Plant species richness elicits changes in the metabolome of grassland species via soil biotic legacy

Christian Ristok1,2 | Yvonne Poeschl1,3 | Jan-Hendrik Dudenhöffer4
Anne Ebeling5 | Nico Eisenhauer1,6 | Fredd Vergara1,2
Nicole M. van Dam1,2 | Alexander Weinhold1,2

1 German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany; 2 Institute of Biodiversity, Friedrich Schiller University Jena, Jena, Germany; 3 Institute of Computer Science, Martin Luther University Halle-Wittenberg, Halle, Germany; 4 Natural Resources Institute, University of Greenwich, Chatham Maritime, UK; 5 Institute of Ecology and Evolution, Friedrich Schiller University Jena, Jena, Germany; 6 Leipzig University, Leipzig, Germany and 7 Department for Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland

Abstract
1. Species-rich plant communities can induce unique soil biotic legacy effects through changing the abundance and composition of soil biota. These soil legacy effects can cause feedbacks to influence plant performance. In addition, soil biota can induce (defensive) secondary metabolites in shoots and roots and thus affect plant–herbivore interactions. We hypothesize that plant diversity-driven soil biotic legacy effects elicit changes in the shoot and root metabolome.

2. We tested this hypothesis by establishing an experiment with four plant species. We grew plants in a sterile substrate inoculated with soil conditioned by different plant species communities: (a) monocultures of either of the four species, (b) the four species in a mixture, (c) an eight species mixture including all four species or (d) a sterile inoculum. After at least 8 weeks in the field, we estimated shoot herbivory. At the same time, we took root and shoot samples for metabolomics analyses by liquid chromatography quadrupole time-of-flight mass spectrometry.

3. We found that shoot and root metabolomes of all plants grown in sterile soil differed significantly from those grown in living soil. The plant metabolomes in living soils differed by species and tissue. Across all species, shoots displayed a greater richness of secondary metabolites than roots. The richness of secondary metabolites differed by species and among living soils. The conditioning species richness significantly affected the Shannon diversity of secondary metabolites in Centaurea jacea. Shoot herbivory positively correlated with the richness and Shannon diversity of secondary metabolites in Leucanthemum vulgare. We detected multiple metabolites that together explained up to 88% of the variation in herbivory in the shoots of C. jacea and Plantago lanceolata.
4. **Synthesis.** Our findings suggest that plant diversity-driven shifts in soil biota elicit changes in the composition and diversity of shoot and root secondary metabolites. However, these plant responses and their effect on shoot herbivores are species-specific. Tracking changes in plant secondary chemistry in response to soil biotic legacy effects will help to understand the mechanisms that govern species-specific plant-plant and plant-herbivore interactions.

**KEYWORDS**
above-ground-below-ground interactions, biodiversity-ecosystem function, chemical diversity, eco-metabolomics, herbivory, Jena Experiment, metabolite profile

## 1 | INTRODUCTION

Each plant species harbours a unique rhizosphere community (Bezemer et al., 2010). In plant communities, each plant species thus contributes to the establishment of soil communities (Wardle et al., 2004). Relationships between plant community diversity and soil biota diversity have been reported to vary from neutral to positive over time in plant diversity experiments (Eisenhauer et al., 2010; Lange et al., 2015; Strecker, Macé, Scheu, & Eisenhauer, 2016). Such plant-induced changes in the soil community can remain operational over time and thus result in soil biotic legacy effects (Kardol, Cornips, van Kempen, Bakx-Schotman, & van der Putten, 2007).

Soil legacy effects can be either positive or negative, depending on whether the conditioned soil biota increase or reduce the performance of individual plants (Ehrenfeld, Ravit, & Elgersma, 2005; Kulmatiski, Beard, Stevens, & Cobbold, 2008; van der Putten et al., 2013). Negative intraspecific legacy effects can result from specialized soil pathogens. Soil pathogen accumulation because of high and species-specific root exudation (Steinauer, Chatzinotas, & Eisenhauer, 2016; van de Voorde, van der Putten, & Bezemer, 2011). At the same time, the same root exudates may suppress root pathogens of neighbouring heterospecific plants, thus providing positive interspecific legacy effects (van de Voorde et al., 2011). Hence, chemical traits of individual plants can shape soil legacy effects through changing soil biota community composition and activity.

These altered soil communities, in turn, can also influence chemical traits of individual plants. For instance, the composition and concentration of defensive plant secondary metabolites, such as glucosinolates, iridoid glycosides or pyrrolizidine alkaloids, can change in response to soil microbial composition, nematodes and mycorrhizal fungi (Bezemer & van Dam, 2005; Hol et al., 2010; Kos, Tuijl, de Roo, Mulder, & Bezemer, 2015b; Wurst, Wagenaar, Biere, & Van der Putten, 2010). These interactions with plant growth facilitators and plant antagonists are thus likely to influence the diversity of secondary metabolites that a plant produces. This additionally implies that soil legacy effects may also affect the entire plant metabolome, that is the entirety of all metabolites synthesized by an organism (Oliver, Winson, Kell, & Baganz, 1998).

Changes in the composition and concentration of plant metabolites are known to affect important ecological functions, such as the resistance to above-ground herbivory. Herbivory can induce metabolite synthesis locally (induced defences) in the attacked plant tissue or systemically throughout the plant (Bezemer & van Dam, 2005). Systemic induction can also elicit changes at the concentration of shoot metabolites as a consequence of interactions with soil biota. This systemic induction can then affect the resistance to above-ground herbivores (van Dam & Heil, 2011). Such a response in above-ground herbivores thus constitutes indirect soil legacy effects. Indirect soil legacy effects can result in increased or reduced performance of specific plant species (Karban, Agrawal, Thaler, & Adler, 1999). This may ultimately affect the fitness of a species and their abundance in the plant community.

Here we analyse soil legacy effects as reflected in shifts in the individual plant metabolome at the end of a plant-soil feedback experiment (Dudenhöffer, Ebeling, Klein, & Wagg, 2018). We defined soil legacy effects on the metabolome as shifts in the composition or the diversity of secondary metabolites. Furthermore, we defined the strength of the legacy effects as the magnitude of the difference in the metabolite profile among different living soils. The plant-soil feedback experiment was designed to test how soils conditioned by different plant communities affect key plant life stages. In the context of the experiment, soil conditioning affected flower production and plant fitness with mostly neutral effects on plant biomass (Dudenhöffer et al., 2018).

Based on the previously reported effects, we hypothesized that (a) plants grown in living soil differ in their metabolome compared to plants grown in sterile soil, and that (b) plants grown in soil conditioned by plant communities that differ in their species richness display different shoot and root metabolomes. In addition, we hypothesized that (c) the individual diversity of secondary metabolites increases with an increasing species richness of the soil conditioning plant community, and that (d) the diversity of secondary metabolites correlates with shoot herbivory. In order to test these hypotheses, we grew four common grassland plant species in three living soils that differed in the diversity of the conditioning plant community. In addition, we added a control group grown in sterile soil.
2 | MATERIALS AND METHODS

2.1 | Experimental design

In summer 2014, we set up a soil legacy experiment with four common central European grassland herb species (Centaurea jacea L., Knautia arvensis (L.) Coult., Leucanthemum vulgare Lam., and Plantago lanceolata L.). We used 3 L pots filled with 2,700 g autoclaved (120°C for 20 min) sand-field soil mixture (50:50, v/v) taken from the ‘Jena Experiment’ (www.the-jena-experiment.de) field site (Thuringia, Germany; 50°55′N, 11°35′E, 130 m a.s.l.) for our study. In order to remove roots and coarse stones, we sieved the field soil through a 5 cm mesh. We added 100 g living or sterile soil inoculum to each pot and thoroughly mixed the inoculum with the sterile sand-field soil substrate. Finally, we added 200 g of the sterile background substrate on top, thus minimizing cross contamination (Dudenhöffer et al., 2018). For each pot, the inoculum comprised only 3.33% (w/w) of the entire substrate, with 96.66% (w/w) being the same sterile standard background substrate. This setup minimized the abiotic effect of any particular soil inoculum (Brinkman, Van der Putten, Bakker, & Verhoeven, 2010). However, this also meant that the autoclaved sterile background may contain pulsed nutrients and toxins (Alphe & Scheu, 1993; Trevors, 1996).

We established three living soil and one sterile soil treatments for each plant species. The living soil inocula had been conditioned by different plant species compositions, that is, monocultures of either of the four species (CR1 – conditioning species richness 1), the four species in mixture (CR4) or an eight species mixture (CR8) including all four plant species supplemented by the four common grass species Festuca rubra L., Helicotrichon pubescens (Huds.) Domort., Phleum pratense L. and Poa pratensis L. All plant communities had been sown in plots of 3.5 m × 3.5 m in summer 2010 and are part of the ‘Trait Based Experiment’ (for more details see Ebeling et al., 2014). More specifically, soil collection for this study was conducted 4 years after establishment of the plots. We collected multiple soil cores from the upper 10 cm of each plot along a transect throughout the length of the plot to account for within-plot variability. We sieved each living soil inoculum through a 1 cm mesh and subsequently stored all soil inocula at 4°C for 24 hr prior to the experimental setup. The sterile soil inoculum was a mixture of equal parts of all living soil inocula sterilized by autoclaving at 120°C for 20 min. This created a common baseline for the sterile soil inoculum treatment.

The full experimental design resulted in 128 pots (Table S1). Each combination of plant species and corresponding soil inoculum treatment was replicated eight times, arranged in eight blocks (Dudenhöffer et al., 2018). The plants of six pots died during the experiment and were thus not available for further analyses; mortality was not related to any experimental treatment (Table S1). Initially, 20 (non-sterilized) seeds per pot were sown (Rieger-Hofmann GmbH, Blaufelden-Rombachhausen, Germany) at a density of 1 cm and subsequently covered with clear plastic cellophane in order to keep humidity in the pots high to encourage germination and seedling establishment. We transferred all pots to two climate chambers (four blocks per chamber) equipped with artificial light (four Osram Powerstar HQI-T 1000/D, E40, 1,000 W, 80,000 lm lamps per chamber) with a photoperiod of 16 hr in light at 20°C and 8 hr in darkness at 16°C. We removed the plastic cellophane once seedlings had established. In mid-November 2014, after 11 weeks, we reduced the number of plants per pot to three individuals by cutting the other plants just below the shoot meristems. The corresponding roots remained in the soil to decompose. Decomposing roots can elicit negative as well as positive effects on plant biomass production (Zhang, Van der Putten, & Veen, 2016). In the context of this experiment, however, we detected mostly neutral effects on plant biomass (Dudenhöffer et al., 2018). We then transferred all pots to an unheated glasshouse located at the Botanical Garden in Jena, Germany. There, the plants were confronted with a natural winter photoperiod and 8°C. In early May 2015, we moved all pots to an open area at the field site of the Jena Experiment maintaining the original eight blocks (for more details see Dudenhöffer et al., 2018). During the field phase of the experiment, the lower half of each pot was covered in a bag that was closed to the bottom. This protected the pot against invasion of external soil biota and below-ground herbivores. At the same time, it allowed only the natural occurring above-ground herbivores and pollinators to interact with all plants. At the end of the experiment, we validated the sterile soil inoculum treatment by assessing the presence of mycorrhizal structures in roots (Dudenhöffer et al., 2018). Only three samples of the sterile treatment displayed the presence of mycorrhizal structures in roots. These samples, however, did not affect any of our consecutive analyses/results and were thus included.

2.2 | Sampling and sample processing

We harvested the shoot and root biomass of one plant per pot at the end of the flowering period, which occurred in July 2015 for K. arvensis, L. vulgare and P. lanceolata, and in September 2015 for C. jacea. We separated the shoot and root biomass by cutting the plants with scissors and removed all flowers from the shoot samples. We counted the total number of leaves and the number of leaves with herbivore damage. Herbivore damage included signs of sucking, chewing and mining on leaves. We washed the roots twice in tap water to remove soil particles, and then dried the samples with paper towels. This process took roughly 30 s, and samples were then immediately stored in paper bags on dry ice to stop further metabolism. In the laboratory, samples were stored in a −80°C freezer, and subsequently freeze-dried (LABCONCO FreeZone Plus 12 Liter, Kansas City, USA) for 72 hr. Dried samples were stored in zip-lock bags filled with silica gel at room temperature until further processing. We measured the dry weight in milligram and ground each sample to a fine homogenous powder using a ball mill (Retsch mixer mill MM 400; Haan, Germany).

2.3 | Metabolome extraction and analysis

We extracted 20 mg dried ground plant tissue of each sample in 1 ml of extraction buffer (methanol/50 mM acetate buffer, pH 4.8; 50/50 [v/v]). The samples were homogenized for 5 min at 30 Hz using a ball mill (Retsch mixer mill MM 400), and subsequently centrifuged (25,155 g, 10 min, 4°C). The supernatant was collected in a 2 ml Eppendorf tube. We repeated the extraction procedure with the remaining pellet and combined the supernatant with the first one. We
centrifuged (25,155 g, 5 min, 4°C) all extracts, transferred 200 µl to an HPLC vial and added 800 µl extraction buffer, resulting in a 1:5 dilution.

We performed chromatographic separation of all diluted extracts by injecting 2 µl on a Thermo Scientific Dionex UltiMate 3000 (Thermo Scientific Dionex, Sunnyvale, CA, USA) UPLC unit, equipped with a C18 column (Acclaim RSLC 120 C18, 2.2 µm, 120 Å, 2.1 × 150 mm, Thermo Fisher Scientific). We applied the following binary elution gradient at a flow rate of 0.4 ml/min and a column temperature of 40°C: 0–2 min, 95% A (water and 0.05% formic acid), 5% B (acetonitrile and 0.05% formic acid); 2–12 min, 5%–50% B; 12–13 min, 50%–95% B; 13–15 min, 95% B; 15–16 min, 95%–5% B; 16–20 min, 5% B.

Metabolites were analysed on a liquid chromatography quadrupole time-of-flight mass spectrometer (LC-qToF-MS; Bruker maXis impact HD; Bruker Daltonik, Bremen, Germany) with an electrospray ionization source operated in negative mode. Instrument settings were as follows: capillary voltage, 2,500 V; nebulizer, 2.0 bar; dry gas temperature, 220°C; dry gas flow, 10 L/min; scan range, 50–1,000 m/z; acquisition rate, 1 Hz. We used sodium formate clusters (10 mM solution of NaOH in 50/50% [v/v] isopropanol/water containing 0.2% formic acid) to perform mass calibration.

2.4 LC-MS data processing

We converted the LC-qToF-MS raw data to the mzXML format by using the CompassXport utility of the DataAnalysis vendor software. Subsequently, we trimmed each data file by excluding the same non-informative regions at the beginning and end of each run using the msconvert function of ProteoWizard v3.0.10095 (Chambers et al., 2012). We performed peak picking, feature alignment and feature group collapse in R v3.3.3 (R Core Team, 2017) using the Bioconductor (Huber et al., 2015) packages ‘xcms’ (Benton, Want, & Ebbels, 2010; Smith, Want, O’Maille, Abagyan, & Siuzdak, 2006; Tautenhahn, Böttcher, & Neumann, 2008) and ‘CAMERA’ (Kuhl, Tautenhahn, Böttcher, Larson, & Neumann, 2012). We performed simulation experiments to analyse the best set of parameters prior to data processing (Table S2). These parameters included, among others, the signal-to-noise ratio which determines the proportion of low-intensity metabolites. Based on our tests, we chose a low signal-to-noise ratio, that is the inclusion of low-intensity metabolites. We used the following ‘xcms’ parameters: peak picking method ‘centWave’ (snthr = 10; ppm = 10; peakwidth = 4, 10); peak grouping method ‘density’ (minfrac = 0.7; bw = 3; mzwid = 0.005); retention time correction method ‘symmetric’. We used ‘CAMERA’ to annotate adducts, fragments and isotope peaks with the following parameters: extended rule set (https://github.com/users/ stanstrup/groups); perfwhm = 0.6; calclso = TRUE; calcCaS = TRUE; graphMethod = lpc. Lastly, we collapsed each annotated feature group, hereafter referred to as ‘metabolite’ which is described by mass-to-charge ratio (m/z) and retention time (rt), using a maximum heuristic approach. This means in detail that the intensity values of the feature, which most often displayed the highest intensity across all samples represent the feature group. We performed pre-processing with ‘xcms’ and ‘CAMERA’ separately for each species and tissue type. We merged the four species-specific feature lists by m/z and rt values, allowing for a retention time window of 10 s and a mass deviation of 5 ppm. We tentatively identified metabolites through the comparison of LC-MS/MS data with literature references. We submitted high-resolution m/z values to the MassBank of North America (MoNA, http://mona.fiehnlab.ucdavis.edu/) spectral database for comparison using a mass tolerance of 0.5 D. In addition, we calculated low-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).

2.5 Statistical analysis

We analysed our data in the statistical software R v3.3.3 using the packages ‘mixOmics’ (Cao et al., 2017), ‘vegan’ (Oksanen et al., 2017), ‘lim4’ (Bates, Mächler, Bolker, & Walker, 2015), ‘ImeRTest’ (Kuznetsova, Brockhoff, & Christensen, 2016), ‘car’ (Fox & Weisberg, 2011) and ‘multcomp’ (Hothorn, Bretz, & Westfall, 2008).

In order to test our hypotheses and to accommodate the experimental design, we analysed the data only within species and tissue. We visualized the differences in metabolome composition between sterile soil and living soil and among the different living soil inoculums by performing Partial Least Squares - Discriminant Analyses (PLS-DAs). Differences in metabolome composition are not only based on the presence, absence, or identity of metabolites. The intensity of the corresponding signals in the mass spectrometer (which is proportional to the concentration of a particular metabolite) also contributes to the metabolome composition. Therefore, we ran pairwise multi-response permutation procedures (MRPP) on log + 1-transformed metabolite intensity data to test for significant differences in the metabolite profile between our different treatments. The MRPP dissimilarity matrix was Bray–Curtis and each analysis was permuted 10,000 times.

We calculated two metrics of metabolite diversity: (a) the number of metabolites within a plant individual (hereafter, richness of secondary metabolites) and (b) the abundance-weighed diversity of metabolites expressed as the Shannon–Weaver index (Hill, 1973) based on plant individual-level metabolite intensities (hereafter, Shannon diversity of secondary metabolites). We used Dunnett’s test for single step comparison to compare the richness and Shannon diversity of secondary metabolites expressed by plants in either living soil against the expression in sterile soil. This analysis is similar to contrasts but corrects for the multiple comparison problem. In order to test if the richness of secondary metabolites increases with increasing conditioning species richness, we calculated a linear mixed effects model. We calculated a similar linear mixed effects model to test if the Shannon diversity of secondary metabolites increases with increasing conditioning species richness. In addition, we analysed if the richness and Shannon diversity of secondary metabolites correlates with above-ground herbivory...
using linear mixed effects models. The linear mixed effects models were based on restricted maximum likelihood estimation and Type I analyses of variance (ANOVA) with Satterthwaite approximation for degrees of freedom. In the first two cases, the richness or Shannon diversity of secondary metabolites was the dependent variable. As explanatory variables, we fitted tissue, the conditioning species richness (CR) of the living soil inocula and the interaction of both. In order to account for the spatial arrangement and non-random design, we applied ‘block’ as the random effect. In the last case, the dependent variable was shoot herbivory (expressed as relative number of damaged leaves in percent). The explanatory variables were either the richness or the Shannon diversity of secondary metabolites, and random effects were the CR of the living soil inocula (random slope) and ‘block’ (random intercept).

In addition, we applied Least Absolute Shrinkage and Selection Operator (LASSO) regression (Bujak, Daghir-Wojtkowiak, Kaliszan, & Markuszewski, 2016; LeWitt, Li, Lu, Guo, & Auinger, 2017; Tibshirani, 1996), to identify the combination of metabolites that could best predict shoot herbivory patterns along the CR gradient. The LASSO algorithm assumes that the herbivory responses can be ‘predicted’ by a linear combination of metabolite intensities. LASSO estimates the coefficients of this linear combination by shrinking coefficients of predictors (here metabolites) using an $l_1$ penalty in order to minimize the mean squared error in the herbivory. Some coefficients are penalized to zero and non-zero coefficients of predictors (metabolites) indicate that these are important ‘features’ for predicting herbivory with the least error. We used the ‘cv.glmnet’ function, including a leave one out cross validation, provided by the ‘glmnet’ (Friedman, Hastie, & Tibshirani, 2010) package to determine the sets of metabolites for each species and tissue type that could explain the herbivory pattern. The cross-validation process returns the most parsimonious model that has a cross-validated error within one standard deviation of the minimum.

3 | RESULTS

3.1 | Soil biota effects on the composition and diversity of plant metabolomes

We compared the metabolomes of plants grown either in sterile or living soil, and observed significant differences across species, above- and below-ground (Figure 1). The metabolomes of plants grown in living soil were more similar to each other than to the metabolomes of plants grown in sterile soil. Based on the results of Dunnett’s test, we found significant differences in the richness and Shannon diversity of secondary metabolites (Table S3) between plants growing in living and sterile soil. The richness of secondary metabolites in the roots of L. vulgare and P. lanceolata was consistently lower in sterile soil compared to living soil plants (Figure 2). When we compared sterile soil plants to plants grown in CR8 soil, we found a lower richness of secondary metabolites in the roots of K. arvensis and in the shoot of C. jacea, but a higher richness of secondary metabolites in the shoot of L. vulgare. The Shannon diversity of secondary metabolites was significantly lower in sterile soil

![FIGURE 1](https://example.com/fig1.png) Per species Partial Least Squares - Discriminant Analysis plots of the metabolites found in shoot (a–d) and root metabolomes (e–h). Plants grew either in sterile soil (black, open circles, n = 6–8) or living soil (blue, closed circles, n = 22–24). Ellipses represent the 95% confidence interval. $p$-values are based on pairwise multi-response permutation procedures. The metabolite intensity matrix was log + 1 transformed for the purpose of data normalization. Abbreviations: expl. var = explained variance [Colour figure can be viewed at wileyonlinelibrary.com]
compared to living soil in L. vulgare and P. lanceolata (with the exception of P. lanceolata plants grown in CR1 soil; Figure 2). Shoot herbivory did not significantly differ between plants grown in sterile soil and plants grown in living soil in either plant species (Table S3).

3.2 | Soil legacy effects on the composition of plant metabolomes

The three living soil inocula differed in their effect on shoot and root metabolomes across all plant species (Figure 3). Consecutive pairwise comparisons revealed that the differences in shoot metabolomes were more prevalent than in root metabolomes. The shoot metabolomes differed significantly between CR4 soil and CR8 soil across all species (Table 1). In P. lanceolata, the shoot metabolomes also differed significantly between all three living soil inocula. Root metabolomes differed between CR1 soil and CR4 soil in samples of C. jacea and L. vulgare (Table 1). In addition, root metabolomes differed between CR4 soil and CR8 soil in samples of C. jacea, L. vulgare and P. lanceolata. The root metabolome of K. arvensis was unaffected by the CR of the soil inoculum (Figure 3f).

3.3 | Soil legacy effects on the diversity of plant metabolomes

We found significant differences in the richness of secondary metabolites between shoots and roots across all species, with a higher richness of secondary metabolites in shoots than in roots (Table 2). In addition, we found significant differences in the Shannon diversity of secondary metabolites between shoot and roots in K. arvensis, P. lanceolata and marginally significant differences in C. jacea (Table 2). In contrast, the Shannon diversity of secondary metabolites between shoot and roots in L. vulgare did not differ (Table 2).

The richness of secondary metabolites significantly increased with increasing CR in K. arvensis and P. lanceolata. In contrast, the richness of secondary metabolites significantly decreased with increasing CR in L. vulgare (Figure 2; Table 2). We observed a tissue-specific response of the richness of secondary metabolites to increasing CR in C. jacea (Table 2). An increase in CR increased the richness of secondary metabolites in C. jacea shoots, but reduced the richness in C. jacea roots (Figure 2).

The Shannon diversity of secondary metabolites significantly responded to an increase in CR in C. jacea, only (Table 2). However, we observed a tissue-specific response with an increase in the Shannon diversity of secondary metabolites with increasing CR in C. jacea shoots, but a decrease in C. jacea roots (Figure 2).

3.4 | Linking richness, Shannon diversity and identity of secondary metabolites to herbivory

We analysed if the amount of above-ground herbivory relates to the richness of shoot secondary metabolites and their Shannon diversity. We found significant positive relationships between above-ground herbivory and the richness ($F_{1,18} = 7.578, p = 0.013$) and Shannon diversity ($F_{1,18} = 9.926, p = 0.005$) of shoot secondary metabolites. The richness of shoot secondary metabolites significantly increased with increasing CR in K. arvensis and P. lanceolata. In contrast, the richness of shoot secondary metabolites significantly decreased with increasing CR in L. vulgare (Figure 2; Table 2). We observed a tissue-specific response of the richness of shoot secondary metabolites to increasing CR in C. jacea (Table 2). An increase in CR increased the richness of shoot secondary metabolites in C. jacea shoots, but reduced the richness in C. jacea roots (Figure 2).

The Shannon diversity of shoot secondary metabolites significantly increased with increasing CR in K. arvensis and P. lanceolata. In contrast, the Shannon diversity of shoot secondary metabolites significantly decreased with increasing CR in L. vulgare (Figure 2; Table 2). We observed a tissue-specific response of the Shannon diversity of shoot secondary metabolites to increasing CR in C. jacea (Table 2). An increase in CR increased the Shannon diversity of shoot secondary metabolites in C. jacea shoots, but reduced the Shannon diversity in C. jacea roots (Figure 2).

**FIGURE 2** Richness (a–d) and Shannon diversity of secondary metabolites (e–h) per species in response to soil inocula that differ in the number of soil conditioning plant species richness. Shoot tissue-specific responses are displayed in green. Root tissue-specific responses are displayed in brown. Responses in sterile soil are displayed in grey but were excluded in the analyses. The shaded area surrounding the regression lines represent the 95% confidence band. The statistical significance of the responses is based on linear mixed effect models. Abbreviations: T, Tissue; C, Conditioning species richness; T × C, The interaction of tissue and conditioning species richness; n.s., not significant. The asterisks denote significance levels: ***< $p < 0.001$, **$p < 0.01$, *$p < 0.05$ [Colour figure can be viewed at wileyonlinelibrary.com]
diversity of secondary metabolites ($F_{1,19} = 17.390, p < 0.001$) in shoots of *L. vulgare* only (Figure 4; Table 3).

In addition, we related shoot herbivory to a combination of specific shoot secondary metabolites and found links between the identities of secondary metabolites and shoot herbivory. By using LASSO, we detected a combination of 15 metabolites in *C. jacea* shoot samples, which explained 88.8% of the total variation in shoot herbivory. Furthermore, we detected a combination of nine metabolites in *P. lanceolata* shoot samples that explained 86.1% of the total variation in shoot herbivory. In contrast, the LASSO regression found no congruent combination of metabolites in samples of *K. arvensis* and *L. vulgare* (Table 4). Because the full dataset contained on average 33.2% low-intensity metabolites, we repeated our analysis with a reduced dataset that only contained metabolites above median intensity. We did this as a sensitivity analysis of the results achieved for the full dataset. In the reduced
**TABLE 2** Statistical parameters resulting from a Type 1 ANOVA on species-specific richness and Shannon diversity of secondary metabolites variables. The four plant species grew in soils conditioned by plant communities differing in species richness. We sampled both plant tissues, the shoot and root. Significant differences ($p < 0.05$) are given in bold.

| Plant species  | Richness of secondary metabolite | Shannon diversity of secondary metabolites |
|----------------|----------------------------------|--------------------------------------------|
|                | NumDF | DenDF | SS      | MS      | $F$  | $p$  | NumDF | DenDF | SS      | MS      | $F$  | $p$  |
| **Centaurea jacea** |       |       |         |         |     |      |       |       |         |         |     |      |
| Tissue         | 1     | 37    | 46,314  | 46,314  | 27.81 | $<0.001$ | 1     | 37    | 0.216  | 0.216  | 4.00 | 0.053 |
| CR             | 1     | 37    | 4,570   | 4,570   | 2.74  | 0.106  | 1     | 37    | 0.271  | 0.271  | 5.02 | 0.031 |
| Tissue × CR    | 1     | 37    | 10,555  | 10,555  | 6.34  | 0.016  | 1     | 37    | 0.396  | 0.396  | 7.33 | 0.010 |
| **Knautia arvensis** |       |       |         |         |     |      |       |       |         |         |     |      |
| Tissue         | 1     | 40    | 346,569 | 346,569 | 379.78 | $<0.001$ | 1     | 40    | 0.130  | 0.130  | 7.06 | 0.011 |
| CR             | 1     | 40    | 4,569   | 4,569   | 5.01  | 0.031  | 1     | 40    | 0.001  | 0.001  | 0.04 | 0.833 |
| Tissue × CR    | 1     | 40    | 2,748   | 2,748   | 3.01  | 0.090  | 1     | 40    | 0.069  | 0.069  | 3.76 | 0.060 |
| **Leucanthemum vulgare** |       |       |         |         |     |      |       |       |         |         |     |      |
| Tissue         | 1     | 42    | 603,301 | 603,301 | 785.78 | $<0.001$ | 1     | 35    | 0.025  | 0.025  | 1.23 | 0.274 |
| CR             | 1     | 42    | 3,376   | 3,376   | 4.40  | 0.042  | 1     | 35    | 0.047  | 0.047  | 2.32 | 0.137 |
| Tissue × CR    | 1     | 42    | 220     | 220     | 0.29  | 0.595  | 1     | 35    | 0.003  | 0.003  | 0.14 | 0.706 |
| **Plantago lanceolata** |       |       |         |         |     |      |       |       |         |         |     |      |
| Tissue         | 1     | 35    | 817,956 | 817,956 | 1,317.62 | $<0.001$ | 1     | 35    | 0.657  | 0.657  | 25.11 | $<0.001$ |
| CR             | 1     | 35    | 3,308   | 3,308   | 5.33  | 0.027  | 1     | 35    | 0.034  | 0.034  | 1.29 | 0.265 |
| Tissue × CR    | 1     | 35    | 757     | 757     | 1.22  | 0.277  | 1     | 35    | 0.022  | 0.022  | 0.84 | 0.367 |

Abbreviations: CR, conditioning species richness; NumDF, numerator degrees of freedom; DenDF, denominator degrees of freedom; SS, sum of squares; MS, mean squares; F, F-value; p, p-value.
dataset, we detected a combination of 11 metabolites in *C. jacea* shoot samples that explained 69.2% of the total variation in shoot herbivory. From the full dataset to the reduced dataset, LASSO retained six metabolites that could best predict herbivory. On the contrary, the set of selected metabolites from the reduced dataset contained five additional metabolites that were not included in the selected metabolite combination from the full dataset. In *P. lanceolata* shoot samples, we detected a combination of seven metabolites in the reduced dataset. These seven metabolites explained 64.2% of the total variation in shoot herbivory (Table 4). All seven metabolites that were part of the linear combination in the reduced dataset were part of the linear combination in the full dataset too.

We identified 10 putative metabolites in *C. jacea* samples and five putative metabolites in *P. lanceolata* samples (Table 5; Figure S1). In addition, we tentatively identified seven metabolites in *C. jacea* and *P. lanceolata* samples that were not part of any linear combination (Table S4; Figure S1).

**4 | DISCUSSION**

We demonstrated that both the root and shoot metabolome of four grassland species reacted to the presence of soil biota. Soils with a different legacy of plant species richness elicited shifts in the metabolite profiles. In addition, we detected combinations of metabolites that best explained the variation in shoot herbivory. Hence, our results point to soil legacy effects as a possible mechanism linking plant communities and above-ground herbivores through changes in secondary metabolites.

**4.1 | Soil biota effects on the composition and diversity of plant metabolomes**

The presence of soil biota had profound effects on shoot and root metabolomes across all four plant species in our study. Plants grown in living soils had substantially different metabolomes than plants grown in sterile soil. Hence, our results support the hypothesis (1) that plants grown in living soil differ in their metabolome compared to plants grown in sterile soil. Our results are in line with work on tomato and ragwort showing that plants grown in sterile soil had reduced phenolic and alkaloid concentrations, respectively, compared to plants grown in living soils (Chialva et al., 2018; Joosten, Mulder, Klinkhamer, & van Veen, 2009). These differences were attributed to the absence of arbuscular mycorrhizal fungi (AMF) in the sterile soil (Chialva et al., 2018; Rivero, Gamir, Aroca, Pozo, & Flors, 2015). AMF colonization can alter the levels of secondary metabolites, such as alkaloids and flavonoids, as well as primary metabolites, such as amino acids and sugars, through compound-specific up- or downregulation (Rivero et al., 2015). Notably, the effects of soil biota on the plant metabolome were reported to be stronger under suboptimal conditions, such as in relatively sandy soil and nutrient poor substrate (Kos, Tuijl, de Roo, Mulder, & Bezemer, 2018).
like we used in our experiment. Our experimental setup also meant that all plants grew in a mostly sterilized substrate (96.66% of the total soil per pot). Sterilization by autoclaving can result in a pulse of nutrients and toxins (Alphei & Scheu, 1993; Trevors, 1996). It may be possible that the plants in our experiment have shown species-specific responses to the pulse in nutrient and toxins. But to address this possibility, we performed all tests within a species, rather than between species. Our results show that the presence of soil biota had a metabolome-wide impact on four different plant species. Hence, our results have strong implications for results obtained in experiments that solely use sterile soil when it comes to their extrapolation to natural systems.

### 4.2 Soil legacy effects on the composition of plant metabolomes

We observed that the CR levels affected the shoot and root metabolomes across all plant species. However, the response to soils with different CR levels was species-specific and tissue-specific. These species-specific and tissue-specific responses support our hypothesis (2) that plant metabolomes change according to the plant diversity-driven soil legacy they encounter. It proved difficult to compare our results to similar studies, because research on plant diversity-driven effects on plant metabolomes is scarce (Peters et al., 2018). In one study, increasing plant diversity was linked to shifts in the above-ground metabolic profile of small-growing herbs but not of tall-growing herbs, with more than 100 detected metabolites that changed in concentration (Scherling, Roscher, Giavalisco, Schulze, & Weckwerth, 2010). In another case, metabolic fingerprinting revealed adaptation to monoculture or plant species mixture history (Zuppinger-Dingley, Flynn, Brandl, & Schmid, 2015). Here, the accumulation of soil pathogens in monocultures was suggested to drive shifts in certain metabolic groups. Our study now adds valuable insights by revealing that plant diversity-driven soil legacy effects induce shifts in the composition and diversity of secondary metabolites. Given the lack of further research on plant diversity-induced shifts in the metabolome, we compared our results to similar studies that focused on single species or single compound classes. These studies on single species or single compound classes confirm our interpretation of plant diversity-induced effects. For instance, bacterial or fungal root pathogens, and non-pathogenic soil bacteria, as well as mycorrhizal fungi caused changes in above-ground defence compounds (Bezem et al., 2005; Hol et al., 2010; van Dam & Heil, 2011). These changes in above-ground defence compounds range from a decrease to an increase depending on the plant species and below-ground interaction partner (Bezem & van Dam, 2005). Although shoot and roots can differ in their response to soil biota, shifts in single compound classes were also reported. For instance, in the roots of *P. lanceolata*, nematodes had no effect on iridoid glycoside concentration, whereas soil micro-organisms increased iridoid glycoside levels (Wurst et al., 2010).

A particular in our study was that within the living soil treatments, the soil legacy of CR 8 soils led to distinct metabolomes expressed across all species and tissues. We attribute this effect in part...
TABLE 4  Comparison between the full metabolite dataset and the reduced metabolite dataset. The reduced metabolite dataset contains only metabolites above median intensity. Displayed are the total numbers of metabolites in both datasets, the number of metabolites selected using Least Absolute Shrinkage and Selection Operator (LASSO) algorithms, and the percentage of explained variance in shoot herbivory by the LASSO-picked metabolites. In addition, displayed are the number of similar metabolites picked by the LASSO algorithms in both the full metabolite dataset and the reduced metabolite dataset.

| Plant species          | Full metabolite dataset | Reduced metabolite dataset |
|------------------------|-------------------------|---------------------------|
|                        | Number of metabolites   | LASSO combination         | Explained variance | Number of metabolites | LASSO combination | Explained variance | Overlapping metabolites |
| Centaurea jacea       | 608                     | 15                        | 88.8%              | 406                  | 11                   | 69.2%              | 6                     |
| Knautia arvensis      | 798                     | –                         | –                  | 499                  | –                     | –                  | –                     |
| Leucanthemum vulgare  | 730                     | –                         | –                  | 475                  | –                     | –                  | –                     |
| Plantago lanceolata   | 766                     | 9                         | 86.1%              | 490                  | 7                     | 64.2%              | 7                     |

TABLE 5  Compounds tentatively assigned in shoot extracts of Centaurea jacea and Plantago lanceolata by LC-MS/MS. The metabolites were detected by LASSO and were part of a linear combination that explains shoot herbivory.

| Source                  | Rt [min] | [M - H]− [m/z] | Putative compound                  | Molecular formula | eV | MS/MS |
|-------------------------|----------|----------------|------------------------------------|-------------------|----|-------|
| C. jacea                | 1.02     | 191.0196       | Quinic acid                        | C_{7}H_{12}O_{6}  | 35 |       |
| C. jacea                | 2.83     | 315.0714       | Quinic acid octenolic ester        | C_{14}H_{24}O_{7} | 35 | 191   |
| C. jacea                | 5.16     | 707.1817       | Chlorogenic acid dimer 1           | C_{32}H_{38}O_{18}| 35 | 191, 163 |
| C. jacea                | 5.26     | 515.1402       | 3,5'-O-dicaffeoylquinic acid       | C_{25}H_{32}O_{12}| 35 | 337, 163 |
| C. jacea                | 5.44     | 707.1818       | Chlorogenic acid dimer 2           | C_{32}H_{38}O_{18}| 35 | 353, 191 |
| C. jacea                | 5.50     | 707.1818       | Chlorogenic acid dimer 3           | C_{32}H_{38}O_{18}| 35 | 353, 191, 163 |
| C. jacea                | 6.64     | 367.1031       | 3-Feruloylquinic acid              | C_{13}H_{20}O_{9} | 35 | 191   |
| C. jacea                | 6.74     | 675.1937       | Chlorogenic acid dimer 4           | C_{22}H_{34}O_{16}| 35 | 337, 191 |
| C. jacea                | 8.04     | 507.1150       | Syringetin-3-O-galactoside         | C_{23}H_{34}O_{13}| 35 | 477   |
| C. jacea                | 8.98     | 521.1297       | Flavonoid glycoside                | C_{24}H_{26}O_{15}| 35 | 359   |
| P. lanceolata           | 1.07     | 407.1190       | Chlorogenic acid derivative        | C_{20}H_{26}O_{6} | 35 | 191   |
| P. lanceolata           | 5.50     | 813.1378       | Verbascoside 1                    | C_{37}H_{50}O_{20}| 35 | 163   |
| P. lanceolata           | 6.99     | 639.1935       | Verbascoside 2                    | C_{29}H_{34}O_{16}| 35 | 415   |
| P. lanceolata           | 8.00     | 637.2141       | Verbascoside 3                    | C_{29}H_{34}O_{16}| 35 | 445   |
| P. lanceolata           | 8.17     | 495.1509       | Iridoid-O-glycoside               | C_{23}H_{28}O_{12}| 35 | 121   |

Abbreviations: eV, Fragmentation energy in electron volt; LASSO, Least Absolute Shrinkage and Selection Operator; MS/MS, mass spectrometry/ mass spectrometry; Rt, Retention time in liquid chromatography in minutes.

to the higher conditioning species richness and to the presence of an additional plant functional group, that is grasses, in the conditioning phase. Our results contrast with those of Kos et al. (2015b). They found that the functional group of the conditioning plant species did not alter the concentration of pyrrolizidine alkaloids and amino acids in Jacobaea vulgaris. We believe that our approach of integrating a comprehensive part of the secondary metabolome in the analysis allowed for detection of plant functional group effects which went unnoticed so far.

4.3  Soil legacy effects on the diversity of plant metabolomes

In addition to analysing the metabolite profile, we analysed the species-specific and tissue-specific richness and Shannon diversity of secondary metabolites. With regard to our hypothesis (3) stating that the individual diversity of secondary metabolites increases with conditioning species richness, we found inconsistent responses of the metabolome to the conditioning species richness treatment. In K. arvensis and P. lanceolata, we observed additional metabolites of low intensity with increasing CR level that did not contribute to the pool of dominating metabolites. This was indicated by an increase in metabolite richness whereas Shannon diversity indicators remained constant. Leucanthemum vulgare lost low-intensity metabolites with increasing CR level. This was indicated by a decrease in the richness at similar Shannon metabolite diversity. Only in C. jacea did we observe similar patterns of richness and Shannon diversity of secondary metabolites. This indicates that metabolites of higher intensity gained or lost intensity and thus changed the pool of dominant metabolites. This induced change in richness and Shannon diversity of secondary metabolites is indicative of the strength of the soil biotic legacy. The strength
of the soil biotic legacy varies with the composition and diversity in the conditioning plant species community. More abundant plant species generally experience stronger negative effects on plant performance than less abundant species (Kos, Veenendrick, & Bezemer, 2013; Maron, Laney Smith, Ortega, Pearson, & Callaway, 2016; van de Voorde, van der Putten, & Bezemer, 2012). This phenomenon is most often attributed to an increase in species-specific soil pathogens with decreasing plant community richness (Kulmatiski et al., 2008; Zuppping-Dingley et al., 2015).

We observed positive and negative intraspecific and interspecific soil legacy effects on the richness of secondary metabolites with increasing CR level. However, the richness and Shannon diversity of secondary metabolites did not reflect directional changes induced by the soil legacy. It is likely that the directional increase in conditioning species richness was not the only factor affecting soil biota. Induced non-directional legacy effects in the soil biota can be the result of shoot and root herbivory in the conditioning plant community (Kostenko, van de Voorde, Mulder, van der Putten, & Bezemer, 2012). Such effects can interfere with the directional soil legacy effect of increasing conditioning species richness.

Notably, given that the experimental setup minimized the effects of soil abiotic conditions by only adding 3.33% soil inoculum to common background substrate, we conclude that our results show that different plant communities can elicit unique metabolite profiles in individual plants via soil legacy effects. In addition, soil legacy effects of plant communities of increasing conditioning species richness induced directional to non-directional changes in the richness and Shannon diversity of secondary metabolites. We conclude that integrating richness and Shannon diversity with metabolite profiles provides a more holistic description of plant secondary chemistry than focusing on only one of these metrics. Hence, we advocate utilizing a comprehensive integration of the secondary plant metabolome rather than single compound classes, as interacting partners, for example herbivores, rarely encounter only a subset of compound classes but rather the full metabolome. Going forward, this knowledge can then help to understand the mechanisms that drive plant–herbivore interactions.

### 4.4 | Linking richness, Shannon diversity and identity of secondary metabolites to herbivory

We related the richness and Shannon diversity of secondary metabolites to the level of shoot herbivory in plants. Herbivory significantly related to the richness and diversity of secondary metabolites in the shoots of *L. vulgare*, only. Previous studies showed negative correlations between metabolite richness and herbivore damage in 12 species of Asteraceae (Macel, de Vos, Jansen, van der Putten, & van Dam, 2014). Hence, we expected to find similar correlations between the richness and Shannon diversity of secondary metabolites and plant herbivory across all species (hypothesis 4). However, our experiment provided limited support for this hypothesis, which we partly attribute to the design of the present study. In our study, we were unable to tease apart constitutive defences and their effect on plant herbivores from herbivory and its effect on plant secondary metabolites via herbivore-induced responses (Bezemer & van Dam, 2005; Macel et al., 2014). In addition, single one-dimensional variables, such as richness or Shannon diversity of secondary metabolites, might not be sufficient to describe the metabolome of a plant in plant–herbivore interactions. Hence, we applied LASSO to detect linear combinations of metabolites that may explain variance in shoot herbivory. We applied LASSO to a full and a truncated dataset to test the robustness of our findings. In both cases, LASSO identified a single linear combination of metabolites that explained variation in shoot herbivory in *C. jacea* and *P. lanceolata*, only. Differences in the linear combination between both datasets in *C. jacea* indicate that low-intensity metabolites contributed to the plant–herbivore interaction. In *P. lanceolata*, seven to nine high-intensity metabolites explained shoot herbivory. We tentatively identified the metabolites of the linear combinations as quinic acid/quinic acid derivatives, chlorgenic acid derivatives, flavonoid glycosides, verbascosides and iridoid glycosides in *C. jacea* and *P. lanceolata*. These metabolites are known for their significant role in plant–herbivore interactions (Bowers & Puttick, 1988; Erb et al., 2009; Leiss, Maltese, Choi, Veroporte, & Klinkhamer, 2009; Sutter & Müller, 2011; Treutter, 2006). In addition, our LASSO outcome hints to two different strategies how plants can adjust their metabolome in plant–herbivore interactions. Either plants concentrate their energy on the synthesis of a few metabolites, in which case LASSO was able to detect a linear combination of metabolites that explains variation in herbivory, or plants spread their energy across the synthesis of many metabolites, in which case LASSO detected multiple equivalent combinations of metabolites. Both strategies may constitute viable approaches to act and react as plant–herbivore interactions unfold. Alternatively, LASSO may not have detected a single linear combination of metabolites in *K. arvensis* and *L. vulgare* because additional herbivore resistance factors exist. Additional resistance factors, such as physical defence, can result in trade-offs between these factors and chemical defence (Eichenberg, Purschke, Ristok, Wessjohann, & Bruehlheide, 2015).

### 5 | CONCLUSIONS

Plant diversity-driven soil legacy affects the performance of plants, as well as the composition and concentration of certain secondary metabolites. We show that soil biota in general induced changes in the root and shoot metabolome of common grassland plant species. In addition, we provide evidence that plant community properties, that is differences in plant species richness, can translate into responses in the composition and diversity of secondary metabolites. We suggest two different strategies of plants to deal with shoot herbivory, based on the species-specific composition of secondary metabolites. Based on our results, we conclude that plant diversity-driven soil legacy can affect plant–herbivore interactions through changes in secondary metabolites. This field
of research may be a key to understand some of the mechanisms that govern species interactions including important ecosystem functions like herbivory.

ACKNOWLEDGEMENTS

We thank Silke Schroeck and Amelie Hauer for assistance at the biomass harvest. We further thank the technical staff and the gardeners of the Jena Experiment for their efforts in maintaining the experimental field site. C.R. acknowledges funding by the German Federal Environmental Foundation (DBU). The Jena Experiment is funded by the German Research Foundation (DFG, FOR 1451) with additional support from the Max Planck Society and the University of Jena. C.R., Y.P., N.E., F.V., N.M.v.D. and A.W. gratefully acknowledge the support of the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig funded by the German Research Foundation (FZT 118). We acknowledge support from the iDiv Open Science Publication Fund.

AUTHORS’ CONTRIBUTIONS

J.H.D., A.E. and C.W. designed and conceptualized the experiment; C.R., Y.P. and A.W. designed and conceptualized the metabolomics analysis; C.R., J.H.D., A.E., F.V. and A.W. collected the data; C.R. and Y.P. analysed the data; C.R., Y.P., N.E., N.M.v.D. and A.W. interpreted the data; C.R. led the writing of the manuscript. All the authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

The data are archived at MetaboLights: https://www.ebi.ac.uk/metabolights/MTBLS544 (Ristok et al., 2019).

ORCID

Christian Ristok https://orcid.org/0000-0002-1446-977X
Yvonne Poensch https://orcid.org/0000-0002-6727-6891
Jan-Hendrik Dudenhöffer https://orcid.org/0000-0003-1548-1436
Anne Ebeling http://orcid.org/0000-0002-3221-4017
Nico Eisenhauer https://orcid.org/0000-0002-0371-6720
Fredd Vergara https://orcid.org/0000-0002-7397-2092
Cameron Wagg https://orcid.org/0000-0002-9738-6901
Nicole M. van Dam https://orcid.org/0000-0003-2622-5446
Alexander Weinhold https://orcid.org/0000-0003-1418-7788

REFERENCES

Alphei, J., & Scheu, S. (1993). Effects of biocidal treatments on biological and nutritional properties of a null-structured woodland soil. In L. Brussaard, & M. J. Kooistra (Eds.), Soil structure/soil biota interrelationships (pp. 435–448). Amsterdam, the Netherlands: Elsevier. https://doi.org/10.1016/B978-0-444-81490-6.50035-2
Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. Journal of Statistical Software, 67(1), 1–48. https://doi.org/10.18637/jss.v067.i01
Benton, H. P., Want, E. J., & Ebbels, T. M. D. (2010). Correction of mass calibration gaps in liquid chromatography–mass spectrometry metabolomics data. Bioinformatics, 26(19), 2488–2489. https://doi.org/10.1093/bioinformatics/btp441
Bezemer, T. M., De Deyn, G. B., Bossinga, T. M., Van Dam, N. M., Harvey, J. A., & Van der Putten, W. H. (2005). Soil community composition drives aboveground plant-herbivore-parasitoid interactions. Ecology Letters, 8(6), 652–661. https://doi.org/10.1111/j.1461-0248.2005.00762.x
Bezemer, T. M., Fountain, M. T., Barea, J. M., Christensen, S., Dekker, S. C., Duysts, H., … van der Putten, W. H. (2010). Divergent composition but similar function of soil food webs of individual plants: Plant species and community effects. Ecology, 91(10), 3027–3036. https://doi.org/10.1890/09-2198.1
Bezemer, T. M., & van Dam, N. M. (2005). Linking aboveground and belowground interactions via induced plant defenses. Trends in Ecology & Evolution, 20(11), 617–624. https://doi.org/10.1016/j.tree.2005.08.006
Bowers, M. D., & Puttick, G. M. (1988). Response of generalist and specialist insects to qualitative allelochemical variation. Journal of Chemical Ecology, 14(1), 319–334. https://doi.org/10.1007/BF01022549
Brinkman, E. P., Van der Putten, W. H., Bakker, E.-J., & Verhoeven, K. J. F. (2010). Plant–soil feedback: Experimental approaches, statistical analyses and ecological interpretations. Journal of Ecology, 98(5), 1063–1073. https://doi.org/10.1111/j.1365-2745.2010.01695.x
Bujak, R., Daghir-Wojtkowiak, E., Kaliszan, R., & Markuszewski, M. J. (2016). PLS-based and regularization-based methods for the selection of relevant variables in non-targeted metabolomics data. Frontiers in Molecular Biosciences, 3, 35. https://doi.org/10.3389/fmolb.2016.00035
Cao, K.-A. L., Rohart, F., Gonzalez, I., Dejean, S. with key contributors Gautier, B., Bartolo, F., … Liquet, B. (2017). mixOmics: Omics data integration project. Retrieved from https://CRAN.R-project.org/packag e=mixOmics
Chambers, M. C., Maclean, B., Burke, R., Amodei, D., Ruderman, D. L., Neumann, S., … Egertson, J. (2012). A cross-platform toolkit for mass spectrometry and proteomics. Nature Biotechnology, 30(10), 918. https://doi.org/10.1038/nbt.2377
Chialva, M., Salvioni di Fossalunga, A., Daghino, S., Ghignone, S., Bagnaresi, P., Chiapello, M., … Bonfante, P. (2018). Native soils with their micro-biotas elicit a state of alert in tomato plants. New Phytologist, 220(4), 1296–1308. https://doi.org/10.1111/nph.15014
Dudenhöffer, J.-H., Ebeling, A., Klein, A.-M., & Wagg, C. (2018). Beyond biomass: Soil feedbacks are transient over plant life stages and alter fitness. Journal of Ecology, 106(1), 230–241. https://doi.org/10.1111/1365-2745.12870
Ebeling, A., Pompe, S., Baade, J., Eisenhauer, N., Hillebrand, H., Proulx, R., … Weiesser, W. W. (2014). A trait-based experimental approach to understand the mechanisms underlying biodiversity–ecosystem functioning relationships. Basic and Applied Ecology, 15(3), 229–240. https://doi.org/10.1016/j.baae.2014.02.003
Ehrenfeld, J. G., Ravit, B., & Elgersma, K. (2005). Feedback in the plant-soil system. Annual Review of Environment and Resources, 30(1), 75–115. https://doi.org/10.1146/annurev.energy.30.050504.144212
Eichenberg, D., Purschke, O., Ristok, C., Wessjohann, L., & Brueelheide, H. (2015). Trade-offs between physical and chemical carbon-based leaf defence: Of intraspecific variation and trait evolution. Journal of Ecology, 103(6), 1667–1679. https://doi.org/10.1111/1365-2745.12475
Eisenhauer, N., Beßler, H., Engels, C., Gleixner, G., Habekost, M., Milcu, A., … Scheu, S. (2010). Plant diversity effects on soil microorganisms support the singular hypothesis. Ecology, 91(2), 485–496. https://doi.org/10.1890/08-2338.1
lanceolata. New Phytologist, 191(4), 1069–1082. https://doi.org/10.1111/j.1469-8137.2011.03768.x
Tautenhahn, R., Böttcher, C., & Neumann, S. (2008). Highly sensitive feature detection for high resolution LC/MS. BMC Bioinformatics, 9, 504. https://doi.org/10.1186/1471-2105-9-504
Tibshirani, R. (1996). Regression shrinkage and selection via the lasso. Journal of the Royal Statistical Society. Series B (Methodological), 58, 267–288.
Treuher, D. (2006). Significance of flavonoids in plant resistance: A review. Environmental Chemistry Letters, 4(3), 147–157. https://doi.org/10.1007/s10311-006-0068-8
Trevors, J. T. (1996). Sterilization and inhibition of microbial activity in soil. Journal of Microbiological Methods, 26(1), 53–59. https://doi.org/10.1016/0167-7012(96)00843-3
van Dam, N. M., & Heil, M. (2011). Multitrophic interactions below and above ground: En route to the next level. Journal of Ecology, 99(1), 77–88. https://doi.org/10.1111/j.1365-2745.2010.01761.x
van de Voorde, T. F. J., van der Putten, W. H., & Bezemer, T. M. (2011). Intra- and interspecific plant-soil interactions, soil legacies and priority effects during old-field succession. Journal of Ecology, 99(4), 945–953. https://doi.org/10.1111/j.1365-2745.2011.01815.x
van de Voorde, T. F. J., van der Putten, W. H., & Bezemer, T. M. (2012). The importance of plant-soil interactions, soil nutrients, and plant life history traits for the temporal dynamics of Jacobaea vulgaris in a chronosequence of old-fields. Oikos, 121(8), 1251-1262. https://doi.org/10.1111/j.1600-0706.2011.19964.x
Van der Putten, W. H., Bardgett, R. D., Bever, J. D., Bezemer, T. M., Casper, B. B., Fukami, T., ... Wardle, D. A. (2013). Plant-soil feedbacks: The past, the present and future challenges. Journal of Ecology, 101(2), 265–276. https://doi.org/10.1111/1365-2745.12054
Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., van der Putten, W. H., & Wall, D. H. (2004). Ecological linkages between aboveground and belowground biota. Science, 304(5677), 1629–1633. https://doi.org/10.1126/science.1094875
Wurst, S., Wagenaar, R., Biere, A., & Van der Putten, W. H. (2010). Microorganisms and nematodes increase levels of secondary metabolites in roots and root exudates of Plantago lanceolata. Plant and Soil, 329(1/2), 117–126. https://doi.org/10.1007/s11104-009-0139-2
Zhang, N., Van der Putten, W. H., & Veen, G. F. C. (2016). Effects of root decomposition on plant-soil feedback of early- and mid-successional plant species. New Phytologist, 212(1), 220–231. https://doi.org/10.1111/nph.14007
Zuppinger-Dingley, D., Flynn, D. F. B., Brandl, H., & Schmid, B. (2015). Selection in monoculture vs. mixture alters plant metabolic fingerprints. Journal of Plant Ecology, 8(5), 549–557. https://doi.org/10.1093/jpe/rtu043

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Ristok C, Poeschl Y, Dudenhöffer J-H, et al. Plant species richness elicits changes in the metabolome of grassland species via soil biotic legacy. J Ecol. 2019;107:2240–2254. https://doi.org/10.1111/1365-2745.13185