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Root volatiles in plant–plant interactions II: Root volatiles alter root chemistry and plant–herbivore interactions of neighbouring plants

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Abstract

Volatile organic compounds (VOCs) emitted by plant roots can influence the germination and growth of neighbouring plants. However, little is known about the effects of root VOCs on plant–herbivore interactions of neighbouring plants. The spotted knapweed (*Centaurea stoebe*) constitutively releases high amounts of sesquiterpenes into the rhizosphere. Here, we examine the impact of *C. stoebe* root VOCs on the primary and secondary metabolites of sympatric *Taraxacum officinale* plants and the resulting plant-mediated effects on a generalist root herbivore, the white grub *Melolontha melolontha*. We show that exposure of *T. officinale* to *C.stoebe* root VOCs does not affect the accumulation of defensive secondary metabolites but modulates carbohydrate and total protein levels in *T. officinale* roots. Furthermore, VOC exposure increases *M. melolontha* growth on *T. officinale* plants. Exposure of *T. officinale* to a major *C. stoebe* root VOC, the sesquiterpene (*E*)-*β*-caryophyllene, partially mimics the effect of the full root VOC blend on *M. melolontha* growth. Thus, releasing root VOCs can modify plant–herbivore interactions of neighbouring plants. The release of VOCs to increase the susceptibility of other plants may be a form of plant offense.

KEYWORDS

associational effects, belowground herbivory, neighbourhood effects, plant–herbivore interactions, plant–plant interactions, volatile priming

1 | INTRODUCTION

Plants emit a variety of volatile organic compounds (VOCs) that can affect the behaviour and performance of other organisms. VOCs induced by herbivory for instance can enhance defences and resistance of neighbouring plants (Arimura et al., 2000; Engelberth, Alborn, Schmelz, & Tumlinson, 2004; Erb et al., 2015; Frost, Mescher, Carlson, & De Moraes, 2008; Karban, Yang, & Edwards, 2014; Pearse, Hughes, Shiojiri, Ishizaki, & Karban, 2013; Sugimoto et al., 2014). As the benefit for the emitter plant is unclear, this phenomenon is commonly regarded as a form of "eavesdropping" by the receiver rather than a form of communication (Heil & Karban, 2010). From the perspective of an emitter plant, it would seem advantageous to use VOCs to suppress rather than enhance defences in neighbours (Heil & Karban, 2010). However, little is known about the capacity of VOCs to suppress defences and enhance herbivore attack rates in neighbouring plants. Broccoli plants

were found to receive more oviposition by diamondback moths after exposure to VOCs from damaged conspecifics (Li & Blande, 2015). Furthermore, exposure to VOCs from damaged neighbours increases herbivore damage on blow wives (*Achyrachaena mollis*) and charlock (*Sinapis arvensis*) (Pearse et al., 2012). Finally, green leafy volatile (GLV) exposure suppresses several defence-related genes in coyote tobacco (*Nicotiana attenuata*; Paschold, Halitschke, & Baldwin, 2006). Clearly, the capacity of VOCs to suppress rather than induce defences requires more attention in order to understand how VOCs influence plant–herbivore interactions of neighbouring plants (Erb, 2018a).

The majority of studies on the effects of VOCs on plant neighbours have focused on the phyllosphere. However, plants also release significant amounts of VOCs into the rhizosphere, which may affect plant defence and plant–herbivore interactions (Delory, Delaplace, Fauconnier, & du Jardin, 2016). Root chemicals, including VOCs, can affect the germination and growth of neighbouring plants (Ens, Bremner, French, & Korth, 2009; Jassbi, Zamanizadehnajari, & Baldwin, 2010) and the behaviour and performance of herbivores (Hu, Mateo, et al., 2018; Robert, Erb, et al., 2012; Robert, Veyrat, et al., 2012). Therefore, it is reasonable to assume that root VOCs may also affect plant–herbivore interactions of neighbouring plants. Root exudates and mycelial networks have been shown to alter plant defences and plant herbivore interactions in neighbouring plants (Babikova et al., 2013; Dicke & Dijkman, 2001), but the specific role of root VOCs in plant–plant interaction has, to the best of our knowledge, not been addressed (Delory et al., 2016).

In this study, we explored the influence of root VOCs on the common dandelion (*Taraxacum officinale* agg.) and its interaction with the common cockchafer *Melolontha melolontha*. In grasslands across Europe, *T. officinale* is often attacked by larvae of *M. melolontha* (Coleoptera, Scarabaeidae; Huber, Bont, et al., 2016), a highly polyphagous root feeder (Hauss & Schütte, 1976; Sukovata, Jaworski, Karolewski, & Kolk, 2015). Previous work found that the interaction between *T. officinale* and *M. melolontha* is modulated by the presence of sympatric plant species (Huang, Zwimpfer, Hervé, Bont, & Erb, 2018). Strong effects were for instance observed for *Centaurea stoebe*, a native European herb that is invasive in the United States. The *M. melolontha* larvae grew significantly better on *T. officinale* plants in the presence of *C. stoebe*, an effect that was found to be mediated through changes in *T. officinale* susceptibility rather than direct effects of *C. stoebe* on the herbivore (Huang et al., 2018). In a companion paper, we describe that *C. stoebe* constitutively produces and releases significant amounts of sesquiterpenes into the rhizosphere (Gfeller et al., 2019). Furthermore, we show that *C. stoebe* root VOCs have neutral to positive effects on the germination and growth of different neighbouring species (Gfeller et al., 2019). Based on these results, we hypothesized that *C. stoebe* root VOCs may play a role in increasing *T. officinale* susceptibility to *M. melolontha*. We tested this hypothesis by exposing *T. officinale* plants to root VOCs from *C. stoebe* and a major *C. stoebe* sesquiterpene and measuring changes in root primary and secondary metabolites and *M. melolontha* growth. This work provides evidence that root VOCs can influence plant–herbivore interactions on neighbouring plants.

2 | METHODS AND MATERIALS

2.1 | Study system

The study system consisted *T. officinale* (Genotype A34) as a receiver plant, *C. stoebe* as an emitter plant and *M. melolontha* as an herbivore of *T. officinale*. The *T. officinale* seeds were obtained from greenhouse-grown A34 plants. The *C. stoebe* L. (diploid) seeds were obtained from a commercial vendor (UFA-SAMEN, Winterthur, Switzerland). The *M. melolontha* larvae were collected from an apple tree yard in Sion, Switzerland (46.21°N, 7.38°E). The larvae were reared on carrot slices under controlled condition (12°C, 60% humidity and constant darkness) for several weeks until the start of the experiments.

2.2 | Impact of *C. stoebe* root VOCs on the interaction between *T. officinale* and *M. melolontha*

To examine whether root VOCs emitted by *C. stoebe* affect the interaction between *T. officinale* and *M. melolontha*, the larvae were restricted to feed on *T. officinale* in the vicinity of *C. stoebe*, another *T. officinale* plant or soil only (combinations = 3, n = 16 per combination). Furthermore, to test how *C. stoebe* influences *T. officinale* growth and chemistry through root VOCs, *T. officinale* plants were grown in the vicinity of *C. stoebe*, another *T. officinale* plant or soil only in the absence of *M. melolontha* (combinations = 3, n = 8 per combination). Seeds of *T. officinale* and *C. stoebe* were germinated in the greenhouse at 50–70% relative humidity, 16/8-hr light/dark cycle, and 24°C at day and 18°C at night. Ten days later, two seedlings of each species were transplanted into a mesh cage (12 × 9 × 10 cm, length × width × height) filled with a mixture of 1/3 landerde (Ricoter, Switzerland) and 2/3 seedling substrate (Klasmann-Deilmann, Switzerland). The mesh cage was made of geotex fleece (Windhager, Austria). Then, two mesh cages were put into a 2-L rectangular pot (18 × 12 × 10 cm, length × width × height). To reduce the interaction between focal and neighbouring plants through root exudates, the mesh cages in each pot were separated by two plastic angles (0.8-cm width), and the pot was cut to produce a gap (12 × 0.5 cm, length × width) in the centre of the bottom paralleling to the longest side of mesh cage. Finally, the gap in the top between two mesh cages was covered by a plastic sheet. A schematic drawing of the setup is shown in Figure 2a. The setup is identical to the one used in the companion paper (Gfeller et al., 2019). Seven weeks after transplantation, a preweighted *M. melolontha* larva was added into the mesh cage with the focal plants (*T. officinale*). The larvae had been starved for 3 days prior to the experiment. After 18 days of infestation, the larvae were removed and reweighted. Then, roots of focal plants were harvested, weighted, flash-frozen in liquid nitrogen, and stored in −80°C for further chemical analyses, including soluble protein and sugars as well as the defensive metabolite sesquiterpene lactone taraxinic acid β-D glucopyranosyl ester (TA-G). Soluble protein was estimated using the Bradford method (Bradford, 1976). Soluble sugars including glucose, fructose and sucrose were measured as described by Velterop and

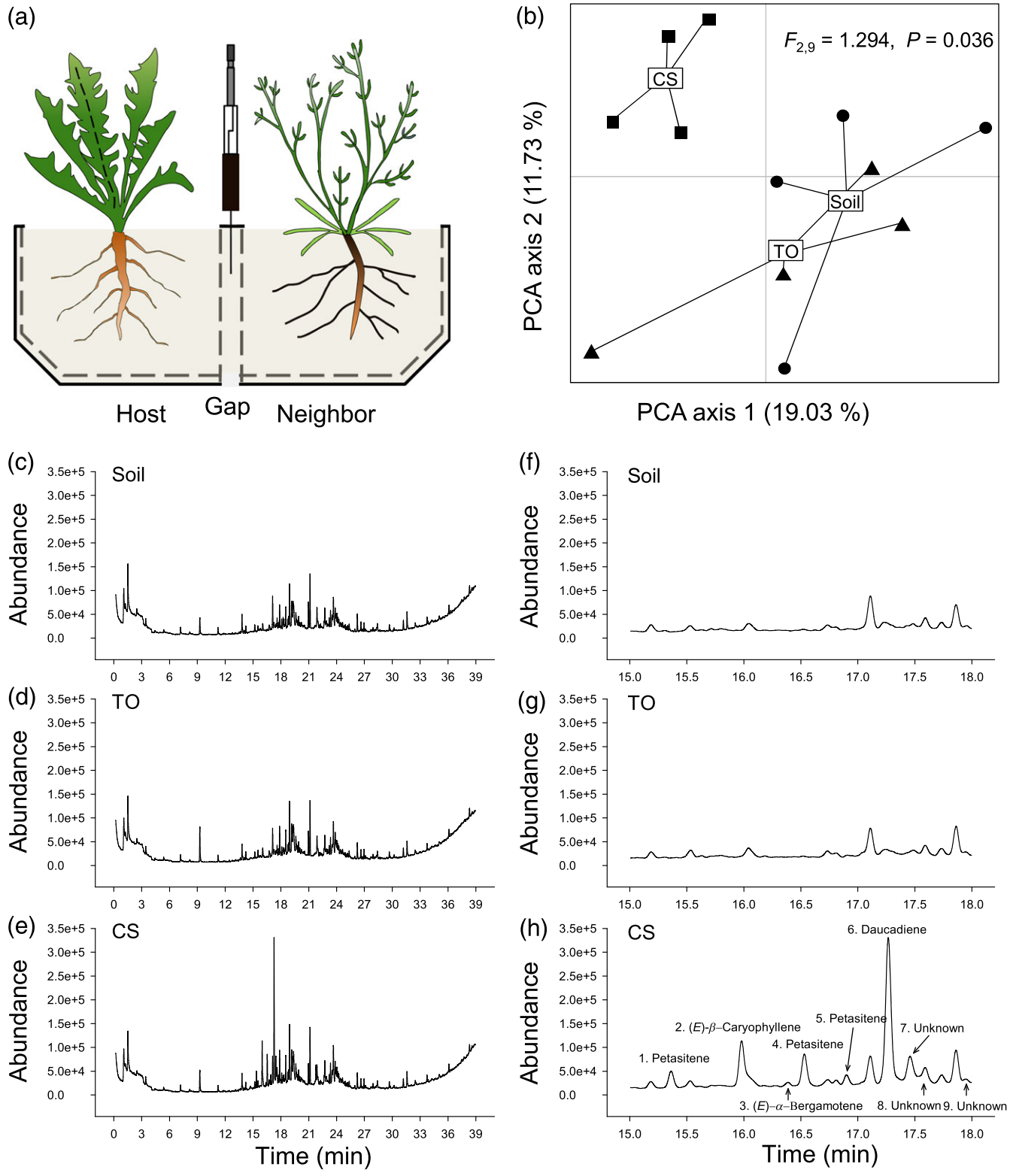

FIGURE 1 Sesquiterpene VOCs from *Centaurea stoebe* diffuse through the rhizosphere. (a) Experimental setup: *Taraxacum officinale* plants were grown in the vicinity of empty soil compartments (soil), *T. officinale* plants (TO), or *C. stoebe* plants (CS), and volatiles were collected in the gap between the plants. (b) The results of a principal component analysis of the volatile organic compound profiles in the gap are shown: The first two axes explained 19.03% and 11.73% of the total variation, respectively. Differences between treatments were visualized by principal component analysis (PCA). Data points represent biological replicates (n = 4). Circles, triangles, and squares indicate neighbour identities. Typical total-ion count gas chromatography mass spectrometry chromatograms of volatiles collected from gap between focal and neighbouring plants from 0 to 39 min (c–e) and from 15 to 18 mins (f–h) are shown [Colour figure can be viewed at wileyonlinelibrary.com]

Vos (2001) and Machado et al. (2013). TA-G was analysed as described by Huber et al. (2015) and Bont et al. (2017). During the experiment, pots were watered daily. Care was taken not to overwater the plants to avoid leachate to cross the air gap between the inner mesh cages. The plant pairs were arranged randomly on a greenhouse table, with distances between pairs equal to distances within pairs. The positions of the pots on the table were rearranged weekly. These two measures resulted in randomized above ground pairings between the two plant species, thus allowing us to exclude systematic effects of above ground interactions on root physiology and resistance.

2.3 | Analysis of root VOC profiles in the gap

To characterize the VOCs that accumulate in the gap when *T. officinale* is exposed to *C. stoebe*, another *T. officinale* or soil only (combinations = 3, n = 8 per combination), we collected and analysed VOCs using solid phase microextraction (SPME) and gas chromatography mass spectrometry (GC-MS). A schematic drawing of the setup is shown in Figure 1a. After 7 weeks of transplantation, VOCs were collected from two randomly selected pots of each combination for one biological replicate (n = 4 per combination). An SPME fibre (coated with 100-µm polydimethylsiloxane; Supelco, Bellefonte, PA, USA) was inserted into the gap of a pot and exposed to VOCs for 60 min at room temperature and then transferred to another pot for 60 min for collection. The incubated fibre was immediately analysed by GC-MS using an Agilent 7820A GC interfaced with an Agilent 5977E MSD (source 230°C, quadrupole 150°C, ionization potential 70 eV, scan range 30–550, Palo Alto, CA, USA). Briefly, the fibre was inserted into the injector port at 250°C and desorbed for 2 min. VOCs were chromatographed on a capillary GC-MS column (HP5-MS, 30 m, 250 µm ID, 2.5 µm film, Agilent Technologies, Palo Alto, CA, USA) with He as carrier gas at a flow rate of 1 mL min^{-1}. The GC temperature program was 60°C for 1 min, increased to 250 at 5°C min^{-1} and followed by 4 min at 250°C. The chromatograms were processed using default settings for spectral alignment and peak picking of PROGENESIS QI (Nonlinear Dynamics, Newcastle, UK). Features were assigned to individual compounds by retention time and peak shape matching and all VOCs were tentatively identified by the use of the NIST search 2.2 Mass Spectral Library (Gaithersburg, MD, USA) as well as retention time and spectral comparison with pure compounds as described (Gfeller et al., 2019). During the experiment, the pots were watered every day and rearranged every week. Plants were not infested by *M. melolontha*.

2.4 | Contribution of (*E*)-β-caryophyllene to plant–plant interactions

(*E*)-β-caryophyllene is one of the major sesquiterpenes released by *C. stoebe* roots and is produced by the root-expressed terpene synthase CsTPS4 (Gfeller et al., 2019). To test whether (*E*)-β-caryophyllene is sufficient to account for the increased growth of *M. melolontha* on *T. officinale* plants, we determined concentration of (*E*)-β-caryophyllene in the air gap between the rhizosphere of *C. stoebe* and *T. officinale* (see above) and then used corresponding synthetic doses to investigate its impact on the interaction between *T. officinale* and *M. melolontha*. A schematic drawing of the setup is shown in Figure 4a.

To check whether we can mimick the (*E*)-β-caryophyllene release of *C. stoebe* with a dispenser containing synthetic (*E*)-β-caryophyllene, we measured (*E*)-β-caryophyllene in the air gap of *T. officinale* plants growing with *C. stoebe* or *T. officinale* plants growing without *C. stoebe* but with an (*E*)-β-caryophyllene dispenser in the air gap (n = 16). Both plant species were 7 weeks old. Dispensers were constructed from 1.5-ml glass vials (VWR) that were pierced by a 1-ul

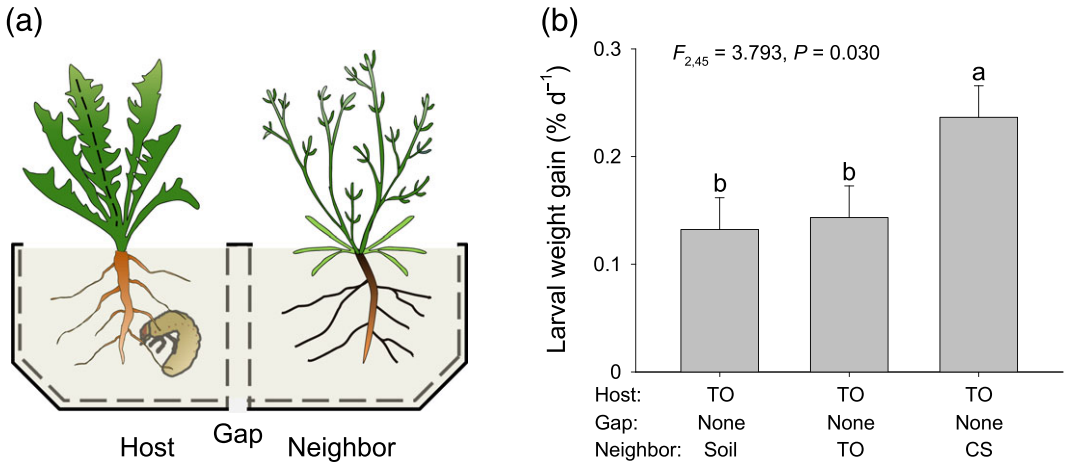

FIGURE 2 Root volatile organic compounds emitted by *Centaurea stoebe* increase *Melolontha melolontha* performance on neighbouring plants. (a) Experimental setup: Individual *M. melolontha* larvae were allowed to feed on *Taraxacum officinale* plants growing in the vicinity of empty soil compartments (soil), *T. officinale* (TO), or *C. stoebe* (CS) for 18 days. (b) Larval performance: Average larval weight gain was calculated as percentage increase in larval weight per day and is shown as mean ± 1 SE (n = 16). Differences between treatments were determined by one-way ANOVAs followed by post hoc multiple comparisons (different letters indicate P < 0.05, least square mean) [Colour figure can be viewed at wileyonlinelibrary.com]

micro-pipette (Drummond) and sealed with parafilm (Bemis). Dispensers were filled with with 100-ul (*E*)-β-caryophyllene (98.5%, GC, Sigma-Aldrich). This device allowed for constant release rates of (*E*)-*β*-caryophyllene. Two days after the dispensers were added, (*E*)-*β*-caryophyllene concentrations were determined by SPME-GC-MS as described above, resulting in eight biological replicates (two pooled setups per replicate).

To test the effect of (*E*)-*β*-caryophyllene on the interaction between *T. officinale* and *M. melolontha*, we conducted an experiment within which *T. officinale* plants were exposed to (a) control dispensers without neighbouring plant, (b) (*E*)-*β*-caryophyllene dispensers without neighboring plant, and (c) control dispensers with *C. stoebe* as a neighbouring plant (*n* = 12 per combination). The experimental setup was as described above. Seven weeks after the transplantation of *C. stoebe*

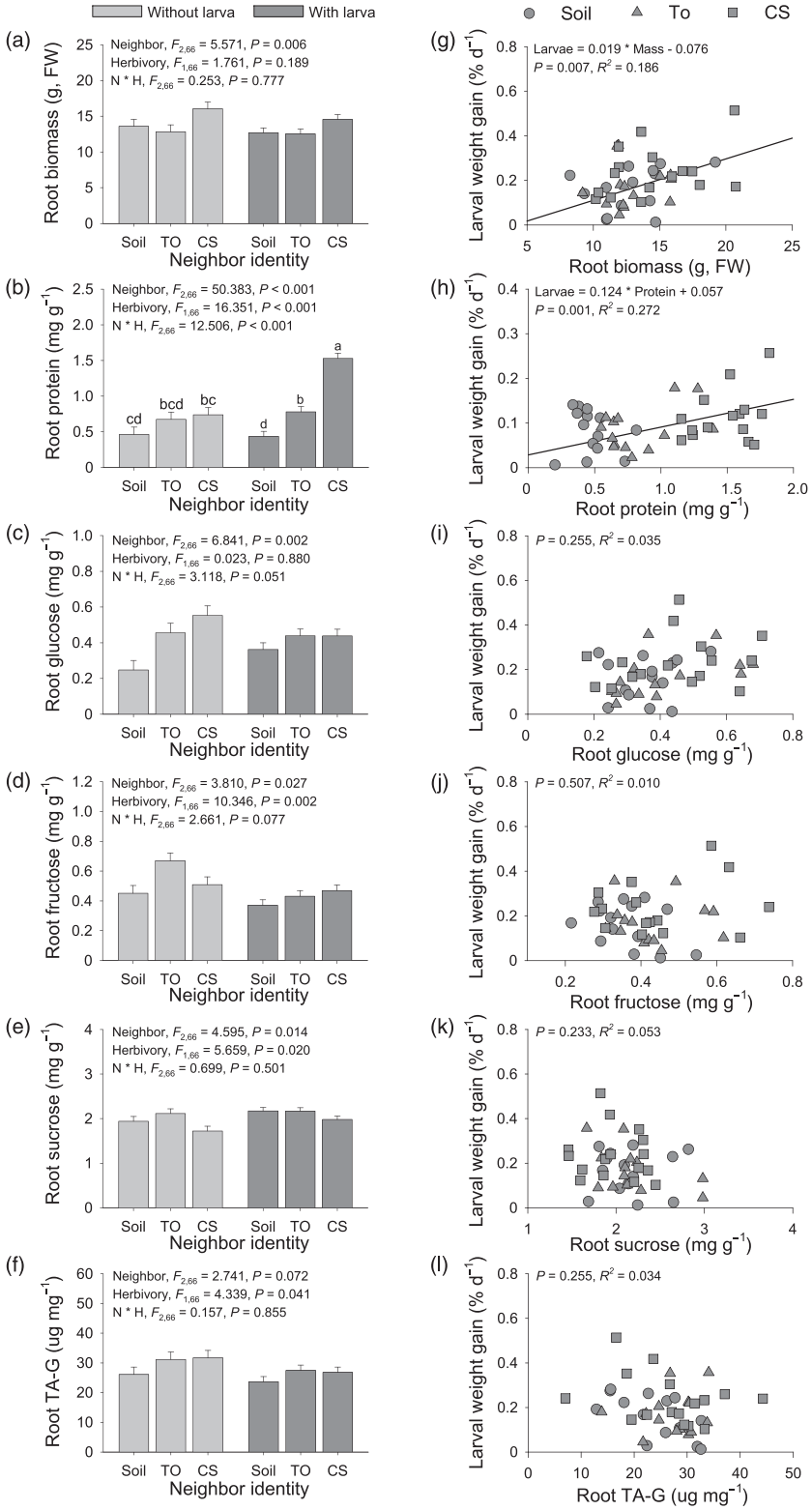

FIGURE 3 Root volatile organic compounds emitted by neighbouring plant influence growth and chemistry of *Taraxacum officinale*. (a) Root biomass, (b) soluble protein, (c) glucose, (d) fructose, (e) sucrose, and (f) taraxinic acid *β*-D glucopyranosyl ester (TA-G) of *T. officinale* growing in the vicinity of empty soil compartment (soil), *T. officinale* (TO), or *Centaurea stoebe* (CS) are shown on the left. The *T. officinale* plants were not attacked (light grey bars, *n* = 8) or attacked by *Melolontha melolontha* larvae (dark grey bars, *n* = 16). Values are means ± 1 SE. Differences between treatments were determined by two-way ANOVAs followed by post hoc multiple comparisons (different letters indicate $P < 0.05$, least square mean). The relationships between larval weight gain and (g) root biomass, (h) soluble protein, (i) glucose, (j) fructose, (k) sucrose, and (l) TA-G of *T. officinale* are shown on the right. Circles, triangles, and squares indicate *T. officinale* growing in the vicinity of soil, TO, or CS, respectively. Pearson coefficients, and R^2 values are shown in the top of the figures. Regression lines and equations are shown for significant correlations

and the addition of the dispensers, one preweighted and starved *M. melolontha* larva was added to the mesh cage in which the *T. officinale* plants were growing. After 18 days, all larvae were recovered from mesh cages and reweighted. During the experiment, the dispensers were replaced every 10 days and pots were rearranged every week.

2.5 | Data analysis

All data analyses were performed with the statistical analysis software R 3.2.0 (R Foundation for Statistical Computing, Vienna, Austria) using "CAR," "LME4," "LSMEANS," "VEGAN," and "RVAIDEMEMOIRE" packages (Bates, Mächler, Bolker, & Walker, 2015; Fox & Weisberg, 2011; Hervé, 2016; Lenth, 2016; Oksanen et al., 2016). Larval weight gain and plant variables were analysed using one- or two-way analyses of variance (ANOVAs using Type II Sums of Squares). ANOVA assumptions were verified by inspecting residuals and variance. Multiple comparisons were carried out using least square mean post-hoc tests (LSM) for significant terms. *P* values were corrected using the false discovery rate method (Benjamini & Hochberg, 1995). To examine the linear associations between larval weight gain and root parameters, Pearson's product–moment correlations were carried out. To examine the overall differences in VOC profiles among different combinations, the relative abundance of the detected features was subjected to principal component analysis (PCA). Monte Carlo tests with 999 permutations were then used to test for significant differences between combinations.

3 | RESULTS

3.1 | Neighbour identity determines VOC profiles in the rhizosphere

PCA analysis revealed that VOC profiles in the air gap between the *T. officinale* rhizosphere and the rhizospheres of the neighbouring treatments differed significantly ($F_{2,9}$ = 1.294, P = 0.036, Figure 1b). VOC profiles of *T.officinale* plants exposed to bare soil or *T. officinale* plants were indistinguishable (P = 0.516, Figure 1b). By contrast, profiles were strongly altered by the presence of *C. stoebe* (P = 0.040, Figure 1b). VOC profiles in the air gap between *T. officinale* and *C. stoebe* were dominated by sesquiterpenes that are released by *C. stoebe* roots (Gfeller et al., 2019), including petasitenes, (*E*)-*β*-caryophyllene and daucadiene (peak area, P < 0.05, Figure 1c–h).

3.2 | Root VOCs of *C. stoebe* increase *M. melolontha* growth on *T. officinale*

The growth of *M. melolontha* was similar on *T. officinale* plants that received below ground VOCs from bare soil or *T. officinale* neighbours (P = 0.791, Figure 2b). By contrast, *M. melolontha* weight gain was significantly higher on *T. officinale* plants that were exposed to root VOCs of *C. stoebe* (P = 0.045, Figure 2b). Thus, *C. stoebe* root VOCs increase *M. melolontha* growth on *T. officinale*.

3.3 | Root VOCs of *C. stoebe* change primary metabolites in *T. offinicale* roots

The *T. officinale* root biomass was significantly affected by the different VOC exposure treatments ($F_{2,66}$ = 5.571, P = 0.006) but not by *M. melolontha* attack ($F_{1,66}$ = 1.761, P = 0.189) or the interaction ($F_{2,66}$ = 0.253, P = 0.777). Root biomass was higher in *C. stoebe* exposed plants compared to plants exposed to *T. officinale* and bare soil (Figure 3a). Root VOC exposure also influenced the concentration of root primary and secondary metabolites (Figure 3b–f). Total root protein concentrations were significantly affected by the VOC source ($F_{2,66}$ = 50.383, P < 0.001), *M. melolontha* attack ($F_{1,66}$ = 16.351, P < 0.001) and their interaction ($F_{2,66}$ = 12.506, P < 0.001). Root protein was the highest in *C. stoebe* exposed plants and lowest in plants

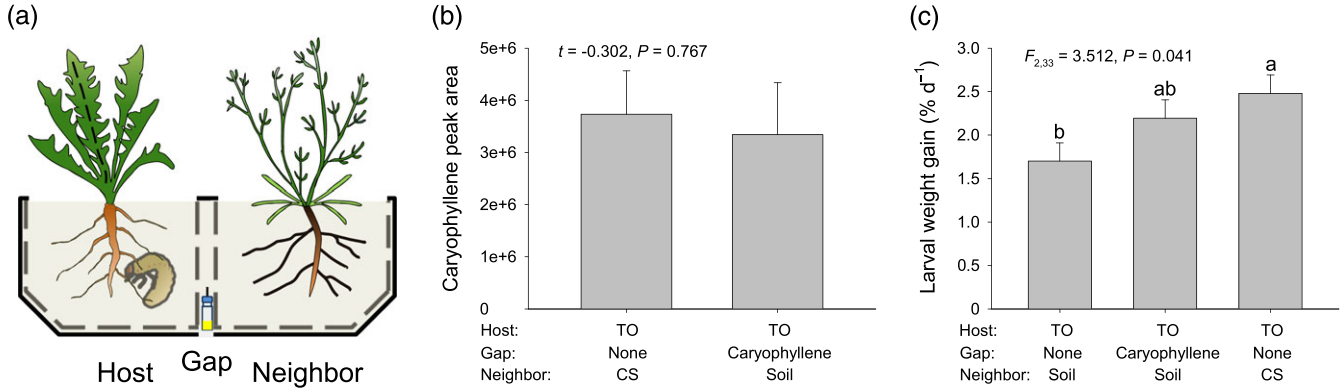

FIGURE 4 (*E*)-*β*-caryophyllene contributes to increased *Melolontha melolontha* growth on neighboring plants. (a) Experimental setup: *Taraxacum officinale* plants were growing in the vicinity of empty soil compartment (soil) or *Centaurea stoebe* (CS) and supplemented with or without synthetic (*E*)-*β*-caryophyllene in the gap. Physiological concentration of (*E*)-β-caryophyllene in gap (b): Control and (*E*)-*β*-caryophyllene dispensers were put in the gap for 2 days before measurements. Values were mean ± 1 SE (n = 8). Differences between treatments were determined by independent sample t tests. Impact of (*E*)-*β*-caryophyllene on *M. melolontha* larval growth (c): The *M. melolontha* larva was allowed to feed on *T. officinale* for 18 days. Values were mean ± 1 SE (n = 12). Differences between treatments were determined by one-way ANOVA followed by post hoc multiple comparisons (different letters indicate P < 0.05, least square mean) [Colour figure can be viewed at wileyonlinelibrary.com]

exposed to bare soil when *M. melolontha* was present (Figure 3b). Root glucose levels were significantly affected by the VOC source ($F_{2,66}$ = 6.841, P = 0.002) but not by *M. melolontha* attack ($F_{1,66}$ = 0.023, P = 0.880) or their interaction ($F_{2,66}$ = 3.118, P = 0.051). Root glucose levels were higher in *C. stoebe* and *T. officinale* exposed plants compared with plants exposed to bare soil (Figure 3c). Root fructose and sucrose were significantly affected by neighbour identify (fructose: $F_{2,66}$ = 3.810, P = 0.027; sucrose: $F_{2,66}$ = 4.595, P = 0.014) and *M. melolontha* attack (fructose: $F_{1,66}$ = 10.346, P = 0.002; sucrose: $F_{1,66}$ = 5.659, P = 0.020) but not by their interaction (fructose: $F_{2,66}$ = 2.661, P = 0.077; sucrose: $F_{2,66}$ = 0.699, P = 0.501). Both root fructose and sucrose levels were higher in plants exposed to *T. officinale*, whereas root fructose levels were lower in plants exposed to bare soil and root sucrose levels were lower in plants exposed to *C. stoebe* (Figure 3d–e). Root fructose levels were higher when *M. melolontha* was absent, whereas root sucrose levels were higher when *M. melolontha* was present (Figure 3d–e). The secondary metabolite TA-G was significantly decreased when *T. officinale* was attacked by *M. melolontha* larvae ($F_{1,66}$ = 4.339, P = 0.041) but was not affected by the VOC source ($F_{2,66}$ = 2.741, P = 0.072) or their interaction ($F_{2,66}$ = 0.157, P = 0.855, Figure 3f). Across treatments, *M. melolontha* larval weight gain was positively correlated with *T. officinale* root biomass (P = 0.007, R^2 = 0.186, Figure 3g) and soluble protein (P = 0.001, R^2 = 0.272, Figure 3h) but not significantly correlated with soluble sugars (glucose, P = 0.255, R^2 = 0.035; fructose, P = 0.507, R^2 = 0.010; sucrose, P = 0.233, R^2 = 0.053; Figure 3i–k) or TA-G (P = 0.255, R^2 = 0.034, Figure 3l).

3.4 | Synthetic (*E*)-*β*-caryophyllene partially mimics *C. stoebe* root VOC effects

The amount of (*E*)-*β*-caryophyllene that accumulated in the air gap supplied with a dispenser was similar to the emission of (*E*)-*β*-caryophyllene into the gap by *C. stoebe* (t = −0.302, P = 0.767, Figure 4b). Similar to the previous experiment, the presence of *C. stoebe* increased *M. melolontha* weight gain compared with bare soil (Figure 4c). The *M. melolontha* growth in the presence of (*E*)-*β*-caryophyllene dispensers was intermediate and not statistically different from the control treatment or the *C. stoebe* treatment (Figure 4c). Thus, (*E*)-*β*-caryophyllene partially mimics *C. stoebe* root VOC effects on *M. melolontha* growth on neighbouring plants.

4 | DISCUSSION

Associational effects triggered by plant VOCs play important roles in determining plant–herbivore interactions in the field (Barbosa et al., 2009; Underwood, 2014). However, to date, most studies focused on above ground interactions through airborne signals and most studies document that leaf VOCs trigger associational resistance in neighbours (Arimura et al., 2000; Engelberth et al., 2004; Erb et al., 2015; Frost et al., 2008; Pearse et al., 2013; Sugimoto et al., 2014). Our results show that root VOCs modulate plant–herbivore interactions and that VOCs may lead to associational susceptibility.

In an earlier study, we found that the presence of *C. stoebe* enhanced the performance of *M. melolontha* larvae feeding on *T. officinale* roots (Huang et al., 2018). In general, physical (e.g., light and contact), chemical (e.g., volatile and exudates), and biological (e.g., arbuscular mycorrhizal fungi) factors may trigger neighbourhood effects and affect plant growth and defence (Babikova et al., 2013; Crepy & Casal, 2015; Erb et al., 2015; Hu, Robert, et al., 2018; Kong et al., 2018; Semchenko, Saar, & Lepik, 2014; Yang, Callaway, & Atwater, 2015). As *C. stoebe* constitutively releases large amounts of sesquiterpenes into the rhizosphere (Gfeller et al., 2019), we hypothesized that root VOCs may be responsible for the plant-mediated changes in *M. melolontha* growth. Using an experimental setup that effectively randomizes above ground cues and eliminates root contact and the exchange of soluble exudates, we found that *C. stoebe* root volatiles diffuse through the rhizosphere and are sufficient to increase the growth of *M. melolontha* on neighbouring *T. officinale*. Thus, this study provides experimental evidence that root VOCs play an important role in below ground associational effects impacting plant–herbivore interactions. Future experiments could for instance address the interactions between VOCs and soluble exudates in below ground associational effects and determine distance-activity relationships of root VOCs.

Plant VOCs can influence herbivore performance directly or indirectly by changing the chemistry of receiver plants (Engelberth et al., 2004; Erb et al., 2015; Huang et al., 2018; Sugimoto et al., 2014; Veyrat, Robert, Turlings, & Erb, 2016; Ye et al., 2018). In our earlier work, we excluded the possibility that *M. melolontha* is directly affected by *C. stoebe* root VOCs or exudates, suggesting that *C. stoebe* increases *M. melolontha* growth through plant-mediated effects. In line with this hypothesis, we demonstrate here that growth and primary metabolism of *T. officinale* roots changes upon exposure to root VOCs of *C. stoebe*. Some of these effects are even stronger when the plants are attacked by *M. melolontha*, suggesting an interaction between root VOC exposure and herbivory. For instance, exposure to *C. stoebe* root VOCs increases root protein content and root growth of *T. officinale* plants. Both parameters are positively correlated with larval performance, indicating that *M. melolontha* growth may be stimulated by enhanced root growth and nutrient levels. Previous studies demonstrated that secondary metabolites such as TA-G protect *T. officinale* against *M. melolontha* (Bont et al., 2017; Huber, Bont, et al., 2016; Huber, Epping, et al., 2016). We found on clear effects of *C. stoebe* VOCs on root TA-G concentrations, implying that *C. stoebe* VOCs do not act by suppressing this plant defence.

The identification of bioactive VOCs from plant-derived blends remains an important bottleneck in chemical ecology. We show that *C. stoebe* releases a complex blend of sesquiterpenes as well as other minor unidentified VOCs from its roots (Gfeller et al., 2019), all of which may be associated with the observed effects on *M. melolontha* growth. Here, we tested whether (*E*)-*β*-caryophyllene, one of the major sesquiterpenes emitted by *C. stoebe*, is sufficient to increase the growth of *M. melolontha* on *T. officinale* in comparison with the full

VOC blend of *C. stoebe*. (*E*)-*β*-caryophyllene is a widespread sesquiterpene in nature that can influence the physiology and behaviour of pathogen, nematodes, and insects (Fantaye, Köpke, Gershenzon, & Degenhardt, 2015; Huang et al., 2012; Rasmann et al., 2005; Robert et al., 2013) and may act as an antioxidant in plants (Palmer-Young, Veit, Gershenzon, & Schuman, 2015). We demonstrate that (*E*)-*β*-caryophyllene exposure leads to *M. melolontha* growth that is intermediate between non-exposed and *C. stoebe* exposed *T. officinale* plants, suggesting that it can partially account for the VOC effects of *C. stoebe*. We propose that other sesquiterpenes emitted by *C. stoebe* such as daucadiene and petasitene may also contribute to enhanced *M. melolontha* growth. More work is needed to test this hypothesis. The identification of TPSs that are likely responsible for sesquiterpene production in *C. stoebe* (Gfeller et al., 2019) represents the first step towards the manipulation and functional assessment of *C. stoebe* root VOCs in vivo (Vaughan et al., 2013).

VOCs of neighbouring plants are well known to increase defences and resistance of neighbouring plants (Arimura et al., 2000; Erb et al., 2015; Sugimoto et al., 2014), and only few documented examples exist where VOCs decrease the resistance of neighbouring plants (Li & Blande, 2015). From the perspective of the sender, inducing susceptibility to herbivores in neighbouring plants may be an advantage, as it may reduce their competitiveness. VOC-induced susceptibility may thus be a form of plant offense. However, several caveats need to be considered. First, many herbivores are mobile, and increasing herbivore growth on neighbouring plants may lead to accelerated migration to the sender plant. Second, herbivore growth, as measured here, is not synonymous with plant damage and may be the result of an increase in performance of the receiver plant, in which case their competitiveness would not be reduced, and the benefit for the emitter would be less evident (Erb, 2018b; Veyrat et al., 2016). Third, the benefits of inducing susceptibility in neighbouring plants may be offset in the absence of herbivores. Indeed, we show that *C. stoebe* VOCs can increase germination and growth of heterospecific neighbouring plants in the absence of herbivores (Gfeller et al., 2019). Therefore, more research is needed to understand the evolutionary and ecological context of the present findings.

In conclusion, the present study shows that root VOCs can influence plant–herbivore interactions on neighbouring plants through plant-mediated effects. Thus, associational effects mediated by below ground VOCs need to be included into models on plant interaction ecology.

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AUTHOR CONTRIBUTIONS

W.H. and M.E. designed the experiments. W.H. carried out greenhouse research. W.H., V.G., and M.E. performed chemical analyses, analysed data, and wrote the manuscript.

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