Letters

Fourier transform infrared spectroscopy and interference of volatile organic compounds on measurements of methane (CH_4) fluxes at tree stems – a general phenomenon for plant systems?

A response to Kohl *et al*. (2021) 'Towards reliable measurements of trace gas fluxes at plant surfaces'

This Letter responds to the comments of Kohl *et al*. (2021) regarding their concerns as to the reliability of methane (CH_4) concentration measurements from stems of tropical trees on volcanic Réunion Island measured by Fourier transform infrared (FTIR) spectroscopy. We acknowledge the points mentioned by Kohl *et al*. (2021) and use this Letter to discuss this topic. We first present the FTIR analyzer settings used during our study on Réunion (Machacova *et al*., 2021), details on how CH_4 concentration data were measured and processed, and results of postprocessing interference analyses. We then show experimental results comparing FTIR and a gas chromatography analyzer for measurements of CH_4 and nitrous oxide (N_2O) fluxes in stems of beech trees. Finally, we examine why we revealed no substantial discrepancies in tree stem CH_4 fluxes as measured by Kohl *et al*. (2019) using an FTIR analyzer.

Based upon our current knowledge, postprocessing analyses and study comparing instruments, it seems unlikely that instrumental biases existed in stem CH_4 measurements caused by potential interference from volatile organic compounds (VOCs). The tree stems investigated on Réunion, which are mostly endemic species, appear to behave as net sinks of atmospheric CH_4.

Measurements of stem CH_4 fluxes on volcanic Réunion Island: FTIR analyzer settings, data processing, consideration of potential VOC interference and results

The greenhouse gas (GHG) fluxes on Réunion Island were measured using a portable DX-4015 FTIR gas analyzer (Gasmet Technologies Oy, Vantaa, Finland), equipped with an official GHG application (GAS-APP-006 spectral library, hereafter termed the 'limited library') to detect water vapor, carbon dioxide (CO_2), CH_4, N_2O, ammonia and carbon monoxide, and extended for nitrogen monoxide and nitrogen dioxide. All gases were explicitly calibrated for our analyzer by the manufacturer. Every morning during the measurement campaign, zero-point calibration of the analyzer was made using nitrogen (N_2, 99.9992% purity) to ensure precise measurements.

The quality of the measured GHG concentrations was monitored continuously during the real-time measurements by evaluating residual values and graphs for each spectrum measurement and analyzed gas compound (Gasmet, 2015). Low residual values denote a reliable analysis, whereas high values indicate nonpreferred gas compounds within the gas sample. Such compounds would not occur in the spectral library and thus could interfere with the individual gas measurements. The residual values were automatically controlled and reported using CALCMET software (v.12.18; Gasmet Technologies Oy). We examined every residual value in detail for CH_4 concentrations obtained from the presented stem flux measurements. The residual values for CH_4 signal across the stem chamber measurements (0.0018 ± 0.0001 absorbance scale, mean $\pm$ SE) were one order of magnitude lower than the 0.01 set as a threshold for reliable analysis (Gasmet, 2015).

In response to the comments of Kohl *et al*. (2021) and the study of Kohl *et al*. (2019), we evaluated potential interference in the stem CH_4 measurements from 11 VOCs commonly occurring in tropical trees (Courtois *et al*., 2012). We reprocessed the obtained spectra using our limited library extended for the following VOCs (hereafter termed the 'extended library'): alpha-pinene, beta-pinene, carvone, delta-3-carene, limonene, methanol, *o*-xylene, *p*-xylene, terpinen-4-ol, terpineol and toluene.

In order to include representatives of all six tree species studied and measurements of CH_4 uptake rates across the entire range detected for the trees, we examined the potential VOC interference on three to five stem flux measurements from the following tree species: *Syzygium borbonicum* ($n = 4$), *Doratoxylon apetalum* ($n = 4$), *Antirhea borbonica* ($n = 5$), *Homalium paniculatum* ($n = 3$), *Mimusops balata* ($n = 3$) and *Labourdonnaisia calophylloides* ($n = 3$). Using the extended library, we postanalyzed two spectra within each chamber measurement: one spectrum obtained *c*. 3 min after chamber closure (i.e. T_0) and another *c*. 3 min before chamber opening (i.e. T_f). We calculated the CH_4 concentration change ($T_0 - T_f$) for spectra reprocessed with the extended library and spectra treated with the limited library. We then compared these two differences for each stem chamber measurement.

Although the absolute CH_4 concentrations changed after reprocessing of the spectra using the extended library (i.e. drift in gas concentrations), the differences in the CH_4 concentrations between T_0 and T_f remained relatively unchanged (0.061 ± 0.004 and 0.059 ± 0.005 ppm for the limited and extended libraries, respectively). This means that extending the library affected quantification of the absolute CH_4 concentrations, but it had no

substantial impact on the slope of CH_4 concentration change in the chamber headspace over time, which is relevant for the flux calculation (for equations, see Machacova *et al.*, 2016). Finally, we calculated how much the pairs of CH_4 concentration differences varied from one another on a percentage scale. Using the percentage values obtained for each tree and the CH_4 fluxes reported in Machacova *et al.* (2021, limited library), we calculated new stem CH_4 uptake rates for all tree species, which are now corrected for potential interference.

The recalculated stem CH_4 uptake was on average only $3.5 \pm 5.0\%$ lower than the uptake calculated initially without consideration of possible VOC interference (Fig. 1). By comparison, Kohl *et al.* (2019) had measured 41% difference on spruce trees. The effect of VOCs on quantification of stem CH_4 fluxes appears to be highly species-dependent. In the cases of *H. paniculatum* and *L. calophylloides*, the inclusion of VOCs in the spectral library increased stem CH_4 uptake, whereas the inclusion resulted in decreased uptake for other tree species (Fig. 1).

After reconsidering potential VOC interference on the CH_4 measurements, all studied tropical tree stems remained net sinks of CH_4 from the atmosphere (Fig. 1). The corrected stem CH_4 uptake (-15.1 ± 1.9 μg CH_4 m^{-2} h^{-1}) did not significantly differ from the stem CH_4 uptake calculated initially (-15.6 ± 2.0 μg CH_4 m^{-2} h^{-1}). As in Machacova *et al.* (2021), the CH_4 uptake remained uniform across the various tree species and did not show significant species-specific variability (Fig. 1).

We performed a similar spectral postanalysis for the Réunion-studied cryptogamic stem covers, which often fully cover the bark. As seen for stems, the potential VOC interference-corrected CH_4 uptake by cryptogams (-7.7 ± 2.4 μg CH_4 m^{-2} h^{-1}) did not vary significantly from the initially calculated uptake (-8.3 ± 3.0 μg CH_4 m^{-2} h^{-1}; Fig. 1).

Therefore, the potential VOC interference is not a significant problem in the tropical tree species and cryptogams studied on Réunion Island, and the results presented in Machacova *et al.* (2021) are reliable. Based on our postprocessing analyses, we may confirm that the mostly endemic tree species and cryptogams investigated on Réunion do behave as net sinks of CH_4 from the atmosphere.

Comparison of FTIR and gas chromatographic approaches for determining CH_4 (and N_2O) fluxes in stems of beech trees under field conditions

We measured CH_4 and N_2O fluxes from five mature beech stems (*Fagus sylvatica*) in a montane beech forest in the White Carpathians during August 2018. For GHG detection, we used the FTIR analyzer with limited library (DX-4040; Gasmet Technologies Oy; Warlo *et al.*, 2018) and a gas chromatography analyzer (GC; Tracera, Shimadzu Corp., Kyoto, Japan; Maier *et al.*, 2018). The CH_4 and N_2O fluxes from tree stems (*c.* 0.4 m aboveground) were measured using static stem chamber systems (Machacova *et al.*, 2017, 2021). For GC analysis, eight gas samples were taken from the systems at time intervals of 0, 20, 40, 70, 105, 150, 200, 260 min after closure. The gas fluxes were quantified based upon the linear changes in GHG concentrations within the chamber headspace over time (Machacova *et al.*, 2016).

The results (Fig. 2) illustrate close agreement between the FTIR and GC analyzer in quantifying CH_4 and N_2O fluxes in beech stems. The stem CH_4 emissions detected using the FTIR (6.0 ± 5.2 μg CH_4 m^{-2} h^{-1}) and GC (6.2 ± 5.3 μg CH_4 m^{-2} h^{-1}) did not differ significantly. Likewise, no significant discrepancy was identified for the low stem N_2O uptake (-0.44 ± 0.17 and -0.53 ± 0.26 μg N_2O m^{-2} h^{-1}, respectively). Such low and nonsignificant variability between the measurement techniques can be expected from diurnal cycles and daily variation. Still, the present analysis cannot separate these temporal effects from potential low VOC interference.

In conclusion, at least in the case of beech trees, it seems that even the FTIR technology used with only the limited library produced

Fig. 1 Fluxes of methane (CH_4) from tree stems and cryptogamic stem covers on volcanic Réunion Island measured with Fourier transform infrared (FTIR) spectroscopy while applying a limited spectral library (gray bars) and a spectral library extended for the interference of 11 volatile organic compounds commonly occurring in tropical trees (dark gray bars). See main text for library details. Fluxes are expressed as medians (solid line) and means (broken line) of measurements from stems of six individual tree species (Syzbor, *Syzygium borbonicum* ($n = 5$); Dorape, *Doratoxylon apetalum* ($n = 5$); Antbor, *Antirhea borbonica* ($n = 5$); Hompan, *Homalium paniculatum* ($n = 3$); Mimbal, *Mimusops balata* ($n = 3$); Labcal, *Labourdonnaisia calophylloides* ($n = 3$)), stems of all studied trees and tree species ('all trees'; $n = 24$), and cryptogams (*Pyrrhobryum spiniforme*, *Leucoloma capillifolium*; $n = 4$). All fluxes, including fluxes from cryptogams, are expressed per m^2 of stem area. Negative fluxes indicate trace gas uptake. Box boundaries mark 25th and 75th percentiles. Flux data were checked for normal distribution (Shapiro–Wilk test) and equality of variances in the different subpopulations. No statistically significant differences were observed between fluxes in individual tree species and in cryptogams detected by FTIR spectroscopy using limited vs extended spectral library at $P < 0.05$ (Mann–Whitney rank-sum test).

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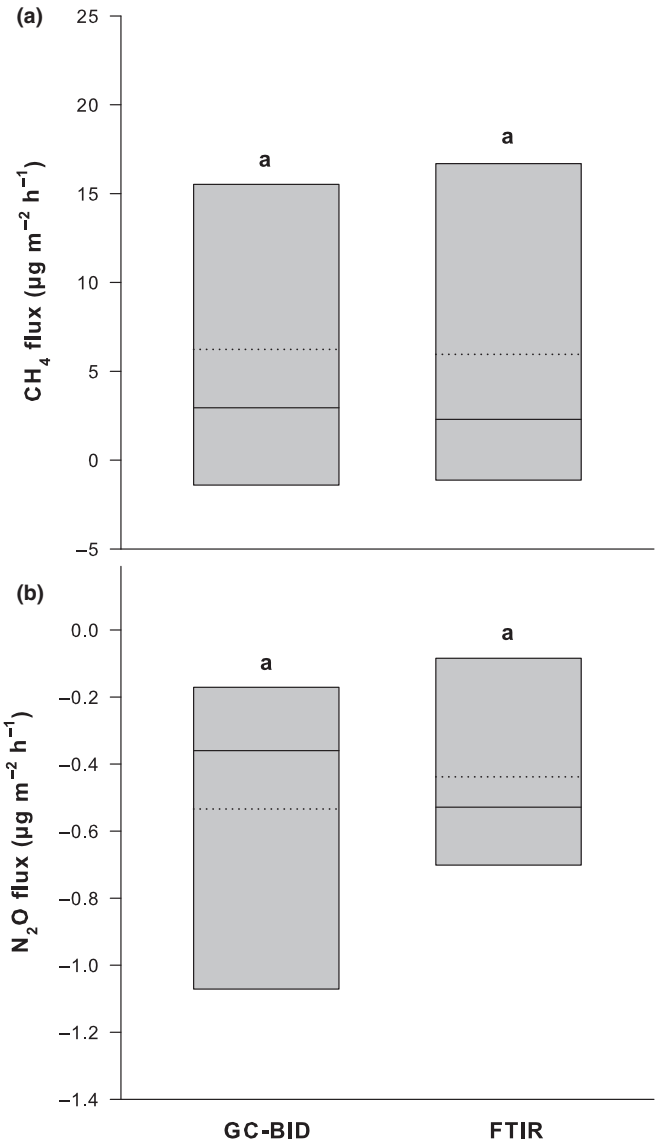

Fig. 2 Methane (CH_4, a) and nitrous oxide (N_2O, b) fluxes in beech stems ($n = 5$) measured using a portable Fourier transform infrared (FTIR) analyzer (DX-4040, limited spectral library) and gas chromatography equipped with a barrier discharge ionization detector (GC-BID). Fluxes are expressed as medians (solid line) and means (broken line) of measurements from stems of five mature beech trees (*Fagus sylvatica*) measured in a temperate beech forest in the White Carpathians. Gas sample uptake for GC analyses was performed one day before measurements with the FTIR analyzer. Measurements were run over three consecutive days. Fluxes are expressed per m^2 of stem area. Positive fluxes indicate trace gas emission, negative fluxes trace gas uptake. Box boundaries mark 25th and 75th percentiles. As indicated by the letter 'a' above the bars, there were no statistically significant differences between the fluxes detected by FTIR spectroscopy vs the GC approach at $P < 0.05$. Flux data were checked for normal distribution (Shapiro–Wilk test) and equality of variances in the different subpopulations. Student's *t*-test was applied for N_2O data. The nonparametric Mann–Whitney rank-sum test was used for CH_4 data.

reliable results comparable with those from the GC approach. Therefore, considerable VOC interference on stem CH_4 flux measurements cannot be generalized for all ecosystems.

Possible reasons for the lack of significant discrepancies between tree stem CH_4 fluxes measured by FTIR with and without correction for potential VOC interference and by GC

In contrast with Kohl *et al.* (2019), we observed a nonsignificant impact of VOCs on the measurements of stem CH_4 fluxes in various tropical tree species and temperate beech trees when using the FTIR analyzer. The reason could be that our measurements were taken under field VOC concentrations. By contrast, the interference with CH_4 concentration studied by Kohl *et al.* (2019) was observed when applying VOCs mostly in ppm mixing ratios that copied industrial conditions rather than field ppb ranges (Asensio *et al.*, 2008). In the qualitative experiment, Kohl *et al.* (2019) tested interference of the following VOCs in a range of mixing ratios stated in ppbv (their Table 2, Experiment 1): methanol (6000–10 000), α-pinene (4000–5000), β-pinene (5000–15 000), Δ^3-carene (3000–7000), R(+)limonene (900–1100), linalool (7000–12 000), *cis*-3-hexen-1-ol (20–60), *trans*-2-hexenyl acetate (500–2000) and toluene (30 000–35 000). Based on field measurements, typical VOC mixing ratios are in a range of only a few ppb (e.g. isoprene and sum of monoterpenes < 4 ppb, methanol maximally in the tens of ppb, toluene 2.5–3.5 ppb). These ratios are seen at the Czech ICOS forest station (mature Norway spruce, September–October 2020; Juráň *et al.*, 2017) and urban area of Brno, Czech Republic (S. Juráň, pers. comm.). The trace gas fluxes from tree stems commonly are measured using various chamber enclosures working on the principle of increasing or decreasing trace gas concentrations in chamber headspace over the closure time resulting from gas emission or uptake by the stems, respectively. For example, Kohl *et al.* (2019, Table 4 therein) present the mixing ratio of only 20 ppbv monoterpenes at the end of stem chamber closure under the typical VOC emission scenario and 320 ppbv under peak events. These mixing ratios are two to four orders of magnitude lower than the aforementioned VOC ratios applied in the same authors' qualitative laboratory tests.

Figs 4(a) and 5(a) in Kohl *et al.* (2019, Experiment 2) present quantitative determination of the VOC effect (tested on β-pinene in the concentration range *c.* 0 to 12 000 ppb) on CH_4 measurements using FTIR technology. Detailed observation of the light blue line belonging to the apparent CH_4 concentration measured with the limited library and the black line presenting β-pinene concentration reveals a time-shift or delay in CH_4 concentration decrease in response to increasing β-pinene concentration (i.e. CH_4 begins to decrease when the β-pinene concentration reaches *c.* 1000 ppb). It seems that lower mixing ratios of β-pinene (below *c.* 1000 ppb) might not affect CH_4 mixing ratio measurements when using the FTIR analyzers, even when only a limited library not including VOCs is utilized. Kohl *et al.*'s (2019) Fig. 4(c) and the linear regression line through the nontransient data (black dots) indeed suggest that there might be interference at near-ambient VOC concentrations. However, the authors excluded data at low VOC concentrations (gray dots) as they occurred after rapid changes in the β-pinene mixing ratio. In our understanding, the nonlinear rather than linear regression trend can indicate unchanged CH_4 mixing ratio detected by gradual increase in the

β-pinene mixing ratio up to 1000 ppb measured with the FTIR analyzer (limited library).

Given the typical mixing ratio of 20 ppbv monoterpenes at the end of stem chamber closure in coniferous Scots pine trees (example from Kohl *et al.*, 2019), which are known to be substantial sources of monoterpenes (Vanhatalo *et al.*, 2020), we are skeptical that this provides clear evidence for significant VOC interference on CH_4 measurements in such a system – even when only a limited library is used.

We raise the possibility that the high VOC mixing ratios biasing field measurements of CH_4 fluxes on spruce stems presented by Kohl *et al.* (2019) might have originated also from organic silicones used as sealing and adhesion material in relation to the chambers, as these are known to be substantial emitters of VOCs when fresh. A second cause for these high ratios might relate to resins exuded from the spruce bark and caused by injuries to surface bark layers during the stem chambers' installation. For their study, Kohl *et al.* (2019) had selected only one tree species (coniferous Norway spruce), which among tree species is a rather strong emitter of monoterpenes (Juráň *et al.*, 2017).

Hence, the question arises as to how transferable to field conditions is the finding by Kohl *et al.* (2019) and whether it can be generalized for all plant systems worldwide. In our opinion, it is complicated to upscale and compare the extraordinary application of very high VOC concentrations to the much lower VOC concentrations typical for forest ecosystems under natural conditions.

In general, the VOC emission rates from tree stems and their chemical compositions are characterized by high interspecies and intraspecies variability and may change over time in response to shifting environmental conditions and stresses (e.g. defensive needs), as well as seasonal dynamics (Courtois *et al.*, 2012; Niinemets *et al.*, 2013; Jones *et al.*, 2014; Juráň *et al.*, 2017; Celedon & Bohlmann, 2019; Rissanen, 2019; Vanhatalo *et al.*, 2020). Likewise, stem CH_4 (and N_2O) exchange, including both gas emission and uptake, is highly variable across various tree species, individual trees and within the stem vertical profile (e.g. Sundqvist *et al.*, 2012; Díaz-Pinés *et al.*, 2016; Machacova *et al.*, 2017, 2019; Warner *et al.*, 2017; Pitz *et al.*, 2018; Welch *et al.*, 2018; Covey & Megonigal, 2019). These facts point to different sensitivities of various tree species to VOC interference. Therefore, we believe more studies focused on low, near-ambient VOC concentrations and various tree species of different forest types across the geographical and climatic gradient are needed to drive more general conclusions about the effect of VOCs on CH_4 concentration measurements in stem chamber systems when using FTIR technology.

In summary, our results show that recalculation for potential interference of commonly occurring VOCs can result in changes in the measured absolute CH_4 concentrations. Nevertheless, the slope of the CH_4 concentration changes over the duration of the stem chamber's closure, and therefore of the slope calculated from that data for stem CH_4 fluxes of tropical trees on Réunion, seems to be much less affected by VOCs (on average by $3.5 \pm 5.0\%$) than the 41% suggested by Kohl *et al.* (2019, 2021). Moreover, the stem CH_4 fluxes measured on beech trees using the FTIR analyzer, even without considering potential VOC interference, were validated by the GC approach.

We acknowledge that VOCs might interfere with CH_4 concentration and stem flux measurements when using FTIR technology. Validation of data and correction for the potential interference should be applied, as proposed in Kohl *et al.* (2019, 2021). Nevertheless, based on our current knowledge, our results and the inconsistencies identified in the study of Kohl *et al.* (2019), it seems that the interference is much less severe than that stated by Kohl *et al.* (2019, 2021). Any such interference is highly species-dependent and cannot be generalized for plant systems all over the world. Based upon our postprocessing analyses and performed measurements, we may say that the mostly endemic tree species and cryptogams investigated on Réunion do behave as important sinks of CH_4 from the atmosphere.

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Data availability

The datasets analyzed during this study are available from the corresponding author upon reasonable request.

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