herbivore relationships is how diversity impacts the population ecology of individual species.
2. In the present study, we explored the relationship between plant species diversity and the performance and fitness of a generalist herbivore, the meadow grasshopper *Chorthippus parallelus* Zetterstedt (Orthoptera, Gomphocerinae). A total of 1620 fourth-instar nymphs of this insect were captured and transferred to cages (10 females and 10 males per cage) on 81 experimental grassland communities in plots containing one to 60 plant species within the Jena biodiversity experiment.
3. Median survival of grasshoppers in the experiment was 14.5 days. Survival was independent of plant species richness and number of plant functional groups in the communities, but increased if plant communities contained grasses. Plant species richness and plant functional group richness had no effect on the number of oothecae laid by females or the number of hatchlings in the next generation.
4. Functional group composition of the plant communities affected most fitness measures. Grass presence increased the number of oothecae laid by females from  $0.78 \pm 0.21$  to  $3.7 \pm 0.41$  per female, and the number of hatchlings in the next generation from  $4.0 \pm 1.3$  to  $16.6 \pm 2.4$ . Certain combinations of plant functional groups increased grasshopper survival.
5. The findings indicate that the fitness of *C. parallelus* is influenced more by plant functional group identity than by plant species richness. In the absence of grasses, grasshoppers performed better if more than just one functional group of plants was present. We call this a ‘rescue effect’ of plant functional group richness.

**Key-words:** dietary mixing, generalist herbivore, grasshopper, plant diversity, plant functional identity, The Jena Experiment.

## Introduction

While a number of recent studies suggest strong and consistent effects of biodiversity on ecosystem processes and trophic interactions (e.g. reviewed in Balvanera *et al.* 2006), the underlying mechanisms are often ambiguous. Although some of these studies have addressed various community-level effects of biodiversity change, the number of investigations on specific interactions is still limited (Balvanera *et al.* 2006;

Scherber *et al.* 2006). So far the effects of plant species extinctions on insect herbivores have mainly been restricted to observational studies, where the abundance of a whole insect community has been measured (Siemann 1998; Haddad *et al.* 2001; Brose 2003), rather than investigation of species-specific herbivore abundances (Haddad *et al.* 2001). Studies focusing on the response of single species to changes in plant diversity are rare (Tahvanainen & Root 1972; Bach 1980; Risch 1981) and often restricted to specialist insect herbivores. For specialists, there are clear predictions concerning how the abundance should decrease with increasing plant diversity (Tahvanainen & Root 1972; Otway, Hector & Lawton 2005).

In contrast, there is only limited knowledge on the effects of resource abundance and diversity on generalist insect

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herbivores. While the 'dietary mixing' hypothesis (Pennings, Nadeau & Paul 1993; Bernays *et al.* 1994) predicts a better performance of generalists on a mixed vs. single-food diet, experiments to test this hypothesis have mostly been restricted to laboratory systems. In species-poor communities, a generalist consumer will be forced to feed on fewer food plants and hence its performance may be negatively affected by plant species loss from the ecosystem. Species-rich communities may, on the other hand, allow a generalist to feed on a broader range of food plants, and this in turn may positively influence its growth, survival and reproduction. There is considerable evidence in the literature that the beneficial effect of dietary mixing in generalist herbivores is mediated both by nutrient complementation and toxin dilution effects (nutrient complementation: Pulliam 1975; Westoby 1978; Rapport 1980; Bernays *et al.* 1994; Simpson & Raubenheimer 2000; Berner *et al.* 2005; toxin dilution: Freeland & Janzen 1974; Bernays & Minkenberg 1997).

Although coextinctions of specialists and their preferred resources have often been reported (e.g. Biesmeijer *et al.* 2006), few studies have addressed what may happen to generalists. There is only one field study that concerns these and related questions within a biodiversity–ecosystem functioning context (Pfisterer, Diemer & Schmid 2003).

Given the lack of experimental evidence for the effects of biodiversity loss on the fitness of generalist herbivores, we chose the meadow grasshopper *Chorthippus parallelus* (Zetterstedt 1821) (Orthoptera: Gomphocerinae) as a model herbivore to study growth, survival and reproduction within grassland communities ranging from one to 60 plant species over a 3-month period during 2004. In addition to plant species richness, we purposely manipulated the number and identity of plant functional groups to assess the consequences of entire plant functional groups lost from an ecosystem (Hooper & Vitousek 1997; Symstad *et al.* 1998; Hector *et al.* 1999; Haddad *et al.* 2001).

This is, we believe, the first study that attempts to tease apart pure species richness effects from functional group identity effects on insect herbivore performance. In order to do this, we have asked three basic questions for which we have tried to find empirical supporting evidence – or not, as the case may be: (i) How does plant species diversity affect the fitness of *C. parallelus*? (ii) Are certain plant functional groups affecting the performance of this grasshopper species differently? (iii) How does plant species identity affect the fitness of *C. parallelus*?

## Materials and methods

### STUDY ORGANISM

Grasshoppers play an important role as primary consumers in grasslands and in agricultural systems (Ingrisch & Köhler 1998). As their performance as a response to balanced and unbalanced food has been studied in considerable detail (Bernays & Chapman 1970a,b; Bernays *et al.* 1994; Simpson & Raubenheimer 2000; Raubenheimer & Simpson 2003), grasshoppers are thus most suitable organisms to

examine the effects of plant species loss on herbivore performance. *Chorthippus parallelus* is a generalist species that is both widespread and abundant in European grasslands (Ingrisch & Köhler 1998). It has been reported to feed on plants within 38 genera of vascular plants, 23 of them belong to the Gramineae and four to the Juncaceae and the Cyperaceae (e.g. Gangwere 1961; Bernays & Chapman 1970a; Ingrisch & Köhler 1998; Franzke 2006; Oswald 2006; Unsicker 2006). The remaining 11 food plant genera belong to another 10 plant families (Ingrisch & Köhler 1998). *C. parallelus* hibernates as oothecae containing on average six to eight eggs (Ingrisch & Köhler 1998). The first nymphs hatch in spring, four juvenile stages being distinguishable, while adults appear from July to November. The adult females oviposit close to tussocks in the top soil layer above or inside the rootstock. *C. parallelus* is the most abundant grasshopper species in the vicinity of the sites investigated in this study (Pratsch 2004).

### EXPERIMENTAL DESIGN

#### The Jena Experiment

The Jena Experiment was established in 2001, on 10 ha of former arable land. The site is located in the floodplain of the River Saale near Jena, Germany (altitude 130 m, 50°57' N 11°37' E). The species pool in the experimental grassland site consists of 60 herbaceous plants that also occur naturally in hay meadows surrounding the study site (*Arrhenatherion* grasslands; for details see Roscher *et al.* 2004). Plant species were assigned to the four functional groups grasses, small herbs, tall herbs and legumes (Roscher *et al.* 2004). The definition of these functional groups is based on life-history and morphological characteristics. Eighty-two experimental grassland plots each with a size of 20 × 20 m were sown with one, two, four, eight, 16 or all 60 plant species, and contained one, two, three or four functional groups. The plant species compositions were chosen at random to ensure that all possible combinations of species richness and number of functional groups occurred (Roscher *et al.* 2004). To account for heterogeneity in abiotic site conditions such as sand content in the soil with increasing distance from the River Saale, all plots were grouped into four blocks each comprising about 20 plots. In order to maintain the plant composition in the Jena Experiment, the plots were weeded and mown twice a year.

#### Caging experiment

In July 2004 two metal cages with a 10-cm border at the bottom, 1 m in height and of 0.5 m diameter, and wrapped with aluminium mesh (3 × 2 mm mesh size) that was coiled up at the top, were installed on each of 81 20 × 20-m plots. The plot with a monoculture of daisy *Bellis perennis* L. was excluded because of insufficient cover of the target species. A 2 × 4-m subplot within each 20 × 20-m plot was selected randomly to install the grasshopper cages. One of the two cages per subplot was randomly selected to serve as a grasshopper-containing cage, while the other served as a control (without grasshoppers).

Shortly before the start of the experiment, both cages per subplot were cleared of terrestrial invertebrates using a vacuum cleaner (1400-W vacuum cleaner, Kärcher A2801 plus, Alfred Kärcher GmbH & Co. KG, Winnenden, Germany).

For oviposition measurements, an extra cage was installed per plot. In blocks four and three, commercial colanders with a diameter of 22 cm, height of 15 cm and 2 mm mesh size were used. Because of delivery difficulties of the manufacturer, the oviposition cages in

blocks 1 and 2 were slightly modified. These cages, 30 cm in height, were made from aluminium mesh fixed using adhesive to a plastic frame of the same diameter as the colanders. Both types of oviposition cage were located between the two larger cages and attached to the ground using long nails. We accounted for potential effects of these different cage types on any of the grasshopper fitness measures, by including them as blocking effects in all statistical analyses.

### Grasshopper sampling

Between 6 and 15 July 2004, about 2000 fourth-instar *C. parallelus* nymphs were sampled using sweep-nets on meadows in the floodplain of the Saale adjacent to the study site. After the nymphs had been separated according to sex, initial body mass was determined by weighing five individuals per sex at a time in one jar using an analytical balance (Sac 51, Scaltec Instruments GmbH, Heiligenstadt, Germany). Grasshoppers were transferred to the cages in groups of 10 individuals (five females and five males) on two consecutive days so that each cage received 10 females and 10 males altogether. In total, 1620 grasshoppers were introduced to the plots.

## FITNESS MEASUREMENTS

### Survival

As an estimate of survival, the number of grasshoppers present in each cage was noted every second day. Survival measurements started on 16 July and ended on 1 September. After the final moult leading to maturity (= maturity moult), it was possible to visually determine the sex of the grasshoppers and thus by this time exact sex ratios in the cages could be determined. For each time interval, the survival data were corrected in the following way: if the number of grasshoppers on a given census day was higher than the number of individuals in the previous census, it was assumed that individuals had been overlooked previously and the number of grasshoppers at that census was adjusted accordingly. Median survival time of grasshoppers was calculated from a Kaplan–Meier survivorship curve fitted separately for each grasshopper cage. Survival data from grasshoppers in both cage types (grasshopper and oviposition cages) was pooled for further analysis.

### Body mass

In addition to weight measurements at the beginning of the experiment, the body weight of adult grasshoppers was determined once in July and twice during August. For adult body weight measurements, three adult females and three adult males were randomly selected from the grasshopper cages. In some cages, where there was high grasshopper mortality, fewer than three adult individuals per sex survived and thus only these individuals could be weighed. Weight gain was calculated with the following formula, using the average of the two adult measurements:

$$\text{Weight gain} = \ln(\text{adult mass}) - \ln(\text{initial mass}).$$

### Fecundity

*C. parallelus* females deposited into the top soil layer. As it was not possible to extract the top soil layer of the large grasshopper cages without causing extensive damage to the vegetation in the experimental

plot, smaller oviposition cages were installed to measure fecundity (see 'Caging experiment'). Two females and one male from the herbivory cage were transferred to these smaller cages after the maturity moult. A plastic cup (10 cm diameter) filled with a mixture of sand and soil (1 : 1) was provided for oviposition in each oviposition cage. At the end of the experiment, these cups were removed and the substrate inside sieved to extract the oothecae. Additionally, the top soil underneath the oviposition cages was dug out by taking soil cores of 22 cm diameter and 10 cm depth. In the laboratory, oothecae were extracted from the soil cores by carefully washing soil off the root balls, and collecting all particles the size of the oothecae in a sieve underneath the water jet. After washing, roots were torn apart to find further egg pods. The number of oothecae from the oviposition pot and the top soil were added together to obtain the number of oothecae per plot as a measure of grasshopper fecundity.

After counting, oothecae were buried into a mixture of sand and soil (1 : 1) and transferred to a refrigerator (c. 5 °C) to initiate diapause. After 7 months, oothecae were removed from the refrigerator and separated in plastic cups (again filled with a mixture of sand and soil) for later measurement of hatching per individual ootheca. Cups, which were covered with fly screen to prevent the hatching grasshoppers from escaping, were checked daily for hatchlings. For data analysis, both the total number of hatched grasshoppers from each oviposition cage (total hatchlings), as well as the mean number of hatchlings per ootheca were used.

## PLANT PARAMETERS

The number of plant species in all cages was determined before grasshoppers were released therein. Grass and legume cover was estimated visually prior to the start of the experiment using an integer cover scale. We used realized species richness and grass and legume cover as covariates in all analyses of grasshopper fitness parameters. A more detailed description of grasshopper effects on the vegetation is given elsewhere (Scherber 2006).

## STATISTICAL ANALYSIS

The data were analysed using SPSS for Windows 13.0. Regression analyses, analyses of variance (ANOVA) and correlations were performed. Assumptions of normality and heteroscedasticity were tested. There were two basic statistical models that were used for all analyses.

First, a standard Analysis of Covariance model (ANCOVA) was used to test for effects of the design variables. This model had the following sequence of terms: grass cover (covariate), block (random factor), number of plant species, number of functional groups, presence of grasses and presence of legumes, grass: legume interaction term.

Second, additional models were used to test for effects of plant functional group composition. These models only contained block (random factor) and functional group composition as a fixed effect. Functional group composition was a factor with six levels: G, GLH, GH, L, LH, H, where mixtures were characterized by containing G = grasses, L = legumes or H = large or small herbs. Thus, in these additional models, we did not differentiate between large and small herbs to facilitate interpretations and comparisons with previous studies.

For the analysis of body mass data, initial grasshopper weight was included as a covariate in the basic ANCOVA model. A reduced ANCOVA model was constructed to test the effects of functional group composition, as described above.

Count data were analysed using generalized linear models (GLIM) with quasi-Poisson errors implemented in R 2.4.0 (R Development Core Team 2005), with terms added sequentially to the null model, and significance for these models was assessed using *F*-tests.

## Results

### PLANT SPECIES RICHNESS IN THE CAGE

The realized plant species richness in the cages was closely correlated with the number of sown plant species on the entire plot ( $n = 81$ ; d.f. = 1;  $P < 0.001$ ,  $r = 0.93$ ). For this reason, sown plant species rather than realized species richness was used for all further analyses.

### GRASSHOPPER SURVIVAL

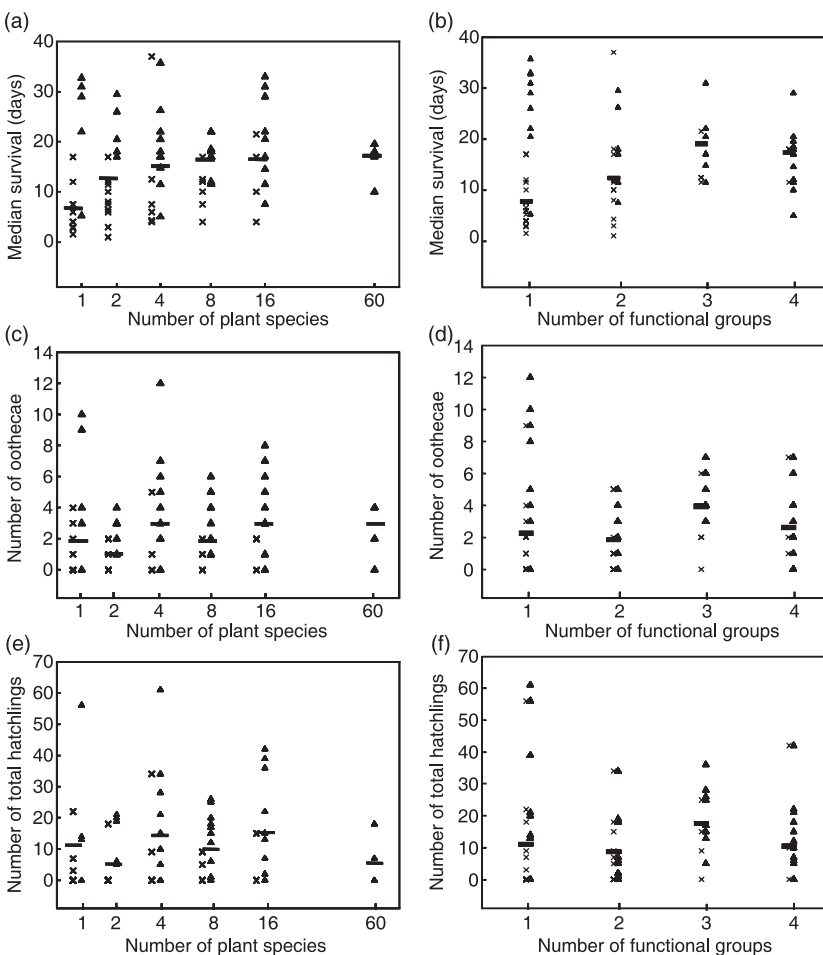
One hundred and ninety-three individuals of the 1620 grasshoppers introduced into the cages at the beginning of the experiment survived until the experiment was terminated after 50 (blocks 1 and 2) and 56 days (blocks 3 and 4). The median survival of all grasshoppers was 14.5 days.

The survival of the grasshoppers was not influenced by the number of plant species in the plot, despite a trend to longer

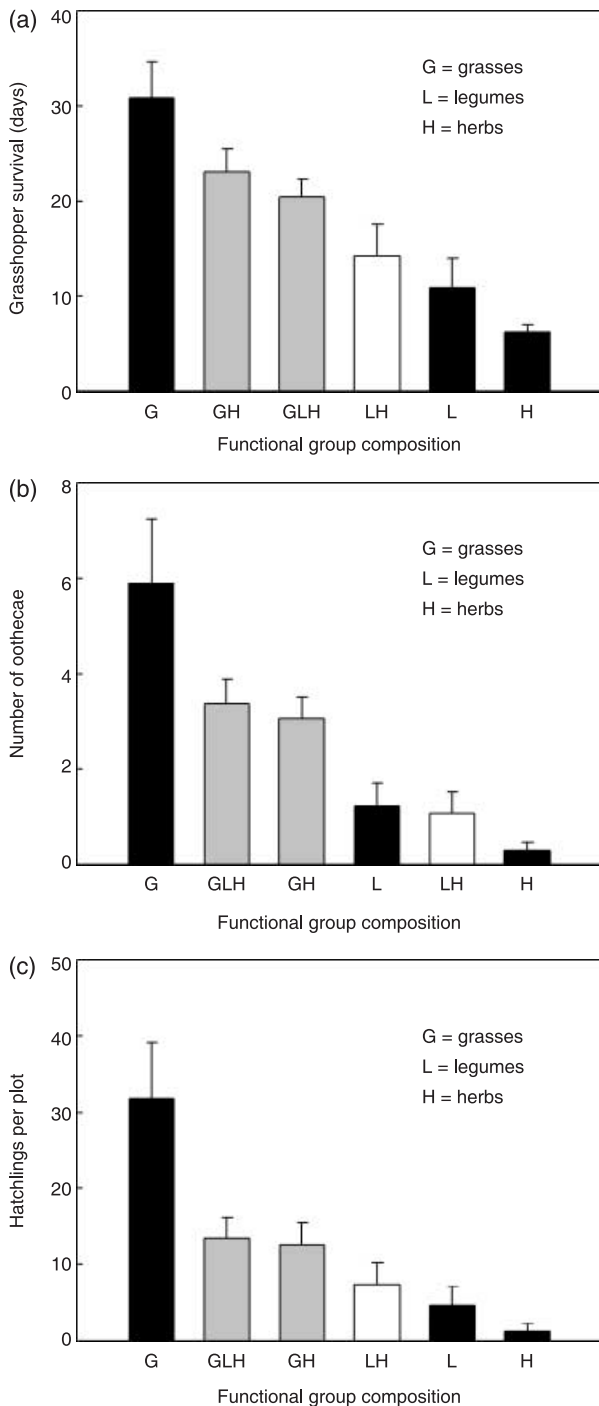
survival at higher levels of species richness ( $F_{5,58} = 2.3$ ;  $P = 0.051$ ; Fig. 1a). Survival tended to be higher in plots with three and four functional groups than in those with only one or two functional groups, but the relationship was not significant ( $F_{3,58} = 2.6$ ;  $P = 0.057$ ; Fig. 1b). The interaction between number of plant species and number of functional groups had no significant effect on grasshopper survival ( $F_{7,58} = 0.703$ ;  $P = 0.669$ ).

The most striking result was the overall positive influence of the grass functional group on grasshopper survival. Individuals of *C. parallelus* caged on plots without grasses survived for 7.5 days (median), whereas the median for survival of those on plots containing grasses was 18 days (separate model without grass cover as covariate:  $F_{1,58} = 39.33$ ; d.f. = 1;  $P < 0.001$ ). The higher the grass cover in the cages at the beginning of the experiment (July 2004), the higher the survival of *C. parallelus* ( $F_{1,41} = 46.9$ ;  $P < 0.001$ ). The presence of legumes did not affect survival ( $F_{1,58} = 1.5$ ;  $P = 0.216$ ) while the interaction between the presence of grasses and legumes was not significant ( $F_{1,58} = 1.9$ ;  $P = 0.166$ ).

Although grasshopper survival was highest in cages containing only grasses (29 days), the functional composition of grassland mixtures had a significant influence on grasshopper survival ( $F_{5,72} = 15.741$ ;  $P < 0.001$ ; Fig. 2a). There were high rates of survival of *C. parallelus* in all plant mixtures containing



**Fig. 1.** The effects of plant species richness (1a, 1c, 1e) and the number of plant functional groups (1b, 1d, 1f) on various measures of grasshopper fitness. Black triangles: plots with grasses, Crosses: plots without grasses. (a, b) Grasshopper survival in days. Horizontal bars represent median survival. (c, d) Number of oothecae per grasshopper female. Horizontal bar represents the mean numbers. (e, f) Total number of hatchlings per female. Horizontal bars represents the mean number of total hatchlings per plot.



**Fig. 2.** (a) Relationship between the functional group compositions and the survival of *C. parallelus* (black bars = only one functional group alone, grey bars = mixtures with grasses, white bar = mixtures without grasses) (N: G = 9, GH = 15, GLH = 19, LH = 12, L = 9, H = 17). (b) Relationship between the functional group compositions and the number of oothecae (black bars = only one functional group alone, grey bars = mixtures with grasses, white bar = mixtures without grasses) (N: G = 9, GH = 15, GLH = 19, LH = 12, L = 9, H = 17). (c) Relationship between the functional group compositions and the number of hatchlings per plot (black bars = only one functional group alone, grey bars = mixtures with grasses, white bar = mixtures without grasses) (N: G = 9, GH = 15, GLH = 19, LH = 12, L = 9, H = 17).

grasses. Survival was poor in plots containing only herbs or legumes (see bars L and GLH, LH in Fig. 2a). In the absence of grasses, the presence of two functional groups, namely legumes and forbs, increased survival of grasshoppers, compared with only one functional group present (see bar LH in Fig. 2a).

The relationship between the number of plant species and grasshopper survival remained nonsignificant ( $F_{5,23} = 0.284$ ;  $P = 0.917$ , Fig. 1a) when only plots with grass species were considered.

Owing to the staggered beginning of the experiment, grasshopper survival showed significant differences between the four blocks (ANOVA:  $F_{3,58} = 5.7$ ;  $P = 0.002$ ). Grasshoppers survived between 8.7 days (block 1) and 19.5 days (block 3) on average.

#### GRASSHOPPER BODY MASS

The initial body mass of female and male grasshopper nymphs in the experiment differed between the four blocks due to the staggered beginning of the experiment. Males introduced into blocks 3 and 4 weighed  $0.065 \pm 0.01$  g, significantly more than those caged in block 1 and 2 with a mass of  $0.05 \pm 0.01$  g (males:  $F_{3,58} = 53.78$ ;  $P < 0.001$ ). The same was true for females that had body mass differences of 0.02 g on average between the blocks (females:  $F_{3,58} = 39.102$ , d.f. = 3;  $P < 0.001$ ). This significant difference in initial body mass found between the blocks made it necessary to use initial body mass as a covariate factor in the ANOVA regarding body mass.

Approximately 10–14 days after caging, grasshoppers went through maturity moult and were weighed again. Body mass in adult grasshoppers could be determined in 497 individuals (239 males and 258 females) coming from 58 of 81 cages. At this time, there was no remaining block effect (males:  $F_{3,32} = 2.232$ ;  $P = 0.102$ ; females:  $F_{3,35} = 1.942$ ;  $P = 0.43$ ). Both females and males had substantially gained biomass by the time of maturity, and females weighed significantly more ( $0.18 \text{ g} \pm 0.01 \text{ g}$ ) than males ( $0.09 \text{ g} \pm 0.01 \text{ g}$ ) (paired *t*-test: d.f. = 47;  $P < 0.001$ ).

There was no effect of plant species richness on weight gain in both sexes of *C. parallelus* (males:  $F_{5,32} = 0.547$ ;  $P = 0.739$ ; females:  $F_{5,35} = 0.913$ ;  $P = 0.484$ ). Functional group richness also had no effect on weight gain in females or males (males:  $F_{3,32} = 1.9$ ;  $P = 0.134$ ; females:  $F_{3,35} = 0.233$ ;  $P = 0.873$ ). The interaction between number of plant species and number of functional groups had no significant effect on grasshopper weight gain (males:  $F_{7,32} = 0.946$ ;  $P = 0.486$ ; females:  $F_{7,35} = 0.286$ ;  $P = 0.955$ ). Similarly, neither the presence of grasses (males:  $F_{1,32} = 0.284$ ;  $P = 0.598$ ; females:  $F_{1,35} = 0.93$ ;  $P = 0.762$ ) nor the initial percentage of grass cover (males:  $F_{1,33} = 1.277$ ;  $P = 0.275$ , females:  $F_{1,35} = 1.6$ ;  $P = 0.23$ ) or the presence of legumes (males:  $F_{1,32} = 0.001$ ;  $P = 0.973$ ; females:  $F_{1,35} = 3.227$ ;  $P = 0.081$ ) affected grasshopper weight gain. The interaction between presence of grasses and legumes was also not significant (males:  $F_{1,32} = 2.01$ ;  $P = 0.159$ ; females:  $F_{1,35} = 0.143$ ;  $P = 0.708$ ). Finally grasshopper weight gain was also not influenced by functional group identity and

functional group composition (males:  $F_{3,32} = 0.735$ ;  $P = 0.601$ ; females:  $F_{3,35} = 1.022$ ;  $P = 0.415$ ). In terms of their effect on female weight gain, grasses came only fourth in functional group ranking, in contrast to their overall prominent effect on survival. When plots with grasses only were considered, the relation between number of species and grasshopper weight gain remained nonsignificant (males:  $F_{5,20} = 0.1$ ;  $P = 0.973$ ; females:  $F_{5,17} = 1.6$ ;  $P = 0.208$ ).

#### GRASSHOPPER FECUNDITY (NUMBER OF OOTHECAE/NUMBER OF HATCHLINGS)

In total, 192 egg pods were laid in the oviposition cages on 53 plots. In many plots, no egg pods were laid and thus the data did not follow a normal distribution. A standard quasi-Poisson model for overdispersed count data (McCullagh & Nelder 1989) was sufficient to include these zero observations, as zero inflation was not severe.

A maximum of up to 12 oothecae was laid per female grasshopper in the cages, with nymphs hatching from oothecae in 47 plots. The number of hatched nymphs ranged from 1 to 61 nymphs per plot and the number of hatchlings per ootheca between 1 and 9 nymphs, with an average of  $4.4 \pm 0.35$ .

The number of plant species did not influence the number of oothecae (GLIM,  $F_{5,71} = 2.1$ ,  $P = 0.07$ ; Fig. 1c). Similarly, the total number of hatchlings was not influenced by the number of plant species present in the plots (GLIM,  $F_{5,71} = 1.591$ ,  $P = 0.34$ ; Fig. 1e).

The number of functional groups did not affect the number of oothecae (GLIM,  $F_{3,68} = 1.9$ ,  $P = 0.13$ ; Fig. 1d). For cages where at least one ootheca had been laid, the relationship was marginally nonsignificant (GLIM,  $F_{3,32} = 14.9$ ,  $P = 0.058$ ). Likewise, the relationship between total hatchlings and the number of functional groups was not significant (GLIM,  $F_{3,68} = 0.7$ ,  $P = 0.52$ ; Fig. 1f) and also the interaction between number of plant species and number of functional groups had no significant effect on number of oothecae (GLIM,  $F_{7,59} = 0.6$ ,  $P = 0.72$ ) or on total number of hatchlings (GLIM,  $F_{7,59} = 0.8$ ,  $P = 0.51$ ).

The presence of grasses increased the number of oothecae (GLIM,  $F_{1,68} = 32.8$ ,  $P < 0.001$ ): the mean number of oothecae in cages without grasses was  $0.78 \pm 0.21$ , while cages with grasses had  $3.7 \pm 0.41$ . On average, only  $4.0 \pm 1.3$  grasshoppers hatched in cages where grasses were absent, compared with  $16.6 \pm 2.4$  when grasses were present (GLIM,  $F_{1,68} = 15.3$ ,  $P < 0.001$ ). With increasing initial cover of grasses, the numbers of *C. parallelus* oothecae significantly increased (regression with square-root transformed number of oothecae:  $F = 25.5$ ;  $P < 0.001$ ). The total number of hatchlings also increased significantly with increasing grass cover (regression:  $F = 34.1$ ; d.f. = 1;  $P < 0.001$ ). The presence of legumes did not affect number of oothecae (GLIM,  $F_{1,66} = 1.6$ ,  $P = 0.2$ ) nor the total number of hatchlings (GLIM,  $F_{1,66} = 0.3$ ,  $P = 0.54$ ). Similarly, the interaction between presence of grasses and legumes had no effect (GLIM number of oothecae,  $F_{1,58} = 2.7$ ,  $P = 0.1$ ; GLIM total number of hatchlings,  $F_{1,58} = 2.5$ ,  $P = 0.11$ ).

The mean number of oothecae (GLIM  $F_{5,75} = 13.3$ ;  $P < 0.001$ ; Fig. 2b) and the total number of hatchlings (GLIM  $F_{5,75} = 8.5$ ;  $P < 0.001$ ; Fig. 2c) were significantly affected by functional group composition, while the number of oothecae was highest in plots containing only grasses (see G bar in Fig. 2b). Plant mixtures containing grasses (GLH, GH Fig. 2b) showed on average fewer oothecae compared with cages containing only grasses, but more than cages without grasses at all (L, LH, H; Fig. 2b). Exactly the same pattern was found for the total number of hatchlings (Fig. 2c). The total number of hatchlings decreased on plots without grasses (L, H, LH, Fig. 2c), but, if the grasshoppers could mix legumes and herbs in their diet, their fitness was seen to increase (LH; Fig. 2c).

Assuming that a grasshopper population of *C. parallelus* will be sustained when there are at least two hatchlings per cage present, populations would persist in the following combinations of functional groups: G (116 hatchlings, averaged over all plots with only grasses), GH (38 hatchlings), GLH (33 hatchlings), L (15 hatchlings) and LH (24 hatchlings).

The relationship between the number of plant species and the number of grasshopper oothecae as well as total number of hatchlings was nonsignificant (GLIM number of oothecae,  $F_{5,35} = 0.8$ ,  $P = 7.4$ ; GLIM total number of hatchlings,  $F_{5,35} = 1.1$ ,  $P = 0.37$ ) when only plots with at least one grass species are considered. Finally, the number of oothecae differed between blocks (GLIM  $F_{3,77} = 1.1$ ,  $P = 0.37$ ) and therefore, the total number of hatchlings also showed a significant block effect (GLIM  $F_{3,77} = 3.2$ ,  $P = 0.02$ ).

## Discussion

The results from this experiment clearly show that, contrary to expectation, the performance of a generalist insect herbivore is independent of the number of plant species present in the system. Of course, it is debatable what degree of polyphagy is needed to detect species richness effects. However, we have shown that *C. parallelus* can survive and reproduce even in plant communities that do not contain the preferred host plant functional group grass at all. This reveals that at least for the response variables measured in this study, it should have been possible to detect plant diversity effects. Additional observations on grasshopper feeding damage to all plant species present in the cages showed that grasshoppers indeed consumed herbs and legumes, but the quantities eaten were rather low.

Thus, from a theoretical point of view, a clear species richness effect on grasshopper survival, body mass and fecundity may have been expected. Nevertheless in our study, other variables proved much more important. Indeed, the design used in this experiment allows such a statement. This is because plant species richness, plant functional group richness and plant functional group identity were manipulated separately from one another as far as possible.

It can therefore be stated with some confidence that plant functional group identity was the most important parameter of those investigated for grasshopper survival, body mass and

fecundity in this experiment. The presence of grasses had an overall positive influence on grasshopper performance. For grasshopper survival, not only was the presence of grasses important but also the number of plant functional groups present in the food plant community.

In the absence of grasses, combinations of particular plant functional groups (e.g. legumes and herbs) increased survival and fecundity compared with treatments with only one of these functional groups, which can be seen as a synergistic effect of the functional groups with regard to grasshopper fitness.

#### EFFECTS OF PLANT SPECIES RICHNESS

The effect of plant species richness on grasshopper fitness parameters was not significant, even though this was only marginally nonsignificant for some fitness parameters. Similar to the effects shown for the grasshopper *Parapleurus alliaceus* (Germar 1817) (Pfisterer *et al.* 2003), survival of *C. parallelus* in our study was not influenced by the number of species in the plant community. In our experiment, fecundity, measured both as the number of oothecae laid by females and the total number of hatchlings, was also independent of plant species richness.

#### EFFECTS OF FUNCTIONAL GROUP RICHNESS

By observing the effect of functional group richness, only survival of the grasshoppers increased with increasing number of plant functional groups present in the plant communities. Fecundity, however, was independent of functional group richness. One possible explanation for the increased survival of grasshoppers in cages with three and four functional groups compared with one or two (Fig. 1b) is that with an increasing number of functional groups, the likelihood that grasses were present increased. Figure 1(b) shows that cages in plots with three and four functional groups where survival was high were those where mixtures contained grasses. Another indication for this is the positive effect of initial grass cover on survival (see below).

#### EFFECTS OF GRASSES AND OTHER PARTICULAR FUNCTIONAL GROUPS

While, as mentioned earlier, grasshoppers feed mainly on grasses, legumes and to a lesser extent other forbs are also important components of their diet (Bernays & Chapman 1970a; Ingris & Köhler 1998; Gardiner & Hill 2004; Unsicker *et al.* 2006). In our experiments, the insects survived much longer when grasses were part of the plant community compared with the situation in which they were absent from cages, and their fecundity was also much higher in the presence of grasses.

It was also found that not only was the presence of grasses important for grasshopper life span, but their survival was also positively correlated with initial grass cover. Survival did not depend on herb or legume presence as long as grasses, as

superior food plants, were available, which is in principle consistent with the study of Miura & Ohsaki (2004) in which they showed that the fitness of the grasshopper *Parapodisma subastris* (Huang, Chunmei 1983) was not affected when it was fed with two qualitatively inferior plants, as long as the superior food plant was provided. However in our study, although grasses were the preferred food plants of *C. parallelus*, there was high variation in survival and fecundity between grass-containing plots (see black triangles in Fig. 1a,c,e). This was especially true for monocultures. Possible reasons for these differences could be the nutritional quality of food plants (e.g. differences in nitrogen content), the presence of defensive compounds, such as silica (Massey, Ennos & Hartley 2007) or mechanical differences in leaf tissue, such as leaf texture or toughness.

#### GRASSHOPPERS AND DIET MIXING

Laboratory studies have shown a positive influence of diet mixing for generalist grasshoppers, thereby underlining the importance of nutrient balancing and/or the toxin dilution hypothesis (Bernays & Chapman 1970a; Chapman & Joern 1990; Waldbauer & Friedman 1991; Bernays *et al.* 1994; Gardiner & Hill 2004; Miura & Ohsaki 2004; Simpson *et al.* 2004; Unsicker *et al.* 2006). In our study, *C. parallelus* showed a significantly increased fitness in the presence of grasses, which can be interpreted as a clear preference for grasses. In the absence of grasses, survival and the total number of hatchlings were higher in particular functional group mixtures (e.g. a combination of legumes and herbs) than on plots with either of these functional groups alone. This synergistic effect of the superior functional groups match with findings of Miura & Ohsaki (2004) who detected that dietary mixing is particularly important when only low-quality plants are available. As we have no precise data on the feeding behaviour of *C. parallelus* in our field experiment, we cannot, however, rule out the possibility that the positive effects of plant functional richness observed are mediated by microclimatic or other rather indirect effects.

#### EVOLUTIONARY IMPLICATIONS

While the results here have shown that *C. parallelus* is a particular type of a generalist, the species has a preference for one functional group but can fall back on alternative foods when and if necessary. Therefore, potentially evolutionary implications of the pattern of diet utilization by *C. parallelus* may be inferred. It is probable that the distinct preference for grasses in contrast to other functional groups is an evolutionary trend towards becoming a grass specialist (hence living up to their common name!). The subfamily Gomphocerinae (slant-faced grasshoppers to which *C. parallelus* belongs) in Europe is believed to be mostly of Angarian origin, where grasshoppers belonging to this subfamily have evolved in tundras, mesophilous grasslands and xerophilic steppes (Uvarov 1929). Their extremely rapid population spread must have taken place as a direct consequence of their ecological adaptation to grassland, i.e.



from herb to grass feeders; indeed, in interglacial periods, they spread with increasing grassland cover all over Europe. According to Bernays (1991), such grass feeding adaptation has possibly evolved independently eight times during the course of their evolution. But as it was also shown in our experiment, the ability to survive and reproduce on inferior food is a stable trait in this species and could serve as an insurance policy against extinction in unpredictable environments. There is ample evidence for the beneficial aspect of diet mixing, to mention only the most two prominent mechanisms, that is: on the one hand, the balancing of nutrients (Pulliam 1975; Westoby 1978; Rapport 1980; Bernays *et al.* 1994, 2005; Simpson & Raubenheimer 2001) plus on the other, the dilution of toxins (Freeland & Janzen 1974; Bernays & Minkenberg 1997). Although the implications are of course speculative, *C. parallelus* may well also mix diets because the relative quality of food types changes over time (e.g. Singer & Bernays 2003) or, because food mixing may minimize exposure to other environmental risks.

#### IN THE CONTEXT OF PLANT SPECIES LOSS

Theoretical treatments on the effects of decreasing diversity on insect herbivore performance have generally proposed different mechanisms for so-called specialists and generalists. For specialists, Root (1973) suggested that specialists should be expected to be more strongly influenced by the loss of particular food plants than by general plant species loss. In contrast, for generalists, Pfisterer *et al.* (2003) argued that declining plant species richness has a negative influence on the fitness of a generalist herbivore due to a decreased likelihood of dietary mixing. As a consequence, random species loss in grassland vegetation poses a threat to generalist herbivores. Because of this, we hypothesize that the loss of particular plant species is a threat for specialist herbivores, while random loss of plant species is also a threat for generalist herbivores. Such a scenario would lead to shifts in the relative abundances of herbivores from large populations of generalist to large populations of specialist. A number of studies have indeed found higher abundances of specialist insects in less diverse plant communities, although the results are equivocal (Elton 1958; Pimentel 1961; Root 1973; Kareiva 1983; Risch, Andow & Altieri 1983; Strong, Lawton & Southwood 1984; Andow 1991; Haddad *et al.* 2001; Otway *et al.* 2005).

The results from a number of experiments on ecosystem services in the past have strongly suggested that it is not the number of plant species but rather the number of plant functional groups, and moreover the diversity of these groups in a plant community, that explain patterns in response to biodiversity loss (Haddad *et al.* 2001; Hooper & Vitousek 1997; Tilman 1997; Symstad *et al.* 1998; Hector *et al.* 1999). If this is true, it may also be useful for theoretical speculations on the relationship between biodiversity and generalist herbivore abundances to include the effects of plant functional groups.

In conclusion, we have demonstrated that in the present study, the performance of *C. parallelus* is highly dependent on the functional group grasses. Although the species could be

considered as a generalist herbivore with a preference for grasses, the effects of particular grass species on fecundity and survival were not that similar. Furthermore, we have shown that the observed grasshopper population was able to survive and reproduce in the presence of food plants from other functional groups, such as legumes and herbs. We call this a 'rescue effect' of plant functional groups. Such functional groups together doubtless have synergistic effects on individual grasshopper fitness in the absence of grasses.

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#### References

- Andow, D.A. (1991) Vegetational diversity and arthropod population response. *Annual Review of Entomology*, **36**, 561–586.
- Bach, C.E. (1980) Effects of plant diversity and time of colonization on a herbivore–plant interaction. *Oecologia*, **44**, 319–326.
- Balvanera, P., Pfisterer, A.B., Buchmann, N., He, J.S., Nakashizuka, T., Raffaelli, D. & Schmid, B. (2006) Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecology Letters*, **9**, 1146–1156.
- Bernays, E.A. (1991) Evolution of insect morphology in relation to plants. *Philosophical Transactions of the Royal Society of London, Series B*, **333**, 257–264.
- Bernays, E.A., Bright, K.L., Gonzalez, N. & Angel, J. (1994) Dietary mixing in a generalist herbivore—tests of 2 hypotheses. *Ecology*, **75**, 1997–2006.
- Bernays, E.A. & Chapman, R.F. (1970a) Experiments to determine the basis of food selection by *Chorthippus parallelus* (Zetterstedt) (Orthoptera – Acrididae) in field. *Journal of Animal Ecology*, **39**, 761–776.
- Bernays, E.A. & Chapman, R.F. (1970b) Food selection by *Chorthippus parallelus* (Zetterstedt) (Orthoptera–Acrididae) in field. *Journal of Animal Ecology*, **39**, 383–394.
- Bernays, E.A. & Minkenberg, O.P.J.M. (1997) Insect herbivores: different reasons for being a generalist. *Ecology*, **78**, 1157–1169.
- Berner, D., Blanckenhorn, W.U. & Körner, C. (2005) Grasshoppers cope with low host plant quality by compensatory feeding and food selection: N limitation challenged. *Oikos*, **111**, 525–533.
- Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J. & Kunin, W.E. (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, **313**, 351–354.
- Brose, U. (2003) Bottom-up control of carabid beetle communities in early successional wetlands: mediated by vegetation structure or plant diversity? *Oecologia*, **135**, 407–413.
- Chapman, R.F. & Joern, A. (1990) *Biology of Grasshoppers*. Wiley, New York.
- Elton, C.S. (1958) *The Ecology of Invasions by Animals and Plants*. Methuen, London.
- Franzke, A. (2006) *Die Wirkung Von Verschiedenen Nahrungsangeboten Auf die Fitness Von Chorthippus Parallelus (Orthoptera: Acrididae)*. Diplomarbeit, Georg-August-Universität, Göttingen.
- Freeland, W.J. & Janzen, D.H. (1974) Strategies in herbivory by mammals—role of plant secondary compounds. *American Naturalist*, **108**, 269–289.
- Gangwere, S.K. (1961) A monograph on food selection in Orthoptera. *Transactions of the American Entomological Society*, **87**, 68–230.
- Gardiner, T. & Hill, J. (2004) Feeding preferences of *Chorthippus parallelus* (Orthoptera: Acrididae). *Journal of Orthoptera Research*, **13**(2), 197–203.
- Haddad, N.M., Tilman, D., Haarstad, J., Ritchie, M. & Knops, J.M.H. (2001) Contrasting effects of plant richness and composition on insect communities: a field experiment. *American Naturalist*, **158**, 17–35.



- Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M.C., Diemer, M., Dimitrakopoulos, P.G., Finn, J.A., Freitas, H., Giller, P.S., Good, J., Harris, R., Hogberg, P., Huss-Danell, K., Joshi, J., Jumpponen, A., Korner, C., Leadley, P.W., Loreau, M., Minns, A., Mulder, C.P.H., O'Donovan, G., Otway, S.J., Pereira, J.S., Prinz, A., Read, D.J., Scherer-Lorenzen, M., Schulze, E.D., Siamantziouras, A.S.D., Spehn, E.M., Terry, A.C., Troumbis, A.Y., Woodward, F.I., Yachi, S. & Lawton, J.H. (1999) Plant diversity and productivity experiments in European grasslands. *Science*, **286**, 1123–1127.
- Hooper, D.U. & Vitousek, P.M. (1997) The effects of plant composition and diversity on ecosystem processes. *Science (Washington, D.C.)*, **277**, 1302–1305.
- Ingrisch, S. & Köhler, G. (1998) *Die Heuschrecken Mitteleuropas*. Westarp Wissenschaften, Magdeburg.
- Kareiva, P.M. (1983) Influence of vegetation texture on herbivore populations: resource concentration and herbivore movement. *Variable Plants and Herbivores in Natural and Managed Systems* (eds R.F. Denno & M.S. McClure). Academic Press, New York.
- Massey, F.P., Ennos, A.R. & Hartley, S.E. (2007) Grasses and the resource availability hypothesis: the importance of silica-based defences. *Journal of Ecology*, **95**(3), 414–424.
- McCullagh, P. & Nelder, J.A. (1989) *Generalized Linear Models*. Chapman & Hall, New York.
- Miura, K. & Ohsaki, N. (2004) Diet mixing and its effect on polyphagous grasshopper nymphs. *Ecological Research*, **19**, 269–274.
- Oswald, A. (2006) *Der Einfluss Unterschiedlicher Futterpflanzen auf die Fitness von Chorthippus parallelus (Orthoptera, Acrididae)*. Friedrich-Schiller-Universität Jena, Jena.
- Otway, S.J., Hector, A. & Lawton, J.H. (2005) Resource dilution effects on specialist insect herbivores in a grassland biodiversity experiment. *Journal of Animal Ecology*, **74**, 234–240.
- Pennings, S.C., Nadeau, M.T. & Paul, V.J. (1993) Selectivity and growth of the generalist herbivore *Dolabella auricollaria* feeding upon complementary resources. *Ecology*, **74**, 879–890.
- Pfisterer, A.B., Diemer, M. & Schmid, B. (2003) Dietary shift and lowered biomass gain of a generalist herbivore in species-poor experimental plant communities. *Oecologia*, **135**, 234–241.
- Pimentel, D. (1961) Species diversity and insect population outbreaks. *Annals of the Entomological Society of America*, **54**, 76–86.
- Pratsch, R. (2004) *Die Konsumentenstruktur (Arthropoda) ausgewählter Wirtschaftswiesen in der Umgebung des Jenaer Biodiversitätsprojektes*. Friedrich-Schiller-Universität Jena, Jena.
- Pulliam, H.R. (1975) Diet optimization with nutrient constraints. *American Naturalist*, **109**, 765–768.
- Rapport, D.J. (1980) Optimal foraging for complementary resources. *American Naturalist*, **116**, 324–346.
- Raubenheimer, D. & Simpson, S.J. (2003) Nutrient balancing in grasshoppers: behavioural and physiological correlates of dietary breadth. *Journal of Experimental Biology*, **206**, 1669–1681.
- R Development Core Team (2005) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, <http://www.r-project.org>.
- Risch, S.J. (1981) Insect herbivore abundance in tropical monocultures and polycultures – an experimental test of 2 hypotheses. *Ecology*, **62**, 1325–1340.
- Risch, S.J., Andow, D. & Altieri, M.A. (1983) Agroecosystem diversity and pest control: data, tentative conclusions, and new research directions. *Environmental Entomology*, **12**, 625–629.
- Root, R.B. (1973) Organization of a plant–arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monographs*, **43**, 95–124.
- Roscher, C., Schumacher, J., Baade, J., Wilcke, W., Gleixner, G., Weisser, W.W., Schmid, B. & Schulze, E.D. (2004) The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. *Basic and Applied Ecology*, **5**, 107–121.
- Scherber, C. (2006) *Invertebrate herbivory, plant diversity and ecosystem processes in experimental grassland*. Dissertation, University of Jena, Germany.
- Scherber, C., Mwangi, P.N., Temperton, V.M., Roscher, C., Schumacher, J., Schmid, B. & Weisser, W.W. (2006) Effects of plant diversity on invertebrate herbivory in experimental grassland. *Oecologia*, **147**, 489–500.
- Siemann, E. (1998) Experimental tests of effects of plant productivity and diversity on grassland arthropod diversity. *Ecology*, **79**, 2057–2070.
- Singer, M.S. & Bernays, E.A. (2003) Understanding omnivory needs a behavioral perspective. *Ecology*, **84**, 2532–2537.
- Simpson, S.J. & Raubenheimer, D. (2000) The hungry locust. *Advances in the Study of Behavior*, **29**, 1–44.
- Simpson, S.J., Sibly, R.M., Lee, K.P., Behmer, S.T. & Raubenheimer, D. (2004) Optimal foraging when regulating intake of multiple nutrients. *Animal Behaviour*, **68**, 1299–1311.
- Strong, D.R., Lawton, J.J.H. & Southwood, T.R.E. (1984) *Insects on Plants: Community Patterns and Mechanisms*. Harvard University Press, Cambridge, MA.
- Symstad, A.J., Tilman, D., Willson, J. & Knops, J.M.H. (1998) Species loss and ecosystem functioning: effects of species identity and community composition. *Oikos*, **81**, 389–397.
- Tahvanainen, J. & Root, R.B. (1972) Influence of vegetational diversity on population ecology of a specialized herbivore, Phyllotreta-Cruciferae (Coleoptera-Chrysomelidae). *Oecologia*, **10**, 321–346.
- Tilman, D. (1997) Distinguishing between the effects of species diversity and species composition. *Oikos*, **80**, 185.
- Unsicker, S.B., Oswald, A., Köhler, G. & Weisser, W.W. (2008) Complementary effects through dietary mixing enhance the performance of a generalist herbivore. *Oecologia*, **156**, 313–324.
- Waldbauer, G.P. & Friedman, S. (1991) Self-selection of optimal diets by insects. *Annual Review of Entomology*, **36**, 43–63.
- Westoby, M. (1978) What are the biological bases of varied diets? *American Naturalist*, **112**, 627–631.

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