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Plant diversity effects on pollinating and herbivorous insects can be linked to plant stoichiometry

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Abstract

Changes in plant diversity have consequences for higher trophic levels, e.g., higher plant diversity can enhance the reproduction and fitness of plant-associated insects. This response of higher trophic levels potentially depends on diversity-related changes in both resource quantity (abundance) and quality (nutritional content). The availability of elemental nutrients in plant resources is one aspect of nutritional quality, but has rarely been addressed as a pathway relating plant diversity to associated insects. Using the experimental plant diversity gradient of a large biodiversity grassland project, the Jena-Experiment, we analysed the %C, %N and %P and the molar ratios of those elements (C:N, C:P and N:P) in a pollinating bee, *Chelostoma distinctum*, and an herbivorous grasshopper, *Chorthippus parallelus*, reared on plots of different plant diversity. Insects showed higher content of C, N and P (% dry mass), and lower C:N and C:P ratios than plants. C:N ratios were significantly higher in grasshoppers than in bees and higher in females than in males of both species. Increasing plant species richness increased the C:N ratio of male bees and female grasshoppers. In both groups, stoichiometry was positively related to plant stoichiometry (male bees: C:P and N:P; grasshoppers: C:N and N:P). Path analysis revealed that diversity-driven changes in plant elemental composition can have consequences for abundance and chemical composition of higher trophic levels, with different responses of the two functional groups.

Zusammenfassung

Die Artenvielfalt von Pflanzen strukturiert die Gesellschaften höherer trophischer Ebenen und verbessert die Fortpflanzung und Fitness von Pflanzen-assoziierten Insekten. Dieser Einfluss auf höhere trophische Ebenen kann von der Quantität (Verfügbarkeit) und Qualität (Zusammensetzung) der Ressourcen abhängen. Wir untersuchten die Stöchiometrie von Interaktionen zwischen Pflanzen und zwei funktionellen Insektengruppen (Herbivoren, Bestäuber) entlang des experimentellen Pflanzendiversitätsgradienten im „Jena-Experiment“. Wir analysierten den prozentuellen Anteil von Kohlenstoff (C), Stickstoff (N)

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und Phosphat (P), sowie die entsprechenden molaren Verhältnisse dieser Elemente (C:N, C:P, N:P) bei einer bestäubenden Bienenart (*Chelostoma distinctum*) und einer herbivoren Heuschreckenart (*Chorthippus parallelus)*, welche wir auf Graslandflächen verschiedener Diversitätsstufen gezüchtet haben. Insekten zeigten höhere prozentuale Anteile in der Trockenmasse (C, N und P) und niedrigere C:N- und C:P-Verhältnisse als Pflanzen. Das C:N-Verhältnis war bei Heuschrecken signifikant höher als bei den Bienen, und in beiden Gruppen bei den Weibchen höher als bei den Männchen. Zunehmende Pflanzenvielfalt erhöhte das C:N-Verhältnis von männlichen Bienen und weiblichen Heuschrecken und die Insektenstöchiometrie beider Gruppen hängte positiv mit der Pflanzenstöchiometrie zusammen (männliche Bienen: C:P und N:P; Heuschrecken: C:N und N:P). Unsere Studie zeigt unterschiedliche Stöchiometrie von antagonistischen und mutualistischen Interaktionen. Daraus ergeben sich verschiedene Schlussfolgerungen für diversitätsgetriebene Veränderungen von der Elementzusammensetzung bei Pflanzen verschiedener trophischer und funktioneller Gruppen.

Keywords: Solitary bee; Carbon; Generalist grasshopper; Grassland; Nitrogen; Plant–insect interactions; Phosphorus; Trophic levels

Introduction

Animals require energy and nutrients for growth and reproduction. Food quality features such as the content of certain fatty acids, the absence of fibre (e.g., lignin) or the content of P and N, have profound impact on consumer ingestion and growth rates (Hartley & Jones 1997; Sterner & Elser 2002). Ecological stoichiometry (ES) is a general theoretical framework investigating the balance of chemical elements in organisms with trophic relations and its consequences for population dynamics and element cycling in ecosystems (Sterner & Elser 2002; Andersen, Elser, & Hessen 2004; Persson, Fink, Goto, Hood, & Jonas 2010).

A general finding within ES is that herbivores have higher N and P contents relative to C as well as lower variability in these contents compared to their plant food (Elser, Fagan, Denno, Dobberfuhl, & Folarin 2000; Frost, Benstead, Cross, Hillebrand, & Larson 2006). This leads to a potential stoichiometric mismatch between the availability of elements in the food and the demand of the consumers, influencing consumption rates of herbivores (Hillebrand, Borer, Bracken, Cardinale, & Cebrian 2009).

In insect consumers, plant chemical composition strongly influences growth, development and performance (Carisey & Bauce 2002). N is an essential element of proteins (Tao & Hunter 2012) and P a key element of nucleic acids. Higher N contents in food plants increase the fitness of herbivorous insects (Awmack & Leather 2002) and herbivore survival, body size, growth rate, and oviposition increases with decreasing C:P ratio (Bertram, Schade, & Elser 2006; Apple, Wink, Wills, & Bishop 2009; Visanuvimol & Bertram 2011).

At the same time, plant diversity can influence herbivore abundance and diversity (Scherber, Eisenhauer, Weisser, Schmid, & Voigt 2010; Borer, Seabloom, Tilman, & Novotny 2012), which can be triggered by higher plant quantity (diversity effects on biomass, see Marquard, Weigelt, Roscher, Gubsch, & Lipowsky 2009) or by changes in nutritional quality (diversity effects on stoichiometry, see Abbas, Ebeling, Oelmann, Ptacnik, & Roscher 2013). Diversity effects on plant elemental content can be caused by (i) increasing overall resource use by complementarity of resource acquisition traits, (ii) mixing effects across plants with different elemental content, or (iii) diversity-induced shifts in allocation patterns between plant organs.

We present a case study linking diversity-induced shifts in plant chemical composition to shifts in the stoichiometry of plant–insect interactions. We studied whether the chemical content of whole plants can mediate plant diversity effects on associated insects, using a pollinating bee and an herbivorous grasshopper in the grassland biodiversity gradient of the Jena-Experiment to test the following hypotheses.

H1. Elemental content differs between insect species and between sexes within the species. This exploratory hypothesis tests for significant differences, which would have to be taken into account in subsequent analyses.

H2. Elemental ratios of consumers are related to the stoichiometry of plants. As there is little information on how much insects can adjust their chemical composition in response to plant chemical composition, the focus of this study is on potential changes in elemental composition of insects. We assume that grasshopper stoichiometry strongly depends on the elemental content of plants, as they directly feed on plant parts, whereas bee stoichiometry is potentially more independent of plant chemical composition.

H3. Elemental content of plants is a significant path linking plant diversity to insect density. Here, we explicitly ask whether plant diversity affects abundance and nutrient content of insects via plant food source quantity or by nutritional quality.

Material and methods

Experimental design

The study was conducted in the grassland plant diversity site of the Jena-Experiment located in Jena (50°55′ N, 11°35′ E), Thuringia in Germany. The species pool consists of a total of 60 common plant species of central

European grasslands, belonging to four functional groups (grasses, legumes, small herbs and tall herbs) and sown in different mixtures and diversity levels (1, 2, 4, 8, 16 and 60 species) on a total of 82 plots with a size of 20 m × 20 m (Roscher, Schumacher, Baade, Wilcke, & Gleixner 2004).

Plant sampling

C, N and P content of plants was analysed from pooled plant aboveground biomass harvested from 2003 to 2009. The harvest was conducted in the centre, 10 m × 15 m, of each of the 82 plots in late May and late August. All plant parts (including flowers) were clipped at 3 cm above ground level in four (May 2003 to May 2007) or three (August 2007 to August 2009) randomly chosen rectangles of 20 cm × 50 cm size. After harvest, sown plant species were separated from detached dead material and of species not sown in the respective plots. Biomass of sown plant species material was dried at 70 °C for 48 h. Samples of pooled plant biomass per plot and harvest were shredded and milled for chemical analyses. After each observation period blossom cover (in %) was estimated of each flowering plant species as a percentage of the plot after bee sampling following Ebeling, Klein, Schumacher, Weisser, and Tscharntke (2008).

Bee and grasshopper sampling

Chelostoma distinctum Stoeckhert (Hymenoptera, Megachilidae) is a common cavity-nesting bee species. In 2005 and 2006, this species occurred in trap nests from 31 plots in a study exposing four trap nests, consisting of plastic tubes. The trap nests were filled with reed internodes and located on each of 78 plots (all plots except the 60 species mixtures) from April to September of both years (Ebeling, Klein, Weisser, & Tscharntke 2012). We sampled 133 nests of *C. distinctum*. The number of brood cells per plot was counted and used as a measure of bee abundance. We assume that brood cells were mainly provisioned by pollen collected by the parents mainly from the experimental plot with their respective nest. Although *C. distinctum* is described to be oligolectic on *Campanula* flowers, it was visiting other flowering species in the study plots (personal observations) leading to a positive relationship of bee abundance and plant diversity in the Jena-Experiment (Ebeling et al., 2012). In late spring the offspring hatched in the laboratory. Hatched bee individuals were dried and milled separately for each plot as well as for females and males and C, N, P were analysed using a mixture of individuals per plot.

The grasshopper *Chorthippus parallelus* Zetterstedt (Orthoptera, Acrididae) is a generalist herbivore and material was derived from a cage experiment conducted within the framework of the Jena-Experiment (Ebeling, Allan, Heimann, Köhler, & Scherer-Lorenzen 2013). Metal cages of 0.5 m diameter and 1 m height, covered with aluminium mesh of 3 mm × 2 mm mesh size, were installed in the centre of 43 plots, excluding plots planted with herbs only as *C. parallelus* avoids herbs as food plants (Unsicker, Oswald, Köhler, & Weisser 2008). All surviving individuals were separated into males and females and dried at 70 °C for 72 h. For each plot a mixture of individuals was used for C, N and P analyses. In cases of low insect abundance, C and N were analysed only.

Chemical analyses and stoichiometry

The dried biomass of plants, bees, and grasshoppers per plot and sex for the insect samples was weighed prior to chemical analyses. For plants, N and C concentrations were determined by an Elemental Analyzer (Vario-EL-III, Elementar, Germany). Plant material was digested with HNO_3 at 200 °C using a microwave system (MARS5Xpress, CEM, Germany) to analyse P photometrically after irradiation with UV and oxidation with $K_2S_2O_8$ with a Continuous Flow Auto-Analyzer (Bran & Luebbe, Germany) (Oelmann, Potvin, Mark, Werther, & Tapernon 2010).

For insects, N and C concentrations were determined by an Elemental Analyzer (Flash-EA-1112 Thermo). For P concentrations insect material was digested with 5 ml oxidation-solution at 120 °C and analysed with a Hitachy-U-3000 spectrophotometer using ascorbic acid and antimony-phosphomolybdate complex. We measured %C, %N, and %P in dried biomass and calculated molar elemental ratios (C:N, C:P, N:P). We used molar ratios rather than weight ratios, as molar ratios reflect the standard approaches in ecological stoichiometry (e.g., Sterner & Elser 2002). To relate the %C, %N, %P and their ratios in bees to the plant C, N, P and their ratios, plant data of May 2005 were used for bee individuals hatched from brood cells established in 2005 and plant data of May 2006 for bee individuals hatched from brood cells established in 2006. Using the May harvest data reflects that the main activity of bees establishing brood cells was in early summer.

To relate the elemental content ratio of grasshoppers, plant data from August 2009, the year when the grasshopper experiment took place was used. We did not measure plant P in 2009 and therefore used the mean of data collected in the years 2003, 2005–2007 to calculate the %P and C:P and N:P ratios.

Statistical analyses

We performed one-way ANOVA to test for significant differences between plant and insect chemical composition and two-way ANOVAs testing effects of species identity and sex as well as their interaction on insect elemental content and ratios (hypothesis 1). Hypothesis 2 (plant diversity effects on elemental contents and ratios of bees and grasshoppers) was tested using multiple linear regressions including plant species richness (log transformed), functional group richness, the presence of legumes in the plant community and the

Table 1. Results of *t*-tests comparing plant and insect elemental composition (bee and grasshopper data were merged to one group).

	C:N	C:P	N:P	%C	%N	%P
t-Value	−41.28	−3.40	9.96	12.57	100.06	6.21
df	144	89	72	189	163	100
p-Value	<0.001	0.001	<0.001	<0.001	<0.001	<0.001

respective elemental ratio or content of plants as explanatory variables. The presence of legumes was examined explicitly because legumes have higher N levels than other plants.

We used path analyses to link plant species richness with density and stoichiometry of insects via food plant quantity (biomass) and quality (C:N) (hypothesis 3). Separate analyses for each insect species and sex were motivated by significant differences (see H1). The full model for grasshoppers included plant species richness (log-transformed), plant biomass (sqrt-transformed), C:N in plants, density of the grasshoppers and grasshopper C:N. The full model for bees was based on plant species richness (log-transformed), blossom cover as measure of food quantity, C:N, density of bees and bee C:N. We performed stepwise model selection based on Akaike's information criterion (AIC) (Johnson & Omland 2004). Path analyses were performed in AMOS (v.20.0 – IBM® SPSS® Amos). All other data-analyses were

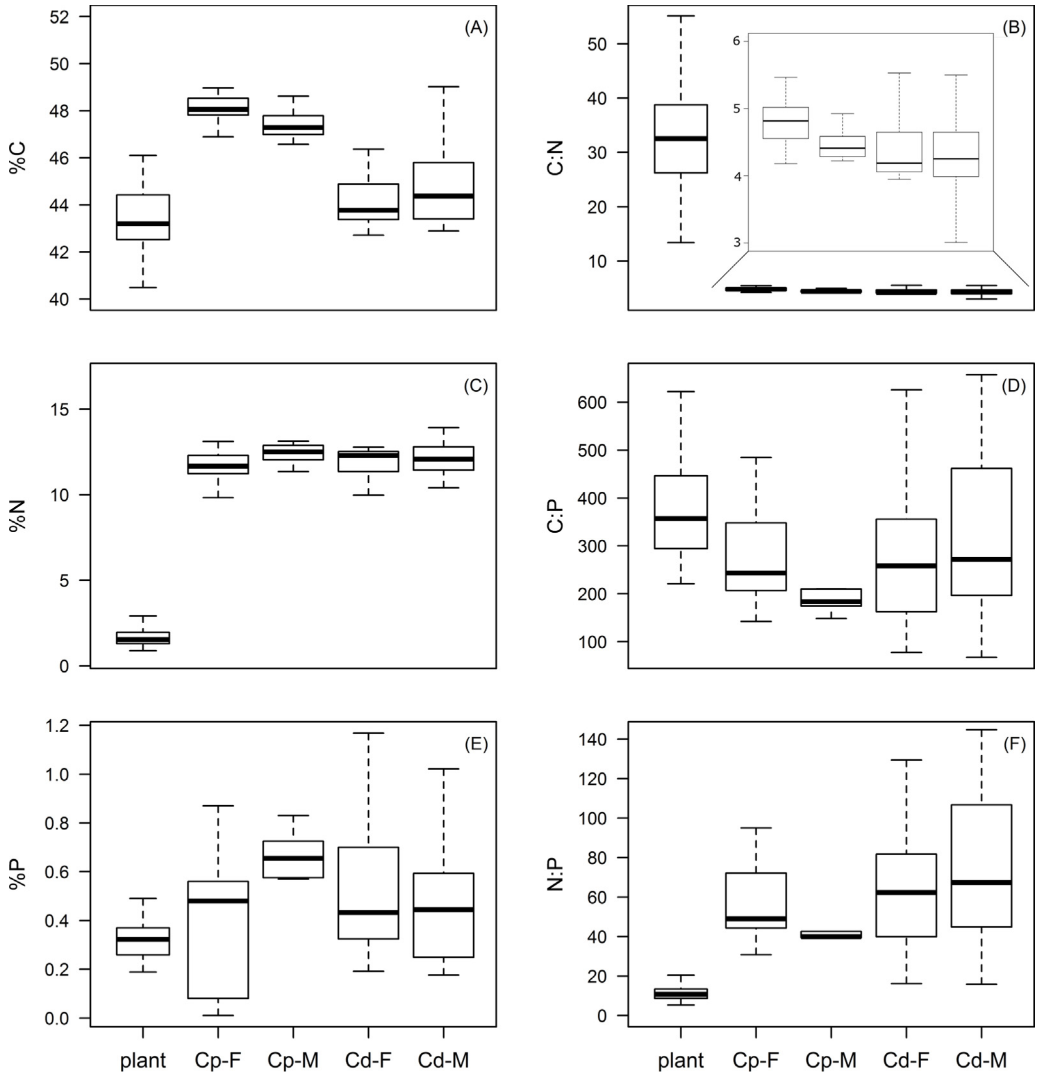

Fig. 1. Boxplots of %C (A), %N (C), %P (E) and C:N (B), C:P (D), N:P (F) molar ratios in dry biomass of plants, of the grasshopper *Chorthippus parallelus* (Cp) and the bee *Chelostoma distinctum* (Cd). Values for males (M) and females (F) are shown separately. Outliers are not presented.

performed with R version 2.13.1 (R Development Core Team 2010) applying functions from the package MASS.

Results

Comparing stoichiometry of taxa and sexes (H1)

Percentages of C, N, P and the N:P ratio were significantly higher, while C:N and C:P ratios were lower in insects than in plants (Table 1). The higher C:N and C:P ratios in plants resulted from low %N and %P values in plants (Fig. 1). %C was significantly higher in grasshoppers than in bees, but %N and %P did not differ between bees and grasshoppers (Table 2 and Fig. 1). Consequently, grasshoppers showed higher C:N ratios than bees, whereas C:P and N:P ratios did not differ (Table 2).

Male bees were more N-rich than females, whereas %C, %P or any of the ratios did not differ between sexes (Table 2). Male grasshoppers were more N- and P-rich than females and showed lower %C values, resulting in lower C:N ratios (Table 2 and Fig. 1). C:P and N:P ratios did not differ between male and female grasshoppers.

Effects of plant species richness and composition (H2)

Plant species richness did not affect the stoichiometry of bees, irrespective of sex. Plant functional group richness altered bee chemical composition depending on sex. With increasing number of functional groups, female bees had marginally increased %C and significantly reduced %N (Fig. 2A), and thus increased C:N ratios (Fig. 2C). In male bees, %P increased with increasing plant functional group richness (Fig. 2B). Ratios of C:P and N:P in male bees were positively related to the corresponding ratios in plants (Fig. 3).

Plant species richness effects on grasshopper stoichiometry were absent except for increasing C:N ratios in female grasshoppers with increasing plant species richness (Fig. 2D). %N of female grasshoppers increased with plant %N ($R^2 = 0.1814$, $n = 36$, $p = 0.004$, estimated coefficient 0.9991 (SD = 0.3334)), resulting in increasing C:N and N:P elemental ratios. There was no relation between plant stoichiometry and the stoichiometry of male grasshoppers (Fig. 3).

Path analysis (H3)

C:N ratio and density of female bees were not influenced by plant species richness (Fig. 4A). In contrast, C:N in male bees, but not their density, increased indirectly with plant species richness. This effect was mediated by a positive relationship between plant richness and blossom cover of flowering plants (Fig. 4B, see Appendix A). C:N ratio of female grasshoppers, but not their density, increased directly with increasing plant C:N, which was higher in highly diverse plant communities (Fig. 4C, see Appendix A). The path model did neither explain variation in C:N nor density in male grasshoppers (Fig. 4D).

Discussion

This is the first study indicating that plant diversity can influence the stoichiometry of higher trophic levels through changes in plant stoichiometry. We specifically found stoichiometric differences between the two insect species and between sexes within the species (accepting H1), also the relation of insect stoichiometry to plant stoichiometry and diversity depended on taxa and sex (partly accepting H2). Specifically, the C:N ratios of female grasshoppers and male bees increased with increasing plant species richness. In path analyses, plant C:N ratios were significantly related to grasshopper density on the plots (partly accepting H3).

We would like to emphasize that our analysis is limited to the community-average elemental content in plants, which has been routinely assessed in this experiment (Abbas et al., 2013), and to only one herbivore and one pollinator species. This imposes two constraints, first we do not know whether this average is indicative of the elemental content of the plant material consumed by the insects, second we ignore other aspects of food quality such as lignin content (which, however, is often correlated to C:N) or content of essential organic molecules. However, our results elucidate a potential nutritional pathway of how plant diversity influences herbivore performance and provide a starting point for further and more mechanistic investigations.

Elemental content differs between insect species and between sexes within the species (H1)

The observed differences in the nutrient content of sexes (for details see Section "Results") necessitated separate analyses for females and males to answer hypotheses H2 and H3. Explaining the sex difference is beyond the scope of our data set, but reproductive effort is one potential basis for this difference. Clearly, energetic investment in male and female offspring differs in bees, as has been shown for a close relative to the species studied here, *Calliopsis persimilis* (Danforth 1990). Sex differences in body stoichiometry have been found for other hymenopterans such as lower %N in females compared to males of the wasp *Ceratosolen fusciceps* Mayr (Zhang & Han, 2010). An alternative explanation is suggested by the overall negative correlation between %P in insects and their body size (Woods, Fagan, Elser, & Harrison 2004). Consequently, higher %P in male grasshoppers may reflect their smaller size compared to females.

Grasshoppers had significantly higher %C than bees and also higher C:N ratios, which corresponds to previous results on insect chemistry (Bertram, Bowen, Kyle, & Schade 2008; Boswell, Provin, & Behmer 2008). Two non-exclusive

Table 2. Results of ANOVAs testing for differences in taxa (T, bees and grasshoppers) and sex (S) (only significant relationships are given).

	Df	Sum Sq	Mean Sq	F value	p-value
C:N					
Taxon	1	3.32	3.32	16.96	<0.001
Sex	1	1.16	1.16	5.96	0.016
T:S	1	0.93	0.93	4.78	0.030
C:P					
T:S	1	119225	119225	2.92	0.091
C					
Taxon	1	316.43	316.43	206.62	<0.001
T:S	1	8.69	8.69	5.67	0.018
N					
Sex	1	6.34	6.33	6.51	0.012
P					
T:S	1	0.33	0.33	3.62	0.059

explanations for this difference exist. First, the elemental composition in cellular and extracellular structures and their proportions drive differences between taxa (Sterner & Elser 2002). Higher %C in grasshoppers compared to bees may reflect the higher proportion of C-rich sclerotic bodyparts (e.g. bigger legs and wings, enlarged pronotum) (Dettner & Peters 1999). Alternatively, body C:N may reflect the stoichiometry of the food resource, as grasshoppers feed

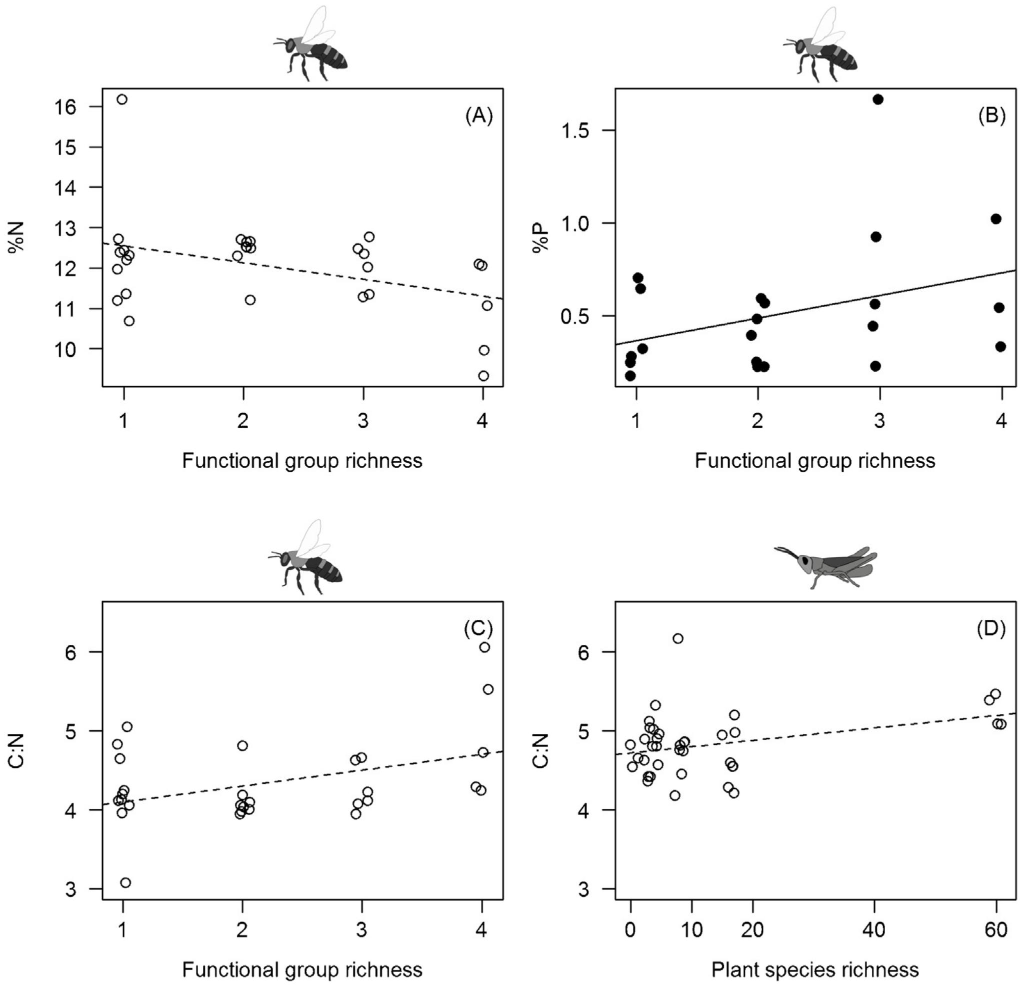

Fig. 2. Effects of plant diversity on %N (A), %P (B) and molar C:N ((C and D)) ratio in animals (only significant relationships shown). Black dots and solid line indicate male animals, open dots and dashed line indicate female animals. (A) $F_{3,27} = 5.0$, $p = 0.033$, $R^2 = 0.126$, (B) $F_{2,18} = 3.24$, $p = 0.029$, $R^2 = 0.183$, (C) $F_{3,27} = 5.25$, $p = 0.030$, $R^2 = 0.132$, (D) $F_{1,35} = 4.9$, $p = 0.034$, $R^2 = 0.098$.

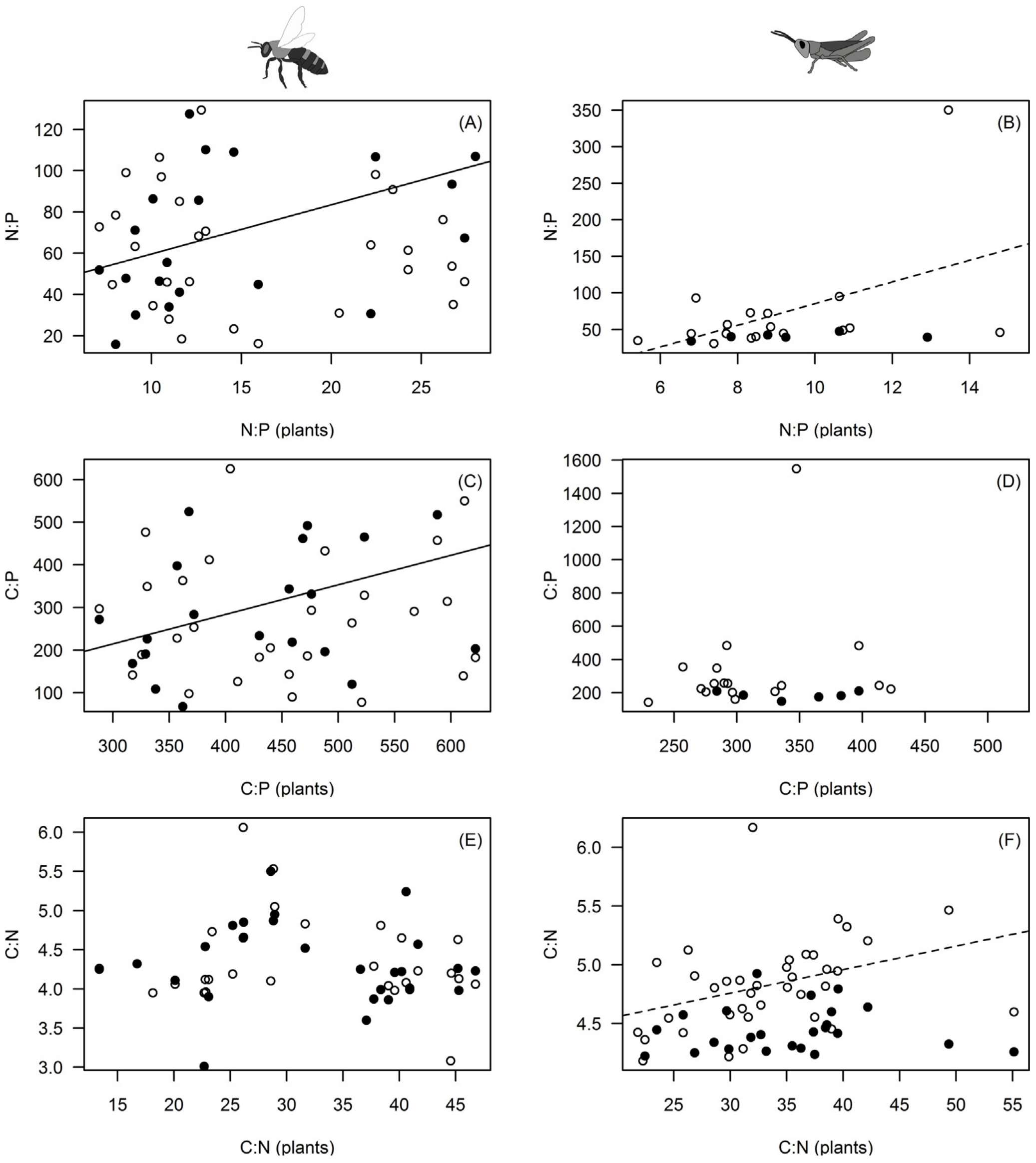

Fig. 3. Effects of plant stoichiometry on molar N:P, C:P and C:N ratios in male and female bees (A, C and E) and grasshoppers (B, D and F). Black dots and solid line indicate male animals, open dots and broken line indicate female animals. Lines show significant relationships. (A) $R^2 = 0.18$, $n = 20$, $p = 0.03$, estimate 2.39 (SD = 1.02), (B) $R^2 = 0.18$, $n = 16$, $p = 0.05$, estimate 14.81 (SD = 7.03), (C) $R^2 = 0.14$, $n = 20$, $p = 0.06$, estimate 0.69 (SD = 0.34), (F) $R^2 = 0.11$, $n = 36$, $p = 0.02$, estimate 0.02 (SD = 0.01).

on C-rich leaves, whereas bees feed on protein-rich pollen. Future studies should analyse the elemental ratios of different plant parts and compare the response of insect stoichiometry to food varying only in nutritional quality. Moreover, gut content could be used as a more precise variable than the overall insect body to reveal stoichiometric coupling between plants, herbivores and pollinators.

Elemental ratios of consumers are related to the stoichiometry of plants (H2)

Our study corroborated previously found stoichiometric differences between plants and their herbivores (Strong, Lawton, & Southwood 1984; Elser et al. 2000), where the content in N and P (in relation to dry weight or C) was lower in the plants than in the insects. Thus, plant stoichiometry can potentially constrain insect elemental content. Our level of inference is weakened by a number of caveats, as (i) we did only measure elemental content as one aspect of nutritional quality ignoring essential organic molecules or lignin content and (ii) we only have information on community average plant stoichiometry, not on the exact stoichiometry of those plant species (and parts) the grasshoppers fed on or of the pollen and nectar ingested by bees. Such detailed studies of plant and insect stoichiometry are warranted as relationships between elemental content in insects and in plant material could already be observed at our coarse level of inference.

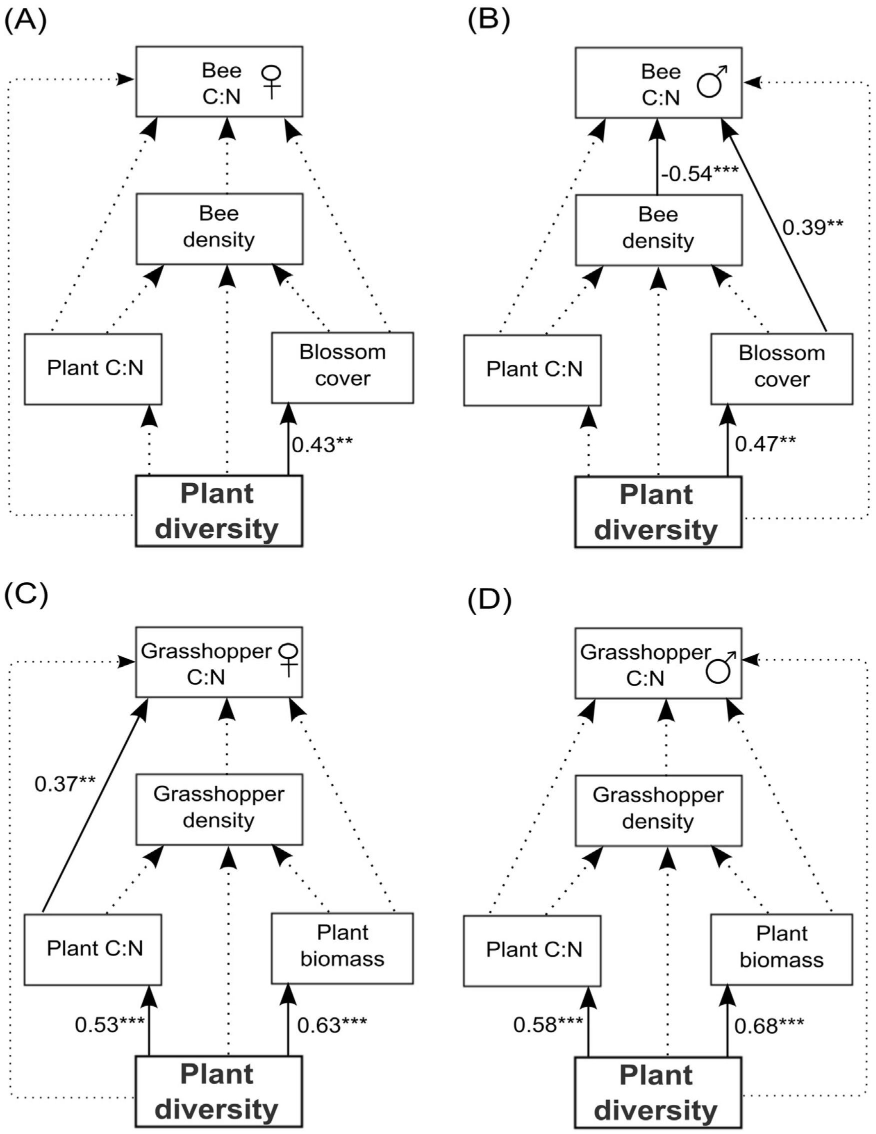

Fig. 4. Path diagram of the final, most parsimonious structural equation models relating plant species richness, plant C:N, plant biomass, animal density and animal C:N for (A) female bees (B) male bees, (C) female grasshoppers, (D) male grasshoppers. Standardised path coefficients are given on top of the path arrows with significances indicated by $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. Paths of marginal significance are not presented here. Detailed information about the final models is given in Appendix A.

If plant diversity affects the stoichiometric mismatch between plants and insects, this may lead to changes in individual feeding rates by compensatory feeding (Suzuki-Ohno, Kawata, & Urabe 2012) and in population level consumption through decreased numerical responses of the consumer (Hillebrand et al. 2009). If plants are rich in N and P, individuals achieve their protein target more easily and reduce consumption rates (Simpson & Raubenheimer 2005). On the population level, however, herbivorous consumption decreases with decreasing content of N and P in autotrophs plants across terrestrial and aquatic ecosystems (Cebrian & Lartigue 2004). Analysing these functional consequences of diversity-driven changes in plant stoichiometry is a logic next step in analysing plant-insect interactions in the face of biodiversity change.

Elemental content of plants is a significant path linking plant diversity to insect density (H3)

Significant relationships between plant diversity and plant C:N in our path analysis corroborated previous analyses from the Jena-Experiment showing that shifts in mean elemental ratios of plants occur with increasing plant species richness or functional group richness, especially by increasing C:N and N:P ratios (Abbas et al. 2013). Theunissen (1994) found that host plant quality decreases with increasing plant species richness due to interspecific competition and thus the attractiveness for consumers decreases. The increase in the C:N ratio of female grasshoppers and bees with increasing plant species richness (grasshoppers) or functional group richness (bees) (see above on H2) may reflect such a reduced quality

(=higher C:N) of the plants. Consequently, the path analysis identified links between insect C:N and insect density on one hand and diversity-mediated changes in food quality (plant C:N) and food quantity on the other hand, which differed between species and sex.

For female grasshoppers, the increasing C:N ratio with increasing plant diversity was mediated by diversity-driven changes in plant C:N. Similar links for male grasshoppers were missing, and grasshopper density was not significantly affected by diversity itself or plant C:N. However, grasshoppers often are generalists and their fitness has been found to be influenced little or not at all by increasing plant species richness (Hudewenz, Klein, Scherber, Stanke, & Tscharntke 2012).

Our linear model revealed a positive influence of plant functional group richness on %P, but not %N, in male bees (see above on H2). In the path analysis, higher C:N in male bees was triggered by diversity-mediated changes in blossom cover, indicating that also food quantity can influence the nutritional status of bees.

Conclusion

Our study analyses the stoichiometry across trophic levels including two functional insect groups along a gradient of plant species richness. We corroborated previous results on different elemental composition of consumers and plants and on stoichiometric differences between insect taxa and sexes. More importantly, we found that plant diversity and stoichiometry influenced at least some aspects of consumer stoichiometry. These results have a preliminary character as discussed above, but they have two important implications for further investigations: First, nutrient stoichiometry might be useful to explain effects of plant composition and plant species richness on higher trophic levels, and second, plant elemental composition potentially has different consequences for different trophic and functional groups.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.baae.2014.02.001.

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