

## Original Article

## Onset of photosynthesis in spring speeds up monoterpene synthesis and leads to emission bursts

J. Aalto<sup>1,2</sup>, A. Porcar-Castell<sup>2</sup>, J. Atherton<sup>2</sup>, P. Kolari<sup>2,3</sup>, T. Pohja<sup>1</sup>, P. Hari<sup>2</sup>, E. Nikinmaa<sup>2</sup>, T. Petäjä<sup>3</sup> & J. Bäck<sup>2</sup><sup>1</sup>SMEAR II Station, University of Helsinki, Korkeakoski 35500, Finland, and Departments of <sup>2</sup>Forest Sciences and <sup>3</sup>Physics, University of Helsinki, Helsinki 00014, Finland

## ABSTRACT

**Emissions of biogenic volatile organic compounds (BVOC) by boreal evergreen trees have strong seasonality, with low emission rates during photosynthetically inactive winter and increasing rates towards summer. Yet, the regulation of this seasonality remains unclear. We measured *in situ* monoterpene emissions from Scots pine shoots during several spring periods and analysed their dynamics in connection with the spring recovery of photosynthesis. We found high emission peaks caused by enhanced monoterpene synthesis consistently during every spring period (monoterpene emission bursts, MEB). The timing of the MEBs varied relatively little between the spring periods. The timing of the MEBs showed good agreement with the photosynthetic spring recovery, which was studied with simultaneous measurements of chlorophyll fluorescence, CO<sub>2</sub> exchange and a simple, temperature history-based proxy for state of photosynthetic acclimation, *S*. We conclude that the MEBs were related to the early stages of photosynthetic recovery, when the efficiency of photosynthetic carbon reactions is still low whereas the light harvesting machinery actively absorbs light energy. This suggests that the MEBs may serve a protective functional role for the foliage during this critical transitory state and that these high emission peaks may contribute to atmospheric chemistry in the boreal forest in springtime.**

*Key-words:* BVOC; chlorophyll fluorescence; hybrid algorithm; monoterpene emission; spring recovery.

## INTRODUCTION

Biogenic volatile organic compounds (BVOC) play an essential role in tropospheric chemistry influencing aerosol growth and formation (Claeys *et al.* 2004; Kulmala *et al.* 2004; Tunved *et al.* 2006; Ehn *et al.* 2014), production and destruction of tropospheric ozone (Atkinson & Arey 2003) and competition for OH with methane (Kaplan *et al.* 2006). It has been suggested that BVOCs are linked to aerosol growth to cloud condensation nuclei (CCN) sizes affecting cloud albedo and thus cooling the climate (Paasonen *et al.* 2013). Simultaneously, the aerosol growth alters the ratio between diffuse and global radiation. Because the biosphere can more efficiently utilize the diffuse radiation, enhancing gross primary

production and uptake of CO<sub>2</sub> (Kulmala *et al.* 2014) again cool the climate. BVOC emissions, including plant terpenoids such as monoterpenes, are an order of magnitude higher than those from anthropogenic sources over northern Europe (Lindfors *et al.* 2000) as well as globally (Guenther *et al.* 1995). Modelling the spatial and temporal dynamics of BVOC emissions is therefore crucial for improving global atmospheric models.

Boreal springtimes are characterized by a rapid increase and large diurnal fluctuations in irradiance and temperature, with irradiance increasing earlier in springtime than temperature. These meteorological changes are known to control the dormancy release in springtime (Rohde & Bhalerao 2007) and recovery of the photosynthetic apparatus, both in terms of light harvesting machinery (Ensminger *et al.* 2004; Porcar-Castell *et al.* 2008a; Porcar-Castell 2011), as well as in terms of overall photosynthetic capacity (Kolari *et al.* 2007, 2014). Interestingly, increased monoterpene emissions (Tarvainen *et al.* 2005; Hakola *et al.* 2006) and also the atmospheric new particle formation events in boreal region (Dal Maso *et al.* 2007) coincide with this period of intensive recovery of photosynthetic capacity. The question remains as to how the high monoterpene emissions and subsequent particle formation events might be linked to the spring recovery of photosynthesis.

As the reviews by Niinemets & Monson (2013) and Monson *et al.* (2012) clearly show, research on the multiple factors affecting BVOC emissions has been intensive in past decades. Large seasonal variation in monoterpene emissions has been reported both using measurements (e.g. Staudt *et al.* 1997; Karl *et al.* 2003; Tarvainen *et al.* 2005; Hakola *et al.* 2006; Keenan *et al.* 2009) and modelling (Staudt *et al.* 2000; Guenther *et al.* 2012). The concept of standard emission potential that is often used in monoterpene emission models is based on the assumption that there is a constant, plant-specific emission potential which does not change seasonally or diurnally and that the variation in emissions mainly originates from incident temperature (Guenther *et al.* 1993), with some modifications to take into account, for example soil moisture and in-canopy processes (Guenther *et al.* 2006). However, diurnal (Aalto *et al.* 2014) and annual (Tarvainen *et al.* 2005; Hakola *et al.* 2006; Keenan *et al.* 2009) variation in monoterpene emission rates can be an order of magnitude or higher, which suggests that also inherent, physiological drivers may be involved in regulation of emissions (Grote & Niinemets 2008). Within the last two decades, it has been

Correspondence: J. Aalto. e-mail: juho.aalto@helsinki.fi

recognized that sensitivity of vegetation to environmental drivers is not constant over a year or growing season (Kolari *et al.* 2014), which has implications to especially carbon assimilation but very likely also to other physiological processes.

Thus, seasonally changing physiological processes should be taken into account when the temporal dynamics of monoterpene emissions is estimated. In case of deciduous trees, seasonal variations in isoprene emission models have been mainly described as a simple function of leaf age and the related enzymatic activity changes (Lehning *et al.* 2001; Grote 2007), and in many cases this is well justified when dealing with species which do not store isoprenoids. In contrast, the effect of seasonality on emissions from evergreen, monoterpene-storing foliage is more difficult to model: it should be manifested in the variation of seasonally varying leaf physiological activity and the related potential for producing, storing and emitting BVOCs. Adequate model descriptions for the seasonality of such evergreen foliage do not exist. According to Tarvainen *et al.* (2005) and Hakola *et al.* (2006), monoterpene emission from boreal vegetation are highest in summer and low or negligible during winter, but they have measured especially high emission rates during spring period (March–April). Furthermore, Lappalainen *et al.* (2013) noticed very high monoterpene air concentrations already in April in a Scots pine (*Pinus sylvestris* L.) forest. Aalto *et al.* (2014) showed that Scots pine buds are strong sources of monoterpenes even before bud break.

These findings indicate that boreal evergreen forests are active and pronounced monoterpene sources well before the high physiological activity starts in spring, although the mechanisms that control the dynamics of these emissions remain under debate. One potential mechanism was proposed by Owen & Peñuelas (2005), who suggested that increased monoterpene emissions may be related to adjustments in pigment contents, primarily to carotenoid synthesis which shares the initial steps of pathway with monoterpene synthesis. A close connection between variation in carotenoid contents and monoterpene emission potential during acclimation of photosynthesis was indeed found in evergreen holm oak (*Quercus ilex*) by Porcar-Castell *et al.* (2009).

We conducted long-term field measurements over several years to study the critical period of spring recovery of photosynthesis. Our aim was to characterize the dynamics of springtime monoterpene emissions and study its synchronization with the recovery of photosynthesis in the evergreen foliage of Scots pine. We hypothesized that monoterpene emission peaks observed during springtime in boreal Scots pine shoots are not directly related to ambient temperatures but rather reflect the physiological process, recovery of photosynthesis in evergreen foliage.

## MATERIAL AND METHODS

### Measurement site

The measurements were carried out during springs 2009, 2010, 2012 and 2013 at the Station for Measuring Forest Ecosystem – Atmosphere Relations (SMEAR) II station

(Hari & Kulmala 2005). The station is located in Hyytiälä, southern Finland (61°N, 24°E, 180 m a.s.l.). The stands surrounding the station are dominated by mature Scots pine (*Pinus sylvestris* L.), with a mixture of Norway spruce (*Picea abies*) and various deciduous species such as downy and silver birches (*Betula pubescens* and *Betula pendula*) and European aspen (*Populus tremula*). The dominant trees of the study stand are 50 years old and the canopy reaches the height of ca. 17 m.

### Measurements of spring recovery of photosynthesis

The spring recovery of photosynthesis was followed using two independent measurements: CO<sub>2</sub> exchange and chlorophyll fluorescence. Two methods were used to cover both the effectiveness of light harvesting machinery alone and the entire photosynthesis including gas exchange. Dynamic enclosure method was used for measuring gas exchange simultaneously with monoterpene emissions. The automated gas exchange measurement system consisted of shoot enclosures, heated fluorinated ethylene propylene (FEP) or polytetrafluoroethylene (PTFE) tubing for sampling and analysers for CO<sub>2</sub>/H<sub>2</sub>O and volatile organic compounds (Kolari *et al.* 2012). The inner surfaces of the acrylic plastic walls of the enclosures were coated with FEP film. The enclosure measurements were conducted in unshaded conditions at the top of the canopy of a Scots pine stand, which was accessed using a scaffolding tower. The CO<sub>2</sub> exchange was measured simultaneously with monoterpene emissions, however, with two times more frequent measurement interval.

The measurements were started already during winter months except in 2010 when the measurements started in the end of March. During a successful measurement day, there were 24–48 chamber closures, usually more than 30. Several shorter or longer breaks occurred during the measurements because of maintenance or various malfunctions either in the measurement system or in the analysers. Two of the measurement shoots were 2 years old (2009 and 2013) and the rest (three shoots, 2010, 2012 and 2013) were 1 year old. All buds were cut off at least 4 weeks before the beginning of the measurements to prevent any growth of the shoots under monitoring. Otherwise all measurement shoots were intact and showed no signs of herbivory or other disturbances.

### Shoot gas exchange measurement system

CO<sub>2</sub> uptake of the shoots was measured with Uras 4 CO<sub>2</sub> analyser (Hartmann and Braun, Frankfurt am Main, Germany) (Altimir *et al.* 2002). Furthermore, air temperature (PT100 or copper-constantan thermocouple) inside the enclosure and photosynthetically active photon flux density (LI-COR LI-190 quantum sensor, LI-COR, Lincoln, NE, USA) were recorded at 5 s interval. The details concerning the measurement system and the flux calculation for CO<sub>2</sub> exchange are given in Altimir *et al.* (2002).

Respiration was estimated based on nighttime CO<sub>2</sub> exchange. Nighttime CO<sub>2</sub> flux was used to model the exponential dependence between temperature and respiration for

each 5 d period. Gross photosynthesis,  $P$  ( $\mu\text{g CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), was calculated by adding the estimated respiration to measured  $\text{CO}_2$  gas exchange. Next, daily maximum photosynthetic capacity  $P_{\text{max}}$  (unit same as for  $P$ ) was estimated by fitting a light response curve to the diurnal observations. A simple Michaelis–Menten type equation was applied as:

$$P = \frac{P_{\text{max}} I}{K_1 + I} \quad (1)$$

where  $I$  refers to photosynthetic photon flux density ( $PPFD$ ,  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ) and  $K_1$  is a parameter adjusting the non-linearity of the light response. Other driving factors such as temperature,  $\text{CO}_2$  concentration or the effect of vapour pressure deficit on stomatal conductance are not taken into account in the Michaelis–Menten approach. To minimize the ranges of variation in those factors, only the morning hours (0600–1200) were chosen for the  $\text{CO}_2$  exchange analysis. Both  $P_{\text{max}}$  and  $K_1$  were fitted for each morning dataset separately minimizing the sum of squares between the model and observations.

To further analyse photosynthetic recovery, we introduce a unitless measure: the relative light use potential ( $RLUP$ ). It describes the potential of the photosynthetic apparatus to utilize light, especially in case of high irradiance, compared with the midsummer situation, expressed as:

$$RLUP(t) = \frac{P_{\text{max}}(t)}{P_{\text{maxA}}} \quad (2)$$

where  $P_{\text{maxA}}$  is the mean  $P_{\text{max}}$  obtained for the period of 20 to 30 June.

## Fluorescence measurements

Chlorophyll fluorescence properties were measured using a Monitoring PAM fluorometer system (Heinz Walz GmbH, Effeltrich, Germany) consisting of two to three independent measuring heads (Porcar-Castell *et al.* 2008b; Porcar-Castell 2011) installed in needles close by the gas exchange enclosure system. Three to four pairs of needles were clipped per measuring head. Prevailing ( $F'$ ) and maximal fluorescence ( $F'_M$ ) were measured every 15, 30 or 60 min using the saturating pulse technique (Schreiber *et al.* 1986). Measuring frequency was adjusted during the season to minimize pulse-induced long-term photoinhibition, using lower frequencies during winter and nights (Porcar-Castell 2011). The duration of the saturating light pulse was 0.8 s and the intensity at the leaf surface was  $>4000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . The data were used to estimate the operating quantum yield of photochemistry in photosystem II (PSII) (Genty *et al.* 1989) as:

$$\phi P = \frac{F'_M - F'}{F'_M} \quad (3)$$

The daily maximum quantum yield of PSII ( $\phi P_{\text{max}}$ ) corresponded to the maximum  $\phi P$  registered during that particular day. This yield was obtained during nighttime and is equivalent to the widely used parameter  $F_v/F_m$  computed for

dark acclimated samples (Kitajima & Butler 1975). Day to day changes in  $\phi P_{\text{max}}$  were used to track the spring recovery of PSII as a proxy of the light harvesting apparatus. The quantum yield data used in this study represent the mean value of two (2009 and 2010) or three (2012 and 2013) measurement heads located to the top branches.

## Monoterpene emission rate measurements and acquisition of emission potentials

The monoterpene volume mixing ratios in shoot enclosures were recorded using proton transfer reaction quadrupole mass spectrometer (PTR-QMS, Ionicon Analytik GmbH, Innsbruck, Austria) coupled with the gas exchange measurement system. All analysers shared the same heated FEP or PTFE sample tubes. Further details concerning the monoterpene measurement are given in Aalto *et al.* (2014). The measurement and volume mixing ratio calculation as well as calibration methods are described in Taipale *et al.* (2008). The monoterpene emission rate calculation and further details concerning the gas exchange measurement system are given in Kolari *et al.* (2012).

In order to analyse the relationship between monoterpene emissions and the prevailing light and temperature, we applied the hybrid algorithm approach presented by Ghirardo *et al.* (2010). It is a quasi-mechanistic emission model based on certain relationships between light and/or temperature and physical and physiological processes behind monoterpene emission and describes the origin of emissions either directly from synthesis processes (*de novo* emissions) or from specialized storage structures (pool emissions).

In the hybrid algorithm, the emission rate  $E$  is a function of two source terms, referred as *de novo* emissions ( $E_{\text{synth}}$ ) and pool emissions ( $E_{\text{pool}}$ ), as follows:

$$E = E_{\text{synth}} + E_{\text{pool}} = E_{0,\text{synth}} C_T C_L + E_{0,\text{pool}} \gamma \quad (4)$$

$E_{0,\text{synth}}$  and  $E_{0,\text{pool}}$  are the emission potentials of the two sources, *de novo* and pool emissions.  $C_T$  and  $C_L$  (unitless) are the synthesis activity factors related to dependence of enzyme activity on temperature ( $C_T$ ) and the dependence of electron transport rate on light ( $C_L$ ), expressed in the form used by Guenther *et al.* (1991, 1993) for modelling isoprene synthesis. Moreover, the temperature activity factor related to pool emissions,  $\gamma$  (unitless), is the same as used by Guenther *et al.* (1991, 1993) and describes the dependence of saturation vapour pressure on temperature as follows:

$$E_{\text{pool}} = E_{0,\text{pool}} \exp[\beta(T - T_0)] \quad (5)$$

Here,  $\beta$  is an empirical coefficient for the temperature dependency of monoterpene evaporation from pools and kept constant ( $0.09 \text{ K}^{-1}$ , Guenther *et al.* 1993).  $T$  ( $^{\circ}\text{C}$ ) is originally leaf temperature, but in this study the temperature measured inside the enclosure was used instead;  $T_0$  is the standard temperature ( $30 \text{ }^{\circ}\text{C}$ ).

The final hybrid formulation for emission rate is converted as follows (Ghirardo *et al.* 2010):

$$E = E_0 [f_{\text{synth}} C_T C_L + (1 - f_{\text{synth}}) \gamma] \quad (6)$$

Here,  $f_{\text{synth}}$  is the fraction of *de novo* emission potential compared with the total emission potential (ranging from 0 to 1) and  $E_0$  ( $\text{ng g}^{-1} \text{s}^{-1}$ ) is total monoterpene emission potential under standardized conditions related to the activity factors:

$$E_0 = E_{0,\text{synth}} + E_{0,\text{pool}} \quad (7)$$

and on the other hand,

$$f_{\text{synth}} = \frac{E_{0,\text{synth}}}{E_0} \quad (8)$$

The parameters  $E_0$  and  $f_{\text{synth}}$  were fitted separately for each day (full 24 h period) and used to track changes in emission potentials throughout the spring period in order to study the light and temperature dependencies in the emission rate data. The algorithm fitting was conducted using MATLAB function 'lsqcurvefit' which allows using initial values as a reasonable starting point for the algorithm fitting ( $E_0=0.1 \text{ ng g}^{-1} \text{ s}^{-1}$ ,  $f_{\text{synth}} = 0.5$ , applied based on the results by Ghirardo *et al.* 2010 and Taipale *et al.* 2011) and returns also 95% confidence intervals. We defined here that the emissions that followed light and temperature (as expressed in temperature and light dependent synthesis activity factors  $C_T$  and  $C_L$ ) originate directly from synthesis (*de novo* emission), and correspondingly, the emissions that followed temperature activity factor  $\gamma$  originate from storages (pool emission).

Because of data gaps the days with monoterpene emission rate data coverage lower than 40% were rejected to ensure sufficient coverage and representativeness both in emission rate and environmental condition data. Other criteria for rejecting were: (1) days with temperature range narrower than 3 °C because low variation in environmental conditions lead to unreliable results when algorithm was fitted to data and (2) days when the values of confidence intervals were several orders of magnitude higher/lower than the fitted  $E_0$ ; those cases were interpreted as days when the emission data did not follow the dependencies used as presumptions in the hybrid algorithm. In total, these criteria caused rejection of 38 d, which is 18% of the measurement days. The days rejected based on the criterion 2 were dominated by freezing ambient temperatures and very low emission rates.

In order to analyse the short- and long-term dynamics in emission rates, we calculated the medians of daily total monoterpene emission potentials for each spring period and study shoot. If the monoterpene total emission potential of a certain day was double or higher when compared with the median of daily total emission potentials of the corresponding spring period/shoot, the day was classified as a monoterpene emission burst (MEB) event. Other days were classified as non-MEB days (lower than double emission potential when compared with the median emission potential) or unclassified (in case of unsuccessful emission potential calculation or data gap). In order to get rid of the interannual variability in emission potentials, the annual and shoot-specific emission potentials were normalized by divid-

ing the daily values with annual springtime mean values of the corresponding shoot; the average of the whole dataset was hence set to 1.

### Temperature-based proxy of photosynthetic recovery

A simple temperature history term,  $S$  (state of photosynthetic acclimation, Mäkelä *et al.* 2004), has been found to be a good proxy for describing the dynamics between the advancement of spring and photosynthetic recovery.  $S$  (°C) gives a proxy for the temperature that the photosynthetic apparatus is acclimated to in the recent past. It is described as follows (Mäkelä *et al.* 2004):

$$\frac{dS}{dt} = \frac{T - S}{\tau} \quad (9)$$

where  $T$  is ambient temperature (°C) and  $\tau$  (h) is time constant related to the slowness of the change of state to new temperature. The initial value of  $S$  was set to the first temperature record of each year and the calculation was conducted with half hour steps using temperatures measured at the Scots pine canopy height. The value of  $S$  at 0900 h was chosen to represent that day. Initially, we chose the same value for  $\tau$  (200 h) as Dal Maso *et al.* (2009), who based it on the study by Kolari *et al.* (2007). We also tested value 100 h for  $\tau$ .  $S$  is hereafter expressed with a subscript indicating the  $\tau$  used in calculation, for example  $S_{200}$  in case of  $\tau = 200$  h. We defined the period of interest as a period when  $-4 < S_{200} < 6$  °C, between the beginning of March and end of May. Based on findings by Kolari *et al.* (2007), this period covers the critical period of photosynthetic spring recovery when photosynthetic capacity increases from the winter stage to about 50% of the summer maximum.

### Ancillary data and statistical methods

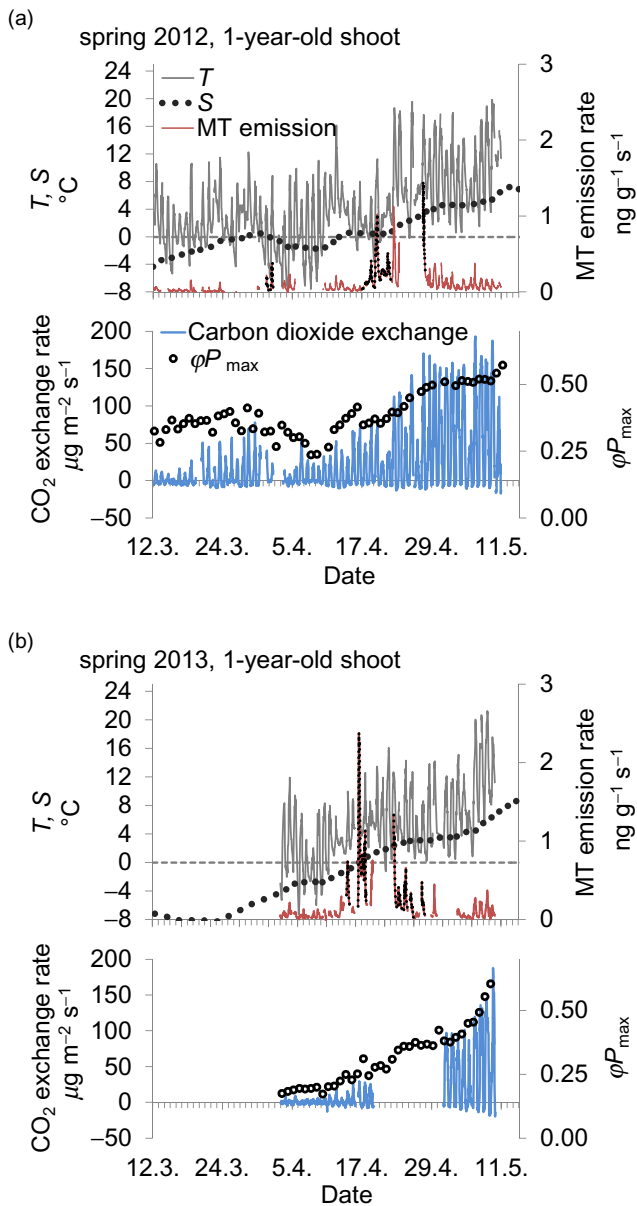
UV-A and UV-B radiation as well as ambient ozone concentration at the Scots pine canopy height records were used as ancillary data to analyse other potential driving factors for emission peaks. UV and  $\text{O}_3$  as well as ambient temperature data were downloaded from the SMEAR database (<http://avaa.tdata.fi/web/smart/smea>, Junninen *et al.* 2009). UV radiation was measured at the height of 18 m using a Solar Light SL501A radiometer (Solar Light Company Inc., Glenside, PA, USA). The  $\text{O}_3$  analyzer was TEI 49 C (Thermo Fisher Scientific Inc., Waltham, MA, USA).

The statistical tests were conducted using the Mann-Whitney U-test with significance level of 0.05. A non-parametric test was selected because the data were not always normally distributed.

## RESULTS

### Photosynthetic recovery in springtime

In the study years, the focal period of photosynthetic spring recovery ( $-4 < S_{200} < 6$  °C; Kolari *et al.* 2007) began between



**Figure 1.** Temperature inside enclosure immediately before closure,  $S_{200}$  (state of photosynthetic acclimation), monoterpene emission rate,  $\text{CO}_2$  exchange rate and maximum quantum yield of PSII ( $\phi P_{\max}$ ) during spring recovery periods 2012 (a) and 2013 (b). The y-axis for temperature,  $S_{200}$ , and  $\text{CO}_2$  exchange rate is on the left side and MT emission rate and  $\phi P_{\max}$  is on the right side of the figures. Only data during period  $-4 < S_{200} < 6$  °C is presented, except for  $S_{200}$  the whole period 12.3.–13.5. The MEB days are highlighted using black and red dashed line for the monoterpene emission rate. Corresponding results concerning all study shoots are represented in supporting information.

the 12 March and 3 April and ended between the 1 and 13 May depending on year (Fig. 1 and supporting information). During this period, temperature variation was remarkable: for example, in 2009 the minimum  $T$  during the period was  $-12$  °C and maximum  $T$  was  $24$  °C. The increase in PPFD was relatively much smaller during the study period and

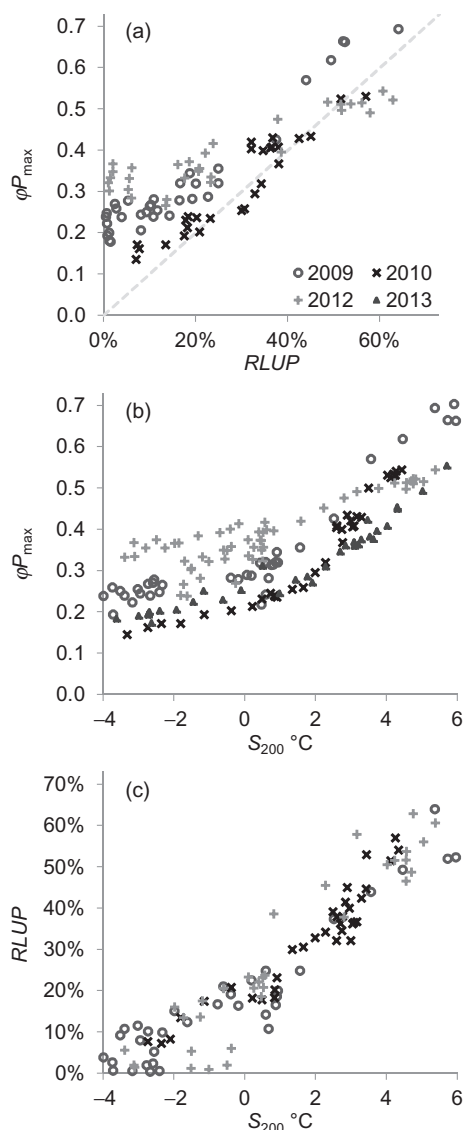
varied from daily maximum irradiances of less than  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  at the beginning of the period to nearly  $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$  at the end of the period. During the spring periods 2009 and 2012, there were some cold spells that caused temporary decrease in  $S_{200}$  values. In 2010 and 2013, no such cold spells occurred and  $S_{200}$  values increased steadily.

The different measures for spring recovery, that is the maximum quantum yield of photochemistry in PSII ( $\phi P_{\max}$ ),  $RLUP$  derived from  $\text{CO}_2$  exchange data and the state of photosynthetic acclimation ( $S_{200}$ ), were correlated. After  $\phi P_{\max}$  and  $RLUP$  exceeded 0.3, they matched close to the 1:1 line but below that their relationship was less clear (Fig. 2a). The relationship between  $S_{200}$  and  $\phi P_{\max}$  was slightly non-linear (Fig. 2b). Clear linear relation was found between  $S_{200}$  and  $RLUP$  (Fig. 2c). Furthermore,  $S_{100}$  was tested against measured photosynthetic recovery (data not shown) and it showed slightly more linear and better agreement on  $\phi P_{\max}$  and  $RLUP$  than  $S_{200}$ .

### Monoterpene emission bursts

High peaks in monoterpene emissions were observed during the critical recovery period in all four spring periods (Fig. 1 and supporting information). Four out of the five study shoots expressed periods of high monoterpene emission rates in early spring, lasting from 1 d to about one week. The days were classified as MEB day or non-MEB days based on the monoterpene emission potentials (Fig. 3) obtained using hybrid algorithm (Eqn 6). The number of classified MEB days varied from 0 to 8 over the recovery period (Table 1). The MEB events were more common in 1-year-old Scots pine shoots (16% of the classified days) than in 2-year-old shoots (10% of the classified days). The mean measured monoterpene emission rate from Scots pine shoots during the MEB days was  $0.29 \text{ ng g}^{-1} \text{ s}^{-1}$ , which is sixfold when compared with non-MEB days ( $0.05 \text{ ng g}^{-1} \text{ s}^{-1}$ ). When comparing the mean daily emission rates between all classified days ( $0.09 \text{ ng g}^{-1} \text{ s}^{-1}$ ) and non-MEB days, the MEB events roughly doubled the springtime monoterpene emissions during the spring recovery period ( $-4 < S_{200} < 6$  °C, Table 1). Importantly, the effect was concentrated in relatively few MEB days with an order of magnitude higher emission rates than in non-MEB days.

Mean monoterpene emission potential obtained using hybrid algorithm ( $E_0$ ) was  $5.48 \text{ ng g}^{-1} \text{ s}^{-1}$  during the MEB days and  $0.76 \text{ ng g}^{-1} \text{ s}^{-1}$  during the non-MEB days. The average  $E_{0,\text{synth}}$  was  $5.22$  and  $0.45 \text{ ng g}^{-1} \text{ s}^{-1}$  and  $E_{0,\text{pool}}$   $0.26$  and  $0.31 \text{ ng g}^{-1} \text{ s}^{-1}$  for MEB days and non-MEB days, respectively. During the MEB days, up to 79% of emissions originated primarily from *de novo* sources and 21% from pools, whereas in the non-MEB days, the situation was opposite: *de novo* sources contributed 22% and the rest originated from pools. Taking into account all data from the spring recovery periods, about 40% of the Scots pine shoot monoterpene emissions originated directly from *de novo* sources and the rest came from pools. However, the variation between shoots and spring periods was high in both intensity and number of



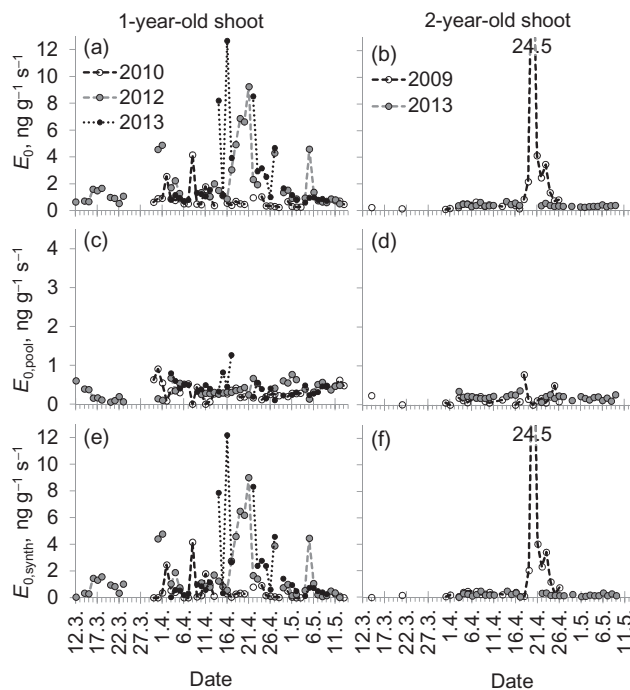
**Figure 2.** The connection between maximum quantum yield of PSII ( $\phi P_{\max}$ ), relative light use potential ( $RLUP$ ) and state of photosynthetic acclimation ( $S_{200}$ ). (a)  $RLUP$  versus  $\phi P_{\max}$ . (b)  $S_{200}$  versus  $\phi P_{\max}$ . (c)  $S_{200}$  versus  $RLUP$ . Data in panel (b) represent springs 2009, 2010, 2012 and 2013 during photosynthetic recovery period when  $-4 < S_{200} < 6$  °C; in (a) and (c), the spring 2013  $RLUP$  data is missing. The dashed line in panel (a) is 1:1 line. Each point represents the value of the variable at 0900 h.

MEB events: only 14% emissions from a 2-year-old shoot in spring 2013 that did not express any clear MEB events came from *de novo* sources, whereas 71% of emissions from a 2-year-old shoot that expressed a very strong MEB period originated from *de novo* sources in spring 2009.

The mean fraction of *de novo* emission potential acquired utilizing hybrid algorithm compared with the total emission potential ( $f_{\text{synth}}$ ) in all classified days was 0.58. Yet, when analysing the MEB days and non-MEB days separately, the mean  $f_{\text{synth}}$  were 0.94 and 0.51, respectively. The variation in  $f_{\text{synth}}$  during the MEB days was low compared with the non-MEB days. The increase in emission rates during the MEB

event was not due to temperature alone, but rather more closely connected to a combined effect of both photosynthetically active radiation and temperature, which was revealed by the hybrid algorithm. The results from the analysis revealed that the  $E_{0,\text{pool}}$  (driven by temperature alone) remained constant, whereas the  $E_{0,\text{synth}}$  (driven by both light and temperature) explained most of the observed variation (Fig. 3, Table 1).

We analysed also other potential differences between MEB days and non-MEB days in environmental drivers (temperature,  $S$  with several time constants,  $PPFD$ , ambient ozone concentration and UV radiation, see supporting information for details) possibly affecting monoterpene emissions, but no statistically significant differences were found. Based on visual estimate only, there is limited evidence that temperatures below  $-4$  °C typically preceded MEB events (Fig. 4). The variation in average thermal conditions between the spring periods was low, but there was some variation in the number of extreme temperature periods, the spring recovery periods 2009 and 2012 showing clearly higher count of cold spells than the spring recovery periods 2010 and 2013 (Table 2).



**Figure 3.** The daily monoterpene emission potentials of the measurement shoots during spring recovery periods 2009–2010 and 2012–2013 obtained fitting hybrid algorithm for each day separately. The figures on the left hand represent the 1-year-old shoots and the figures on the right hand represent the 2-year-old shoots. (a and b) Total monoterpene emission potentials ( $E_0$ ). (c and d) Monoterpene pool emission potentials ( $E_{0,\text{pool}}$ ). (e and f) Emission potential for *de novo* emissions ( $E_{0,\text{synth}}$ ). The legend for (a) is valid also for (c) and (e) and the legend for (b) is valid for (d) and (f).

**Table 1.** Average monoterpene emission rates, CO<sub>2</sub> exchange rates, environmental conditions and emission potentials of the study shoots expressing monoterpene emission burst (MEB) events during the spring recovery period ( $-4 < S_{200} < 6$  °C)

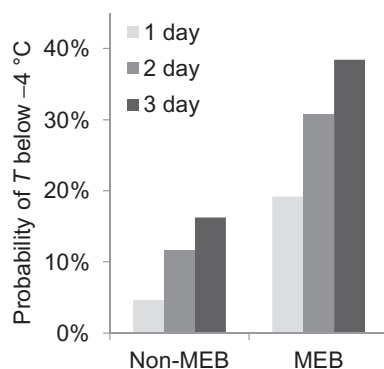
Classification	Unit	2009, 2-year-old		2010, 1-year-old		2012, 1-year-old		2013, 1-year-old	
		Non-MEB	MEB	Non-MEB	MEB	Non-MEB	MEB	Non-MEB	MEB
<i>n</i> of classified days	<i>n</i>	24	6	33	3	58	9	33	8
Emission rate measurements									
Maximum E	ng g <sup>-1</sup> s <sup>-1</sup>	0.66	3.19	0.34	0.71	1.12	1.43	0.45	2.70
Median E	ng g <sup>-1</sup> s <sup>-1</sup>	<0.005	0.07	0.02	0.03	0.05	0.17	0.05	0.27
Mean E	ng g <sup>-1</sup> s <sup>-1</sup>	0.02	0.21	0.03	0.07	0.08	0.23	0.07	0.47
Other measurements/environmental conditions									
Mean PPFD	μmol m <sup>-2</sup> s <sup>-1</sup>	170	339	207	205	203	152	283	226
Mean T inside enclosure	°C	3.6	5.5	5.3	6.0	5.1	3.0	5.3	5.9
Mean daily maximum T	°C	8.3	13.0	10.6	13	11.4	7.7	11.1	11.6
Mean daily minimum T	°C	-0.4	-2.0	1.1	1.6	0.3	-0.5	0.0	2.3
Mean φP <sub>max</sub>	unitless	0.33	0.31	0.36	0.24	0.44	0.43	0.33	0.33
Mean CO <sub>2</sub> exchange	μg m <sup>-2</sup> s <sup>-1</sup>	5.6	6.2	26.8	13.5	23.6	15.3	n.a.	n.a.
Mean RLUP	unitless	0.16	0.18	0.33	0.26	0.27	0.30	n.a.	n.a.
Obtained utilizing hybrid algorithm									
Median E <sub>0</sub>	ng g <sup>-1</sup> s <sup>-1</sup>	0.39	2.97	0.53	2.56	1.08	4.90	1.00	4.31
Median E <sub>0, pool</sub>	ng g <sup>-1</sup> s <sup>-1</sup>	0.09	0.12	0.29	0.00	0.37	0.30	0.41	0.36
Median E <sub>0, synth</sub>	ng g <sup>-1</sup> s <sup>-1</sup>	0.21	2.86	0.14	2.47	0.82	4.59	0.57	3.68
Median f <sub>synth</sub>	unitless	0.71	0.95	0.34	1.00	0.65	0.94	0.59	0.95
Mean E <sub>pool</sub>	unitless	0.70	0.10	0.9	0.06	0.72	0.31	0.73	0.28
Mean E <sub>synth</sub>	unitless	0.30	0.90	0.1	0.94	0.28	0.69	0.27	0.72

The data concerning 2-year-old shoot in spring 2013 is not shown because it did not expressed any MEB events.

E<sub>pool</sub> and E<sub>synth</sub> refer to the estimated emission rates from pool and *de novo* sources and are based on the hybrid algorithm.

The results of statistical testing for mean monoterpene emission rate and other measurements/environmental conditions between the MEB and non-MEB days are indicated with an asterisk in case of statistically significant difference, whereas hyphen refers to significance level over 0.05.

n.a., not applicable.



**Figure 4.** The probability of temperature below  $-4\text{ }^{\circ}\text{C}$  1, 2 or 3 d prior to monoterpene emission burst days (MEB) and non-MEB days.

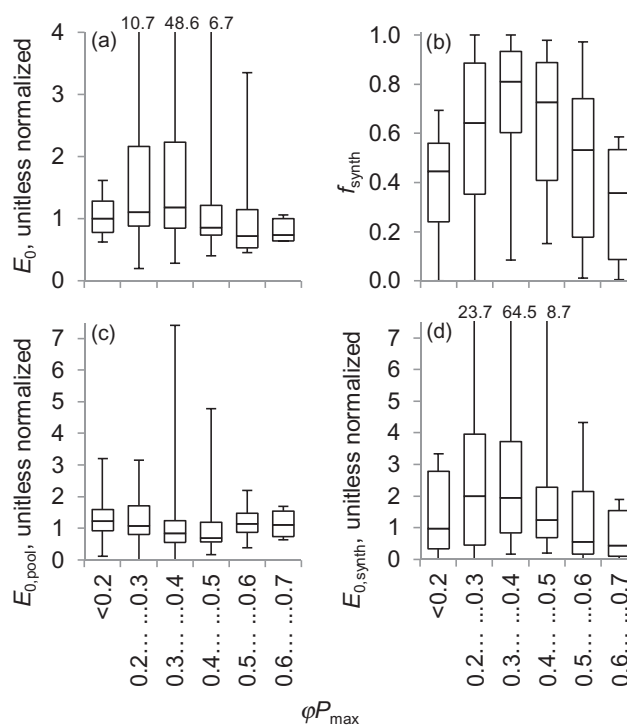
### The relation between monoterpene emission potentials and the onset of photosynthesis

Surprisingly, the highest values of hybrid algorithm-based  $E_{0,\text{synth}}$  and  $f_{\text{synth}}$  coincided with relatively low efficiency of photosynthetic apparatus (Fig. 5a,b,d). The period with highest  $E_{0,\text{synth}}$  coincided with the maximum quantum yield of PSII ( $\phi P_{\text{max}}$ ) values from 0.2 to 0.5 (Fig. 5a,b,d). Variation in  $E_{0,\text{synth}}$  was highest at the same time, suggesting that there were also days of lower  $E_{0,\text{synth}}$  occurring during the critical period, although the median during that period was doubled when compared with the  $E_{0,\text{synth}}$  outside the period. The median values of  $f_{\text{synth}}$  during the photosynthetic recovery period were higher than 0.6 and values around 0.9 were common. On the other hand, the stage of photosynthetic recovery had no effect on  $E_{0,\text{pool}}$  (Fig. 5c).

The increase in emission potential and especially in  $E_{0,\text{synth}}$  by a factor of 2–10 during MEB days took place when  $0 < S_{200} < 2\text{ }^{\circ}\text{C}$  (Fig. 6a) and during that time, nearly 50% of the days could be classified as MEB days. On the other hand, when  $-2 < S_{200} < 0\text{ }^{\circ}\text{C}$  or  $2 < S_{200} < 4\text{ }^{\circ}\text{C}$ , only 15–20% of the days were MEB days. The MEB events seemed to be most intensive when  $S_{200}$  was just below 0. When  $-2 < S_{200} < 4\text{ }^{\circ}\text{C}$ , the mean  $E_{0,\text{synth}}$  was 3.5 times higher when compared with the mean  $E_{0,\text{synth}}$  when  $-4 < S_{200} < -2\text{ }^{\circ}\text{C}$  and  $4 < S_{200} < 6\text{ }^{\circ}\text{C}$ .

	2009	2010	2012	2013	1981–2010
March mean $T$ , $^{\circ}\text{C}$	-3.3	-3.0	-0.6	-6.5	-3.4
April mean $T$ , $^{\circ}\text{C}$	3.5	3.8	2.2	2.4	2.3
May mean $T$ , $^{\circ}\text{C}$	10.8	10.9	9.6	12.5	8.9
Period $-4 < S_{200} < 6$	12.3.–1.5.	25.3.–13.5.	12.3.–11.5.	3.4.–9.5.	
Mean ambient $T$ , $^{\circ}\text{C}$	1.0	4.0	2.3	3.6	
Standard deviation of daily mean ambient $T$ , $^{\circ}\text{C}$	5.0	3.0	3.3	3.5	
Number of periods with ambient $T < -4\text{ }^{\circ}\text{C}$	13	1	13	5	
Number of periods with ambient $T > 10\text{ }^{\circ}\text{C}$	8	10	13	9	

All temperatures are ambient temperatures: in case of the study periods 2009–2013 downloaded from the SMEAR database (<http://avaa.tdata.fi/web/smart/smea>, Junninen *et al.* 2009) and in case of the normal period 1981–2010 from Pirinen *et al.* (2012).



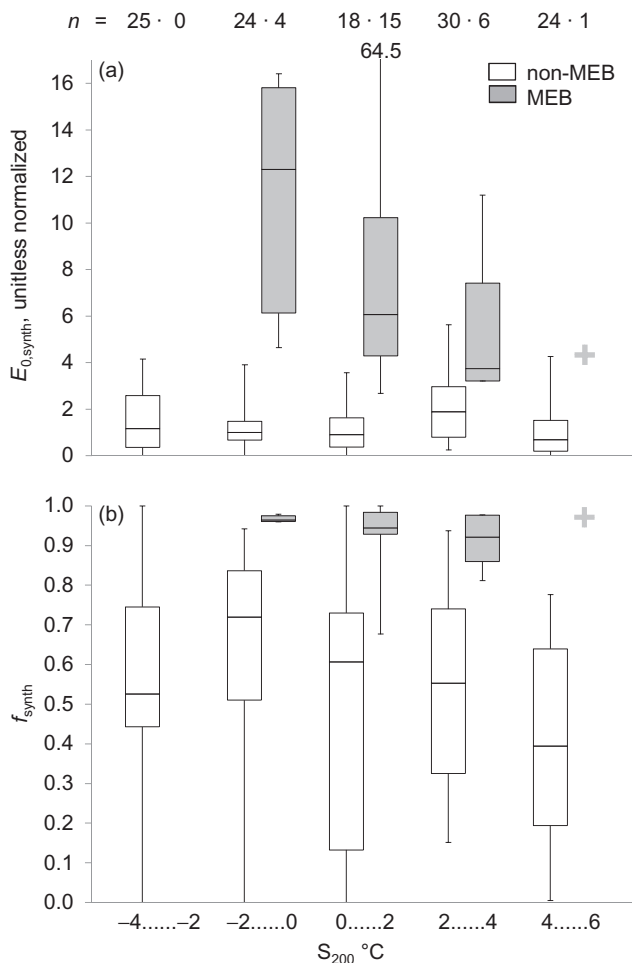
**Figure 5.** The relation between the maximum quantum efficiency of photosystem II and emission potential during spring recovery of photosynthesis. (a, c and d) Represent the mean total monoterpene emission potential ( $E_0$ ), pool emission potential ( $E_{0,\text{pool}}$ ) and synthesis emission potential ( $E_{0,\text{synth}}$ ), respectively, in unitless normalized form. (b) Represents the mean fraction of *de novo* monoterpene emission potential ( $f_{\text{synth}}$ ) compared with total monoterpene emission potential. The error bars represent the minimum and maximum values and boxes express the first, second (median) and third quartiles. The values above panels (a) and (d) express the whole area of variation in case of cut error bars.

## DISCUSSION

Field measurements from Scots pine shoots over several years clearly indicate that unexpectedly high MEB are frequently observed during the onset of the photosynthetic spring recovery period and that these bursts cannot be

**Table 2.** Comparison between the thermal conditions during the photosynthetic spring recovery periods ( $-4 < S_{200} < 6\text{ }^{\circ}\text{C}$ ) 2009, 2010, 2012 and 2013





**Figure 6.** The relative difference in *de novo* monoterpene emission potential and *de novo* fraction between the monoterpene emission burst (MEB) days and non-MEB days in relation to  $S_{200}$ . (a) *De novo* emission potential ( $E_{0,\text{synth}}$ ) based on values normalized using median *de novo* emission potential from period  $-4 < S_{200} < 6$  °C. (b) *De novo* fraction ( $f_{\text{synth}}$ ). The boxes represent the first, second (median) and third quartiles and error bars refer to minimum and maximum values. The filled boxes represent the MEB days and non-filled represent the non-MEB days.  $n$  for each bar is marked above panel (a), representing the number of days classified as either MEB days or non-MEB days. The values for MEB days  $4 < S_{200} < 6$  °C is marked without error bars and filling because they represent only 1 d. The single value above panel (a) expresses the whole area of the variation in case of cut error bar.

explained by incident temperature alone. Instead, there are strong implications that the bursts are related to physiological changes during the spring recovery period. We found evidence that the origin of these high emissions in MEB days was recently synthesized monoterpenes: the *de novo* emission potentials were an order of magnitude higher than those related to non-MEB days and consisted of ca. 90% of the total emission potential. In contrast, in the end of the study period, after the early stages of the photosynthetic spring recovery period, the *de novo* fraction was only 40–50%, which is in good agreement with the findings by Ghirardo *et al.* (2010) and Taipale *et al.* (2011).

### Timing, magnitude and interannual variation of MEB events

Based on previous observations, early springtime monoterpene emissions from the same boreal Scots pine site as well as from subarctic Scots pines are characterized with high daytime emission rates, up to  $0.7 \mu\text{g g}^{-1} \text{h}^{-1}$  (Tarvainen *et al.* 2005; Hakola *et al.* 2006). Our measured maximum emission rates match reasonably well with these values, ranging from 0.3 (for non-MEB days) to  $0.6 \mu\text{g g}^{-1} \text{h}^{-1}$  (for MEB days). There was large year-to-year variation in number and magnitude of MEB events: the MEB days were most abundant in 2009 and 2013 and lowest during 2010. Based on a general weather pattern during those years (Table 2), it seems that in a relatively warm and steadily progressing spring period the MEB events tend to be rare, whereas large temperature variations and cold spells during spring period increase the probability of MEB days to some extent.

The timing of MEB events was remarkably similar between the studied years: the events took place always during the early stages of photosynthetic spring recovery, in early to mid-April. Such high emission peaks are not usual later in the spring period or summer (see also Aalto *et al.* 2014). This implies that they are either a consequence of the spring recovery itself or related to conditions affecting the recovery processes. They occurred at a time when daily mean temperatures are still relatively low and minima can be clearly below zero, so temperature has potentially an impact on their occurrence. On the other hand, there were also non-MEB days at the same time, indicating the existence of special triggers behind MEB events.

### Direct temperature effect on MEB events

Diel changes in temperature, and especially relatively low temperature minima, were probably linked to the occurrence of MEB events. Very often, cold nights or longer cold spells were coinciding with a MEB event, although the effect was not straightforward and occurred with a time delay of 2 to 3 d. Such an effect of low temperatures on monoterpene emissions should be considered as a potential triggering factor for high emission rates in springtime.

Temperatures below  $-4$  °C expose non-woody plant tissues to freezing (Brown *et al.* 1974) and they also seemed to increase the risk of MEB events in pine shoots. Copolovici *et al.* (2012) showed that cold stress, particularly freezing temperatures lead to increase in terpenoid emissions along with a decline in photosynthesis due to photoinhibition and changes in stomatal conductance. Freezing can be manifested as cellular damage, which is one potential explanation for increased emissions proposed by Copolovici *et al.* (2012). On the other hand, also photosynthetic recovery after freezing may be the reason for the increased monoterpene emissions. During freezing and thawing, a number of processes linked to quality and quantity of sugars and to membrane transport properties take place that could also influence the emissions (Pearce 2001). However, neither cold spells nor freezing

temperatures alone were able to explain all variation in monoterpene emission potentials.

Statistical testing revealed few explanations for MEB events (Table 1 and supporting information). The decrease of  $S$ , in practice cold spells or cold nights, was the clearest difference between MEB and non-MEB days, but the difference was not statistically significant. It seemed that the decrease of  $S$  took place some days before a MEB event because the mean value of  $\max dS/dt$  during both MEB and non-MEB days were positive in time frame of 1 to 2 d and negative only if 2 to 3 preceding days were taken into account. Furthermore, low temperatures – much lower than  $S$  values – had some effect on the emergence of MEB events, but again the difference between MEB and non-MEB days was not statistically significant and three preceding 24 h periods had to be taken into account to achieve the clearest effect. Both effects – the decrease of  $S$  and the difference between  $S$  and ambient temperature – are by theory mutually dependent so it is hard to conclude which one is the primary explanatory factor. All other tested potential triggers were clearly weaker than those related to  $S$  values and low temperatures.

The models where temperature is driving the monoterpene pool emissions often use a constant value for temperature dependence,  $\beta$  ( $0.09 \text{ K}^{-1}$ ; Guenther *et al.* 1993), despite that the temperature dependence of emissions caused by evaporation from pools actually varies somewhat between seasons, compounds, tree species (Guenther *et al.* 1993; Tarvainen *et al.* 2005) and within canopy (Helmig *et al.* 2007). This temperature dependence relates especially to emissions originating from storage pools, which in case of conifer foliage are quite large (Ghirardo *et al.* 2010; Niinemets *et al.* 2010). However, the observed MEB events were particularly related to considerably increased *de novo* monoterpene synthesis rates and the pool emission potentials did not change during the MEB events, implying that the incident temperature evidently had only minor effect on the emission bursts.

### MEB events and photosynthetic recovery in springtime

Emission models have traditionally used temperature and light as the main drivers for terpenoid emissions (e.g. Guenther *et al.* 2012, 2012) and the seasonal effects are considered to be well constrained by the temperature and light modification of emissions. However, we have shown here that even if the average emissions may follow temperature well, the transient bursts of terpenoids during spring recovery period cannot be explained with a normal temperature dependence. Given the fact that the state of acclimation,  $S$ , can be directly estimated using temperature history data from meteorological stations, or land-surface temperature from remote sensing platforms, we anticipate that this index has a high potential to improve our capacity to model BVOC emissions at multiple scales.  $S$  has been found to be a simple and effective proxy for tracking the photosynthetic spring recovery (Mäkelä *et al.* 2004; Kolari *et al.* 2007) and this was confirmed in our study as well. The time constant  $\tau$  plays a central role in the performance of  $S$  as a proxy. In Mäkelä

*et al.* (2004), the best value for  $\tau$  was 330 h, whereas in this study shorter  $\tau$  was used because of more linear relationship between measured photosynthetic recovery and  $S$  during the early recovery period which was a focus of this study.  $S_{200}$ , which was used for defining the study period, was found to be slightly less applicable in describing the photosynthetic spring recovery than  $S_{100}$ . The key findings were independent on  $\tau$  (100 or 200 h), thus it is safe to use  $S_{200}$  for defining the study period and as a basis for further applications. The event number was highest when  $S_{200}$  was close to a threshold,  $0^\circ \text{C}$ , corresponding to about 30% of the maximum photosynthetic efficiency (Kolari *et al.* 2007) and the probability of MEB events was lower either earlier or later in springtime. This implies that the recovery process may in general proceed in a linear manner, but that a sudden change may occur when the  $S_{200}$  reaches a threshold level.

### Potential functional role of monoterpene emission bursts

The evergreen Scots pine needles tolerate very low temperatures in a winter hardened state, but are very sensitive to them during the photosynthetic spring recovery (Ensminger *et al.* 2004; Porcar-Castell 2011). An important, physiologically relevant feature linking the MEB events to spring recovery may thus be related to the dynamics in protective mechanisms during the transition phase between winter and summer.

Boreal springtime conditions are very challenging for evergreen foliage due to the combination of low temperatures and high irradiance levels. Low temperatures inhibit the carbon reactions, whereas high irradiance ensures that evergreen foliage continues to absorb excitation energy in excess of its utilization rates (Öquist & Huner 2003). Excess energy can be harmlessly dissipated as heat using a number of protective mechanisms that involve sustained structural and biochemical changes in the photosystems, these adjustments are visible through the accumulation of the de-epoxidized form of the xanthophyll cycle pigment zeaxanthin, as well as the accumulation of specific antenna proteins (Öquist & Huner 2003; Ensminger *et al.* 2004; Verhoeven 2014). When temperatures start to increase, these sustained structural and biochemical changes are reversed and the photosystems shift towards more reversible/flexible mechanisms for protection (Demmig-Adams *et al.* 2006; Porcar-Castell *et al.* 2008a; Verhoeven 2014) based on the dynamic operation of the xanthophyll cycle. Indeed, a transient increase in xanthophyll-cycle pigments has been repeatedly observed in Scots pine foliage during this transition phase (Porcar-Castell *et al.* 2008a,b, 2012), suggesting that the photosynthetic apparatus attempts to up-regulate this protective mechanism.

The role of BVOCs in abiotic stresses has been subject to intensive research during the last years (see e.g. Niinemets 2009, Fineschi & Loreto 2012). Mostly, these studies have concentrated on moderate or high stress levels inducing emissions of, for example, isoprenoids and C6 aldehydes. Physiological factors controlling induced emissions range from genetic to energy and carbon intermediate availability

(Rosenstiel *et al.* 2003; Li & Sharkey 2013; Monson 2013). Our results further corroborate the findings that metabolic regulation of isoprenoid emissions is a complicated process and that many factors can be involved, depending on the severity and timing of stresses. We suggest that the MEB events are an important metabolic feature, linking spring recovery and terpenoid emissions. The 'metabolic' hypothesis, aiming at explaining the physiological and ecological role of terpenoids in protecting plants against environmental stresses was proposed already more than 10 years ago and was reviewed by Loreto & Schnitzler (2010). On the one hand, because carotenoid and monoterpene synthesis share a common precursor, it is likely that up-regulation of carotenoid synthesis during this transient phase results in what has been called 'opportunistic' emissions of BVOCs (Owen & Peñuelas 2005; Porcar-Castell *et al.* 2009); on the other hand, accumulation of reducing power during this transient phase when light reactions have recovered, but the carbon (dark) reactions are lacking behind, could result in enhanced BVOC emissions, in what has been called the safety-valve hypothesis (Peñuelas & Llusia 2004; Owen & Peñuelas 2005). So far, field studies have not been available to test the metabolic hypotheses, which require good temporal resolution and high measurement accuracy for both BVOC emissions and the other physiological parameters. We propose, based on our data from several years, that the MEB events during the onset of photosynthetic spring recovery are likely serving a protective functional role activated in the metabolism during this critical transitory state. Further investigations on mechanisms and functionality of the relationship will require studies that assess the relationship between BVOC emissions, photosynthesis and leaf pigment concentrations at high temporal resolution during this period.

### Implications of monoterpene emission bursts on canopy scale concentrations

Taking into account that the monoterpene emission bursts roughly doubled the cumulative emissions from Scots pine branches over the whole spring period, the bursts can have considerable short-term effects on the atmospheric composition, especially inside the canopy and close to it (see e.g. Lappalainen *et al.* 2013). The observed variation between years and branches indicates that shoot and needle position or age may have an impact on the occurrence of MEB events. Small-scale variations in light or temperature are likely to be responsible for the observed variation, although the effect of ageing of the evergreen foliage on their monoterpene emissions is currently not well known and thus cannot be totally excluded. However, the phenomenon was nevertheless observed over 4 years, in both 1- and 2-year-old shoots, and at a fairly similar time each year, and thus we consider that it is very likely that the MEB events are consistently seen during spring period at least in some shoots at the top of the pine canopy.

We report here for the first time large bursts of monoterpenes from intact pine foliage, but it is well known that the emission spectrum from Scots pine foliage is consist-

ing of many other, even more reactive compounds than monoterpenes, such as several sesquiterpenes (e.g. Hakola *et al.* 2006; Duhl *et al.* 2008; Bäck *et al.* 2012). Sudden bursts of sesquiterpenes have been seen to originate, for example, due to rough handling or herbivore infestations (reviewed in Duhl *et al.* 2008). Because the analysis method used in this study (PTR-QMS) is only capable of distinguishing the total sum of monoterpenes, it remains unclear if the MEB events include similar monoterpene spectrum as the average emissions over the period. Earlier, Bäck *et al.* (2012) reported large differences in monoterpene emission blend (chemotype) between individual Scots pine trees of the same stand as measured here. Moreover, there is strong evidence that the composition of stress-induced emissions differs from those emitted in a constitutive manner (compounds that are constantly emitted with a strong temperature dependence) (Loreto & Schnitzler 2010; Niinemets *et al.* 2010). As the MEB events may well be fundamentally stress-induced during the spring recovery, it is likely that they also include emissions of some very reactive compounds. This may have important implications when reactivity and other effects on tropospheric composition and new particle formation are considered. The potential that also sesquiterpenes or other reactive compounds may be included in the bursts makes them even more relevant for atmospheric chemistry.

Another important consequence in the observation of springtime MEB events is that their timing coincides with the reported new particle formation events in a boreal forest. Our findings are in good agreement with the study by Dal Maso *et al.* (2009), who found a peak in activity of atmospheric new particle formation in early spring, coinciding with  $S_{200}$  ca. 0 °C. They hypothesized that the BVOCs produced at the start-up of photosynthetic production have more potential for atmospheric particle formation than the BVOCs produced later in the year, indicating that potentially some unidentified, reactive compounds may be emitted during the MEB events. Dal Maso *et al.* (2009) also suggested that the peak in O<sub>3</sub> concentration, coinciding with the S value 0 °C could indicate a stronger BVOC source, acting as oxidative agent in the boundary layer. In this study, we were able to show that (1) the timing of increased monoterpene emissions coincide with  $S_{200}$  values equivalent to those presented by Dal Maso *et al.* (2009) and (2) the increase in monoterpene emissions is very likely related to the photosynthetic spring recovery, causing an increase in the recently synthesized monoterpene emissions. Our finding emphasizes the occurrence of a strong source for BVOCs during the spring period, in addition to those already known (e.g. Aalto *et al.* 2014, Aaltonen *et al.* 2011, 2012), potentially important for the local atmospheric chemistry and may contribute to climate through cloud formation (Kulmala *et al.* 2004; Paasonen *et al.* 2013).

### CONCLUSIONS

Significant episodes of monoterpene emissions (MEB) are observed in boreal Scots pine foliage every spring period, during the early stages of photosynthetic recovery. The

episodes are mainly caused by a transient increase in monoterpene synthesis, taking place when the photosynthetic carbon uptake is starting, but the light use efficiency is still far from the summertime maximum. The episodes are not directly caused by high daytime temperatures, however, large diel temperature range and cold spells in between warmer periods may induce MEB events in the following days. The link to photosynthetic recovery gives reason to assume that the increase in monoterpene synthesis is caused directly or indirectly by protective processes, which gives support to the metabolic hypothesis (protection mechanism against excess energy) for the role of monoterpenes. This finding also links the photosynthetic spring recovery to atmospheric processes via terpenoid synthesis and emissions. This calls upon seasonality models describing processes behind seasonal acclimation to revise the terpenoid emission models. State of photosynthetic acclimation, *S*, has potential to be assimilated into BVOC emission models because it offers an easy proxy for both the photosynthetic recovery and for the high terpenoid emissions from the widespread coniferous, evergreen species.

## ACKNOWLEDGMENTS

The authors acknowledge the financial support from the Academy of Finland Center of Excellence Program (grant numbers: 1118615 and 272041), the Nordic Center of Excellence CRAICC, the EU FP7 Expeer I3, funds of the University of Helsinki (Project number 490117) and the Graduate school 'Atmospheric Composition and Climate Change: From Molecular Processes to Global Observations and Models – Doctoral Programme (ACCC)'. The work was partly supported by the Office of Science (BER), U.S. Department of Energy via Biogenic Aerosols - Effects on Clouds and Climate (BAECC). We wish to thank the staff at the SMEAR II station and the Hyytiälä Forest Research Station for practical advice and assistance in constructing the field measurements and their maintenance over the years.

## REFERENCES

- Aalto J., Kolari P., Hari P., Kerminen V.-M., Schiestl-Aalto P., Aaltonen H., ... Bäck J. (2014) New foliage growth is a significant, unaccounted source for volatiles in boreal evergreen forests. *Biogeosciences* **11**, 1331–1344.
- Aaltonen H., Pumpanen J., Pihlatie M., Hakola H., Hellén H., Kulmala L., ... Bäck J. (2011) Boreal pine forest flow biogenic volatile organic compound emissions peak in early summer and autumn. *Agricultural and Forest Meteorology* **151**, 682–691.
- Aaltonen H., Pumpanen J., Hakola H., Vesala T., Rasmus S. & Bäck J. (2012) Snowpack concentrations and estimated fluxes of volatile organic compounds in a boreal forest. *Biogeosciences* **9**, 2033–2044.
- Altimir N., Vesala T., Keronen P., Kulmala M. & Hari P. (2002) Methodology for direct field measurements of ozone flux to foliage with shoot chambers. *Atmospheric Environment* **36**, 19–29.
- Atkinson R. & Arey J. (2003) Gas-phase tropospheric chemistry of biogenic volatile organic compounds: a review. *Atmospheric Environment* **37**, 197–219.
- Bäck J., Aalto J., Henriksson M., Hakola H., He Q. & Boy M. (2012) Chemodiversity of a Scots pine stand and implications for terpene air concentrations. *Biogeosciences* **9**, 689–702.
- Brown M.S., Pereira E.S.B. & Finkle B. (1974) Freezing of nonwoody plant tissues. *Plant Physiology* **53**, 709–711.
- Claeys M., Grahlan B., Vas G., Wang W., Vermeylen R., Pashynska V., ... Maenhaut W. (2004) Formation of secondary organic aerosols through photooxidation of isoprene. *Science* **303**, 1173–1176.
- Copolovici L., Kännaste A., Pazouki L. & Niinemets Ü. (2012) Emissions of green leaf volatiles and terpenoids from *Solanum lycopersicum* are quantitatively related to the severity of cold and heat shock treatments. *Journal Plant Physiology* **169**, 664–672.
- Dal Maso M., Sogacheva L., Aalto P.P., Riipinen I., Komppula M., Tunved P., ... Kulmala M. (2007) Aerosol size distribution measurements at four Nordic field stations: identification, analysis and trajectory analysis of new particle formation bursts. *Tellus* **59B**, 350–361.
- Dal Maso M., Hari P. & Kulmala M. (2009) Spring recovery of photosynthesis and atmospheric particle formation. *Boreal Environment Research* **14**, 711–721.
- Demmig-Adams B., Ebbert V., Mellman D.L., Mueh K.E., Schaffer L., Funk C., ... Adams W.W. III (2006) Modulation of PsbS and flexible versus sustained energy dissipation by light environment in different species. *Physiologia Plantarum* **127**, 670–680.
- Duhl T.R., Helmig D. & Guenther A. (2008) Sesquiterpene emissions from vegetation: a review. *Biogeosciences* **5**, 761–777.
- Ehn M., Thornton J.A., Kleist E., Sipilä M., Junninen H., Pullinen I., ... Mentel T.F. (2014) A large source of low-volatility secondary organic aerosol. *Nature* **506**, 476–479.
- Ensminger I., Sveshnikov D., Campbell D.A., Funk C., Jansson S., Lloyd J., ... Öquist G. (2004) Intermittent low temperatures constrain spring recovery of photosynthesis in boreal Scots pine forests. *Global Change Biology* **10**, 995–1008.
- Fineschi S. & Loreto F. (2012) Leaf volatile isoprenoids: an important defensive armament in forest tree species. *iForest Biogeosciences and Forestry* **5**, 13–17.
- Genty B., Briantais J.-M. & Baker N.R. (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* **990**, 87–92.
- Ghirardo A., Koch K., Taipale R., Zimmer I., Schnitzler J.-P. & Rinne J. (2010) Determination of *de novo* and pool emissions of terpenes from four common boreal/alpine trees by <sup>13</sup>C<sub>2</sub> labelling and PTR-MS analysis. *Plant, Cell & Environment* **33**, 781–792.
- Grote R. (2007) Sensitivity of volatile monoterpene emission to changes in canopy structure – a model based exercise with a process-based emission model. *Plant Biology* **173**, 550–561.
- Grote R. & Niinemets Ü. (2008) Modeling volatile isoprenoid emissions – a story with split ends. *Plant Biology* **10**, 8–28.
- Guenther A., Monson R.K. & Fall R. (1991) Isoprene and monoterpene emission rate variability: observations with eucalyptus and emission rate algorithm development. *Journal of Geophysical Research* **96**(D6), 10799–10808.
- Guenther A., Hewitt C.N., Erickson D., Fall R., Geron C., Graedel T., ... Zimmerman P. (1995) A global model of natural volatile organic compound emissions. *Journal of Geophysical Research* **100**(D5), 8873–8892.
- Guenther A.B., Zimmermann P.R., Harley P.C., Monson R.K. & Fall R. (1993) Isoprene and monoterpene emission rate variability: model evaluations and sensitivity analyses. *Journal of Geophysical Research* **98**(D7), 12609–12617.
- Guenther A., Karl T., Harley P., Wiedinmyer C., Palmer P.I. & Geron C. (2006) Estimates of global terrestrial isoprene emissions using MEGAN (Model of Emissions of Gases and Aerosols from Nature). *Atmospheric Chemistry and Physics* **6**, 3181–3210.
- Guenther A.B., Jiang X., Heald C.L., Sakulyanontvittaya T., Duhl T., Emmons L.K. & Wang X. (2012) The Model of Emissions of Gases and Aerosols from Nature version 2.1 (MEGAN2.1): an extended and updated framework for modeling biogenic emissions. *Geoscientific Model Development* **5**, 1471–1492.
- Hakola H., Tarvainen V., Bäck J., Ranta H., Bonn B., Rinne J. & Kulmala M. (2006) Seasonal variation of mono- and sesquiterpene emission rates of Scots pine. *Biogeosciences* **3**, 93–101.
- Hari P. & Kulmala M. (2005) Station for Measuring Ecosystem-Atmosphere Relations (SMEAR II). *Boreal Environment Research* **10**, 315–322.
- Helmig D., Ortega J., Duhl T., Tanner D., Guenther A., Harley P., ... Sakulyanontvittaya T. (2007) Sesquiterpene emissions from pine trees: Identification, emission rates and flux estimates for the contiguous United States. *Environmental Science and Technology* **41**, 1545–1553.
- Junninen H., Lauri A., Keronen P., Aalto P., Hiltunen V., Hari P. & Kulmala M. (2009) Smart-SMEAR: on-line data exploration and visualization tool for SMEAR stations. *Boreal Environment Research* **14**, 447–457.

- Kaplan J.O., Folberth G. & Hauglustaine D.A. (2006) Role of methane and biogenic volatile organic compound sources in late glacial and Holocene fluctuations of atmospheric methane concentrations. *Global Biogeochemical Cycles* **20**, GB2016.
- Karl T., Guenther A., Spirig C., Hansel A. & Fall R. (2003) Seasonal variation of biogenic VOC emissions above a mixed hardwood forest in northern Michigan. *Geophysical Research Letters* **30**, 2186–2189.
- Keenan T., Niinemets Ü., Sabate S., Gracia C. & Peñuelas J. (2009) Seasonality of monoterpene emission potentials in *Quercus ilex* and *Pinus pinea*: implications for regional VOC emission modeling. *Journal of Geophysical Research* **114**(D22202), 22202–22212.
- Kitajima M. & Butler W.L. (1975) Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. *Biochimica et Biophysica Acta* **376**, 105–115.
- Kolari P., Lappalainen H.K., Hänninen H. & Hari P. (2007) Relationship between temperature and the seasonal course of photosynthesis in Scots pine at northern timberline and in south boreal zone. *Tellus* **59B**, 542–552.
- Kolari P., Bäck J., Taipale R., Ruuskanen T.M., Kajos M.K., Rinne J. & Hari P. (2012) Evaluation of accuracy in measurements of VOC emissions with dynamic chamber system. *Atmospheric Environment* **62**, 344–351.
- Kolari P., Chan T., Porcar-Castell A., Bäck J., Nikinmaa E. & Juurola E. (2014) Field and controlled environment measurements show strong seasonal acclimation in photosynthesis and respiration potential in boreal Scots pine. *Frontiers in Plant Science* **5**, 717.
- Kulmala M., Suni T., Lehtinen K.E.J., Dal Maso M., Boy M., Reissell A., ... Hari P. (2004) A new feedback mechanism linking forests, aerosols, and climate. *Atmospheric Chemistry and Physics* **4**, 557–562.
- Kulmala M., Nieminen T., Nikandrova A., Lehtipalo K., Manninen H.E., Kajos M.K., ... Kerminen V.-M. (2014) CO<sub>2</sub>-induced terrestrial climate feedback mechanism: from carbon sink to aerosol source and back. *Boreal Environment Research* **19B**, 122–131.
- Lappalainen H.K., Sevanto S., Dal Maso M., Taipale R., Kajos M., Kolari P. & Bäck J. (2013) A source-orientated approach for estimating daytime concentrations of biogenic volatile organic compounds in an upper layer of a boreal forest canopy. *Boreal Environment Research* **18**, 127–144.
- Lehning A., Zimmer W., Zimmer I. & Schnitzler J.-P. (2001) Modeling of annual variations of oak (*Quercus robur* L.) isoprene synthase activity to predict isoprene emission rates. *Journal of Geophysical Research* **106**, 3157–3166.
- Li Z. & Sharkey T.D. (2013) Metabolic profiling of the methylerythritol phosphate pathway reveals the source of post-illumination isoprene burst from leaves. *Plant, Cell & Environment* **36**, 429–437.
- Lindfors V., Laurila T., Hakola H., Steinbrecher R. & Rinne J. (2000) Modeling speciated terpene emissions from the European boreal forest. *Atmospheric Environment* **34**, 4983–4996.
- Loreto F. & Schnitzler J.-P. (2010) Abiotic stresses and induced BVOCs. *Trends in Plant Science* **15**, 154–166.
- Mäkelä A., Hari P., Berninger F., Hänninen H. & Nikinmaa E. (2004) Acclimation of photosynthetic capacity in Scots pine to the annual cycle of temperature. *Tree Physiology* **24**, 369–378.
- Monson R.K. (2013) Metabolic and gene expression controls on the production of biogenic volatile organic compounds. In *Biology, Controls and Models of Tree Volatile Organic Compound Emissions* (eds Ü. Niinemets & R.K. Monson), pp. 153–179. Springer, Berlin, Germany.
- Monson R.K., Grote R., Niinemets Ü. & Schnitzler J.-P. (2012) Tansley review. Modeling the isoprene emission rate from leaves. *New Phytologist* **195**, 541–559.
- Niinemetts Ü. (2009) Mild versus severe stress and BVOCs: thresholds, priming and consequences. *Trends in Plant Science* **15**, 145–153.
- Niinemetts Ü., Monson R. K., Arneth A., Ciccioli P., Kesselmeier J., Kuhn U., ... Staudt M. (2010) The leaf-level emission factor of volatile isoprenoids: caveats, model algorithms, response shapes and scaling. *Biogeosciences* **7**, 1809–1832.
- Niinemetts Ü. & Monson R.K. (2013) State-of-the-art of BVOC research: what do we have and what have we missed? A synthesis. In *Biology, Controls and Models of Tree Volatile Organic Compound Emissions* (eds Ü. Niinemets & R.K. Monson), pp. 509–528. Springer, Berlin, Germany.
- Owen S.M. & Peñuelas J. (2005) Opportunistic emissions of volatile isoprenoids. *Trends in Plant Science* **10**, 420–426.
- Öquist G. & Huner N.P.A. (2003) Photosynthesis of overwintering evergreen plants. *Annual Review of Plant Biology* **54**, 329–355.
- Paasonen P., Asmi A., Petäjä T., Kajos M.K., Äijälä M., Junninen H., ... Kulmala M. (2013) Warming-induced increase in aerosol number concentration likely to moderate climate change. *Nature Geosciences* **6**, 438–442.
- Pearce R.M. (2001) Plant freezing and damage. *Annals of Botany* **87**, 417–424.
- Peñuelas J. & Llusia J. (2004) Plant VOC emissions: making use of the unavoidable. *Trends in Ecology and Evolution* **19**, 402–404.
- Pirinen P., Simola H., Aalto J., Kaukoranta J.-P., Karlsson P. & Ruuhela R. (2012) Climatological statistics of Finland 1981–2010. *Finnish Meteorological Institute Reports* **2012**: **1**, 1–96.
- Porcar-Castell A. (2011) A high-resolution portrait of the annual dynamics of photochemical and non-photochemical quenching in needles of *Pinus sylvestris*. *Physiologia Plantarum* **143**, 139–153.
- Porcar-Castell A., Juurola E., Ensminger I., Berninger F., Hari P. & Nikinmaa E. (2008a) Seasonal acclimation of photosystem II in *Pinus sylvestris*. II. Using the rate constants of sustained thermal energy dissipation and photochemistry to study the effect of the light environment. *Tree Physiology* **28**, 1483–1491.
- Porcar-Castell A., Pfündel E., Korhonen J.F.J. & Juurola E. (2008b) A new monitoring PAM fluorometer (MONI-PAM) to study the short and long-term acclimation of photosystem II in field conditions. *Photosynthesis Research* **96**, 173–179.
- Porcar-Castell A., Peñuelas J., Owen S.M., Llusia J., Munné-Bosch S. & Bäck J. (2009) Leaf carotenoid concentrations and monoterpene emission capacity under acclimation of the light reactions of photosynthesis. *Boreal Environment Research* **14**, 794–806.
- Porcar-Castell A., Garcia-Plazaola J.I., Nichol C., Kolari P., Olascoaga Gracia B., ... Nikinmaa E. (2012) Physiology of the seasonal relationship between Photochemical Reflectance Index and photosynthetic Light Use Efficiency. *Oecologia* **170**, 313–323.
- Rohde A. & Bhalerao R. P. (2007) Plant dormancy in the perennial context. *Trends in Plant Science* **12**, 217–223.
- Rosenstiel T.N., Potosnak M.J., Griffin K.L., Fall R. & Monson R.K. (2003) Increased CO<sub>2</sub> uncouples growth from isoprene emission in an agriforest ecosystem. *Nature* **421**, 256–259.
- Schreiber U., Schliwa U. & Bilger W. (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynthesis Research* **10**, 51–62.
- Staudt M., Bertin N., Hansen U., Seufert G., Ciccioli P., Foster P., ... Fugit J.-L. (1997) Seasonal and diurnal patterns of monoterpene emissions from *Pinus pinea* (L.) under field conditions. *Atmospheric Environment* **31**, 145–159.
- Staudt M., Bertin N., Frenzel B. & Seufert G. (2000) Seasonal variation in amount and composition of monoterpenes emitted by young *Pinus pinea* trees – implications for emission modelling. *Journal of Atmospheric Chemistry* **35**, 77–99.
- Taipale R., Ruuskanen T.M., Rinne J., Kajos M.K., Hakola H., Pohja T. & Kulmala M. (2008) Technical note: quantitative long-term measurements of VOC concentrations by PTR-MS – measurement, calibration, and volume mixing ratio calculation methods. *Atmospheric Chemistry and Physics* **8**, 6681–6698.
- Taipale R., Kajos M.K., Patokoski J., Rantala P., Ruuskanen T.M. & Rinne J. (2011) Role of *de novo* biosynthesis in ecosystem scale monoterpene emissions from a boreal Scots pine forest. *Biogeosciences* **8**, 2247–2255.
- Tarvainen V., Hakola H., Hellén H., Bäck J., Hari P. & Kulmala M. (2005) Temperature and light dependence of the VOC emissions of Scots pine. *Atmospheric Chemistry and Physics* **5**, 989–998.
- Tunved P., Hansson H.-C., Kerminen V.-M., Ström J., Dal Maso M., Lihavainen H., ... Kulmala M. (2006) High natural aerosol loading over Boreal forests. *Science* **312**, 261–263.
- Verhoeven A. (2014) Sustained energy dissipation in winter evergreens. *New Phytologist* **201**, 57–65.

Received 20 January 2015; received in revised form 19 March 2015; accepted for publication 22 March 2015

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Figure S1.** Temperature inside enclosure immediately before closure, S200 (state of photosynthetic acclimation),

monoterpene emission rate, CO<sub>2</sub> exchange rate and maximum quantum yield of PSII ( $\phi P_{max}$ ) during spring recovery periods 2009 (a), 2010 (b), 2012 (c) and 2013 (d,e). The y-axis for temperature,  $S_{200}$  and CO<sub>2</sub> exchange rate is on the left side and the MT emission rate and  $\phi P_{max}$  is on the

right side of the figures. Only data during period  $-4 < S_{200} < 6$  °C is presented, except in case of  $S_{200}$  the whole period 12.3.09–13.5.09.

**Table S1.** Test for the possible triggers for the MEB events.