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LETTER

Functional trait diversity across trophic levels determines herbivore impact on plant community biomass

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Abstract

Understanding the consequences of trophic interactions for ecosystem functioning is challenging, as contrasting effects of species and functional diversity can be expected across trophic levels. We experimentally manipulated functional identity and diversity of grassland insect herbivores and tested their impact on plant community biomass. Herbivore resource acquisition traits, i.e. mandible strength and the diversity of mandibular traits, had more important effects on plant biomass than body size. Higher herbivore functional diversity increased overall impact on plant biomass due to feeding niche complementarity. Higher plant functional diversity limited biomass pre-emption by herbivores. The functional diversity within and across trophic levels therefore regulates the impact of functionally contrasting consumers on primary producers. By experimentally manipulating the functional diversity across trophic levels, our study illustrates how trait-based approaches constitute a promising way to tackle existing links between trophic interactions and ecosystem functioning.

Keywords

Biodiversity experiment, body size, complementarity effect, functional trait diversity, grassland, herbivore, incisor strength, insect communities, plant–herbivore interactions, trophic interactions.

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INTRODUCTION

How biodiversity regulates ecosystem functioning has stimulated much research in the past decade (Hooper *et al*. 2005) and led to important progress towards answering this question (Cardinale *et al*. 2012). Pioneering biodiversity experiments (e.g. Tilman *et al*. 1997) have shown a general positive relationship between species diversity and ecosystem functioning. However, it is increasingly recognised that it is not the number of species *per se* that influences ecosystem functioning, but rather the functional traits of species within communities and their diversity (Hooper *et al*. 2005). On one hand, the traits of the dominant species are hypothesised to determine biodiversity effects on ecosystem functioning because dominant species represent the largest amount of biomass within communities (mass-ratio hypothesis, Grime 1998). On the other hand, functional diversity, i.e. the diversity and the abundance of traits in a community (Mouillot *et al*. 2011), may affect ecosystem functioning largely because of niche differences between co-occurring species (Hooper *et al*. 2005). Increasing niche differences between species is hypothesised to improve resource utilisation and enhance ecosystem functioning (Chesson 2000; Maire *et al*. 2012).

Most biodiversity experiments conducted in terrestrial systems have manipulated species diversity within one single trophic level, primary producers (Cardinale *et al*. 2012). Some studies have considered multiple interacting trophic levels and tested how plant species diversity impacts higher trophic levels (i.e. bottom-up effect, Scherber *et al*. 2010a; Haddad *et al*. 2009). In contrast, few studies have explicitly manipulated functional diversity of higher trophic levels (top-down effects, Cardinale *et al*. 2012; but see Duffy 2002; Hillebrand & Cardinale 2004 for aquatic ecosystems; see Fontaine *et al*. 2005 for bee-plant interactions). Recent experimental evidence from aquatic systems (e.g. Best *et al*. 2013; Lefcheck & Duffy in press) suggests that functional diversity in higher trophic levels predicts ecosystem functioning with more accuracy than species diversity. Considering the role of trophic interactions for ecosystem functioning (Belovsky & Slade 2000; Duffy *et al*. 2007), testing the impact of functional diversity within and across multiple trophic levels represents an important step forward to improve our ability to scale up biodiversity effects to ecosystems (Reiss *et al*. 2009; Schmitz *et al*. 2015). To achieve this goal, it would require to explicitly identify the functional traits that mediate trophic interactions between adjacent trophic levels and determine their impact at the ecosystem scale (trait-mediated interactions, Schmitz *et al*. 2015).

Herbivores play a major role for plant diversity and functioning of grassland ecosystems (Díaz *et al*. 2007; Haddad *et al*. 2009). Yet, studies investigating plant–herbivore interactions at the community and ecosystem scale have mostly

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focused on single herbivore species (e.g. Scherber *et al.* 2010b) or on large groups of vertebrate herbivores (sheep or cattle; see Díaz *et al.* 2007 for review). However, herbivore communities, and in particular herbivorous insect communities, can be extremely species rich (Novotny *et al.* 2006) and functionally diverse, e.g. they exhibit a large variation in body size and feeding niches (Jarman & Sinclair 1979; Ibanez *et al.* 2013a; Kartzinel *et al.* 2015). How functional diversity within herbivore communities determines their impact on plant communities has never been quantitatively tested.

Grasshoppers represent the largest proportion of arthropod biomass in temperate grasslands (Blumer & Diemer 1996), significantly impacting plant biomass and ecosystem functioning (Belovsky & Slade 2000). Previous work demonstrated that the impact of single grasshopper species on plant biomass can be precisely predicted from mandibular traits (i.e. incisor strength) (Deraison *et al.* 2015) because of mechanistic linkages between incisor strength and leaf biomechanical properties (Ibanez *et al.* 2013a). Across grasshopper species, clear feeding niche differences have been reported (Deraison *et al.* 2015), with grasshoppers characterised by strong incisor strength consuming tough leaves while grasshoppers with weaker incisor strength preferentially consuming tender leaves. Feeding niche differences between grasshoppers suggest that complementarity may determine herbivore community impact on plant community biomass (Deraison *et al.* 2015). Increasing the functional diversity of mandibular traits within a grasshopper community could increase the number of plant species eaten, thus increasing overall plant biomass consumption (complementarity hypothesis; Hooper *et al.* 2005; Duffy *et al.* 2007). However, the complementarity hypothesis only holds if local plant resources are limiting for herbivores. In absence of resource limitation, increasing the functional diversity of mandibular traits would in contrast decrease herbivore impact on plant biomass. At a fixed density of herbivores within communities, an increase in mandibular trait diversity would increase the range of plant species potentially eaten and result in a lower herbivory pressure at the level of plant individuals (see the consumer niche breadth effect, Thébault & Loreau 2003; Duffy *et al.* 2007). This effect is likely to be modulated by plant diversity itself, an important parameter controlling for resource availability (Otway *et al.* 2005; Duffy *et al.* 2007).

We conducted a grasshopper diversity experiment using six functionally contrasting grasshopper species, and manipulated both species richness and functional diversity (FD, Laliberté & Legendre 2010) of grasshopper incisor strength while controlling for variations in grasshopper functional identity (defined as the abundance-weighted mean trait value of the community, Mouillot *et al.* 2011). Throughout a 2-year experiment, we sampled plant biomass to assess the impact of grasshoppers. We hypothesised that the functional identity and diversity of grasshopper and plant communities were better predictors of grasshopper impact on plant biomass than species identity and diversity. Specifically, increasing functional diversity of incisor strength would: (1) increase feeding niche differences between grasshoppers at the community level; (2) translate into a higher impact of grasshopper communities on plant community biomass.

MATERIAL AND METHODS

Field site

The experiment was set up for two consecutive years (from 26 June 2012 to 4 October 2013) in a species-rich calcareous grassland (76 plant species at the field scale) located in the long-term ecological research (LTER) site 'Zone Atelier Plaine et Val de Sèvre' (46°11′ N, 0°28′ W) in central-western France. The grassland was managed extensively by a local farmer (no fertilisation, cutting frequency twice a year). Vegetation was dominated by grasses (average cover in June 2012: 38.5%; e.g. *Arrhenatherum elatius*, *Bromus erectus*, *Dactylis glomerata*), forbs (30.5%; e.g. *Daucus carota*, *Salvia pratensis*, *Verbena officinalis*) and legumes (7.5%; *Lotus corniculatus*, *Ononis repens*, *Trifolium pratense*) (see Deraison *et al.* 2015 for additional information on site selection).

Grasshopper diversity experiment

We designed a grasshopper diversity experiment to disentangle the effects of species diversity from effects of functional identity (community-weighted mean) and diversity (FD) on plant community biomass (Dias *et al.* 2013). We focused on a mandibular trait, incisor strength, which has been shown to reflect with high accuracy the feeding niche of single grasshopper species (see Ibanez *et al.* 2013a; Deraison *et al.* 2015). Grasshopper feeding preferences can be explained by chemical and biomechanical properties of plant leaves (e.g. leaf dry matter content and C : N ratio), and differences in grasshopper feeding niche are correlated with differences in incisor strength suggesting for complementarity. The impact of grasshopper species may also depend on multiple interacting traits. For instance, grasshopper species can vary greatly in body size and stoichiometric balance, from which contrasting effects on plant biomass can be expected (Hillebrand *et al.* 2009). We therefore tested the effects of grasshopper body size and stoichiometric balance (i.e. carbon nitrogen ratio, C : N ratio) in our experiment.

Grasshopper species selection and trait measurements

We selected six grasshopper species: *Chorthippus biguttulus* L. (hereafter Cb), *Chorthippus dorsatus* Zetterstedt (Cd), *Calliptamus italicus* L. (Ci), *Euchorthippus elegantulus* Zeuner (Ee), *Pezotettix giornae* Rossi (Pg) and *Pseudochorthippus parallelus* Zetterstedt (Pp). The six species are numerically dominant in the study area, representing 90% of grasshopper total abundance.

Grasshopper traits were measured in 2011 in a companion study (Deraison *et al.* 2015) on 10 individuals per sex and per grasshopper species. Briefly, incisor strength was calculated following Ibanez *et al.* 2013a:

$$\mathrm{IS} = \mathrm{A} \times \frac{\mathrm{L_a}}{\mathrm{L_i}} \times \frac{1}{\mathrm{R_i}} \qquad (1)$$

where A is the mandible section area (proxy of muscle force), $\mathrm{R_i}$ is the incisor region length, $\mathrm{L_a}$ the adductor muscle lever and $\mathrm{L_i}$ the incisor lever. Grasshopper body size was measured as the length from the head to the femur apex of the posterior legs (Deraison *et al.* 2015). Grasshopper C : N ratio was determined using a CHONS microanalyser (Carlo Erba Reagents, Paris, France). For each grasshopper trait, we used the mean value calculated for each species and sex in subsequent analyses.

Experimental design

The experiment was laid out as a randomised block design with five blocks, 14 treatments and a total of 70 cages (see supporting information in Appendix S1 for more information on the experimental design). Cages measured 1 m³ and were made from transparent insect proof netting (PE 22.30, 920 × 920 μ; DIATEX, Saint Genis Laval, France). All treatments were randomly assigned within each block. Grasshopper density was fixed at 24 individuals per m² with a constant sex ratio of one. 24 individuals per grassland m² is a density at which a significant effect on plant biomass can be expected (Scherber *et al.* 2010b; Deraison *et al.* 2015). It is also a realistic density observed in the study area. For instance, adult density can range between 0 and 60 individuals per m² and a density above 20 individuals per m² can be observed in 10% of the grasslands (Badenhausser, pers. obs.).

The grasshopper diversity treatments had the following four levels: (1) a control treatment with no grasshoppers; (2) six single-species treatments; (3) 6 three-species treatments and (4) a six-species treatment. We used the three-species treatments to create contrasting grasshopper assemblages characterised by different functional identities (community-weighted means) and diversities (FD, Laliberté & Legendre 2010). The community-weighted mean represents the mean value of a particular trait across a local grasshopper community, weighted by the relative abundance of each species. FD is similar to the variance of the community trait distribution. Based on the six species pool, we simulated all possible species assemblages in the three-species treatments and selected final assemblages according to a set of defined constraints: (1) a similar individual density for each species in each mixture; (2) a balanced distribution of species across treatments; (3) mean incisor strength variation across mixtures independent from the variation of FD-incisor strength and (4) mean incisor strength and FD-incisor strength independent from variation of mean grasshopper body size and mean grasshopper C : N ratio across communities. Overall, correlations between mean incisor strength and FD-incisor strength were not significant across treatments ($r^2 = 0.16$, $F_{1,11} = 2.12$, $P = 0.17$) (Appendix S1). Community-weighted mean and FD metrics were computed using the package FD in R (R Development Core Team 2013), version 3.2.1.

Our design allowed us to disentangle the effects of functional diversity of incisor strength and functional identity, quantified on multiple grasshopper traits (incisor strength, body size, grasshopper C : N ratio), while controlling for independent variations of grasshopper species diversity (See Appendix S1 for community-trait values).

Experimental protocols

Before cages had been stocked with grasshoppers, botanical surveys were conducted in all 70 cages in early summer 2012 and 2013 to estimate the initial cover of each plant species. Cover was visually estimated in nine quadrats (10 × 10 cm) regularly spaced every 20 cm within each cage. We also removed aboveground invertebrates and predators (e.g. spiders) from all cages using a vacuum cleaner. Before the beginning of the experiment, young adults of each grasshopper species were collected from 25 grasslands in the study area in order to avoid potential effects of developmental plasticity on grasshopper traits (Thompson 1992). Each individual was sexed and randomly transferred into the different treatments. In total, 1560 individuals were caught to initiate the experiment.

During the experiment, we checked every 2 weeks: (1) grasshopper density by counting carefully the number of living individuals inside each cage; (2) the presence of other aboveground invertebrates (e.g. spiders) which were removed by hands if present. This inspection did not disturb the vegetation patch. We replaced dead grasshopper individuals in order to keep the species density constant. At the end of August 2012 and 2013, grasshopper replacement was stopped. One month later, we removed grasshoppers by hand from each cage and at that time we estimated grasshopper survival as the proportion of living individuals at the end of the experiment.

Then we measured grasshopper herbivory for each plant species per cage to quantify the feeding preferences within grasshopper assemblages (see Deraison *et al.* 2015 for a detailed methodology). Each year, herbivory was measured on individual plant species that represented 80% of the total plant cover in each cage. We randomly sampled 10 tillers for grasses or 10 stems for legumes and other forbs. We visually estimated the proportion of leaf area which had been consumed by grasshoppers (in steps of 5%) for each leaf belonging to each harvested tiller or stem. We then averaged observed herbivory for each plant species in each cage. Observed herbivory ranged from 0 (when leaves were intact) to 100% when leaves had been entirely consumed, leaving only the plant stem.

Finally, aboveground plant biomass was measured in each cage at the beginning of October 2012 and 2013. We harvested all plant biomass above a cutting height of 5 cm from the soil surface within a 50 × 50 cm quadrat to mimic the effect of mowing. Plant material was oven-dried at 60 °C for 72 h and weighed. Grasshopper impact was then calculated by comparing the plant biomass within each grasshopper treatment with plant biomass in the control treatment (no herbivore) using the log response ratio (LNRR) (Suding *et al.* 2003):

$$\mathrm{LNRR} = \ln\left(\frac{\text{Biomass harvested in control treatment}}{\text{Biomass harvested in herbivory treatment}}\right) \qquad (2)$$

A LNRR of zero means that the grasshopper species had no impact on plant biomass, i.e. the harvested biomass was similar in the control cages compared with the cages with grasshoppers. When LNRR > 0, the grasshopper species had removed plant biomass. An increasing value shows a higher grasshopper impact.

Plant trait measurements

We quantified the functional structure of plant communities within each cage by measuring three plant traits which influence the impact of grasshoppers on plant biomass: leaf carbon nitrogen ratio (C : N ratio), leaf dry matter content (LDMC) and plant height. LDMC and leaf C : N ratio are positively related to leaf toughness, a major determinant of the feeding preferences of grasshopper species (Ibanez *et al.* 2013a). Plant height is related to the aboveground biomass. Plant traits were measured in the experiment in early June 2013. We selected plant species representing more than 80% of the total biomass per cage. Trait measurements were carried out on eight individuals per species and cage using standardised protocols (Cornelissen *et al.* 2003).

To quantify the quality and the diversity of plant resources for grasshoppers within each cage, we computed a community-weighted mean and plant functional diversity for each plant community in each cage. Plant functional diversity was computed using the three traits together, following Laliberté & Legendre (2010).

Feeding preferences of grasshopper communities

Using herbivory data, we quantified the average feeding preference and niche breadth for each grasshopper assemblage as a function of the selected plant traits (LDMC, C : N ratio) following Ibanez *et al.* (2013b). The average feeding preference is defined as the average trait value of the plant consumed by grasshoppers. It is calculated as the sum of trait values of the leaves eaten weighted by the percentage of herbivory observed for a focal plant species *i*. It was calculated for each plant trait:

$$\text{Average feeding preference} = \sum\nolimits_{i=1}^{n} \frac{a_i}{\sum\nolimits_{i=1}^{n} a_i} \times trait_i \qquad (3)$$

where a_i is the observed herbivory (%) of the plant species i and n is the total number of plant species per cage over the 2-year experiment.

Feeding niche breadth is defined as the range of trait values of the plants consumed by grasshoppers. It reflects the diversity of plant species consumed by grasshoppers as a function of the trait values of the plant species *i*, weighted by the observed herbivory (a_i) on plant species *i*. It was calculated for each plant trait:

$$\text{Feeding niche breadth} = \sum\nolimits_{i=1}^{n} \frac{a_i}{\sum\nolimits_{i=1}^{n} a_i} \times z_i \qquad (4)$$

where z_i is the square difference between the mean trait value of the plant species i and the mean trait value of the entire plant community.

Data analysis

Grasshopper traits and feeding niche

We tested the relationship between functional identity (community-weighted mean) and diversity (FD) of grasshopper communities and the feeding niche. Using linear regression, we tested the relationship between the community-weighted means and FD of grasshoppers and grasshopper average feeding niche preference and niche breadth. This analysis was conducted for all grasshopper traits (incisor strength, body size and C : N ratio) and plant traits (LDMC and C : N ratio).

Grasshopper community impact on plant biomass

We used four models to link the diversity of grasshopper communities to their impact on plant community biomass (LNRR, see eqn 2). The first model (treatment model) tested the effect of grasshopper species treatments (13 levels). The second model (species diversity model) tested the effect of grasshopper species richness, i.e. the number of grasshopper species within each treatment (1, 3 or 6 species). The third and the fourth model (functional diversity models) evaluated the effects of the functional trait diversity of grasshopper communities by explicitly distinguishing the effects of grasshopper functional identity (mean incisor strength) and diversity (FD-incisor strength). Functional identity tested for the effect of the trait of the dominant species (mass-ratio hypothesis, Grime 1998) similarly to the sampling-effect in experiments focused on plants (Hooper *et al.* 2005). Functional diversity tested for the effect of species feeding niche differences. A positive effect of functional diversity suggests a higher complementarity effect between species on plant biomass. The fourth model integrated information on the local plant community within each cage, i.e. plant community functional identity (community-weighted means) for three selected traits (mean LDMC, mean plant C : N ratio, mean plant height) and plant functional diversity. All of these explanatory variables were not correlated (Appendix S2).

We used linear mixed effect models for the analysis of LNRR data. All models included the cage number (from 1 to 70) to account for repeated measurements over the two successive years as random factors. In addition, year and block were included as fixed effects. Grasshopper survival per cage was added as a covariate to correct for potential effects on plant biomass (Appendix S3). In the third and fourth models we also included mean body size and mean grasshopper C : N ratio to take into account for their variation across treatments and isolate the net effect of mean incisor strength and FD-incisor strength. However, as mean body size and mean grasshopper C : N ratio were correlated ($r > 0.7$, Appendix S1), we tested competing models with one of these two variables. In all models, interaction terms between year and explanatory variables were included but they were not significant. All covariates were standardised (mean-centred and divided by the standard deviation) to interpret parameter estimates on a comparable scale. For each model, we generated all possible models and simplified them step-by-step using Maximum Likelihood test. Selected models were compared using the Akaike information criterion, corrected for small sample sizes (AICc). Model residuals were inspected for constant variance and normality. Finally, post hoc analyses were performed to assess differences between treatments (Tukey′s honestly significant differences, Hothorn *et al.* 2008). Parameters of the best model were estimated using restricted maximum likelihood.

RESULTS

Relationships between grasshopper functional identity, functional diversity and feeding niche

Grasshopper traits explained observed feeding preferences between and within grasshopper communities. Increasing the functional diversity of grasshopper incisor strength increased the feeding niche breadth of grasshopper communities estimated with LDMC (Fig. 1a). Mean incisor strength correlated positively with the average trait value of the plant consumed by grasshoppers (Fig. 1b,c). Grasshopper communities with strong incisors preferentially ate plants with high LDMC and C : N ratio. In contrast, grasshopper communities characterised by a low mean incisor strength mainly consumed plants with low LDMC and C : N ratio. Mean incisor strength was not correlated with the feeding niche breadth. Consistently, average feeding preference and niche breadth were also not correlated. This suggests an absence of link between the mean incisor strength of grasshopper communities and the range of plant trait values consumed by herbivores. Grasshopper body size or C : N ratio was not correlated with any feeding niche metrics.

Herbivore community impact on plant biomass

The grasshopper experimental treatments (model 1) significantly impacted plant community biomass (LNRR) (Fig. 2a). Grasshopper impact was lowest in the single species treatment *P. giornae* (0.16 ± 0.14 SE) and highest in the *C. dorsatus* and six-species treatments (1.09 ± 0.19 SE). Plant biomass was 136.00 g m^{-2} (± 21.29 SE) in cages without herbivores. With herbivores, plant biomass ranged from 52.68 g m^{-2} (± 10.42 SE) in the six-species mixture to 121.07 g m^{-2} (± 17.26 SE) in the single-species treatment *P. giornae* (Appendix S4). Consistently, the species diversity model (model 2) showed a positive and significant relationship between herbivore species richness and herbivore impact (Fig. 2b).

Grasshopper impact (LNRR) was best explained by models 3 and 4 (lowest AICc, Tables 1 and 2). Herbivore functional identity and diversity both explained the effect of herbivore community on plant biomass (Figs 3 and 4, Table 2). Herbivore communities with strong incisor and high functional diversity (FD-incisor strength) had the strongest negative impact on plant biomass. Grasshopper C : N ratio was also selected in the final model and had a negative effect on plant biomass. Alternative models including grasshopper body size was not selected (ΔAICc > 2). Model 4 indicated that tall and functionally diverse plant community was less impacted by herbivores (high mean plant height, functional diversity, Figs 3 and 4). Other plant community traits, i.e. mean LDMC and mean plant C : N ratio, were not selected in model 4 (Table 2). In all analyses, high herbivore survival had a positive impact on plant biomass.

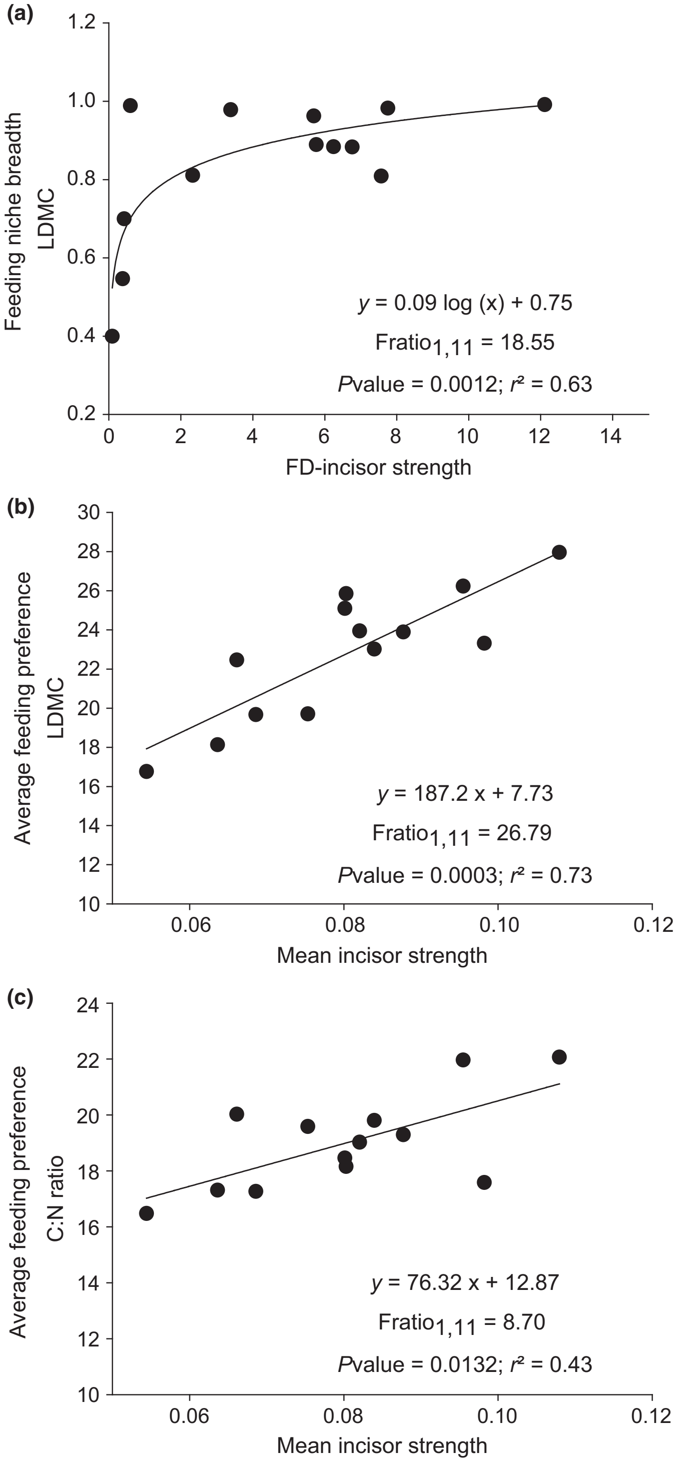

Figure 1 Relationship between grasshopper functional identity (community-weighted means) and diversity (FD) and the feeding niche breadth (panel a) and feeding preference for leaf dry matter content (LDMC, panel b) and plant C : N ratio (panel c). We indicated regression parameters in each panels.

DISCUSSION

The use of functional traits has recently been proposed to scale up trophic interactions from the species up to the community and the ecosystem levels (Reiss *et al.* 2009; Schmitz *et al.* 2015). While promising, experimental evidences demonstrating the explicit linkage between traits, trophic interactions and ecosystem functioning are scarce for

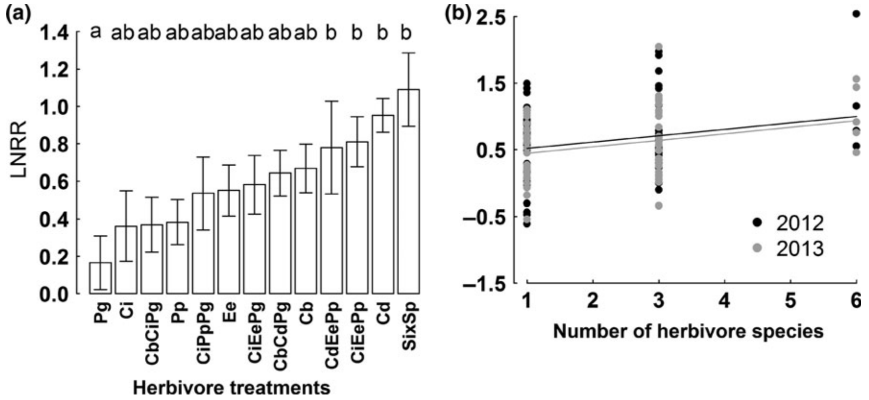

Figure 2 Average log response ratio (LNRR) (± SE) over the 2 years (a) for each herbivore treatment (Herbivore treatment: $F_{1,12} = 3.16$, $P < 0.001$; Survival: $F_{1,1} = 10.97$, $P < 0.001$; Year: $F_{1,1} = 0.21$, $P = 0.76$; Block: $F_{1,4} = 4.14$, $P = 0.002$; Year : Block: $F_{1,4} = 3.00$, $P = 0.02$) and (b) against the number of herbivore species per cage (Number of herbivore species: $F_{1,2} = 9.13$, $P = 0.004$; Herbivore survival: $F_{1,1} = 8.53$, $P = 0.004$; Year: $F_{1,1} = 0.30$, $P = 0.65$; Block: $F_{1,4} = 2.83$, $P = 0.02$; Year : Block: $F_{1,4} = 3.22$, $P = 0.01$). Significant differences ($P < 0.05$) between herbivore treatments are represented by different letters (a, b) calculated by multiple comparisons for parametric linear mixed effect models. Abbreviations are Cb: *Chorthippus biguttulus*, Cd: *Chorthippus dorsatus*, Ci: *Calliptamus italicus*, Ee: *Euchorthippus elegantulus*, Pg: *Pezotettix giornae*, Pp: *Pseudochorthippus parallelus*. SixSp refers to the six species mixture.

Table 1 Model selection results testing the effect of herbivore and plant communities on plant community biomass (LNRR, see eqn 2 in the main text). All models included the cage number (from 1 to 70 cages) as a random effect to account for repeated measurements over the two successive years of the experiment. Model 1 (treatment model) tested the effect of herbivore species treatments. Model 2 (species diversity model) tested the effect of herbivore species diversity. Models 3 and 4 (functional diversity models) evaluated the effects of the functional composition of herbivore communities. Model 4 also included the functional composition of the local plant community. I(Year–Block) represents the interaction term between year and block

Model	Selected model	AICc
1	Year + Block + I(Year–Block) + Herbivore treatment + Herbivore survival	198.9
2	Year + Block + I(Year–Block) + Number of grasshopper species + Herbivore survival	199.2
3	Year + Block + I(Year–Block) + Mean incisor strength + Mean grasshopper C : N ratio + FD-incisor strength + Herbivore survival	186.3
4	Year + Block + I(Year–Block) + Mean incisor strength + Mean grasshopper C : N ratio + FD-incisor strength + Mean plant height + Plant functional diversity + Herbivore survival	179.1

Table 2 Results of linear mixed effect models testing the effect of the functional composition of herbivore communities (models 3 and 4) and plant communities (model 4) on plant community biomass (log response ratio). d.f., degrees of freedom. I(Year–Block) represents the interaction term between year and block

	Model 3: Herbivore functional diversity			Model 4: Herbivore functional diversity and functional characteristics of local plant communities		
Model parameters	d.f.	*F* value	*P* value	d.f.	*F* value	*P* value
Year	1	0.17	0.778	1	0.83	0.250
Block	4	4.11	0.002	4	4.36	0.002
I(Year–Block)	4	2.97	0.018	4	3.02	0.017
Mean incisor strength	1	13.63	0.049	1	15.66	0.005
Mean grasshopper C : N ratio	1	0.01	0.050	1	0.01	0.021
FD-incisor strength	1	7.75	0.001	1	8.91	0.006
Herbivore survival	1	14.99	0.0001	1	2.73	0.006
Mean plant height				1	14.57	0.006
Plant functional diversity				1	10.46	0.001
Residual	116			114		
AICc	186.3			179.1		

multi-species assemblage (Schmitz *et al.* 2015). By explicitly manipulating trait identity and diversity in experimental communities, our study provides novel insights on how functional trait diversity across trophic levels determines the outcome of trophic interactions on primary producers. We demonstrate that resource acquisition traits in functionally diverse insect communities represent a novel niche dimension which regulates herbivore impact on grassland ecosystems. Our results echoed recent findings in aquatic systems highlighting the role of functional diversity in upper trophic levels on ecosystem functioning (e.g. Best *et al.* 2013; Lefcheck & Duffy in press). Most of these experiments have manipulated species diversity and quantified the effect of functional diversity using *post hoc* analyses (Duffy *et al.* 2007). Experiment manipulating functional diversity had generally manipulated contrasting trophic groups (e.g. Best *et al.* 2013) or continuous traits for which no clear linkage between trait and feeding preferences have been quantitatively assessed (i.e. mass-related traits or mobility, Lefcheck & Duffy in press). In contrast, our study was specifically designed to (1) test the effect of functional diversity of important traits *a priori*, for which a mechanistic link with the species feeding niche has been demonstrated and (2) control for the effect of functional identity on multiple inde-

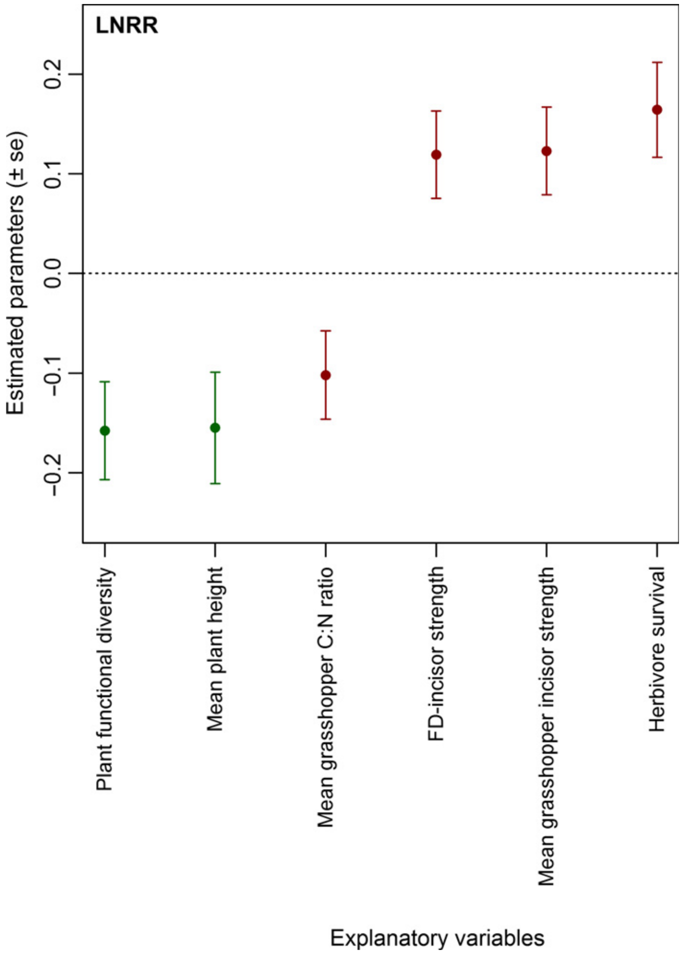

Figure 3 Scaled estimates (± SE) for the herbivore and plant community functional composition on log response ratio (LNRR) (model 4). See Table 2 for model parameters.

pendent traits (i.e. incisor strength, body size, C : N ratio), an important issue in functional diversity experiments (Dias *et al.* 2013).

The functional identity and diversity of herbivore communities jointly determined their impact on plant biomass, suggesting that mass-ratio and complementarity processes are equally important to explain consumer impact on primary producers. In contrast, previous studies focusing on plant functional diversity and productivity often reported a negligible impact of complementarity as compared to mass-ratio processes (de Bello *et al.* 2010 for a review; but see Mouillot *et al.* 2011). As opposed to primary producers, interspecific interactions between herbivores may be limited due to complementarity in feeding preferences (Deraison *et al.* 2015), resulting in maximised resource utilisation (Chesson 2000; see the limiting similarity theory, MacArthur & Levins 1967). In contrast, plants generally compete for the same few resources (inorganic N, light, Grime 1998). In that case, resources are generally preempted by the most competitive species resulting in asymmetric competition between species (Maire *et al.* 2012). Complementarity in resource acquisition may thus be a fundamental property of diverse herbivore communities (Duffy 2002; see also recent evidence for large African herbivores, Kartzinel *et al.* 2015), ultimately determining their impact on plant communities.

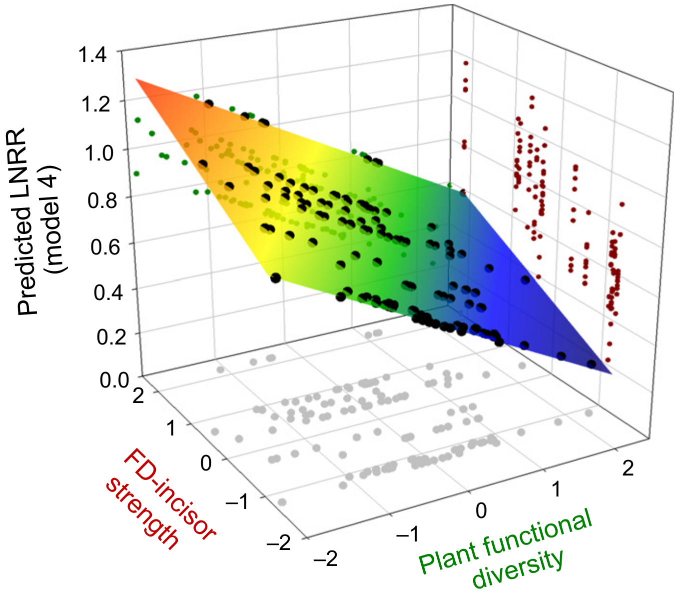

Figure 4 Predicted impact of grasshopper FD-incisor strength (scaled) and plant functional diversity (scaled) on plant community biomass (Predicted log response ratio (LNRR)) (see parameters of model 4 in Fig. 3).

Increasing the diversity of resource acquisition traits (i.e. incisor strength) within herbivore communities increased the feeding niche breadth at the community scale and translated into a higher impact on plant community biomass, following the complementarity hypothesis. Our results therefore confirm the theoretical predictions that increasing niche differences within upper trophic levels increase consumer impact on primary producers competing for limiting resources (Thébault & Loreau 2003; Sapijanskas & Loreau 2010). Mechanistically, increasing functional diversity of mandibular traits is hypothesised to increase herbivore impact on plant biomass by increasing the diversity of plants eaten, i.e. shifting from a specialist towards generalist herbivore communities. This community-level pattern thus mirrors species-based theoretical predictions on the impact of specialist vs. generalist consumers (Duffy *et al.* 2007). The reduced mortality observed when herbivores interacted with other functionally contrasting species (see the positive effect of herbivore trait dissimilarity on grasshopper survival in interspecific treatment relatively to intra-, Appendix S3) is also in line with the complementarity hypothesis as it is a theoretical expectation that individual performance increases in interspecific treatments relatively to intra- when niche differences between species are maximised (Chesson 2000). We cannot formally distinguish whether this pattern is due to competition (MacArthur & Levins 1967) or to facilitation (Hooper *et al.* 2005) as we used constant herbivore densities in our experimental design. Further experiments are needed to evaluate the relative importance of competition and facilitation in multispecies herbivore assemblages. However, in both cases, the observed complementarity in herbivore feeding niche, highlighted in our study, is fundamental to explain their impact on plant community biomass.

Importantly, our study also shows that an increase in plant functional diversity may reduce the impact of herbivore communities on plant community biomass (Fig. 4). This result is supported by other studies suggesting that increasing species and functional diversity within plant communities decreases the impact of their consumers at the community scale (e.g. Otway *et al*. 2005; Duffy *et al*. 2007). Resource limitation increased at high plant diversity for each single consumer species because it increased the probability of occurrence of non-preferred plants, thus diluting their impact at the whole community scale (Thébault & Loreau 2003; Ives *et al*. 2005; Haddad *et al*. 2009). These findings are in agreement with previous studies which demonstrated that species-rich plant communities may support highly diverse arthropod communities exploiting different resources (Schaffers *et al*. 2008; Scherber *et al*. 2010a). This is also supported by survival data as grasshopper interactions shifted from intraspecific interactions > inter- to intra = inter as plant functional diversity increased (Appendix S3), a pattern suggesting for density-dependent regulation (*sensu* Chesson 2000).

The effect of herbivore functional identity on plant biomass was highlighted by the concomitant effects of community-weighted incisor strength and herbivore C : N ratio. In a previous study (Deraison *et al*. 2015), we provided a detailed analysis on the relationship between incisor strength and single grasshopper species impact on plant biomass. Consistently with our findings observed at the species level, grasshopper communities with high incisor strength had the highest impact on plant biomass because grasshopper species with strong incisors targeted the most dominant plants in our experiment. Interestingly, we also found an effect of grasshopper community-weighted C : N ratio on plant biomass. This may be due to food preferences which can be determined in part by the stoichiometric match between herbivores and plants (Behmer & Joern 2008). However, recent findings (Ibanez *et al*. 2013a; Deraison *et al*. 2015) suggested no links between herbivore C : N ratio and food preferences. More likely, the effect of grasshoppers on plant biomass might be explained by the negative correlation between grasshopper C : N ratio and body size (Table S1). Tall species are predicted to exhibit higher metabolic rates (metabolic theory, Brown *et al*. 2004) that are often correlated with lower C : N ratio (Hillebrand *et al*. 2009). In that case, large grasshoppers may need to consume more plant biomass than small ones to achieve a higher nutrient demand (Hillebrand *et al*. 2009). Consistently, we found that herbivores with low C : N ratio exhibited higher mortality in intraspecific treatments (Appendix S3), and suggested that large insect herbivores tend to be more food limited than small ones. Nevertheless, we note that community-weighted body size was not selected in any final models explaining the overall impact of herbivores on plant communities, a result that is at odds with metabolic theory (Brown *et al*. 2004). Our results suggest that accounting for other traits related to resource acquisition and to a lesser extent to herbivore stoichiometry may allow a better description of interactions in plant–herbivore networks and their ecosystem level consequences.

While this study focused on insect herbivore communities, the mechanisms on which our interpretation relies, based on acquisition trait differences and feeding niche complementarity, can be easily extended to other systems. Our findings may apply directly to mammalian herbivore communities as strong differences in feeding preferences have been recently demonstrated (Kartzinel *et al*. 2015). In this context, craniodental anatomy (Codron *et al*. 2008), digestive morphology and capacity (Belovsky 1986) may represent good candidate traits (see also Mouillot *et al*. 2005 for similar evidence in fish communities). Similarly, our findings could apply to other ecosystem functions. As mandible trait diversity regulated herbivore disturbance on plant community, clear predictions could be made on how functionally diverse herbivore communities impact C–N cycling (Belovsky & Slade 2000). Also, important traits such as bee tongue length or carabid beetle mandible strength and their respective diversity in species-rich insect communities may determine pollination success (Fontaine *et al*. 2005) and pest regulation (Bohan *et al*. 2011). In communities in which intraguild predation occurs (e.g. carabid beetles and spider communities, Rusch *et al*. 2015), predation could be controlled by differences in body size between co-occurring species (Woodward & Hildrew 2002). Overall, manipulating the functional diversity of both resource acquisition and size-related traits provides exciting and promising avenues to reveal how trophic interactions shape ecosystem functioning in complex systems.

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AUTHORSHIP

All co-authors contributed to the original idea of the experiment and to the experimental design. HD, IB and NG conducted the experiment. HD & NG collected plant and grasshopper trait data. HD, IB and NG performed the analyses. NG and HD wrote the first draft of the manuscript. All co-authors substantially contributed to revisions.

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