



Fakultät Wissenschaftszentrum Weihenstephan für Ernährung,  
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**Drought effects on isoprenoid emissions of  
*Pinus sylvestris* L. and *Castanea sativa* MILL. assessed by the  
Tree Drought Emission Monitor**

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*In memory of my father Thomas*

*who awoke my interest to all the little technical things and*

*did not live to see the completion of this thesis*



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## II. Summary

Isoprenoids play an important role in plant communication and in defense against diverse stressors in- and outside of the plants. The emission of isoprenoids into the atmosphere is strongly dependent on temperature and light. After emission into the atmosphere, they contribute to photochemical processes such as ozone or secondary aerosol production, which can lead to further positive temperature feedback on the climate system. The isoprenoid emission potential is altered by stress events, such as droughts, which influence these emissions. These events are likely to increase in frequency and/or intensity under climate change. Drought stress response strongly differs between species and even within provenances and less is known on isoprenoid emission at this detailed level during phases of drought stress and recovery. Thus, within this PhD project, the plant chamber system “Tree DEMON” – Tree Drought Emission MONitor” was used to investigate the effect of soil water deficit on isoprenoid emissions on Scots pine (*Pinus sylvestris* L.) and sweet chestnut (*Castanea sativa* Mill.).

The Tree DEMON was newly developed from scratch and tested to perform studies on tree seedlings in four plant chambers. It was able to measure gas exchange of CO<sub>2</sub>, water vapor and isoprenoids in parallel under steady-state conditions, which were guaranteed by a multi-step air-conditioning system and by running the system in an environmentally controlled climate chamber. Further this location reduced external disturbances. Gas exchange of CO<sub>2</sub> and water vapor was measured by an infrared gas analyzer, while isoprenoid emissions were sampled onto adsorption tubes. Sampled compounds were thermally desorbed from the tubes and analyzed by a GC-MS/FID system. This setup allowed screening and treatment studies lasting from one day to several weeks.

Two drought experiments with Scots pine (study I and II) and one with sweet chestnut seedlings (study III) were performed: (I) In the first study, the effect of drought and re-watering on gas exchange of three provenances was investigated. (II) The second study looked at the gas exchange of one provenance under normal conditions, followed by a drought and re-watering period at a higher temporal resolution. Additionally, seedlings were labelled with <sup>13</sup>CO<sub>2</sub> three times corresponding to the different soil water content to better distinguish between isoprenoids emissions of from pools and *de novo* sources by using an extended standardizing algorithm. (III) In the third experiment, sweet chestnut seedlings were screened for their emissions composition and put under drought stress to study the effect of changing water availability on gas exchange.

## Summary

All three studies showed that drought reduces gas exchange of CO<sub>2</sub>, water vapor and isoprenoid emission and confirmed a reliable and stable usage of the Tree DEMON.

In study I the three provenances showed distinct isoprenoid emission patterns and were classified into a non- $\Delta^3$ -carene, with either higher  $\alpha$ -/ $\beta$ -pinene or  $\beta$ -myrcene fraction, and a  $\Delta^3$ -carene dominated type, but not uniquely linked to provenances. Isoprenoid emission rates, net-photosynthesis and transpiration were reduced during summer drought stress and significantly recovered after rewetting. Furthermore a seasonal emission increase towards autumn was observed. Compared with the German provenance, the Spanish and Italian provenances revealed higher isoprenoid emission rates and more plastic responses to drought stress and seasonal development, which points to a local adaptation to climate.

Conforming to observations in study I, decreasing soil water content led also in study II to an expected decrease of isoprenoids, water vapor and CO<sub>2</sub> gas exchange. However, during re-watering, water vapor and CO<sub>2</sub> gas exchange recovered fast to pre-drought levels, whereas isoprenoids increased to a lower level compared to the initial non-stressed phase.  $\delta^{13}\text{C}$  content after labelling was different at each monoterpene and ranged from 0.5 to 95% for unstressed trees and around  $36\pm 5\%$  of the total emission rate originated from *de novo* synthesized isoprenoids. During full drought, the *de novo* fraction was reduced to 3%. For the re-watering phase *de novo* emissions recovered only partly back to 20%, while pool emissions reached pre-drought conditions. Thus, emissions of *de novo* synthesized isoprenoids of Scots pine are down regulated by soil drought rather than isoprenoids emissions from pools.

Study III identified first time several chemo species within sweet chestnut by a screening study. The drought experiment showed a faster reduction to almost zero for CO<sub>2</sub> and water vapor gas exchange, while isoprenoid emissions and were reduced delayed in time.

The Tree DEMON showed to be a versatile and reliable machine and studies benefit strongly from the capability to measure at four trees in parallel. It gave new insights on the plastic adjustment of isoprenoid emissions during drought and showed the presence of different chemo species for Scots pine and sweet chestnut. Both facts should be considered in future isoprenoid models, since drought affects tree species/provenances differently and the presence of multiple chemo species may lead to different lifetime and reactions of isoprenoid emissions. Further, the results provided new emission rate information (quantitative and qualitative) for inventory data and could be used to improve and test emissions models.



### III. Zusammenfassung

Isoprenoide spielen eine wichtige Rolle für die Pflanzenkommunikation und für die Abwehr von Stressfaktoren inner- und außerhalb der Pflanze. Diese werden abhängig von Temperatur und Licht von der Pflanze in die Atmosphäre emittiert. Dort tragen sie zu photochemischen Prozessen bei, z.B. Bildung von Ozon oder Aerosolen, und führen damit zu einer Rückkopplung auf das Klimasystem. Das Emissionspotential der Pflanzen wird dabei von Stressereignissen, wie etwa Dürre, verändert, was zu Emissionsänderungen führen kann. Aufgrund des globalen (Klima-)Wandels wird von einer Zunahme der Frequenz und Intensität von Dürreereignissen ausgegangen.

Die Trockenstressantwort von Pflanzen unterscheidet sich stark zwischen Arten als auch Provenienzen. Es ist jedoch bisher nur wenig über die Veränderung der Isoprenoid-Emissionen zwischen unterschiedlichen Provenienzen und Arten während Trockenheits- und Erholungsphasen bekannt. Im Rahmen dieser Arbeit wurde deshalb ein Pflanzenkammersystem „Tree DEMON – Tree Drought Emission MONnitor“ entwickelt, um den Effekt von Bodenwasserdefizit auf Isoprenoid Emissionen von Waldkieferprovenienzen (*Pinus sylvestris* L.) und Edelkastanie (*Castanea sativa* Mill.) zu untersuchen.

Die Tree DEMON Apparatur wurde von Grund auf neu entwickelt und getestet, um verschiedene Studien an Baumsämlingen durchzuführen. Das Vierkammersystem ermöglichte es, parallel den Gasaustausch von CO<sub>2</sub>, Wasserdampf und Isoprenoiden der Pflanzen im Flussgleichgewicht zu messen. Des Weiteren wurde die Apparatur in einer Klimakammer mit konstanten Umweltparametern aufgestellt und eine mehrstufige Luftaufbereitung verwendet, um ein stabiles Flussgleichgewicht und den Ausschluss von externen Störgrößen zu garantieren. Der Gaswechsel von CO<sub>2</sub> und Wasserdampf wurde mit einem Infrarot Gasanalysator gemessen, wohingegen die Isoprenoide auf Adsorptionröhren angereichert wurden. Die gesammelten Isoprenoide wurden von den Röhren thermal desorbiert und mit einem GC-MS/FID System analysiert. Dieser Versuchsaufbau ermöglicht Screening- und Behandlungsversuche, die von einem Tag bis mehrere Wochen andauern können.

In dieser Arbeit wurden zwei Studien mit Waldkiefersämlingen (I und II) und eine mit Edelkastaniensämlingen (III) durchgeführt. In der ersten Studie wurde der Dürre- und Wiederbewässerungseffekt auf den Gaswechsel und Chemospezies zwischen drei Provenienzen untersucht. Die zweite Studie (II) untersuchte den Gaswechsel bei nur einer Provenienz unter verschiedenen Bewässerungsniveaus (Normal-Dürre-Normal) mit zeitlich höher aufgelösten Messintervallen. Zusätzlich wurden die Sämlinge innerhalb jedes Bewässerungsniveaus mit <sup>13</sup>CO<sub>2</sub> markiert, um

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besser zwischen gespeicherten (Pool) und neu synthetisierten (*de novo*) Emissionen differenzieren zu können. In der dritten Studie (III) wurden die Edelkastanien nach ihren Chemospezies unterschieden und unter Trockenstress gesetzt. Dabei wurde ebenfalls der Gaswechsel bei unterschiedlichen Bewässerungslevels untersucht.

In allen drei Studien führte die reduzierte Verfügbarkeit von Wasser zu einem reduzierten Gaswechsel von CO<sub>2</sub>, Wasserdampf und Isoprenoiden. Dies zeigte die zuverlässige und stabile Nutzung des Tree DEMON Systems. In Studie I zeigten die drei Provenienzen unterschiedliche Emissionsmuster, welche in zwei nicht- $\Delta^3$ -Carene emittierende Klassen, mit entweder hohen  $\alpha$ -/ $\beta$ -Pinene oder  $\beta$ -Myrcen Anteilen, und einer  $\Delta^3$ -Carene dominierenden Klasse eingeteilt werden konnte. Isoprenoid-Emissions-, Nettphotosynthese- und Transpirationsraten wurden während der simulierten Sommerdürre reduziert, erholten sich signifikant nach einer Erholungsphase. Ein saisonaler Anstieg der Emission konnte im Herbst beobachtet werden. Die spanischen und italienischen Provenienzen hatten gegenüber der deutschen Herkunft höhere Emissionsraten und eine plastischere Reaktion auf die Dürre als auch eine saisonale Anpassung. Beide Punkte deuten auf eine lokale Anpassung an das Klima.

Übereinstimmend mit Studie I führte auch in Studie II die Dürre zu der erwarteten Reduktion von Emissionen und des Gaswechsel von CO<sub>2</sub> und Wasserdampf. Während der Wiederbewässerung war zu sehen, dass sich der Gaswechsel von CO<sub>2</sub> und Wasserdampf schnell auf das Vorstressniveau erholte, wohingegen die Isoprenoid-Emissionen auf einem niedrigeren Niveau blieben. Die <sup>13</sup>C-Markierung ergab für jedes Isoprenoid eine unterschiedliche %<sup>13</sup>C-Anteil, welcher für nicht gestresste Bäume 0.5-95% reichte. Daraus ermittelte sich ein *de novo* Anteil von 36±5% für die gesamten Isoprenoid-Emissionen aller Bäume im nicht gestressten Zustand. Während der stärksten Dürrephase reduzierte sich dieser Anteil auf 3% und konnte sich in der Erholungsphase auf 20% erhöhen. In dieser Studie konnte gezeigt werden, dass *de novo* synthetisierten Isoprenoide stärker herunter werden reguliert werden als aus Pools emittierte Isoprenoide.

Studie III zeigte erstmalig in einem Screening Versuch das Vorhandensein mehrere Chemospezies für die Edelkastanie. Im Dürreversuch erfolgte eine starke Reduktion des Wasserdampf- und CO<sub>2</sub> Gasaustausches, wohingegen bei den Isoprenoid Emissionen eine zeitlich verzögerte Reduktion beobachtet wurde.

Der Tree DEMON erwies sich als flexibel einsetzbare und zuverlässige Messapparatur und die einzelnen Studien profitierten stark von der Möglichkeit vier Bäume parallel zu messen. Diese Systemeigenschaft führte einerseits zu neuen Einblicken in der plastischen Anpassung der

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Isoprenoid Emission während einer Dürre und andererseits konnte die generelle Existenz unterschiedlicher Chemospezies in den Isoprenoid Emissionen der Waldkiefer und Edelkastanie bestätigt werden. Beide Ergebnisse sollten in zukünftigen Isoprenoid Emissionsmodellen berücksichtigt werden, da die Dürre auf die Emission zwischen Baumarten als auch Provenienzen unterschiedlich starken Einfluss hat und das Vorhandensein mehrerer Chemospezies die Emissionszusammensetzung ändert. Beides ändert die Lebenszeit der emittierten Produkte und beeinflusst stark die Folgereaktionen. Des Weiteren können die erhobenen Emissionsdaten zu Emissionsinventurdatensätzen beitragen und zur Verbesserung und Validierung von Emissionsmodellen verwendet werden.

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## 1 Introduction

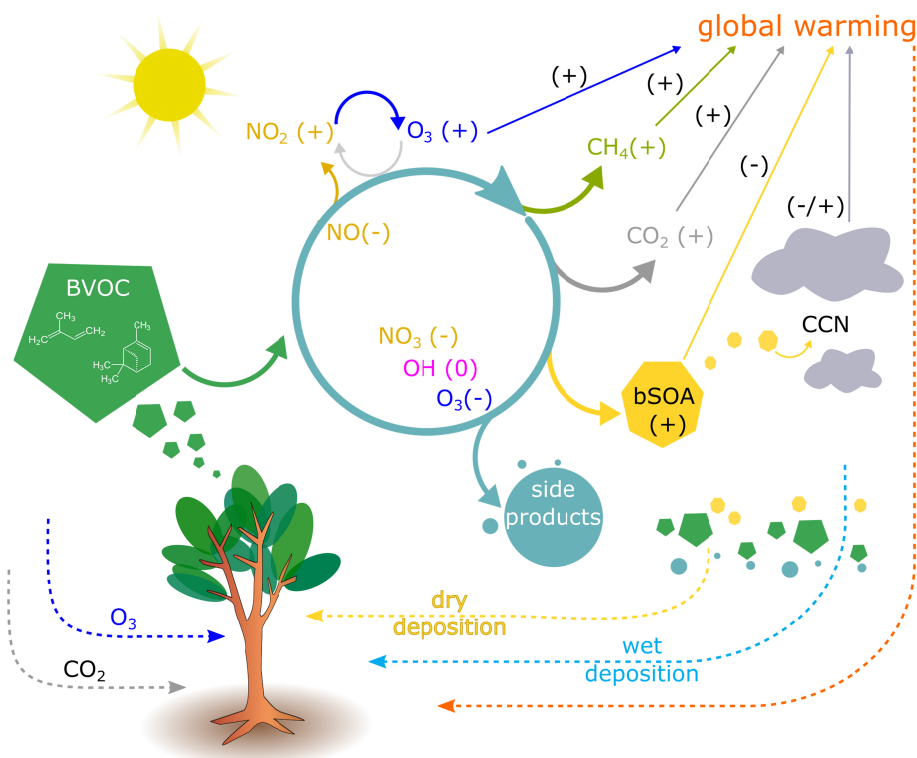
Biogenic volatile organic compounds (BVOC) are hydrocarbons released by living organisms into the environment. BVOC are emitted by many forms of organisms found in all domains of life (e.g., Shaw et al. 2007, Insam and Seewald 2010, Loreto et al. 2014) and serve for various ecological functions such as communications or defense (e.g., Paré and Tumlinson 1999, Mithofer and Boland 2012, Ueda et al. 2012). They also affect atmospheric processes (Atkinson and Arey 2003, Mellouki et al. 2015). Within the plant kingdom, trees are one major producer of BVOC (Kesselmeier and Staudt 1999). BVOC, typically not including methane, can be split into several subgroups according to their chemical class. Isoprenoids (also see *Figure 3* for exemplary structures), such as isoprene (5C atoms), monoterpenes (MT) (10C atoms), and sesquiterpenes (SQT) (15C atoms) are one major group within BVOC (Kesselmeier and Staudt 1999, Guenther et al. 2012). Besides, other volatile compounds, groups such as alcohols and carbonyls, contribute to the BVOC.

### 1.1 Global budget of BVOC

The global BVOC budget to the atmosphere was estimated by a global emission model (MEGAN) to be around 1087 Tg yr<sup>-1</sup> for the year 2000 (Guenther et al. 2012). The total emitted BVOC were modelled to be around 535 Tg yr<sup>-1</sup> isoprene and 162 Tg yr<sup>-1</sup> MT. Other VOC emissions were estimated to be around 390 Tg yr<sup>-1</sup> (including CO). The model simulation also showed that the BVOC amount emitted into the atmosphere is highly variable between ecosystems and their contributing species. Several recent model estimations (Guenther et al. 2012, Sindelarova et al. 2014, Messina et al. 2016) revealed that major shares of isoprene and MT are contributed by tropical forest ecosystems (around 78% according to Guenther et al. 2012), which is caused by high radiation, temperature and water availability leading to high productivity rates all year round. In case of MT emission the deciduous broadleaf ecosystems are the second largest emitters whereas conifer dominated ecosystems only contribute to around 9% due to a restricted vegetation period. However, large uncertainties in these model calculations are induced by difficulties in modeling plant stress and seasonal changes (Niinemets et al. 2010a). With respect to global change, these budgets may significantly change in the future until 2100 (Lathi re et al. 2005, Pacifico et al. 2012) due to change of factors such as climate, species distribution, land use and more frequent stress events (Pe uelas and Staudt 2010).

## 1.2 Role in atmospheric processes and feedback function

Due to their high volatility, isoprene and MT are reaction partners within various atmospheric processes causing feedbacks on plants and global warming (see *Figure 1*, Arneth et al. 2010).



**Figure 1 Schematic diagram of important reactions (simplified) of BVOCs in the atmosphere and their potential feedback on plants;** (bSOA = biogenic secondary aerosols, CCN = cloud condensation nuclei, BVOC = biogenic volatile organic compounds). Synthesis from processes described by Peñuelas and Staudt (2010), Arneth et al. (2010) and Blande (2014).

These processes restrict the lifetime of most emitted compounds within the atmosphere between some minutes to some hours (Atkinson and Arey 2003, Mellouki et al. 2015). Atkinson and Arey (2003) and Mellouki et al. (2015) provided an overview of several key processes: BVOC increases the oxidation from NO to NO<sub>2</sub>, which is necessary for O<sub>3</sub> formation and thus increasing its formation potential. O<sub>3</sub> has direct effects on plant tissue, leading to damage and oxidative stress (Felzer et al. 2007) and increase of BVOC emissions (Loreto et al. 2004). Other important reactions take place with OH radicals and O<sub>3</sub> in which BVOC are reduced to further side products. These side production reactions can lead to formation of biogenic secondary organic aerosols (bSOA), decrease and increase of OH radicals, and other follow-up products (see e.g., Aschmann et al. 1998, Chen et al. 2011, Iinuma et al. 2013). Reduced availability of OH radicals also led to a decreased reduction of CH<sub>4</sub> (Lelieveld et al. 2008) which increases radiative forcing.



SOA act as cloud condensation nuclei (CCN) (Pierce et al. 2012). Here, both clouds originating from bSOA-based CCN and bSOA itself can backscatter or adsorb incoming radiation and thus alter radiative forcing (Makkonen et al. 2012). Furthermore, bSOA can be filtered out as dry deposit or be solved within droplets. The dry deposit SOA might affect human health (Poschl 2005, Gaschen et al. 2010) and can react on plant tissue again (Blande 2014). The follow up reactions can lead to atmospheric feedback loops (e.g., by increased temperature) altering the emission potential of plants (Peñuelas and Staudt 2010).

### 1.3 Synthesis and storage of isoprenoid in plants

In order to understand the sources of BVOC emission a short overview on the potential pathways in plant tissue and their storage are given in the next two subchapters. Isoprenoid precursors are synthesized in specific enzymatic pathways into isoprene, MTs or SQTs (see 1.3.1), which can be either stored for longer or shorter time spans, or are emitted into the atmosphere (see 1.3.2 and 1.4)

#### 1.3.1 Isoprenoid synthesis pathways

Isopentenyl diphosphate (IDP) and dimethylallyl diphosphate (DMDP) are precursors for isoprenoid synthesis. Both are synthesized in two different pathways (Lichtenthaler et al. 1997b) as shown in *Figure 2*. The mevalonic acid (MVA) pathway, e.g., described by Dudareva et al. (2013), is located in the cytosol. It starts with acetoacetyl-CoA, which is then converted by three enzymatic reactions to MVA and by three further reactions to IDP. IDP can further be used to synthesize farnesyl diphosphate, which is the precursor for sesquiterpenes (Dewick 1999).

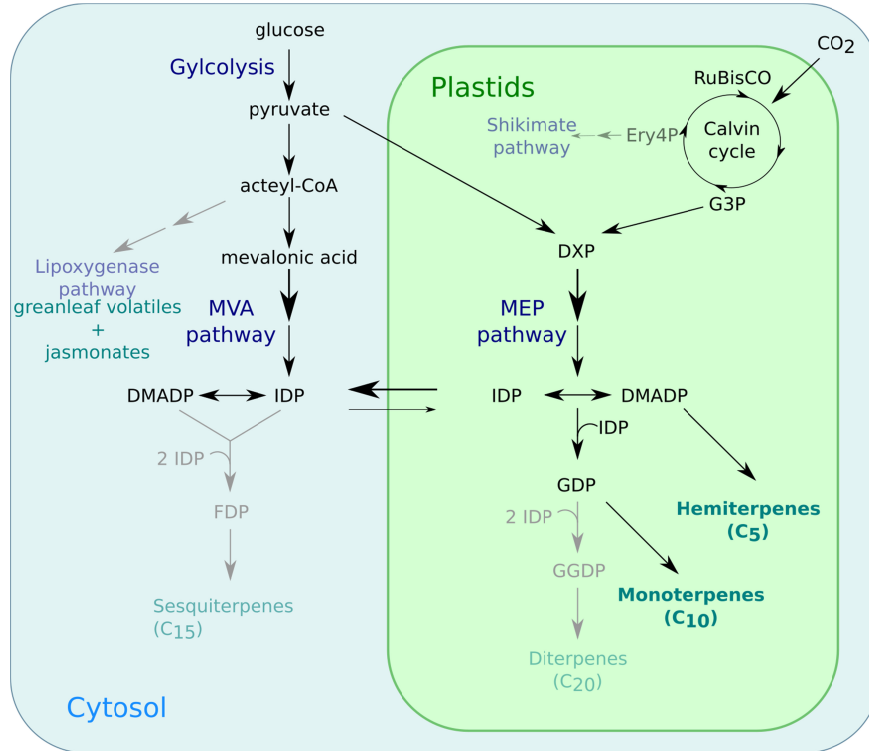
The second pathway, the 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate pathway (MEP/DOXP pathway), is located in the plastids, such as chloroplasts or leucoplasts (Gutensohn et al. 2013a). The MEP pathway begins with condensation of pyruvate and glyceraldehyde-3-phosphate (G3P) to 1-deoxy-D-xylulose 5-phosphate (DXP) and in six further enzymatic steps DXP converted to IDP and DMAPD (Lichtenthaler et al. 1997a, Rohmer 1999). In case of isoprene DMAPD is converted by the isoprene synthase.

It was shown for the MVA and MEP precursor's substrates, as well as IDP, in various studies with isotopic <sup>13</sup>C-labeling (e.g., Dudareva et al. 2005, Hemmerlin et al. 2003, Yang et al. 2012, Mendoza-Poudereux et al. 2015), that these could be transferred from plastids to the cytosol. Opitz et al. (2014) showed even a bidirectional transfer between both.

Additionally Pazouki and Niinemets (2016) suggested the exchange of higher order intermediate products. This was already shown by Gutensohn et al. (2013b) and Dong et al. (2016) case

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of geranyl diphosphate (GDP) which was transferred between cytosol and plastids and between chloroplast and mitochondria, respectively. Yet, plastids have the highest GDP potential, thus also the capability to synthesize, e.g., monoterpenes. This exchange could play a role during different stress phases, when photosynthesis and its products are reduced.



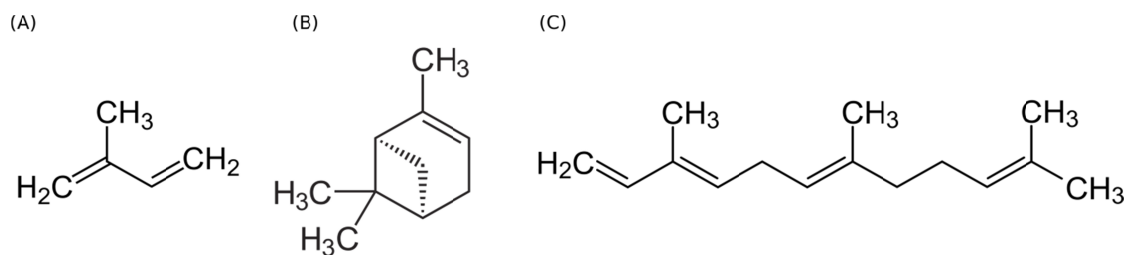
**Figure 2 Simplified schematic of the terpenoids synthesis with their pathways**

Figure and caption retrieved and transformed from Niinemets et al. 2013, Licensed under <https://creativecommons.org/licenses/by/3.0/> Retrieved 10.01.2017. Arrows represent enzymatic reaction, black signatures the intermediate products, bold forest green signature the resulting volatile group, and dark blue signature the specific pathways. Greyed out arrows and signatures are indicating to further not discussed volatile end products and their pathways. (Abr : acetyl-CoA = acetyl coenzyme A; DMADP = dimethylallyl diphosphate; DXP = 1-deoxy-D-xylulose 5-phosphate; Ery4P = erythrose 4-phosphate; FDP = farnesyl diphosphate; G3P = glyceraldehyde-3-phosphate; GGDP = geranylgeranyl diphosphate; GDP = geranyl diphosphate; IDP = isopentenyl diphosphate; LOX = lipoxygenase; MEP-pathway = methylerythritol 4-phosphate pathway; MVA = mevalonic acid)

Both pathways need substrates such as pyruvate, G3P, and acetate to synthesize the product IDP/DMADP. From the energetic point of view ATP is needed for phosphorylation and NADPH/NADH for reduction processes. While the MEP pathway has more steps and shows higher carbon losses in form of CO<sub>2</sub>, the MVA pathway is shorter, but requires more reduction power (Kesselmeier and Staudt 1999). Substrate and energy can be provided by the chloroplasts

or mitochondria (only energy) and from stored carbon pools via glycolysis (Mendoza-Poudereux et al. 2015).

In case of monoterpene synthase, which mostly succeeds after the MEP pathway within the plastids, IDP and DMPD are forming geranyl diphosphate (GDP) by head to tail condensation as universal monoterpene precursor substrate (e.g., Dewick 1999, Kreuzwieser et al. 1999). GDP further acts as substrate for the monoterpene synthesis. Here, different kinds of monoterpene synthases can produce various kinds of monoterpenes (Pazouki and Niinemets 2016).



**Figure 3 Exemplary chemical structures of non-oxygenated terpenoids.** (A) Isoprene, (B) (+)- $\alpha$ -pinene, (C)  $\alpha$ -farensene.

These can have acyclic, monocyclic and bicyclic structures (see also *Figure 3*). Also further rearrangement, oxidation, hydroxylation and dehydration or adding functional subgroups, such as ketones, alcohols, and ethers can form new monoterpene.

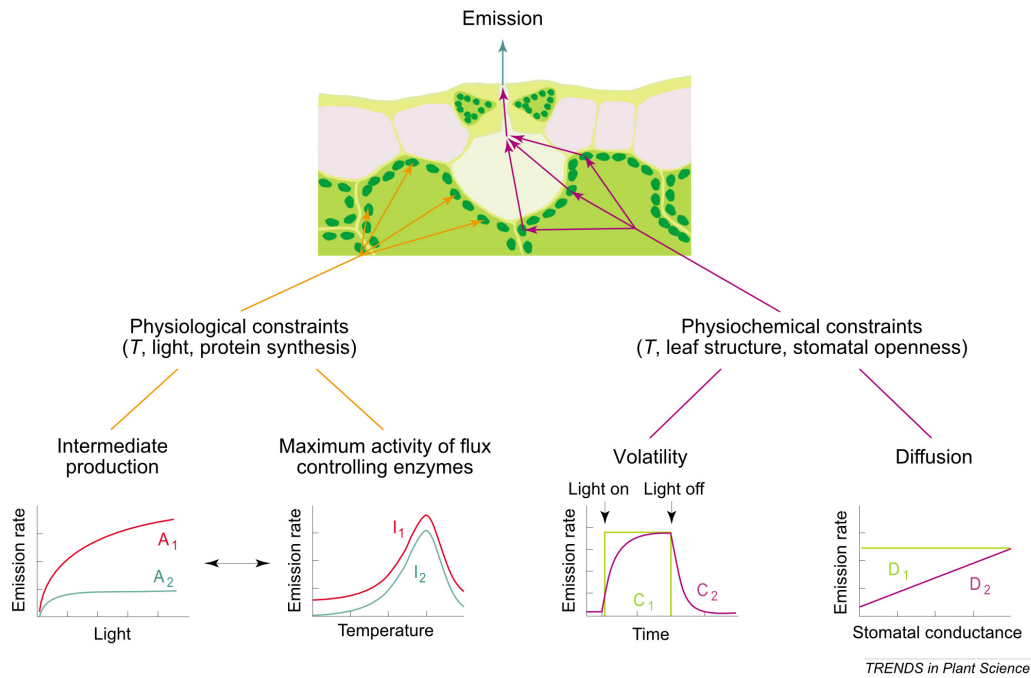
### 1.3.2 Isoprenoid storage and pools

The newly synthesized isoprenoids are stored over a short time within the cytosol. Depending on their hydrophilic or lipophilic character the compounds are stored in liquid aqueous or lipid phase (Niinemets et al. 2004). The pool size is controlled by multiple factors impacting synthesis and emission processes (see *1.4*).

Furthermore, some plants developed specialized storage organs which are coupled to specialized isoprenoid producing cells. These are found in form of secretory glands in broad-leaf species in leaf and wood (e.g., McCaskill et al. 1992, Pickard 2008). In case of conifer species these organs are referred to as resin ducts and are located in leaves, bark, and wood (Trapp and Croteau 2001). These specialized organs can be induced, e.g., by damage of wood by bark beetles (Nagy et al. 2000), or constitutively pre-aligned in specific plant organs (Trapp and Croteau 2001).

## 1.4 Control and regulation of isoprenoid emissions

The emission potential of the plant is controlled by multiple factors from in- and outside the plant tissue. A typical emission path would be the transition of a stored or newly (*de novo*) synthesized isoprenoid (see 1.3.1) from liquid to gaseous phase within the leaf and diffusion through the stomata into the atmosphere as shown in *Figure 4*.



**Figure 4 Control and regulation of isoprenoid emissions.** Plant-generated volatile organic compound (VOC) emission is limited by either physiological or physicochemical factors. The physiological constraints determine the availability of VOC precursors and maximum activity of rate-controlling enzymes, whereas the physicochemical factors limit the volatility (air-phase partial pressure, aqueous- and lipid-phase concentrations), the diffusion within the gas, aqueous and lipid phases of organic compounds within the leaves, and the gas-phase diffusion at the leaf–atmosphere interface. (*T* represents temperature.) The figure shows four representative situations in which the VOC emission is constrained by different limitations. Precursor availability mostly explains the light dependence, whereas the maximum activity of enzymes controlling the pathway flux primarily controls the temperature dependence of isoprenoid emissions. In these scenarios, precursor production is larger for A1 than for A2, and the emission rate is higher for I1 than for I2 owing to a higher total enzyme content and activity. Volatility significantly modifies emissions of hydrophobic compounds such as non-oxygenated monoterpenes, whereas gas-phase diffusion can strongly curb the emission of water-soluble compounds such as methanol, formaldehyde and formic acid. Compound C1 has high volatility and immediately responds to modifications in the rate of compound synthesis, whereas there is a significant time lag between the rates of synthesis and emission for the less volatile compound C2. Compound D1 supports a larger gas-phase partial pressure for a common aqueous-phase concentration than compound D2, explaining the stomatal insensitivity of the emission of compound D1. Figure and caption reprinted from Niinemets et al. 2004 (Figure 1), Copyright (2004), with permission from Elsevier. See Niinemets et al. 2004 for further references within the caption.

## Introduction

This emission path depends on different physiological and physiochemical properties of the emitting plant tissue (Niinemets et al. 2004). From the physiological side, emissions are limited by the synthesis capability within the plant. The synthesis capacity is strongly driven by light, which determines to a high degree the carbon fixation in the photosynthesis apparatus, and by temperature, which regulates the enzyme activity. Both light and temperature are driving the amount of precursor compounds and thus the final isoprenoid synthesis (see 1.3.1).

Furthermore, emissions are controlled by the physiochemical properties of the emitted compound (see *Figure 4*) and of the gaseous and aqueous leaf phases (e.g., cytosol, sub-stomatal air space). The most important compound property is the gas-aqueous phase partition coefficient  $K_H^{pc}$ , which is also known as Henry's law volatility coefficient [ $\text{Pa m}^3 \text{ mol}^{-1}$ ] (Henry 1803) and is defined by Eq. 1:

$$K_H^{pc} = pc^{-1} \quad (1)$$

where  $p$  [Pa] is the partial pressure of the compound within the gaseous space above the aqueous space with compound concentration  $c$  [ $\text{mol m}^{-3}$ ]. Here, high water soluble compounds, e.g., methanol, have a low  $K_H^{pc}$  and thus are less volatile, whereas high hydrophobic compounds, such as non-oxygenated isoprenoids (e.g.,  $\alpha$ -pinene), with a high  $K_H^{pc}$  are less water soluble and thus are high volatile.

The partial pressure of a volatile compound within the gaseous space in the plant is defined by the concentration in the liquid phase and the compounds'  $K_H^{pc}$ . Thus, higher temperatures increase  $K_H^{pc}$ , which leads to decreased compound solubility of the aqueous medium and therefore to a higher compound volatility. The concentration within the aqueous phase is mostly controlled by synthesis or supply of new compounds and the size of the aqueous phase. For the final emission the compound has to pass the stomata. The rate of diffusion is determined by the compounds' partial pressure difference  $\Delta p$  outside the leaf and the intercellular chamber. Typically, compounds with a high volatility/high  $K_H^{pc}$  are insensitive to stomata conductance, therefore in case of stomata closure (decreased conductance) the diffusion equilibrium can be recovered faster due to faster built up of higher partial pressure in gaseous space within the leaf and thus emission can be sustained. Compounds with low  $K_H^{pc}$  are more dependent on liquid volume availability. The control of the emission by the stomata, therefore, depends on the compound type, the timespan until a new equilibrium between leaf in- and outside gaseous phase is reached.

### 1.5 Constitutive and induced emissions

Emissions can be divided into two types depending on their type of occurrence: constitutive and induced emissions. Constitutive emissions are emitted by the plant permanently during optimum and stress conditions. They vary strongly between species (Oderbolz et al. 2013) and between stress levels and types (Niinemets 2010a) (see also *Table 1*). The BVOC emission inventory by Oderbolz et al. (2013) showed that most conifer trees (e.g., *Abies*, *Picea* species) are constitutively emitting monoterpenes, while only some deciduous tree species (e.g., *Quercus*, *Populus* or *Eucalyptus* species) showed constitutive isoprene and monoterpene emissions. The constitutive emissions split further into *de novo* synthesized emissions (strongly dependent on light) and pool emissions (strongly dependent on temperature) from permanent storage organs as found in resin ducts in Scots pine needles and wood. The pool sizes (either liquid phase or specialized storage organs) are controlled by the compound synthesis rate and storage size and are limited by physiological constraints (see *I.3* and *I.4*). Important roles of constitutive emitted isoprenoids are, e.g., to allure or distracting insects (Dicke 2003, Schiestl 2010), removal of O<sub>3</sub> within the canopy (Calfapietra et al. 2013), act as antioxidants (Loreto et al. 2004, Vickers et al. 2009) and reduction of thermal damages (Singsaas et al. 1997).

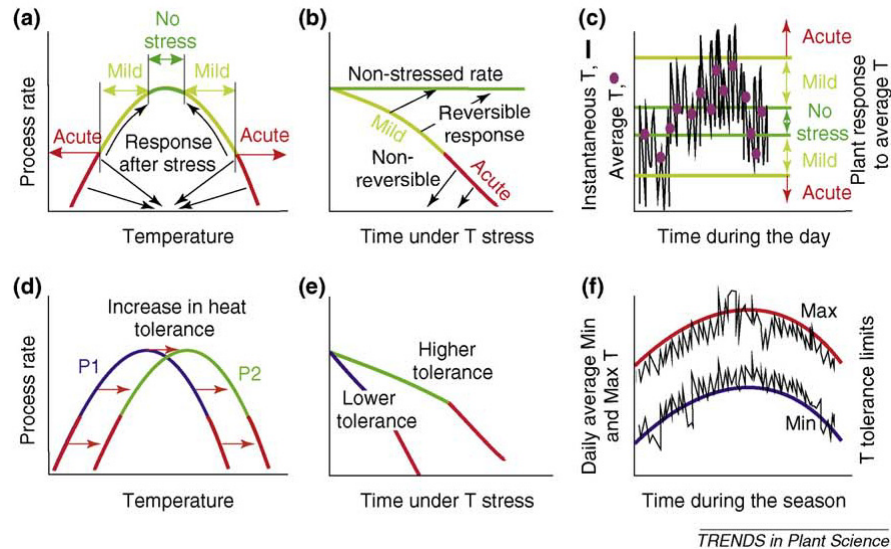
Induced emissions are typically emitted not at all or only at low amounts by the plant at non-stressed states, but external abiotic or biotic stressors can induce those emissions (see *I.6*). Often this short-term response is strongly related to a stress event, such as extreme heat (e.g. Joó et al. 2011), herbivory (e.g., Litvak and Monson 1998, Kessler and Baldwin 2001, Joó et al. 2011, Trowbridge et al. 2014, Faiola et al. 2015), or high ozone levels (e.g., Loreto et al. 2004, Pellegrini et al. 2012). The emission response of the plant can be temporally and quantitatively very variable and strongly depends on factors such as the strength of the initial stressor, the plants' potential adaptation to the stressor and co-occurring factors.

### 1.6 Stress concepts and stress response to isoprenoid emissions

Stress is a physiological response of the plant triggered by abiotic or biotic stimuli (stressors). First of all the strength and the duration of the stress determine if the stress reaction is not present, mild or acute (see *Figure 5a, b*). If, however, the stressor has a longer or stronger impact on the plant, the tree will suffer from acute (intensive) stress. This can lead to various kinds of damages, such as extensive built up of reactive oxygenated species (Vickers et al. 2009), mechanical damage on cell walls (Le Gall et al. 2015) or programmed cell death (Lam et al. 2001). Here, a recovery might not be possible anymore, and the plant might die in part or completely. In case of short extremes (see *Figure 5c*), the stress response is also more driven by average external conditions, rather by the short extreme itself, and thus damage effects can be buffered by

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the plant protection mechanisms. Such a mechanism was shown for example during a heat-drought event when isoprene emitting poplar kept their photosynthesis system of longer intact than non-emitting Poplars (Vanzo et al. 2015).



**Figure 5 Simplified representation of the basic concepts of stress in plants: (a–c) mild and acute stress, (d–f) tolerance and acclimation exemplified for temperature stress. (a)** Plant physiological processes (photosynthesis, growth, BVOC synthesis) respond to temperature according to a response curve with a maximum. In the vicinity of the optimum, plants experience no stress (dark green line), but plant physiological activity decreases above and below the temperature optimum. In the case of mild temperature stress (light green lines), plant physiological activity plastically returns to the pre-stress value after the stress, whereas in the case of acute stress (red lines), plant physiological activity further decreases upon return to optimum conditions. The response to any given level of stress depends on the duration of the stress episode, so-called dose response. **(b)** A stress that was initially mild (light green) can turn into acute stress (red) under prolonged exposure. The dose response has major implications for interpreting plant reactions to rapid fluctuations in leaf temperature which commonly occur during the day as a result of changes in cloudiness and convective heat exchange. **(c)** (color codes as in a) In rapidly fluctuating environments, plant response is driven by average temperature rather than by rapid pulses of temperature that result in low doses of heat and chilling stress. **(d, e)** Plants with greater heat tolerance (P2, dark green versus P1, dark blue) sustain acute heat stress (highlighted by red) at higher temperatures **(d)** and can tolerate given level of heat stress for longer time periods without sustained damage **(e)**. Analogously, acclimation to higher temperature improves plant performance under heat stress. **(f)** Temperature acclimation is particularly significant for plants in seasonal climates where daily average maximum and minimum temperatures strongly vary during the growing season. As a result of temperature acclimation, seasonal variations in temperature infrequently exceed the threshold for plant damage (red line shows heat tolerance and blue line cold tolerance limits). Often there is a trade-off between tolerance and acclimation to a certain type of stress, and tolerance to some other stress such as heat versus cold stress (acute cold stress shown by red lines, d, f). Figure and caption reprinted from Niinemets et al. 2010a (Figure 1), copyright (2010), with permission from Elsevier. See Niinemets et al. 2010a for further references.

## Introduction

However, some short extreme events, such as late frost events (Menzel et al. 2015), can lead to strong acute stress, which affect the plant organs permanently, e.g. by leaf destruction. Since plants, especially trees, are constantly exposed to different stressors, stress tolerance can increase over the time (see *Figure 5d*). This acclimation was observed for example after a longer drought stress phase by development of smaller leaves/limited above ground biomass in order to reduce transpiration (e.g., Royo et al. 2001, Seidel and Menzel 2016). The tolerance level (see *Figure 5e*) can also differ between trees because of reduced fitness or a long term adaption linked to selection processes. In case of Scots pine, a species with a wide distribution area, many provenances exist which are adapted to specific regional climatic conditions as well as specific stressors (see e.g., Luoma 1997, Mátyás et al. 2004, Taeger et al. 2013).

Tolerance levels are also often depending on seasonal development of a plant (see *Figure 5f*). Frost tolerance during winter is set up by storage of frost inhibiting substances or in case of deciduous trees the release of leaves (Charrier et al. 2015). During summer leaves become more heat tolerant due to rearrangement of part of photosynthesis system or enhanced production of isoprenoids (Pétron et al. 2001, Sharkey 2005). A review article by Niinemets (2010a) perfectly summarizes the varying responses constitutive in BVOC emissions of plants to different stress phases and types (see *Table 1*).

**Table 1 Stress response of constitutive emissions** (rate relative to initial) observed at short- and long-term stress and after end of stress. Table reprinted from Niinemets et al. 2010a (Figure 4c), copyright (2010), with permission from Elsevier. See Niinemets et al. 2010a for corresponding references for each stress type are listed. Original emission response symbols replaced by ↑ Increase, ↔ no response, ↓ decrease. Table was reformatted for better readability.

Stress	Short term	Long term	After stress
Drought	↔	↓	↑↔↓
Heat	↑	↓	↑↓
Salinity	↔	↓	↑
Shading	↓	↓	↓
Ozone	↔↓	↑↓	↑↓
Wounding	↑↓	↑↓	↑↓
Herbivory	↑↓	↑↓	↑↓
Fungal infestation	↔↓	↓	↓



### 1.7 Drought stress and its impacts on isoprenoid emissions

The definition of drought can be linked to meteorological, agricultural, hydrological or socio-economic criteria (Wilhite et al. 2014): For example, meteorological drought is observed when precipitation deviates negatively (amount, intensity, length and timing) from the long term normal. Agricultural drought is defined as soil water content being inadequate to sustain the physiological requirements of a particular crop or plant at a specific time. In general, agricultural drought conditions are reached after meteorological drought but before hydrological drought which is defined by shortages in water supply present at the surface (e.g., rivers, lakes) and/or subsurface (e.g., groundwater). Socio-economic drought is reached when human activities are hindered by lowered precipitation and corresponding water availability.

Extreme droughts are reoccurring events as shown for example by tree ring analyses for the Mediterranean area (Cook et al. 2016) or for Northern Africa (Touchan et al. 2011). Both studies showed a significant increase of drought frequency for the last 20 years in their study region. Spinoni et al. (2015) listed extreme drought events in Europe during the last 60 years, where several droughts occurred at different times/spatial scales and magnitude: e.g., observed in the heat wave in August 2003 over Europe, 2010 in European Russia, 2012 in Portugal. The most recent drought year for Europe was in 2015 (Ionita et al. 2017), where multiple drought events occurred over different regions in specific months. This study also reported that five out of six hottest summers occurred after the year 2000. Future climate projections simulate an increase of drought frequency by 20% until 2100 in Southern and Central Europe (Prudhomme et al. 2014). Since a drought event normally extends over a larger geographical scale, isoprenoid emissions are decreasing over a large regional scale as shown by model calculations by Müller et al. (2008) and Sindelarova et al. (2014).

The strength and duration of drought (see also *Figure 5a, c*) determines the response of a plant to decreasing water availability, as described by Chaves et al. (2003), Bréda et al. (2006) and Rennenberg et al. (2006). Important drought responses which have a high impact on isoprenoid emission are: The closure of stomata limit water loss reduces gas exchange of CO<sub>2</sub> and water vapor. This leads to reduced uptake of CO<sub>2</sub> and photosynthesis. Since isoprenoid synthesis is closely related to the chloroplastic pathway (see *1.3.1*) this could lead to shortage of potential precursors for isoprenoid synthesis and reduce potential emission as shown in many studies (e.g., Llusià and Peñuelas 1998, Šimpraga et al. 2011, Bourtsoukidis et al. 2014, Wu et al. 2015). However, according to Brilli et al. (2007) isoprene synthesis substituted photosynthesized fixed carbon by local stored carbon pools to some degree – but this has first to be shown for other isoprenoids.

## *Introduction*

Decreasing transpiration, and thus latent heat, increases leaf temperature and consequently the emission from storage pools equally increase due to increased Henry's law volatility coefficient of the compounds within the leaf (see *I.4*). In case of mixed type emitters (pool and *de novo*) such as many conifers (e.g., *Pinus sylvestris* L. or *Pinus Halepensis* MILL.) drought might have opposite impacts over the two pathways. This was however not investigated so far. Depending on the type of isoprenoid emission, emissions of hydrophobic compounds are not limited by the stomata conductivity, while for hydrophilic compounds emission can be reduced (see also *I.4*).

During a long-term and intensive drought, severe damages are caused by xylem embolism (Meinzer and McCulloh 2013), carbon starvation (Hartmann 2016), and/or increased reactive oxygen species (Cruz de Carvalho 2014). These can cause later on diebacks, e.g., by defoliation (Poyatos et al. 2013) or even of complete tree stands (Allen et al. 2010).

## 2 Aims and outlines

Subsequent to chapter 1, the complex nature of BVOC emissions, its dependency on environmental and plant physiological factors as well as different responses on stress, required an appropriate system which enabled treatment studies and supported the measurement of emissions of multiple tree seedlings at once.

One major aim of this thesis was the construction, evaluation and iterative improvement of the Tree Drought Emission MONitor (Tree DEMON), a four-chamber plant gas exchange system (see 3.1, 4.1 - Lüpke et al. 2017b, 4.2 - Lüpke et al. 2016). Parallel to the Tree DEMON development, a complete lab infrastructure for BVOC emission analysis was set up and appropriate analytical methods were implemented (see following chapters 3.2, 4.1 - Lüpke et al. 2017b, 4.2 - Lüpke et al. 2016 as well as and 4.3 - Lüpke et al. 2017a) and substantially improved over time. An adsorption tube calibration system was equally developed and successfully used.

After the development and construction of the Tree DEMON and lab infrastructure set up several studies were performed from 2013 till 2015. These studies aimed for a better understanding of drought effects on isoprenoid emissions which is needed to improve processes or parameterization of models (Grote et al. 2010, Guenther et al. 2012, Grote et al. 2014). Also it should be tested how far stress can be indirectly assessed by changes in the isoprenoid emission (Niinemets 2010b). These studies had to exclude co-occurring stressors, such as extreme heat, insect attacks or photorespiration (especially under strong light conditions) which are often present under field drought conditions and may bias the pure drought effect. Therefore the Tree DEMON was placed in controlled environment of a climate chamber.

Seedlings of *Pinus sylvestris* L. (Scots pine) were chosen for the first study due to the overlying large scale experiment within the project E<sup>3</sup> (see also Seidel et al. 2016, Seidel and Menzel 2016), which provided a large choice of provenances and tree individuals. Especially provenances growing at the edge of the species' distribution (e.g., Southern and Western Europa) have been affected by drought (see e.g., Rebetez and Dobbertin 2004, Galiano et al. 2010, Vacchiano et al. 2012), which already caused local diebacks (Allen et al. 2010). Scots pine as a mixed type isoprenoid emitter (Shao et al. 2001, Ghirardo et al. 2010) is also known to emit a significant amount of isoprenoids in Europe (Oderbolz et al. 2013). Since future climate scenarios predict an increasing drought frequency, it is important to assess the drought response on isoprenoid emissions of important European tree species in more detail.

## *Aims and outlines*

In the first study (performed 2013) isoprenoid emissions and gas exchange of three provenances of Scots pine during a drought and a re-watering phase were investigated (see 4.2; Lüpke et al. 2016) in order to check if the drought - isoprenoid emission response varied among provenances. Furthermore chemo species were determined within the provenances.

The second study with Scots pine (performed 2015, Lüpke et al. 2017a) was performed to fill gaps regarding the drought effect on *de novo* and storage emission, which could not be answered in the first experiment. Here, only one provenance was investigated in depth during variable water regimes (normal – drought – normal) and their effects on gas exchange of MT, CO<sub>2</sub> and water vapor. In order to better separate pools and *de novo* emissions, three <sup>13</sup>C labeling at different soil moisture states were performed and an improved standardization algorithm was applied.

*Castanea sativa* Mill. (sweet chestnut) was selected as second tree species since it is a primary light dependent emitter with no isoprenoid storage organs. Its isoprenoid emission has been investigated only rudimentary in two studies before (Pio et al. 1993, Aydin et al. 2014) and the effect of drought stress on the emission was not investigated so far. In study III (performed 2014) a BVOC screening and drought study was performed and results were used as a case study in Lüpke et al. (2017b).

For both species some general key question were investigated:

- How do isoprenoid emissions quantitative and qualitative differ between both species? (Study I to III)
- Does drought affect gas exchange and emissions of both species differently? (Study I to III)

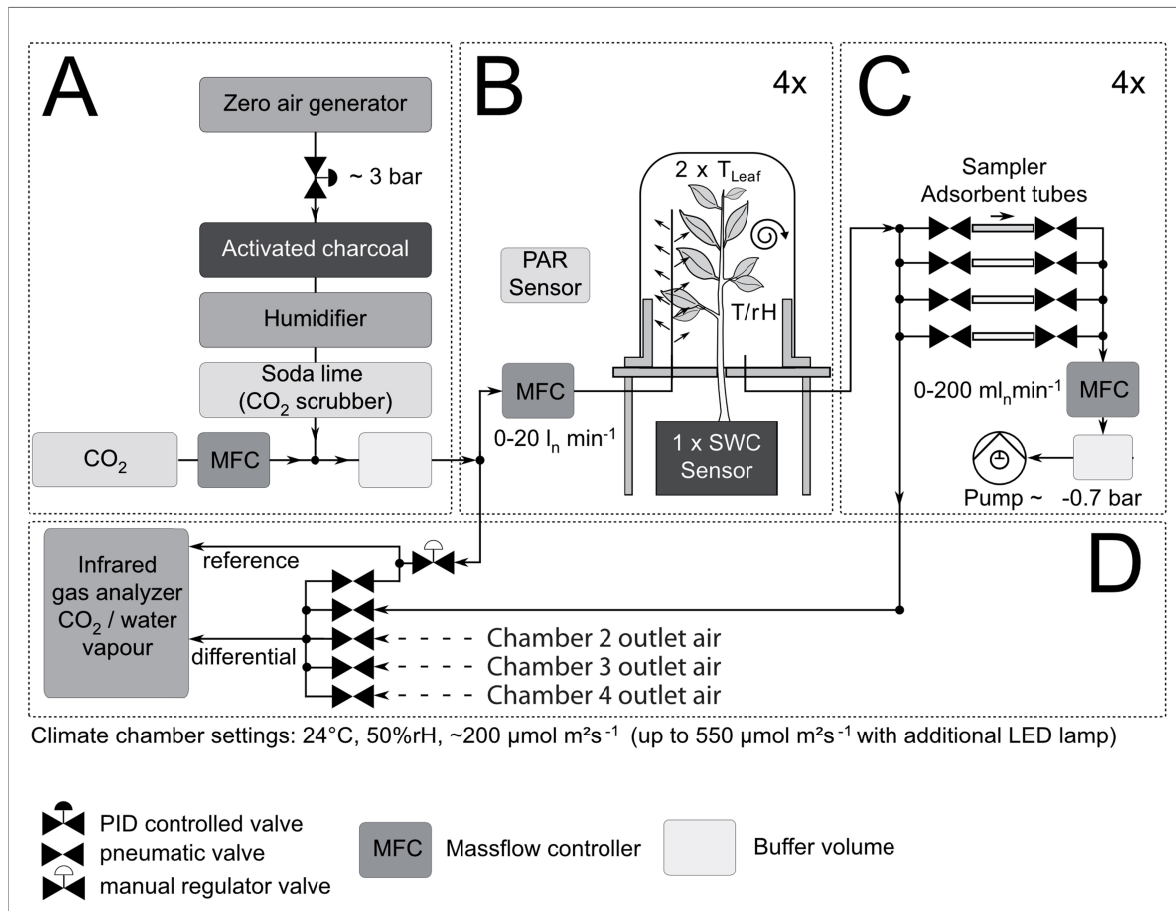
Specifically for Scots pine following question were answered:

- How do provenances affect the drought stress response on isoprenoid emissions? (Study I)
- Are pool and de novo emissions affected differently strong by drought and re-watering? (Study II)

### 3 Overview of methods

#### 3.1 Tree Drought Emission MONitor

The Tree Drought Emission MONitor (Tree DEMON) was developed, built and used within the framework of this thesis in order to assess BVOC emissions from tree seedlings. It was laid out as a dynamic plant-enclosure system (Figure 6), in which air was constantly exchanged. Therefore, under constant environmental settings a steady state of the gas exchange could be reached, while the risk of condensation of water vapor and high chamber temperatures was reduced.



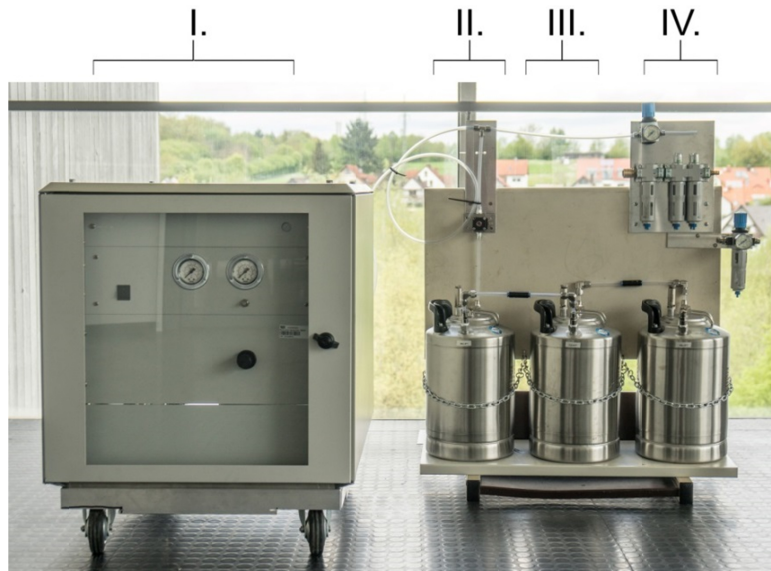
**Figure 6 System schematic** Figure and caption retrieved and transformed from Lüpke et al. 2017b, Licensed under <https://creativecommons.org/licenses/by/3.0/>; Retrieved 10.01.2017). The Tree DEMON gas exchange system design. (A) Purge air supply and conditioning; (B) chambers with sensors; (C) BVOC sampler unit containing four sampler strings with four ports each; (D) CO<sub>2</sub> and water vapor gas exchange unit. Arrows indicate the direction of air flow. MFC mass flow controller, T/rH temperature (T) and relative humidity sensor (rH), leaf temperature sensor ( $T_{\text{leaf}}$ ), photosynthetically active radiation sensor (PAR), soil water content (SWC) sensor. A PID (proportional–integral–derivative) controlled valve was used to stabilize pressure to a constant level

## Overview of methods

Several minimum design criteria in terms of material selection, environmental parameter control and recording were given by Ortega and Helmig (2008) and Niinemets et al. (2011) in order to achieve reproducible results for BVOC emission measurements (see also Lüpke et al. 2016 and Lüpke et al. 2017b for detailed parts/material requirements and specifications).

### 3.1.1 Air supply conditioning

The air supply (see *Figure 6a* and *Figure 7*) was conditioned in several steps (see also Lüpke et al. 2016, Lüpke et al. 2017b for a more detailed technical description) to achieve VOC free air with a CO<sub>2</sub> concentration of ~400 μmol mol<sup>-1</sup> and a relative humidity of ~20% at 24°C at a stable system pressure. This was required to generate steady-state conditions (stable CO<sub>2</sub> and H<sub>2</sub>O concentration at inlets) and to improve the measurement stability of the gas exchange. Furthermore the air conditioning reduced the number of air samples taken at inlet air, since it was considered as VOC free.

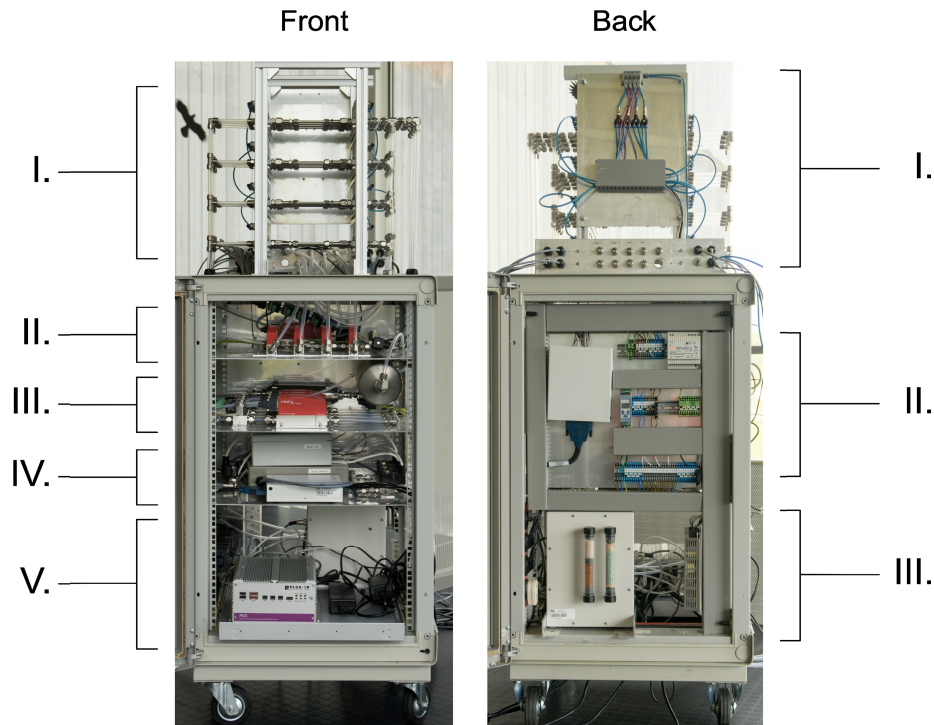


**Figure 7 Air supply conditioning:** (I.) VOC purging: Dried compressor air was delivered to a zero-air generator to remove with a platinum catalyzer CH<sub>4</sub> and VOC. Then (II.) the pressure was stabilized by a PID (proportional-integrative-derivative) controlled proportional valve. Residual VOC was filtered with an additional active charcoal in a 10-l canister. (III.) Dry air was humidified with an ultrapure water bubbler in a 10l canister. (IV.) Supply air CO<sub>2</sub> was purged by soda lime to 0 μmol mol<sup>-1</sup> in a 10l canister. CO<sub>2</sub> was re-added mass flow controlled to guarantee a constant CO<sub>2</sub> concentration within an equilibrium canister (see rack description III.).

## Overview of methods

### 3.1.2 System housing

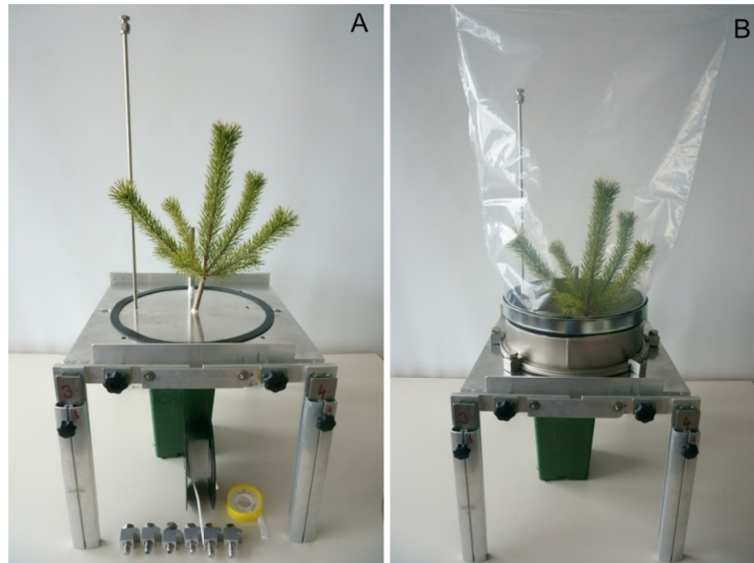
The 19-inch rack housing included following system parts (see also *Figure 8*). It included the distribution system of inlet air to the chambers, sampling of VOCs, gas exchange measurement of H<sub>2</sub>O and CO<sub>2</sub> by an infrared gas analyzer, power supplies, and controlling and recording of all sensors, valves and mass flow controllers:



**Figure 8 Tree DEMON system housing:** **Front I:** Sampler unit with four ports for each chamber. Each port consisted of two valves separating a sample tube from chamber outlet air until start of mass flow controlled sampling. **Front II:** Five mass flow controllers controlled flow rates of supply air to the four plant chambers and of CO<sub>2</sub> to the CO<sub>2</sub> addition. A pressure transducer regulated the system pressure by a proportional valve (see also *Figure 6a* and *Figure 7*). One 50µm filter ensured that the inlet air stream leading to the mass flow controller was free of contaminating particles. Further a manual valve distributed inlet air to the reference measurement channel of the CO<sub>2</sub>/H<sub>2</sub>O infrared gas analyzer (IRGA). **Front III:** Four mass flow controllers set the sampling flow rate (see level I) with each having a 50µm particle filter installed. Outlet air was connected to a vacuum pump ensuring sufficient under-pressure monitored by a pressure transducer. The 2-liter canister was used for mixing and homogenizing the added CO<sub>2</sub>. **Front IV:** I/O (Input/Output) connector block for digital and analog channels, relay boards and 5 magnetic valves for sequentially delivering the outlet air of the four chambers/once inlet air to the CO<sub>2</sub> and H<sub>2</sub>O gas analyzer. **Front V:** A computer with measurement cards was running the control and measurement software. Additional power supplies for mass flow controllers and IRGA were installed. **Back I:** Connectors for chamber and supply in- and outlet air tubing, and sensors connection to the chambers were provided for quick access. **Back II:** I/O connector block sampled/set digital and analog I/O channels. Power supply and connections for sensors (temperature, humidity, pressure transducer) and valves were provided for easy accessibility. **Back III:** CO<sub>2</sub> and H<sub>2</sub>O gas exchange was measured with a two channel IRGA.

### 3.1.3 Plant chambers

The plant chamber consisted of two main parts: the carrier platform with a base plate and sensors (see *Figure 9A* and caption) and the transparent top chamber on a flange (*Figure 9B*).



**Figure 9 Plant chamber:** (A) Base plate with carrier: The base plate consisted of two parts. One base plate contained air in- and outlet and a combined temperature and humidity sensor. Air was mixed by distributing the inlet air via multiple micro outlets along a stainless steel tube into the chamber. The other base had no additional hardware installed and was used to seal properly the stem. The carrier was height adjustable to install various types of plants and pots. (B) Top chamber: The top chamber consisted of a transparent PVDF (polyvinylidene fluoride) film from a gas sampling bag fixed on a stainless steel flange with a volume of 30-liter. This made the upper part on the lower side static and easier to seal and on the top side transparent and more elastic. The materials were inert and had low gas permeability.

An external photosynthetic active radiation (PAR) sensor was placed at mid-height in the mid position of the four plant chamber. In order to enhance PAR radiation an additional multispectral LED lamp was installed above the plant chambers. In 2013 soil moisture was measured by a mobile time domain reflection (TDR) probe before and after installation into the plant chamber, while in 2014 a TDR sensor was installed in pot basement of each pot to measure soil water content continuously.

### 3.1.4 Software and hardware control

The software controlling the Tree DEMON was written in LabVIEW language “G” – a dataflow programming language. It is a graphical language and the execution is determined by graphical block diagrams which are built by different function nodes connected with “wires” propagating the data. The language is able to run code parallel in a multithread/-CPU environment by an inbuilt multiplexer.



## Overview of methods

LabVIEW was used because most measurement devices used in the Tree DEMON were provided with specific virtual instruments (VI) or could be implemented with on board software tools. Since a VI was often provided by manufactures or 3<sup>rd</sup> party developers, a much faster and time saving implementation of the hardware was achieved. Furthermore, LabVIEW provided many tools and predefined software code as well as examples for a fast prototype building. At last, the easy set-up of a graphical user interface allowed programming a complete solution to run, control and monitor the Tree DEMON.

In total four different measurement sources were implemented: an external USB thermocouple sensor board (USB-TEMP), internal PCI-express DAQ measurement card with two I/O connectors covering digital and analog connectors, the digital MODBUS<sup>®</sup> connected mass flow controllers and the IRGA connected via a serial rs232 connection. Depending on the purpose and specification of the hardware, they were grouped into different loops running at different execution speeds. Execution speed (timing) was given by the specific task within the loop, e.g., mass flow control, which was limited by the minimum acquisition time given by the specific hardware (e.g., MODBUS<sup>®</sup> frequency). Since LabVIEW is able to perform tasks in parallel, it was possible to run multiple loops to acquire data and to control hardware. Commands and data between loops were transferred via a queue or notification system. The following loops were implemented: mass flow control, timing (sampling, lamp) and relay control, data storage, sensor data acquisition (IRGA, USB-TEMP and NI-DAQ) with PID for pressure set and GUI interface for setup and hardware control.



**Figure 10** User interface of the Tree DEMON: (A) actual sensor data and switch states. (B) settings for sampling time/ids and flow rate configuration

A graphical user interface presented current data (higher frequency) as well as data time series and allowed the user to adjust custom settings such as sample time and duration, flow rates or lamp duration (see *Figure 10*). The software was continuously optimized and adapted for new experimental setting, e.g., finer control LED light or input error handling.

### 3.1.5 Time line of Tree DEMON development and improvement

The Tree DEMON was planned and built within the first 1.5 years (2012 to mid of 2013) of this thesis. After selection of the appropriate built parts (see Lüpke et al. 2016, Lüpke et al. 2017b), these were installed into a 19-inch rack housing, the plant chambers and the air conditioning system. In 2014, the BVOC sampler unit was upgraded from magnetic valves to pneumatic valves and beginning of 2015 the air supply system underwent a security overhaul by exchanging the filter tanks of the air supply conditions. The software was improved steadily from 2013 to 2015.

### 3.1.6 Environmental control

Since BVOC emissions are sensible to changing environmental parameters such as light and temperature, the whole Tree DEMON system was placed in an environmental controllable climate chamber at TUM experimental station Dürnast near Freising. The used climate chamber was set to a constant temperature of 24°C and a ramped light program to simulate day and night (see Lüpke et al. 2016, Lüpke et al. 2017b and Lüpke et al. 2017a for detailed settings). Light intensity was increased by an extra multispectral LED lamp. Due to this controlled environment, multiple replications in the experiments were possible.

Even in the controlled climate environment an increased plant chamber temperature was still caused by the radiation of illumination, the greenhouse effect of plant chambers and the drought-included reduction of plant transpiration. A regulation of individual plant chamber temperature and humidity was initially planned, but was not accomplished within the framework of this thesis. However, Soutschek (2015) tested in a related Master thesis a first prototype to control chamber temperature with a thermoelectric system. The built device was able to regulate the plant chamber air temperature in a range of 3.8°C, but only with a slow adjustment speed. Therefore, in all experiments temperature variation and its effects on BVOCs were compensated by specific standardization algorithms (see 3.4.2)

### 3.1.7 System evaluation

Tree DEMON was positively evaluated by Lüpke et al. (2017b), covering several aspects, such as repeatability of sampling, system stability, spectral characteristics of chamber and LED light. In addition, the performance of the system was demonstrated in two case studies: a screening study and a drought stress study with *Castanea sativa* MILL. (see 3.5, 4.1).

## 3.2 Chemical analysis

### 3.2.1 Adsorbent tube and thermal desorption

BVOC emissions are ranging typically in the lower parts per billion (ppb) to part per trillion (ppt) range and need to be pre-concentrated on adsorbent tubes (AT) before analysis. An AT can be filled with a specific adsorbent material to trap non-polar or polar, light or heavy volatile compounds from the gas phase and to release them with thermal extraction. Since there is no universally applicable adsorbent material, specific materials (single or multiple in row) were selected for trapping target compounds. For the experiments, the main target compounds were isoprenoids ranging from C5 (e.g., isoprene) to C10 (monoterpenes such as  $\alpha$ -pinene) up to potentially C15-molecules (sesquiterpenes such  $\alpha$ -farnesene). Therefore, a two-bed configuration was selected with 40 mg of Carbograph 5TD (C3 to C8, 60/80 mesh), a graphitized carbon black, and 70 mg of Tenax<sup>®</sup> TA (C6 to C26, 60/80 mesh), a porous polymer. An inert silica coated stainless steel was used as tube material which was less sensitive to wall reaction than stainless steel and more robust than glass (see Lüpke et al. 2016 for technical details).

The adsorbed compounds on the tube material were automatically extracted by a thermal desorption unit (ATD 650, Perkin Elmer, USA) in two steps. In the first step the compounds were thermally (280°C) extracted from the adsorbent tube for 15 minutes onto a cold trap (-30°C) filled with an adsorbent material (Perkin Elmer Air toxics). In the second step the cold trap was ballistically heated (40°C s<sup>-1</sup>) to 300°C so that all compounds were desorbed at once and transferred via a heated glass line to the separation column of the gas chromatograph (GC). Splits of the carrier gas flows in both steps allowed diluting the desorbed amount and reduced the risk of overloading the column and transferring too much humidity coming from the air sample. In a last step the AT was thermally reconditioned for reuse. During the course of the experiments, the thermal desorption method was constantly enhanced and fine-tuned; therefore the exact settings are presented in the respective publications.

### 3.2.2 Gas chromatography

The extracted compounds were separated in a GC by a multi-ramp temperature program over a 30 m column (5% diphenyl 95% dimethyl polysiloxane). The separation is based on the principle that compounds in the carrier gas are interacting differently with the so called stationary phase of the capillary column. The ongoing adsorption and desorption processes of a compound in the stationary and carrier gas phase during its transport through the separation column depends on the physicochemical properties of a compound and the polarity of the stationary phase leading to a specific transport time. The transport or retention times of different compounds are

## Overview of methods

sensitive to temperature and pressure changes in the column. Thus, the separation of a compound mix can be optimized by using appropriate stationary phase polarity, column temperature and carrier gas pressure. The compounds leaving the separation column with the carrier gas (He) are detected here using flame ionization detection and mass spectroscopy.

### 3.2.3 *Flame ionization detector*

Half of the extracted compounds were combusted at 300°C in a hydrogen flame while ions formed during this process were detected as electrical signal (Otto 2006). The detected signal is proportional to the concentration of combusted material. Benefits of the FID comprise a high linearity over a large detection range and low maintenance efforts. However, using an FID compounds are identified by retention time only, thus a known compound mixture has to be used beforehand to allocate the exact retention time and the signal response to each compound of interest.

### 3.2.4 *Mass spectrometry*

Mass spectrometry (MS) was performed with a Perkin Elmer SQ8 single quad with an electron ionization source EI (70eV). Briefly about the functional principle of the system (Hübschmann 2009): A compound entering the MS is ionized by electrons of the ion source which results in negative, positive as well as neutral molecule species. Negative ions and electrons are filtered out. First a quadrupole pre-filter removes non-target ions and thus keeps background noise lower. The pre-filtered positively ionized molecule and its fragments are further filtered by their mass to charge ratio ( $m/z$ ) by the mass analyzer (quadrupole mass filter). Due to quasi-parallel full scan mode, the MS was able to scan over a large  $m/z$  range (e.g.  $m/z$  30 to 330) and in a single ion mode to scan for specific  $m/z$ . The filtered ions are then transferred to a conversion dynode producing electrons, which are amplified, detected and converted to a measurement signal.

EI ionization of a molecule produces a compound specific fragmentation pattern. Using constant ionization energy, typically 70eV the fragmentation pattern of a molecule could be compared with reference patterns in the NIST library to identify the molecule (Stein 2008). For the studies full scan as well as single ion modes were used to detect, identify and quantify compounds.

In the  $^{13}\text{C}$  labeling study (Lüpke et al. 2017a) the MS response was used to calculate labelling ratios of isoprenoids. Each built-in  $^{13}\text{C}$  atom increased the  $m/z$  ratio of a specific molecule fragment by one and consequently the amount of built-in  $^{13}\text{C}$  atoms in the target molecule could be determined (see Lüpke et al. 2017a).

### 3.2.5 Calibration

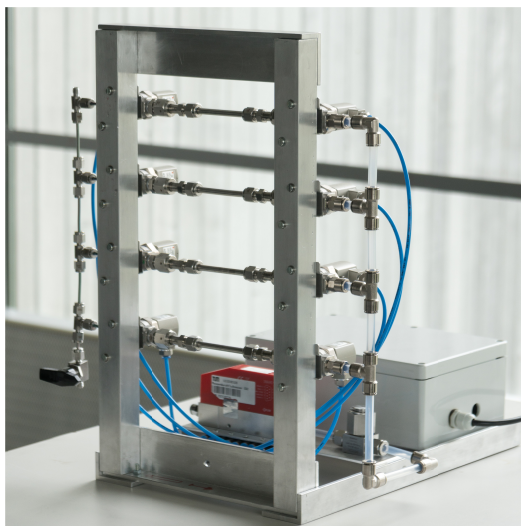
A multicomponent gas standard (NPL, UK) containing 10 isoprenoids ( $\sim 2 \text{ nmol mol}^{-1}$ ) was used as calibration standard. Further  $\Delta^2$ -carene (Siad GmbH, Austria) with around  $87 \text{ nmol mol}^{-1}$  was added as internal gas standard to each sample. A multipoint calibration was performed with 3 to 4 volume levels to provide different loadings on the AT together with the added internal standard. The internal standard compensated for variability of the ATs and of the analytical system. In Eq. 2 a linear calibration curve was fitted with the calibration data (*Area* is the integrated signal of the respective detector (MS/FID), *N* is the loaded compound amount [ $\text{nmol mol}^{-1}$ ] on the AT, *a* is the relative response factor and *b* is the intercept).

$$N_{\text{sample}} = a * \frac{\text{Area}_{\text{sample}} N_{\text{int}}}{\text{Area}_{\text{int}}} + b \quad (2)$$

In case of compounds with no available standards the calibration curve of a structural equivalent compound within the calibration standard was used. Sample concentration  $\chi$  [ $\text{nmol mol}^{-1}$ ] was calculated by dividing the sample amount by sample molar volume  $V_m$  [ $\text{mol}$ ] in Eq. 3:

$$\chi_{\text{sample}} = \frac{N_{\text{sample}}}{V_{m \text{ sample}}} \quad (3)$$

Calibrations were performed shortly before and after each experiment and after maintenance of the TD-GC/FID-MS system.



**Figure 11 External calibration unit:** Four sample ports each containing an adsorption tube. In- and outlet tubing and vacuum pump are not shown. Box in the background contains a mass flow controller, power supply, relays and an Arduino microcontroller as control interface. A LabVIEW program was controlling the system.

## Overview of methods

In 2013 calibration standards were added using the sample system of the Tree DEMON, from 2014 on a self-built external calibration system was used (see *Figure 11*). This system was almost identically to the BVOC sample system used in the Tree DEMON system of 2014. The external calibration unit allows a higher flexibility and the smaller tubing diameter of the intake lines speeded-up the equilibrium time between gas phase and tubing surface when starting a new calibration.

### 3.3 Leaf area estimation

Leaf area  $A_{\text{leaf}}$  of Scots pine was determined by harvesting the whole above ground biomass of the seedlings, drying the material for 48 h at 60°C and scanning a subset of needle leaves. ImageJ calculated the area of the scanned leaf by using a scanned reference scale and the specific leaf area (SLA) was calculated together with the dry mass. Total leaf area was determined upscaling the SLA with the total leaf mass. In case of sweet chestnut this procedure was slightly adapted (see Lüpke et al 2017a for details) and a non-destructive method was additionally implemented. The non-destructive method was based on allometric relationships between the leaf area and its length and width. Thus, these parameters were measured to calculate an allometric function as shown by Serdar and Demirsoy (2006) on the same tree species.

### 3.4 Gas exchange of CO<sub>2</sub>, water vapor and isoprenoids

#### 3.4.1 CO<sub>2</sub> and water vapor gas exchange calculation

In and outlet CO<sub>2</sub> and water vapor concentrations were measured with a two channel non-dispersive infrared gas analyzer. This device measures continuously the gas concentration of CO<sub>2</sub> and water vapor of an air sample by the amount of absorption of an infrared beam at a target gas specific wavelength (Long et al. 1996). In order to determine photosynthesis and transpiration rates, the calculation was done after Caemmerer and Farquhar (1981) with Eq. 4 and 5. Transpiration rate  $E$  [mmol m<sup>-2</sup> s<sup>-1</sup>] was calculated from water vapor mole fraction differences  $W_{\text{diff}}$  [mmol mol<sup>-1</sup>] of chamber inlet and outlet where the inlet air referred to reference  $W_{\text{ref}}$  [mmol mol<sup>-1</sup>] based on the molar air flow  $F_m$  [mol s<sup>-1</sup>] per leaf area  $A_{\text{Leaf}}$  [m<sup>2</sup>] with Eq. 4:

$$E = (W_{\text{diff}})F_m A_{\text{Leaf}}^{-1} (1 - (W_{\text{diff}} + W_{\text{ref}}))^{-1} \quad (4)$$

Net photosynthesis rate  $A$  was determined in a similar manner as the transpiration rate but using CO<sub>2</sub> mole fractions of both channels  $C_{\text{ref}}$  and  $C_{\text{diff}}$  [μmol mol<sup>-1</sup>] which had to be corrected by the plant transpiration according to Eq. 5:

$$A = C_{\text{diff}} F_m A_{\text{Leaf}}^{-1} - \frac{E}{1000} (C_{\text{diff}} + C_{\text{ref}}) \quad (5)$$

### 3.4.2 Isoprenoid emission rate calculation and standardization

The emission rate  $EM$  [ $\text{nmol m}^{-2} \text{s}^{-1}$ ] is calculated by Eq. 6 after Niinemets et al. (2010):

$$EM = (\chi_{out} - \chi_{in})F_M^{-1}A_{Leaf}^{-1} + \chi_{out} E \quad (6)$$

with  $\chi_{in/out}$  being in-/outlet-air compound concentration, the molar air flow rate  $F_M$  [ $\text{mol s}^{-1}$ ], leaf area  $A_{leaf}$  [ $\text{m}^2$ ]. Due to the intensive air filtering  $\chi_{in}$  was assumed as zero, but regularly checked by inlet air samples. In the second term of Eq. 6 the mass balance was corrected by the additional transpired water vapor of the plant, since only the flow rate into the chamber was measured.

Different standardization algorithms were applied to compensate for temperature/light variability between the chambers (see Lüpke et al. 2017b) which made reported emission rates comparable to literature data and allowed proper statistical analyses. The pure temperature based algorithm of Guenther et al. (1995) (Eq. 7) was used on pool dominated/stored emission:

$$EM_{Std} = \frac{EM}{e^{\beta(T_L - T_{std})}} \quad (7)$$

It is based on an exponential functional containing the empirical determined value  $\beta$  of  $0.09 \text{ K}^{-1}$  ( $\beta$  can range for Scots pine from  $0.076$  to  $0.18 \text{ K}^{-1}$ , Niinemets et al. 2010b), leaf temperature  $T_L$  in K and standard temperature of  $278.15 \text{ K}/303.15 \text{ K}$  depending on the target standard temperature. This algorithm was used in the 2013 experiment (Lüpke et al. 2016) to standardize the emission rates to  $25^\circ\text{C}$ , since *de novo* shares could not be determined.

Light and temperature (typically *de novo* synthesized) depending emission rates were standardized by algorithms in Eq. 8 proposed by Guenther (1997) and by Niinemets et al. (2010b):

$$EM_{Std} = \frac{EM}{f(T_L) * f(Q)} \quad (8)$$

In Eq. 8 the measured *de novo* emission rate was standardized by the correction term for leaf temperature  $f(T_L)$  to  $30^\circ\text{C}$  (similar to the divisor term in Eq. 7) and for the correction term  $f(Q)$  to light level of  $1000 \mu\text{mol PAR m}^{-2} \text{s}^{-1}$ . The parameters for  $f(Q)$  and  $f(T_L)$  used were the same as proposed by Guenther (1997). This algorithm was used in Lüpke et al. (2017b) based on the terms and parameters of Niinemets et al. (2010b).

In Lüpke et al. (2017a) a mixed standardization algorithm was applied to incorporate pool and *de novo* emitted compounds which is typical for many conifers. This algorithm had been used before by Ghirardo et al. (2010) and Harley et al. (2014) and combined Eq. 7 and Eq. 8 to Eq. 9

## Overview of methods

with a scale factor  $f_{denovo}$  representing the *de novo* share of the emission.  $f_{denovo}$  was derived by labeling the plants with  $^{13}\text{CO}_2$  (Ghirardo et al. 2010, Harley et al. 2014, Lüpke et al. 2017a).

$$EM_{Std} = \frac{EM_{sample}}{(f_{denovo} f(T_L) * f(Q)) + ((1 - f_{denovo}) * e^{(\beta(T_L - T_{std}))})} \quad (9)$$

Parameters are similar to Eq. 7 and Eq. 8.

### 3.5 Experimental setups

Several pre-studies and three successful experiments were conducted from 2013 till 2015. Selected species with their origin (provenance) as well as the sample designs are listed in *Table 2*.

**Table 2 Overview on experimental setups of the major experiments** (prov = provenance, rep. = replication, N = number of individuals). \*Out of the 3 rep. only 1 was successful.

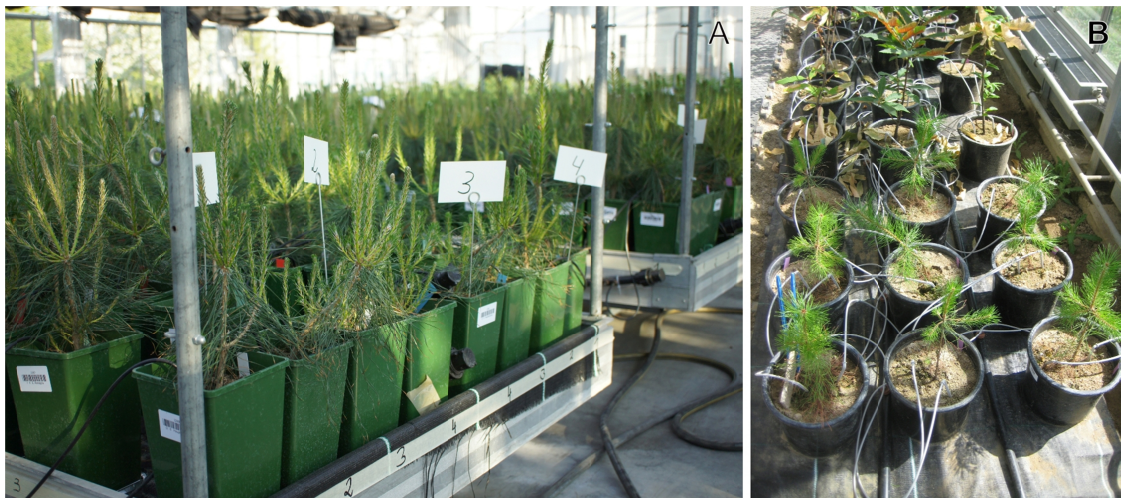
Study year	2013	2014	2015
<b>Publication</b>	Lüpke et al. (2016)	Lüpke et al. (2016)	Lüpke et al. (2017a)
<b>Species</b>	<i>Pinus sylvestris</i> L.	<i>Castanea sativa</i> Mill.	<i>Pinus sylvestris</i> L.
<b>Provenances</b>	Spain: "Alto Ebro" Germany: "Mittel-/ Ostdeutsches Tiefland"; Italy: "Emilia Romagna"	Germany: "übriges Bundesgebiet" without North Germany; not clear specified	Germany: "Mittel-fränkisches Hügelland" Middle Franconia (Bavaria)
<b>Soil type</b>	100% humus	30% humus 70% sand	30% humus 70% sand
<b>Pot size</b>	2-l	5-l	5-l
<b>Total trees</b>	~300 per prov	~ 50	~ 50
<b>Selected trees and setup</b>	36 (3 prov. x 3 rep. x 2 trees x 2 groups)	Screening exp.: 20 (5 rep. x 4 trees) Drought exp.: 12 (3 rep x 2 trees x 2 groups)*	8 (2 rep. x 2 trees x 2 groups)
<b>Irrigation during exp.</b>	Dripping water system	Manual	Manual

In the respective winters before the experiment (2012/2013), all seedlings were planted at an age of one (deciduous) or two (conifers) into pots (see *Figure 12*). All experiments were conducted only after leaves were fully developed to exclude leaf growth effects. In all studies the effect of changing water regimes on the isoprenoid emissions was the main focus.



## Overview of methods

In the first study (2013) 2-l pots were used due to the size of the overlying experiment (~3000 trees) which required simple handling and room for the pots. Drought treatment of the trees was controlled by the dripping irrigation system of the greenhouse. During drought/after re-watering) isoprenoid emissions of Scots pine three provenances were measured with the Tree DEMON. Here each day a new subset of trees (two controls/two treated) were installed into the Tree DEMON for measurement.



**Figure 12 Experimental greenhouse setups:** (A) Scots pine seedlings (2013) with dripping watering system. Randomized provenances were placed in treatment lines (1 to 4). (B) Experimental setup of Scots pine seedlings and sweet chestnut (2014) with dripping watering system. Plants were placed in a 3 x 3 raster.

For the other two studies (2014/2015) tree seedlings of five species (*Fagus sylvatica* L., *Quercus robur* L., *Betula pendula* ROTH, *Castanea sativa* MILL. and *Pinus sylvestris* L.) were planted into 5 l pots with a humus sand mixture at end of 2013 and trees were placed in a greenhouse (see *Figure 12*). After a pre-screening study of all deciduous trees (data not shown) *Castanea sativa* MILL. was selected for the 2014 experiment, since emission patterns/amounts were suitable for further treatment studies. Here, 20 tree seedlings, size-wise suitable for the Tree DEMON, were first screened to identify potential chemo species and standard emission rates. Afterwards 12 out of these 20 trees were selected for a drought experiment with three replications. Each replication was run completely in the Tree DEMON and lasted around 16 days. Soil water content was regulated by manual watering. Due to insect infestation, a climate chamber failure and a damage of the pressure tanks (air conditioning part) of the Tree DEMON (see *Figure 7* for new tanks), only one of the three replications were successful. The screening and part of the drought experiment was used as case study to show the Tree DEMON performance in Lüpke et al. (2017b).

### *Overview of methods*

In 2015 eight *Pinus sylvestris* L. seedlings, size-wise suitable for the Tree DEMON, were used for a drought experiment with isotopic  $^{13}\text{CO}_2$  labelling. Similar to the 2014 experiment, soil water content was controlled manually and each four plants were installed for around 16 days in the Tree DEMON.

## 4 Abstracts and contributions to the individual publications

### 4.1 The Tree Drought Emission MONitor (Tree DEMON), an innovative system for assessing biogenic volatile organic compounds emission from plants

Marvin Lüpke, Rainer Steinbrecher, Michael Leuchner and Annette Menzel (2017): *Plant Methods* 13:14; doi: [10.1186/s13007-017-0166-6](https://doi.org/10.1186/s13007-017-0166-6). (Lüpke et al. 2017b)

Background: Biogenic volatile organic compounds (BVOC) emitted by plants play an important role for ecological and physiological processes, for example as response to stressors. These emitted compounds are involved in chemical processes within the atmosphere and contribute to the formation of aerosols and ozone. Direct measurement of BVOC emissions requires a specialized sample system in order to obtain repeatable and comparable results. These systems need to be constructed carefully since BVOC measurements may be disturbed by several side effects, e.g., due to wrong material selection and lacking system stability.

Results: In order to assess BVOC emission rates, a four plant chamber system was constructed, implemented and throughout evaluated by synthetic tests and in two case studies on 3-year-old sweet chestnut seedlings. Synthetic system test showed a stable sampling with good repeatability and low memory effects. The first case study demonstrated the capability of the system to screen multiple trees within a few days and revealed three different emission patterns of sweet chestnut trees. The second case study comprised an application of drought stress on two seedlings compared to two in parallel assessed seedlings of a control. Here, a clear reduction of BVOC emissions during drought stress was observed.

Conclusion: The developed system allows assessing BVOC as well as CO<sub>2</sub> and water vapor gas exchange of four tree specimens automatically and in parallel with repeatable results. A canopy volume of 30 l can be investigated, which constitutes in case of tree seedlings the whole canopy. Longer lasting experiments of e.g., 1–3 weeks can be performed easily without any significant plant interference.

**Contributions:** I did the finalized design/construction/programming of the Tree DEMON and performed system test shown in this publication. *Rainer Steinbrecher* provided support for the system and its evaluation, *Michael Leuchner and Annette Menzel* provided methodological support. All co-authors reviewed and improved the draft manuscript before submission. About 85% of the work was done by myself.

#### **4.2 Impact of summer drought on isoprenoid emissions and carbon sink of three Scots pine provenances**

Marvin Lüpke, Michael Leuchner, Rainer Steinbrecher and Annette Menzel (2016): *Tree Physiology* 36: 1382–1399, doi: [10.1093/treephys/tpw066](https://doi.org/10.1093/treephys/tpw066). (Lüpke et al. 2016)

Scots pine (*Pinus sylvestris* L.) provenances cover broad ecological amplitudes. In a greenhouse study, we investigated the impact of drought stress and rewetting on gas exchange for three provenances (Italy: Emilia Romagna; Spain: Alto Ebro; Germany: East-German lowlands) of 2-year old Scots pine seedlings. CO<sub>2</sub>, water vapor and isoprenoid exchange of stressed and control trees were quantified with a four-chamber dynamic-enclosure system in the controlled environment of a climate chamber.

The three provenances showed distinct isoprenoid emission patterns and were classified into a non- $\Delta^3$ -carene, with either high  $\alpha$ -/ $\beta$ -pinene or  $\beta$ -myrcene fraction, and a  $\Delta^3$ -carene dominated type. Isoprenoid emission rates, net-photosynthesis and transpiration were reduced during summer drought stress and significantly recovered after rewetting. A seasonal increase of isoprenoid emission rates towards autumn was observed for all control groups. Compared with the German provenance, the Spanish and Italian provenances revealed higher isoprenoid emission rates and more plastic responses to drought stress and seasonal development, which points to a local adaptation to climate. As a result of drought, net carbon uptake and transpiration of trees was reduced, but recovered after rewetting.

We conclude from our study that Scots pine isoprenoid emission is more variable than expected and sensitive to drought periods, likely impacting regional air chemistry. Thus, a provenance-specific emission assessment accounting for reduced emission during prolonged (summer) drought is recommend for setting up biogenic volatile organic compound emission inventories used in air quality models.

**Contributions:** I had the idea for and designed the study. *Michael Leuchner* and *Rainer Steinbrecher* provided methodological support and supported me together with *Annette Menzel* in data analysis and writing of the manuscript. All co-authors reviewed the draft manuscript before submission. About 80% of the work was done by myself.

#### 4.3 Quantification of monoterpene emission sources of a conifer species in response to experimental drought

Marvin Lüpke, Rainer Steinbrecher, Michael Leuchner and Annette Menzel (2017): re-submission currently under review in *AOB Plants* (Lüpke et al. 2017a) – resubmitted at 05.08.2017

Monoterpene emissions of conifer tree species, emitted from *de novo* synthesis and storage pools, play an important role in plant ecology and physiology. During drought stress both emission sources are affected differently and with conventional measuring techniques they are difficult to separate. We investigated  $^{13}\text{C}$  labelled monoterpene (MT) emission of eight 3-year-old Scots pine seedlings in a drought stress experiment using a dynamic gas exchange chamber system (Tree DEMON). MT, water vapor and  $\text{CO}_2$  gas exchange were measured for a 2-day normal watering, a 11-day treatment and a 3-day re-watering period. In each period all trees were  $^{13}\text{C}$  labelled once for five hours. Results showed the expected decrease of MT, water vapor and  $\text{CO}_2$  gas exchange with decreasing soil water content. However, during re-watering water vapor and  $\text{CO}_2$  gas exchange recovered fast to pre-drought levels, whereas MT increased to a lower level compared to the initial non-stressed phase. The  $^{13}\text{C}$  labelling showed highly variable  $\%^{13}\text{C}$  values for different monoterpenes, which ranged compound-specific from 0.5 to 95% for unstressed trees. Overall, around  $36\pm 5\%$  of the total emission rate originated from *de novo* synthesized MTs during the 2-day prior to stress period. During full drought, the *de novo* fraction was reduced to 3%. For the re-watering phase *de novo* emissions recovered only partly to 20%, while pool emissions reached pre-drought conditions. Thus, emissions of *de novo* synthesized MTs of Scots pine are down regulated by soil drought rather than MT emissions from pools.

**Contributions:** I had the idea for and designed the study. *Michael Leuchner* and *Rainer Steinbrecher* provided methodological support. *Annette Menzel* helped in data analysis and writing of the manuscript. All co-authors reviewed the draft of the manuscript before submission. About 80% of the work was done by myself.

## 5 Discussion

### 5.1 Tree DEMON - a tool for stress assessment?

The Tree Drought Emission MONitor was successfully used for several experiments (Lüpke et al. 2016, Lüpke et al. 2017b, Lüpke et al. 2017a). Its core features, gas exchange measurements, were tested in depth and proved to work reliably and reproducibly (Lüpke et al. 2017b). During its use from 2013 until 2016 the system was steadily improved in respect to changed requirements or missing features. Major improvements were the exchange of the sampler system (magnetic to pneumatic valves), the installation of soil moisture sensors in 2014 and the exchange of the air supply filter tanks in 2015. While the first upgrades improved the overall sampler performance and system functionality, the second upgrade was necessary due to security aspects (TÜV - German Association for Technical Inspection approval).

#### 5.1.1 Key benefits

Compared to commercial single chamber systems and other self-built chambers (see list in Ortega and Helmig 2008), the Tree DEMON has four independent plant chambers allowing two group studies with one replication (Lüpke et al. 2016, Lüpke et al. 2017a) and screening studies with high throughput (Lüpke et al. 2016). This kind of studies required a good parallel sampling performance and was proved by the repeatability and comparability test of the sampler (with and without chamber) in Lüpke et al. (2017b).

The Tree DEMON measured only non-invasively plant physiological parameters such as gas exchange (CO<sub>2</sub>, water vapor and a wide range of VOC) and leaf temperature. Therefore, it was possible with a non-invasively biomass estimation method (see Serdar and Demirsoy 2006, Lüpke et al. 2017b) to reuse Sweet chestnut seedlings and perform multiple samplings during the vegetation season or even different kinds of experiments. Other methods, e.g., leaf water potential measurement, would involve damage to the plants and thus introduce a bias to any results.

VOC filtering and conditioning of supply air, also used in other systems, e.g., by Komenda (2001) or Joó et al. (2010), enabled periodical instead of permanent inlet air samples. This doubled the analytical capacity of air samples and allowed performing long-run (Lüpke et al. 2017a) or high-throughput screening experiments (Lüpke et al. 2016, Lüpke et al. 2017b). This approach also reduced overall costs and simplified handling compared to a supply by synthetic air gas bottles.

## Discussion

The selected chamber layout enabled the study of whole canopies of the tree seedlings. Thus, potential disturbance effects, e.g., mechanical disturbance or risk of infection, were reduced and a more representative biomass could be investigated compared to single leaf studies or small branch studies (e.g., Blanch et al. 2007, Ghirardo et al. 2010, Harley et al. 2014).

The software running the Tree DEMON offered a convenient user interface to control and set all important parameters and thus to reduce user errors and help monitoring the experiment. Furthermore, data from all sensors were aggregated into one data file, which speeded up post processing for data analysis.

### 5.1.2 Key limitations

For the experiments, an environment with constant temperature was selected, since conditioning of air supply as well as gas exchange were more stable under these conditions. Even so small variations of air and leaf temperature were observed in the plant chambers due to a small temperature inhomogeneity in the climate chamber, drought treatment effects and greenhouse effects due to radiation. These temperature differences were compensated for by the standardization algorithm (see e.g., Eq. 7, of Guenther et al. 1995). In order to perform fast changing temperature programs or a placement of the Tree DEMON in natural environments (e.g. outdoor field conditions) additional technical upgrades would be necessary (see 6). An individual regulation of the plant chamber temperature was tested with a prototype thermoelectric cooler (Soutchek, 2014), but in its first version it was only able to regulate temperature in a very small range and at a slow rate. The likely reason for this mediocre cooling performance was the large chamber volume, which made the system more inert to temperature changes, and high inlet flow rates, which would require a higher cooling capacity to reach a target temperature. In consequence this system inertness would also apply to other environmental parameters, thus for experiments requiring a fast reaction time smaller chambers or high inlet flow rates would be required (see e.g. system by Ghirardo et al. 2010, Harley et al. 2014).

The size of the plant chamber limited tree age to up to four years and the pot size to up to five liters. Therefore tree seedlings were selected according to their maximal canopy size still fitting into the plant chambers, which may reduce the number of selectable individuals. The limitation of the pot size might influence plant growth, root growth and water storage (Poorter et al. 2012), but this was not relevant for the emission studies in this thesis which were predominantly short-term and for which watering and nutrient supply were controlled.

The setting and controlling of the soil water content underlay several limitations. In Lüpke et al. (2016) SWC could only be measured before and after installation in plant chamber, thus data

included a temporal uncertainty. Later on, one SWC sensor per pot was installed and used in Lüpke et al. (2017b) and Lüpke et al. (2017a), thus a continuous measurement was possible. Since the sensor only represented a part of the pot, the total SWC might either be under- or overestimated, depending of the distribution of water in the pot.

### 5.2 Methodical adjustments

#### 5.2.1 Emission quantification

In the experiments (Lüpke et al. 2016, Lüpke et al. 2017a, Lüpke et al. 2017b,) different approaches to quantify emissions were used. In Lüpke et al. (2016) only compound specific  $m/z$  (e.g.,  $m/z$  93) from the MS was used, since the FID was unreliable due to wet zero gas air. Since the MS tended to be more sensitive and more variable, an internal standard was used to compensate the fluctuations. Later on, the installation of a gas dryer stopped this issue, thus for the studies Lüpke et al. (2017a) and Lüpke et al. (2017b) the FID was used for primary quantification, since this sensor type is known for its better linearity over a large measurement range and long-term stability (Otto 2006).

#### 5.2.2 Emission standardization

Due to the small variations in temperature and in the rare cases of a lamp failure resulting in varying light conditions emission rates were corrected by different standardization algorithms (see 3.4.2 for details, Guenther et al. 1995, Schuh et al. 1997, Niinemets et al. 2010b, Guenther et al. 2012, Harley et al. 2014). The optimal correction algorithm was selected based on the available information on the emissions' dependency on light or temperature and about *de novo* shares which could be derived by  $^{13}\text{C}$  labelling. All used standardization algorithms contained empirical parameters, which were derived from data of previous emission studies and varied within and between species (Niinemets et al. 2010b). Thus, an individual tree assessment could reduce uncertainty of the estimated standardized emission rates. This individual assessment was however not performed due to limited experimental capacity, temperature and light limitation of the climate chamber (up to  $24^{\circ}\text{C}$ /up to  $550 \mu\text{mol m}^{-2}\text{s}^{-1}$ ) and untested behavior of the Tree DEMON within changing temperature conditions.

### 5.3 Emission type, potential and chemo species

The  $^{13}\text{C}$  labelling method proved that Scots pine was a mixed-type emitter with emission both coming from storage and *de novo* synthesis as shown in Lüpke et al (2017b) and confirmed by several other studies (Shao et al. 2001, Tarvainen et al. 2005, Holzke et al. 2006, Ghirardo et al. 2010). In contrast, sweet chestnut was a pure *de novo* emitting species and highly light



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dependent as shown by pre-screening tests (data not shown) and by Lüpke et al. (2017b). Both species were identified as MT emitters, which corresponded well to emission inventories by Kesselmeier and Staudt (1999) or Simon et al. (2006). Both species emitted MTs at a similar magnitude ( $30^{\circ}\text{C}$   $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) in Lüpke et al. (2017b) and Lüpke et al. (2017a). Their emission composition was however different: Those of Scots pine were dominated by  $\alpha$ -pinene,  $\Delta^3$ -carene,  $\beta$ -pinene and myrcene and in case of sweet chestnut they were dominated by  $\alpha$ -pinene,  $\beta$ -pinene, and different isomers of ocimenes.

### 5.3.1 Scots pine

MT emissions of different provenances of Scots pine have not been investigated in detail so far. Lüpke et al. (2016) were the first to show for three provenances (see 3.5) differences in the MT emission composition with four distinct chemo species and varying emission rates among the provenances studied. A major distinction characteristic of the Scots pine chemo species types was the presences or absence of  $\Delta^3$ -carene, which was in case of  $\Delta^3$ -carene absences further split ratios of  $\alpha$ -/ $\beta$ -pinene and the presence of myrcene. While some provenances showed a consistent chemo species, such as the Brandenburg provenance, the other two varied much more. Equally, the Scots pine provenance used in Lüpke et al. (2017a) showed at least two distinct chemo species.

The emitted MT compounds identified in Lüpke et al. (2016) and Lüpke et al. (2017a) are well in accordance with other studies (e.g., Janson 1992, Shao et al. 2001, Komenda 2002, Holzke et al. 2006, Ghirardo et al. 2010, Bäck et al. 2012). It was however expected that Scots pine provenances would reveal different chemo species since also resin composition studies revealed multiple chemo species (Tobolski and Hanover 1971, Manninen et al. 2002). Furthermore also within local stands high variation of the chemical composition in emissions was observed by e.g., Holzke et al. (2006), Bäck et al. (2012) and in resin by Kannaste et al. (2013). Differences both between and within provenances were observed in the two Scots pine studies of this thesis. Reasons for different chemo species might be related to an evolutionary adaptation against local specific pests (see e.g., Iason et al. 2011, Achotegui-Castells et al. 2013). In addition the chemical composition may drive or be driven by the understory vegetation (Iason et al. 2005), thus the high spatial distribution of Scots pine and the corresponding high variety of understory vegetation may have led to different chemo species.

Looking at the overall total emission rates reported in Lüpke et al. (2016), a strong variation between provenances was measured with higher emission rates of the two southern Europe provenances from Spain and Italy during summer and autumn. This strong difference might be

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related to an occurrence of herbivores all year round, which are not present in regions with regularly cold winters. A split into pool and *de novo* emission would have helped to gain more insight, but this was not possible by the method of day night differences due to very short installation times and potential installation effects. Also  $f_{denovo}$  could not be determined in this study. In the second experiment on Scots pine (Lüpke et al. 2017)  $f_{denovo}$  was determined by multiple  $^{13}\text{C}$ -labelling. However, the revealed values were below the reported fractions of Ghirardo et al. (2010). The results also showed variable  $f_{denovo}$  between individual trees and compounds which was probably related to different leaf to wood ratio (availability of resin ducts) and natural variation between the trees.

### 5.3.2 Sweet chestnut

The screening study of sweet chestnut trees (Lüpke et al. 2017b) revealed three distinct chemo species. These were a trans- $\beta$ -dominated type, an intermediate type with terpinene and a  $\beta$ - and  $\alpha$ -pinene dominated type. Emission rates varied strongly within each chemo type, thus no pattern between specific chemo species was visible. The MTs identified partly matched the results of other studies (Pio et al. 1993, Aydin et al. 2014), but different chemo species had not been identified before.

### 5.3.3 Importance of the results for modelling

Large-scale BVOC emission models require the plants' emission potential (emission rate and capacity at standard conditions) and its type (temperature or light dependent) for the correct calculation (see e.g., Arneth et al. 2008, Guenther et al. 2012, Acosta Navarro et al. 2014). Current models are based on inventory data which list the emission potential and type at different taxonomic ranks or plant functional types ( see e.g., Kesselmeier and Staudt 1999, Steinbrecher et al. 2009, Guenther et al. 2012). The sweet chestnut and first Scots pine study showed however that at provenance level pronounced differences were present and thus should be considered. This would require large-scale screening studies constituting a time and cost intensive process.

Results of BVOC emission models are further incorporated into atmospheric chemistry models (Makkonen et al. 2012, Smolander et al. 2014). Smolander et al. (2014) included the effect of chemo species into an atmospheric chemistry model and showed that specific process/yield rates of major compounds ( $\Delta^3$ -carene/ $\alpha$ -pinene dominance) significantly affected specific intermediate and end products or processes such as OH reactivity. Thus models, which consider specific compounds reactions, could perform more accurately by including chemo species or provenances information.

## 5.4 Effect of drought stress on isoprenoid emissions, CO<sub>2</sub> and water vapor gas exchange

The effect of drought stress on isoprenoid emission was investigated on the two species, namely Scots pine in chapter 4.2 (Lüpke et al. 2016) and 4.3 (Lüpke et al. 2017a) as well as sweet chestnut in chapter 4.1 (Lüpke et al. 2017b). Drought led to an expected decrease of BVOC emissions of both species and hereby confirmed results of other drought – isoprenoid emission studies on other tree species, such as *Pinus halepensis* MILL. (Llusià and Peñuelas 1998), *Populus alba* L. (Brilli et al. 2007), *Quercus ilex* L. (Llusià et al. 2011), *Fagus sylvatic* L. (Šimpraga et al. 2011), *Quercus robur* L. (Bourtsoukidis et al. 2014), *Pinus sylvestris* L. (Wu et al. 2015).

### 5.4.1 Sweet chestnut

In case of sweet chestnut only one replicate of the drought study could be used (Lüpke et al. 2017b). This replication showed that *de novo* emitted MT followed in a lagged manner the decreasing photosynthesis rate during increasing drought. The lag might be explained by the use of stored carbohydrates for MT synthesis as observed by Brilli et al. (2007) for isoprene synthesis during drought. Continuing drought stress led to leaf die off at one out of two trees which resulted in an almost complete cessation of MT emission. Compared to Scots pine, photosynthesis, transpiration and emission response of sweet chestnut to decreasing water regimes was stronger and thus revealed relative anisohydric behavior, which complied with a study of Gomes-Laranjo et al. (2012).

### 5.4.2 Scots pine

In both Scots pine studies (Lüpke et al. 2017b, Lüpke et al. 2017a) drought strongly reduced photosynthesis, transpiration and increased leaf temperature by around 2 to 2.5°C compared to the control groups. This is in accordance with other conifer studies (e.g., Irvine et al. 1998, Blanch et al. 2007, Bansal et al. 2013). However, both Scots pine studies differed in the drought strength applied, the recovery time and sample interval.

In Lüpke et al. (2016) the drought was lasting 6 weeks with periodically little watering and plants were sampled only once within the drought phase and after a longer recovery phase. The watering management led to a slight variability of SWC at each sample, but otherwise it would not have been possible to investigate larger sample sizes and cover three provenances. In general, the two southern provenances of Spain and Italy responded stronger to drought by much more reduced gas exchange than the German provenance and both southern provenances showed also a stronger recovery. All three provenances reduced MT emission during drought,

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but the two southern were able to increase their emissions faster during recovery. This response pointed onto a higher plasticity/resilience of the southern provenances on drought as equally shown by a thermography study on the same provenances by Seidel et al. (2016). A closer look at the single compound response to drought revealed different dependencies on SWC, which might be related to the different share of *de novo* and pool emission of each compound (see Lüpke et al. 2017a, Ghirardo et al. 2010) and was investigated in more detail in Lüpke et al. (2017a).

In Lüpke et al. (2017a) fewer specimens were investigated over 16 days and were sampled on a daily basis with continuously changing water regime (normal-drought-normal). During the course of this experiment Scots pine showed a relative isohydric response by keeping transpiration high until SWC threshold was reached. Compared to older trees (Irvine et al. 1998, Poyatos et al. 2008) and the first study (Lüpke et al. 2016), this reaction was more abrupt, which might be related to sandy soil type. But it confirms the general isohydric behavior of Scots pine (Irvine et al. 1998, Leo et al. 2014, Salmon et al. 2015) and also shows that anisohydric and isohydric are not sharply separated but by a smooth transition (Klein et al. 2014). The  $^{13}\text{C}$ -labelling method also proposed by Ghirardo et al. (2010) and Harley et al. (2014) allowed a better separation of pool and *de novo* emissions and a mixed standardization algorithm could be applied.

The  $^{13}\text{C}$ -labelling showed that during drought stress the *de novo* emissions were more affected than pool emissions. Thus, a fixed  $f_{denovo}$  could not be used for the standardization algorithm since with changing stress conditions  $f_{denovo}$  is not static because the actual synthesis rate decreases. Thus in this study for the first time a correct scaled  $f_{denovo}$  was estimated with purely *de novo* emitted 1,8-cineole in a non-linear model for its use in the mixed standardization algorithm. During re-watering  $\Delta^3$ -carene and  $\beta$ -pinene revealed quite high night values. This increased emission from pools might be related to refilling of water in the xylem and an increased oleoresin pressure of resin ducts. This behavior was shown in emission increase from dry winter conditions (stem contains less water) to spring conditions (stem is refilled) by Vanhatalo et al. (2015). Additionally, a study of Rissanen et al. (2016) showed positive relation of xylem water content with oleoresin pressure, which was positively correlated with stem emissions.

### 5.4.3 Stress response strategies of Scots pine and Sweet chestnut

Both tree species showed a different stress response (isohydric/anisohydric) and BVOC emission types. Scots pine maintained MT emissions also during the short extreme drought states via storage pools, while in the case of sweet chestnut (at one tree observed) emission declined completely and even leaves fell, which returned after two months of normal watering, however.

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This anisohydric behavior might be related to a more risk taking strategy which was also observed during summer drought in 2003 in Switzerland (Barthold et al. 2004). This behavior might be an adaptation to its natural distribution area, which is defined by a warm temperate climate (annual mean of 8 – 15°C, at least 6 months with average 10°C) with moderate precipitation (600 – 800 mm) and warm and drier summers (Conedera et al. 2016).

In contrast, the evergreen conifer Scots pine covers a wider climatic region (Houston Durrant et al. 2016), which ranges from annual average temperatures from -3 to 15°C and precipitation from ~450mm up to 3000 mm. As a pioneer species, Scots pine can grow on disturbed sites with good light conditions. Its needles are sustained for at least two years and can last for up to six years (Praciak 2013) and their built-up is more resource intensive (Givnish 2002), because stronger cell walls or built of resin ducts with stored isoprenoids. Also it is known that pines built-up more constitutive resin ducts in their woody parts than other conifers (Mumm et al. 2003). Scots pine probably follows a stress endurance strategy and tries to keep as much needles as possible intact during stress phases by strongly controlling stomata opening and conductance (Martínez-Sancho et al. 2017). Thus stored isoprenoid enabled the plants to pass on *de novo* synthesis of compounds, since stored compounds are able to maintain functions such as acting as defense against various pests (Falldt et al. 2006, Heijari et al. 2011), wounding protection (Loreto et al. 2000), antimicrobial (Trombetta et al. 2005), antioxidant (Loreto et al. 2004) and sustain the photosynthesis system longer intact (Copolovici et al. 2005). Although synthesis is reduced during drought stress, a low amount of *de novo* compounds can still be sustained via pathways using stored carbon. Their share on the total emission was however relatively low as shown by Brillì et al. (2007) and indicated by the <sup>13</sup>C labelling results in Lüpke et al. (2017a). The alternative carbon sources might play an important role at mild stress phases to compensate for reduced availability of recently photosynthetically fixed carbon Brillì et al. (2007).

## 6 Outlook

The Tree DEMON was laid out as a modular system with standard components. Thus an easier adaptation to a new research question is possible. Several possible improvements of the Tree DEMON came up during this thesis to enlarge the range of applications. An individual control of the plant temperature, humidity and pot soil water content would enhance manipulation possibilities. Different plant chamber sizes and formats would enable to cover different tree sizes or plant parts, e.g., to separate emission from stem, roots and leaves. In case of outdoor field application the system should be downsized for better portability and heated tubing would be required in order to avoid water condensation. Dependence on the field site infrastructure requirements on the air supply could be lowered by e.g., exclusion of VOC filters by in- and outlet sampling. This additionally offers the possibility to investigate VOC uptake of plants – a less investigated field.

The performed experiments did not differentiate between plant parts due to a whole canopy concept. However, it is known for Scots pine that different plant parts contain varying amounts and types of MTs (Ghirardo et al. 2010) and cover different emission types (stem – storage, leaf – mixed or *de novo*). Thus, drought stress might affect these different ways: e.g., relation oleoresin pressure to xylem water content, different availability of carbon for synthesis in bark and leaves. Future studies should also investigate the point between mild and full drought and the recovery time in more detail, since the performed studies were temporal course or not long enough. This additional information would be required to understand the detailed drought and recovery process better.

Due to the technical issues of the sweet chestnut experiments and its preliminary descriptive results, a re-run of the experiment would be necessary to get statistically tested results and more representative data for further modelling.

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