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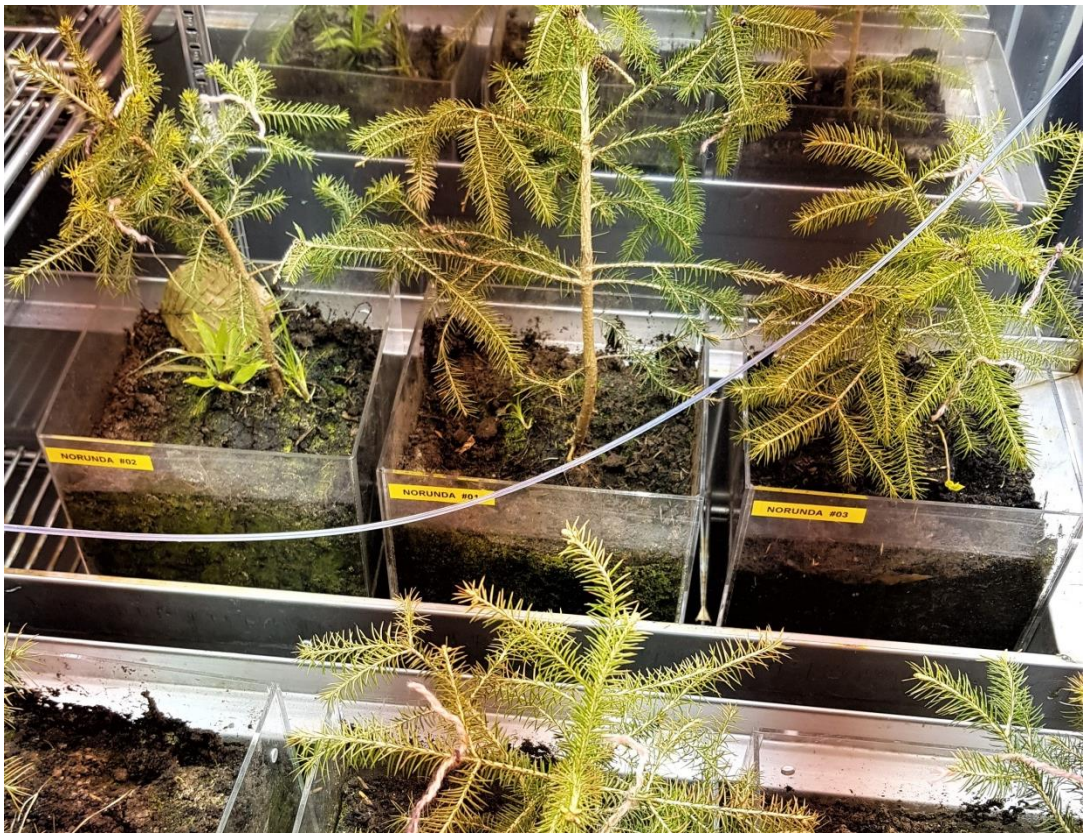
Early season BVOC emissions for young potted Norway spruce (*Picea abies)*



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Early season BVOC emissions for young potted Norway spruce (Picea abies)
Utsläpp av BVOC från ung planterad gran (Picea abies) under tidig säsong
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Master thesis, 30 credits, in Physical Geography and Ecosystem Analysis

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Abstract

Biogenic volatile organic compounds (BVOC) are emitted naturally from the biosphere to the atmosphere and are a part of the global carbon emissions. BVOCs are contributing to around 5-10% of the total net carbon exchange to the atmosphere. The BVOC emissions from the biosphere are mainly from plants and other organisms and consists of many different compounds, such as isoprene, monoterpenes (MT) and methanol. BVOCs have a high chemical reactivity and reactions with atmospheric compounds leads to oxidization of BVOCs, which contributes to formation of cloud condensation nuclei and secondary organic aerosols, affecting the solar radiation penetration and particle composition of the atmosphere, making the study of BVOCs important. The compounds produced are affected by different environmental factors such as light, temperature and stress. This thesis analyzed the relationship of BVOC emissions affected by light and mechanical stress on young potted Norway spruce (*Picea abies*) by using proton transfer reaction – time of flight mass spectrometry combined with a portable photosynthesis system with a conifer chamber.

Despite the choice of instrument, no sesquiterpene emissions could be detected. The results of the thesis reveal differences in emissions for the different spruces, where MT emissions for spruce 2 were twice as high than for spruce 6. However, isoprene and methanol emissions were not found for spruce 2 but emitted from spruce 6 indicating differences between the spruces. Differences between branches of the same spruce were found as well, where branch 1 on spruce 2 emitted MT in a range between 0.13 and 0.24 μg gdw^{-1} h^{-1}, emissions in line with published research, while the other branches had emissions closer to 0 μg gdw^{-1} h^{-1}. The light response measurements revealed the increase with light of emissions to be 10 to 40 times lower for all compounds compared to published research. The analyze of mechanical stress by cutting the needles revealed expected results with induced emissions for all compounds, and up to two orders of magnitude for MT compared to before mechanical damage. Isoprene revealed higher induced emissions directly after cut in light conditions compared to dark conditions. The decay of the emissions after cut showed no difference in light or dark conditions for the compounds.

The results of this thesis are compared to published literature analyzing mature spruce stands during an entire season, which makes the comparison uncertain, however, some of the spruces in this thesis did show emissions on the lower end of the published research.

Keywords: BVOC, PTR-TOFMS, photosynthesis, Norway spruce

Sammanfattning

Naturliga utsläpp av biogena flyktiga organiska ämnen (BVOC) förekommer från biosfären till atmosfären och bidrar till de globala kolutsläppen. BVOC bidrar till ungefär 5–10% av den totala netto kol utbytet till atmosfären. BVOC har en hög kemisk reaktivitet och reaktioner som sker med föreningar i atmosfären leder till oxidering av BVOC, vilket i sin tur bidrar till bildande av molnkondensationskärnor och sekundära organiska aerosoler. Dessa påverkar solstrålningens permeabilitet och partikelsammansättningen av atmosfären, vilket gör forskningen på BVOC viktig att bedriva. BVOC utsläppen från biosfären kommer huvudsakligen från växter och organismer och består av många olika kemiska föreningar, som exempelvis isopren, monoterpener (MT) och metanol. Föreningarna som produceras påverkas av olika abiotiska faktorer som solljus, temperatur och stress. Denna studie syftar till att analysera förhållandet mellan BVOC utsläpp och solljus samt mekanisk stress på ung planterad gran (*Picea abies*) genom att använda proton transfer reaction – time of flight mass spectrometry kombinerat med ett portabelt fotosyntes system med en barr kammare.

Trots valet av instrument för studien kunde inga utsläpp av seskviterpen uppmätas. Resultatet i denna studie visar på olikheter i utsläpp för olika granar, där MT utsläpp för gran 2 var dubbelt så stora som för gran 6. Dock uppmättes inga utsläpp av isopren eller metanol för gran 2, medan det uppmättes för gran 6, vilket visar på olikheter mellan granarna. Olika utsläppshalter kunde även bekräftas mellan olika grenar på samma gran, där gren 1 på gran 2 uppmätte utsläpp av MT i ett intervall mellan 0.13 och 0.24 $\mu g\ gdw^{-1}\ h^{-1}$, vilket är i linje med resultat från annan publicerad forskning. Andra grenar på gran 2 uppmätte utsläpp närmre 0 $\mu g\ gdw^{-1}\ h^{-1}$. VOC utsläppens reaktion på solljus visade endast ökning som var 10 till 40 gånger lägre än annan publicerad forskning. Analysen av mekanisk stress genom att skära i barren nådde förväntat resultat med ökade utsläpp för alla föreningar, där MT utsläppen ökade med upp till två storleksordningar jämfört med uppmätta utsläpp innan barren blev skadade. Isopren uppmätte en större ökning av utsläpp direkt efter skada under ljusa förhållanden jämfört med mörka. Minskningen av utsläppen efter skada visade inte på några skillnader i mörker eller ljus för de analyserade föreningarna.

Andra publicerade forskningsartiklar rörande samma ämne fokuserar främst på vuxna granskogar och har ofta pågått under en hel växtsäsong, vilket gör jämförelser med denna studie aningen osäkra. Några av granarna i denna studie visar dock på liknande mängd utsläpp som den lägre randen av uppmätta utsläpp i de jämförda forskningsartiklarna.

Nyckelord: BVOC, PTR-TOFMS, fotosyntes, Rödgran

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List of Abbreviations

BVOC: biogenic volatile organic compound

VOC: volatile organic compound

MT: monoterpenes

SQT: sesquiterpenes

OH: hydroxyl radical

CCN: cloud condensation nuclei

SOA: secondary organic aerosol

IRGA: infrared gas analyzer

RGB: red green blue

PTR-TOFMS: proton transfer reaction – time of flight mass spectrometry

PAR: photosynthetically active radiation

SLA: specific leaf area

1. Introduction

The biosphere is naturally emitting biogenic volatile organic compounds (BVOCs) to the atmosphere, contributing to the global emissions of carbon. The atmosphere consists of several volatile organic compounds (VOCs), where emissions of BVOCs constitutes of about 85% of all the atmospheric VOCs (Guenther et al. 1995; Hallquist et al. 2009; Sindelarova et al. 2014). BVOCs are generally considered hydrocarbon trace gases emitted to the atmosphere, excluding methane (Kesselmeier and Staudt 1999; Ghimire et al. 2016). The majority of BVOCs are emitted from the biosphere from both plants and other organisms, contributing to around 5-10% of the total net carbon exchange to the atmosphere (Niinemets and Monson 2013). The emission of BVOCs consists of several classes of compounds, such as isoprenoids, alkanes, alcohols, acids etc. (Kesselmeier and Staudt 1999). The isoprenoids consisting of isoprene (C_5H_8), monoterpenes ($C_{10}H_{16}$) and sesquiterpenes ($C_{15}H_{24}$) are the most abundant compounds (Wang et al. 2017a). Plants can emit BVOCs from all plant organs, where green leaves, such as conifer needles, contributes to most of the emissions. The greatest variety of compounds are emitted from flowers and fruits whereas the emissions from woody parts of the plant consist mostly of isoprenoids, especially from woody plants such as conifers (Laothawornkitkul et al. 2009; Niinemets and Monson 2013). The most important compounds emitted from coniferous trees are monoterpenes (MT) and sesquiterpenes (SQT) (Kesselmeier & Staudt, 1999; Bäck et al., 2012; Persson et al., 2016).

By emitting BVOCs the plants release some of its fixed carbon, which in term yields high metabolic costs (Niinemets and Monson 2013). However, some of the emissions are thought to protect the plant from biotic (herbivore and insect attacks) and abiotic (extreme light and temperature, and pollutants such as ozone) stresses and can also serve as communication within and between plants, and to interact with the environment. Consequently, the gains from emitting BVOCs seems to overcome the loss of carbon for the plant (Dudareva et al. 2006; Niinemets and Monson 2013). BVOCs does not only affect the communication and protection of plants, they also impact the atmospheric chemistry. The highly reactive BVOCs are, when emitted, oxidized in photochemical reactions by the atmospheric compounds ozone (O_3), hydroxyl radical (OH) and by the nitrate radical (NO_3) (Niinemets and Monson 2013; Scott et al. 2018). These reactions influence the atmospheric composition by affecting the concentration of O_3 and methane (CH_4), two greenhouse gases. Altering the concentration of these might affect global warming (Scott et al. 2018). BVOCs also affect the atmosphere in other ways, one of them being reactions with nitrogen oxides (NOx), which typically reacts with isoprene, affecting tropospheric O_3. In atmospheres with low NOx concentration the vegetation typically

consumes O_3, but with high NOx concentrations O_3 is being produced (Laothawornkitkul et al. 2009). Another way BVOCs affect the atmosphere is by oxidation of BVOCs, typically from the bigger molecules like MT, contributing to the formation of cloud condensation nuclei (CCN) and secondary organic aerosols (SOA), affecting solar radiation penetration and the particle composition of the atmosphere where SOA causes the natural blue haze, but also some health problems (Sharkey et al. 2007; Niinemets and Monson 2013; Scott et al. 2018).

Leaf emissions of BVOCs can be affected by several factors, such as light intensity, temperature, leaf structure and stomatal conductance (Laothawornkitkul et al. 2009). Stomatal conductance however does not affect the isoprenoid emissions since they are less soluble in water. Isoprenoid emissions are more affected by light intensity and temperature. Because of the light and temperature dependency of isoprenoid emissions, they tend to have diurnal patterns, where the emissions increase during the morning, peaks midday and decrease during the evening and night (Niinemets and Monson 2013). Some plant species have the availability to store BVOCs in storage compartments of the leaf, which affects the light and temperature dependency, as emissions of compounds not stored in storage pools tend to be more light dependent and the emissions of compounds stored can be considered light-independent (Laothawornkitkul et al. 2009). Seasonal trends of BVOC emissions have also been observed in some forests, boreal forests being one of them (Hakola et al. 2003; Hakola et al. 2006; Niinemets and Monson 2013; Bourtsoukidis et al. 2014a). The seasonality of the emissions depends as well on light and temperature, where emissions typically are highest during the growing season (summer) and lower during winter (Hakola et al. 2003).

Conifer trees mostly emit MT, Scots pine (*Pinus sylvestris*) for example is not considered an isoprene emitter, and Norway spruce (*Picea abies*) is considered a low isoprene emitter (Hakola et al. 2003). Therefore, BVOC emissions from boreal forests mainly consists of terpene emissions such as MT and SQT and the boreal forest emissions alone contributes to 10% of the global MT emissions (Sindelarova et al. 2014).

1.1 Research objectives and questions

In this study BVOC emissions for Norway spruce during the early season (spring) were analyzed using real-time trace gas analysis with proton transfer reaction – time of flight mass spectrometry (PTR-TOFMS, IONICON Analytik Gesellschaft, Innsbruck, Austria). In addition to this, photosynthesis will also be analyzed.

Current literature on BVOCs are mainly focusing on the growing season of vegetation, and even though some studies have been made on seasonality, not many have focused on Norway spruce in early season. In general, the entire research area of

BVOCs is not as studied compared to other areas, which makes this study a good contribution to science with the focus on BVOC emissions from Norway spruce during spring conditions. SQTs are generally interesting to study because of their high reactivity once emitted, however the emissions are hard to capture because of their volatility (Hakola et al. 2006). A controlled environment using real time detection measurements might facilitate the study of SQTs, as well as all isoprenoid emissions in general. Using real-time trace gas detection makes it possible to investigate other studies on how short-term abiotic changes affect BVOCs.

The aim of this thesis is therefore to further investigate BVOC emissions coupled with photosynthetic activity for Norway spruce during early season. The following research questions will be addressed in this study, (1) how might emissions differ within one spruce when looking at different branches? (2) How do the emissions respond to light, based on response curves and stress exposure? (3) How do BVOC emissions as well as carbon assimilation respond to stress in terms of needle damage?

2. Background

2.1 Light and temperature dependency

Carbon assimilation in plants, photosynthesis, is light dependent and the light dependency can be described by light response curves which reveals how much the carbon assimilation relates to the amount of irradiance (Monson et al. 1992; Bonan 2008). The light response curve is individual for different plant species (Jones 2014). A study by Urban et al. (2007) investigated how cloudy or clear skies affected the carbon assimilation, amongst other factors, of a Norway spruce forest and found a clear relationship with irradiance and carbon assimilation. Photosynthesis also has a strong response to temperature, with effects that can be reversible up to a certain temperature threshold, but beyond the threshold the damages caused by high temperatures are irreversible (Berry and Björkman 1980; Monson et al. 1992). The photosynthetic processes of Norway spruce have been observed to be optimal at a temperature around 25°C (Šigut et al. 2015). Thermotolerance of photosynthesis has been revealed to be improved with occurring emissions of isoprene (Sharkey et al. 2001)

Isoprene is closely related to light as isoprene is dependent on products from photosynthesis such as the terpene precursor and phosphorylation energy (ATP) (Kesselmeier and Staudt 1999). Since no isoprene storage pool exists, isoprene is emitted *de novo* and will be emitted in a close relationship with light intensity, and no emissions will occur without light being present. However, all BVOC emissions have been shown to, in some part, be dependent on temperature as well, and since isoprene emissions are dependent on photosynthetic activity, the emissions are also affected by

temperature since it influences the synthase activity (Kesselmeier and Staudt 1999; Laothawornkitkul et al. 2009; Bourtsoukidis et al. 2014a).

Unlike isoprene, MT and SQT can be stored in different needle storage compartments like resin ducts or glands after synthesis and could thus be considered as light-independent (Kesselmeier and Staudt 1999). By having these storage compounds, the plant can store the BVOCs and emit them later when needed, for example when exposed to stresses such as mechanical wounding or herbivory (Räisänen et al. 2008; Niinemets and Monson 2013). However, studies have shown that MT can, as isoprene, be emitted *de novo* in some cases, and can thus not be entirely considered as light-independent (Kesselmeier and Staudt 1999; Bäck et al. 2012; Niinemets and Monson 2013; Wang et al. 2017a). A study by van Meeningen et al. (2017) investigated the light response of isoprenoids on Norway spruce amongst other species and the results revealed that isoprene emissions are strongly linked to available light, as well as some MT emissions.

Bourtsoukidis et al. (2014a) studied the BVOC emissions over a Norway spruce forest and observed a high temperature dependency for emissions of acetone and MT. Studies have also shown that SQT emissions are temperature dependent (Kesselmeier and Staudt 1999). Temperature dependency for MT emissions have also been observed over Scots pine forests (Räisänen et al. 2008; Rinne et al. 2007). The temperature dependent nature of the terpenoid emissions could be explained by the occurrence of storage pools, as temperature will affect vapor pressure and stomatal resistance from the storage pools, giving emissions an exponential increase with temperature (Kesselmeier and Staudt 1999).

The impact of heat stress on BVOC emissions was investigated in a study by Kleist et al. (2012) where they concluded that BVOC emissions did respond to temperature increase, but extremely high temperatures can cause irreversible effects on the emissions. The study confirmed increased MT emissions from Scots pine due to heat-induced damage.

2.2 Mechanical stress

Research analyzing mechanical stress on vegetation, such as wounding, reveals induced emissions of BVOC (Loreto et al. 2000; Loreto et al. 2006; Brilli et al. 2011; Rinnan et al. 2013). Loreto et al. (2000) investigated how mechanical stress affected Mediterranean pine (*Pinus pinea*) in the Mediterranean area. The study revealed induced MT emissions when needles were exposed to mechanical damage by cutting the needles, the emissions were still induced after 24 hours. The induced emissions differed depending on if the needles were cut in light or dark conditions, where the light conditions revealed larger emissions of MT. Other studies analyzing the wounding of leaves confirm the same results with a burst of BVOC emissions, such as

methanol and isoprene, induced by mechanical damage to the leaves (Loreto et al. 2006; Brilli et al. 2011). According to Loreto et al. (2006) and Brilli et al. (2011), the burst of methanol could be due to an aqueous pool being depleted and evaporating. Rinnan et al. (2013) were analyzing the BVOC emissions from heath ecosystems during the off season and could also confirm that cutting the vegetation induced the emissions.

Photosynthesis has been shown to be suddenly induced with mechanical stress in terms of cutting a leaf but later decreasing to levels lower than initial photosynthetic rate (Loreto et al. 2006; Brilli et al. 2011).

2.3 Measuring BVOC and photosynthesis

To generally measure photosynthesis, a portable photosynthesis system (LI-6400, LICOR, Lincoln, NE, USA) has been used in several studies (Persson et al. 2016; van Meeningen et al. 2017). By using a dual infrared gas analyzer (IRGA) placed in the sensor head, the system analyzes both CO_2 and H_2O, measuring differences in gas concentrations between a reference chamber and a sample chamber directly connected to the enclosed leaf chamber head (LI-COR Biosciences, Inc. 2011). This enables measurements of changes in leaf dynamics in real time (LI-COR Biosciences, Inc. 2011). By measuring CO_2 and H_2O at reference and sample cells simultaneously, the LI6400XT can minimize noise and gives fast response times. The internal calculations for e.g. photosynthesis of the LI6400XT are based on the equations presented by von Caemmerer and Farquhar (1981). The LI6400XT makes it possible to control the environment in the leaf chamber, by adjusting CO_2, H_2O, temperature and light. By using different components on the sensor head of the IRGA, the measurements can be designed to fit the experiments, such as using different leaf chambers and light sources.

BVOC emissions can be measured and quantified by using proton transfer reaction – time of flight mass spectrometry (PTR-TOFMS, IONICON Analytik Gesellschaft, Innsbruck, Austria), which was developed by the University of Innsbruck and is described in detail in Hansel et al. (1995) and Graus et al. (2010). The PTR-TOFMS uses an ion source producing H_3O+ ions from water vapor via a hollow cathode discharge. The ions are led through the PTR-drift tube, where sample gas with VOCs is continuously injected via an inlet system and VOCs undergo a proton transfer reaction with the H_3O+ ions (Ionicon Analytik Gesellschaft m.b.H 2012). The reactions in the drift tube are performed under controlled pressure, temperature and applied voltage (Brilli et al. 2011; Ionicon Analytik Gesellschaft m.b.H 2012). The analyzing system of the PTR-TOFMS contains a transfer lens system, which pulse the ions to the orthogonal time-of-flight region, where the ions are separated according to their mass to charge (m/z) ratio. By using a soft method of

proton transfer reaction to detect VOCs, the fragmentation of these can be kept low and gives an efficient ionization with most VOCs (Ionicon Analytik Gesellschaft m.b.H 2012). The high purity of the H_3O+ ion and the fact that the ions does not react with any major components of pure air, makes it easier to calculate absolute concentrations, as well as to find trace gases in air samples. Since the samples are injected directly in the reaction chamber, the drift tube, the use of a PTR-TOFMS does not call for any preparations of samples, which can be a big advantage in field measurements (Ionicon Analytik Gesellschaft m.b.H 2012). The acquisition of the raw data can be done by the software TofDaq (Tofwerk AG, Switzerland).

Using a ventilated (dynamic) enclosure technique instead of a static enclosure gives more realistic emission rates, since the environmental parameters can be kept fairly constant to ambient values, making the leaf chamber experiment resemble closer to natural conditions (Ortega and Helmig 2008). Combining the LI6400XT with the PTR-TOFMS gives a dynamic enclosed leaf chamber, with the possibility to control the environment within the chamber, making it a good fit for this study and the experiments within.

2.4 Species description

Norway spruce (*P. abies*) is a common conifer tree in the northern hemisphere of Europe and Asia (Anderberg, 2018). It is occurring naturally in all of Sweden except the most southern parts, where it appears as a part of planted forests, and the arctic-alpine areas. Norway spruce can grow to become a tall tree, with one of the tallest in Sweden being measured to 51 meters with a width of 5.3 meters, however because of intense deforestation many Norway spruces does not grow that tall. If grown freely, a Norway spruce can get up to 400 years old (Anderberg, 2018). The isoprenoid emissions for Norway spruce are varying seasonally, with the emissions mainly being MT in May, isoprene in June and SQT in July (Hakola et al. 2003, 2006).

Some pests may occur on Norway spruce, with the Eastern spruce gall adelgid (*Adelges abeitis*) being amongst the common ones (Stimmel, 1980). It is identified by small, short pineapple shaped growths at the base of a branch. The damage caused by the galls is making the branches more prone to breakage and lowers the aesthetic quality of the trees (Stimmel, 1980).

2.5 Genotypic variation

In order to accurately model BVOC emissions to investigate their impact on the environment it is important to consider genotypic variation within species as this could result in high uncertainties in model estimations (Persson et al., 2016). Genotypic variation can be proved to influence the BVOC emissions of a species, where differences in genotype within a species have been revealed to affect the

emission patterns (Bäck et al., 2012). However, the species still emit the same compounds of BVOCs, but the chemotypes might differ. It has been stated that the chemotypes hardly differs between branches on the same individual tree throughout the different seasons, which makes it possible to conclude that environmental factors do not affect the chemotypes of individuals (Bäck et al., 2012), making it interesting to further study the genotypic variation within species. Persson et al. (2016) conducted a study on Norway spruce amongst other species and used genetically identical individuals from two different provenances. Amongst the results of the study it was found that there was no evident intra-genotypic difference between the species studied, but the spruces from different provenances showed different emission patterns. However, the study was conducted on four individuals, which might not be enough to confirm the results (Persson et al. 2016).

3. Method

To answer the given research questions, a setup using the portable photosynthesis system (LI-6400/LI-6400XT, LICOR, Lincoln, NE, USA) with the opaque conifer chamber with an RGB (red, green, blue) light source (6400-22L) combined with the proton transfer reaction – time of flight mass spectrometry (PTR-TOFMS, IONICON Analytik Gesellschaft, Innsbruck, Austria) were used. This enables the control of temperature and photosynthetically active radiation (PAR) to investigate light response curves, as well as real time detection of BVOCs, which makes it possible to investigate potential emission bursts due to mechanical needle stress. The final set up, illustrated in Figure 1a and b, consisted of a 0.67 meter Teflon tube attached between the conifer chamber and the PTR-TOFMS inlet, giving the total tube length of about 2.10 meters, including the tube from the PTR-TOFMS. A catalytic converter serving as a zero-air generator with an ozone scrubber was also used attached to the LI-6400XT, to get clean air in the system to yield a clearer BVOC signal. The flow from the LI-6400XT was set to 300 μmol s^{-1} (~0.4 l/min) and the flow from the PTR-TOFMS was set to 200 SCCM (Standard Cubic Centimeters per Minute) (0.2 l/min).

3.1 Initiating measurements

Seven seedlings of Norway spruces, about 5 years old, were analyzed in this study. They were collected from the Norunda research site year 2016 and were put in square pots with the dimensions of 19.5 cm x 19.5 cm, using soil from Norunda and later filled with different soil. After being potted the spruces were brought to a garden outside Lund and had since then been placed under a beech tree in order to get protection from rainfall as well as sunlight. The spruces were brought in from winter conditions and placed in a climate chamber provided by the department of Physical Geography and Ecosystem Science at Lund University. In addition to the spruces, two PAR sensors (LI-190R, LICOR, Lincoln, NE, USA) connected to a data logger (CR1000, Campbell Scientific inc., Logan, UT, USA) were installed to monitor the performance of the climate chamber. The potted spruces were put in the chamber on the 20-02-2018 with the temperature being set to a range of 5-11 °C , the humidity to 60% and the light to a range of 0-3 (where, 0 = 0 μmol m^{-2} s^{-1}, 1 = 250 μmol m^{-2} s^{-1}, 2 = 400 μmol m^{-2} s^{-1} and 3 = 600 μmol m^{-2} s^{-1}) to simulate day and night conditions in order for the spruces to adjust to warmer temperatures simulating early spring season. To simulate spring season, after about 20 days, the temperature was adjusted to a range of 10-17 °C, the humidity still at 60% and the light still at a range of 0-3, but different day and night cycles in order to match measuring schedule. The spruces were at first watered once a week with approximately 300 ml of tap water. To give the spruces time to adjust, 23 days passed before starting the first measurements.

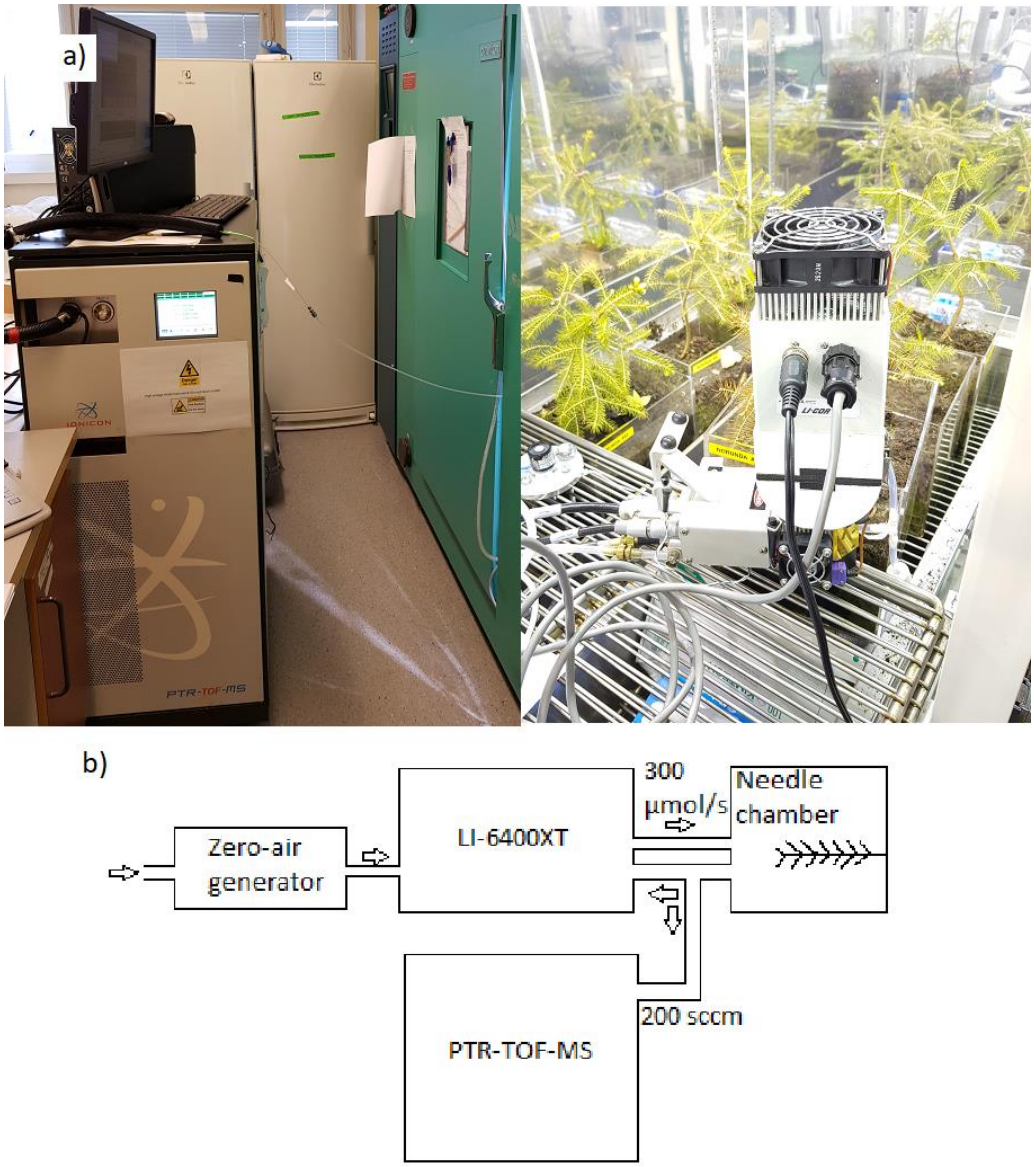

Figure 1. a) Experiment set-up with the PTR-TOFMS linked to the needle chamber and the LI-6400XT inside a climate chamber, **b)** flow scheme of the set-up using a catalytic converter as a zero-air generator to get clean air in to LI-6400XT. The reference air from the LI-6400XT was set to constant temperature (30°C) and CO_2 (400 ppm) with a flow rate of 300 µmol/s (~0.4 l/min). The needles in the chamber were irradiated with different levels of photosynthetically active radiation (PAR) depending on the ongoing experiment. Sample air from the needle chamber was then extracted from the needle chamber to the PTR-TOFMS with a flow of 200 sccm (0.2 l/min) to analyze BVOCs, with the rest of the air going back into the LI-6400XT for analyzing carbon assimilation.

The first measurements were started on the 13-03-2018 using the portable photosynthesis system to measure the photosynthetic activity of the spruces. This was done inside the climate chamber, to avoid stressing the spruces. Three branches were measured on each spruce, selected by which fit into the conifer chamber. The branches were measured for approximately 20 minutes in order to achieve stable values, before switching out the branches. Before each new measurement the IRGAs were matched to get more accurate values. The logger on the LI-6400 was set to log values every 10 seconds. To know which branches that had been measured a string

was tied around the branch to mark the area that was inside the chamber and in order to distinguish between branches on one spruce, the strings were color coded. To account for the biomass being measured to more accurately estimate photosynthesis, an initial guess of the leaf area was done without harvesting the needles, however, the correct biomass was accounted for when harvesting the needles at the end of the measurements. The spruces were numbered 1-7 and the branches on each spruce were numbered 1-3, henceforth the measured spruces will therefore be referred to example S1B1 (spruce 1 branch 1) etc. The temperature and light settings throughout the measurements were set to 30 °C and 1000 μmol m^{-2} s^{-1} (when not analyzing light response) to apply standardized conditions, and not to overly stress the plant with higher temperatures.

3.2 Measuring BVOC emissions

The BVOC measurements were conducted on initially seven selected spruces, three branches on each spruce. One spruce was excluded due to low photosynthesis values as well as no visible BVOC emissions, and further on during the experiments one spruce was selected to be used in whole-plant chamber measurements, leaving five spruces left to for the study. In order to measure BVOC emissions from the spruces the portable photosynthesis system was used together with the PTR-TOFMS, as described above.

Blank measurements were taken prior to the first measurements every day in order to later distinguish between background emissions and the emissions from the needles. They were conducted with the PTR-TOFMS connected to the empty conifer chamber and measured for approximately 10-20 minutes. In order to keep track of the measurements and to compare the collected data from the LI-6400XT and the PTR-TOFMS, the log number of the LI-6400XT was noted as well as the time of the computer connected to the PTR-TOFMS. For all measurements except cutting the needles, approximately 30 minutes passed after inserting a new branch in the conifer chamber, before starting the first measurements in order to avoid induced emissions caused by rough handling.

The PTR-TOFMS found over 500 peaks in the compound mass spectrum, however, the analysis was restricted to the peaks of the compounds displayed in Table 1, with the assumption that a signal for these compounds was expected to be found.

Table 1. Description of investigated BVOC compounds with their chemical formula, their assumed protonated mass and molar mass used in this study, where MT is assumed two protonated masses due to fragmentation (Park et al. 2013).

Compound	Chemical formula	Exact protonated mass (amu)	Molar mass (g/mol)
Methanol	CH_5O	33.032	32.04
Isoprene	C_5H_8	69.071	68.12
Monoterpenes	$C_{10}H_{16}$	81.079 + 137.131	136.23
Sesquiterpenes	$C_{15}H_{24}$	205.195	205.36

3.2.1 Respiration and light response curve

Measurements of respiration and light response curves were done in combination. The climate chamber was programmed to have nighttime during local morning and lunch time, for the spruces to be adjusted to dark conditions, to get more accurate respiration and light response curve measurements. Respiration was measured for approximately 10 minutes before initiation of the light response curve. The light response curve was then measured starting with low light levels increasing slightly with measurements at light levels of 0, 5, 10, 15, 20, 25, 30, 40, 50, 100, 200, 300, 500, 700, 1000, 1500 μmol m^{-2} s^{-1}. Each light level was measured for approximately 5 minutes, after which photosynthesis was assumed stable. The temperature setting was held constant with the LI-6400XT set to 30°C.

3.2.2 Cutting needles

To induce mechanical stress simulating for example herbivory, the needles on a branch were cut in half and the BVOC emissions were measured. Around 10% of the needles on a branch were cut to get a better comparison of the emissions between the branches. The needles that were cut were stored for biomass sampling later. After being cut, the branch was quickly inserted into the conifer chamber and measurements were started. The branch was measured for around 30 minutes, until the peak of BVOCs went down, with light levels set to 0 μmol m^{-2} s^{-1} in dark conditions and 1000 μmol m^{-2} s^{-1} in light conditions with temperature set to 30°C.

This setup was used both during dark conditions and light conditions, to compare the light levels impact on the mechanically induced emissions. Seven branches were measured in dark conditions, with six light response curves, and seven branches were measured in light conditions. During dark measurements the climate chamber was programmed to keep dark conditions, as when doing the respiration and light curve measurements. After the dark cutting was done, a light response curve was started using the same method as previous, in order to compare the light response with and without mechanical stress. Cutting measurements in light conditions were done the same way, but with the climate chamber being programmed to have light

conditions. If the chamber did not have light conditions, the branch was inserted into the conifer chamber with lights on for about one hour to adjust to light conditions before continuing, to get more accurate light measurements.

3.3 Harvesting needles

In order to estimate the biomass from the needles, they were harvested and collected in aluminum containers. The containers were weighted three times before adding the needles to get the average tare weight, and three times after adding the needles to get the average weight of the needles. To get the dry weight used as the biomass, the needles were dried in an oven at 75°C until the weight was stable, after about 24h. The needles were then quickly weighted the same way as previously. The cut needles were dried and weighted similarly and the total biomass of one branch was calculated by adding the dry weight of the harvested needles and the dry weight of the cut needles of that branch.

3.4 Data analysis

The measured BVOC concentration data from the PTR-TOFMS were processed using the widget tool PTRwid to detect the different peaks and calibrate the mass following the method from Holzinger (2015). The photosynthesis data were extracted from the LI-6400XT, and both BVOC data and photosynthesis were analyzed using MS Excel. All measurements were conducted at standardized temperature and pressure (30°C and 1000 kPa).

The compounds selected for analysis are presented in Table 1 and identified using the assumed protonated mass stated in the table. All compounds but SQT were identified, hence it could not be further analyzed.

3.4.1 Calculating the emission rate of BVOC

The output from the PTRwid tool gives the BVOC concentration in volume mixing ratio, $C_{vmr,}$ (µmol mol^{-1}). With standard temperature (30°C) and pressure (1000 kPa), the assumption that one mol of air, mol_{air} (l mol^{-1}), occupies 22.4 liters was applied to convert the volume mixing ratio to concentration of each compound, C (µg C l^{-1}), with the following formula (Eq. 1):

$$C = \frac{C_{mm} \cdot C_{vmr}}{mol_{air}} \qquad \text{(Eq. 1)}$$

where C_{mm} is the molar mass (g mol^{-1}) of the specific compounds given by Table 1.

With the output from Eq 1, the emission rate ER (µg C g_{dw}^{-1} h^{-1}) from the measured BVOC compounds was calculated using the dynamic enclosure emission rate equation (Eq. 2) following Ortega et al. (2008):

$$ER = \frac{[C_{out} - C_{in}]Q}{m_{dry}} \quad \text{(Eq. 2)}$$

where C_{out} is the concentration of each compound within the chamber, and C_{in} is the concentration of the compounds of the inlet air (µg C l^{-1}), the background value, Q is the flow rate through the chamber (l h^{-1}) and m_{dry} is the dry weight of biomass (g) of the enclosed needles.

Emission rates are defined as positive fluxes calculated from Eq. 2, assuming BVOC emissions from the plant to the atmosphere.

3.4.2 Calculating carbon assimilation

The output from the LI-6400XT gives the reference, C_r and sample, C_s IRGA mole fraction of CO_2 (µmol CO_2 mol^{-1} air), as well as the reference, W_r and sample, W_s IRGA mole fraction of water vapor (mmol H_2O mol air^{-1}) (LICOR Biosciences, 2011). The equation for net carbon assimilation, A_n (µmol CO_2 m^{-2} s^{-1}) derived by von Caemmerer and Farquhar (1981) was used (Eq. 3):

$$A_n = \frac{F\left(C_r - C_s\left(\frac{1000 - W_r}{1000 - W_s}\right)\right)}{100S} \quad \text{(Eq. 3)}$$

where S is the leaf area (m^2) and F is the flow rate (µmol s^{-1}).

To scale the dry needle biomass (g) to a leaf area, S (cm^2), a specific leaf area (SLA) of 38.4 cm^2/g at a canopy height of 20m derived by Wang et al. (2017b) based on a research campaign on adult Norway spruce in the area of Norunda research station was applied in this study, with the assumption that this SLA can be applied to the young potted spruces used in this study. By multiplying the SLA with the dry needle biomass, the leaf area, S was derived and applied in Eq. 3.

Net carbon assimilation is defined as positive output calculated from Eq. 3, as carbon being stored in the plant, the negative output from Eq. 3 is thus defined as respiration, carbon emitted from the plant.

3.4.3 Averaging and outliers

The emission rates of BVOCs were plotted, using an averaged background value C_{in} using the last 100-120 readings from the blank measurements for before inserting branches S2B1, S2B3, S7B1 and S6B1. The averaged background value for each compound, isoprene, MT and methanol was applied to Eq. 2.

The compounds starting with a negative emission value were adjusted to fit the zero line, by adding a number to all data making the first value of the time series zero to get a better detection of the BVOC emissions during the time series. The

number was individually selected for each dataset and compound based on how far off zero the first value was.

Light response curves were made by averaging the data for each light level (see section 3.2.1) where the values were considered stable, around mid-measurement. The first minute was excluded and assuming that the needles had adjusted to the new light level after one minute, the following three minutes were included in the averaging, skipping the last measured minute to avoid including values from opening the chamber to switch branches.

Creating a table for pre and post cutting the needles were based on the time series after the cut. The emissions were noted directly after cut, after 3 minutes, 5 minutes, 10 minutes and after 30 minutes to catch the decay of emissions for the different compounds. The decay functions were selected based on the best fit of a trendline applied to the data, either an exponential function, $y=c\ exp^{(bx)}$ or a logarithmic function, $y=b\ ln(x)+c$ was used for the decay rate of emissions.

S6B1 had three outliers in the carbon assimilation data, which were removed at light levels: 25, 700 and 1000 $\mu mol\ m^{-2}\ s^{-1}$.

4. Results

4.1 Emission differences between branches of the same spruce

The different patterns of BVOC emission and carbon assimilation can be seen in Figure 2 and 3, which illustrates the difference in emission and assimilation between branches of the same spruce, analyzed as a light response curve. The photosynthetic activity (carbon assimilation) for spruce 2 was increasing with higher light level for all branches (Figure 2). The compensation point for branch 1 was around 30 μmol m^{-2} s^{-1} and 500 μmol m^{-2} s^{-1} for branch 3, while branch 1 had a positive net carbon assimilation from 0 μmol m^{-2} s^{-1} and revealed no compensation point in the measurements. At the highest light level, 1500 μmol m^{-2} s^{-1} there was a range in net assimilation from 10-30 μmol CO_2 m^{-2} s^{-1}. Spruce 6 revealed a more irregular pattern for carbon assimilation (Figure 3), with the highest net assimilation reaching around 5 μmol CO_2 m^{-2} s^{-1} for branches 1 and 3, and around 35 μmol CO_2 m^{-2} s^{-1} for branch 2 at light level 1500 μmol m^{-2} s^{-1}.

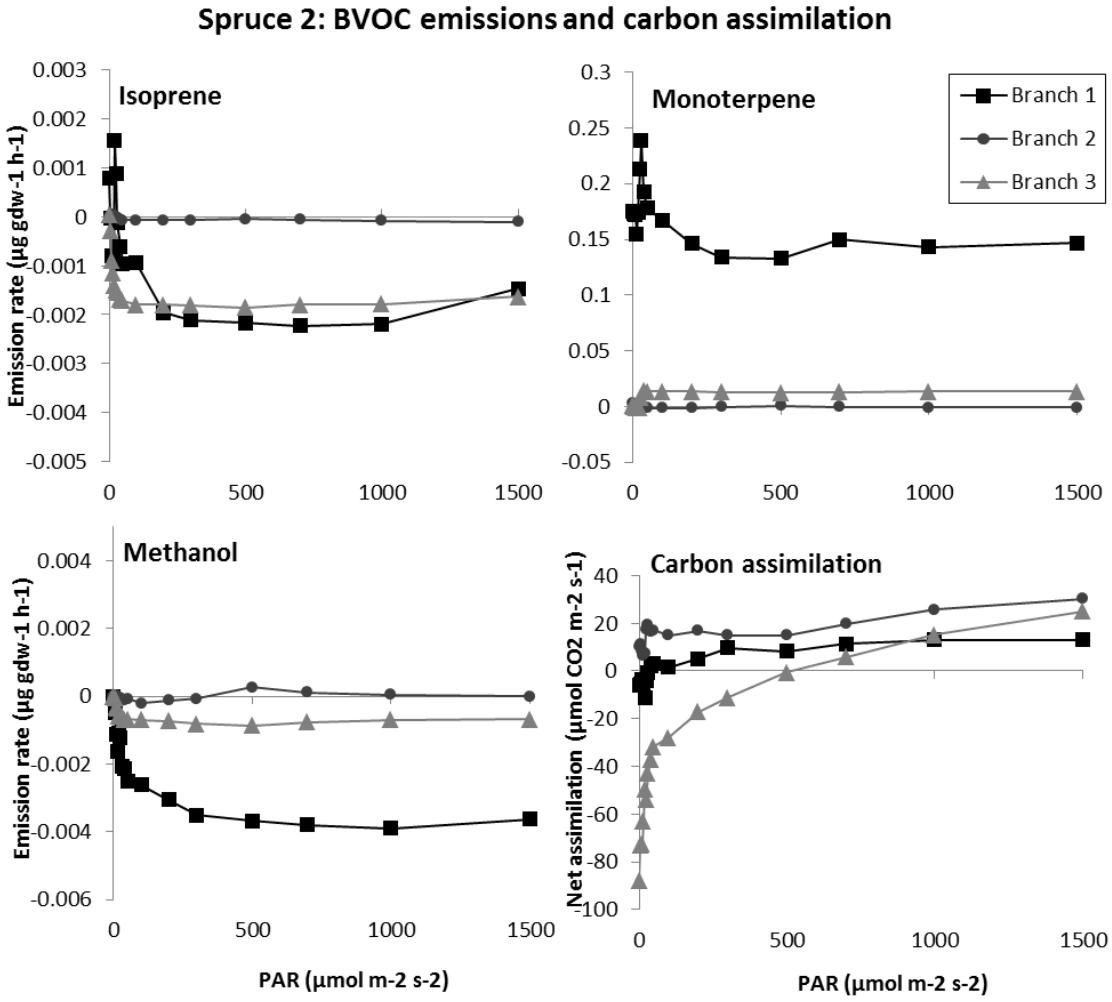

Figure 2. BVOC emission and carbon assimilation patterns for three different branches on the same spruce, spruce 2.

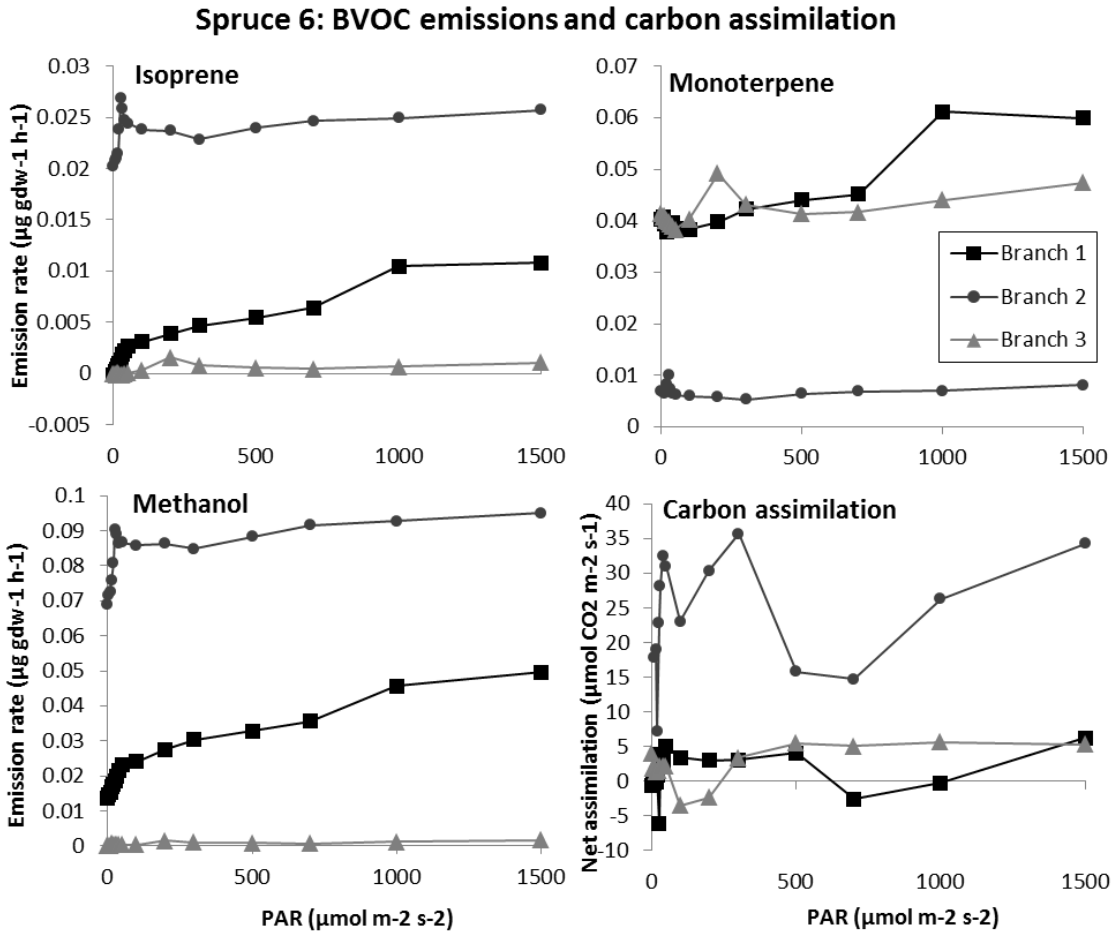

Figure 3. BVOC emission and carbon assimilation patterns for three different branches on the same spruce, spruce 6.

Spruce 2 was not emitting any isoprene or methanol from any branch, as seen in Figure 2, however, branch 1 was emitting MT in a range between 0.13 and 0.24 µg gdw^{-1} h^{-1} and branch 3 revealed emissions around 0.01 µg gdw^{-1} h^{-1} after light level 30 µmol m^{-2} s^{-1}, while branch 2 had zero emission. A higher emission activity was seen for spruce 6, emissions were detected for all analyzed compounds (Figure 3). The MT emissions for branch 1 and 2 were revealing a similar pattern with emissions ranging from 0.04 to 0.06 µg gdw^{-1} h^{-1} while branch 3 was emitting around 0.006 µg gdw^{-1} h^{-1}. The isoprene emissions from spruce 6 were indicating an expected light response for two branches, however, the amount of emission differed between the branches, where branch 2 was emitting the highest amount, around 0.02-0.025 µg gdw^{-1} h^{-1}, branch 1 was emitting less, increasing from 0 to 0.01 µg gdw^{-1} h^{-1}, and branch 3 was emitting close to 0 µg gdw^{-1} h^{-1}. Methanol emission revealed a similar pattern as isoprene, where branch 2 was emitting the highest amount, ranging from 0.07 to 0.09 µg gdw^{-1} h^{-1}, branch 1 was emitting around 0.01 to 0.05 µg gdw^{-1} h^{-1} and branch 3 with emissions close to 0 µg gdw^{-1} h^{-1}.

A comparison between spruce 2 and spruce 6 confirmed that MT emissions were twice as high for spruce 2, while isoprene and methanol were not emitted at all from spruce 2 but emitted from spruce 6.

4.2 Light response

Some examples of the light response for the BVOC compounds and the carbon assimilation are presented in Figure 4, 5 and 6. Figure 4 illustrates S7B2, which revealed BVOC emissions lower than 0.002 μg gdw^{-1} h^{-1} for all compounds. Looking at carbon assimilation, the compensation point was around 150 μmol m^{-2} s^{-1} and the assimilation increased after that point, reaching a highest net assimilation of around 40 μmol CO_2 m^{-2} s^{-1} at light level 1500 μmol m^{-2} s^{-1}.

S6B1 is presented in Figure 5, illustrating a pattern representing a general light response curve for carbon assimilation and isoprene. The compensation point for this branch was around 15 μmol m^{-2} s^{-1} and the net assimilation reached the highest value of around 6 μmol CO_2 m^{-2} s^{-1} at light level 1500 μmol m^{-2} s^{-1}. Another branch from spruce 6, S6B2, is presented in Figure 6 and revealed a low response to light for the BVOC emissions, and an unstable carbon assimilation response ranging between 7 and 35 μmol CO_2 m^{-2} s^{-1}.

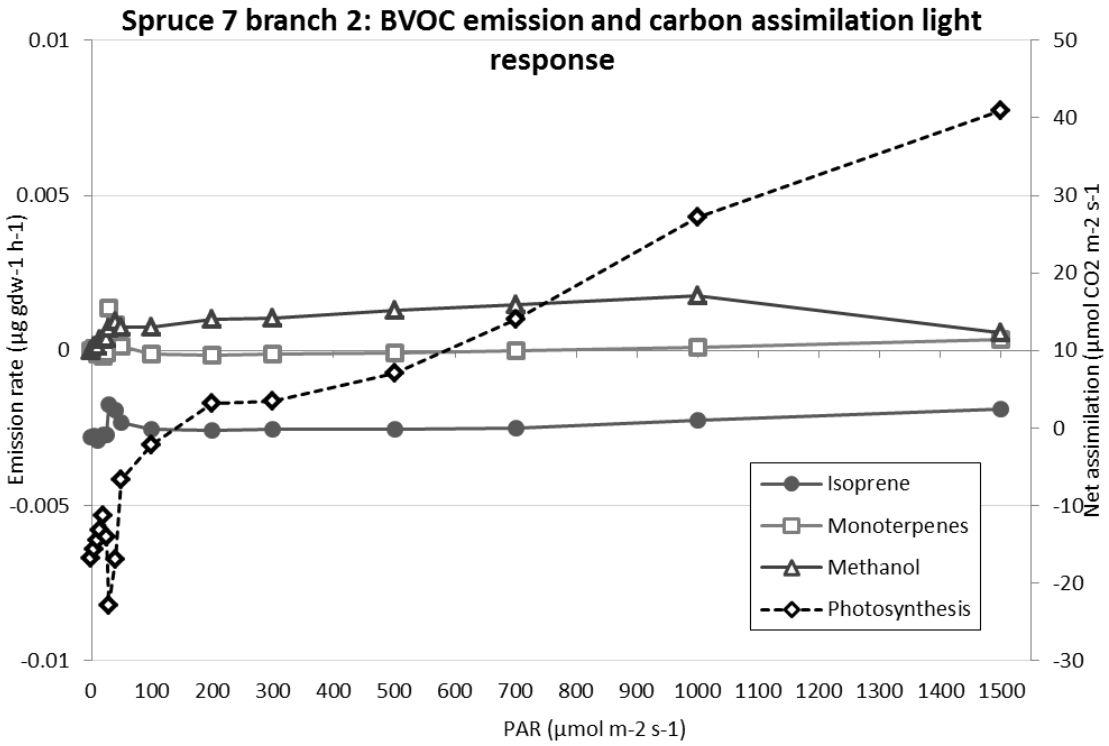

Figure 4. Light response of BVOC emissions and carbon assimilation for S7B2, with light levels from 0 to 1500 μmol m^{-2} s^{-1}.

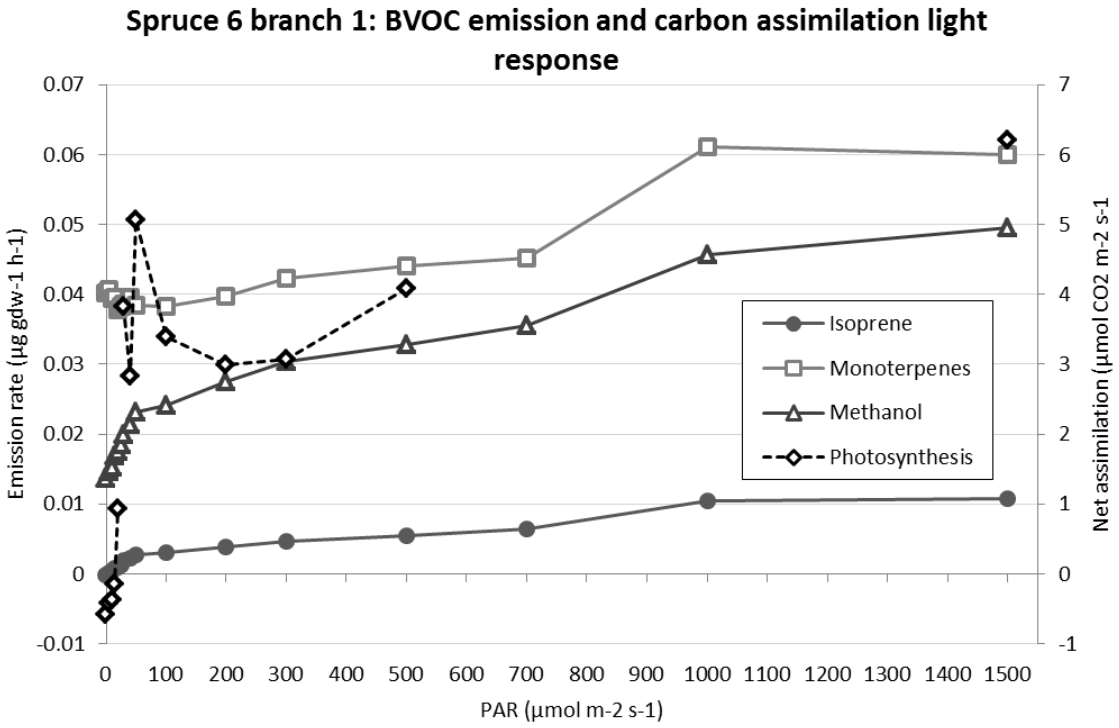

Figure 5. Light response of BVOC emissions and carbon assimilation for S6B1, with light levels 0 to 1500 µmol m^{-2} s^{-1}. The missing values for photosynthesis are removed outliers.

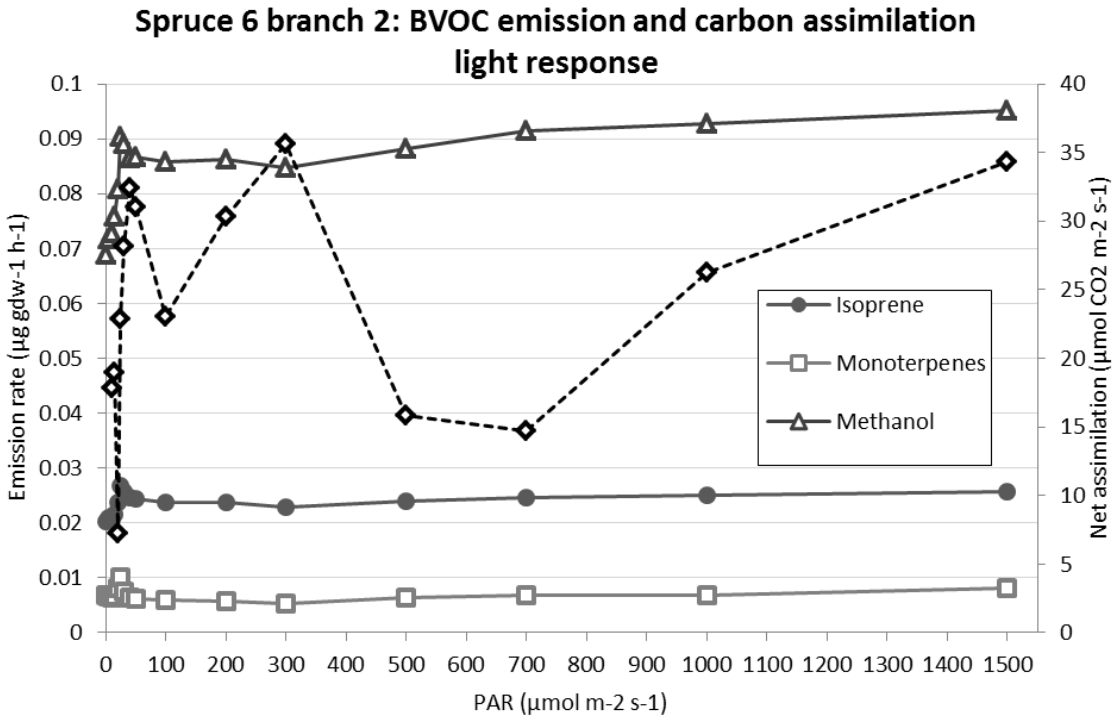

Figure 6. Light response of BVOC emissions and carbon assimilation for S6B2, with light levels 0 to 1500 µmol m^{-2} s^{-1}.

4.3 Mechanical stress

One example from the cutting needle experiments is visually presented in Figure 7 illustrating the induced emissions from S2B1, cut in dark conditions at 0 μmol m^{-2} s^{-1} PAR, and the decay of emissions over 30 minutes. The start of the cut is illustrated with the first arrow in Figure 7, and the last arrow after around 30 minutes illustrates when the emission values were assumed stable and the last post cut value were taken. The emissions directly after the cut, after 3 minutes, 5 minutes, 10 minutes and lastly 30 minutes as well as the rate of the decay of MT emissions following an exponential function of $y=c\ exp^{(bx)}$ and isoprene, methanol and carbon assimilation following a logarithmic function of $y=b\ ln(x)+c$ are presented in Table 2 and 3. The factor c in the equations state the initial highest emission, directly after cut, followed by the decay rate, b, stating how fast the emission rate is decaying and the emission rate at a given time can be found by applying that time in the factor x. S2B1 in Figure 7 was initially only emitting MT as seen in Figure 2, and cutting the needles was inducing the MT emissions from around 0.17 to 5.37 μg gdw^{-1} h^{-1}. Figure 7 and Table 3 reveals that isoprene and methanol were induced after cut for S2B1, from close to 0 μg gdw^{-1} h^{-1}, to around 0.02 μg gdw^{-1} h^{-1} and 0.05 μg gdw^{-1} h^{-1} respectively.

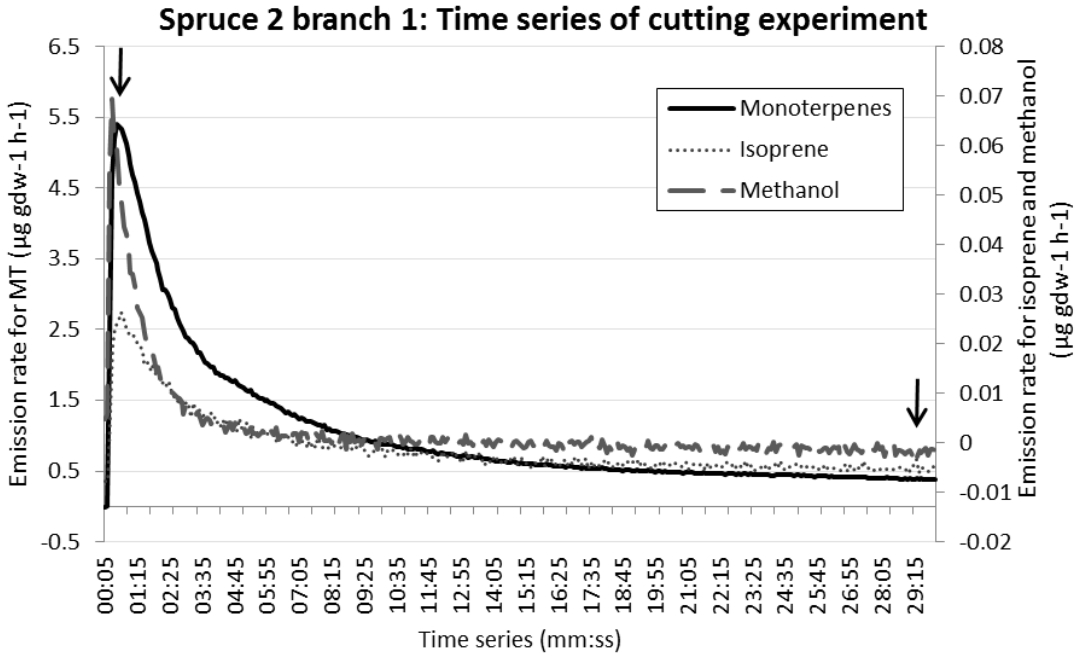

Figure 7. A time series of the BVOC emission rate from S2B1 after cutting the needles. The first arrow around 5 s marks the start of the cut, the second arrow around 30 minutes marks the end of the measurement. MT emissions follow the primary y-axis and isoprene and methanol emissions follow the secondary y-axis.

Table 2 displays the results when the needles were cut at light level 1000 μmol m^{-2} s^{-1}. The result reveals an increase in MT emissions for all measured branches, as well as a slight increase in isoprene emissions after needle damaging for almost all

branches. Methanol emissions reveals an initial increase for all the measured branches except S3B3 and S6B2. The emissions ceased to values around 0 μg gdw^{-1} h^{-1} after 30 minutes except for branch S6B3, where the emissions remained higher compared to before the cut, 0.032 compared to 0.001 μg gdw^{-1} h^{-1}. The methanol emissions after cut for S6B3 were initially 0.049 μg gdw^{-1} h^{-1} decreasing to 0.032 μg gdw^{-1} h^{-1} after 3 minutes, where it stabilized and remained the same for the measured 30 minutes. The same pattern was observed for S6B2, however, with lower emissions around 0.007 μg gdw^{-1} h^{-1}. Carbon assimilation had a similar pattern with initial high negative values ranging from -600 to -1900 μmol CO_2 m^{-2} s^{-1} when measuring directly after cut as the chamber had been opened which could explain the high negative values as an artifact of this. After the chamber had been flushed from ambient air, the emissions were rapidly increasing and after 10 minutes all values were positive, indicating carbon assimilation. Branches S6B2, S6B3 and S2B2 revealed a decrease in net assimilation 30 minutes after cut compared to before, and branches S2B3, S3B3 and S7B3 revealed an increase in net assimilation after cut compared to before. Because of data loss, the measured emissions before the cut of branch S1B3 were unknown, however, the result after the cut revealed high net assimilation rates of around 100 μmol CO_2 m^{-2} s^{-1}. All branches but S6B2 and S7B3 revealed an increase in net assimilation after 10 minutes, before decreasing after 30 minutes. MT emissions were decaying exponentially while isoprene, methanol and carbon assimilation were having a logarithmic decay.

Table 3 gives the results of the cut needles in dark conditions at 0 μmol m^{-2} s^{-1} PAR. Due to data loss there are only four complete measurements done both before and after cutting. However, the results for MT emissions revealed a similar pattern as in light conditions, with an initial increase in emissions after cutting with emissions still induced after 30 minutes. Isoprene and methanol revealed a small initial increase after cut for all branches with isoprene ranging from 0.001 to 0.025 μg gdw^{-1} h^{-1} and methanol from 0.005 to 0.04 μg gdw^{-1} h^{-1}, only to decay fast with values close to 0 μg gdw^{-1} h^{-1} again after 5 minutes. The carbon assimilation measurements in dark conditions (Table 3) confirms a similar result as in light conditions, with high negative values directly after cut (artifact from opening the chamber) between -100 and -2000 μmol CO_2 m^{-2} s^{-1}, decaying logarithmically over time. A decrease in respiration after 30 minutes compared to before cutting the needles was observed in three of four cases, where S6B1 indicated an increase in respiration after 30 minutes. S6B1 also revealed a pattern of positive assimilation rates of 4.91 and 9.31 μmol CO_2 m^{-2} s^{-1} after 5 and 10 minutes, starting to respire at -4.11 μmol CO_2 m^{-2} s^{-1} after 30 minutes. S1B1 confirmed a similar pattern after 10 minutes, however, the increase in assimilation was from -131.7 to 13.55 μmol CO_2 m^{-2} s^{-1}, and down to -4.07 μmol CO_2 m^{-2} s^{-1}.

A comparison of the decay rate functions indicates no obvious difference between needle damaging in dark and light conditions for any of the compounds. However, comparing the first measurements after cut reveals higher emissions of isoprene for measurements in light conditions for all branches but S2B1 with high emissions in dark. Methanol and MT revealed low differences directly after cut in light and dark conditions.

Table 2. Comparison of BVOC emission (µg gdw^{-1} h^{-1}) and carbon assimilation (µmol CO2 m^{-2} s^{-1}) before and after cutting the needles directly after cut, after 3 minutes, 5 minutes, 10 minutes and 30 minutes at T 30°C and PAR 1000 µmol m^{-2} s^{-1}. The decay function values c and b and the R^2-value of the compounds are also presented, where the decay was assumed exponential for MT with the function $y=c\ exp^{(bx)}$ and logarithmic for isoprene, methanol and carbon assimilation with the function $y=b\ ln(x)+c$.

	Pre cut	After cut	3 min	5 min	10 min	30 min	c	b	R^2
				Monoterpenes (µg gdw^{-1} h^{-1})					
S1B3	-	1.195	0.921	0.664	0.428	0.231	1.995	-0.405	0.97
S2B2	-0.001	3.423	1.862	1.403	0.800	0.340	6.152	-0.546	0.97
S2B3	0.014	3.573	1.774	1.602	0.823	0.413	5.887	-0.508	0.96
S3B3	0.001	1.001	0.563	0.384	0.247	0.148	1.531	-0.465	0.99
S6B2	0.007	1.258	0.857	0.701	0.524	0.351	1.680	-0.304	0.98
S6B3	0.044	1.909	1.321	1.029	0.733	0.480	2.682	-0.335	0.99
S7B3	0.000	0.163	0.160	0.149	0.139	0.118	0.905	-0.079	0.90
				Isoprene (µg gdw^{-1} h^{-1})					
S1B3	-	0.008	0.007	0.005	0.004	0.002	0.009	-0.004	0.90
S2B2	0.000	0.012	0.008	0.004	0.001	-0.001	0.013	-0.008	0.98
S2B3	-0.002	0.017	0.007	0.007	0.002	0.001	0.016	-0.01	0.94
S3B3	0.021	0.007	0.003	0.002	0.001	0.001	0.006	-0.004	0.93
S6B2	0.025	0.008	0.004	0.004	0.003	0.003	0.007	-0.003	0.86
S6B3	0.001	0.011	0.006	0.005	0.003	0.004	0.010	-0.005	0.90
S7B3	0.000	0.002	0.000	0.000	0.000	-0.001	0.002	-0.002	0.84
				Methanol (µg gdw^{-1} h^{-1})					
S1B3	-	0.020	0.003	0.003	0.002	0.001	0.016	-0.011	0.78
S2B2	0.000	0.031	0.000	-0.001	-0.002	-0.003	0.024	-0.02	0.77
S2B3	-0.001	0.016	0.001	0.001	-0.001	-0.001	0.013	-0.01	0.80
S3B3	0.072	0.015	0.002	0.001	0.001	0.001	0.012	-0.008	0.76
S6B2	0.093	0.021	0.007	0.005	0.005	0.007	0.018	-0.009	0.71
S6B3	0.001	0.049	0.032	0.031	0.033	0.032	0.045	-0.01	0.67
S7B3	0.000	0.010	0.002	0.000	0.000	0.000	0.008	-0.006	0.84
				Carbon assimilation (µmol CO_2 m^{-2} s^{-1})					
S1B3	-	-667	108.48	137.03	140.11	102.14	-483	467	0.70
S2B2	25.81	-1015	-21.57	10.60	16.92	10.28	-788	614	0.73
S2B3	15.18	-1935	-1.08	17.44	35.29	15.63	-1488	1164	0.71
S3B3	79.37	-	-	-	-	90.74	-	-	-
S6B2	26.26	-1537	-88.58	-43.35	3.91	5.74	-1217	925	0.75
S6B3	5.67	-212	-2.15	3.57	5.42	3.72	-164	129	0.73

S7B3	7.39	-1325	-7.45	18.13	27.08	31.00	-1024	807	0.73

Table 3. Comparison of BVOC emission (µg gdw^{-1} h^{-1}) and carbon assimilation (µmol CO2 m^{-2} s^{-1}) before and after cutting the needles directly after cut, after 3 minutes, 5 minutes, 10 minutes and 30 minutes at T 30°C and PAR 0 µmol m^{-2} s^{-1}. The decay function values c and b and the R^2-value of the compounds are also presented, where the decay was assumed exponential for MT with the function $y=c\ exp^{(bx)}$ and logarithmic for isoprene, methanol and carbon assimilation with the function $y=b\ ln(x)+c$.

	Pre cut	After cut	3 min	5 min	10 min	30 min	c	b	R^2
	Monoterpenes (µg gdw^{-1} h^{-1})								
S1B1	-	0.822	0.521	0.394	0.264	0.155	0.990	-0.402	0.99
S1B2	-	1.401	0.890	0.676	0.435	0.213	2.305	-0.448	0.97
S2B1	0.175	5.371	2.099	1.548	0.849	0.387	9.016	-0.617	0.97
S3B1	-	0.673	0.421	0.317	0.210	0.071	1.265	-0.519	0.92
S3B2	-	1.361	0.831	0.601	0.377	0.206	2.185	-0.457	0.99
S7B1	0.000	0.246	0.149	0.114	0.073	0.045	0.366	-0.411	0.99
S7B2	0.000	0.112	0.080	0.060	0.036	0.024	0.173	-0.388	0.99
S6B1	0.040	1.042	0.589	0.432	0.303	0.204	1.427	-0.393	0.98
	Isoprene (µg gdw^{-1} h^{-1})								
S1B1	-	0.005	0.002	0.001	0.000	0.000	0.005	-0.003	0.96
S1B2	-	0.007	0.002	0.001	0.000	-0.003	0.007	-0.006	0.95
S2B1	0.001	0.025	0.004	0.002	-0.001	-0.005	0.022	-0.018	0.91
S3B1	-	0.006	0.002	0.000	0.000	-0.002	0.006	-0.005	0.96
S3B2	-	0.007	0.001	0.000	-0.002	-0.002	0.006	-0.006	0.94
S7B1	0.000	0.001	-0.001	-0.002	-0.003	-0.003	0.001	-0.003	0.98
S7B2	0.000	0.001	-0.003	-0.003	-0.003	-0.004	0.000	-0.003	0.81
S6B1	0.000	0.005	0.002	0.000	-0.002	0.000	0.005	-0.004	0.84
	Methanol (µg gdw^{-1} h^{-1})								
S1B1	-	0.005	0.000	0.000	0.000	0.000	0.004	-0.003	0.70
S1B2	-	0.025	0.003	0.003	0.001	0.000	0.021	-0.015	0.80
S2B1	0.000	0.052	0.003	0.001	0.001	-0.002	0.041	-0.032	0.77
S3B1	-	0.026	0.012	0.010	0.009	0.004	0.024	-0.012	0.92
S3B2	-	0.022	0.003	0.001	0.000	0.000	0.018	-0.013	0.81
S7B1	0.000	0.016	0.000	-0.001	-0.001	-0.001	0.012	-0.01	0.75
S7B2	0.000	0.019	0.000	-0.001	-0.001	-0.001	0.015	-0.012	0.74
S6B1	0.014	0.045	0.014	0.011	0.011	0.012	0.038	-0.02	0.75
	Carbon assimilation (µmol CO_2 m^{-2} s^{-1})								
S1B1	-	-2115	-193.4	-131.7	13.55	-4.07	-1707	1275	0.78
S1B2	-	-738	-35.51	-15.17	-11.30	-9.36	-578	435	0.73
S2B1	-5.96	-88.65	-29.58	-25.89	-25.65	-5.55	-79	46	0.86
S3B1	-	-415	-45.28	-59.88	-64.51	-44.32	-329	213	0.69
S3B2	-	-743	-62.85	-40.88	-52.32	-68.62	-581	405	0.70
S7B1	-52.70	-456	-55.06	-42.90	-32.27	-30.53	-367	254	0.75
S7B2	-16.80	-782	-44.95	-23.46	-15.40	-12.12	-615	459	0.74
S6B1	-0.58	-532	-12.94	4.91	9.31	-4.11	-412	318	0.72

After measuring the cut branch for 30 minutes, light response measurements were started. The light response for carbon assimilation before cutting and after cutting are presented in Figure 8. Two individual branches, S2B1 and S7B1 were analyzed. The results revealed a similar light response curve pattern before and after the cutting measurements for both branches. The compensation point was reached later after cutting for both branches, at 45 μmol m^{-2} s^{-1} compared to 30 μmol m^{-2} s^{-1} for S2B1 and 1200 μmol m^{-2} s^{-1} compared to 800 μmol m^{-2} s^{-1} for S7B1. The highest net assimilation value occurred at 1500 μmol m^{-2} s^{-1} in all cases. The net assimilation for S2B1 reached a higher value after being cut, around 14 μmol m^{-2} s^{-1} compared to 13 μmol m^{-2} s^{-1}. S7B1 revealed the opposite, with the uncut reaching the highest net assimilation of around 9 μmol m^{-2} s^{-1} compared to the cut value of around 3 μmol m^{-2} s^{-1}.

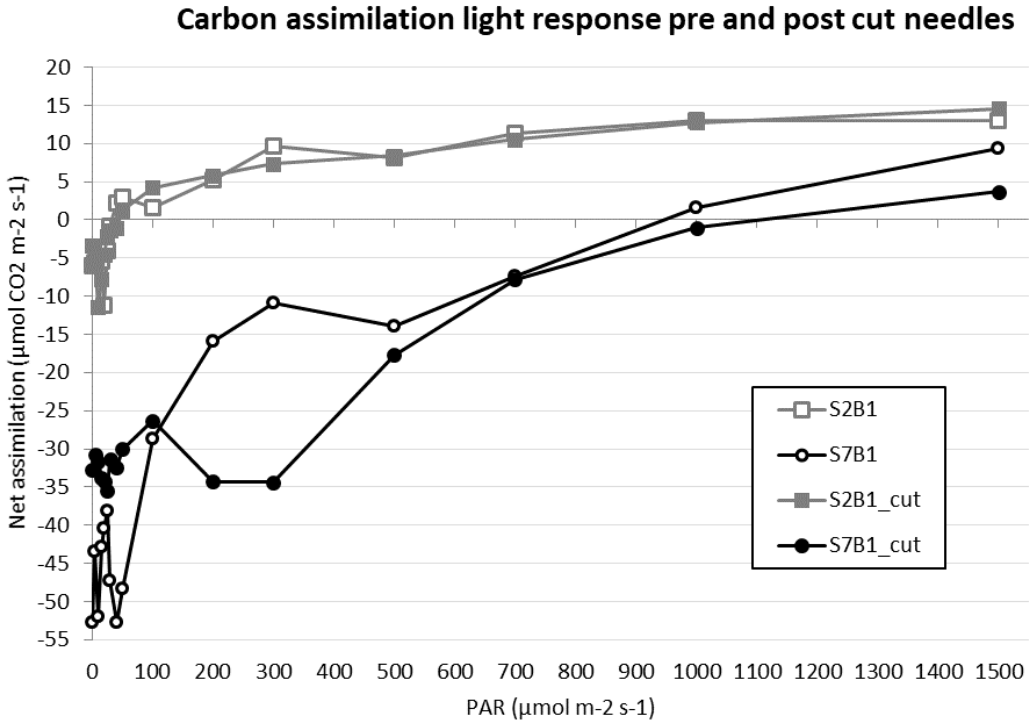

Figure 8. A light response curve comparing carbon assimilation for pre and post cut needles, where light response measurements for the cut needles are started 30 minutes after cutting. S2B1 and S7B1 are being represented in the figure before and after the needles were cut.

5. Discussion

The attempt to quantify the BVOC emission from young potted Norway spruce show unexpected results. Out of the ten analyzed branches, MT emissions were only found for five branches, isoprene was only found for three branches and methanol was found for the same three branches as isoprene. The lack of SQT throughout the study was unexpected since the choice of instrument, PTR-TOFMS, was selected to have a better chance at capturing these emissions, and since SQT emissions were found in other studies of Norway spruce (Hakola et al. 2003; Bourtsoukidis et al. 2014a; van Meeningen et al. 2017). The general low emission rates could be explained by the young age of the spruces, as well as the early season, which could imply that the spruces were inactive during the measurements, or that the compounds were stored to fill up an empty storage pool of the needle instead of directly emitted. The photosynthetic rate of the spruces could be used as a measure of the activity, were the results of the light response revealed a positive net assimilation for all branches at light levels of 1000 μmol m^{-2} s^{-1} and higher, ranging between 4 to 79.4 μmol CO_2 m^{-2} s^{-1} in this study. Comparing these results to a study by van Meeningen et al. (2017), net assimilation rates for mature Norway spruces was found to be ranging from 3.6 to 12.1 μmol CO_2 m^{-2} s^{-2} in light levels from 500 to 1000 μmol CO_2 m^{-2} s^{-2}. This is indicating that the young spruces in this study had a net assimilation rate comparable to a mature spruce. However, the assimilation rates in this study were scaled using an SLA value from a mature Norway spruce stand in Norunda calculated by Wang et al. (2017b), which could yield errors when applicating to much younger spruce. A comparison to a mature Norway spruce might also be inaccurate, however it is an indication that the young spruces in the study were active in the form of carbon assimilation. However, all branches were photosynthetically active after 1000 μmol m^{-2} s^{-1}, but not all branches did emit any BVOC.

The branches that revealed emissions of BVOCs were within the range of emissions detected in other studies analyzing the seasonality of BVOC emissions for Norway spruce, and thus covering the early season emissions. The highest MT emissions in this study was from spruce 2, where branch S2B1 emitted MT in a range between 0.13 and 0.24 μg gdw^{-1} h^{-1}, branch S2B3 emitted MT around 0.01 μg gdw^{-1} h^{-1}. Lower MT emissions were revealed for Spruce 6 ranging from 0.01 to 0.06 μg gdw^{-1} h^{-1}. The highest isoprene emissions were found for branches S6B2 and S3B3 ranging between 0.02 to 0.025 μg gdw^{-1} h^{-1} as well as the highest methanol emissions ranging between 0.06 to 0.09 μg gdw^{-1} h^{-1}.

Hakola et al. (2003) analyzed the seasonality of BVOC emissions over a boreal forest in southern Finland. They found that for Norway spruce the emission potential for both isoprene and MT were highest during the growing season where the highest emission potential for MT was 1.4 μg g_{dw}^{-1} h^{-1} in May. The study found that

Norway spruce still had some MT emission during winter, and during spring (April) they found a total emission rate of around 0.2 μg g_{dw}^{-1} h^{-1} including both isoprene and MT. Another study by Hakola et al. (2006) was done on Scots pine with similar results, however the potential emission rate for MT in April was ranging from 0.14 to 0.36 μg g_{dw}^{-1} h^{-1} for two different branches, suggesting higher total emission rates compared to the study on Norway spruce. The detected April values in both studies are comparable to the MT emission for S2B1 in this study, however, the isoprene emission is higher compared to the young spruces.

In a study by van Meeningen et al. (2017) isoprenoid emissions and the response to changing light conditions were analyzed and revealed the mean average actual BVOC emissions from Norway spruce to range from 0.05 and 1.26 μg g_{dw}^{-1} h^{-1} for isoprene and between 0.01 and 0.29 μg g_{dw}^{-1} h^{-1} for MT. Bourtsoukidis et al. (2014a) investigated the seasonality of VOC emissions over a mature Norway spruce dominated forest in Germany and found the median spring (April) emissions of methanol, 0.0337 μg g_{dw}^{-1} h^{-1}, isoprene, 0.0037 μg g_{dw}^{-1} h^{-1} and MT, 0.2031 μg g_{dw}^{-1} h^{-1}. The study found the lowest emission for MT to be 0.0109 μg g_{dw}^{-1} h^{-1}. The high end of MT emission for both studies are comparable to the MT emission from S2B1, while the low emissions are comparable to the other low emitting branches in this study, S2B3, S6B1, S6B2 and S6B3. The detected isoprene emissions found in the study by van Meeningen et al. (2017) was higher compared to the isoprene emission from the young spruces in this study, however, the detected isoprene emissions from the study by Bourtsoukidis et al. (2014a), are lower and can be compared to the isoprene emissions from S6B1, S3B3 and S6B2. The methanol emissions detected in the study by Boursoukidis et al. (2014a) are comparable to the emissions from S6B1, S3B3 and S6B2.

The results of the measurements on the spruces in this study showed no apparent signs of SQT emissions, however, van Meeningen et al. (2017) investigated four different mature (around 40 year old) Norway spruces in July, and only found SQT emissions in one of the spruces used in the study with mean average actual emission of 0.16 μg g_{dw}^{-1} h^{-1}. The study by Hakola et al. (2003) found that Norway spruce start emitting SQT late in summer and another study by Hakola et al. (2006) analyzing Scots pine also found emissions of SQT later in the year. However, the study by Bourtsoukidis et al. (2014a) did find SQT emissions during spring time (April) with the median emission being 0.1186 μg g_{dw}^{-1} h^{-1}, and the lowest measured emission being 0.0016 μg g_{dw}^{-1} h^{-1}. The reason for the lack of SQT emissions from the spruces used in this study could be the seasonality, however, some findings of SQT emissions in the previous mentioned studies are contradicting the assumption that the lack of SQT emissions is because of the early season. It could also be because of the young spruces used in this study, the three mentioned studies were measuring on

mature Norway spruce, and one on mature Scots pine, which could then influence the SQT emissions.

5.1 Emission differences between branches

Emission rates of MT have been shown to differ significantly between tree individuals of Scots pine when comparing to the emissions of the entire canopy (Räisänen et al. 2009). The same emission rate differences have been revealed for Scots pine as well in a study by Hakola et al. (2006). This is in line with the results of this study, revealing that at least isoprene, MT and methanol emission rates can differ between branches of the same individual tree of Norway spruce, presented in Figure 2 and 3.

Spruce 2 did not reveal any emissions for isoprene or methanol, but two branches, S2B1 and S2B3 were showing emissions of MT. The last branch S2B2 did not emit any MT, indicating differences among the branches and S2B1 revealed emissions of MT up to 20 times higher compared to the other branches.

Emissions of isoprene and methanol compounds were found for spruce 6. The emission differences between the branches are apparent and in line with the findings for spruce 2. The highest emissions of isoprene and methanol are detected from branch S6B2. Isoprene and methanol emissions are also revealed for S6B1, however, the emissions from S6B2 are almost twice as high for both isoprene and methanol compared to S6B1. The last measured branch S6B3 is not showing any emissions of isoprene or methanol, but MT emissions are detected at the same level as for S6B1. The lowest MT emissions were found for S6B2, with emission rates four times lower than the other branches of spruce 6. However, the isoprene and methanol emission were highest for S6B2.

5.2 Light response curve

The light response for BVOC emissions in this study indicated a slight light dependency for the compounds of isoprene and methanol, in the cases where those emissions did occur, for branches S6B1, S6B2 and S3B3 (S3B3 is not presented in the figures). The light response of isoprene for S6B1 revealed increased emissions from 0 $\mu g\ g_{dw}^{-1}\ h^{-1}$ at a light level of 0 $\mu mol\ m^{-2}\ s^{-1}$, to the highest emission of 0.01 $\mu g\ g_{dw}^{-1}\ h^{-1}$ at the light level 1500 $\mu mol\ m^{-2}\ s^{-1}$. The total increase of isoprene emission for S6B2 and S3B3 is 0.005 $\mu g\ g_{dw}^{-1}\ h^{-1}$, which is two times lower than for S6B1. The methanol emissions revealed a higher increase with higher light levels, S6B1 increasing from 0.013 $\mu g\ g_{dw}^{-1}\ h^{-1}$ to 0.05 $\mu g\ g_{dw}^{-1}\ h^{-1}$, and S6B2 from 0.067 $\mu g\ g_{dw}^{-1}\ h^{-1}$ to 0.095 $\mu g\ g_{dw}^{-1}\ h^{-1}$. Comparing the light response for isoprene with a study by van Meeningen et al. (2017) analyzing four mature Norway spruce, the increases in that study are ranging from 10 to 40 times higher compared to the spruces this study.

A study by Bourtsoukidis et al. (2014a) found that methanol had a clear diurnal cycle during April and revealed that methanol had the lowest temperature dependency of the compounds analyzed. The results in this study found increase of methanol emission with light, which is in line with the study by Boutsoukidis et al. (2014a), as the diurnal cycles would be affected mostly by irradiation.

The study by van Meeningen et al. (2017) also found that Norway spruce emit MT in darkness ranging from 0.01 to 0.22 μg g_{dw}^{-1} h^{-1}, which can be seen for S6B1, S6B3, S2B1 and S2B3, where MT emissions are within the same range in dark conditions. Van Meeningen et al. (2017) found MT (α-pinene) to be light dependent for many of the measured individuals, but as the PTR-TOFMS in this study cannot distinguish between different MT compounds, the light dependency of the emissions is hard to compare. However, a slight light dependency can be seen for MT emissions of S6B1 and S6B3, where the emissions are 0.04 μg g_{dw}^{-1} h^{-1} in dark conditions, increasing to 0.06 and 0.05 μg g_{dw}^{-1} h^{-1} respectively with increased light level, which is still a lower increase compared to the results by van Meeningen et al. (2017). MT emissions for S2B3 are 0 μg g_{dw}^{-1} h^{-1} at light levels lower than 30 μmol m^{-2} s^{-1} after which the MT emissions are increasing to around 0.01 μg g_{dw}^{-1} h^{-1}. The study by Bourtsoukidis et al. (2014a) also found that the emission response for some MT emissions was greatly reduced during night time compared to day time, while the temperature dependency decreased during the night, indicating that some light dependency can be seen for MT. Light dependency in MT emissions have also been revealed for Scots pine (Tarvainen et al. 2005). The results for S2B1 are different however, the emissions are up to five times higher compared to the other branches, and show no sign of light dependency, contradicting the results and studies found that MT are light dependent.

The carbon assimilation is found the be light dependent for all spruces, which is in line with the results of other studies (Monson et al. 1992; Bonan 2008; Jones 2014). The carbon assimilation for S6B1 and S6B2 can be seen to fluctuate a lot between light levels, which was also found to be the case for one spruce in the study by van Meeningen et al. (2017). Exposure to stress might be the explanation for this.

5.3 Cutting the needles

Cutting the needles revealed initially increased emissions for all analyzed BVOC compounds. The induced emissions of isoprene are higher at light level 1000 μmol m^{-2} s^{-1} compared to when cutting the needles in dark conditions, as expected because of the light dependency of isoprene. However, the light level did not affect the initial induced emissions directly after cut of methanol or MT. An explanation for this could be the storage pools being depleted while cutting the needle, independent of irradiance, as observed for methanol emissions in the study by Brilli et al. (2011). The

decay rate of the BVOC compounds is not obviously different in dark or light conditions. However, the induced emissions of isoprene and methanol are back to around 0 μg g_{dw}^{-1} h^{-1} already after 5 minutes in dark conditions, compared to in light conditions where emissions of methanol are close to 0 μg g_{dw}^{-1} h^{-1} for three out of seven spruces after 10 minutes and the isoprene emissions are close to 0 μg g_{dw}^{-1} h^{-1} after 30 minutes. The difference in time when isoprene stops emitting in light and dark conditions could be because of the initial induced emissions, they are higher in light conditions, and since the decay rate does not differ, the emissions will stop sooner in dark conditions. That isoprene emissions seem to occur in dark conditions could be explained by the need to open the chamber to cut the needles, hence allowing ambient air to enter and the PTR-TOFMS is thus not only analyzing the needle emissions, but also ambient air. The induced emissions of isoprene, methanol and carbon assimilation were decaying logarithmically.

In light conditions, the emissions for both isoprene and methanol before cutting the needles were lower for S6B3 and increased four times for isoprene and 30 times for methanol compared to 30 minutes after cut. However, a decrease in emissions of isoprene and methanol was seen for S6B2 and S3B3, where the emissions were eight and 13 times lower respectively 30 minutes after cut compared to the emissions before cut. When cutting in dark conditions, the emissions of isoprene and methanol showed no change in the emissions before cutting and 30 minutes after cut. The study by Bourtsoukidis et al. (2014a) found that methanol increased during mechanical stress, however, during the stress events, the emissions were still much lower than when peaking during the growing season. The initial increase for all branches except S6B2 and S3B3 is in line with those findings, however a lasting increase in emission of methanol was only found for S6B3.

Boutsoukidis et al. (2014a) also found enhanced MT emissions up to two order of magnitude when exposed to mechanical stress or strong winds. The MT emissions after cutting the needles revealed induced emissions directly after cut in line with those results. The induced MT emissions were decaying exponentially both in light conditions as well as in dark conditions with the emissions 30 minutes after cut being about 2 to 5 times higher than the emissions before cut. Other studies on Scots pine show a similar increase in MT emissions after a stress event, Räisäinen et al. (2009) analyzed MT emissions over a boreal Scots pine forest and noticed an occurrence of European pine sawfly during the study, however, the outbreak was not considered severe but they could still conclude that the herbivory increased the total flux of MT over the forest. Another study on Scots pine was conducted by Hakola et al (2006) in which they looked at the seasonality of MT emissions and compared two branches of the same trees, one of which they debudded. Their results show that

immediately after the buds were cut, MT emissions increased up to seven times, lasting for approximately a week.

Carbon assimilation in this study shows an initial decrease after cut, releasing a lot of carbon as respiration (negative values) in both dark and light conditions. This could be explained the same as for isoprene, the need to open the chamber and allowing ambient air to enter, the high increase in negative values could then be an artifact of this. After 5 minutes in the light conditions, the net assimilation was positive, and for 50% of the spruces the net carbon assimilation was higher compared to before the cut. The higher net assimilation was still observed 30 minutes after cut.

A similar result was seen for S6B1 and S1B1 during dark conditions, the initial net assimilation was negative as respiration occurred in dark conditions (and the fact that the chamber was opened), however, 5 and 10 minutes into the measurements the net assimilation turns positive and after 30 minutes the needles begin to respire again.

Photosynthesis has been shown to suddenly increase after mechanical stress, which could be the case seen in the results (Loreto et al. 2006; Brilli et al. 2011). However, the photosynthesis returns to lower values than initially after a while, in line with literature. This can also be seen when analyzing the light response curve of carbon assimilation, comparing to before and after cut. The compensation point for both S7B1 and S2B1 were reached at higher light levels after cut than before cut. The net assimilation rate reached the same level for S2B1 for both measurements, whereas the highest level of net assimilation was twice as high for S7B1 before cutting the needles compared to after cut.

5.4 Sources of error/limitations of study

One major limitation to this study is the season as well as the time limit, the spruces were taken into the climate chamber from outside winter conditions, which could be one of the reasons to why low BVOC emissions were found since the spruces did not have enough time to acclimatize to warmer conditions before measurements needed to be initiated. The spruces in the study were young potted spruces and the measurements were conducted inside a climate chamber, which in turn is another limitation when comparing the results to other studies which mostly is analyzing mature spruces in its natural conditions. Laboratory studies are however an advantage when conducting measurements because of the ability to control the environment, making it possible to conduct measurements under standardized conditions from the start.

An unfortunate event of data loss led to the study only analyzing the light response of Norway spruce which is a limitation as well, since Norway spruce is considered a high emitter of MT, which in term are considered highly dependent on

temperature (Kesselmeier & Staudt 1999; Hakola et al. 2003). Temperature measurements would be a good addition to the results. The data loss also led to a less branches included in the final analyze, it is an advantage to have a lot of data to compare results with to decrease the uncertainty of the study.

The background concentration values used in the calculation of the emissions could also be a source of error, if the BVOC concentration in the background is found to be high or close to the measured concentration, it could give uncertainties in the peak calculations (Holzinger 2015). A cleaner background signal would ultimately lead to a better detection of BVOC emissions, as the peak signal would be clearer. Another factor affecting the detection of BVOC emissions is the time, the branches were only given 30 minutes to stabilize after being inserted into the chamber compared to other studies where the branches were given at least 1 h to stabilize (Grabmer et al. 2006; van Meeningen et al. 2017). A study by Boursoukidis et al. (2014b) revealed substantially increased emissions due to mechanical stress after inserting a branch into a needle chamber. The uncertainty that the change in emissions is due to anything other than the branches reaction to the different tested conditions would thus be lower if given more time to stabilize. Some branches were indicating negative emission rate, in order to give a better detection of the compounds, a zero-line adjustment was made, moving the baseline from negative values to zero making the response curves positive. The necessity of this adjustment can be because of the previously mentioned stabilization time, the spruces could still be adjusting from the mechanically induced stress and the BVOC compounds reflecting the induced stress, giving negative values while stabilizing, before increasing with experimentally tested conditions as well as time. The negative emission rate for the concerned branches, as well as the emission rate in general, are generally low and could be considered zero.

The length of the tube between the needle chamber and the PTR-TOFMS and the material of the tube are also factors affecting the signal of BVOC, as well as change in temperature in the room. A change in temperature of the TOF-MS can lead to a change in the ions path over time, affecting the peak detection of the compounds (Graus et al. 2010). Temperature differences between the needle chamber, climate chamber and the heated drift tube can affect the deposition of BVOCs along the tube yielding sampling loss. A longer tube also enhances the risk of higher losses of BVOC due to sorption. The results in the study by Bourtsoukidis et al. (2014b) confirmed higher measurement accuracy and lower VOC sampling losses by using a glass tube between the chamber and PTR-MS inlet, as well as isolating and heating the lines to prevent temperature differences influencing the VOCs.

Another factor influencing the BVOC emissions is the needle development, a study by Grote et al. (2013) found that the leaf development stages have different emission capacity. The emission rate has also been found to change with needle age

for Scots pine (Räisänen et al. 2008). The final days of measurements, new needles had grown on the spruces, which then could affect the BVOC emissions. The measurements were conducted within a couple of days to minimize the errors because of this, in order to get a better comparison between the different spruces.

Future studies on Norway spruce is essential in understanding how the trees are affected by different environmental factors enhanced by climate change. Temperature as a factor would be a good addition to the research as BVOC emissions are highly dependent on it, as well as more in debt studies on the stress effect on spruces such as insect attacks. To find a method to avoid opening the chamber when analyzing mechanical stress by damaging the needles would be a good improvement to this study. Looking at light dependency, a method analyzing the different compounds of MT instead of the total MT would give more substance to the results, as some MT compounds have a higher light dependency than others. However, this is a tradeoff when using TOF-MS to get a better detection of highly volatile compounds.

6. Conclusion

Measurements in this study were performed on young potted Norway spruce to analyze the affects of light and stress on emissions of BVOC compounds MT, isoprene and methanol during the early season. The study reveals BVOC emissions on the lower end of published results, indicating low activity of the spruces. The low activity could be explained by the young age of the spruces, as well as the early season, a majority of the published results are analyzing mature spruce stands during the growing season. This could also be an explanation to the lack of SQT amongst the BVOC compounds detected, other published researched that detected SQT emissions from Norway spruce was analyzing mature spruces, and also found the emissions later in the season. The results did however confirm a difference in amount and compounds emitted between the branches of the same spruce, as well as between the spruces, indicating uncertainties when assuming emissions from one spruce based on one branch. The study revealed low light response for the analyzed compounds compared to published literature, however, since most literature researched mature spruces, the comparison might be inaccurate. The light response measurements confirmed a light response for carbon assimilation, in line with published research. Simulating mechanical stress by damaging the needles did show expected results as the compounds increased when the needles were cut, as is in line with published research. Isoprene revealed a higher increase in emission directly after cut in light conditions compared to dark, which could be explained by the light dependency of isoprene. The decay rate of the emissions was not affected by differences in light levels. The carbon assimilation confirms published research by increased in emissions 30 minutes after cut compared to before cut for 50% of the cases, as well as reaching the compensation point later when performing light response measurements after the cut compared to before the cut.

Limitations to the study do occur, and improvements could be made for future research on young Norway spruce. Addition of temperature analyzes could help get a better understanding of the nature of BVOC emissions, as well as improvements to the instruments and methods used.

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