



RESEARCH PAPER

Tracking sucking herbivory with nitrogen isotope labelling: Lessons from an individual trait-based approach

Felix Neff^{a,b,*}, Marco M. Lehmann^c, Marco Moretti^d, Loïc Pellissier^{e,f},
Martin M. Gossner^{b,g}

^aAgroecology and Environment, Agroscope, Zürich 8046, Switzerland

^bForest Entomology, Swiss Federal Research Institute WSL, Birmensdorf 8903, Switzerland

^cForest Dynamics, Swiss Federal Research Institute WSL, Birmensdorf 8903, Switzerland

^dConservation Biology, Swiss Federal Research Institute WSL, Birmensdorf 8903, Switzerland

^eLandscape Ecology, Institute of Terrestrial Ecosystems, ETH Zürich, Zürich 8092, Switzerland

^fLandscape Ecology, Swiss Federal Research Institute WSL, Birmensdorf 8903, Switzerland

^gDepartment of Environmental Systems Science, Institute of Terrestrial Ecosystems, ETH Zürich, Zürich 8092, Switzerland

Received 22 March 2022; accepted 10 June 2022
Available online 11 June 2022

Abstract

Response and effect traits help to understand how changes in ecological communities (e.g. in response to land use) relate to changes in ecosystem functioning. In grasslands, plants and insect herbivores are involved in many ecosystem processes such as herbivory and plant biomass production. Simultaneous changes in the trait composition of both plants and herbivores should affect herbivory rates, with consequences for plant growth and potentially biomass production. The mechanisms underlying these links are little understood for grasses and sucking insects, which build a major part of grassland communities. In a mesocosm experiment, we manipulated the composition of grasses and sucking herbivores (Hemiptera) to study the role of plant traits, herbivore traits and their interaction on herbivory and plant growth. Because sucking herbivory is generally difficult to quantify, we developed a novel experimental setting, in which we labelled plants with ¹⁵N isotope. This allowed to quantify ¹⁵N uptake and thus sucking rates of individuals. We found that herbivory and simultaneous plant growth reduction are most strongly linked to herbivore species identity. Unexpectedly, herbivory did not increase with herbivore size, but was highest for small species and for thin-bodied Heteroptera. Additionally, herbivory and plant growth reduction depended on the interacting herbivore and plant species, indicating trait matching, which could, however, not be explained with commonly used traits. This indicates that mechanisms linking ecological communities and ecosystem processes are highly context-specific. To understand how global change affects ecosystem functioning, studies need to cover all functionally relevant groups, including plant sap suckers.

© 2022 The Authors. Published by Elsevier GmbH on behalf of Gesellschaft für Ökologie. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>)

Keywords: Ecosystem functioning; Grasses; Hemiptera; Multitrophic interactions; Trait matching

*Corresponding author at: Agroecology and Environment, Agroscope,
Zürich 8046, Switzerland
E-mail address: mail@felixneff.ch (F. Neff).

Introduction

Global change drivers such as land-use change and intensification shift the composition of ecological communities across ecosystems and trophic levels, with consequences for ecosystem functioning (Allan et al., 2015). In semi-natural grasslands, which are important hotspots of biodiversity in temperate regions (Habel et al., 2013), intensive agricultural use has been shown to be a major filter in the assembly of both plant and insect communities (Neff et al., 2019; Socher et al., 2012). At the same time, the rates of different ecosystem processes have been found to be strongly affected by land-use intensity (Ambarlı et al., 2021) and these changes have been related to shifts in diversity or composition of ecological communities (Wang et al., 2020). The underlying mechanisms by which changes in community composition affect ecosystem processes are, however, understudied.

An important ecosystem process in semi-natural grasslands is insect herbivory, which might be strongly linked to plant growth and thus affects plant biomass production. Plant biomass production in these systems is an important provisioning service contributing to agricultural production (Bengtsson et al., 2019). Insect herbivory might either reduce plant biomass production through reduced plant growth (Crawley, 1989) or stimulate plant growth (Dungan et al., 2007). Plant biomass production and insect herbivory are tightly linked to plant and insect communities (Lavorel et al., 2013), but how changes in these multi-trophic communities affect ecosystem processes is still poorly understood.

The use of effect traits, i.e. species or individual morphological or physiological characteristics that affect ecosystem processes, can improve the understanding of the mechanisms linking ecological communities and ecosystem processes (Lavorel & Garnier, 2002). For example, plants characterised by high specific leaf area (SLA) and leaf nitrogen content (LNC) tend to be associated with faster plant growth and contribute to higher plant biomass production (Funk et al., 2017; Wright et al., 2004). At the same time, plant biomass production was found to be more strongly reduced by larger grasshoppers (Moretti et al., 2013) with stronger mandibles (Deraison et al., 2015). Additionally, traits of organisms belonging to different trophic levels might have interactive effects on ecosystem processes through trait matching (Schleuning et al., 2015). For example, plant biomass consumption depends on the interaction between plant toughness and the grasshopper's mandible strength (Ibanez, Lavorel, et al., 2013). Thus, we need to better understand how traits of organisms at different trophic levels jointly affect ecosystem processes to predict how shifts in communities affect ecosystem functioning.

Such questions have rarely been studied at the level of single species or functional groups (but see Ibanez et al. 2013). Furthermore, studies addressing similar

questions so far never addressed herbivores that feed by sucking plant saps, which are, however, accounting for a large share of herbivore communities in grasslands (e.g. Risch et al. 2015) and can significantly reduce plant growth (e.g. Meyer & Whitlow 1992). This is not least because sucking herbivory rates are genuinely hard to quantify, given that feeding marks are hard to see and may not well be related to uptake rates (Schowalter, 2011). However, sucking herbivore communities are substantially affected by intensive land use, which changes their trait composition, e.g. by filtering for smaller species (Neff et al., 2019). How these changes in trait composition affect insect herbivory and relate to plant growth, and consequently plant biomass production are still open questions.

Here, we manipulated the trait composition of plants and herbivores in a fully crossed mesocosm experiment to study how traits are related to insect herbivory and plant growth and whether there is indication for trait matching between the two trophic levels. We focused on hemipteran species sucking on grasses, both of which are important functional groups in semi-natural grasslands (Neff et al., 2021). To overcome the difficulty of assessing sucking herbivory, we developed a novel experimental setting, where plants were labelled with a heavier isotope of nitrogen (^{15}N), which enabled us to track the flow of nitrogen in the system (e.g. Steffan et al. 2001). Stable isotope techniques are increasingly used in insect ecology (e.g. Quinby et al. 2020), and also to study nutrient flows in food webs or to assess herbivory (e.g. Schallhart et al. 2012, Porras et al. 2020). Here, labelling of plants with ^{15}N allowed us to quantify herbivory rates of single sucking herbivores, which has to our knowledge not been done before, but provides large potential for more mechanistic studies on insect herbivory. The grass species included in the experiment were chosen to cover a gradient in palatability inferred from three traits (leaf dry matter content (LDMC), SLA, LNC), which have commonly been used to relate plant palatability to chewing herbivory (e.g. Schädler et al. 2003). Herbivore species were chosen to cover a trait space defined by three potential effect traits (body volume, body shape, rostrum length). We were interested in the interplay of these traits in determining insect herbivory and changes in plant growth.

We predicted that herbivory rates would be highest on plant species characterised by high palatability and for the largest herbivore species, resulting in reduced plant growth, unless there is a stimulation of compensatory plant growth by herbivory. Additionally, if there is trait matching evident for the trophic relations between these two groups, we predicted that highest herbivory and consequently highest plant growth reduction should be observed at certain combinations of plant and herbivore traits. For example, we expect that plants with thicker leaves (i.e. low SLA; Wilson et al. 1999) are better accessible to herbivores with longer rostra and thus deeper leaf penetration potential, inducing trait matching.

Materials and methods

Plant and herbivore material

Plant species were restricted to Poaceae and were selected based on three traits, which are essential determinants of the global leaf economic spectrum (Wright et al. 2004) and are related to palatability and herbivory rates (e.g. Schädler et al. 2003): LDMC, SLA and LNC. Three species were selected from each of three clusters of species sharing similar traits (Fig. 1A): *Agrostis capillaris*, *Arrhenatherum elatius* and *Poa trivialis* in the high palatability cluster (low LDMC, high SLA and high LNC); *Cynosurus cristatus*, *Festuca arundinacea* and *Holcus lanatus* in the medium palatability cluster (low LDMC, high SLA and low LNC); and *Deschampsia cespitosa*, *Festuca ovina* agg. and *Sesleria caerulea* in the low palatability cluster (high LDMC, low SLA and low LNC) (Appendix A for details).

Insect herbivore species were selected from grass feeding Hemiptera (suborders Auchenorrhyncha and Heteroptera) based on three morphometric traits related to sucking herbivore effects, i.e. body volume, rostrum length and body shape (see Appendix A for inclusion rationales). Species were selected to cover the trait space (Fig. 2A) and included *Aelia acuminata*, *Lygus* spp., *Notostira* spp., *Trigonotylus caelestialium*, *Stenodema laevigata*, *Deltocephalus pulicaris*, and *Laodelphax striatella* (Appendix A for details).

Experimental design and setup

The mesocosm experiment was performed in experimental cages in August/September 2019 with a completely randomized design with two crossed treatment factors (Appendix B: Figs 1–3): plant palatability (three factor levels) and herbivore species identity (seven species and one control treatment without herbivores). Each treatment combination was replicated five times (3 plant treatments × 7 herbivore treatments × 5 replicates = 105 cages), except for the control treatments, to which some additional cages originally planned to contain further herbivore species were added, resulting in up to eight replicates (3 plant treatments × 1 herbivore control × 7–8 replicates = 23 cages). Because some cages were built with a plexiglass that was unexpectedly preventing plant growth, these cages were excluded from analyses. These cages had been randomly assigned to the study treatments and together with other, minor incidents, we ended up with two to five (seven for some controls) replicates per treatment combination and a total of 94 experimental cages (see Appendix B: Table 1 for a complete overview of replicates), which still enabled robust analyses given the fully crossed experimental design.

At the start of the experiment, each cage contained an individual of each of the three plant species selected for the palatability cluster, which were labelled with ¹⁵N to track the flow of nitrogen in the system, and two individuals of a herbivore species (Appendix B: Fig. 1). Different measures were taken on plants and herbivores at the start and the end

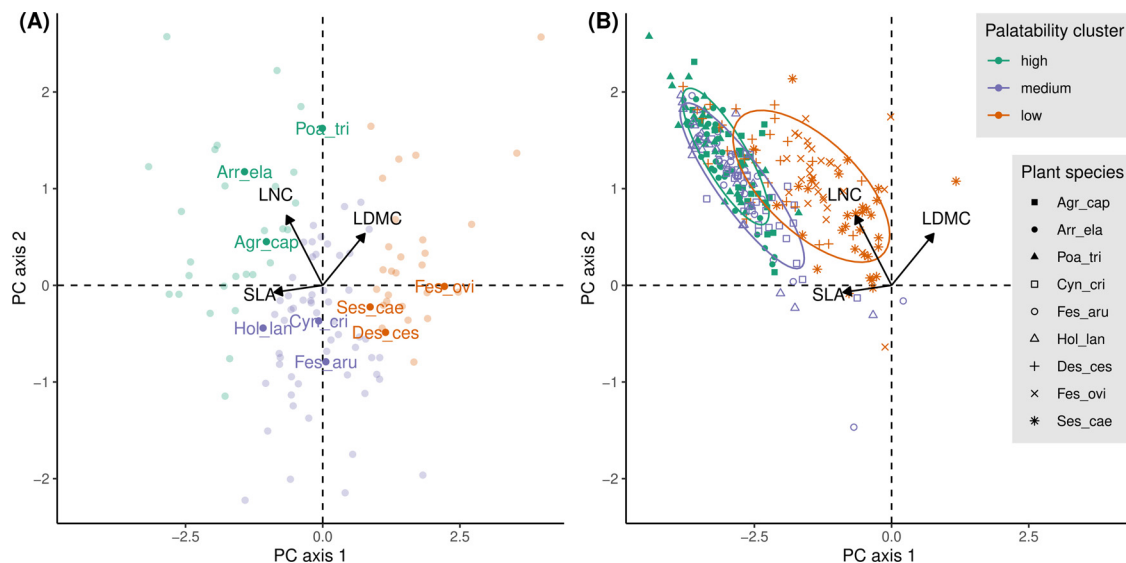


Fig. 1. (A) Allocation of Poaceae species in a two-dimensional trait space derived from principal component analysis (PCA) on LDMC, SLA and LNC (derived from a trait data base; Kattge et al., 2020). Each point is a species, colours represent assignment to three different palatability clusters (low, medium, high palatability). Arrows show PCA loadings of the three trait variables. Study species are indicated by strong colours and labels. (B) Allocation of study plants in the same two-dimensional trait space as in (A). Each point is an individual, colours show the palatability cluster and shapes represent the species. Ellipses show cluster allocations based on a multivariate normal distribution for a confidence level of 0.66. Agr_cap: *Agrostis capillaris*, Arr_ela: *Arrhenatherum elatius*, Poa_tri: *Poa trivialis*, Cyn_cri: *Cynosurus cristatus*, Fes_aru: *Festuca arundinacea*, Hol_lan: *Holcus lanatus*, Des_ces: *Deschampsia cespitosa*, Fes_ovi: *Festuca ovina*, Ses_cae: *Sesleria caerulea*.

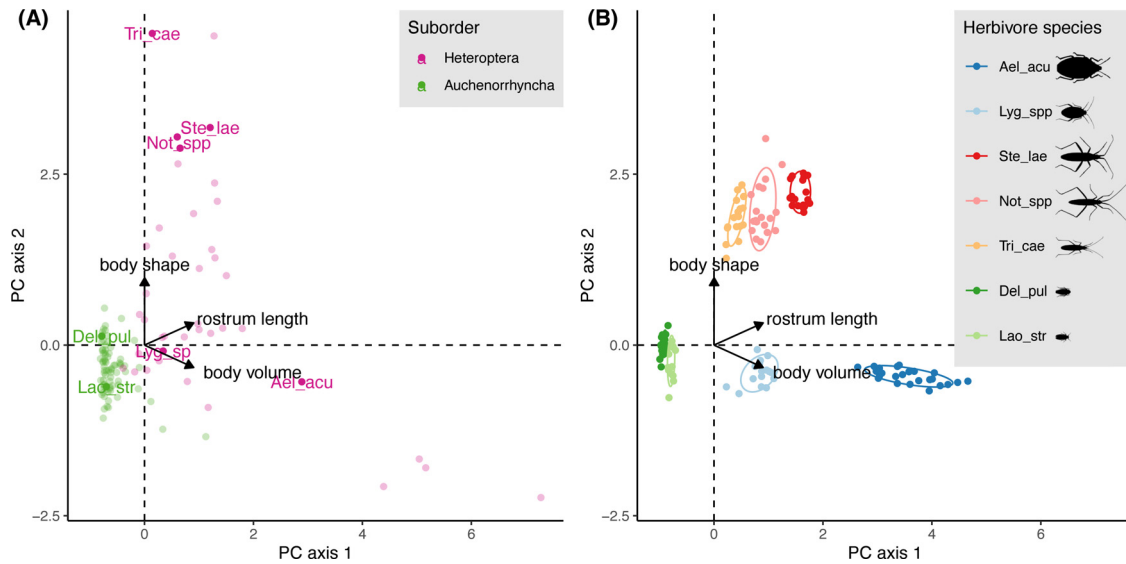


Fig. 2. (A) Allocation of Heteroptera and Auchenorrhyncha species that feed on Poaceae in a two-dimensional trait space derived from principal component analysis (PCA) on body volume, rostrum length and body shape. Species selection is based on a study by Neff et al. (2019), from which also species-level trait values are derived. Arrows show PCA loadings of the three trait variables. Colours indicate the two suborders. The herbivore species that were selected for the experiment are indicated by strong colours and labels. (B) Allocation of study specimens in the same two-dimensional trait space as in (A). Each point is an individual, colours show the species. Ellipses show cluster allocations based on a multivariate normal distribution for a confidence level of 0.66. Here and in other figures, insect icons in the legend show the outline of the study species true to scale. Ael_acu: *Aelia acuminata*, Lyg_spp: *Lygus* spp., Ste_lae: *Stenodema laevigata*, Not_spp: *Notostira* spp., Tri_cae: *Trigonotylus caelestialium*, Del_pul: *Deltocephalus pulicaris*, Lao_str: *Laodelphax striatella*.

of the experiment (Appendix B: Table 2), which were used to quantify traits, plant growth and herbivory rates. For details on the experimental setup, see Appendix A.

Estimation of ecosystem processes

For each plant individual, we predicted dry mass at the end of the experiment ($m_{end,pred}$) that would have been expected in the absence of herbivores from estimated dry mass at the beginning of the experiment and growth observed for control plants not affected by herbivores (Appendix A). Predicted dry mass was related to measured dry mass at the end of the experiment (m_{end}) to determine relative deviation from expected growth g_{off} as

$$g_{off} = \frac{m_{end,pred} - m_{end}}{m_{end}} \quad (1)$$

with positive values representing lower than expected growth and negative values representing higher than expected growth. These values were used as proxies of plant growth reduction.

Insect herbivory was estimated based on uptake of ^{15}N by herbivores (u_{abs}) and mean ^{15}N concentration of the available plants (C_{15N}), which were determined from $\delta^{15}N$ ratios, nitrogen content and biomass of plant and herbivore samples (Appendix A). ^{15}N uptake by herbivores relative to available ^{15}N in plants (u_{rel}) was determined as

$$u_{rel} = u_{abs} C_{15N} \quad (2)$$

and was used as a proxy of insect herbivory.

Statistical analyses

All analyses were conducted in R v3.5.2 (R Core Team, 2018). Linear mixed effects models were used to relate herbivory and plant growth reduction to (i) herbivore species and plant palatability cluster identities and (ii) herbivore traits and plant traits. The identity models contained herbivore species identity, plant palatability cluster identity, their interaction as well as the potentially confounding variables herbivore survival and distance to light (integer denoting the row at which the cage was positioned; Appendix B: Fig. 4) as fixed effects and a random effect for the cage. Herbivory was analysed at the level of individual herbivores, with survival indicating whether the individual was found alive at the end of the experiment (0/1), whereas plant growth reduction was analysed at the level of the individual plants, with survival indicating the number of individual herbivores that were found alive at the end of the experiment (0–2). Herbivory was log-transformed prior to analyses to meet distributional assumptions. The trait models had the same structure as the identity models, but herbivore species and plant palatability cluster identities were replaced with herbivore and plant PC axes. Based on the principal

component analyses that were used for the selection of herbivore and plant species, study specimens were placed on the same PC axes based on their measured trait values. PERMANOVA from the package ‘vegan’ (Oksanen et al., 2018) was used to check whether plant palatability clusters for the study plants were also represented by their PC axis values based on measured traits (9999 permutations). The two PC axes per trophic level were then included in the models. Additionally, all possible interactions between herbivore PC axes and plant PC axes ($n = 4$) were included in the model. Backward model selection (based on χ^2 tests) was used to find the optimal interaction structure for each model. Only interactions but no main effects were excluded during model selection. As for the identity models, herbivory was analysed at the level of individual herbivores, whereas plant growth reduction was analysed at the level of individual plants. Trait values of the respective other level were aggregated at cage level by taking mean values. The effect of sex on herbivory was tested in both the identity and trait models, but was not found to be significant, which is why it was excluded from the final models. All linear mixed effects models were run through the package ‘glmmTMB’ (Magnusson et al., 2020).

Results

Plant palatability clusters were represented by traits measured for the study plants (PERMANOVA: $P < 0.001$ for all pairwise comparisons based on PC axes), although variation within the clusters was quite large (Fig. 1B). On average, the study plants had higher LNC than plants in the data base, indicating a fertilisation effect caused by the ^{15}N labelling (Appendix B: Fig. 5). All study plants were strongly enriched in ^{15}N compared to plants of the same species that were not included in the experiment (Appendix B: Fig. 5). Dry mass of control plants without herbivores present increased by $230\% \pm 16\%$ (mean \pm SE) relative to predicted dry mass at the start of the experiment, while dry mass of plants with herbivores present increased by $160\% \pm 8\%$, which was significantly less than for control plants (LMM: $\chi^2 = 14.36$, $P = 1.5\text{e-}04$; Appendix B: Fig. 6).

The traits measured on the study herbivore specimens matched closely the expected trait ranges (Fig. 2B). Mortality among retrieved study specimens was 52.7% ($n = 68$). Additionally, 14.0% ($n = 21$) of individuals could not be retrieved at the end of the experiment and were thus recorded as dead, resulting in an overall survival rate of 40.7% ($n = 61$), which differed greatly among study species (Appendix B: Table 3). All specimens, including the ones that had died, had clearly elevated ^{15}N concentrations, indicating (premortem) feeding activity of all specimens (Appendix B: Fig. 7). Average absolute ^{15}N uptake by herbivores was estimated to $0.590\mu\text{g}$ ($0.006 - 2.939\mu\text{g}$ [5% and 95% quantiles]), which relative to plant content of ^{15}N corresponds to $97.7\mu\text{g}$ ($1.09 - 476.8\mu\text{g}$) of dry plant material

that was taken up (Appendix B: Fig. 8). Average dry mass of herbivores was 3.73mg ($0.214 - 16.44\text{mg}$; Appendix B: Fig. 9). Differences in relative ^{15}N uptake between herbivore species were ranging from $27.1\mu\text{g}$ ($1.68 - 103.2\mu\text{g}$) of dry plant material for *Stenodema laevigata* to $188.9\mu\text{g}$ ($0.294 - 518.0\mu\text{g}$) for *Trigonotylus caelestialium* (Appendix B: Fig. 8). Signs of herbivory on the plants were recorded on 25 plants (11.3%), 12 of which were on plants that were with *T. caelestialium*.

Effect of plant and herbivore species on herbivory and plant growth reduction

Herbivory (^{15}N uptake of herbivores relative to average plant ^{15}N content) was strongly affected by the interaction of plant palatability cluster identity and herbivore identity (LMM: $\chi^2 = 41.40$, $P = 4.2\text{e-}05$; Appendix B: Table 4), while plant growth reduction (relative deviation in plant growth from control) was marginally significantly related to the interaction (LMM: $\chi^2 = 18.55$, $P = 0.10$; Appendix B: Table 5). Also, there was a significant effect of herbivore identity on herbivory (LMM: $\chi^2 = 52.09$, $P = 1.8\text{e-}09$; Appendix B: Table 4). Apart from the interactive effects, plant palatability cluster identity did neither show a significant relation to herbivory nor plant growth reduction. Herbivory but not plant growth reduction was higher for surviving individuals (Appendix B: Table 4). Model predictions from both process models indicate that the higher herbivory, the higher plant growth reduction (Fig. 3). Highest predicted herbivory and plant growth reduction were observed for *T. caelestialium* on plants of the medium and high palatability cluster and for *Notostira* spp. on plants of the low palatability cluster (Fig. 3). While for *Notostira* spp., no difference in herbivory rates were found between the two species *N. elongata* and *N. erratica* (student’s t-test: $P = 0.39$), there was a tendency for higher herbivory rates in *Lygus rugulipennis* compared to *L. pratensis* (student’s t-test: $P = 0.068$). Accounting for the different *Lygus* species in the analyses of herbivory rates did, however, not change the overall picture (Appendix B: Fig. 10).

Effect of plant and herbivore traits on herbivory and plant growth reduction

Herbivory was highest for specimens with small body volume (low herbivore PC axis 1 values) and thin bodies (high herbivore PC axis 2 values) (Fig. 4, Appendix B: Table 6). Neither plant PC axes nor the interactions between herbivore and plant PC axes were significantly related to herbivory. Plant growth was reduced most strongly by large herbivores (herbivore PC axis 1) on plants with high LNC (plant PC axis 2) or by small herbivores on plants with low

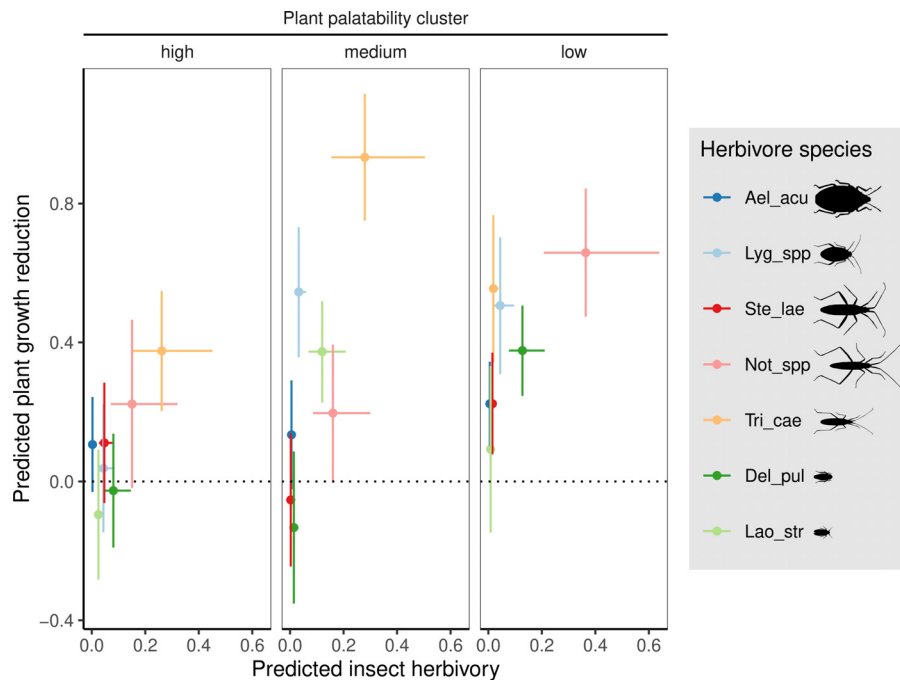


Fig. 3. Predictions from models analysing the joint effect of plant and herbivore treatment and their interaction on herbivory and on plant growth reduction. Predictions are shown for different plant palatability clusters (panels; high, medium, low palatability) and for different herbivore species (colours). Model predictions and standard errors are shown. Herbivory was log-transformed prior to modelling to meet distributional assumptions, but transformed back for the illustration of this figure. Models also account for herbivore survival and cage position. *Ael_acu*: *Aelia acuminata*, *Lyg_spp*: *Lygus* spp., *Ste_lae*: *Stenodema laevigata*, *Not_spp*: *Notostira* spp., *Tri_cae*: *Trigonotylus caelestialium*, *Del_pul*: *Deltocephalus pulicaris*, *Lao_str*: *Laodelphax striatella*. Detailed model results in [Appendix B](#): Tables 4 and 5.

LNC, as was indicated by a significant interaction between the two PC axes ([Fig. 5](#), [Appendix B](#): Table 7).

Discussion

Plant growth was clearly inhibited by herbivore presence and tended to be most strongly reduced in settings that showed highest herbivory rates, supporting the potential of sucking herbivores to affect plant biomass production. Because sucking herbivores withdraw photosynthates from the plants, they potentially reduce their ability for growth. Furthermore, herbivory can lead to plant stress-responses such as lowered photosynthesis ([Sulaiman et al., 2021](#)), also resulting in lowered plant growth. Alternatively, plants may hold their C uptake constant but invest a large part of their photosynthetically obtained C into defence (and thus respiration) or store it in the roots, which would reduce the relative amount of C available for aboveground growth ([Dyer et al., 1991](#); [Walling, 2000](#)). Although these different mechanisms can explain the observed plant growth reduction, it might still be unexpected, given that in non-outbreak situations, insect herbivory is often expected to increase plant productivity ([Dyer et al., 1993](#)). However, such stimulation in growth might only be apparent once herbivory pressure is reduced again ([Hawkins et al., 1986](#)), which was not the case here with herbivores being present during the whole

experiment. Also, the study design only allowed us to study the plants for two weeks after infestation with herbivores, which might not be long enough to observe compensatory growth. Thus, although the observed reduction in plant growth with increasing herbivory was considerable, more work needs to be done to understand its quantitative impact in real-world ecosystems.

Process rates differed between herbivore species, but the observed relations did not match our expectation that large herbivore species would consume more and reduce plant growth more, as is the case for grasshoppers ([Moretti et al., 2013](#)). Although survival was included in our models, this result may still have been partly influenced by differences in survival rates between herbivore species. As survival rates were high for very different species such as large bugs (e.g. *Aelia acuminata*) and small leafhoppers (e.g. *Deltocephalus pulicaris*), we expect other factors to be more important in explaining the observed species differences. Increasing consumption rates and thus herbivory increasing with body size are generally expected due to higher metabolic rates ([Brown et al., 2004](#)). While the positive relation between body size and metabolic rates in herbivores is undisputed (e.g. [Ehnes et al. 2011](#)), other factors can affect metabolic rates of herbivores. For example, species that are engaged in regular activities with high metabolic demand (e.g. flying, producing sounds) tend to have higher metabolic rates ([Reinhold, 1999](#)). The smaller species included in our study

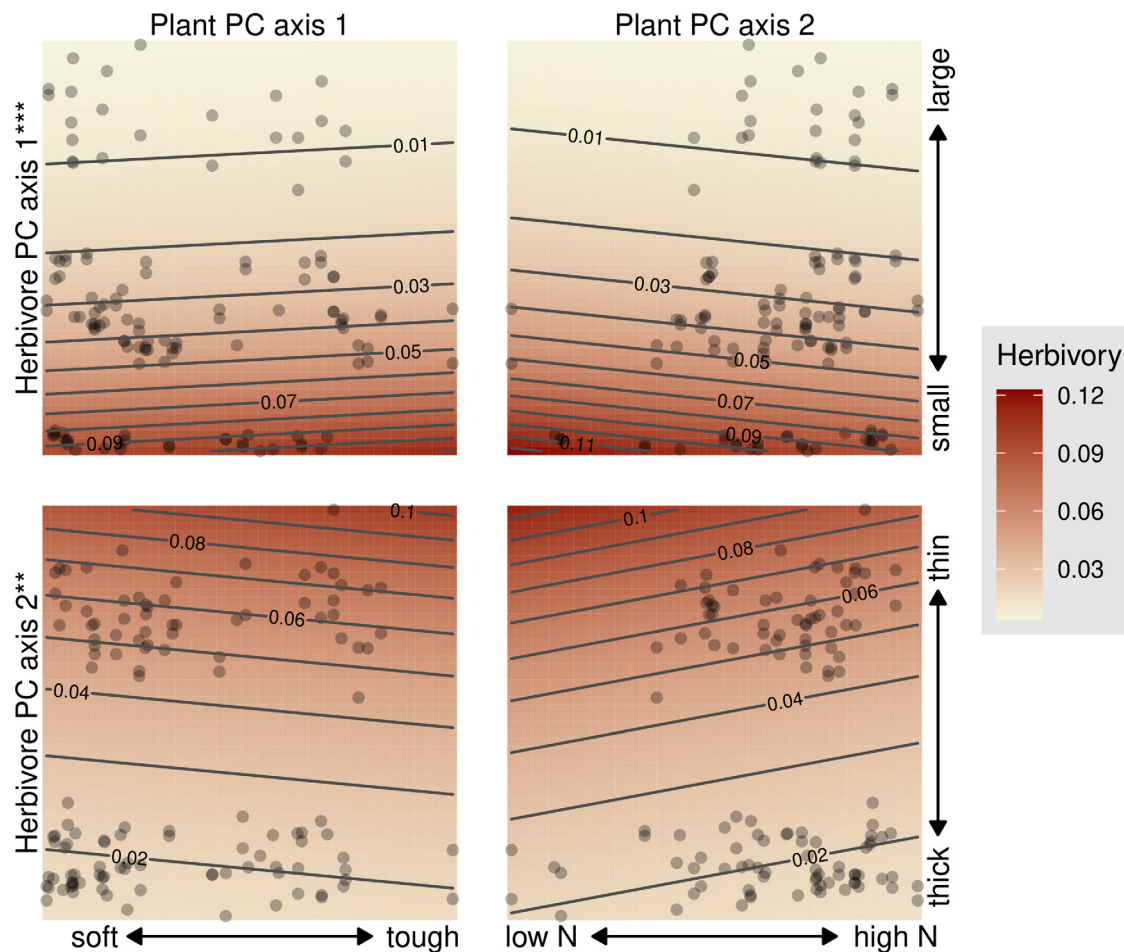


Fig. 4. Predicted herbivory from a model analysing the joint effect of plant and herbivore traits and their interactions (colour gradient). Plant and herbivore traits were represented by PC axes (Fig. 1 and Fig. 2), whose meaning is indicated by the arrows below and right to the graphs. Points show the spread of underlying observations in trait space. All non-significant interactions were excluded from the model, leaving no interaction between plant and herbivore traits in this model. Models also account for herbivore survival and cage position. Significance of variables is indicated next to axis labels (***: $p \leq 0.001$, **: $p \leq 0.01$, *: $p \leq 0.05$). Herbivory was log-transformed prior to modelling to meet distributional assumptions, but transformed back for the illustration of this figure. Detailed model results in Appendix B: Table 6.

have shorter generation times (Biedermann & Niedringhaus, 2004; Wachmann et al., 2004–2012), which might require more activities with high metabolic demand in a shorter time to fulfil their life cycle. Measures such as metabolic rate should be further addressed as potential effect traits related to herbivory and plant growth reduction.

Mechanical plant palatability traits such as LDMC and SLA have been related to leaf toughness and are thus regularly postulated to be negatively related to plant palatability for chewing herbivores (e.g. Descombes et al. 2020). The lack of clear relationships in this study suggests that those traits are less related to accessibility of leaf tissue and transport vessels for sucking herbivores and that other traits such as nutrient contents could be stronger determinants (Pres-tidge, 1982). Because LNC is of essential value for sucking herbivores, given it is generally a major limiting nutrient in their diet (Elser et al., 2000), the lacking relation between LNC and herbivory in our study is surprising. It might, however, be related to the elevated LNC of all study plants

compared to values reported in previous studies, which was a consequence of the fertilization imposed by the labelling. Thus, the plant palatability clusters that were defined based on literature traits were partly blurred. Consequently, all herbivores might have met their nitrogen demand in all palatability clusters, such that differences in consumption rates rather reflect differences in physiological needs of herbivores than of plant palatability defined by the three investigated plant traits. While the results of our study question the usefulness of commonly used plant palatability traits for sucking herbivores, further work needs to investigate which traits might be more relevant for this important group of insect herbivores.

Both herbivory and plant growth reduction depended on the combination of herbivore species and plant palatability cluster. This indicates trait matching, but because the interactive effect could at best weakly be explained by the investigated traits, other traits might be involved to explain the specialisation of sucking herbivores to certain grasses. In

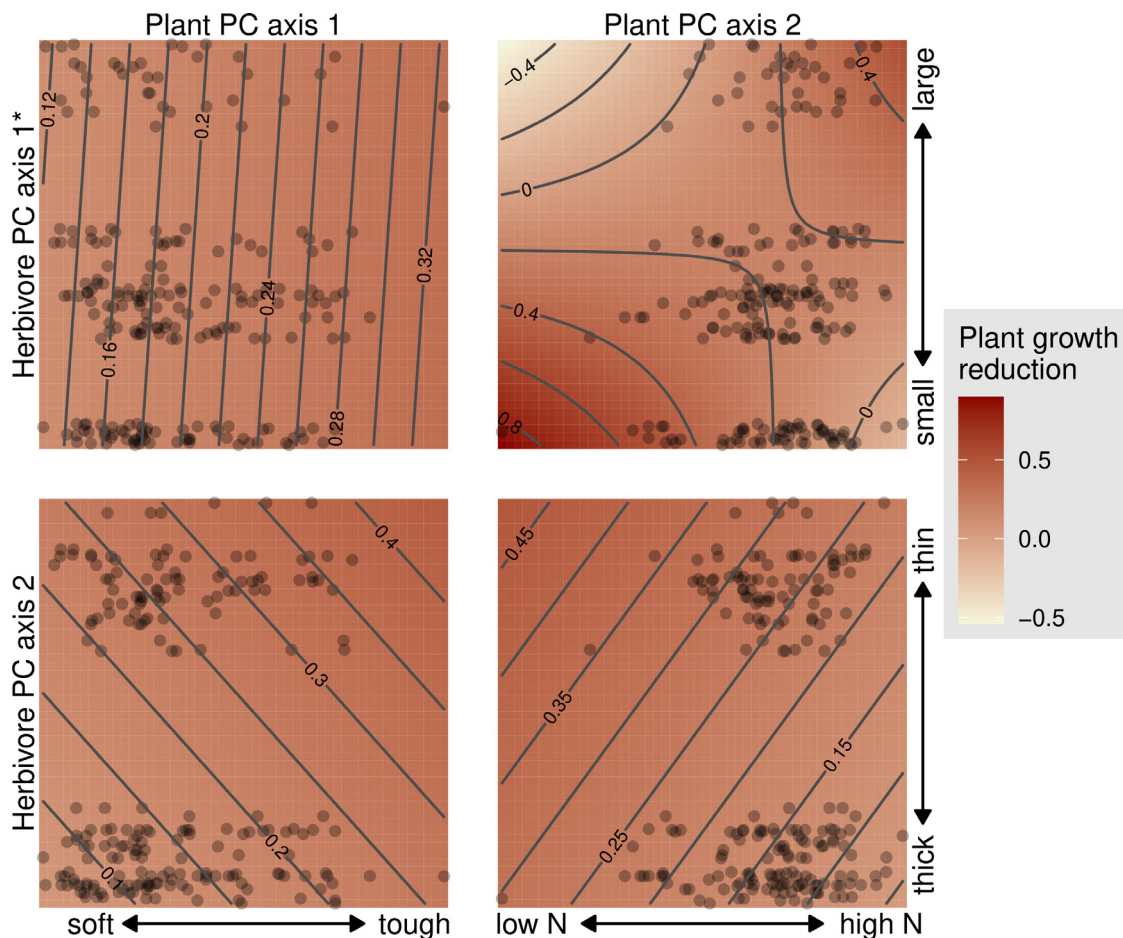


Fig. 5. Predicted plant growth reduction from a model analysing the joint effect of plant and herbivore traits and their interactions (colour gradient). Plant and herbivore traits were represented by PC axes (Figs. 1 and 2), whose meaning is indicated by the arrows below and right to the graphs. Points show the spread of underlying observations in trait space. All non-significant interactions were excluded from the model, leaving only the interaction between herbivore PC axis 1 and plant PC axis 2 in the model. Models also account for herbivore survival and cage position. Significance of variables is indicated next to axis labels (***: $p \leq 0.001$, **: $p \leq 0.01$, *: $p \leq 0.05$). Detailed model results in Appendix B: Table 7.

dicotyledons, specialisation is often explained by the highly diversified composition in terms of secondary compounds, which is postulated to be an evolutionary response to herbivores (Ehrllich & Raven, 1964). Grasses, however, lack this diversity in secondary compounds (Tschardt & Greiler, 1995), posing the question of what is mainly driving specialisation. A probable factor are once more varying nutrient levels among grass species and individuals, with herbivores being physiologically adapted to very specific host stoichiometries (Denno & Roderick, 1990). Furthermore, grasses are known to use elevated silicon concentrations as defence against herbivores (Vicari & Bazely, 1993). Thus, differences in silicon concentrations could explain the observed patterns, although their efficacy against sucking herbivory is not well understood so far (Keeping & Kvedaras, 2008). Investigating trait matching by assessing host and herbivore stoichiometries and additional host defence structures could be a way forward to extend this concept to sucking herbivores.

By labelling plants with ^{15}N isotope, we successfully quantified sucking herbivory at the level of single individuals, which is otherwise hard to observe. As such, the method provides great potential for future mechanistic studies on insect herbivory. We show that different herbivore species differently affect herbivory and plant growth and find indications for interactive effects between herbivores and plants in determining process rates, which suggest trait matching. Such relationships are in line with previous studies from grasslands with grasshoppers and indicate the importance of plant and herbivore community shifts for ecosystem functions such as plant biomass production. However, the traits generally recognised to be involved in the relationships among plants, grasshoppers and ecosystem processes had little explanatory power in our model system. This suggests that new traits should be addressed to understand the consequences of changes in multi-trophic community composition, e.g. in response to land-use intensification, for ecosystem functioning.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank S. Bänziger, H. Berner, G. Casciano, C. Cattaneo, N. Feddern, G. Flückiger, U. Graf, T. Juchli, M. Laski, L. Neff, S. Neff, M. Oetli, A. Perret-Gentil, G. Reiss, D. Schneider, B. Sneider, G. Szemes, M. Tran and B. Wermelinger for their help in setting up the experiment and in collecting and analysing samples. We are grateful to the fenaco Genossenschaft and to J. Heinze for providing seeds for the experiment. We thank two anonymous reviewers for their valuable inputs. This study was funded by the SNF [310030E-173542/1, granted to MMG] and by the SNF Ambizione project ‘TreeCarbo’ [No. 179978, granted to MML].

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:[10.1016/j.baae.2022.06.004](https://doi.org/10.1016/j.baae.2022.06.004).

References

- Allan, E., Manning, P., Alt, F., Binkenstein, J., Blaser, S., Blüthgen, N., Böhm, S., Grassein, F., Hölzel, N., Klaus, V. H., Kleinebecker, T., Morris, E. K., Oelmann, Y., Prati, D., Renner, S. C., Rillig, M. C., Schaefer, M., Schloter, M., Schmitt, B., & Fischer, M. (2015). Land use intensification alters ecosystem multifunctionality via loss of biodiversity and changes to functional composition. *Ecology Letters*, *18*(8), 834–843. doi:[10.1111/ele.12469](https://doi.org/10.1111/ele.12469).
- Ambarlı, D., Simons, N. K., Wehner, K., Kämper, W., Gossner, M. M., Nauss, T., Neff, F., Seibold, S., Weisser, W., & Blüthgen, N. (2021). Animal-mediated ecosystem process rates in forests and grasslands are affected by climatic conditions and land-use intensity. *Ecosystems*, *24*(2), 467–483. doi:[10.1007/s10021-020-00530-7](https://doi.org/10.1007/s10021-020-00530-7).
- Bengtsson, J., Bullock, J. M., Egoh, B., Everson, C., Everson, T., O’Connor, T., O’Farrell, P. J., Smith, H. G., & Lindborg, R. (2019). Grasslands-more important for ecosystem services than you might think. *Ecosphere*, *10*(2), e02582. doi:[10.1002/ecs2.2582](https://doi.org/10.1002/ecs2.2582).
- Biedermann, R., & Niedringhaus, R. (2004). *Die Zikaden Deutschlands—Bestimmungstabellen für alle Arten*. Wissenschaftlich Akademischer Buchvertrieb-Fründ.
- Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology*, *85*(7), 1771–1789. doi:[10.1890/03-9000](https://doi.org/10.1890/03-9000).
- Crawley, M. J. (1989). Insect herbivores and plant population dynamics. *Annual Review of Entomology*, *34*(1), 531–562. doi:[10.1146/annurev.en.34.010189.002531](https://doi.org/10.1146/annurev.en.34.010189.002531).
- Denno, R. F., & Roderick, G. K. (1990). Population biology of planthoppers. *Annual Review of Entomology*, *35*(1), 489–520. doi:[10.1146/annurev.en.35.010190.002421](https://doi.org/10.1146/annurev.en.35.010190.002421).
- Deraison, H., Badenhauer, I., Börger, L., & Gross, N. (2015). Herbivore effect traits and their impact on plant community biomass: An experimental test using grasshoppers. *Functional Ecology*, *29*(5), 650–661. doi:[10.1111/1365-2435.12362](https://doi.org/10.1111/1365-2435.12362).
- Descobes, P., Kergunteuil, A., Glauser, G., Rasmann, S., & Pellissier, L. (2020). Plant physical and chemical traits associated with herbivory *in situ* and under a warming treatment. *Journal of Ecology*, *108*(2), 733–749. doi:[10.1111/1365-2745.13286](https://doi.org/10.1111/1365-2745.13286).
- Dungan, R. J., Turnbull, M. H., & Kelly, D. (2007). The carbon costs for host trees of a phloem-feeding herbivore. *Journal of Ecology*, *95*(4), 603–613. doi:[10.1111/j.1365-2745.2007.01243.x](https://doi.org/10.1111/j.1365-2745.2007.01243.x).
- Dyer, M. I., Acra, M. A., Wang, G. M., Coleman, D. C., Freckman, D. W., McNaughton, S. J., & Strain, B. R. (1991). Source-sink carbon relations in two *Panicum coloratum* ecotypes in response to herbivory. *Ecology*, *72*(4), 1472–1483. doi:[10.2307/1941120](https://doi.org/10.2307/1941120).
- Dyer, M. I., Turner, C. L., & Seastedt, T. R. (1993). Herbivory and its consequences. *Ecological Applications*, *3*(1), 10–16. doi:[10.2307/1941781](https://doi.org/10.2307/1941781).
- Ehnes, R. B., Rall, B. C., & Brose, U. (2011). Phylogenetic grouping, curvature and metabolic scaling in terrestrial invertebrates. *Ecology Letters*, *14*(10), 993–1000. doi:[10.1111/j.1461-0248.2011.01660.x](https://doi.org/10.1111/j.1461-0248.2011.01660.x).
- Ehrlich, P. R., & Raven, P. H. (1964). Butterflies and plants: A study in coevolution. *Evolution*, *18*(4), 586. doi:[10.2307/2406212](https://doi.org/10.2307/2406212).
- Elser, J. J., Fagan, W. F., Denno, R. F., Dobberfuhl, D. R., Folarin, A., Huberty, A., Interlandi, S., Kilham, S. S., McCauley, E., Schulz, K. L., Siemann, E. H., & Sterner, R. W. (2000). Nutritional constraints in terrestrial and freshwater food webs. *Nature*, *408*(6812), 578–580. doi:[10.1038/35046058](https://doi.org/10.1038/35046058).
- Funk, J. L., Larson, J. E., Ames, G. M., Butterfield, B. J., Cavender-Bares, J., Firn, J., Laughlin, D. C., Sutton-Grier, A. E., Williams, L., & Wright, J. (2017). Revisiting the Holy Grail: Using plant functional traits to understand ecological processes. *Biological Reviews*, *92*(2), 1156–1173. doi:[10.1111/brv.12275](https://doi.org/10.1111/brv.12275).
- Habel, J. C., Dengler, J., Janišová, M., Török, P., Wellstein, C., & Wiezik, M. (2013). European grassland ecosystems: Threatened hotspots of biodiversity. *Biodiversity and Conservation*, *22*(10), 2131–2138. doi:[10.1007/s10531-013-0537-x](https://doi.org/10.1007/s10531-013-0537-x).
- Hawkins, C. D. B., Whitecross, M. I., & Aston, M. J. (1986). Long-term effects on cowpea plant growth of a short-term cowpea aphid infestation. *Canadian Journal of Botany*, *64*(8), 1727–1732. doi:[10.1139/b86-231](https://doi.org/10.1139/b86-231).
- Ibanez, S., Bison, M., Lavorel, S., & Moretti, M. (2013). Herbivore species identity mediates interspecific competition between plants. *Community Ecology*, *14*(1), 41–47. doi:[10.1556/comec.14.2013.1.5](https://doi.org/10.1556/comec.14.2013.1.5).
- Ibanez, S., Lavorel, S., Puijalon, S., & Moretti, M. (2013). Herbivory mediated by coupling between biomechanical traits of plants and grasshoppers. *Functional Ecology*, *27*(2), 479–489. doi:[10.1111/1365-2435.12058](https://doi.org/10.1111/1365-2435.12058).
- Kattge, J., Bönisch, G., Díaz, S., Lavorel, S., Prentice, I. C., Leadley, P., Tautenhahn, S., Werner, G. D. A., Aakala, T.,

- Abedi, M., Acosta, A. T. R., Adamidis, G. C., Adamson, K., Aiba, M., Albert, C. H., Alcántara, J. M., C. C. A., Aleixo, I., Ali, H., . . . , & Wirth, C. (2020). TRY plant trait database – enhanced coverage and open access. *Global Change Biology*, 26(1), 119–188. doi:10.1111/gcb.14904.
- Keeping, M. G., & Kvedaras, O. L. (2008). Silicon as a plant defence against insect herbivory: Response to Massey, Ennos and Hartley. *Journal of Animal Ecology*, 77(3), 631–633. doi:10.1111/j.1365-2656.2008.01380.x.
- Lavorel, S., & Garnier, E. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: Revisiting the Holy Grail. *Functional Ecology*, 16(5), 545–556. doi:10.1046/j.1365-2435.2002.00664.x.
- Lavorel, S., Storkey, J., Bardgett, R. D., de Bello, F., Berg, M. P., Le Roux, X., Moretti, M., Mulder, C., Pakeman, R. J., Díaz, S., & Harrington, R. (2013). A novel framework for linking functional diversity of plants with other trophic levels for the quantification of ecosystem services. *Journal of Vegetation Science*, 24(5), 942–948. doi:10.1111/jvs.12083.
- Magnusson, A., Skaug, H., Nielsen, A., Berg, C., Kristensen, K., Maechler, M., . . . Brooks, M. (2020). *Generalized linear mixed models using template model builder* (1.0.2.1). <https://CRAN.R-project.org/package=glmmTMB>.
- Meyer, G. A., & Whitlow, T. H. (1992). Effects of leaf and sap feeding insects on photosynthetic rates of goldenrod. *Oecologia*, 92(4), 480–489. doi:10.1007/BF00317839.
- Moretti, M., de Bello, F., Ibanez, S., Fontana, S., Pezzatti, G. B., Dziock, F., Rixen, C., & Lavorel, S. (2013). Linking traits between plants and invertebrate herbivores to track functional effects of land-use changes. *Journal of Vegetation Science*, 24(5), 949–962. doi:10.1111/jvs.12022.
- Neff, F., Blüthgen, N., Chisté, M. N., Simons, N. K., Steckel, J., Weisser, W. W., Westphal, C., Pellissier, L., & Gossner, M. M. (2019). Cross-scale effects of land use on the functional composition of herbivorous insect communities. *Landscape Ecology*, 34(8), 2001–2015. doi:10.1007/s10980-019-00872-1.
- Neff, F., Brändle, M., Ambarlı, D., Ammer, C., Bauhus, J., Boch, S., Hölzel, N., Klaus, V. H., Kleinebecker, T., Prati, D., Schall, P., Schäfer, D., Schulze, E. D., Seibold, S., Simons, N. K., Weisser, W. W., Pellissier, L., & Gossner, M. M. (2021). Changes in plant-herbivore network structure and robustness along land-use intensity gradients in grasslands and forests. *Science Advances*, 7(20), eabf3985. doi:10.1126/sciadv.abf3985.
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2018). *Community Ecology Package*. 2.4-6 <https://CRAN.R-project.org/package=vegan>.
- Porras, M. F., López-Londoño, T., Rost, J., Biddinger, D., Calvin, D., & Rajotte, E. G. (2020). A method for a long-term marking of spotted lanternfly (Hemiptera: Fulgoridae) using a stable isotope of nitrogen. *Environmental Entomology*, 49(4), 993–997. doi:10.1093/ee/nvaa067.
- Prestidge, R. A. (1982). The influence of nitrogenous fertilizer on the grassland Auchenorrhyncha (Homoptera). *Journal of Applied Ecology*, 19(3), 735–749. doi:10.2307/2403278.
- Quinby, B. M., Creighton, J. C., & Flaherty, E. A. (2020). Stable isotope ecology in insects: A review. *Ecological Entomology*, 45(6), 1231–1246. doi:10.1111/een.12934.
- R Core Team. (2018). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.
- Reinhold, K. (1999). Energetically costly behaviour and the evolution of resting metabolic rate in insects. *Functional Ecology*, 13(2), 217–224. doi:10.1046/j.1365-2435.1999.00300.x.
- Risch, A. C., Schütz, M., Vandegehuchte, M. L., Van Der Putten, W. H., Duyts, H., Raschein, U., Gwiazdowicz, D. J., Busse, M. D., Page-Dumroese, D. S., & Zimmermann, S. (2015). Aboveground vertebrate and invertebrate herbivore impact on net N mineralization in subalpine grasslands. *Ecology*, 3312–3322. doi:10.1890/15-0300.1.
- Schädler, M., Jung, G., Auge, H., & Brandl, R. (2003). Palatability, decomposition and insect herbivory: Patterns in a successional old-field plant community. *Oikos*, 103(1), 121–132. doi:10.1034/j.1600-0706.2003.12659.x.
- Schallhart, N., Tusch, M. J., Wallinger, C., Staudacher, K., & Traugott, M. (2012). Effects of plant identity and diversity on the dietary choice of a soil-living insect herbivore. *Ecology*, 93(12), 2650–2657. doi:10.1890/11-2067.1.
- Schleuning, M., Fründ, J., & García, D. (2015). Predicting ecosystem functions from biodiversity and mutualistic networks: An extension of trait-based concepts to plant-animal interactions. *Ecography*, 38(4), 380–392. doi:10.1111/ecog.00983.
- Schowalter, T. D. (2011). *Insect ecology: An Ecosystem Approach* (3rd ed.). Academic Press. doi:10.1016/B978-0-12-381351-0.00019-6.
- Socher, S. A., Prati, D., Boch, S., Müller, J., Klaus, V. H., Hölzel, N., & Fischer, M. (2012). Direct and productivity-mediated indirect effects of fertilization, mowing and grazing on grassland species richness. *Journal of Ecology*, 100(6), 1391–1399. doi:10.1111/j.1365-2745.2012.02020.x.
- Steffan, S. A., Daane, K. M., & Mahr, D. L. (2001). 15N-enrichment of plant tissue to mark phytophagous insects, associated parasitoids, and flower-visiting entomophaga. *Entomologia Experimentalis et Applicata*, 98(2), 173–180. doi:10.1046/j.1570-7458.2001.00772.x.
- Sulaiman, H. Y., Liu, B., Kaurilind, E., & Niinemets, Ü. (2021). Phloem-feeding insect infestation antagonizes volatile organic compound emissions and enhances heat stress recovery of photosynthesis in *Origanum vulgare*. *Environmental and Experimental Botany*, 189, 104551. doi:10.1016/j.envexpbot.2021.104551.
- Tscharntke, T., & Greiler, H. J. (1995). Insect communities, grasses, and grasslands. *Annual Review of Entomology*, 40(1), 535–558. doi:10.1146/annurev.en.40.010195.002535.
- Vicari, M., & Bazely, D. R. (1993). Do grasses fight back? The case for antiherbivore defences. *Trends in Ecology & Evolution*, 8(4), 137–141. doi:10.1016/0169-5347(93)90026-L.
- Wachmann, E., Melber, A., & Deckert, J. (2004–2012). *Wanzen Band 1–5*. Goecke & Evers.
- Walling, L. L. (2000). The myriad plant responses to herbivores. *Journal of Plant Growth Regulation*, 19(2), 195–216. doi:10.1007/s003440000026.
- Wang, X., Li, F. Y., Tang, K., Wang, Y., Suri, G., Bai, Z., & Baoyin, T. (2020). Land use alters relationships of grassland productivity with plant and arthropod diversity in Inner

- Mongolian grassland. *Ecological Applications*, 30(3), e02052. doi:[10.1002/eap.2052](https://doi.org/10.1002/eap.2052).
- Wilson, P. J., Thompson, K., & Hodgson, J. G. (1999). Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. *New Phytologist*, 143(1), 155–162. doi:[10.1046/j.1469-8137.1999.00427.x](https://doi.org/10.1046/j.1469-8137.1999.00427.x).
- Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J. H. C., Diemer, M., Flexas, J., Garnier, E., Groom, P. K., Gulias, J., Hikosaka, K., Lamont, B. B., Lee, T., Lee, W., Lusk, C., & Villar, R. (2004). The worldwide leaf economics spectrum. *Nature*, 428(6985), 821–827. doi:[10.1038/nature02403](https://doi.org/10.1038/nature02403).

Available online at www.sciencedirect.com

ScienceDirect