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Additive effects of plant chemotype, mutualistic ants and predators on aphid performance and survival

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Bottom-up, exclusion experiment, *Lasius niger*, *Metopeurum fuscoviride*, mutualism, relative effects, top-down, tritrophic interaction.

ABSTRACT

1. Cascading effects in ecological systems acting across three or more trophic levels can be either of a resource-based (bottom-up) or natural enemy-based (top-down) nature. But, due to their complexity these effects are often considered separately and their relative strength, acting simultaneously, remains unknown.

2. In a semi-natural field experiment using tansy (*Tanacetum vulgare* L.) and the specialised tansy aphid *Metopeurum fuscoviride* Stroyan as a model system, we compared the effects of four distinct plant chemotypes (i.e. bottom-up), defined by the bouquet of their volatile terpenoids, on aphid population dynamics by manipulating the presence/absence of mutualistic ants and presence/absence of naturally-occurring predators (i.e. top-down).

3. Predators reduced aphid abundance and colony survival but did not reduce initial growth rate due to a time lag until predators arrived on the plants. Ants directly benefited initial aphid growth rates and abundance, even in the absence of predators, but not the number of days an aphid colony persisted on the plant.

4. Plant chemotype directly affected aphid growth rate and final abundances across the different plants and also the abundances of tending ants and predators indirectly mediated by aphids. We found that tending ants were more abundant on one plant chemotype. Although ant abundance did not affect aphid population development, it became clear that ants had a preference towards aphids on certain chemotypes. However, a higher number of predators led to a lower number of aphids.

5. The results confirm the importance of plant chemical variation, acting through multiple effects on many species in arthropod communities, and support results from field studies. In a natural population, with a diverse selection of host-plant variants, aphid populations and their interacting species can therefore be structured at the level of an individual plant. This has implications for research in metacommunity ecology, which often consider host-plants to be homogenous within a given local population and rarely consider spatial effects at local scales.

INTRODUCTION

Herbivore populations and communities are considered to be regulated by bottom-up (resource-based) and top-down (e.g. predation) processes (Kareiva & Sahakian, 1990; Hunter & Price, 1992; Forkner & Hunter, 2000; Walker & Jones, 2001; Wimp & Whitham, 2001; Ode, 2006; Hanley & La Pierre, 2015). The relative importance of each process often depends on the developmental stage of the herbivore; for example, larvae and adults can experience a different set of selection pressures (Walker & Jones, 2001). Many research papers investigated such dynamics from a single perspective only, or emphasised only one of the two driving forces, hence limiting our understanding of population and community dynamics in terrestrial systems (Walker & Jones, 2001).

Top-down control by predators is assumed to play a crucial role in regulating herbivore populations, by negatively affecting their densities via direct predator-prey interactions (Schmitz, Hambäck & Beckerman, 2000), or indirectly by affecting the prey's behaviour (e.g. predator avoidance behaviour; Clegg & Barlow, 1982). This can alter herbivore population growth, distribution (Roitberg, Myers & Frazer, 1979) and stabilise associated communities (Halaj & Wise, 2001).

As herbivores directly depend on the availability and quality of their host plants (i.e. food source), variation in plant traits (e.g. nutritional quality, genotype or phenotype) can also directly influence herbivore population growth and distribution among host-plants (bottom-up effects; e.g. Dixon, Chambers & Dharma, 1982; Awmack & Leather, 2002; Johnson, 2008). In natural systems, it is unlikely that either bottom-up or top-down forces act in isolation, rather there is a combination of these forces (Hunter and Price, 1992). For instance, predators can be influenced by the direct influence of plants on herbivore densities that then affects predator density (density-mediated interactions; Bailey et al. 2006), or plants can alter herbivore traits that change their susceptibility to predation (trait-mediated interactions; e.g. by sequestering plant secondary compounds; Brower et al. 1967; Mooney and Singer, 2012). Furthermore, predator-prey interactions can be indirectly affected by host-plant variation through direct effects of the plants acting on predator density

(Gassmann & Hare, 2005; Poelman et al. 2009). Plants have also been shown to directly influence predator traits, for example the availability of plant extrafloral nectar can increase the longevity and searching efficiency of parasitoid wasps (Siekmann, Tenhumberg & Keller, 2001). These mechanisms can indirectly influence herbivore abundances (density-mediated or trait-mediated indirect effects on herbivores; Mooney and Singer, 2012). This means that bottom-up effects can affect herbivore populations not just directly but also indirectly, via effects of plant variation on predator populations, thus changing the outcome of species interactions (Wimp & Whitham, 2001; Johnson & Agrawal, 2005; Whitham et al., 2006; Johnson, 2008; Whitham et al., 2012; Williams & Avakian, 2015; Zytynska & Weisser, 2016).

Bottom-up effects mediated by intraspecific variation among plants can arise through various genetically-based traits leading, for example, to differences in plant growth habit or metabolic diversity (Kareiva & Sahakian, 1990; Johnson & Agrawal, 2005; Johnson, 2008; Mooney & Agrawal, 2008; Williams & Avakian, 2015; Bálint et al., 2016; Zytynska & Weisser, 2016; Züst & Agrawal, 2017). Host plant biochemistry is a key factor in affecting herbivore performance and often mediates herbivore preferences (Bernays & Chapman, 1994; Karban & Baldwin, 1997; Rosenthal & Berenbaum, 1992). One extensively studied type of variation in plant chemical diversity is the metabolic variation in secondary metabolites such as volatile organic compounds (VOCs; Keskitalo, Pehu & Simon, 2001; Lee, Sugawara, Yokoi & Takahata, 2010; Eller, de Gouw, Graus & Monson, 2012; Holopainen & Blande, 2012; Azam et al., 2013). Plant VOCs can either be stored in specific morphological structures, constitutively synthesised and emitted (Clancy, Zytynska, Senft, Weisser & Schnitzler, 2016) or emitted after induction by abiotic or biotic stress (Holopainen & Gershenzon, 2010). Plants use these volatile compounds for direct defence (e.g. Martin & Bohlmann, 2005) or for internal, intra- or interspecific communication (e.g. Riedlmeier et al., 2017) as well as for communicating with higher trophic levels (reviewed in Paré & Tumlinson, 1999; de Vos & Jander, 2010; Holopainen & Blande, 2012). One example is the recruitment of predators or parasitoids by herbivore-infested plants (plant-natural enemy-herbivores; e.g. Ninkovic, Al Abassi & Pettersson, 2001; Linhart, Keefover-Ring, Mooney, Breland & Thompson, 2005; Bálint et al., 2016). Some herbivore species (Prudic, Khera, Sólyom & Timmermann, 2007; Opitz & Müller, 2009; Goodey, Florance, Smirnoff & Hodgson, 2015; Erb & Robert, 2016) have also evolved to take advantage of host-plant derived secondary metabolites (including non-volatile defensive compounds, e.g. salicin derivatives or glucosinolates, and volatile defensive compounds, e.g. benzaldehyde) to use them in their own defence strategies against predation (Gauld, Gaston & Janzen, 1992; Dyer, 1995). Thus, plant within-species variation in the abundance and composition of secondary metabolites, like VOCs, leading to so called different plant chemotypes (i.e. a group of plants with similar chemical profiles; Holopainen, Hiltunen & von Schantz, 1987; Keefover-Ring, Thompson & Linhart, 2009; Ghirardo, Heller, Fladung, Schnitzler & Schroeder, 2012; Clancy, Zytynska, Senft, Weisser & Schnitzler, 2016), can have multiple effects on herbivore populations and the associated arthropod community.

Plant-aphid-predator systems are ideal for simultaneously studying bottom-up and top-down effects. Aphids (Hemiptera; Aphididae) are specialised insects feeding on the phloem sap of particular plants. Due to the highly specific nature of this interaction, plant within-species variation can have dramatic effects on the plant-aphid relationship. For instance, variation among plant genotypes or chemotypes (e.g. varying in camphor, β-pinene and linalool) can directly affect aphid performance (Underwood & Rausher, 2000; Linhart, Keefover-Ring, Mooney, Breland & Thompson, 2005; Krauss et al., 2007; Kleine & Muller, 2011; Utsumi, Ando, Craig & Ohgushi, 2011; Williams & Avakian, 2015). Furthermore, aphids are prey to a number of specialised and generalised predators, able to control aphid populations in a top-down manner (reviewed in Diehl, Sereda, Wolters & Birkhofer, 2013).

Many aphid species are able to establish mutualistic relationships with ants. These plant-aphid-predator systems then gain in complexity (Stadler & Dixon, 2005). In exchange for aphid-produced honeydew, ants provide aphids with hygienic and protective services. Ants influence the predator-aphid relationship by attacking or carrying away (i.e. showing antagonistic behaviour) aphidophagous predators like syrphid larvae or ladybirds (reviewed in Way, 1963), and thereby increasing aphid fitness (Nixon, 1951; Addicott, 1978; Buckley, 1987; Flatt & Weisser, 2000; Stadler & Dixon, 2005).

However, ants are not always beneficial for aphids but can also act as predators (Billick, Hammer, Reithel & Abbot, 2007; Singh, Zytynska, Hanna & Weisser, 2016). In many aphid systems, both mutualistic ants and predators can be influenced by intra-specific variation in the host plant. With this, both density-mediated and trait-mediated indirect effects on the third trophic level can come into effect. Moreira & Mooney (2013), for instance, could show that plant genetic diversity directly influenced aphid abundance which in turn affected the abundance of mutualistic ants and parasitoids (i.e. density-mediated indirect effects on the third trophic level). Host-plant mediated changes in aphid traits (e.g. through plant architecture or biochemical diversity) are also known to affect the aphids' susceptibility to predatory attacks (e.g. increased hiding places) or altering ant preferences (and thus density) via variation in aphid-honeydew composition across plants (i.e. trait-mediated indirect effect on the third trophic level; Cushman, 1991; Fischer & Shingleton, 2001; Johnson, 2008; Kareiva & Sahakian, 1990; Züst & Agrawal, 2017). The availability of ants can be crucial for some aphid species (e.g. obligate myrmecophilous species) and limit their realised distribution across different host plants (Wimp & Whitham, 2001; Senft, Weisser & Zytynska, 2017).

In summary, aphids are subjected to a number of forces (e.g. plant chemotype, mutualistic ants and predators) defining their fitness. However, to which extent these different bottom-up and top-down forces contribute to observed patterns remains elusive.

One aphid-plant system that has been studied extensively is the tansy-aphid system. It offers ideal conditions to test relative effects of bottom-up and top-down effects (Stadler, 2004). It consists of (i) common tansy (*Tanacetum vulgare* L.; Asteraceae), an aromatic plant with a high chemical diversity regarding quantity and quality of stored and emitted VOCs (i.e. different plant chemotypes; Clancy, Zytynska, Senft, Weisser & Schnitzler, 2016; Forsén & Von Schantz, 1973; Rohloff, Mordal & Dragland, 2004); (ii) the highly specialised aphid *Metopeurum fuscoviride* Stroyan (Homoptera, Aphidoidea), an obligate myrmecophilous species, commonly tended by (iii) ants such as *Lasius niger* L. (Formicidae); and (iv) predated on by various common aphidophagous predators. In field studies on this system, the occurrence of aphids, tending ants and aphidophagous predators were associated with differences in the blend of volatile terpenoids across different plant chemotypes (Bálint et al., 2016; Clancy, Zytynska, Senft, Weisser & Schnitzler, 2016). This bottom-up effect of plant chemotype may therefore mediate effects of mutualists and predators on aphid populations (i.e. indirect effects of the chemotype), which could contribute to the distinct distribution of aphids observed in field surveys (Senft, Weisser & Zytynska, 2017); e.g. through higher predation pressure or reduced protection by ants on certain plant chemotypes.

So far, findings of potential plant chemotype, ant and predator effects on the aphid populations in the tansy-aphid system are mostly based on observational data, and thus remain correlational. Herein, we carried out a manipulation experiment that allowed us to explore how and to what extent direct and indirect bottom-up and top-down forces affect the aphid populations on tansy plants. While tansy terpenoid production (chemical volatiles), and thus also differences between chemotypes, has a genetic basis (i.e. different genotypes express different chemical phenotypes; Holopainen, Hiltunen, Lokki, Forsén & Schantz, 1987), all our hypotheses are based on differences regarding variation in the plant’s volatile chemical profile. Firstly, we hypothesised that aphid population growth rates (and subsequent abundance and colony survival) will vary across plant chemotypes. Secondly, we hypothesised that ants will benefit aphid populations by increasing growth and survival rates, whereas predators will have a negative effect on these parameters. Finally, the combined effect of ants and predators on aphid population dynamics (growth, abundance and colony survival) will depend on the plant chemotype, explained by effects of chemotype on the abundance of ants and predators on each plant.

MATERIAL AND METHODS

PLANTS PROPAGATION AND GC-MS PROFILING OF CHEMOTYPES

We used plants grown from seed collected from a field site of tansy (*Tanacetum vulgare* L.; Asteraceae) that was previously the focus of two studies about the spatio-temporal dynamics of tansy aphids (Senft, Weisser & Zytynska, 2017), and the chemical diversity of tansy plants and how this affects early aphid colonisation (Clancy, Zytynska, Senft, Weisser & Schnitzler, 2016). Seeds were collected in late autumn 2013 from dried flower heads of plants growing at a distance between 3-21 m on a field site with 172 plant patches in Altenhausen, north of Freising in southern Germany (N 48°25'1.51"; E 11°46'1.19"). Tansy plants have a low rate of self-fertilisation (Lokki, Sorsa, Forsén & Schantz, 1973). Therefore, the chemotypic profile of mother plants and their offspring can be different. We first grew 18 plants from seed collected from eight mother plants (1-3 seedlings per mother plant); these were chosen because they differed in their effects on aphids in the field (Clancy, Zytynska, Senft, Weisser & Schnitzler, 2016). Plants were grown in individual pots (13 cm in diameter) containing commercial potting soil (Einheitserde®, Type SP ED63 T, Sinntal-Altengronau, Germany) and kept under greenhouse conditions (21.6°C mean ambient temperature, 67% mean relative humidity, 16:8 hours (light:dark) at Dürnast Experimental Station, Technical University of Munich, Freising, Germany) prior to the experiment. We analysed the chemical composition of all 18 plants using gas chromatography mass-spectrometry (GC-MS; see Table S1 in Supporting Information), based on 22 volatiles 'putatively emitted from storage' (i.e. constitutively released from undisturbed glands on the plant's surface), which differentially defines tansy plants due to the variation in terpenoids (monoterpenes, oxygenated monoterpenes and sesquiterpenes) as in (Clancy, Zytynska, Senft, Weisser & Schnitzler, 2016). From this, four plant individuals were chosen for the current experiment, based on chemotype effects in the field, including two plants low in 4-Terpineol (higher aphid colonisation) and two high in this compound (lower aphid colonisation). Within these groups, the two plants were further chosen to be different in other aphid-related compounds from field data such as (E)-Dihydrocarvone. By comparing the chemical profiles (based on the relative concentrations of the 22 volatile terpenoids 'putatively emitted from storage'; Table S1) of the experimental plants to the profiles of field plants (data from Clancy, Zytynska, Senft, Weisser & Schnitzler, 2016) and by clustering them, we could confirm that the chemical profiles of the four experimental plants reflected the diversity of the chemical profiles of 172 plants from field sites (further information about the chemotype identification and the clustering analysis of experimental and field plants can be found in Appendix S1). The field and experimental plants clustered into the four main classes as previously described (Clancy, Zytynska, Senft, Weisser & Schnitzler, 2016). Two out of four experimental plants belonged to class 2 chemotypes (experimental chemotypes 2.1 and 2.2) and two to class 4 chemotypes (experimental chemotypes 4.1 and 4.2; Fig. 1). Class 2 chemotypes were dominated mostly by L-camphor (55.9% ± 2.4%; Fig. 1) and supported early aphid colonisation in the field (Clancy, Zytynska, Senft, Weisser & Schnitzler, 2016). The terpenoid profiles of class 4 chemotypes were not clearly dominated by a single volatile, however (Z)-β-terpineol (from 0.0 % to 55.0 %) and eucalyptol (from 2.2 % to 33.1 %) were most abundant (Fig. 1). Early aphid colonisation in the field was less likely on plants belonging to chemotype class 4 (Clancy, Zytynska, Senft, Weisser & Schnitzler, 2016).

We then used vegetative propagation to obtain 20 clonal replicates of each of the four individual experimental chemotypes. This was achieved simply through splitting mother plants into multiple sections. Daughter plants were repotted in separate pots and regrowth occurred. We have previously shown that this method produces stable chemotype clones in tansy (Clancy, Zytynska, Senft, Weisser & Schnitzler, 2016). Three weeks after the last splitting event, and a week prior to the experiment, all but the longest stem were trimmed.

APHIDS AND ANTS

Metopeurum fuscoviride aphids were collected from various tansy plants (mixed aphid genotypes) grown in the vicinity of the Weihenstephan campus of the Technical University of Munich, Freising, Germany. To avoid a bias due to variation among aphid genotypes, aphids were collected all in one glass jar and randomly selected for the experiment (note, crowding does not induced winged morph production in this species; Mehrparvar, Zytynska & Weisser, 2013). Collected aphids were used the same day for the experiment (see experimental design section).

Lasius niger L. ant colonies (five colonies with each >2000 workers) were also collected around the University campus. All ant nests contained a high number of brood (> 500). The colonies were housed in 10 L buckets and placed on the experimental field site near Dürnast Experimental Station, five days prior to the start of the experiment.

EXPERIMENTAL DESIGN

To test the effects of plant chemotype, mutualistic ants and predators on aphid performance (aphid growth rate, abundance and survival), we used a fully-factorial randomised block design with four chemotypes (chemotypes 2.1, 2.2, 4.1 and 4.2) from two different chemotype classes (class 2 chemotypes are known to support early aphid colonisation while class 4 chemotypes did not support early aphid colonisation in the field), two ant treatments (presence and absence) and two predator treatments (presence and absence). Each of the 16 treatments was replicated five times (i.e. five blocks), resulting in 80 tansy plants. Each of the five blocks contained one repeat of each treatment in randomised order and a different *L. niger* ant colony (i.e. each of the five ant colonies was connected to 8 of the 16 plants in each block; Figure S1).

EXPERIMENTAL SETUP

The experiment was performed near Dürnast Experimental Station (N 48°24'32", E 11°43'20") within a rectangular meadow with an approximate size of 90 x 30 m from mid-June until mid-July 2015. Only the part where the tansy plants were placed was mown (7 x 7 m). High grass and a variety of flowering plants surrounded the experimental area, ensuring sufficient habitat for natural enemies. The plants were transferred from the greenhouse to the field site and watered twice a day with tap water in case of no rain. Tube-like cages with a height of 30 cm and a diameter of 13 cm made out of PET transparencies (IP 2912, H. Brunner GmbH, Achern, Germany) were placed on top of each pot and sealed (Fig. 2). The cages contained a fine mesh on one side to allow ventilation and the top of each cage was closed with a removable mesh lid. PVC-tubes (1 m length) were connected from the ant colonies to the cages allowing ants to enter the cages. Insect glue (Raupenleim grün, Schacht, Braunschweig, Germany) was used around the top of the cages and a fine mesh sealing the bottom of the pot to prevent ants from entering the cages opportunistically (Fig. 2).

Ants were allowed to access the plants immediately after placing the plants outside (June 21st). At the beginning the mesh lids on top of the cages were closed to prevent predators from accessing the plants until start of the experiment. One day later (June 22nd) we added 10 * 1st instar larvae, 10 * 2nd/3rd instar larvae and 5 * 4th instar larvae/adult aphids to each plant, allowing them to settle overnight. On the next day (June 23rd) the aphids were counted and missing aphids (same age structure) were replaced the next day. On June 25th (day zero of the experiment) the numbers of aphids per plant were re-counted and the experimental cages were opened in the predator treatments. Aphids, ants and predators in each cage were counted with a mechanical counter on days 2, 4, 6, 8, 11, 14, 17 and 20. The order of counting the cages (from 9 a.m. to 4 p.m.) was randomised every day to avoid diurnal effects. If no ant could be encountered on a plant, five ants were collected from the corresponding colony and transferred to the plant (these ants were not counted). At the end of the experiment on July 15th the aboveground biomass of each tansy plant was measured by drying plants at 70 °C for three days for dry weight determination.

STATISTICAL ANALYSIS

To quantify aphid population performance we used three measures: (1) initial growth rate (up to day eight), which reflects the reproduction potential of a colony; (2) final abundance (day 20), which reflects the overall success; and, (3) survival, reflecting the persistence of a colony.

For each cage a per capita initial growth rate (Agrawal, Underwood & Stinchcombe, 2004) was calculated by subtracting the natural log of the aphid abundance on day eight (time when ant attendance dropped; Figure S2) by the log of the initial aphid abundance and then divided by the number of days. Linear models were used to analyse the aphid growth rate. Explanatory variables were either chemotype class (Class 2 and Class 4) or individual chemotypes (2.1, 2.2, 4.1 and 4.2), ant treatment (presence/absence) and predator treatment (presence/absence). In the full model chemotype was allowed to interact with the ant and the predator treatment. As a fixed factor we used experimental block (1-5) and plant biomass as a covariate. Non-significant interactions and explanatory variables were removed from the full model using the backwards stepwise method. In the result section only minimal adequate models are presented. The strength of each effect was estimated by calculating the percentage of variance explained by each predictor (i.e. predictor sum of squares divided by the total sum of squares, multiplied by 100%).

To analyse treatment effects on the final aphid abundance, we calculated the log of the difference between the final and the initial aphid abundance. As described above, we used two linear models (log-transformed to achieve linearity) with the same variables as for the growth rate analysis.

The survival of aphid colonies was analysed using the survival functions "survfit" (package "survival"; Therneau, 2015) with standard settings in R, version 3.2.2 (R Development Core Team, 2014). Estimates of the different survival curves (censored data) were calculate using the Kaplan-Meier method by the "survfit" function and a cox proportional hazard model was used to analyse the effects of the different treatments (chemotypes, ants (presence/absence) and predators (presence/absence)) on aphid colony survival. A full model, containing all interactions, was fit to the data and the backwards stepwise method was applied to remove non-significant terms.

We further analysed the abundance of ants and predators on each day by using a generalised linear mixed effect model fit by maximum likelihood (GLMER) in the R package "lme4" (Bates, Maechler, Bolker & Walker, 2015) with a poisson error distribution and log link function. In this analysis each plant identity was included as the random factor due to repeated observations over the time course of the experiment. Continuous explanatory variables were centred and scaled using the scale function. As explanatory variables we used predator or ant treatment, respectively, and chemotype or chemotype class. To distinguish between density- and trait-mediated effects of chemotype (mediated by aphids) on ant or predator abundances we established two models: one with the number of aphids (during the day of observation) as covariate and one without (Mooney & Singer, 2012; Moreira & Mooney, 2013). Experimental block was used as fixed effect, plant biomass and day of observation were used as covariates in both models. A full model, containing all explanatory variables and covariates, was fit to the data and the backwards stepwise method was applied to remove non-significant terms. Significance levels were calculated after model comparison through likelihood ratio tests.

We used linear models to determine if (i) the mean number of ants was correlated with aphid colony growth rate (until day 8), i.e. are more ants associated with a higher aphid colony growth rate?; (ii) a higher aphid colony growth rate led to higher aphid colony peak population sizes (log transformed), and, (iii) a higher mean predator abundance led to a decrease final aphid abundance. All statistical analyses were carried out in R, version 3.2.2 (R Development Core Team, 2014).

RESULTS

The initial aphid population size was 40.0 ± 1.3 aphids (mean ± SE). The number of ants visiting aphid colonies decreased over time with more ants tending until day eight (X^2_1=74.79, P<0.001; Figure S2 and Table S2). Final plant biomass varied across chemotypes ($F_{3,76}$=5.98, P=0.001), and was therefore included as a covariate in further models (i.e. plant biomass was highly correlated with plant growth rate (calculated as length growth per day): LM $F_{1,78}$=147.45, p<0.001).

Over the experimental period, five different kinds of aphidophagous predators were observed: syrphid larvae (Syrphidae; $N_{cumulative}$=24) on 14 plants (35% of plants where predators were allowed to enter); ladybirds and ladybird larvae (e.g. *Coccinella septempunctata* L., Coccinellidae; $N_{cumulative}$=13) on two plants (5%); lacewing larvae (Chrysopidae; $N_{cumulative}$=6) on five plants (12.5%); spiders (Araneae; $N_{cumulative}$=6) on three plants (7.5%); and parasitoids indirectly encountered through mummies (hardened shell of the host aphid after successful parasitism by a parasitoid wasp; Braconidae; $N_{cumulative}$=43) on eleven plants (27.5%). Despite the relatively high proportion of plants with mummies, the abundance per plant remained low, ranging between one and eight, and therefore parasitoids did not strongly contribute to any top-down regulation effect on aphid colony sizes, and thus were removed from further models.

APHID COLONY GROWTH AND ABUNDANCE

To test the influence of plant chemotype, ants, and predators on the performance of aphids, we analysed the initial growth rate (until day eight, after which ant tending was reduced; Figure S2) of aphid colonies on each plant. Plant chemotype explained 9.5 % of the total variation in the model ($F_{3,72}$=3.36, P=0.023; Table 1). Here, the individual chemotype within the overall class was important since the aphids responded to the two chemotypes within class 2 differently. The difference was mainly driven by chemotype 2.1 which had, by far, the highest growth rate both in the presence and absence of ants (Fig. 3a).

The presence of ants on a plant had an overall positive effect on aphids by increasing colony growth rates, accounting for 13.6 % of the total variation ($F_{1,71}$=14.41, P<0.001; Table 1, Fig. 3a), but there was no evidence that higher mean ant abundances (within the ant presence treatment) on a plant resulted in higher aphid colony growth rates (LM $F_{1,38}$=1.81; P=0.187). We observed higher numbers of ants on plant chemotype 2.1 (X^2_3=7.66, P=0.053; Fig. 3b; Figure S3, Table S2). This chemotype effect on ant abundance is not only explained by the variable aphid density (i.e. actual aphid abundance; X^2_1=9.60, P=0.002) across the plants (i.e. density-mediated indirect chemotype effect), as the effect of chemotype on ants remains significant after including aphid abundance in the model. This shows that there is a potential trait-mediated indirect effect of plant chemotype on ants (X^2_3=7.93, P=0.047; i.e. chemotype effects on aphid traits indirectly affects ants), leading to even higher ant abundances on chemotype 2.1, as expected with a simple increase in aphid numbers (i.e. higher ant-aphid ratio).

Predators on the other hand did not influence aphid colony growth, likely due to the low number of predators in the system until day eight (Table 1; Figure S4). None of the interactive terms in our full model (i.e. all possible two- and three-way interactions between chemotypes, ants and predators) were significant and thus removed from the model.

A higher aphid growth rate inevitably led to a higher peak population size (LM $F_{1,78}$=4.04, *P*=0.048). The mean aphid peak population size during the experiment was 242.1 ± 12.0 (ranging between 80 and 541). The different aphid populations reached their peaks between experimental day 6 and day 20.

As a measure for the impact of the different treatments on aphid abundances across the whole experiment (i.e. beyond the peak population size) we calculated the log of the difference between the final and the initial aphid abundance. Ants increased the final abundance of aphids ($F_{1,76}$=4.03, *P*=0.048; Table 1), but only explained 3.2 % of the total variation in the model. Predators had a strong negative impact, accounting for 20.5 % of the total variation ($F_{1,75}$=25.58, *P*<0.001; Table 1); the final abundance of aphids was around four times higher when predators were absent. Aphids on chemotype class 2 plants had higher abundances at the end of the experiment, compared to those on plants of chemotype class 4 ($F_{1,77}$=5.11, *P*=0.027; Table 1). In contrast to aphid growth rate, this effect was not driven by individual plant chemotypes. Chemotype class accounted for 4.1 % of the variation in the model. Again, none of the interactive terms (i.e. possible two- and three-way interactions between chemotypes, ants and predators) were significant indicating that additive rather than interactive effects are present in our system.

APHID COLONY SURVIVAL AND PREDATION EFFECTS

The first plants without aphids (local extinction) were observed on day eleven (N=2). At the end of the experiment (day 20) 18 plants were without aphids. Predators decreased the survival of aphid colonies (Cox proportional hazards model: LRT=7.91, P= 0.005), but this did not vary across plant chemotypes (non-sig. term) and ants did not benefit colony survival (non-sig. term). None of the interactive terms in our full survival model (i.e. all possible two- and three-way interactions between chemotypes, ants and predators) were significant and thus removed from the model.

The abundance of predators increased over time (X^2_1=12.57, P<0.001; Figure S4 and Table S3). Predators were more abundant on larger plants (X^2_1=9.08, P=0.003; Table S3) and on chemotype 4.2 (X^2_3=10.94, P=0.012; Fig. 3c; Figure S3 and Table S3). Plant chemotype was significant when the covariate aphid abundance was both included in and excluded from the model showing that chemotype effects were rather trait-mediated indirect effects on predators than mediated through aphid density (Table S3). Neither the ant presence treatment nor the abundance of ants reduced predator abundances on the plants (Table S3 and Figure S5). In general, a higher mean predator abundance led to a lower final aphid abundance (LM $F_{1,38}$=15.99, P<0.001; Figure S6).

DISCUSSION

We found that plant chemical variation influenced aphid population dynamics across the host plants. There was a positive direct effect of certain plant chemotypes on aphid population growth and through this the final abundance of aphids, but not on aphid colony survival. Further, aphid population dynamics were indirectly affected via chains of direct interactions (Fig 4; Wootton, 1994). This means that plant chemical variation altered aphid densities, and higher aphid densities led to increased ant abundances (i.e. density-mediated indirect effect), but more ants did not lead to more aphids; yet, the presence of ants had a strong positive effect on aphid numbers. In addition, beyond effects via aphid densities, we also found trait-mediated indirect effects of plant chemotype on ants. Plant chemotype also affected predator abundances; however, higher aphid densities did not lead to higher predator abundances, suggesting trait-mediated indirect effects of plant chemotype on predators. The presence of, and higher numbers of predators were more able to reduce aphid population sizes. We did not detect any interaction modifications (i.e. higher-order interactions; Wootton, 1994), since plant chemotype did not alter the overall beneficial effect of ants on aphids or negative effect of predators on aphids, but simply enhanced these effects via chemotype effects on the ants and predators.

BOTTOM-UP EFFECT OF PLANT CHEMOTYPE ON APHID PERFORMANCE

Our results confirm previous field observations of tansy aphids that showed a beneficial effect of tansy plants in terpenoid chemotype class 2 (with camphor as dominating compound; Clancy, Zytynska, Senft, Weisser & Schnitzler, 2016); Clancy, Zytynska, Senft, Weisser and Schnitzler (2016) could show that under natural conditions colonisation by winged aphids in the early part of the season was more likely on chemotype class 2 (43% probability of early aphid colonisation) than on plants from chemotype class 4 (17%) containing (Z)-β-terpineol and/or eucalyptol (syn. 1,8 cineol) as dominating volatile terpenoids. As shown here, the mechanism underlying this could be the increased growth rate when feeding on these chemotypes that would inevitably lead to higher number of aphids within a short period of time, and thus enhance the chance of successfully establishing a colony in the field. However, whether this is driven by a reduced plant defence of chemotype class 2 against aphids, or other characteristics being beneficial for aphid population development (e.g. higher defence capability of aphids against predators or diseases, like fungal infections; e.g. Züst & Agrawal, 2017) remains to be elucidated in future experiments.

BOTTOM-UP EFFECT OF PLANT CHEMOTYPE ON THE ASSOCIATED COMMUNITY

The number of ants observed on plants varied with plant chemotype. Ants visited aphid colonies on plant chemotype 2.1 more frequently indicating a potential ant preference for aphids on this chemotype, especially as ants had the free choice between colonies on all four chemotypes. This plant-derived effect on ants is indirectly mediated by aphids. While the number of aphids had an effect on ant abundance in the statistical model (i.e. density-mediated indirect effect with more aphids leading to more ants), chemotype still explained residual variation also when accounting for aphid abundance. This suggests that the plant chemotype also has a trait-mediated indirect effect on ant abundance (i.e. more ants per aphid; Mooney & Agrawal; 2008; Mooney & Singer, 2012, Moreira & Mooney, 2013). The latter implicates that the plant chemotype affects aphid traits. Here, it is conceivable that differences in aphid growth rates among plant chemotypes mediate this effect (high growth/high reward) or that aphid honeydew production/quality differs among chemotypes, for instance by differently sequestering plant secondary compounds (beneficial or disadvantageous in terms of ant recruiting; Brower et al., 1967; Fischer & Shingleton, 2001; Vantaux, Van den Ende, Billen & Wenseleers, 2011; Pringle, Novo, Ableson, Barbehenn & Vannette, 2014; Züst & Agrawal, 2017). Whether ants are indirectly more attracted to this particular chemotype or repelled by others remains unknown. It also remains unknown whether direct effects of the plant chemotype on the third trophic level (i.e. directly affecting ant density or ant traits) come into play, as the experimental design did not allow to test for such direct effects. However, it is known that plants can repel ants with certain odour profiles making aphid colonies more susceptible to predatory attacks (Ghazoul, 2001; Junker, Gershenzon & Unsicker, 2011). As described for another obligate myrmecophilous aphid species (*Chaitophorus populicola* Thomas, Wimp & Whitham, 2001), aphids could be restricted to certain plant hosts via host-plant effects on ant preference.

Predators were also indirectly affected by plant chemotype effects on aphid traits (i.e. not a density-mediated indirect effect via variable aphid colony sizes; Mooney & Singer, 2012), resulting in higher abundances on chemotype 4.2. As described for ants, it remains unknown whether chemotype indirectly affects predator abundances mediated via aphid traits (e.g. susceptibility to attacks) or whether the plant chemotype directly affects predator density. Nevertheless, due to a higher predation pressure on these chemotypes (i.e. more predators lead to a higher reduction of aphids),

aphids probably have a reduced chance to establish a new colony on these chemotypes under natural conditions. This supports our field observations (Clancy, Zytynska, Senft, Weisser & Schnitzler, 2016).

TOP-DOWN EFFECT OF ANTS AND PREDATORS ON APHID DYNAMICS

In our system, ants increased aphid population growth and final abundance, confirming previous work showing the beneficial function of ants in this mutualistic system (Stadler, 2004; Mehrparvar, Mansouri & Weisser, 2014). Despite the exclusion of ants, the obligate myrmecophilous aphid species *Metopeurum fuscoviride* was still able to maintain a relatively high colony growth rate. As consequence however, they often suffered from fungal infections (pers. observation), probably as these aphids are not able to remove honeydew efficiently and the nidus remained within the colony (Buckley, 1987; Nielsen, Agrawal & Hajek, 2010).

Ant attendance decreased over time and made *Metopeurum* colonies prone to predatory attacks. Such a decrease (between June and July) is not unusual and reflects observations in the field under natural conditions where *L. niger* encounters started to decrease in the beginning of July (Senft, Weisser & Zytynska, 2017).This may be associated with the mating flight of ants (mostly between July and August; see Hart, Hesselberg, Nesbit,& Goodenough, 2017). According to Edwards (1951), ants change their foraging behaviour from protein to sugar sources when their larvae start to pupate. This might change again, during/after mating flights, when a vast number of winged ant individuals leave the nest and thus the need of sugar-rich sources (i.e. honeydew) decreases abruptly; this might lead to the abandonment of aphid colonies. As shown elsewhere (Addicott, 1979; Rico-Gray & Oliveira, 2007), after promoting the growth of low-density aphid colonies, the positive impact of ants decreased with an increasing population of aphids. It is assumed that ants are not able to respond to the rapid increase of aphid densities or they already have sufficient resources from ‘medium-sized’ colonies. In consequence, predators were able to reduce aphid abundance and lower colony survivorship across all treatments, even on plants where ants had access (as in Stadler 2004).

Interestingly, our data suggests that there is a positive relationship between plant biomass and predator abundance as well as a negative relationship to aphid population development. Plants with higher biomass had more predator encounters and lower aphid abundances at the end of the experiment: For example, tansy chemotype 2.2 plants had a higher biomass compared to the other chemotypes, yet conferred lower aphid population growth rates. However, the causal relationship between these remains unknown, but we cannot rule out a potential positive fitness effect of lower aphid numbers on plant biomass production (Halaj & Wise, 2001).

INTRASPECIFIC VARIATION IN CHEMOTYPES

Our results highlighted the extent of variation within the main chemotype classes (i.e. between the individual chemotypes). For example we found higher growth rates of aphid colonies on chemotype 2.1 but not on chemotype 2.2. Therefore, it is not just the main compounds differentiating the plants, but also the minor compounds, or the terpenoid pattern, which affected the associated ecological community (Clancy, Zytynska, Senft, Weisser & Schnitzler, 2016). We also found inconsistencies in the preferences of ants and predators for aphid colonies on individual plant chemotypes within their respective chemotype class. Differences in aphid, ant and predator responses between individual tansy plants (beyond chemotype class) could also be caused by additional differences for example in phenotype (e.g. trichome density; Johnson, 2008) or metabolomic diversity of non-volatile compounds (Kleine & Müller, 2011, Clancy et al. resubmitted). Although, it is known that the tansy chemotype is genetically determined and associated with genetic variation and a number of morphological traits (e.g. shoot height, number of flower heads, corymb height or flowering time) (Keskitalo, Pehu & Simon, 2001).

CONCLUSION

By disentangling multiple factors under a controlled manipulated experimental design, we were able to quantify effects of plant chemotype (bottom-up), mutualistic ants and predators (top-down) on aphid population dynamics (Fig. 4). We showed that, while direct effects between pairs of species had the strongest effect on structuring the community, effects of plant chemotype could also act indirectly on aphid populations through chain of direct interactions via the higher trophic levels. This work confirms results from field studies, highlighting the ecological and evolutionary consequences of plant chemical variation for natural communities. Such variation can lead to structuring of communities at the plant level, with each plant variant (here, chemotype) having its own specific effect on the interacting herbivore, mutualist and predator populations, i.e. individual plant-specific community dynamics. In a natural population of a patchily-distributed host plant, individual variation such as chemical profile differences between plants can create a heterogeneous habitat for associated herbivores even within a single field site. Where this occurs, the community of herbivores and their associated mutualists and natural enemies may vary at the level of a single plant. This could lead to metacommunity dynamics at smaller scales than is often considered in current literature (often regional scales are used). We suggest that effects of within-species plant variation should be incorporated into studies of metacommunity dynamics, especially when the system is focused on sessile plant-specialist herbivores.

AUTHORS' CONTRIBUTION

This study was designed by MS, SZ, JPS and WWW. Data was collected and analysed by MS. The chemotype analysis was done by MC and JPS. A first draft was written by MS, and all authors contributed critically to the drafts and gave final approval for publication.

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DATA ACCESSIBILITY

In case of acceptance, the data supporting the results will be archived in an appropriate public repository such as Dryad or the institutional repository (*mediaTUM)* of the Technical University of Munich.

REFERENCES

Addicott, J.F. (1978) Competition for mutualists: aphids and ants. *Canadian Journal of Zoology,* **56**, 2093-2096.

Addicott, J.F. (1979) A multispecies aphid-ant association: density dependence and species-specific effects. *Canadian Journal of Zoology,* **57**, 558-569.

Agrawal, A.A., Underwood, N. & Stinchcombe, J.R. (2004) Intraspecific variation in the strength of density dependence in aphid populations. *Ecological Entomology,* **29**, 521-526.

Awmack, C.S. & Leather, S.R. (2002) Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, **47**, 817-844.

Azam, M., Song, M., Fan, F., Zhang, B., Xu, Y., Xu, C. & Chen, K. (2013) Comparative Analysis of Flower Volatiles from Nine Citrus at Three Blooming Stages. *International Journal of Molecular Sciences,* **14**, 22346-22367.

Bailey, J. K., Wooley, S. C., Lindroth, R. L. & Whitham, T. G. (2006) Importance of species interactions to community heritability: a genetic basis to trophic-level interactions. *Ecology Letters*, **9**, 78–85.

Bálint, J., Zytynska, S.E., Salamon, R.V., Mehrparvar, M., Weisser, W.W., Schmitz, O.J., Benedek, K. & Balog, A. (2016) Intraspecific differences in plant chemotype determine the structure of arthropod food webs. *Oecologia,* **180**, 797-807.

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015) lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-8.

Bernays, E.A. & Chapman, R.F. (1994) *Host-plant selection by phytophagous insects*. New York, NY: Chapman & Hall.

Billick, I., Hammer, S., Reithel, J.S. & Abbot, P. (2007) Ant-aphid interactions: Are ants friends, enemies, or both? *Annals of the Entomological Society of America*, **100**, 887-892.

Brower, L.P., Van Zandt Brower, J. & Corvino, J.M. (1967) Plant poisons in a terrestrial food chain. Proceedings *of the National Academy of Sciences of the United States of America*, **57**, 893–898.

Buckley, R.C. (1987) Interactions Involving Plants, Homoptera, and Ants. *Annual Review of Ecology and Systematics,* **18**, 111-135.

Clancy, M.V., Zytynska, S.E., Senft, M., Weisser, W.W. & Schnitzler, J.-P. (2016) Chemotypic variation in terpenes emitted from storage pools influences early aphid colonisation on tansy. *Scientific Reports,* **6**, 38087.

Clegg, J.M. & Barlow, C.A. (1982) Escape behaviour of the pea aphid *Acyrthosiphon pisum* (Harris) in response to alarm pheromone and vibration. *Canadian Journal of Zoology*, **60**, 2245-2252.

Cushman, J.H. (1991) Host-Plant Mediation of Insect Mutualisms: Variable Outcomes in Herbivore-Ant Interactions. *Oikos,* **61**, 138-144.

de Vos, M. & Jander, G. (2010) Volatile communication in plant–aphid interactions. *Current Opinion in Plant Biology,* **13**, 366-371.

Diehl, E., Sereda, E., Wolters, V. & Birkhofer, K. (2013) Effects of predator specialization, host plant and climate on biological control of aphids by natural enemies: a meta-analysis. *Journal of Applied Ecology,* **50**, 262-270.

Dixon, A.F.G., Chambers, R.J. & Dharma, T.R. (1982) Factors affecting size in aphids with particular reference to the black bean aphid, aphis fabae. *Entomologia Experimentalis et Applicata*, **32**, 123-128.

Dyer, L.A. (1995) Tasty Generalists and Nasty Specialists? Antipredator Mechanisms in Tropical Lepidopteran Larvae. *Ecology,* **76**, 1483-1496.

Edwards, R.L. (1951) Change in the foraging behavior of the garden ant Lasius niger. *Entomologist's Monthly Magazine London*, **87**, 280.

Eller, A.S., de Gouw, J., Graus, M. & Monson, R.K. (2012) Variation among different genotypes of hybrid poplar with regard to leaf volatile organic compound emissions. *Ecological Applications,* **22**, 1865-1875.

Erb, M. & Robert, C.A.M. (2016) Sequestration of plant secondary metabolites by insect herbivores: molecular mechanisms and ecological consequences. *Current Opinion in Insect Science*, **14**, 8-11.

Fischer, M. & Shingleton, A. (2001) Host plant and ants influence the honeydew sugar composition of aphids. *Functional Ecology,* **15**, 544-550.

Flatt, T. & Weisser, W.W. (2000) The effects of mutualistic ants on aphid life history traits. *Ecology,* **81**, 3522-3529.

Forkner, R.E. & Hunter, M.D. (2000) What goes up must come down? nutrient addition and predation pressure on oak herbivores. *Ecology,* **81**, 1588-1600.

Forsén, K. & Von Schantz, M. (1973) Chemotypen von *Chrysanthemum vulgare* '(L.) Bernh in G. Bendz & J. Santesson (Eds.), *Chemistry in Botanical Classification: Nobel Symposia Medicine and Natural Sciences* (*pp.* 145-147). New York and London: Academic Press.

Gauld, I.D., Gaston, K.J. & Janzen, D.H. (1992) Plant allelochemicals, tritrophic interactions and the anomalous diversity of tropical parasitoids: the" nasty" host hypothesis. *Oikos*, 353-357.

Ghazoul, J. (2001) Can floral repellents pre-empt potential ant–plant conflicts? *Ecology Letters,* **4**, 295-299.

Ghirardo, A., Heller, W., Fladung, M., Schnitzler, J.P. & Schroeder, H. (2012) Function of defensive volatiles in pedunculate oak (*Quercus robur*) is tricked by the moth *Tortrix viridana*. *Plant, Cell & Environment,* **35**, 2192-2207.

Goodey, N.A., Florance, H.V., Smirnoff, N. & Hodgson, D.J. (2015) Aphids pick their poison: Selective sequestration of plant chemicals affects host plant use in a specialist herbivore. *Journal of Chemical Ecology*, **41**, 956-964.

Gassmann, A.J. & Hare, J.D. (2005) Indirect cost of a defensive trait: variation in trichome type affects the natural enemies of herbivorous insects on *Datura wrightii*. *Oecologia*, **144**, 62–71.

Grevstad, F.S. & Klepetka B.W (1992) The influence of plant architecture on the foraging efficiencies of a suite of ladybird beetles feeding on aphids. *Oecologia*, **92**, 399-404.

Halaj, J. & Wise, D.H. (2001) Terrestrial trophic cascades: how much do they trickle? *The American Naturalist,* **157**, 262-281.

Hanley, T.C. & La Pierre, K.J. (Eds.) (2015) *Trophic Ecology: Bottom-up and Top-down Interactions Across Aquatic and Terrestrial Systems*. Cambridge, UK: Cambridge University Press.

Hart, A. G., Hesselberg, T., Nesbit, R. and Goodenough, A. E. (2018) The spatial distribution and environmental triggers of ant mating flights: using citizen-science data to reveal national patterns. *Ecography*, **41**, 877-888.

Holopainen, J.K. & Blande, J.D. (2012) Molecular Plant Volatile Communication. In C. López-Larrea (Ed.) *Sensing in Nature* (pp. 17-31). New York, NY: Springer US.

Holopainen, J.K. & Gershenzon, J. (2010) Multiple stress factors and the emission of plant VOCs. *Trends in Plant Science,* **15**, 176-184.

Holopainen, M., Hiltunen, R. & von Schantz, M. (1987) A study on tansy chemotypes. *Planta Medica,* **53**, 284-287.

Holopainen, M., Hiltunen, R., Lokki, J., Forsén, K., & Schantz, M. V. (1987). Model for the genetic control of thujone, sabinene and umbellulone in tansy (*Tanacetum vulgare* L.). *Hereditas*, **106 (2)**, 205-208.

Hunter, M.D. & Price, P.W. (1992) Playing Chutes and Ladders: Heterogeneity and the Relative Roles of Bottom-Up and Top-Down Forces in Natural Communities. *Ecology,* **73**, 724-732.

Johnson, M.T.J. (2008) Bottom-up effects of plant genotype on aphids, ants, and predators. *Ecology,* **89**, 145-154.

Johnson, M.T.J. & Agrawal, A.A. (2005) Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). *Ecology,* **86**, 874-885.

Junker, R.R., Gershenzon, J. & Unsicker, S.B. (2011) Floral odor bouquet loses its ant repellent properties after inhibition of terpene biosynthesis. *Journal of Chemical Ecology,* **37**, 1323-1331.

Karban, R. & Baldwin, I.T. (1997) *Induced responses to herbivory*. Chicago, Il: University of Chicago Press.

Kareiva, P. & Sahakian, R. (1990) Tritrophic effects of a simple architectural mutation in pea plants. *Nature,* **345**, 433.

Keefover-Ring, K., Thompson, J.D., & Linhart, Y.B. (2009) Beyond six scents: defining a seventh *Thymus vulgaris* chemotype new to southern France by ethanol extraction. *Flavour and Fragrance Journal*, **24**, 117-122.

Keskitalo, M., Pehu, E. & Simon, J.E. (2001) Variation in volatile compounds from tansy (Tanacetum vulgare L.) related to genetic and morphological differences of genotypes. *Biochemical Systematics and Ecology,* **29**, 267-285.

Kleine, S. & Muller, C. (2011) Intraspecific plant chemical diversity and its relation to herbivory. *Oecologia,* **166**, 175-186.

Krauss, J., Härri, S.A., Bush, L., Husi, R., Bigler, L., Power, S.A. & Müller, C.B. (2007) Effects of fertilizer, fungal endophytes and plant cultivar on the performance of insect herbivores and their natural enemies. *Functional Ecology,* **21**, 107-116.

Lee, J., Sugawara, E., Yokoi, S. & Takahata, Y. (2010) Genotypic variation of volatile compounds from flowers of gentians. *Breeding Science,* **60**, 9-17.

Linhart, Y.B., Keefover-Ring, K., Mooney, K.A., Breland, B. & Thompson, J.D. (2005) A chemical polymorphism in a multitrophic setting: thyme monoterpene composition and food web structure. *The American Naturalist,* **166**, 517-529.

Lokki, J., Sorsa, M., Forsén, K. A. J., & Schantz, M. V. (1973) Genetics of monoterpenes in *Chrysanthemum vulgare*. *Hereditas*, **74**(2), 225-232.

Martin, D. & Bohlmann, J. (2005) Molecular Biochemistry and Genomics of Terpenoid Defenses in Conifers. *Recent Advances in Phytochemistry,* **39***,* 29-56.

Mehrparvar, M., Mansouri, S.M. & Weisser, W.W. (2014) Mechanisms of species-sorting: effect of habitat occupancy on aphids' host plant selection. *Ecological Entomology,* **39**, 281-289.

Mehrparvar, M., Zytynska, S.E. & Weisser, W.W. (2013) Multiple cues for winged morph production in an aphid metacommunity. *PloS one,* **8,** e58323.

Mooney, K.A. & Agrawal, A.A. (2008) Plant genotype shapes ant-aphid interactions: implications for community structure and indirect plant defense. The American Naturalist*,* **171**, E195-205.

Mooney K.A. & Singer M.S. (2012) Plant variation in herbivore-enemy interactions in natural systems. In Ohgushi T., Schmitz O., Holt R.D. (Eds.) *Ecology and Evolution of Trait-Mediated Indirect Interactions: Linking Evolution, Community, and Ecosystem*. Cambridge, UK: Cambridge University Press.

Ness, J.H., Morris, W.F. & Bronstein, J.L. (2009) For ant-protected plants, the best defense is a hungry offense. *Ecology*, **90**, 2823–2831.

Nielsen, C., Agrawal, A.A. & Hajek, A.E. (2010) Ants defend aphids against lethal disease. *Biology Letters,* **6**, 205-208.

Ninkovic, V., Al Abassi, S. & Pettersson, J. (2001) The influence of aphid-induced plant volatiles on ladybird beetle searching behavior. *Biological Control,* **21**, 191-195.

Nixon, G.E.J. (1951) The association of ants with aphids and coccids. London, UK: Commonwealth Institute of Entomology.

Ode, P.J. (2006) Plant chemistry and natural enemy fitness: effects on herbivore and natural enemy interactions. *Annual Review of Entomology,* **51**, 163-185.

Opitz, S. E., & Müller, C. (2009). Plant chemistry and insect sequestration. *Chemoecology*, **19**, 117-154.

Paré, P.W. & Tumlinson, J.H. (1999) Plant volatiles as a defense against insect herbivores. *Plant Physiology,* **121**, 325-332.

Poelman, E.H., Oduor A.M., Broekgaarden C., Hordijk C. A., Jansen J.J., Van Loon J.J., Van Dam N.M., Vet L.E., & Dicke M. (2009). Field parasitism rates of caterpillars on *Brassica oleracea* plants are reliably predicted by differential attraction of Cotesia parasitoids. *Functional Ecology* **23**, 951-962.

Pringle, E.G., Novo, A., Ableson, I., Barbehenn, R.V. & Vannette, R.L. (2014) Plant-derived differences in the composition of aphid honeydew and their effects on colonies of aphid-tending ants. *Ecology and Evolution,* **4**, 4065-4079.

Prudic, K. L., Khera, S., Sólyom, A., & Timmermann, B. N. (2007). Isolation, identification, and quantification of potential defensive compounds in the Viceroy butterfly and its larval host–plant, Carolina willow. *Journal of Chemical Ecology*, **33**, 1149-1159.

R Development Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rico-Gray, V. & Oliveira, P.S. (2007) *The ecology and evolution of ant-plant interactions*. Chicago, Il: University of Chicago Press.

Riedlmeier, M., Ghirardo, A., Wenig, M., Knappe, C., Koch, K., Georgii, E., Dey, S., Parker, J.E., Schnitzler, J.-P. & Vlot, C. (2017) Monoterpenes support systemic acquired resistance within and between plants. *The Plant Cell***,** **29**, 1440–145.

Rohloff, J., Mordal, R., & Dragland, S. (2004) Chemotypical variation of tansy (*Tanacetum vulgare* L.) from 40 different locations in Norway. *Journal of Agricultural Food Chemistry*, **52**, 1742-1748.

Roitberg, B.D., Myers, J.H. & Frazer, B.D. (1979) The Influence of Predators on the Movement of Apterous Pea Aphids between Plants. *Journal of Animal Ecology*, **48**, 111-122.

Rosenthal, G.A. & Berenbaum, M.R. (1992) *Herbivores: their interactions with secondary plant metabolites: ecological and evolutionary processes*. New York, NY: Academic Press.

Schmitz, O.J., Hambäck, P.A & Beckerman, A.P. (2000) Trophic cascades in terrestrial systems: a review of the effects of carnivore removals on plants. *American Naturalist,* **155**, 141-153.

Senft, M., Weisser, W.W. & Zytynska, S.E. (2017) Habitat variation, mutualism and predation shape the spatio-temporal dynamics of tansy aphids. *Ecological Entomology***, 42**, 389-401.

Siekmann, G., Tenhumberg, B., Keller, M.A. (2001) Feeding and survival in parasitic wasps: sugar concentration and timing matter. *Oikos* **95**, 425–430.

Singh, A., Zytynska, S.E., Hanna, R. & Weisser, W.W. (2016) Ant attendance of the cotton aphid is beneficial for okra plants: deciphering multitrophic interactions. *Agricultural and Forest Entomology*, **18**, 270-279.

Stadler, B. (2004) Wedged between bottom-up and top-down processes: aphids on tansy. *Ecological Entomology,* **29**, 106-116.

Stadler, B. & Dixon, A.F. (2005) Ecology and evolution of aphid-ant interactions. *Annual Review of Ecology, Evolution, and Systematics*, **36**, 345-372.

Therneau T (2015). A Package for Survival Analysis in S. version 2.38, URL: https://CRAN.R-project.org/package=survival.

Underwood, N. & Rausher, M.D. (2000) The effects of host-plant genotype on herbivore population dynamics. *Ecology,* **81**, 1565-1576.

Utsumi, S., Ando, Y., Craig, T.P. & Ohgushi, T. (2011) Plant genotypic diversity increases population size of a herbivorous insect. *Proceedings of the Royal Society B: Biological Sciences,* **278**, 3108-3115.

Vantaux, A., Van den Ende, W., Billen, J. & Wenseleers, T. (2011) Large interclone differences in melezitose secretion in the facultatively ant-tended black bean aphid *Aphis fabae*. *Journal of Insect Physiology,* **57**, 1614-1621.

Walker, M. & Jones, T.H. (2001) Relative roles of top-down and bottom-up forces in terrestrial tritrophic plant–insect herbivore–natural enemy systems. *Oikos,* **93**, 177-187.

Way, M.J. (1963) Mutualism between ants and honeydew-producing Homoptera. *Annual Review of Entomology*, **8**, 307-344.

Whitham, T.G., Bailey, J.K., Schweitzer, J.A., Shuster, S.M., Bangert, R.K., LeRoy, C.J., Lonsdorf, E.V., Allan, G.J., DiFazio, S.P., Potts, B.M., Fischer, D.G., Gehring, C.A., Lindroth, R.L., Marks, J.C., Hart, S.C., Wimp, G.M. & Wooley, S.C. (2006) A framework for community and ecosystem genetics: from genes to ecosystems. *Nature Reviews Genetics,* **7**, 510-523.

Whitham, T.G., Gehring, C.A., Lamit, L.J., Wojtowicz, T., Evans, L.M., Keith, A.R. & Smith, D.S. (2012) Community specificity: life and afterlife effects of genes. *Trends in Plant Science,* **17**, 271-281.

Williams, R.S. & Avakian, M.A. (2015) Colonization of Solidago altissima by the specialist aphid Uroleucon nigrotuberculatum: effects of genetic identity and leaf chemistry. *Journal of Chemical Ecology,* **41**, 129-138.

Wimp, G.M. & Whitham, T.G. (2001) Biodiversity consequences of predation and host plant hybridization on an aphid-ant mutualism. *Ecology,* **82**, 440-452.

Wootton, J.T. (1994) The nature and consequences of indirect effects in ecological communities. Annual Review of Ecology, Evolution, and Systematics, **25**: 443-466.

Züst, T. & Agrawal, A.A. (2017) Plant chemical defense indirectly mediates aphid performance via interactions with tending ants. *Ecology***, 98**, 601-607.

Zytynska, S.E. & Weisser, W.W. (2016) The Effect of Plant Within-Species Variation on Aphid Ecology. In A. Vilcinskas (Ed.) *Biology and Ecology of Aphids* (pp. 152-170). Boca Raton, FL: CRC Press.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Table S1. Volatile organic compounds of the different chemotypes

Appendix S1. Chemotype identification and clustering

Figure S1. Experimental Design – Setup

Figure S2. Number of ants during the experiment

Table S2. Effects on ant abundances.

Figure S3. Ant abundance and predator abundance on different tansy chemotypes

Figure S4. Number of predators during the experiment

Table S3. Effects on predator abundances.

Figure S5. Predator abundance in dependency of ants presence and abundance.

Figure S6. Mean predator abundance and final aphid abundance.

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TABLES AND FIGURES

Table 1. **Effects of plant chemotype class, terpenoid chemotype, ants and predators on aphid colonies (growth rate and abundance).**

Response variable:	**growth rate$^{\#1}$**			**log(final-initial)^{1}**		
	df	**F**	**P**	**df**	**F**	**P**
<u>covariates</u>						
block	**4,75**	**2.63**	**0.041**	**-**	**-**	**-**
biomass	-	-	-	**1,78**	**15.04**	**<0.001**
<u>variables</u>						
chemotype class (n=2)	x	x	x	**1,77**	**5.11**	**0.027**
Individual chemotype (n=4)	**3,72**	**3.36**	**0.023**	x	x	x
ants (presence/absence)	**1,71**	**14.41**	**<0.001**	**1,76**	**4.03**	**0.048**
predators (presence/absence)	**-**	**-**	**-**	**1,75**	**25.58**	**<0.001**

Notes: $^{\#}$ Growth rate until day eight. ^{1} Models used were linear models. All full models contained interaction terms of main variables, but were not retained in the final models (not significant). All analysis were performed in R. "-" shows where a term was not retained in the most parsimonious model. "x" shows terms that were not included in the model.

***Figure 1*. Chemotype clustering, chemotype profiles and experimental plants**. Hierarchical cluster analysis of relative ‘likely emitted from storage’ volatile concentrations from 172 plants from a small-scale field site and the four experimental mother plants (data from the field plants were used from Clancy, Zytynska, Senft, Weisser & Schnitzler, 2016). Four main classes were identified. Stacked bars show the mean relative concentrations of terpenoids in the different classes as well as the relative concentration of each experimental mother plant (2.1, 2.2, 4.1 and 4.2).

***Figure 2.* Experimental design: cages for predator and/or ant exclusion.** Fine mesh was used to avoid aphid and ant movement between plants. Insect glue (Raupenleim grün) is a sticky substance, across which ants and other walking invertebrates cannot pass, this was used to minimise access to plants by naturally-occurring ants while allowing access to flying predator species (particularly important for the ant absence, predator present treatment combination).

***Figure 3.* The effect of plant chemotype on aphid growth rate, ant and predator abundance. a)** Plant chemotype and ants affected aphid growth rates (N = 10). Aphids on chemotype 2.1 had a higher growth rate compared to aphids on other chemotypes and the presence of ants increased aphid growth rate across all chemotypes. **b)** Plant chemotype affected ant abundance (N = 10, Observations = 9) with significant higher number of ants on chemotype 2.1 (independent of aphid abundance) than on chemotype 2.2 **c)** Plant chemotype affected predator abundance (N = 10, observations = 9) with more predators found on chemotype 4.2 than on 4.2. Mean values ± SE are shown.

***Figure 4.* The tansy chemotype acts directly on all species in the community.** Aphid population development parameters are directly affected by the associated species community (mutualistic ants and predators; arrow strength indicates strength of effect, “+” and “-“ signs indicate positive or

negative effects). Plant chemotype indirectly affected higher trophic levels via aphids through trait-mediated indirect effects (i.e. ants and predators) and density-mediated indirect effects (i.e. ants) mediated by higher number of aphids on certain chemotypes.

Fig. 1

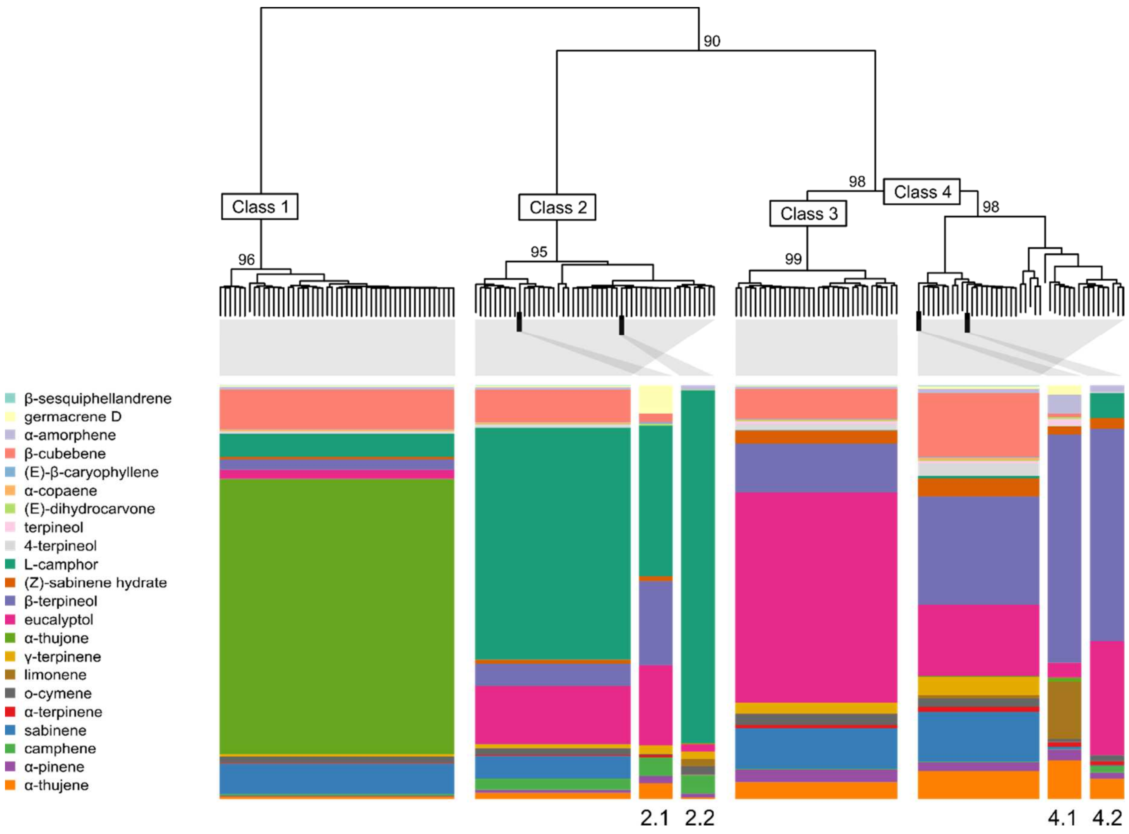

90
98
Class 4
98
Class 1
Class 2
Class 3
96
95
99
β-sesquiphellandrene
germacrene D
α-amorphene
β-cubebene
(E)-β-caryophyllene
α-copaene
(E)-dihydrocarvone
terpineol
4-terpineol
L-camphor
(Z)-sabinene hydrate
β-terpineol
eucalyptol
α-thujone
γ-terpinene
limonene
o-cymene
α-terpinene
sabinene
camphene
α-pinene
α-thujene
2.1
2.2
4.1
4.2

Fig. 2

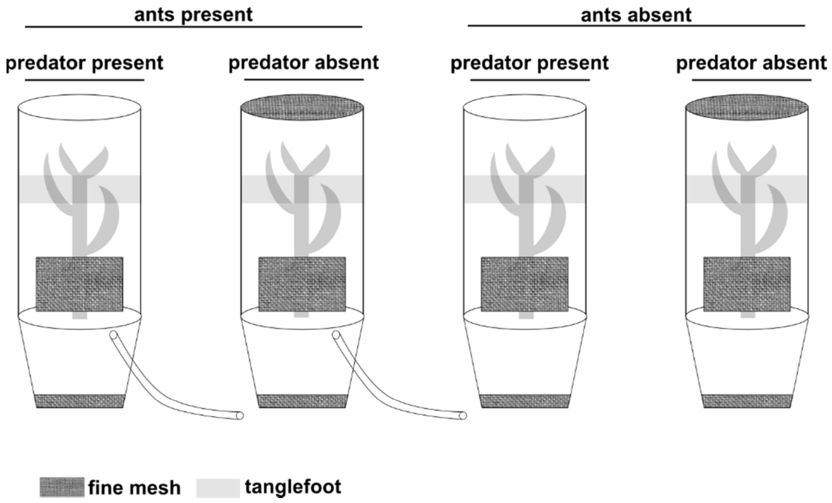
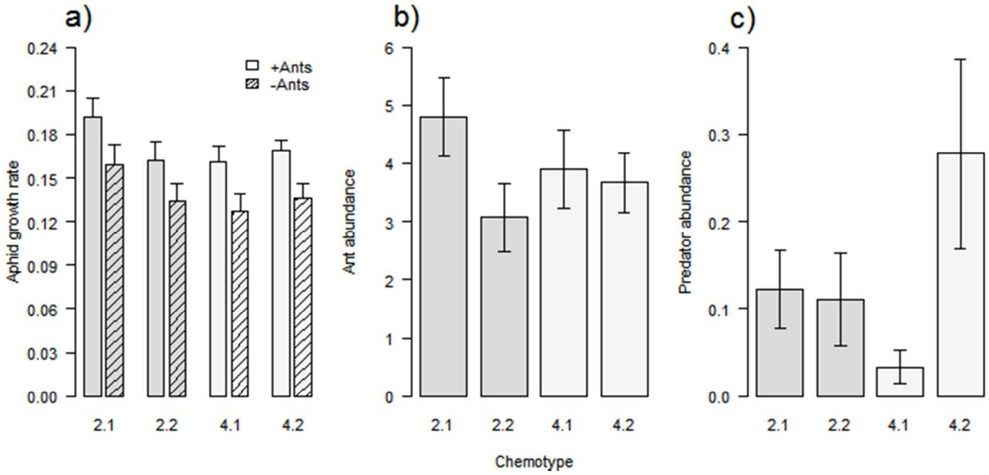
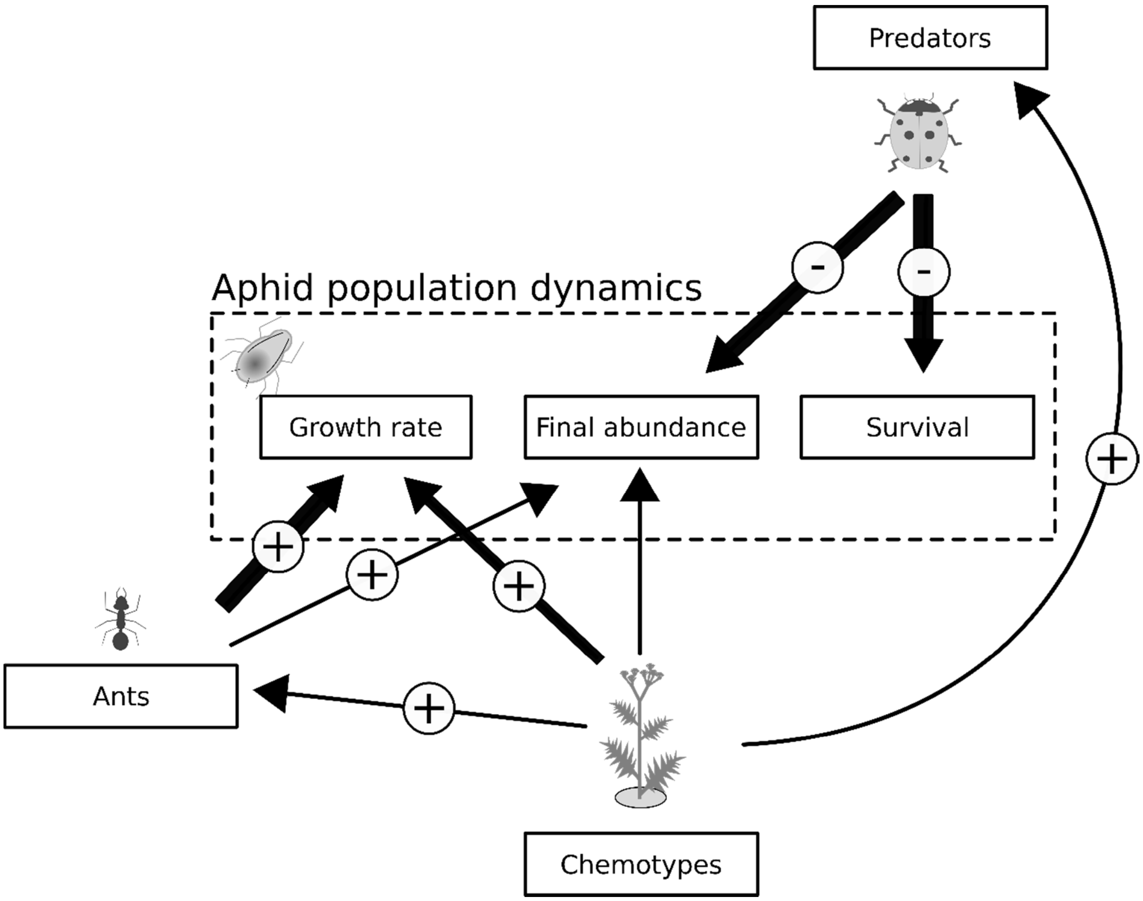

ants present
ants absent
predator present
predator absent
predator present
predator absent
fine mesh
tanglefoot

Fig. 3


a)
b)
c)
Aphid growth rate
0.24
0.21
0.18
0.15
0.12
0.09
0.06
0.03
0.00
+Ants
-Ants
Ant abundance
6
5
4
3
2
1
0
Predator abundance
0.4
0.3
0.2
0.1
0.0
2.1
2.2
4.1
4.2
Chemotype

812 Fig. 4


Predators
Aphid population dynamics
-
-
Growth rate
Final abundance
Survival
+
+
+
+
Ants
+
Chemotypes

813

Functional Ecology

Supporting Information

Additive effects of plant chemotype, mutualistic ants and predators on aphid performance and survival

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Table S1. Volatiles identified in the total hexane extracts of the experimental tansy plants. Volatiles were grouped according to Clancy et al. (2016): "stored compounds" and compounds "putatively emitted from storage glands". All values are relative amounts of the respective group.

		Chemotype	**2.1**	**2.2**	**4.1**	**4.2**
		Chemotype Class	**2**	**2**	**4**	**4**
	Amount of „stored compounds“ [ticks]		0.014215	1.013205	2.553993	0.135082
	Compound	**Family**				
Stored compounds	β-Pinene	MT	3.780576488	0.20418072	0.136157182	2.27706606
	Verbenyl-Acetate	MT-Ac	0	0.355335795	2.359124957	0.760634873
	Bornyl-Acetate	MT-Ac	33.94748315	0	0	6.566077876
	Dihydrocarvyl-Acetate	MT-Ac	0	82.05113325	74.70823192	0
	Isopulego- Acetate	MT-Ac	2.971821027	0	0	3.150671174
	Myrtenyl-Acetat	MT-Ac	0	0	0	0
	α-Terpinyl Acetate	MT-Ac	0	0.02591537	0.092239364	0
	Sabinol	O-MT	0	0	0.008661984	0.260474133
	Berbenol	O-MT	0	0	0	0
	Camphenol-6	O-MT	0.811247976	0.691464973	0	0
	Borneol	O-MT	5.864887332	0.141250055	0.043427139	17.82190699
	Myrtenol	O-MT	0	0	0.696770919	0
	(Z)-Carveol	O-MT	22.5875627	13.82971747	15.35932412	32.0582497
	γ-Elemene	SQT	0	0.01276062	0.025349804	0.113085675
	Unknown Sesquiterpene #1	SQT	4.742905844	0	0	0.700306801
	Unknown Sesquiterpene #2	SQT	0	1.046197913	1.952434726	14.04411823
	Unknown Sesquiterpene #3	SQT	0	0.038422335	0.06479748	0
	γ-Muurolene	SQT	0	0	0	0
	δ-Cadenine	SQT	0	0.531509921	1.931044807	0
	Germacrene B	SQT	0	0	0.746225488	9.024984277
	Isolongifolene	SQT	3.007066061	0.60194158	0.234077527	8.738162606
	Cedrene-13-ol, 8	SQT	2.1257653	0.453565893	1.453715281	4.362638709
	α-Cadinol	SQT	20.16068412	0	0	0
	allo-Aromadendrene	SQT	0	0.016604104	0.1884173	0.121622893

		Chemotype	**2.1**	**2.2**	**4.1**	**4.2**
		Chemotype Class	**2**	**2**	**4**	**4**
	Amount of „putatively emitted“ compounds [ticks]		0.073570	0.364745	0.350769	0.457566
	Compound	**Family**				
putatively emitted from storage glands	α-Thujene	MT	3.894415957	0.370040491	9.448758574	4.958082825
	α-Pinene	MT	1.767227394	0.984858588	2.604891969	1.495541237
	Camphene	MT	4.503712056	4.544791954	0.152330613	1.613817363
	Sabinene	MT	0	0	0.467075903	0.193684794
	α-Terpinene	MT	0.601954971	0.054242696	1.329645492	0.952374496
	o-Cymene	MT	0.178865365	2.05362998	0.694537397	1.454855996
	Limonene	MT	0	1.717378775	13.66023105	0
	γ-Terpinene	MT	2.097420111	1.807786961	0	0
	α-Thujone	MT	0	0.069123659	1.091229029	0
	Eucaliptol	O-MT	19.36099692	1.634569378	3.569476651	27.56789981
	(Z)-β-Terpineol	O-MT	20.25304062	0	55.00626509	51.17954904
	(Z)-Sabinene Hydrate	O-MT	1.25431434	0.397651011	1.988486641	2.562051467
	L-Camphor	O-MT	36.22001257	85.15932415	0	6.071621272
	4-Terpineol	O-MT	0	0	0.333043321	0.344941483
	Terpineol	O-MT	0	0	1.371315206	0
	(E)-Dihydrocarvone	O-MT	0.472168035	0.070214402	0.408956571	0
	α-Copaene	SQT	0	0	0.043523549	0
	(E)-β-Caryophyllene	SQT	0.360690013	0.154435908	0	0
	β-Cubebene	SQT	2.25684378	0	1.062006746	0
	α-Amorphene	SQT	0	0.981952051	4.487645007	1.605580224
	Germacrene D	SQT	6.778337875	0	2.280581183	0
	β-Sesquiphellandrene	SQT	0	0	0	0

Abbreviations: MT: monoterpene; MT-Ac: acetylated monoterpene; O-MT: oxygenated monoterpene; SQT: sesquiterpene.

***Appendix S1.* Additional information about identification of plant chemotypes and clustering of chemotypes into chemotype classes.**

We compared the chemical profiles (based on the relative concentrations of the 22 volatile terpenoids ‘putatively emitted from storage’; Table S1) of the experimental plants to the profiles of field plants (data from Clancy, Zytynska, Senft, Weisser & Schnitzler, 2016), using an ANOSIM in CAP4 (Henderson & Seaby, 2007) that confirmed that the chemical profiles of the 4 experimental plants reflected the diversity of the chemical profiles of 172 plants from field sites (ANOSIM, R=0.261, *P*=0.106). To confirm the classification of the four experimental plants (as described in Clancy, Zytynska, Senft, Weisser & Schnitzler, 2016), we performed the same clustering analysis using the proportion of chemicals present in each experimental and field plant. With the package ‘pvclust’ in R (Suzuki & Shimodaira, 2015), version 3.1.1 (R Development Core Team, 2014), using the Ward.D2 method, the correlation distance method and 1000 bootstrap replications. The field and experimental plants clustered into the four main classes as previously described (Clancy, Zytynska, Senft, Weisser & Schnitzler, 2016; ANOSIM R=0.922, *P*<0.001; Fig. 1). Two out of four experimental plants belonged to class 2 chemotypes (experimental chemotypes 2.1 and 2.2) and two to class 4 chemotypes (experimental chemotypes 4.1 and 4.2).

Clancy, M.V., Zytynska, S.E., Senft, M., Weisser, W.W. & Schnitzler, J.-P. (2016) Chemotypic variation in terpenes emitted from storage pools influences early aphid colonisation on tansy. *Scientific Reports,* **6**, 38087.

Henderson, P. & Seaby, R. (2007) Community analysis package 4.0. *Pisces Conservation Ltd, Lymington, UK*.

R Development Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Suzuki, R. & Shimodaira, H. (2015) pvclust: Hierarchical Clustering with P-Values via Multiscale Bootstrap Resampling. In: R package version 2.0-0. http://CRAN.R-project.org/package=pvclust.

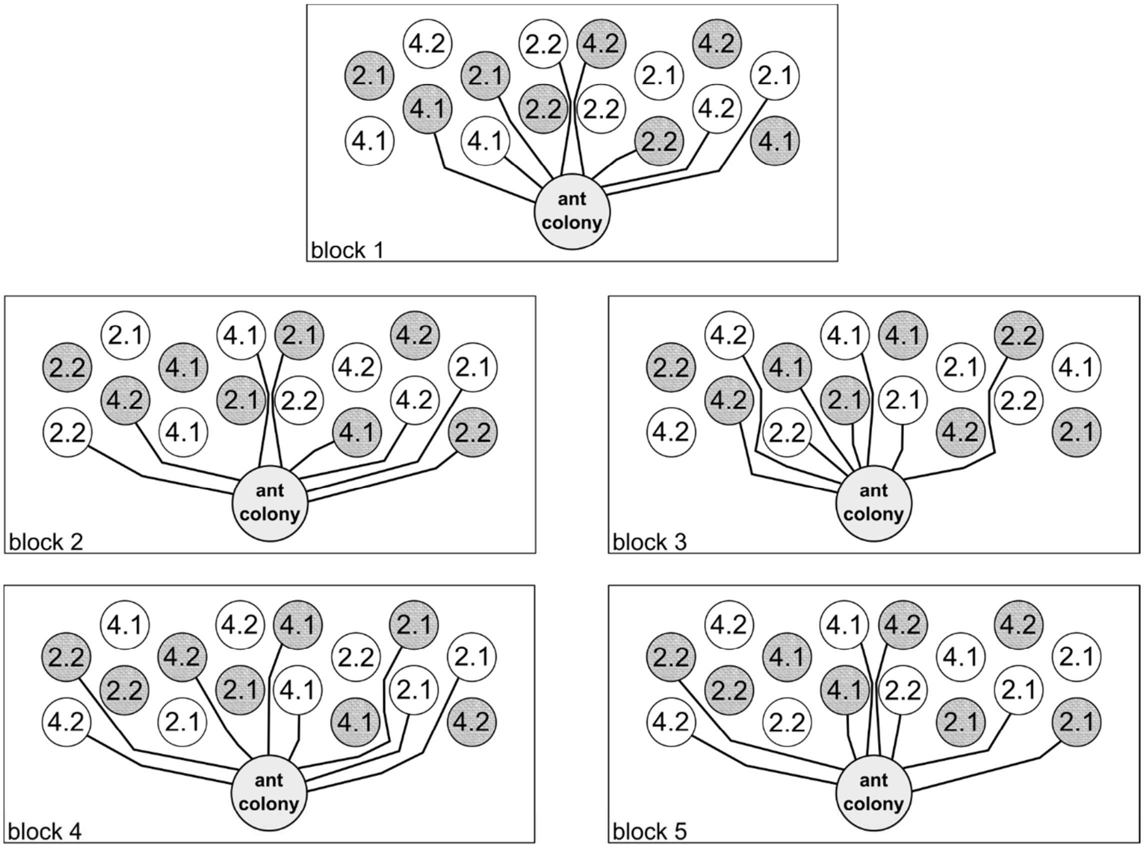


Figure S1. **Experimental design: random block design:** Four experimental tansy chemotypes (2.1, 2.2, 4.2 and 4.2), two ant treatments (presence and absence: connections to ant colonies) and two predator treatments (presence (dark cages) and absence (white cages). Each of these 16 treatments was repeated five times, resulting in 80 plants over five treatment blocks. Each block contained one repeat and a different *L. niger* ant colony. The ants of the different colonies had access (via PVC-tubes of the same length) to their respective experimental plants (8 plants per block). The position of the plants within one block was completely randomised.

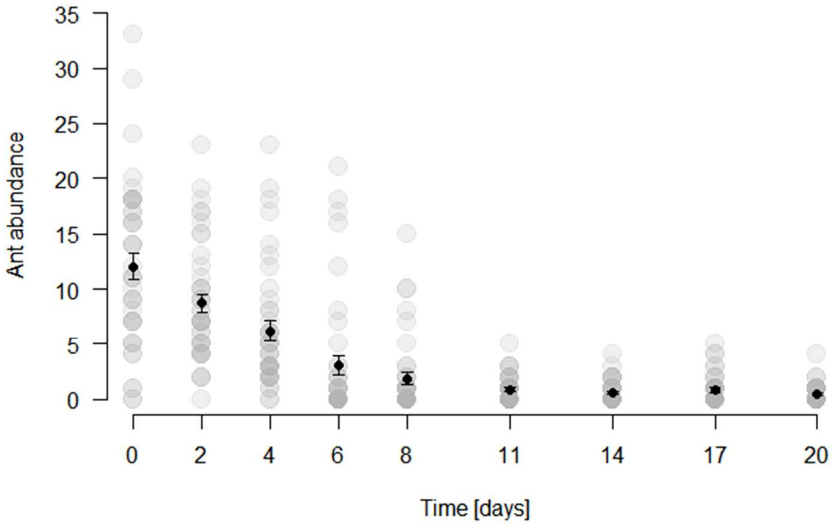


Figure S2. **Number of ants in all 'ant present' treatments.** Grey points are individual observations (superimposing leads to darker grey scale). In black the mean values ± SE are shown for each day.

***Table S2*. Effects on ant abundances.**

Response variable:	**ant abundance[1]** **density-mediated**			**ant abundance[1]** **trait-mediated**		
	$N_{obs.}=360$, $N_{plants}=40$, $N_{times}=9$			**$N_{obs.}=360$, $N_{plants}=40$, $N_{times}=9$**		
	df	**χ^2**	**P**	**df**	**χ^2**	**P**
fixed factor						
block	**4**	**37.38**	**<0.001**	**4**	**34.43**	**<0.001**
covariates						
biomass	-	-	-	-	-	-
time	**1**	**74.79**	**<0.001**	**1**	**66.37**	**<0.001**
aphid abundance	**1**	**9.60**	**0.002**	**x**	**x**	**x**
variables						
predators (presence/absence)	**-**	**-**	**-**	**-**	**-**	**-**
chemotypes	**3**	**7.93**	**0.047**	**3**	**7.66**	**0.053**

Notes: [1] Models used were generalised linear mixed model fit by maximum likelihood (Laplace Approximation) with a poisson error distribution and plant as random intercept and a random slope in time. Significance levels were calculated by using model comparison through likelihood ratio tests. "-" shows where a term was not retained in the most parsimonious model. "x" shows terms that were not included in the model. Two models with and without controlling for aphid abundances (as a covariate) were run to distinguish between density-mediated and trait-mediated effects on ant abundances (Mooney and Singer (2012)). N_{obs}: number of observations, N_{plants}: number of plants, N_{times}: number of observations pre individual plant.

Mooney K.A., Singer M.S. (2012) Plant variation in herbivore-enemy interactions in natural systems. In: T. Ohgushi, Schmitz O. & Holt R.D. (Eds.) *Ecology and Evolution of Trait-Mediated Indirect Interactions: Linking Evolution, Community, and Ecosystem*. Cambridge, UK: Cambridge University Press.

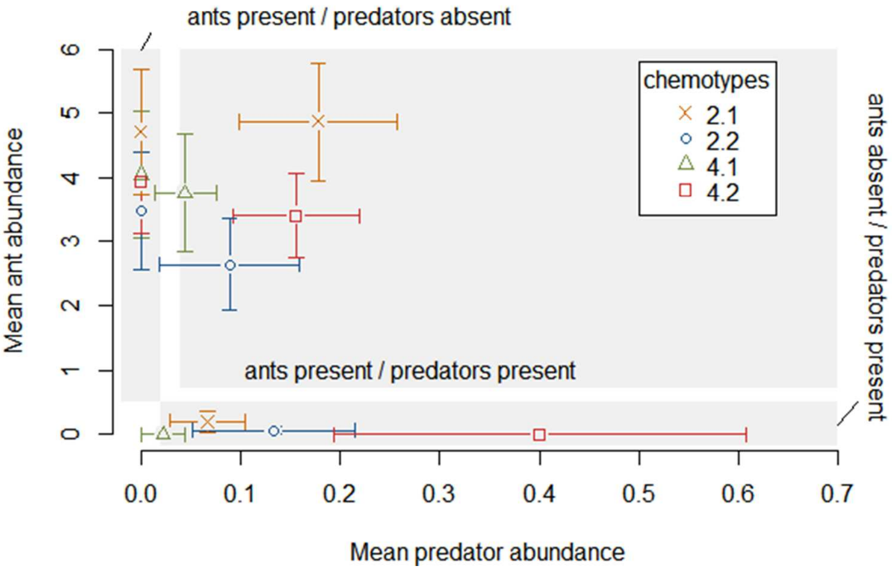


***Figure S3.* Ant abundance and predator abundance on different tansy chemotypes**. Plant chemotype had an effect on ant abundance (GLMER model “ant abundance” X^2_3=7.93, P=0.047; **Table 2**) and on predator abundance (GLMER model “predator abundance” X^2_3=10.75, P=0.013; see also **Table 2).** The different ant and predator treatment combinations are separated by grey rectangles. Mean values (N = 5, Observations = 9) and standard errors (SE) are shown.

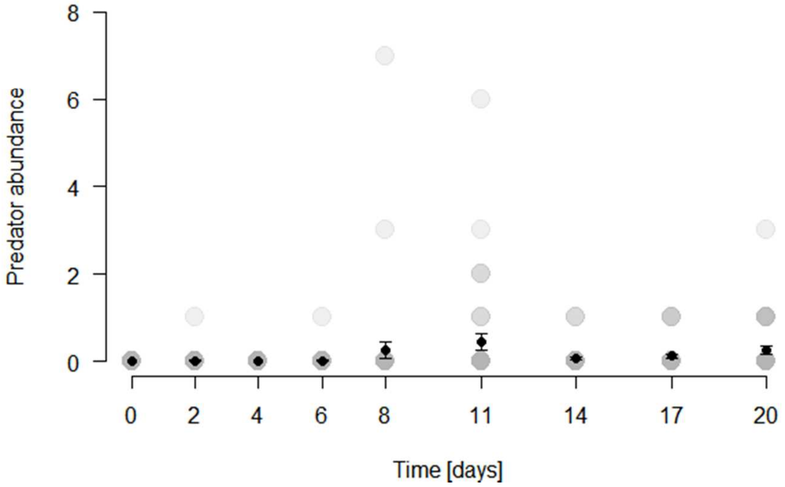


Figure S4. **Number of predators in all 'predator present' treatments.** Grey points are individual observations (superimposing leads to darker grey scale). Overall, the number of visiting predators might be higher because observations were done within a short time frame every second/third day. In black the mean values ± SE are shown for each day.

***Table S3*. Effects on predator abundances.**

Response variable:	**predator abundance[1]** density-mediated			**predator abundance[1]** trait-mediated		
	$N_{obs.}=360$, $N_{plants}=40$, $N_{times}=9$			**$N_{obs.}=360$, $N_{plants}=40$, $N_{times}=9$**		
				df	**χ^2**	**P**
fixed factor						
block	4	9.47	0.050	4	9.41	0.051
covariates						
biomass	**1**	**9.08**	**0.003**	**1**	**10.90**	**<0.001**
time	**1**	**12.57**	**<0.001**	**1**	**13.24**	**<0.001**
aphid abundance	**1**	**0.25**	0.619	**x**	**x**	**x**
variables				**-**	**-**	**-**
ants (presence/absence) or ant abundance	**-**	**-**	**-**	**-**	**-**	**-**
chemotypes	**3**	**10.94**	**0.012**	**3**	**10.81**	**0.013**

Notes: [1] Models used were generalised linear mixed model fit by maximum likelihood (Laplace Approximation) with a poisson error distribution and plant as random intercept and a random slope in time. Significance levels were calculated by using model comparison through likelihood ratio tests. “-“ shows where a term was not retained in the most parsimonious model. “x” shows terms that were not included in the model. Two models with and without controlling for aphid abundances (as a covariate) were run to distinguish between density-mediated and trait-mediated effects on predator abundances (Mooney and Singer (2012)). N_{obs}: number of observations, N_{plants}: number of plants, N_{times}: number of observations pre individual plant.

Mooney K.A., Singer M.S. (2012) Plant variation in herbivore-enemy interactions in natural systems. In: T. Ohgushi, Schmitz O. & Holt R.D. (Eds.) *Ecology and Evolution of Trait-Mediated Indirect Interactions: Linking Evolution, Community, and Ecosystem*. Cambridge, UK: Cambridge University Press.

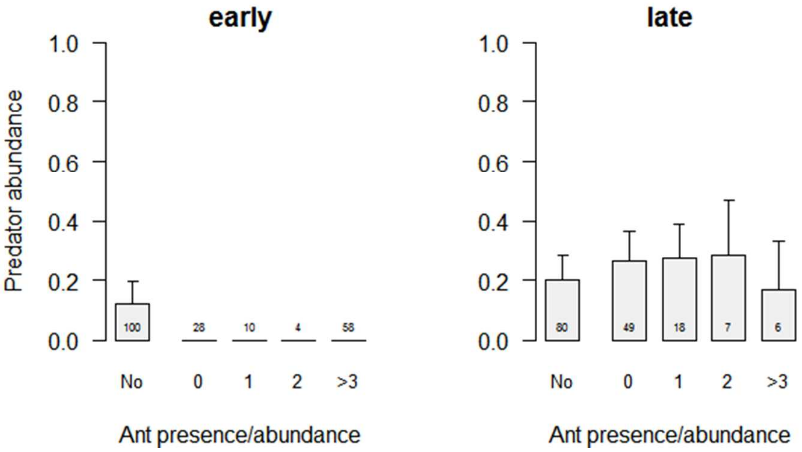


Figure S5. **Mean predator abundance in dependency of either 'ant absence' ("No") or ant abundance within the 'ant presence' treatment (0, 1, 2, > 2 ants) during the first part of the experiment (until day eight) and the late part of the experiment (day 11 until the end of the experiment).** Means and SE are shown. Numbers within bars show the number of observations.

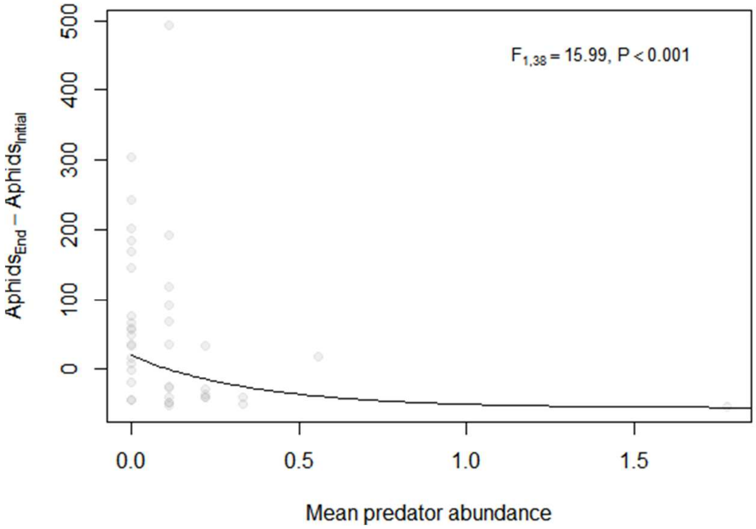
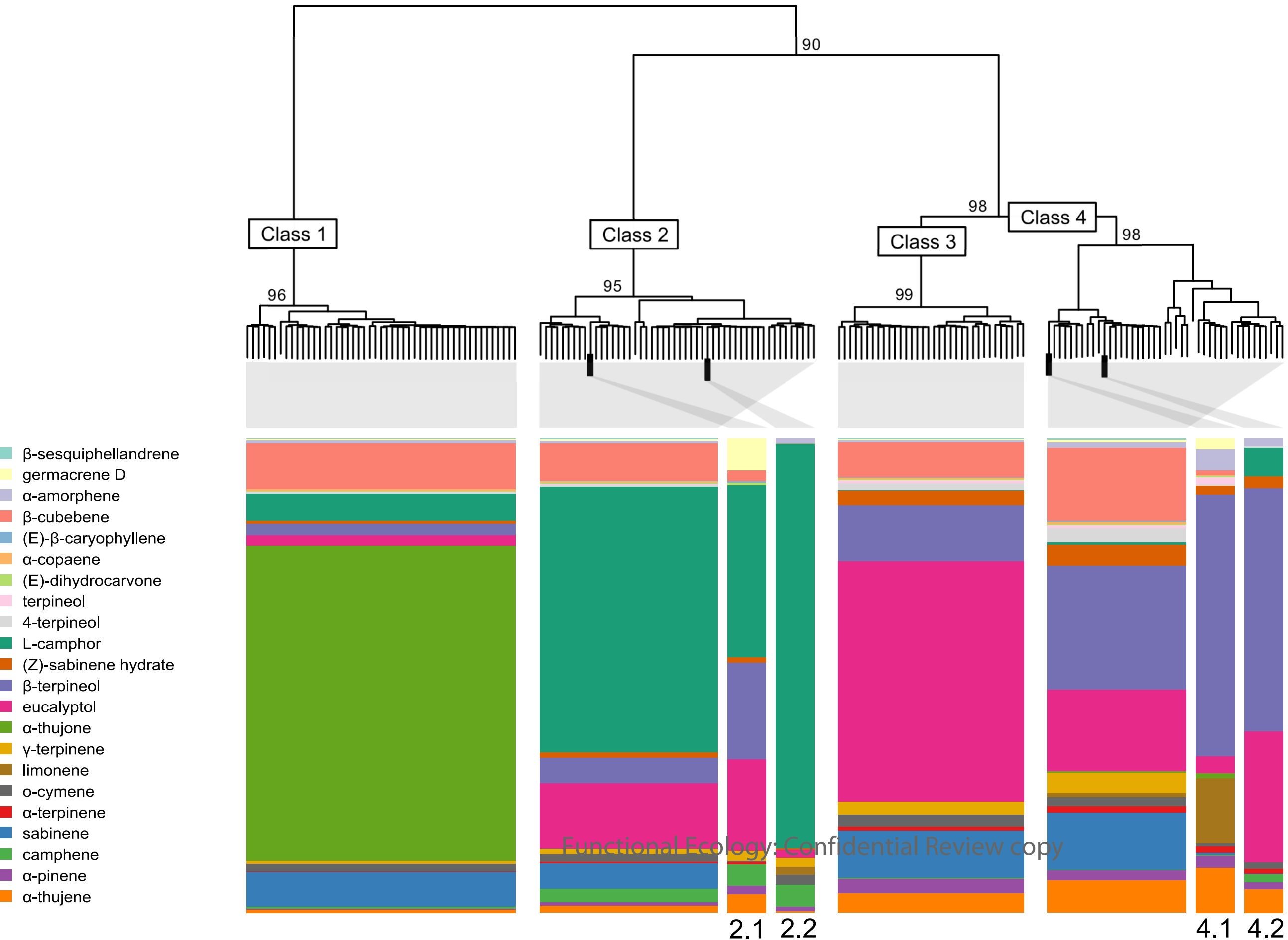


Figure S6. **The relationship between the mean predator abundance and the number of aphids at the end of the experiment (difference between final and initial aphid abundance).** We used a linear model with log-transformed aphid numbers in dependency of the mean number of predators during all observational days. Only plants within the predator present treatment were considered.



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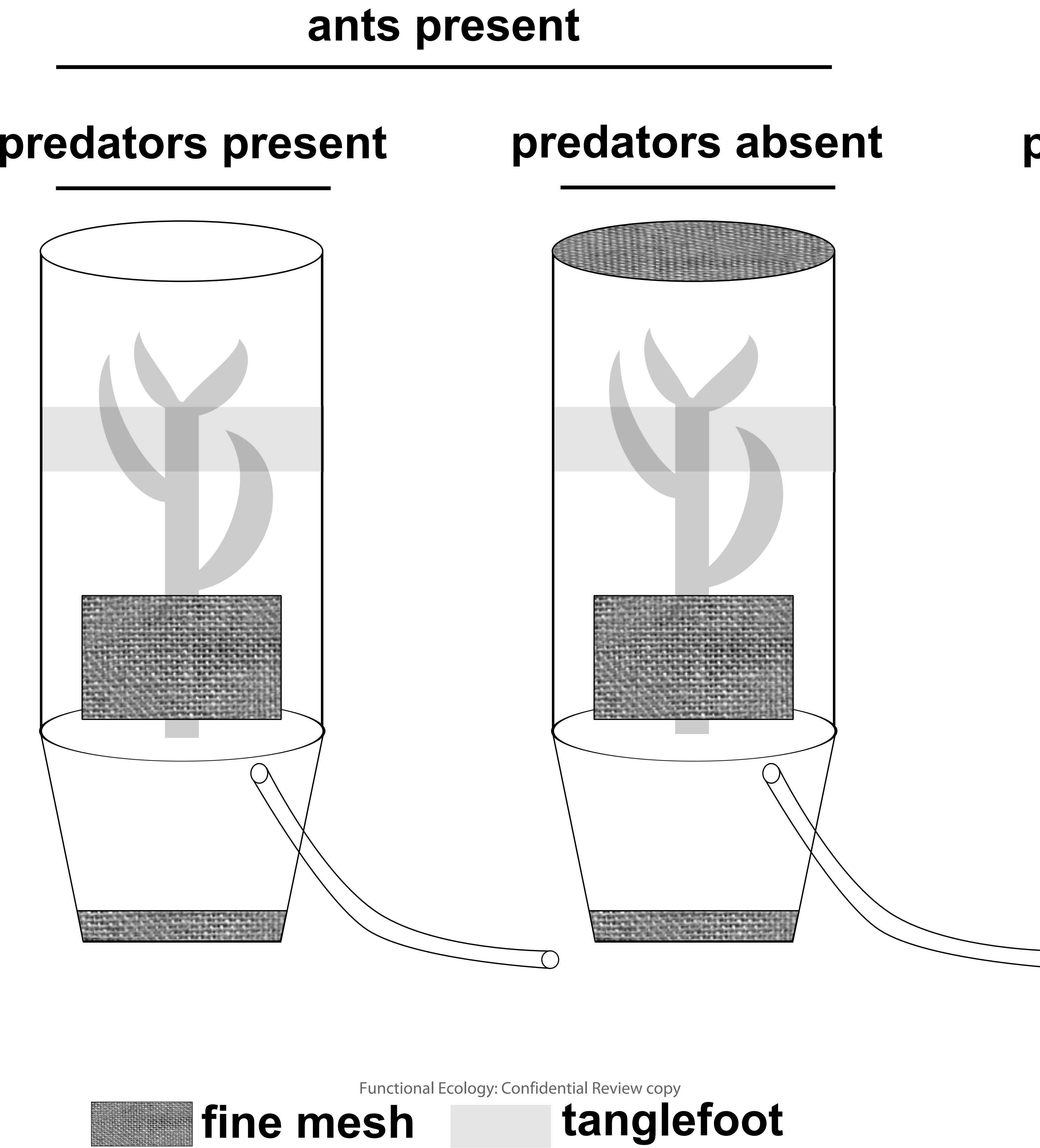
ants present
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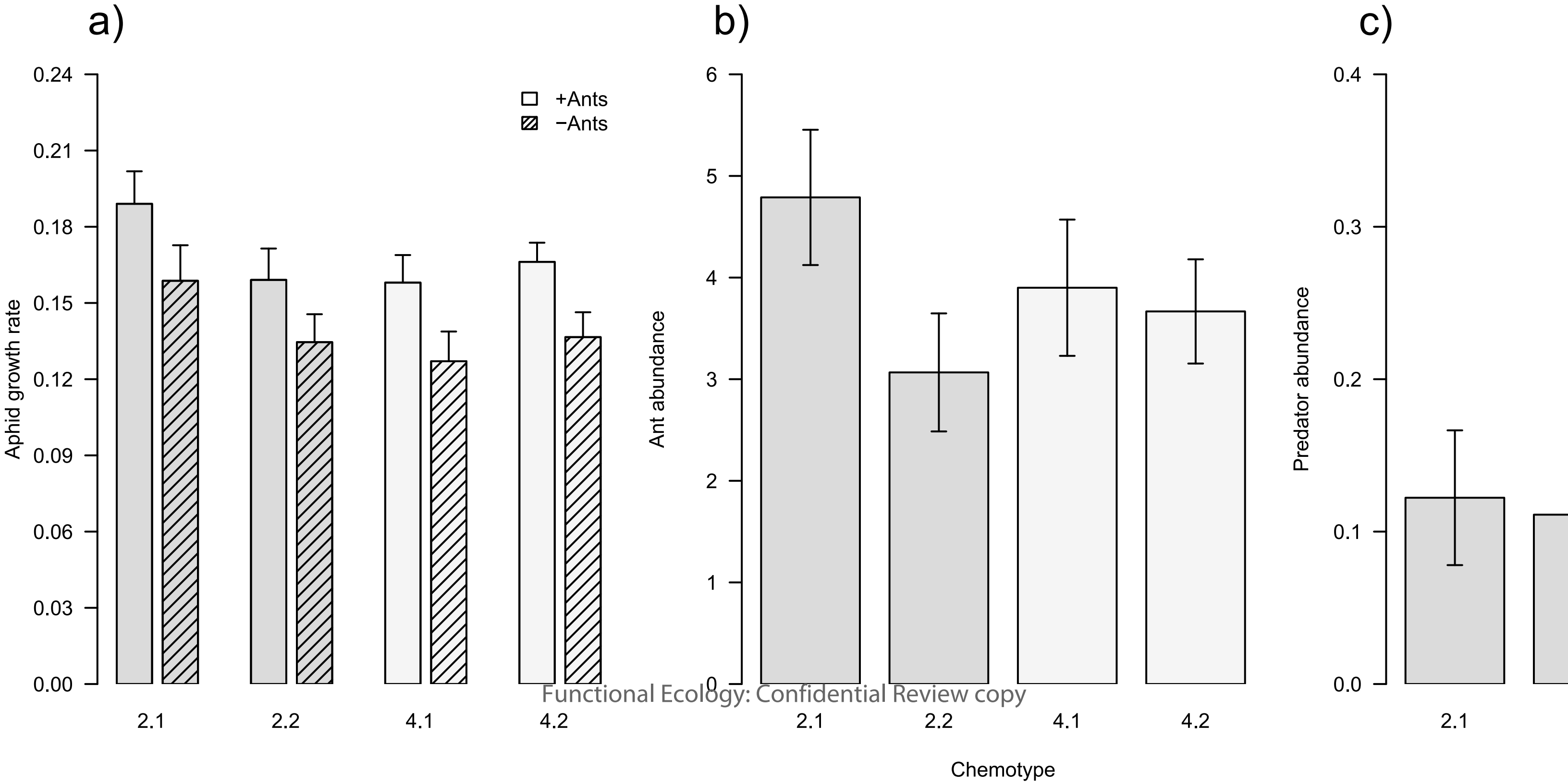
a)
Aphid growth rate
0.24
0.21
0.18
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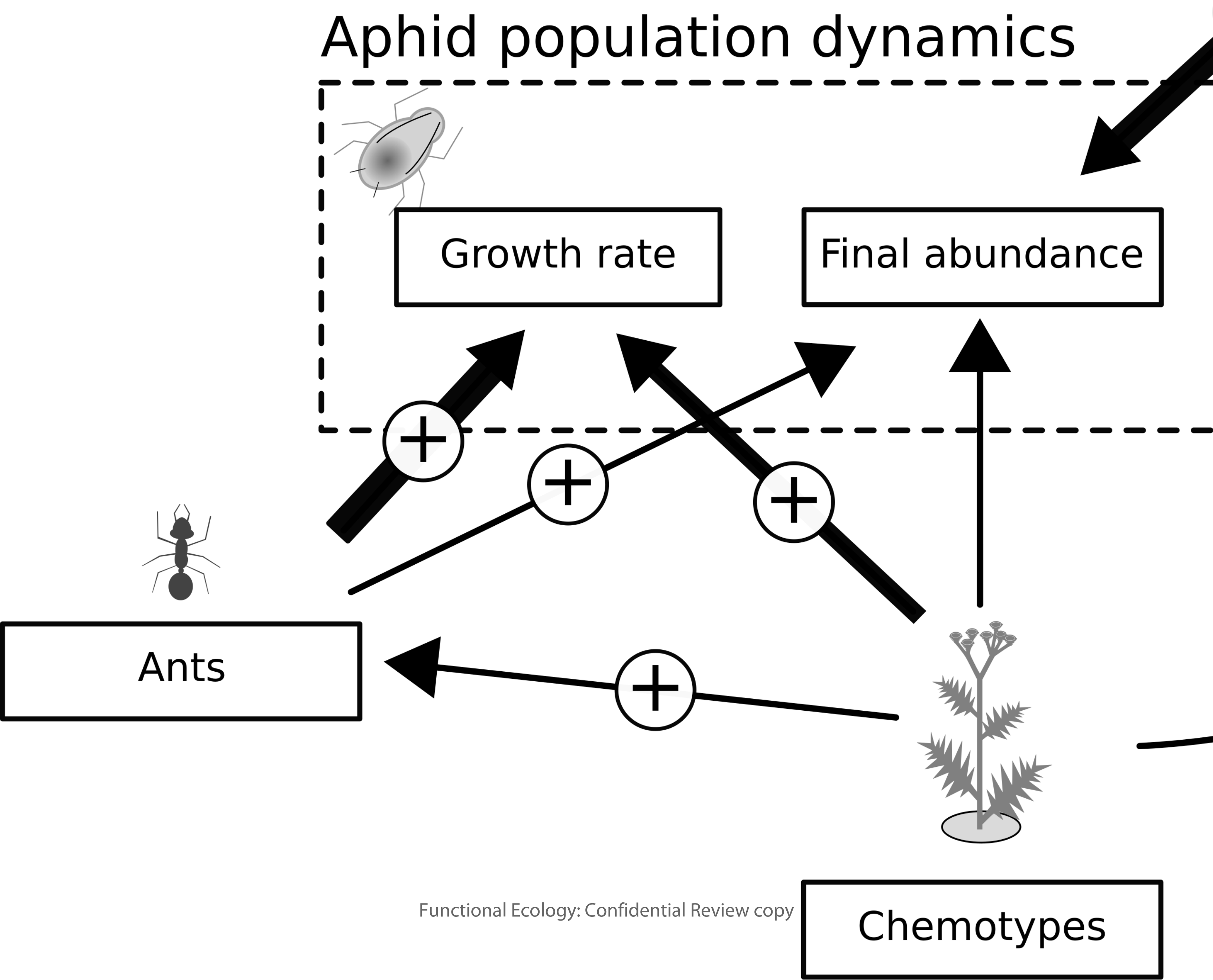
☐ +Ants
▨ −Ants
2.1
2.2
4.1
4.2
b)
Ant abundance
6
5
4
3
2
1
0
2.1
2.2
4.1
4.2
Chemotype
c)
Predator abundance
0.4
0.3
0.2
0.1
0.0
2.1


Aphid population dynamics
Growth rate
Final abundance
Ants
Chemotypes
+
+
+
+