Field-deployable branch enclosure system for biogenic volatile organic compounds emitted from conifers

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Abstract. Biogenic volatile organic compounds (BVOCs), emitted primarily from terrestrial plants, significantly influence atmospheric chemistry and climate change. Conifers are major sources of BVOCs in temperate regions. However, their unique physiology, particularly the storage of terpenes within their tissues, makes accurate measurements of BVOC emissions challenging owing to contact-induced release. We developed a portable dynamic branch enclosure system specifically optimized for BVOC measurement in conifers, which enables measurements of multiple trees in a single day. The system uses filtered ambient air as a purge gas to reduce logistical challenges and features a foldable bag design to minimize excessive BVOC release induced by physical contact. It provides BVOC- and ozone-free air, maintains stable internal temperature and humidity conditions closely approximating those of natural environments, and ensures repeatable measurements of BVOC emissions. ~~Field testing with Japanese cedar (*Cryptomeria japonica*) revealed significant individual variations in BVOC emission rates and compositions.~~ Field testing with Japanese cedar (*Cryptomeria japonica*) demonstrated the system's robust field performance, successfully capturing both significant inter-individual variability and the dynamic diurnal patterns of BVOC emissions. ~~Field testing with Japanese cedar (*Cryptomeria japonica*) successfully captured significant individual variations in BVOC emission rates and compositions.~~ The system's ability to reliably resolve these differences under field conditions demonstrates its applicability for advancing our understanding of BVOC dynamics in diverse ecosystems.~~These findings demonstrate the system's reliability and applicability for assessing BVOC emissions under field conditions, offering a practical solution for advancing our understanding of BVOC dynamics in diverse ecosystems.~~

1. Introduction

Biogenic volatile organic compounds (BVOCs), emitted primarily from terrestrial plants, play a significant role in atmospheric chemistry. Through atmospheric degradation processes, BVOCs contribute to the formation of tropospheric ozone and secondary organic aerosols (Chatani et al., 2015; Ghirardo et al., 2016; Laothawornkitkul et al., 2009), which significantly affect air quality. These atmospheric constituents can alter radiative forcing and precipitation patterns, influencing the global climate (Randall et al., 2013; Rotstayn et al., 2009).

Among terrestrial plant species, conifers are major BVOC emitters in temperate regions, and their emissions have

important implications for regional air quality and climate (Peñuelas ~~&~~and Staudt, 2010). They release a diverse array of BVOCs, notably monoterpenes (MTs), sesquiterpenes (SQTs), and diterpenes (DTs) (Chatani et al., 2018; Guenther et al., 1993, 2012; Matsunaga et al., 2011). Monoterpenes (C_{10} compounds) are highly volatile and major BVOCs in temperate and boreal regions (Guenther et al., 1993). Sesquiterpenes (C_{15}) have a notable role in atmospheric processes, particularly in particle formation, as their oxidation produces ultra-low volatility organic compounds that act as efficient nucleators (Dada et al., 2023). Diterpenes (C_{20}) have even lower volatility but high reactivity, which may make them particularly important for particle formation, although these properties also pose challenges for their observation.

The profiles of BVOC emissions from plants are determined by a complex interplay of factors, including environmental conditions such as temperature and drought (Birami et al., 2021), genetic factors, and biotic stresses such as plant–microbe interactions (Saunier et al., 2020). Moreover, some plant species alter their emission profiles in response to BVOCs emitted by neighbouring plants (Arimura et al., 2012). However, how these factors influence the composition and rates of BVOC emission remains insufficiently understood (Tani et al., 2024).

Recent comprehensive reviews have further underscored the critical and distinct roles of these terpene classes in biosphere-atmosphere interactions (Bourtsoukidis et al., 2024, 2025; Yañez-Serrano et al., 2024). For instance, diterpenes are now understood to be particularly potent contributors to SOA formation, potentially having a disproportionately large impact relative to their emission rates (Yañez-Serrano et al., 2024). Moreover, these reviews highlight that the emission rates and composition of MTs and SQTs can vary significantly among individuals, which may reflect diverse adaptive strategies to environmental stresses (~~Yañez-Serrano et al., 2024;~~ Bourtsoukidis et al., 2024, 2025). To untangle the complex factors governing these emissions, a dual approach of broad-scale analysis and detailed, individual-level data collection is essential. Quantifying emission characteristics at the level of individual plants is crucial for understanding how emissions change in response to varying environmental conditions. This understanding is essential for predicting future changes in BVOC–aerosol–climate feedbacks.

One challenge hindering individual-level BVOC measurements under natural conditions is the logistical difficulty associated with conventional sampling methods. Since conifers store terpenes within their needles (Saito et al., 2022), physical contact can trigger a burst release of these compounds, making it difficult to accurately measure their emission rates. The cuvette method, which clamps an individual leaf, is generally unsuitable for conifers for this reason. The dynamic branch enclosure method (Ortega et al., 2008; Ortega ~~&~~and Helmig, 2008) reduces direct leaf contact but still contacts the stem during device installation, which is particularly important consideration for conifers such as cedars that store terpenes in both leaves and stems. Several studies using dynamic branch enclosure methods on coniferous trees allowed a stabilization period after device installation of approximately 24 h (Helin et al., 2020; Helmig et al., 2013; Hiura et al., 2021; Matsunaga et al., 2011, 2012, 2013), 48 h (Bouvier-Brown et al., 2009), or 1–2 weeks (Praplan et al., 2020) to mitigate the effects of excessive emissions associated with physical contact during device installation. During the stabilization period, it is essential to either maintain continuous gas flow (Helin et al., 2020; Helmig et al., 2013; Hiura et al., 2021; Matsunaga et al., 2011, 2012, 2013) or avoid fully sealing an enclosure (Praplan et al., 2020) to sustain normal physiological conditions and prevent condensation before measurements are taken. However, when gas cylinders are used as the purge gas source in locations without a power supply, numerous cylinders must be transported to the site to maintain continuous gas flow throughout the measurement period, posing significant logistical challenges for field measurements (Hiura et

al., 2021; Matsunaga et al., 2011, 2012, 2013). Instead of gas cylinders, pumps can be used to supply purge air by drawing in external air. However, to our knowledge, no studies have clearly shown whether a pump-based system can be entirely powered by a portable battery, which is essential in remote areas without a power supply. These difficulties highlight the need for simplified, field-deployable techniques for BVOC measurement.

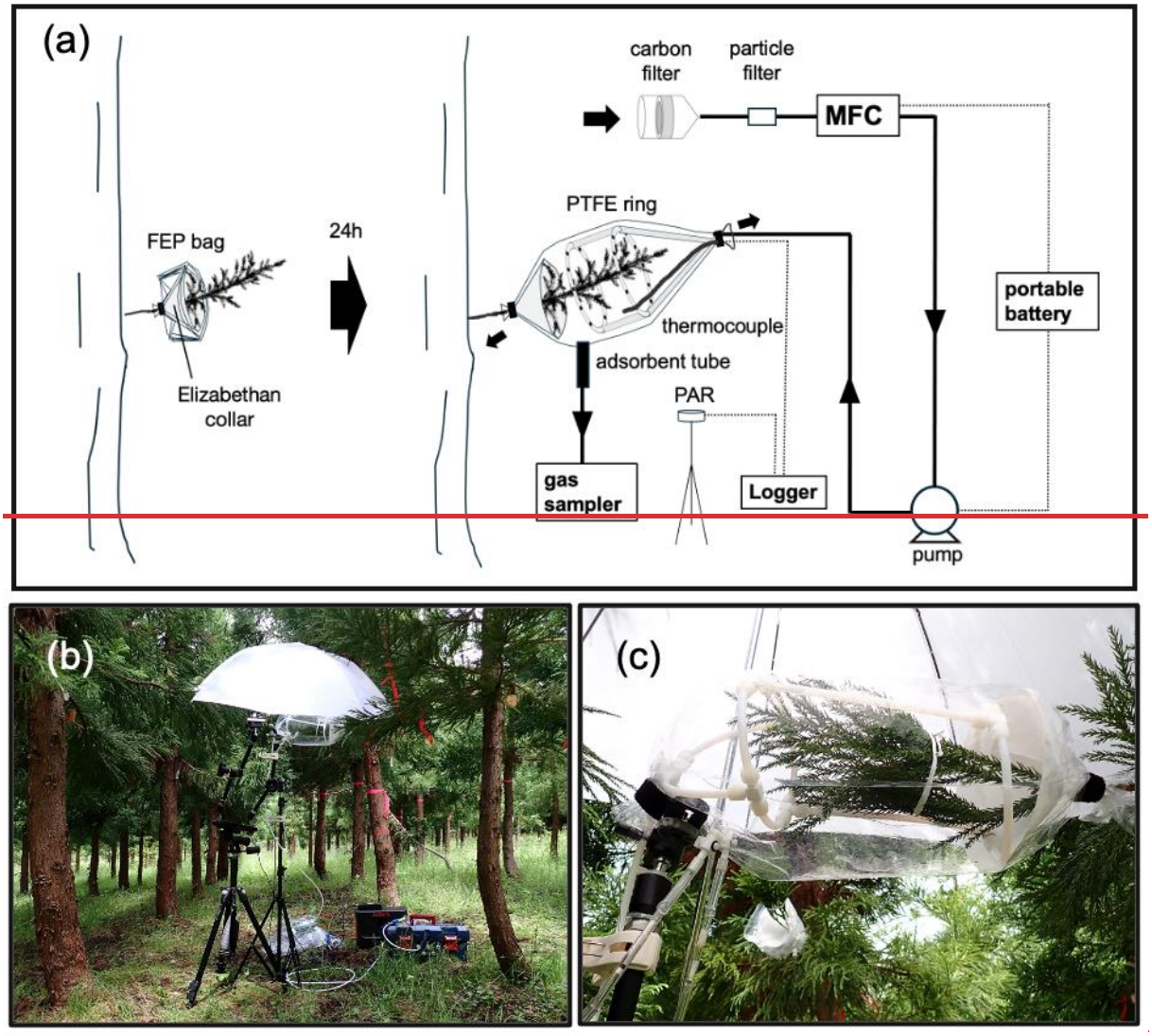
Here, we developed a simplified dynamic branch enclosure system for measuring BVOC emissions from conifers, which enables measurements of multiple trees in a single day. Our system addresses the limitations of conventional methods by using filtered ambient air as the enclosure purge gas and minimizing purge air consumption with a foldable bag design. The introduction of a foldable bag eliminates the need for purge gas during equipment setup. It enables a single person to measure BVOCs from up to five trees per day using the dynamic branch enclosure method. We describe the design and evaluation of our enclosure system and demonstrate its applicability through measurements of BVOC emissions from Japanese cedar (*Cryptomeria japonica*), one of the dominant conifer species in Japanese forests.

2. Methodology

2.1 Branch enclosure system

2.1.1 System design and components

The branch enclosure system is designed to deliver VOC- and ozone-free air while ensuring stable internal conditions for BVOC measurements (Fig. 1a). It uses a diaphragm pump (FD-15, IBS, Osaka, Japan) and an activated carbon cartridge (CHC-50, Advantec, Tokyo, Japan) to purify the purge air. The chamber consists of an open-ended fluorinated ethylene propylene (FEP) bag (25 cm width × 60 cm long, GL Sciences Inc., Tokyo, Japan) to enclose the branch under observation. To ensure circulation and homogenization of air inside the bag, purified air is introduced through 6 mm PTFE tubing formed into 2 rings, each containing 12 holes (approximately 1.5 mm in diameter) for the air to exit from. The 2 rings also provide structural integrity to avoid contact with the branch. The PTFE rings are supported by a tripod equipped with a flexible arm to minimize stress on the branch (Fig. 1b). A mass flow controller (D-6361-DR/FAS, Bronkhorst Japan, Tokyo, Japan) maintains a constant purge air flow rate of 5 L/min. Power for the mass flow controller and the diaphragm pump is supplied by a portable battery (JE-1000D, 30.4Ah/35.2V DC, Jackery, Tokyo, Japan). The temperature inside the bag and the nearby photosynthetically active radiation (PAR) are monitored by a PTFE-coated thermocouple (GL Sciences Inc., Tokyo, Japan) and a PAR sensor (MIJ-14PAR Type 2/K2, Environmental Measurement Japan, Fukuoka, Japan), respectively, using a data logger (Thermic model 2400A, ETOdenki, Tokyo, Japan). All purge lines were made with 6 mm PTFE tubing. The enclosure bag and PAR sensor were secured at an appropriate height on a tripod. A photographic white umbrella was used to diffuse and reduce the intensity of direct sunlight on the enclosure during purging and sampling.~~A parasol was used to reduce solar radiation and prevent leaf temperature increases during purging and sampling.~~


(a)
carbon filter
particle filter
MFC
PTFE ring
FEP bag
24h
thermocouple
portable battery
adsorbent tube
Elizabethan collar
PAR
gas sampler
Logger
pump
(b)
(c)

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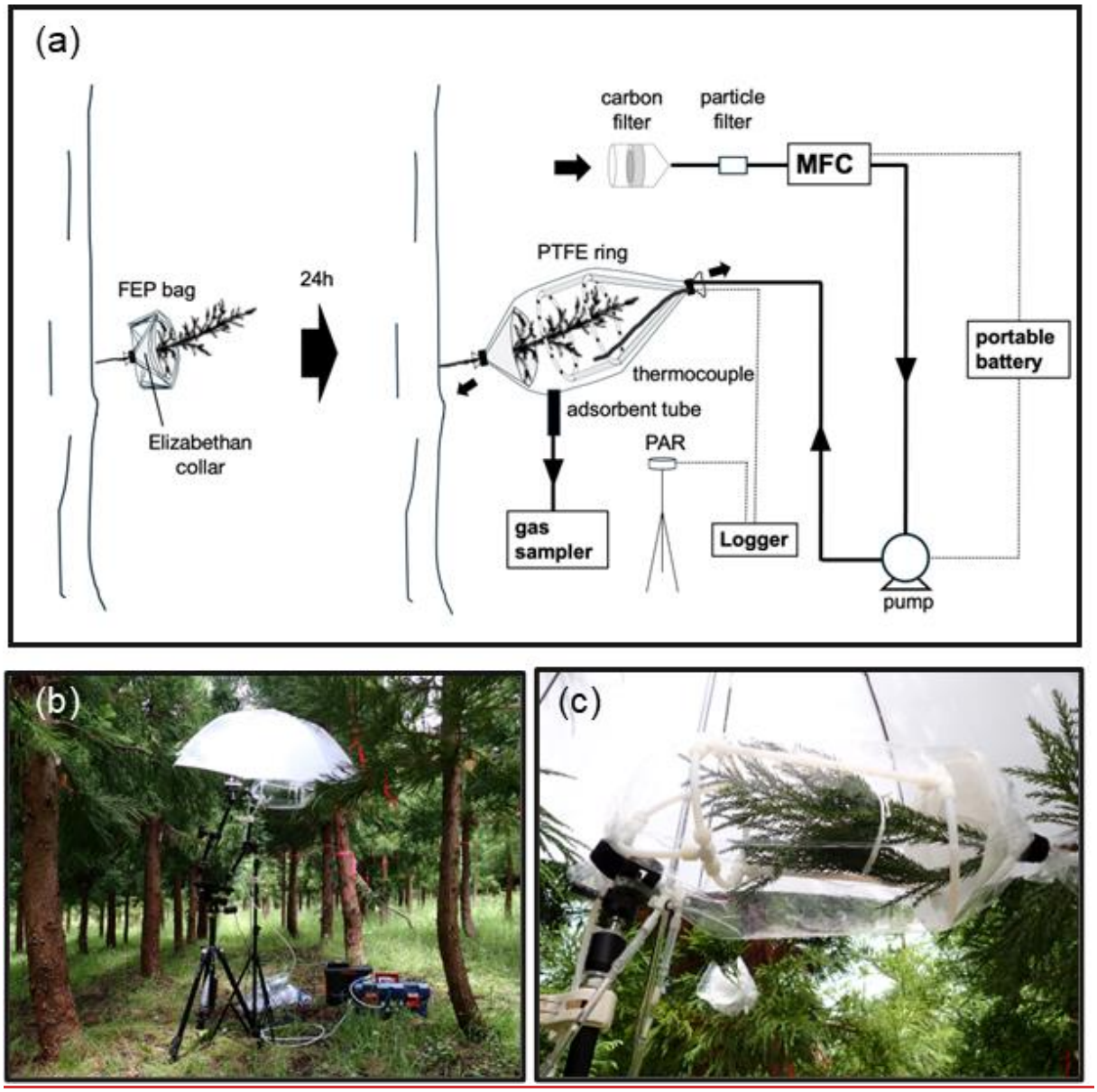


Figure 1: (a) Schematic of Branch enclosure and associated apparatus, (b) enclosure setup, and (c) close-up of the enclosure. MFC, mass flow controller; Logger, data logger; PAR, photosynthetically active radiation sensor.

2.1.2 System operation and sampling protocol

The system is designed for efficiently sampling multiple trees in a single day. This is achieved by pre-installing the support collars on each target tree the day before, and then moving the main portable enclosure apparatus between these collars on the sampling day. In this study, five sets of collars were used to sample five trees. The detailed procedure for enclosing a single branch is as follows:~~The sampling procedure is as follows:~~

1. Pre-attachment: One day before sampling, an open-ended FEP bag and a funnel-shaped support, referred to as an 'Elizabethan collar', are attached to a branch (Fig. 1a). The collar is used to minimize contact between the bag and the branch. The trunk end of the FEP bag is secured around the branch with hook-and-loop tape. The bag is folded back so that it remains off the collar.
2. Enclosure deployment: On the sampling day, the PTFE ring is inserted over the branch, the branch is enclosed

by unfolding the FEP bag, and the distal end of the bag is secured around the branch with hook-and-loop tape. After the branch is enclosed, the inside of the bag is purged with the purified air for at least 1 h before BVOC collection. The bag is secured to maintain a slight positive pressure inside, ensuring that purge air is discharged from both ends.

3. BVOC collection: Air in the bag is drawn through an adsorbent tube (6 mm OD × 90-mm-long glass tube; Supelco, Bellefonte, PA, USA) at 200 mL/min for 10 min by a portable sampler (GSP-300FT-2, Gastec, Kanagawa, Japan). Two types of sorbent tubes are used: one for MTs, packed in series with Tenax TA (20/35 mesh, approximately 100 mg; Supelco) and Carbopack B adsorbents (20/40 mesh, approximately 50 mg; Supelco), and another for SQTs and DTs, packed with HayeSep Q adsorbent (60/80 mesh, Hayes Separations Inc., Bandera, TX, USA). After sampling, compounds collected on the HayeSep Q adsorbent tubes are immediately extracted with approximately 2 mL of hexane (special grade; Fuji Film Wako, Osaka, Japan). The solvent is transferred into 2 mL glass vials, stored in a cooler box (approximately 0 °C) for 1 day, and then frozen (approximately below −30 °C) until analysis. The MT sorbent tubes are also stored in a cooler box and then frozen until analysis.

2.2 Measurements

2.2.1 Monoterpenes

The MT adsorbent tubes were analysed by a custom-built thermal desorption unit coupled to a gas chromatograph (GC) equipped with a mass selective detector (MSD) and a flame ionization detector (FID) (Agilent 6890/5973, Agilent Technologies, Santa Clara, CA, USA). The adsorbent tubes were purged at 40 °C with nitrogen at 40 mL/min for 1 min, then heated to 250 °C. The desorbed analytes were transferred in helium at 10 mL/min to a focusing trap packed with 2 mg Tenax TA and 2 mg Carboxen 1000 (Supelco) maintained at −130 °C. The trap was then heated to 180 °C, and desorbed analytes were injected onto an HP-5 column (60 m, 0.32 mm I.D., 1 μm film thickness; Agilent) in the GC oven. The GC oven temperature was held at 35 °C for 2 min, increased to 160 °C at 4 °C/min, then to 300 °C at 45 °C/min, and held at 300 °C for 10 min. Analytes eluting from the column were split between the MSD and FID. Methyl salicylate was also analyzed using this method. MSD analysis was performed in SIM/SCAN mode. Peaks were identified by comparing retention times with those of standards (Table S1) prepared in methanol. MTs were quantified using the FID, based on calibration curves constructed from the peak areas of standards introduced at various concentrations.

2.2.2 Sesquiterpenes and diterpenes

Following solvent extraction and concentration (Matsunaga et al., 2012), SQTs and DTs were measured by GC-MSD/FID (Agilent 6890/5973). An internal standard (approximately 10 ng of cyclopentadecane dissolved in hexane at a concentration of around 10 ng/μL; Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) was added to the hexane extract, which was then concentrated to approximately 30 μL by using nitrogen blowdown at 60 mL/min. A 1μL aliquot of the concentrated extract was manually injected into the GC. The GC oven temperature was programmed to hold at 60 °C for 2 min, ramp to 120 °C at 30 °C/min, increased to 150 °C at 2 °C/min, and finally ramped to 320 °C at 5 °C/min, holding at 320 °C for 10 min. The separated compounds were split between MSD and FID, with MSD operated in SIM/SCAN mode; quantification was performed using the SIM mode signals.

Peaks were identified by comparing retention times with those of standards (Table S1). SQTs and DTs were quantified by using calibration curves based on the peak areas of standards introduced at various concentrations.

2.3 Calculation of basal emission rate

The rate of BVOC emission (E, in ng (gdw)$^{-1}$ h^{-1}) was first calculated using a mass-balance equation:

~~The basal emission rate (E_S, ng (gdw)$^{-1}$ h^{-1}), representing emission rate at a standard temperature of 30 °C (T_S), was calculated by following the G93 algorithm (Guenther et al., 1993, 2012), as:~~

$$E = \frac{F_{in} \times (M - M_{blank}) \times 60}{F_{samp} \times W_{dry} \times t_{samp}} \quad \frac{F \times (C_{out} - C_{in})}{W_{dry}} \tag{1}$$

$$E_S = \frac{E}{\exp[\beta(T - T_S)]} \tag{2}$$

where F is the flow rate of purge air through the enclosure (L h^{-1}); ~~where E is the rate of BVOC emission, F is the airflow rate inside the enclosure (L/hour),~~ C_{out} is the BVOC concentration in the air exiting the enclosure (ng L^{-1}), determined from the mass of the compound collected on a sorbent tube divided by the total volume of air sampled; ~~concentration of BVOCs in the enclosure exhaust air, calculated by dividing the mass of the measured BVOCs (ng) by sampling flow rate (L/hour),~~ C_{in} is concentration of BVOCs in the ~~enclosure~~ incoming purge air, determined from a blank measurement (an empty enclosure); ~~calculated from the sampling of air in the enclosure without a branch inside,~~ W_{dry} is the dry weight of the enclosed branch (g dw), estimated from Shirota (2000).

To allow for comparison across measurements taken at different temperatures, this measured emission rate (E) was then normalized to a basal emission rate (E_s, in ng (gdw)$^{-1}$ h^{-1}) at a standard temperature (T_s, 30°C = 303.15 K), following the algorithm of Guenther et al. (1993):

$$E_s ~~E_S~~ = \frac{E}{\exp[\beta(T - T_s)]} \tag{2}$$

where, ~~T_s is the standard temperature (30 °C), and~~ T is the temperature inside the enclosure, and β is an empirical coefficient that quantifies the temperature sensitivity of emissions. ~~where E is the rate of BVOC emission, F_{in} is the airflow rate inside the enclosure (L/min), F_{samp} is the sampling flow rate (L/min), M is the mass of the measured BVOCs (ng), M_{blank} is the mass of the BVOCs in the blank (ng), W_{dry} is the dry weight (g dw), estimated from Shirota (2000), t_{samp} is the sampling time (min), T_s is the standard temperature (30 °C), and T is the temperature inside the enclosure. The species-specific coefficient β quantifies the sensitivity of BVOC emissions to temperature changes. It should be noted that these values were not empirically derived from our own dataset, as the measurements were conducted over a narrow temperature range.~~ The ~~values of~~ β values used were ~~for Japanese cedar were applied; were~~ 0.17 for MTs, 0.20 for SQTs, and 0.21 for DTs (Matsunaga et al., 2011, 2012, 2013). It should be noted that these β values were not empirically derived from our own dataset, as the measurements were conducted over a narrow temperature range that was unsuitable for robust parameterization.

3. Method evaluation

3.1 Performance of activated carbon cartridge

We evaluated the efficiency of the activated carbon cartridge at removing BVOCs and ozone. Cedar branches collected from trees growing in the premises of the National Institute for Environmental Studies were chopped into pieces enclosed in a FEP bag. BVOC emission rates were measured with and without the activated carbon cartridge (n = 4–6). Ozone removal efficiency was similarly evaluated by measuring ambient ozone with and without the cartridge using a UV absorption ozone monitor (1006-AHJ, Dasibi, Glendale, CA, USA). Ozone was measured for 10 min a day over 2 days.

Total BVOC concentration was significantly lower with the cartridge than without (P =0.0117, Welch's two-sample t-test; Table 1). Ozone concentrations in the air passing through the activated carbon cartridge were significantly lower than ambient (Table 1). This confirms that the activated carbon cartridge effectively removes ozone, consistent with findings from a previous study (Namdari et al., 2021). These findings confirm that our system is capable of supplying ozone- and BVOC-free air to the enclosure.

Table 1. Ozone mixing ratio and total concentration of the measured BVOCs with and without the activated carbon cartridge. Both new and used activated carbon cartridges were tested. The used cartridge had been employed for approximately 8 hours in field experiments. Results obtained with the used cartridges are shown in parentheses. The limit of detection (LOD) was 1 ppb for ozone and 0.77 ng L^{-1} for total BVOCs. The LOD for total BVOCs was calculated by taking the root sum of squares (RSS) of the individual compound LODs (Table S1) and dividing by the 2 L sampling volume.

	Ozone (ppb)	Total BVOCs (ng L^{-1})
No cartridge	21 ± 1	2400 ±1800
Activated carbon cartridge	<LOD (<LOD)	<LOD (<LOD)

3.2 Environmental control

We evaluated the effect of purge gas source on environmental conditions inside the bag enclosure, using purge gas from ambient air (as described in section 2.1.2) and from gas cylinders (compressed air, Tomoe Shokai Co., Ltd., Tokyo, Japan) as used in previous studies (Hiura et al., 2021; Matsunaga et al., 2011). The experiments were conducted at Tsukuba University Mountain Science Centre. During the experiments, the temperature and relative humidity inside the bag were measured every minute with a hygrometer (2119A, ETO denki, Tokyo, Japan) and a PTFE-coated thermocouple (GL Sciences Inc., Tokyo, Japan). Those outside the bag were obtained from a meteorological station at Tateno, Tsukuba, operated by the Japan Meteorological Agency.

Our system is designed to maintain internal conditions that closely reflect the ambient environment. The humidity within the enclosure more closely reflected the external humidity when purge air was supplied via our air delivery system compared to using dry cylinder gas (Table 2). This reduces the atmospheric water demand on the branch, avoiding potential artifacts caused by an artificially high vapor pressure deficit (Stoy et al., 2021; Núñez et al., 2002). ~~Byron et al., 2022; Haberstroh et al., 2018).~~

The temperature within the enclosure remained consistent with the external temperature, regardless of whether purge air was supplied via the air delivery system or from a cylinder (Table 2). In some cases, Praplan et al. (2020) found that prolonged direct sunlight causes an enclosure to heat up, resulting in a maximum temperature difference

of 27.5°C. In this study, it is assumed that the use of a sunshade prevented direct sunlight, thereby mitigating the temperature increase. This overall stability in both temperature and humidity is attributed to two key design features. First, the photographic white umbrella was used to diffuse and reduce the intensity of direct sunlight, preventing overheating of the enclosed branch. Second, the high air exchange rate, with a residence time of approximately 1.6 minutes, effectively prevents the accumulation of both heat and transpired water vapor.

Table 2. Temperature and relative humidity inside and outside the bag enclosure when air supply was drawn from cylinder and ambient air

	Air source			
	Cylinder		Ambient air	
	Inside enclosure	Outside enclosure	Inside enclosure	Outside enclosure
Humidity (%)	40 ± 1	70 ± 3	59 ± 2	63 ± 0.9
Temperature (°C)	30.4 ± 0.6	29.0 ± 0.4	30.0 ± 1	27.7 ± 0.4

3.3 Measurement repeatability

We evaluated the repeatability of BVOC emission rate measurements using a 1-m-long branch collected from a 10-year-old cedar tree at the Tsukuba University Mountain Science Centre. ~~After trimming its base, we cut the branch under water to maintain its vascular integrity.~~ After trimming the base, the branch was re-cut under water to maintain its vascular integrity, and the cut end was kept submerged throughout the experiment. This underwater cutting technique is a standard method to prevent air from the xylem vessels, which can cause cavitation and disrupt water transport (Ogasa et al., 2016; Umebayashi et al., 2016). Indeed, measurements on detached branches represent a well-established approach in BVOC research (e.g., Jardine et al., 2020), including for coniferous species with large storage pools similar to *C. japonica* (Mochizuki et al., 2011; Miyama et al., 2018). Furthermore, Monson et al. (2007) demonstrated that this method maintains stable rates of photosynthesis, stomatal conductance, and isoprene emission for detached branches, showing no significant differences from branches that remained attached to the tree. As *C. japonica* emits stored rather than de novo synthesized BVOCs, and the distance from the cut site to the enclosed section of the branch was sufficiently long (60 cm), the effect of cutting on our measurements is considered negligible. Following the procedure described in section 2.1.2, air in the enclosure bag was collected in adsorbent tubes (9 tubes for MTs, 5 tubes each for SQTs and DTs). Blank samples were also taken from the empty enclosure.

The relative standard deviations (RSDs) of MTs ranged from 4.1% to 19%, with an average of 10%. That of SQT was 5.6% (Fig. 2). That of individual-level BVOCs in the field was 159% (data not shown).

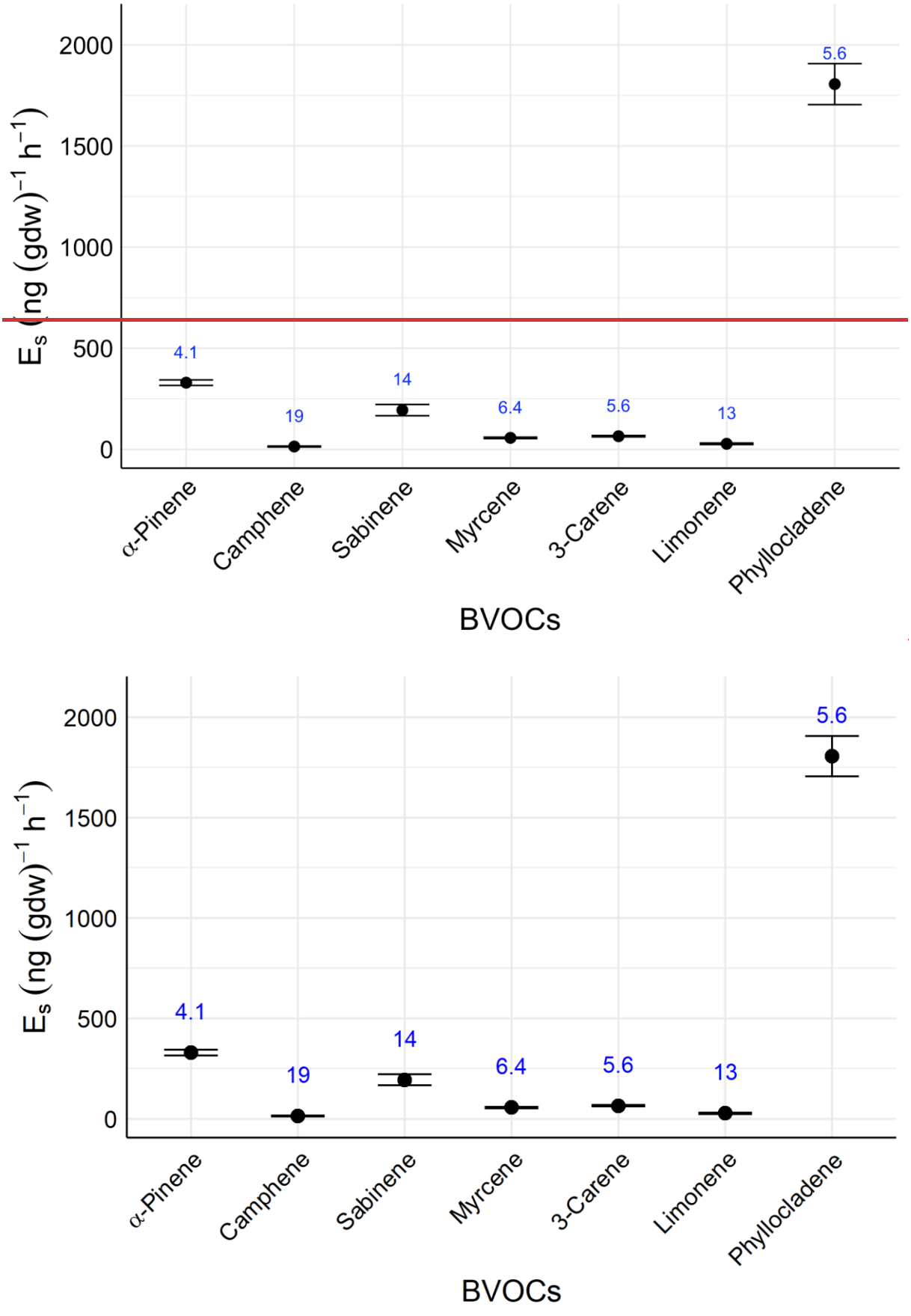


Figure 2: Basal emission rates of terpenes (*E*s) and relative standard deviations (%, *n* = 5, blue)

These findings suggest that RSDs of the new measurement method are sufficiently low compared with the variability in individual-level BVOC emission rates, confirming the method's reproducibility in assessing individual emission rates. The RSDs are comparable to those reported in previous studies with other tree species (Li et al., 2019).

In addition to the tests on excised branches, we conducted a reproducibility experiment on an intact branch of a live seedling. The same branch was measured twice, and the results are compared in Figure S1. The measurements showed reasonable consistency for all detected compounds, supporting the stability of our system. We note, however, that this test was conducted on a young seedling with a limited number of emitted compounds. ~~In addition, we conducted a validation using branches attached to the main stem, rather than excised branches. Two measurements were conducted from the same branch, and the results of the first and second measurements were compared. These results are presented in Figure S1. For all detected compounds, the reproducibility appears to be satisfactory. However, it should be noted that the experiment was conducted using young seedlings, and the number of detected compounds was relatively limited.~~

3.4 System stabilization time

We conducted an indoor experiment to evaluate the time required for the excessive release of BVOCs due to contact during the attachment of the enclosure system to subside. A cut cedar branch placed in a thermostatic chamber was used. Measurements were taken with a TDU-GC-MS system, as in section 2.2.1, but equipped with a dual trapping system for online preconcentration and refocusing of BVOCs. Air was drawn from the enclosure at 45 mL/min into the TDU for 12 min and analysed by GC-MS. Measurements were taken every 45 min over 3 days. Throughout the experiment, the chamber was maintained at a constant 25 °C. As the temperature remained constant, no temperature correction was applied in the calculation of the basal emission rate.

We also investigated effects of extending the pre-attached FEP bag on basal emission rates during the measurement day. Using the same materials as before, we tied a FEP bag to a cedar branch, folded it, and left it for approximately 24 h. The bag was then extended to cover the branch again. Samples were taken every 45 min, 20 times in total, while the bag was purged with air, and we compared basal emission rates before and after the bag was extended. Installation of an enclosure (i, Fig. 3) initially triggered an excessive release of BVOCs. Emission decreased to approximately 5% of its peak value within 24 h of installation.

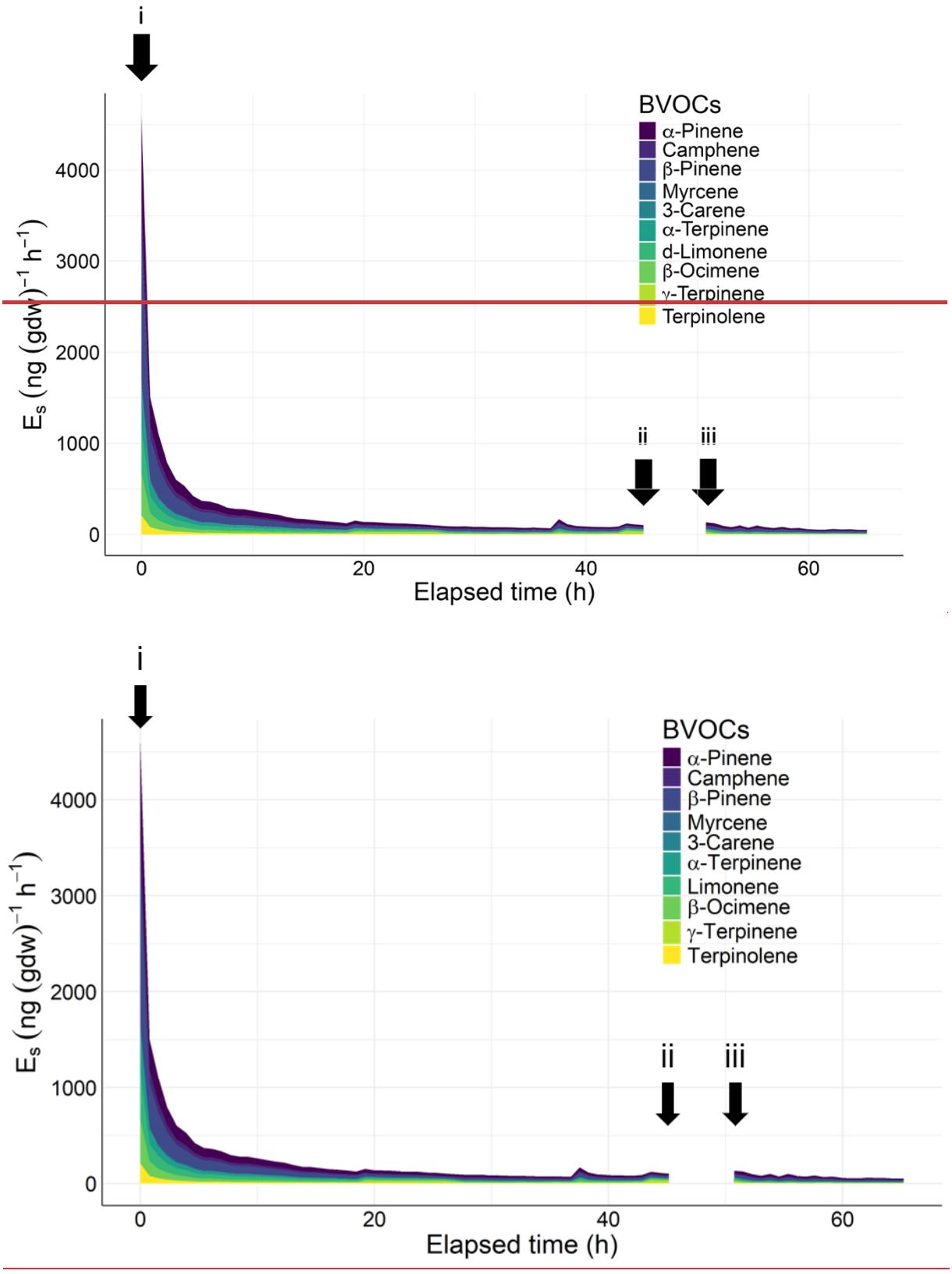


Figure 3. BVOC emission rates from a tree branch over time under different enclosure conditions. A branch was covered with a bag (0 h, i), and continuous measurements were taken until 45 h. The bag was folded back (ii), and this state was maintained until 52 h (iii). The bag was unfolded over again to cover the branch, and measurements were

resumed until 72 h. Total amount of terpenes emitted was 3.75 µg/gdw. Considering that the amount of terpene stored in the leaves of Cryptomeria is approximately 15.877mg/gdw (Saito et al., 2022), this represents only 0.02% of its storage pool.

These results indicate that allowing 24 h after enclosure installation mitigates potential overestimation of BVOC emission rates due to the installation. Previous studies allowed 24 h or more (Helin et al., 2020; Helmig et al., 2013; Hiura et al., 2021; Matsunaga et al., 2011). Our results suggest that 24 h is enough.

The results also reveal that the emission rate 1 h after bag unfolding (Fig. 3, iii) was virtually unchanged from the pre-unfolding level (ii), so the potential effect of excessive emissions during the bag-unfolding process was sufficiently accounted for. Notably, Mochizuki et al. (2011) showed that the excessive emission of MTs induced by vibration stimulus in Japanese cedar subsided within approximately 20 min. We anticipate that the effects of minor vibrations during bag unfolding will subside within 1 h.

4. Field deployment

We deployed the developed branch enclosure system in two field settings to evaluate its performance. The first involved multiple Japanese cedar individuals from different regional populations growing in a common garden. The second consisted of a diurnal measurement on a mature individual tree.

4.1 Measurements in a common garden

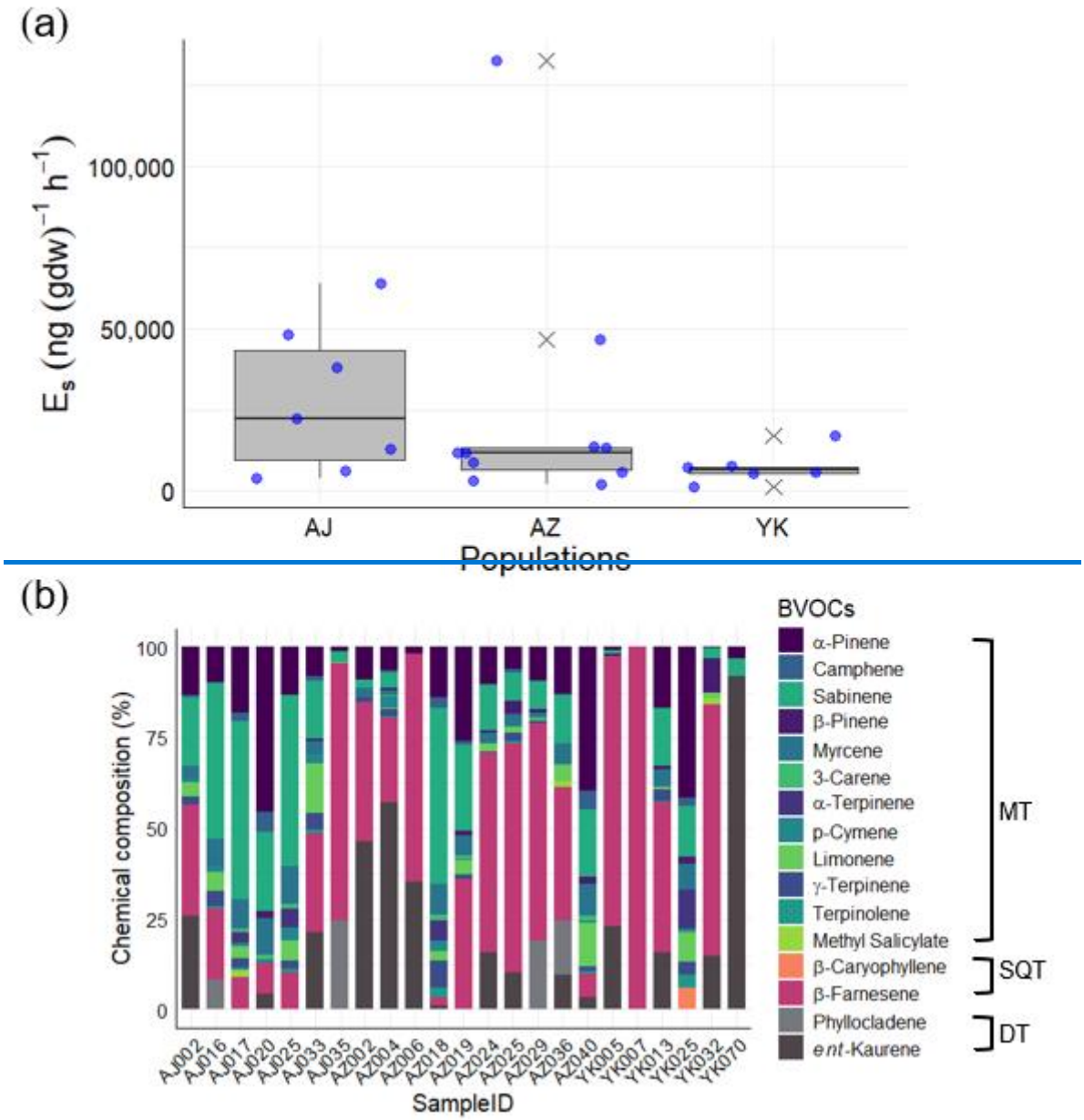
The enclosure system was tested on Japanese cedar trees grown in a common garden setup at the Kawatabi Field Centre, Tohoku University, using cedar trees grown from cuttings of natural populations collected across Japan (Tsumura, 2022). Geographical differentiation in several functional traits of Japanese cedar has been revealed through common garden experiments (Hiura, 2022). We used cuttings derived from three populations: 9 individuals from Ajigasawa (40.67° N, 140.20° E, 297m a.s.l.), 9 from Azoji (34.48° N, 131.96° E, 1060m a.s.l.), and 6 from Yakushima (30.33° N, 130.46° E, 1267m a.s.l.). Details of each population are provided in Kimura et al., (2014). The mean annual temperature at Kawatabi is 10.5 °C and the mean annual precipitation is 1697 mm. ~~All field measurements were conducted on live, intact branches of these rooted trees.~~ Field measurements were conducted on live, intact branches, with one south-facing branch selected per tree at approximately 1.3 m above the ground. Sampling was performed during daytime (9:00–15:00) from 29 May to 9 June 2023, using a consistent protocol for all individuals.~~We selected one south-facing branch per tree at approximately 1.3 m above the ground. The sampling protocol was applied consistently across all individuals during daytime from 29 May to 9 June 2023.~~We selected one branch per tree, approximately 1.3 m above the ground and facing south, and sampled during daytime from 29 May to 9 June 2023.~~

Measurements at the Kawatabi Field Centre detected 14 compounds, principally α-pinene, sabinene, β-farnesene, and *ent*-kaurene (Fig. 4). While ~~T~~total emission rates did not ~~significantly~~ differ significantly among populations (ANOVA, $P = 0.417$, $F=0.913$), we observed substantial inter-individual variation.~~.~~ Specifically, the total emission

rates varied by several orders of magnitude among individuals. Such high variability in emission rates is a well-documented characteristic of *C. japonica* (Saito et al., 2022; Matsunaga et al., 2011; Miyama et al., 2019; Tani et al., 2024), supporting the biological origin of this variation.

In addition to the total rates, there was also considerable variation in the emission compositions (Fig. 4b). ~~However, we observed considerable individual variation in both the total emission rates, which spanned several orders of magnitude, and in the emission compositions (Fig. 4b).~~ For example, in some trees (AJ016, AJ017, AJ020, AJ025, AJ033, AZ018, AZ019, AZ040, YK025) MTs accounted for more than 50% of the emission composition. In contrast, other individuals (AJ002, AJ035, AZ002, AZ004, AZ006, AZ024, AZ025, AZ029, AZ036, YK005, YK007, YK013, YK032, YK070) showed profiles where SQTs and DTs accounted for more than 50% of the emission composition.~~Such high inter-individual variability is a well-documented characteristic of *C. japonica* (Saito et al., 2022; Matsunaga et al., 2011; Miyama et al., 2019; Tani et al., 2024), supporting the biological origin of the variation observed in our study.~~

~~Measurements at the Kawatabi Field Centre detected 14 compounds, principally α-pinene, sabinene, β-farnesene, and *ent*-kaurene (Fig. 4).~~


(a)
E_s (ng (gdw)$^{-1}$ h^{-1})
100,000
50,000
0
AJ
AZ
YK
Populations
(b)
Chemical composition (%)
100
75
50
25
0
AJ002
AJ016
AJ017
AJ020
AJ025
AJ033
AJ035
AZ002
AZ004
AZ006
AZ018
AZ019
AZ024
AZ025
AZ029
AZ036
AZ040
YK005
YK007
YK013
YK025
YK032
YK070
SampleID
BVOCs
α-Pinene
Camphene
Sabinene
β-Pinene
Myrcene
3-Carene
α-Terpinene
p-Cymene
Limonene
γ-Terpinene
Terpinolene
Methyl Salicylate
β-Caryophyllene
β-Farnesene
Phyllocladene
ent-Kaurene
MT
SQT
DT

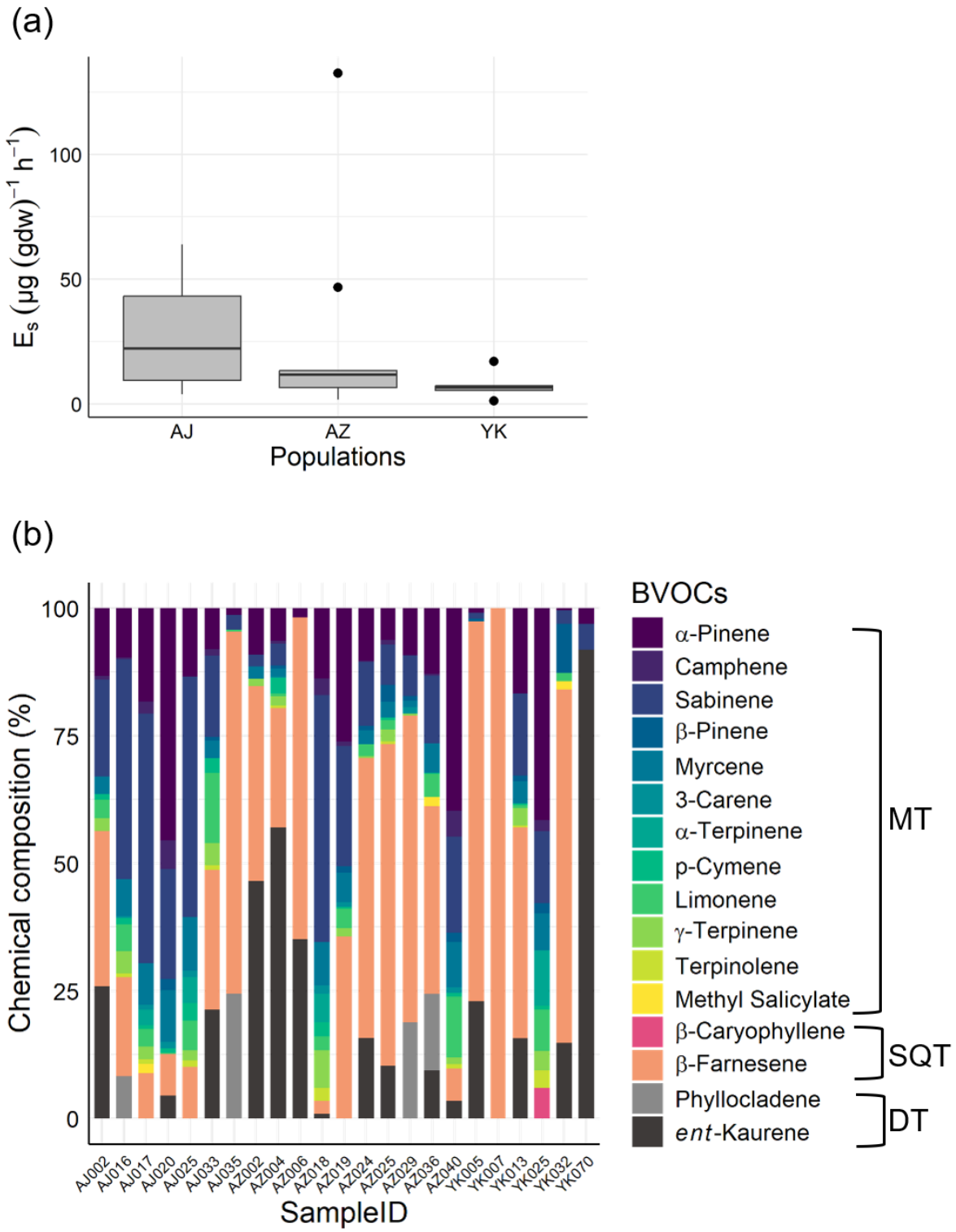


Figure 4 Field experiment results showing BVOC emissions from Japanese cedar (*Cryptomeria japonica*). (a) Total BVOC emission rates from different populations (Yakushima [YK], Azouji [AZ], Ajigasawa [AJ]). (b) Chemical composition of BVOCs measured from individual trees from three populations. ~~**This data is consistent with our previous data, although the detection limit has been revised.**~~

~~Total emission rates did not significantly differ among populations (P = 0.417; F=0.913; ANOVA). There was~~

~~considerable individual variation in emission compositions (Fig. 4b). For example, in some trees (AJ016, AJ017, AJ020, AJ025, AJ033, AZ018, AZ019, AZ040, YK025) MTs accounted for more than 50% of the emission composition. In other trees (AJ002, AJ035, AZ002, AZ004, AZ006, AZ024, AZ025, AZ029, AZ036, YK005, YK007, YK013, YK032,YK070) SQTs and DTs accounted for more than 50% of the emission composition.~~

Among all trees, β-farnesene was the most dominant SQT detected. Concentrations of β-farnesene varied significantly. Among trees emitted β-farnesene (all individuals with the exception of YK025, YK070), β-farnesene accounted for 2.6~100% of total emissions. β-Farnesene is a crucial volatile organic compound, exerting significant influence on interactions between plants, aphids, and predator insects (Wang et al., 2024). β-Farnesene emission can be triggered by parasitism (Kivimäenpää et al., 2020). This compound is also suggested to be involved in responses to abiotic stresses, and it may help prevent leaf necrosis caused by local ozone reduction (Palmer-Young et al., 2015). Therefore, we can infer that trees with high β-farnesene emission rates may be experiencing some form of stress. BVOC emissions are broadly influenced by various biotic and abiotic factors (Holopainen ~~&~~and Gershenzon, 2010; Loreto ~~&~~and Schnitzler, 2010). Therefore, the variations in overall emission characteristics observed here may be attributed to environmental differences in the field and responses to biotic stress. However, the main objective of this study was the development of methods, and the sample size is not large enough to draw any conclusions about factor determining BVOC emissions. Further sampling will be necessary to clarify these factors, while detailed analyses of the relationships with phyllosphere microbial communities are being conducted elsewhere (Ishizaki et al., in submission).

4.2 Diurnal variation in BVOC emissions

To evaluate the diurnal variation of BVOC emissions and assess the system's response to environmental conditions, we conducted additional measurements on a Japanese cedar tree (height: approximately 10 m) growing on the premises of the National Institute for Environmental Studies, Japan. Based on preliminary observations indicating that the BVOC profile of this individual was dominated by monoterpenes (MTs), the measurements focused specifically on MT emissions. Sampling was conducted at multiple time points over a 24-hour period on 5 August 2025, from the lowermost branch of the tree.

Throughout the night and morning (01:00–11:00), the emission rate increased with rising air temperature (Fig. 5a). The relationship between temperature and emissions during this pre-noon period (Fig. 5b) exhibited a typical exponential response, consistent with established models (e.g., Guenther et al., 1993). The temperature response coefficient (β, $°C^{-1}$) calculated from this data using Equation (2) was 0.191. It is worth noting that our β value was derived using non-linear regression, which differs from the log-transformed linear regression method used in some previous studies on this species (e.g., Matsunaga et al., 2011; Okumura et al., 2013). For comparison, applying the linear method to our data yields a β of 0.143, which agrees well with their reported values (0.09–0.17). This result demonstrates that our method can quantitatively and accurately assess the standard environmental responses of plants.

In the afternoon, however, as a heatwave caused the temperature to exceed 40°C, the emission rate surged dramatically, deviating from the morning trend (Fig. 5c). Notably, even after the temperature decreased to approximately 30°C in the evening, the emission rate did not return to the level predicted by the standard temperature dependency (Fig. 5b). This hysteresis suggests that the surge was not a simple thermal response but

was likely triggered by factors such as heat-induced physiological damage, as reported by Nagalingam et al. (2024). Although a mechanistic investigation of this single case is outside the scope of this methodological paper, it highlights the system's capability to capture plant responses to extreme weather events. This interesting phenomenon warrants further investigation.

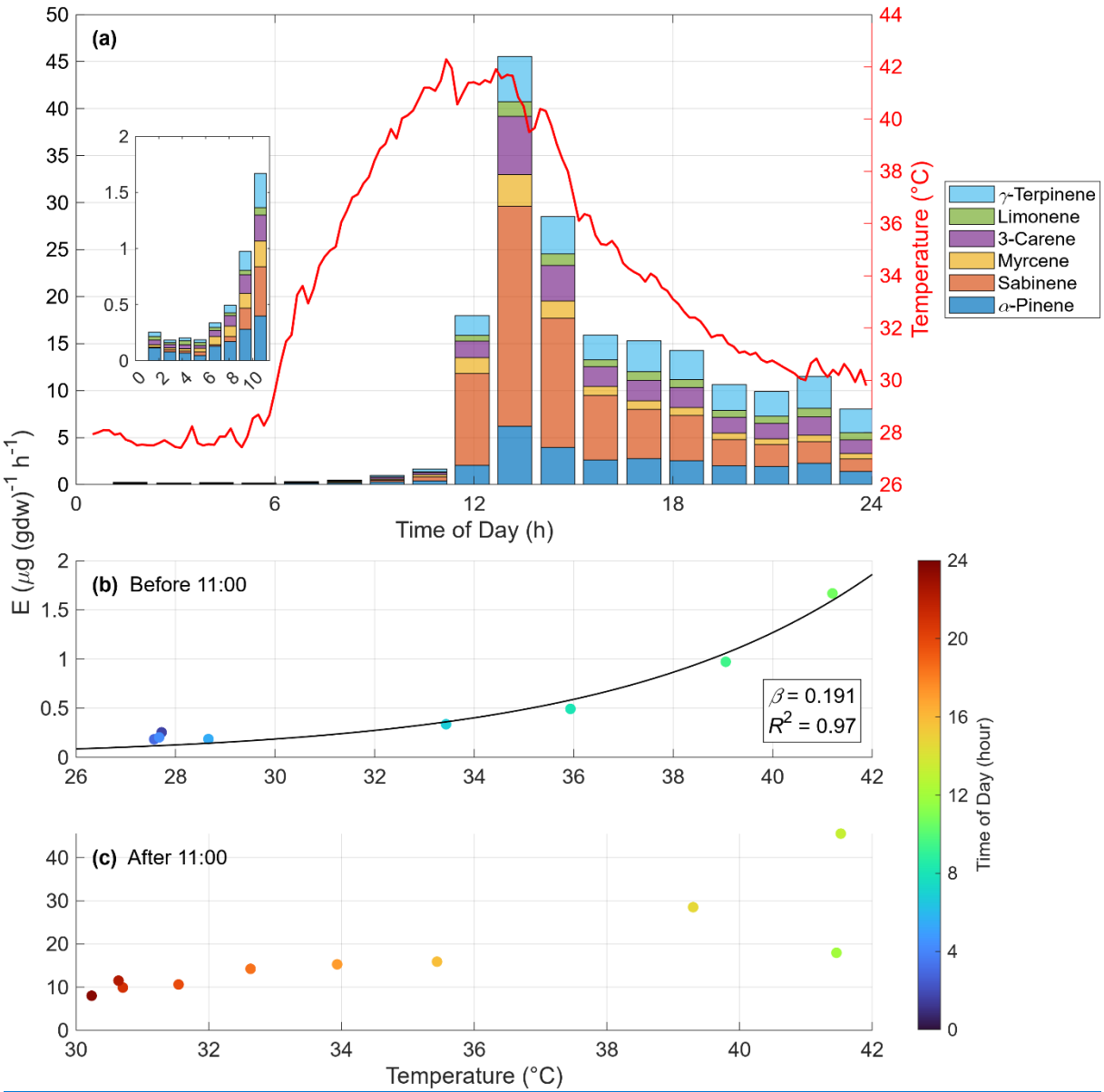


Figure 5. Diurnal variation of monoterpene emissions from a field-grown Japanese cedar (Cryptomeria japonica) tree. (a) Time series of emission rates (E; stacked bars) for monoterpene compounds and air temperature in the enclosure bag (red line), with an inset highlighting the 01:00–11:00 period. (b, c) Scatter plots of the emission rate versus temperature for data (b) before 11:00 and (c) after 11:00, respectively. The color of the markers indicates the time of day (hour).

5. Conclusion

We successfully developed a portable, field-deployable system for measuring BVOC emissions from conifers. By using scrubbed ambient air as a purge gas and implementing a foldable bag design, the system addresses the

logistical and methodological challenges associated with conventional techniques. The system maintained stable environmental conditions, provided BVOC- and ozone-free air, and ensured high measurement repeatability, making it suitable for field applications.

Field testing revealed significant variability in BVOC emissions both among individual trees and across geographical origins. These findings highlight the complex interplay of multiple factors in determining BVOC emission characteristics. This variability emphasizes the critical need for individual-level assessments to enhance our understanding of BVOC dynamics in diverse ecosystems.

By providing a practical and reliable tool for BVOC measurement under natural conditions, the system can advance our understanding of the ecological roles and atmospheric effects of BVOCs, supporting studies of their spatiotemporal patterns and climatic interactions.

Data availability

Data are available at 10.5281/zenodo.14965367, or upon request by contacting the corresponding authors.

Supplement

The supplement related to this article is available on-line at:

Author contributions

SJA and TS developed and maintained the instruments. YS and YT designed and managed the common gardens. YO and TS collected and analysed the samples. TIK provided assistance with data analysis. TS and TH supervised the research. YO analysed the data and wrote the manuscript with contributions from all coauthors.

Competing interests

The authors declare that they have no conflict of interest.

Disclaimer

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Supplement

Table S1 List of compounds studied in this work, including purity and supplier of the standard chemicals used and limits of detection (LOD). Cyclopentadecane (98%, Tokyo Chemical Industry Co.) was used as an internal standard.

Tokyo Chemical Industry Co., Tokyo Chemical Industry Co., Ltd., Tokyo, Japan; Sigma-Aldrich, Sigma-Aldrich, Tokyo, Japan; ChromaDex, ChromaDex, Los Angeles, CA, USA; Toronto Research Chemicals, Toronto Research Chemicals, Toronto, ON, Canada; Fujifilm Wako, Fujifilm Wako, Osaka, Japan; MP Biomedicals Japan, MP Biomedicals Japan, Tokyo, Japan; N.A., Not Available.

Compound	Purity	Supplier	LOD (ng)
α-pinene	99%	Tokyo Chemical Industry Co.	0.127
camphene	80%	Tokyo Chemical Industry Co.	0.107
(±)-limonene	95%	Tokyo Chemical Industry Co.	0.079
myrcene	97.60%	Sigma-Aldrich	0.033
α-terpinene	95%	Sigma-Aldrich	0.280
3-carene	≥98.5%	Sigma-Aldrich	0.020
p-cymene	99%	Sigma-Aldrich	0.039
γ-terpinene	97%	Sigma-Aldrich	0.044
thujopsene	97%	Sigma-Aldrich	0.810
sabinene	97.90%	ChromaDex	0.113
terpinolene	97.50%	ChromaDex	0.155
β-farnesene	98.50%	ChromaDex	0.763
β-pinene	96%	Toronto Research Chemicals	0.089
β-ocimene	>90%	Toronto Research Chemicals	0.195
copaene	94%	Toronto Research Chemicals	0.326
β-caryophyllene	95%	Toronto Research Chemicals	0.254
methyl salicylate	98%	Fujifilm Wako	0.421
longifolene	89%	MP Biomedicals Japan	0.172
ent-kaurene	N.A.	transferred from Tokushima Bunri University	0.550
phyllocladene	N.A.	transferred from Yamagata University	0.501

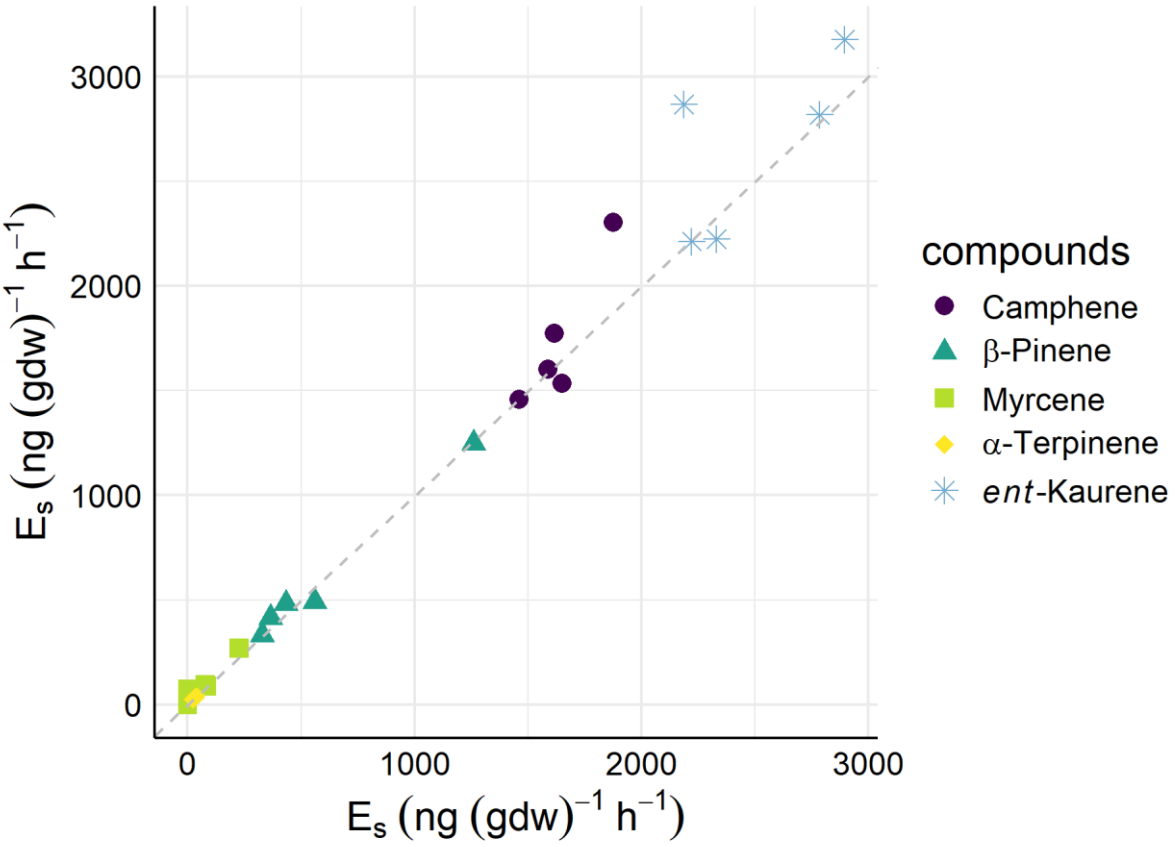

Figure S1 Correlations of the duplicate measurements of BVOC emission rates from Japanese cedar (Cryptomeria Japonica). Emission rates were measured twice for each of five individuals, using the same branch in both measurements. The black dashed line is the 1:1 line.

【AC1】

We sincerely thank Dr. Praplan for the careful review and encouraging comments. Our detailed responses are provided below, with the *reviewer's comments* and our replies distinguished by formatting. The line numbers referenced in our responses correspond to those in the revised manuscript.

Comment1:- lines 211-212: 'After trimming its base, we cut the branch under water to maintain it vascular integrity.' Could the author explain a little bit more how the branch was cut under water and possibly provide a reference demonstrating how vascular integrity is maintained by doing so?

Response1: We agree that our explanation regarding the procedure of cutting branches under water was insufficient. We have added following sentences in the main manuscript.

L232: After trimming the base, the branch was re-cut under water to maintain its vascular integrity, and the cut end was kept submerged throughout the experiment. This underwater cutting technique is a standard method to prevent air from the xylem vessels, which can cause cavitation and disrupt water transport (Ogasa et al., 2016; Umebayashi et al., 2016). Indeed, measurements on detached branches represent a well-established approach in BVOC research (e.g., Jardine et al., 2020), including for coniferous species with large storage pools similar to C. japonica (Mochizuki et al., 2011; Miyama et al., 2018). Furthermore, Monson et al. (2007) demonstrated that this method maintains stable rates of photosynthesis, stomatal conductance, and isoprene emission for detached branches, showing no significant differences from branches that remained attached to the tree. As *C. japonica* emits stored rather than de novo synthesized BVOCs, and the distance from the cut site to the enclosed section of the branch was sufficiently long (60 cm), the effect of cutting on our measurements is considered negligible.

Comment2: - lines 226-227: 'at least one terpene was detected in each category'. Why did the author decided not to included all the detected terpenes (one in each category) in Fig. 2? There seems to be only MTs and one DT.

Response2: Thank you for this comment, which has highlighted an ambiguity in our manuscript. First, Figure 2 does present all of the terpenes that were detected in the experiment. We recognize that our wording was confusing. Our original intention was to state that both the thermal desorption and solvent extraction methods successfully

detected compounds, thus validating the use of both techniques. However, we agree that this sentence was not essential and could be misinterpreted. To improve the clarity of the manuscript, we have removed this sentence entirely.

Comment3:- Figure 4: I am not sure to understand the boxplot (panel (a)) as there are datapoints scattered horizontally (why?) and some blue dots are on the same levels as gray crosses. It is not clear from the caption if the crosses are outliers, but if they are, why are there blue dots (not outliers?) at the same height? In panel (b), the three colors used for MTs are very similar and make it difficult to see what compounds are present in the emissions from the figure.

Response3: We agree that the presentation was confusing, and we have revised both panels accordingly.
For Figure 4 (a): Our original figure superimposed a jittered scatter plot onto the boxplot, which caused the confusing horizontal distribution and resulted in outliers appearing twice (once as a boxplot outlier, once as a scatter point). We acknowledge this was misleading. We have revised the figure to a standard boxplot format, showing only the outliers as individual points, which makes the plot much clearer. Additionally, we changed the y-axis units to $\mu g\ (gdw)^{-1}\ h^{-1}$ to avoid the large numbers (e.g., 50,000) of the original ng-based scale and improve readability.
For Figure 4 (b): We also agree that the colors for the monoterpenes were too similar. We have addressed this by selecting a new, more distinct color palette in the revised figure to ensure each compound can be easily distinguished.

Comment4: - lines 296-303: The authors mention the possible effect of stress, but state that it is not the objective of their study to look closer at the factors determining BVOCs emissions. The sample size, they argue, is 'not large enough', but I believe that it is still a decent enough sample size as they have shown using various statistical tools. As a suggestion (more than a request for revision), I think that it would be nice to include something about the environmental conditions (e.g. temperature and its effect on the emission rates) as the sensors (for temperature, radiation, etc.) are part of the dynamic branch enclosure system and it would be good to demonstrate what conclusions could be made with the acquired dataset. I understand, however, if the authors have planned to demonstrate this in a subsequent manuscript with a larger dataset and more solid conclusions.

Response4: Thank you for your constructive comments. We agree that exploring the system's ability to capture environmental responses is a crucial aspect of its validation.
In our main field campaign (Chapter 4.1), the primary objective was to assess inter-individual variation. For this reason, we normalized all emission rates to a standard temperature to minimize temperature-induced variability and better resolve the underlying biological differences between trees.
However, we also recognize the importance of demonstrating the system's capability to track environmental drivers, a point also raised by Reviewer #2. Therefore, in response to the reviews, we conducted an additional field experiment specifically designed to monitor the diurnal variation of BVOC emissions from a single, intact tree.
These new results have been added as a new section (4.2) and figure (Fig. 5). This new section provides a clear demonstration of what can be concluded from our dataset regarding environmental responses, directly addressing your suggestion. While a more detailed investigation with a larger dataset is part of our future plans, we believe this addition significantly strengthens the manuscript by validating the system's performance under dynamic, field conditions. We appreciate your encouragement.

Comment5: In addition to my previous comments, I would like to add that, for the dataset published, it would be good to have for the BVOC data the inclusion as metadata of what units apply the numbers that are reported.

Response5: Thank you for your comments. As per your comment, we have added units to the published dataset.

【AC2】

We sincerely thank the reviewer for the careful review and constructive comments. Our detailed responses are provided below, with the *reviewer's comments* and our replies distinguished by formatting. The line numbers referenced in our responses correspond to those in the revised manuscript.

General Comments

Comment1: ***System validation and applicability.*** *The central claim of the manuscript is that the system enables portable measurements from multiple trees within a single day. However, most of the performance evaluations (e.g., reproducibility and stabilization time) are based on measurements from cut branches, which do not represent intact physiological conditions. Cut branches are known to alter emission profiles, especially in species with large internal storage pools such as conifers. Given that this system is meant to overcome such limitations, a convincing demonstration under field conditions using live, rooted trees is essential. Otherwise, this system would not differ significantly from simple, well-stablished chamber-based measurements in laboratory. The authors should also clarify whether the system is designed to be reused across trees or if multiple enclosure collars need to be installed in advance. Discussing a field-based example of multi-tree sampling in practice would help substantiate this important advantage.*

Response1: We sincerely thank Reviewer #2 for this insightful and crucial feedback. We agree that demonstrating the system's performance on live, intact trees in the field is essential to substantiate our central claim of portability and multi-tree sampling.

1. Why we used cut branches for method validations

We wish to clarify our rationale for using excised branches for some of the initial validation tests. To precisely evaluate the system's intrinsic performance (e.g., reproducibility), stable emission rates were required, which is very difficult to achieve in the field due to fluctuating environmental conditions. We did consider using potted saplings in a controlled environment, but found them unsuitable because their BVOC emissions were too low. Consequently, we opted for excised branches from mature trees, as they provided the most realistic and stable conditions for these specific validation purposes.

Our approach aligns with a large body of literature. Measurements on detached branches

are a well-established method in BVOC research for short-term experiments where emission rates are not significantly affected by excision (e.g. Jardine et al., 2020), especially for coniferous species with large internal storage pools like Cryptomeria japonica (e.g., Mochizuki et al., 2011; Miyama et al., 2018; Jardine et al., 2020).
To minimize any potential artifacts from this widely used method, we followed standard best-practice protocols. As detailed in the manuscript (L232), we used long branches and performed underwater re-cutting to maintain vascular integrity (Monson et al., 2007). We believe this approach ensured that our initial validation was both robust and reliable.

2. Additional measurements on live, rooted tree
Second, we conducted additional field measurements on live, rooted trees. These new results (section 4.2), which we detail in response to your next comment, validate our system's performance under field conditions.

3. Clarification on field operation
Third, regarding field usability, the collars are indeed reusable. They are installed the day before measurement and can be moved between trees. In this study, five measurement sets were used during the field campaign. As such, this system allows for the sampling of multiple individuals in settings where electricity is not available. To clarify, the following sentences were added:

L114: The system is designed for efficiently sampling multiple trees in a single day. This is achieved by pre-installing the support collars on each target tree the day before, and then moving the main portable enclosure apparatus between these collars on the sampling day. In this study, five sets of collars were used to sample five trees. The detailed procedure for enclosing a single branch is as follows:

*Comment2: **Ambiguity in field deployment.** The field deployment data show emission rates spanning up to six orders of magnitude among individuals of the same species. While biological variability is expected, such a wide range raises questions about system consistency. The authors attribute this variability to individual differences, but without clearer evidence that the technique itself is not contributing to it (e.g. via consistently different handling of the samples from the three areas - perhaps this is why the same tree species are so consistently different among the different locations), this interpretation remains uncertain. If the final field deployment data were also based on detached*

branches, then the system has not yet been demonstrated under its intended real-world conditions, and the results would not validate the system's field applicability as claimed.

Response2: We thank the reviewers for raising this important point and for the opportunity to clarify our field methodology and results.

1. **Clarification on field measurement conditions**

   First, we would like to clarify that all field measurements in section 4 were conducted from intact branches of live, rooted trees, not from excised branches, as shown in the photo in Figure 1(b).

2. **Evidence of system stability and consistent methodology**

   To demonstrate the stability of our system, we have added new data from repeated measurements conducted on the same branch (see new Figure S1 in the Supplement). These results show high reproducibility, which strongly indicates that our measurement system is stable and reliable, and that the system itself is not the source of the large variability observed between different trees.

   Furthermore, all measurements were conducted in a common garden. In this setting, trees from different provenances grow under identical environmental conditions, and were sampled during the same period. The sampling protocol and handling were applied consistently across all individuals. This experimental design makes it highly unlikely that the observed differences are artifacts of location or inconsistent methodology.

   We have revised the sentences in 3.3. measurement repeatability:

   L258: In addition to the tests on excised branches, we conducted a reproducibility experiment on an intact branch of a live seedling. The same branch was measured twice, and the results are compared in Figure S1. The measurements showed reasonable consistency for all detected compounds, supporting the stability of our system. We note, however, that this test was conducted on a young seedling with a limited number of emitted compounds.

3. **Consistency with known biological variability**

   The high degree of variability we observed is well-documented characteristic of Japanese ceder. Several previous studies have reported similarly large, order-of-magnitude differences in BVOC emission rates among individuals of this species (Saito et al., 2022; Matsunaga et al., 2011; Miyama et al., 2019; Tani et al.,2024). Therefore, our results are consistent with the known biological variability for this species.

In summary, based on (a) our standardized experimental design that minimized

methodological artifacts, (b) the demonstrated stability of our system from repeated measurements, and (c) the consistency of our results with the known high biological variability of the species, we attribute the observed range of emission rates to the inherent genetic and physiological differences among the individual trees.
To make clear the above points, we have revised the sentences in 4. Field deployment:

L311: Field measurements were conducted on live, intact branches, with one south-facing branch selected per tree at approximately 1.3 m above the ground. Sampling was performed during daytime (9:00–15:00) from 29 May to 9 June 2023, using a consistent protocol for all individuals.

L314: Measurements at the Kawatabi Field Centre detected 14 compounds, principally α-pinene, sabinene, β-farnesene, and ent-kaurene (Fig. 4). While total emission rates did not differ significantly among populations (ANOVA, P = 0.417, F=0.913), we observed substantial inter-individual variation. Specifically, the total emission rates varied by several orders of magnitude among individuals. Such high variability in emission rates is a well-documented characteristic of C. japonica (Saito et al., 2022; Matsunaga et al., 2011; Miyama et al., 2019; Tani et al., 2024), supporting the biological origin of this variation.
In addition to the total rates, there was also considerable variation in the emission compositions (Fig. 4b). For example, in some trees (AJ016, AJ017, AJ020, AJ025, AJ033, AZ018, AZ019, AZ040, YK025) MTs accounted for more than 50% of the emission composition. In contrast, other individuals (AJ002, AJ035, AZ002, AZ004, AZ006, AZ024, AZ025, AZ029, AZ036, YK005, YK007, YK013, YK032, YK070) showed profiles where SQTs and DTs accounted for more than 50% of the emission composition.

Comment3: ***Lack of environmental response validation.*** *A core requirement for validating a new BVOC enclosure system is demonstrating that it can reproduce known patterns such as diurnal variations and emission responses to temperature and light. The manuscript does not include any environmental-driven validation. Without observing characteristic temporal emission patterns (e.g. the temperature and light-driven increases during day), it is difficult to distinguish between physiological emissions and stress-induced pulses caused by handling or storage depletion. At least a clear diurnal*

cycle from a rooted field-grown tree is required for validating such new measurement technique.

Response3: Thank you for your constructive comments. In direct response to your feedback, we conducted an additional measurement specifically designed to monitor the diurnal variation of BVOC emissions from a live, rooted tree. We have added these new results to the manuscript as a new section (chapter 4.2) and figure (Fig. 5).

The new measurements revealed two distinct phases. During the pre-noon period, emissions systematically tracked the rise in temperature, showing a clear temperature dependency, and the calculated temperature coefficient (β) is consistent with the established literature for this species. This demonstrates the system's consistency and its ability to monitor a standard physiological process within a single individual. It provides strong evidence that the large variability observed in the common garden study was indeed due to inter-individual differences, not system instability.

In contrast, the afternoon was characterized by a non-linear emission surge during a heatwave, a response that deviated from the initial temperature dependency. This highlights the system's capability to also capture stress-related environmental responses to extreme events.

This new experiment complements our initial work in the common garden (Fig. 4), where the primary objective was to compare intraspecific variation across a consistent midday period (09:00–15:00), rather than to characterize diurnal cycles.

We are confident that the addition of this dedicated diurnal study, which confirms the system's ability to measure meaningful biological responses, significantly strengthens the manuscript and fully addresses your concern.

L346:

4.2 Diurnal variation in BVOC emissions

To evaluate the diurnal variation of BVOC emissions and assess the system’s response to environmental conditions, we conducted additional measurements on a Japanese cedar tree (height: approximately 10 m) growing on the premises of the National Institute for Environmental Studies, Japan. Based on preliminary observations indicating that the BVOC profile of this individual was dominated by monoterpenes (MTs), the measurements focused specifically on MT emissions. Sampling was conducted at multiple time points over a 24-hour period on 5 August 2025, from the lowermost branch of the tree.

Throughout the night and morning (01:00–11:00), the emission rate increased with rising

air temperature (Fig. 5a). The relationship between temperature and emissions during this pre-noon period (Fig. 5b) exhibited a typical exponential response, consistent with established models (e.g., Guenther et al., 1993). The temperature response coefficient (β, $°C^{-1}$) calculated from this data using Equation (2) was 0.191. It is worth noting that our β value was derived using non-linear regression, which differs from the log-transformed linear regression method used in some previous studies on this species (e.g., Matsunaga et al., 2011; Okumura et al., 2013). For comparison, applying the linear method to our data yields a β of 0.143, which agrees well with their reported values (0.09–0.17). This result demonstrates that our method can quantitatively and accurately assess the standard environmental responses of plants.

In the afternoon, however, as a heatwave caused the temperature to exceed 40°C, the emission rate surged dramatically, deviating from the morning trend (Fig. 5c). Notably, even after the temperature decreased to approximately 30°C in the evening, the emission rate did not return to the level predicted by the standard temperature dependency (Fig. 5b). This hysteresis suggests that the surge was not a simple thermal response but was likely triggered by factors such as heat-induced physiological damage, as reported by Nagalingam et al. (2024). Although a mechanistic investigation of this single case is outside the scope of this methodological paper, it highlights the system's capability to capture plant responses to extreme weather events. This interesting phenomenon warrants further investigation.

Specific Comments

*Comment4: **L24-26**. Please note that observing significant individual variation cannot not demonstrate system reliability (quite the opposite actually). This should be reworded to avoid conflating biological variation with instrument performance.*

Response4: Thank you for this crucial point. We completely agree that our original wording conflated biological variation with system reliability, which was a logical error. We have reworded this sentence in the abstract to correct this and to more accurately describe our findings.

The revised sentence now reads:

L24: Field testing with Japanese cedar (Cryptomeria japonica) demonstrated the system's robust field performance, successfully capturing both significant inter-individual variability and the dynamic diurnal patterns of BVOC emissions. The system's ability to

reliably resolve these differences under field conditions demonstrates its applicability for advancing our understanding of BVOC dynamics in diverse ecosystems.

Comment5: ***L37-42.*** *Please consider expanding this paragraph and referencing recent review articles covering emission behaviour of monoterpenes (eg. https://doi.org/10.1038/s43247-023-01175-9) , sesquiterpenes (e.g. https://doi.org/10.1111/gcb.70258), and diterpenes (e.g. https://doi.org/10.21203/rs.3.rs-5407662/v1), to provide more context on their chemical properties and relevance.*

Response5: Thank you very much for introducing interesting references. We have added the suggested review articles in the paragraph.

L48: Recent comprehensive reviews have further underscored the critical and distinct roles of these terpene classes in biosphere-atmosphere interactions (Bourtsoukidis et al., 2024, 2025; Yañez-Serrano et al., 2024). For instance, diterpenes are now understood to be particularly potent contributors to SOA formation, potentially having a disproportionately large impact relative to their emission rates (Yañez-Serrano et al., 2024). Moreover, these reviews highlight that the emission rates and composition of MTs and SQTs can vary significantly among individuals, which may reflect diverse adaptive strategies to environmental stresses (Bourtsoukidis et al., 2024, 2025). To untangle the complex factors governing these emissions, a dual approach of broad-scale analysis and detailed, individual-level data collection is essential.

Comment6: ***L154-163. A.*** *The emission rate equation differs from more commonly used formulations (e.g., E = F × (Cout − Cin)/(dry weight mass)). Please elaborate on the reasoning behind this approach and its comparability.*

Response6: Thank you for your constructive comments. Our original formulation was non-standard and not sufficiently clear. To improve clarity, we have revised the manuscript to use the standard mass-balance equation as follows:

L166: The rate of BVOC emission (E, in ng $(gdw)^{-1}$ h^{-1}) was first calculated using a mass-balance equation:

$$E = \frac{F \times (C_{\text{out}} - C_{\text{in}})}{W_{\text{dry}}} \quad (1)$$

where F is the flow rate of purge air through the enclosure (L h^{-1}); Cout is the BVOC concentration in the air exiting the enclosure (ng L^{-1}), determined from the mass of the compound collected on a sorbent tube divided by the total volume of air sampled; Cin is concentration of BVOCs in the incoming purge air, determined from a blank measurement (an empty enclosure); Wdry is the dry weight of the enclosed branch (g dw), estimated from Shirota (2000).

To allow for comparison across measurements taken at different temperatures, this measured emission rate (E) was then normalized to a basal emission rate (Es, in ng $(gdw)^{-1}$ h^{-1}) at a standard temperature (Ts, 30°C = 303.15 K), following the algorithm of Guenther et al. (1993):

$$E_s = \frac{E}{\exp[\beta(T - T_s)]} \quad (2)$$

where T is the temperature inside the enclosure, and β is an empirical coefficient that quantifies the temperature sensitivity of emissions. The β values used were 0.17 for MTs, 0.20 for SQTs, and 0.21 for DTs (Matsunaga et al., 2011, 2012, 2013).

Comment7: ***B.*** *The use of basal emission rate (ES) calculated using fixed β values from the literature assumes consistent temperature sensitivity across all conditions. This approach is not appropriate in a study designed to evaluate natural emissions. Empirical derivation of temperature responses would provide more convincing validation.*

Response7: Thank you for this valid point. We agree that empirically deriving the β value from our own data would be the most robust approach.
However, our field campaign was conducted over a short period of 11 consecutive days, resulting in a limited temperature range. The coefficient of variation for the measured temperatures was only 0.15 (RSD), indicating low temperature variability.
With such small temperature range and limited size of our dataset, it was not suitable for a robust empirical derivation of β. Therefore, we used established β values from the literature as the more reasonable and necessary step for normalization in this context.
We have revised the manuscript to clearly state this limitation and provide the context of the narrow temperature range.

L179: It should be noted that these β values were not empirically derived from our own dataset, as the measurements were conducted over a narrow temperature range that was unsuitable for robust parameterization.

Comment8: ***L195-196.*** *The statement that the system reduces "desiccation stress" based on chamber humidity is incorrect. Drought stress is primarily soil-driven, and relative humidity in the enclosure does not replicate root water availability. Please rephrase or remove this statement.*

Response8: Thank you for this accurate and important correction. Our use of "drought stress" was incorrect. We have removed this term from the manuscript. Our intended meaning was that using ambient air, which is more humid than dry cylinder air, reduces the atmospheric water demand on the branch. To describe this phenomenon accurately, we have revised the text to refer to vapor pressure deficit, a key driver of BVOC emissions.

L212: The humidity within the enclosure more closely reflected the external humidity when purge air was supplied via our air delivery system compared to using dry cylinder gas (Table 2). This reduces the atmospheric water demand on the branch, avoiding potential artifacts caused by an artificially high vapor pressure deficit.

Comment9: ***L204/Table 2.*** *With the flows used, one would have expected higher humidity inside the chamber as the result of evapotranspiration from a living branch.*

Response9: Thank you for your comments. The moderate humidity levels observed were an expected result of our experimental design.
We used a photographic white umbrella to diffuse and reduce the intensity of direct sunlight on the enclosure. This, combined with our system's efficient air exchange (residence time of approx. 1.6 minutes), prevents both overheating and the accumulation of transpired water vapor, which explains the stable conditions observed.

L104: A photographic white umbrella was used to diffuse and reduce the intensity of direct sunlight on the enclosure during purging and sampling.
L220: This overall stability in both temperature and humidity is attributed to two key design features. First, the photographic white umbrella was used to diffuse and reduce the intensity of direct sunlight, preventing overheating of the enclosed branch. Second, the high air exchange rate, with a residence time of approximately 1.6 minutes, effectively prevents the accumulation of both heat and transpired water vapor.

Comment10: ***L210-216.*** *The orders of magnitude of phyllocladane stronger emissions is perhaps an indication that we are seeing the effects of stress and not natural emissions.*

Response10: You are correct that such a large emission warrants careful consideration. While we acknowledge that stress can influence BVOC emissions, we believe the primary reason for these particularly high phyllocladane emissions is biological rather than a measurement artifact. Our interpretation is based on previous findings (Saito et al., 2022) which show that significant phyllocladane emission from *C. japonica* is a trait specific to individuals that have this compound stored in their tissues. The high emissions were observed only in certain individuals, which is consistent with this understanding.
As this paragraph focuses specifically on the reproducibility of the measurement system, we feel that a detailed discussion of the biological interpretation of specific compound emissions is beyond its scope. However, we appreciate you bringing this to our attention.

Comment11: ***Chapter 3.4 / Figure 3.*** *The sharp emission peak followed by exponential decay likely reflects the depletion of storage pools in a severed branch rather than natural stabilization. Comparing late-stage emissions to initial peaks does not validate reproducibility but rather shows a system with low emission rates as the storage pools are emptying. Demonstrating that with isoprene, which is mainly de novo produced, would have been more convincing.*

Response11: Thank you for your comments. First, regarding the use of isoprene, this was not possible as our study species, Cryptomeria japonica, does not emit de novo synthesized BVOCs such as isoprene and relies exclusively on storage pools. Second, we argue that the observed decay is not due to storage pool depletion. The total amount of terpenes emitted during the measurement (3.75 µg/gdw) accounts for only 0.024% of the estimated total storage (approx. 15.9 mg/gdw, based on Saito et al., 2022). This fraction is too small to cause depletion. Instead, we interpret the pattern as a transient emissions pulse caused by mechanical disturbances of installing the chamber, a phenomenon reported by Mochizuki (2011). Therefore, the subsequent stabilization demonstrates the system's ability to measure a steady baseline emission rate once this initial disturbance subsides.

Comment12: ***Chapter 4.*** *As mentioned above, please clarify whether the field deployment involved measurements from branches still attached to living trees. This is a key point for assessing whether the system has been tested in realistic conditions.*

Response12: Thank you for seeking this clarification. We confirm that all field deployment measurements were conducted on intact branches of living trees. We have now made this point more explicit in the manuscript to avoid any ambiguity.

L311: Field measurements were conducted on live, intact branches, with one south-facing branch selected per tree at approximately 1.3 m above the ground.