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RESEARCH ARTICLE

Temporal changes in plant–soil feedback effects on microbial networks, leaf metabolomics and plant–insect interactions

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Abstract

1. The importance of plant–soil feedbacks (PSF) for above-ground and below-ground multitrophic interactions is well recognized. However, most studies only condition soil for a short time before testing the feedback response. Here we investigate the influence of time of conditioning on soil microbiome composition, plant growth and metabolomics, and plant–insect interactions. We used soil collected from large outdoor mesocosms with monocultures of six species and investigated the temporal changes in the soil over a full year.
2. Every 2 months, we assessed the legacy effects of the soils on plant growth of one of the species (*Jacobaea vulgaris*) in a climate-controlled chamber. Each time we used tissue culture plants that were genetically identical. We also measured leaf herbivore performance and leaf metabolomes, as well as the abiotic and biotic soil properties.
3. We show that the monoculture soils harboured different microbiomes, but that these varied over time. Growth of the test plants also varied over time and plants grew consistently less well in their own soil. The soil legacy effects on the leaf metabolome were less consistent and varied strongly over time. Networking analysis showed that soil bacteria had stronger effects on the leaf metabolome than fungi early on. However, after 12 months of conditioning, only soil fungal community composition explained the metabolomic profiles of the leaves. Insect herbivory was not affected by soil conditioning, but decreased with increasing time of conditioning.
4. *Synthesis*. Our results show that the biomass response of the test plants to soil conditioning remained consistent throughout the year, even though both the soil microbiome and leaf metabolomic responses to conditioned soil varied greatly over time. These soil-induced changes in the metabolome of plants over time can be an important driver of above-ground multitrophic interactions in nature. Our study demonstrates that the duration of conditioning has a strong impact

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on plant and soil properties, which highlights that temporal variation is an important aspect to consider in future studies investigating plant–soil interactions.

KEYWORDS
ecological omics, ecometabolomics, insect herbivory, *Jacobaea vulgaris*, *Mamestra brassicae*, plant–herbivore interactions, plant–soil (below-ground) interactions, soil legacy effects, temporal variation

1 | INTRODUCTION

Characteristics of the soil greatly impact the plant that grows in it, as well as other plant-associated organisms. For example, plant biomass, foliar metabolomes and the performance of herbivorous insects all depend on the microbial community that is present in the soil (Heinen et al., 2018; Huberty, Choi, et al., 2020; Zhu et al., 2018). However, plants also impact biotic and abiotic parameters in the soil, such as the microbial community. Via their effects on soil, plants can influence the performance of other plants that grow later in this soil. This is called plant–soil feedback (PSF) (Bever, 1994; Van der Putten et al., 2013). While many studies have provided evidence for PSFs, the temporal dynamics of PSF processes (i.e. how the strength of PSF varies in time) are still poorly understood (Bezemer et al., 2018; Hawkes et al., 2013; Lepinay et al., 2018).

Most knowledge of PSFs is based on greenhouse studies in which soil is conditioned by a plant for several weeks only. The impact of a plant on the soil microbial community can change considerably over time (Hannula, Kielak, et al., 2019), but how the duration of conditioning by the first plant affects the performance of the succeeding plant and the herbivores on the plant is not well known. If pathogens accumulate over time in the soil, the negative soil-mediated effect on another plant may increase over time (Luo et al., 2019), but could also result in temporal variation in the defence induction patterns of plants (Heil & Bostock, 2002). This could lead to more pronounced metabolomic changes in plants in response to soil conditioning over time, and to stronger responses of insect herbivores to soil conditioning.

Soil properties that are important for plant growth such as nutrient availability and the composition of soil microbial communities all change over time. This could be due to changes in the climate (e.g. seasonal differences), but also due to plant-mediated effects. Rhizodeposition patterns, for example, change with the age of the plant (Dechassa & Schenk, 2004), and the chemical composition of roots can vary over time due to changes in plant phenology or environmental temperatures (Huang et al., 2020). In PSF experiments where seedlings are planted in soil without any other plants at the start, over time, root biomass will increase, and therefore the root surface that is in contact with the soil increases. This suggests that the influence of the plant on soil nutrients and microbial communities in the soil will increase over time (Latz et al., 2015; Micallef et al., 2009). We therefore expect that PSFs will become stronger with increasing time of conditioning. Such temporal PSF effects on biomass have been reported for the duration of short (2–8 weeks) conditioning periods (Lepinay et al., 2018). However, how these effects change over the course of a year, and how this influences plant metabolomes and plant–herbivore interactions is unknown.

Jacobaea vulgaris is a biennial herb, which is native to Europe and Asia and invasive in North America, Australia and New Zealand (Bain, 1991). This species can grow in diverse habitats such as sand dunes, woodlands and grasslands and therefore has the capacity to grow in a broad range of soils (Bezemer et al., 2006). Plants of this species grow less well in own soil than in soils conditioned by other plant species, likely caused by an accumulation of soil pathogens in its own soil (Bezemer et al., 2006; Van de Voorde et al., 2011; Wubs & Bezemer, 2016; Wubs & Bezemer, 2018). Previous work has shown that the growth and chemical composition of the plant *J. vulgaris* depends on microbial characteristics of the soil in which the plant grows (Bezemer et al., 2006, 2013; Huberty, Martis, et al., 2020; Joosten et al., 2009; Kostenko et al., 2012). Aphids and caterpillars feeding on the foliage of the plant also respond to soil legacies and this is related to changes in plant chemical composition (Kos et al., 2015b; Kostenko et al., 2012). As the metabolic changes in *J. vulgaris* are related to changes in the soil (Huberty, Martis, et al., 2020) and as plant-mediated effects on the soil during the conditioning phase change over time (Hannula, Kielak, et al., 2019), we expect that plant–herbivore interactions on the succeeding plant will also be time dependent and change with the duration of soil conditioning.

In this study, we test in a controlled set-up how inoculation with soil from six monocultures (one conspecific and five heterospecific soils) over time influences growth and chemical composition of genetically identical clones of *J. vulgaris*, and the performance of a generalist herbivorous caterpillar, *Mamestra brassicae*. After establishment of the monocultures, soil was collected every 2 months, and each time abiotic properties of the soil were determined. The *J. vulgaris* test plants were grown each time in identical sterilized bulk soil inoculated with 10% monoculture soil and on 100% sterilized bulk soil in the same climate-controlled growth cabinet. Metabolic profiles of the test plants were analysed at 2, 8 and 12 months and correlated to the microbiomes of the soils. This enabled us to build correlation networks which make it possible to link specific changes in the microbial community of the soils to metabolomic changes in the plants. Furthermore, the abundance of bacteria and fungi in the soil was determined with qPCR (Figure 1).

We address the following questions (i) Are the soil legacy effects of conspecifically and heterospecifically conditioned soils on plant biomass, herbivore performance and metabolomic profiles consistent over time? (ii) Do PSFs get stronger with increasing time of soil conditioning? (iii) Do changes in microbial communities in the soil or in soil nutrients explain the PSF effects on plant growth, metabolomics and herbivory?

2 | MATERIALS AND METHODS

2.1 | Monocultures

In all, 30 containers (48 cm × 80 cm × 50 cm) were filled with 200 L soil on the 3rd and 4th of April 2017. The soil was sieved through a 32-mm sieve

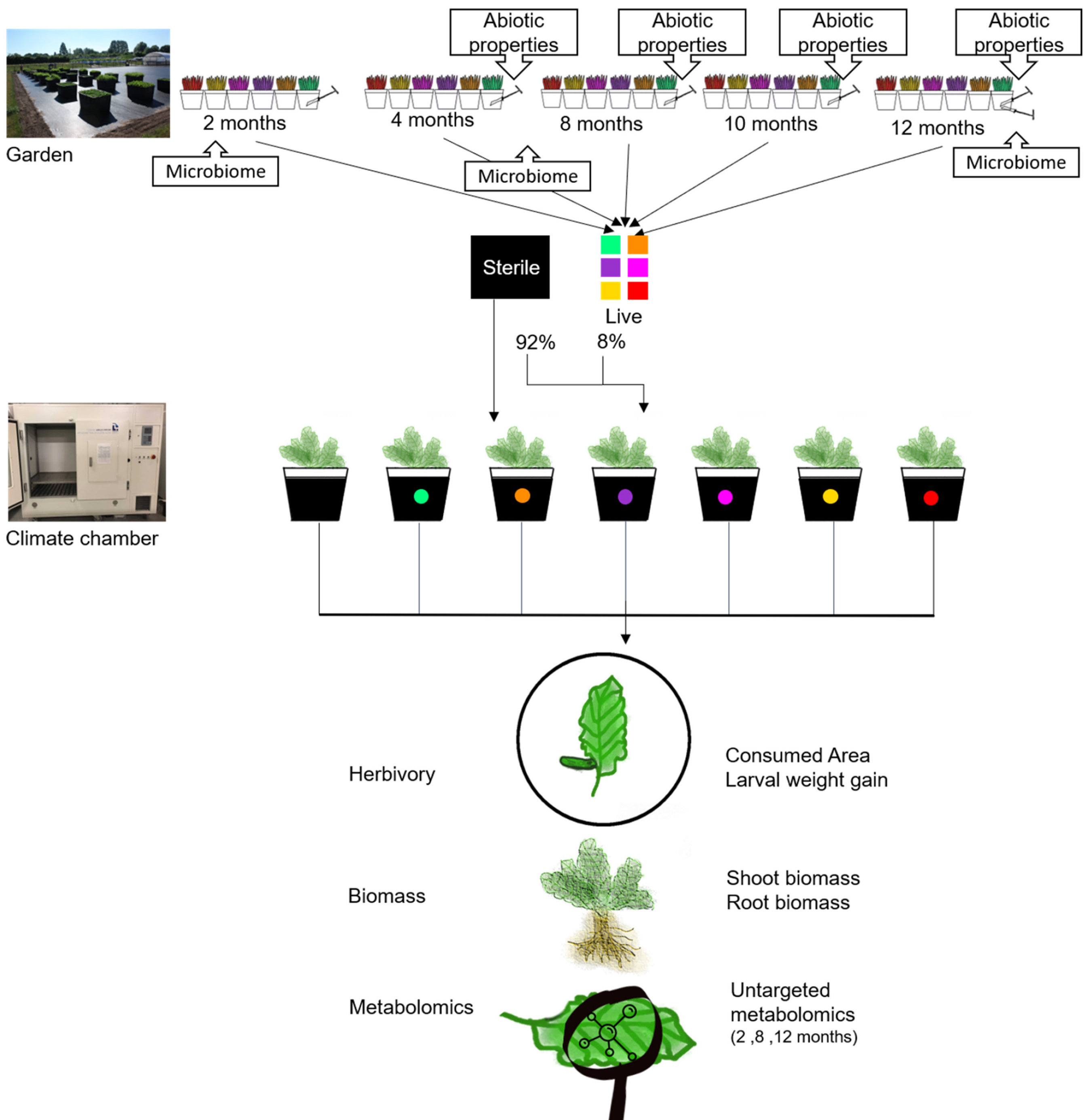

FIGURE 1 Conceptual framework of the experimental design: Soils were conditioned in large containers by monocultures by six plant species in a common garden over the course of 1 year. Soil samples were collected five times and soil abiotic characteristics were measured. The soil was mixed with sterilized bulk soil and *Jacobaea vulgaris* was grown in a climate chamber in different rounds. After 6 weeks of growth, one leaf was clipped and used for a detached leaf assay with *Mamestra brassicae*, biomass of shoot and roots was determined and leaf metabolomics analysis was done for the 2, 8 and 12 month rounds. Samples to determine fungi and bacteria communities in the soil were taken at 2, 6 and 12 months. Samples to quantify bacteria and fungi were taken at 1, 2, 3, 4, 5, 6, 9, 10 and 12

to remove large stones and roots. The soil originated from a grassland near Lange Dreef, Driebergen, The Netherlands (52°02′N, 5°16′E) and is described as holtpodzol, sandy loam (84% sand, 11% silt, 2% clay, ~3% organic matter, 5.9 pH, 1.15 g N kg^{-1}, 0.06 g P_2O_5 kg^{-1}, 0.94 g K kg^{-1}). To inoculate the soil with a diverse microbial community, the soil in each container was topped with 5 cm of soil collected from a natural grassland 'De Mossel' (Natuurmonumenten, Ede, The Netherlands, 52°04′N, 5°45′E) which was sieved through a 10-mm sieve. This soil is characterized as holtpodzol, sandy loam (83% sand, 10% silt, 4% clay, 3% organic matter, 5.2 pH, 1.06 g N kg^{-1}, 0.08 g P_2O_5 kg^{-1}, 0.74 g K kg^{-1}).

Seeds of the species used in this experiment were sown in steamed (60°C) potting soil and grown for 3 weeks in a greenhouse (70% relative humidity, light/dark 16/8 h, 21/16°C), with supplemented light from 400 W metal halide lamps (225 μmol m^{-2} s^{-1} photosynthetically active radiation, 1 lamp per 1.5 m^2). All seeds originated from Cruydt-Hoeck (Nijberkoop, The Netherlands), except for *Jacobaea vulgaris*. Seeds for this species were collected in De Mossel in 2014. The plant species used consisted of three grasses, *Holcus lanatus*, *Festuca ovina*, *Alopecurus pratensis*, and three forbs, *Hypochaeris radicata*, *Jacobaea vulgaris*, *Taraxacum officinale*. We selected these plant species as they were identified in previous studies as plant species that create soil legacy effects that influence *J. vulgaris* (Van de Voorde et al., 2011; Wubs & Bezemer, 2016; Zhang et al., 2021).

The seedlings were planted at a density of 100 seedlings per container on the 1st of May 2017. Plants that died were replaced within the first 2 weeks. Seedlings from other species were weeded out regularly. For each plant species, five containers were established. The containers were placed in a randomized block design in a common garden at the Netherlands Institute Of Ecology (NIOO-KNAW) in Wageningen, the Netherlands (51°59′N, 5°40′E). The containers were watered regularly during the summer months. No permission was needed to set up these containers at the NIOO-KNAW.

2.2 | Soil sampling

Every 2 months, four soil cores (0.7 cm diameter, 10 cm depth) were taken from each container. The soil was mixed and large pieces of roots were removed. Details about the sampling dates are provided in the supporting information (Table S1). Photos of the monocultures at each sampling point are presented in Figure S1. After the final sampling point (May 2018), total above-ground biomass was removed from each container, dried at 40°C and total shoot biomass per container was determined (Figure S2). Due to a technical failure of the growth cabinet used for the plant growth experiment (see below) in January 2018, the 6-month sampling event was excluded from further analysis.

2.3 | Soil abiotic characteristics in the monocultures

Every 2 months, the soil abiotic characteristics were determined for each soil, starting at 4 months. For each soil, a subsample was dried (40°C) and sieved through a 2-mm mesh. 30 ml of 0.01 M $CaCl_2$ was added to 3 g of soil and the mixture was shaken for 2 h at 250 rpm and centrifuged at 3000 rpm for 5 min. 15 ml of the supernatant was filtered through a Whatman Puradisc Aqua 30 syringe filter with cellulose acetate membrane. To measure soil extractable nutrients (i.e. Fe, K, Mg, P, S, Zn), 12.87 ml of this filtrate was transferred to a 15 ml tube and 130 μl HNO_3 was added, vortexed and analysed by inductively coupled plasma-optical emission spectrometer (ICP-OES, Thermo Scientific iCAP 6500 Duo Instrument with axial and radial view and CID detector microwave digestion system). The remaining of the filtrate was transferred to a 15 ml tube and pH was determined. $NO_2^+NO_3$ and NH_4 were measured on a QuAAtro Autoanalyser (Seal analytical). For each sampling round, five samples from the sterilized bulk soil from the bag that was used for the plant growth experiment (see below) were also analysed except for the first round of analyses (4 months).

2.4 | Soil bacterial and fungal communities

During the course of the experiment, soil samples were collected every month from each container for molecular identification of the bacterial and fungal communities. These samples have been sequenced and the results have been published elsewhere (Hannula, Kielak, et al., 2019). Here we use the data from soil samples collected at 2, 6 and 12 months. This coincided with the two of the three sampling rounds for which leaf metabolomics were analysed (see below). For one of the rounds of the feedback experiment (8 months), the soil microbial sampling of 6 months was used as there was no sampling at 8 months (Table S1). A detailed description of the collection of the soils and the data processing are found in the supplementary information methods S1 and in Hannula, Kielak, et al. (2019). Bacterial and fungal biomass was estimated based on qPCR against a known standard as described in Hannula, Kielak, et al. (2019). In addition to what is presented in Hannula, Kielak, et al. (2019), here we report bacterial and fungal data for all measured time points.

2.5 | Plant–soil feedback test

For the PSF tests, we used clonal *J. vulgaris* grown in tissue culture for 17 years and formerly collected from Meijendel, the Netherlands. Per time point we asexually propagated a total of 60 *J. vulgaris* plants on MSO medium with 100 mg/L benzylaminopurine (BAP) in a climate room (16 h:8 h light:dark photoperiod, 20°C). After 4 weeks of growth, they were individually put on MSO medium without BAP for 10 days to form roots. Time intervals of growth on the two different media were the same for all time points. For each time point, 35 equally sized (approximately 4 cm) *J. vulgaris* plantlets were selected for the PSF experiment and one plantlet was planted per pot.

The plants were grown in pots filled with 50 g (dry weight) of soil collected from the containers mixed with 410 g sterilized

bulk soil. To calculate how much wet soil from each sample was needed, a subsample was weighed, dried (40°C) and soil moisture was determined. After collection and prior to filling the pots, the soil samples were kept for 3 days at 4°C. The bulk soil consisted of homogenized soil that was used to fill the containers (Lange Dreef, Driebergen) which was gamma-irradiated (>25 Kgray). For each pot, the live soil and bulk soil were mixed separately. At each round, five additional pots were filled with 100% bulk soil and these pots served as sterile control. To allow the microbial community to establish before proceeding with the planting, the pots were put in a climate chamber without light for 7 days (16°C, humidity 70%).

The PSF experiment was carried out in the same growth cabinet each round to assure uniform conditions (16 h:8 h light:dark photoperiod, 21, 16°C, humidity 70% and PAR 290 μmol/m^2/s). During the 6-month round, the lights in the cabinet were accidentally switched off for an unknown period of time. Therefore, the data from this sampling round were not included in the analyses. Plants were watered twice a week. To ensure similar soil moisture in all pots, each time we weighed each pot and added water until it reached a weight of 505 g. Water was autoclaved to avoid introduction of microbes through the water. After 6 weeks of growth, the plants were harvested. For this, plants were carefully removed from the pots and roots were washed. After that the two largest leaves of each plant were cut and fresh weight was recorded. For the time points at which metabolomic profiles were measured, the largest leaf was immediately wrapped in aluminium foil and then flash-frozen in liquid nitrogen and stored at −80°C. The largest but one leaf was always used for a non-choice bioassay using the caterpillar *M. brassicae* (see below). The roots were cut off the shoots and wrapped in aluminium foil and flash frozen in liquid nitrogen. The remainder of the shoots were put in a bag and frozen at −80°C. All frozen samples were lyophilized for 7 days and then stored in an exicator with silica gel upon use.

2.6 | Herbivory assay

The stem of the largest but one leaf of each plant was wrapped in wet cotton wool and parafilm to keep the leaf hydrated. *Mamestra brassicae* (Lepidoptera: Noctuidae) eggs were obtained from the University of Wageningen, the Netherlands and upon hatching the caterpillars were reared on artificial diet (as described in Hannula, Zhu, et al., 2019). For each round, we recorded the biomass of each of 40 L2 caterpillars. Then caterpillars were individually placed on one of the leaves in a petri dish (100 × 15 mm). The petri dishes were randomly ordered and placed in a climate chamber with no light, 20°C, humidity 70%. After 4 days, the caterpillars were weighed again and leaf consumption of each leaf was assessed by drawing the consumed area of every leaf on an acetate sheet and scanning the sheet with an Epson STD4800. Food consumption (in mm^2) was then determined with the program WinFOLIA (Version: 2016b Pro; Regent Instruments Canada Inc.). Mean weight gain per day of *M. brassicae* was also calculated. A few leaves were desiccated (1 for 4 months, 1 for 8 months, 3 for 12 months). These samples were excluded from the analysis.

2.7 | Metabolomics ^{1}H NMR analysis of *J. vulgaris* leaves

Metabolomic profiling was performed using leaf samples from the 2-, 8- and 12-month conditioning rounds. For the metabolomics analysis, the samples were extracted according to an adapted version of the protocol described by Kim et al. (2010). The lyophilized leaf was ground with a metal ball bearing in a TissueLyser (Retsch Mixer Mill MM 400) for 3 min at 30 s^{-1}. Of this powder, 20 mg was weighed and extracted with 600 μl of Methanol-d_4, sonicated for 10 min and centrifuged for 10 min at 13.000 ppm. 250 μl of supernatant was transferred to an NMR tube (103.5 × 3 mm, inside-ø 2.24 ± 0.05 mm). We used a Bruker AV-600 MHz NMR spectrometer (Bruker), operating at a frequency of 600.13 MHz to record the ^{1}H NMR spectra. CH_3OH-*d*4 was used as an internal lock and ^{1}H NMR spectra were recorded with pulse width (PW) = 30° (11.3 μs), Relaxation delay (RD) = 1.5 s and 128 scans with 10 min and 26 s acquisition time with 0.16 Hz/point. To reduce the signal of H_2O frequency during the recycle delay, we used a pre-saturation sequence. FIDs were Fourier transformed by a line broadening of 0.3 Hz. We manually baseline corrected the spectra and calibrated them to the solvent at 0.60 ppm before phasing them in TOPSIN (v.3.0. Bruker). The data were bucketed with scaling to total intensity and a bucket width of 0.04 ppm in AMIX software (v. 3.9.12 Bruker BioSpin GmbH). This is a pre-processing step which is often used in metabolomics to reduce the effect of small shifts of signals between signals (Kim et al., 2010).

The residual signals from the solvents in the regions between 4.70–4.90 ppm and 3.32–3.28 ppm were excluded. Pre-processing led to a data matrix with 246 buckets per sample. Each bucket contained the intensity of the signal from the NMR within the size of the buckets and corresponds directly to the molar level of a compound leading to a signal in this region of the NMR. Molecules which have more than one H atom will therefore lead to signals in several buckets across the NMR spectra. The chemical environment of the H atom is defined by the neighbouring atoms and determines the chemical shift and the splitting pattern of a signal.

2.8 | Statistical analysis

All analyses were performed in R Studio (RStudio Team, 2016) using the packages 'VEGAN' (Oksanen et al., 2018) and 'MIXOMICS' (Cao et al., 2020). Networks were constructed in R Studio and processed in Cytoscape version 3.7.2 (Shannon et al., 2003). Heatmaps were created with metaboanalyst (Chong et al., 2018).

Dry weight of shoots and roots, weight gain of *M. brassicae* and the total area consumed were analysed each with a two-way

ANOVA with the factors 'Time' and 'Monoculture'. Since each round, a new experiment was started and the individuals measured were not the same, time was not included as a repeated measure factor in these analyses. Data were then analysed per round. Plants grown in 100% sterilized soil were excluded from the dataset for this analysis. For each round, we reran the ANOVA including the 100% sterilized soil, and tested with a Dunnett's t test if the monoculture soils significantly differed from the sterilized soil. All assumptions of ANOVA were fulfilled.

Soil abiotic characteristics and the abundance of fungi and bacteria were analysed in the same way and details about these analyses are shown in the supplementary information method S2.

2.9 | Metabolomic analysis

We visualized metabolomic changes in the leaves with Principal component analyses (PCA), one PCA was constructed for all data, followed by individual PCAs for each of the three rounds separately. Statistical significance was inferred from a permutational analysis of variance (PERMANOVA) based on Bray–Curtis dissimilarities with the factors 'Monoculture', 'Time' and 'Total plant biomass'. Then separate PERMANOVAs were conducted for each round, with the factors 'Monoculture' and 'Shoot biomass'. For these analyses, the metabolome data were normalized by the control by dividing, for each sample, the intensity of each bucket by the mean intensity of that bucket for plants grown in sterilized soil. Permutations were set to 999. Information on the construction of the heatmap is found in the supplementary information (method S3).

2.10 | Bacterial and fungal community analyses

Bioinformatic analysis were performed as described by Hannula, Kielak, et al. (2019); Hannula, Zhu, et al. (2019). Changes in the composition of the fungal and bacterial communities were depicted with a PCA. Statistical significance was inferred from a permutational analysis of variance (PERMANOVA) based on Bray–Curtis dissimilarities with the factors 'Monoculture' and 'Time'. Data were then analysed per round with the factor 'Monoculture'. Bacterial and fungal copies (qPCR) were each analysed with an ANOVA with the factors 'Time' and 'Monoculture'. Then they were analysed per sampling round with an ANOVA with the factor 'Monoculture'. Fungal copies were log-transformed to meet the assumption of normality.

2.11 | Relationship between soil characteristics and plant and herbivore responses

The relationship between the composition of soil abiotic characteristics and features measured in the plant (shoot and root biomass, *M. brassicae* weight gain per day and damage) was analysed with redundancy analysis (RDA). The relationship between the metabolome and the abundance of bacteria/fungi was analysed with RDA. Soil characteristics were standardized prior to the analysis. Univariate variables measured in the plant (shoot and root biomass, *M. brassicae* weight gain per day and damage) and abundance of bacteria and fungi in the soil were compared with Pearson correlations.

The relationships between fungal community, bacterial community, soil abiotic characteristics and leaf metabolome composition were analysed with co-inertia analysis. Significances were tested with a permutation test with 999 permutations. For these analyses, the filtered OTUs were used.

Circos plots were constructed to display correlations between bacteria, fungi and the NMR buckets differing the most between the monocultures. For bacteria and fungi, the OTUs were labelled to the finest taxonomic rank known. When possible, the buckets were assigned to chemical groups (sugars, phenolic compounds, TCA-related compounds, aliphatics). Compounds that could be identified from the NMR spectra were assigned by chemical shift and splitting pattern. Buckets which could not be assigned to a chemical group were labelled with their chemical shift (ppm). To make the circos plots, the 40 variables in each measured community (bacteria, fungi, metabolome) that were most influential in a sparse Partial Least Squares (sPLS) were selected and correlated to each other. Only correlations with Pearson correlation coefficients higher than 0.8 were plotted. These correlations use the latent components as proxy (González et al., 2012). The components were set to two components each. This was done per round and for each monoculture soil. Correlation circle plots were constructed to display the correlation of the selected variables and the components of the sPLS.

To further explore the relationship between the metabolome of the plant and the bacterial and fungal communities in the soil, relevance network analyses were carried out. A sparse sPLS with regression (three sPLS components, 20 variables each) was run and then a network was constructed for all rounds and for each round separately. Information on the construction of the correlation networks is shown in supplementary information methods S4.

3 | RESULTS

3.1 | Changes in plant biomass, herbivore performance and metabolomic profiles of *J. vulgaris*

3.1.1 | Biomass of *J. vulgaris* test plants

Over time, *J. vulgaris* consistently grew less well in its own soil than in other soils. Moreover, shoot and root biomass of the test plants varied greatly over time and between monoculture soils (Table 1, Figure 2). Shoot and root biomass was lowest in soil collected after 8 months, but this was also true for plants grown in sterile soil (Figure 2). In the 8- and 10-month rounds, biomass did not differ between monocultures (Figure 2, Table 1). Biomass did not vary much

among the soils of the five other species. Overall, biomass was highest in 100% sterilized soil.

3.1.2 | Herbivory

Weight gain and food consumption of *M. brassicae* varied greatly between rounds (Table 1), but there was no effect of monoculture soil (Figure 3) except on weight gain in the 12-month round (Table 1). *M. brassicae* performed consistently less well when plants were grown in soils conditioned for longer than 4 months than in younger soils, except when the soil was conditioned by *T. officinale*.

3.1.3 | Leaf metabolomes

Metabolomes differed significantly between rounds and were related to plant biomass, but did not differ between monoculture soils (PERMANOVA; Table 2). The leaf metabolomes differed strongly among the three sampling rounds (Table 2, Figure 4). The 2-month and 8-month sampling rounds were separated most clearly along the first PCA axis, while the 2- and 12-month rounds were more similar in composition along the first axis, but were separated along the second axis (Figure 4). Separate PCAs for each round show that there was considerable variation in metabolome profiles, but that the metabolomes of plants grown in sterilized soil varied less than those of plants grown in conditioned soils (Figure S3). Separate PERMANOVAs per round revealed that the soil effects were less strong in the 2-month round than in the later rounds (measured as R^2; Table 2). The correlation between total plant biomass and metabolome profiles also increased over time (Table 2). Phenolic compounds varied most strongly between the monoculture soil treatments, but formic acid was the only compound that responded consistently among rounds (Figure S4).

The variation in the composition of the metabolome was significantly related to the consumed area by caterpillars in the 8-month round (Table S2).

3.2 | Biotic and abiotic changes within the soil over time

3.2.1 | Bacteria and fungi

Bacterial composition in the soil differed strongly among the three measured rounds, but also among the different monocultures (Figure S5a, Table S3). Fungal composition also differed strongly between monocultures and differed between the rounds (Figure S5b, Table S3). The biomass of bacteria in the soil peaked at 6 months for five of the six species, but 1 month earlier in *J. vulgaris* monocultures (Figure S6a, Table S4). Fungal copy numbers were higher in both *T. officinale* and *J. vulgaris* monocultures than in other monocultures after 6 and 12 months (Figure S6b, Table S4).

3.2.2 | Soil abiotic properties

Potassium and to a lesser extent magnesium and phosphate availability were higher in monoculture soils of *J. vulgaris* than in other soils and this was to a lesser extent also true for *T. officinale* soil (Figure S7, Table S5). Organic matter; iron (Fe) and sulphur (S) varied over time (Figure S7, Table S5).

TABLE 1 Results of ANOVA testing the effects of monoculture soil (*Holcus lanatus*, *Festuca ovina*, *Alopecurus pratensis*, *Hypochaeris radicata*, *Jacobea vulgaris* and *Taraxacum officinale*) and the different rounds (2, 4, 8, 10 and 12). Hereafter, the effect of monoculture soils for each round on shoot and root biomass of *Jacobaea vulgaris* and larval weight gain and food consumption of *Mamestra brassicae* was tested for each round separately. *F*-values, degrees of freedom (*df*) and *p*-values are presented. Significant *p*-values are presented in bold

Round	Factor	Shoot	Root	Larval weight gain	Consumed area
	Round (R)	$F_{(4120)} = 42.16$, $\mathbf{p < 0.001}$	$F_{(4120)} = 34.62$, $\mathbf{p < 0.001}$	$F_{(4,98)} = 62.03$, $\mathbf{p < 0.001}$	$F_{(4,98)} = 49.14$, $\mathbf{p < 0.001}$
	Monoculture (M)	$F_{(5120)} = 5.39$, $\mathbf{p < 0.001}$	$F_{(5120)} = 7.55$, $\mathbf{p < 0.001}$	$F_{(5,98)} = 1.34$, $p = 0.25$	$F_{(5,98)} = 0.06$, $p = 0.99$
	R × M	$F_{(20,120)} = 0.71$, $p = 0.80$	$F_{(20,120)} = 0.64$, $p = 0.87$	$F_{(20,98)} = 1.16$, $p = 0.30$	$F_{(20,98)} = 1.38$, $p = 0.15$
2	M	$F_{(5,24)} = 3.12$, $\mathbf{p = 0.026}$	$F_{(5,24)} = 2.96$, $\mathbf{p = 0.032}$	$F_{(5,24)} = 1.38$, $p = 0.27$	$F_{(5,24)} = 0.89$, $p = 0.50$
4	M	$F_{(5,24)} = 2.65$, $\mathbf{p = 0.048}$	$F_{(5,24)} = 5.15$, $\mathbf{p = 0.003}$	$F_{(5,23)} = 0.65$, $p = 0.66$	$F_{(5,22)} = 2.25$, $p = 0.09$
8	M	$F_{(5,24)} = 0.41$, $p = 0.84$	$F_{(5,24)} = 0.50$, $p = 0.77$	$F_{(5,14)} = 0.62$, $p = 0.69$	$F_{(5,11)} = 0.43$, $p = 0.81$
10	M	$F_{(5,24)} = 0.77$, $p = 0.58$	$F_{(5,24)} = 0.45$, $p = 0.81$	$F_{(5,22)} = 2.41$, $p = 0.07$	$F_{(5,22)} = 0.12$, $p = 0.99$
12	M	$F_{(5,24)} = 2.55$, $\mathbf{p = 0.050}$	$F_{(5,24)} = 3.44$, $\mathbf{p = 0.017}$	$F_{(5,22)} = 2.67$, $\mathbf{p = 0.049}$	$F_{(5,19)} = 0.67$, $p = 0.65$

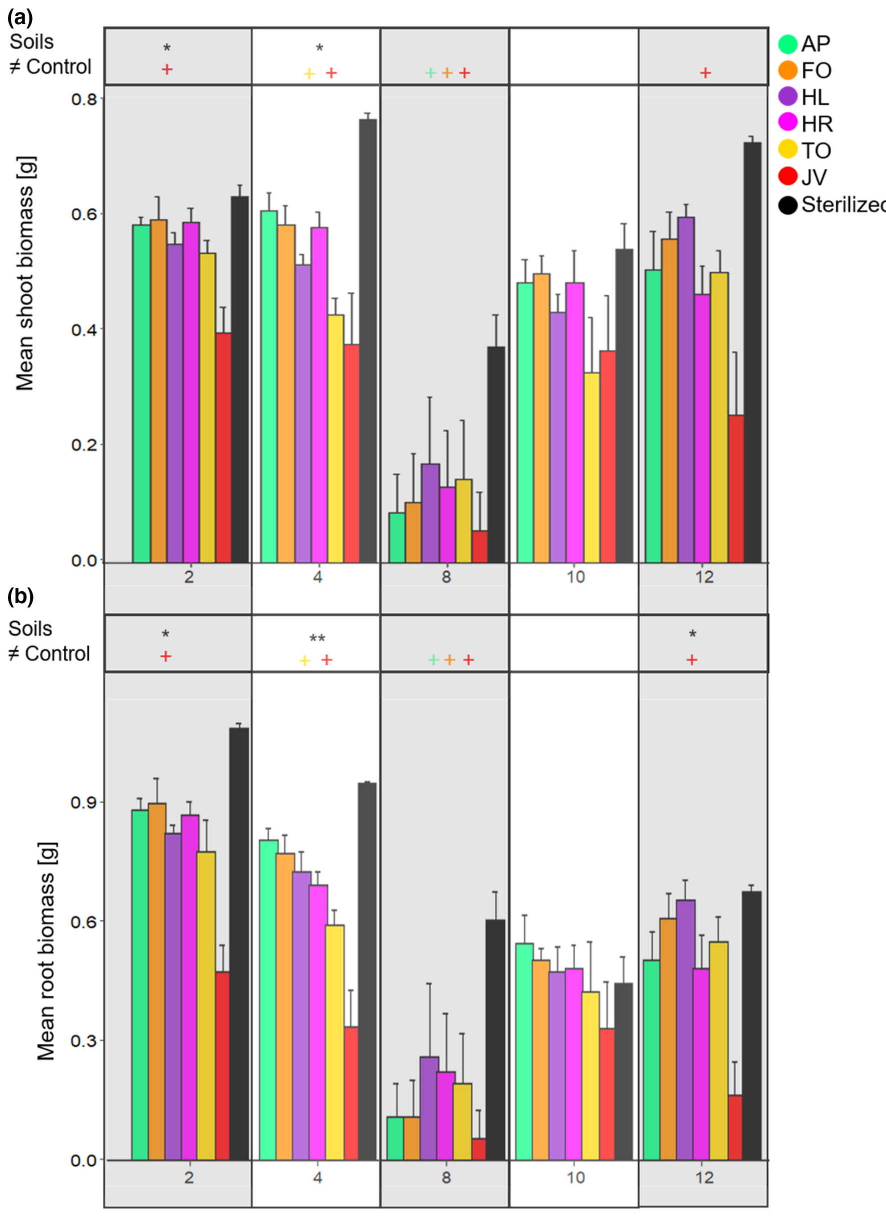

FIGURE 2 Mean (± SE) shoot (a) and root (b) biomass of *Jacobaea vulgaris* in monoculture soils collected over the course of 1 year (AP, *Alopecurus pratensis*; FO, *Festuca ovina*; HL, *Holcus lanatus*; HR, *Hypochaeris radicata*; TO, *Taraxacum officinale*; JV, *J. vulgaris* and in 100% sterilized soil (control). For each round, results of an ANOVA testing the effects of monocultures are also depicted in the figure (*$p < 0.05$, **$p < 0.01$). Plants grown in sterilized soil were excluded for this analysis. A Dunnet post-hoc test, following a separate ANOVA was used for the comparison of each treatment with the 100% sterilized soil was tested ($p < 0.05$ is depicted as + with the colour indicating the species)

3.3 | Correlating soil characteristics and plant and insect responses

Bacterial composition in the soil correlated in the 8-month round, and fungi in the 12-month round to metabolome changes (Table 3). The composition of both the bacterial and the fungal communities significantly correlated to root biomass in the 12-month round and for the fungal community this was also the case for shoot biomass. Soil abiotic characteristics did not correlated to metabolome profiles, but were significantly correlated to larval weight gain in the 8-month round and to leaf consumption by *M. brassicae* in the 10-month round (Table 3).

The correlations between soil microbial communities and metabolomes varied among the sampling rounds (Figure 5). After 2 months, we observed positive correlations between soil fungi, bacteria and metabolic compounds selected through sPLS. After 8 months, the number of positive correlations increased, while after 12 months there were fewer positive correlations and at this timepoint also negative correlations between microbes and the metabolome appeared. The identity of metabolic compounds that correlated with soil microbes varied between the rounds. However, in each round, changes in compounds related to the TCA cycle such as malate and malic acid were linked to OTUs of bacteria and fungi. The patterns also varied between monocultures (Figure S8). Notably, there were more negative correlations between fungal OTUs and the metabolome for forb soils than for grass soils. For most monoculture soils, there were more strong correlations between the metabolome and bacteria than between metabolome and fungi and these correlations were mostly positive (Figure S9). Bacterial and fungal communities in the soil were strongly linked in all rounds, as depicted by the positive correlations in the correlation network (Figure S10).

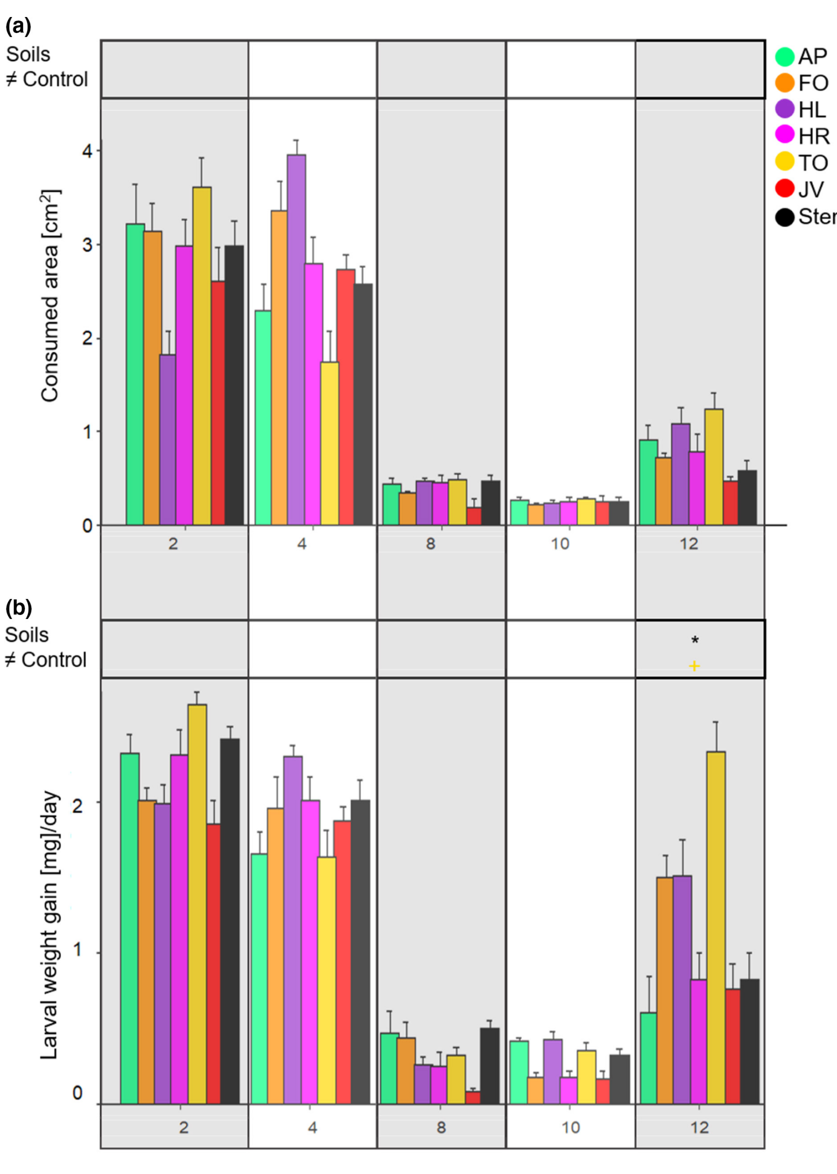

FIGURE 3 Mean (± SE) area consumed (a) and larval weight gain (b) of *M. brassicae* larvae on leaves of *Jacobaea vulgaris* grown in monocultures soils (AP, *Alopecurus pratensis*; FO, *Festuca ovina*; HL, *Holcus lanatus*; HR, *Hypochaeris radicata*; TO, *Taraxacum officinale*; JV, *J. vulgaris*) and in 100% sterilized soil (control) in each round. For each round, results of an ANOVA testing the effects of monocultures are also depicted in the figure (*$p < 0.05$). Plants grown in sterilized soil were excluded for this analysis. A Dunnet post-hoc test, following a separate ANOVA was used for the comparison of each treatment with the100% sterilized soil was tested ($p < 0.05$ is depicted as + with the colour indicating the species)

We further investigated how changes in the metabolome were correlated to changes in the bacterial and fungal communities in the soil using relevance networks (Figures S11 and S12). These networks showed similar patterns as the circus plots. The relevance networks further showed that certain bacteria and fungi are correlated to only one compound in the metabolome of *J. vulgaris*, while others are correlated to more different compounds.

4 | DISCUSSION

Our study on plant–soil feedbacks, plant growth, metabolomics and insect herbivory highlights several important aspects. First, although the magnitude of soil effects on biomass varies depending on the duration that identical monoculture soils were conditioned, the PSF patterns on plant growth (conspecific versus heterospecific soil) are generally similar over time. However, the effects of soil conditioning and time of conditioning on herbivory and on the leaf metabolome were not consistent among the different rounds of the experiment and were strongly influenced by the time of conditioning. Metabolomic changes in the leaves were linked to microbial and fungal communities in the soils, but this was so particularly after 8 months of conditioning and less after 2 or 12 months of conditioning. Below we discuss these findings in more detail.

Our results show that generally soil legacy effects on plant biomass stayed the same independent of the duration of the conditioning even though biomass of *J. vulgaris* test plants grown in soils collected at different times varied. In general, except after 10 months of conditioning, *J. vulgaris* produced most biomass above-ground and below-ground in 100% sterilized soil and least in conspecific-conditioned soil. Such better performance in sterile than in live soil has been reported in multiple studies (Joosten et al., 2009; Kos et al., 2015a; Wang et al., 2019; Xue et al., 2018). *J. vulgaris* has a negative conspecific feedback (e.g. Bezemer et al., 2018; Van de

Voorde et al., 2012) probably resulting from an accumulation of pathogens in conspecific conditioned soil (Bezemer et al., 2006; Van de Voorde et al., 2011; Wubs & Bezemer, 2016). Here we show that the build-up of this negative conspecific soil effect occurs relatively quickly, already after 2 months of conditioning. Our results also show that these soil legacy effects remain remarkably consistent when the conditioning monoculture is aging. The strength of PSF moderately differed depending on the age of the conditioning community, but the overall patterns stay the same over the course of a year. Therefore, concerning biomass responses, we reject our hypothesis that the PSF effects get stronger over time, but rather that at least for *J. vulgaris*, the relative effects of heterospecific and conspecific soil conditioning on plant growth remain consistent over time. If generally true, this is an important result, since it highlights that the time of conditioning is not a major factor influencing the outcome of PSF experiments.

Root biomass responses to soil legacies were more pronounced than those of shoot biomass. Biomass of the roots of the test plants decreased considerably with aging of the soils, while shoot biomass first declined, but was similar in 12-month old soil as in the beginning of the experiment. These findings strengthen the view that root biomass of *J. vulgaris* is more sensitive to PSF than shoot biomass (Kos et al., 2015a; Wang et al., 2019). This can be explained simply by the proximity of the roots to the soil and therefore a more direct influence of the soil microbes on the roots than on the shoots. However, the stronger influence on the roots can also be the result of a change in resource allocation from the roots to the shoots within the plant in response to stressful conditions, such as pathogenic microbes in the soil.

The species-specific differences of conditioned soils were less pronounced for soils that were conditioned for 8 or 10 months. This might be because these samples were taken in early January and March, the winter period. During winter, the functions of the microbial communities in the soil might be reduced due to cold temperatures, or might converge since decomposition processes may

TABLE 2 Results of permutational multivariate analysis of variance (PERMANOVA) testing the effect of monoculture soil (*Holcus lanatus*, *Festuca ovina*, *Alopecurus pratensis*, *Hypochaeris radicata*, *Jacobaea vulgaris* and *Taraxacum officinale*), round (2, 8 and 12 months) and total plant biomass on the leaf metabolome of *J. vulgaris*. Hereafter, the effect of monoculture soils and plant biomass on the metabolome was tested for each round separately. The analyses are based on Bray–Curtis distances. Permutations were set to 999. Pseudo *F*-values, degrees of freedom (*df*), explained variance (R^2) and *p*-values are presented. Significant *p*-values are presented in bold

Round	Factor	F	R^2	p
	Round (R)	$F_{(2,39)} = 30.37$	0.35	**0.001**
	Monoculture (M)	$F_{(5,39)} = 0.78$	0.02	0.58
	Total Biomass (B)	$F_{(1,39)} = 35.83$	0.21	**0.001**
	M × R	$F_{(10,39)} = 1.26$	0.07	0.28
	M × B	$F_{(5,39)} = 1.28$	0.04	0.27
	R × B	$F_{(2,39)} = 1.58$	0.02	0.21
2	M	$F_{(5,18)} = 0.75$	0.12	0.63
	B	$F_{(1,18)} = 1.70$	0.05	0.19
	M × B	$F_{(5,18)} = 1.61$	0.26	0.15
8	M	$F_{(5,5)} = 7.05$	0.29	**0.006**
	B	$F_{(1,5)} = 57.15$	0.47	**0.001**
	M × B	$F_{(5,5)} = 4.81$	0.20	**0.023**
12	M	$F_{(5,16)} = 1.68$	0.22	0.16
	B	$F_{(1,16)} = 11.42$	0.29	**0.001**
	M × B	$F_{(5,16)} = 0.62$	0.08	0.77

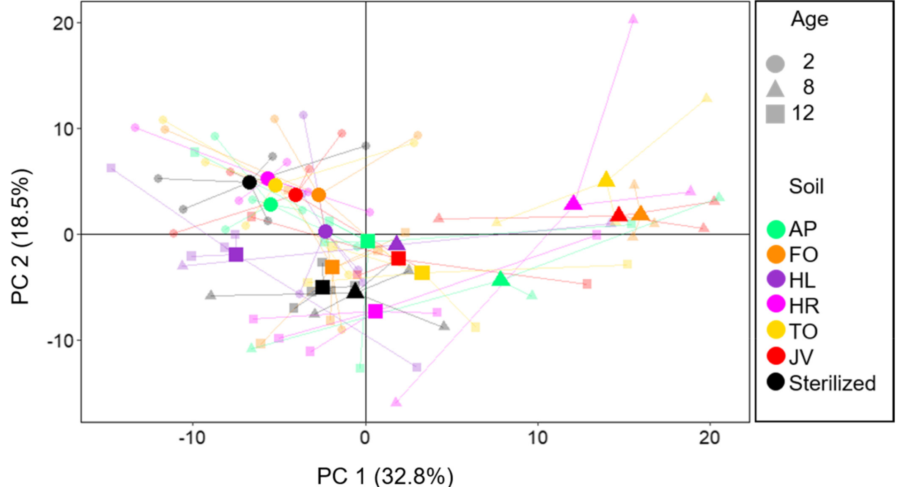

FIGURE 4 Principal component analysis (PCA) depicting the composition of the metabolome of *Jacobaea vulgaris* grown in monoculture (AP, *Alopecurus pratensis*; FO, *Festuca ovina*; HL, *Holcus lanatus*; HR, *Hypochaeris radicata*; TO, *Taraxacum officinale*; JV, *J. vulgaris* and in 100% sterilized soil (control) in the different rounds (2 months circles, 8 months triangles, 12 months squares). Centroids connected to the sample scores of the replicates for the first two axes of an unconstrained principal component analysis (PCA) are presented. The percentage explained variance by each axis is also depicted. PCAs depicting metabolomic profiles at each round are presented in Figure S3

TABLE 3 Relationships between properties measured in the plant (shoot and shoot biomass, metabolome and insect performance) and soil characteristics (soil abiotic properties, bacterial composition, fungal composition, bacteria abundance and fungi abundance) overall and for each round. The relationships with metabolomes were analysed with co-inertia analysis. For the co-inertia analysis, the RV coefficient and the significance tested with permutation tests (999 permutations) ae presented. Relationships between univariate variables (abundance data) were analysed with Pearson correlations. The correlation coefficient is presented. All other relations were analysed with redundancy analysis (RDA). For the RDAs, the explained variance and the significance tested with permutation tests (999 permutations) are displayed. *, ** indicate significant effects in the tests at $p < 0.05$; $p < 0.01$, respectively. Significant p-values are presented in bold

Round	Variable	Abiotic characteristics	Bacteria community	Fungi community	Bacteria abundance	Fungi abundance
	Shoot biomass (SB)	6.26***	1.55***	1.52**	0.18*	−0.17*
	Root biomass (RB)	4.11***	2.62***	1.81***	0.12	−0.18*
	Metabolome (M)	0.14	0.15*	0.11	0.02	0.03
	Larval weight gain (WG)	1.86	1.89**	2.57***	0.15	−0.03
	Consumed area (CA)	0.90	1.83*	7.25***	0.27*	−0.14
2	SB	6.40[a]	3.40	4.16	0.27	−0.15
	RB	5.97[a]	3.10	4.42	0.33	−0.09
	M	0.14[a]	0.42	0.43	0.03	0.01
	WG	1.05[a]	3.53	3.68	−0.09	0.01
	CA	0.09[a]	3.35	3.57	0.23	0.06
4	SB	6.05			−0.24	−0.44*
	RB	10.45			−0.48	−0.44
	M					
	WG	2.61			−0.17	0.01
	CA	3.79			−0.08	−0.22
8	SB	3.42	7.46[a]	7.06[b]	0.03	−0.03
	RB	2.73	7.37[b]	7.02[b]	−0.01	0.08
	M	0.26	0.52*[b]	0.45[b]	0.03	0.06
	WG	15.98*	6.81[b]	5.44[b]	−0.17	0.26
	CA	11.93	6.05[b]	6.76[b]	0.07	0.01
10	SB	12.48			−0,21	0.08
	RB	5.38			−0.22	0.10
	M					
	WG	2.62			0.08	0.15
	CA	13.64*			0.08	0.05
12	SB	5.16	3.94	5.27**	0.01	−0.10
	RB	6.46	4.08*	4.64*	−0.01	−0.10
	M	0.18	0.35	0.39*	0.03	0.07
	WG	6.06	3.66	4.07	−0.15	0.19
	CA	6.45	4.10	4.00	−0.23	0.12

[a]Communities were measured in soil after 6 months of conditioning.

[b]Soil abiotics were not measured after 2 months and data collected for 4 months were used.

dominate due to litter accumulation in all monocultures during the previous months. This litter effect could also be an explanation for the stronger links between the microbiome and the metabolome of *J. vulgaris* that we show in this study after 8 and 10 months of conditioning. In our study, it is not possible to disentangle seasonal from time effects and future studies should address this with long-term experiments carried out over multiple years.

We did not find any significant difference in the performance of *M. brassicae* feeding on the plants grown in differently conditioned soils except for the plants grown in conditioned soil of *T. officinale* after 12 months. At this time, the daily weight gain was higher on leaves from plants grown in *T. officinale* soil than on leaves from other plants. However, we did not find a correlation between the metabolome composition and the herbivore performance after 12 months. Multiple studies have found effects of PSFs on insect performance (Badri et al., 2013; Bezemer et al., 2013; Heinen et al., 2019). The abundance of the specialized aphid *Aphis jacobaeae*, for example, differed greatly among *J. vulgaris* plants grown in 10 differently

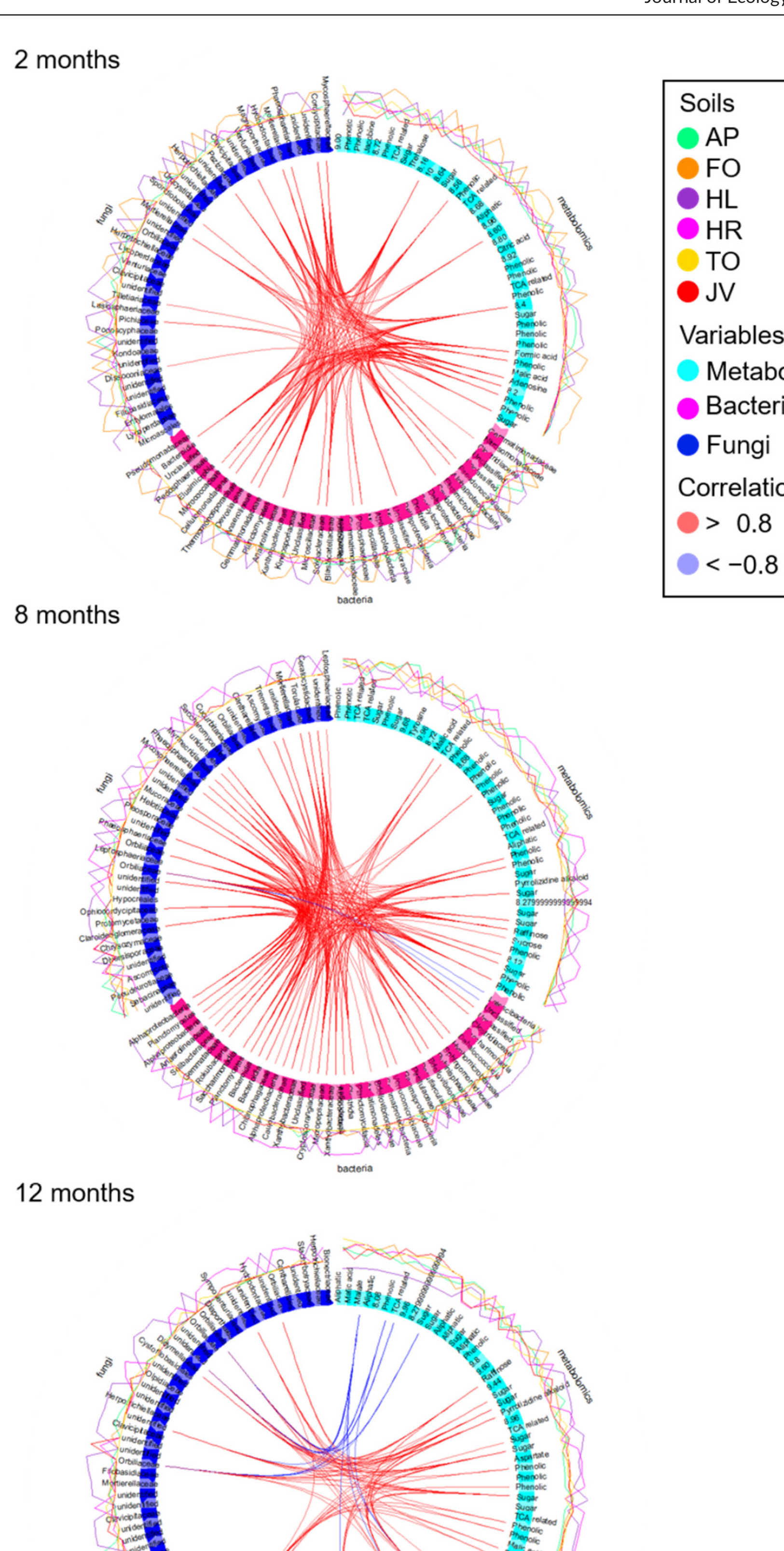

FIGURE 5 Circos plots visualizing the correlation between bacterial and fungal communities in the soil and metabolomic changes in *Jacobaea vulgaris* plants grown in these soils for each round. Displayed are correlations that are higher than 0.8 (red) or lower than −0.8 (blue). Lines outside the circles show the concentration of each compound in the plants in six monocultures. For the bacteria and the fungi, the abundance of each OTU in each monoculture is displayed. Correlations within each measured community (metabolome, bacteria and fungi) are not depicted. Plant species abbreviations are AP, *Alopecurus pratensis*; FO, *Festuca ovina*; HL, *Holcus lanatus*; HR, *Hypochaeris radicata*; JV, *J. vulgaris*; TO, *Taraxacum officinale*

conditioned soils (Kos et al., 2015b). However, the abundance of the generalist aphid species (*Brachycaudus cardui*) did not differ in the same experiment. This shows that the outcome of PSF studies investigating herbivore responses to PSF are variable and depend on the used insect species, test plant species and conditioning plant species. We observed that the performance of the herbivore was poorer on plants grown in soils conditioned for 8 months or longer, except on plants grown in soil conditioned by *T. officinale*. This could be due to the induction or priming of defensive responses in *J. vulgaris* in soils conditioned for a longer period. Although we did not measure plant hormones, which are a classical marker for priming, we detected a metabolomics pattern that may be related to priming in our study. Tricarboxylic acid (TCA) cycle compounds are among the most reactive metabolites upon priming (Pastor et al., 2014) and we found that more signals in the NMR spectra differed for tricarboxylic acid (TCA) cycle-related compounds in plants grown in soils conditioned for more than 4 months. However, this pattern can also be related to the lower weight of the plants in general on soils conditioned for more than 8 months, especially since we showed in an earlier study that in *J. vulgaris*, TCA-related compounds tend to be negatively correlated with biomass (Huberty, Martis, et al., 2020). While we cannot draw conclusions from our study about priming in the plant, the metabolomics approach we used identified pathways that should be investigated further, to examine plant physiological responses to soil conditioning. In our study, variation in herbivore responses among different times could have resulted from differences in herbivore batches used at the different times. However, all batches were treated and kept under the same conditions. Other studies on timing of particular treatments and insect herbivory have taken the approach that the treatments were initiated at different times and that herbivory in all treatments was tested at the same time (e.g. Wang et al., 2015; Wang et al., 2017). In our study, this was not feasible, as the effect of timing of conditioning would then interact with seasonal effect (e.g. some containers should be started in winter while others should be set up in the summer). Importantly, even though the absolute amount of herbivory varied considerably between rounds, our study also shows that the response of plant growth (biomass) of *J. vulgaris* to soil conditioning was relatively constant over time. While we cannot be conclusive about what drove the temporal differences in herbivore responses (seasonal effects on soils that cause temporal variation in plant quality or temporal variation in herbivore batches), we did not see strong effects of soil conditioning on herbivory overall, suggesting that the soil legacy effects on plants are more important than soil legacy effects on herbivores feeding from those plants.

The metabolome of *J. vulgaris* varied among the different rounds of the experiment and this occurred in conditioned soil, but also in sterilized soil. While the metabolome of plants grown in sterilized soil was similar in the 8- and 12-month sampling rounds, it was different in the 2-month round. One explanation for this pattern is that *J. vulgaris*, although kept under the same conditions in a climate chamber without seasons, has an imprinted seasonal rhythm, which might lead to growth differences between seasons or that this occurred due to epigenetic variation in the plantlets. The metabolites which were most influenced by growing in soils conditioned by different monocultures differed between the rounds. However, formic acid concentrations always differed among plants that grew in the different soils. Formic acid is well known for its antifungal properties below-ground (Rizaludin et al., 2021) and variation in the concentration in *J. vulgaris* shoots can be an indication of plant responses to the different microbiomes. With this, we show that apart from plant growth, PSFs can also impact plant metabolomes. In this study, we focus on leaf metabolomes. The reason to analyse leaf tissues was to link the plant metabolomes to leaf herbivore performance. Clearly, changes in the soil and in the soil microbiome can also influence root metabolomes (Mangeot-Peter et al., 2020). However, recent studies indicated that the effects of growing in different soil microbiomes on the metabolome were stronger above-ground than below-ground (Ristok et al., 2019).

The metabolomic patterns varied greatly between the rounds. This can be related to the high temporal variability that is typical for plant metabolomes. The temporal scales at which the metabolome and the biomass of a plant change are very different. Biomass changes are long-term changes while metabolomic changes can occur within seconds (Peters et al., 2018). This makes it difficult to relate both, but our study highlights the importance of long-term experiments with repeated metabolomics measurements for drawing reliable conclusions about plant–soil feedback effects on leaf metabolomics.

Correlations among changes in the soil microbiome and changes in the metabolome of the plants grown in these soils suggest that specific bacteria which are influenced by the host plant can influence the metabolome of the plants that grow later in the soil. Streptomycetaceae presence, for example, in our study was related to pyrrolizidine alkaloids in the plant. Streptomycetaceae are well known to promote plant growth and produce antibiotics (Olanrewaju & Babalola, 2019; Viaene et al., 2016). Our results suggest that Streptomycetaceae in the soils probably increase the concentrations of secondary metabolites such as PAs in plants, which grow in these soils. Our analysis identifies bacteria and fungi that correlate to specific effects in the metabolome of *J. vulgaris*. This represents a first important step for future studies investigating the connections between specific microbes and the metabolome. Furthermore, we show that several bacteria and fungi can be linked to single compounds in the metabolome while others influence a complex set of compounds. This indicates that in the soil community certain bacteria and fungi have specific effects on the metabolome of a plant.

The composition and abundance of the inoculated microbial community will change over time when the *J. vulgaris* plants grow in the inoculated soil. The assumption is that over time the impact of the current plant is increasing and the impact of the previous plant on the soil microbial community is declining (Hannula et al., 2021). However, other work in our group with the plant Chrysanthemum, another species of the Asteraceae family, has shown that the microbial composition in pots inoculated with soils from different monocultures still differs after Chrysanthemum plants have been

grown in these inoculated soils for 2–3 months (Ma et al., 2020; Pineda et al., 2020). As the metabolome in our study was determined at the end of the experimental growth period, future studies should also sequence the microbial community in soils after the test plants have grown in those soils rather than sequencing the inocula as we did in the current study, to examine linkages between the plant metabolome and the soil microbial community at the time of sampling.

Available nutrients in the soil did only explain certain herbivore performance traits, but not the changes within the plant. When relating changes in the soil to changes in the plant, we face the problem of different temporal and spatial scales at which they act. The microbiome of the soil as well as the metabolome of the plant can change quickly, while plant biomass is less variable and changes become visible only after longer time periods. This is a hurdle when linking more classical ecological parameters with new multivariate techniques that cannot be totally overcome and that researchers should be aware of when conducting such studies (Peters et al., 2018).

In conclusion, we show that soil legacy effects of six different monocultures on above- and below-ground biomass of a common test plant differ in magnitude over time, but that the overall response pattern for home versus away soil remains similar over time. Herbivory was not strongly affected by soil conditioning. Interestingly, we find that plant metabolomic responses to soil conditioning are highly inconsistent and became more apparent in the later sampling rounds. This indicates that the effects of soil legacies on plant metabolomic profiles become more pronounced when the soil is conditioned for a longer period of time, which is in stark contrast with the relatively consistent responses we observed in plant biomass. This strongly suggests that soil legacy effects may have farther-reaching impacts than on plant growth alone. As plants are the primary resources for most organisms, soil legacy effects, through plant-metabolomic processes, may have lasting impacts higher in the ecological food web.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

M.H. and T.M.B. designed and planned the experiment; R.H., K.S., R.J., S.E.H. and M.H. carried out the experiments and kept the containers running for 1 year; M.H. processed the metabolomics samples and analysed the results with T.M.B. and Y.H.C.; S.E.H. processed and analysed the sequencing data; M.H. wrote the first version of the manuscript and all co-authors critically added to the manuscript.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/1365-2745.13872.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in figshare at https://doi.org/10.6084/m9.figshare.19322561.v1 (Huberty et al., 2022).

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