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Grassland odorscape: a new tool to explore the ecosystemic services

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Abstract. Plants, insects and microorganisms are the protagonists of the ecosystem services provided by permanent grasslands. All of them emit volatile organic compounds (VOCs) to communicate. The result is a chemical landscape, also called odorscape. To explore this pool of information and enhance our understanding on the process behind the ecosystem services, we trapped volatile compounds on two mountain permanent pastures chosen for their contrasting grazing and fertilisation management. Measurements were made in July over two periods of three consecutive days using solid-phase microextraction (SPME) fibers fixed on sticks. The VOCs adsorbed on the fibers were then analyzed by gas-chromatography-mass spectrometry (GC-MS). We also recorded floristic composition, trapped the pollinator insects and took pictures of the canopy to quantify grassland colors using image analysis. We obtained rich and complex VOC profiles. They contained a total of 67 peaks corresponding to VOCs belonging to various chemical families. Nevertheless, the differences between the two grasslands chemical signatures were less marked than expected, considering their differences in floristic species richness (83 *vs.* 65 species). Certain compounds however, were specific to one of the two pastures and others were positively (e.g. benzaldehyde) or negatively (e.g. α-pinene) correlated to the presence of pollinators. Measuring the odorscape constitutes a promising tool to better understand a range of services provided by grasslands.

Keywords. Grassland – Chemical landscape – Volatile organic compounds – Volatilome – Biodiversity.

Le paysage odorant des prairies : un nouvel outil pour explorer les services écosystémiques

Résumé. *Les plantes, les insectes et les microorganismes des prairies émettent des composés organiques volatils (COV) pour communiquer. Il en résulte un paysage chimique aussi appelé paysage odorant que nous avons voulu explorer pour mieux comprendre les processus à l'origine des services écosystémiques rendus par l'écosystème prairial. Nous avons ainsi capté les COV dans deux prairies permanentes pâturées, contrastées du point de vue de l'intensité de leur conduite et de leur niveau de fertilisation. Les mesures ont été faites en juillet à 2 périodes de 3 jours consécutifs en utilisant des fibres SPME (Micro-Extraction en Phase Solide) fixées sur des piquets. Les COV ont été analysés par chromatographie en phase gazeuse couplée à la spectrophotométrie de masse (GC-MS). Des relevés botaniques et des piégeages des insectes pollinisateurs ont été effectués. L'analyse d'image à partir de photos a été utilisée pour quantifier les couleurs présentes dans les parcelles. Nous avons obtenu des profils riches en COV, contenant un total de 67 pics correspondant à des familles chimiques variées. La différence entre les profils des deux prairies a été moins importante que prévue compte tenu de leur composition botanique contrastée. Nous avons néanmoins mis en évidence des COV spécifiques aux prairies et montré que certains COV étaient corrélés positivement (ex. benzaldéhyde) ou négativement (ex. α-pinène) avec les insectes pollinisateurs. La mesure du paysage chimique des prairies s'avère un outil prometteur pour rendre compte des services écosystémiques.*

Mots-clés. *Prairies – Paysage chimique – Composés organiques volatils – Volatilome – Biodiversité.*

I – Introduction

Permanent grasslands shelter a diversity of microbes, plants and animals, all of which emit volatile organic compounds (VOCs) into the atmosphere. The role of plant VOCs is to attract pollinating insects, but also to communicate with other plants when they undergo heat or drought stresses, or to attract the predators of herbivore insects when they are attacked. COVs from insects and microorganisms are either by-product of their metabolism or signal molecules. The resulting chemical landscape, also called odorscape, likely contains a high amount of information on the biotic status of the grasslands and on the ecosystemic services they provide. While most of the studies conducted up to now focused on the interaction between a given plant and a given insect isolated in closed chambers, Leppik and Frerot (2014) have characterized the chemical landscape of maize crop *in situ* using solid phase micro extraction fibers (SPME) located in the field. In a recent study (Cornu *et al*., 2015), we applied this technique to permanent grasslands in order to (1) check its feasibility in such a heterogeneous ecosystem and (2) to explore pasture odorscape as an innovative tool to assess and enhance the possible ecological roles and environmental services it offers. Accordingly, VOC measurements were implemented in two mountain semi natural grasslands displaying contrasting diversities of flowering plants.

II – Materials and methods

The experiment was conducted at the INRA farm of Marcenat located in an upland area of central France on volcanic soils (Cantal, France – 45.3046N, 2.8378E; altitude 1070-1190m). The region is characterized by a low annual mean temperature of 7.4°C (2002-2013), and a high mean annual precipitation of 1167mm.yr^{-1}. Two plots, close to each other (1.3 km) were chosen for their contrasting floristic diversity and management: "Montagne", a large 8.98 ha pasture that had received no fertilizer in the last 20 years and had always been grazed at a low stocking rate, resulting in a high botanical diversity, and "La Prade", a 1 ha pasture, flat and wet in places, well fertilized (30 NU.ha^{-1}.yr^{-1} and 40 m3.ha^{-1}.yr^{-1} of slurry) and grazed at a higher stocking rate, inducing moderate botanical diversity.

Measurements were made in July in two periods of 3 days: P1 (1, 3 and 4 July) while cows were grazing and P2 (16, 17 and 18 July) when cows had been turned out of the pastures. On each pasture, three 10 × 12 m areas, representative of the different plant communities were excluded from grazing. These exclosures were removed just after the third day of measurement in each period. Sward structure measurements, VOC trapping and insect counts were performed on the same three consecutive days in each of the six exclosures, in both periods. Botanical analyses were performed at the end of P1. All the species and their abundances were determined in three 1 m^2 quadrats distributed along the diagonal of each exclosure. Species present in the exclosure but not in the quadrat were also identified. Flowering intensity was estimated during the 2 periods based on the relative abundance of dicotyledonous flowering stems using a stick at 50 locations over each exclosure (first contact). In addition, in P1, eight pictures of the canopy were taken vertically in each exclosure and the average number and percentage of blue-red pixels, known to be attractive for pollinators, were quantified using the imageJ software (Schneider *et al*., 2012) to quantify. Insects were trapped using pan traps (Westphal *et al.*, 2008) except for bumblebees which were netted. Flies were discarded, wild bees were sorted, identified and counted, and the remaining hymenoptera were counted as "others".

The SPME fibers were cleaned and conditioned by heating in a gas chromatograph injector before using. They were fixed on a metal stake in the middle of the exclosure, at the maximum canopy height, at in the same place in P2 as in P1. Each day of measurement, the 6 fibers were installed between 08:30 and 10:00 and removed in the same order between 16:30 and 18:00. VOCs were then analyzed by gas chromatography coupled to mass spectrometry (GC/ MS). Peak area information was extracted from the raw GC-MS data and expressed as a percentage of the total chromatogram area.

Data were analysed using Statistica Software (Statsoft, Maisons-Alfort, France). The effects of the day of measurement and of the exclosure were tested separately by means of univariate ANOVAs. A two-way ANOVA was performed to test the effect of the grassland (G), the period (Per) and their interaction (G × Per). The variables for which the grassland and the period had the most significant effects were subjected to a principal component analysis (PCA). Correlations between VOCs and insects were tested using parametric statistical analysis and the highlighted relationships were checked using non-parametric Spearman rank correlations.

III – Results and discussion

During P1, temperature was close to the average for July (15-17°C with a maximum at 25°C) but quite rainy while in P2, weather was hot (18-22°C and a maximum at 29°C) with no precipitation. Montagne had a higher average number of species per square meter than La Prade (30 *vs*. 19) and forbs were twice as abundant (average 51% *vs*. 24% respectively). Flowering intensity was much greater on Montagne over the two periods compared with La Prade (P1: 26 *vs*. 5%; P2: 15 *vs*. 4% respectively), as was the abundance of the 'blue-red' color of the canopy (0.9 *vs*. 0.4% of pixels). Regarding the chemical signatures, a total of 67 VOC were detected over the six exclosures and the two periods: 10 hydrocarbons, 8 alcohols, 10 aldehydes, 8 ketones, 7 acids, esters, lactones or anhydrides, and 24 unidentified compounds (UI). Among these were 13 benzene compounds and 11 terpenoids. These compounds were recovered on both plots, except UI-21 found only in Montagne. Compared with a maize field, our grasslands emitted a greater number of compounds, belonging to a wider range of chemical families. Four major VOCs together represented 40% of the total peak area: UI-7, propan-2-one, 1-ethoxy-2-propanol and butyrolactone. Propan-2-one may have an abiotic origin as well as a microbial, plant or animal origin (Sharkey, 1996). It is also known as a signal molecule toward insects (Davis *et al.*, 2013). The compounds originating from ruminants that could have been expected, such as volatile fatty acids from C2 to C7, sulfur compounds, phenolic compounds and indole derivatives were not found. Only benzyl alcohol and benzoic acid were recovered, and these compounds could also have originated from plants. The range of the number of compounds trapped in the exclosures was similar on Montagne (60 to 67) and on La Prade (63 to 66). However, the two-way ANOVA showed that six compounds were significantly more abundant on Montagne than on La Prade: terpinene, UI-3 and UI-14 ($p<0.01$) propan-2-one, UI-21 and 2-phenylethyl acetate ($p<0.05$). Only benzoic acid was significantly more abundant on La Prade than on Montagne ($p<0.05$). Therefore, although the two plots have contrasted botanical compositions, these differences did not induce broad variations in their VOCs profiles as expected. By contrast, a marked and significant effect of the period was observed: 19 compounds had a higher relative abundance in P1 than in P2 including terpenes, 1-ethoxy-2-propanol, Δ-hexenol, Δ-hexenyl acetate, and 2-phenylethyl acetate while 12 VOCs were more abundant in P2 than in P1, among which most ketones, the aldehydes and 1-butanol. These differences observed between P2 and P1 were probably due to the effects of temperature and humidity changes between P1 and P2 associated with those of plant maturation and cow grazing. Among the compounds that were more abundant in P1 when cows were grazing beside the exclosures, compounds such as longifolene and bisabolene probably reflected the higher percentage of dicotyledonous flowering plants in P1 compared with P2.

The PCA performed with the peaks most significantly influenced by the grassland (terpinene, UI-14 and benzoic acid), the period (1-butanol and Δ-bisabolene) or both (UI-3) discriminates well the two periods on the PC 1 while the two plots tend to be separated along the PC 2 (Fig. 1). PC 1 clearly separates P1 from P2, mainly because of β-bisabolene and UI-14 which have higher relative abundances in P1. Together, the two first PC explained 68% of the variance.

Significant correlations ($p<0.05$) between VOCs and insect counts were found. There was a negative correlation between the number of wild bee species and limonene (R = -0.39) and between the number of “other hymenoptera” and α-pinene (R = -0.35), Δ-hexenyl acetate (R = -0.41) and limonene (R = -0.46). Butyrolactone, already cited as a constituent of insect pheromones, correlated with the number of pollinators and the number of bee species. Lastly, a negative correlation was observed between limonene and the number of wild bee species, which could reflect the vegetative stage of the plants, limonene being emitted by leaves and stems rather than by flowers. However, the trapping conditions were probably less efficient than in maize crops, where the headspace may be protected against wind, retained and to some extent concentrated by the height of the plants. In the future, we need to improve our method to reduce the variability between measurements.

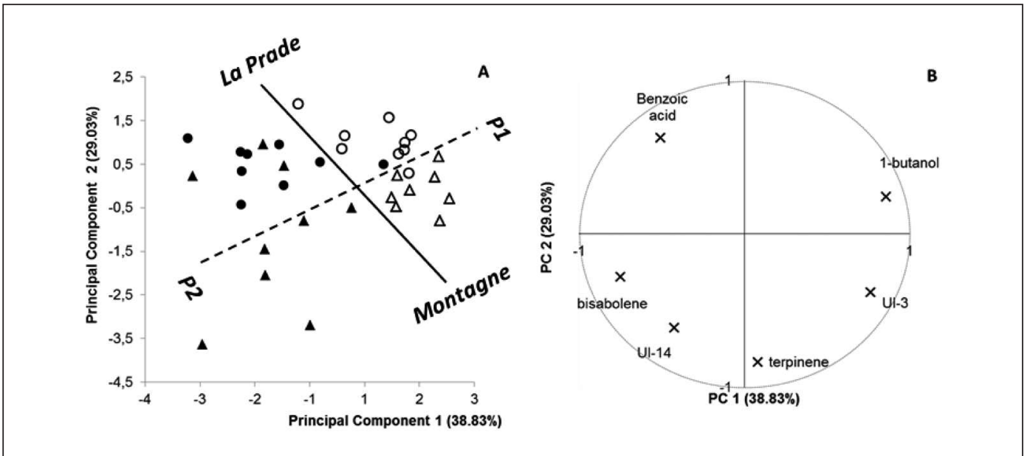

Fig. 1. Representation of Montagne and La Prade grasslands at both periods on the 1 × 2 plane of the PCA. A: Observations (2 grasslands× 3 exclosures × 3 repetitions× 2 periods) and B: Variables included in the PCA. P1: filled symbols, P2: empty symbols, Montagne : triangles, La Prade : circles.

IV – Conclusions

Our study has demonstrated that SPME-GC-MS method is able to provide grassland VOC profiles. The correlations found between wild pollinators and certain VOCs, even if they need to be confirmed, provide clues to finding “semiochemicals” linking grasslands to the insects they host. These first encouraging results open the way to new knowledge to assess and enhance grassland value in terms of ecological services. In the future, microbial activity in soil, pollinating attractiveness and relationships between grasslands odorscape and milk sensorial quality would be an interesting track to explore with this innovative tool.

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References

Cornu A., Farruggia A., Leppik E., Pinier C., Fournier F., Genoud D. and Frerot B., 2015. Trapping the pasure odorscape using open-air solid phase micro extraction, a tool to assess Grassland vaue. *Plos One,* Nov 4, 1-14.

Davis T.S., Crippen T.L., Hofstetter R.W. and Tomberlin J.K., 2013. Microbial volatile emissions as insect semiochemicals. *Journal of Chemical Ecology,* 39, 840-859. doi: 10.1007/s10886-013-0306-z PMID: 23793954.

Leppik E. and Frerot B., 2014. Maize field odorscape during the oviposition flight of the European corn borer. *Chemoecology,* 24, 221-228**.**

Schneider C.A., Rasband W.S. and Eliceiri K.W., 2012. NIH Image to ImageJ: 25 years of image analysis, *Nature methods,* 9, 671-675.

Sharkey T., 1996. Emission of low molecular mass hydrocarbons from plants. *Trends in Plant Science,* 1, 78-82.

Westphal C., Bommarco R., Carre G., Lamborn E., Morison N., et al., 2008. Measuring bee diversity in different European habitats and biogeographical regions. *Ecological Monographs,* 78, 653-671.