Highly diverse emission of volatile organic compounds by Sitka spruce and determination of their emission pathways

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Supplementary Information

Set-up

Individual cylindrical Teflon enclosures were made for each spruce tree, using clear 0.1 mm thick Teflon FEP foil (Ortega and Helmig, 2008). The enclosures were designed to encompass all except the bottom few branches of the trees, and to minimise contact between the contained branches and the interior wall of the enclosure (Ortega and Helmig, 2008; Janson et al., 1999). The tops of the enclosures were fitted with a ¼" inlet port and a ½" outlet port. A length of ¼" Teflon tubing extended 20 cm from the inlet port into the enclosure to ensure homogenisation (Niinemets et al., 2011). The enclosures were sealed around the trees a week before emissions sampling was commenced. A separate, Empty enclosure, was made for background measurements (Hayward et al., 2004) (Fig. S3). ¼

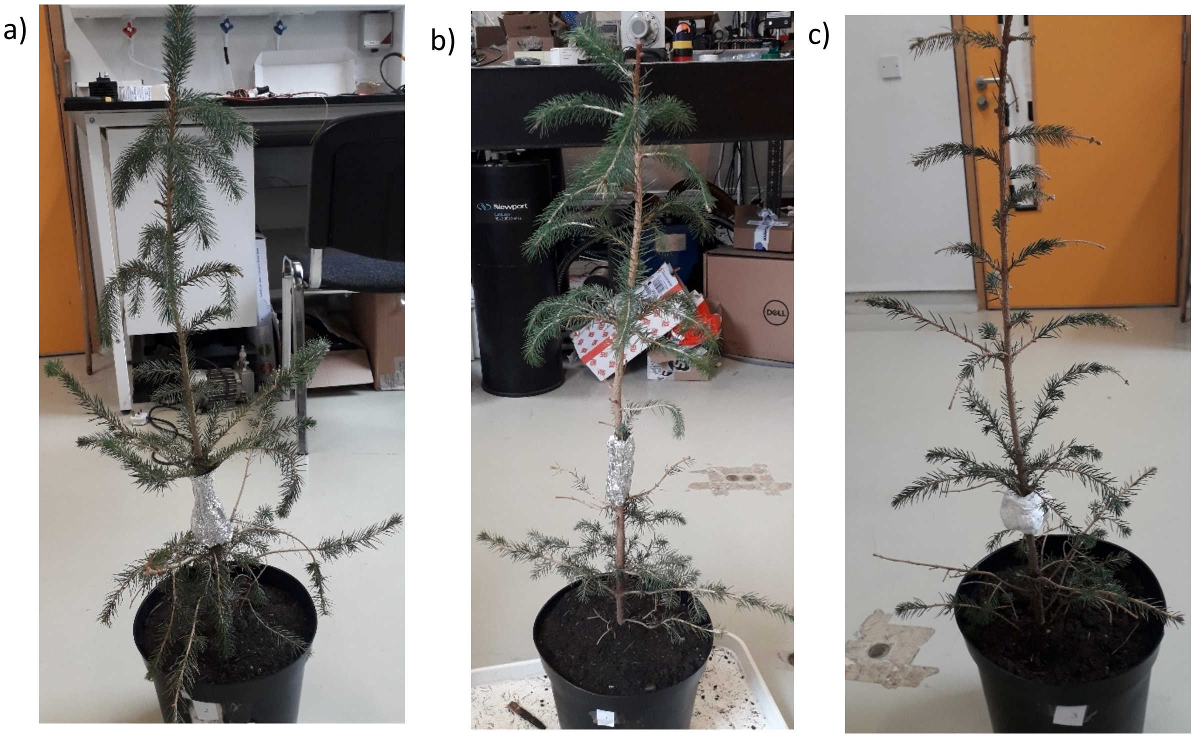


Figure S1 *Three Sitka spruce saplings used in this work: a) Spruce 1, b) Spruce 2 and c) Spruce 3.*

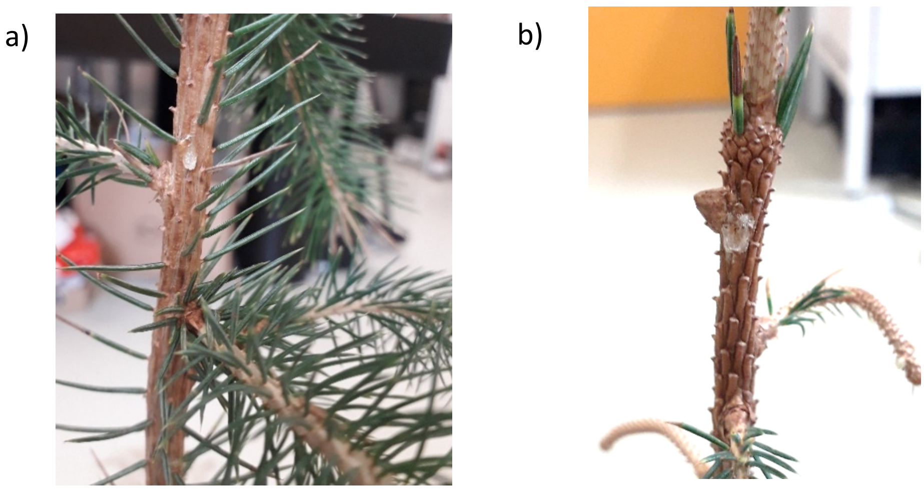


Figure S2 *Resin deposits on a) Spruce 1 and b) Spruce 3.*

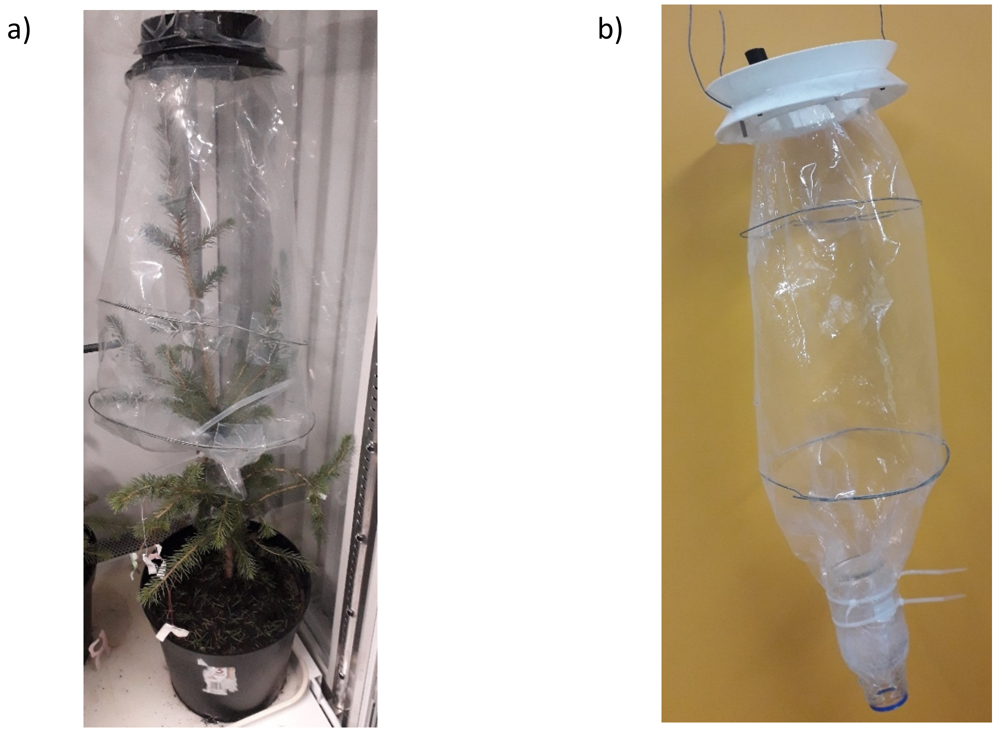


Figure S3 *Enclosure around a) Spruce sapling and b) empty enclosure.*

Plant growth cycles

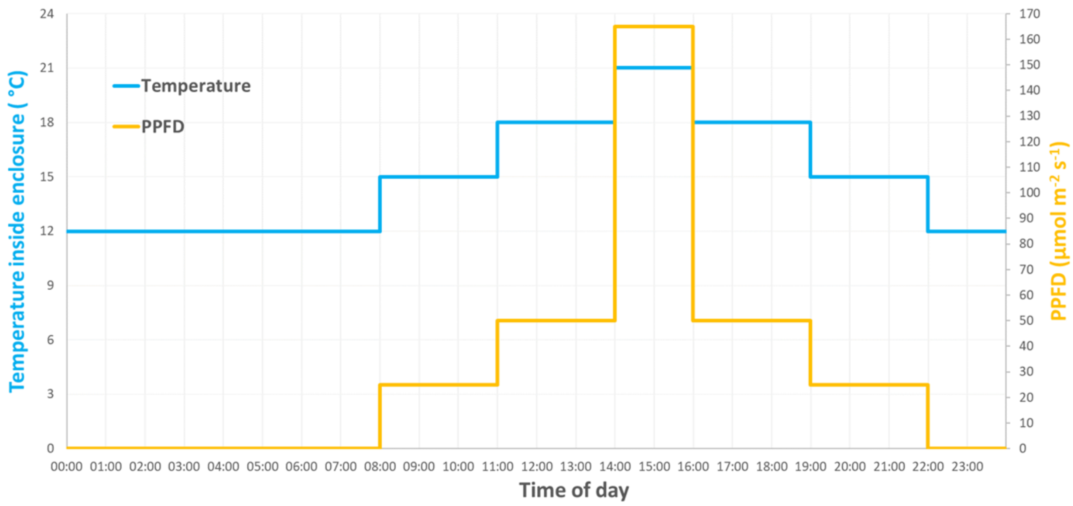


Figure S4 *Temperature and PPFD variation during the Daily Cycle.*

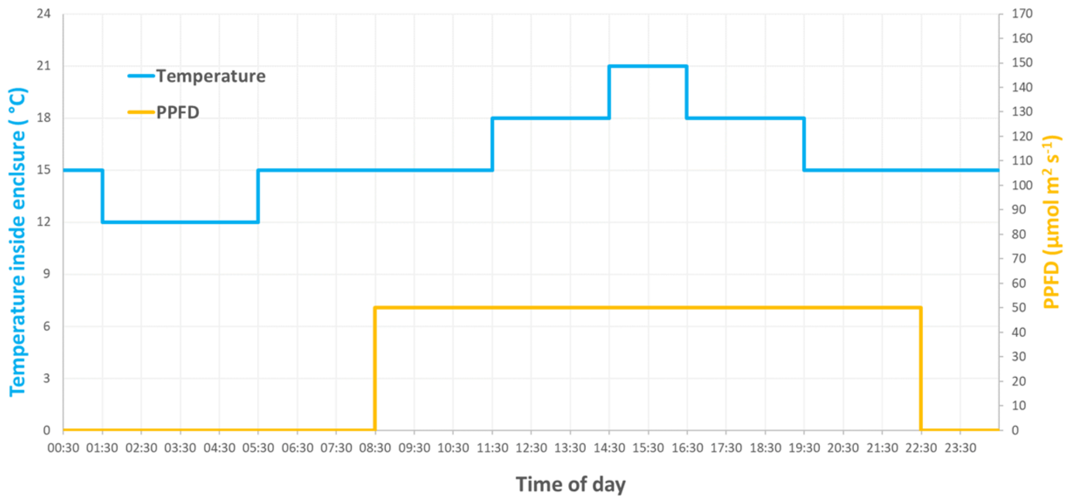


Figure S5 *Temperature and PPFD variation during the Temperature Cycle.*

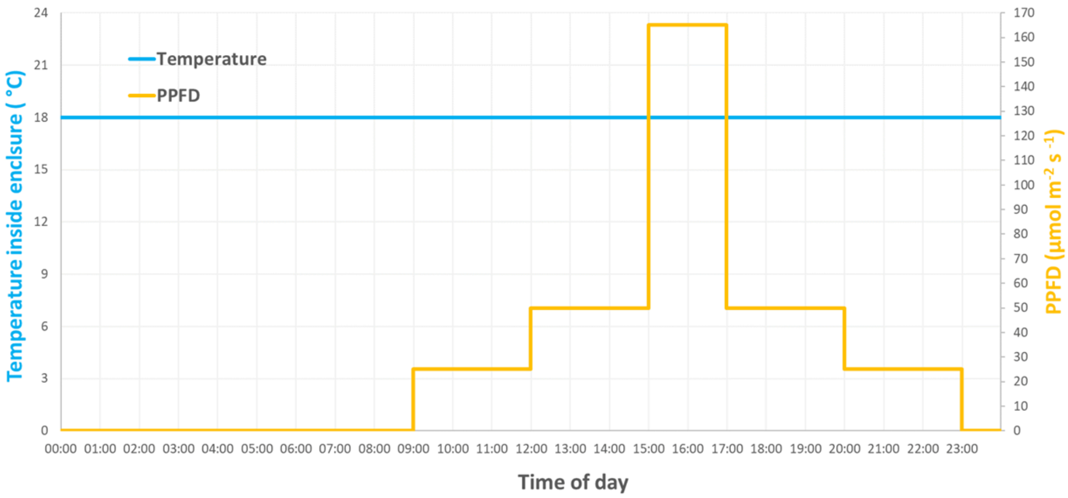
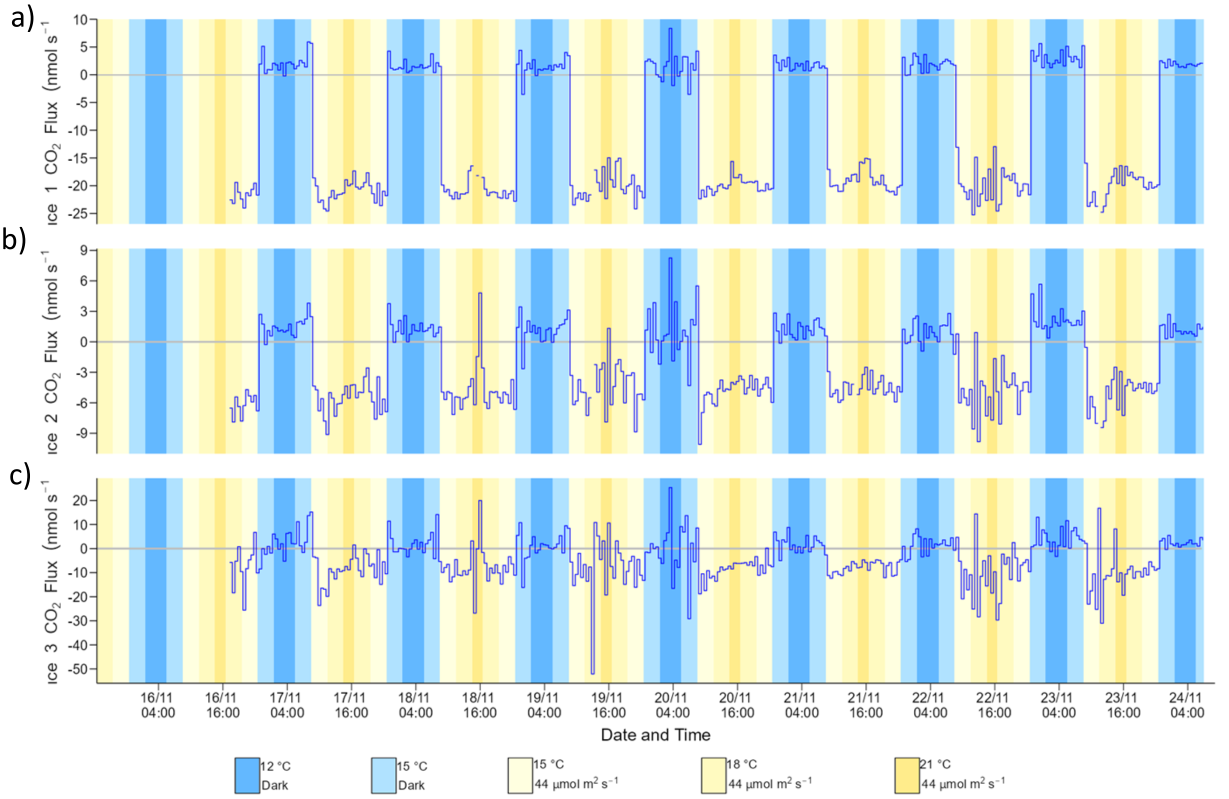


Figure S6 *Temperature and PPFD variation during the Light Cycle.*

Biomass measurements

Table S1 *Dried biomass measurements for each spruce tree.*

	Dried Needle Mass **g**	**Dried Needle and Branch Mass** **g**
Spruce 1	8.03	18.50
Spruce 2	9.80	19.56
Spruce 3	3.77	11.33



Temperature Cycle

Figure S7 *Time series of the CO_2 flux for a) Spruce 1, b) Spruce 2 and c) Spruce 3 during the Temperature Cycle.*

Light Cycle

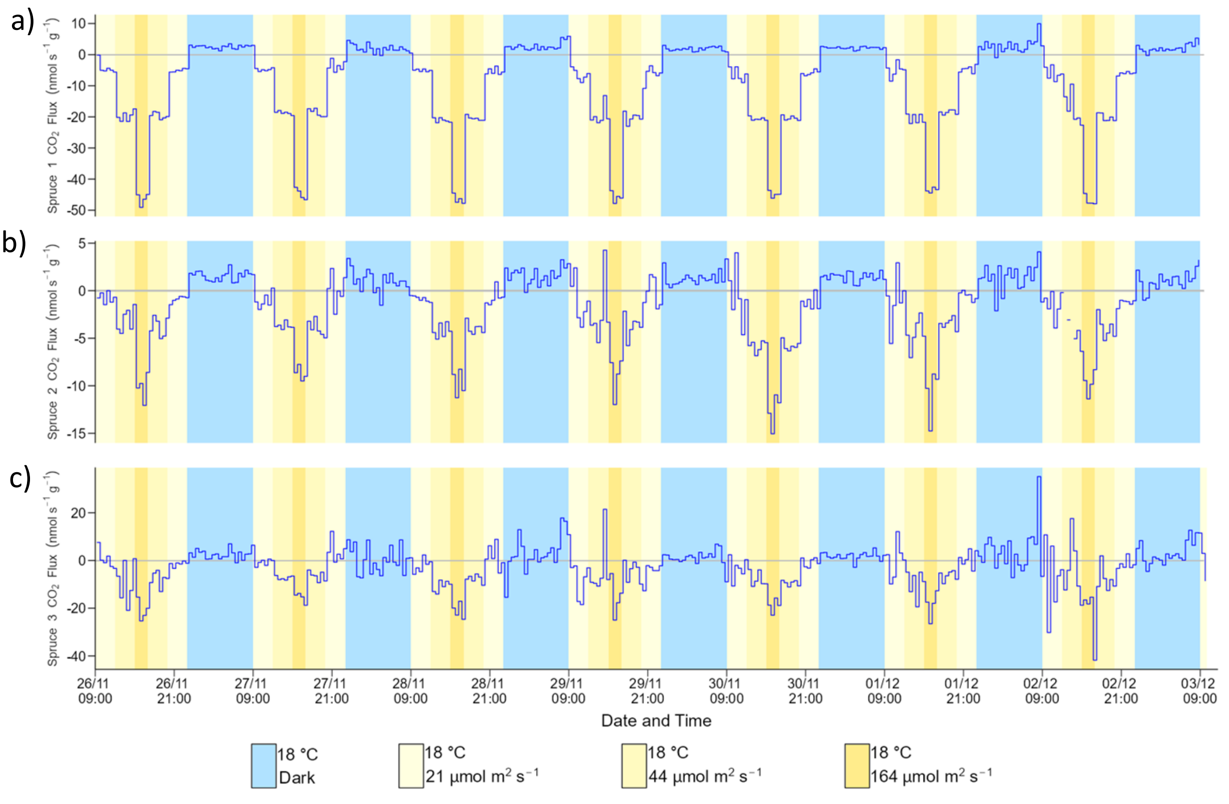


Figure S8 *Time series of CO_2 flux for a) Spruce 1 b) Spruce2, and c) Spruce 3 during the Light Cycle.*

BVOC emissions

Table S2 *BVOC emissions from Spruce 1 and Spruce 2 detected with ToF-CIMS and TD-GC/MS.*

Formula	MW	Name	Spruce 1		Spruce 2	
	g mol^{-1}		ToF-CIMS	TD-CG/MS	ToF-CIMS	TD-GC/MS
C_3H_9N	59.11		✓			
$C_2H_4O_2$	60.05	Acetic acid				✓
C_5H_8	68.12	Isoprene	✓	✓		✓
C_4H_6O	70.09	Methacrolein				✓
$C_3H_6O_2$	74.08	Propanoic acid		✓		✓
$C_4H_{10}O$	74.12	1-Butanol				✓
C_6H_8	80.13				✓	
C_5H_6O	82.10	2-Methyl-furan		✓		✓
C_5H_8O	84.12	*E*-3-Penten-2-one				✓
$C_5H_{10}O$	86.13	2-Methyl-3-buten-2-ol		✓		
		2-Pentanone		✓		✓
		Pentanal				✓
$C_4H_8O_2$	88.11	Butanoic acid				✓
$C_3H_8N_2O$	88.11		✓			
$C_5H_{12}O$	88.15	3-Methyl-1-butanol		✓		✓
C_7H_8	92.14		✓		✓	
C_6H_6O	94.11	Phenol				✓
C_6H_8O	96.13				✓*	
$C_6H_{10}O$	98.15	*E*-2-Hexenal			✓	✓
$C_5H_8O_2$	100.13		✓			
$C_6H_{12}O$	100.16	Hexanal				✓
		2-Hexanone				✓
C_7H_{16}	100.20	Heptane		✓		✓
$C_6H_{14}O$	102.18	1-Hexanol		✓		✓
C_7H_8O	108.14		✓		✓	
C_8H_{12}	108.18		✓		✓	
$C_7H_{10}O$	110.15				✓	
$C_5H_{10}NO_2$	117.15		✓			
C_8H_8O	120.15	Acetophenone				✓
C_9H_{12}	120.19		✓		✓	
$C_7H_{12}N_2$	124.18		✓			
$C_8H_{12}O$	124.18				✓	
$C_8H_{16}O$	128.21	1-Octen-3-ol				✓
$C_{10}H_{10}$	130.19		✓			
$C_8H_{18}O$	130.23	2-ethyl-1-hexanol				✓
$C_{10}H_{14}$	134.22	o-Cymene		✓		✓

C_9H_8O	132.16	(*E*)-Cinnamaldehyde	✓	✓	✓	✓
$C_6H_{14}O_3$	134.18		✓			
$C_8H_8O_2$	136.15	Methyl benzoate		✓		✓
$C_9H_{12}O$	136.19				✓	
$C_{10}H_{16}$	136.24	Monoterpene	✓		✓*	
		Myrcene		✓		✓
		β-Phellandrene		✓		✓
		δ-Limonene		✓		✓
		α-Pinene		✓		✓
		Camphene		✓		✓
$C_8H_{12}O_2$	140.18				✓	
$C_9H_{10}O_2$	150.17				✓	
C_3H_9N	150.22		✓			
$C_7H_4O_4$	152.10		✓			
$C_8H_8O_3$	152.14	Methyl salicylate		✓	✓	✓
$C_{10}H_{16}O$	152.23	Piperitone	✓	✓	✓	✓
		Camphor	✓	✓	✓	✓
$C_{10}H_{18}O$	154.25	Eucalyptol		✓	✓	✓
$C_{12}H_{16}$	160.25		✓			
$C_{11}H_{14}O$	162.23		✓			
$C_5H_{10}O_6$	166.13		✓			
$C_{10}H_{14}O_2$	166.22		✓		✓*	
$C_{10}H_{16}O_2$	168.24		✓			
$C_{10}H_{20}O_2$	172.26	Isopentyl isovalerate	✓	✓		✓
$C_{12}H_{16}O$	176.26				✓	
$C_{12}H_{20}O$	180.29		✓			
$C_{10}H_{16}O_3$	184.23				✓	
$C_{13}H_{12}O$	184.24		✓			
$C_{14}H_{16}O$	200.28		✓		✓	
$C_{10}H_{18}O_4$	202.25		✓		✓	
$C_{15}H_{24}$	204.36	*E*-β-Farnesene			✓	✓
$C_{10}H_{14}O_5$	214.22		✓			
$C_{17}H_{26}O$	246.39		✓		✓	
$C_{20}H_{24}$	264.40		✓		✓	
$C_{13}H_{30}O_5$	266.37		✓		✓	
$C_{18}H_{34}O$	266.46		✓		✓	
$C_{20}H_{24}O_2$	296.40		✓		✓	

** Isomers with different temporal trends detected by ToF-CIMS as different ions. Counted as two distinct BVOCs. Monoterpenes are ordered according to decreasing concentration, which were inferred from TD-GC/MS chromatogram peak areas. Yellow and red colours helped to visually identify compounds emitted by Spruce 1 and 2 respectively*

Chlorophyll fluorescence

Maximum photosystem II efficiency (F_v/F_m) was determined by measuring chlorophyll fluorescence. F_v/F_m reflects the ability of photosystem II to reduce its electron acceptors in the photosynthetic electron transport chain and it is used to infer plant health (Henriques, 2009). The chlorophyll fluorescence was measured on three days during the *Daily Cycle* (Table 2).

Table S3 *Chlorophyll fluorescence measurements for Spruce 1 and Spruce 2 during the Daily Cycle. Each value is the average of three measurements taken from three branches on the same tree.*

Date	**PPFD**	**Temperature**	**Spruce 1**		**Spruce 2**	
	µmol m^{-2} s^{-1}	**°C**	**F_v/F_m**	**STD**	**F_v/F_m**	**STD**
04/11/2021	0	12	0.85	0.02	0.75	0.01
08/11/2021	0	12	0.79	0.02	0.83	0.03
11/11/2021	0	12	0.81	0.02	0.82	0.08

Chlorophyll fluorescence was measured in the dark period. The F_v/F_m values were similar for both Spruce 1 and Spruce 2 and was centred around 0.81 (unitless). However, for both trees values below 0.8 were recorded on one of the days, suggesting that there was some extent of photoinhibition due to stress. Lower F_v/F_m ratios indicate that all chlorophyll is not taking part in photosynthesis, which could be due to damage, and implies the plant is stressed (Maxwell and Johnson, 2000). It is unlikely the trees were suffering from stress, as the temperatures used in this study are representative of the natural environment, and the PPFD was an order of magnitude lower than that measured for the ambient atmosphere.

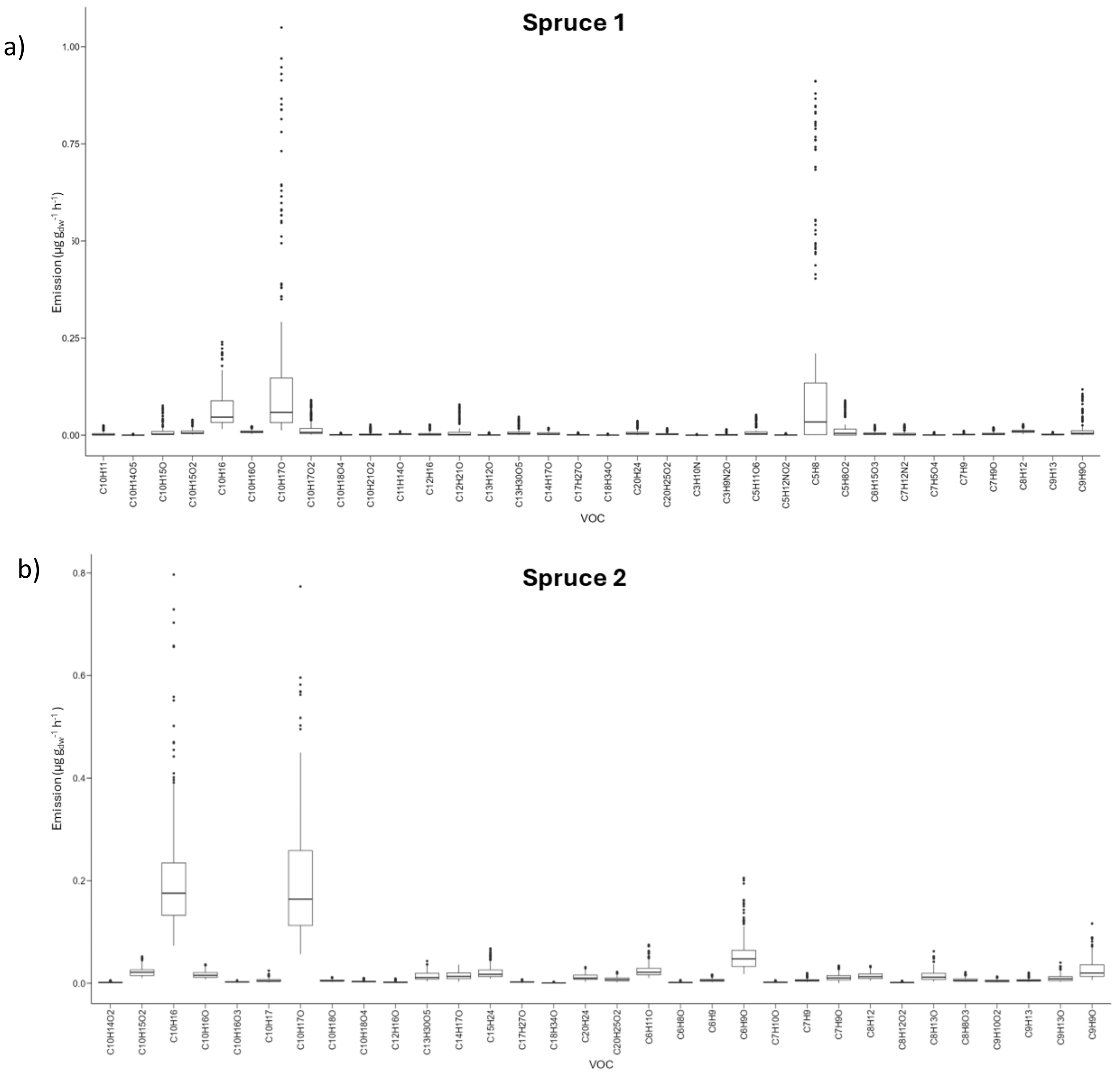


Figure S9 *Box plot for a) Spruce 1 and b) Spruce 2 (bottom) VOC emissions (in µg g_{dw}^{-1} h^{-1}). The bar represents the median, the bottom and top limit of the box the 25th and 75th quantiles, and the end of bottom and top bars the 5th and 95th quantiles.*

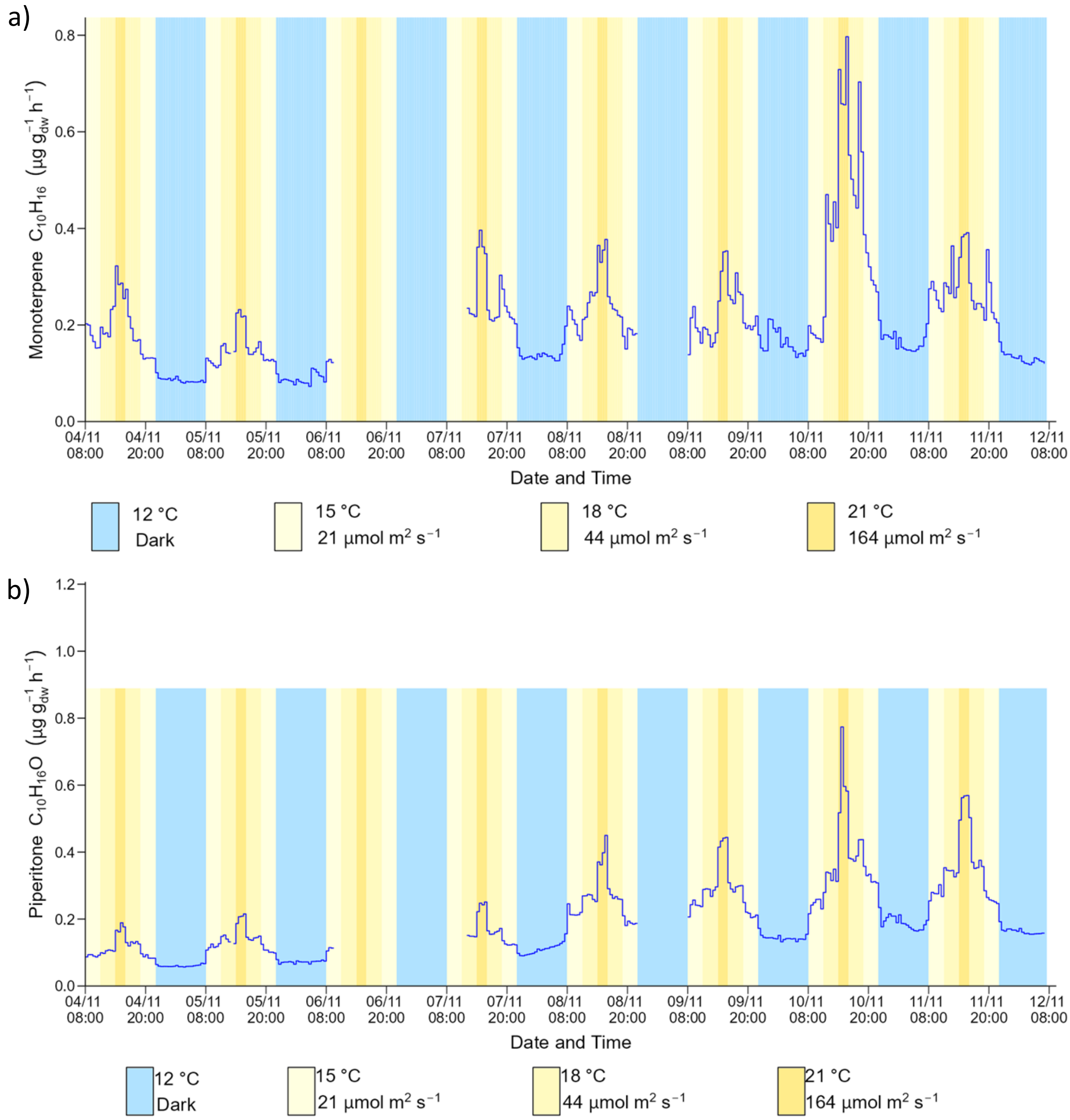


Figure S10 *Time series of a) piperitone ($C_{10}H_{16}O$) and b) monoterpenes ($C_{10}H_{16}$) emission fluxes from Spruce 2 during the Daily Cycle.*

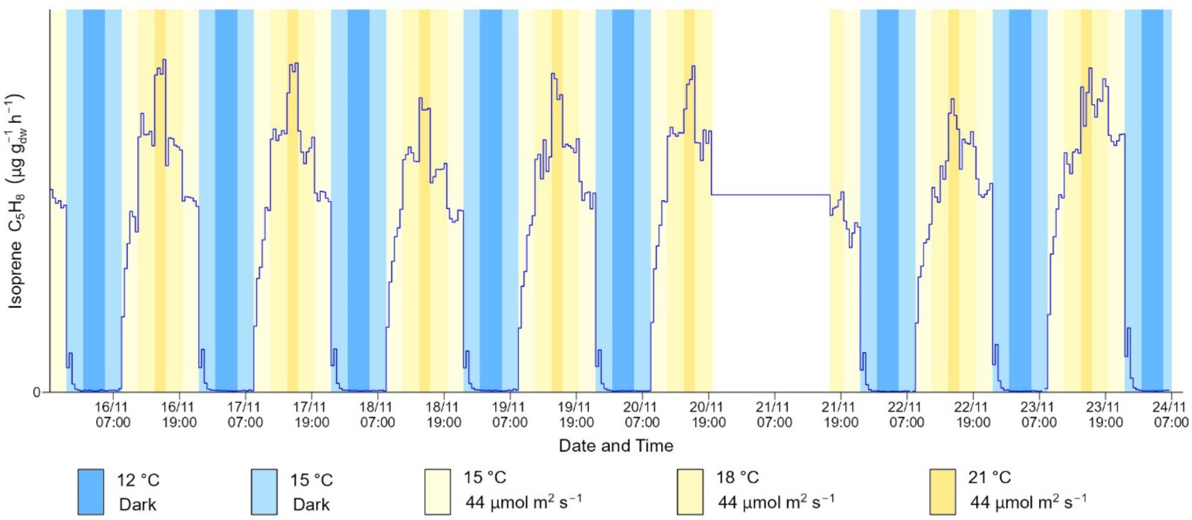


Figure S11 *Time series of the isoprene (C_5H_8) emission flux from Spruce 2 during the Temperature Cycle.*

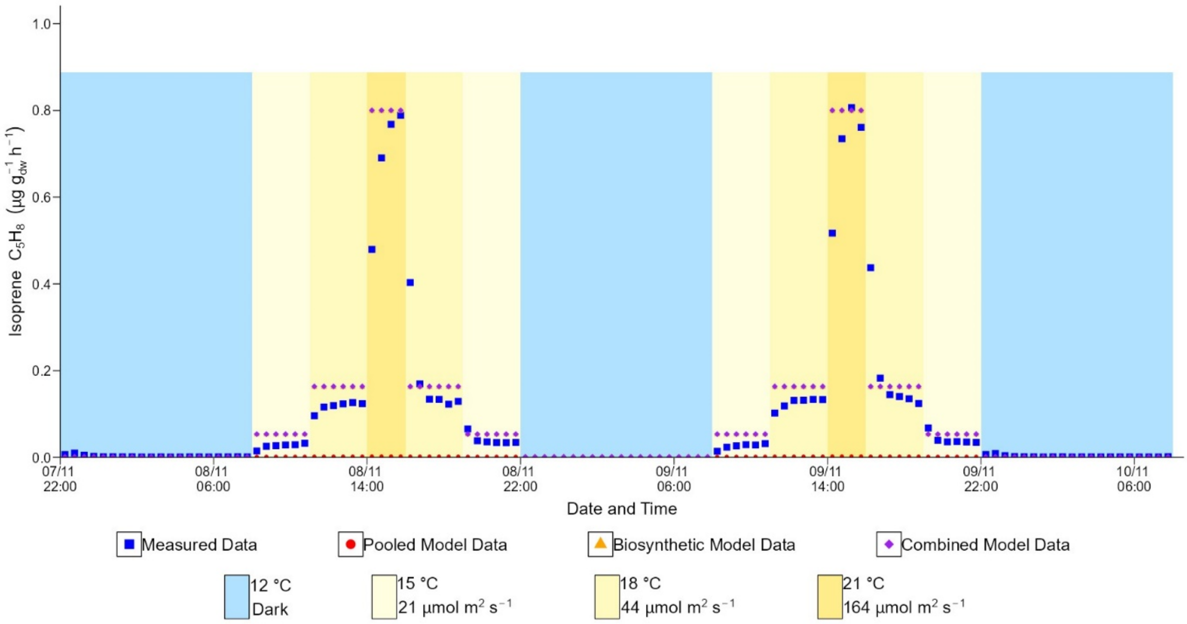


Figure S12 *Measured and all modelled data for the emission of isoprene from Spruce 1. As pooled emission was negligible, results from biosynthetic and combined models are overlaying.*

Table S4 *Chlorophyll fluorescence measurements for Spruce 1 and Spruce 2 during the Temperature Cycle. Each value is the average of three measurements taken from three branches on the same tree.*

Date	Light Intensity $\mu mol\ m^{-2}\ s^{-1}$	Temperature °C	Spruce 1	Spruce 2
16/11/2021	0	12	0.815	0.810
16/11/2021	44	18	0.762	0.773
19/11/2021	0	12	0.821	0.831
19/11/2021	44	18	0.820	0.785
22/11/2021	0	12	0.815	0.760
22/11/2021	44	18	0.785	0.805

The F_v/F_m values obtained for Spruce 1 and Spruce 2 during the *Temperature Cycle* are listed in Table S2.5. The values in the dark were centred around 0.8 and were similar to those observed during the *Daily Cycle*. One branch on Spruce 2 had lower measurements, and may indicate that Spruce 2 was stressed.

Table S5 *Chlorophyll fluorescence measurements for Spruce 1 and Spruce 2 during the Light Cycle. Each value is the average of three measurements taken from three branches on the same tree.*

Date	Light Intensity µmol m^{-2} s^{-1}	Temperature °C	Spruce 1	Spruce 2
28/11/2022	0	18	0.804	0.828
28/11/2022	164	18	0.653	0.742
30/11/2021	0	18	0.694	0.768
30/11/2021	164	18	0.718	0.751
02/12/2021	0	18	0.726	0.769
02/12/2021	164	18	0.750	0.724

The F_v/F_m values recorded during the *Light Cycle* for Spruce 1 and Spruce 2 were slightly lower than those measured during the other cycles. This indicates that although Spruce 2 was stressed, it was not suffering any additional stress during the *Light Cycle*.

Table S6 Contribution of CO_2 uptake, respiration and BVOC emissions to the carbon balance for Sitka spruce.

Carbon balance	Carbon flux mg C day^{-1} g^{-1}	Respiration %	BVOC emission %
Absolute uptake	- 4.07	6.8	0.2
Respiration	0.28	-	-
BVOC emission	0.01	-	-
Net uptake	- 3.78	7.4	0.2

Table S7: Table of calculated annual emission fluxes for piperitone, isoprene and monoterpenes from Sitka spruce in Ireland.

BVOC	Annual emission flux		
	tonne $year^{-1}$	g C ha^{-1} $year^{-1}$	g C m^{-2} $year^{-1}$
isoprene	13,000	34,000	3.3
piperitone	8,200	19,000	1.9

monoterpenes	1,600	4,200	0.4
total	22,800	57,200	5.6

Standardisation

Table S8 *BVOC emission fluxes from Spruce 1 standardised to 1000 µmol m^{-2} s^{-1} and 30°C in µg h^{-1}g^{-1}. BVOC emissions ordered in order of decreasing standardised emission flux.*

Formula	BVOC	Standardisation Method	Standardised Emission Flux µg h^{-1} s^{-1}
$C_{10}H_{16}O$	Piperitone	Combined	17.290
C_5H_8	Isoprene	Biosynthesis	6.306
$C_{10}H_{16}$	Monoterpene	Combined	0.925
$C_{10}H_{14}O$		Combined	0.859
$C_5H_8O_2$		Biosynthesis	0.467
$C_{10}H_{16}O_2$		Combined	0.455
C_9H_8O	(*E*)-Cinnamaldehyde	Biosynthesis	0.334
$C_{13}H_{30}O_5$		Combined	0.276
$C_{12}H_{20}O$		Biosynthesis	0.271
$C_{10}H_{16}O$	Camphor	Combined	0.199
$C_5H_{10}O_6$		Biosynthesis	0.196
$C_{20}H_{24}$		Combined	0.177
$C_{10}H_{14}O_2$		Combined	0.166
$C_{10}H_{10}$		Biosynthesis	0.155
$C_{10}H_{20}O_2$	Isopentyl isovalerate	Biosynthesis	0.148
C_8H_{12}		Combined	0.147
$C_{14}H_{16}O$		Combined	0.138
$C_7H_{12}N_2$		Biosynthesis	0.127
$C_3H_8N_2O$		Biosynthesis	0.082
$C_6H_{14}O_3$		Combined	0.082
$C_{12}H_{16}$		Biosynthesis	0.081
$C_{11}H_{14}O$		Combined	0.077
$C_{20}H_{24}O_2$		Combined	0.074
$C_7H_4O_4$		Combined	0.067
C_7H_8O		Combined	0.052
$C_{17}H_{26}O$		Combined	0.046
C_7H_8		Combined	0.040

C_9H_{12}		Combined	0.030
$C_{13}H_{12}O$		Biosynthesis	0.025
$C_{10}H_{18}O_4$		Combined	0.020
$C_{10}H_{14}O_5$		Combined	0.016
$C_5H_{10}NO_2$		Combined	0.015
$C_{18}H_{34}O$		Combined	0.014
C_3H_9N		Combined	0.012

Table S9 *BVOC emission fluxes from Spruce 2 standardised to 1000 µmol m^{-2} s^{-1} and 30°C in µg h^{-1}g^{-1}. BVOC emissions ordered in order of decreasing standardised emission flux.*

Formula	**BVOC**	**Standardisation Method**	**Standardised Emission Flux µg h^{-1} s^{-1}**
$C_{10}H_{16}$	Monoterpene	Combined	1.226
$C_{10}H_{16}O$	Piperitone	Combined	1.029
C_6H_8O		Pooled	0.192
$C_{20}H_{24}$		Pooled	0.134
C_9H_8O	(*E*)-Cinnamaldehyde	Combined	0.120
$C_6H_{10}O$	*E*-2-Hexenal	Pooled	0.104
$C_{15}H_{24}$	*E*-β-Farnesene	Combined	0.097
$C_8H_{12}O$		Combined	0.073
$C_{14}H_{16}O$		Combined	0.071
$C_{10}H_{14}O_2$		Pooled	0.066
$C_{10}H_{16}O$	Camphor	Combined	0.061
C_7H_8O		Combined	0.056
$C_{13}H_{30}O_5$		Combined	0.055
C_8H_{12}		Combined	0.051
$C_9H_{12}O$		Combined	0.046
C_9H_{12}		Combined	0.035
$C_8H_8O_3$	Methyl salicylate	Combined	0.033
$C_{20}H_{24}O_2$		Combined	0.033
C_7H_8		Combined	0.031
$C_{10}H_{16}$		Pooled	0.029
$C_{10}H_{18}O_4$		Combined	0.025
C_6H_8		Combined	0.023

$C_{10}H_{18}O$	Eucalyptol	Combined	0.022
$C_{17}H_{26}O$		Combined	0.018
$C_9H_{10}O_2$		Combined	0.018
$C_{12}H_{16}O$		Combined	0.012
$C_7H_{10}O$		Combined	0.009
$C_{10}H_{16}O_3$		Combined	0.008
C_8H_{12}		Combined	0.007
C_6H_8O		Combined	0.007
$C_{10}H_{14}O_2$		Pooled	0.006
$C_{18}H_{34}O$		Combined	0.003

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