

Research

Natural rubber reduces herbivory and alters the microbiome below ground

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Introduction

Tissue integrity and wound sealing are vital for all organisms as wounds constrain tissue functioning and facilitate the entry of pathogens (Savatin *et al.*, 2014). In vertebrates, wounding often involves the disruption of blood vessels, and consequently the draining blood carries defensive cells, antibodies, and coagulants to the site of wounding. Similar wound sealing and defensive processes are evident in laticifers, specialized cells found in *c*. 10% of all flowering plants. The laticifers' cytoplasm, called latex, exudes upon damage and can thereby deter or impair the growth of herbivores, or even kill them outright (Dussourd, 1995; Konno *et al.*, 2004; Bont *et al.*, 2017). Consequently, latex may reduce plant damage and improve plant fitness under herbivory (Agrawal & Konno, 2009; Konno, 2011; Huber *et al.*, 2016a,b; Salomé Abarca *et al.*, 2019; Castelblanque *et al.*, 2021; Gracz-Bernaciak *et al.*, 2021).

Apart from defense against herbivores, laticifers are hypothesized to mediate plant-microbe interactions (Agrawal & Konno, 2009). Experimental evidence for this notion is, however, scarce. Mutants

Summary

• Laticifers are hypothesized to mediate both plant-herbivore and plant-microbe interactions. However, there is little evidence for this dual function.

• We investigated whether the major constituent of natural rubber, *cis*-1,4-polyisoprene, a phylogenetically widespread and economically important latex polymer, alters plant resistance and the root microbiome of the Russian dandelion (*Taraxacum koksaghyz*) under attack of a root herbivore, the larva of the May cockchafer (*Melolontha melolontha*).

• Rubber-depleted transgenic plants lost more shoot and root biomass upon herbivory than normal rubber content near-isogenic lines. *Melolontha melolontha* preferred to feed on artificial diet supplemented with rubber-depleted rather than normal rubber content latex. Likewise, adding purified *cis*-1,4-polyisoprene in ecologically relevant concentrations to diet deterred larval feeding and reduced larval weight gain. Metagenomics and metabarcoding revealed that abolishing biosynthesis of natural rubber alters the structure but not the diversity of the rhizosphere and root microbiota (ecto- and endophytes) and that these changes depended on *M. melolontha* damage. However, the assumption that rubber reduces microbial colonization or pathogen load is contradicted by four lines of evidence.

• Taken together, our data demonstrate that natural rubber biosynthesis reduces herbivory and alters the plant microbiota, which highlights the role of plant-specialized metabolites and secretory structures in shaping multitrophic interactions.

of *Euphorbia lathyris* that lack laticifers showed enhanced resistance toward the fungal leaf pathogen *Botrytis cinerea*, possibly due to the presence of microbial growth stimulating factors in laticifers (Castelblanque *et al.*, 2021). Whether latex mediates plant-microbe interactions upon exuding through wounds is, however, unclear. Considering that laticifers are among the most common defensive reservoirs in plants (Lewinsohn, 1991), this knowledge gap significantly constrains our understanding of plant constitutive defenses against microbes.

Latex typically contains high concentrations of specialized metabolites (Sessa *et al.*, 2000; Agrawal & Konno, 2009; Huber *et al.*, 2015; Salomé-Abarca *et al.*, 2021) that are known to serve as defenses against herbivores (Steppuhn *et al.*, 2004; Züst *et al.*, 2012; Kerwin *et al.*, 2015; Huber *et al.*, 2016b; Li *et al.*, 2018). These and other specialized metabolites are also increasingly recognized as mediators of plant–microbe interactions (Schütz *et al.*, 2021). For instance, specialized metabolites show growth-promoting or growth-inhibiting activities toward microbes (Pang *et al.*, 2021), and transgenic plants deficient in specialized metabolites can be more susceptible to pathogens

(Tierens et al., 2001; Bednarek et al., 2009; Koprivova et al., 2019; Chen et al., 2020). Furthermore, abolishing the production of specialized metabolites, such as coumarins, benzoxazinoids, and triterpene derivatives, through genetic manipulations has revealed their role in altering the composition and function of the root and rhizosphere microbiome (Hu et al., 2018; Stringlis et al., 2018; Cotton et al., 2019; Huang et al., 2019; Voges et al., 2019; Jacoby et al., 2021). As specialized metabolites may alter both plant-herbivore and plant-microbe interactions, and as herbivores and microbes may facilitate or inhibit each other (Friedli & Bacher, 2001; Koornneef & Pieterse, 2008; Robert-Seilaniantz et al., 2011; Savatin et al., 2014; Willsey et al., 2017; Hilleary & Gilroy, 2018), it is critical to study the impact of plant specialized metabolites on the microbiome in the presence or absence of herbivores, which is, however, rarely done (Hu et al., 2018).

A latex metabolite that has frequently been hypothesized to mediate both plant-microbe and plant-herbivore interactions is natural rubber, which consists of over 90% cis-1,4-polyisoprene. Natural rubber is among the economically most important plant polymers (Mooibroek & Cornish, 2000) and has likely convergently evolved implicating important ecological functions (Metcalfe, 1967; Agrawal & Konno, 2009). The most commonly postulated function involves herbivore defense since natural rubber is sticky and thus may entrap entire insects or glue their mouthparts (Dussourd & Eisner, 1987; Dussourd, 1995). Apart from herbivore defense, natural rubber may also contribute to wound sealing, thereby preventing the entry of pathogenic microorganisms. Wound sealing may be particularly important below ground, as the soil harbors a rich microbiota including pathogens (Tringe et al., 2005; Fierer & Jackson, 2006). Whether natural rubber alters plant-herbivore and plant-microbe interactions in planta is, however, unclear.

The Russian dandelion (Taraxacum koksaghyz, Asteraceae)-an obligate outcrossing diploid-accumulates particularly high quantities of high-molecular-weight natural rubber in its laticifers (Ulmann, 1951). Taraxacum koksaghyz, native to Kazakhstan and the western part of Xinjiang (China), is increasingly cultivated as a rubber crop in Europe, where it is attacked by the soil-dwelling herbivorous larvae of the May cockchafer, Melolontha melolontha (Scarabeidae, Coleoptera; Härdtl, 1953). Melolontha melolontha, native to Europe, is polyphagous like many Scarabeidae larvae that occur in the native range of T. koksaghyz (Gninenko, 1998; Malysh et al., 2006; Jackson & Klein, 2006), although its preferred host plants are dandelions (Hauss & Schütte, 1976).

Here, we address the hypotheses that *cis*-1,4-polyisoprene: improves resistance of T. koksaghyz to M. melolontha herbivory; alters the plant microbiome; and reduces pathogen load under M. melolontha attack. Using transgenic rubber-deficient lines, chemical supplementation, and behavioral assays, we demonstrate that cis-1,4-polyisoprene helps protect T. koksaghyz from M. melolontha herbivory. Furthermore, amplicon sequencing and shotgun metagenomics revealed that the biosynthesis of this polyisoprene may alter the root and rhizosphere microbiome but not the pathogen load and that the changes in the microbiome depend on the presence of the *M. melolontha* larva.

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Materials and Methods

Plant material and growth conditions

Taraxacum koksaghyz and Daucus carota ssp. sativus (Nantaise2/ Jubila, Kiepenkerl; Bruno Nebelung GmbH, Everswinkel, Germany) were cultivated in a glasshouse with temperature 20-25°C, 20 klx light intensity (high-pressure sodium lamp, HPS 600 Watts, Greenbud, enhanced yellow and red spectrum) with a 16 h photoperiod. Two independently transformed TkCPTL1-RNAi lines (RNAi-'rubber-depleted') and their respective rubber-bearing near isogenic lines (NILs-'normal rubber content') were used (Niephaus et al., 2019). For details on lines and cultivation, see Methods S1 and S2.

Insect material

Melolontha melolontha L. larvae were collected from meadows in Switzerland (46.692442°N, 9.410272°E; Urmein, Viamala region) and Germany (49.936182°N, 9.279764°E; Mespelbrunn, Spessart region). Experiments were performed with the third instar larvae (L3). Insects were reared individually in 180-ml plastic beakers filled with a mix of potting soil and grated carrots in 24 h darkness at 12–14°C. For experiments, larvae were starved for 72 h in the dark at room temperature. All ex planta experiments were performed in the dark to avoid light disturbance.

Data analysis

Data analysis was performed with R v.4.1.2 (R Core Team, 2020) and visualized with GGPLOT2 (Wickham, 2016). Details of the procedures are described in the individual sections.

Resistance of rubber-depleted TkCPTL1-RNAi and rubberbearing NIL plants under M. melolontha herbivory

To test whether natural rubber benefits T. koksaghyz under root herbivory in vivo, two rubber-depleted (TkCPTL1-RNAi-A and -B) lines and their respective normal rubber content NIL controls (NIL-A and -B) (plant genotype - rubber-depleted or normal rubber content) were subjected to M. melolontha herbivory. Taraxacum koksaghyz stores up to 2-12% natural rubber of root dry weight (DW; Ulmann, 1951). The concentration of cispolyisoprene in the roots of RNAi plants is reduced by c. 90% after transgenic knockdown of the expression of a cisprenyltransferase-like subunit (CPTL1), a protein that is required for rubber chain elongation in the laticifers (Niephaus et al., 2019). We cultivated plants for 10 wk, a time point at which NIL plants accumulate natural rubber to 2-3% per root DW (Niephaus et al., 2019). Half of the replicates were then infested with one preweighed M. melolontha larva. After 15 d, plant roots and shoots were harvested and weighed, and larval weight gain was measured. Experiments with lines from the A and B genetic backgrounds were performed at different time points. Since the A lines suffered high infestation of white flies and thrips during the experiment, they were excluded from the analysis. Plants that spontaneously flowered, heavily wilted due to loss of the main root during larval feeding, or whose larva died during the experiment were also excluded from analysis (remaining dataset n = 11-18). The shoot and root fresh weights (FW) were analyzed with linear models, testing for the effect of plant genotype, herbivory treatment, and their interaction. Pairwise comparisons between treatments within each plant genotype were adjusted using the FDR method with the package EMMEANS (Lenth, 2022). Difference in larval weight gain between normal rubber content and rubber-depleted plants was analyzed with a Wilcoxon signed-rank test.

Choice experiment with carrot seedlings supplemented with latex

To investigate whether natural rubber alters M. melolontha feeding preference, we recorded the choice of *M. melolontha* larvae between carrot seedlings coated with latex of either TkCPTL1-RNAi or NIL plants similar to as described (Huber et al., 2016b). The coating approach allows to test the effect of latex metabolites independent of associated changes in the remaining root tissue. Carrot seedlings were used as a homogenous food source that is readily accepted by *M. melolontha* larvae. Roots of 5-wk-old carrot seedlings were covered entirely with latex of freshly cut 10-wk-old TkCPTL1-RNAi or NIL T. koksaghyz roots of the A and B genetic backgrounds. Seedlings painted with TkCPTL1-RNAi and NIL latex were pairwise arranged on opposite sides of 180-ml beakers filled with vermiculite (n = 23 Å-pairs; n = 28 B-pairs). One larva was then placed in the center of each beaker. Larval position (TkCPTL1-RNAi side, NIL side, inactive = no choice) was recorded every hour until 6-7 h after start of the experiment. Experiments on A and B plants were performed at two separate time points. Choice data were analyzed for each line and time point separately using a binomial test.

Choice experiment with carrot seedling supplemented with *cis*-1,4-polyisoprene

As transgenic plants may exhibit side effects due to RNAi silencing, we test whether cis-1,4-polyisoprene is sufficient to deter M. melolontha feeding. To this end, we assessed the choice of the larvae between carrot seedlings supplemented with ecologically relevant concentrations of purified cis-1,4-polyisoprene (purification protocol in Methods \$3) or a solvent control. Soil adhering to 8wk-old carrot seedlings was washed off, and seedlings were arranged in pairs of homogeneously looking root sizes (n = 47). Carrot roots were dipped three times either in 1% w/v purified cis-1,4-polyisoprene dissolved in chloroform or chloroform (solvent control), resulting in c. 1.1% cis-1,4-polyisoprene based on root FW, similar to concentrations of natural rubber in roots of wild-type (WT) T. koksaghyz (0.2-1.2% of root FW (Ulmann, 1951)). Carrot seedlings of each treatment were placed on opposite sides of 180-ml beakers filled with vermiculite. One M. melolontha larva was placed in the center of each beaker, and larval position (cis-1,4-polyisoprene side, solvent control side,

inactive = no choice) was recorded after 1, 2, 3, 4, and 6 h. Choice data were analyzed for each line and time point separately using a binomial test.

Nonchoice experiment with artificial diet supplemented with *cis*-1,4-polyisoprene

To investigate whether *cis*-1,4-polyisoprene may also alter larval performance, we fed *M. melolontha* larvae for five consecutive days with artificial diet supplemented with isolated cis-1,4polvisoprene (Methods \$3) or solvent as a control. Artificial diet cubes of 400 mg (Huber et al., 2016b) were supplemented with either 1.2 ml of a 1.1% w/v cis-1,4-polyisoprene solution in chloroform or chloroform as a solvent control, resulting in 3% cis-1,4-polyisoprene in the rubber-supplemented cubes. Cubes were incubated under a fume hood for 1 h to allow chloroform to fully evaporate before the feeding assays. Preweighed larvae were allowed to feed solitarily on a diet cube for 24 h inside a 180-ml plastic beaker covered with a moist tissue (n = 26 per treatment). The procedure was repeated with the same individuals for five consecutive days. Larvae that did not feed throughout the experiment were excluded from the analysis. Daily weight gain was analyzed using a linear mixed effect model with day and individual larva as random effects using the package LME4. Total larval weight gain after 5 d and total weight gain per total consumed diet were analyzed with Wilcoxon signed-rank tests.

Role of triterpenes in *T. koksaghyz* resistance and *M. melolontha* behavior

As a complementary approach to assess whether any variation in herbivore resistance between the RNAi and NIL plants is due to the polyisoprene rather than the threefold increase in triterpene content in the RNAi-silenced plants (Niephaus *et al.*, 2019), we specifically assessed the effect of triterpenes in the interaction of *T. koksaghyz* to *M. melolontha.* To this end, we tested herbivore resistance of two independent triterpene-reduced *TkOSC*-RNAi lines (oxidosqualene cyclase knockdown; *TkOSC*-RNAi-L2 and -L3; 73–80% reduced pentacyclic triterpene content, but normal *cis*-1,4-polyisoprene levels) (van Deenen *et al.*, 2019). Second, we assessed whether lupeol, a *T. koksaghyz* triterpene, alters *M. melolontha* growth when added to artificial diet in physiologically relevant amounts (Unland *et al.*, 2018; Pütter *et al.*, 2019). Details on the experimental setup and statistical analysis can be found in Methods S4.

Microbial colonization the of root-soil continuum upon herbivory

To investigate whether natural rubber biosynthesis alters the microbial colonization of roots and the rhizosphere upon damage, we compared root and rhizosphere microbiomes of rubberdepleted *Tk*CPTL1-RNAi-A and normal rubber content NIL-A plants ('plant genotype') under control conditions, *M. melolontha* herbivory and mechanical wounding ('treatment') using amplicon sequencing and shotgun metagenomics. Mechanical wounding was included to impose a uniform damage treatment that is unaffected by TkCPTL1-RNAi silencing. All plants were propagated in soil originating from an agricultural field in which T. koksaghyz had been cultivated for several years (for details, see Methods S_5). Plants were either noninfested (NIL n = 10, TkCPTL1-RNAi n = 10, infested with one preweighed M. melolontha larva (NIL n = 9, TkCPTL1-RNAi n = 10), or wounded using a metal stick pricking into the pots 10 times every third day (NIL n = 9, R TkCPTL1-RNAi n = 9). At 14 d after infestation, plants were harvested, root and shoot FW determined, and biomass data analyzed as described above. For assessing the microbiome, we chose a subset of six plants per treatment that showed visual signs of damage upon herbivory or wounding. To obtain the rhizosphere microbiome fraction, roots were washed in three consecutive steps with 30 ml sterile water similar to as described (Hu et al., 2018), and washing fractions were combined. To obtain the root microbiome (ecto- and endophytes), washed roots were subsequently frozen in liquid nitrogen, stored at -20° C, lyophilized and then ground to a fine. Details can be found in the Methods S6.

DNA extraction, library preparation, and sequencing

DNA was isolated from the lyophilized rhizosphere washing fraction and root powder using the DNeasy Powersoil Pro kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions (n = 6 per treatment). To account for possible contaminations, a negative control was included where the root powder was replaced by the same amount of ultrapure water. Extracted DNA was quantified and checked for purity using a spectrophotometer (Pearl 3435; Implen, Munich, Germany) and submitted to Novogene (Beijing, China) for library preparation and sequencing.

Shotgun metagenomics The genomic DNA was randomly fragmented by sonication, and then DNA fragments were endpolished, A-tailed, and ligated with full-length adapters for Illumina sequencing, followed by PCR amplification with P5 and indexed P7 oligos. The PCR products as the final construction of the libraries were purified using the AMPure XP reagents. Then, libraries were checked for size distribution by an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), and quantified by qPCR. Libraries were then sequenced on an Illumina NovaSeq 6000 instrument (Illumina, San Diego, CA, USA) on a S4 150PE flow cell.

Amplicon sequencing DNA extracted from the rhizosphere and roots was processed to generate an amplicon library targeting the 16S (bacteria) and ITS2 (fungi) regions of the rRNA. Briefly, after samples passed QC, libraries were built by amplifying the 16S rRNA gene (515F and 806R primers) or the ITS2 rRNA gene (ITS3-2024F and ITS4-2409R primers). Purified PCR products (AMPure XP; Beckman Coulter Inc., Brea, CA, USA) were used for a second PCR to ligate Illumina adapters. Libraries were purified again, pooled at equimolar ratio, and sequenced on Illumina NovaSeq 6000 instrument (Illumina) on a SP 250PE flow cell. Bioinformatics data processing and analysis

Shotgun metagenomics Raw data were processed with TRIM-GALORE v.0.6.6 (Krueger, 2020) to remove adapters and lowquality reads, and reads obtained from the host plant were discarded through BOWTIE2 v.2.4.4 (Langmead & Salzberg, 2012) using the *T. koksaghyz* genome (Lin *et al.*, 2018). Reads were merged into contigs using MEGAHIT v.1.2.9 (Li *et al.*, 2015), and functional annotation was performed using PROKKA v.1.14.6 (Seemann, 2014). Raw reads were mapped against the output from PROKKA using BOWTIE2 and SAMTOOLS (Danecek *et al.*, 2021) obtaining a count matrix of gene frequency for each sample.

Amplicon sequencing Demultiplexed forward and reverse reads were merged using the PEAR 0.9.1 algorithm with default parameters (Zhang *et al.*, 2014). Data QC, operational taxonomic unit (OTU) clustering, and chimera removal were carried out using VSEARCH 2.14.2 (Rognes *et al.*, 2016). Taxonomy was assigned to each OTU using VSEARCH by querying the SILVA database (v.138; Quast *et al.*, 2013). Singletons and OTUs coming from amplification of plastidial DNA were discarded from the downstream analyses.

Microbiome data analysis

Diversity analysis Shannon and Simpson diversity indices were calculated for each sample using the package PHYLOSEQ (McMurdie & Holmes, 2013). Then, two different generalized linear models were fit for Shannon and Simpson diversity indexes using Bayesian Hamiltonian Markov chain Monte Carlo in the package RSTANARM (Goodrich et al., 2020), testing the effect of plant genotype, treatment, and their interactions on the diversity metric. Weakly informative normally distributed priors were used for both the intercept (mean = 0, scale = 2.5) and the coefficients (mean = 0, scale = 2.5), with autoscaling turned on, running four chains with 2000 iterations each and discarding the first 1000 as burn-in, for a total of 4000 observations. Comparisons between groups were performed using the function hypothesis of the BRMS package (Bürkner, 2017). The equivalent of a two-tailed P-value (pMCMC) was estimated by calculating and doubling the frequency at which any of the 4000 observations disagreed with (had opposite sign to) the posterior estimate.

Multivariate analysis In order to perform the structure analysis, the OTU counts were normalized using DESEQ2 (Love *et al.*, 2014), and then fit to a Bayesian generalized linear multivariate multilevel model in the BRMS package (Bürkner, 2017), testing the effect of plant genotype, treatment, and their interaction; running four chains with 2000 iterations each and discarding the first 1000 as warm-up, for a total of 4000 observations. Comparisons between groups were performed using the function hypothesis of the BRMS package; the equivalent of a two-tailed *P*-value (pMCMC) was estimated as reported above.

Differential taxa Each plant genotype (NIL-A and TkCPTL1-RNAi-A) and each treatment (herbivory or wounding) were

contrasted toward the respective control group using DESEQ2, keeping the OTUs that resulted being differentially abundant within each treatment/control combination (FDR-adjusted P < 0.05). At the same time, a similar set of data was generated, but the counts were randomized within the OTU table, while keeping the number of reads within each sample constant. This enabled to test whether the number of differentially abundant OTUs in each group was different from random using a chi-squared test. Similarly, we tested whether treatment (within each of the plant genotypes) influences the relative abundance of OTUs grouped for each microbial genus. To this end, data were aggregated at the 'Genus' taxonomic level, and filtered to remove those genera that represent <1% of the community within each group. Then, the influence of treatment on the relative abundance of each bacterial genus for each plant genotype was tested separately by fitting a linear model using the LME4 package. We used a chi-squared test to test the effect of plant genotype on the number of unique OTUs of each treatment (wounding, herbivory) compared with the respective control group for each compartment (root, rhizosphere) separately.

Magnitude of change Each plant genotype (NIL-A and *Tk*CPTL1-RNAi-A) and each treatment (herbivory or wounding) was contrasted toward the respective control group using DESEq2. Then, abs(log₂FC) values were then compared by fitting linear mixed effect models each including one of the four combinations of genotypes (NIL-A and *Tk*CPTL1-RNAi-A) and treatments (herbivory or wounding) and *geneID* as random factor with the package LME4. Pairwise comparisons were then tested using the package EMMEANS. Gene content analysis The gene count table was normalized using DESEQ2 and then fit to a Bayesian generalized linear multivariate multilevel model as described above. Also, genes differentially abundant between each treatment (herbivory or wounding)/control combination, within each plant genotype, were identified using DESEQ2 and their number was tested compared with a random null model as described above.

Results

Cis-1,4-polyisoprene benefits *T. koksaghyz* under *M. melolontha* attack

To test whether the biosynthesis of cis-1,4-polyisoprene benefits plant performance upon root herbivory, we subjected plants of transgenic cis-1,4-polyisoprene-depleted TkCPTL1-RNAi lines ('rubber-depleted') and the corresponding NIL lines ('normal rubber-content') to M. melolontha herbivory. Rubber-depleted TkCPTL1-RNAi plants suffered a stronger reduction in shoot growth under *M. melolontha* herbivory than the rubber-bearing NIL plants (P = 0.03, Fig. 1a). In NIL plants, *M. melolontha* herbivory did not affect shoot growth (P = 0.9, Fig. 1a), whereas in TkCPTL1-RNAi plants, M. melolontha reduced shoot biomass by 33% (P = 0.003, Fig. 1a). A similar pattern was observed for root biomass: in NIL plants, M. melolontha herbivory did not affect root biomass (P = 0.4, Fig. 1b), whereas in TkCPTL1-RNAi plants, M. melolontha reduced root biomass by 34% (P = 0.02, Fig. 1b). Melolontha melolontha larvae gained approximately twofold more weight on the TkCPTL1-RNAi than on the



Fig. 1 Silencing the biosynthesis of the major natural rubber metabolite, *cis*-1,4-polyisoprene, reduces the performance of *Taraxacum koksaghyz* under *Melolontha melolontha* herbivory. (a) Shoot and (b) root fresh weight accumulation of *T. koksaghyz* after 15 d of *M. melolontha* herbivory in rubber-depleted *Tk*CPTL1-RNAi lines and rubber-bearing near isogenic lines (NIL) plants ('plant genotype'). *P*-values of linear models are shown on top of the panels. *P*-values of pairwise comparisons were adjusted using the FDR method. (c) *Melolontha melolontha* weight gain on *Tk*CPTL1-RNAi and NIL plants. *P*-values of Wilcoxon signed-rank test is shown. df, degrees of freedom; SS, sum squares, *n* = 11–18. Error bars indicate SE.

NIL plants, but these differences were statistically not significant due to the large within-group variation ($P \ge 0.13$, Fig. 1c). Originally, RNAi and control NIL lines of two different genetic backgrounds, A and B, were tested. Only data for the B background, however, are presented here. Plant resistance in the A background could not be analyzed as growth was dwarfed due to high levels of white fly and thrips infestation. However, in a follow-up experiment, RNAi plants tended to be more resistant than the corresponding NIL plants also in the A background (Fig. S1).

To corroborate the role of natural rubber in herbivore defense, we performed a series of *in planta* and *ex planta* experiments. First, we assessed *M. melolontha* feeding preference between carrot seedlings coated with latex of *Tk*CPTL1-RNAi or their respective NIL plants. For both the A and the B lines, larvae preferred to feed on carrot seedlings painted with latex of *Tk*CPTL1-RNAi plants 4 h and 7 h after the start of the experiment, respectively (P < 0.05), with *c*. 75% of the larvae choosing the rubber-deficient plants (Fig. 2a). Larval choice showed an



(b) Nonchoice experiment



Fig. 2 Ecologically relevant concentrations of *cis*-1,4-polyisoprene deter *Melolontha melolontha* feeding and reduce food quality. (a) Choice of *M. melolontha* larvae between carrot seedlings supplemented with latex of rubber-depleted TkCPTL1-RNAi plants and normal rubber content near isogenic lines (NIL) plants, or between seedlings supplemented with 1.1% *cis*-1,4-polyisoprene or a solvent control. *P*-values of binomial tests are shown. The number of active larvae is indicated inside the bars. Data were recorded at time points when larvae first showed significant choice behavior. (b) Diet consumption, larval weight gain, and larval weight gain per consumed diet of *M. melolontha* larvae feeding for five consecutive days on artificial diet with 3% *cis*-1,4-polyisoprene or solvent control. Boxplots show the median and the interequartile range (IQR) of the data distribution, with whiskers extending to 1.5 times the IQR. Individual points show raw data points with outliers as individual points outside the whiskers. *P*-values refer to a linear mixed effect model in the left panel, and Wilcoxon signed-rank tests in the middle and right panels. df, degrees of freedom; *n* = 24–26.

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expected temporal pattern, with a gradual increase in the number of larvae on the rubber-depleted side, followed by a neutral distribution of the larvae (Fig. S2).

Second, we assessed *M. melolontha* feeding preference between carrot seedlings coated with ecologically relevant concentrations of purified *cis*-1,4-polyisoprene or a solvent control. Five hours after the start of the experiment, larvae preferred to feed on control rather than on *cis*-1,4-polyisoprene supplemented roots (P = 0.014, Fig. 2a), with 69% of the larvae feeding on the control roots.

Third, we measured larval growth and food consumption over five consecutive days in a nonchoice assay in which larvae were offered artificial diet supplemented with either an ecologically relevant concentration of *cis*-1,4-polyisoprene or a solvent control. Surprisingly, larvae consumed on average 13% more *cis*-1,4polyisoprene supplemented diet than the control diet (P = 0.013, Fig. 2b). Nevertheless, larvae lost threefold more weight within the 5 d on the *cis*-1,4-polyisoprene supplemented diet (P = 0.01, Fig. 2b). Consequently, *cis*-1,4-polyisoprene supplementation reduced larval weight gain per mg consumed diet (P = 0.048, Fig. 2b).

Finally, we specifically tested the effect of pentacyclic triterpenes – which are elevated threefold in the rubber-depleted TkCPTL1-RNAi compared with NIL lines (Niephaus *et al.*, 2019)–on plant resistance. *Taraxacum koksaghyz* resistance and *M. melolontha* weight gain did not differ between two independent triterpene-reduced *TkOSC*-RNAi lines and their respective triterpene-containing NIL lines. Furthermore, lupeol, a major *T. koksaghyz* triterpene (Pütter *et al.*, 2019), did not alter diet consumption or larval weight gain when supplemented in ecologically relevant concentrations to artificial diet (Figs S3–S5).

Taken together, these data provide complementary evidence that *cis*-1,4-polyisoprene deters *M. melolontha* feeding and thereby benefits *T. koksaghyz* under *M. melolontha* attack.

Natural rubber biosynthesis alters the structure of the *T. koksaghyz* root and rhizosphere microbiome upon tissue damage via *M. melolontha* herbivory and mechanical wounding treatment

To test whether natural rubber may alter the plant microbiome, we assessed the root and rhizosphere microbiome of rubberdepleted *Tk*CPTL1-RNAi and the normal rubber content control NIL lines in the A genetic background using plants grown in natural field soil under control, *M. melolontha* herbivory and mechanical wounding treatments.

We first tested the hypothesis that natural rubber and the damage treatments (wounding or herbivory) alter the microbial diversity. Based on both Shannon and Simpson indices, neither the RNAi silencing nor the damage treatments altered the rhizosphere or root microbial diversity (Fig. S6; Tables S1–S4).

Next, we tested whether RNAi silencing and the damage treatments alter the root and rhizosphere microbial structure. In the rhizosphere, the structure of the bacterial but not fungal community was affected by both the plant genotype and the damage treatment (P(NIL control-RNAi control) = 0.0005 for bacteria and 0.466 for fungi; P(NIL control-NIL herbivory or NIL wounding) = 0.0005 for bacteria and > 0.5 for fungi; Table 1; Figs S7, S8). Interestingly, the effect of the damage treatment depended on the plant genotype: in NIL plants, both herbivory and wounding influenced the structure of the rhizosphere bacterial community, whereas in TkCPTL1-RNAi plants, only herbivory had such an effect (pMCMC = 0.01). By contrast, in NIL plants, neither herbivory nor wounding affected the structure of the rhizosphere fungal community (pMCMC > 0.47), whereas in TkCPTL1-RNAi plants, both herbivory and wounding altered the rhizosphere fungal community (pMCMC < 0.03, Table 1; Figs S9, S10). These changes were also reflected in the microbial gene pool of the rhizosphere obtained from shotgun metagenomic sequencing, which was altered by both the plant genotype

Table 1 *P*-values (pMCMC) of multivariate analysis investigating the structure of the rhizosphere and root microbiome of rubber-deficient *Tk*CPTL1-RNAi and normal rubber content NIL *Taraxacum koksaghyz* plants growing in natural field soil under control conditions, *Melolontha melolontha* herbivory or mechanical wounding.

Pairwise comparison Sequencing method	デ社 Rhizosphere			Root	
	16S amplicon (bacteria)	ITS amplicon (fungi)	Shotgun metagenomic	16S amplicon (bacteria)	ITS amplicon (fungi)
NIL wounding – NIL control	0.0005	0.5	0.0815	0.0005	0.5
NIL herbivory – NIL wounding	0.7	0.7	0.0005	0.0055	0.7
RNAi herbivory – RNAi control	0.0115	0.02	0.0005	0.0590	0.2
RNAi wounding – RNAi control	0.4	0.03	0.0005	0.0005	0.1
RNAi herbivory – RNAi wounding	0.1	0.9	0.0005	0.0005	0.7
NIL control – RNAi control	0.0005	0.5	0.0005	0.0005	0.7
NIL herbivory – RNAi herbivory	0.0070	0.2	0.0005	0.0005	0.1
NIL wounding – RNAi wounding	0.5	0.4	0.0005	0.0005	0.1

P-values of pairwise comparisons were obtained by fitting a Bayesian generalized linear multivariate multilevel model using normalized operational taxonomic unit counts from 16S, ITS and shotgun metagenome datasets for rhizosphere samples, and from 16S and ITS datasets from root samples. Bold entries indicate *P*-values < 0.05. n = 6.

(Table 1; Fig. 3a) and the treatment within each plant genotype (Table 1; Fig. S11).

Similar to the rhizosphere in the plant roots, the bacterial but not the fungal community structure was altered by the plant genotype, treatment, and their interaction (Figs S7–S10). Specifically, in NIL plants, both herbivory and wounding influenced the structure of the root bacterial community (pMCMC < 0.001), while in *Tk*CPTL1-RNAi plants, only wounding had this effect (pMCMC < 0.001; Table 1; Fig. S9). None of the treatments altered the structure of the fungal community in either plant genotype (Table 1; Fig. S10).

Since multivariate analysis only reveals differences in microbial community structure, but not in the extent of these changes, we calculated the magnitude of change in the rhizosphere and root microbiomes (absolute \log_2 fold changes) upon wounding or herbivory in each plant genotype. In the rhizosphere, the bacterial microbiota showed a larger magnitude of change upon both herbivory and wounding in *Tk*CPTL1-RNAi than NIL plants (Fig. 3b; Table S5), whereas the fungal microbiota exhibited a higher magnitude of change upon herbivory but lower magnitude of change upon wounding in *Tk*CPTL1-RNAi than NIL plants (Fig. S12; Table S5). In the roots, a similar pattern was observed: The bacterial community exhibited a higher magnitude of change upon herbivory or wounding in *Tk*CPTL1-RNAi than NIL plants (Fig. 3b; Table S6), while the fungal community was unaffected by the plant genotype (Fig. S12; Table S5).

(a) Microbial frequencies



Fig. 3 Abolishing the biosynthesis of *cis*-1,4-polyisoprene through RNAi alters the *Taraxacum koksaghyz* microbiome dependent on the presence of damage by the herbivore and mechanical wounding treatment. (a) Canonical analysis of principal coordinates ordination (Bray–Curtis distance matrix) on microbial gene frequencies from rhizosphere samples obtained from shotgun metagenomic sequencing, reporting the effect of plant genotype (near isogenic lines (NIL) (green), *Tk*CPTL1-RNAi (orange)) under control, herbivory, and mechanical wounding. Percentages in parentheses report the variance explained by the respective axis. Statistical analysis on the pairwise comparisons is found in Table 1 under 'shotgun metagenomic'; *n* = 6. (b) The magnitude of changes in the abundance of each bacterial operational taxonomic unit (OTU) (absolute log₂ fold changes) upon wounding and herbivory differed between *Tk*CPTL1-RNAi and NIL plants. Data were analyzed using a linear mixed-effects model for bacterial rhizosphere samples ($\chi^2 = 1900$, df = 3, *P* < 0.001) and bacterial root samples ($\chi^2 = 250.64$, df = 3, *P* < 0.001). *P*-values of pairwise comparisons were adjusted using the FDR method; *n* = 6.

Abolishing biosynthesis of natural rubber did not increase the microbial colonization or pathogen load in *T. koksaghyz* roots upon injury

As both the abundance of *cis*-1,4-polyisoprene (plant genotype) and herbivory or mechanical wounding treatment shaped the structure of the rhizosphere and root microbiota, we used four approaches to test the hypothesis that biosynthesis of the dominant metabolite of natural rubber restricts the entry of potentially pathogenic microorganisms into the roots.

First, we used shotgun metagenomics to test whether plant genotype and treatment influenced the microbial load in plant roots (i.e. the percentage of sequence reads that did not align to the plant genome relative to the reads that aligned to the host plant). While the percentage of microbial reads in the roots showed a tendency to be altered by the treatment (P = 0.051, linear model), these changes were independent of plant genotype (P > 0.5, linear model, Fig. 4a).

Second, if natural rubber restricts the colonization of roots, we would expect a higher number of OTUs unique to the herbivore or wounding treatment (i.e. not found under control conditions) in rubber-depleted *Tk*CPTL1-RNAi than normal rubber content NIL plants, particularly in roots. Contrary to our expectations, the numbers of bacterial OTUs unique to the herbivore and wounding treatments were lower in the roots but higher in the rhizosphere in the *Tk*CPTL1-RNAi compared with NIL plants (Fig. 4b). The number of fungal OTUs unique to the herbivore or wounding treatment did not differ between *Tk*CPTL1-RNAi and NIL plants (Fig. 4b).

Third, we assessed whether taxa that are differentially abundant between NIL and *Tk*CPTL1-RNAi plants under wounding or herbivory are pathogens. Although we found taxa that were differentially abundant in each plant genotype by treatment combination, none of those taxa appeared to be agents of plant diseases (Notes S1). Also, the pathogens and endophytes (pathogens: *Botrytis* spp.; endophytes: *Curtobacterium* sp., *Leifsonia* sp., *Methylobacterium* sp., *Microbacterium* spp.) that had been recorded in the field for these *T. koksaghyz* lines (F. Eickmeyer, pers. comm.) and were present in our dataset were similarly abundant across the damage treatments and plant genotypes in both root and rhizosphere samples (Notes S1; Tables S6–S12).

Fourth, to obtain more insights into the function of the plant genotype-dependent taxonomic changes upon wounding and herbivory, we tested whether the changes in the microbial gene frequencies of each plant genotype by treatment combination obtained by shotgun metagenomic sequencing compared with the control treatment of the respective plant genotype were significantly different from random. We found a higher number of differentially abundant genes in all the groups than what we would expect by chance (Table \$13). Within each plant genotype by treatment combination, we classified each differentially abundant gene according to its likely functional role within the community. Most of the differentially abundant genes were related to metabolic functions (Table \$14). However, we also found a group of differentially abundant genes that have been previously reported in plant pathogens as pathogenicity or virulence factors. The number of these pathogen-related genes that were enriched in the wounding or herbivory treatment was at least three times higher in NIL than in TkCPTL1-RNAi plants (Table 2).

Taken together, these data provide evidence that biosynthesis of natural rubber alters the microbiome and that these changes depended on the presence of a herbivore or wounding; yet, natural rubber biosynthesis did not reduce pathogen load when roots were damaged.

Discussion

Cis-1,4-polyisoprene reduces herbivory

Natural rubber has long been hypothesized to be defensive against herbivores, but experimental evidence for this notion is scarce. In this study, we provide parallel lines of evidence that natural rubber reduces herbivory. First, rubber-deficient TkCPTL1-RNAi lines silenced in cis-1,4-polyisoprene biosynthesis were preferred by *M. melolontha* and lost more biomass upon herbivory than normal rubber content NIL lines. While the benefits of natural rubber biosynthesis on plant performance under herbivory were weaker in genetic background A than B, likely because M. melolontha herbivory was less severe in the former, the preference of *M. melolontha* for rubber-deficient plants was consistent in both transgenic lines. Second, purified cispolyisoprene reduced both larval weight gain and the attractiveness of the food. The observed temporal patterns in the choice of M. melolontha-with first a gradual increase in the number of larvae on the polyisoprene-deficient side, followed by redistribution of the larvae in an undirected manner-is typical for M. melolontha responding to deterrent compounds (Huber et al., 2021); this temporal pattern likely emerges because feeding reduces the attractiveness of the food. Third, T. koksaghyz triterpenes - which are elevated in rubber-depleted TkCPTL1-RNAi lines (Niephaus et al., 2019)-did not alter herbivore growth and plant resistance.

Our results are in line with a recent study showing that the addition of a *trans*-1,4-polyisoprene to artificial diet deters feeding and larval growth of the longhorn beetle that naturally feeds on the *trans*-1,4-polyisoprene-producing *Eucommia* trees (Pan *et al.*, 2015). As specialized metabolites may, however, function differently in isolation than when surrounded by their native matrix (Niemeyer, 2009; Chen *et al.*, 2020; Huber *et al.*, 2021), *in planta* experiments as carried out in our study are critical to infer ecological functions (Steppuhn *et al.*, 2004; Huber *et al.*, 2016b; Erb, 2018). Future work that elucidates how the branching structure, molecular weight, and concentration of *cis*-1,4-polyisoprene affects plant–environment interactions would provide exciting insights into why this polymer – and specialized metabolites in general – is so variable in nature.

Several mechanisms may contribute to the defensive properties of natural rubber under herbivore attack. Natural rubber is thought to contribute to the stickiness of latex, and thereby may trap insects or immobilize their mouth parts (Agrawal & Konno, 2009). *Melolontha melolontha* is too large to be entrapped by latex; however, we did not observe that the larva's mouth parts were glued together. Nevertheless, it is possible that smaller



Fig. 4 Abolishing the biosynthesis of *cis*-1,4-polyisoprene through RNAi does not increase microbial colonization or pathogen load in *Taraxacum kok-saghyz* roots. (a) The percentage of microbial reads in rhizosphere and root samples for each treatment (control, herbivory, or wounding) and plant geno-type (rubber-deficient *Tk*CPTL1-RNAi, 'RNAi' (orange); normal rubber content near isogenic lines, 'NIL' (green), plants). Boxplots show the median and the interequartile range (IQR) of the data distribution, with whiskers extending to 1.5 times the IQR. *P*-values of linear models are shown on top of the panels; n = 6. (b) The number of bacterial and fungal operational taxonomic units (OTUs) in rhizosphere and roots of rubber-deficient *Tk*CPTL1-RNAi and normal rubber content NIL *T. koksaghyz* plants that are unique to the herbivore and wounding treatment (i.e. not found under control conditions). Statistics refer to χ^2 tests; n = 6.

Table 2 Microbiome functional analysis.

Contrast	Microbial genes
NIL herbivory – NIL control	Acetyl-coenzyme A synthetase ¹ ATP-dependent RNA helicase DeaD ² ATP-dependent RNA helicase SrmB ³ Beta-N-acetylglucosaminidase/beta- glucosidase ⁴ Beta-xylosidase ⁵ Levanase ⁶ Mannitol 2-dehydrogenase ⁷ Multidrug efflux pump subunit AcrB ⁸ Sensor protein KdpD ⁹ Translation initiation factor IE-2 ¹⁰
NIL wounding – NIL control	Acetyl-coenzyme A synthetase ¹ Acyl-CoA dehydrogenase ¹¹ ATP-dependent RNA helicase DeaD ² ATP-dependent RNA helicase RhIE ¹² Beta-xylosidase ⁵ Bifunctional chorismate mutase/prephenate dehydratase ¹³ Cystathionine gamma-synthase ¹⁴ Extracellular serine protease ¹⁵ Glucans biosynthesis protein G ¹⁶ Nitrilotriacetate monooxygenase component A ¹⁷
RNAi herbivory – RNAi control	Acyl-CoA dehydrogenase ¹¹ ATP-dependent RNA helicase SrmB ³ nutative sensor histidine kinase TcrY ¹⁸
RNAi wounding – RNAi control	-

Microbial genes previously reported in the literature to be associated with pathogenicity or virulence that were found significantly more abundant in the rhizosphere of treated plants (herbivory or wounding) than the respective control group. 'NIL' indicates plants with normal rubber content and 'RNAi' indicates rubber-depleted plants. ¹Gu et al. (2019): ²Li et al. (2008); ³Granato et al. (2016); ⁴Huang et al. (2021); ⁵Guzha et al. (2022); ⁶Versluys et al. (2018); ⁷Jennings et al. (1998); ⁸Burse et al. (2007); ⁹Yang et al. (2018); ¹⁰Zhang et al. (2013); ¹¹Ruswandi et al. (2005); ¹²Hausmann et al. (2021); ¹³Kim et al. (2020); ¹⁴Fu et al. (2013); ¹⁵Figaj et al. (2019); ¹⁶Page et al. (2001); ¹⁷Agarwal et al. (2021); ¹⁸Cai et al. (2017).

insects could be affected by the stickiness of natural rubber. Alternatively, natural rubber may act as a matrix that facilitates the distribution and penetrance of other latex specialized metabolites inside the herbivore. Indeed, cis-polyisoprenes help to disperse antimicrobial triterpenes in vitro (Salomé-Abarca et al., 2021). In our experiment, addition of cis-polyisoprene to artificial diet was sufficient to reduce larval weight gain in the absence of other defensive metabolites, indicating that the polymer at least partially acts by itself. Furthermore, addition of cis-1,4-polyisoprene reduced *M. melolontha* weight gain per unit of consumed diet. We thus hypothesize that cis-1,4-polysioprene, being chemically inert, impedes nutrient uptake possibly by binding to nutrients or by agglutinating the epithelium of the herbivore's midgut. Experiments that assess herbivore physiology and localize cis-1,4polyisoprene inside the organism are needed to elucidate the mode of action of natural rubber in herbivore defense. Such efforts could also help to resolve the ongoing controversy whether most plant polymers act as defenses by reducing food quality

(Barbehenn & Peter Constabel, 2011; War et al., 2012; Perko-Cis-1,4-polyisoprene alters the microbiome but not the Apart from herbivore defense, laticifers and in particular natural rubber are hypothesized to mediate plant-microbe interactions (Agrawal & Konno, 2009). We found that abolishing the biosynthesis of cis-1,4-polysioprene altered the structure of both the root and the rhizosphere microbiome in noninfested, undamaged plants. This is similar to recent work showing that silencing the biosynthesis of other specialized metabolites, for instance benzoxazinoids, alters the root and rhizosphere microbiome (Stringlis et al., 2018; Cotton et al., 2019; Koprivova et al., 2019; Jacoby et al., 2021; Pang et al., 2021). Our work therefore suggests that many specialized metabolites that are typically considered to be defenses against herbivores may in addition shape plant-microbe At least two mechanisms may account for the observed rubber-associated changes in the microbiome structure in the absence of plant damage: First, natural rubber may structure the microbiota through direct exposure, as on the one hand, endophytes may inhabit laticifers (Gunawardana et al., 2015) and on the other hand, natural rubber is released to the soil when roots decay. We, however, did not observe any changes in the abundance of known T. koksaghyz endophytes with variation in plant rubber content. Second, abolishing the biosynthesis of cis-1,4-polysioprene through RNAi may alter the microbial structure through changes in metabolic fluxes, particularly in triterpene biosynthesis, which are known to be elevated in our RNAi lines (Niephaus et al., 2019). Triterpenes may promote or inhibit microbial growth (Pacheco et al., 2012), and disrupting triterpene biosynthesis in A. thaliana altered the root microbiome (Huang et al., 2019). Assessing the effect of natural rubber on the growth of selected microbes and complementing the rhizosphere of rubberdeficient plants with relevant cis-1,4-polyisoprene concentrations could help to differentiate among these possibilities. The major role of natural rubber in plant-microbe interactions

vich & Ward, 2022).

interactions.

pathogen load below ground

is hypothesized to be wound sealing, thereby preventing the entry of pathogens. We obtained mixed evidence for this hypothesis: On the one hand, abolishing the biosynthesis of cis-1,4polysioprene altered the structure of the rhizosphere and root microbiota upon damage by both M. melolontha herbivory and mechanical damage. Furthermore, rubber-deficient TkCPTL1-RNAi plants exhibited a larger magnitude of change in the root microbiota upon wounding or herbivory than normal rubber content NIL plants. These results support the role of natural rubber in wound sealing and emphasize that plant-microbe and plant-herbivore interactions should be studied in concert when assessing the function of specialized metabolites (Hu et al., 2018; Kudjordjie et al., 2021).

On the other hand, silencing the biosynthesis of cis-1,4polysioprene did not alter the percentage of microbial reads in plant roots upon damage, nor increase the number of OTUs in

roots unique to damaged plants, nor increase the number of pathogenicity-related microbial genes that are enriched in roots upon damage. These finding contrast with recent *in vitro* assays showing that *cis*-1,4-polyisoprene forms a physical barrier to bacteria and to a lower extent also to fungi including *Botrytis* spp. (Salomé-Abarca *et al.*, 2021). The different conditions *in vitro* and *in planta*, as well as variation in the amount, molecular weight and branching structure of *cis*-1,4-polyisoprene may contribute to differences in the inferred role of this polymer in pathogen defense. Future experiments that assess the performance of rubber-depleted and normal rubber content plants in combination with manipulation of the soil microbiota similar to as described by (Hu *et al.*, 2018 and Kudjordjie *et al.*, 2021) will be critical to improve our understanding of the role of natural rubber in pathogen defense.

In this study, we have shown that biosynthesis of *cis*-1,4polysioprene reduces herbivory and structures the microbiome and that the changes in the microbiome are modulated by the presence of *M. melolontha* herbivory and mechanical wounding. However, four lines of evidence reject the notion that rubber biosynthesis reduces microbial colonization or pathogen load. Taken together, latex metabolites may mediate both plant–herbivore and plant–microbe interactions, highlighting the role of plant specialized metabolites and secretory structures in shaping the complex trophic interactions in nature.

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Competing interests

None declared.

Author contributions

MH conceived the study. MH, LB, ND, and BM designed the experiments. LB performed experiments. LB, AM, and MH analyzed data. MH, CSG, and DP supervised students. DP, JG,

MH, and SX contributed resources. LB, AM, and MH wrote the initial draft, and all authors contributed to its final version.

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Data availability

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The data that support the findings of this study are openly available in NCBI SRA under the BioProject nos. PRJNA779274 (16S amplicon metagenomics), PRJNA779290 (ITS amplicon metagenomics) and PRJNA779369 (shotgun metagenomics). Public code repository can be found here: https://github.com/amalacrino/dandelion_microbiome. Raw data and R codes of the herbivore and plant performance data can be found in the Methods S7 and Table S15.

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Supporting Information

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

Fig. S1 Resistance of rubber-deficient RNAi and NIL plants (A line) grown in natural field soil.

Fig. S2 Choice experiments ex planta, including all time points.

Fig. S3 Biomass accumulation of triterpene-reduced plants under herbivory (Line 2).

Fig. S4 Biomass accumulation of triterpene-reduced plants under herbivory (Line 3).

Fig. S5 Larval weight gain and diet consumption of larvae feeding on lupeol-supplemented diet.

Fig. S6 Diversity analysis (Shannon and Simpson indexes) for 16S ITS rhizosphere and root.

Fig. S7 Canonical analysis of principal on bacterial metabarcoding reporting effect of genotype on rhizosphere and root.

Fig. S8 Canonical analysis of principal on fungal metabarcoding reporting effect of genotype on rhizosphere and root.

Fig. S9 DCA on bacterial metabarcoding reporting effect of treatment.

Fig. S10 DCA on fungal metabarcoding reporting effect of treatment.

Fig. S11 Canonical analysis of principal on microbial gene content on rhizosphere reporting the effect of treatment.

Fig. S12 Magnitude of changes in abundance for fungal operational taxonomic unit.

Methods S1 Plant material and growth conditions.

Methods S2 Identification of transgenic rubber-depleted plants.

Methods S3 Isolation of pure cis-1,4-polyisoprene.

Methods S4 Details on triterpene experiments.

Methods S5 Origin and storage of soil used for microbiome experiment.

Methods S6 Details on sample handling of microbiome study and statistical analysis.

Methods S7 R codes of statistical analysis of plant and herbivore performance data.

Notes S1 Taxa analysis.

 Table S1 Diversity analysis. Model coefficients Shannon diversity.

Table S2 Diversity analysis. Model coefficients Simpson diversity.

Table S3Diversity analysis.Pairwise comparisonsShannonindex.

Table S4 Diversity analysis. Pairwise comparisons Simpsonindex.

Table S5 Magnitude of changes in abundance of bacterial andfungal operational taxonomic unit.

Table S6 Number of observed vs random operational taxonomic units in rhizosphere.

Table S7 Number of observed operational taxonomic units thatare differently abundant in rhizosphere 16S data.

Table S8 Number of observed operational taxonomic units thatare differently abundant in rhizosphere ITS data.

Table S9 Number of observed vs random operational taxonomicunits in roots.

Table S10 Number of observed operational taxonomic units thatare differently abundant in roots 16S data.

Table S11 Relative abundance of bacterial genus in plant rhizosphere.

Table S12 Relative abundance of bacterial genus in plant roots.

Table S13 Functional analysis reporting the number of observedgenes differently abundant.

Table S14 Genes that are differentially abundant between her-
bivory or wounding and the respective controls.

Table S15 Raw data of plant and herbivore performance data.

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