Plant diversity effects on herbivory are mediated by soil biodiversity and plant chemistry

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Article

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Abstract

Insect herbivory is a key process in ecosystem functioning. While theory predicts that plant diversity modulates herbivory, the mechanistic links remain unclear. We postulated that the plant metabolome mechanistically links plant diversity and herbivory.

In autumn and in spring, we assessed aboveground herbivory rates and plant metabolomes of seven plant species in experimental plant communities varying in plant species and resource acquisition strategy diversity. In the same plots, we also measured plant individual biomass as well as soil microbial and nematode community composition.

Herbivory rates decreased with increasing plant species richness. Path modelling revealed that plant species richness and community resource acquisition strategy affected soil community composition. In particular, changes in nematode community composition affected plant metabolomes and thereby herbivory rates.

These results provide experimental evidence that soil community composition plays an important role in reducing herbivory rates with increasing plant diversity by changing plant metabolomes.

Introduction

Insect herbivory is an essential ecosystem process removing up to 31% of biomass in grasslands within a single season¹. Plant diversity can influence the abundance and diversity of insect herbivores^{2–5}, including herbivory rates^{6,7}. Plant traits, in particular those associated with resource acquisition and competition, are considered to provide mechanistic links between plant diversity and herbivory⁸. However, a recent study shows that the commonly used plant traits fail to explain a substantial part of the variance in most ecosystem processes⁹.

Several factors may explain differences in herbivory across plant diversity gradients. On the one hand, higher levels of plant diversity may increase niche diversity by increasing spatial heterogeneity and the variety of food sources. Hence, more diverse plant communities may support more insect herbivores and increase herbivory rates⁶. On the other hand, increased plant diversity may lead to dilution effects which decrease herbivory rates, as it will be more difficult for specialized insect herbivores to localize their host plant^{10,11}. Lastly, the abundance of predatory and parasitoid arthropods, which can reduce herbivore populations, is higher in more diverse plant communities^{7,12–14}.

In addition to these ecological mechanisms, differences in herbivory rates across plant species richness gradients may also be explained through changes in plant chemistry. Plant metabolomes, *i.e.* the composition of all metabolites produced by an individual plant¹⁵, change in response to abiotic and biotic variation. This metabolomic response to environmental conditions co-determines the defensive status of a plant¹⁶. For instance, plants increase the synthesis of defensive secondary metabolites following an attack by herbivores^{17,18}. These induced responses can affect plant defences both locally, *i.e.* in the attacked tissue, and systemically, *i.e.* throughout the whole plant¹⁹. In addition to herbivory-induced changes, plant

diversity itself can affect plant metabolomes. Plant-plant interactions, such as resource competition, can alter the metabolome by triggering the production of allelopathic compounds²⁰. Lastly, soil legacy effects, which may result from systemically induced changes triggered by soil biota, such as microbes and nematodes^{19,21} can also affect plant metabolomic profiles²². Taken together, the plant metabolome both affects and reflects above- and belowground interactions with insect herbivores, other plants, and soil biota. Hence, we argue that measuring the plant's metabolome will provide unique mechanistic insights into the effects of plant diversity on herbivory.

Here, we analyse metabolomes of three grass species and four forb species differing in their resource acquisition strategies. The plants were grown in 34 experimental plant communities that varied in plant diversity, *i.e.* species richness and functional trait diversity²³. We test if and how plant diversity alters the secondary metabolome and how this relates to herbivory rates. Moreover, we investigate if plant diversity-associated changes in plant metabolomes link to differences in soil communities. We hypothesize that (1) the sown plant species richness as well as the resource acquisition strategy of the plant community affect the herbivory rates of individual plants. In addition, we hypothesize that (2) plant species richness and plant community resource acquisition traits affect directly or indirectly, via the soil biota, the plant metabolome and thereby herbivory.

Results

The effects of plant species richness and resource acquisition strategy

Herbivory rates decreased significantly with plant species richness (**Fig. 1a**, $F_{1,425} = 5.24$; $p = 0.023$). In addition, herbivory rates were lower in May, *i.e.* at the beginning of the vegetation period, than in August (**Fig. 1**, $F_{1,432} = 229.39$; $p < 0.001$).

We found no significant effect of the community's spatial resource acquisition strategy on herbivory rates ($F_{1,103} = 2.35$; $p = 0.128$). In other words, the relative abundance of tall-statured species with large leaves and deep roots *versus* the relative abundance of small-statured species with small leaves and shallow roots did not significantly affect the plant-individual herbivory rates (**Fig. 1b**). However, when we tested for the effect of spatial resource acquisition strategy of the plant community within functional groups, we found a significant effect on the herbivory rate in grasses ($F_{1,179} = 3.93$; $p = 0.049$). Grasses growing in communities that predominantly contained tall-statured plants with large leaves suffered lower herbivory rates (**Supplementary Fig. S1a**). In contrast, we observed no significant effect of spatial resource acquisition strategy on the herbivory rate in forbs ($F_{1,127} = 0.42$; $p = 0.519$; **Supplementary Fig. S2a**). When we tested for the effect of the temporal resource acquisition strategy of the plant communities on herbivory rates, we only found a marginally significant relationship ($F_{1,107} = 3.50$; $p = 0.064$). More specifically, plant-individual herbivory rates tended to be greater in communities containing mostly later growing and flowering species (**Fig. 1c**). Separate analyses for each functional group showed that the effect was significant for grasses

($F_{1,133} = 6.32$; $p = 0.013$; **Supplementary Fig. S1b**), but not for forbs ($F_{1,251} = 0.98$; $p = 0.324$; **Supplementary Fig. S2b**).

In addition, we tested for the effects of plant species richness on plant metabolome composition and on metabolite diversity, *i.e.* richness or Shannon diversity of secondary metabolites (see **Supplementary Material & Methods** for a detailed description). We found a significant effect of plant species richness on the metabolome composition across most plant species in both or at least in one sampling season, except for *Holcus lanatus* (**Table 1**). Consecutive pairwise comparisons revealed that the metabolome of plants grown in monocultures most frequently differed from the metabolome of plants grown in more diverse communities (**Supplementary Table S1**). However, due to limitations in the experimental design, we could not calculate the pairwise comparisons for the lowest (monoculture) and highest (8 species mixture) sown plant species richness level (see **Supplementary Material & Methods**).

We did not find an effect of plant species richness on the richness of secondary metabolites. Rather, we observed an effect of plant functional group identity on the richness of secondary metabolites ($F_{1,5} = 6.69$; $p = 0.049$; **Supplementary Table S2a**). Forb species had significantly greater numbers of secondary metabolites (396 ± 8.1 ; mean $\pm$ SE) than grass species (311 ± 8.5 ; mean $\pm$ SE). We also discovered that the effect of plant species richness on the Shannon diversity of secondary metabolites depended on the functional group identity ($F_{1,138} = 5.35$; $p = 0.022$; **Supplementary Table S2a**). Increasing sown plant species richness increased the Shannon diversity of secondary metabolites in grasses, while it reduced the Shannon diversity of secondary metabolites in forbs (**Supplementary Fig. S3**).

Moreover, we analysed the extent to which resource acquisition strategy affects metabolite diversity. We found that some traits associated with spatial resource acquisition strategy can increase or decrease the richness of metabolites dependent on sampling season and functional group identity. The community-weighted means of plant height, rooting depth, and root length density all reduced the richness of metabolites in grasses early in the season (May), while they increased the richness of metabolites in grasses in August (**Supplementary Fig. S4**). We observed a similar effect of plant height on the richness of metabolites in forbs. In contrast, rooting depth and root length density increased the richness of metabolites in forbs in May, but reduced the richness in August (**Supplementary Table S2; Supplementary Fig. S5**). In addition, we discovered that leaf area, a trait associated with spatial resource acquisition, and flowering onset, associated with temporal resource acquisition, had similar effects on the Shannon diversity of metabolites. Both traits increased Shannon diversity of metabolites in grasses in both sampling seasons (**Supplementary Fig. S6**). In forbs, both traits reduced the Shannon diversity in May, while they increased the Shannon diversity of metabolites in August (**Supplementary Table S2; Supplementary Fig. S7**).

Analysing the significant paths in our *full-model* PLS-PM (**Fig. 2b**; Goodness of Fit (GoF) = 0.13), we observed that nematode community composition was negatively related to plant species richness and resource acquisition traits, while the nematode community itself positively affected plant metabolomes. Plant species richness was negatively correlated with the relative abundance of predatory, omnivorous, and plant feeding nematodes (**Supplementary Fig. S8**). The spatial resource acquisition trait leaf area was negatively correlated with the relative abundance of bacterial feeders, fungivores, omnivores, and plant feeders, while rooting depth and root length density negatively correlated with predators, but positively correlated with plant feeders. Conversely, the temporal resource acquisition traits growth starting date and flowering start were negatively correlated with plant feeders (**Supplementary Fig. S8**). In addition, we discovered that nematode community composition positively affected plant metabolome composition, which had a negative effect on plant individual herbivory rates. We extracted the 100 most important metabolite mass spectra that characterized the metabolome, *i.e.* the metabolites with the highest weight relations, and could assign molecular formulas and structures to 13 mass spectra (**Supplementary Table S3; Supplementary Fig. S9 – S21**). The assigned compounds were mainly phenolics, their precursors or their derivatives, which are all products of the shikimic acid pathway in plants. Moreover, these compounds are known to respond to phytopathogenic nematode infection²⁴ as well as to play a role in plant-herbivore interactions^{25–27}. As part of the nematode community, the relative abundance of bacterial feeders, predators, omnivores, and plant feeders showed positive correlations with the concentration of the assigned compounds. The assigned compounds, especially sinapic acid, a flavonol, the chlorogenic acid dimers, and quinic acid, were negatively correlated with plant herbivory (**Supplementary Fig. S22**). Our most parsimonious model predicted 30% of the total variation in the secondary metabolome, and 23% of the total variation in herbivory rates.

Our *full-model* PLS-PM also revealed that plant herbivory was negatively affected by the resource acquisition traits of the plant community and positively affected by plant individual biomass (**Fig. 2b**). Plant biomass also negatively affected the plant metabolome, while itself being positively affected by the resource acquisition traits, and negatively affected by plant species richness, the nematode community, and the microbial community. Lastly, the microbial community was positively affected by the plant species richness.

Based on our observations that functional group identity can affect the plant's metabolome as well as herbivory rate, we calculated two additional PLS-PMs: a *grasses-only* model (**Fig. 2c**; GoF = 0.19) and a *forbs-only* model (**Fig. 2d**; GoF = 0.19). In comparison to the *full-model* PLS-PM, in the *grasses-only* model both the microbial community and the nematode community positively affected the plant metabolome. In contrast to the *full-model*, the resource acquisition traits did not affect the nematode community and their effect on herbivory was positive, while the effect on plant individual biomass was negative. In the *forbs-only* model, the plant metabolome was negatively affected by the nematode community, but positively affected by plant individual biomass, while itself positively affecting herbivory. Moreover, plant individual biomass was not affected by the microbial or nematode community, nor did individual plant biomass affected herbivory. Taken together, our comparisons suggest that in grasses and forbs different mechanisms link plant diversity and soil community composition with plant metabolomes and herbivory.

Discussion

Our study highlights for the first time how different facets of biodiversity in plant communities jointly form a mechanistic explanation for aboveground herbivory rates. We showed that increasing the sown plant species richness or establishing plant communities of tall-statured species with larger leaves and deeper roots reduces herbivory rates of individual plants. In contrast, herbivory rates increased in plant communities containing mostly late growing and flowering species. These results confirm our first hypothesis. Using partial least squares path modelling, we revealed that these plant diversity effects on herbivory rates are mediated by soil biota, in particular nematode community composition. These plant diversity-driven differences in soil communities affected plant metabolomes, thus supporting our second hypothesis. Compared to previous studies^{5,19,28} our study yields novel mechanistic insights by highlighting how belowground communities shape plant metabolomes as a significant driver of aboveground herbivory.

The abundance, diversity, and community structure of soil biota are commonly determined by the species identity and traits of individual plants as well as the diversity of the plant community^{28–32}. In line with these previous findings, we found that plant species richness and variation in functional traits associated with resource acquisition affected soil microbial and nematode community composition in our path model. The positive effect of plant species richness on the microbial community is likely due to an increased and more diverse influx of organic matter in the form of rhizodeposits^{31,33,34}. The observed negative effect of resource acquisition traits on most functional nematode guilds was mainly driven by the community-weighted leaf area, growth starting time, and flowering onset. This suggests that the abundance and seasonality of resource influx from the plant community into the soil determines nematode community structure³⁵. In contrast, rooting depth and root length density were positively correlated with phytophagous nematodes, suggesting that in addition to the seasonality of resource influx, phytophagous nematodes are mainly affected by root architecture³⁵. The shifts in soil community composition were related to significant changes in plant metabolomes. Specifically, the abundance of bacterial feeders, predators, and phytophagous nematodes positively correlated to the concentration of defence-related metabolites in individual plants. Bacterial feeding nematodes positively contribute to the mineralization of nitrogen in the soil, which supports plant growth and potentially the synthesis of defence-related metabolites³⁶. Predatory nematodes control plant parasitic nematodes, thus indirectly supporting plant growth as well³⁶. In contrast, phytophagous nematodes induce systemic defence response reactions in plants, which can explain the positive correlation between the nematode abundance and the concentration of defensive secondary metabolites in leaves^{19,21}. Interestingly, we identified several phenolic compounds that are produced via the shikimic acid pathway. This pathway is also leading to the production of salicylic acid³⁷, a plant defence signalling hormone that is induced systemically by root feeding nematodes²¹. Our path models showed that plant diversity mostly affected belowground community composition, which likely affected the shoot metabolome via the systemic induction of metabolites^{17,19,38–40}. The observed changes in the plant metabolome were significantly related to herbivory rate, and the composition of the plant metabolome, especially the concentration of specific secondary metabolites therein, can affect herbivore resistance¹⁶.

This underscores earlier findings that plant diversity affects soil community composition, especially for plant growth facilitators and plant antagonists, which can alter plant metabolomes and thereby herbivory rates^{17,22,41–43}.

We also discovered that the richness and Shannon diversity of secondary metabolites in grasses and forbs varied differently to changes in resource acquisition associated community-weighted traits. These contrasting responses are likely due to differences in defensive strategies. Grasses possess silica crystals providing mechanical protection from herbivory⁴⁴. High levels of silica may trade-off with carbon-based defences, such as phenolics⁴⁵, commonly found in herbs⁴⁶. Moreover, grasses and forbs differ in their associations with soil biota, such as the symbiosis with mycorrhizal fungi, which can contribute to diverging metabolomics responses to similar resource acquisition strategies^{22,47}. While the difference between grasses and forbs was not the focus of our study and we only studied a small subset of species in each functional group, our results still point towards the importance of including functional group identity to improve predictive models analysing plant-herbivore interactions and multitrophic control.

While the present experiment provides novel insights into the above-belowground controls of herbivory, it also calls for future studies. Many of the observed relationships between soil community composition, plant metabolome, and herbivory rates are correlational and may result from reciprocal effects. Manipulative experiments are needed to disentangle the individual or interactive roles of plant and soil biodiversity in driving changes in plant metabolomes and herbivory rates⁴⁸. Such studies should also be conducted in the presence and absence of aboveground herbivores to explore if aboveground herbivory modulates the plant and soil biodiversity effects on the metabolome, for example via locally induced responses⁴⁸. Preferably, these studies should consider the roles of specialist versus generalist herbivores as well as different feeding types of herbivores⁴⁹.

Taken together, the present study shows that changes in plant species richness and community resource acquisition traits alter belowground communities, thus driving changes in the shoot metabolome and herbivory rate. By including metabolomic analyses, we advanced our knowledge on the mechanisms linking plant diversity and herbivory rates via the changes in plant metabolomes⁴⁸. It thereby expands our capability to better characterize the complex nature of multitrophic interactions above and below the ground.

Methods

Experimental design

The Trait-Based Experiment²³ was established in 2010 within the 'Jena Experiment' (www.the-jena-experiment.de) field site, Thuringia, Germany; 50°55' N, 11°35' E, 130 m a.s.l.⁵⁰. We sampled 34 plots that differed in plant species richness (1, 2, 4 and 8 species) and plant functional trait dissimilarity. The

functional trait dissimilarity was based on traits that reflect spatial and temporal resource acquisition strategies. We chose plant height, leaf area, rooting depth, and root length density to reflect spatial resource acquisition. To reflect temporal resource acquisition, we chose growth starting date and flowering onset. The spatial resource acquisition strategy across plant communities ranged from small-statured species with small leaves and shallow roots to tall-statured species with large leaves and deep roots. The temporal resource acquisition strategy across plant communities ranged from early-growing and -flowering species to late-growing and -flowering species. The plots that were sampled, were arranged in three blocks, mown twice per year, and weeded three times per year. For more details on the design, see²³.

Secondary metabolome sampling and sample processing

On 24 – 25 August 2015 and 31 May – 01 June 2016, we sampled aboveground biomass of seven common central European grassland species (grasses: *Dactylis glomerata* L., *Holcus lanatus* L., *Phleum pratense* L., forbs: *Geranium pratense* L., *Leucanthemum vulgare* (Vaill.) Lam., *Plantago lanceolata* L., and *Ranunculus acris* L.). We sampled 444 plant individuals across both sampling seasons and all species, *i.e.* three individuals per species and plot. One sample was lost during sample processing. We harvested the shoot biomass by cutting the plants ca. 1 cm above the ground and removed all inflorescences. All samples were taken between 15.00 and 19.00 h each sampling day to minimize diurnal variation. All samples were processed, extracted, and analysed according to Ristok *et al.* (2019)²² with slight changes (see **Supplementary Material & Methods** for a detailed description).

LC-MS data processing and metabolite prediction

We followed the LC-MS data processing protocol described in Ristok *et al.* (2019)²² with minor changes (see **Supplementary Material & Methods** for a detailed description). We predicted metabolite structures through the comparison of LC-MS data with literature references. We submitted high-resolution mass-to-charge values to the MassBank of North America (MoNA, <http://mona.fiehnlab.ucdavis.edu/>) spectral database. We used a mass tolerance of 0.5 D for comparison. In addition, we calculated high-resolution molecular weights, molecular formulae for putative molecular ions in neutral form, and particle weights for mass spectrometry generated fragments using ChemDraw Ultra 8.0 (www.cambridgesoft.com).

Leaf herbivory rate assessment

For each plant individual, we counted the total number of leaves and the number of leaves with herbivore damage. We noted and categorized herbivore damage for each damaged leaf per plant sample. Herbivore damage included signs of sucking, chewing, and mining on leaves. The damage categories were 1-10%, 10-20%, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90%, and 90-100%. We multiplied the number of leaves in each category with the damage level of that category (0.1 for 1-10%, 0.2 for 10-20%, etc.). Next, we

took the sum across all categories. Finally, we calculated the herbivory rate for each sample by dividing the summed herbivory by the total number of leaves.

Soil sampling

In each plot, we took soil samples to a depth of 10 cm using a metal corer (diameter 2 cm) on 27 August 2015 and 06 June 2016. We pooled and homogenized five subsamples per plot to account for spatial heterogeneity. We sieved soil samples to 2mm. We stored one part at – 20 °C for phospholipid fatty acid analysis and the other part at 4 °C for nematode extraction.

Phospholipid fatty acid analysis

We determined the soil microbial community via phospholipid fatty acid (PLFA) analysis. We extracted PLFAs following the Frostegård et al. (1991)⁵¹ protocol with detailed description in Wagner et al. (2015)⁵². We analysed all samples on a gas chromatograph (see **Supplementary Material & Methods** for a detailed description). We used the following PLFA-markers, expressed as ng g⁻¹ dry weight soil, as bacterial markers: (a) gram-negative bacteria: cy17:0 and cy19:0; (b) gram-positive bacteria: i15:0, a15:0, i16:0, and i17:0; and (c) widespread in bacteria: 16:1 ω 5 and 16:1 ω 7. We used the following PLFA markers as fungal markers: (a) saprophytic fungi: 18:1 ω 9t, 18:2 ω 6t, and 18:2 ω 6c; and (b) arbuscular mycorrhizal fungi: 20:1^{52,53}. We summed up all markers within a respective group to receive a representative value.

Nematode extraction and identification

We extracted nematodes from 25 g soil fresh weight using a modified Baermann method^{52,54}. Then, we counted all nematodes at 100x magnification and identified at least 100 randomly chosen nematodes (if available) at 400x magnification using a Leica DMI 4000B light microscope. Nematodes were identified to genus or family level following Bongers (1994)⁵⁵. We classified all nematodes according to their respective trophic group, *i.e.* plant feeders, fungal feeders, bacterial feeders, predators, and omnivores. In addition, we assigned all nematodes a c-p score (colonization-persistence gradient) that ranged from 1 to 5⁵⁶. Finally, we combined the information of the trophic group and c-p score to create functional nematode guilds^{57,58}.

Statistical analysis

We analysed our data in the statistical software R v3.5⁵⁹ (<http://www.r-project.org>) using the packages 'lme4'⁶⁰, 'lmerTest'⁶¹, 'effects'⁶², and 'plspm'⁶³

To test for the effects of sown plant species richness and resource acquisition strategy (spatial and temporal), we calculated community mean scores (CMS) based on the original PCA species scores calculated when the experiment was designed^{23,64}. We based our CMS calculations on the relative species-specific cover for each plant community recorded in August 2015 and May 2016, respectively. In short, the six functional traits plant height, leaf area, rooting depth, root length density, growth starting date, and flowering onset were analysed in a standardized PCA. The first PCA axis arranged species according to their spatial resource acquisition strategy. The second PCA axis arranged species according to their temporal resource acquisition strategy²³. Plots with high community mean scores on the first PCA axis (CMS_PCA1) were mostly dominated by tall-statured species with deep sparse roots and large leaves. In contrast, plots with negative community mean scores on the first PCA axis contained a high proportion of small-statured species with dense shallow roots and small leaves. Plots with high community mean scores on the second PCA axis (CMS_PCA2) contained mostly late growing and late flowering species⁶⁴.

We tested our first hypothesis that either the sown plant species richness or any of the resource acquisition strategies affect the plant-individual herbivory rate by calculating linear mixed effects models. We fitted the herbivory rate (log-transformed) as the response variable. As predictor variables, we fitted sampling season (categorical; August 2015 or May 2016), plant functional group identity (categorical; grass or forb), and either sown plant species richness (metric; 1, 2, 4 or 8) or either CMS_PCA1 or CMS_PCA2 (metric), as well as the three-way interaction. We fitted plot nested in block and species identity as independent random effects. Model simplification was achieved by model comparison using the Akaike Information Criterion (AIC). All linear mixed effects models were based on restricted-maximum likelihood estimation and Type I analysis of variance with the Satterthwaite approximation for degrees of freedom.

To test our second hypothesis that plant species richness and plant community resource acquisition traits affect directly or indirectly, via the soil biota, the plant metabolome and thereby herbivory, we calculated partial-least squares path models (PLS-PM)⁶⁵. We hypothesized direct links from the experimental design variables sown plant species richness and resource acquisition traits to microbial and nematode community, as well as to plant individual biomass, plant metabolome, and herbivory (for a detailed description of each latent variable see **Supplementary Table S4**). In addition, we hypothesized links from the microbial and nematode community to plant biomass and the composition of the plant metabolome, as well as from plant biomass to secondary metabolome and herbivory. Finally, we hypothesized a link from plant metabolome to herbivory (**Fig. 2a**). To explore the effect of plant functional group identity, we calculated three separate PLS-PMs: (a) the *full model* using all data, *i.e.* across all seven plant species, all plots, and both sampling seasons; (b) the *grasses-only model* using only data of the three grass species; and (c) the *forbs-only model* using only data of the four forb species. All data were scaled and we used bootstrapping ($n = 200$) to calculate confidence intervals for the effect sizes within the path models. Finally, we simplified all path models, *i.e.* we reduced the number of moderators, until the most parsimonious solution was achieved⁶⁵. Across all three models, the latent variable *resource acquisition traits* was characterized by, *i.e.* moderators with the highest weight relation as part of the linear combination⁶⁵, the community-weighted

means of leaf area, rooting depth, root length density, growth starting date, and flowering start. The latent variable *soil microbial community* was characterized by gram-negative, gram-positive, and general bacteria, as well as saprophytic. The effects of the latent variable *nematode community* were mostly driven by bacterial feeders with a colonization-persistence score (c-p) of 1 and 3, predators (c-p 4 and 5), fungivores (c-p 4), omnivores (c-p 4), and plant feeders (c-p 2 and 3).

Declarations

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Authors' contributions

CRi, AW, NE, and NMvD conceived the study. CRi, CRo, FV, and AW collected the data. MC identified and computed the nematode indices. CRi and YP analysed the data. CRi, AW, NE, and NMvD interpreted the data. CRi wrote the manuscript under guidance of AW, NE, and NMvD. All authors contributed critically to the drafts and gave final approval for publication.

References

1. Meyer, S. T. *et al.* Consistent increase in herbivory along two experimental plant diversity gradients over multiple years. *Ecosphere* **8**, e01876 (2017).
2. Haddad, N. M., Tilman, D., Haarstad, J., Ritchie, M. & Knops, J. M. H. Contrasting Effects of Plant Richness and Composition on Insect Communities: A Field Experiment. *Am. Nat.* **157**, 19 (2001).
3. Hertzog, L. R., Meyer, S. T., Weisser, W. W. & Ebeling, A. Experimental Manipulation of Grassland Plant Diversity Induces Complex Shifts in Aboveground Arthropod Diversity. *PLOS ONE* **11**, e0148768 (2016).
4. Loranger, H. *et al.* Invertebrate herbivory increases along an experimental gradient of grassland plant diversity. *Oecologia* **174**, 183–193 (2014).
5. Scherber, C. *et al.* Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. *Nature* **468**, 553–556 (2010).

6. Ebeling, A. *et al.* Plant Diversity Impacts Decomposition and Herbivory via Changes in Aboveground Arthropods. *PLoS ONE* **9**, e106529 (2014).
7. Wan, N.-F. *et al.* Global synthesis of effects of plant species diversity on trophic groups and interactions. *Nat. Plants* **6**, 503–510 (2020).
8. Loranger, J. *et al.* Predicting invertebrate herbivory from plant traits: polycultures show strong nonadditive effects. *Ecology* **94**, 1499–1509 (2013).
9. van der Plas, F. *et al.* Plant traits alone are poor predictors of ecosystem properties and long-term ecosystem functioning. *Nat. Ecol. Evol.* 1–10 (2020) doi:10.1038/s41559-020-01316-9.
10. Castagneyrol, B., Jactel, H., Vacher, C., Brockerhoff, E. G. & Koricheva, J. Effects of plant phylogenetic diversity on herbivory depend on herbivore specialization. *J. Appl. Ecol.* **51**, 134–141 (2014).
11. Finch, S. & Collier, R. H. Host-plant selection by insects – a theory based on ‘appropriate/inappropriate landings’ by pest insects of cruciferous plants. *Entomol. Exp. Appl.* **96**, 91–102 (2000).
12. Schuldt, A. *et al.* Multiple plant diversity components drive consumer communities across ecosystems. *Nat. Commun.* **10**, 1460 (2019).
13. Haddad, N. M. *et al.* Plant species loss decreases arthropod diversity and shifts trophic structure. *Ecol. Lett.* **12**, 1029–1039 (2009).
14. Hines, J. *et al.* Chapter Four - Towards an Integration of Biodiversity–Ecosystem Functioning and Food Web Theory to Evaluate Relationships between Multiple Ecosystem Services. in *Advances in Ecological Research* (eds. Woodward, G. & Bohan, D. A.) vol. 53 161–199 (Academic Press, 2015).
15. Oliver, S. G., Winson, M. K., Kell, D. B. & Baganz, F. Systematic functional analysis of the yeast genome. *Trends Biotechnol.* **16**, 373–378 (1998).
16. van Dam, N. M. & van der Meijden, E. A Role for Metabolomics in Plant Ecology. in *Annual Plant Reviews Volume 43* (ed. Hall, R. D.) 87–107 (Wiley-Blackwell, 2011).
17. Bezemer, T. M. & van Dam, N. M. Linking aboveground and belowground interactions via induced plant defenses. *Trends Ecol. Evol.* **20**, 617–624 (2005).
18. Karban, R. & Baldwin, I. T. *Induced Responses to Herbivory*. (University of Chicago Press, 1997).
19. van Dam, N. M. & Heil, M. Multitrophic interactions below and above ground: en route to the next level. *J. Ecol.* **99**, 77–88 (2011).
20. Fernandez, C. *et al.* The Impact of Competition and Allelopathy on the Trade-Off between Plant Defense and Growth in Two Contrasting Tree Species. *Front. Plant Sci.* **7**, (2016).
21. Wondafraash, M., Van Dam, N. M. & Tytgat, T. O. G. Plant systemic induced responses mediate interactions between root parasitic nematodes and aboveground herbivorous insects. *Front. Plant Sci.* **4**, (2013).
22. Ristok, C. *et al.* Plant species richness elicits changes in the metabolome of grassland species via soil biotic legacy. *J. Ecol.* **107**, 2240–2254 (2019).
23. Ebeling, A. *et al.* A trait-based experimental approach to understand the mechanisms underlying biodiversity–ecosystem functioning relationships. *Basic Appl. Ecol.* **15**, 229–240 (2014).

24. Ohri, P. & Pannu, S. K. Effect of phenolic compounds on nematodes- A review. *J. Appl. Nat. Sci.* **2**, 344–350 (2010).
25. Erb, M. *et al.* Signal signature of aboveground-induced resistance upon belowground herbivory in maize. *Plant J.* **59**, 292–302 (2009).
26. Leiss, K. A., Maltese, F., Choi, Y. H., Verpoorte, R. & Klinkhamer, P. G. L. Identification of Chlorogenic Acid as a Resistance Factor for Thrips in Chrysanthemum. *Plant Physiol.* **150**, 1567–1575 (2009).
27. Treutter, D. Significance of flavonoids in plant resistance: a review. *Environ. Chem. Lett.* **4**, 147–157 (2006).
28. Wardle, D. A. *et al.* Ecological Linkages Between Aboveground and Belowground Biota. *Science* **304**, 1629–1633 (2004).
29. Bezemer, T. M. *et al.* Divergent composition but similar function of soil food webs of individual plants: plant species and community effects. *Ecology* **91**, 3027–3036 (2010).
30. Eisenhauer, N. *et al.* Plant diversity effects on soil microorganisms support the singular hypothesis. *Ecology* **91**, 485–496 (2010).
31. Lange, M. *et al.* Plant diversity increases soil microbial activity and soil carbon storage. *Nat. Commun.* **6**, 6707 (2015).
32. Strecker, T., Macé, O. G., Scheu, S. & Eisenhauer, N. Functional composition of plant communities determines the spatial and temporal stability of soil microbial properties in a long-term plant diversity experiment. *Oikos* **125**, 1743–1754 (2016).
33. Steinauer, K., Chatzinotas, A. & Eisenhauer, N. Root exudate cocktails: the link between plant diversity and soil microorganisms? *Ecol. Evol.* **6**, 7387–7396 (2016).
34. Eisenhauer, N. *et al.* Root biomass and exudates link plant diversity with soil bacterial and fungal biomass. *Sci. Rep.* **7**, 44641 (2017).
35. Yeates, G. Effects of Plants on Nematode Community Structure. *Annu. Rev. Phytopathol.* **37**, 127–149 (1999).
36. Freckman, D. W. & Caswell, E. P. The Ecology of Nematodes in Agroecosystems. *Annu. Rev. Phytopathol.* **23**, 275–296 (1985).
37. Dempsey, D. A., Vlot, A. C., Wildermuth, M. C. & Klessig, D. F. Salicylic Acid biosynthesis and metabolism. *Arab. Book* **9**, e0156 (2011).
38. Agrawal, A. A. & Weber, M. G. On the study of plant defence and herbivory using comparative approaches: how important are secondary plant compounds. *Ecol. Lett.* n/a-n/a (2015) doi:10.1111/ele.12482.
39. Ehrlich, P. R. & Raven, P. H. Butterflies and Plants: A Study in Coevolution. *Evolution* **18**, 586–608 (1964).
40. Coley, P. D. Herbivory and Defensive Characteristics of Tree Species in a Lowland Tropical Forest. *Ecol. Monogr.* **53**, 209–234 (1983).
41. Hol, W. H. G. *et al.* Reduction of rare soil microbes modifies plant-herbivore interactions. *Ecol. Lett.* **13**, 292–301 (2010).

42. Kos, M., Tuijl, M. A. B., de Roo, J., Mulder, P. P. J. & Bezemer, T. M. Species-specific plant–soil feedback effects on above-ground plant–insect interactions. *J. Ecol.* **103**, 904–914 (2015).
43. Wurst, S., Wagenaar, R., Biere, A. & Van der Putten, W. H. Microorganisms and nematodes increase levels of secondary metabolites in roots and root exudates of *Plantago lanceolata*. *Plant Soil* **329**, 117–126 (2010).
44. Massey, F. P. & Hartley, S. E. Physical defences wear you down: progressive and irreversible impacts of silica on insect herbivores. *J. Anim. Ecol.* **78**, 281–291 (2009).
45. Cooke, J. & Leishman, M. R. Tradeoffs between foliar silicon and carbon-based defences: evidence from vegetation communities of contrasting soil types. *Oikos* **121**, 2052–2060 (2012).
46. Larson, R. A. The antioxidants of higher plants. *Phytochemistry* **27**, 969–978 (1988).
47. Chialva, M. *et al.* Native soils with their microbiotas elicit a state of alert in tomato plants. *New Phytol.* (2018) doi:10.1111/nph.15014.
48. Peters, K. *et al.* Current Challenges in Plant Eco-Metabolomics. *Int. J. Mol. Sci.* **19**, (2018).
49. Mithöfer, A. & Boland, W. Recognition of Herbivory-Associated Molecular Patterns. *Plant Physiol.* **146**, 825–831 (2008).
50. Roscher, C. *et al.* The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. *Basic Appl. Ecol.* **5**, 107–121 (2004).
51. Frostegård, Å., Tunlid, A. & Bååth, E. Microbial biomass measured as total lipid phosphate in soils of different organic content. *J. Microbiol. Methods* **14**, 151–163 (1991).
52. Wagner, D., Eisenhauer, N. & Cesarz, S. Plant species richness does not attenuate responses of soil microbial and nematode communities to a flood event. *Soil Biol. Biochem.* **89**, 135–149 (2015).
53. Ruess, L. & Chamberlain, P. M. The fat that matters: Soil food web analysis using fatty acids and their carbon stable isotope signature. *Soil Biol. Biochem.* **42**, 1898–1910 (2010).
54. Ruess, L. Studies On the Nematode Fauna of an Acid Forest Soil: Spatial Distribution and Extraction. *Nematologica* **41**, 229–239 (1995).
55. Bongers, T. *De Nematoden van Nederland [The nematodes of The Netherlands]. Second edition.* (KNNV Publishing, 1994).
56. Bongers, T. & Bongers, M. Functional diversity of nematodes. *Appl. Soil Ecol.* **10**, 239–251 (1998).
57. Cesarz, S. *et al.* Nematode functional guilds, not trophic groups, reflect shifts in soil food webs and processes in response to interacting global change factors. *Pedobiologia* **58**, 23–32 (2015).
58. Ferris, H., Bongers, T. & de Goede, R. G. M. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. *Appl. Soil Ecol.* **18**, 13–29 (2001).
59. R Core Team. *R: A Language and Environment for Statistical Computing.* (R Foundation for Statistical Computing, 2017).
60. Bates, D., Maechler, M., Bolker, B. M. & Walker, S. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7. (2014).
61. Kuznetsova, A., Brockhoff, P. B. & Christensen, R. H. B. lmerTest: Tests in Linear Mixed Effects Models. (2016).

62. Fox, J. & Weisberg, S. *An R Companion to Applied Regression*. (Sage, 2019).

63. Sanchez, G., Trinchera, L. & Russolillo, G. plspm: Tools for Partial Least Squares Path Modeling (PLS-PM). (2017).

64. Fischer, F. M. *et al.* Plant species richness and functional traits affect community stability after a flood event. *Philos. Trans. R. Soc. B Biol. Sci.* **371**, 20150276 (2016).

65. Sanchez, G. PLS path modeling with R. *Berkeley Trowchez Ed.* (2013).

Table

Table 1 Overall differences, given as P-values, in the species-specific and sampling season-specific shoot metabolomes of plants grown in plant communities of four different species richness levels. Represented are multi-response permutations procedures p-values based on 10,000 permutations. Significant differences (p < 0.05) are given in bold.

Plant species	<i>Geranium pratense</i>		<i>Leucanthemum vulgare</i>		<i>Plantago lanceolata</i>		<i>Ranunculus acris</i>	
Sampling Season	Aug '15	May '16	Aug '15	May '16	Aug '15	May '16	Aug '15	May '16
p - Value	0.0001	0.0979	0.0062	0.6334	0.0184	0.1616	0.0009	0.0218
Plant species	<i>Dactylis glomerata</i>		<i>Holcus lanatus</i>		<i>Phleum pratense</i>			
Sampling Season	Aug '15	May '16	Aug '15	May '16	Aug '15	May '16		
p - Value	0.0171	0.0077	0.5824	0.5342	0.2619	0.0026		

Figures

Figure 1

The plant-individual herbivory rate (log-transformed) in response to (a) sown species richness, (b) community spatial resource acquisition strategy, and (c) community temporal resource acquisition strategy of the Trait-Based Experiment. For clarity, the placement of the symbols corresponding to the sampling season in panel 'a' have been slightly shifted along the x-axis. The relationship in August is displayed in circles and red colour. The relationship in May is displayed in triangles and black colour. Regression line estimates are based on linear mixed effect models with plot nested in block, and species identity as independent random terms. The shaded band displays the standard error.

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Figure 2

Hypothesis-based conceptual partial-least-squares path model (A) as well as path model including data across both sampling campaigns and all plant species (B), only across all grasses (C), and only across all herbs (D). Species richness represents the plot-level sown plant species richness. Resource acquisition traits represent the community-weighted mean traits maximum plant height, leaf area, rooting depth, root length

density, growth starting date, and flowering start. Microbial community represents PLFA-based estimates on plot-level gram-negative, gram-positive, and undefined bacteria, as well as arbuscular mycorrhizal fungi and all other fungi abundance. Nematode community represents plot-level summed relative abundance of functional nematode guilds, i.e. bacterial-feeding, carnivorous, fungal-feeding, omnivorous, and plant-feeding. Biomass represents plant-individual aboveground dry biomass. Metabolome represents plant-individual secondary metabolite composition. Herbivory represents plant-individual herbivory rate expressed as the proportion of damaged leaves to the total number of leaves. All data is scaled. Black arrows display significantly positive relationships. Red arrows display significantly negative relationships. Number on arrows are path coefficients.

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