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Emissions of volatile organic compounds from aboveground and belowground parts of rapeseed (*Brassica napus* L.) and tomato (*Solanum lycopersicum* L.)

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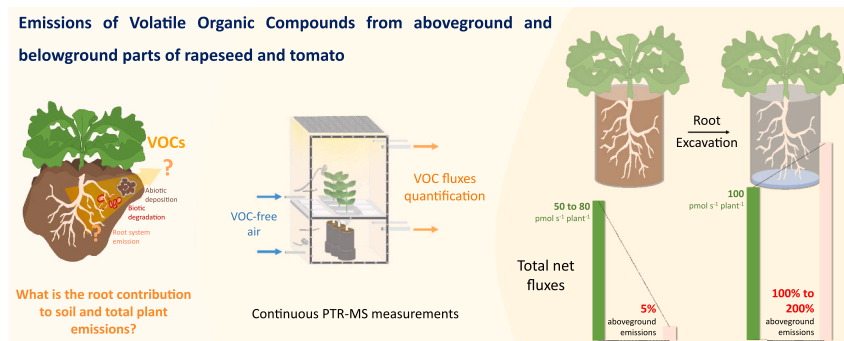
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HIGHLIGHTS

- A new chamber for measuring plant above- and belowground VOC fluxes was developed.
- Roots contributed to only 5 % of total plant VOC emissions when soil was present.
- Roots contributed as much as shoots when soil was removed.
- Methanol was the main VOC emitted by shoots and roots.

GRAPHICAL ABSTRACT



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ABSTRACT

Root systems represent a source of Volatile Organic Compounds (VOCs) that may significantly contribute to the atmospheric VOC emissions from agroecosystems and shape soil microbial activity. To gain deeper insights into the role of roots in the VOC emissions from crops, we developed a dynamic chamber with isolated aboveground and belowground compartments, allowing for simultaneous measurements of VOC fluxes from both compartments in controlled conditions. We continuously monitored VOC emissions from intact plants of rapeseed (*Brassica napus* L.) and tomato (*Solanum lycopersicum* L.) i) over 24 h when plants were rooted in soil, and ii) over 6 h following soil removal. The measurements were performed using a highly sensitive Proton Transfer Reaction – Time of Flight – Mass Spectrometer and a Thermic Desorption- Gas Chromatography – Mass Spectrometer. Net VOC emissions measured at the soil surface represented <5 % of the aboveground emissions and were higher during the day than at night. However, when soil was removed, belowground VOC emissions became up to two times higher than aboveground emissions. This large increase in VOC emissions from roots observed after soil removal was almost exclusively due to methanol emissions. Differences in VOC composition between plant species were also detected with and without soil: rapeseed emitted more sulphurous and nitrogenous compounds and tomato more mono- and poly-unsaturated hydrocarbons. Our results suggest that roots may be a largely

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underestimated VOC source and that the soil is a strong sink for root-borne methanol. Root VOC emissions should be considered when agricultural practices involve roots excavation.

1. Introduction

Biogenic aboveground and belowground Volatile Organic Compounds (VOCs) play a key role in plant fitness and interactions with other organisms (Dudareva et al., 2006; Delory et al., 2016; Massalha et al., 2017). All plant organs emit numerous VOCs, which can readily diffuse in their environment and trigger changes in the beneficial or deleterious (micro)organism activity near the plant. Aboveground plant VOCs have been extensively studied since they are the primary source of VOCs in the atmosphere, contributing to ozone and secondary organic aerosol formation and, thus, to air quality and climate (Guenther et al., 1995; Laothawornkitkul et al., 2009). In contrast, belowground constitutive VOC emissions received less attention. However, they may provide a wealth of information on the biotic belowground interactions and might significantly contribute to total ecosystem VOC fluxes. Most of the studies on belowground VOCs have been performed in the context of plant stress and little information on VOC emissions from non-stressed plants is available (van Dam et al., 2012; Danner et al., 2015; Murungi et al., 2018; Ehlers et al., 2020; Gulati et al., 2020; Lee Díaz et al., 2022). Furthermore, the results from different studies are difficult to compare due to different VOC measurement protocols. For example, the use of ambient or VOC-free air, the type and selectivity of the VOC analyzers can vary from one study to another (Tang et al., 2019). As a result, numerous VOCs are not consistently measured across studies and a general insight into the VOC root emission profiles is still lacking.

The few studies reporting VOC measurements at the soil surface suggest that VOC emissions from roots are much lower than total aboveground emissions (Asensio et al., 2008; Gray et al., 2014; Trowbridge et al., 2020). However, since the soil can act as both a source and sink of VOCs (Lin et al., 2007; Peñuelas et al., 2014; Tang et al., 2019; Rinnan and Albers, 2020; Jiao et al., 2023; Yang et al., 2024), the VOC root emissions may be poorly estimated when measurements are performed at the soil surface. VOCs can be emitted and consumed in the rhizosphere, the soil zone directly in contact with the roots and especially rich in microorganisms (Peñuelas et al., 2014). Moreover, VOCs can be retained in the soil liquid phase or at the gas-liquid and liquid-solid soil interfaces, and can also be transformed by soil microorganisms (Kim et al., 2005; Insam and Seewald, 2010; Peñuelas et al., 2014). Measurements of root VOC emissions with and without soil could therefore improve our understanding of root VOCs dynamics. Up to now, contrasting results have been reported. For example, while the quantification of isoprenoids revealed strong emissions from excavated pine root systems (Lin et al., 2007), no significant difference was observed between the monoterpene and isoprene fluxes of pine forest soil with or without active roots (Gray et al., 2014).

Our study focuses on crop soils, which occupy a large part of terrestrial surface and undergo frequent disturbances due to agricultural practices (e.g. ploughing, harvesting or mechanical removal of weeds). Such disturbances may expose roots to the surface possibly increasing the transfer of root VOCs into the atmosphere. We chose rapeseed and tomato because of their large agricultural interest and their different VOC emission profiles. Rapeseed, the world's fourth most important source of oilseed (FAO, 2022) is known to emit many sulfur and halogenated VOCs (Acton et al., 2018; Jiao et al., 2020), whereas tomato, the most produced vegetable worldwide (FAO, 2022), releases mostly terpenes that are stored in glandular trichomes (Dehimeche et al., 2021).

To investigate root VOC emissions from these two crops, we developed a dynamic chamber device with isolated aboveground and belowground compartments allowing for simultaneous measurements of VOCs, CO₂, and H₂O fluxes in each compartment, under controlled environmental conditions (air humidity, temperature, light, and CO₂). A

highly sensitive Proton Transfer Reaction Quadrupole-ion-guide Time-Of-Flight Mass Spectrometer (PTR-Qi-TOF-MS) was used to characterize the constitutive emissions from the shoots and the roots of potted tomato and rapeseed plants and a Thermic Desorption Gas Chromatography Mass Spectrometry (TD-GC-MS) to confirm compound identification. Our main hypothesis was that roots can significantly contribute to the total VOC emitted by the plant. To investigate this, we continuously monitored aboveground and belowground VOC emissions from intact plants, first in the presence of soil around the roots and then in its absence. We also hypothesized that VOC emission profiles differ between these two plant species, but are similar between aboveground and belowground parts. The latter hypothesis is based on the fact that VOC transport within the plant can occur actively or passively (Escobar-Bravo et al., 2023), which may lead to the homogenization of the profiles of emitted VOC between the above- and below-ground parts of a plant species.

2. Material and method

2.1. Plant growth

Tomato (*Solanum lycopersicum* L., var. F1 DEFIANT 73430) and winter rapeseed (*Brassica napus* L., var. AVISO) plants were grown from seed in 2.6 L aluminium pots (23 cm height, 12 cm diameter) filled with 3 kgDW of a mixture of 2/3 (vol.) agricultural soil and 1/3 sand (see the initial soil characteristics in Table A1), using one plant per pot. Pots were installed in a climate-controlled growth chamber in two separate blocks, with 17 to 27 replicates per plant species and 10 additional pots with bare soil, to obtain an average plant density of 36 plants m⁻² for both species. The day/night temperature was set at 24/18 °C with a photoperiod of 14 h (light intensity between 250 and 350 μmol m⁻² s⁻¹ Photosynthetic Photon Flux Density). The pots were watered regularly to maintain a soil humidity of 21 % g_{water}/100 g_{dry soil} equivalent to 60 % of the estimated field capacity, and were moved periodically to minimize the impact of spatial environmental variations within the chamber. The air was humidified with ultrasonic humidifiers to maintain a relative humidity (RH) between 70 and 85 %. 38 days after sowing, 10 mL of nutrient solution was added to the pot surface with 81.6 g L⁻¹ NH₄NO₃, 42.08 g L⁻¹ K₂HPO₄, and 37.29 g L⁻¹ K₂SO₄, which is equivalent to a fertilizer input of 100 kg N ha⁻¹, 26 kg P ha⁻¹, 125 kg K ha⁻¹ and 24 kg S ha⁻¹ for a density of 350,000 plants per ha. Plants were grown for about 8 weeks before gas exchange measurements.

2.2. Experimental set-up

The gas exchange measurements were made using a custom-made bi-compartmented dynamic chamber (288 L and 144 L volume of above- and belowground compartment respectively) made of poly(methyl methacrylate), covered with fluoroethylenepropylene (FEP) film, and connected to 6 air inlets and outlets via PFA tubes (Fig. 1). The separation between the aboveground and belowground compartments was achieved by a system of embeddable polyoxymethylene plates completed with film collars: one main plate with square openings through which the plant could pass, and closing plates for sealing the collars surrounding the plant stems to the main separating plate, thus completing the separation between the compartments (Fig. 1b, and Fig. A2). Film collars made of FEP film, perfluoroalkoxy (PFA) tube, and Polytetrafluoroethylene (PTFE) paste had been attached to the base of the seedling stem when the plants were roughly 20 cm tall (see Fig. A1, and Fig. 1). Throughout the measurements, VOC- and NO_x-free air (FDGSi SAS air generator, Lieusaint, France) flowed continuously through

the aboveground and belowground compartments at a rate of 18 and 9 L min^{-1} , respectively. This resulted in an air residence time of approximately 16 min in each compartment. The CO_2 and water contents of the inlet air were adjusted by first replenishing it with pure CO_2 from a gas bottle ($\text{H}_2\text{O} < 50$ ppm, $\text{CO} < 5$ ppm, $\text{NO} + \text{NO}_2 < 2$ ppm, total sulfur < 1 ppm) and then by passing it through a gas washing bottle filled with distilled water. To avoid the condensation on the walls of the aboveground compartment during the rise in air humidity when the stomata were open, the air humidity at the entrance to the compartments was reduced in the morning by decreasing the air flowing through the air humidification system. Thus, RH ranged throughout the measurements from 46 % to 94 % and from 67 to 82 % in the above- and below-ground compartments, respectively. Pressure regulators were installed at the compartment outlets to ensure a slight overpressure in the belowground compartment thus preventing air contamination by the upper compartment (Fig. 1c). Each compartment was ventilated with a fan to homogenize the air. A bank of LED (Colasse SA, Seraing, Belgium) with a

wavelength range between 400 and 700 nm (0.26 % UV) was installed above the chamber, delivering over day time between 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the top and the bottom of the aboveground compartment respectively. The temperature in the chamber was regulated via the air conditioning system of the room. Mean air temperature evolved at night between 20 and 21 °C, and at daylight in the range 20–24 °C and 20–23 °C in the above- and below-ground compartment respectively.

2.3. Gas measurements protocol

Gas exchange measurements were performed within a 12-day interval for each species, with non-vernalized rapeseed and tomato plants in the BBCH-scale phenological stages 2 (formation of side shoots, 8 to 11 leaves) and 7 (presence of green fruits), respectively (Meier, 2001). In each of the four experimental replicates, VOC fluxes were measured on a group of three new plants that had been installed together in the

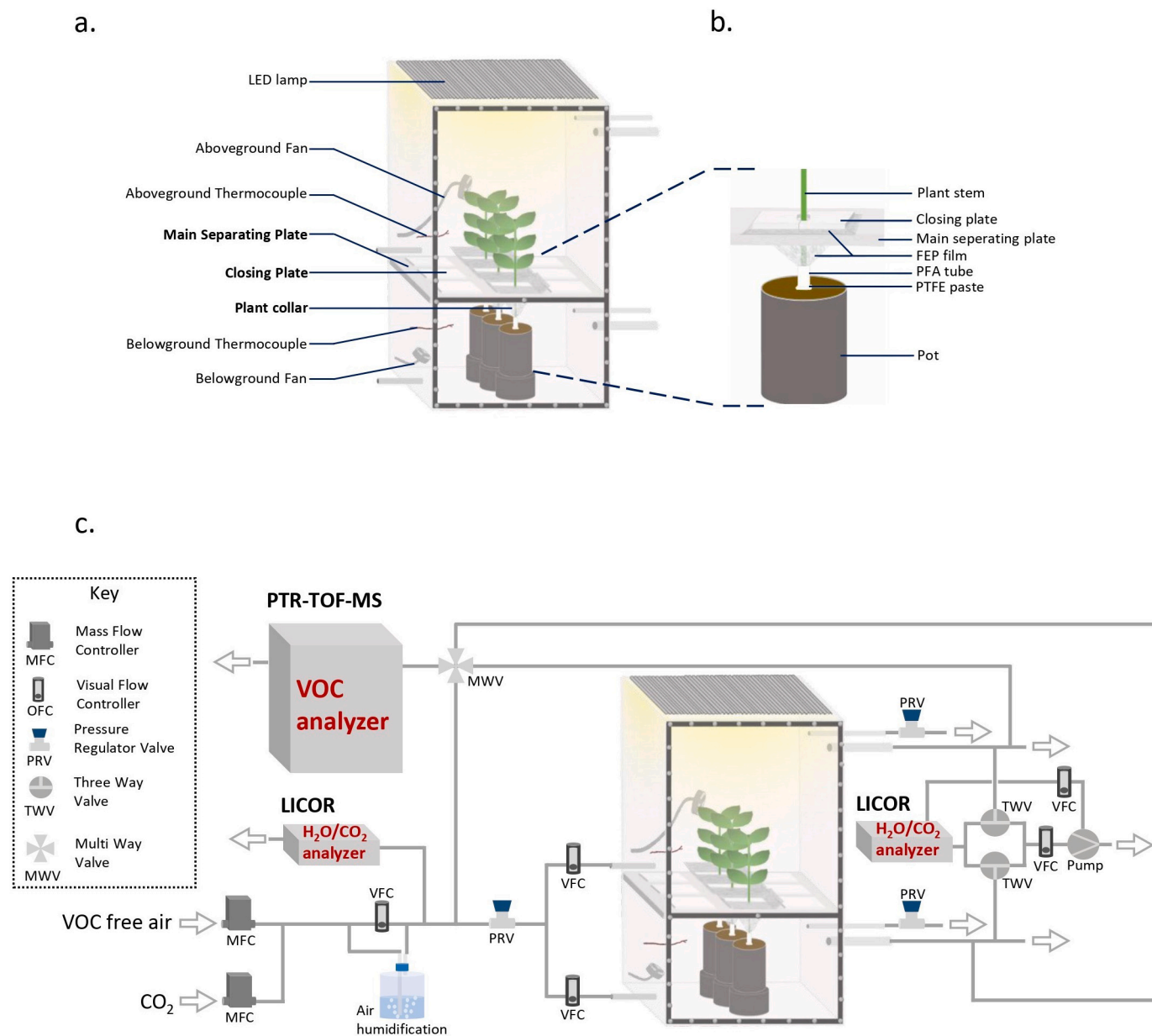


Fig. 1. Schematics of (a) the two-compartment dynamic chamber comprising (b) a non-invasive separation system of embeddable plates and plant collar. (c) General set-up for continuous measurements of VOC fluxes from aboveground and belowground parts of multiple plants. FEP refers to Fluoroethylenepropylene, PFA to Perfluoroalkoxy, and PTFE to Polytetrafluoroethylene.

chamber the previous evening. Before installing the plants, algae were removed from the top soil layer by scraping the first millimeter of the soil. The plants were left in the chamber to acclimate for 12 h under the same experimental conditions, before starting the measurements at 7:30 am (30 min before lighting). The measurements recorded over the following 24 h were used to calculate the constitutive belowground and aboveground VOC fluxes of the potted plants. The pots were then removed from the chamber, the soil around the roots was gently removed, and the root systems were cleaned with tap water. Subsequently, the plants were reintroduced into the chamber for 1 h of plant acclimatization and air renewal in the compartments, followed by around 4.5 h of gas exchange measurements in the morning. During this second period, the soil-free root systems were suspended within the empty aluminium pots, containing about 100 mL of tap water at the bottom to prevent root desiccation. The small part of the root system reaching the pot's bottom was immersed in water. This period was used to determine the VOC fluxes from excavated plants.

The VOC concentrations of the air streams were obtained from the raw data of a HR-PTR-Qi-ToF-MS (Ionicon Analytik GmbH, Innsbruck, Austria). Continuous air analysis (temporal resolution of 1 s, m/z (mass-to-ionic-charge) range of 10–510) was performed with a drift tube pressure of 3.8 mbar, a drift temperature of 80 °C, and a voltage of 848 V, corresponding to an E/N ratio of 120 Td (1 Td = 10–17 V cm²). The m/z axis was calibrated using the protonated water ($H_3^{18}O^+$, m/z 21.022), acetone ($C_3H_7O^+$, m/z 59.049) and diiodobenzene (PerMaSCal Ionicon, $C_6H_4I_2H^+$, m/z 330.941, the main ion, and $C_6H_5I^+$, m/z 203.943, a fragment). The PTR-Qi-ToF-MS was automated to measure firstly the air at the compartment inlet for 5 min and then the air at the outlet of each compartment for 15 min. VOCs were also trapped on cartridges (Tenax TA adsorbent) by sampling the air stream at 0.5 L min⁻¹ during 2 h of the day period. These cartridges were then analysed by Thermic Desorption – Gas Chromatography – Mass Spectrometry (TD-GC-MS) to complete VOC identification (description in A3). To ensure complete trapping of all VOCs on the Tenax cartridges, additional cartridges were placed in a downstream position. None of the investigated VOCs were detected in these downstream cartridges. CO₂ and H₂O concentrations were recorded with two infrared absorption gas analyzers, IRGA (LI-840 A, Licor, Lincoln, NE, USA), one continuously measuring the air entering the compartments and the second alternately measuring the air leaving the aboveground and belowground compartments (in line with the PTR-ToF-MS). Solenoid valves and pump system ensured that a continuous air flow of 0.3 L min⁻¹ at the outlet of each compartment passed through the tubes leading to the IRGA, either to be analysed or to reach an exhaust, avoiding pressure variation inside the compartments (Fig. 1). The PTR-Qi-ToF-MS is equipped with an internal pump drawing off the air at a rate of around 80 mL min⁻¹, equivalent to a maximum of 1 % of the airflow through the compartments. Consequently, the variation in air flow associated with this draw-off was deemed negligible and was not compensated for.

The PTR-Qi-ToF-MS was calibrated several times during the experiments. To that purpose, we used a standard gas cylinder containing methanol, acetonitrile, acetaldehyde, acetone, acetic acid, isoprene, methyl vinyl ketone, 2-butanone, benzene, toluene, o,p,m-xylene, 1,2,4-trimethyl benzene, a-pinene, 1,2,4-trichlorobenzene at around 1ppmV (SIAD). The gases from this cylinder were diluted with synthetic air (alphagaz 1, Air Liquide, Paris, France) using two fluor-inert coated mass flow controllers (Bronkhorst, AK Ruurlo, Netherlands) to generate different concentrations. A mean calibration factor was calculated as the slope of the regression between the measured and the theoretical mixing ratios. The obtained calibration factors were applied for the mixing ratio calculations for the 14 compounds (see Table A2). For the ions that were not present in the calibration standard gas cylinder, a transmission curve was determined using 10 non-fragmenting compounds of the mentioned calibrations (Fig. A2 and the corresponding proton transfer reaction rate coefficients-k). For non-calibrated other masses, a generic value of $k = 2 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ was used (Cappellin et al., 2012). TD-GC-MS protocol is

explained in A.3. VOC identification was obtained by comparison between VOC mass spectrums and the NIST (National Institute of Standards and Technology) Mass Spectrum database (Stein et al., 2012), as well as comparison of their retention indices (RI) with those of Adams, 2005 and RI references from the NIST chemistry webbook. The Li840 was calibrated using a standard gas mixture for CO₂ (395.8 ± 1.2 ppm) and a Li610 dew temperature generator for H₂O.

Besides gas exchange measurements with plants, control measurements on bare soil and in the empty chamber were also carried out, by introducing aluminium pots - filled with bare soil or not - with their collars.

The cited ions $C_2H_5O^+$ (m/z 45.033), CH_3S^+ (m/z 49.011), $C_4H_9O_2^+$ (m/z 89.060), $C_3H_9OS^+$ (m/z 93.036), $C_2H_8O_2S^+$ (m/z 95.015), $C_6H_{11}O^+$ (m/z 99.088), and $C_6H_{10}NS^+$ (m/z 128.053) were assigned to the VOCs acetaldehyde, methanethiol, acetoin, 2-(methylthio)ethanol, dimethyl sulfone, hexanal, and cyclopentyl isothiocyanate based on literature (see Appendix B). However, it is important to note that these identifications are tentative and require confirmation. The other cited VOCs were identified by PTR-MS or GC-MS without ambiguity.

2.4. Plant areas and dry weights characterisation

After gas measurements, the total leaf surface of each plant was measured using a planimeter (LI-3100 Area Meter, Licor). Total root length and surface were determined by processing root images obtained by a scanner (Epson 12,000 XL) at 600 dpi with the package MorphoLibJ (Legland et al., 2016) from ImageJ for homogenizing the background, and then RhizoVision Explorer (version 2.0.3) software (Seethepalli et al., 2021) for computing root traits. The dry mass of each organ was measured after oven drying at 80 °C for 72 h.

The dry biomass and surface of exchange of the aboveground and belowground parts of between 59 and 77-day old tomato and rapeseed plants are indicated in Table A3. While the surface of leaves and stem developed per plant was equivalent between the two species, the total dry weight of aboveground parts was 60 % higher in rapeseed than in tomato. Comparatively, the root dry weight and root surface were twice higher in rapeseed than in tomato, leading to a shoot-to-root biomass ratio of 5.4 for rapeseed and 7.4 for tomato. When the root system was carefully extracted from the soil to proceed to a second period of measurements on excavated plants, a significant number of small roots remained in the soil, leading to a loss of about 30 % in root dry mass and about 70 % in total root surface.

2.5. Calculations

Spectra obtained by PTR-Qi-ToF-MS were analysed using PTR Viewer (version 3.4.4, Ionicon Analytik). Only peaks with a height >10 cps (counts per second) and a m/z >40 were selected, except for formaldehyde (m/z 31.018) and methanol (m/z 33.033). The data were then processed using the R software (Version 4.1.0 – © 2009–2022 RStudio). The mean flux values in each compartment were calculated using the following formula:

$$F_i = Q \times \frac{P}{R \times T} \times (\chi_{i \text{ out}} - \chi_{i \text{ in}}) \quad (1)$$

where F_i is the flux of VOC i in mol h⁻¹, Q is the flow rate through the compartment in m³ h⁻¹, P the pressure in the compartment in Pa, R the perfect gas constant, T the temperature in the compartment in Kelvin, $\chi_{i \text{ out}}$ and $\chi_{i \text{ in}}$ the mixing ratios of the VOC i at the compartment outlet and inlet respectively. Standard deviations (SD) were calculated from measurements of the different repetitions. The mean fluxes were calculated for three distinct periods: i) a 13-h daylight period and ii) a 9-h night period when plants were still in the soil, after removing the one-hour air renewal time succeeding the light environment change, and iii) a 15-min day period after plants had been extracted from soil and

installed for 4.5 h in the chamber.

$$F_{Net\ i} = F_{Main\ i} - F_{Control\ i} \quad (2)$$

The mean net fluxes $F_{Net\ i}$ in each compartment were estimated according to Eq. (2), where $F_{Main\ i}$ and $F_{Control\ i}$ are the mean fluxes of the main (plants in and extracted from the soil) and control (bare soil and empty chamber) modalities respectively. Mean net fluxes of bare soil and excavated roots were obtained from their mean fluxes after subtracting the corresponding mean fluxes of the empty chamber. To obtain the net mean fluxes of non-excavated roots, bare soil mean fluxes were subtracted from the non-excavated ones. For fluxes that derive from subtraction or sums of fluxes with their own SD directly calculated from experimental replicates, i.e. net and total fluxes, the Monte Carlo method was used to calculate the resulting SD (Papadopoulos and Yeung, 2001). For example, for calculating the SD of the mean net flux of belowground non excavated plants, 1000 randomly simulated values of the mean belowground bare soil flux coupled to 1000 randomly simulated values of the mean belowground plant flux were generated given their respective mean and standard deviation, considering normal distributions. The SD of the mean net flux was then calculated on the 1000 subtraction results from Eq. (2).

2.6. Statistical analysis

The significance of the average daytime and night-time peak fluxes of

the main modalities (plants in and extracted from the soil) was evaluated against the equivalent average fluxes of the control modalities (bare soil and empty chamber, respectively) using a Wilcoxon test with the function `wilcox.test` function from the package `stats` (version 4.1.0) (Fay and Proschan, 2010; Cassell, 2016). Only peaks with a Wilcoxon test $p < 0.06$ were considered significant and retained. Net mean fluxes of CO_2 and water were obtained similarly.

The diversity (Shannon index) of the VOC profiles were estimated with the package `vegan` (version 2.6.4) from R (Oksanen, 2013). It corresponds to $H = -\sum_i F_{norm\ Ni} \log(F_{norm\ Ni})$, where $F_{norm\ Ni}$ is the net mean flux of the emitted VOC_i normalized over the net mean total emission.

2.7. VOC background from the chamber

The empty chamber emitted the ions putatively identified as formaldehyde ($m/z\ 31.018 - \text{CH}_3\text{O}^+$) and formic acid ($m/z\ 47.013 - \text{CH}_3\text{O}_2^+$) from roughly 7 to 20 pmol s^{-1} and methanol ($m/z\ 33.033 - \text{CH}_5\text{O}^+$), acetaldehyde ($m/z\ 45.033 - \text{C}_2\text{H}_5\text{O}^+$), acetic acid ($m/z\ 61.029 - \text{C}_2\text{H}_5\text{O}_2^+$), and acetone ($m/z\ 59.049 - \text{C}_3\text{H}_7\text{O}^+$) at $<7\ \text{pmol s}^{-1}$. Since fluctuating emissions of the ion putatively identified as ethanol ($m/z\ 47.049 - \text{C}_2\text{H}_7\text{O}^+$) were detected from inlet airflow, this ion peak was removed from the analysis.

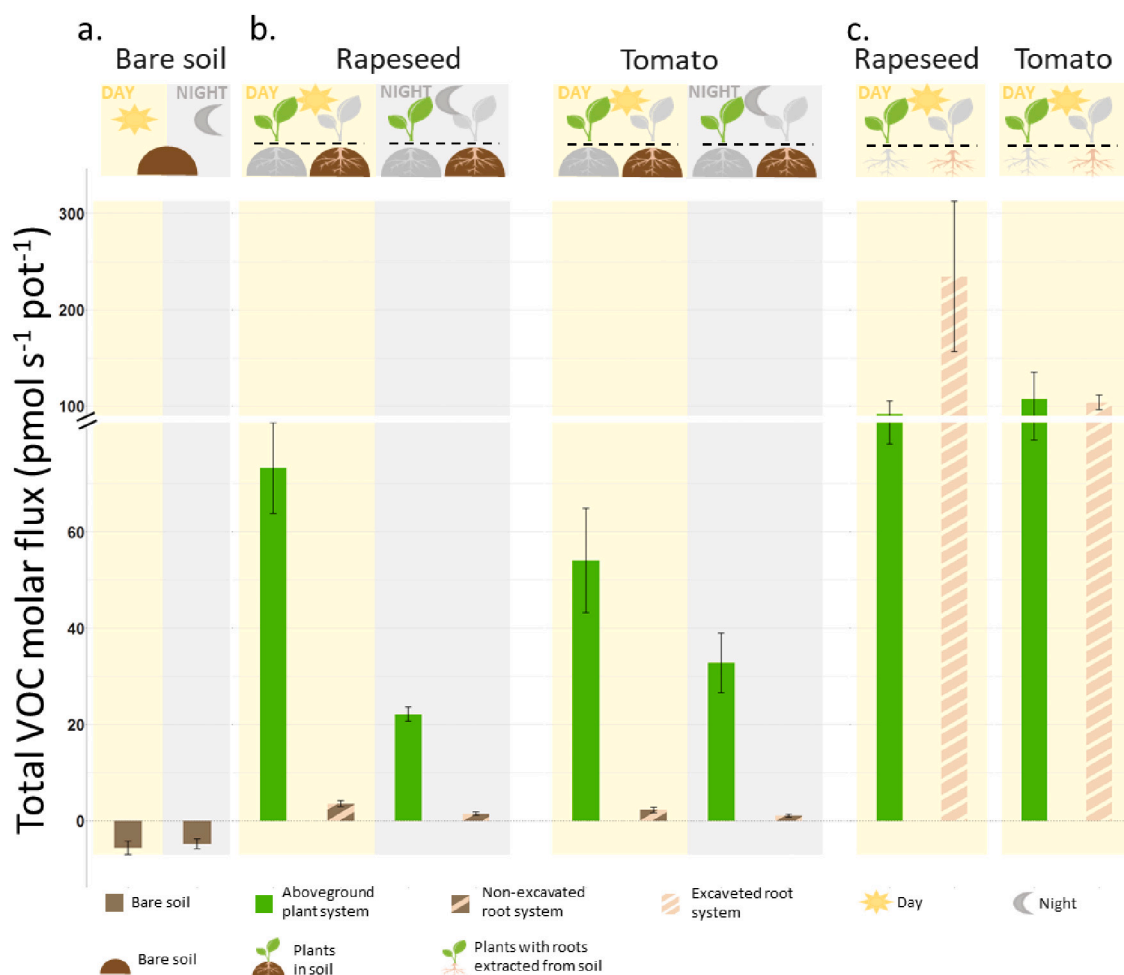


Fig. 2. Total mean net VOC fluxes during day and night periods, (a) over bare soil surface, (b) from aboveground and belowground parts of rapeseed and tomato plants in soil, and (c) of the same plants after root extraction from soil. Error bars correspond to standard errors ($n = 4$ for all modalities except excavated tomato, for which $n = 3$) calculated with the Monte Carlo method. In (a) and (c) the net fluxes were obtained by subtracting the fluxes from the empty chamber, while in (b) the net fluxes were obtained by subtracting the fluxes from bare soil in the chamber.

3. Results

In this section, only the most important results on VOC fluxes are presented. The fluxes of the approximately 90 ions that were significant in one or more modalities and their identification are given in Appendix B.

3.1. Total VOC fluxes

3.1.1. Molar fluxes

Plants were always net VOC emitters, regardless of the compartment or the treatment (Fig. 2). However, the net VOC fluxes emitted by the non-excavated roots of rapeseed and tomato remained very low compared to aboveground VOC emissions (Table 1). On the contrary, molar root VOC fluxes were 2 to 3 times higher than those from aboveground parts after root excavation. Compared to the net fluxes from non-excavated plants, root VOC emissions increased by about 50-fold, while aboveground fluxes increased by a maximum of 2-fold.

VOC emissions from non-excavated roots represented <5 % of the whole plant emissions when expressed per pot ($\text{pmol s}^{-1} \text{pot}^{-1}$). However, when expressed per dry weight of the emitting tissues ($\text{pmol s}^{-1} \text{g}^{-1}$), belowground VOC emissions amplitude were around 25–30 % of the aboveground emissions (Table 1). No correlation was found between belowground VOC emissions per plant, root dry weight, or root surface when considering the variability among the four replicates of a given species. We note that belowground total VOC fluxes from excavated roots were very similar between species when expressed per dry weight or root surface, while they were 2 times higher for rapeseed than tomato when expressed per plant (Table 1).

The number of repetitions was too small to perform a paired statistical test for assessing the significance of differences between day and night fluxes. However, a diminution of total emissions was observed at night for each of the repetitions and compartment of non-excavated plants.

The soil alone was a weak sink for the VOCs present in the empty belowground compartment (Fig. 2). Apart from acetaldehyde and acetone, all VOCs emitted by the chamber material (see 2.7) were significantly deposited to the bare soil with fluxes between 0.06 and 0.2 $\text{nmol m}_{\text{soil surface}}^{-2} \text{s}^{-1}$. Total net fluxes of VOC to the bare soil averaged roughly $6 \pm 1 \text{ pmol s}^{-1} \text{pot}^{-1}$. For each repetition, the sink term of bare soil was slightly lesser during the night than the day.

3.1.2. Fluxes of carbon emitted as VOCs and comparison to CO_2 fluxes

When we expressed the VOC fluxes in moles of carbon instead of moles of compounds, the VOC emissions from tomato became higher than those from rapeseed before excavation, i.e. tomato emitted more volatile organic carbon than rapeseed (Table 1). After excavation, the emission of volatile organic carbon from roots was 2 times higher than from shoots in the case of rapeseed, while it was 3 times lower than from

shoots in the case of tomato.

Carbon emissions as VOCs by non-excavated roots were insignificant compared to their CO_2 emission (about 0.005 % and 0.010 % for rapeseed and tomato, respectively), but represented roughly 0.4 % of the C respired as CO_2 after excavation (Fig. A3). By comparison, the carbon emitted as aboveground VOCs during the day represented between 0.1 % and 0.5 % of the CO_2 respired by shoots during the night and between 0.02 % and 0.08 % of the net CO_2 fixed by the plant during the day. The species with the higher increase of aboveground VOC flux after excavation, i.e. tomato, showed a concomitant decrease by 30 % of its CO_2 assimilation, while around a 10 % decrease was observed for rapeseed after root excavation.

3.2. Main VOC profiles of plant emissions

Methanol (m/z 33.033, CH_3O^+) was the main VOC emitted by plants, apart from nocturnal emissions from unexcavated rapeseed roots (Fig. 3). Before root excavation, methanol emissions from roots accounted for 33 % of total VOC emissions from rapeseed and 56 % from tomatoes. After excavation, the methanol contribution to belowground VOC emissions rose to 94 % and 95 % for rapeseed and tomato, respectively. By contrast, the proportions of methanol in the aboveground emissions remained relatively unchanged before and after root excavation (around 90 % and 70 % for rapeseed and tomato, respectively). Besides methanol, the main plant VOCs were methanethiol, acetic acid (m/z 61.029, $\text{C}_2\text{H}_5\text{O}_2^+$), monoterpenes (m/z 137.133, $\text{C}_{10}\text{H}_{17}^+$), and acetaldehyde (Fig. 3). Although plant VOC profiles exhibited between around 20 and 60 ions, the three most emitted VOCs of each modality contributed to >70 % of total VOC emissions.

Whether roots were excavated or not, rapeseed emitted more sulphurous and nitrogenous VOCs from above- or belowground than tomato, which was a greater emitter of mono- and poly-unsaturated hydrocarbons. This indicates that a large number of the VOCs observed are specific to the two plant species and not ubiquitous. Root VOCs identified by TD-GC-MS were also detected in aboveground collected VOCs, except for acetophenone and benzeneacetaldehyde (m 120.058, $\text{C}_8\text{H}_8\text{O}$) and benzenepropanenitrile (m 131.073, $\text{C}_9\text{H}_9\text{N}$), solely identified in excavated rapeseed roots. After excavation, the VOC profile of the aboveground tomato showed a greater VOC richness and evenness, resulting in a higher Shannon index (1.2 ± 0.1) than aboveground rapeseed and excavated roots (< 0.6) (Fig. A4).

Methanol, monoterpenes, sesquiterpenes (m/z 205.195, $\text{C}_{15}\text{H}_{25}^+$), acetaldehyde, and benzaldehyde (m/z 107.049, $\text{C}_7\text{H}_7\text{O}^+$) were the only compounds found in all root emissions, regardless of modality. Acetic acid (m/z 61.029, $\text{C}_2\text{H}_5\text{O}_2^+$) and acetoin (m/z 89.060, $\text{C}_4\text{H}_9\text{O}_2^+$) were exclusively detected in non-excavated root emissions.

The $\text{C}_6\text{H}_{11}^+$ (m/z 83.086) molecule, commonly considered as a product of Green Leaf Volatile (GLV) fragment (Pang, 2015), which contributed to around 0.1 % of tomato non-excavated aboveground

Table 1

Total net VOC fluxes during day and night periods, expressed by pot, dry weight or surface, and as carbon fluxes by pot for aboveground and belowground plant parts. Results are presented as Mean \pm standard deviation ($n = 4$ for all modalities except excavated tomato: $n = 3$) calculated with the Monte Carlo method. The net fluxes from excavated plants were obtained by subtracting the fluxes from the empty chamber, while those from non-excavated plant were obtained by subtracting the fluxes from bare soil in the chamber.

Species	Total VOC molar flux unit	Aboveground			Belowground		
		Non-excavated roots		Excavated roots	Non-excavated roots		Excavated roots
		Day	Night	Day	Day	Night	Day
Rapeseed	$\text{pmol s}^{-1} \text{pot}^{-1}$	73 ± 9	22 ± 1	90 ± 10	3.5 ± 0.6	1.4 ± 0.3	230 ± 80
	$\text{pmol s}^{-1} \text{g}^{-1} \text{dry wt}$	2.7 ± 0.4	0.8 ± 0.1	3.4 ± 0.5	0.7 ± 0.2	0.3 ± 0.1	70 ± 20
	$\text{pmol s}^{-1} \text{m}^{-2}$	400 ± 60	120 ± 10	500 ± 90	5 ± 1	1.9 ± 0.5	1100 ± 500
	$\text{pmol-C s}^{-1} \text{pot}^{-1}$	100 ± 10	25 ± 2	120 ± 10	4.9 ± 0.7	1.8 ± 0.3	260 ± 80
Tomato	$\text{pmol s}^{-1} \text{pot}^{-1}$	50 ± 10	32 ± 6	110 ± 30	2.2 ± 0.6	1.0 ± 0.3	104 ± 8
	$\text{Pmol s}^{-1} \text{g}^{-1} \text{dry wt}$	3.1 ± 0.6	1.9 ± 0.4	6 ± 2	1.0 ± 0.2	0.4 ± 0.1	70 ± 5
	$\text{pmol s}^{-1} \text{m}^{-2}$	250 ± 60	150 ± 30	500 ± 100	6 ± 2	3 ± 1	1000 ± 200
	$\text{pmol-C s}^{-1} \text{pot}^{-1}$	160 ± 20	70 ± 7	370 ± 40	6 ± 1	2.2 ± 0.4	120 ± 9

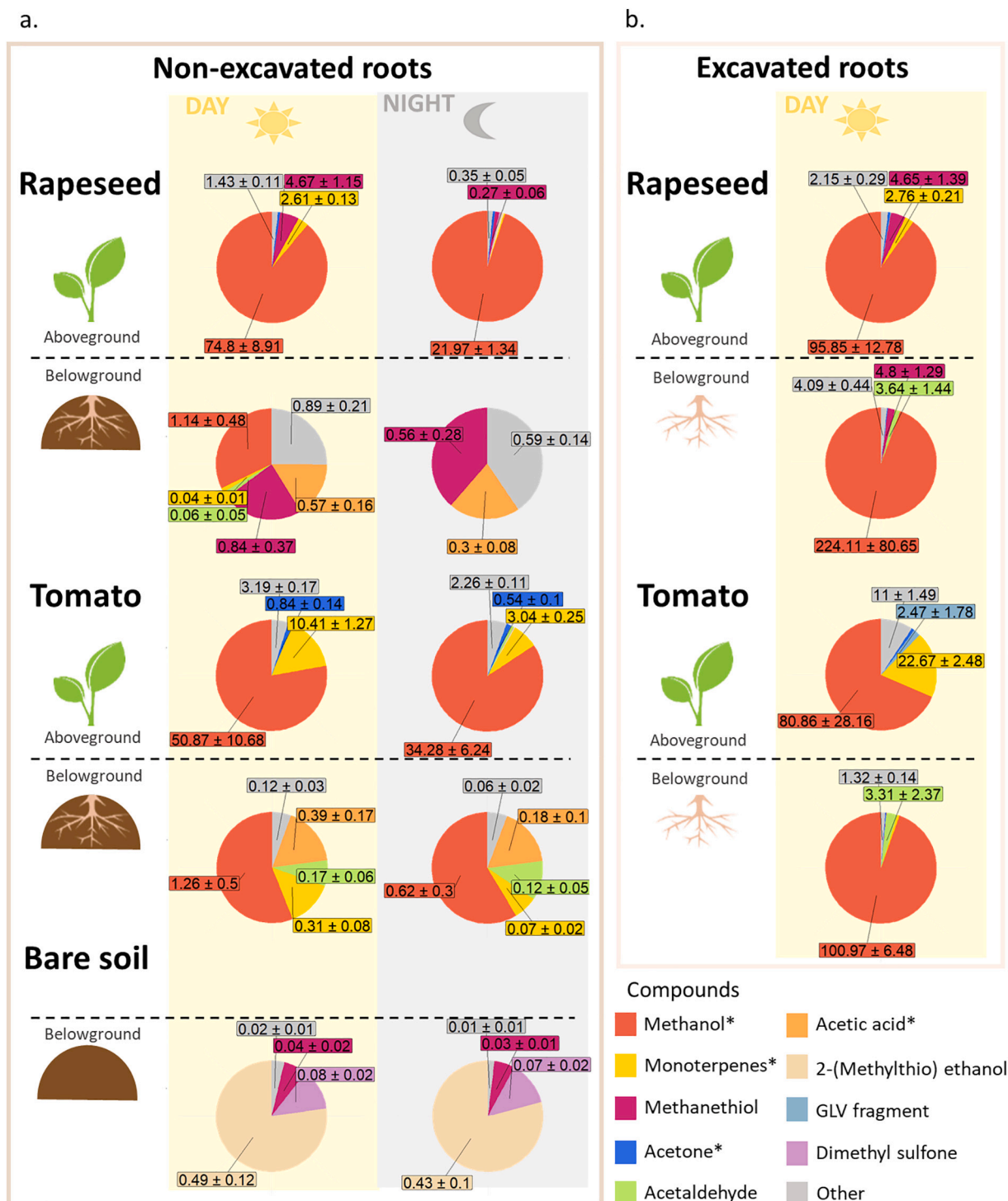


Fig. 3. VOC emission profile from (a) bare soil, non-excavated rapeseed and tomato plants, and (b) excavated rapeseed and tomato plants. Only the 3 major ions contributing to the net emissions of each modality were retained, resulting in 9 ions presented with their putative names. Minor VOCs were included into the “other” category. Figures in labels correspond to net mean fluxes in $\text{pmol s}^{-1}\text{pot}^{-1}$ with their associated standard errors ($n = 4$ for all modalities but excavated tomato, to which $n = 3$) calculated with the Monte Carlo method. GLV = Green Leaf Volatiles. *VOC identified with no ambiguity (see Appendix B).

emissions, became the third most emitted VOC from aboveground tomato after excavation, accounting for about 2 % of all aboveground VOC emissions. This compound and nonanal (m/z 143.143, $\text{C}_9\text{H}_{19}\text{O}^+$), which also is a GLV (ul Hassan et al., 2015), were emitted from roots after excavation but not from non-excavated roots.

The two main ions identified in bare soil emissions, 2-(methylthio) ethanol and dimethyl sulfone, were not significantly emitted by roots (Fig. 2). However, the presence of roots significantly increased the

diversity and quantity of VOCs emitted at the soil surface. Rapeseed and tomato roots added 13 and 19 ions, respectively, to the 10 emitted by the bare soil. Net non-excavated root emissions were 3 to 4-fold higher than net bare soil emissions.

When expressing VOC emissions in mole carbon, methanol remained the main contributor to rapeseed VOC emissions (Fig. A5). However, monoterpenes were the main VOCs emitted by tomato, apart from excavated roots where methanol contributed for three-quarters of fluxes.

Besides these two ions, the main plant VOCs contributing to carbon emissions were methanethiol, acetaldehyde, acetic acid, acetone, phenylethyl isothiocyanate (m/z 164.053, $C_9H_9NS^+$), and GLV fragment. Phenethyl isothiocyanate and GLV, known to be induced by stress (van Dam et al., 2012; Ameje et al., 2018), were mainly detected in rapeseed roots and aboveground tomato, respectively, after their excavation.

Ratios between excavated and non-excavated root VOC fluxes show that methanol emissions increased by 60 to 240 after root excavation. The other main VOCs of the non-excavated root emissions (i.e. methanethiol, dimethyl disulphide (m/z 94.998, $C_2H_7S_2^+$) and acetaldehyde for rapeseed and monoterpenes, acetaldehyde and C_3H_6 (m/z 43.054) for tomato) increased all after the excavation. However, the few VOCs that increased similarly were minor compared to methanol as they contribute, at best, to 1 % of the total plant VOC emissions (Fig. A6).

4. Discussion

Our set-up enabled to compare the constitutive VOC fluxes from above- and belowground compartments of the same plants and follow their emissions during day and night time. To our knowledge, our study is the first to compare VOC fluxes from unexcavated and excavated root systems together with aboveground emissions from plants. Contrary to our hypothesis, roots appeared as very small contributors to total plant VOC emissions when the soil was undisturbed. Root VOC profiles were composed of species-specific VOCs and ubiquitous VOCs, simultaneously found in both compartments and species. However, after their excavation, roots emitted almost exclusively methanol, in a quantity equal to superior to the aboveground parts of the plant.

4.1. Contribution of undisturbed roots to net VOC fluxes from soil and plants

Our results show that the contribution of roots to the total VOC emissions by common agricultural plants remained very low when root systems were left undisturbed in the soil. The scarce studies quantifying constitutive VOCs from root systems reported contrasting results regarding their contribution to the total VOC fluxes from the soil (Peñuelas et al., 2014; Tang et al., 2019). However, the total VOC fluxes from non-excavated roots reported here were of the same order of magnitude as fluxes obtained in similar conditions (plant in pot, zero air stream, non-selective ion detection, laboratory measurements) as reported by Acton et al. (2018) and Dehimeche et al. (2021) (Table 2). When expressed as total carbon emitted as VOCs (Table 2), non-excavated root fluxes were one order of magnitude higher than those found by Gray et al. (2014) from forest soil under ambient air. Nonetheless, they reported a 10^5 higher carbon flux from soil respiration than from soil VOCs, which is coherent with the ratio of 10^5 to 10^6 that we found. Asensio et al. (2007) reported a general increase in the VOC sink of the soil when oak root systems were present. Trowbridge et al. (2020)

Table 2

Root VOC fluxes reported from previous studies and corresponding VOC fluxes calculated in this experiment. The VOC flux of the Danner et al., 2015 study was calculated from a graphic readout of measured concentration (ppb) and set-up characteristics.

Species	Field/laboratory measurement	Analyser	VOC(s)	flux	flux (this study)	Unit	reference
Rapeseed	laboratory	PTR-MS	All significant (methanol not significant)	4	14.4	$ng\ m_{soil}^{-2}\ surface\ s^{-1}$	Acton et al., 2018
Tomato	laboratory	GC-MS	All significant (methanol not characterized)	1 to 10	10.5	$ng\ m_{soil}^{-2}\ surface\ s^{-1}$	Dehimeche et al., 2021
<i>Brassica rapa</i>	laboratory	PTR-MS	Methanol	0.1	0.007	$ng\ g_{dry\ wt}^{-1}\ s^{-1}$	Danner et al., 2015
Multiple species	Subalpine coniferous forest	PTR-MS	All significant	0.56	5.2/6.4	$ng-C\ m_{soil}^{-2}\ surface\ s^{-1}$	Gray et al., 2014
Multiple species	Subalpine coniferous forest	PTR-MS	Methanol	0.5 (max 1.7)	3.2/3.6	$ng\ m_{soil}^{-2}\ surface\ s^{-1}$	Gray et al., 2014
Multiple species	hardwood forest	PTR-MS (after VOC collection in bags)	Methanol	-0.5	3	$ng\ m_{soil}^{-2}\ surface\ s^{-1}$	Trowbridge et al., 2020

also found net VOC depositions in a forest soil dominated by oak and beech. In these studies, aboveground parts of the plants may have strongly participated in the VOC composition of the ambient air, thus modifying the air/soil VOC concentration gradients and the soil behavior (Rinnan and Albers, 2020; Jiao et al., 2023). Depending on the plant species, roots can also modify the soil structure, pH, carbon content, and community of microorganisms (Nye, 1981; Angers and Caron, 1998), which all can affect the sink/source role of the soil for VOCs (Insam and Seewald, 2010; Peñuelas et al., 2014; Tang et al., 2019). In our experiment, as few VOCs were shared between non-excavated root and bare soil fluxes, the presence of roots added new VOCs to the VOC blend emitted by the soil and doubled or tripled its net emission compared to the bare soil.

Many VOC were shared between the above- and below-ground VOC profiles of a given plant species but not between species, partly confirming our second hypothesis. However, some VOCs, among the most emitted (e.g. methanol, acetaldehyde or acetone), were ubiquitous. Acetaldehyde, the second main VOC emitted by excavated roots, was detected only in the presence of roots, although it is also known to be emitted by leaves, especially upon wounding (Loreto et al., 2006). Acetic acid and acetoin that were detected only from non-excavated root emissions may come from soil microbial activity. Gulati et al. (2020) also detected acetic acid from emissions at tomato-planted soil surface but not in soil near the tomato roots. Nonetheless, its high partitioning into water (Sander, 2014) can make its detection particularly sensitive to humidity, which may be why it was not consistently detected in belowground VOC. Acetoin has already been detected by Abis et al. (2020) in soil microbial emissions. Further studies may confirm whether these VOCs are consistently emitted by root systems.

Interestingly, cyclopentyl isothiocyanate was found in rapeseed non-excavated root emissions but not in aboveground ones. Isothiocyanates are known to be produced during glucosinolate degradation upon brassica plant damage (Halkier and Gershenzon, 2006; van Dam et al., 2012). In a meta-analysis van Dam et al. (2009) reported higher concentrations and a greater diversity of glucosinolates, in roots than in shoots. These authors postulated that roots may have higher constitute levels of glucosinolates due to a stronger pathogenic pressure from soil microorganisms. These observations may explain the presence of cyclopentyl isothiocyanate in constitutive root emissions.

Fluxes from non-excavated plants were more homogenous between compartment and species when expressed by tissue dry mass rather than by plant. Thus, the VOC emission of roots may be linked to their biomass, as it has already been considered for leaf VOC emissions in the same vegetation type (Guenther et al., 1995).

4.2. Diurnal dynamic of root VOC emissions

Many studies on VOC soil fluxes (with or without roots) have reported a positive correlation to temperature (Schade and Custer, 2004;

Gray et al., 2014; Bachy et al., 2018), which can result from biotic and/or abiotic processes (Stotzky and Schenck, 1976; Peñuelas et al., 2014; Tang et al., 2019). Our results may show a diurnal root VOC flux variation with higher emissions during the day than at night. This could be partly due to the 3 °C rise in temperature that we observed during the illumination period. Gray et al. (2014) reported a positive temperature correlation only when photosynthate transport from shoots to roots was not blocked, indicating that the VOC fluxes from the forest floor were mainly linked to biological processes in the root system and its associated rhizosphere. However, a higher number of repetitions in this study would have helped determine the biotic or abiotic nature of the diurnal variation observed for non-excavated root emissions.

4.3. Methanol emission by root systems

Methanol was generally the first contributor to belowground emissions in our experiment and showed a spectacular increase after roots' excavation from soil. Interestingly, methanol emissions from non-excavated root systems have been barely reported. The use of GC-MS, such as in the study of Dehimeche et al. (2021), may be unsuitable for detecting such a low-mass polar compound. Its non-detection by Acton et al. (2018) is more surprising considering the high degree of similitude between our experimental set-ups. However, higher methanol emissions from non-excavated *Brassica rapa* roots have already been reported by Danner et al. (2015) (Table 2). As only a few VOCs were characterized by Danner et al. (2015), the relative importance of methanol to total VOC root emissions cannot be assessed. Field studies showed that methanol was the first contributor to VOC exchanges from planted soils (Gray et al., 2014; Trowbridge et al., 2020). While Gray et al. (2014) observed net methanol emissions, Trowbridge et al. (2020) reported net deposition (Table 2).

Considering the methanol emission from excavated roots, it is likely that the net methanol emissions from non-excavated roots originated from the roots themselves, rather than from soil microorganisms. Methanol is known to be predominantly produced by the demethylesterification of cell wall pectin in plants and, to a lower extent, from the demethylation of macromolecules (Dorokhov et al., 2018). The demethylesterification process occurs through pectin methyltransferase (PME) activity, which is associated with the root elongation zone and root cap (Stephenson and Hawes, 1994; Palin and Geitmann, 2012). These results suggest that roots, similar to leaves, have a high capacity for constitutive methanol production. Soil microorganisms can oxidize methane into methanol, which can then be further transformed by micro-organisms into formaldehyde and ultimately into CO₂ (Hogendoorn et al., 2020). Methanol is also emitted by dead plant material (Warneke et al., 1999; Gray et al., 2010) and from pectin degradation by microorganisms that are usually phytopathogenic (Hayat et al., 2010; Gupta et al., 2015). Since the litter was removed in our experiment and the plants were young and healthy, these sources are unlikely to contribute significantly to the observed methanol emissions. A microbial origin cannot be excluded entirely, because a few non-pathogenic bacteria are also known to degrade pectin (Hayat et al., 2010).

After excavation, total root VOC fluxes increased by approximately 50 times, essentially driven by the increased methanol emission in both species. To our knowledge, only Steeghs et al. (2004) have detected direct methanol emission from roots, using artificially-grown root cultures plunged in a liquid culture medium. However, no quantitative flux was reported. Our measurements corresponded to a first attempt to quantify VOC emissions from the entire root systems separated from the soil, although their excavation led to the loss of one and two-thirds of their original masses and surfaces, respectively. Moreover, the excavation process generated wounding and may have induced stress by excluding roots from their natural soil environment. Indeed, two VOCs suggest that the roots were stressed by excavation: isothiocyanate (C₉H₉NS) emitted only from excavated rapeseed roots and GLV from both species. Isothiocyanates have already been detected in artificially-

wounded brassica roots (Crespo et al., 2012; van Dam et al., 2012). Lee Díaz et al. (2022) detected the GLV nonenal in the emissions of tomato roots upon herbivory feeding but not for non-stressed roots. Mechanical damage and pathogen attacks can also increase methanol emissions from aboveground organs (Dorokhov et al., 2018). However, stress-induced methanol emissions may not always be observed in all plant species (Rasulov et al., 2019), nor in belowground parts of *Brassica rapa*, as mentioned by Danner et al. (2015). Since mechanical damage on the leaf results in a transient methanol emission burst (Loreto et al., 2006; Brilli et al., 2011), methanol additional root emission generated by physical damage can be considered unlikely to participate in the methanol root fluxes measured 5.5 h after excavation. The effect of soil removal on methanol root emissions is nonetheless unknown.

Considering the massive increase in methanol root emissions after excavation compared to the other VOCs and its predominant contribution to total VOC fluxes for both species, methanol could either be an exceptional stress-induced root VOC, or the main VOC emitted by roots in the absence of soil. The net methanol emission measured for non-excavated roots and the methanol sink behavior of the bare soil throughout the 36 h measurements suggest that methanol was constitutively emitted by roots and was mostly retained or transformed within the soil. Since the root epidermis has no cuticle, the polar methanol mainly generated outside the lipid cell membrane during the demethylation of cell wall pectin may be expected to diffuse from the root to the rhizosphere and soil readily. Once in the soil, methanol can be dissolved in the liquid phase, be adsorbed at gas/liquid and liquid/solid interfaces, and/or be consumed by microorganisms (Stotzky and Schenck, 1976; Kim et al., 2005; Insam and Seewald, 2010; Peñuelas et al., 2014; Tang et al., 2019). Studies on the fate of VOCs in soils are still lacking, so we cannot estimate the degree of significance of these soil abiotic and biotic processes in this behavior (Bachy et al., 2018). However, methanol dissolution into water appears somewhat uncertain since belowground methanol emissions did not rise throughout the measurement period, as could have been expected from the decrease in soil water content due to plant transpiration and soil evaporation. Given the far lower increases of the other main VOC root fluxes after excavation, if the methanol sink was explained by adsorption at soil interfaces, this would mean that this soil presented a remarkable affinity for methanol. To our knowledge, the methanol adsorption in soil has not yet been characterized. However, Albers et al. (2018) found a very fast and high mineralization of methanol compared to other VOCs in different soil types, explained mainly by microbial degradation. This microbial consumption may be even more critical in the rhizosphere, which is the preferential soil zone for methylotrophic communities. Indeed, a meta-analysis from Ling et al. (2022) demonstrated an increase in methylotrophy and methanol oxidation by 47 % and 78 %, respectively, when moving from the bulk soil to the rhizosphere. Macey et al. (2020) showed that rhizosphere methylotroph communities assimilated pea root exudates. In both the phyllosphere and rhizosphere of germinating and young *Medicago truncatula* plants, the microbial methanol metabolism provided a competitive advantage for wild-type strain AM1 of *Methylobacterium extorquens* compared to methylotrophy mutants that are unable to grow with methanol as a unique source of energy and carbon (Sy et al., 2005). These results tend to confirm the consumption of root methanol by at least some root methylotrophs. Moreover, Stacheter et al. (2013) found a higher methanol oxidation activity from root-associated soils than from root-free soils, even at nanomoles of methanol per gram of soil, while roots alone were not a net sink for methanol.

Overall, our results suggest that roots release large amounts of VOC, mainly as methanol, which may be mostly retained and/or transformed within the soil. Supposing that all methanol released from excavated roots was consumed by the rhizosphere, the consumption rate would be around 250 nmol per gram of dry root and per hour. This is in the same order of magnitude as the methanol consumption rate of 87 ± 12 nmol g DW⁻¹ h⁻¹ reported by Kanukollu et al., (2022) for *Taraxacum officinale* rhizosphere. In terms of carbon loss, the total VOC flux of excavated

roots was 66 and 76 $\mu\text{g C g DW}^{-1} \text{ day}^{-1}$ for rapeseed and tomato, respectively. If these fluxes represent the normal constitutive emissions by roots, they could account for an important portion of the total release of organic matter by roots, i.e. rhizodeposition. Comparatively, the exudation of total organic acids by tomato roots varies between 5 and 500 $\mu\text{g C g DW}^{-1} \text{ day}^{-1}$ (Imas et al., 1997; Kuijken et al., 2015), and that of dissolved organic carbon by rapeseed roots between 700 and 1470 $\mu\text{g C g DW}^{-1} \text{ day}^{-1}$ (Svenningsson et al., 1990). Assuming that other rhizodeposition processes such as the secretion of mucilage and the release of cap cells amount to <10 % of total C rhizodeposition (Nguyen, 2003; Rees et al., 2005) the VOC emissions measured here on excavated roots would represent ca. 10 % of the total rhizodeposited C. However, these estimates should be considered as an upper limit, as the emissions from bare roots may have been enhanced by the excavation stress. The significant difference in root emissions observed with and without soil highlights that any cropping practice in which root systems are partially or entirely excavated (e.g. ploughing, harvesting or mechanical removal of weeds) can lead to large VOC bursts, potentially altering plant-organism interactions and air quality in agroecosystems.

4.4. A new set-up for investigating plant VOC emissions

Our set-up likely limited the variability between the repetitions since the VOC fluxes of three plants were simultaneously measured. It enabled to monitor time-variations of belowground VOC emissions in an unprecedented manner, as discussed below. It can be used for VOC flux measurements of plants at different phenological stages, up to one-meter tall plants. The experimental parameters were set to optimize the measurement of very low VOC fluxes while limiting the disturbance of natural processes. First, we injected VOC-free air in both compartments to diminish the relative measurement uncertainties, especially for soil VOC fluxes. However, VOC-free air leads to the overestimation of the plant VOC emissions and hides the bidirectional VOC exchanges between soil and atmosphere which represents a non-negligible process (Rinnan and Albers, 2020). Our results should therefore be considered as an upper estimation of total VOC emission potential rather than of actual VOC exchange with the atmosphere. Second, a quite long residence time was also set in the chamber to maximise VOC concentrations. Consequently, RH was high and varied during the course of the day, especially in the aboveground compartment. Peak intensities of VOCs can fluctuate depending on the RH (Trefz et al., 2018), and plant transpiration decreases at high RH, lowering the transport of dissolved hydrophilic VOCs. Third, a small overpressure was set in the belowground compartment, resulting in a weak air flux from the below- to the aboveground compartment to ensure no contamination from the above- to belowground compartment. Fourth, to avoid root desiccation after excavation, a few centimeters of water were introduced in the bottom of the pots where hydrophilic VOC could have been partly retained, thus minimizing the actual VOC emissions from roots.

Fifth, the excavation of roots, leading to one-third mass loss, likely created mechanical stress response in the entire plant system. In addition to the increase in VOCs emitted upon stress (such as GLV), a diminution by nearly one-third of the CO_2 assimilated was observed for aboveground tomato after excavation. In comparison, aboveground rapeseed gas emissions were more negligible. Our study was a first attempt to quantify root VOC emissions without the soil surrounding the roots. Characterizing the constitutive VOC root emissions before their alteration in soil without impairing the root system activity is complex. Further studies may provide additional insight into the root VOCs that are emitted upon root disturbances to verify if methanol root emission can be induced by stress. These experiments may include the response of above- and belowground VOC emissions after mechanical root wounding, soil temperature variation, soil water content decrease. Studies on root emissions at the soil surface may be performed with different soil types to evaluate the role of soil on root VOC emissions.

5. Conclusion

Our study aimed to assess whether roots can significantly contribute to the total VOC emitted by crop plants and whether VOC profiles would differ between aboveground and belowground compartments. The results showed that root VOC profiles were largely species specific and comprised many VOCs also emitted by the aboveground parts. Only a small number of VOCs, among the most emitted ones, were detected in both species. Root systems contributed only to a small extent to total VOC emissions from tomato and rapeseed when growing in soil but emitted as much VOC as the aboveground part of the plant when excavated from the soil. Methanol contributed almost exclusively to belowground VOC emissions of both excavated plant species. Roots may therefore increase total crop VOC emissions as soon as they come into contact with open air, highlighting the possible impact of soil mechanical disturbances on air quality. These results suggest that the rhizosphere may be a strong biotic sink for root-borne methanol.

CRedit authorship contribution statement

Auriane Voyard: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Raluca Ciuraru:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Conceptualization. **Florence Lafouge:** Writing – review & editing, Data curation, Conceptualization. **Céline Decuq:** Writing – review & editing, Formal analysis, Data curation. **Alain Fortineau:** Visualization, Validation, Methodology. **Benjamin Loubet:** Writing – review & editing, Supervision, Software, Conceptualization. **Michael Staudt:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Conceptualization. **Frédéric Rees:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.177081>.

Data availability

Data will be made available on request.

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