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Functional Ecology

**Title:** Geographical variation of floral scents in generalist entomophilous species with variable pollinator communities

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**Conflict of Interest**

The authors declare no conflict of interest.

**Author contribution**

NDM and MD conceived and designed the VOCs study project. FM, NH, YP, BS conceived the general aspects of the ARSENIC project and NH, YP and BS selected the study sites. HLC, and BS to a lesser degree, conducted the VOCs extractions in the field and BA conducted the preliminary analysis. BB processed the VOCs analysis and helped with the identification. NDM, NH, YP, and BS conducted the pollinator sampling and the fieldwork. ES contributed to the greenhouse survey. NDM conducted the VOCs extractions in the greenhouse, the statistical

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analysis presented in the manuscript and prepared the manuscript. MD and FM supervised the analysis and edited the manuscript, and all authors revised the manuscript.

**Data accessibility**

The data used in this study are available in Zenodo repository: https://doi.org/10.5281/zenodo.5719263

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**Abstract**

1. Floral scents are considered major attractants for pollinator species and scent composition is likely to be under pollinator-mediated selection. Relatively few studies have investigated geographical variation in floral scents in plant species with generalist pollination, and little is known about the mechanism behind this variation, often interpreted as the result of local adaptation to pollinators without considering phenotypic plasticity. One possible way to investigate this question is to compare floral bouquets both in natural populations and in populations reared under controlled conditions.

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2. We compared the identity and proportions of volatile organic compounds (VOCs), collected on plants both in the field and in the greenhouse, emitted by four generalist plant species found in six calcareous grasslands distributed along an environmental and diversity gradient. We recorded pollinator visits in the field to document geographical variation of pollinator communities associated to each plant species. We then analysed the relation between VOCs and pollinator variation using a redundancy analysis (RDA).
3. We found that VOC composition differed among species and among populations, but also between field and greenhouse. The RDA showed a significant effect of the variation in pollinator communities associated with the variation in the VOCs emission, even after removing potential confounding effects, suggesting some stability in the compounds attractive to a given pollinator species. However, only one species showed significant geographical differences among populations tested in both conditions, suggesting local genetic adaptation. Another species showed geographical variation in VOC proportion in the field, but these results were not confirmed by the greenhouse extractions, suggesting an effect of phenotypic plasticity.
4. Our findings suggest that geographic variation of floral scent may, in some cases, be genetically based and possibly explained by pollinator-mediated selection, even in generalist pollinated species, which represent the large majority of pollination systems. However, our results also showed significant phenotypic plasticity, suggesting that studies of floral volatiles must be conducted in natural populations to obtain a representative picture of the signal that can be potentially perceived by pollinators.

**Keywords:** Environmental gradient, floral traits, greenhouse experiment, intraspecific trait variation, network analysis, plant-insect interactions, population differentiation, Volatile Organic Compounds.

**Translated Abstract (Résumé)**

1. Les odeurs florales sont considérées comme des composants attractifs majeurs pour les pollinisateurs et la composition des odeurs est devrait être soumise à une sélection médiée par les pollinisateurs. Relativement peu d'études se sont focalisées sur la variation géographique des odeurs florales chez les espèces végétales à pollinisation généraliste, et peu de choses sont connues sur les causes de cette variation, souvent interprétée comme le résultat d'une adaptation locale aux pollinisateurs, sans tenir compte de l’effet de la plasticité phénotypique. Une façon d'étudier cette question est de

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comparer les bouquets floraux à la fois dans les populations naturelles et dans les populations cultivées en conditions contrôlées.

2. Nous avons comparé l'identité et les proportions des composés organiques volatils (COV), prélevés sur les plantes à la fois sur le terrain et en serre, au sein de quatre espèces végétales généralistes présentes dans six prairies calcaires réparties le long d'un gradient environnemental et de diversité. Nous avons mesuré les visites des pollinisateurs sur le terrain afin de documenter la variation géographique des communautés de pollinisateurs associées à chaque espèce de plante. Nous avons ensuite analysé la relation entre les COV et la variation des pollinisateurs en utilisant une analyse de redondance (RDA).
3. Nous avons constaté que la composition des COV différait entre espèces et entre populations, mais aussi entre le terrain et la serre. La RDA a montré un effet significatif de la variation des communautés de pollinisateurs associée à la variation de l'émission des COV, même après avoir éliminé les effets confondants potentiels, suggérant une certaine stabilité dans les composés attractifs pour une espèce de pollinisateur donnée. Cependant, une seule espèce a montré des différences géographiques significatives entre les populations testées dans les deux conditions, ce qui suggère une adaptation génétique locale. Une autre espèce présente une variation géographique dans la proportion de COV sur le terrain, mais ces résultats n'ont pas été confirmés par les extractions en serre, ce qui suggère un effet de plasticité phénotypique.
4. Nos résultats suggèrent que la variation géographique de l'odeur florale peut, dans certains cas, avoir une base génétique et peut-être expliquée par la sélection médiée par les pollinisateurs, même chez les espèces avec une pollinisation généralistes, qui représentent la grande majorité des systèmes de pollinisation. Cependant, nos résultats ont également montré un rôle significatif de la plasticité phénotypique, suggérant que les études sur les bouquets floraux doivent être menées dans des populations naturelles pour obtenir une image représentative du signal potentiellement perçu par les pollinisateurs.

Mots clés : Gradient environnemental, traits floraux, expérience en serre, variation intraspécifique des traits, analyse de réseau, interactions plantes-insectes, différenciation des populations, Composés organiques volatils.

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## INTRODUCTION

Because plants are sessile organisms, they have to cope with the problem of distant reproduction by dispersing pollen. A majority of angiosperms depend on animals for pollen dispersal (Ollerton et al., 2011) and invest in a suite of floral signals attractive to pollinators. Volatile Organic Compounds (VOCs) are considered as major non-visual attractants for many pollinator species (Schiestl 2010). Floral scents are often a complex mix of several compounds, which are produced by biochemical pathways of secondary metabolism (Junker & Parachnowitsch, 2015; Knudsen et al., 2006). Several studies have shown that both VOC identity and proportions can mediate interactions with floral visitors (Beyaert & Hilker, 2014; Burger et al., 2013; Raguso, 2008). Indeed, the inhibition of a particular group of compounds can mediate a shift between two groups of pollinators or modify their behaviour (Byers, Bradshaw, & Riffell, 2014; Larue-Kontic & Junker, 2016; Shuttleworth & Johnson, 2010). Floral scent composition is thus expected to be under pollinator-mediated selection (Delle-Vedove et al., 2017; Schiestl & Johnson, 2013).

Pollinator-mediated selection has been invoked to –at least partly- explain similarities and apparent evolutionary convergence of floral scents among plants sharing the same species or groups of pollinators (Farré-Armengol et al., 2020 and references therein). At a lower taxonomical level, one expects divergent floral scents among populations of the same plant species if pollinator assemblages vary geographically. Although some studies have provided direct evidence of selective pressures exerted by pollinators on floral scents (Chapurlat et al., 2019; Gross et al., 2016; Majetic et al., 2009a; Parachnowitsch et al., 2012) and of the capacity of floral scents to rapidly evolve under a particular pollination regime (Gervasi & Schiestl, 2017), whether and how floral scents show adaptation to local pollinators remains largely unknown, in particular in species with generalist pollination (Friberg et al., 2019; Petrén et al., 2021; Suinyuy et al., 2015).

Floral scents emitted by plants with generalist pollination (*i.e.* visited by widely different pollinator species) have been less investigated compared to specialized systems, probably because documenting the effective variation in pollinators community, and how these pollinators respond to floral scents, is particularly difficult in these systems (Farré-Armengol et al., 2020). Moreover, when a large spectrum of insect species participate to pollination, the strength of selection exerted by each pollinator species may be relatively weak, thus decreasing the likelihood of any detectable pattern of local adaptation (as in host/parasite

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interactions: Kawecki & Ebert, 2004; Lajeunesse & Forbes, 2002). *In natura*, geographical variation in floral scents has been found in several generalist plant species (Doubleday et al., 2013; Zych et al., 2019), and has often been interpreted as the result of divergent pollinator-mediated selection (reviewed in Delle-Vedove et al., 2017). However, some crucial information is lacking from most of these studies, such as (i) how pollinator communities effectively vary among study populations, and (ii) whether the variation in floral scents documented in the field is explained by a geographical variation among scent-emitting genotypes (Delle-Vedove et al., 2017).

Indeed, several studies have shown evidence for phenotypic plasticity of floral odour, related to environmental conditions. Both total emission rates and composition have been shown to vary with nitrogen supply (Friberg et al., 2017, Holopainen & Gershenzon, 2010; Majetic et al., 2017), temperature (Farré-Armengol et al., 2014) and water availability (Campbell et al., 2019). The level of plasticity varies among compounds, with even some VOCs specifically found in some environmental conditions but not in others (Campbell et al., 2019; Farré-Armengol et al., 2014). Despite recent advances and increasing interest in floral scent variation, little is known about the relative importance of genetic adaptation vs. phenotypic plasticity (Delle-Vedove et al., 2017). Studies using common garden approaches to provide the genetic bases of floral scent variation (Dobson et al., 1997; Dötterl, Wolfe, & Jürgens, 2005; Doubleday et al., 2013; Parachnowitsch et al., 2012; Petrén et al., 2021; Zych et al., 2019) usually describe scent composition obtained in experimental (garden or laboratory) populations, which may not be representative of scents produced in natural populations. On the other hand, studies that focus on natural conditions commonly explain variation as the result of local adaptation to pollinators, often excluding phenotypic plasticity (Breitkopf et al., 2013; Huber et al., 2005). Only the comparison between natural populations and controlled conditions can help ascertain the origin of the variation (Delle-Vedove et al., 2017). This has been done by only a few studies which found quite high resemblance between bouquets among conditions (Chapurlat et al., 2018; Friberg et al., 2013; Majetic, Raguso, & Ashman, 2009b). Although this limit exists for both specialized and generalist pollination systems, the fact that floral scent studies have been overall biased towards specialized systems (Farré-Armengol et al., 2020) makes our understanding of local adaptation of plants to their generalist pollinators even more fragmentary.

Here, we analysed floral scents emitted by four species, belonging to four different plant families, with generalist pollination. We first investigated VOCs variation in six natural

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populations in three separated regions, distributed along an environmental and diversity gradient in France, where we also characterized the pollinator communities. Then, we reared the plant species, from seeds collected on the field sites, in a common garden under the same conditions and we compared floral bouquets to those extracted in the field. We therefore addressed the following questions (see Supporting Information, Fig. S1): (i) How does the community of visiting insects vary among regions? (ii) How do floral scents vary within and among regions? (iii) How do floral scents co-vary with pollinator communities? (iv) Does phenotypic plasticity alone explain the variation in floral scents detected in the field (*i.e*. does such variation not persist when investigated in a common garden experiment)?

## MATERIAL AND METHODS

### Study systems and study species

We selected six calcareous grassland areas of 1 hectare each in three different regions (Hauts-de-France, Normandie and Occitanie) along an environmental and diversity gradient in France (two populations in each region, more details in Fig. S2). Due to the small geographical distance between sites in the same region (max. 30 km), we treated these as different populations. The six populations are included in the European NATURA 2000 network, and the access did not require any permission.

We focused on four perennial plant species typical and representative of the calcareous grassland habitat, belonging to four different plant families with different inflorescence types and phenologies (Table S1). We chose these species for their high abundance and their presence in at least two of the three regions (Fig. S2): *Globularia vulgaris* (Plantaginaceae) was present in Occitanie and Normandie, *Anthyllis vulneraria* (Fabaceae, with colour dimorphism, Fig. S3) and *Ranunculus bulbosus* (Ranunculaceae) were found in Occitanie and Hauts-de-France, *Pilosella officinarum* (Asteraceae) was found in all sites except one. All four species are capable of cross-pollination, produce both nectar and pollen and are generalist entomophilous species, mainly visited by solitary bees and hoverflies (Appendix S1 – Method S1), thus considered as potentially attractive to local pollinators (Honnay et al., 2007; Navarro, 2000; Percival, 1961; Steinbach & Gottsberger, 1994). *P. officinarum* is the only potentially apomictic species that can also reproduce sexually in the field, relying on insect visitors for pollination, especially on solitary bees (Bishop & Davy, 1994).

### Pollinators

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We performed pollinator sampling in the same sites and on the same day as VOCs extraction (see details below). Pollinators were sampled on plant individuals not used for scent collections. The sampling period range from April to June 2017, which corresponded to the flowering peaks of the four species. We collected pollinators once a month, for two consecutive months (*i.e.*, two days of sampling in total for each plant species and population) to account for pollinator diversity turnover. We sampled pollinators visiting receptive flowers, using hand nets for 4 hours each day, two hours in morning and two in the afternoon, to account for daily variability. All sampled pollinators were put in a killing vial, pinned in the laboratory, and identified by expert taxonomists (see de Manincor et al., 2020). We focused on native wild bees (Family Apoidea, group Anthophila) and hoverflies (Family: Syrphidae) because they are among the most abundant and efficient pollinator groups in temperate habitats and in our sites in particular (de Manincor et al., 2020; Klecka et al., 2018; Michener, 2007). Moreover, both groups are known to visit and pollinate our focal plant species, as confirmed by the literature (Appendix S1 – Method S1).

**Floral scents**

*Overview* – We studied VOCs emitted in the field during spring 2017 on the same days we sampled pollinators for each of the four species (*i.e.*, two days of extraction in total for each plant species and population). At the end of the flowering season, we collected seeds from at least 20 different plants per species spatially separated in the field, to grow the focal species in greenhouse and repeat the study in controlled conditions during spring 2018, after overwintering. This was done for all species except *G. vulgaris*, for which plants did not flower in the greenhouse.

*Plant sampling* – Floral scent extractions were performed in the field between April and June 2017, two sessions of extraction per plant species overlapping with their flowering peak, and at the greenhouse between April and May 2018 (Table S2). Floral scents were collected when the selected plant species had reached receptivity, *i.e.* when they attracted pollinators. In the field, we selected 5-15 individuals per site and species, according to availability in flowering plants. In the greenhouse, because of the limited number of successfully germinated seeds and flowering individuals, we selected from 5 to 8 plant individuals per site and per species, all belonging to different maternal families. Although relatively low, these numbers are similar to those usually used for this type of study (Burkle & Runyon, 2019; Campbell et al., 2019; Filella et al., 2013; Kantsa et al., 2018; Majetic et al., 2015). All surveyed plants in the

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greenhouse were excluded from pollination events since they were placed in a separated chamber cell, and we controlled for temperature, water availability, light, and soil content. Odour collection was always performed under natural light in the field and at maximum ambient temperature and minimum humidity (always during sunny days, see Table S2 for more details). In the greenhouse, floral scents were collected under a mix of natural and artificial lights, and we settled the cell temperature around 20°C. All scent collections, in both environments, were performed between 12:00 to 17:00 h, corresponding to the period of insect maximum activity in the field.

*Scent extraction and chemical analysis* – Scents were extracted in the field and in greenhouse using the dynamic headspace technique (Proffit et al., 2008; Soler et al., 2011). For each individual, the inflorescence was enclosed in a Nalophan® bag for one hour. One control (empty bag) was sampled for each combination of site, species and date, in order to collect VOCs occurring in the ambient air. Two traps – one for compound identification and one for compound quantification – were inserted into the bag for 20 minutes. After floral scent extraction, each trap was put into a vial and stored at -20°C until further analyses. VOCs were then identified and quantified using Gas Chromatography coupled with Mass Spectrometry. Sample identification was based on their retention indices and mass spectra, which were compared to those recorded in databases (Adams, 2007). We subtracted VOCs occurring in ambient air from the controls to the samples collected on the same days and under the same conditions of collection. The compounds quantities were calculated relatively to the internal standard and transformed in relative amounts (proportions). All VOCs samples have been treated in the same way, under both conditions. Detailed methods used for scent extraction and analysis are available in Appendix S1 – Method S2.

**Statistical analyses**

Here we provided a general description of the statistical analyses. All details are available in Appendix S1 – Method S3.

*Geographical variation in pollinator communities* – We analysed variation in pollinator communities at three taxonomic levels separately: (i) Family, (ii) Genus and (iii) Species. For each taxonomic level, we used a Permutational Multivariate Analysis of Variance (PERMANOVA) to tease apart the effects of region and identity of the focal plant species on Bray-Curtis distances between pollinator abundances.

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*Variation in floral scents* – For each plant species, three datasets were analysed separately: (i) field scent data, (ii) greenhouse scent data, and (iii) a merged dataset including both field and greenhouse, restricted to the populations that were studied in both conditions. We assessed the importance of explanatory factors (populations and conditions) using a PERMANOVA performed on Bray-Curtis distances between scents and, when the population effect was found significant, we performed a multiple pairwise comparisons.

*Co-variation of floral scents and pollinators* – To test whether changes in floral scents in the field were associated to some variation in the pollinator communities (at the species level), we performed a redundancy analysis (RDA). We used the VOCs table (VOCs proportions) as the response variable, and the number of pollinator visits, the populations and the identity of plant species as explanatory variables. We used the function *varpart* of the package *vegan* (Oksanen et al., 2019) to assess the amount of variation in the VOCs table explained by the three explanatory tables. We used a biplot graph to represent the results of the RDA analysis and to show compounds important in pollinator variation among plant species.

All analyses were conducted in R version 4.0.2 (R Development Core Team 2018).

## RESULTS

### Variation in pollinators among regions

On the same days VOCs were collected in the field, we recorded a total of 641 pollinator interactions with our focal plants, from 624 sampled pollinator individuals, corresponding to 6 families, 31 genera and 88 species overall (Table S3). The main pollinators were native wild bees of the family Apidae and Halictidae (group Anthophila, n = 240 and n = 225, respectively). We found significant geographical variation in pollinator community composition at the pollinator species level (Table 1), with very few pollinator species shared among regions (Table S3 and Figs S4-S7). We did not find any significant geographical variation between regions at the pollinator family or genus levels (Table 1).

In general, we observed clear and significant variation in pollinator communities among the four focal plant species, independently of the taxonomic level, indicating that each plant species has its own pollinator assemblage (Fig. 1) which also significantly varies among regions (Table 1).

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**Overall characteristics of floral scents**

In total, we detected 38 volatile compounds (24 in the field and 22 under greenhouse conditions), belonging to four different chemical families, mainly fatty acid derivatives and monoterpenes, including six unknown compounds (Table S4). Floral scents of surveyed species shared many volatile compounds, with 21 VOCs out of the 24 being detected in all four species in the field (except (*E*)-Hex-3-enol, (*E*)-β-Ocimene not produced by *P. officinalis* and Sabinene only produced by *G. vulgaris*), and 18 out of 22 in the greenhouse (Table S5). Overall, the major compounds (average proportion >10% in at least one population, occurrence in more than half of sampled individuals) found in natural populations were partly similar among species. This included Limonene (major compound in all four species), (*E*)-β-Ocimene (major compound in *A. vulneraria* and *R. bulbosus*) and Nonanal (rare in *A. vulneraria*, major in the three other species, Fig. S8). However, every species presented a specific floral bouquet, with some species-specific volatile compounds and among-species differences in VOC proportions (Fig. 2, Table S5 for details).

**Variation in floral scents among populations and regions in the field**

The patterns of variation among populations and regions differed among species (Table 2), with either very stable composition of floral scents among all sampled sites, variation among sites with no obvious regional trend, or relatively strong variation in the VOC proportions among regions. To provide a global picture of scent variation among sites and conditions, Figure 2 presents the average proportions of major compounds, as defined above, and some other compounds explaining most of the statistical differences among populations and/or conditions (greenhouse vs. field), based on Simper analyses (Table 3). Complete information on average proportions of all VOCs in each sample, and among-individual variation, is available in Table S5. In addition, within and between-population variations can be visualized in Figs 3 and S9-S11.

*Globularia vulgaris* was the only species in which no significant geographical variation was detected (Fig. S9). In *P. officinarum*, a significant population effect was detected in the field (Table 2), but only half of the pairwise comparisons remained significant after p-value adjustment, with no particular regional trend (Figs 2, S10, Table 3). In contrast, in both *A. vulneraria* and *R. bulbosus*, we found some geographical variation of floral scent composition, which included significant differences between regions (Tables 2-3, Figs 3,

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S11). This variation included differences in terms of VOC occurrence and/or proportions and was not entirely explained by differences in the variance of among individuals (Tables S5-S6). Although significant differences sometimes occurred between sites from the same region in both these species, most of the geographical variation was explained by the higher proportion of Ocimenes in the southern populations, whereas scents from the northern populations were dominated by (*Z*)-Hex-3-enyl acetate and Nonanal (Fig. 2, Table S5).

**Co-variation of floral scents and pollinators in the field**

Variation in VOCs extracted in the field was significantly associated with the pollinator community: the pollinator individual fraction explained 8.4% of VOC variation, even after removing potential confounding effects, *i.e.* species and site effects (model poll | pop + sp, Table 4). In other words, similar floral scents produced by two different species and/or in different sites were associated to similar pollinator species assemblages. All other effects tested in the RDA were significant, confirming that different populations and different species are associated to different VOCs (Table 4). As visible in the biplot graph (Fig. 4), the three compounds closest to the first two axes of the principal component analysis underlying the RDA (Limonene, (*E*)-β-Ocimene, and Nonanal) seemed associated to specific pollinator species, according to the considered plant species and location. Nonanal was associated with the presence of one solitary bee (*Andrena flavipes*) and two hoverflies (genus *Cheilosia*), in the northern populations of *R. bulbosus*, while (*E*)-β-Ocimene was associated with *A. simontornyella*, in the southern populations. Limonene was associated with the presence of five pollinators: two solitary bees (*A. combinata* and *A. labialis*) associated to *A. vulneraria*; and three bee species of the family Apidae associated to *P. officinarum* (*Ceratina cyanea*, *Bombus lapidarius* and *B. pascuorum*, with the latter also visiting *A. vulneraria* and *R. bulbosus*).

**Variation in floral scents among sites and regions in the greenhouse**

In all three species that were also studied in the greenhouse, we found significant differences in the composition of floral scents between field and greenhouse conditions (Tables 2-3, Figs S12-14). Some of this variation, but not all of it, was explained by the high variance among individuals grown in the greenhouse (Table S6). Most VOCs, in particular those that were not identified as major compounds, were found in one condition but not in the other (Fig. 2, Table S5). Nonanal, a major compound for *R. bulbosus* and *P. officinarum* (Fig. 2) was only

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detected in the field. Other major compounds were found in both conditions (*e.g.* (*E*)-β-Ocimene or Limonene), but sometimes with marked differences in terms of relative proportions (Table S5).

Even when grown in homogeneous environment, plants from different populations sometimes exhibited significant differences in scent composition. However, patterns of geographical variation were congruent between field and greenhouse studies in *R. bulbosus* only (Table 3, Fig. 3). In this species, as found in the field, the southern population reared in greenhouse emitted high proportions of (*E*)-β-Ocimene against high relative abundances of (*Z*)-Hex-3-enyl acetate in northern populations (Fig. 2, Table S5).

In contrast, although populations of *P. officinarum* showed significant differences in scent composition when grown in the greenhouse, this geographical variation was not consistent with the results found in the field (except between FAL and F, Table 3). Finally, the two populations of *A. vulneraria* included in the greenhouse study did not show any significant geographical variation in floral scents composition (Fig. S11). However, the proportions of some major volatile compounds detected in the greenhouse were globally consistent with results obtained under field conditions, with a dominance of Limonene in southern populations and (*Z*)-Hex-3-enyl acetate in the northern one (Fig. 2, Table S5).

Two compounds, (*Z*)-Hex-3-enyl acetate and Nonanal, which explained part of the observed geographical variation (Table 3), are sometimes considered as potential contaminants or stress compounds. However, when running the same analyses without each or both compounds, results did not differ from the results presented above, with marginal differences only in the pairwise analysis for *P. officinarum* (Table S8). The same results were obtained when we ran additional test with only a subset of selected compounds (the three compounds that were the most associated with pollinators and only the compounds found both in the field and under greenhouse conditions: see Tables S7-S8 for details).

## DISCUSSION

Focus on floral scent in plants with generalist pollination is not the norm for much of floral scent biology studies, although they represent the large majority of pollination systems (Farré-Armengol et al., 2020; Waser et al., 1996), especially in temperate habitats. In this study, we focused on four plant species from four different families, widely distributed in

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different regions at different latitudes, and associated with a marked variation in the communities of pollinator species (both among plant species and among regions). Our approach also showed an important variation in floral scents between growth conditions, which is not often investigated or considered by studies comparing *in natura* volatile compounds between spatially separated populations (Majetic et al., 2009b).

In all four plant species, we observed strong regional differences in the pollinator communities at the species, but not at the genus, level. Preliminary analyses on the pollinator diversity sampled in the six study sites across two consecutive years (and associated with the entire plant communities), showed that the pollinator variation was much stronger among regions than within regions and, between-year β diversity was quite low (result not showed, but for reference see de Manincor, 2019). So, there is a clear and significant geographical variation in the pollinator community, independently from temporal turnover. This opens the question of whether relatively closely related insect pollinators would exhibit different preferences for floral scents, possibly leading to divergent selective pressures among plant populations of the same species, but very little is known on this particular topic. In generalist pollination systems, some variation in the response to VOCs among pollinators was found in several studies, but it always involved insects belonging to different families or orders (Larue-Kontic & Junker, 2016; Shuttleworth & Johnson, 2010). To our knowledge, only a few studies found closely related insects reacting differently to floral scents, and they mostly deal with specialized pollination systems (Friberg et al., 2019; Suinyuy et al., 2015), such as the mutualistic interaction involving *Ficus* species (Soler et al., 2011; Souto-Vilarós et al., 2018) or closely related orchids (Breitkopf et al., 2013).

In the current study, we found the patterns of geographical variation in floral scents to strikingly depend on the species under scrutiny. We did not detect any regional variation in the floral scent composition of *G. vulgaris*, even though pollinator communities were clearly different among regions. This suggests that local adaptation to different pollinator communities is not necessarily expected, possibly because different pollinator species may react to the same signal. A possible explanation is the early phenology of this species: a recent study showed that early-blooming species produce generalist attractants, while the uniqueness and originality of floral compounds increase throughout the season with the increasing number of species (Burkle & Runyon, 2019). In *P. officinarum*, variation in floral scent was found in the field, but with no clear geographical trend, possibly because this

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species is predominantly apomictic. Importantly, the variation in floral scents reported here cannot be interpreted in the light of pollination biology only. Several studies have shown that both generalist and specific pollinators detect and/or are attracted by only a portion of the compounds contained in a given floral bouquet (Burger et al., 2013; Dötterl, Füssel, et al., 2005; Dötterl & Vereecken, 2010; Milet-Pinheiro et al., 2013). This means that at least some of the observed variation might be either neutral or under selective pressures mediated by agents different from pollinators (such as herbivores, see Schiestl 2010; Delle-Vedove et al., 2017).

The other two species, *A. vulneraria* and *R. bulbosus*, showed strong patterns of regional variation in floral scents. Interestingly, some of the dominant compounds involved in this variation were the same for the two species, including a prevalence of β-Ocimenes in southern populations. In a recent meta-analysis, Farré-Armengol et al. (2020) found a prevalence of monoterpenes in floral scents, associated with dry conditions, a possible indication of their role in protection against oxidative stresses. β-Ocimene is also known to play important roles in pollination (Farré-Armengol et al., 2017), meaning that local insect visitors in the southern populations of the two species could use these compounds as a possible signal for locating flowers.
Indeed, (*E*)-β-Ocimene was one of the three compounds explaining pollinator variation (Fig. 4) and it was associated with the presence of one solitary bee, *Andrena simontornyella*, which is characteristic of southern France (Le Féon et al., 2020). In contrast, Nonanal was emitted at high proportions in the northern populations of *R. bulbosus* and was associated with two hoverflies of the genus *Cheilosia*, that also use this species as host plant for their larvae (Doczkal, 2000, 2002). Thus, Nonanal emission by *R. bulbosus* may be also used by insects for host plant localization, probably together with other compounds, as shown by other studies (Cha et al., 2011; Fraser et al., 2003).

As already underlined, pollinator mediated selection is not the only force acting on floral scent composition, which should also depend on physiological constraints, selection mediated by other agents (e.g. herbivores) and genetic drift (Delle-Vedove et al., 2017). However, it must be noted that geographical variation in the emission of some dominant volatile compounds in some of the studied plant species was explained by a marked variation in the pollinator communities, suggesting that floral scent variation is associated with the presence of specific pollinators. These findings suggest that chemical mediation can vary among

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populations in generalist plant species with common pollinators. Whether such local preference was elicited by an adaptation of local pollinators to high frequencies of a given set of compounds in some areas, or by an adaptation of plants to innate preference of local pollinators need to be further investigated.

One of the objectives of the current study was to investigate the possible role of phenotypic plasticity in the observed variation in floral scents among regions. In all three investigated species, scent composition was found to vary with growth condition, involving both qualitative and quantitative variation. Even if our work is not based on a true "reaction-norm" study (*i.e.* we did not use clonal individuals as in Majetic *et al.*, 2009b; Friberg et al., 2017), individuals reared in greenhouse were likely from the same genetic pool as the plants surveyed in wild populations. Although some differences could have been elicited by the small sample size in the greenhouse study, the fact that some compounds were found in most individuals in one of the two conditions while completely absent from the other, suggests that the culture condition influenced the emitted floral scents. In particular, patterns of variation between field and greenhouse for the Nonanal occurrence were quite surprising, but additional tests ran without it (Tables S7-S8) suggest that this compound alone did not drive the observed difference between growth conditions. Such difference seems particularly strong compared to previous studies, which mainly focused on specialized pollination systems (Friberg et al., 2013), and this would definitely require further investigation.

Most of the observed differences in floral scents between the two conditions may result from (i) abiotic parameters that differed between field and greenhouse and (ii) the fact that insect visitation was prevented in the greenhouse but not in the field, leading to possible changes in scent after pollination as found by several studies (Delle-Vedove et al., 2017; Proffit et al., 2018; Schiestl, 2010; Schiestl & Ayasse, 2001; Terry et al., 2007). Even though plants that were sampled in the field were bagged early in the morning before scent extraction and were retained only if not faded, it is possible that the effect of culture condition results from some post-pollination changes, in addition to a plasticity response to abiotic factors. Overall, this strengthens the idea that investigating floral scents only in controlled conditions can produce a biased picture of signals potentially perceived by pollinators in the wild. Given the observed high impact of plasticity of floral scents in our study species, further studies investigating whether VOCs involved in pollinator attraction express lower levels of plasticity (*i.e.*, expected to be more canalized) compared to those involved in other functions,

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such as stress response or defence against herbivores (*i.e.*, expected to be more plastic), would help in better understanding the patterns of variation in floral scents composition in nature.

Whether phenotypic plasticity explains geographical variation in floral scents seems again to depend on the species. The clearest case was *R. bulbosus*, for which greenhouse and field studies showed rather consistent results. Even if the emission of some compounds depended on the growth condition, our results suggest a genetic basis of a part of the floral scent variation observed among populations, which was not clear for the other two species. Whereas *A. vulneraria* showed geographical variation of both floral scents and flower colour in the wild, only colour variation has been assessed in the greenhouse study (Appendix S1 – Method S4, Fig. S15). Studies that investigated co-variation of floral colour and scents have found mixed results to date (Delle-Vedove et al., 2011; Dormont et al., 2019; Kantsa et al., 2017; Majetic et al., 2008). In our case, it seems that at least a part of floral scent variation is not genetically based and may not be entirely correlated with colour variation.

With this study, we show that geographical variation in floral scents in plant species with generalist pollination can occur and that it is likely to have some genetic basis in at least one of the surveyed species (*R. bulbosus*). The observed differences at the insect species level seem to be linked to differences in floral scents, and the presence of some pollinators was associated to the production of specific compounds. Alternative approaches are now needed to test more directly whether geographical floral scent variation in generalist species results from local adaptation to pollinators. One promising perspective is the measurement of selection gradients, that have been recently proven successful even on complex traits such as floral scents (Majetic et al., 2009a; Ehrlén et al., 2012; Parachnowitsch et al., 2012; Gross et al., 2016).

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**FIGURE LEGEND**

**Figure 1** Pollinator interactions for all the four focal plant species *Anthyllis vulneraria* (AV, in pink), *Globularia vulgaris* (GV, in blue), *Pilosella offcinarum* (PO, in gold) and *Ranunculus bulbosus* (RB, in green). The shared pollinator species are shown in the middle of the image and their interactions have multiple colours depending on the plant species with which they interact. Full insect species names are provided in Table S3b.

**Figure 2** Average proportions of major and some minor volatile compounds that explained most of the statistical differences (based on Simper analyses, Table 3) among populations and/or in the field (*F*) and greenhouse (*G*) conditions, in the four plant species *Globularia vulgaris*, *Anthyllis vulneraria*, *Pilosella officinarum* and *Ranunculus bulbosus*. For *G. vulgaris* we could only analyse scents extracted in the field. We considered as "major" those volatile compounds with an average proportion higher than 10% in at least one population and occurring in more than half of the sampled individuals. In the “Other compounds” we included all “minor” compounds found in both *F* and *G* conditions. Information about the whole floral bouquets of each species and population, and the average proportion of each compound are provided in Table S5. Within and between-population variations are represented with NMDS Figures 3, S9-S11.

**Figure 3** Non-metric multidimensional scaling showing floral scents pattern of variation among and within-populations, both under field (on the left) and greenhouse (on the right) conditions, for *Ranunculus bulbosus*. Significant geographical differences have been found under both conditions between the southern populations (BF and F, orange and dark orange circles) and northern populations (LAR and R, light blue and dark blue triangles). Stress values were 0.16 in both conditions.

**Figure 4** Biplot graph obtained from the RDA, evidencing significant compounds important in pollinator variations among plant species. VOCs are represented as black arrows in the plane of the first two axes of the principal component analysis (PCA) underlying the RDA and point in the direction of increasing values for that VOC. The compounds most closely associated to the first two axes of the PCA are Nonanal, (*E*)-β-Ocimene and Limonene. The dashed grey lines represent the projection of vectors of pollinator occurrences on plants on the VOC PCA, and the black points represent the individual data points (*i.e.* VOC emission by site and plant species). The highlighted plant species (in blue) were visited by pollinators associated with the three compounds mentioned above.

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## TABLES

**Table 1** Results of PERMANOVA analysis to test the variation in pollinator communities at three taxonomic levels: (i) Family, (ii) Genus and (iii) Species. For each category we tested if there was a geographical variation (effect "Region"), if this variation was linked to the identity of the four focal plant species (effect "Plant Species") and we tested the interaction among the two factors ("Region*Plant Species"). Significant results are shown in bold.

| Taxonomic level | Model | Effect | Df | F.Model | R2 | Pr(>F) | p-adjusted |
|---|---|---|---|---|---|---|---|
| (i) Family (n = 6) | poll_fam ~ Region | Reg | 2 | 0.99 | 0.13 | 0.425 | 0.425 |
| | poll_fam ~ Plant species | **Plant sp** | 3 | 3.94 | 0.50 | 0.002 | **0.003** |
| | poll_fam ~ Region * Plant species | **Reg*Plant sp** | 3 | 4.57 | 0.26 | 0.006 | **0.009** |
| (ii) Genus (n = 31) | poll_gen ~ Region | Reg | 2 | 1.42 | 0.18 | 0.109 | 0.12 |
| | poll_gen ~ Plant species | **Plant sp** | 3 | 2.09 | 0.34 | 0.002 | **0.003** |
| | poll_gen ~ Region * Plant species | **Reg*Plant sp** | 3 | 2.80 | 0.27 | 0.00 | **< 0.0001** |
| (iii) Species (n = 88) | poll_sp ~ Region | **Reg** | 2 | 1.53 | 0.19 | 0.016 | **0.021** |
| | poll_sp ~ Plant species | **Plant sp** | 3 | 1.97 | 0.33 | 0.00 | **0.001** |
| | poll_sp ~ Region * Plant species | **Reg*Plant sp** | 3 | 2.21 | 0.24 | 0.00 | **< 0.0001** |

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**Table 2** Results of PERMANOVA analysis on the floral bouquet (bouquet) in the four focal species (AV, *Anthyllis vulneraria*; GV, *Globularia vulgaris*; PO, *Pilosella officinarum* and RB, *Ranunculus bulbosus*). We performed three types of analyses: for each species we tested the effect of populations (i) in field and (ii) greenhouse conditions, separately. We then tested (iii) if there were differences in the floral bouquet between field vs greenhouse conditions (type environment, effect condition) and between populations (effect population) that were tested in both conditions. Significant results are shown in bold.

| Condition | Model | Effect | Species | Df | F.Model | R2 | Pr(>F) | p-adjusted |
|---|---|---|---|---|---|---|---|---|
| (i) Field | bouquet_AV ~ Population | Population | **AV** | 3 | 3.23 | 0.24 | 0.00 | **< 0.0001** |
| | bouquet_GV ~ Population | | GV | 3 | 1.33 | 0.12 | 0.16 | 0.17 |
| | bouquet_PO ~ Population | | **PO** | 4 | 2.15 | 0.23 | 0.00 | **< 0.0001** |
| | bouquet_RB ~ Population | | **RB** | 3 | 5.92 | 0.32 | 0.00 | **< 0.0001** |
| (ii) Greenhouse | bouquet_AV ~ Population | Population | AV | 2 | 1.28 | 0.18 | 0.24 | 0.24 |
| | bouquet_PO ~ Population | | **PO** | 2 | 3.48 | 0.32 | 0.01 | **0.01** |
| | bouquet_RB ~ Population | | **RB** | 1 | 2.66 | 0.18 | 0.03 | **0.03** |
| (iii) Field vs Greenhouse | bouquet_AV ~ Condition + Population | Condition | **AV** | 1 | 29.16 | 0.39 | 0.00 | **< 0.0001** |
| | | Population | | 2 | 3.55 | 0.94 | 0.00 | **< 0.0001** |
| | bouquet_PO ~ Condition + Population | Condition | **PO** | 1 | 46.39 | 0.53 | 0.00 | **< 0.0001** |
| | | Population | | 2 | 3.66 | 0.84 | 0.01 | **< 0.0001** |
| | bouquet_RB ~ Condition + Population | Condition | **RB** | 1 | 29.76 | 0.47 | 0.00 | **< 0.0001** |
| | | Population | | 1 | 5.94 | 0.09 | 0.00 | **0.0021** |

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**Table 3** Results of pairwise comparisons between populations (BF and F in Occitanie region; CG and FAL in Normandie region; LAR and R in Hauts-de-France region) and in the three conditions following the PERMANOVA analysis (Table 2). We first tested the effect of populations (i) in field and (ii) greenhouse conditions, separately, in the four focal species (AV, *Anthyllis vulneraria*; GV, *Globularia vulgaris*; PO, *Pilosella officinarum* and RB, *Ranunculus bulbosus*). We then tested if there were differences in the floral bouquet (iii) between field vs. greenhouse condition (type environment, effect condition) and between populations that were tested in both conditions (type environment, effect population). We also present the results of the Simper analysis, and we listed the volatile compounds which explained at least 30% (cumulative) of the statistical difference between paired comparisons. The volatile compounds are listed in order of contribution to the detected variation. Significant results are shown in bold. The (*) symbol indicates that two groups of plants show a difference in the variance of their floral scents, based on the *betadisper* analysis (Table S6).

| Condition | Effect | Species | Paired comparison | Df | Pseudo-F | R2 | p-value | p.adjusted | VOCs involved in the variation (Simper analysis) |
|---|---|---|---|---|---|---|---|---|---|
| (i) Field | Population | **AV** | **BF vs F (*)** | **1** | **2.70** | **0.14** | **0.00** | **< 0.0001** | *(Z)-Hex-3-enol; Limonene; 6-methyl-Hept-5-en-2-one; (E)-β-Ocimene; (Z)-Hex-3-enyl acetate* |
| | | | **BF vs LAR (*)** | **1** | **4.68** | **0.23** | **0.00** | **< 0.0001** | *Limonene; (E)-β-Ocimene; (Z)-β-Ocimene; Cyclohexen-1-yl ethanone; α-Terpenyl acetate* |
| | | | **BF vs R (*)** | **1** | **4.05** | **0.22** | **0.00** | **< 0.0001** | *Limonene; (E)-β-Ocimene; Nerylacetone; (Z)-Hex-3-enyl acetate; Nonanal* |
| | | | **F vs LAR** | **1** | **3.89** | **0.19** | **0.00** | **< 0.0001** | *(E)-β-Ocimene; (Z)-Hex-3-enol; 6-methyl-Hept-5-en-2-one; (Z)-Hex-3-enyl acetate; α-Terpenyl acetate* |
| | | | **F vs R** | **1** | **2.77** | **0.16** | **0.00** | **< 0.0001** | *(Z)-Hex-3-enol; (E)-β-Ocimene; 6-methyl-Hept-5-en-2-one; Nerylacetone; (Z)-Hex-3-enyl acetate* |
| | | | LAR vs R | 1 | 1.58 | 0.1 | 0.13 | 0.13 | |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| GV | BF vs CG | 1 | 0.62 | 0.04 | 0.74 | 0.74 | |
| | BF vs F | 1 | 1.36 | 0.06 | 0.23 | 0.34 | |
| | BF vs FAL | 1 | 1.34 | 0.08 | 0.19 | 0.34 | |
| | CG vs F | 1 | 1.63 | 0.12 | 0.17 | 0.34 | |
| | CG vs FAL | 1 | 0.7 | 0.1 | 0.68 | 0.74 | |
| | F vs FAL | 1 | 2.52 | 0.17 | 0.07 | 0.34 | |
| **PO** | CG vs F | 1 | 1.15 | 0.09 | 0.33 | 0.35 | |
| | **CG vs FAL (*)** | **1** | **2.24** | **0.16** | **0.02** | **0.05** | *6-methyl-Hept-5-en-2-one; (E)-β-Farnesene; α-Pinene; Unknown compound 4; Unknown compound 3* |
| | CG vs LAR | 1 | 1.27 | 0.08 | 0.24 | 0.3 | |
| | **CG vs R** | **1** | **2.14** | **0.12** | **0.03** | **0.05** | *Limonene; Nonanal; Non-1-ene; Unknown compound 4; Unknown Sesquiterpene* |
| | FAL vs LAR | 1 | 2.39 | 0.19 | 0.05 | 0.08 | |
| | **FAL vs R (*)** | **1** | **5.42** | **0.33** | **0.00** | **0.01** | *Limonene; Non-1-ene; Unknown compound 1; 6-methyl-Hept-5-en-2-one; (E)-β-Farnesene* |
| | **F vs FAL** | **1** | **5.35** | **0.4** | **0.01** | **0.04** | *(Z)-β-Ocimene; (E)-β-Farnesene; Non-1-ene; 6-methyl-Hept-5-en-2-one; Limonene* |
| | F vs LAR | 1 | 1.78 | 0.15 | 0.07 | 0.09 | |
| | **F vs R** | **1** | **2.33** | **0.17** | **0.01** | **0.04** | *(Z)-β-Ocimene; Nonanal; Nerylacetone; Limonene; Geranylacetone* |
| | LAR vs R | 1 | 1.09 | 0.08 | 0.35 | 0.35 | |
| **RB** | **BF vs F** | **1** | **2.23** | **0.1** | **0.04** | **0.04** | *α-Pinene; α-Terpenyl acetate; (E)-β-Farnesene; Geranylacetone; Unknown compound 1* |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | **BF vs LAR** | 1 | **6.53** | **0.22** | **0.00** | **< 0.0001** | *(Z)-Hex-3-enyl acetate; Unknown compound 3; (E)-β-Ocimene; Nonanal; (Z)-β-Ocimene* |
| | | | **BF vs R (*)** | 1 | **6.05** | **0.21** | **0.00** | **< 0.0001** | *(E)-β-Ocimene; (Z)-β-Ocimene; (Z)-Hex-3-enyl acetate; Unknown Sesquiterpene; Cyclohexen-1-yl ethanone* |
| | | | **F vs LAR** | 1 | **7.09** | **0.32** | **0.00** | **< 0.0001** | *(Z)-Hex-3-enyl acetate; Nonanal; Unknown compound 3; Geranylacetone; (E)-β-Ocimene* |
| | | | **F vs R** | 1 | **10.77** | **0.42** | **0.00** | **< 0.0001** | *Nonanal; Cyclohexen-1-yl ethanone; (E)-β-Ocimene; Unknown compound 1; Unknown Sesquiterpene* |
| | | | **LAR vs R** | 1 | **5.91** | **0.25** | **0.00** | **< 0.0001** | *Unknown compound 3; (Z)-Hex-3-enyl acetate; Cyclohexen-1-yl ethanone; (E)-β-Farnesene; Unknown compound 2* |
| (ii) Greenhouse | Population | AV | BF vs F | 1 | 0.92 | 0.1 | 0.51 | 0.51 | |
| | | | BF vs LAR (*) | 1 | 1.48 | 0.16 | 0.21 | 0.39 | |
| | | | F vs LAR (*) | 1 | 1.31 | 0.14 | 0.26 | 0.39 | |
| | | | | | | | | | |
| | | **PO** | **CG vs F (*)** | **1** | **5.65** | **0.34** | **0.00** | **0.01** | *p-Cymene; Limonene; Unknown compound; Hexadec-1-ene; Tetradec-1-ene* |
| | | | CG vs FAL | 1 | 0.99 | 0.11 | 0.42 | 0.42 | |
| | | | **F vs FAL (*)** | **1** | **4.75** | **0.3** | **0.02** | **0.02** | *p-Cymene; Limonene; Dodecan-1-ol; Geranylacetone; 6-methyl-Hept-5-en-2-one* |
| | | | | | | | | | |
| | | **RB** | **F vs LAR** | **1** | **2.66** | **0.18** | **0.04** | **0.04** | *(E,E)-α-Farnesene; (Z)-Hex-3-enyl acetate; (E)-β-Ocimene ; Limonene; (E,E)-2,6-dimethylOcta-1,3,5,7-tetraene* |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| (iii) Field vs Greenhouse | Condition | **AV** | **field vs greenhouse (*)** | **1** | **25.94** | **0.39** | **0.00** | **< 0.0001** | *Sabinene; (E)-β-Ocimene; β-Pinene; Limonene; Myrcene; p-Cymene* |
| | Population | | BF vs F (*) | 1 | 1.5 | 0.05 | 0.17 | 0.17 | |
| | | | BF vs LAR (*) | 1 | 2.37 | 0.08 | 0.05 | 0.08 | |
| | | | F vs LAR | 1 | 2.32 | 0.08 | 0.05 | 0.08 | |
| | | | | | | | | | |
| | Condition | **PO** | **field vs greenhouse** | **1** | **40.26** | **0.53** | **0.00** | **< 0.0001** | *(Z)-Hex-3-enyl acetate; β-Pinene; p-Cymene; Non-1-ene; Nonanal; Tetradecanal* |
| | Population | | CG vs F (*) | 1 | 2.37 | 0.09 | 0.08 | 0.12 | |
| | | | CG vs FAL | 1 | 1.26 | 0.05 | 0.26 | 0.26 | |
| | | | F vs FAL | 1 | 2.84 | 0.12 | 0.06 | 0.12 | |
| | | | | | | | | | |
| | Condition | **RB** | **field vs greenhouse** | **1** | **25.42** | **0.47** | **0.00** | **< 0.0001** | *Nonanal; Unknown compound 3; (Z)-Hex-3-enyl acetate; (E)-Caryophyllene; Sabinene; Unknown compound 1; (E)-β-Ocimene* |
| | Population | | **F vs LAR** | **1** | **3.22** | **0.1** | **0.04** | **0.04** | *(Z)-Hex-3-enyl acetate; Nonanal; Unknown compound 3; (E)-β-Ocimene; 6-methyl-Hept-5-en-2-one; Unknown compound 1* |

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**Table 4** Results of the RDA analysis of VOCs emitted by the four focal plant species (*Anthyllis vulneraria*, *Globularia vulgaris*, *Pilosella officinarum*, *Ranunculus bulbosus*) in the six sites, presented as correlations between each combination of explanatory variables and the VOC table, together with their statistical significance obtained by permutation of table rows. Each fraction corresponds to an explanatory table used in the analysis. poll: pollinator table (pollinator visits to each focal plant species); pop: population table (sites); sp: identity of the four focal plant species; *df*: degree of freedom; *R*2adj: adjusted coefficient of determination. *p*-values are indicated for testable fractions only.

| Fraction | df | $R^2$adj (%) | p-value |
|---|---|---|---|
| poll | 15 | 0.29 | **< 0.0001** |
| pop | 5 | 0.08 | **< 0.0001** |
| sp | 3 | 0.12 | **< 0.0001** |
| poll + pop | 15 | 0.29 | **< 0.0001** |
| poll + sp | 15 | 0.29 | **< 0.0001** |
| pop + sp | 8 | 0.21 | **< 0.0001** |
| poll + pop + sp | 15 | 0.29 | **< 0.0001** |
| **Controlling 1 table** | | | |
| poll \| sp | 12 | 0.17 | **< 0.0001** |
| poll \| pop | 10 | 0.21 | **< 0.0001** |
| pop \| sp | 5 | 0.08 | **< 0.0001** |
| sp \| pop | 3 | 0.13 | **< 0.0001** |
| **Individual fractions** | | | |
| poll \| pop + sp | 7 | 0.08 | **< 0.0001** |
| Residuals | - | 0.70 | - |

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**SUPPORTING INFORMATION**

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

Appendix S1 – Method S1: Plant species additional information

Appendix S1 – Method S2: Scent extraction and floral scent analysis

Appendix S1 – Method S3: Statistical analyses

Appendix S1 – Method S4: *Anthyllis vulneraria* colour dimorphism and floral reflectance analysis

Figure S1: Diagram presenting the stages of the study and the statistical analyses

Figure S2: Map of site and region locations and species occurrence.

Figure S3: *Anthyllis vulneraria* populations colour dimorphism

Figure S4: *Anthyllis vulneraria* interaction networks

Figure S5: *Pilosella officinarum* interaction networks

Figure S6: *Globularia vulgaris* interaction networks

Figure S7: *Ranunculus bulbosus* interaction networks

Figure S8: Proportions of emission due to Limonene, (*E*)-β-Ocimene and Nonanal

Figure S9: Non-metric multidimensional scaling showing floral scents dissimilarities **among populations** for *Globularia vulgaris*

Figure S10: Non-metric multidimensional scaling showing floral scents dissimilarities **among populations** for *Pilosella officinarum*

Figure S11: Non-metric multidimensional scaling showing floral scents dissimilarities **among populations** for *Anthyllis vulneraria*

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Figure S12: Non-metric multidimensional scaling showing floral scents dissimilarities **among conditions** for *Anthyllis vulneraria*

Figure S13: Non-metric multidimensional scaling showing floral scents dissimilarities **among conditions** for *Pilosella officinarum*

Figure S14: Non-metric multidimensional scaling showing floral scents dissimilarities **among conditions** for *Ranunculus bulbosus*

Figure S15: Bee and hoverfly vision of the two-colour dimorphism in *Anthyllis vulneraria* populations.

Table S1: Floral morphology and phenology of the four focal species.

Table S2: Environmental conditions recorded during the floral scent extraction in the two tested conditions (field and greenhouse).

Table S3: Number of insect visitors, at the family, genus and species level, recorded (a) for the four focal species and (b) for each species in the different populations.

Table S4: List of Volatile Organic Compounds (VOCs) identified in the field and greenhouse conditions.

Table S5: List of VOCs (proportion mean ± sd) emitted by the four focal species.

Table S6: Results of *betadisper* analysis.

Table S7: Additional PERMANOVA results with/without specific compounds.

Table S8: Additional pairwise results with/without specific compounds.

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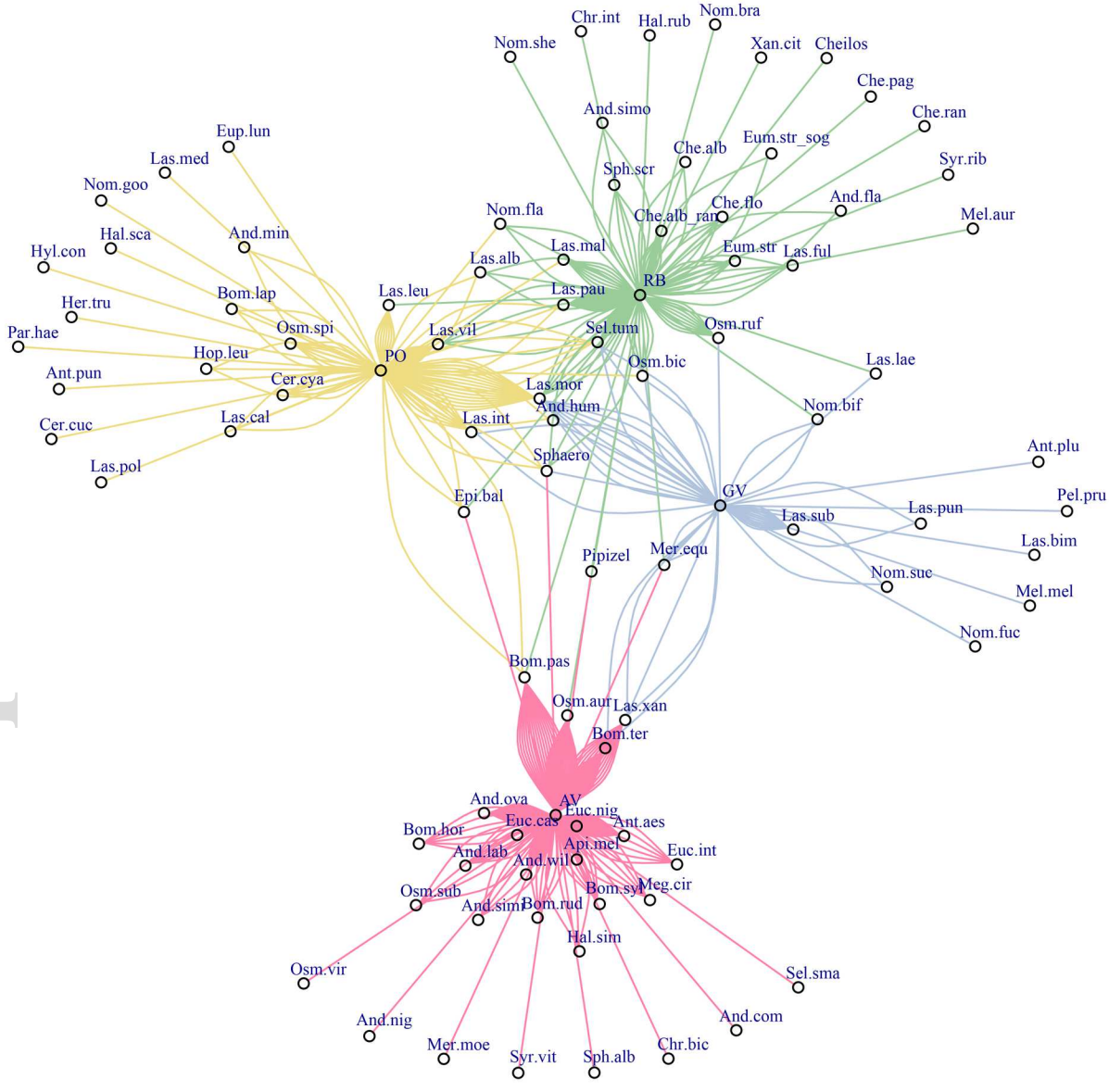

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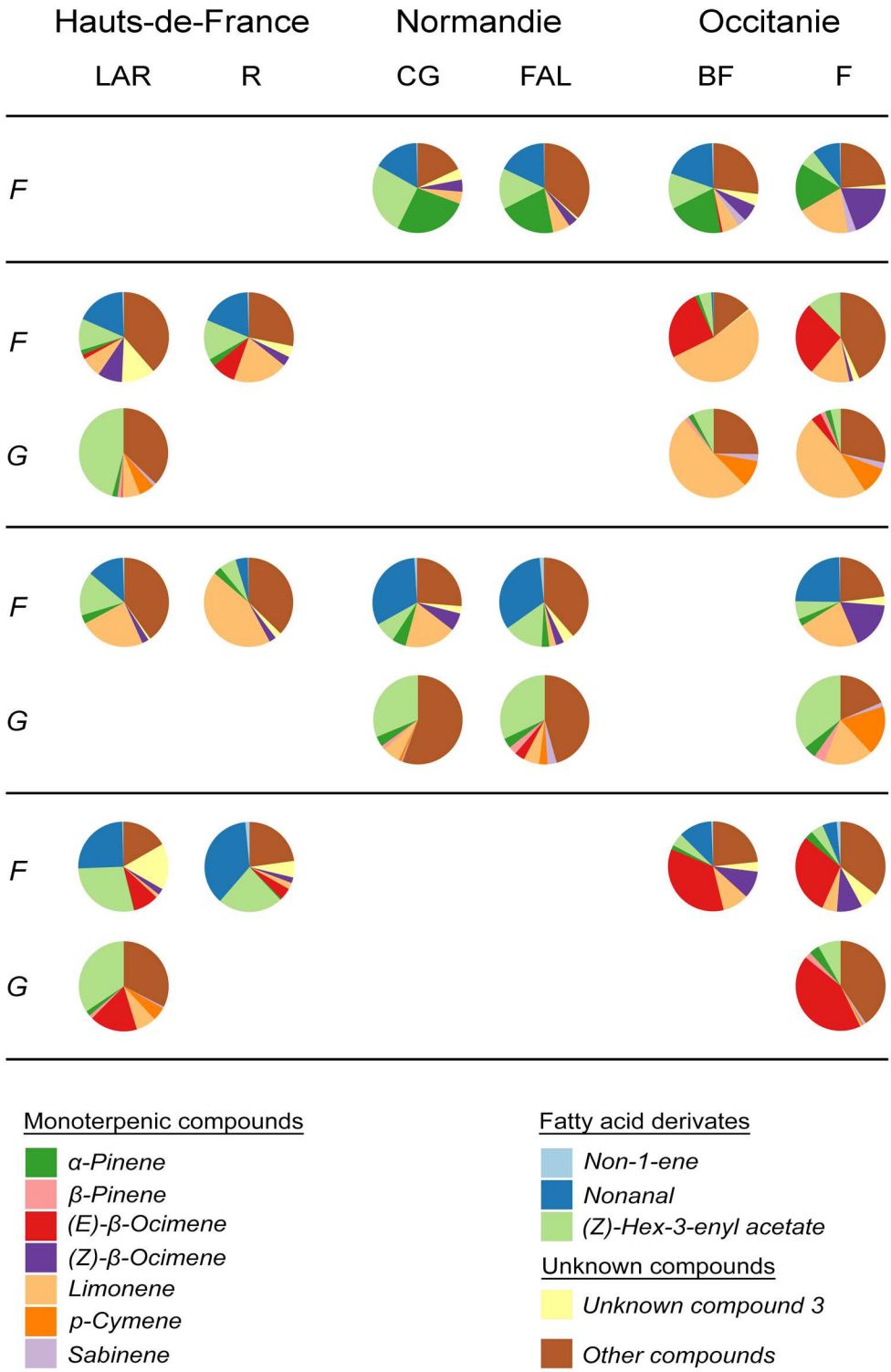

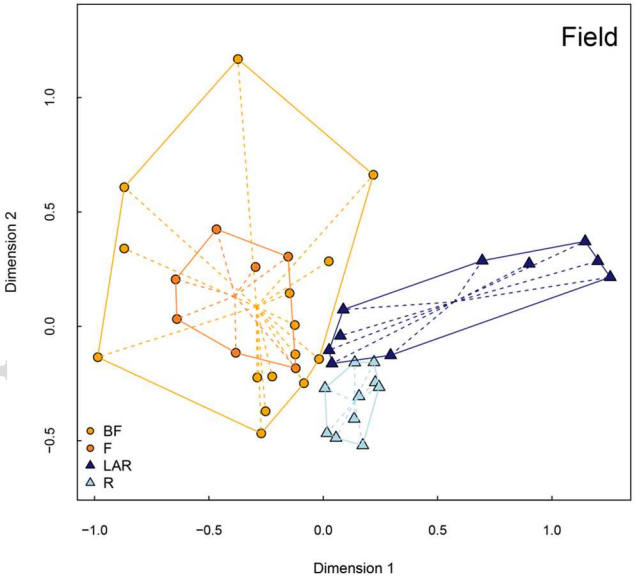
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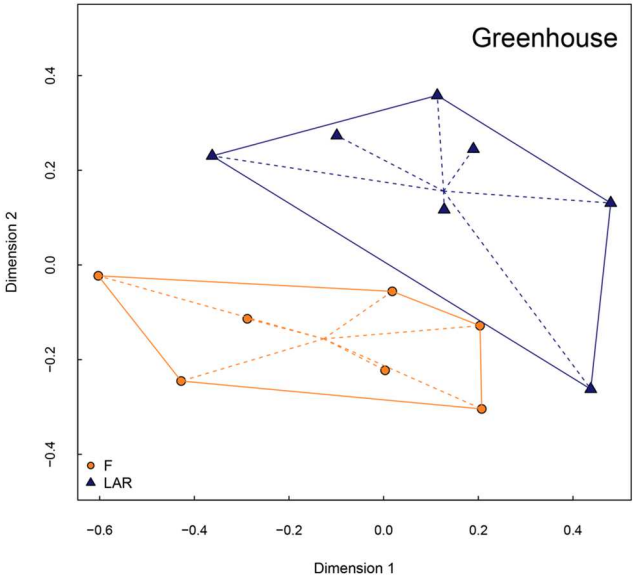
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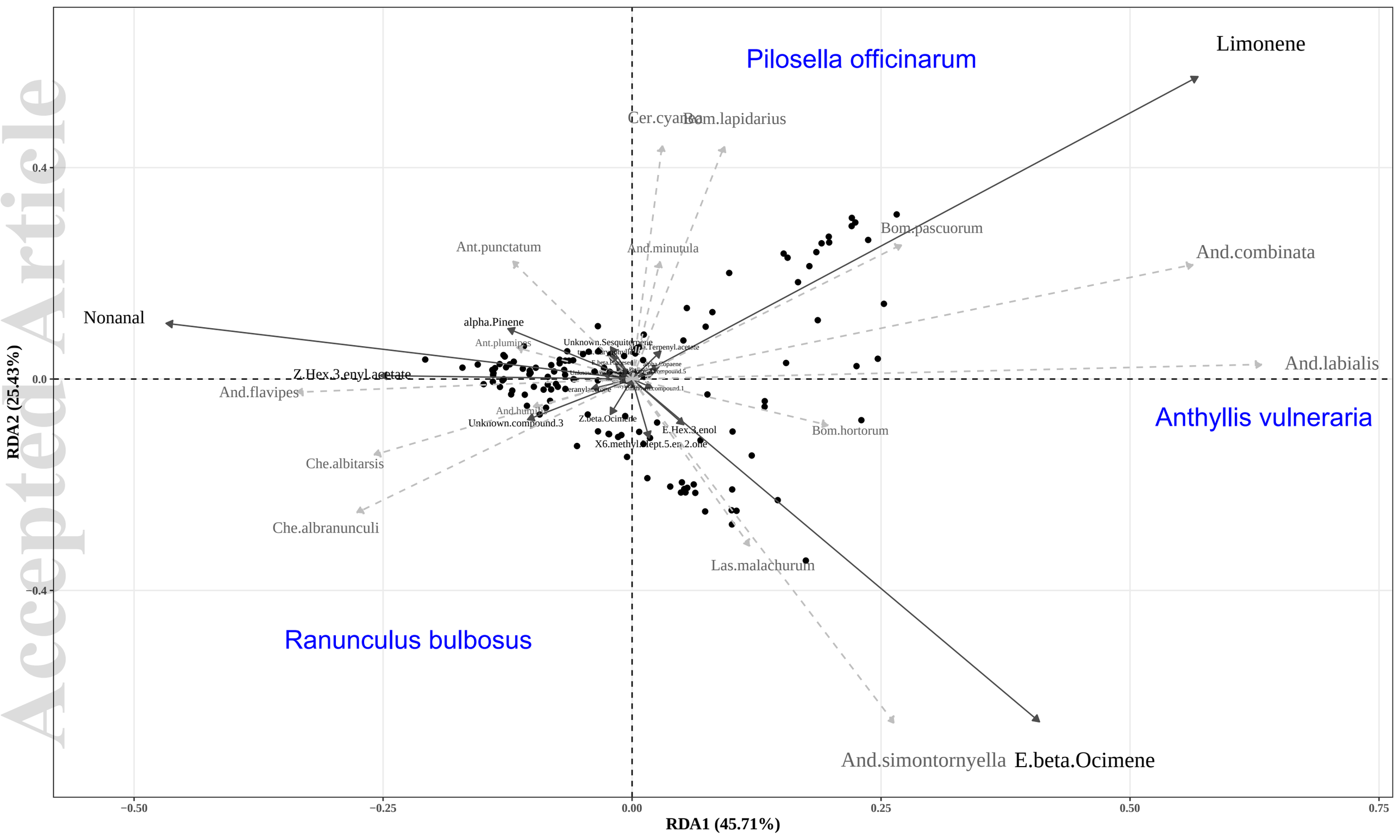
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