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Title: Herbivore effect traits and their impact on plant community biomass: an experimental test using grasshoppers

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Summary

1. Using trait-based approaches to study trophic interactions may represent one of the most promising approaches to evaluate the impact of trophic interactions on ecosystem functioning. To achieve this goal, it is necessary to clearly identify which traits determine the impact of one trophic level on another.

2. Using functionally contrasting grasshopper species, we tested the ability of multiple traits (morphological, chemical and biomechanical) to predict herbivore impact on the biomass of a diverse plant community. We set up a cage experiment in an old species rich grassland field and evaluated how multiple candidate grasshopper effect traits mediated herbivore impact on plant biomass.

3. Grasshoppers had different impact on plant community biomass (consuming up to 60 % of plant community biomass). Grasshopper impact was positively correlated with their incisive strength while body size or grasshopper C:N ratio exhibited low predictive ability. Importantly, the strong relationship between the incisive strength and the impact was mediated by the grasshopper feeding niche, which was well predicted in our study by two simple plant traits (leaf dry matter content, leaf C:N ratio). Feeding niche differences between grasshoppers were explained by differences in incisive strength, highlighting the fundamental linkage between grasshopper effect traits and their niche.

4. Our study contributes to the development of the trait-based approach in the study of trophic interactions by providing a first experimental test of the relationship between herbivore effect traits, their impact on plant community biomass, and in a larger extent on ecosystem functioning. By comparing the relative importance of multiple interacting grasshopper traits, our study showed that incisive strength was a key effect trait which determined grasshopper feeding niche and its relative impact on plant community biomass.

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Key-words: plant insect interactions, effect traits, functional traits, grasshoppers, feeding niche, herbivory, grassland, C:N ratio, leaf dry matter content, body size, incisive strength

Introduction

Functional traits have been hypothesized to reflect the species niche (McGill *et al.* 2006; Devictor *et al.* 2010) as they determine how species respond to their environment (sensu Grinnell 1917) and how they impact their local environment (sensu Elton 1927). Based on these properties, a trait-based response-effect framework has been proposed to explore the consequences of environmental changes on ecosystem functioning (Lavorel & Garnier 2002; Suding *et al.* 2008). Basically, traits can be used to upscale individual species responses to environmental change at the community level (Suding, Goldberg & Hartman 2003; Gross *et al.* 2009) and to quantify how community functional changes (response traits) may in turn impact ecosystem functioning (effect traits, see Suding *et al.* 2008). To date, the trait-based approach has been mainly developed for primary producers (de Bello *et al.* 2010), often ignoring the impact of upper trophic levels on plant community dynamics and on ecosystem functioning (Belovsky & Slade 2000; Hillebrand & Matthiessen 2009). Integrating trait-based approaches in a multitrophic perspective may represent one of the most promising challenges to evaluate the impact of trophic interactions on ecosystem functioning (Reiss *et al.* 2009).

Herbivores play a major role in regulating plant diversity and ecosystem functioning (Olff & Ritchie 1998; Belovsky & Slade 2000). The majority of the studies that have used traits to investigate plant-herbivore interactions have focused on how plant functional traits respond to the herbivory pressure of one or few large herbivores such as sheep or cattle (see Diaz *et al.* 2007 for a review). Fewer studies have tested the effect of different herbivore functional groups on herbivory rates, e.g. considering different groups of invertebrates (Tanentzap *et al.*

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2010; Loranger *et al.* 2012). Recently, some studies have gone one step further using continuous traits to investigate the relationship between plants and insect herbivore communities. On one side, some studies suggested that community plant traits can determine insect herbivore species abundance according to a particular set of trait values (van der Plas, Anderson & Olff 2012; Frenette-Dussault, Shipley & Hingrat 2013). On the other side, complementary studies showed that local insect communities can have in turn different impacts on ecosystem functioning such as productivity or N cycling (e.g., Moretti *et al.* 2013). A central hypothesis emerging from these studies is that plant biomass consumption can be predicted from insect herbivore effect traits. However, which effect traits are likely to explain the impact of functionally contrasting herbivores on plant community biomass remains unclear. Particularly, how functionally contrasting insect herbivores, differing in multiple potential effect traits, may impact plant communities has, to our knowledge, never been experimentally tested.

An important component of grassland ecosystems is grasshoppers (Baldi & Kisbenedek 1997). These insect herbivores may consume up to 30% of the total plant biomass depending on species identity and on their dynamics over the growing season (Raynal 1989; Blumer & Diemer 1996). Grasshoppers are characterized by strong functional differences between species (Whitman 2008; van der Plas *et al.* 2012), hence different impacts on plant biomass might be expected. Different candidate traits may be considered as grasshopper effect traits. First, grasshopper body size is predicted to be linked to the quantity of biomass consumed in order to sustain species metabolic demands (see the "metabolic theory" in Brown *et al.* 2004; Schmitz & Price 2011). Large grasshoppers are predicted to have a higher impact on plant biomass than small ones (Moretti *et al.* 2013). Second, the strength of the mandibles has been suggested as a key trait for grasshoppers, reflecting their ability to cut hard leaves (Clissold

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2007). Grasshoppers with low mandibular strength might not be able to eat tough leaves and this may limit their impact on plant biomass. On the other hand, grasshoppers with higher mandibular strength may consume a larger range of plant types, and thus may have a higher impact on plant biomass (following a threshold rule, see Seath 1977; Lucas 2004). Mandibular traits have been recently shown to reflect the feeding preferences of grasshopper species (hereafter defined as grasshopper feeding niche) (Ibanez *et al.* 2013a). If feeding niche differences between grasshoppers (as reflected by their differences in mandibular traits) are important, the relationship between herbivore effect traits and their impact on plant biomass should be determined by the local resource availabilities, namely the trait values and the abundance of plant species within communities. This hypothesis suggests a weak functional response of grasshoppers, i.e. grasshopper feeding niche corresponds to an intrinsic property of the species which is independent of plant species abundance (Ibanez *et al.* 2013b). In this context, the stoichiometric balance of herbivores should also mediate their impact (Behmer & Joern 2008; Joern, Provin & Behmer 2012). For instance, the match between the carbon and nitrogen ratio of plants and grasshoppers should determine their feeding choice and ingestion rate (Hillebrand *et al.* 2009).

In this study, we experimentally tested how multiple effect traits mediate the impact of grasshoppers on plant community biomass. Specifically, two non-exclusive hypotheses were tested: (i) Grasshopper impact on plant community biomass increases as body size or incisive strength increases. In other words, differences in trait values between grasshopper species translate directly into a proportional impact on plant biomass; (ii) Grasshopper species impact is mediated by the match between their feeding niche and resource availability, defined as the abundance and the traits of plant species within communities. In that case, differences in trait values between grasshoppers reflect their feeding niche differences. As the impact of

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grasshoppers is likely to emerge from the interactions of multiple traits (e.g. body size, mandibular traits, insect carbon nitrogen ratio), we aimed to quantify the relative influence of multiple interacting traits on the impact of grasshopper species on plant community biomass and whether this impact was direct or mediated by species feeding niche differences.

Material and methods

STUDY SITE AND GRASSHOPPER SPECIES SELECTION

The study area was located in a large long term ecological research (LTER) site ("Zone Atelier Plaine et Val de Sèvre" - 46°11'N, 0°28'W) in central-western France. The LTER site covered approximately 450 km² of an intensively managed agricultural plain, mostly dedicated to cereal crop production with up to 12% of the land surface covered by grasslands. Since 2003, grasshopper species richness and density in grasslands have been surveyed annually within the LTER site (Badenhausser 2012). Thirty grasshopper species (Caelifera) have been recorded in the study area, and among them the genera *Chorthippus* (Gomphocerinae) and *Euchorthippus* (Gomphocerinae) dominate (Badenhausser 2012). Species from these genera are mostly considered grass-feeders (Bernays & Chapman 1970). Other abundant species in the study area, e.g. *Calliptamus italicus* L. and *Pezotettix giornae* Rossi, are known to feed on legumes and forbs (Unsicker *et al.* 2008). In this study, we selected the six numerically dominant grasshopper species, i.e., those accounting for 80% of the individuals recorded from 2003 to 2011 in the study area (Badenhausser 2012): *Chorthippus biguttulus* L., *Chorthippus dorsatus* Zett, *C. italicus*, *Euchorthippus elegantulus* Zeuner*, P. giornae* and *Pseudochorthippus parallelus* Zett. These species are characterized by contrasted size and habitat preferences (Badenhausser 2012).

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THE GRASSHOPPER EXPERIMENT

Field site selection

The experiment was performed between 27th July and 5th October 2012 in an-old grassland. It was a typical species rich calcareous grassland established at least 20 years ago on shallow soil and managed using extensive options (no fertilization, cutting frequency once or twice a year). In early 2012, vegetation was dominated by grasses (average cover: 38.5%; e.g. *Arrhenatherum elatius* L.*, Bromus erectus* Huds, *Dactylis glomerata* L.), forbs (30.5%; e.g. *Daucus carota* L., *Salvia pratensis* L.*, Verbena officinalis* L.) and legumes (7.5%; *Lotus corniculatus* L., *Medicago arabica* L., *Ononis repens* L.*, Trifolium pratense* L.). This grassland was chosen because of its high plant diversity (76 plant species in total) in order to offer a wide range of plant types. The large range of plant trait values associated with the leaf-economic spectrum (Reich, Walters & Ellsworth 1997; Wright *et al.* 2004) suggested also high plant functional diversity: leaf dry matter content (LDMC, %) ranged from 13% to 36% and carbon nitrogen (C:N, unit less) ratio from 8 to 37 (see Appendix S1 in Supporting Information).

Experimental design

The experiment was performed using a randomized block design (Hurlbert 1984) with seven treatments and five blocks for a total of 35 cages. Each cage corresponded to a 1 m^3 enclosure made from transparent insect proof netting (PE 22.30, 920 × 920 µ, DIATEX, France). The seven treatments consisted of one control treatment with no grasshoppers and six herbivory treatments each being a monospecific treatment with one of the six grasshopper species. Treatments were applied once within each of the five blocks to avoid pseudoreplication (Hulbert 1984) for a total of 5 independent replicates per treatment (7 treatments × 5 blocks = 35 cages).

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Grasshopper density per cage was fixed at 24 individuals of the same species (12 males and 12 females), a density at which a significant effect on plant biomass can be expected (Raynal 1989; Scherber *et al.* 2010). This is a realistic density in the study area. As an example, adult grasshopper density in a random sample of 100 grasslands ranged from 0 to 60 individuals/m² in 2004 (Badenhausser 2012), with an averaged density > 20 individuals/m² in 10% of these grasslands (Badenhausser *pers. data*).

Before adding the grasshoppers, we removed aboveground invertebrates and predators (e.g. spiders) from all cages using a vacuum cleaner. Young adults of each grasshopper species were collected from the surrounding area just before the beginning of the experiment. Each individual was sexed and randomly transferred into the different cages. During the experiment, we checked every two weeks: (i) grasshopper survival by counting the number of living individuals in each cage; (ii) the presence of other aboveground invertebrates (e.g. spiders) which were removed manually if present. We replaced dead grasshopper individuals in order to keep the density constant using adult grasshoppers caught at that time in the study area. At the end of the experiment, grasshoppers were removed by hand from each cage. In total, 720 individuals were caught to initiate the experiment.

Herbivore impact

Before the start of the experiment, a botanical survey was conducted in the 35 cages to estimate the initial abundance of plant species in late June 2012. We visually estimated the percent cover of each plant species in nine quadrats (10 cm × 10 cm) regularly spaced every 20 cm within each cage.

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We measured grasshopper herbivory per plant species observed at the end of the experiment within each cage to quantify the realized feeding niche of the six grasshopper species. To do so, we selected in each cage the plant species that represented 80% of the total plant cover and we randomly sampled for each plant species 10 tillers for grasses or 10 stems for legumes and other forbs. We then visually estimated the proportion of leaf area which was consumed by herbivores (in steps of 5%) for each leaf belonging to each harvested tiller or stem. We finally averaged the observed herbivory by 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. At the end, an observed herbivory for 22 plant species was available for each grasshopper species over a total of 40 plant species present within the experimental cages (See Fig. S2 for observed herbivory per species).

In each cage, aboveground plant biomass was quantified at the end of the experiment. We harvested all plant biomass above a cutting height of 5 cm from the soil surface in a 50 cm × 50 cm quadrat to mimic the effect of mowing. Plant material was oven-dried at 60°C for 72h and weighed. Observed herbivore impact was then calculated by comparing the total biomass within each herbivory treatment with the total biomass in the control treatment (no herbivores) in the same block (see raw data in Appendix S3) using the log response ratio (LNRR) (Suding *et al.* 2003) as follows:

$$Observed\ LNRR = \ln\left(\frac{Biomass\ harvested\ in\ control\ treatment}{Biomass\ harvested\ in\ herbivory\ treatment}\right) \qquad (eqn\ 1)$$

An observed LNRR of zero means no grasshopper impact on plant biomass, i.e. the harvested biomass was similar in the control cages compared with the cages with herbivores. Values of

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observed LNRR above zero, indicates that the grasshoppers had removed plant biomass, with increasing values related to a higher herbivory impact.

Plant biomass production was also monitored every three weeks in each cage during the time of the experiment by harvesting two small quadrats (15 cm × 15 cm) located outside the 50 cm × 50 cm quadrat used for the final biomass measurement. Plant biomass did not vary during the experiment (data not shown) likely due to a dry summer (no major rain events from July to September 2012). Hence, differences in total biomass at the end of the experiment in the 50 cm × 50 cm quadrats can be interpreted with confidence as the direct effect of grasshoppers on plant biomass in the herbivory treatments.

GRASSHOPPER TRAIT MEASUREMENTS

To test how herbivore effect traits may explain the impact of grasshopper species on plant community biomass, we measured a set of three candidate effect traits: (i) body size; (ii) mandibular traits such as the incisive strength; (iii) chemical traits, i.e. C:N ratio. Grasshopper body size and incisive strength were measured on 10 individuals per sex and per grasshopper species. Selected individuals were randomly chosen from a pool of individuals collected in the study area between 2009 and 2012 and stored in alcohol. The body size (BS) was measured as the length from the head to the femur apex of the posterior legs (Defaut 2012). The incisive strength (IS) was calculated using the formula of Ibanez *et al*. 2013a:

$$IS = A * \frac{La}{Li} * \frac{1}{Ri} \qquad \textit{(eqn 2)}$$

where Ri is the incisive region length, A the mandible section area, La the length of the adductor muscle lever and Li the length of the incisive lever. All morphological

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measurements were performed using a stereo microscope (Leica Microsystems M50) equipped with an integrated high definition microscope camera (Leica IC80 HD). Grasshopper C:N ratio was quantified using individuals of each species and sex. We used individuals collected within cages at the end of the experiment. After being collected, grasshoppers were kept 24h in the freezer and then oven dried (60°C during 48h). We used five replicates per species and per sex, each replicate consisting of four ground individuals. Carbon and nitrogen content of each sample was determined using a CHONS microanalyser (Carlo Erba 1500). Interspecific differences in trait values accounted for more than 80% of the total variability observed between measured individuals for all traits (data not shown), suggesting a relative low intraspecific variability for morphological traits. We thus used the mean trait value per species for subsequent analyses.

PLANT FUNCTIONAL TRAITS

To test whether plant functional traits can predict grasshopper feeding niche (i.e. which plant species were preferred and in which quantity as a function of plant traits), we selected seven leaf traits related with leaf shape, leaf physiological or biomechanical properties. These traits were: leaf complexity (leaf perimeter / leaf length ratio), leaf thickness, leaf nitrogen and carbon content, leaf C:N ratio, specific leaf area and LDMC. Plant trait data came from a local database from the LTER site "Zone Atelier Plaine et Val de Sèvre" from which we extracted a mean trait value per plant species present in our experiment (see Method S1 for more information on plant trait measurements). As with grasshopper traits, intraspecific variability for measured plant traits was low (below 20%, data not shown) and most of the variability was explained by species differences.

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DATA ANALYSES

All statistical analyses were performed using the R environment for statistical computing (R Development Core Team 2011) version 3.0.2.

Correlation between grasshopper traits

To link grasshopper effect traits to their impact on plant community biomass, we first evaluated how traits were correlated across grasshopper species using a principal component analysis (PCA). This approach approximated the functional niche of grasshopper species (Devictor *et al.* 2010) defined as their relative position in the functional trait space (See Appendix S1). Grasshopper body size (BS) correlated negatively with the carbon nitrogen (C:N) ratio (Fig. S1A). In contrast, incisive strength (IS) varied independently from BS. Consequently, we selected BS and IS as effect traits to test herbivore impact on plant community biomass in the following analyses, each trait reflecting an independent leading dimension of the species niche (Gross, Suding & Lavorel 2007).

Grasshopper impact on plant biomass and direct relationship with traits

We used a linear mixed model to test for the effect of the selected grasshopper species on plant community biomass (Observed LNRR, see *eqn 1*), performed with the function lmer (package lme4, Bates *et al.* 2014). The model had the following form:

$$Observed\ LNRR \sim \alpha + \beta_1 * grasshopper\ species\ identity + b * block + \varepsilon \qquad (eqn\ 3)$$

where α is the intercept, β_1 is the fixed effect coefficients for the species identity regressor, b the random effect coefficients for the intercepts 'block' (i.e., the random effects vary by block) and ε the residual error for each observation in each block. To test if grasshopper traits

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can explain observed grasshopper impact on plant biomass, we then substituted grasshopper identity by the trait values of each grasshopper species in the following linear mixed model:

$$Observed\ LNRR \sim \alpha + \beta_1 * IS + \beta_{2*} BS + \beta_3 * grasshopper\ survival + b_* block + \varepsilon \qquad (eqn\ 4)$$

where α is the intercept, β_1 and β_2 are the fixed effect coefficients for grasshopper functional trait regressors, β_3 are the fixed effect coefficient for grasshopper survival regressor, b the random effect coefficients for the intercepts 'block' and ε the residual error for each observation in each block. IS (incisive strength) and BS (body size) are the species mean trait values observed for each grasshopper species. All the explanatory variables were standardized (mean-centred and divided by the standard deviation) to interpret parameter estimates on a comparable scale (Schielzeth 2010). We selected the best model with the function dredge (library MuMIn Barton 2013) using the Maximum Likelihood test and Akaike information criterion for model selection corrected for small sample size (AICc) (Burnham & Anderson 2002). Residuals were inspected and met parametric test assumptions. We also provided R^2 value as an index of model fit following Nakagawa & Schielzeth (2013). Note that, to correct for a potential effect of grasshopper survival on biomass consumption, we included mean grasshopper survival per cage throughout the experiment in the models. On average, grasshopper mean survival was 0.80 ± 0.03. However, some grasshopper species (e.g. *P. parallelus*) showed relatively lower survival especially in late August (mean survival: 0.55 ± 0.01) (Appendix S4). While we regularly replaced dead individuals to keep grasshopper density constant in cages during the experiment, mortality could act as a potential bias on biomass consumption (Figs. S3 & S4).

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Grasshopper feeding niche

To test whether grasshopper impact was mediated by their feeding niche, we first modelled each species' feeding preferences using: (i) observed herbivory on the 22 plant species at the end of the experiment as the response variable; (ii) plant functional trait values as explanatory variables (see also Fig. S2). We then tested which grasshopper traits explained feeding niche differences.

Quantification of grasshopper feeding niche as a function of plant functional traits - We selected two independent plant traits that well explained functional differences between plant species (Fig. S1B). These traits reflected two major leaf properties that may impact herbivore choice, namely the LDMC and the C:N ratio of the leaves. High LDMC is related with high leaf toughness which may reflect a biomechanical barrier against herbivory (Seath 1977; Lucas 2004) and may influence grasshopper food selection (Ibanez *et al.* 2013a). C:N ratio determines the stoichiometric relationships between plants and herbivores and may influence the quantity of leaves that a herbivore needs to eat to achieve nutrient regulation but may also affect the selective choice of herbivores (Hillebrand *et al.* 2009).

Grasshopper feeding niche was quantified using the herbivory data observed at the end of the experiment. Maximum herbivory observed across species ranged from 60 % (e.g. *P. giornae*) up to 100% (e.g. *C. italicus*). To account for these differences when comparing feeding preferences across grasshoppers, observed herbivory was standardized between 0 and 100% for each grasshopper species before analysis to represent the relative feeding preferences of each species. For each grasshopper species, we modelled their feeding preferences as:

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$$\textit{Standardized herbivory} \sim \alpha + \beta_1 * LDMC + \beta_2 * C{:}N + b*block + \varepsilon \qquad \textit{(eqn 5)}$$

where α is the intercept, β_1 and β_2 are the fixed effect coefficients for plant functional trait regressors, b the random effect coefficients for the random effect intercepts 'block' (i.e., the random effects vary by block) and ε the residual error for each observation in each block. We used polynomial functions for plant species traits (not shown in *eqn 5*) as the relationship between plant traits and feeding preferences of grasshoppers may not be necessarily linear (Ibanez *et al.* 2013a). The best models were selected using the AICc model selection procedure. Plant species abundance within cages may also impact the feeding preferences of grasshoppers. This may occur if grasshoppers develop a functional response to local resource availability (Ibanez *et al.* 2013b). We thus integrated species abundance within each cage as explanatory variable. Plant abundance was however not retained in any of the final models.

Feeding niche differences between grasshoppers - We used a randomization procedure to test whether grasshopper species had different feeding niches, i.e. whether they differed in their food selectivity as a function of plant traits. To do so, we first used *eqn 5* for each grasshopper species to generate a predicted herbivory as a function of LDMC and C:N ratio values (namely quantitative description of the herbivore feeding niche). We generated plant trait combinations from the range of LDMC and C:N values observed in the experiment. The null hypothesis assumed that herbivore food selection was random, i.e. determined only by plant species abundance in the experiment and not by plant traits. From this predicted data set, we randomly shuffled 10000 times the feeding preferences across plant trait combinations (LDMC and C:N ratio) for each grasshopper species independently and calculated after each randomization event a pairwise null feeding preferences for each pair of grasshoppers:

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Difference in feeding preferences = (predicted preferences$_{ij}$ of grasshopper species a – predicted preferences$_{ij}$ of grasshopper species b) (*eqn 6*)

where predicted feeding preferences is a function of the plant trait values *i* for LDMC and j for C:N ratio (see *eqn 5*).

From the 10000 randomizations, we calculated a mean difference in the feeding preferences for each pair of grasshopper species and a 95% confidence interval which represented the null envelope. We then compared the null envelope to the observed difference between each pair of grasshopper species using *eqn 6* for each combination of plant trait values. When the difference of feeding preferences for a given pair of grasshopper species was above the null envelope, it indicated a significant difference in feeding niche which supported significant niche differentiation between grasshoppers. When the difference of feeding preferences for a given pair of grasshopper species was below the null envelope, this indicated that grasshoppers were more similar in their feeding choice than expected by chance, supporting a feeding niche equivalence between grasshoppers.

Linking feeding niche to grasshopper traits – We tested whether the differences in trait values between grasshoppers reflected their differences in feeding niche. To do so, we calculated an average feeding niche difference between each pair of grasshoppers based on the feeding preferences observed on the 22 plant species. Then we used linear models to test whether differences in trait values between grasshoppers explained their feeding niche differences. Models included differences in BS, IS and grasshopper C:N ratio either together or separately. We used an (AICc)-based model selection procedure to select the best model.

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Direct or mediated effect of grasshopper traits on plant community biomass

To test between the two competing hypothesis (i.e. whether grasshopper effect traits had a direct impact on plant community biomass or whether this effect was mediated by the feeding niche), we used a confirmatory path analysis (Shipley 2013). The models tested integrated direct and indirect pathways between grasshopper effect traits, the feeding niche, grasshopper survival and plant community biomass. To model the effect of grasshopper feeding niche on plant community biomass, we used a synthetic variable (*Predicted log-response ratio - LNRR*) which included the standardized herbivory on the 22 plant species for each grasshopper species (Fig. S2) and the plant initial abundance at the beginning of the experiment in each cage. Predicted LNRR in each cage k was then:

$$Predicted\ LNRR_k = log\left(\frac{\sum_{i}^{n} standardized\ herbivory_i * Initial\ relative\ abundance_{i_k}}{n}\right) \quad (eqn\ 7)$$

with n the number of plant species per cage k (n ranged from 6 to 11 species per cage). Predicted LNRR assumed that grasshoppers should have the highest impact when their preferred plant species had high abundance, i.e. initial food availability matches grasshopper feeding niche.

Using the confirmatory path analysis (Shipley 2013), we tested two sets of hypotheses (See Fig. 5 and Appendix S5 for more information and for detailed set of hypotheses): (i) grasshopper impact is directly linked to their effect traits (the body size and the incisive strength); (ii) grasshopper impact is mediated by their feeding niche. This analysis was based on a d-sep approach which used an acyclic graph that summarizes the hypothetical relationships between variables to be tested using the C statistic. We followed Grace &

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Bollen (2005), using standardized path coefficients, to quantify the direct and indirect effects of the path coefficients.

Results

LINKING HERBIVORE EFFECT TRAITS TO THEIR IMPACT ON PLANT COMMUNITY BIOMASS

When compared with the control treatment (no grasshopper), grasshopper impact ranged from a non-significant impact (*P. giornae*, *P. parallelus*) to up to 60% of the total plant biomass (*C. dorsatus*) (see data per species in Fig. S3A). Grasshopper species had a significant and species-specific impact on plant biomass ($P = 0.0008$, Model $r^2 = 0.44$, Appendix S3B).

Observed grasshopper species impacts (LNRR) were well predicted by their functional traits and their survival (Fig. 1, model $r^2 = 0.41$. See also Table S6A for detailed results of model selection). However, only IS had a significant impact on plant biomass ($P = 0.002$) while the effect of BS was marginally significant ($P = 0.06$, Fig. 1). We also found a significant and positive effect of species differences in survival ($P = 0.001$), indicating that grasshoppers that experienced higher mortality had also less impact (e.g., *P. parallelus,* Fig. S4), albeit dead individuals were replaced each two-week period.

LINKING PLANT TRAITS TO GRASSHOPPER FEEDING NICHE

The feeding niche of each grasshopper species was well predicted by simple plant traits (LDMC and C:N ratio of the leaves) ($r^2 > 0.70$ for all models, Table 1 & Table S6B). All models included non-linear relationships between plant traits and feeding preferences (Fig. 2). The distribution of feeding preferences was sometimes complex with bimodal shapes (e.g.

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P. parallelus) indicating the presence of one or two peaks in the selection of preferred plants depending on the grasshopper species.

Grasshoppers showed strong feeding niche differences (Fig. 3). *C. italicus* and *P. giornae* feeding niche differences were in general lower than expected by chance, indicating that the two species had a similar niche. These species showed marked differences compared to the Gomphocerinae species (*Chorthippus, Pseudochorthippus* and *Euchorthippus*) (Fig. 3). The feeding niche of *C. italicus* and *P. giornae* differentiated from other grasshopper species with respect to LDMC and to a lesser extent to C:N ratio of the leaves (Fig. 3). *C. italicus* and *P. giornae* mostly targeted low LDMC values (mostly legume and other forb species) while other grasshopper species selected leaves with higher LDMC (e.g. *C. biguttulus*, *E. elegantulus* and *P. parallelus*). In addition, *C. italicus* and *P. giornae* tended to select leaves with higher C:N ratio compared to *C. biguttulus, C. dorsatus.* When comparing *E. elegantulus* with *C. biguttulus, C. dorsatus* and *P. parallelus*, we found large differences in the feeding preferences according to the LDMC of the leaves. *E. elegantulus* only selected leaves with high LDMC while the three other species were able to select leaves with low LDMC. Finally, *C. dorsatus, C. biguttulus* and *P. parallelus* showed marked overlap in their feeding niches, suggesting feeding niche equivalence. However, they differentiated in some regions of the trait space. Notably, *P. parallelus* showed a clear peak in feeding preferences for intermediated levels of C:N ratio and LDMC which was not the case for *C. biguttulus*. The observed feeding niches of *C. dorsatus, C. biguttulus* and *P. parallelus* suggested that these species had a higher niche breadth compared to other species since they ate a wider range of plant species, although with different selectivity.

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LINKING GRASSHOPPER TRAITS TO FEEDING NICHE

We observed a significant and positive correlation between the average grasshopper feeding niche differences and their differences in incisive strength (IS; $r^2 = 0.63$, P = 0.0004, Fig. 4 & Table S6C). The more the IS difference increased, the stronger the difference of feeding niche was. This result did not hold if we considered BS or grasshopper C:N ratio. Differences in mandibular traits alone were able to explain observed differences in feeding preferences between grasshopper species.

DIRECT AND MEDIATED EFFECT OF GRASSHOPPER TRAITS ON PLANT BIOMASS

The confirmatory path analysis supported the hypothesis (ii) that the effect of grasshopper effect traits on plant community biomass was mediated by their feeding niche ($\chi^2 = 11.30$, d.f. = 12, P = 0.5; Fig. 5 & Table S5), whereas the hypothesis (i), that grasshopper traits directly impact plant biomass community, was not supported by the data ($\chi^2 = 60.28$, d.f. = 16, P < 0.0001; Table S5). In the selected model, the feeding niche (i.e. the predicted LNRR) was positively correlated with incisive strength ($r^2 = 0.59$, Fig. 5) indicating that grasshopper species with stronger incisive strength have higher predicted LNRR values. The feeding niche was positively correlated with the observed impact at the end of the experiment ($r^2 = 0.38$; Fig. 5). This result indicated that when preferred plants of a given grasshopper species were available at high abundance grasshopper impact on plant community biomass was highest. Similarly with the first analysis (Fig. 1), grasshopper survival had a positive effect on herbivore impact. Survival was also slightly and negatively impacted by predicted LNRR likely due to an effect of *P. parallellus*, a grasshopper characterized by a high predicted LNRR and a low survival. We did not find any direct or indirect significant relationship between BS and predicted LNRR. It was thus excluded from the final path analysis (Fig. 5).

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Discussion

In this study, we used an experimental approach to test whether different grasshopper species with contrasted functional traits have contrasting impact on plant community biomass. Our study provided a formal demonstration of the existence of different effect traits across grasshopper species. By quantifying the realized feeding niche of grasshoppers in field conditions, an important result of our study was to demonstrate a clear linkage between grasshopper traits and their feeding niche which in turn determined their impact on plant community biomass. This result supported our second hypothesis that the effect of grasshopper traits was mediated by their feeding niche.

GRASSHOPPER IMPACT ON PLANT BIOMASS IS NOT RELATED TO THEIR BODY SIZE

When evaluating the relative importance of multiple grasshopper traits on plant biomass (Fig. 1), incisive strength was the most important factor explaining grasshopper impact while the effect of the body size was not significant (Figs. 1 & 5). This result was somehow intriguing as body size is known to be a key trait related to herbivore metabolism and stoichiometry (Whitman 2008). However, grasshopper species were characterized by strong feeding niche differences (Figs. 2 & 3) which were independent from their body size. This result suggested that for a given size, grasshoppers can develop complementary strategies for food acquisition (Unsicker *et al.* 2008). In our experiment, food availability was inherently constrained by the functional characteristic of dominant plant species within cages. In this context, the largest grasshopper (e.g. *C. italicus*) ate only rare and subordinate forb species (e.g. Fig. 2; Fig. S2) limiting its impact on plant community biomass. The feeding niche appeared as a fundamental property of the grasshopper species (see also Ibanez *et al.* 2013b). It constrained their ability to eat non-preferred plant species even provided at high abundance, suggesting low functional response to food availability (Unsicker *et al.* 2008; Ibanez *et al.* 2013b).

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In real field conditions, one could expect a positive linkage between body size and herbivore impact if larger grasshoppers are able to reach favourable habitat that match their resource requirements. This hypothesis is supported by a recent correlational study (Moretti *et al.* 2013) which suggested that larger insect herbivores tended to have higher impact on plant biomass in the field. However, few studies if none have experimentally tested this assumption and whether feeding niche can explain the abundance of grasshoppers in real field conditions. An alternative hypothesis would be that grasshoppers which mostly target rare or subordinate species (such as *C. italicus*) can compensate for low resource availability at the local field scale (Behmer 2009) with high movement abilities. This hypothesis is supported by a previous study (Behmer, Raubenheimer & Simpson 2001) which has observed for some locust species a preference for rare plant species. This strategy would clearly match the ecology of *C. italicus*, a species phylogenetically related to locust species (Uvarov 1977). *C. italicus* dominates agricultural landscapes and reaches high abundance in artificial grasslands such as alfalfa crops (Badenhausser 2012). The feeding niche of *C. italicus* indicated that it may mostly focus on weed species (other forbs in Fig. 2) avoiding dominant grass and legume species. While this species may have a limited impact at the local field scale, it may have a large impact at the landscape scale due to its high movement capacity (Uvarov 1977).

GRASSHOPPER IMPACT ON PLANT BIOMASS IS MEDIATED BY THE FEEDING NICHE

The grasshopper feeding niche was well predicted by two leaf traits: the LDMC and the C:N ratio (Figs. 2 & 3). It is surprising that only two simple plant traits can predict with such a high accuracy (Model $r^2 > 0.70$, Table 1) the feeding niche of grasshoppers considering the multiple strategies that plants can develop to deter herbivores (Moles *et al.* 2013). These two important plant traits are related to complex plant functions and may be integrative of plant

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defence against herbivory (Carmona, Lajeunesse & Johnson 2011). For instance, LDMC and C:N ratio are strongly related to plant growth rate (Gross *et al.* 2007). A large body of literature suggested that plants with slow growth rate invest more into constitutive defence against herbivory than into chemical secondary compounds (Coley 1988; Herms & Mattson 1992). In addition, leaf C:N ratio is also a key trait for nutrient regulation (Sterner & Elser 2002) which may influence food selection by herbivores (Berner, Blanckenhorn & Körner 2005; Hillebrand *et al.* 2009; Cease *et al.* 2012). In contrast, fast growing species, especially forbs unlike to grasses, tend to favour secondary compounds (Coley 1988; Moles *et al.* 2013) characterized by low metabolic cost which can be toxic to some herbivores and harmless to others (Dethier 1954; Fraenkel 1959). While LDMC and C:N ratio generally correlate across plant species at large scales (Reich *et al.* 1997; Wright *et al.* 2004), this correlation is often system dependent at local scales (Gross *et al.* 2007). In our study, they clearly varied independently within the local plant species pool (mainly because of the presence of many forb species other than legumes in our species pool, see Fig. S1B). It allowed us to investigate the interactive effect of C:N ratio and LDMC on grasshopper feeding niche.

By comparing grasshopper feeding preferences, we found evidence for highly contrasted feeding niches (Fig. 3). This suggests a high niche complementarity between grasshoppers, every species targeting plants within a specific range of trait values. High LDMC and C:N ratio are associated with high leaf toughness (Ibanez *et al.* 2013a): a barrier trait against herbivory (Seath 1977; Lucas 2004; Santamaría & Rodríguez-Gironés 2007). In that case, the distribution of herbivory would follow a threshold rule, whereby grasshoppers characterized with weak incisive strength are not able to eat tough plants which may limit their access to the resource. Our study clearly showed that grasshopper feeding niche did not follow such a rule but rather we found clear patterns of niche complementarity between species in

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accordance with Ibanez *et al.* (2013a). This result was also supported by a strong relationship between grasshopper incisive strength and feeding preferences (Fig. 4). However, while differences in incisive strength differentiated herbivore feeding preferences (e.g. *C. italicus* and *P. giornae* vs. *E. elegantulus* and *C. dorsatus* in Fig. 3), it did not explain why species with strong mandibular strength did not eat tender leaves (e.g. *E. elegantulus*). Other factors such as plant secondary compounds could explain such differences (Ali & Agrawal 2012). For instance, *E. elegantulus* mostly targets slow-growing grass species (high LDMC and C:N ratio) generally characterized by low concentration of secondary compounds (Clissold *et al.* 2009) and might not be able to eat other plant types due to higher toxicity.

Stoichiometric match between grasshoppers and plants could also partly explain niche complementarity across herbivores (Joern *et al.* 2012; Ibanez *et al.* 2013a). For instance, we found a strong relationship between grasshopper body size and their C:N ratio (Fig. S1A) with bigger grasshoppers characterized by lower C:N ratio. This relationship would predict that larger grasshoppers characterized by higher metabolic rate would target higher nitrogen rich plant species (Hillebrand *et al.* 2009). We did not find such a clear relationship as neither grasshopper C:N ratio nor body size were able to explain observed differences in the feeding niche between grasshoppers (Fig. 4). While incisive strength explained in large part such differences, we recognised that we may have underestimated the importance of stoichiometric relationship between plants and grasshoppers in determining their feeding niche. For instance, we measured C:N ratio of the whole plant leaves and of the whole grasshoppers. Such measurement integrated structural components of the organisms which is not always directly related to their metabolic needs (Maire *et al.* 2013). Further studies might be needed to investigate in more detail the relationship between mandibular traits of grasshoppers and less tractable physiological traits such as their nutritional balance or grasshopper metabolic

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rates. Such a study may help to shed light on the fundamental mechanisms that determine plant-herbivore interactions. For instance, some grasshoppers showed a complex feeding niche with bimodal shape (e.g., *P. parallelus*, Fig. 2). This species is known to improve its fitness in grasslands characterized by high plant species diversity (Bernays & Chapman 1970; Unsicker *et al.* 2008) suggesting for complex nutrient regulation. Why some grasshoppers need contrasted food types while others peaked only on specific trait values remains unclear.

Microhabitat (Joern 1982) and the predation risk (Hawlena *et al.* 2011) might also be important explaining herbivore impact on primary producers. For instance, we found that grasshopper survival was an important parameter explaining their impact on plant biomass. While most of the grasshoppers survived during the time of the experiment, *P. parallelus* mortality was not negligible. This species originated from north Europe and has been reported to be strongly dependent on microclimate (Uvarov 1977). While this species had a very similar feeding niche compared to *C. dorsatus* (species with the highest impact on plant biomass, Fig. S3) dry summer conditions may have resulted in important mortality which may have limited its ability to consume plant resources. Alternatively, differences in survival across species may reflect contrasted phenology between grasshoppers (Badenhausser *et al.* 2009). Integrating grasshopper phenology and its match with plant dynamics may help to anticipate grasshopper impact on plant community over the growing season.

Conclusion

Several recent studies (e.g. Suding *et al.* 2008; Lavorel *et al.* 2013) have proposed the use of a trait-based approach to investigate interactions across contrasted trophic levels and their importance for biodiversity dynamics (van der Plas *et al.* 2012) and ecosystem functioning (Moretti *et al.* 2013). Our study contributes to the development of a trait-based approach with

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a multitrophic perspective by providing a first experimental test on the relationship between herbivore effect traits, their impact on plant community biomass and in a larger extent on ecosystem functioning. By comparing the relative importance of multiple interacting grasshopper traits (morphological traits, chemical traits, and mandibular traits), our study showed that incisive strength was a key effect trait which determined grasshopper feeding niche and their impact on plant community biomass (Fig. 5). Some direct consequences are that: (i) the effect of incisive strength on plant community biomass is likely to be context dependent: grasshopper impact is mediated by the match between their feeding niche (as reflected by mandibular traits) and plant functional traits and abundance; (ii) trait-based models such as the response-effect framework (Lavorel *et al.* 2013) need to integrate both herbivore and plant functional traits and their interactions to predict the effect of herbivores on primary producers.

Overall, our study demonstrated that grasshoppers can have a non-negligible impact on plant communities (pre-empting up to 60% of plant biomass). Because grasshoppers targeted specific plant traits which have a considerable importance on ecosystem functioning (C:N ratio; LDMC), they are likely to profoundly alter important ecosystem functions such as productivity, decomposition and carbon nitrogen cycling. In this context, the strong feeding niche complementarity observed in this study between grasshoppers suggests that herbivore diversity within communities might be a key parameter which should determine herbivore impact on ecosystem functioning. How a species rich grasshopper community may impact multiple ecosystem functions remains to be explored.

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Data Accessibility

Data are accessible at http://doi.org/10.5061/dryad.5q33h (Deraison *et al.* 2014).

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Table 1. Linking herbivore feeding niche to plant functional traits. Results of the polynomial models estimating the feeding preferences of the six grasshopper species independently as a function of plant traits (22 plant species): the leaf dry matter content (LDMC) and the C:N ratio (CN).

	(1) *C. biguttulus*				(2) *C. dorsatus*			
Model r^2	0.72				0.73			
Model parameters	d.f.	Parameter estimate	F ratio	P	d.f.	Parameter estimate	F ratio	P
LDMC	1	3.80	18.92	0.0004	1	2.50	14.63	0.0015
$LDMC^2$	1	0.33	5.99	0.0248				
$LDMC^3$	1	-0.02	2.05	0.1696				
CN	1	0.32	0.17	0.6869	1	3.39	4.02	0.0623
CN^2	1	-0.03	0.05	0.8189	1	0.44	2.25	0.1533
CN^3	1	-0.03	6.02	0.0245	1	-0.12	10.91	0.0045
Error	18				16			
	(3) *C. italicus*				(4) *E. elegantulus*			
Model r^2	0.86				0.78			
Model parameters	d.f.	Parameter estimate	F ratio	P	d.f.	Parameter estimate	F ratio	P
LDMC	1	-1.71	6.26	0.0222	1	6.54	33.20	<.0001
$LDMC^2$	1	0.39	19.22	0.0004	1	0.21	3.12	0.0924
$LDMC^3$					1	-0.05	7.66	0.0119
CN	1	1.54	9.17	0.0072				
CN^2	1	-0.31	16.87	0.0007				
CN^3								
Error	18				20			
	(5) *P. giornae*				(6) *P. parallelus*			
Model r^2	0.83				0.80			
Model parameters	d.f.	Parameter estimate	F ratio	P	d.f.	Parameter estimate	F ratio	P
LDMC	1	-2.60	79.59	<.0001	1	5.14	26	<.0001
$LDMC^2$	1	0.14	4.81	0.0410	1	-0.56	6.36	0.0219
$LDMC^3$					1	-0.07	8.69	0.0090
CN	1	0.57	2.96	0.1014	1	2.53	6.78	0.0185
CN^2					1	0.22	2.94	0.1045
CN^3					1	-0.04	12.5	0.0025
Error	19				17			

We provided model r^2 and parameter estimated F ratio and P value of each selected variables.

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Figure Legends

Fig. 1. Impact of grasshopper species on plant biomass (LNRR, -) (n = 30) as a function of grasshopper body size (BS, mm) and incisive strength (IS, -). Model $r^2 = 0.41$; BS: F $ratio_{1,26} = 3.00$, Pvalue = 0.06; IS: F $ratio_{1,26} = 6.43$, P value = 0.002; Survival: F $ratio_{1,26} = 10.69$, Pvalue = 0.001

Fig. 2. Predicted grasshopper feeding niche as a function of plant traits (LDMC & C:N ratio) from *eqn 5* (see Table 1 detailed model parameters). LDMC is expressed in % and C:N ratio is unit less. The response surface indicates model predictions for each grasshopper. White colours indicate high predicted feeding preferences closed to 1, dark colours indicate low predicted feeding preferences closed to 0. As an illustration, coloured dots represents the predicted feeding preferences for all plant species recorded in the experiment (n = 40). Size of the circles is proportional to the predicted feeding preferences for each plant species as a function of LDMC and C:N ratio. Green circles refer to grass species, red circles to legume species and blue circles to other forb species.

Fig. 3. Grasshopper feeding niche differences (pairwise comparison) as a function of plant traits (C:N ratio, LDMC). The red area indicates that the difference of feeding niche between herbivores is above the null envelope (95% confidence interval), suggesting niche differentiation. The blue area indicates that the feeding niche difference between grasshoppers is below the null envelope, suggesting feeding niche similarity. Yellow/green areas represent the null envelop, i.e. non-significant feeding niche difference between grasshoppers. Letters represent the grasshopper species abbreviation: Cb : *C. biguttulus*, Cd : *C. dorsatus*, Ci : *C. italicus*, Ee : *E. elegantulus*, Pg : *P. giornae*, Pp : *P. parallelus*.

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Fig. 4. Relationship between incisive strength and averages feeding niche differences between grasshoppers. (Model adjusted $r^2 = 0.63$, IS differences: $Fratio_{1,13} = 22$, Pvalue = 0.0004). See Fig. 3 for species abbreviation.

Fig. 5. Graph of the path analysis examining the relationship between grasshopper traits and their feeding niche (predicted LNRR) on plant community biomass (observed LNRR). Path coefficients are shown for each pair of connected variables. The thickness of the arrows indicates the interaction strength and is proportional to the standardized path coefficients. The R^2 value indicates the total proportion of variation of a given path coefficient that could be explained by the model.

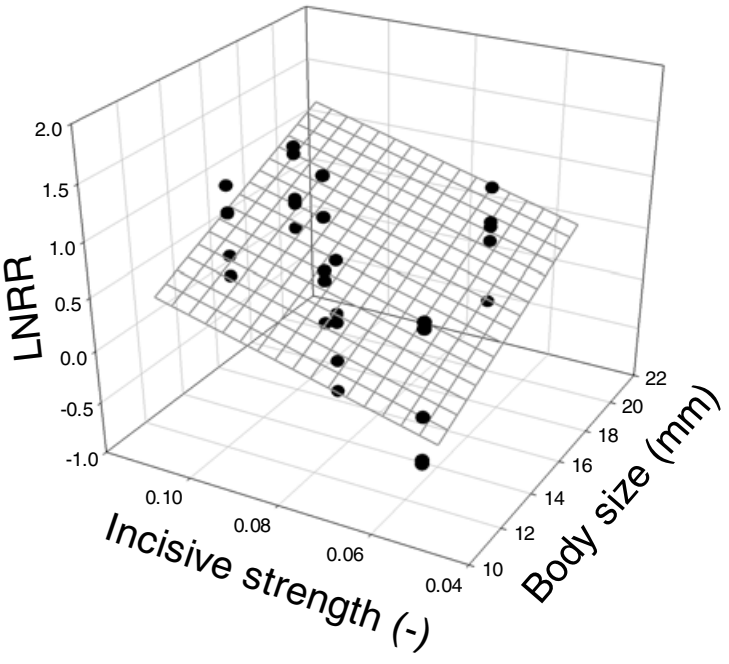

Figure 1.

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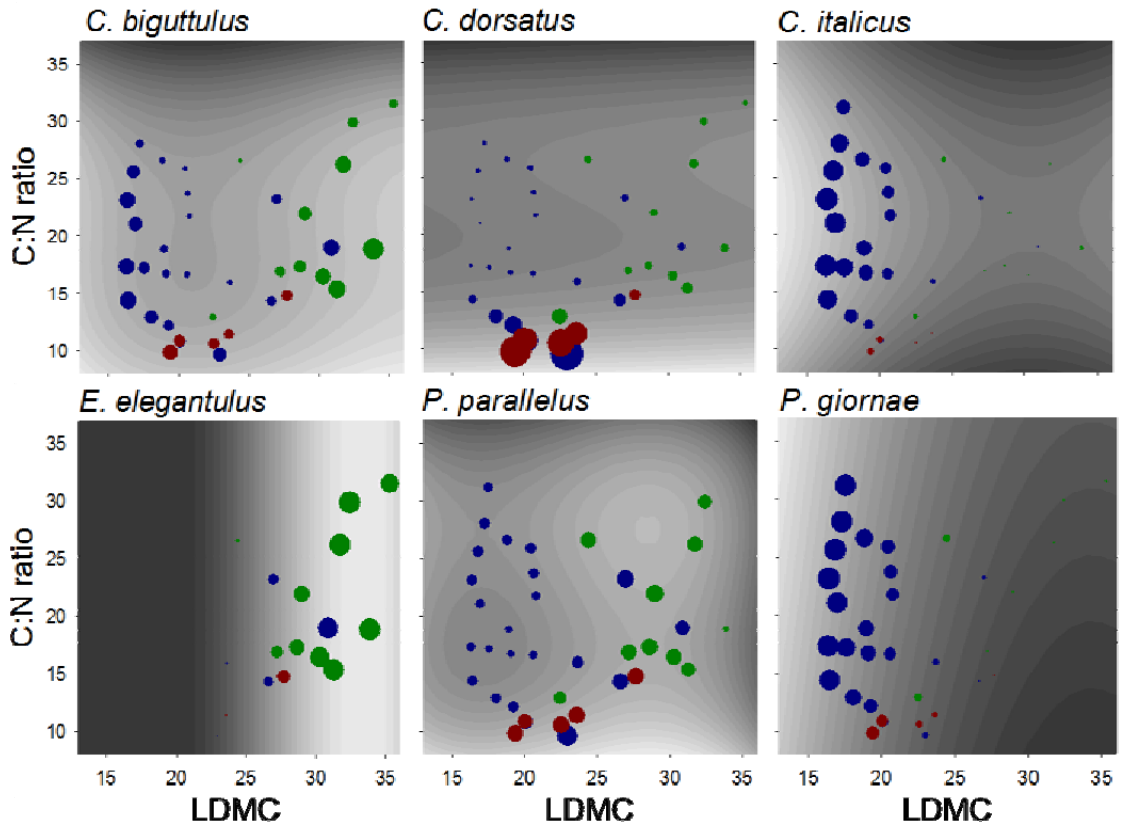

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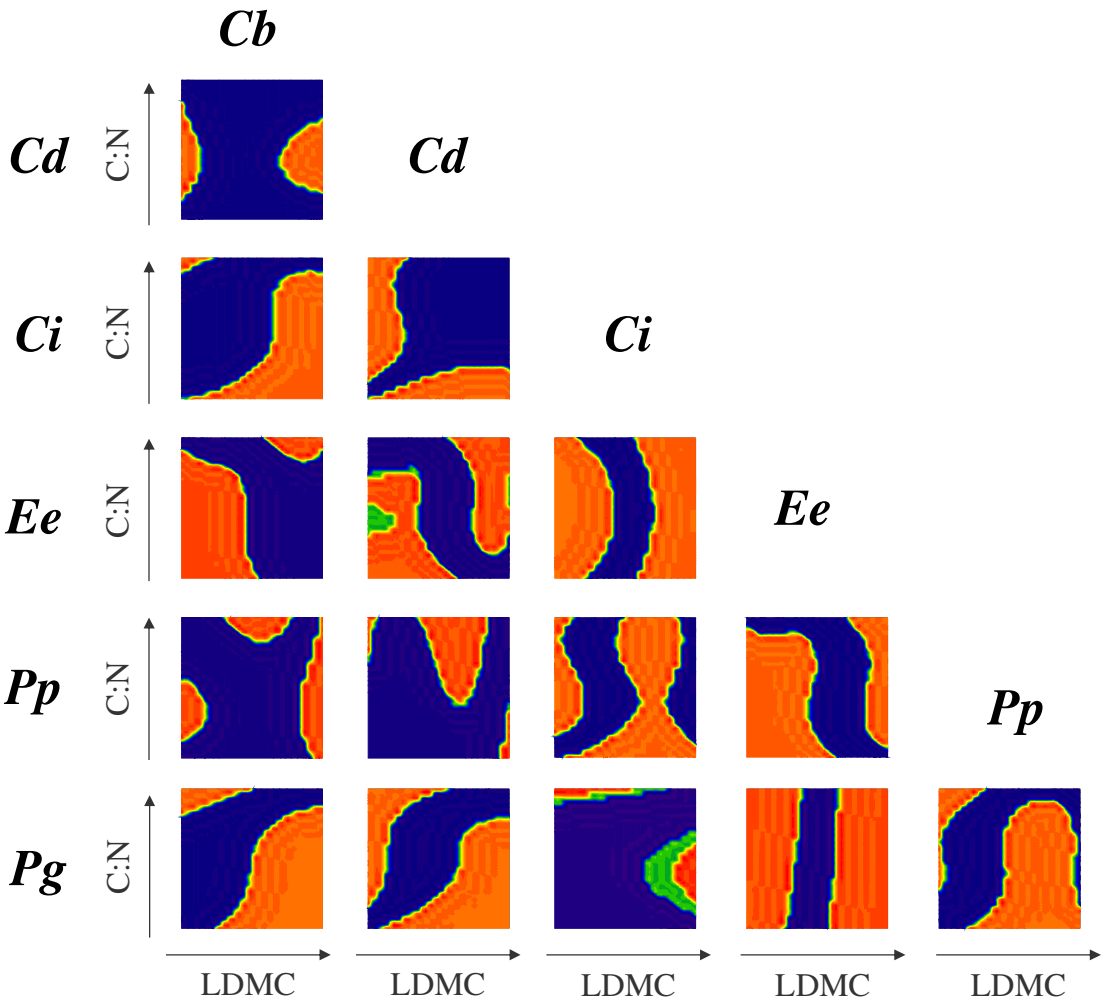

Figure 3.

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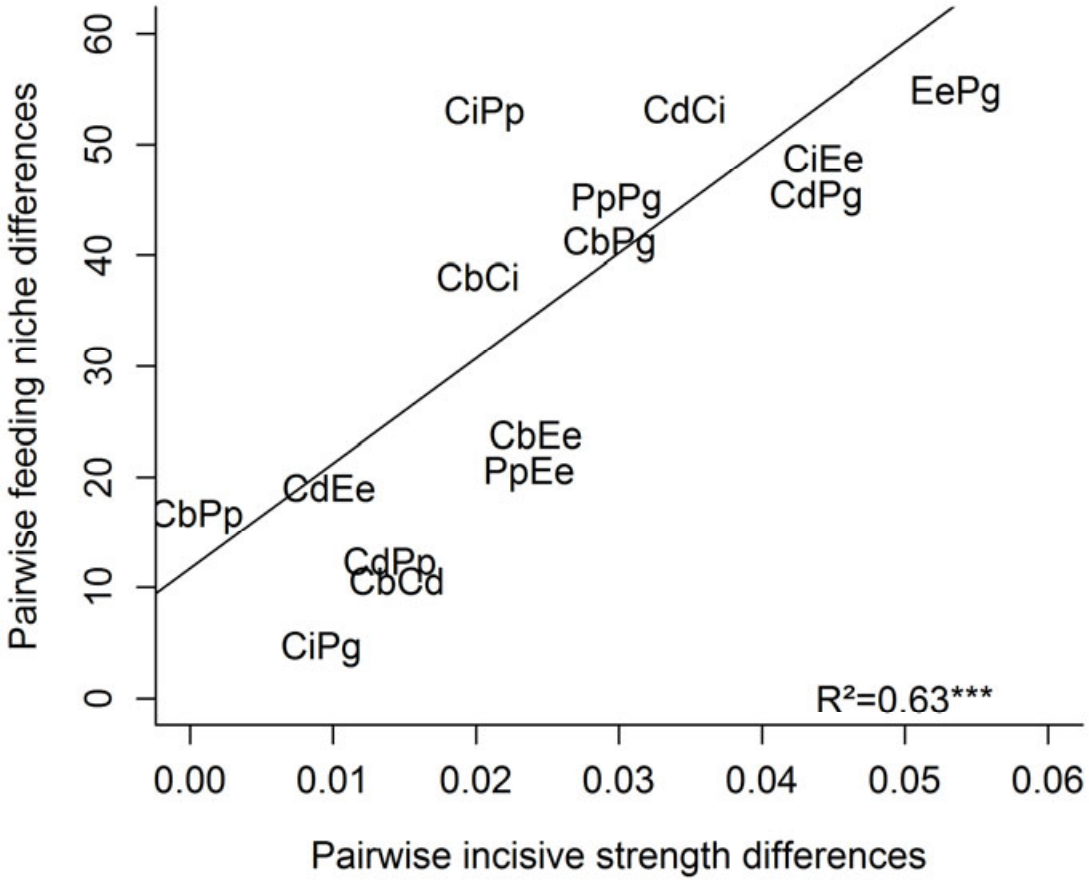

Figure 4.

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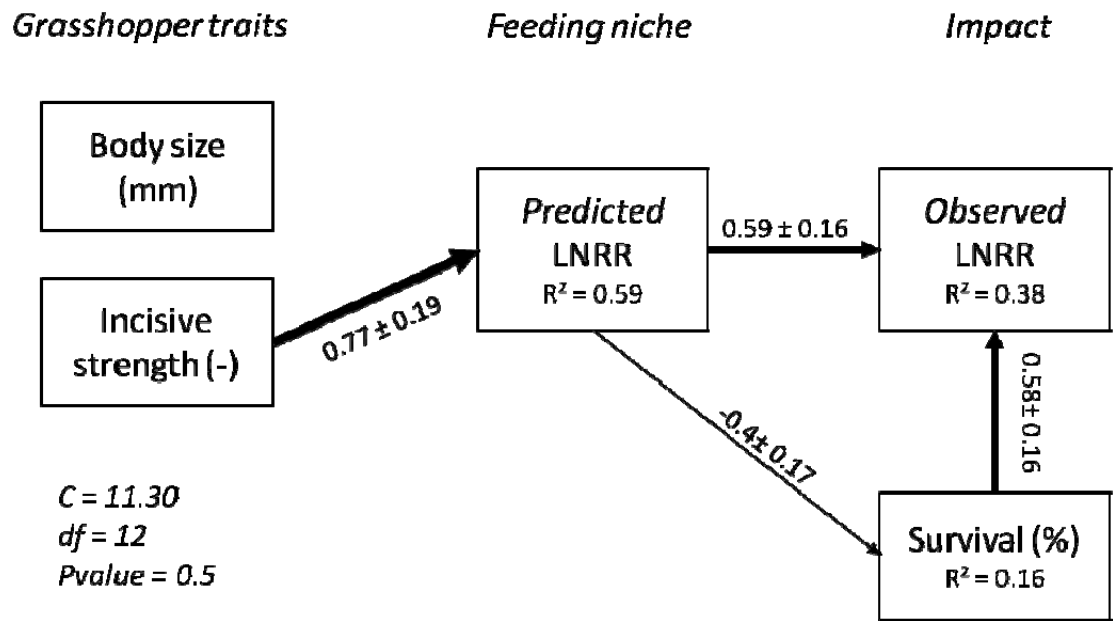

Figure 5.

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