Methodological and Ecological Caveats in Deciphering Plant Volatile Emissions: the Case Study of Tomato Exposed to Herbivory and Resource Limitation

Alan Kergunteuil (alan.kergunteuil@inrae.fr)
Institut National de Recherche pour l'Agriculture l'Alimentation et l'Environnement
https://orcid.org/0000-0002-3607-4912
VICTOIRE COQUERET
Institut National de Recherche pour l'Agriculture l'Alimentation et l'Environnement
ROMAIN LARBAT
Institut National de Recherche pour l'Agriculture l'Alimentation et l'Environnement
STÉPHANE ADAMOWICZ
Institut National de Recherche pour l'Agriculture l'Alimentation et l'Environnement
JACQUES LE BOT
Institut National de Recherche pour l'Agriculture l'Alimentation et l'Environnement
CHRISTOPHE ROBIN
Institut National de Recherche pour l'Agriculture l'Alimentation et l'Environnement

Research Article

Keywords: Volatile organic compounds, Tuta absoluta, Solanum lycopersicon, Nitrogen limitation, Methyl salicylate, Terpenes

Posted Date: April 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-419451/v1

Abstract

Over recent years, major progress in experimental approaches have bring insights about the ecological functions of volatile organic compounds (VOCs) released by plants. However, deciphering volatile emissions in a methodologically robust and ecologically relevant manner remains a challenging issue. A surge in interest is required to characterize potential blind spots in volatile sampling that could result in dramatic bias in our understanding of VOCs. In parallel, ecologists need to account for various environmental factors in order to address appropriately the sources of variations of VOCs. Here we use two common porous polymers, polydimethylsiloxane (PDMS) and Porapak Q, to collect VOCs released by tomato exposed to herbivory in combination with nitrogen shortage. We dissect two key features of volatile blends, i.e., their composition and their diversity. Upon nitrogen limitation, Porapak Q stresses the up-regulation of a common defensive compound (methyl salicylate), while herbivory induces three terpenes involved in the recruitment of natural enemies of *Tuta absoluta* (2-carene, □-pinene and β-phellandrene). This study suggests that the combination of resource availability and herbivory governs the differential production of generalist and specific VOCs that are active against a broad spectrum or particular herbivore species, respectively. But PDMS was found unsuitable to observe such patterns in the composition of VOC emissions. Additionally, Porapak Q was found more sensitive than PDMS to track the increase in the diversity of stress-related VOC emissions upon nitrogen limitation. This suggests that plants growing with poor resources release more information in surroundings. We discuss particular implications for tri-trophic-mediated plant defences.

Introduction

Plants release in their surroundings a large number of volatile organic compounds (VOCs) that play a key role in ecological networks. Ecological research in plant-insect interactions has shown that VOCs induce and/or prime anti-herbivore defences in adjacent plants (Li 2016), affect host-plant location by herbivores (Conchou et al. 2019), attract natural enemies (Dicke and Baldwin 2010) and influence hyper-parasitoids (Cusumano et al. 2019). In other words, variations in volatile profiles influence several trophic levels and, ultimately, determine major ecosystem processes (Hunter 2016). With this in mind, ecologists expend considerable efforts in addressing sources of variations in VOCs. However, methodological and ecological issues make this purpose challenging. On the one hand, the restricted tools ecologists have access to explore VOCs emissions likely undermine our ability to account for the natural complexity of volatile profiles released by plants. On the other hand, while plants are rarely exposed to individual stress in nature, our understanding of VOCs is mainly based on disparate studies that address separately the influence of different stresses on volatile emissions. Taken together, we advocate accounting for both sampling issues and realistic combinations of factors driving volatile emissions in order to unravel the roles of VOCs in a robust and relevant manner.

Over the last two decades, great progress has been made in the development of sensitive methods for headspace collection of VOCs (Tholl et al. 2020). However, most of these methods rely on porous

files. We hypothesize that the use of single

porous polymers, as it is generally done, results in blind spots that hamper the functional interpretation of plant volatile profiles. Because the affinity between porous polymers and individual VOCs determines the fraction of volatilomes collected, sampling methods may conduct in important pitfalls that make our understanding of plant VOCs highly versatile. Among the most common porous polymers, polydimethylsiloxane (PDMS) is used in two widespread sampling procedures, i.e., solid-phase microextraction (SPME) and stir-bar sorptive extraction (SBSE) (Bicchi and Maffei 2012). In addition, Porapak Q (copolymers of ethylvinylbenzene and divinylbenzene) has gained popularity for its suitability with solvent extraction (Tholl et al. 2020). In animal ecology, Noushini et al. (2020) have recently shown that sampling methods entail major biases when interpreting insect semiochemicals. In the same line, we need a surge in interest in plant ecology to compare stress-related volatile emissions across different porous polymers. This endeavour may improve the robustness of our functional interpretation of VOCs.

Another important avenue to better understand the functions of VOCs revolves around the integration of induced responses to multiple stress (Pareja and Pinto-Zevallos 2016). Although insect herbivory (Rowen and Kaplan 2016) and plant nutrition (Islam et al. 2017; Ormeño et al. 2008) influence VOCs emissions, there are still major gaps in knowledge about joint effects of both factors on plant volatile profiles (but see Chen 2013 in cotton; De Lange and Rodriguez-Saona 2019 in cranberry). This line of research is required to test classic hypotheses of plant defence theory linking soil fertility with the production of anti-herbivore defensive compounds (Stamp 2003). For instance, the growth:differentiation hypothesis stands that plants growing with poor resources increase their investments in anti-herbivore compounds due to the high cost of tissue replacement upon harsh conditions (Herms and Mattson 1992).

However, little has been done to investigate how the combination between herbivory and resource availability affects the production of generalist and specific defensive compounds that are active against a broad spectrum or particular herbivore species, respectively. In the absence of herbivores, resource availability helps to predict the quantity of defensive metabolites (Le Bot et al. 2009). But the quality of such compounds remains the poor relative of the growth:differentiation hypothesis. We hypothesize that plants experiencing resource limitation without herbivory may invest in the production of generalist defensive compounds. In contrast, after herbivores start feeding, a large body of evidence points the elicitation of species-specific VOCs (Rowen and Kaplan 2016). As a consequence, plant defences are enhanced according to herbivore identity (de Moraes et al. 1998; Mc Cormick et al. 2012), even if the influence of resource availability in such patterns remains understudied. Beyond the composition of VOC profiles, we believe that their diversity, measured as Shannon entropy, may provide complementary knowledge about the roles of VOCs in plant defences. Shannon entropy is largely used in the assessment of biological diversity but only few ecologists keep in mind that such index is rooted in information theory (Shannon 1948). In brief, Shannon entropy has been initially developed to estimate the amount of information a message contains. Thereby, studying Shannon entropy of VOC profiles may help to understand how plants exposed to multi-stress adjust information they release in surroundings and, in turn, determine the breadth of VOC-mediated ecological interactions.

Over the last decade, although 69 studies interested in plant-insect interactions have analysed tomato's VOCs emissions, none of them has integrated joint effects of herbivory and nutrient. Only one study has compared volatile profiles across different methods of collection but the authors focused on performance in VOC extraction rather than functional interpretation of VOCs (Shu et al. 2010). In this context, we hope that our approach may help to dissect (i) potential blind spots in sampling procedures of VOCs that play a key role in plant-insect interactions (ii) how nitrogen resources and herbivory affect the production of anti-herbivore VOCs, whether they are active against a broad spectrum or specific herbivores and (iii) how nitrogen resources and herbivory affect the information amount conveyed by VOC profiles.

Methods And Materials

Sampling Procedures Commonly Used to Explore Volatiles Released by Tomato. Between 2010 and 2020, tomato has been widely used to explore plant VOCs emissions from plant-insect interaction perspectives. We performed a Web of Science query on February 9th 2021 with the following combination of terms: *(tomato* OR Solanum lycopersicum) AND ('plant volatile' OR 'volatile organic compound' OR VOC) AND (insect OR herbivore OR pest)*. We applied additional criteria on the 251 results by removing (i) studies based on electroantennography, electronic nose, specific families of VOCs, leaf exudates, root VOCs and plant samples that were grounded prior VOC collection and (ii) measures interested in VOCs released by essential oils, mutant plants or plants infested with nematodes, bacteria or viruses. In total, 19 studies used Porapak Q, 19 studies used Tenax, 14 studies used PDMS-based SPME or SBSE, 9 used Super Q, 6 used HayeSep Q and 2 studies used charcoal to collect VOCs (Table S1). Only three studies used a combination of two porous polymers (Tenax and Carboxen) but they did not aim at comparing the respective fractions of VOCs collected. Overall, we report 210 records of VOC profiles. The average number of VOCs collected per plant is relatively low, i.e., 17.67, and displays a high standard deviation, i.e., 13.49 (Table S1). This variability raises critical questions when comparing VOC emissions across studies.

Plant Material and Growth Conditions. Tomato seeds (*Solanum lycopersicum* L. cv. Better Bush VFN Hybrid, breeder: Tomato Growers, Florida, USA) were sown on humid filter paper in Petri dishes. Five days after sowing (DAS), seedlings were transferred for six days to a hydroponic system adapted to young germinating plantlets set in a growth chamber with the following conditions: 16 h hemeroperiod, 23°C/18°C (day/night) in air and 25°C in solutions, 60 ± 20 % air humidity. The full nutrient solution supplied $N\text{-}NO_3^-$ at a low concentration of 0.3 mM. Better Bush is an early dwarf cultivar, easy to manage and well adapted to hydroponics. It is responsive to scarification elicitation (Royer et al. 2013) and polyphenol concentration is enhanced by nitrogen limitation (Larbat et al. 2012; Larbat et al. 2014).

Eleven days after sowing, the plantlets were transferred to a Nutrient Film Technique (NFT) system set in the same growth chamber. The entire experiment comprised 32 plants. Half of the plants were maintained at low nitrogen (0.3 mM $N\text{-}NO_3^-$), which has been shown limiting for tomato growth (Adamowicz and Le Bot 2008), while the other half received a full nutrient solution with high $N\text{-}NO_3^-$

concentration (7 mM). Nitrogen concentration and pH were measured and adjusted manually with increasing frequency as growth proceeded, up to once a day for high nitrogen availability and twice a day for nitrogen limitation. For more details on the composition of nutrient solutions and on their maintenance, see (Royer et al. 2013). The limited nitrogen concentration does not induce deficiency symptoms on plants during the experiment (Adamowicz and Le Bot 2008; Royer et al. 2016). All the plants could not be grown concurrently because of the limited space inside the growth room and a limited number of VOC trapping systems. Therefore, the experiment was run over four successive periods using four herbivore-damaged and four control plants each time grown under low or high nitrogen conditions.

Tuta absoluta *Larvae and Leaf Infestation*. Prior to the experiment, *T. absoluta* adults were reared on tomato plants in an insectarium set in a climate chamber adjacent to the main experiment. A large population of larvae developed on the leaves. Infestation was made the 39th day after sowing. When plants were at the vegetative stage with 6–8 developed leaves, 12 third instar larvae selected from this population were deposited on the three terminal leaflets of the 4th true leaf for 8 plants exposed to high and low nitrogen regimes. Control plants were slightly touched with a paintbrush to mock larval deposition. Larvae were allowed to feed on leaves until plant harvest just over two days later.

Collection of plant volatiles. One day before the larval deposition, plants were set in PET-bags (polyethylene terephthalate / Albal®, Cofresco Frischhalteprodukte, Minden Germany; 31 cm diameter and 55 cm high). A metal wire, set inside the bag around each plant, prevented contacts between the bag and the leaves. For each plant studied, VOCs were sequentially collected on two adsorbent phases, polydimethylsiloxane (PDMS) tubes (Carl Roth) and Porapak Q filters (ARS, Mecanopoly, Florida, USA), after 47h and 48h of herbivory, respectively. Thereby, we aimed at collecting as many different volatile compounds as possible.

A push-pull system with two vacuum pumps (Thomas, Gardner Denver Inc, Milwaukee, WI USA) was used to pump air into and out of the bag (Tholl et al. 2020). The inlet and outlet airflow were adjusted with air regulators at 1.5 L min^{-1} and 1L min^{-1}, respectively. Air was pulled in from the outside through a particle filter at the side of each plastic box and pushed into the PET bag through a charcoal filter and polytetrafluoroethylene tubing. Air pulled out the system went through adsorbent phases. The bag bottoms and tops were wrapped around inlet and outlet tubing and sealed by cable binders.

PDMS tubes had an external diameter of 2 mm and a length of 5 mm (Kallenbach et al. 2014). PDMS tubes were attached with metal strings and hung inside the bags wrapping the tomato shoots for passive trapping. VOC collections on PDMS were performed 47 hours after infestation during a 40 min period. After the collection period, PDMS tubes were stored in glass vials at -20°C until thermodesorption. Porapak filters consist of glass tubes containing 30 mg Porapak Q (ARS, Florida, USA). VOCs were collected on Porapak 48 hours after infestation during a 2h period. After VOC collection, the volatile compounds were eluted with 200 mL of dichloromethane containing 10 ng mL^{-1} of an internal standard (n-nonylacetate; Sigma-Aldrich, Seelze, Germany). Eluates were then stored in glass vials at − 20°C until analysis.

Volatile Analysis and Quantification. Compounds collected with PDMS were analysed with gas chromatography-mass spectrometry using a thermal desorption unit (TD20, Shimadzu) connected to a quadrupole GC-MS (QP2010Ultra, Shimadzu) with transfer line temperature: 240°C, ion source temperature: 220°C, and mass range: 33–400 m/z. Volatiles were desorbed from PDMS tubes placed in 89 mm glass TD tubes (Supelco), through a liquid nitrogen flow at a rate of 60 mL min^{-1} at 200°C for 8 min. Compounds were separated with helium at a flow rate of 40 cm sec^{-1} through a 30 m x 250 mm, 0.25 mm Rtx-5MS column. The oven temperature was held at 40°C for 5 minutes and then increased to 185°C at a rate of 5°C min^{-1}. At the end of the run, it increased to 280°C at 30°C min^{-1} for 0.83 minute to clean the column. Every ten samples, a blank tube was included. Data processing was performed using the program Shimadzu GC-MS solution software (v2.72) provided by the manufacturer and exported *via* its export function into an MS Excel macro file. The relative proportion of each molecule in the volatile blend was computed by dividing its peak area by the total peak area of all identified molecules.

Analyses of the compounds trapped with the Porapak filters were performed using gas chromatography (Agilent 6890 series, Agilent Santa Clara, CA, USA) coupled either to a quadrupole mass selective detector (Agilent 5973, interface temp. 270°C, quadrupole temp. 150°C, source temp. 230°C; electron energy 70 eV) or a flame ionization detector (FID, temp. 300°C). Compounds were separated in a 30 m x 250 mm x 0.25 mm DB5-MS column (Wilcom GmbH, Heppenheim, Germany) using He (mass detector) or H_2 (FID) carrier gases. One microliter of the sample was injected without splitting at an initial oven temperature of 40°C (2 min hold) followed by a ramp to 155°C (1°C min^{-1}), a ramp to 300°C (60°C min^{-1}), and a hold for 3 min. A semi-quantification of all VOCs was performed using their flame ionization detection peak area referenced to the area of the internal standard, using the same method as GC-MS. Emission rates (*X*) were calculated in $pg.h^{-1}$:

$$X = RF \times \frac{PA(x)}{PA(IS)} \times \frac{\vec{v}_{in}}{2 \times \vec{v}_{out}} \times 1000$$

with *RF* the response factor corresponding to the compound (*RF* = 1, when unknown), *PA(x)* the compound *X* peak area, *PA(IS)* the internal standard peak area, the factor "*2*" corresponds to the trapping time (hours), and the bag inlet and Porapak filter outlet flow rates, respectively.

The following compounds were identified by comparison of retention times and mass spectra with authentic standards: (*E*)-β-caryophyllene, α-copaene, α-humulene, (*Z*)-β-ocimene, α-phellandrene, α-pinene, α-terpinene. In the absence of standards, compounds were tentatively named by comparison with mass spectra libraries of Wiley and the National Institute of Standards and Technology, as in McCormick et al. (2016). The compounds were tentatively named only when they matched with more than 85 % identity. Putative identification of compounds with similar or unknown chemical structures were indexed with Arabic numerals.

Statistical Analyses. All the statistical analyses were performed with R, version 4.0.3 (R core team 2020).

In order to identify specific signatures in the composition of VOC profiles, we performed a discriminant analysis of principal components (Yang and Yang 2003). For this purpose, we first carried out a principal component analysis with the package 'vegan' (Oksanen et al. 2020; function 'rda'). Then, we used the principal component scores of each individual profile to conduct a linear discriminant analysis (LDA; function 'lda'). This approach resolves collinearity computational issues that generally impede classical LDA. Moreover, we believe that performing ordination based on principal components, rather than specific productions of VOC, is particularly relevant in metabolomic. Indeed, principal components can be considered as composite dimensions accounting for positive and negative correlations between single metabolites (i.e., shared pathway and trade-offs, respectively). In brief, LDA is a supervised classification procedure that aims to predict the probability of belonging to a given category based on predictor variables. The suitability of the LDA is assessed with the percentage of correct classification. In our case, correct classifications were optimal for LDA performed on scores deriving from the first 6 principal components (78% and 66% of correct classifications for Porapak Q and PDMS, respectively).

We estimated the diversity of plant volatile profiles based on Shannon entropy computed with the package 'vegan' (Oksanen et al. 2020; function 'diversity'). Significant differences in the diversity of VOC profiles across the four treatments (i.e., nitrogen by herbivory factorial matrix) were assessed with linear models. We performed multiple comparison via the package 'multcomp' (Hothorn et al. 2008; function 'glht'; *Tukey's HSD* tests).

Results

The Two Porous Polymers Collect Different Fractions of VOC Profiles. Porapak Q and PDMS collect an equal number of compounds, i.e., 33 VOCs (Table 1 and Table 2). However, two third of these VOCs were specific to each porous polymer. Only 12 compounds were common to Porapak Q and PDMS: 2 aldehydes (decanal and nonanal), 1 alkane (decane), 5 monoterpenes (p-cymene, β-phellandrene, α-pinene, α-terpinene and terpinolene) and 4 sesquiterpenes ((*E*)-β-caryophyllene, α-humulene, copaene and δ-elemene).

Table 1

Plant volatile emissions ($pg.h^{-1}$ ± sem) captured with Porapak Q

		Herbivore-free plants		Herbivore-infested plants	
Volatile organic compound a	**Chemical class**	**High nitrogen**	**Low nitrogen**	**High nitrogen**	**Low nitrogen**
propanoic acid ester #1	acid ester	10.91 ± 2.79	11.39 ± 1.65	8.3 ± 0.73	13.74 ± 2.36
propanoic acid ester #2	acid ester	35.14 ± 7.7	15.03 ± 3.05	23.03 ± 4.08	16.68 ± 3.57
pentanoic acid ester	acid ester	16.85 ± 3.62	9.97 ± 3.6	14.97 ± 2.62	17.7 ± 4.6
decanal	aldehyde	9.1 ± 1.19	7.29 ± 1.74	6.34 ± 1.14	9.25 ± 2
nonanal	aldehyde	19.2 ± 4.25	15.1 ± 3.49	12.01 ± 1.31	19.02 ± 4.03
decane	alkane	28.24 ± 6.85	29.01 ± 4.95	48.03 ± 27.75	34.61 ± 6.76
alkane #1	alkane	6.57 ± 1.5	6.26 ± 1.2	10.65 ± 5.08	7.1 ± 1.14
heneicosane	alkane	33.71 ± 10.93	35.55 ± 7.44	35.53 ± 13.08	42.8 ± 9.27
cyclohexanone	ketone	19.52 ± 5.07	13.85 ± 3.69	14.31 ± 2.45	21.03 ± 4.04
cyclopentanone	ketone	25.79 ± 5.77	27.86 ± 3.36	20.22 ± 1.84	33.12 ± 5.11
ascaridole	monoterpene	7.13 ± 1.65	4.43 ± 2.02	10.61 ± 3.02	5.84 ± 1.71
2-carene	monoterpene	1332.45 ± 196.94	705.97 ± 176.56	1674.09 ± 389.75	814.13 ± 148.38
m-cymene	monoterpene	202.09 ± 34.85	112.54 ± 28.25	253.34 ± 73.68	138.45 ± 34.07
p-cymene	monoterpene	36.47 ± 5.65	18.48 ± 4.29	40.58 ± 9.85	24.76 ± 5.73
1,3,8-p-menthatriene	monoterpene	41.2 ± 9.27	21.1 ± 7.13	49.64 ± 13.23	28.69 ± 8.23

a - Compounds in bold were identified by comparison of retention times and mass spectra with authentic standards. In the absence of standards, tentative identification was done by comparison with mass spectra from the libraries of Wiley and the National Institute of Standards and Technology (more than 85% of identity was required to assign a tentative name) .

A semi-quantification of all VOCs was performed using their flame ionization detection peak area

		Herbivore-free plants		Herbivore-infested plants	
(Z)-β-ocimene	monoterpene	16.77 ± 4.24	9.04 ± 3.51	14.33 ± 3.25	15.71 ± 4
β-phellandrene	monoterpene	4873.96 ± 837.64	2551.33 ± 646.5	6005.39 ± 1477.33	3003.76 ± 565.54
α-pinene	monoterpene	160.08 ± 25.13	82.95 ± 18.95	199.44 ± 46.86	99.7 ± 18.37
β-pinene	monoterpene	68.31 ± 18.08	37.12 ± 10.22	67.36 ± 14	51.06 ± 11.45
α-terpinene	monoterpene	426.14 ± 76.01	214.16 ± 55.05	520.37 ± 130.39	256.03 ± 51.1
terpinolene	monoterpene	22.1 ± 5	9.68 ± 2.81	24.11 ± 6.44	12.94 ± 2.9
aristolene	sesquiterpene	9.82 ± 2.02	4.54 ± 1.9	8.77 ± 1.27	8.49 ± 2.42
(E)-β-caryophyllene	sesquiterpene	176.27 ± 36.6	127.96 ± 33.67	169.38 ± 27.65	207.45 ± 43.07
α-copaene	sesquiterpene	19.67 ± 5.62	12.05 ± 4.77	19.39 ± 3.64	12.48 ± 3.61
β-elemene	sesquiterpene	3.47 ± 1.85	2.14 ± 1.26	3.92 ± 1.25	3.83 ± 1.21
δ-elemene	sesquiterpene	20.32 ± 4.04	10.93 ± 4.19	17.53 ± 3.32	19.92 ± 5.37
α-humulene	sesquiterpene	30.17 ± 5.79	20.75 ± 5.68	28.13 ± 4.69	34.55 ± 7.06
methyl salicylate	other	1.73 ± 1.22	18.01 ± 7.26	2.65 ± 1.6	7.33 ± 1.7
undecene	other	8.87 ± 2.21	9.25 ± 1.44	7.18 ± 0.76	11.12 ± 1.96
unknown #1	other	20.8 ± 5.48	21.11 ± 3.32	18.42 ± 3.03	26.69 ± 4.72
unknown #2	other	171.19 ± 55.73	405.76 ± 118.89	189.25 ± 45.64	414.74 ± 71.54
unknown #3	other	10.46 ± 2.61	8.72 ± 2.05	6.01 ± 1.06	14.98 ± 4.21

a - Compounds in bold were identified by comparison of retention times and mass spectra with authentic standards. In the absence of standards, tentative identification was done by comparison with mass spectra from the libraries of Wiley and the National Institute of Standards and Technology (more than 85% of identity was required to assign a tentative name) .

A semi-quantification of all VOCs was performed using their flame ionization detection peak area

		Herbivore-free plants		Herbivore-infested plants	
unknown #4	other	15.94 ± 3.68	7.47 ± 1.96	21.4 ± 5.52	9.67 ± 2.95

a - Compounds in bold were identified by comparison of retention times and mass spectra with authentic standards. In the absence of standards, tentative identification was done by comparison with mass spectra from the libraries of Wiley and the National Institute of Standards and Technology (more than 85% of identity was required to assign a tentative name) .

A semi-quantification of all VOCs was performed using their flame ionization detection peak area referenced to the area of the internal standard.

Table 2
Relative plant volatile emissions (% ± sem) captured with polydimethylsiloxane (PDMS)

		Herbivore-free plants		Herbivore-infested plants	
Volatile organic compound a	**Chemical class**	**High nitrogen**	**Low nitrogen**	**High nitrogen**	**Low nitrogen**
decanoic acid ester	acid ester	0.39 ± 0.1	0.14 ± 0.04	0.27 ± 0.07	0.21 ± 0.08
nonanoic acid ester	acid ester	1.13 ± 0.85	0.21 ± 0.06	2.93 ± 1.4	0.27 ± 0.07
octanoic acid ester	acid ester	0.97 ± 0.22	0.48 ± 0.12	0.35 ± 0.15	0.53 ± 0.17
decanal	aldehyde	0.4 ± 0.11	0.44 ± 0.06	0.46 ± 0.11	0.6 ± 0.1
nonanal	aldehyde	1.31 ± 0.35	1.71 ± 0.41	1.07 ± 0.3	1.8 ± 0.31
n-decane	alkane	3.19 ± 0.63	2.07 ± 0.41	2.62 ± 0.49	3.4 ± 0.62
tetramethylhexane	alkane	0.35 ± 0.14	0.12 ± 0.05	0.18 ± 0.08	1.84 ± 1.69
p-cymene	monoterpene	0.27 ± 0.04	0.3 ± 0.03	0.23 ± 0.03	0.38 ± 0.14
ectocarpene	monoterpene	1.23 ± 0.58	0.07 ± 0.03	0.13 ± 0.04	0.72 ± 0.66
alloocimene	monoterpene	0.44 ± 0.04	0.33 ± 0.05	0.32 ± 0.04	0.32 ± 0.06
α-ocimene	monoterpene	0.22 ± 0.07	0.19 ± 0.09	0.17 ± 0.04	0.17 ± 0.03
α-phellandrene	monoterpene	2.76 ± 0.85	2.84 ± 0.65	2.89 ± 0.87	4.06 ± 0.53
β-phellandrene	monoterpene	59.3 ± 2.51	62.22 ± 1.36	61.34 ± 1.97	58.53 ± 2.38
α-pinene	monoterpene	0.43 ± 0.06	0.64 ± 0.09	0.45 ± 0.12	0.6 ± 0.09

a- Compounds in bold were identified by comparison of retention times and mass spectra with authentic standards. In the absence of standards, tentative identification was done by comparison with mass spectra from the libraries of Wiley and the National Institute of Standards and Technology (more than 85% of identity was required to assign a tentative name) .

The relative proportion of each compound in individual volatile blends was computed by dividing its unds.

		Herbivore-free plants		Herbivore-infested plants	
α-terpinene	monoterpene	0.66 ± 0.1	0.72 ± 0.06	0.59 ± 0.07	0.58 ± 0.08
terpinolene	monoterpene	15.33 ± 0.95	15.05 ± 1.11	14.56 ± 1.14	12.56 ± 1.3
alloaromadendrene	sesquiterpene	0.14 ± 0.05	0.16 ± 0.04	0.13 ± 0.04	0.13 ± 0.05
(E)-β-caryophyllene	sesquiterpene	5.91 ± 1.34	6.05 ± 0.93	5.43 ± 0.9	5.46 ± 0.79
α-copaene	sesquiterpene	0.25 ± 0.05	0.23 ± 0.04	0.2 ± 0.05	0.16 ± 0.03
δ-elemene	sesquiterpene	0.34 ± 0.08	0.16 ± 0.04	0.31 ± 0.08	0.12 ± 0.03
α-gurjunene	sesquiterpene	0.22 ± 0.06	0.22 ± 0.09	0.21 ± 0.04	0.59 ± 0.43
α-humulene	sesquiterpene	0.86 ± 0.09	1.13 ± 0.12	1.02 ± 0.19	1.06 ± 0.22
bergamiol	terpenoid	0.03 ± 0.01	0.02 ± 0	0.03 ± 0.01	0.02 ± 0.01
4-caranol	terpenoid	0.05 ± 0.02	0.07 ± 0.02	0.06 ± 0.01	0.05 ± 0.02
trans-3-caren-2-ol	terpenoid	0.77 ± 0.14	1.39 ± 0.27	0.83 ± 0.19	2.32 ± 0.7
3-decyn-2-ol	terpenoid	0.3 ± 0.04	0.16 ± 0.03	0.19 ± 0.04	0.19 ± 0.03
geraniol	terpenoid	0.62 ± 0.07	0.74 ± 0.17	0.79 ± 0.12	0.5 ± 0.1
menthol	terpenoid	0.21 ± 0.11	0.04 ± 0.01	0.56 ± 0.33	0.05 ± 0.01
2,5-dimethyl-3,4-hexanediol	other	0.23 ± 0.05	0.35 ± 0.09	0.2 ± 0.06	0.19 ± 0.05
6,10-dimethyl-5,9-undecadien-2-one	other	0.32 ± 0.14	0.32 ± 0.12	0.24 ± 0.05	0.2 ± 0.04

a- Compounds in bold were identified by comparison of retention times and mass spectra with authentic standards. In the absence of standards, tentative identification was done by comparison with mass spectra from the libraries of Wiley and the National Institute of Standards and Technology (more than 85% of identity was required to assign a tentative name) .

The relative proportion of each compound in individual volatile blends was computed by dividing its unds.

		Herbivore-free plants		Herbivore-infested plants	
4-methyl-1-decene	other	0.09 ± 0.02	0.09 ± 0.06	0.09 ± 0.03	0.07 ± 0.01
4-methylmannitol	other	0.63 ± 0.28	0.62 ± 0.28	0.68 ± 0.37	1.94 ± 0.81
unknown #1	other	0.36 ± 0.15	0.54 ± 0.2	0.47 ± 0.16	0.41 ± 0.15

a- Compounds in bold were identified by comparison of retention times and mass spectra with authentic standards. In the absence of standards, tentative identification was done by comparison with mass spectra from the libraries of Wiley and the National Institute of Standards and Technology (more than 85% of identity was required to assign a tentative name) .

The relative proportion of each compound in individual volatile blends was computed by dividing its peak area by the total peak area of all identified compounds.

Changes in the Composition of Stress-Related VOC Profiles. Linear discriminant analyses account for most of inter-treatment variability, both with Porapak Q (95%; Fig. 1a) and PDMS (98%; Fig. 1b). In the legend of Fig. 1, only the 3 VOCs harbouring the highest correlations with the 6 principal components are shown (c.f., Tables S2 and S3 for exhaustive correlations between VOC emissions and principal components).

For VOCs captured on Porapak Q, we report a significant discrimination between the four treatments along LD1 and LD2 (Fig. 1a). This discrimination is mainly based on PC2, PC3 and PC4. Low and high nitrogen regimes result in opposite patterns of VOC emission in herbivore-free plants. Non-infested plants exposed to limited nitrogen display high emissions of methyl salicylate and unknown #1 along with reduced production of propanoic ester #2 (i.e., negative correlations with PC4). On the contrary, uninfested plants with non-limiting nitrogen reduced the release of methyl salicylate and unknown #1 while they enhance the emission of propanoic ester #2 (i.e., positive correlations with PC4). The influence of herbivory on VOC emissions depends on nitrogen regimes. Upon high nitrogen supply, herbivory triggers higher emissions of three terpenes, 2-carene, □-pinene and β-phellandrene (i.e., positive correlations with PC2). When nitrogen supply is limited, herbivory is associated with a general relaxation of VOCs such as alkane #1, decane and ascaridole (i.e., positive correlations with PC3).

With PDMS, volatile profiles discriminate mainly based on PC6 and PC3. Only herbivore-infested plants exposed to nitrogen limitation segregate along LD2 (Fig. 1b). These plants are characterized by reduced emissions of terpinolene and 4-caranol and high releases of 4-methylmannitol (i.e., negative correlations with PC3). The three other treatments partially overlap along LD1 although herbivore-free plants exposed to high and limited nitrogen supply clearly differ in VOC emissions. First, herbivore-free plants with non-limiting nitrogen mainly produce terpinolene and 4-caranol parallel to a decrease in the emission of 4-methylmannitol (i.e., positive correlations with PC3). Second, herbivore-free plants under restricted

nitrogen regime produce preferentially geraniol and reduce the emission of alloocimene and octanoic acid ester (i.e., positive correlations with PC6).

Changes in the Diversity of Stress-Related VOC Profiles. Porapak Q enables to point variations in the diversity of VOC profiles between treatments (Fig. 2a). Although herbivory does not influence the Shannon entropy of plant volatile emissions, our results show that nitrogen limitation results in significantly higher diversity of VOC profiles. The Shannon entropy increases by 18% between high and low nitrogen nutrition (1.47 ± 0.04 and 1.73 ± 0.04, respectively). However, PDMS does not allow to track such variations (Fig. 2b).

Discussion

This study highlights two pitfalls in exploring the role of plant volatiles. Because porous polymers differ in their affinity with VOCs, they entail important blind spots that hamper the functional interpretation of plant volatile emissions. Additionally, we stress the importance to consider the interplay between biotic and abiotic stresses to better understand variations in the composition and the diversity of VOC profiles. More particularly, we show that the combination of nitrogen limitation and herbivory governs the differential production of generalist and specific anti-herbivore VOCs. Hence, our results not only endorse the growth:differentiation hypothesis, they also help to complete it by dissecting the relationships between soil fertility and the nature of defensive compounds produced by plants. Finally, we argue that accounting for Shannon entropy of VOC profiles may bring insights to estimate the information amount plants release in surroundings and, thereby, how they adjust the breadth of VOC-mediated ecological interactions in response to stress.

In our case, Porapak Q is more efficient than PDMS to collect VOCs playing key roles in plant defences against herbivores (e.g., see below for methyl salicylate and 2-carene). Moreover, our results indicate that nitrogen limitation is an important driver in the production of anti-herbivore VOCs. To our knowledge, this is the first study reporting the effect of nitrogen limitation on the up-regulation of methyl salicylate, a common compound involved in anti-herbivore defences (Heil and Ton 2008; Rodriguez-Saona et al. 2011). In the same line, Copolovici et al. (2014) have shown that abiotic stress can elicit plant volatile defences against herbivores. This pattern endorses the growth:differentiation hypothesis (Herms and Mattson, 1992). So far, while resource availability has been shown to explain general patterns in the distribution of major plant defensive ecotypes (Fine et al. 2006; Woods et al. 2012), little has been done to finely dissect the nature of anti-herbivore compounds produced by plants growing in poor and fertile soils. Here, we corroborate our initial hypothesis predicting that herbivore-free plants exposed to nitrogen limitation enhance the production of generalist VOCs, such as methyl salicylate, that prevent infestation from a large spectrum of herbivores. Indeed, methyl salicylate plays an important role in the acquisition of systemic resistance against insects and pathogens (Heil and Ton 2008) but also in the recruitment of a wide range of generalist natural enemies of herbivores (Rodriguez-Saona et al. 2011). Once herbivory becomes effective, plants switch the production of VOCs towards more specific anti-herbivore

oluta results in the overexpression of three

terpenes (2-carene, □-pinene and β-phellandrene) which have been shown to attract *Macrolophus pygmaeus*, a predator of the tomato leafminer (de Backer et al. 2017). However, the up-regulation of these specific terpenes involved in the recruitment of natural enemies is restricted to plants growing with high nitrogen resources. This pattern reflects how resource availability likely influence the cost-benefit balance of synthetizing anti-herbivore specialized metabolites.

In addition, the diversity of VOC profiles brings complementary knowledge to better understand how plant growing with low nitrogen resources likely maintain tritrophic-mediated defences. In line with the composition of VOC profiles, Porapak Q is more sensitive than PDMS to record variations in the diversity of plant volatile emissions. Based on the former porous polymer, we show that Shannon entropy of plant volatile emissions increases with nitrogen limitation independently from herbivory. Because this pattern suggests an increase in the information amount conveyed by VOC, we can reasonably think that it results in larger VOC-mediated interactions between plants and associated arthropod communities. Although such mechanism could benefit plants through the recruitment of a wide range of natural enemies, demonstrating its adaptive nature remains a challenging issue. For this purpose, Kaplan (2012) has highlighted the necessity to integrate the complexity of food webs. Of course, plants are not omniscient and we cannot exclude that larger VOC-mediated ecological interactions may also benefit plant antagonists at the expense of plant fitness. Further studies exploring how plants adjust the quantity of information they release in surroundings should account for the composition of plant-associated arthropod communities.

The present study shows that, parallel to authentic variations in plant volatile emissions, differences in stress-related VOC profiles arise from chemical collection efficiencies. In our case, PDMS was found inefficient to collect two VOCs playing a key role in tomato's defences against *T. absoluta*, i.e., methyl salicylate and 2-carene. In addition, although □-pinene and β-phellandrene were collected on both PDMS and Porapak Q, only the latter enables to point their induction in response to herbivory. Therefore, studies based on particular porous polymers may fail in interpreting the ecological function of VOCs. In this context, we advocate for a shift in practice in plant ecology, from single- to multi-bed adsorbent tubes containing different porous polymers. However, such sampling methods need to be finely optimized as it has been shown in atmospheric sciences (Ho et al. 2018). Moreover, this study stresses the necessity to integrate resource availability when addressing the functions of VOCs in anti-herbivore plant defences. Here, we show that nitrogen resources are a major source of variations in volatile emissions that helps to better understand plant strategies in the production of generalist and specific anti-herbivore VOCs, but also in the diversity of volatile profiles. Altogether, this study points the need to improve sampling procedures and, at the same times, account for realistic combination of factors driving VOC emissions in order to explore the ecological function of VOCs.

Declarations

Funding - The Ph.D. grant of Victoire Coqueret was co-funded by INRA (Département Environnement et thanks to the Regional Council of Lorraine for its

financial support “projet émergent” (grant 15P03127). Our thanks go to the EIR-Agreenium (International Research School) and the Deutscher Akademischer Austauschdienst, DAAD, for their financial support to VC during her period at the MPI-CE in Jena.

Conflicts of interest– The authors have no relevant financial or non-financial interests to disclose.

Availability of Data and Material - Data and R script used to analyse data are archived at the repository ‘Data Portal INRAE’. They are freely available: https://doi.org/10.15454/WWSSOO

Authors’ contributions - VC, CR, RL, JLB, SA and AK conceived the ideas. VC, CR, RL, JLB and SA designed the experiment. VC, CR and RL designed the methodology. VC collected the data. AK and VC analysed the data. AK led the writing of the manuscript. All authors contributed critically to the drafts and gave a final approval for publication.

Acknowledgements - We thank Aude Fauvet (INRAE), Claude Gallois (Université de Lorraine), Michael Reichelt, Sandra Lackner, Franziska Eberl and Carlos Sanchez Acros (MPI for Chemical Ecology) for their advice on various practical aspects of this study. We are grateful to Jonhatan Gershenzon and Sybille B. Unsicker for their help in conducting experiments. We thank Sergio Rasmann and XX anonymous reviewers for their valuable suggestions for the improvement of the manuscript. Larvae were provided by Philippe Bearez and Nicolas Desneux (INRAE ISA Sophia Antipolis).

References

Adamowicz S, Le Bot J (2008) Altering young tomato plant growth by nitrate and CO2 preserves the proportionate relation linking long-term organic-nitrogen accumulation to intercepted radiation. New Phytologist 180:663–672. https://doi.org/10.1111/j.1469-8137.2008.02605.x

Bicchi C, Maffei M (2012) The plant volatilome: methods of analysis. In Normanly J (ed) High-throughput phenotyping in plants: methods and protocols, Humana Press, New York, Heidelberg, Dordrecht, London, pp 289–310

Chen Y (2013) Analyzing blends of herbivore-induced volatile organic compounds with factor analysis: “Revisiting cotton plant, *Gossypium hirsutum* L., defense in response to nitrogen fertilization”. Journal of Economic Entomology 106:1053–1057. https://doi.org/10.1603/EC12178

Conchou L, Lucas P, Meslin C, Proffit M, Staudt M, Renou M (2019) Insect odorscapes: from plant volatiles to natural olfactory scenes. Frontiers in Physiology 10:972. https://doi.org/10.3389/fphys.2019.00972

Copolovici L, Kännaste A, Remmel T, Niinemets Ü (2014) Volatile organic compound emissions from *Alnus glutinosa* under interacting drought and herbivory stresses. Environmental and Experimental Botany 100:55–63. https://doi.org/10.1016/j.envexpbot.2013.12.011

Cusumano A, Harvey JA, Dicke M, Poelman EH (2019) Hyperparasitoids exploit herbivore-induced plant volatiles during host location to assess host quality and non-host identity. Oecologia 189:699–709. https://doi.org/10.1007/s00442-019-04352-w

De Backer L, Bawin T, Schott M, Gillard L, Markó IE, Francis F, Verheggen F (2017) Betraying its presence: identification of the chemical signal released by *Tuta absoluta*-infested tomato plants that guide generalist predators toward their prey. Arthropod-Plant Interactions 11:111–120. https://doi.org/10.1007/s11829-016-9471-7

De Lange ES, Rodriguez-Saona C (2019) Does enhanced nutrient availability increase volatile emissions in cranberry? Plant Signaling and Behavior 14:1–5. https://doi.org/10.1080/15592324.2019.1616517

de Moraes C, Lewis W, Paré P, Alborn H, Tumlinson J (1998) Herbivore-infested plants selectively attract parasitoids. Nature 393:570–573. https://doi.org/10.1038/31219

Dicke M, Baldwin IT (2010) The evolutionary context for herbivore-induced plant volatiles: beyond the "cry for help". Trends in Plant Science 15:167–175. https://doi.org/10.1016/j.tplants.2009.12.002

Fine PVA, Miller ZJ, Mesones I, Irazuzta S, Appel HM, Stevens MHH, Sääksjärvi I, Schultz JC, Coley PD (2006) The growth-defense trade-off and habitat specialization by plants in Amazonian forests. Ecology 87:150–162. https://doi.org/10.1890/0012-9658(2006)87[150:tgtahs]2.0.co;2

Heil M, Ton J (2008) Long-distance signalling in plant defence. Trends in Plant Science 13:264–272. https://doi.org/10.1016/j.tplants.2008.03.005

Herms DA, Mattson WJ (1992) The dilemma of plants: to grow or defend. Quarterly Review of Biology 67:283–335. https://doi.org/10.1086/417659

Ho SSH, Wang L, Chow JC, Watson JG, Xue Y, Huang Y, Qu L, Li B, Dai W, Li L, Cao J (2018) Optimization and evaluation of multi-bed adsorbent tube method in collection of volatile organic compounds. Atmospheric Research 202:187–195. https://doi.org/10.1016/j.atmosres.2017.11.026

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biometric Journal 50:346–363

Hunter MD (2016) The phytochemical landscape: linking trophic interactions and nutrient dynamics. Princeton University Press, Princeton, Oxford

Islam MN, Hasanuzzaman ATM, Zhang ZF, Zhang Y, Liu TX (2017) High level of nitrogen makes tomato plants releasing less volatiles and attracting more *Bemisia tabaci* (Hemiptera:Aleyrodidae). Frontiers in Plant Science 8:466. https://doi.org/10.3389/fpls.2017.00466

Kallenbach M, Oh Y, Eilers E, Veit D, Baldwin I, Schuman M (2014) A robust, simple, high-throughput [illegible] experiments. The Plant Journal 78:1060–1072.

https://doi.org/10.1111/tpj.12523

Kaplan I (2012) Trophic complexity and the adaptive value of damage-induced plant volatiles. Plos Biology 10:e1001437

Larbat R, Le Bot J, Bourgaud F, Robin C, Adamowicz S (2012) Organ-specific responses of tomato growth and phenolic metabolism to nitrate limitation: organ-specific responses to nitrate limitation. Plant Biology 14:760–769. https://doi.org/10.1111/j.1438-8677.2012.00564.x

Larbat R, Paris C, Le Bot J, Adamowicz S (2014) Phenolic characterization and variability in leaves, stems and roots of Micro-Tom and patio tomatoes, in response to nitrogen limitation. Plant Science 224:62–73. https://doi.org/10.1016/j.plantsci.2014.04.010

Le Bot J, Bénard C, Robin C, Bourgaud F, Adamowicz S (2009) The 'trade-off' between synthesis of primary and secondary compounds in young tomato leaves is altered by nitrate nutrition: experimental evidence and model consistency. Journal of Experimental Botany 60:4301–4314. https://doi.org/10.1093/jxb/erp271

Li T (2016) Neighbour recognition through volatile-mediated interactions. In Blande J & Glinwood R (eds) Deciphering chemical language of plant communication, Springer International Publishing, pp 153–174

McCormick A, Reinecke A, Gershenzon J, Unsicker S (2016) Feeding experience affects the behavioral response of polyphagous gypsy moth caterpillars to herbivore-induced poplar volatiles. Journal of Chemical Ecology 42:382–393. https://doi.org/10.1007/s10886-016-0698-7

McCormick A, Unsicker SB, Gershenzon J (2012) The specificity of herbivore-induced plant volatiles in attracting herbivore enemies. Trends in Plant Science 17:303–310. https://doi.org/10.1016/j.tplants.2012.03.012

Noushini S, Park SJ, Jamie I, Jamie J, Taylor P (2020) Sampling technique biases in the analysis of fruit fly volatiles: a case study of Queensland fruit fly. Scientific Reports 10:1–14. https://doi.org/10.1038/s41598-020-76622-0

Oksanen J, Blanchet F, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2020) *vegan: Community Ecology Package* (p. R package version 2.5-7). R package version 2.5-7. Retrieved from https://cran.r-project.org/package=vegan%0A

Ormeño E, Baldy V, Ballini C, Fernandez C (2008) Production and diversity of volatile terpenes from plants on calcareous and siliceous soils: effect of soil nutrients. Journal of Chemical Ecology 34:1219–1229. https://doi.org/10.1007/s10886-008-9515-2

Pareja M, Pinto-Zevallos D (2016) Impacts of induction of plant volatiles by individual and multiple stresses across trophic levels. In Blande J & Glinwood R (eds) Deciphering chemical language of plant

R core team (2020) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing,. Retrieved from https://www.r-project.org/

Rodriguez-Saona C, Kaplan I, Braasch J, Chinnasamy D, Williams L (2011) Field responses of predaceous arthropods to methyl salicylate: a meta-analysis and case study in cranberries. Biological Control 59:294–303

Rowen E, Kaplan I (2016) Eco-evolutionary factors drive induced plant volatiles: a meta-analysis. New Phytologist 210:284–294. https://doi.org/10.1111/nph.13804

Royer M, Larbat R, Le Bot J, Adamowicz S, Nicot P, Robin C (2016) Tomato response traits to pathogenic *Pseudomonas* species: does nitrogen limitation matter? Plant Science 244:57–67. https://doi.org/10.1016/j.plantsci.2015.12.008

Royer M, Larbat R, Le Bot J, Adamowicz S, Robin C (2013) Is the C:N Ratio a reliable indicator of C Allocation to primary and defence-related metabolisms in tomato? Phytochemistry 88:25–33. https://doi.org/10.1016/j.phytochem.2012.12.003.

Shannon C (1948) A mathematical theory of information. The Bell System Technical Journal 27:379–423. https://doi.org/10.1002/j.1538-7305.1948.tb01338.x

Shu JP, Sun DB, Liu SS (2010) Comparative performance of different methods used to collect tomato plant volatiles. Allelopathy Journal 26:71–82

Stamp N (2003) Out of the quagmire of plant defense hypotheses. The Quarterly Review of Biology 78:23–55. https://doi.org/10.1017/CBO9781107415324.004

Tholl D, Weinhold A, Röse U (2020) Practical approaches to plant volatile collection and analysis. In Pichersky E & Dudareva N (eds) Biology of Plant Volatiles, CRC press, Boca raton, FL, USA, pp 3–26

Woods EC, Hastings AP, Turley NE, Heard SB, Agrawal A (2012) Adaptive geographical clines in the growth and defense of a native plant. *Ecological Monographs* 82:149–168.

Yang J, Yang JY (2003) Why can LDA be performed in PCA transformed space? Pattern Recognition 36:563–566. https://doi.org/10.1016/S0031-3203(02)00048-1

Figures

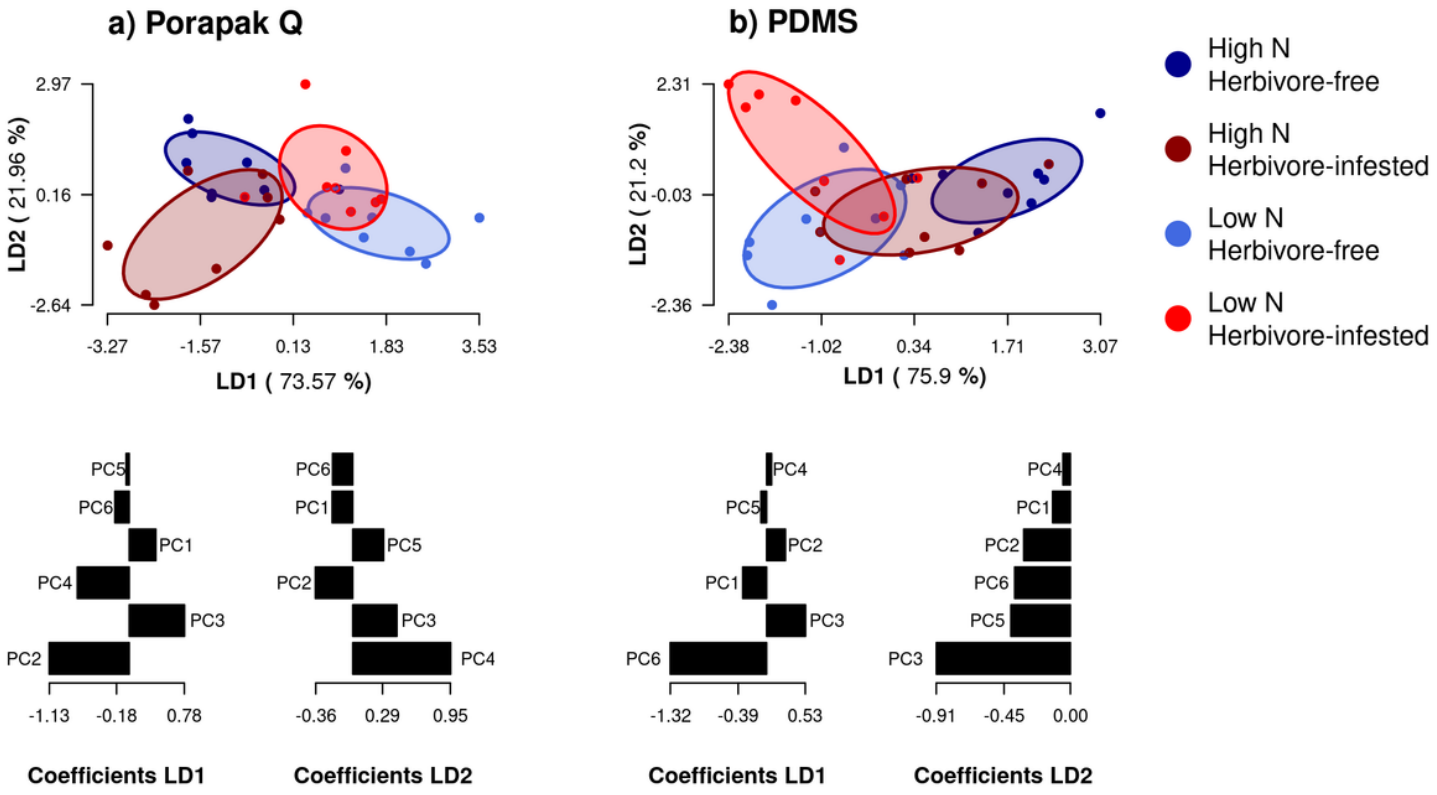

Figure 1

COMPOSITION OF VOLATILE PROFILES RELEASED BY PLANTS EXPOSED TO DIFFERENT NITROGEN AND HERBIVORY REGIMES a) Discriminant analysis of principal components of volatile profiles captured on Porapak Q (correct classification: 78%) and discriminant coefficients of principal components. Each principal component is correlated with specific emissions of volatile (see Table S2 for exhaustive correlations) : PC1 – β-pinene (-0.93), (Z)-β-ocimene (-0.92), pentanoic acid ester (-0.89) ; PC2 - 2-carene (0.65), α-pinene (0.64), β-phellandrene (0.61) ; PC3 – alkane #1 (-0.82), decane (-0.79), ascaridole (-0.44) ; PC4 - methyl salicylate (-0.63), propanoic acid ester #2 (0.48), unknown #1 (-0.45) ; PC5 – propanoic acid ester #1 (0.32), propanoic acid ester #2 (0.31), decanal (-0.31) ; PC6 – propanoic acid ester #2 (-0.65), methyl salicylate (-0.47), unknown #1 (-0.22) b) Discriminant analysis of principal components of volatile profiles captured on polydimethylsiloxane (correct classification: 66%) and discriminant coefficients of principal components. Each principal component is correlated with specific emissions of volatile (see Table S3 for exhaustive correlations) : PC1 – (E)-β-caryophyllene (-0.87), α-copaene (-0.77), β-phellandrene (0.76) ; PC2 – nonanoic acid ester (0.68), nonanal (0.68), decanal (0.67) ; PC3 - terpinolene (0.75), 4-methylmannitol (-0.54), 4-caranol (0.48) ; PC4 - tetramethylhexane (0.81), p-cymene (0.63), trans-3-caren-2-ol (-0.50) ; PC5 - menthol (-0.55), nonanoic acid ester (-0.45), α-pinene (-0.43) ; PC6 - alloocimene (-0.49), geraniol (0.44), octanoic acid ester (-0.43)

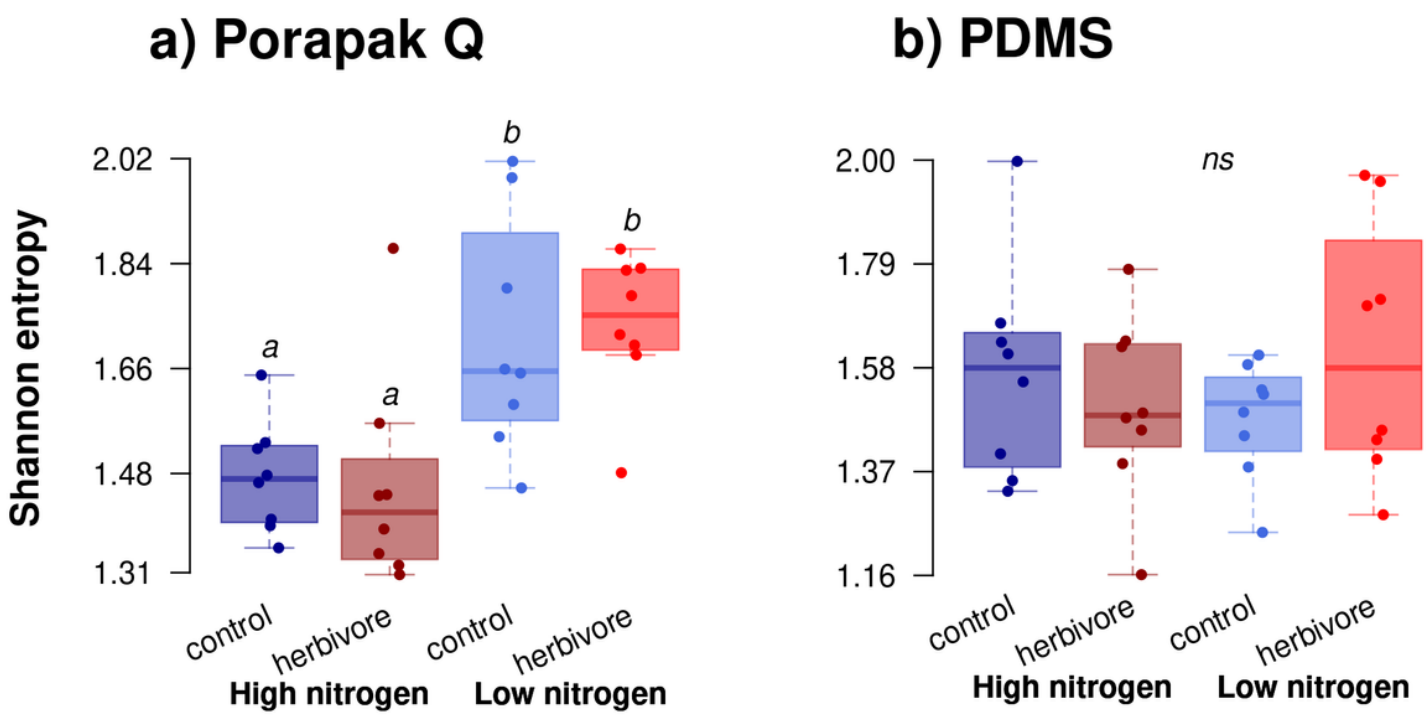

Figure 2

DIVERSITY OF VOLATILE PROFILES RELEASED BY PLANTS EXPOSED TO DIFFERENT NITROGEN AND HERBIVORY REGIMES a) Shannon entropies of volatile profiles captured with Porapak Q. Different letters indicate significant differences between treatments (<0.05). b) Shannon entropies of volatile profiles captured with polydimethylsiloxane. ns: non-significant (<0.05).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial.docx