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Tiivistelmä – Referat – Abstract

Continuously progressive climate change has built a need for sustainable energy source. In the near future we need an alternative source to fossil fuels. At the same time we should secure lower carbon emissions to the atmosphere and increase carbon sinks and accumulate bigger carbon pools to biosphere. Wood based second-generation biofuels are potential option for a sustainable source of energy and therefore an alternative for fossil fuels and also for first-generation biofuels which are produced from food suitable sources. The high cellulose content of wood drives the use as an energy source but long investment time to raw material production impairs the wood's possibility to be a quick solution to current climate and energy challenges. Hybrid aspen (Populus tremula x tremuloides) as a fast growing tree is one of the species under research to produce cellulose faster. Institute of Biotechnology at University of Helsinki has developed a method for gene manipulation of aspen to enhance the cambial development and tree growth which would shorten the rotation time of harvesting raw material. Simultaneously we need to predict possible side effects that may come with the use of gene manipulation. The wood based production of bioenergy creates a sink for atmospheric carbon dioxide until logging. Furthermore many of the Populus species including aspen emit biogenic volatile organic compounds (BVOC) which slow the climate change due to the secondary particle (SOA) formation. This study investigates seasonal BVOC emission profile and concentration of aspen Populus tremula in natural forest environment during years 2010, 2011 and 2013. The other part of the study concentrates on emissions from gene manipulated hybrid aspen species Populus tremula x tremuloides in greenhouse environment. The results will show the seasonal profile of BVOC emissions and other trace gas exchange (CO 2, H2O) and their concentration on leaf level. Small part of the atmospheric carbon that tree takes in as CO2 is released back in air as BVOCs and based on these results from three year time aspen releases carbon 0.44-0.57 % per year as BVOVs. The emission profiles show clearly that temperature and light conditions affect to BVOC emission volume. Furthermore, the leaf development phase has a huge effect on seasonal emission profile. The other part of the study that investigate the differences in BVOC emissions between genetic manipulated trees and control trees. Based on these measurements there is no significant difference in BVOC emissions between gene manipulated and wild type hybrid aspen on leaf level. Since environmental conditions affect emission profile and volumes, the climate change with increasing temperature may increase aspen's seasonal BVOC emissions. Based on measurements of this study the potential use of gene manipulated aspen does not increase BVOC emissions on leaf level but on canopy level the result may be different.

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FIELD AND GREENHOUSE STUDIES ON BVOC EMISSION PATTERNS FROM POPLARS (TREMBLING AND HYBRID ASPEN)

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February 2019

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Abstract

Continuously progressive climate change has built a need for sustainable energy source. In the near future we need an alternative source to fossil fuels. At the same time we should secure lower carbon emissions to the atmosphere and increase carbon sinks and accumulate bigger carbon pools to biosphere. Wood based second-generation biofuels are potential option for a sustainable source of energy and therefore an alternative for fossil fuels and also for first-generation biofuels which are produced from food suitable sources. The high cellulose content of wood drives the use as an energy source but long investment time to raw material production impairs the wood's possibility to be a quick solution to current climate and energy challenges. Hybrid aspen (Populus tremula x tremuloides) as a fast growing tree is one of the species under research to produce cellulose faster. Institute of Biotechnology at University of Helsinki has developed a method for gene manipulation of aspen to enhance the cambial development and tree growth which would shorten the rotation time of harvesting raw material. Simultaneously we need to predict possible side effects that may come with the use of gene manipulation. The wood based production of bioenergy creates a sink for atmospheric carbon dioxide until logging. Furthermore many of the Populus species including aspen emit biogenic volatile organic compounds (BVOC) which slow the climate change due to the secondary particle (SOA) formation. This study investigates seasonal BVOC emission profile and concentration of aspen Populus tremula in natural forest environment during years 2010, 2011 and 2013. The other part of the study concentrates on emissions from gene manipulated hybrid aspen species Populus tremula x tremuloides in greenhouse environment. The results will show the seasonal profile of BVOC emissions and other trace gas exchange (CO_2, H_2O) and their concentration on leaf level. Small part of the atmospheric carbon that tree takes in as CO_2 is released back in air as BVOCs and based on these results from three year time aspen releases carbon 0.44-0.57 % per year as BVOVs. The emission profiles show clearly that temperature and light conditions affect to BVOC emission volume. Furthermore, the leaf development phase has a huge effect on seasonal emission profile. The other part of the study that investigate the differences in BVOC emissions between genetic manipulated trees and control trees. Based on these measurements there is no significant difference in BVOC emissions between gene manipulated and wild type hybrid aspen on leaf level. Since environmental conditions affect emission profile and volumes, the climate change with increasing temperature may increase aspen's seasonal BVOC emissions. Based on measurements of this study the potential use of gene manipulated aspen does not increase BVOC emissions on leaf level but on canopy level the result may be different.

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1. Background

1.1. Motivation

Continuously increasing CO_2 concentration in the atmosphere is causing the greenhouse effect which is warming the troposphere and inducing the climate change. The use of fossil fuels sustains the growing energy production but also it increases the amount of released carbon to the atmosphere. Searching the means of controlling the climate change are ongoing. One alternative for fossil fuels are wood based second-generation biofuels. These biofuels are sustainable source of energy and they do not create a competitive situation in land use between food agriculture and energy production as first-generation biofuels has created (Solomon, 2010). Wood has a high cellulose content and is suitable as energy source but long investment time due to the long rotation time is a disadvantage (Nieminen et. al., 2011). Poplars, due to their fast growth, are a good source for cellulose. Institute of Biotechnology at University of Helsinki have developed a method for gene manipulation of hybrid aspen (Populus tremula x populous tremuloides) to enhance the cambial development and tree growth which would shorten the rotation of logging time (Nieminen et. al., 2008). The gene manipulated aspen would increase the bioenergy potential of forests. Poplar trees are also known to have large BVOC emissions (Günther at. al., 1995) that may have climatological effects. The BVOC emissions take part to secondary aerosol (SOA) formation which affect cloud formation (Vehkamäki and Riipinen, 2012). Aerosols modify cloud properties, such as lifetime and albedo, which define how much solar radiation can be reflected, which leads to cooling. On the other side, clouds can also absorb some of the outgoing infrared radiation emitted by Earth, which has a warming effect (Spracklen et. al., 2008).

1.2. Poplar trees and aspen

Aspen is a species of deciduous trees that belongs to a poplar genus. Aspen has pervaded in many parts of Europe and Asia. It is also found in Africa but only in Algeria. North America has similar species on its own, Populus tremuloides. Aspen tree grows fast and the economic value of it has varied during times. Recently it is acknowledged that as a fast growing tree aspen and all poplars can act as a fairly effective carbon sink for atmospheric carbon. Aspen can be found in both deciduous and conifer forested areas even from the highest latitudes and altitudes. Aspen is a

unique tree in terms of exploiting the whole growing season despite the fact that its leaves burst rather late in May. Young trees have chlorophyll also on the surface of the stems which induces photosynthesis to start early in spring (Koivisto et al., 1997). Aspen also forms a wide clones and all trees in a same forest may originate from the same mother tree reproduced by roots. Another unique feature is that the shape and the structure of the leaves that allows them to react quickly to air current and even the smallest air movement propels them. The sensitivity of leaf movement accelerates the evaporation which simultaneously accelerates the nutrient intake from the soil. Therefore the movement of leaves may accelerate BVOC emissions together with evaporation. It is notable that in this study leaves were not able to move naturally due to the chamber system applied.

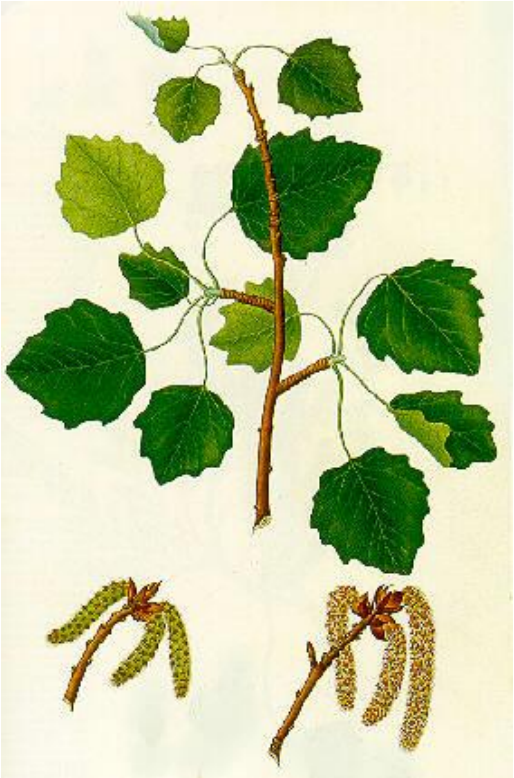

Figure 1. Aspen leaves, gynoecium (left) and stamen.

In Finland aspen is classified as a key species for biodiversity of nature. This tree provides surrounding for good living conditions of multiple animal and fungus species during the long life cycle. Aspen is also one of the target species in research to achieve efficient cellulose based bioenergy source for production of so called second-generation biofuels (Nieminen et al., 2012). The study of engineered transgenic Populus tremula x tremuloides by Immanen et al. (2016) has shown that cytokine hormones act as major regulators of cambial activity which leads to stimulated cambial cell division and increased production of biomass, up to 80% in dry weight. The BVOC's that

aspen produces are in order of magnitude isoprene, methanol, monoterpenes and acetone. This study focuses on isoprene and methanol emissions and represent the results of monoterpene and acetone emission shortly. The emission profile varies during the growing season. The largest methanol emissions are released when the leaves are young and in developing phase (Nemecek-Marshall 1995, Hüve et al., 2007). Isoprene emissions start to grow rapidly after the leaves have reached the mature phase.

On poplar species cambial development and growth rate is regulated by cytokine signaling (Nieminen et al 2008). Immanen et al. (2016) has developed transgenic hybrid aspens (Populus tremula x tremuloides) in their study of cytokinin and auxin signalling in cambial development. Similar transgenic hybrid aspens were used in this study together with control trees without gene manipulation which represent the natural wild type. The control trees were clones with each other and therefore all variation in growth is expected to be a result of environmental factors which were fairly well controlled in this study as the measurements were done in greenhouse. Cyto+ trees differ from control trees by one transferred gene but were clones until the gene transformation. The transferred gene IPT7 increases the cytokine production of trees and is targeted on xylem. Induced cytokine production stimulates the cambial cell division activity above normal level. As a results of Immanen et al., (2016) study the gene transferred trees increased the production of lignocellulosic trunk biomass in environment controlled greenhouse. The gene transformation is not expected to affect BVOC production since it is targeted on xylem growth. This study confirms expectation. The gene transferred trees produce more branches and leaves due to the increased xylem activity which then may affect total BVOC volumes on canopy level.

Emitting isoprene is characteristic feature for fast-growing perennial plants. Emission rates depend on the temperature and photosynthetically active radiation (PAR) (Günther et al. 1995). Isoprene emissions correlate with increasing temperature and PAR (Sharkey et al. 2008). Low concentrations of CO_2 stimulates emissions (Rasulov et al. 2009).

The reasons for isoprene and other BVOC emission are suggested to be versatile. Plants face several abiotic stress factors continuously in their living environments. These stress factors are high radiation, temperature, drought and oxidation. Vickers et al. (2009) suggest that by emitting isoprenoids including isoprene, the plants protect themselves against these stress factors.

BVOC emissions also participate in interactions between plant and insects (De Moraes et. al., 2001). BVOCs repel herbivores or attract pollinators. Plants induce emissions when herbivores take contact. These induced emissions can act as direct defense or as an indirect defense against stress and pathogens. During direct defense actions the emissions repell directly herbivores. Indirect defense attracts natural enemies of herbivores to reach the plant and attack against herbivores. BVOCs also prevent fungal and bacterial infections. (Dicke and Vet, 1999)

Production of isoprene is tightly tied up to plants components in metabolism with three carbons (C3) which are produced in the glycolysis cycle. This sugar metabolism cycle produces derivatives such as pyruvate, acetyl 1-CoA and glycerylaldehyde-3-phosphate (G3P) (Vickers et al. 2009). Karl et al (2002) presented multiple origins for isoprene with labelled $^{13}CO_2$ in on-line study. Plant utilizes these three molecules to produce different isoprenoids which then poses multiple roles in the plant. Primary roles of isoprenoids in metabolism are hormones that regulate growth, photosynthetic components such as chlorophyll and plastoquinones and structural components which are sterol membrane components. Secondary roles are also in metabolism and in the defense system that include phytoalexins and antioxidants such as tocopherols and carotenoids (Karl et al., 2009). As indicated before, isoprene is one of the components in the plant's defense system against outside stress factors.

Plant uses two pathways for synthesizing different isoprenoids from pyruvate, acetyl 1-CoA and G3P. The pathways are cytosolic mevalonic acid (MVA) pathway and chloroplastic 2-C-methyl-d-erythritol 4-phosphate (MEP) pathway. The cytosolic MVA pathway produces mainly sesquiterpenes, triterpenes, homoterpenes, precursors for sterols and ubiquinone. The chloroplastic pathway produces hemiterpenes i.e. isoprene, monoterpenes, diterpenes, tetraterpenes and higher order of isoprenoids. (Vickers et al. 2009)
Both synthesis the MVA and the MEP are able to produce isopentenyl pyrophospate (IPP), five-carbon building block, which links these pathways together (Vickers et al. 2009). An isomer for IPP is dimethylallyl pyrophosphate (DMAPP). Modifacation reaction between two isomers is catalysed enzyme called isopentyl diphosphate isomerase (IDI). DMAPP has 5 carbons which can now convert to isoprene on demand (figure 2) (Vickers et al. 2009).

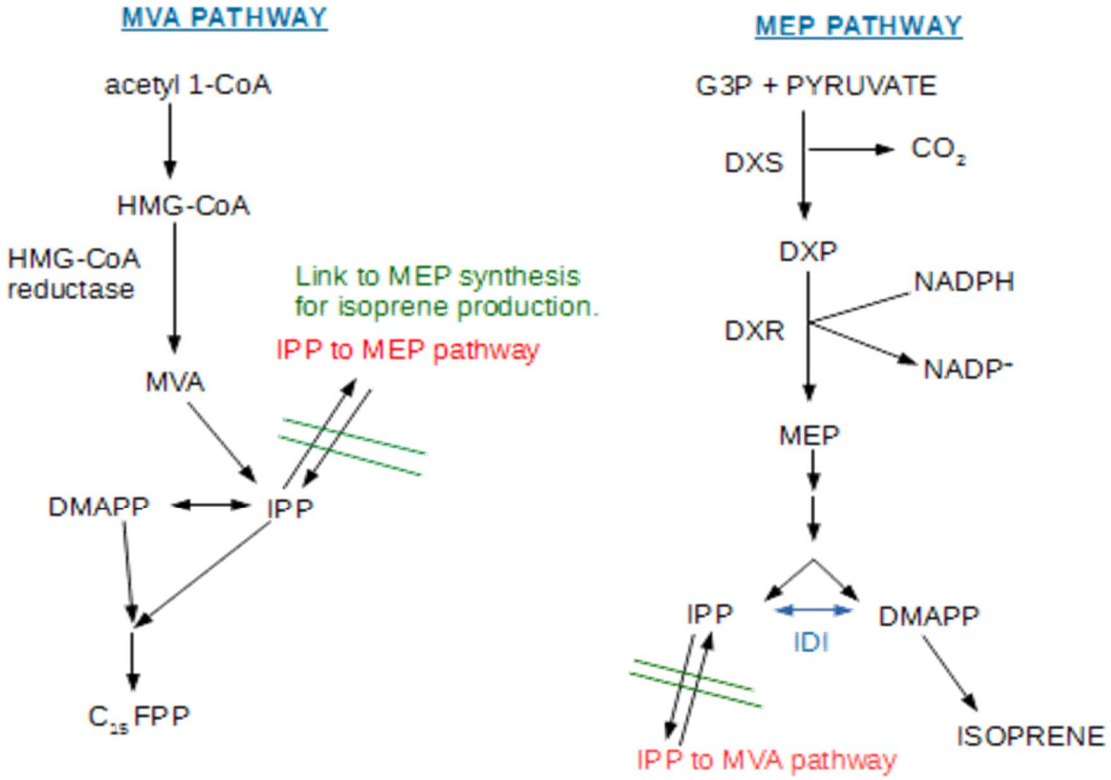

Figure 2. The MEP and MVA pathways. In MVA pathway acetyl 1-CoA reacts to through HMG-CoA to MVA and to IPP where it can be moved to MEP pathway to produce isoprene. In MEP pathway the G3P and pyruvate react together to form DXP, MEP and IPP or DMAPP to produce isoprene. This figure is drawn by K. Tiusanen and follows the MVA and MEP pathways presented in book of Monson and Niinemets, 2013.

The basal rate of emissions for different poplar species were modelled by Günther et al (1993, 1995) and had notable differences between the species. In the model species have similar instantaneous response to temperature. Rasulov et al (2009) research indicate that DMADP pool size is the controlling factor in the isoprene emission levels. While the light intensity, CO_2 and O_2 concentrations stimulate the isoprene emissions, the emission levels are dependent on the DMADP pool size. It also shows that the level of energetic metabolites which are formed via photosynthesis determines the DMADP pool size.

The BVOC's also has an important effect in atmosphere in reactions with other reactive oxygen species (ROS) which are gaseous components for example ozone (O_3), hydroxyl radicals (OH), nitrate

(NO_3) molecules. As an active component the BVOC's have climatological effects. Study by Vehkamäki and Riipinen (2012) demonstrate how gas molecules such as oxidized BVOC's are able to cluster and grow in size to nucleation point in atmosphere. After nucleation point these gas molecule are able to clusters can grow their particle size and form secondary organic aerosols (SOA) that can participate on cloud formation. Therefore worldwide isoprene emissions has an effect on cloudiness, air quality and climate (Kulmala et. al., 2004).

Poplars emit mainly methanol and isoprene. Methanol (CH_3OH) is an alcohol with one methyl group and one hydroxyl group. It is a light and volatile compound. Methanol has a polar nature and solubilizes in water. This water solubility partly explains the negative values detected when measuring methanol flux. The negative flux is a result of condensed water vapor inside the chamber.

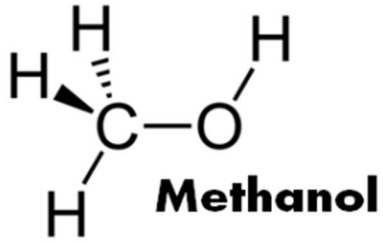

Figure 3a. Methanol molecule.

IUPAC name	Methanol
CAS number	67-56-1
Molecular formula	CH_3OH
Molar mass	32.04 g mol^{-1}
Boiling point	64.7°C
Melting point	-97.6°C
Density	0.792 g cm^{-3}
Vapor pressure	13.02 kPa @ 20°C

Figure 3b. Chemical and physical properties of methanol.

Water solubility of methanol influences the production and reservoirs within trees and plants. Methanol emissions in plants are a result of growth and demethylation of pectin. The emissions generally decline while leaves are developing to mature phase (Nemecek-Marshall 1995).

This study present similar emission pattern from both greenhouse and Hyytiälä measurements. Hüve et al. (2007) were able to observe diurnal emission pattern when studying developing leaves in different stages. They suggest that cumulative daily emissions correlate with total daily leaf

growth rates but the diurnal variations also depend on stomatal conductance. In their study the morning peaks in emissions were explained with stomatal opening and reservoirs release. The emissions from mature leaves originate from the root growth, which shows similar pectin demethylation reactions (Folkers et al. 2008).

Global atmospheric methanol budget is estimated to be 103 Tg per year (Millet et al. 2008). This estimation in done with 3-D chemical transport model (GEOS-Chem). In troposphere methanol reacts in gas phase with OH to produce CH_2O and HO_2. It is suggested that these compounds increase tropospheric ozone levels. The important role of methanol in the upper troposphere is to control oxidants. (Tie et al. 2003)

Isoprene (C_5H_8, 2-methyl-1.3-butadiene) is the main emistted BVOC for the majority poplars. In atmospheric conditions isoprene is highly volatile organic compound (VOC). It has two double bonds in structure, therefore it reacts easily with other components. In atmosphere isoprene goes through oxidation in reaction with hydroxyl radical (OH), ozone (O_3), nitrate (NO_3) radical or via photolysis (Hallquist et al.2009). Isoprene is acknowledged to be harmful for human health and is suspected to cause cancer and genetic defects (Haynes). European Chemicals Agency have set limit values for inhalation exposure. Systematic inhalation exposure of Isoprene is studied with rats and the limit for Lowest Observable Adverse Effect Concentration LOAEC (rat) is 19.503 mg/L in air. Nevertheless this study does not refer biologic origin atmospheric isoprene emissions to be harmful.

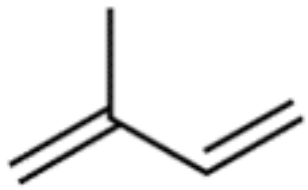

Figure 4. Isoprene molecule.

	Isoprene
IUPAC name	2-Methyl-buta-1,3-diene
CAS number	78-79-5
Molecular formula	C_5H_8
Structure	$CH_2=C(CH_3)CH=CH_2$
Molar mass	68.1 g mol^{-1}
Boiling point	34°C
Melting point	-146°C
Density	0.681 g cm^{-3}
Vapor pressure	53.2 kPa @ 20°C

Figure 5. Chemical and physical properties of isoprene.

Biosphere vegetation is a main source of atmospheric isoprene which is classified as a trace gas. The BVOCEM, MEGAN and LPJ-GUESS models estimate that the global biogenic isoprene emissions are 378 - 496 Tg C yr^{-1} (Arneth et. al., 2011) depending on the emphasis of the model. Isoprene is released also from anthropogenic sources such as a byproduct of oil industry. Roughly half (50%) (Guenther et al. 2012) of the worldwide VOC emissions consist of isoprene. Methane (CH_4) is still the most common as it is usually left out from the VOC budgets.

As a hydrocarbon molecule with two double bonds isoprene is a very reactive specie and it undergoes oxidation reaction in the presence of OH, O_3 or NO_3. Chemical lifetime of isoprene in the presence of OH is 1.7 hours and in the presence of NO_3 is 1.2 hours but in the presence of O_3 is 1.2 days (Holloway and Wayne, 2010). This shows that most likely isoprene is oxidized by OH in daytime and NO_3 during night. Ozone acts as an oxidizing agent if OH and NO_3 are not available although overall 1.2 days is not a long reaction time in the atmospheric conditions. Reaction times also show that almost all isoprene oxidizes rapidly which generally leads to the formation of nitrated or oxygenated compounds. These new compounds have lower vapor pressures than origin products i.e. isoprene and can take part to formation of secondary organic aerosols (SOA) (Vehkamäki and Riipinen, 2012).

The chain structure and two double bonds of isoprene offer total of four potential carbons where e.g. OH can attack in initial step of oxidation. Two of the first adducts are allylic and have two forms of isomers. Hence the first step in oxidation reaction of isoprene can lead to a six different peroxide radicals RO_2 (1.2.). After first step these radicals undergo extension reactions with NO (1.3.), HO_2 or RO_2. Now possibilities for next steps extends. Following equations presents simplified pathways for reaction of Isoprene (RH) and OH to radicals (R) and further reaction to peroxide radical.

$$RH + OH \rightarrow R + H2O \quad (1.1.)$$

$$R + O2 \rightarrow RO2 \quad (1.2.)$$

Peroxide radicals of isoprene as other VOCs participates in cyclic reaction in the presence of NO_x to produce ozone. These cyclic reactions increase concentrations of tropospheric ozone rapidly especially in the areas that receive high volume of solar radiation. Most of the NO_x emissions have anthropogenic origin and therefore the pollution which O_3 and VOCs forms, is typically found from the large cities. Natural photochemical smog is found in places where vegetation emits large quantities of terpenes, for example in parts of California. The peroxide radical reaction with NO produces NO_2 (1.3.) which breaks to NO and one ground state oxygen atom by solar radiation (1.4.). Reactions as follows:

1. Peroxide radical reaction with nitrogen monoxide:

$$RO_2 + NO \rightarrow RO \text{ (alkoxide radical)} + NO_2 \quad (1.3.)$$

2. Nitrogen dioxide reaction with solar radiation:

$$NO_2 + h\nu \rightarrow NO + O \text{ (ground state oxygen)} \quad (1.4.)$$

Produced nitrogen monoxide can now react again with peroxide radical as in reaction 1.3.

3. All ground state oxygen atoms form ozone in practice.

$$O + O_2 + M \rightarrow O_3 + M \quad \text{where catalyst } M = N_2 \text{ or } O_2 \quad (1.5.)$$

When NO_x compounds are not available, other reactions initiates with HO_2 and RO_2 radicals. These reactions do not increase the ozone concentration in the atmosphere and actually can serve as ozone sink. (Holloway and Payne, 2010)

1.3. Research questions

This study examines the effect of cytokine manipulation on BVOC emissions on hybrid aspen Populus tremula x tremuloides and reports the seasonal emission profiles and released carbon via BVOCs from Hyytiälä forest station.

The first part of the study examines the seasonal data which is measured from mature aspen tree Populus tremula in natural growing conditions at Hyytiälä forest station. The goal is to characterize seasonal patterns of emissions and differences between seasons 2010, 2011 and 2013 in natural environment. The study also attempts to analyze the reasons behind the variation of emissions that occur between these years. This thesis will present the seasonal figures from three years collected data of aspens BVOC emission profiles during growing season. Other presented figures are environmental variables such as temperature, light conditions and water conditions which are known to have an effect on BVOC emissions (Günther et al., 1995). The interesting part is also the carbon flow out from the tree in form of BVOCs and trees effectiveness of sequestering carbon from atmosphere via photosynthesis. One goal of this thesis is to calculate how large part of carbon is released back to atmosphere and consumed by BVOC production from CO_2 exchange?

The second part of this study is concentrating to the measurements that were executed in greenhouse at Viikki during two first weeks of June in 2017. The first aim of greenhouse measurements was to examine if the gene manipulated aspen produce BVOC's or not. Then if the emission are detected the next aim was to study the differences between emission profiles and quantities between wild type hybrid aspen (control) and gene manipulated hybrid aspen (cyto+). The gene manipulation pursues to grow the trees faster and produce more biomass for bioenergy production in shorter time. Faster growing biomass is expected also to create an effective and faster sink for atmospheric carbon. The research questions for this part are then set as follows: Is gene manipulated hybrid aspen more effective BVOC producer then control tree? Furthermore how gene manipulation impact on BVOC profile?

2. Materials and Methods

2.1. Seasonal data from Hyytiälä forest station

Seasonal data of BVOC emissions is measured with a PTR-MS which is connected to dynamic chambers. Altogether the measurement equipment includes chamber, sample tubing and gas analyzer. Measurement data is gathered in Hyytiälä SMEAR II field station which located in Southern Finland (61°51'N, 24°17'E, 180 meters above sea level). Station site is located in boreal forest region. The field is proportionately homogenous Scots Pine forest. For this study the data collected continuously during growing seasons in years 2010, 2011 and 2013. Figure 6 gather in table the length of measurements, leaf area, dry mass and mean values of temperature, precipitation and emissions flow.

Year	Chamber type	Chamber number	Measurement start date	Measurement end date	Number of measured days	Leaf area m^2	Dry mass; leaf g	Mean season temperature °C (1.6.-30.9.)	Precipitation mm (1.5.-31.8)	isoprene mean flow rate ng g_{dw}^{-1} s^{-1}
2010	slide	179	4.6.	25.10.	144	0,012	0,62	14.9	327	2.11
2011	slide	184	26.5.	26.10.	154	0,013	0,71	15.2	314	1.35
2013	cylinder	296	1.3.	28.6.	120	0,121	5,1	14.7	262	0.97
2013	slide	197	17.6.	5.8.	50	0,009	0,39	14.7		3.82
2013	slide	199	5.8.	17.10.	74	0,008	0,42	14.7		1.37

Figure 6. Data table from Hyytiälä measurements, showing year, chamber type, start and end dates of measurements, number of measurement days, leaf area and dry mass, mean temperature measured outside the chamber on the mast at 16.8 m high, precipitation between May 1st and August 31st to describe summer time differences and mean flow rate of isoprene.

Hyytiälä seasonal data is measured with a dynamic slide chambers during years 2010, 2011 and 2013. Chambers were situated at south side on treetop of aspen which was chosen for the trial tree at the field station. The shoots in the chambers have stayed the same while leaves renew themselves during the years 2010 and 2011. In 2013 measurements began already in March which is three months earlier than in years 2010 and 2011. In 2013 the first period of the data from March to June was gathered with cylinder chamber. The rest of the year's 2013 data was measured with slide chambers as was in years 2010 and 2011. The chambers were set approximately 1.5 meters below treetop leaving about 16 meters distance to ground. The locations of measurement chambers adapt tree growth each year and therefore are a bit higher (18 m) in year 2013 compared to year 2010.

The chambers were set on the tip of healthy shoots. Most of the time chambers are open and having normal gas exchange with atmosphere. Measurements were done 2 – 3 times in an hour, approximately one measurement between every 20 or 30 minutes. Just before closing the chamber the device measures gas concentrations from ambient air. Then chamber is then closed for 180 seconds during the emission measurement. Right after measurement the chamber opens to normalize the conditions before next measurement.

Slide chamber measuring method is used to measure gas exchange of individual leaves. Slide chamber is a small box of volume 1 dm^3 made of acrylic plastic that is attached on its place on the side from the measured leaf. Leaves are tied between two frames which prevents the free movement. Leaves are set beside each other as much as possible, therefore the shading effect, reducing photosynthetically active radiation, is limited. Otherwise leaves lie in normal natural conditions and can have normal gas exchange with the surroundings. For measuring the emissions from leaves the box slides over the frames and closes. The PTR-MS device measures the gas concentrations from air just before closing the chamber. Right after chamber closing the device measures the gas emissions from the leaves and calculations reflect the change of the concentrations during closure time. Chamber is closed for 180 s at a time in every 20 - 30 minutes for the whole growing season or measuring time. Figure 7 show an open slide chamber and in figure 8 there is a closed slide chamber.

In this study most of the data is from measurements with slide chamber. The data for years 2010 and 2011 are all from slide chamber measurements. Also 2013 data is from slide chamber measurements between June 28th and the end of the growing season.

Figure 7. Open slide chamber. Picture by Juho Aalto.

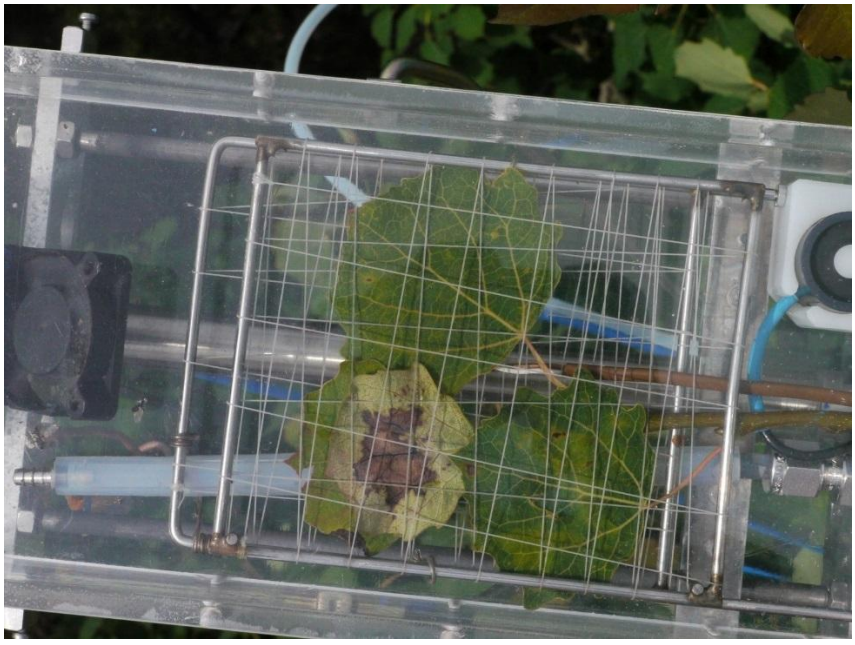

Figure 8. Closed slide chamber. Picture by Juho Aalto.

In spring 2013 from March to 28th June the measured data comes from cylinder chamber measurement (figure 9). Cylinder chamber with volume of 4.5 dm^3 and set into an upright position. In this method leaves are free for movement but wind does not get into the chamber and ventilation solely based to fan and there is a possibility for leaves to create a shade to each other. This shade effect is usually removed from the data with suitable coefficient. The coefficient factor (0.85) was added to this measured dataset by Juho Aalto.

In cylinder chamber the box closes from the foot of the leaf which is opposite direction than in slide chamber. In year 2013 the measurements included emissions from the bud phase and also right after the leaves had flushed. The cylinder chamber were changed to a slide chamber in June because the leaves started to get brownish. The reason for browning leaves remain unknown but it is clear that growing conditions inside the chamber was not favorable during that summer. Also the slide chamber had to move in the beginning of August in 2013 as the leaves got brown color again. The proper data is collected only if the shoot has live leaves in the middle of the growing season.

Figure 9. Open cylinder chamber.
Picture by Juho Aalto.

2.2. Collecting data in greenhouse at Viikki

Greenhouse experiment took place in 5. - 16. June 2017 at Helsinki University Viikki greenhouse estate. The environmental conditions in greenhouse varied from warm to hot, temperature was between 21 – 38 °C. Since the measurement days were in early June there was also light almost 20 hours per day. During some of the days the weather was cloudy which kept the emissions on moderate level but does not show in the structure of emission profiles. The plants were well watered all time.

The measurements were carried out with proton reaction mass spectrometer (PTR-MS) which was combined to manual frame chamber. The inlet flow suction from chamber to PTR-MS was 0.150-0.168 L/min depending on the day. The substitute air flow to chamber that was used to clean the

chambers just before starting the measurement was VOC free air which originated in in zero air generator. During measurements the chambers were open to ambient air. The air flow volume varied between 1.6-2.1 L/min and differences were took into account in calculations. The air flow was different daily and therefore measured every morning. The measuring system may have caused the differences in airflow. One leaf from the lower part of the tree sprout was set in the chamber. The trees were set to adjust in place for 1-3 days before measuring began. The fan and the thermometer was placed inside the chamber. PAR was measured right above the chambers. The area of the leaves and the tree height were measured during time when the trees were handled as it is. Figure 11 summarizes the measurement points, leaf areas and tree heights of the measured trees.

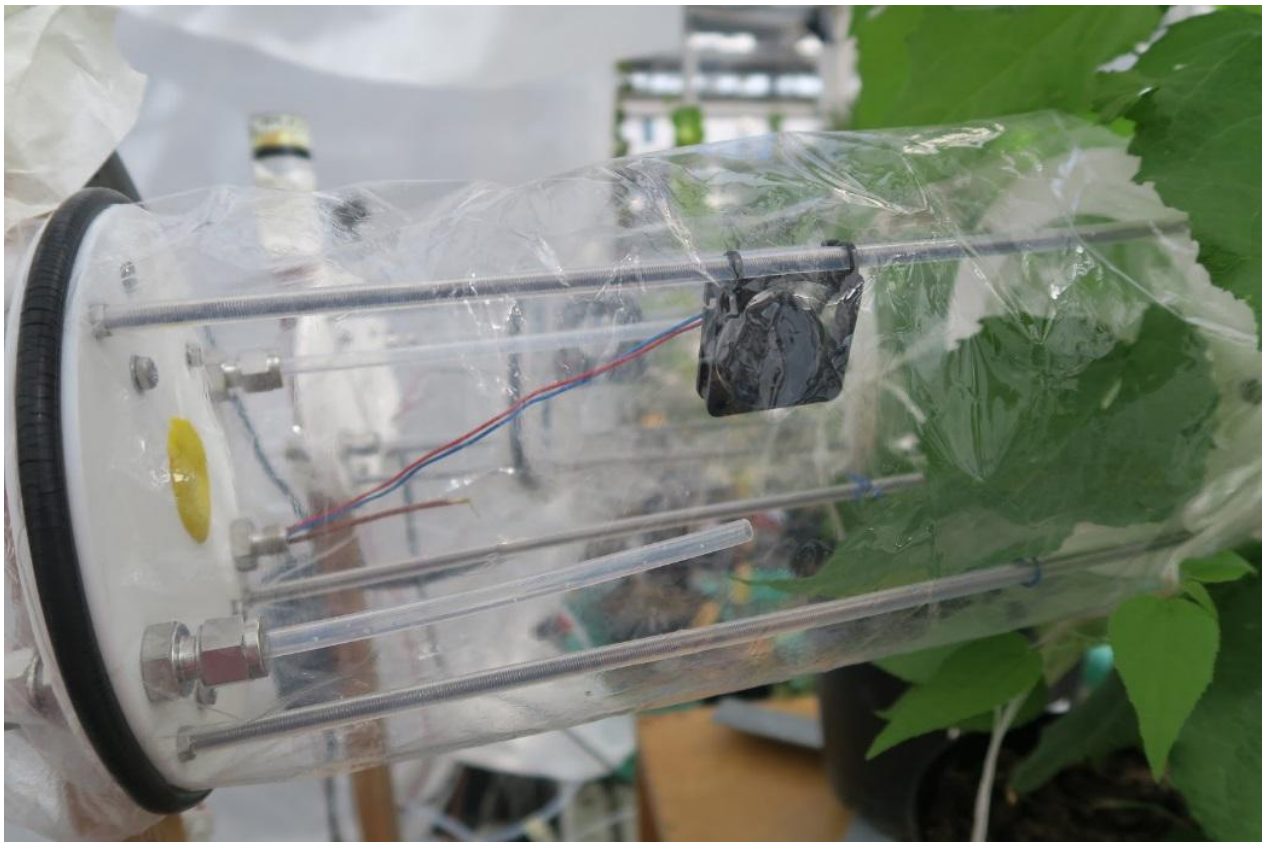

Figure 10. Closed frame chamber from Viikki measurements.

Measurement day	control tree number	control tree height cm	control tree leaf area cm^2	control tree number of measurement points	cyto+ tree number	cyto+ tree height cm	cyto+ tree leaf area cm^2	cyto+ tree number of measurement points
5.6.2017	1	35	110.071	7	1	39	119.161	8
6.6.2017	1	35	110.071	8	1	39	119.161	9
8.6.2017	2	32	135.952	15	2	34	151.035	11
9.6.2017	2	32	135.952	12	2	34	151.035	11
12.6.2017	3	40	109.759	12	3	45	132.360	15
13.6.2017	3	40	109.759	14	3	45	132.360	11
15.6.2017	4	57	170.430	15	4	53	109.759	10
16.6.2017	4	57	170.430	10	4	53	109.759	12

Figure 11. Information table of Viikki measurements: The date of measurement, tree number, tree height, measured leaf area (one side) and measurement points per day.

Figure 12. The measurement setup in Viikki. One leaf attached inside the chamber. One control tree and one cyto+ tree were measured for two days in a row.

The chamber type used in Viikki was frame chambers (figures 10 and 12). The tree branches were set inside the chamber which is closed with transparent Teflon enclosure. The volume of the chamber is approximately 8 L and is equipped with thermometer and inlet and outlet ports. Outside the chamber was placed the photosynthetically active photon flux density sensor. This model is manual operating and therefore effects to quantity of measurement points. With this method the study received 5 - 15 measurement points for each tree during the 8 days of measurements. There was one leaf from lower part of the tree placed inside the frame chamber with operating ventilation inside during measuring. One control tree and one cyto+ tree were measured at a time for two days in a row. All together the measurements were executed for 4 control trees and 4 cyto+ trees.

2.3. PTR-MS (Proton-transfer-reaction mass spectrometry)

PTR-MS is a method for simultaneous real time BVOC measurement in atmospheric conditions. PTR-MS is also used in industry applications and several other processes to detect VOCs from air. This method is particularly effective in measurements of environmental gases in atmosphere because it is highly sensitive. It measures sub ppbv concentrations in seconds. PTR-MS uses a proton donor H_3O^+ ions which does not react with major constituents of air (e.g. N_2, O_2) and it is able to detect very large variety of VOCs. The base of PTR-MS is this specific mass selected reagent H_3O^+ which is ionized from water. This particular H_3O^+ reagent can react continuously and consecutively with analyzed gas. Moreover H_3O^+ reaction with multiple organic molecules is exothermic and fast proceeding which makes it highly effective reagent in such proton donation reactions. Other great benefit is that H_3O^+ is selective to organic constituents found in air and only slightly fragmentation occurs. Consequently PTR-MS is effective even with very low concentrations of VOCs. (Mayhew et al. 2017)

At the starting point of measurements the PTR-MS ionizes water to hydronium ion (H_3O^+) in a hollow cathode. Then hydronium ions are lead to a drift tube reactor where incoming air that contains the BVOCs is driven through the air inlet. A proton transfer reaction occurs in the drift tube reactor in medium vacuum. Overall reaction is:

$$H_3O^+ + R \rightarrow RH^+ + H_2O \qquad (1.6.)$$

Where R represents the BVOC molecule. Other common molecules in ambient air (e.g. N_2, O_2) have lower proton affinity than water therefore they are not ionized in this process. On the other hand

BVOC's have much higher proton affinity than water and they undergo this proton transfer reaction. Ambient air acts only as a buffer gas. (Mayhew et al. 2017)

In the next step the air flow moves toward quadrupole mass analyzer. The mass analyzer has an electrostatic potential which guides molecules only with certain charge through. Then mass analyzer determines the count rates for substances that are proportional to concentrations of $[RH^+]$ and $[H_3O^+]$. Subsequently system parameters such as drift voltage, pressure and temperature are available to calculate average time t. The reaction rate k coefficient can be found from literature and BVOC concentrations can be calculated as follows. Concentrations of protonated BVOCs corresponds to reaction kinetics equation that is a second-order elementary reaction. The rate of the reaction is (Mayhew et al. 2017):

$$-d[H_3O^+]/dt = k\,[H_3O^+]\,[R] \qquad (1.7.)$$

The assumption that $[R] \gg [H_3O^+]$ is valid because R is a neutral gas and becomes a constant in this pseudo first order reaction. Using integration the yield of the reaction is:

$$[H_3O^+]_t = [H_3O^+]_0\, e^{-k[R]t} \qquad (1.8.)$$

The concentration of $[H_3O^+]$ links the concentration of protonated BVOC $[RH^+]$ to equation:

$$[RH^+]_t = [H_3O^+]_0 - [H_3O^+]_t \qquad (1.9.)$$

With rearranging these two above equations gets:

$$[RH^+]_t \approx [H_3O^+]_0\,(1 - e^{-k[R]t}) \qquad (1.10.)$$

A well working approximation for this equation is then:

$$[RH^+]_t \approx [H_3O^+]_0\,[R]kt \quad \text{if} \qquad (1.11.)$$

$$[RH^+]_t \ll [H_3O^+] \approx [H_3O^+]_0 = \text{constant} \qquad (1.12.)$$

Where $[H_3O^+]_0$ is hydronium ion concentration in the begin of reaction and $[RH^+]_t$ is protonated BVOC concentration in the end of reaction. In other words at the beginning the hydronium ion concentration is much larger than the protonated and measured RH^+ ion in the end. Therefore it is reasonable to assume that all BVOCs in the air go through reaction. Moreover the concentration of $[RH^+]$ depends only on the concentration of **[**R] **and** $[H_3O^+]_0$ the latter is now constant and known along with the reaction coefficient k of **[**R] and the reaction time t. One remaining unknown factor

is concentration of [R] in the air and now can be calculated with known parameters. (Mayhew et al. 2017)

The outcome from PTR-MS is a spectrum of measured ion counts (y-axis) as a function of m/z (x-axis) values of the product ions, where m is the mass of the ion and z is the charge of the ion. The charge is always z=1 in PTR-MS method. (Mayhew et al. 2017) This study concentrates only on four masses out of many the PTR-Ms can detect. Aspen's PTR-MS analysis outcome is mostly methanol m/z = 33, acetone m/z = 59, isoprene m/z = 69 and monoterpene m/z= 137.

Figure 13 gives an overall visual outlook of functions of PTR-MS device. The different parts are water inlet, hollow cathode discharge, air inlet, drift tube, quadrupole mass analyzer and secondary electron multiplier. (Mayhew et al. 2013)

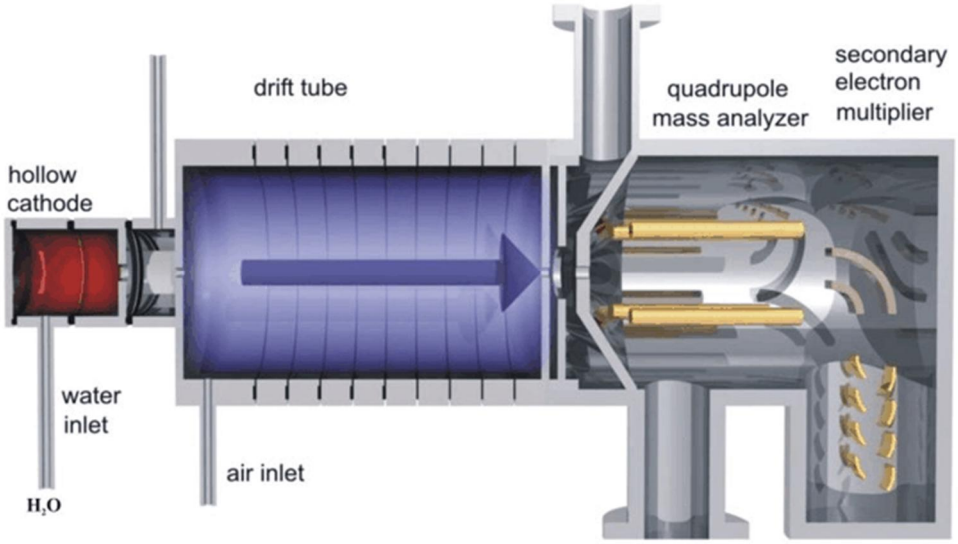

Figure 13. PTR-MS device.

https://www.uibk.ac.at/ionen-angewandte-physik/umwelt/research/pics/animation.gif

Collected PTR-MS data gives concentrations and emissions of protonated BVOC masses. Concentrations are measured from open chamber right before closing it and all measuring time and are presented in parts per billion [ppb] units. The results are expressed as protonated weight mass flux in ng g_{dw}^{-1} s^{-1} per leaf dry mass. Moreover the Hyytiälä data was processed from raw data to ready for analyzing package by Juho Aalto and includes time, two different temperatures,

photosynthetic active radiation, transpiration as water flux, relative humidity, air pressure and both concentrations and fluxes of carbon dioxide. The CO2 fluxes were measured in 2010 and 2011 with URAS 4 infrared gas analyzer (Hartmann and Braun, Frankfurt am Main, Germany) and in 2013 with LI-COR LI-840A infrared gas analyzer (LI-COR, Lincoln,, Nebraska, USA). The data from Viikki was built from separate measurements of temperature, PAR and PTR-MS.

In Hyytiälä the ambient temperature was measured outside the chamber and added to dataset by Juho Aalto and second temperature is from inside the chamber. In Viikki the temperature was measured from inside the chamber. All temperatures are presented in celsius degrees. The slide chamber measured photosynthetic active radiation inside and cylinder and frame chambers measured PAR outside. Some gaps in the PAR data from Hyytiälä data have been filled with values from another PAR sensor nearby (PAR_{gp}). Gap filled PAR_{gf} is added the missing values from other PAR detector nearby. In partly cloudy days may be variation between another detector (PAR_{gf}) and chamber (PAR). In sunny and cloudy days this gap filling method works well. The unit for PAR flux is presented in $\mu mol\ m^{-2}\ s^{-1}$.

In Hyytiälä data the transpiration is a measure of all-sided leaf area of H_2O flux. The transpiration is presented in $mg\ m^{-2}\ s^{-1}$. The CO_2 exchange is a measure of all-sided leaf area where negative sign stands for efflux and the unit is $\mu g\ m^{-2}\ s^{-1}$. Relative humidity was calculated from water vapour concentration inside the chamber. Air pressure unit is hPa. Data include gaps because of measurement system malfunction, maintenance breaks and other technical reasons.

Soil water potential was received from open data AVAA portal Smart Smear.

In Viikki measurements the leaf mass was weighted from another set of trees than measured was made. Six leaves per cyto+ and six leaves per control tree was collected and calculated the average weight. All leaves that were measured were also photographed and their area were determined with Image J program.

2.4. Emission model

Alex Günther (1997) has developed an equation which can be used to estimate atmospheric large scale emissions from plant foliage vegetation. In this study the model is run to normalize all the fluxes from different measurements to achieve comparable results both seasonal scale and tree scale to see differences between control trees and cyto+ trees. The model includes four factors

which affect BVOC emissions. There are instantaneous factors such as photosynthetic active radiation (PAR) and temperature (T) and emission activity factor γ decribes their effect. Landscape variations accounts as other factors. These are foliar density, D, and landscape average emission potential, ε. The last part of the equation is emission activity factor δ to account for longer than >1 h term controls. The equation of emission flux, F, is then:

$$F = \varepsilon D \gamma \delta \qquad (1.13.)$$

Where γ and δ are nondimensional parameters. Foliar density D is given in grams dry mass per square meter (g m^{-2}) and emission potential ε is in micrograms per gram per hour ($\mu g\ g^{-1}\ h^{-1}$). (Günther 1997) Since this study concerns leaf level emissions and is based on measured data the foliar density and landscape average emission potentials are left out from the comparison built.

PAR and temperature affect directly the diurnal and seasonal variations of isoprene emissions. These two factors controls emissions in two different regimes according to Günther et al. (1999) and according to Rasulov et al. (2009), however, PAR and T together control the intensity of emissions which seems to be more precise description. First isoprene emissions are controlled by volatilization of stored compounds. Secondly isoprene production itself controls produced emissions. These variations can be calculated with following emission activity factor γ equation by Günther et al. (1991, 1994):

$$\gamma = \left[\frac{\alpha\, C_{L1}\, L}{\sqrt{1+\alpha^2\, L^2}}\right]\left[\frac{\exp\left(\frac{C_{T1}\,(T-T_S)}{R\,T_S\,T}\right)}{C_{T3}+\exp\left(\frac{C_{T2}\,(T-T_M)}{R\,T_S\,T}\right)}\right] \qquad (1.14.)$$

Where variables in equation are following:

L = PAR in μmol photons $m^{-2}\ s^{-1}$

T = leaf temperature in Kelvin, chamber temperature represents leaf temperature in this study

T_s = leaf temperature in standard conditions in Kelvin, ambient temperature represents this variable in calculations of this study

R = 8,314 $JK^{-1}\ mol^{-1}$

α = 0,0027

$C_{L1} = 1{,}066$

$C_{T1} = 95\,000\ J mol^{-1}$

$C_{T2} = 230\,000\ J mol^{-1}$

$C_{TM} = 314\ K$

$C_{T3} = 0{,}961$ rather than 1

The above algorithm is applicable when it is assumed that emissions are controlled by isoprene production. Another equation is useful if assumed that emissions are controlled by volatilization of stored products and Günther et al. proposal is then:

$$\gamma = \exp(\beta [T - Ts]) \qquad (1.15.)$$

Where β is an empirical coefficient $0.09°C^{-1}$. This study is counting on the fact that isoprene emissions are controlled by PAR and temperature during growing season in site so it neglects the equations for stored products.

Emission activity factor δ is based on seasonal variations of isoprene emissions. Many research studies (Günther et. at., 1997) have become to conclusion that during winter the isoprene emissions are almost zero which is seen in this study as well.

Günther algorithm describes general seasonal behaviour as follows:

$$\delta = 0 \qquad \text{if } J < J_0 \text{ or } J > J_0 + J_d \qquad (1.16.)$$

$$\delta = \sin([J - J_0] / J_d) \qquad \text{if } J_0 < J < J_0 + J_d \qquad (1.17.)$$

Where

J= current day of the year

J_0 = the date of annual onset of isoprene emission

J_d = the duration of isoprene emission in days

In this study the general seasonal behavior of Günther algorithm is compensated with more accurate calculations of emission activity factor for 5 days periods during the growing season based on the measured values and this general description of δ is left out. The activity factor was

calculated as follows: 1) Night time data was excluded. 2) The daytime data was divided into five days periods. 3) Then calculations follow equation 1.14. to get activity factor γ for each period. 4) Last, the measured emission is multiplied with activity factors γ to receive normalized emission rates.

3. Results and discussion

3.1. Seasonal BVOC emissions from Hyytiälä measurements

The data of year 2011 is not as solid as 2010 data. There is a gap in 2011 during weeks from middle July to middle August in emissions. In year 2013 the data was collected with two different chambers from 3 different shoots on a tree. In figures 30, 36 and 39 each measured dataset is marked with different color. On the results of BVOC emissions are normalized and therefore presented with same color. The daytime emissions are only presented as the night time emissions are close to zero.

Figure 14 shows isoprene emissions from season 2010 which were high and showing two peaks up to almost 90 ng g^{-1} s^{-1} in first half of July and seasonal average is 4.88 ng g^{-1} s^{-1}. Isoprene emission profile indicate that young and developing leaves emit none or very little isoprene. Once leaves are mature enough the isoprene emissions grow rapidly toward highest point. The seasonal profiles refers the fact that isoprene synthesis initiates after leaves have reached some particular point in their development. In year 2011 the isoprene emission peak is below 70 ng g^{-1} s^{-1} and does not reach similar levels than 2010, average being 2.89 ng g^{-1} s^{-1}. In August 2011 isoprene emissions drop to low levels approximately below 10 ng g^{-1} s^{-1}. These results show that in year 2013 isoprene emissions (figure 16) were lower than in years 2010 and 2011 with average 0.17 ng g^{-1} s^{-1} and most of the seasonal isoprene emissions remain under 4 ng g^{-1} s^{-1}. This large difference may originate from the differences between the measurements or the fact that the chosen leaves were not capable to stay green in the chambers during the measurements.

In 2010 the isoprene emissions start to grow rapidly on June 20th. In 2011 isoprene emissions also start to grow rapidly again on 20th June reaching their peak soon after. This is shown in figures 17 and 18 which compare isoprene emissions against PAR June 14th, 20th and 28th. These days represent the times very close to summer solstice and 7 days before and after. The days were chosen to look into emissions with similar day length and similar light conditions. The emissions

are close to zero on June 14th but start to grow on June 20th and have grown much on June 28th. This is clearly the point when the leaves reaches their mature phase.

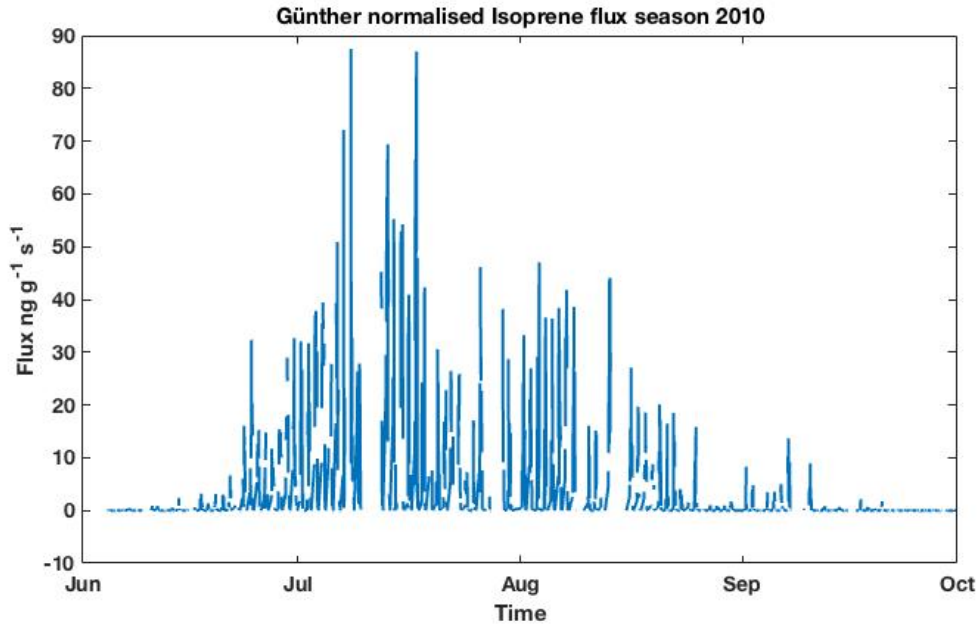

Figure 14. Seasonal daytime (June – October) isoprene emissions, year 2010.

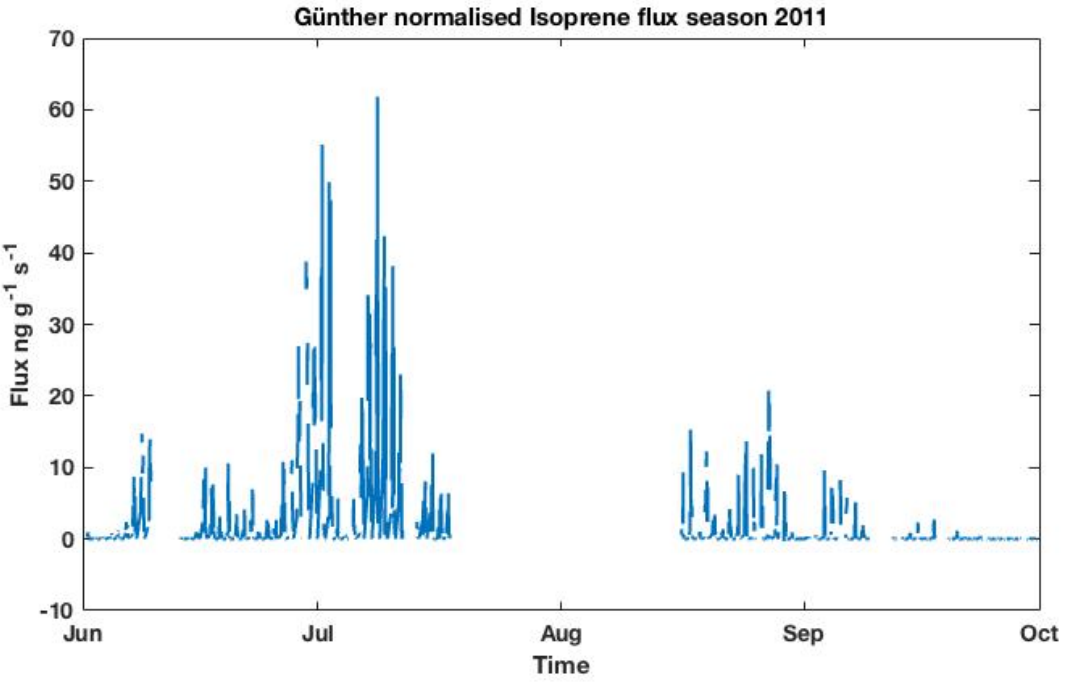

Figure 15. Seasonal daytime (June – October) isoprene emissions, year 2011.

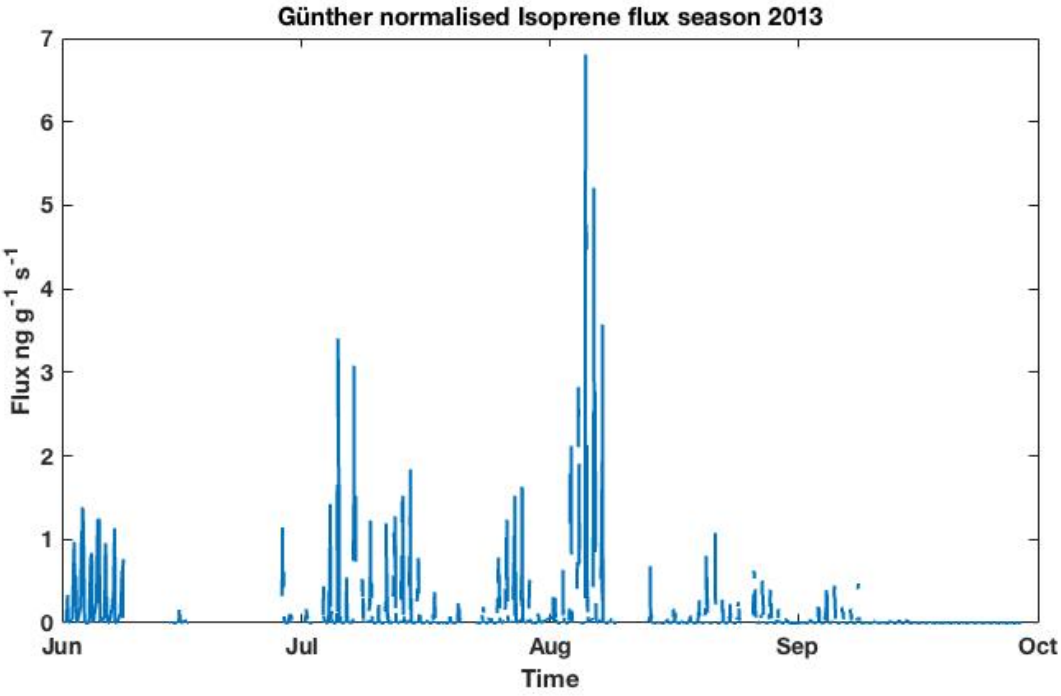

Figure 16. Seasonal daytime (June – October) isoprene emissions, year 2013.

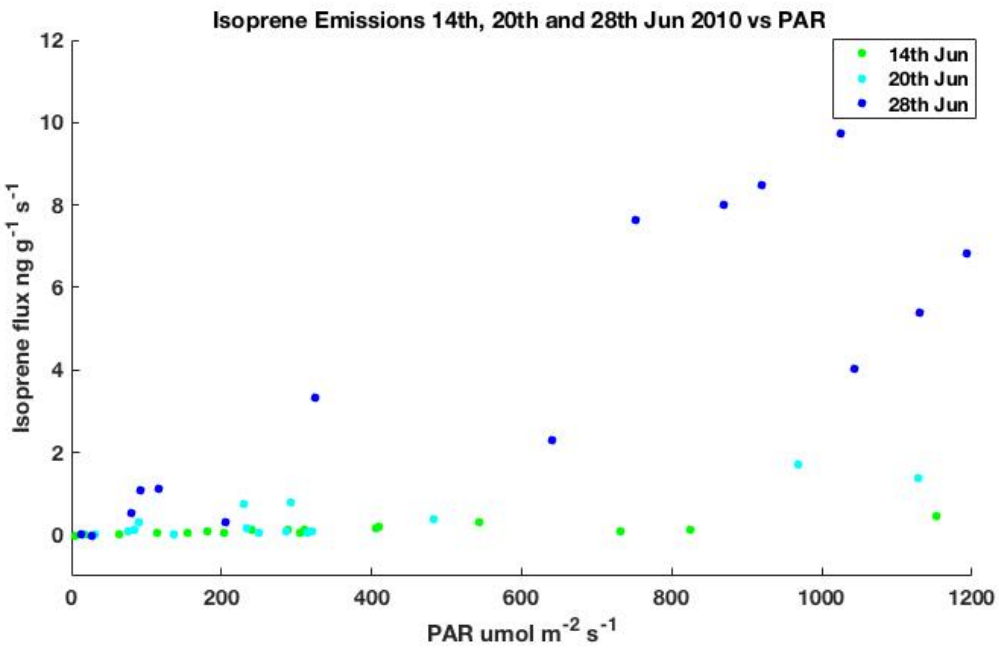

Figure 17. Daytime isoprene emissions in year 2010 against PARon June 14, 20 and 28th.

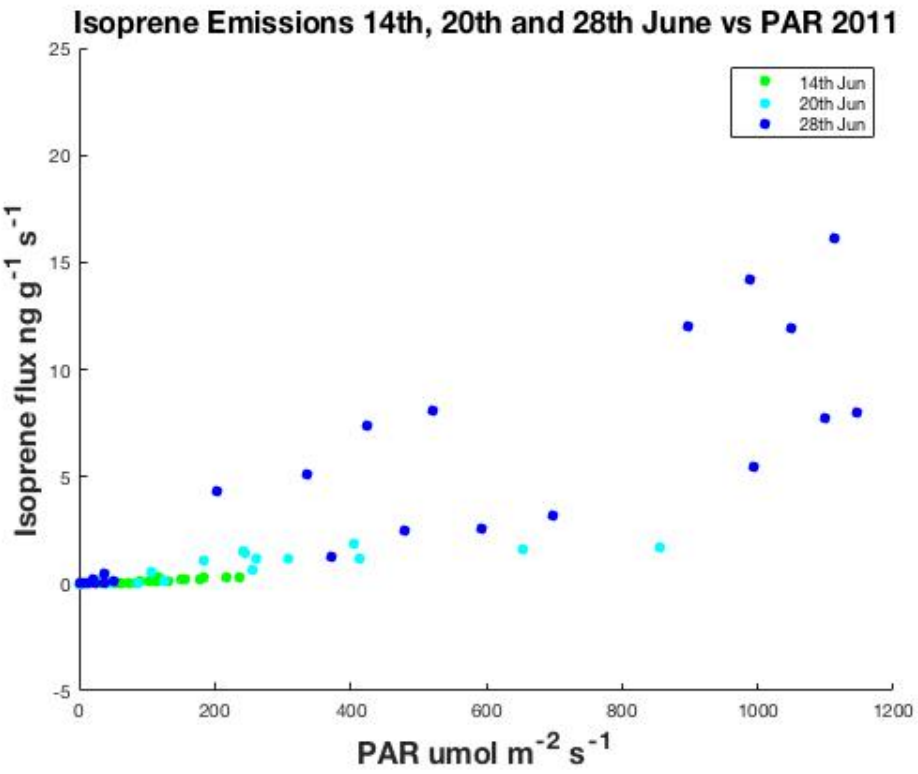

Figure 18. Daytime isoprene emissions in year 2011against PARon June 14, 20 and 28th.

In year 2010 methanol flux is the largest in June (figure 19), nearly 3 ng g^{-1} s^{-1} when leaves are in developing phase. Methanol flux decreases to less than 1 ng g^{-1} s^{-1} level in the end of June at the same time with growth of isoprene flux. The seasonal average in 2010 was 0.14 ng g^{-1} s^{-1}. The 2010 methanol figure starts from June 23rd as raw data included many negative values which suggest that the fan has not worked properly and has also affected acetone results but not isoprene. Negative values on methanol fluxes is outcome of condensed water inside the chamber and solubility of methanol to water therefore some data is removed.

In year 2011 methanol emission (figure 20) shows similar pattern of emissions than 2010. At the developing phase of the leaves methanol emissions are at their highest at 2.5 -3.5 ng g^{-1} s^{-1}. The methanol emissions decrease when isoprene emissions grow and seasonal average 0.07 ng g^{-1} s^{-1} is showing lower level than 2010 for the season.

In 2013 normalized methanol emissions (figure 21) do not show clear similar pattern for emissions to be highest in June and then decreasing when leaves are reached mature phase. The seasonal

methanol emissions stayed at a level between 0 – 0.33 ng g^{-1} s^{-1} and the average value was as low as 0,009 ng g^{-1} s^{-1}. Methanol is showing a peak in early days of August. The results are in line with isoprene results since the methanol emissions are similarly lower than in 2010 and 2011. The beginning of June is different showing almost zero emissions which is probably the result of different method used at that time.

Similar dynamic chamber system is known to underestimate the methanol emissions 5-30% which is good keep in mind when reading the results (Kolari et al. 2012). Kolari et al. (2012) have studied the accuracy of chamber measurements and concluded on the concern of isoprene emissions that they are similar in shape as theoretical dynamics and follow the predicted line but the concentration never reached the steady-state concentration. This method is based on the rate of change of concentration right after the chamber is closed. They also showed that with rising relative humidity the systematic errors rise as well. Underestimation of fluxes is usually depending on the chamber, but the length and the contamination of the sample tubing may also affect the results. Chamber closure time is important factor to achieve reliable results and therefore 180 seconds is a good compromise. Kolari et al. (2012) concludes that chamber measurements offer a system for analyzing BVOCs with good accuracy.

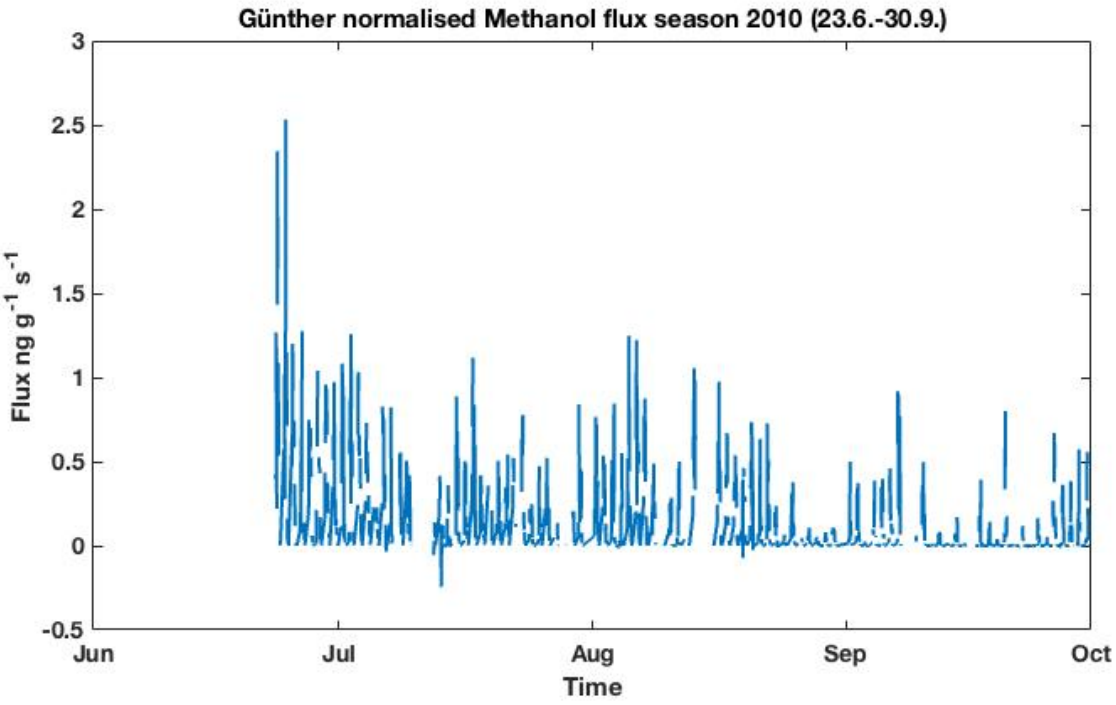

Figure 19. Seasonal daytime (June 23rd – October) methanol emissions, year 2010.

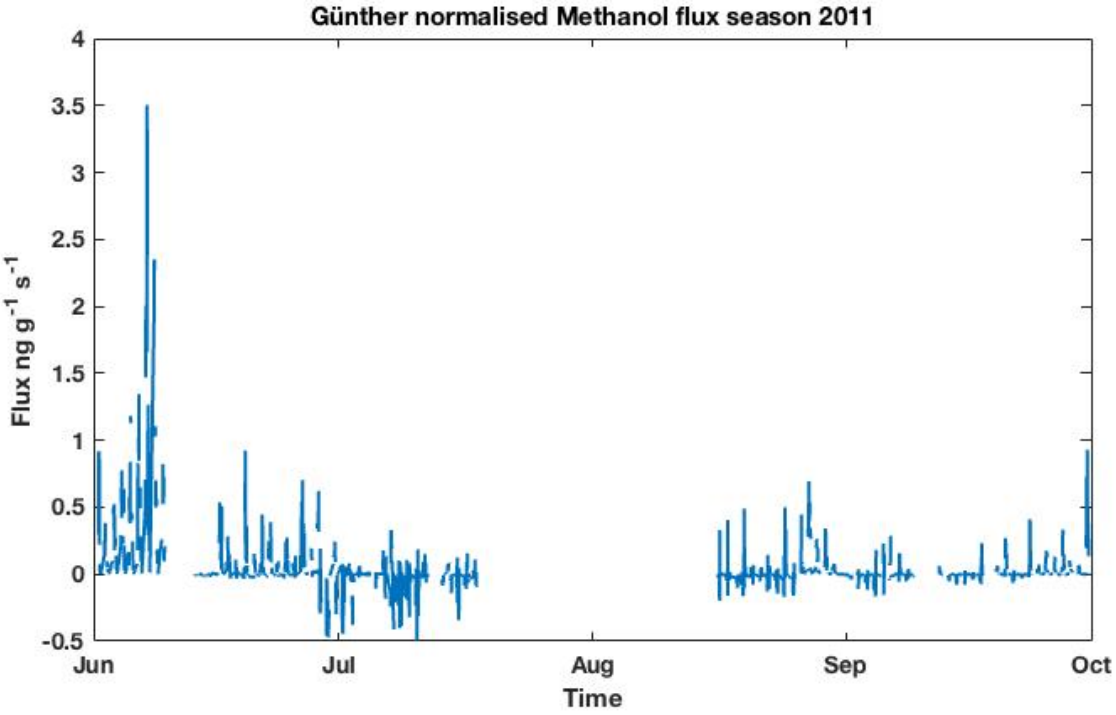

Figure 20. Seasonal daytime (June – October) methanol emissions, year 2011.

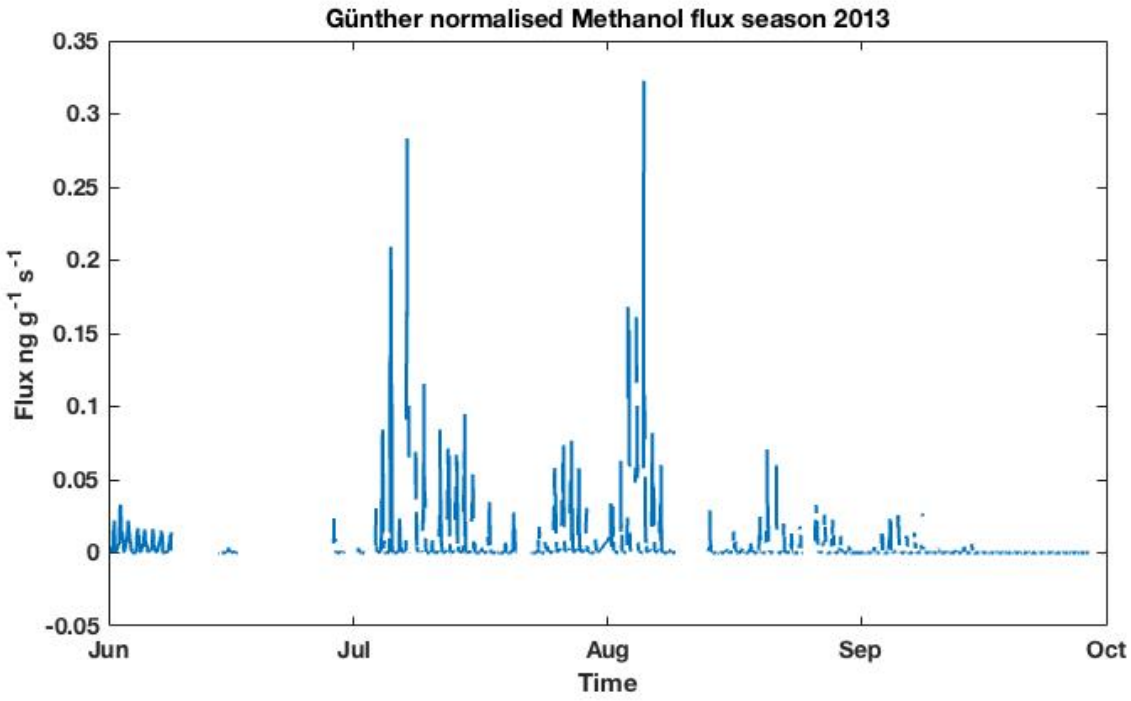

Figure 21. Seasonal daytime (June – October) methanol emissions, year 2013.

Aspen has low monoterpene emissions. In year 2010 the monoterpene emission average is 0.18 ng g^{-1} s^{-1} which is presented on figure 22. There is one observable peak in the mid July at same time with high isoprene emissions. In 2011 monoterpenes (figure 23) show higher peaks in emissions in first half of June which was not seen in 2010. Altogether the 2011 seasonal emissions remaining considerably lower levels than in 2010 in whole and average being 0.13 ng g^{-1} s^{-1}. Monoterpene emissions in year 2013 (figure 24) were similarly much lower than isoprene and methanol but in line with years 2013 results having average at 0.004 ng g^{-1} s^{-1} level.

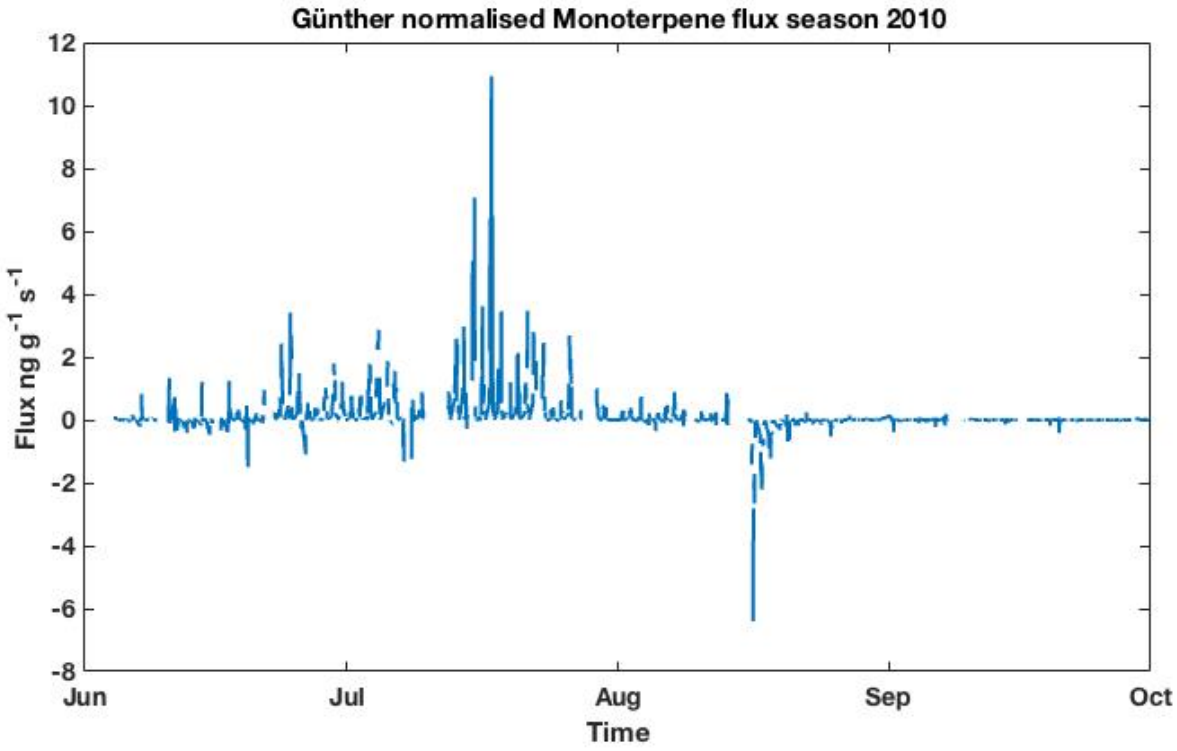

Figure 22. Seasonal daytime (June – October) monoterpene emissions, year 2010.

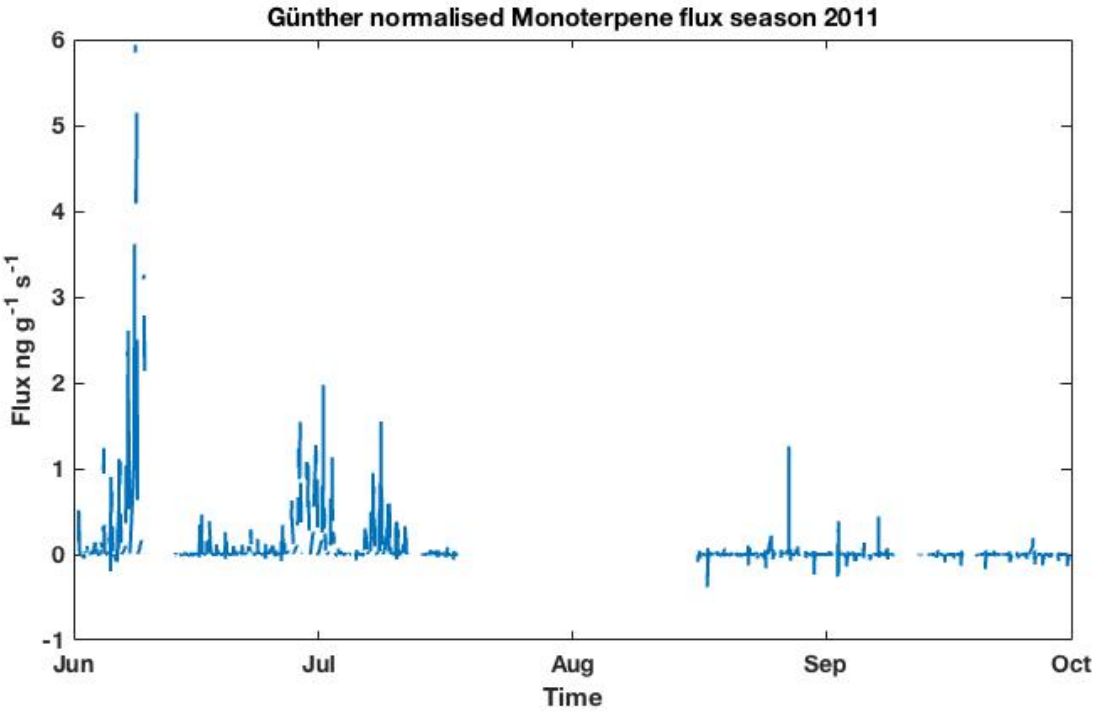

Figure 23. Seasonal daytime (June – October) monoterpene emissions, year 2011.

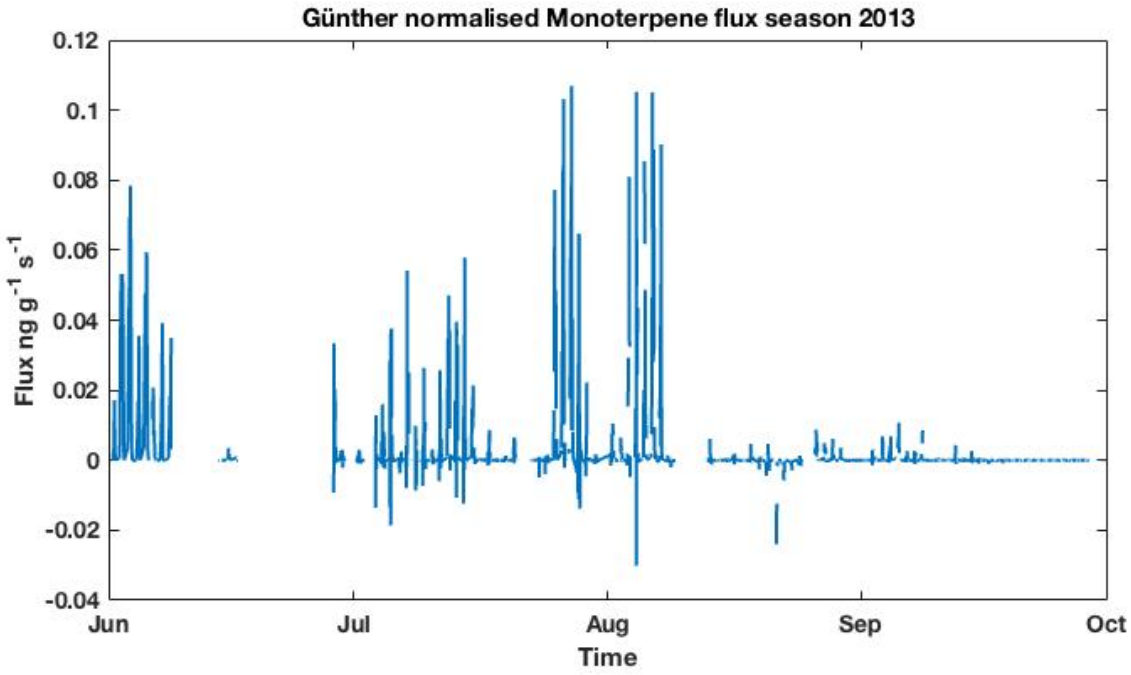

Figure 24. Seasonal daytime (June – October) monoterpene emissions, year 2013.

In year 2010 the seasonal acetone emissions remain lower than 2.5 ng g^{-1} s^{-1} with average being 0.23 ng g^{-1} s^{-1} and emissions remain at same level through the whole growing season (Figure 25). In 2011 acetone (figure 26) show higher peaks in emissions in first half of June which was not seen in 2010. Seasonal emissions remaining considerably lower levels than in 2010 in whole and average is 0.12 ng g^{-1} s^{-1}. In year 2013 acetone emissions (figure 27) are also showing low emissions with average 0.02 ng g^{-1} s^{-1}.

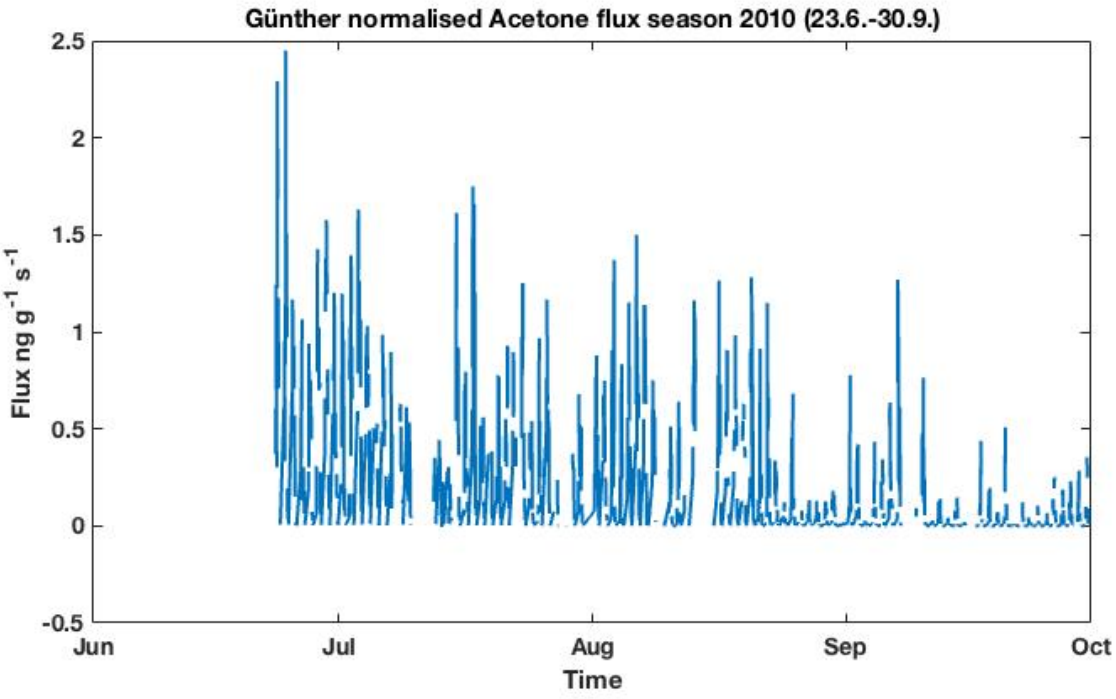

Figure 25. Seasonal daytime (June – October) acetone emissions, year 2010.

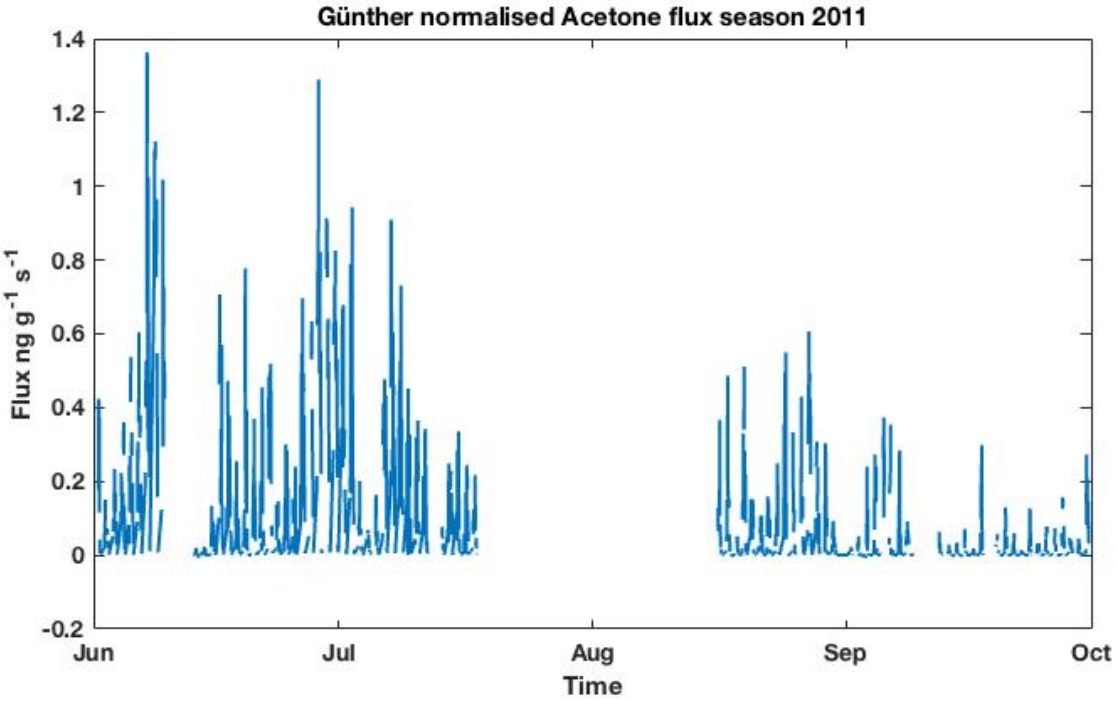

Figure 26. Seasonal daytime (June – October) acetone emissions, year 2011.

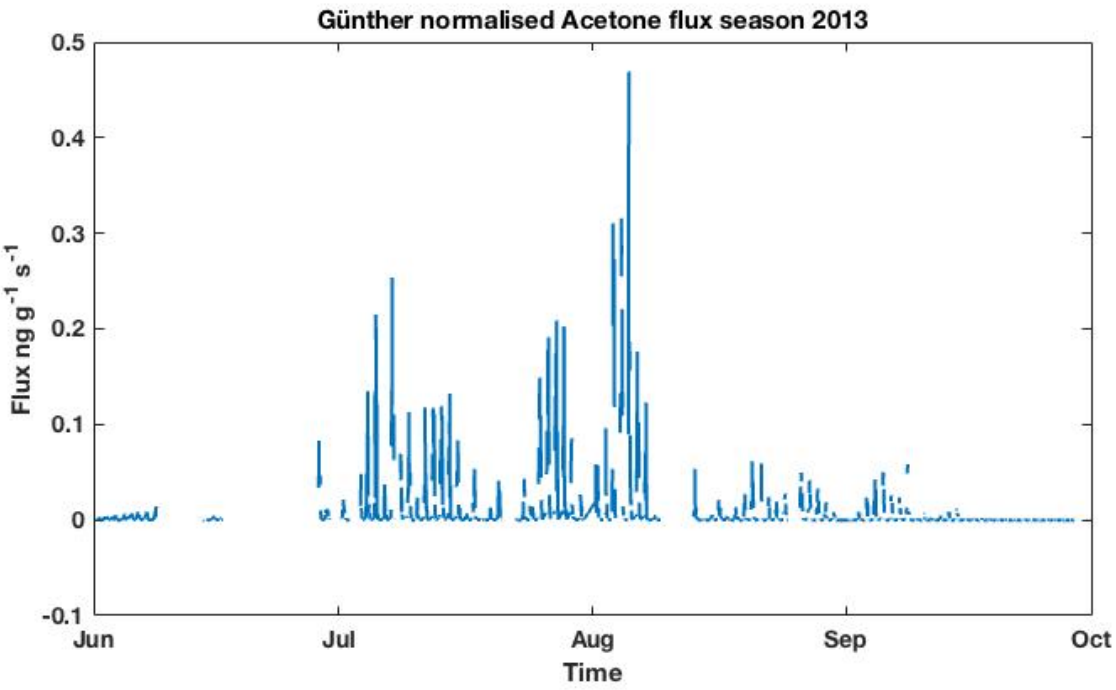

Figure 27. Seasonal daytime (June – October) acetone emissions, year 2013.

The data of this study also displays zero emissions during winter based on year 2013 where chamber was set up in March at Hyytiälä and showing almost zero emissions before growing season started. Emissions start to grow quickly at begin of the season and more precisely when the leaves are mature enough and reaches the maximum emission level rapidly in the beginning of July according to data of this thesis. Right after maximum they begin to decrease and reaches zero before end of the season. Data of this study shows this seasonal pattern very clearly. Maximum is reached in the end June or latest at the beginning of July. Therefore emission activity factor changes daily depending on the date of the growing season. In this study the emission factor is calculated for 5 days periods.

3.2. Seasonal environmental conditions during Hyytiälä measurements

Measured seasonal changes in environmental conditions show exceptionally hot and dry summer in year 2010. During the high summer season (July) with fully developed mature leaves the daytime temperature stayed above 20°C and many occasions peaked above 30°C (figure 28). Growing season

temperature in 2011 (figure 29) is moderate, the average daily highest temperature remaining closer to 20°C than 30°C even during the normally hottest month July. Temperature (figure 30) was moderate staying well around 20°C or less during the growing season in year 2013. During season 2013 the temperature rose above 30°C only in two days at the end of June.

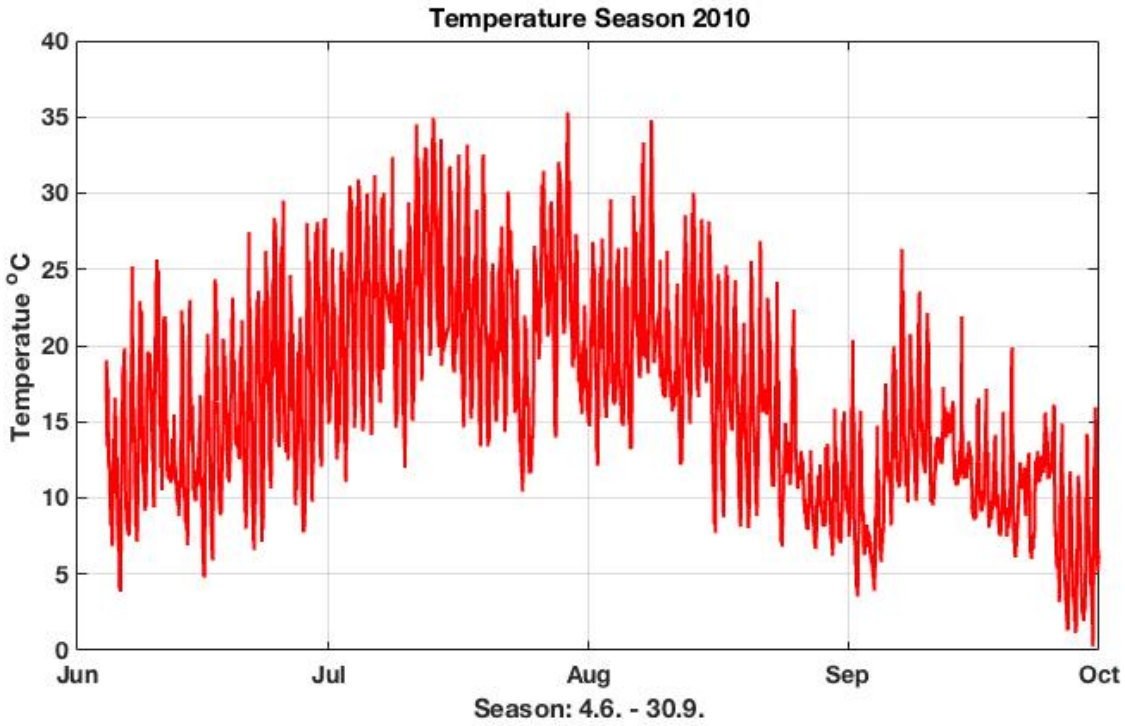

Figure 28. Seasonal (June – October) variation in temperature, year 2010.

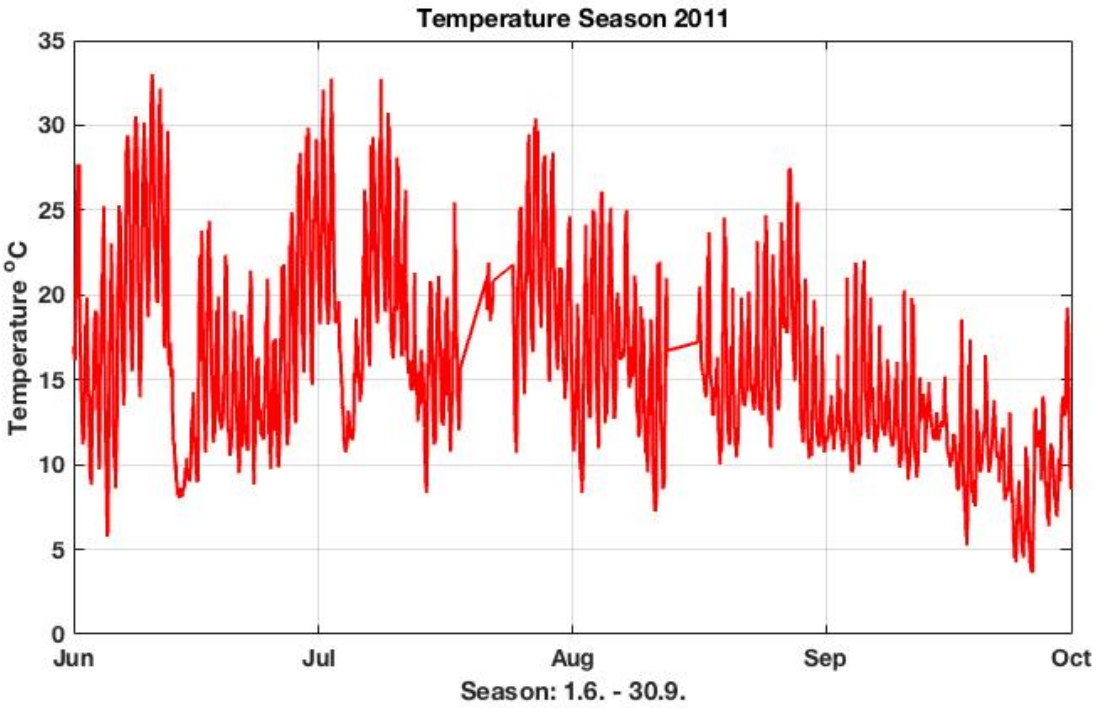

Figure 29. Seasonal (June – October) variation in temperature, year 2011.

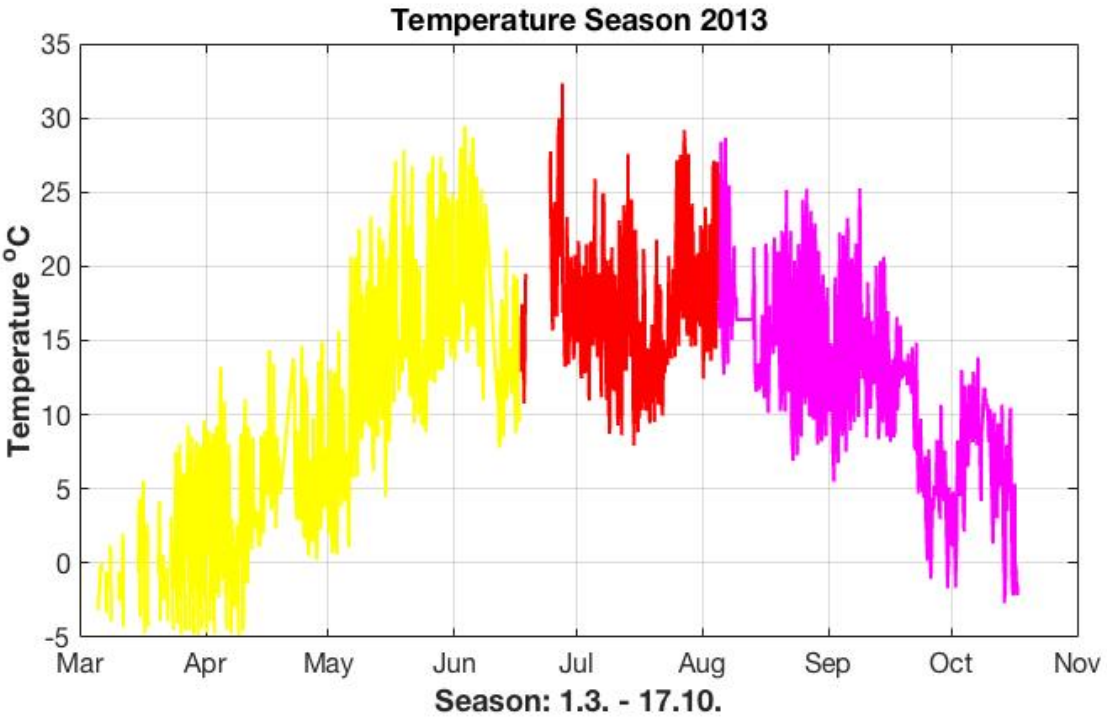

Figure 30. Seasonal (March – October) variation in temperature, year 2013. Different colors showing the measurement periods for each different chamber. Measurement time with cylinder chamber is yellow. First period with slide chamber measurement is red and moved slide chamber the second period is magenta.

In year 2010 soil water content in horizon A decreased in June and July reaching very low level in turn of August and remained under 0.2 m^3 m^{-3} the whole month until middle September (figure 31). This lack of water is weakly seen in water flux (figure 34). The water flux decreases rapidly in the end of August and remains low in September. In year 2011 soil water content (figure 32) decreases below 0.1 m^3 m^{-3} at beginning of August but rises already in middle August. Also the soil water content data is very incoherent in 2011 and only the big picture of the seasonal change is weakly interpreted. Measured H_2O flux (figure 35) is at its maximum 50 mg m-2 s-1 in first weeks of July and decreases steadily reaching low levels in September. Over all the 2011 H_2O flux is lower than in year 2010 despite the fact that soil water content is higher but the temperature stays lower than 2010.

In year 2013 soil water content (figure 33) is in lower level already in the beginning of season than in years 2010 and 2011. Soil water content start to decrease in early July and reached the lowest point just in early days of August being nearly as low as 0.06 m^3 m^{-3}. The water flux shows peaks in middle June and in the end of July and also at the beginning of August (figure 36) the maxima coincide with peaks in the temperature. The water flux shows slightly different levels with each different chamber systems but being mostly between 10 - 30 mg m^{-2} s^{-1} during the growing season. The peak in May should be ignored due to the measurement failure that continued for couple of weeks in May.

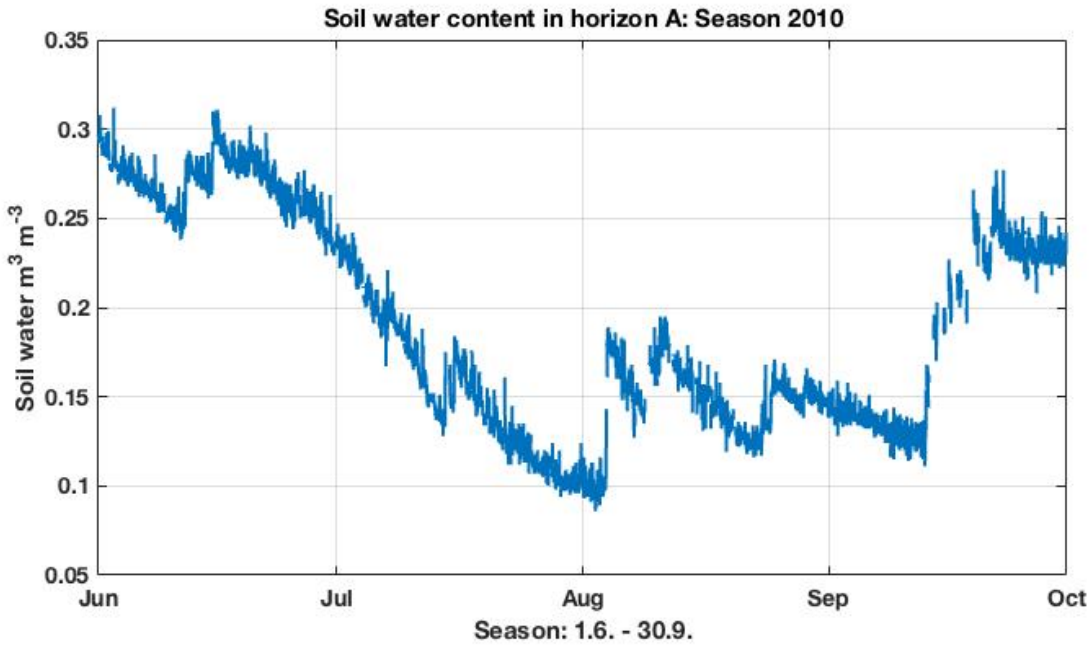

Figure 31. Seasonal (June – October) variation in soil water content in horizon A, year 2010.

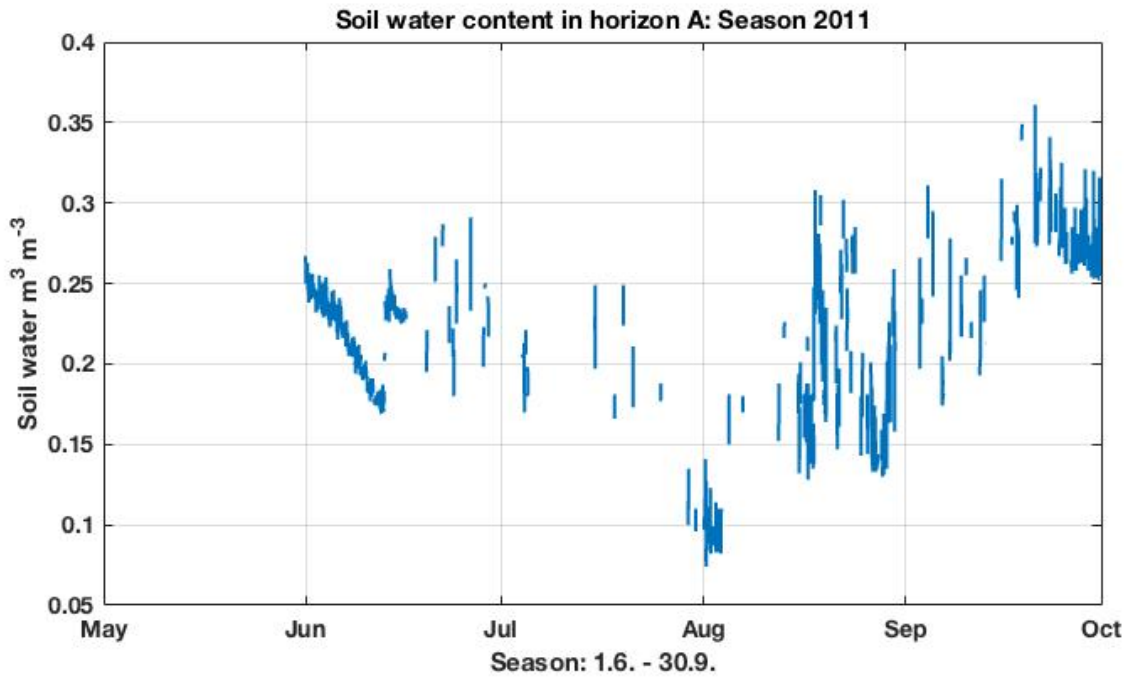

Figure 32. Seasonal (June – October) variation in soil water content in horizon A, year 2011.

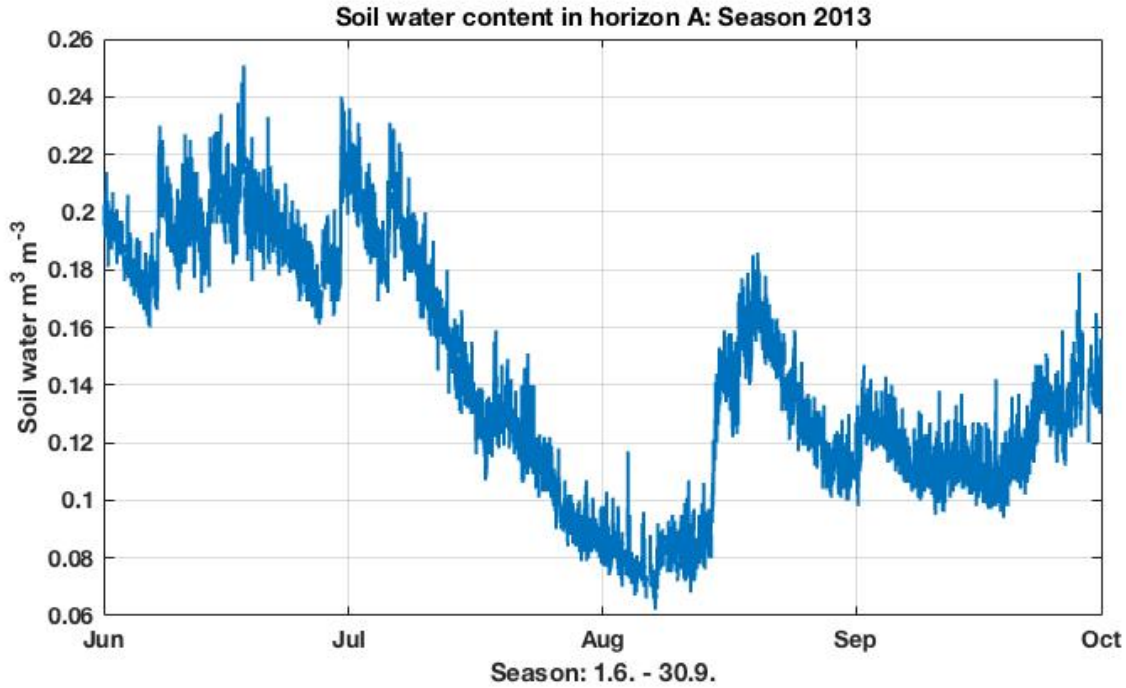

Figure 33. Seasonal (June – October) variation in soil water content in horizon A, year 2013.

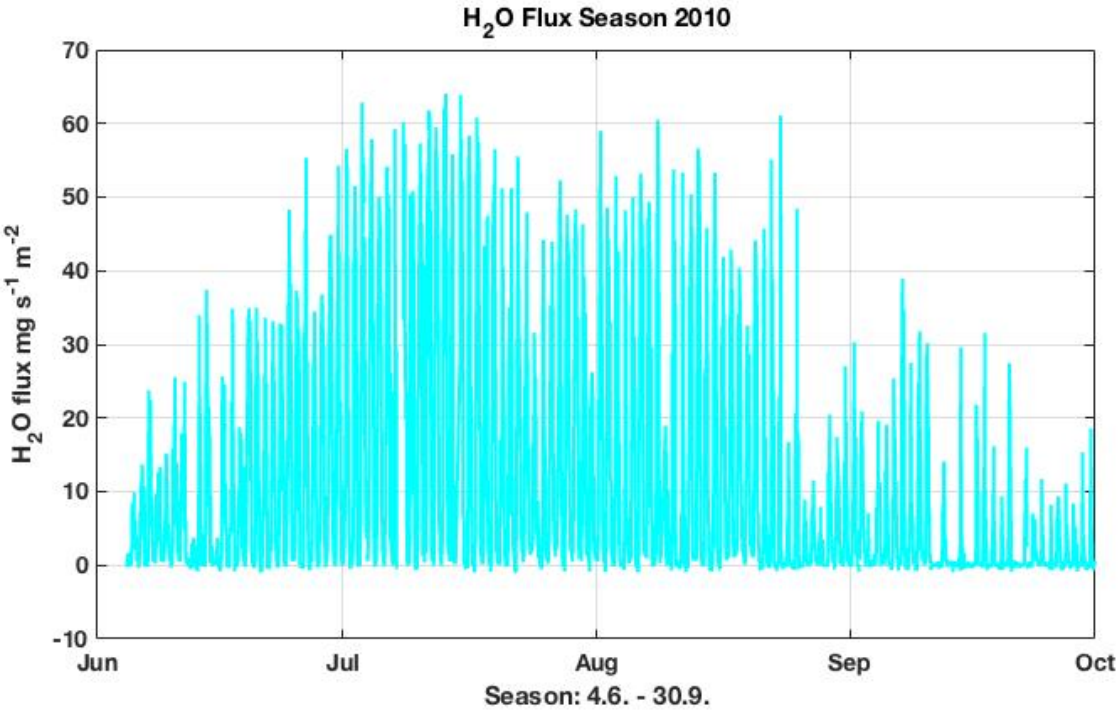

Figure 34. Seasonal (June – October) variation in H_2O flux, year 2010.

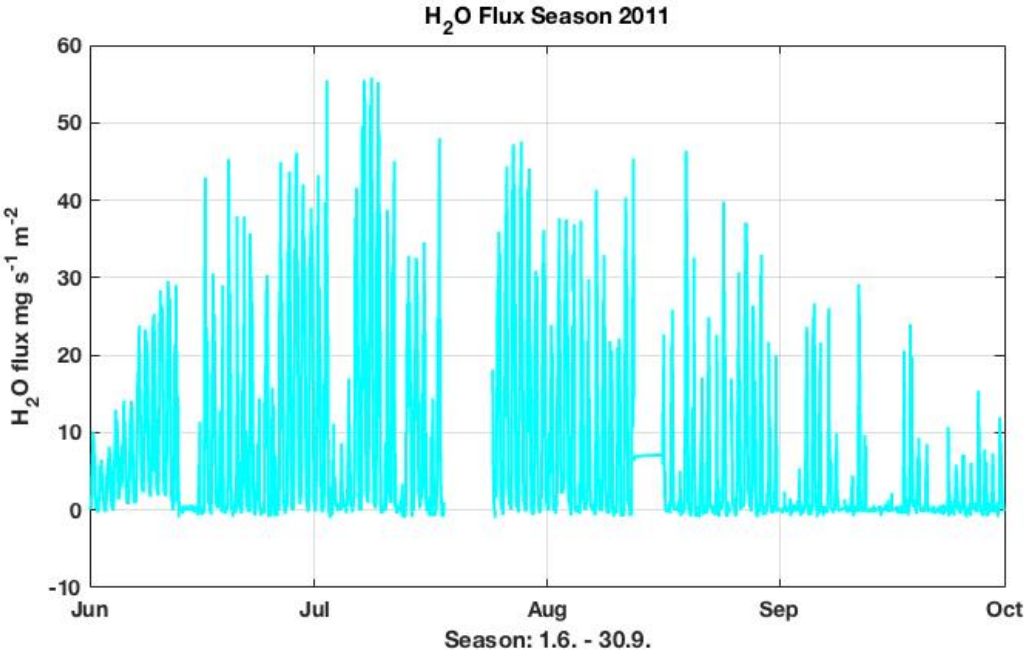

Figure 35. Seasonal (June – October) variation in H_2O flux, year 2011.

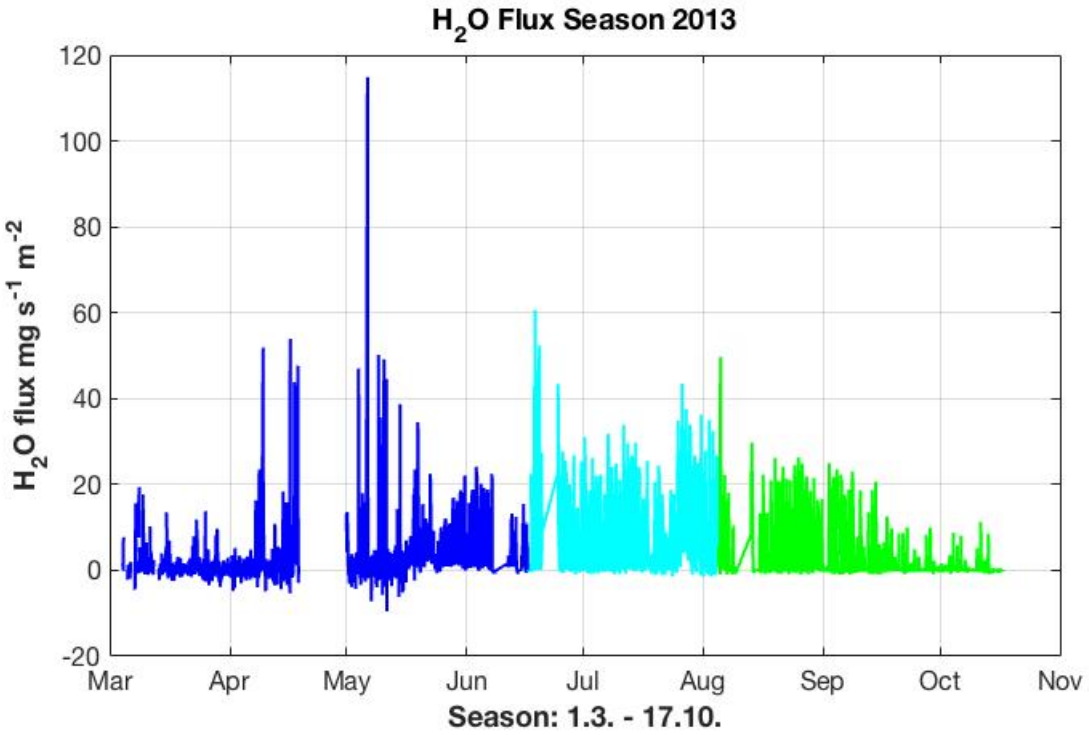

Figure 36. Seasonal (March – November) variation in H_2O flux, year 2013. Different colors showing the measurement periods for each different chamber. Measurement time with cylinder chamber is blue. First period with slide chamber measurement is cyan and moved slide chamber the second period is

In the end of August leaves have reached the old age and all fluxes decreases rapidly while only CO_2 emission levels remains in moderate level. For the whole season 2010 carbon dioxide exchange was at the highest compared to 2011 and 2013, almost reaching 450 $\mu g\ m^{-2}\ s^{-1}$ in the first half of July, remaining high and the daily highest point being > 250 $\mu g\ m^{-2}\ s^{-1}$ until middle August (figure 37). The CO_2 flux in year 2011 (figure 38) remains lower level, showing peaks at 350 $\mu g\ m^{-2}\ s^{-1}$ in first half of July. CO_2 flux is approximately closer 150 $\mu g\ m^{-2}\ s^{-1}$ during the intense growing season. CO_2 flux (figure 39) is lowest in 2013 from measured years staying below 200 um $m^{-2}\ s^{-1}$ for the whole season. CO_2 timeline present one very high peak in the early August which strikes simultaneously with temperature and H_2O peaks and also with BVOC peaks.

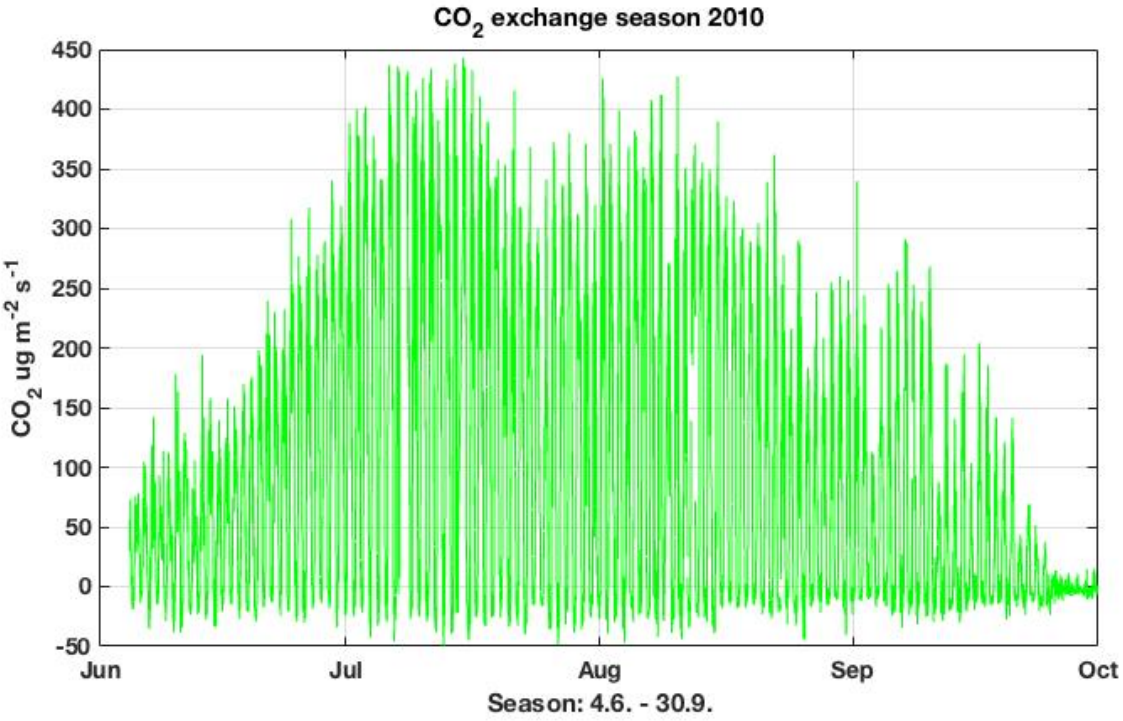

Figure 37. Seasonal (June – October) variation in CO_2 flux, year 2010.

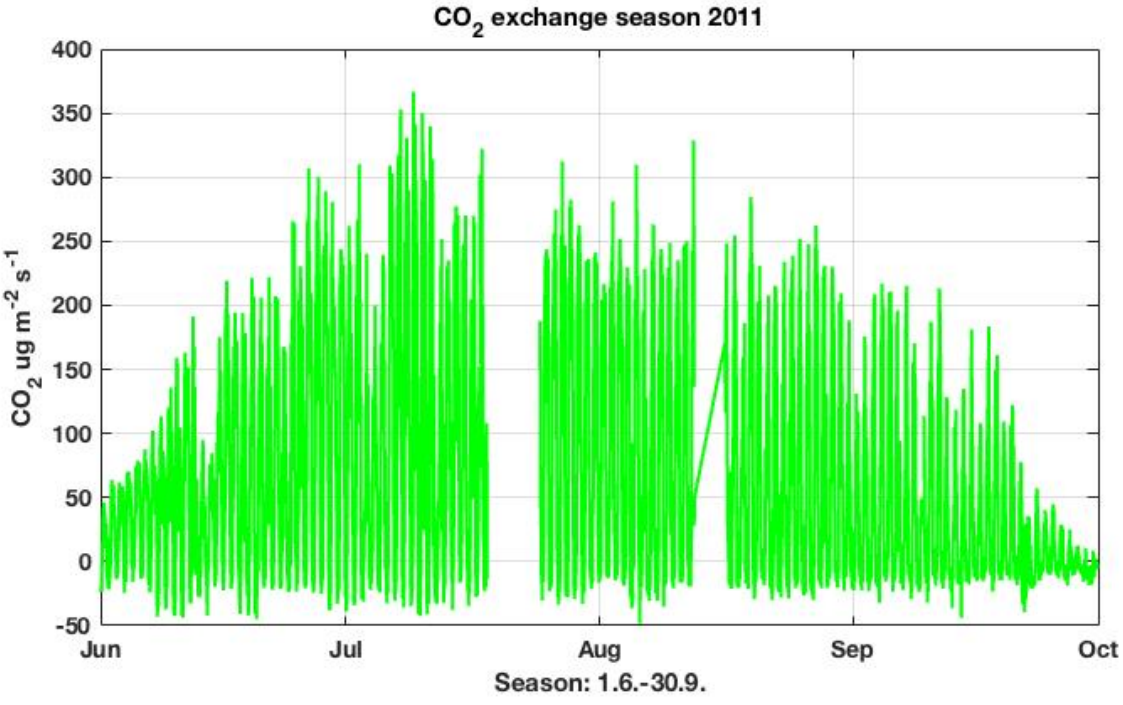

Figure 38. Seasonal (June – October) variation in CO_2 flux, year 2011.

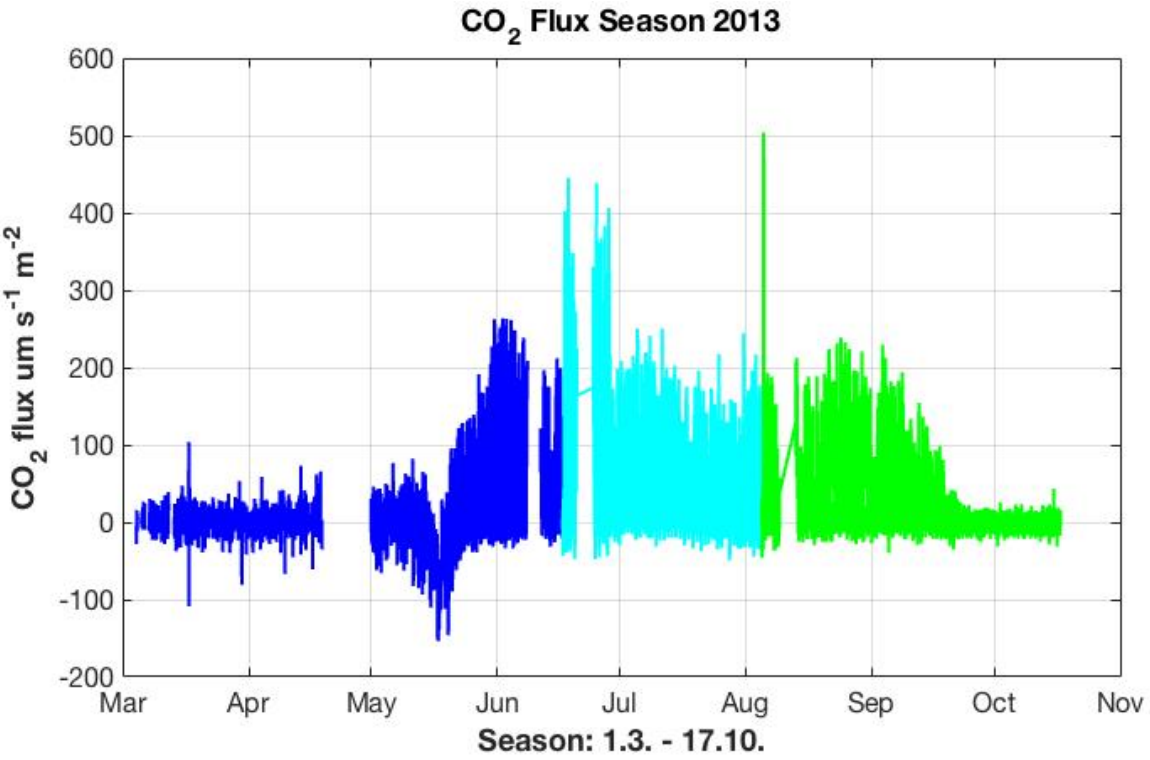

Figure 39. Seasonal (March - November) variation in CO_2 flux, year 2013. Different colors showing the measurement periods for each different chamber. Measurement time with cylinder chamber is blue. First period with slide chamber measurement is cyan and moved slide chamber the second period is green.

3.3. Hyytiälä seasonal carbon loss via BVOCs

Calculations of the carbon loss via BVOC emissions are based on the seasonal measured CO_2 exchange and all presented BVOC emissions which include methanol, isoprene, monoterpenes and acetone which are majority in quantity of emissions (figure 40). In the mid July 2011 there was a small gap in the measured data which may have a slight effect on this result. In year 2010 the BVOC production consumed 0.51% of total absorbed carbon calculated from mean values of CO_2 and BVOC fluxes. In year 2011 the carbon loss via BVOC emissions were 0.57%. This percentage is most probably affected slightly by two short gaps seen in CO_2 data and one longer gap in BVOC data during the growing season. The carbon loss in year 2013 is calculated from June 28th to October being and is 0.44%. This result represents the emissions from similar chamber type than years 2010 and 2011 were measured.

Year	BVOC% of total C
2010	0.51%
2011	0.57%
2013	0.44%

Figure 40. Seasonal (June 1st – September 30th 2010 and 2011 and June 28th – September 30th 2013) carbon loss via BVOC's in percentages.

3.4. Viikki greenhouse measurements

The data was collected during 8 days in June 2017 and the aim was to compare differences of normalized emissions between control trees and cyto+ trees. The number of measurement points varied daily from 7 to 15 depending on day. When investigating the results it was clear that first two days results, June 5th and 6th results were not in line with other day's results. The foreline pump of the PTR-MS device had lost its pressure during night time on June 5th and 6th and although it seemed to work well after some repair the measured results did not suggest proper results. The measured emissions on 5th and 6th showed very high concentrations of all BVOCs compared to other day's results which indicated to wrong pressure and flow which were used in calculations. Therefore it was decided to reject the results from those days and only put forward results from 6 days measurements. The valid results were measured on 8th, 9th, 12th, 13th, 15th and 16th of June 2017 and presented here. Figure 41 demonstrate cyto+ trees and control trees normalized mean emission levels for all measured compounds; isoprene, methanol, acetone and monoterpene emission levels hence the study's main focus is on isoprene and methanol emissions.

The trees were approximately one month old sprouts and at a time of measurements they were in fast growing stage. Measurements showed expected emission pattern for methanol and isoprene emissions. Methanol emissions were as its peak during the morning hours and decreased toward the afternoon. This is expected due to the methanol accumulation in stomatal area during the night and its release after stomatal opening (Hüve et al., 2007). Figure 41 show normalized mean fluxes for measured BVOCs. All trees were producing more methanol than isoprene as their leaves were in developing stage. Control tree's measured methanol emissions varied between maximum ~1600 ng g^{-1} s^{-1} and minimum ~200 ng g^{-1} s^{-1} and cyto+ tree's max.~1500 ng g^{-1} s^{-1} and min. ~100 ng g^{-1} s^{-1}.

Large differences between 8th and 9th and also 12th and 13th may originate from stress which they suffered when placed inside the chambers as a very young sprouts. The other reason may be that the sprouts just were in very early developing stage. Similar differences are not seen on June 15th and 16th which may indicate that the measured leaves had reached their mature phase.

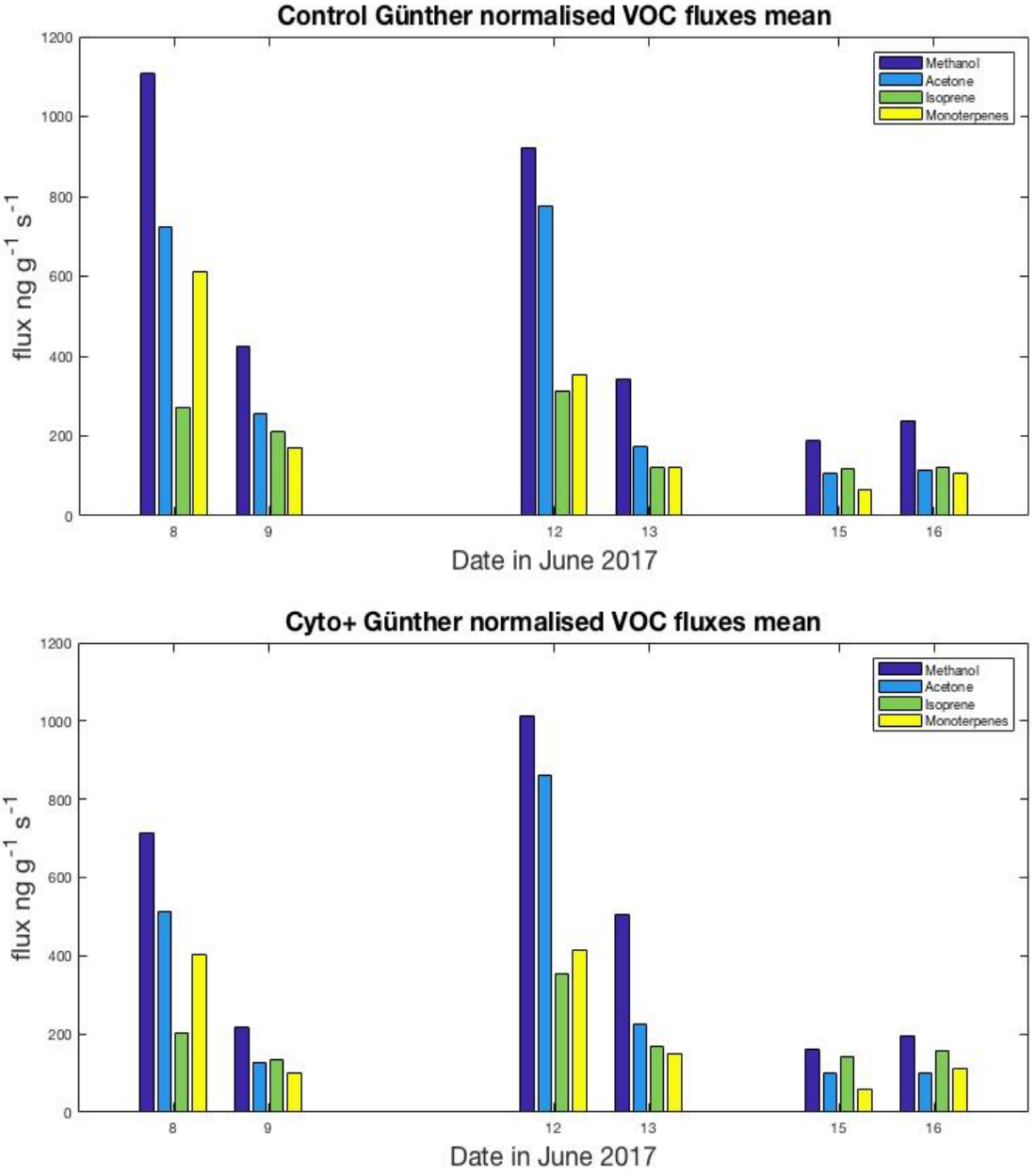

Figure 41. Control and cyto+ tree's isoprene, methanol, monoterpene and acetone mean emissions on June 8, 9, 12, 13, 15 and 16th.

Isoprene emissions were between max. ~500 ng g^{-1} s^{-1} and min. ~100 ng g^{-1} s^{-1} on both control and cyto+ trees. The flux rates are very high compared to Hyytiälä rates which may be the result of greenhouse environment or superfast development of the plants. These greenhouse aspens grow from cell cultivation to 1,5 m long trees in approximately 8 weeks. The isoprene emission result is expected to reflect with methanol emissions which are higher than isoprene emissions at this stage. Isoprene emissions are expected to increase when leaves reaches the mature phase that is presumably close already on June 15th and 16th. Figures 42 and 43 display the emission profile June 16th of methanol and isoprene. In the measured morning hours the methanol emissions are at highest level and decreases during the day. Isoprene goes through a contradictory diurnal pattern presenting low levels in the morning while increasing toward afternoon. This profile is well known and expected for isoprene emissions that are light and temperature dependent (Günther et al., 1995) but it not clear that methanol and acetone emissions are dependent on PAR and T. Some of the measurement days the temperature was as high as 38°C but any emission decreasing was not observed which is seen in natural environment above 35°C (Sharkey et al., 2008). The reason is probably because the plants were well watered all the time and did not suffer any drought.

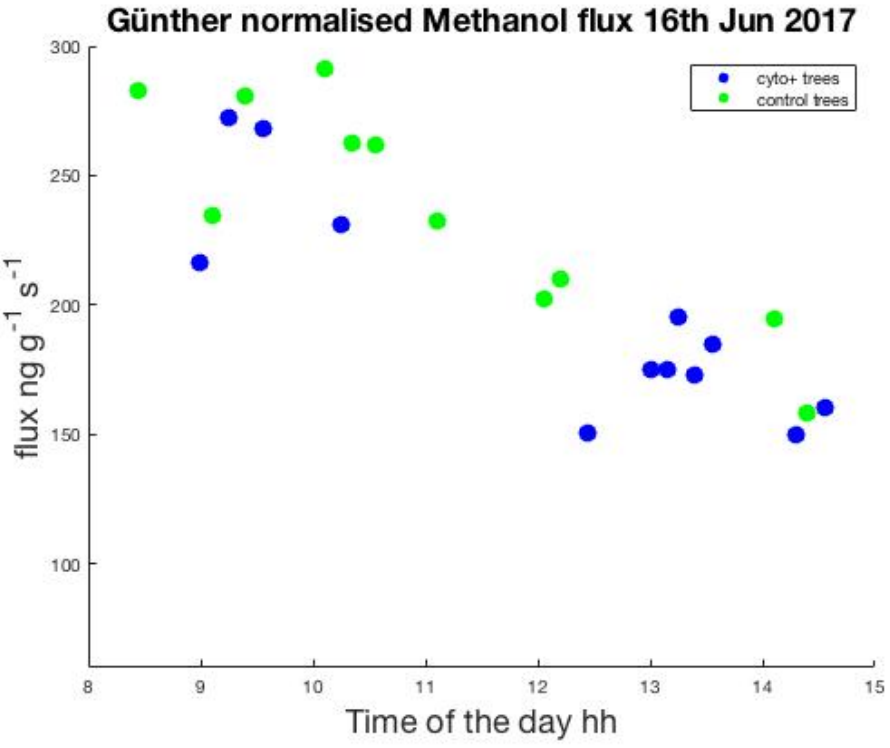

Figure 42. Cyto+ and control tree's Günther normalized methanol emissions on June 16th.

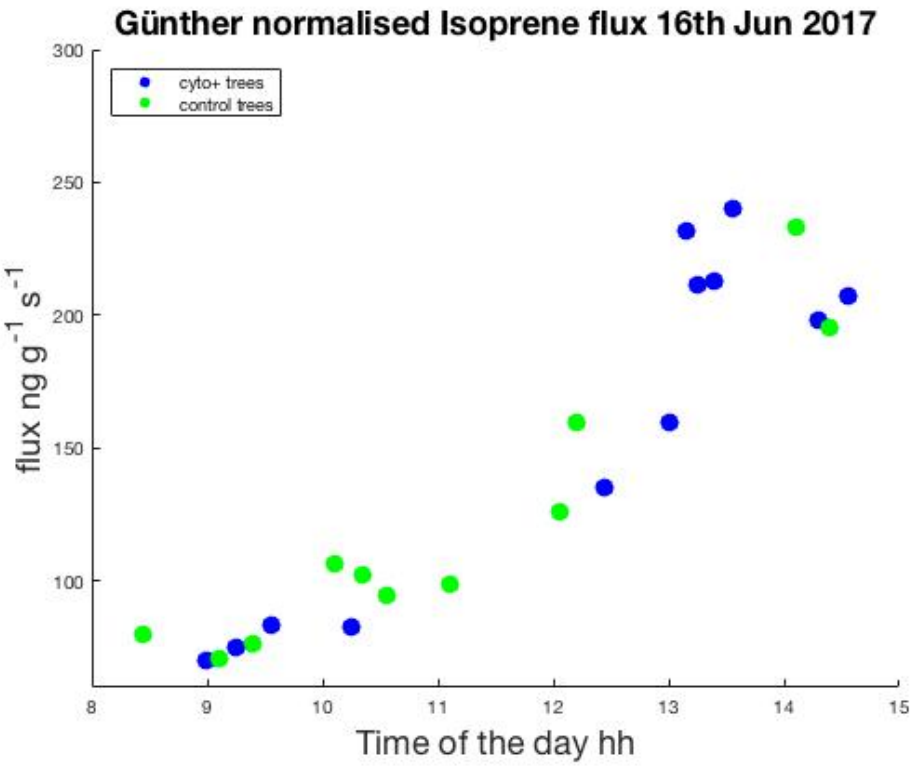

Figure 43. Cyto+ and control tree's Günther normalized isoprene emissions on June 16th.

When comparing the cyto+ trees and control trees emission profiles and quantities the results show no significant differences in methanol, isoprene, acetone or monoterpene emissions (figure 41). Based on these measurements the gene manipulation on aspen does not affect BVOC emissions on leaf level. On canopy level the emission volume and effect may be different due to the increased amount of branches and leaves. Therefore it is possible that BVOC emissions increase if cyto+ trees are grown in large scale plantations.

4. Conclusions

Climate change and concern for environment has generated many approaches for new bioenergy sources and alternatives for fossil fuels. As first-generation biofuels have shown straight competition between food agriculture and energy production the second-generation wood based bioenergy is a functional option (Solomon, 2010).

Aspen as a fast growing tree is one option in producing more cellulose based sustainable bioenergy and biofuels in the future. Furthermore on this study investigated gene manipulated aspen would be even better option with its predicted faster logging cycle. At the same time climate change forces us to look for more efficient sinks for atmospheric carbon. The gene manipulated faster growing aspen could be one answer to that necessity. This thesis has examined the BVOC emissions from aspen with two different approaches. The BVOCs under scope are isoprene, methanol, monoterpenes and acetone. In this study all BVOC emission measurements were done with PTR-MS that was attached to slide, cylinder or frame chamber.

The first part investigated seasonal variations, concentrations and profiles from natural environment growing aspen (Populus tremula). One perspective was to look the seasonal profiles and find out more about the environmental factors that may affect BVOC emissions. The measured data was wide consisting of whole seasons in years 2010, 2011 and 2013. It was clear that light and temperature affect emissions and emissions are dependent on leaf development. The profile showed the biggest methanol emissions right after the leaves had emerged and decreased together with the leaf development. Once the leaves have reached the mature phase the isoprene emissions started to grow rapidly and reached their peak in two week time staying high until August and then started to decrease. The highest levels of isoprene emissions varied between years. The warmest season (2010) showed also the highest emission volumes. Isoprene synthetization requires that part of the carbon uptaken as CO_2 is consumed to produce BVOCs instead of carbohydrates. Emitting plants use 1 - 2 % of the photosynthetic carbon fixation to BVOC synthetization and this percentage increases up to 50% if plants experience stress (Sharkey and Yeh, 2001). In this study the consumption of BVOC production took less than 1% from total CO_2 exchange. The carbon share was calculated from mean values of total CO_2 exchange and BVOC emissions for all seasons. The carbon consumption to BVOCs from total carbon intake varied depending on the season between 0.44 - 0.57 %.

The second part examined the emission differences, volumes and profiles from wild type hybrid aspen (Populus tremula x tremuloides) and gene manipulated version of the same tree in greenhouse environment. As a result of gene manipulation the trees have shown to grow faster due to enhanced production of cytokine hormone (Nieminen et al., 2008, Immanen et al., 2016). The measured trees both control and cyto+ were approximately one month old sprouts. Altogether one

leaf from six pairs of control and cyto+ trees was measured. The results show expected pattern and profile for all emissions. Methanol emissions were at highest in the mornings. At the beginning of measurements methanol was showing a decreasing trend towards end which indicates that leaves had developed to mature phase. Major isoprene emission growth was not seen as the measurements ended at point were the growth was expected. Control tree's methanol emissions varied between 200 and 1600 ng g^{-1} s^{-1}. Cyto+ tree's methanol emissions varied between 100 and 1500 100 ng g^{-1} s^{-1}. Both trees had similar isoprene emission levels which were between 500 - 100 ng g^{-1} s^{-1}. Monoterpene and acetone levels stayed low on both trees as expected. Based on these measurements there are no differences in BVOC emissions between gene manipulated aspen and wild type aspen. This measurement period was short and results may need repetition due to the reason that measurements were done with really young trees and only for two weeks. Longer time period would create better emission profile and verify the high concentrations that were emitted in greenhouse environment in this study.

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