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Research article

Geographic distribution of terpenoid chemotypes in *Tanacetum vulgare* mediates tansy aphid occurrence but not abundance

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Oikos
2024: e10320
doi: 10.1111/oik.10320

Subject Editor: Nina Fatouros
Editor-in-Chief:
Gerlinde B. De Deyn
Accepted 16 February 2024

Intraspecific variation of specialized metabolites in plants, such as terpenoids, are used to determine chemotypes. Tansy *Tanacetum vulgare* exhibits diverse terpenoid profiles that affect insect communities. However, it is not fully known whether patterns of their chemical composition and associated insects vary beyond the community scale. Here, we investigated the geographic distribution of mono- and sesquiterpenoid chemotypes in tansy leaves and their relationships with specific insect communities across Germany. We sampled tansy leaves from ten plants with and five plants without aphids in each of 26 sites along a north–south and west–east transect in Germany. Hexane-extracted metabolites from leaf tissues were analyzed by gas chromatography-mass spectrometry (GC-MS). Plant morphological traits, aphid occurrence and abundance, and occurrence of ants were recorded locally. The effect of plant chemotype, plant morphological parameters, and abiotic site parameters such as soil types, temperature and precipitation on insect occurrences were analyzed. Plants clustered into four monoterpenoid and four sesquiterpenoid chemotype classes. Monoterpene classes differed in their latitudinal distribution, whereas sesquiterpenes were more evenly distributed across the transect. Aphid and ant occurrence was influenced by monoterpenoids. Plants of monoterpenoid class 1 were colonized by aphids and ants significantly more often than expected by chance, whereas in other classes there were no significant differences. Aphid abundance was affected by soil type, and average annual temperature positively correlated with the occurrence of ants. We found significant geographic patterns in the distribution of tansy chemodiversity and show that monoterpenoids affect aphid and ant occurrence, while the soil type can influence aphid abundance. We show that geographic variation in plant chemistry influences insect community assembly on tansy plants.

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Keywords: chemical diversity, *Formica rufa*, geographical gradients, *Lasius niger*, *Metopeurum fuscoviride*, *Myrmica rubra*, plant–herbivore interactions, specialized metabolites

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Introduction

Within the field of biogeography, researchers aim to understand how environmental differences affect the distribution of organisms (Tivy 2018). Geographic location affects plant communities at interspecific, but also at intraspecific levels (Moreira et al. 2012). High intraspecific variation can be observed in specialized phytochemical compounds that vary considerably within plant families and can even vary within species (Kleine and Müller 2011, Kessler and Kalske 2018). For instance, the abundance of pyrrolizidine alkaloids in *Senecio jacobaea* (Macel and Klinkhamer 2010) and the abundance of specific monoterpenoids in *Pinus banksiana* (Taft et al. 2015), *Melaleuca alternifolia* (Bustos-Segura et al. 2017) and *Gossypium hirsutum* (Clancy et al. 2023) have been used for the classification of chemotypes. Chemotypes are defined as groups of conspecific plants that can be distinguished by the often heritable composition of specialized compounds of a specific system-relevant class (Müller et al. 2020). For example, individuals of common thyme *Thymus vulgaris* can be categorized into chemotypes based on the dominant monoterpenoids such as geraniol, α-terpineol, sabinene hydrate, linalool, carvacrol or thymol (Thompson et al. 2003). Chemotypes can be dominated by one or several dominant compounds or differing blends lacking clearly dominating compounds, but are rather mixed chemotypes (Dussarrat et al. 2023). Specialized metabolites in plants have many ecological functions and can strongly influence ecological interactions such as the attraction of herbivore predators, pollinators, and mycorrhizal fungi, defence against herbivores and pathogens, communication with other plants, and protection against abiotic stressors, such as UV-B radiation and drought (Dixon and Paiva 1995, Dicke et al. 2009, Mofikoya et al. 2019, Grof-Tisza et al. 2022). Importantly, several studies have shown patterns in distribution of plant chemotypes across spatial and abiotic gradients. For instance, in *T. vulgaris* it has been found that the composition of secondary compounds differs significantly between sites in the south of France, and has been shown to depend on soil composition, winter ambient temperatures (Thompson et al. 2007, 2019), and chemotypic differences are associated with herbivory (Linhart et al. 1999). Furthermore, chemotype frequency of *Pinus banksiana* plants in the US was observed to be correlated with abiotic factors, such as precipitation (Taft et al. 2015). However, how specialized plant metabolism and interactions between plants and their antagonists might contribute to our understanding of geographic variation is currently poorly understood.

Secondary plant metabolites have likely evolved in part to mediate interactions between plants and their associated organisms (Wetzel and Whitehead 2019). While these metabolites mediate plant–insect interactions, not all interactions are favourable for the plant. That is, in addition to potentially affecting how insect herbivores perform on a plant, secondary metabolites can also serve as important host-recognition cues. Some secondary metabolites are highly volatile. These volatile organic compounds (VOCs) commonly serve as informational cues for insect herbivores (Ghirardo et al. 2012) but can also attract natural enemies of herbivores (Heil and Bueno 2007, Baldwin 2010) and indirectly contribute to herbivore defence. However, many more variables appear to mediate plant–insect interactions, than just plant chemistry. Another crucial aspect in how insects may perceive and locate their host plants and which ultimately contributes to shaping insect communities is the host plant's morphology. For instance, in the perennial shrub *Baccharis pilularis*, plant architecture affected the composition of herbivore communities and morphology correlated with herbivory levels (Rudgers and Whitney 2006). This study found that erect plants attracted more moth galls, while a higher density of a gall-forming midge was found on prostrate plants (Rudgers and Whitney 2006). Where a plant grows, i.e. its immediate surrounding vegetation, also matters for interactions between plants and their interaction partners. For instance, a plant's immediate surroundings can shape its volatile emissions and, in turn, influence insect herbivores (Kigathi et al. 2019, Ziaja and Müller 2023). However, few studies in chemical ecology consider the influence of geographic variation, plant architecture and community context. Therefore, our objective was to investigate complex interactions between these factors to better understand plant–insect interaction patterns, using tansy plants and associated insects as a model system.

Tansy, *Tanacetum vulgare* (Asteraceae), is an aromatic herb endemic to Eurasia that exhibits a considerable variation in its terpenoid composition (Keskitalo et al. 2001, Kleine and Müller 2011, Clancy et al. 2016). Terpenoids (isoprenoids) represent a large group of plant-specialized metabolites whose backbones consist of two common five-carbon isoprene units (Rosenkranz and Schnitzler 2016). A widespread range of terpenoid synthases and subsequent modifying enzymes lead to numerous monoterpenoids and sesquiterpenoids (Degenhardt et al. 2009, Lange and Srividya 2019). These terpenoids can be stored in glandular trichomes on the leaf surface in tansy (Guerreiro et al. 2016), or they can be induced and immediately emitted through biotic stresses such as herbivory (Clancy et al. 2016). The blends of the stored terpenoids vary between individuals, and tansy plants can be classified into different chemotypes according to those differences (Kleine and Müller 2011). Chemical (terpenoid) composition in tansy has been found to differ quite strongly between and within populations in various studies, but geographical patterns or their drivers have not received much attention in this system (Wolf et al. 2011, 2012). Tansy chemotypes characterized by the dominance of volatile terpenoids were found to correlate with the plant-associated insect communities within single field sites in several studies (Kleine and Müller 2011, Clancy et al. 2016). In addition to the volatile terpenoid pattern of tansy on a small scale, non-volatile metabolomic profiles also correlated with the abundance of aphids in the field (Clancy et al. 2018) and to the local genetic population structure of associated aphids (Zytynska et al. 2019). Although these discoveries strongly suggest a role of intraspecific chemical diversity in mediating relationships between tansy and its specialized aphids, it is not clear how mono- and sesquiterpenoid profiles are related and how these different terpenoid classes may influence insect colonization patterns.

Aphids are sap-sucking plant-parasitic insects comprising up to 4000 species (Eastop 1986). They are exposed to many external forces that significantly impact their populations, such as predation and parasitism, environmental conditions, geography, and climate (Eastop 1986, Loxdale and Balog 2018). Furthermore, plant-related variables, such as host-plant chemistry, have strong effects on the behaviour and abundance of aphids, and may serve as host-finding cues for aphids. Plant chemical composition, for example, can determine aphid feeding preference (Neuhaus-Harr et al. 2024) or alter the predation rates on aphids (Stadler 2004, Linhart et al. 2005). However, while there are abundant studies on large-scale variation in insect–plant interactions (Tscharntke and Brandl 2004, Rand and Louda 2006, Hortal et al. 2010), only a few have linked this variation to variation in plant chemistry (Watt et al. 1997, Berenbaum and Zangerl 1998). This is despite the fact that chemical variation in a single host plant species across space is common (Kessler and Kalske 2018, Wetzel and Whitehead 2019). As plant chemistry differs across geographical ranges, this may have ecological implications for associated insects.

We assessed terpenoid variation in tansy on a spatial scale and investigated consequences for plant–insect interactions. We sampled tansy along a north-west to south-east gradient in Germany, in which plant leaf samples, plant morphological traits, information on aphid abundance, ant species occurrence, abiotic site parameters, and the coordinates of the sampling sites were collected to answer the following questions:

1) How do terpenoid compounds in tansy cluster in chemotypes across a northwest–southeast transect in Germany, and how are mono- and sesquiterpenoid chemotypes linked to one another? We hypothesize that there are geographical differences in the distribution of this intraspecific variation of tansy in Germany, as different chemical distributions have been reported in the literature within Germany and beyond. We expect that mono- or sesquiterpenoid profiles are not interrelated due to the involvement of different biosynthetic pathways.
2) How do chemotypes, plant growth variables, and site variables affect aphid and ant occurrence and aphid abundance on the plants? We predict that chemotype and plant architecture significantly affect associated insect communities.

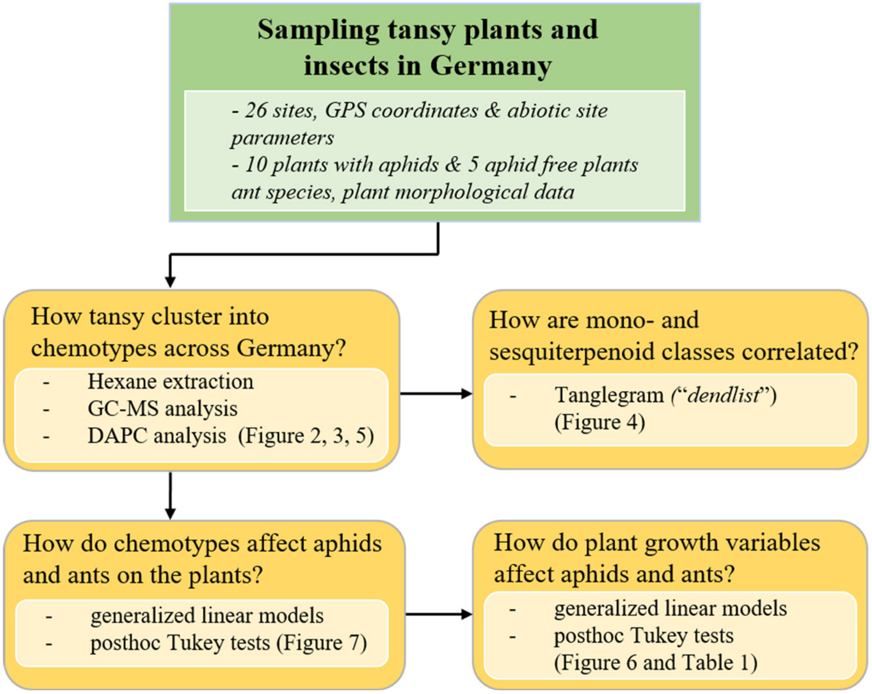

Figure 1. Flowchart of the chemical and ecological analyses conducted in this study. The major aims of our study are provided in yellow boxes and light-colored boxes indicate the analysis conducted. Arrows represent chronological steps taken in our analytical approach. References to respective output for each goal are given in each box.

Material and methods

Sampling of tansy plant populations and insect community

The overview of sampling, chemical and ecological analyses conducted in this study are summarized in the flowchart in Fig. 1. Tansy plants were sampled from 26 sites along a northwest–southeast transect in Germany (Supporting information). The GPS coordinates of each sampling location were recorded on-site, with sites mainly located by country roads, train tracks, and agricultural edges. The sampling site around Bremen was the most northern site chosen (53°04′32.952″N, 08°48′25.794″E), while the sampled site near Freising was the most southern one (48°23′41.5464″N, 11°41′41.9964″E), spanning roughly 700 km across Germany. The site near Bielefeld was the most western (52°01′08.7636″N, 08°31′51.6252″E), while Leipzig was the most eastern sampling site (51°20′26.2752″N, 12°22′29.0388″E), covering roughly 300 km across Germany. The sampling survey took place from the 23 June to the 23 July in 2014. From each site, five plants without aphids and up to ten plants colonized by the specialized herbivore aphid *Metopeurum fuscoviride* (minimum three stems occupied) were sampled. As sampling took place during peak aphid colonization, the abundance of aphids on most plants restricted the sample collection, and often a maximum number of five plants without aphids could be found in every site. For each plant, the presence of ants, as well as the ant species (*Formica rufa*, *Lasius niger* and *Myrmica rubra*) were recorded or marked as 'unknown species' when they could not be identified. For plants with aphids, aphid abundance was calculated by counting the number of colonies, and estimating the size of each colony (XS: < 10 aphids, S: 10–50 aphids, M: 50–200 aphids, L: > 200 aphids). A subset of plants was randomly selected for chemical analysis.

Plant morphological measurements and abiotic site parameters

Plant morphological traits and geographic locations were recorded for each plant. Plant morphological traits included 1) the height of the tallest stem; 2) the number of stems; 3) the plant's diameter at its widest width; and 4) the relative height of the surrounding vegetation, which was assessed by taking the weighted % cover within a 2 m radius of five vegetation categories that were ranked by their height (i.e. bare soil (1), grass (2), small herbs (3), tall herbs (4), shrubs (5)). As derived parameters, 5) the volume ($\text{radius}^2 \times \pi \times \text{height}$), 6) the plant bushiness (plant volume divided by the number of stems), and 7) the emission potential of volatiles (volume × total terpenoid concentration) were estimated. Abiotic site parameters included 1) the annual precipitation (reflecting the sum of rain over a year in mm), 2) the average annual temperature (in degrees Celsius) in every region. Meteorological data were obtained from the German weather service (Kaspar 2023). Furthermore, 3) we analyzed one sample of soil per site, including the percentage of sand, silt and clay.

Hexane extraction of terpenoids and GC–MS analysis

Leaf material collection and extraction were performed as described by Clancy et al. (2016). The detected compounds were identified by comparison of the mass spectra using the National Institute of Standards and Technology (NIST), Mass Spectral Library (NIST 11) and Wiley 275 GC/MS Library (Wiley, New York), and confirmed by comparison of the Kovats retention indices as reported by Guo et al. (2019, 2020) based on chromatography retention times of a saturated alkane mixture (C9–C25; Sigma-Aldrich). The potential changes in the GC–MS sensitivity were corrected by normalizing to the internal standard (monoterpene δ-2-carene). The compounds were quantified using, using six dilutions of the external standards: sabinene, α-pinene, linalool, methylsalicylate, β-caryophyllene, α-humulene, geraniol and bornyl acetate. The chemical structure of the identified compounds was sketched using ChemDraw professional (ChemDraw ver. 21.0.0).

Statistical analysis

Clustering plants into chemotypes

The plants were clustered into classes separately according to their monoterpenoid or sesquiterpenoid profiles by using the '*hclust*' function with the '*ward.D2*' method of correlation distance in the R 'factoextra' package (Kassambara and Mundt 2020). The '*statistical meta-analysis*' function in the online software MetaboAnalyst ver. 5.0 (Pang et al. 2021) was used to compute the heatmap of the monoterpenoid and sesquiterpenoid compounds contributing to the differentiation of the classes. The discriminant analyses of principal components (DAPC; Jombart et al. 2010), which is informed by the same cluster analysis was then applied to visualize the separation of monoterpenoid and sesquiterpenoid chemotype classes. Four different categorical groupings, monoterpenoids and sesquiterpenoids classes that were already identified by using '*hclust*' were used to compute DAPC in an effort to enhance a discrimination between predefined classes. The data was first analyzed using an unconstrained principal component analysis (PCA) (Supporting information) and following, a discriminant analysis was used to infer the separation. By combining the PCA with DA, DAPC maximizes the variance between the classes. The number of retained principal components (PCs) was determined by cross-validation using the '*xvalDapc*' function in the 'adegenet' package ver. 2.1.1 (Jombart 2008) in R to avoid unstable assignments of individuals to clusters. Guo et al. (2021) previously employed this technique of DAPC to characterize different fungi species using the complete fungal volatilomes.

Additionally, to compare the relationship between mono- and sesquiterpenoid classes, a tanglegram of both dendrogram trees was obtained by using the '*dendlist*' function in the 'dendextend' package (Galili 2015). We also tested the associations between the selected monoterpenoids using correlation analysis with a Spearman method at a 99% confidence interval by using in 'ggpubr' package (Kassambara 2020).

All other graphs were made using the package 'ggplot2' in R (Wickham 2016). For better resolution, the resulting images were edited using the image processing software 'Inkscape' (ver. 1.1.1).

Phytochemical diversity analysis

Hill diversity is a measure of diversity that focuses on three components: richness, evenness, and disparity. To compare the phytochemical diversity indices for the chemotype classes, we calculated the functional Hill diversity (FHD) for all samples, separately for monoterpenoids and sesquiterpenoids, using the 'chemodiv' R package (Petrén et al. 2023a). FHD was calculated at diversity orders from $q=0$ to $q=3$. For increasing q-values, the measure puts more weight on abundant compounds; at $q=0$, the relative abundances of compounds are not taken into account; at $q=1$, the weight is proportional to their abundance, and at $q > 1$, more weight is put on abundant compounds of which the upper limit is set to $q=3$. Dissimilarities between compounds were calculated based on PubChem Fingerprints (Kim et al. 2021), which quantify dissimilarities based on the structural properties of the molecules. Each compound's chemical identifiers (SMILES and InChIKey) were extracted from the PubChem open database (https://pubchem.ncbi.nlm.nih.gov).

Distribution of terpenoid chemotypes across a geographical transect in Germany

We carried out a permutational multivariate analysis of variance (PERMANOVA, '*adonis2*' function in 'vegan' R package; Oksanen et al. 2020) using chemical distance matrices (999 permutations, Bray–Curtis method) versus the latitude and longitude across each pairwise combination.

Morphological differences across chemotypes and geographical transect

To test whether plant morphology differed between chemotype classes, we used a one-factorial ANOVA. We tested the number of stems, plant volume, emission potential, plant height, radius, and bushiness. Furthermore, we used a one-factorial ANOVA to test whether the plant morphology was related to soil type. To test whether plants differed morphologically across a latitudinal or longitudinal gradient in Germany, we conducted Pearson correlations, using the Holm–Bonferroni method for correction.

Effect of chemotypes on associated insect community

To test whether aphid occurrence or ant occurrence was influenced by chemotypes, plant morphology, and site variables, we set up a generalized linear model (GLMs) with binomial distribution when the response variable was occupancy (1/0). For aphid abundance, the number of colonies per plant was multiplied with the minimum number of aphids in each colony category. The response variable, the number of aphids, was log-transformed to ensure that the assumption of normality was met, and a linear model with a normal distribution (LM) was used. The 'emmeans' R package with Tukey adjustment was used to assess post hoc pairwise comparisons among factor levels following model fit (Russell 2021).

Prior to running the generalized models and to rule out multicollinearity, we excluded all variables with a variance inflation factor higher than five ('*VIF*' function in 'car' R package, Fox and Weisberg 2019). We omitted 'plant radius', 'plant volume', and 'total terpenoid concentration' and 'soil type'. As predictor variables, we included 'monoterpenoid class', 'sesquiterpenoid class', and their interaction, 'monoterpenoid concentration', 'sesquiterpenoid concentration', 'emission potential', 'bushiness', 'height of surrounding vegetation', 'plant height', 'the number of stems', 'annual temperature at the site', 'annual precipitation at the sampled site', 'latitude' and 'longitude' in all models. In the model for aphid abundance we additionally included the presence of all three ant species '*F. rufa*', '*L. niger*' and '*M. rubra*', as well as the 'soil type' of the respective site.

To test whether the FHD affected aphid occurrence and abundance, we used a one-way analysis of variance test. FHD of mono- and sesquiterpenoids were log-transformed to meet normality assumptions. Statistical models were carried out using R ver. 4.1.3 (www.r-project.org).

Results

Monoterpenoid and sesquiterpenoid tansy chemotypes

We analyzed 278 plants and identified 30 monoterpenoids and 21 sesquiterpenoids. The molecular schemes of some mono- and sesquiterpenoids are depicted in Fig. 2a–3a. Plants clustered into four distinct monoterpenoid (Fig. 2b) and four sesquiterpenoid chemotype classes (Fig. 3b). The eastern Hanover sampling site was excluded from further analyses because two unknown sesquiterpenoid compounds found there could not be annotated by library search and Kovats index comparisons, and this site specifically showed exceedingly high concentrations of β-thujone and bicyclosesquiphellandrene, causing severe outliers (Supporting information).

A heatmap of monoterpenoids shows the separating features of α- and β-thujone in class 1, camphor and camphene in class 2, trans-verbenol and trans-chrysanthenyl acetate in class 3, and α-terpinene, α- and β-phellandrene in class 4 (Fig. 2b). Monoterpenoid classes comprised 69, 25, 37 and 147 plants, for classes 1, 2, 3 and 4, respectively. Discriminant analysis of principal components models (DAPC) showed a discrimination among the different monoterpenoid classes (Fig. 2c). Specifically, class 1 was dominated by β-thujone (approximately 75%), class 2 was defined by a mixture of approximately 40% camphor and 20% sabinene, class 3 was dominated by trans-chrysanthenyl acetate (approximately 60%), and class 4 comprised a group of plants with a mix of compounds (Fig. 2d). The total concentration of monoterpenoids varied from 0.02 to 112 pmol g^{-1} leaf fresh with classes 1 and 2 having the highest monoterpenoid concentrations and class 4 having the lowest (Fig. 2e). Additionally, we found that some monoterpenoids were closely linked to each other. Regardless of the chemotype categorization, camphene

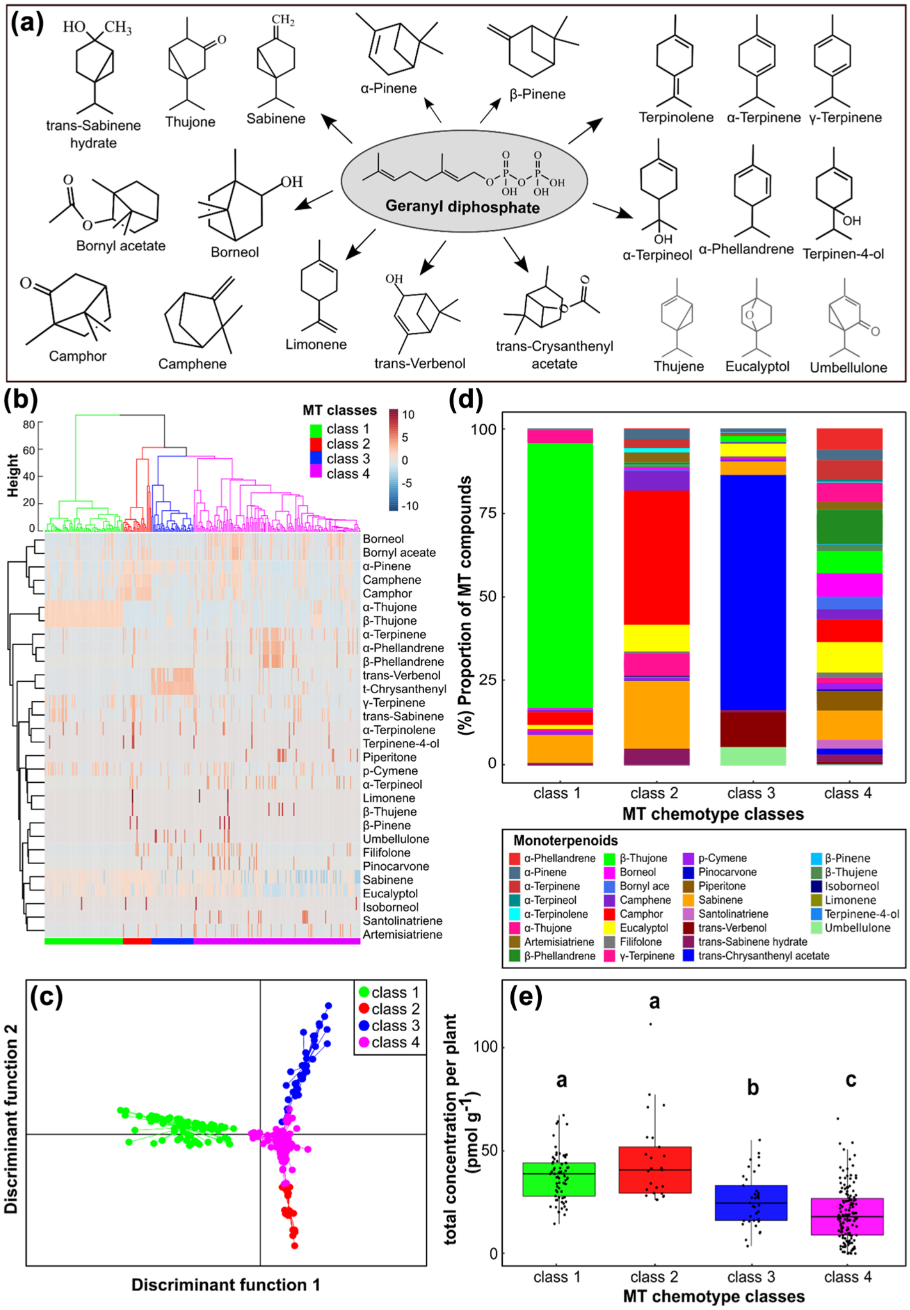

Figure 2. Visualization of the monoterpenoid (MT) chemotype classes using different statistical approaches. (a) Schematic illustration of monoterpenoid products synthesized from geranyl diphosphate via carbocationic reactions mechanism. The compounds that are biosynthetically linked stand in the same row, e.g. sabinene, thujone and trans-sabinene hydrate. (b) Hierarchical cluster analysis of monoterpenoid compounds across 278 individual plants. Four main classes were identified and each cluster is highlighted by a different color; class 1 green, class 2 red, class 3 blue, and class 4 magenta. The variety and separating features of monoterpenoids found in each class is displayed in the heatmap. (c) Discriminant analysis of principal components plot shows separations among the MT chemotype classes. (d) Proportion (in percentage) of each representative compound of each class is provided in a stacked barplot. Data is normalized to logarithmic scale and Pareto matrix. (e) Total concentration of monoterpenoids with significant differences (Tukey test, $p < 0.05$) indicated by letters.

and camphor concentrations were associated in monoterpenoid classes 1, 2 and 4 ($R^2 = 0.42$, $p < 0.001$; Supporting information), and borneol and bornyl acetate concentrations showed a positive correlation ($R^2 = 0.50$, $p < 0.001$; Supporting information) among the many plants.

Plants clustered into four sesquiterpenoid chemotype classes, with the compound pattern of each sesquiterpenoid class presented in a heatmap (Fig. 3b). Here, 26, 63, 11 and 175 plants were classified into classes 1, 2, 3 and 4, respectively. A DAPC model showed a discrimination among the

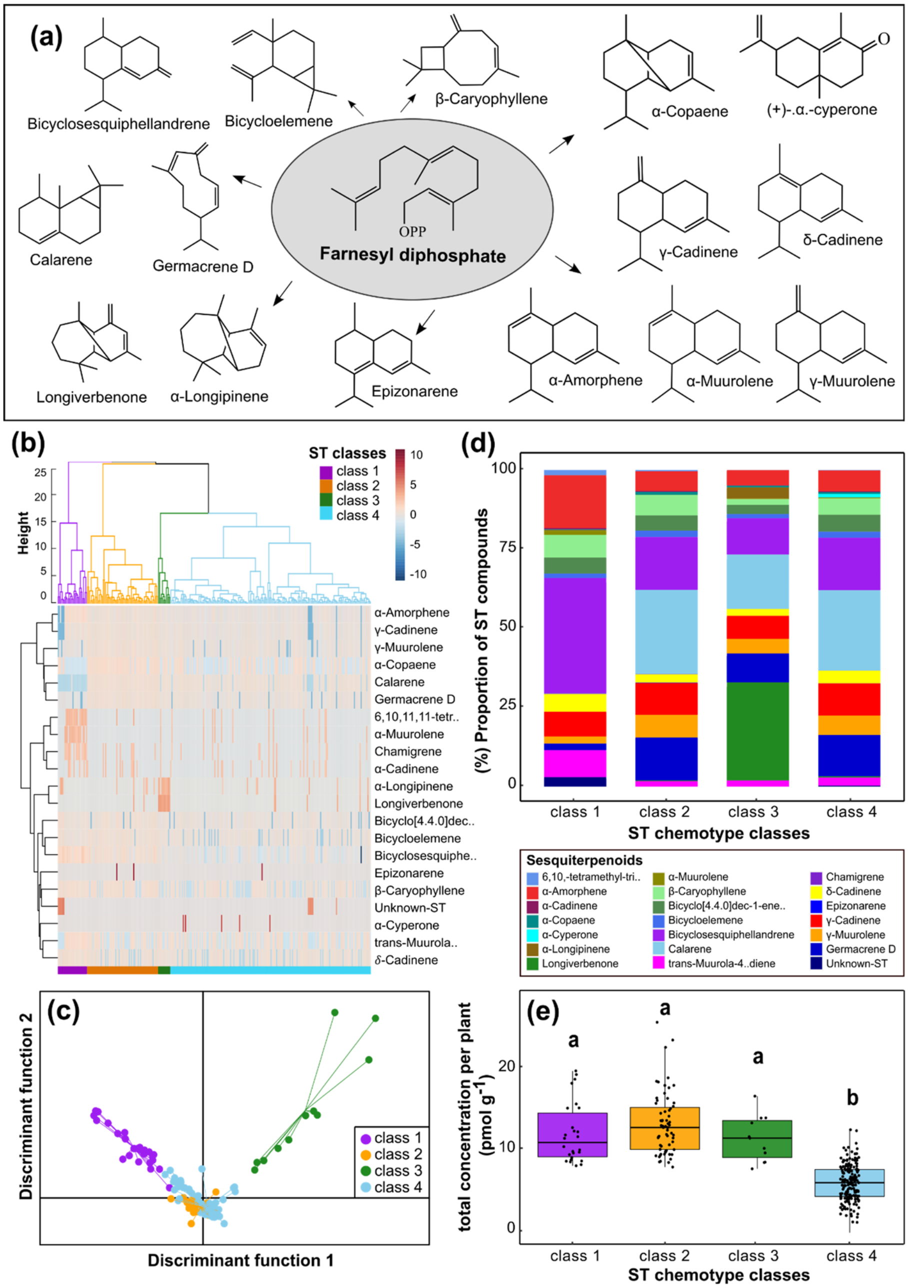

Figure 3. (a) Schematic illustration of sesquiterpenoid (ST) products synthesized from farnesyl diphosphate. All the sesquiterpenoids are the main products of farnesyl diphosphate. (b) Hierarchical cluster analysis of sesquiterpenoid compounds across 278 plants; four main classes were classified – class 1 purple, class 2 orange, class 3 forestgreen, and class 4 lightblue. The variation of the sesquiterpenoid compounds of each class is depicted in the heatmap. Data is logarithmically transferred and Pareto scaled. (c) Discriminant analysis of principal components indicates separations among the sesquiterpenoid classes. (d) Proportional composition of the compounds, provided in percentage for each class. (e) Total concentration of sesquiterpenoids with significant differences (Tukey test, $p < 0.05$) indicated by the letters on the top.

sesquiterpenoid classes (Fig. 3c). In contrast to distinct monoterpenoid composition between classes, sesquiterpenoids did not show much variation in their profile content. Instead, sesquiterpenoid profiles showed a consistent presence of bicyclosesquiphellandrene, β-caryophyllene, γ-muurolene and α-amorphene (Fig. 3d). The plants belonging to sesquiterpenoid class 1 were characterized by the highest proportion of bicyclosesquiphellandrene (36%). Even though classes 2 and 4 did not show a distinct variation in relative sesquiterpenoid composition, as they were characterized by calarene (25%), germacrene D (13%), and γ-cadinene (10%), their sesquiterpenoid profiles were expressed at significantly different total concentrations (Fig. 3e). Furthermore, although sesquiterpenoid class 3 contained fewer individuals, it was predominantly formed by longiverbenone (31%). The total concentration of sesquiterpenoids ranged from 1.3 to 25.5 pmol g^{-1} (Fig. 3e), with sesquiterpenoid class 4 showing significantly ($p < 0.05$) lower concentrations compared to the other classes. The tanglegram of monoterpenoid and sesquiterpenoid chemotypes across all tansy individuals showed no clear correlation between monoterpenoid and sesquiterpenoid profiles (Fig. 4a).

Diversity metrics of chemotype classes

Quantifying the FHD enabled us to study the diversity of tansy monoterpenoid and sesquiterpenoid chemotype classes in novel ways that differ in the weight placed on abundant compounds through compound richness, evenness and disparity (Petrén et al. 2023b). Overall, chemotypes that are dominated by individual compounds (and hence have a lower evenness)

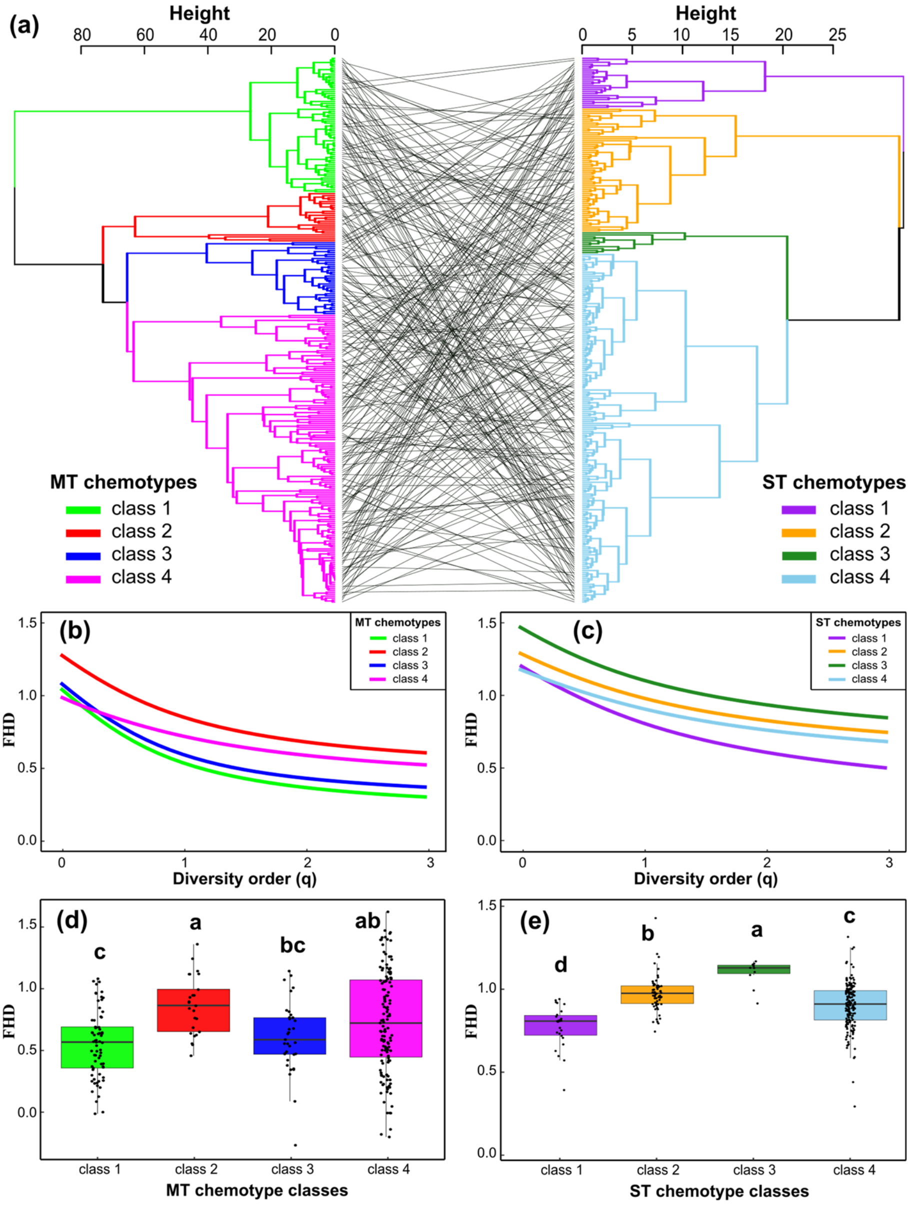

Figure 4. (a) A tanglegram of the chemotype trees demonstrating that monoterpenoid (MT; on the left) and sesquiterpenoid (ST; on the right) chemotype classes are not correlated. The opposite scenario (MT chemotypes associated with ST chemotype) would have been indicated by linkage or cross-correlation between the same plant. The diversity profile shows the functional Hill diversity (FHD) at diversity orders from $q=0$ to $q=3$ for MT (b) and ST (c) chemotype classes. For increasing q-values, the measure is less sensitive to the relative abundances of compounds; at $q=0$ the relative abundances of compounds are not taken into account; at $q=1$ equal weight is put on all compounds. The boxplots show variation in FHD for the MT (d) and ST (e) classes in detail for $q=1$. Note that values are log-transformed in figures and analyses. Significant differences ($p < 0.05$) between classes are indicated by the letters above the boxes. Mean number of monoterpenoids per sample: class 1 = 5.9, class 2 = 7.2, class 3 = 5.7, class 4 = 5.6. Mean number of sesquiterpenoids per sample: class 1 = 10.3, class 2 = 10.7, class 3 = 12.0, class 4 = 9.7.

are indicated by deeper curves that tend to have lower FHD values between FHD order 2 and 3, (Fig. 4b–c). Curves associated with Hill numbers quantify the relative importance of compounds to the structure of the chemotype class.

Functional Hill diversity among monoterpenoid classes was the lowest for monoterpenoid classes 1 and 3, which were at their lowest at higher orders of q, emphasizing the role of abundant compounds in structuring these monoterpenoid classes (β-thujone and trans-chrysantenyl-acetate, respectively, Fig. 2d). Monoterpenoid class 2 had the highest FHD, but a rather deep curve, indicating that abundant compounds are likely important in this class as well (camphor, Fig. 2d). Class 4 had an intermediate mean FHD, with a shallow curve (Fig. 4b, d), which indicates that dominant compounds play a less important role in this class (indicated by an even distribution of chemical compounds in Fig. 2d). Notably, the diversity at the level of chemotypes, rather than individual plants, appeared to be higher in class 4, as an effect of larger differences between samples in their composition (Fig. 2b).

Within the sesquiterpenoid classes, class 1 and 3 had a deeper curve (Fig. 4c), with low and high FHD (Fig. 4e), emphasizing the stronger role of dominant compounds in shaping the structure of the sesquiterpenoid classes (bicyclosesquiphellandrene and longiverbenone, respectively, Fig 3d). Sesquiterpenoid classes 2 and 4 had more similar and shallower curves with intermediate mean FHD (Fig. 4c, e), indicating a more similar chemical profile, with a more even distribution of compounds (Fig. 3d). Overall, the FHD of sesquiterpenoids differed significantly between all classes (Fig. 4e).

North–south and west–east gradient in monoterpenoid and sesquiterpenoid chemotypes

The distribution of tansy monoterpenoid classes differed significantly across Germany. Monoterpenoid classes 1 and 2 were found more frequently in the east and south, while monoterpenoid classes 3 and 4 were more frequently observed in north and west Germany (Fig. 5a). A PERMANOVA test showed that monoterpenoid compositions significantly varied depending on the latitude ($R^2 = 0.01$; $p < 0.002$; Fig. 5c) and longitude ($R^2 = 0.01$; $p < 0.001$; Fig. 5e, Supporting information) coordinates, even though the explained variance is low. Biosynthetically linked monoterpenoids, such as β-thujone and sabinene, camphor and camphene, increased substantially with decreasing latitude and increasing longitude towards the far south. Contrastingly, trans-crysanthenyl acetate and trans-verbenol showed an opposite trend, with the highest concentration reported in plants at high latitudes towards more northern sites (Supporting information).

In contrast, sesquiterpenoid classes were more homogeneously distributed across Germany (Fig 5b), indicating independence of the geographic effects on mono- and sesquiterpenoid chemotypes (Fig 5d, f). Sesquiterpenoid compositions did not significantly differ in their geographic distribution (lat.: $R^2 = 0.002$; $p = 0.56$; lon.: $R^2 = 0.002$; $p = 0.58$; Supporting information).

Additionally, we ran PERMANOVA tests to see whether the soil type differed across mono- and sesquiterpenoid classes. Soil type was analyzed in 25 of the 26 sites. Loam and silt loam were found in four sites, while loamy sand occurred in seven sites and sandy loam in eight sides (Supporting information). Sand was only found in one site (Bergen, north Celle; Supporting information). We found that 'soil type' was significantly associated with mono- ($R^2 = 0.06$; $p < 0.001$) and sesquiterpenoids ($R^2 = 0.03$; $p = 0.004$), although the explained variance was low (Supporting information). Because of this, a PCA analysis did not show a clear clustering of soil type for mono- and sesquiterpenoid compounds (Supporting information). Not all monoterpenoid classes were found on all soil types. For example, only monoterpenoid classes 3 and 4 were found growing on sand (Supporting information). However, the distribution of sesquiterpenoid chemotypes was more even over all soil types (Supporting information). Similarly, the number of plants from the monoterpenoid classes differed significantly among the percentage of sand, silt and clay, while sesquiterpenoid classes did not differ across different percentages (Supporting information).

Plant morphology differences between chemotypes and across Germany

The number of stems per plant differed marginally significantly between monoterpenoid classes ($F_3 = 2.55$, $p = 0.056$; Supporting information). A post hoc test showed that the number of stems was lower in plants from monoterpenoid class 1 compared to class 4 (Fig. 6a). Plant volume and emission potential differed significantly between plants of different monoterpenoid chemotypes ($F_3 = 2.71$, $p = 0.045$; $F_3 = 8.71$, $p < 0.001$; Supporting information). Plant volume was significantly lower in plants belonging to monoterpenoid class 1 than in class 2, but neither differed from monoterpenoid classes 3 and 4 (Fig. 6b). Plants belonging to monoterpenoid class 2 had significantly higher emission potential than all other monoterpenoid classes (Fig. 6c).

Sesquiterpenoid classes showed substantial differences in the number of stems ($F_3 = 3.69$, $p = 0.012$; Supporting information). Specifically, plants from sesquiterpenoid class 1 had a significantly higher number of stems than sesquiterpenoid class 2 and 3 (Fig. 6d). However, there were no differences in their volume or emission potential (Fig. 6e–f). Plant height, plant radius, and plant bushiness did not differ significantly across monoterpenoid nor sesquiterpenoid classes. F-statistics and p-values for all measured plant traits are in the Supporting information.

Plant traits also varied across the geographical gradient. Plant height differed significantly along the latitudinal gradient ($t_{(369)} = -3.19$ $cor = -0.16$, $p = 0.009$, Supporting information), with plants typically growing taller in the north. Plant bushiness differed across the longitudinal gradient ($t_{(369)} = -2.87$, $cor = -0.15$, $p < 0.01$, Supporting information), with plants growing bushier in the west. Most plant variables, such as radius and number of stems, were positively

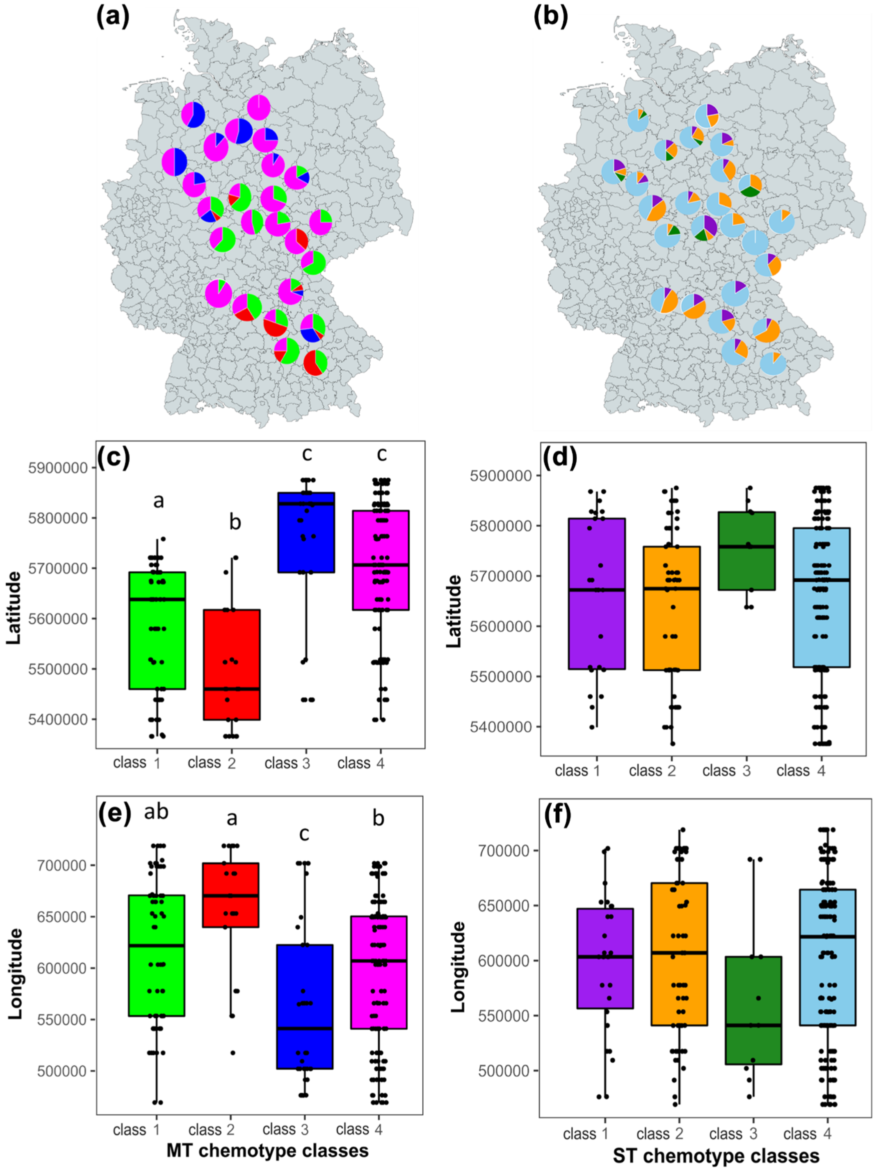

Figure 5. Proportion of monoterpenoid (MT) and sesquiterpenoid (ST) classes within each sampled site. Monoterpenoid classes are color-coded as following: green – class 1, red – class 2, blue – class 3, magenta – class 4 (a). Sesquiterpenoid classes are color-coded as following: purple – class 1, orange – class 2, dark green – class 3, light blue – class 4 (b); monoterpenoid classes found over different latitude (c) and longitude (e) and sesquiterpenoid classes found over different latitude (d) and longitude (f); significant differences are indicated on top of the boxplots ($p < 0.05$).

correlated (i.e. higher plants tended to be bushier; Supporting information).

Furthermore, we found that the number of stems, plant height, plant bushiness and plant radius varied over the different soil types, while plant volume and emission potential did not differ across different soil types (Supporting information). On loamy sand and sandy loam, plants had significantly more stems than plants growing on silt loam (Supporting information). Plants on loam grew significantly higher than plants on loamy sand and silt loam (Supporting information). Similarly, plants growing on loam were significantly bushier than plants growing on sandy loam and silt loam, respectively (Supporting information). Plants growing on loamy sand or sandy loam however, had a larger radius compared to plants growing on silt loam (Supporting information).

Effects of site conditions and plant variables on tansy aphids and associated ants

Modelling the effects on aphid occurrence with a binomial GLM, we found that aphid occurrence was affected by chemical and morphological plant traits. Specifically, the monoterpene class ($\chi^2_{(3)} = 11.99$, $p = 0.007$, Fig. 7a) and, marginally significantly, the number of stems ($\chi^2_{(1)} = 3.76$,

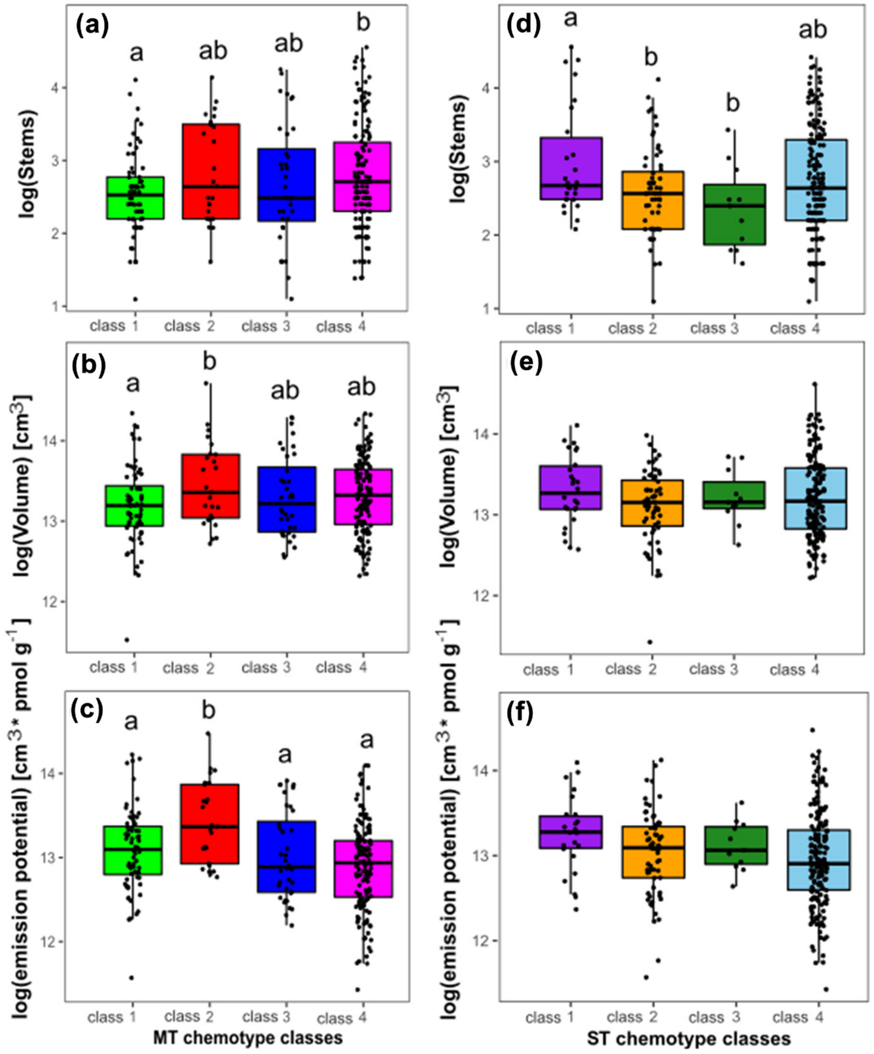

Figure 6. Plant traits with a significant difference across monoterpenoid classes: number of stems (a), plant volume (b) and emission potential (c). Plant traits across different sesquiterpenoid classes: number of stems (d), plant volume (e) and emission potential (f). Emission potential was calculated by the plant volume × the concentration of all terpenoid compounds in that specific plant. Degrees of freedom (DF), statistic (F-value) and p-value for plant trait variation across monoterpenoid and sesquiterpenoid classes using a one-factorial ANOVA are indicated in the Supporting information.

$p = 0.053$) influenced the occurrence of *M. fuscoviride* aphids (Table 1). Even though the aphid occurrence responds to monoterpenoid classes, the FHD of neither the monoterpenoids nor the sesquiterpenoids affected *M. fuscoviride* presence or numbers significantly (Supporting information).

Sesquiterpenoid classes did not influence aphid presence (Fig. 7b, Table 1, Supporting information). In contrast to this, a linear model with aphid abundance revealed that aphid abundance was not affected by monoterpenoid classes, but was significantly influenced by the soil type of the respective site ($F_4 = 20.81$, $p = 0.013$, Table 1). Furthermore, the height of the surrounding vegetation marginally significantly influenced aphid abundance ($F_1 = 5.71$, $p = 0.059$, Table 1). Correlation tests supported our findings that the abundance of *M. fuscoviride* was not correlated with any plant variables nor to single (dominant) compounds of the monoterpenoid classes (spearman correlation with 0.95-confidence level; Supporting information).

Three species of ants were observed regularly in all sites and often on the same plant (*F. rufa*, *L. niger* or *M. rubra*, Supporting information). All plants with aphids appeared to have ant present. A binomial GLM with ant presence as dependent variable showed that monoterpenoid class, but not sesquiterpenoid class, significantly affected probability of ant presence ($\chi^2_{(3)} = 10.62$, $p = 0.014$; Table 1, Supporting information), and post hoc Tukey tests indicated that monoterpenoid class 1 had significantly higher probability of ant presence than monoterpenoid class 4. Furthermore, ants were more likely to be present at sites with higher temperatures ($\chi^2_{(1)} = 3.91$, $p = 0.048$; Table 1, Supporting information). This was independent of the species.

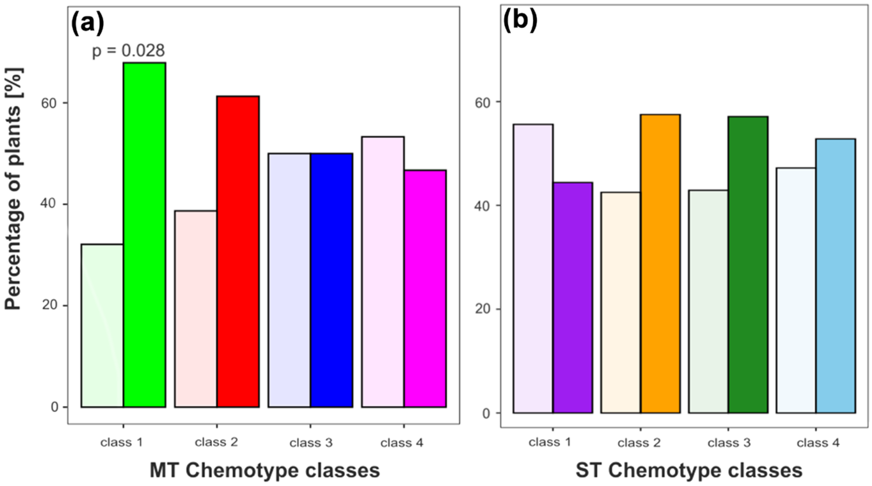

Figure 7. Bar charts indicate the percentage of plants without *M. fuscoviride* aphids (transparent) and with aphids (solid) within monoterpenoid (MT) classes (a) and sesquiterpenoid (ST) classes (b). A binomial test showed that plants of monoterpenoid class 1 (a) were colonized by this aphid species significantly more often than expected by chance (binomial test: 95% conf. interval = 0.52–0.82, p = 0.028; Supporting information). Sesquiterpenoid classes (b) did not influence aphid occurrence (Supporting information).

Parasitized aphids were commonly observed in all sites (Supporting information). However, we did not observe clear drivers of the probability of parasitism except latitude and longitude. Specifically, parasitism was more common in the southwest than in the northeastern sites (Supporting information).

Discussion

We demonstrated that *T. vulgare* plants exhibit variation in distinct mono- and sesquiterpenoid chemotypes across a wide geographical range in Germany. Our results show that the chemical composition of monoterpenoids differed significantly across geographical coordinates, demonstrating that the monoterpenoids profile of tansy was more dissimilar with increasing geographical distance. While monoterpenoid chemotypes displayed different local dominance patterns, sesquiterpenoid chemotypes were homogeneously distributed across Germany. We further demonstrated that monoterpenoid classes, but not sesquiterpenoid classes, are involved in shaping aphid *M. fuscoviride* and ant *L. niger*, *F. rufa* and *M. rubra* occurrence patterns. We additionally found that monoterpenoid chemotypes are influenced by soil type (silt, sand and clay content) whereas sesquiterpenoid chemotypes did not seem to be affected by soil content. Furthermore, soil type affected the number of aphids while mean annual temperature had a positive influence on ant occurrence, suggesting that chemical, morphological and geographic factors structure the wider ecological community.

Tansy chemodiversity has been investigated in different geographical regions of Europe. For instance, a study from Finland revealed that Finland's central and southern regions were home to tansy chemotypes with higher concentrations of camphor (Keskitalo et al. 2001). Interestingly, we also found that plants from monoterpenoid class 2, which is dominated by camphor, were more frequent in southern Germany. Tansy seems to differ in its terpenoid profile between and within countries. For example, tansy plants from Finland showed a unique davadone D chemotype, while myrcene-tricyclene chemotypes were more common in the south and southwest compared to the rest of the country (Keskitalo et al. 2001). Moreover, a study from Lithuania found that tansy exhibited different dominant compounds (such as eucalyptol, trans-thujone and myrtenol) between different locations (Judzentiene and Mockute 2005). In line with these findings, we observed plants from the β-thujone chemotype more prevalent and plants from the trans-chrysanthenyl acetate chemotype less prevalent in the south of Germany compared to the northern German sites. These findings suggest that differences in terpenoid profiles are common and likely increase at larger geographic scales and that different regions bolster different dominance patterns of terpenoid compounds. Perhaps the different soil types occurring in different sites, could partly explain the patterns of monoterpenoid chemotype classes, as previous studies have found that e.g. soil properties influenced essential oil composition in *Thymus pulegioides* (Vaičiulytė et al. 2017, 2022). As our study only assessed soil type at the site level, variation in soil type and relationships with individual plants within sites could not be captured, and hence small-scale variation in soil type as driver of local chemical variation warrants future study.

Our study found that on a German-wide scale, tansy plants could be grouped into distinct chemotypes using their mono- and sesquiterpenoid profiles. Hierarchical cluster analyses revealed four monoterpenoid and four sesquiterpenoid classes that were not strongly associated with one another. This lack of alignment between individuals of mono- and sesquiterpenoid chemotypes strongly emphasizes differing and unrelated biosynthesis pathways for these two compound classes. Sesquiterpenoids are generally produced through the cytosolic mevalonate pathway (MVA), whereas

Table 1. Degrees of freedom (DF), statistic (χ^2 or F-value) and p-value for chemical, morphological, geographic, and biotic variables included, when applicable, in a binomial generalized linear model with aphid occurrence or ant occurrence as response variable, and in a GLM with aphid abundance as response variable. Bold letters indicate significant p-values ($p < 0.05$) and italic letters indicates marginally significant values ($0.05 < p < 0.01$).

	df	Aphid presence χ^2 (p-value)	Ant presence χ^2 (p-value)	Aphid abundance F-value (p-value)
MT class	3	**11.99 (0.007**)**	**10.62 (0.014*)**	2.18 (0.710)
MT concentration	1	0.56 (0.453)	1.07 (0.301)	0.05 (0.863)
ST class	3	2.79 (0.426)	2.16 (0.541)	5.36 (0.339)
ST concentration	1	0.31 (0.577)	0.10 (0.756)	0.44 (0.600)
MT class:ST class	8	8.26 (0.409)	10.08 (0.259)	8.07 (0.647)
Emission potential	1	0.38 (0.536)	1.05 (0.306)	0.08 (0.825)
Bushiness	1	0.21 (0.643)	2.21 (0.137)	0.19 (0.729)
Height of surrounding vegetation	1	0.10 (0.753)	0.94 (0.332)	*5.71 (0.059)*
Height	1	1.85 (0.173)	0.14 (0.706)	30.81 (0.476)
Stems	1	*3.76 (0.053)*	0.63 (0.426)	2.55 (0.206)
Soil type	4	–	–	**20.81 (0.013*)**
Mean annual temperature	1	0.83 (0.362)	**3.91 (0.048*)**	3.63 (0.132)
mean annual precipitation	1	0.16 (0.694)	0.61 (0.434)	4.21 (0.105)
Latitude	1	0.52 (0.471)	0.53 (0.466)	0.05 (0.861)
Longitude	1	0.31 (0.577)	0.01 (0.301)	0.22 (0.712)
Formica rufa	1	–	–	3.55 (0.136)
Lasius niger	1	–	–	2.59 (0.202)
Myrmica rubra	1	–	–	0.01 (0.953)
Residuals	150	–	–	237.19

the plastidial methylerythritol phosphate (MEP) pathway yields multiple monoterpenoid products (Davis and Croteau 2000). However, the MVA and MEP can provide isopentenyl diphosphate precursors for monoterpenoid and sesquiterpenoid biosynthesis (Dudareva et al. 2005). This may explain why some individuals of monoterpenoid chemotype classes link with sesquiterpenoid chemotype classes. The diversity of terpenoid compounds in plants is generated by terpene synthases, a diverse family of enzymes that catalyze terpenoid compounds from single substrates (Bohlmann et al. 1998). For instance borneol and bornyl acetate showed a significant positive correlation in the same plant where they both were active, indicating that they are likely produced from the synthase of the bornyl diphosphate enzyme (Supporting information). A similar result was observed between camphor and camphene. Interestingly, the chemotypes also differed in their phytochemical diversity in compound richness, evenness, and dissimilarity, which may impact interactions between plants and insects (Whitehead et al. 2021, Neuhaus-Harr et al. 2024).

We assessed the impacts of monoterpenoid and sesquiterpenoid composition on interactions with a specialized insect herbivore, i.e. *M. fuscoviride*, and three species of ants *F. rufa*, *L. niger* and *M. rubra*. Plants belonging to monoterpenoid class 1 were significantly more likely to be colonized by aphids, whereas equal occupancy of plants was observed for all sesquiterpenoid classes. This finding is in line with other studies, since our monoterpenoid class 1 contains β-thujone as a dominant compound, which has been associated with an increased abundance of another tansy specialist aphid, *Macrosiphoniella tanacetaria* (Kleine and Müller 2011). Interestingly, previous studies also found higher abundance and earlier colonization rates of *M. fuscoviride* on plants with camphor as dominant compound, which would resemble monoterpenoid class 2 in our study (Clancy et al. 2016, Senft et al. 2019). This could explain why we observed the tendency of higher aphid presence on monoterpenoid class 2 plants, even though this finding was not significant. However, other studies have shown that not only dominant but also minor compounds within a blend significantly affect plant–insect interactions (McCormick et al. 2014, Clancy et al. 2016).

Preference, and therefore presence, appears to be affected by terpenoids, a finding in line with the idea that volatile terpenoids serve as cues for finding host plants (Bruce et al. 2005, Ninkovic et al. 2021). It is possible that monoterpenoids are more helpful to aphids as cues for host plant identification than sesquiterpenoids, as plants exhibited much higher concentrations of monoterpenoids compared with sesquiterpenoids. Clancy et al. (2016) observed that the emission of terpenoids, presumably evaporated/released from glandular cells (Devrnja et al. 2021), affected *M. fuscoviride* colonization. Given the higher volatility of monoterpenoids (Mofikoya et al. 2019), higher concentrations could be expected in the near ambient air of the plants' canopy. This fits with the observation that the influence of terpenoids on aphid presence on individual chemotypes is mainly determined by monoterpenoids and not by sesquiterpenoids. If monoterpenoids are used as host-finding cues, the significant low concentration of monoterpenoids in monoterpene class 4 could perhaps be the reason why we see a tendency of low aphid presence in these plants. Another reason, why mono- and not sesquiterpenoids could be used as host finding cues by aphids, could be the differentiation of the monoterpenoid profiles in terms of their (dominant) compounds. Profiles were very distinct in monoterpenoids, while the sesquiterpenoid classes were chemically more similar. FHD had no effect on aphid presence and abundance. Functionally related terpenoids seem to have the same effect on aphids compared to functionally unrelated terpenoids.

Furthermore, not only aphids, but also ants might use monoterpenoids as cues. As *M. fuscoviride* is a facultative ant-tended species that benefits strongly from mutualism (Flatt and Weisser 2000), chemotypes might structure aphid colonization and population indirectly via ant preference. Hence, it is unsurprising that we found a higher ant occupancy of plants belonging to monoterpenoid class 1 compared to plants from monoterpenoid class 4, similar as in the aphids. It has been found that the presence of ants before aphid appearance led to a stronger likelihood of aphid colonization (Senft et al. 2018). However, it could also be likely that aphid presence attracts ants, which take a lot of time to wander around until they find suitable food sources. Even though we could not confirm whether ant presence was shaping aphid presence, or vice versa, and this requires manipulative studying, it does suggests that plant chemotypes mediate the strong relationship between those insects and tansy (Mehrparvar et al. 2017). Furthermore, aphids may have an increased preference for plants of specific chemotypes (Neuhaus-Harr et al. 2023), but also that chemotypes could affect aphid survival and population growth, e.g. via interactions with ants (Mehrparvar et al. 2017). Furthermore, we recognized that geographically changing environmental factors affected the abundance of ants, as their abundance increased with the average annual temperature of the site. This has already been shown for Mediterranean ant species (Cerdá et al. 1998). For example, *L. niger*, known for its thermal tolerance and preference for temperatures in the 18–26°C range, increases their foraging activity at higher temperatures (Blanchard et al. 2021), which could ultimately influence aphid presence and abundance. Hence, chemical cues, such as mono- or sesquiterpenoids might only be one factor shaping aphid communities.

Although we show an effect of chemotypes on plant occupancy by aphids, we did not find such links with aphid abundance. One caveat of our study might be that the observation of insect and plant growth and chemistry resemble only a snapshot in time. Although tansy chemotypes has been found to be stable over time (Clancy et al. 2016), the abundance of aphids fluctuates throughout a season. Single observation points cannot capture the dynamics of an aphid colony over time. As we sampled in peak aphid colonization, this might be one reason why we did not find an effect of tansy chemotypes on aphid abundance, as it is known that e.g. early colonization of aphids is influenced by chemotypic variation (Clancy et al. 2016). Future studies using chemotypes from different geographic locations are needed to assess the effects on aphid colony dynamics in a more controlled manner.

Furthermore, we did not observe links between individual terpenoid compounds and aphid abundance. Several studies now show links between terpenoid composition on aphid preference, and presence on a plant for various aphid species in this model system (Neuhaus-Harr et al. 2024). However, aphid presence on a plant can vary strongly in their abundance, from very few aphids to thousands. These differences in aphid abundance partially may occur after a plant is colonized, and hence can also be shaped by other factors than plant chemotype (Neuhaus-Harr et al. 2024), such as predation pressure (Senft et al. 2019) or resource availability. Indeed, it has been found that coccinellid beetles, which prey heavily on aphids, are more abundant on plants with high β-thujone contents (Kleine and Müller 2011), which might explain why aphids on plants with high β-thujone did not show higher abundances.

We also found that aphid abundance was affected by the soil type. As monoterpenoid classes differed significantly between soil types, the effect of soil types on aphid abundance might be mitigated via the host plant. Other morphological traits, such as the number of stems, the plant volume or the emission potential differed significantly among the mono- or sesquiterpenoid classes and the soil type. Hence, it is possible that morphological traits also play a role in mediating herbivore densities on individual plants and that soil properties influence herbivores indirectly through shaping plant morphology. Previous studies suggested that both the chemotype and other associated plant traits are crucial for host plant selection and performance of herbivorous insects. For instance, in *Brassica oleracea*, high levels of glucosinolates prolonged the development time of the specialist *Pieris rapae* and reduced survival in the generalist *Mamestra brassicae* (Gols et al. 2008). In *Salix sachalinensis*, leaf pubescence reduced overall leaf consumption by the willow leaf beetle *Melasoma lapponica* (Hayashi et al. 2005). Furthermore, Carmona et al. (2011) showed that herbivore susceptibility depends on defence traits, including morphological and chemical traits. Our findings that sap-sucking aphids are influenced by terpenoid composition and the soil type, while ants are influenced by temperatures, support this general observation of the mediating role of plant chemical and morphological traits observed in other plant–insect systems, but also shows how the geographic location intermingles with these factors.

Conclusion

The intraspecific profiles of secondary plant metabolites in different tansy individuals provide a unique perspective for studying the relationships between plants and their environment. Evidently, secondary metabolites play a mediating role between plants and their living environment, and it is also becoming increasingly clear that in many plant species the distribution of plant chemotypes shows relationships with the abiotic environment. Using locally balanced groups of aphid-colonized and uncolonized tansy plants on a transect in Germany, we were able to show that there is a strong geographical clustering of certain chemotypes in tansy and that the composition of monoterpenoids influences the colonization of plants by aphids and ants over large spatial distances. Although correlations between monoterpenoid blends and soil type were found, the causes of chemotypic spatial clustering between and within sites warrant further studies. If clustering with soil type is widespread, it is important to understand whether and how this is adaptive for the plant in a variable biotic and abiotic environment. To this end, a combination of targeted surveys in extreme environments and manipulative studies in common gardens under different environmental conditions can advance our understanding of the evolution and ecology of chemotypic clustering.

Acknowledgements – The authors thank Ina Zimmer, Kerstin Koch and Baris Weber for technical support during the measurements. Additionally, the authors are grateful to fellow PhD candidate Moritz Popp for his help on this work. Open Access funding enabled and organized by Projekt DEAL.

Funding – This work was supported by grants of the Deutsche Forschungsgemeinschaft DFG within the Research Unit FOR3000 to JJR (JU2856/5-1), and WWW (WE3081/40-1) and joint DFG projects to (WWW (WE3081/25-2) and JPS (SCHN653/7-2).

Author contributions

Humay Rahimova and **Annika Neuhaus-Harr** contributed equally to this publication and share first authorship. **Humay Rahimova:** Data curation (equal); Formal analysis (lead); Methodology (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Annika Neuhaus-Harr:** Formal analysis (lead); Methodology (lead); Validation (equal); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Mary V. Clancy:** Conceptualization (equal); Data curation (equal); Investigation (equal); Methodology (equal); Writing – review and editing (equal). **Yuan Guo:** Data curation (equal); Formal analysis (equal); Methodology, Validation (equal); Writing – review and editing (equal). **Robert R. Junker:** Formal analysis (equal); Software (lead); Validation, Writing – review and editing (equal). **Lina Ojeda-Prieto:** Investigation, Methodology, Writing – review and editing (equal). **Hampus Petrén:** Formal analysis (equal); Methodology, Resources (equal); Writing – review and editing. **Matthias Senft:** Data curation (equal); Investigation (equal); Methodology, Writing – review and editing. **Sharon E. Zytynska:** Conceptualization (equal); Data curation (equal); Methodology, Supervision (equal); Writing – review and editing (equal). **Wolfgang W. Weisser:** Conceptualization (lead); Funding acquisition (equal); Project administration (lead); Supervision (lead); Writing – review and editing (equal). **Robin Heinen:** Methodology (equal); Project administration (equal); Supervision (lead); Writing – original draft (equal); Writing – review and editing (lead). **Jörg-Peter Schnitzler:** Conceptualization (equal); Funding acquisition (equal); Project administration (equal); Supervision (equal); Writing – review and editing (equal).

Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.9p8cz8wqk (Rahimova et al. 2024).

Supporting information

The Supporting information associated with this article is available with the online version.

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