Dissertationes Forestales 253

Long-term dynamics of BVOC production, storage and emission in boreal Scots pine

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Academic dissertation

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TIIVISTELMÄ

Kasvit tuottavat osana sekundaarimetaboliaansa tuhansia erilaisia haihtuvia orgaanisia yhdisteitä eli VOC-yhdisteitä, joita ne hyödyntävät erityisesti puolustusyhdisteinä. Alhaisista pitoisuuksistaan huolimatta nämä yhdisteet osallistuvat ilmakehässä moninaisiin kemiallisiin prosesseihin, jolloin niiden vaikutus ulottuu paljon yksittäisen kasvin kasvuympäristöä laajemmalle. Mänty (*Pinus sylvestris* L.) tuottaa erityisesti mono- ja seskviterpeenejä, joista valtaosa varastoituu pihkaan. Pihka on tiehyissä paineenalaisena. Tässä tutkimuksessa paineen havaittiin korreloivan positiivisesti sekä ilman lämpötilan että versojen transpiraationopeuden kanssa. Lisäksi sekä korkean pihkanpaineen että ilman korkean lämpötilan havaittiin lisäävän monoterpeenien haihduntanopeutta rungosta.

Monoterpeenisyntaasiaktiivisuus kuvaa neulasten maksimaalista kykyä tuottaa monoterpeenejä. Ympäristötekijöiden vuodenaikaisvaihtelun ja neulasten iän todettiin selittävän suurimman osan neulasten monoterpeenisyntaasiaktiivisuuksien sekä monoterpeenivarastojen ja -päästöjen vaihtelusta. Männynneulasten monoterpeenipitoisuuden vaihtelu vuodenaikojen, eri-ikäisten neulasten ja eri puiden välillä oli puolestaan verrattain pientä. Monoterpeenisyntaasiaktiivisuus oli suurempaa alle vuoden ikäisillä neulasilla kuin tätä vanhemmilla. Saman puun neulasten monoterpeenisyntaasiaktiivisuuksien ja monoterpeenivarastojen yhdistekohtainen koostumus ei heijastunut päästöjen koostumukseen: esimerkiksi δ-3-kareenia oli päästöissä selvästi suurempi osuus kuin varastoissa ja syntaasiaktiivisuuksissa.

VOC-yhdisteiden päästöjä on mitattu puiden yhteyttävistä osista jo pitkään, mutta tässä tutkimuksessa seurattiin ensikertaa puiden puuosien päästöjä usean vuoden ajan. Mittaukseen käytettiin automaattista kammiomittausjärjestelmää ja siihen liitettyä protoninvaihtoreaktiomassaspektrometriä.

Männyn rungosta havaittiin vapautuvan ilmaan monoterpeenejä ja metanolia. Kummankin aineen päästöissä näkyi vuodenaikaisvaihtelua: Metanolipäästöt olivat suurimmillaan keskellä kasvukautta. Monoterpeenipäästöt puolestaan olivat korkeimmillaan paitsi kesien kuumimpina päivinä, myös keväällä puiden yhteytyskapasiteetin palautuessa lepokauden jälkeen. Tutkittujen puiden monoterpeenipäästöjen enantiomeerikoostumuksessa esiintyi vuorokausivaihtelua. Puiden vapauttamien yhdisteiden määrän, yhdisteiden reaktiivisuuden, metsän puulajikoostumuksen ja puiden eri kemotyyppien runsauden havaittiin heijastuvan latvuskerroksen yläpuolisen ilman terpeenikoostumukseen.

Asiasanat: monoterpeeni, metanoli, pihka, runko, syntaasiaktiivisuus, mittauskammio

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ABSTRACT

Plants synthesise thousands of biogenic volatile organic compounds (BVOCs) as part of their secondary metabolism. Scots pine (*Pinus sylvestris*) particularly produces mono- and sesquiterpenes, which are mainly stored in oleoresin in resin ducts. In this study, the monoterpene emission rate from stems was found to increase as a function of increasing resin pressure, which was positively correlated with the air temperature and foliage transpiration rate.

Monoterpene synthase activity describes the maximum monoterpene production potential. The seasonal cycle and needle age were observed to explain the majority of the variation in needle monoterpene synthase activities, monoterpene storage pools and monoterpene emissions from shoots. Variation in the monoterpene concentration between seasons, different needle age classes and different trees was observed to be minor. Monoterpene synthase activity was higher in <1-year-old needles compared to older ones. Within a single tree, the compound-specific composition of monoterpene synthase activities and monoterpene storages was not reflected in the composition of emissions. For example, the share of δ-3-carene was substantially higher in the emissions than in the storage pools and synthase activities.

An automated enclosure measurement system including a proton transfer reaction mass spectrometer was utilized to follow the VOC emissions from the woody compartments of trees over several years. This was the first study to quantify such emissions for an extended period. Scots pine stems were observed to emit monoterpenes and methanol into the ambient air. The fluxes displayed a seasonal cycle: methanol emissions were highest in the midst of the growing season, whereas monoterpene emissions peaked not only on the hottest summer days, but also in the spring when the photosynthetic capacity of trees recovered. The emissions of some monoterpenes exhibited distinct diurnal patterns in their enantiomeric compositions. The above-canopy air terpene concentrations reflected the emission rates from trees, the atmospheric reactivities of the compounds, the tree species composition of the measurement site and the abundances of different tree chemotypes.

Keywords: monoterpene, methanol, resin, stem, synthase activity, measuring chamber

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In Viikki, 15th March 2018

Anni Vanhatalo

List of original articles

This thesis consists of an introductory part and five original research articles, of which three have been published in peer-reviewed journals, one is a discussion paper and one is a manuscript. The articles are referred to in the text by their Roman numerals.

I Vanhatalo A., Aalto J., Chan T., Hölttä T., Kabiri K., Kolari P., Hellén H., Bäck J. Scots pine stems as dynamic sources of monoterpene and methanol emissions. A manuscript.

II Vanhatalo A., Chan T., Aalto J., Korhonen J. F., Kolari P., Hölttä T., Nikinmaa E., Bäck J. (2015). Tree water relations can trigger monoterpene emissions from Scots pine stems during spring recovery. Biogeosciences 12: 5353–5363. https://doi.org/10.5194/bg-12-5353-2015.

III Vanhatalo A., Ghirardo A., Juurola E., Schnitzler J.-P., Zimmer I., Hellèn H., Hakola H., Bäck J. (2018). Long-term dynamics of monoterpene synthase activities, monoterpene storage pools and emissions in boreal Scots pine. Under review in Biogeosciences Discussions. https://doi.org/10.5194/bg-2018-17.

IV Rissanen K., Hölttä T., Vanhatalo A., Aalto J., Nikinmaa E., Rita H., Bäck J. (2016). Diurnal patterns in Scots pine stem oleoresin pressure in a boreal forest. Plant, Cell and Environment 39: 527–538. http://dx.doi.org/10.1111/pce.12637.

V Yassaa N., Song W., Lelieveld J., Vanhatalo A., Bäck J., Williams J. (2012). Diel cycles of isoprenoids in the emissions of Norway spruce, four Scots pine chemotypes, and in Boreal forest ambient air during HUMPPA-COPEC-2010. Atmospheric Chemistry and Physics 12: 7215–7229. https://doi.org/10.5194/acp-12-7215-2012.

Table of contents

Terms and abbreviations

Branch enclosure	A measuring chamber enclosing a leafless piece of a living branch
BVOC	Biogenic volatile organic compound
CO_2	Carbon dioxide
De novo synthesis	'From new', VOC synthesis from simple precursors
Deposition	Mass flux to an object
DW	Dry weight
Emission	Mass flux from an object
Enantiomer	One of two molecules that are mirror images of each other
Exchange	Net mass flux, sum of deposition and emission
FEP	Fluorinated ethylene propylene
Flux	The transfer of matter, either positive (emission) or negative (deposition)
GC-MS	Gas chromatograph-mass spectrometer
H_2O	Water
H_3O^+	Hydronium ion
Isoprene	C_5H_8 carbohydrate, highly volatile and abundant in plant emissions
Isoprenoid	Organic compound with two or more C_5 components, e.g. monoterpenes
Monoterpene	$C_{10}H_{16}$ carbohydrate abundant in plant emissions
m/z	Mass-to-charge ratio
O_3	Ozone
OH	Hydroxyl group or ion
ppb	Parts per billion per volume
PTFE	Polytetrafluoroethylene
PTR-MS	Proton transfer reaction-mass spectrometer
Sesquiterpene	$C_{15}H_{24}$, a carbohydrate with a short atmospheric lifetime
Shoot enclosure	A measuring chamber enclosing a living branch tip with foliage
SOA	Secondary organic aerosol
Stem enclosure	A measuring chamber enclosing a section of a living tree stem
Terpene	An organic compound composed of isoprene units
VOC	Volatile organic compound

1 INTRODUCTION

1.1 Boreal forests & atmosphere

Boreal forest constitutes about one third of the world's forest cover. The biome is named after *Boreas*, the god of the North Wind in Greek mythology. The biome is characterised by strong seasonal cycles, winter snow cover, relatively low biodiversity, and a highly variable intensity of human activities. Moreover, forest dynamics in natural boreal forests is driven by recurrent disturbances by wind, snow, fire and insects, among others (Gauthier et al. 2015). The disturbances largely vary in both their intensity and spatial coverage, which causes the characteristic structural variation in boreal forests (Gauthier et al. 2015).

The number of tree species in the boreal region is relatively low compared to areas closer to the Equator. Many abundant and commercially important tree species are pines. Globally, there are 114 pine species. They are grouped into two subgenera: *Pinus* species (hard pines) have two vascular bundle bunches in their needles, while *Strobus* species (soft or white pines) have only one. The specification of many pines is still ongoing, and many pine species hybridize with each other. However, *Pinus* is among the oldest extant conifer genera.

In Northern Europe, large forested areas are dominated by Scots pine (*Pinus sylvestris* L., Fig. 1). The species thrives on sites of contrasting fertility and nutrient availability (Sarvas 1964, Oleksyn et al. 2002). The species is widely utilized commercially, mainly in the timber, pulp and paper industries. The species is also ecologically an essential resource for many organisms.

Figure 1. The wide distribution area of Scots pine (grey) over the northern hemisphere reflects the numerous site types and climates to which the species is adapted. The measurement site of this study at Hyytiälä, Finland, is marked with a black dot.

Due to the vast distribution, boreal forest affects the atmosphere, the gaseous envelope surrounding the Earth, very significantly and in many different ways. The role of boreal forest as atmospheric carbon sink is relatively well known and accounted for in climate change scenarios. However, the impacts of boreal forests are not only limited to carbon but extend far beyond. Across the boreal biome, surface albedo (the ratio of reflected to incident radiation) is seasonally highly variable, which affects the radiative balance of the Earth (e.g. Betts and Ball 1997, Moody et al. 2007). Simultaneously, boreal forests are a large source of natural aerosols (e.g. Tunved et al. 2006), which affect the radiative balance by scattering and absorbing solar radiation (direct effect) and by modulating cloud properties (indirect effect, e.g. IPCC 2013, Rosenfeld et al. 2014). Furthermore, by producing aerosol precursors, boreal forests and especially coniferous trees feed back to the growing conditions of trees (Kulmala et al. 2014). However, the aerosol–cloud–climate interactions are still not very well understood (e.g. Rosenfeld et al. 2014). The uncertainties, especially in the indirect effects (aerosol–cloud interactions) and the related radiative forcing, have remained large, and they still contribute the largest uncertainty to the total radiative forcing estimate (IPCC 2013).

2 SCIENTIFIC BACKGROUND

2.1 Trace compounds of the atmosphere

Volatile organic compounds (VOCs) are a vast group of carbon-containing compounds. Their concentration in ambient air is minimal: only in the range of parts per billion (ppb) or trillion (ppt) per volume in ambient well-mixed air, or even less. Atmospheric VOCs have multiple sources, but plant-derived emissions clearly dominate globally. The annual biogenic (from biological sources) volatile emissions are estimated to be around 1 Pg (10^{15} g), out of which half are comprised of a single compound, isoprene (Guenther et al. 2012). In the past, VOCs were often considered as by-products of essential plant metabolism. Nowadays, their importance in numerous plant functions is recognised, as later discussed in detail. In addition to biogenic sources, many human activities such as traffic and industry release large amounts of VOCs into the atmosphere (Blake et al. 2009).

Although VOCs are present in only small amounts in the atmosphere, they have a marked impact on atmospheric processes due to their reactive nature. VOCs start to degrade as soon they are released from plant tissues. As volatiles emitted by plants undergo transformations with other compounds in the atmosphere, their molecular mass increases, they become less volatile, and they are thus more likely to condense in liquid or solid phase. At this point, they are called extremely low volatility compounds (ELVOCs), which have only recently been quantified as measurement techniques have advanced (Ehn et al. 2014).

Individual volatiles have considerably different reactivities (i.e. how much substances react once mixed with other substances), and they consequently have different fates in the atmosphere. Their average lifetimes (the period when a compound exists before reacting further) in the atmosphere vary from a few seconds to months, largely depending on the atmospheric circumstances. In addition to reactivity, vapour pressure and volatility are widely used concepts in VOC studies. The volatility of a compound (the tendency of a compound to vaporize) depends on its vapour pressure within a plant tissue, where it is freshly synthesised or stored for the longer term. The temperature and concentration within the tissue, in turn, control the vapour pressure of the compound (Lerdau et al. 1997). The higher the vapour pressure, the easier the compound escapes from plant tissue and the less it can be stored within a plant for future use. In plant tissues, solubility (the amount of a substance that dissolves in a given amount of solvent) is an important factor defining how much of a specific compound tissues can contain. VOCs show a wide array of solubilities in water and lipid solutions.

The role of volatiles in the climate system lies in their consequential effects: VOC emissions, e.g. monoterpene emissions from Scots pines, react in the atmosphere with oxidants such as hydroxyl radicals (OH), ozone (O_3) and nitrate radicals (NO_3) (e.g. Seinfeld and Pandis 2016), but they can also contribute to the oxidant budget through Criegee intermediates (Mauldin III et al. 2012). The oxidation of VOCs decreases their saturation vapour pressure and results in the formation of secondary organic aerosols (SOA). As SOA particles grow to approximately 100 nm in diameter, they become capable of acting as cloud condensation nuclei (CCN) (e.g. Andreae and Rosenfeld 2008). Cloud droplets form around CCN, and their number affects the cloud properties (e.g.

Andreae and Rosenfeld 2008, Rosenfeld et al. 2014). Clouds generally have a cooling effect on the climate, as they reflect solar radiation back into space.

There are many ways to classify the compounds in the air and in plant emissions: they can be classified based on their origin (natural, human sources), functional groups (alcohols, amines, alkenes), chemical composition (C_5, C_{10}, C_{15} compounds) and so on. Trace gases are often defined as those constituents of the atmosphere whose concentration changes do not affect atmospheric composition. Examples of trace gases include argon, ozone, nitrous oxides (NO_X), carbonyl sulphide (COS) and dimethyl sulphide (DMS). As methane is not usually considered as a trace gas, methanol is the most abundant trace gas in the Earth's atmosphere. The following paragraphs discuss some compound groups that are interesting atmospherically, and especially from a plant perspective.

Terpenoids are one abundant group of trace gases in the atmosphere. They are the largest group of known plant metabolites, comprising more than 40 000 different chemical structures (Bohlmann and Keeling 2008). Some plant terpenoids are primary metabolites (essential for development, growth and reproduction) (e.g. Alba et al. 2012, Fineschi et al. 2013), such as sterols and carotenoids, whereas the majority of them serve as secondary metabolites, increasing plant fitness. The wide variety and complexity of volatile compounds has made it difficult to group them. The diversity most likely reflects the very many biotic functions of terpenoids in nature.

Isoprenoids, on the other hand, are organic compounds that have two or more C_5 components, i.e. isoprene units. Monoterpenes, sesquiterpenes, diterpenes, triterpenes and polyterpenes are all isoprenoids (Fig. 2). In terpenes, isoprene units are joined together "head-to-tail".

Monoterpenes are composed of two isoprene units (C_{10}). They may be acyclic (no ring structure) or mono-, bi- or tricyclic, chiral or achiral (stereoisomerism), oxidized or non-oxidized. Emission inventories have shown that monoterpenes dominate the atmospheric emissions together with isoprene (Kesselmeier and Staudt 1999). Guenther et al. (2012) estimated that mono- and sesquiterpenes together comprise about 18% of global biogenic VOC (BVOC) emissions.

Monoterpene lifetimes in the atmosphere vary from less than a minute to several hours, largely depending on the atmospheric conditions (Kesselmeier and Staudt 1999,

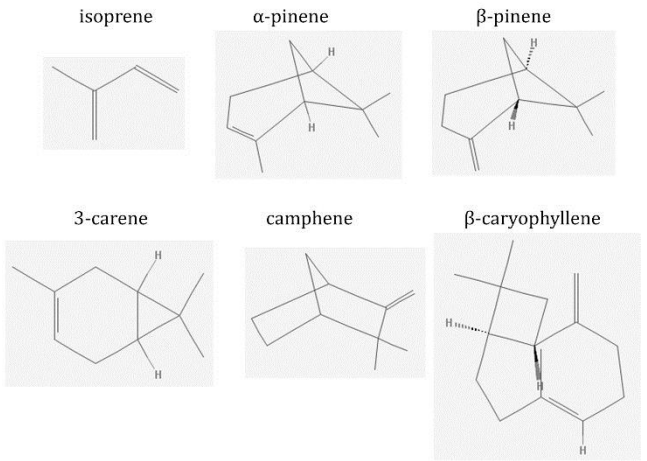

Figure 2. Some abundant volatiles in plant VOC emissions: isoprene is a hemiterpene and β-caryophyllene is a sesquiterpene. The rest of the examples are monoterpenes.

Atkinson 2000, Hewitt et al. 2011). Large amounts of secondary organic aerosols are formed from terpenes emitted from coniferous trees once air masses move over boreal areas (Tunved et al. 2006).

Sesquiterpenes are a group of terpenoids that are formed of three isoprene units (C_{15}). About 5 000 sesquiterpenes have been detected among plant secondary metabolites (Wink 2003). Sesquiterpenes are generally very reactive compounds, and their lifetimes in the atmosphere are short, only in the order of minutes (Kesselmeier and Staudt 1999). Due to their high reactivity, sesquiterpenes have a more important role in atmospheric processes than their low concentration in ambient air suggests.

Many vitamins and pigments are also terpenes: vitamin A, for instance, is an oxygenated diterpene (C_{20}) and β-carotene (C_{40}) is the compound giving the orange colour in carrots. An example of triterpenes (C_{30}) are glycosides, the protective compounds exploited as antibiotics (e.g. streptomycin). Polyterpenoids ($C_{>45}$) are very large compounds: for example, latex (the bark exudate of rubber trees, genus *Hevea*) is a polyterpene composed of some 4 000 isoprene units. Diterpenes and other larger compounds are rather non-volatile due to their high molecular mass, and this is why they are regarded as less important from the atmospheric point of view than isoprene and mono- and sesquiterpenes.

The above-mentioned VOC groups constitute only a part of the volatiles in plant emissions and in the atmosphere. Thus, in addition to the above-mentioned groups, there are also other commonly used descriptive groupings, including AVOC (anthropogenic VOC), NMVOC (non-methane VOC), OVOC (oxygenated VOC) and ELVOC (extremely low volatility organic compounds).

2.2 Plant volatile emissions – the scent blend of the flora

The term ‘volatilome’ is occasionally used as a synonym for the emission blend of a certain plant. The blends vary considerably depending on the species and their growing conditions, and a large amount of intra-specific variation also occurs. Plants have generated a wide spectrum of scents, fragrances, aromas, odours and smells, mainly for three reasons: defence, pollination and communication.

The role of volatiles in the carbon cycle of ecosystems is minor. In the day, about 0.05–0.5% of the carbon fixed in photosynthesis is released as VOCs into the atmosphere (Grabmer et al. 2006). According to another estimate (Harrison et al. 2013), 1–2% of the net primary production of terrestrial plants is released to the atmosphere as isoprene and monoterpenes. In highly stressful conditions, however, the share may rise considerably.

The global mean BVOC emission for vegetated areas is estimated to be 0.7 g C m^{-2} yr^{-1} (Guenther 2002). In the tropics, carbon loss can exceed 100 g C m^{-2} yr^{-1} (Guenther 2002). However, the role of volatiles in the climate is far greater than what the carbon loss from plants or their low concentration in air appear to suggest.

In general, broadleaved and coniferous trees are strong VOC emitters, especially isoprenoid emitters, whereas grasses and crops have lower emissions (Guenther 2012). Exceptions, however, always occur. Nevertheless, very few plant species emit ample amounts of both isoprene and monoterpenes. It has been hypothesized that this trade-off is because isoprene and monoterpene synthesis compete for the same substrate and reducing power (Harrison et al. 2013). The dominance of either isoprene or monoterpenes seems to be related to the ecological strategies of the plant species. There is some

evidence that predominantly isoprene-emitting species have a higher photosynthetic capacity, higher specific leaf area and shorter lifespan of leaves compared to plant species mainly emitting monoterpenes (Harrison et al. 2013).

2.2.1 Chemodiversity and plant chemotypes

Intra-species variability in plant volatile emissions is substantial, both qualitatively and quantitatively. One good example of quantitative variation was provided in the study by Schuh et al. (1997), who observed that sunflower (*Helianthus annuus* L.) sesquiterpene emission rates vary by 3–4 orders of magnitude between plant individuals. This variability is rather large given that the studied plants were in the same vegetative stage and they were exposed to exactly the same light and temperature conditions during their growth. Another study on qualitative intra-species variability revealed a wide range of monoterpene emission spectra among Scots pines at a single site (Bäck et al. 2012). The emissions of some trees contained no δ-3-carene, whereas it comprised more than 80% of the emissions of some other trees.

Chemotypes are defined as chemically differing groups among individuals of the same species, i.e. distinct chemical phenotypes (e.g. Keefover-Ring et al. 2009, Kännaste et al. 2013). For example, the above-mentioned pines can be classified in pinene, carene and intermediate chemotypes. The chemotype is strongly under genetic control and is thus an inheritable trait. In practice, all the chemotype determinations of plants are based on their foliar or shoot VOC emissions.

The volatile emission blends of plants are mixtures of many compounds. The mixtures have several assets from the plants' perspective. For example, the synergistic effects of two or more compounds may be stronger or last longer than just one compound at the same dose (Gershenzon and Dudareva 2007). The emission blends of species belonging to the same genus usually resemble each other. However, exceptions occur, such as in the genus *Quercus*: some oak species exclusively emit monoterpenes, some only isoprene and some neither of them (Kesselmeier and Staudt 1999).

2.2.2 Plethora of emitted compounds

The physiological and ecological functions of volatiles are highly compound-specific. Some compounds protect plants against oxidative stress, whereas others may be important in communication between organisms. Here, I briefly discuss the compounds that are handled in this thesis in the order of their molecular mass, starting from the lightest one, methanol.

Methanol is the simplest alcohol, formed of a methyl group coupled with a hydroxyl group (CH_3OH, MeOH). It is moderately soluble in water, and may thus easily be transported in plant transpiration flow in the xylem sap (e.g. Seco et al. 2007). Plants emit large amounts of methanol during their growth, and emissions are highest during the most active growing period. Methanol is produced in the demethylation of pectin during cell wall expansion (Fall and Benson 1996). More precisely, the primary cell wall rigidity loosens due to enzymatic activity and allows cell extension (Galbally and Kirstine 2002). Within plant tissues or on their surfaces, methanol may also oxidise to formaldehyde, the most abundant carbonyl in the atmosphere (Muir and Shirazi 1996, Galbally and Kirstine

2002). In addition to plant growth, other significant methanol sources include biomass decay and burning, anthropogenic activities as well as atmospheric and oceanic reactions (Galbally and Kirstine 2002 and references therein). Methanol is an abundant trace compound in the lower atmosphere: its concentration may reach tens of ppb, and it has a relatively long atmospheric lifetime ranging from a few days up to a year (Atkinson 2000).

Isoprene (2-methyl-l,3-butadiene) is a small hemiterpene formed of five carbon atoms (C_5). It is the most volatile compound in plant emissions, and it cannot therefore be stored in plant tissues. Isoprene is synthetized via the same MEP (2-C-methyl-D-erythritol 4-phosphate) pathway as monoterpenes and many other essential plant metabolites (Lichtenthaler 1999). Isoprene is a lipophilic compound that stabilizes cellular membranes at temperatures that are well below threshold for heat damage. Moreover, isoprene reduces the formation of reactive oxygen species (Velikova et al. 2012). In addition, isoprene is an effective antioxidant: its emissions protect plants against oxidative stress caused, for example, by O_3 and NO_X.

Globally, annual biogenic isoprene emissions have been estimated to be 523–800 Tg yr^{-1} (Guenther et al. 2012). The Finnish isoprene emission estimate is 4–11 Gg yr^{-1} (Oderbolz et al. 2013). As a general rule of thumb, the vegetation in tropical and temperate areas has a tendency to primarily emit isoprene, whereas the boreal flora tends to produce monoterpenes (Unger 2014). However, wetlands dominated by isoprene-emitting *Sphagnum* species and some other moss species are also significant isoprene sources in the northern hemisphere (e.g. Hanson et al. 1999, Ekberg et al. 2011).

Monoterpenes have diverse ecological functions, including defence, deterrence, attraction and signalling (see e.g. Gershenzon and Dudareva 2007). They attract pollinators and the predators of herbivores, repel herbivores, and act as fungicides and foraging inhibitors. Many of the functions are highly compound-specific, and some of these ecological interactions are extremely specialised. For example, stereoisomers of the same compound may have very different functions, although they both have exactly the same molecular composition. Monoterpenes also comprise the largest volatile fraction of conifer resins, as discussed below. Monoterpene emissions from plants are temperature and/or light dependent, depending on the compound and plant species in question. For example, in Scots pine foliage, emissions of the majority of monoterpenes are only dependent on temperature, but 1,8-cineole emission is dependent on both temperature and light intensity (Tarvainen et al. 2005). The global annual monoterpene emission estimates vary between 30 and 177 Tg yr^{-1} (Guenther et al. 2012). Oderbolz et al. (2013) estimated annual Finnish monoterpene emissions to equal 105–230 Gg yr^{-1}, depending on the applied vegetation data. In the same study, Scots pine was stated to be the largest monoterpene source in Europe, accounting for 17–40% of the annual monoterpene emissions of the continent.

Sesquiterpenes are synthesised in the cytosol of plant cells. The sesquiterpene emission rates of plants usually correlate positively with temperature, but their relationship with light and leaf stomatal control remains unclear (Duhl et al. 2008). The most commonly found sesquiterpene in plant emissions is β-caryophyllene, the next most common being α- and β-farnesene and α-humulene (Duhl et al. 2008). Sesquiterpene emission estimates for Finland vary between 9 and 23 Gg yr^{-1} (Oderbolz et al. 2013) i.e. an order of magnitude less than for monoterpenes. However, global and regional sesquiterpene emission estimates are highly uncertain due to the lack of comprehensive emission studies (Duhl et al. 2008). In their extensive review on sesquiterpene emissions

from vegetation, Duhl et al. (2008) concluded that substantial intra- and inter-species variability exists, as well as variability related to the environmental and phenological states of plants.

2.2.3 Variability in synthesis and emissions

The regulation of VOC synthesis can be divided in short (seconds–minutes), medium (hours–days) and long (weeks–years) time scales (Harrison et al. 2013). Short-term variability in VOC synthesis is mostly related to substrate availability, whereas in the long term, transcriptional dynamics play crucial role (Harrison et al. 2013). *De novo* VOC emissions are directly coupled to photosynthetic activity, but emissions from specific (e.g. glandular hairs or resin ducts) and non-specific (e.g. lipid cell membranes) storage pools are continuous. Moreover, emissions can be classified as constitutive (primary) VOC emissions taking place constantly all the time or induced (secondary) emissions occurring only after an inducing stress factor such as herbivore damage.

Temperature controls both VOC synthesis through enzymatic activity and VOC emissions through diffusion. Solar radiation, on the other hand, increases the synthesis through improved substrate (precursor) availability, but does not increase emissions if temperature remains constant. The synthesis rate is also limited by substrate availability, which is closely linked to photosynthesis. The monoterpene synthase activity (paper III) is defined as the maximum potential (rate) of the needles to synthesise monoterpenes. Thus, the potential may be far higher than the realized synthesis rate due, for instance, to low temperatures or limited substrate availability.

As described in the preceding sections, the volatile emissions of plants are highly variable. The rate of VOC emission from a plant tissue to the atmosphere depends on multiple factors, including compound pools in tissues, the *de novo* synthesis rate, the properties of the released compound and the diffusion resistance of plant tissues (e.g. Ghirardo et al. 2010, Wildhalm et al. 2015). Monoterpene emission, for example, is controlled by the compound-specific vapour pressure within tissues. The vapour pressure, in turn, is controlled by the prevailing temperature and compound concentration (e.g. Lerdau et al. 1997, Schuh et al. 1997, Tarvainen et al. 2005). VOC concentrations in plant tissues are largely influenced by emission barriers. Thus, VOCs synthesised in plant cells must cross multiple cellular barriers until they are released into the ambient air (Wildhalm et al. 2015). At minimum, they must cross a cell membrane, cell wall and cuticle. Lipid cell membranes form a barrier for hydrophobic VOCs, such as monoterpenes (Wildhalm et al. 2015). On the plant surface, VOCs exit plant tissues through the cuticle or leaf stomata. Thus, the openness of stomata regulates VOC release to the atmosphere (e.g. Niinemets and Reichstein 2003). Stomatal regulation is, however, very compound-specific: methanol is under strong stomatal control due to its low vapour pressure, but, for example, isoprene is practically not at all under such control because it has a high vapour pressure and a large diffusion gradient to the ambient air.

Emission models (e.g. Guenther et al. 1993, Guenther 1997, Ghirardo et al. 2010) describe volatile emissions with functions dependent on the temperature and/or light intensity. A widely used concept in emission modelling is the emission potential (standard emission factor, normalized emission). It relies on the exponential relationship between emission rates and light intensity and/or temperature (Guenther et al. 1993). The most commonly used standard conditions are 30 °C and 1 000 µmol m^{-2} s^{-1}, while other

conditions are applied more seldom. Emission potentials enable meaningful comparisons between different sampling conditions.

Moreover, the seasonal and ontogenetic cycles of plants are reflected in their VOC dynamics. Plant age (whole plant or plant organ) affects the VOC emission strength and composition, especially in the case of long-lived species such as trees. However, the issue is currently rather poorly understood. In the case of trees, emission surveys are often conducted in laboratory conditions with small saplings, while mature forest-grown trees may show very different emissions. Komenda and Koppmann (2002) measured differing emission rates from young (3–4-yr-old) and mature (40-yr-old) Scots pines: the standardized emission rates were 0.06–0.64 μg g^{-1} DW h^{-1} for young and 0.24–3.7 μg g^{-1} DW h^{-1} for mature pines. Street et al. (1997) found the total VOC emissions of a mature tree (a 7-yr-old eucalyptus in this case) to be 5 times higher than those from a 1-yr-old sapling. Additionally, foliage age, or the more physiologically expressed physiological/ontogenetic status, affects the emission strength and composition. Buds and newly emerged leaves are far more noteworthy emission sources than their share of the total tree biomass would imply. In a study by Aalto et al. (2014), the emissions of several VOCs were one to two orders of magnitude higher from bursting buds and rapidly growing shoots than those from mature foliage of Scots pine. The widely used Model of Emissions of Gases and Aerosols from Nature version 2.1 (MEGAN2.1) nowadays takes into account the foliage age: leaves are divided into four categories (new, growing, mature and senescing) which all have different compound-specific parametrizations in the model (Guenther et al. 2012).

Plants experience dramatic physiological changes during repeated seasonal cycles, particularly in the boreal region: The ambient air temperature varies annually in the range of >50 °C, photosynthetically active radiation (PAR) is several times more abundant in summer than in winter, and water availability is limited in winter due to freezing temperatures and in some cases also in summer due to low soil moisture. This is all evidently reflected in the functions of plants and their volatile emissions.

Plants experience a wide range of biotic (caused by any living organisms) and abiotic (related to the non-living physical and chemical constrains of the environment) stress factors. All of them influence the plant VOC emission strength and spectrum, as numerous studies on the topic have revealed. Ozone is one of the best-known stress factors. It damages plant foliage and thus reduces growth, which is why it has been classified as the globally most harmful air pollutant for plants (e.g. Sandermann 1996, Krupa et al. 2001).

Plant responses to stress factors are multiple. A plant encountering serious stress may have volatile emission rates orders of magnitude higher than its intact counterparts. Biotic stress factors such as herbivores and pathogens usually increase VOC synthesis and emissions, as these compounds frequently act as defensive secondary metabolites (e.g. Bergström et al. 2014). Many volatiles taste bad, which deters herbivores (Iason et al. 2011). Moreover, the type of herbivory has an effect: chewing insects (such as caterpillars) induce higher volatile emission rates than sap-sucking insects (e.g. aphids), and specialized herbivores induce greater emissions than generalists (Rowen and Kaplan 2016).

Mechanical damage can be caused by both abiotic and biotic factors. Abiotic factors include heavy rain, hail, heavy snowfall and large snow loads on plants. Freezing inside plant tissues can also be regarded as abiotic mechanical damage. Wind may affect emission rates in both the short and longer term. Trees are well adapted to the day-to-day

movement of branches by wind. However, intense wind with possible hail or snow predisposes trees to structural damage and may, for instance, break resin ducts within branches, stems and even roots. Wind flexes stems, which may be reflected in a prolonged period of increased emissions. Haase et al. (2011) observed a mean increase of 93% in the ambient air monoterpene mixing ratios as a storm passed over the measurement site. They also estimated that the strongest storm events could result in monoterpene emission rates of 119–1 240 g km^{-2} h^{-1}, which is an order of magnitude higher than in clear weather. Moreover, mechanical damage may induce VOC synthesis and subsequent emissions. Rough handling has been reported to increase the monoterpene emissions of Monterey pine (*Pinus radiata* D. Don) by 10–50-fold (Juuti et al. 1990).

2.2.4 VOCs of different tree compartments

The majority of studies on plant volatiles have concentrated on the foliar tissues. However, other vegetative and reproductive tissues also produce and emit volatiles. Monoterpene concentrations in woody tree parts have received some attention (e.g. Smith 1977, Hanover 1992, Fäldt et al. 2001, da Silva Rodrigues-Correa et al. 2012), probably due to their potential for commercial applications, but there have been very few studies on the emissions from woody parts. Rhoades (1990) aptly stated: "-- there does not appear to be any published report of types or amounts of volatiles emitted by the boles of intact trees." In close to 30 years, the number of reports has increased only slightly.

Rhoades himself (1990) sampled volatiles from eight intact lodgepole pine (*Pinus contorta* Douglas ex Loudon) stems *in situ* and found considerable intra-species variation. Later, Heijari et al. (2011) sampled volatiles from intact and weevil-damaged bark (in practice, the stem) of Scots pine seedlings. Haapanala et al. (2012) measured monoterpenes from the stumps of Scots pine, Norway spruce (*Picea abies* (L. Karst.) and birch species. Similarly, Kivimäenpää et al. (2012) quantified VOC emissions from fresh Scots pine stumps. The latter two examples represent highly stressful conditions.

Emission measurements from living woody tissues are scarce, probably due to technical and/or methodological constraints, but the number of studies on concentrations in tissues is higher. Sjödin et al. (1996) investigated the monoterpene concentrations of different Scots pine tissues. They classified them qualitatively into three distinct groups based on monoterpene contents: 1) stem and root xylem and root phloem, 2) needles and 3) branch tissues excluding needles. Similarly, Moore and Hanover (1987) found large differences in monoterpene concentrations between different tissues of blue spruce (*Picea pungens* Engelm). Moreover, the conifer resin composition differs between sapwood and heartwood (Back 2002, Celedon and Bohlmann 2018).

The terpene blend may also vary within a plant tissue depending on its position in the tree and canopy (Moore and Hanover 1987 and references therein). In blue spruce, the lower canopy needles had almost twice the monoterpene concentrations of the middle and top canopy needles, whereas there was no height-related variation in bark monoterpene concentrations (Moore and Hanover 1987).

In addition to plant tissues, a significant VOC emission source consists of exposed resin drops and patches on plant surfaces. Freshly exuded resin flows (Fig. 3) may constitute a notable proportion of the monoterpene flux, even in non-stressed forest ecosystems (Eller et al. 2013). Scots pine buds and immature cones are almost invariably more or less covered by resin, and exposed resin may thus contribute considerably to the

Figure 3. Oleoresin flow protects conifer tissues against pathogens and herbivores such as insects (one beetle covered by crystallized resin barely visible in the lower right corner of the photo). Here, the oleoresin of Scots pine is flowing after phloem sample collection.

terpene fluxes of boreal forests. Moreover, forestry practices produce an abundance of resinous surfaces, as the studies by Haapanala et al. (2012) and Kivimäenpää et al. (2012) have demonstrated.

Currently, the widely employed VOC emission modelling tool 'MEGAN' (Guenther et al. 2012) takes into account only leaf parameters, and not at all the other tree organs such as stems, branches or reproductive organs. Thus, the considerable variability in VOC emissions from various tree tissues is currently poorly accounted for in emission model parametrizations.

2.3 Coniferous and especially pine resin

Coniferous resin is composed of volatile mono- and sesquiterpenes and non-volatile resin acids (Savage et al. 1996, Phillips and Croteau 1999, Back 2002). It acts as an efficient deterrent against herbivorous insects and mammals, which, together with antimicrobial effects, are thought to be the main functions of coniferous resin.

Out of all the substances produced by plants, tree resins are among the most durable against abrasion, as the existence of amber (fossilized coniferous resin from the Tertiary period) demonstrates. Resin is a tough material for many organisms to exploit as a source of nutrition, but some have still succeeded in this, and cannot actually live on any other substance. Globally, several fungi mainly belonging to the Ascomycetes are known to live on resin exudates and resin-impregnated wood and bark (Tuovila 2013). In Finland, the only known resinicolous species is the microscopic fungus *Chaenothecopsis montana* Rikkinen (Tuovila 2013).

Trees store resin for future defence purposes. Pines store their resin in resin ducts, but other tree species may also store resin in blisters or cavities. The genus *Pinus* is thought to be one of the oldest of the coniferous families, but it is considered to have one of the most developed resin duct systems (Lewinsohn et al. 1991, Strömvall and Petersson 2000). The resin duct (also called resin canal) network extends over the whole tree from the roots, trunks, branches and twigs to needles and cones. It consists of radial and axial ducts, which connect bark, sapwood and heartwood (Celedon and Bohlmann 2018). Resin

duct dimensions in pine trunks vary from 50 µm (radial) to 200 µm (longitudinal). Longitudinal ducts have a larger volume than radial ones, although radial ducts are more abundant in cross-section. In needles, resin ducts lie longitudinally in the mesophyll near the epidermis. In a cross-section of a needle, the ducts lie in a circle around the central vascular bundle. Some species, e.g. Norway spruce, form traumatic resin ducts axially in the secondary xylem as a consequence of an injury (e.g. Nagy et al. 2000).

Resin ducts are surrounded by epithelial cells, which are living in sapwood and dead in heartwood (e.g. Celedon and Bohlmann 2018). Monoterpene synthesis for resin production occurs in the plastids of these epithelial cells (Back 2002, Lewinsohn et al. 1991 and references therein). The epithelial cells of *Pinus* remain alive for several years, unlike in many other conifer genera (Wu and Hu 1997). The same cells also cause the resin pressure (paper IV). In the resin ducts of southern pines, pressures of 7–12 bar have been measured (Strömvall and Petersson 2000). The measurements of mature Scots pines have indicated pressures of 3–9 bar, with higher pressures at the base of the stem than within the canopy (paper IV).

There are no estimates in the literature on how large the resin storage pools are in a mature Scots pine. Based on the available information on resin contents and biomass distributions, some estimates can be made: the stem wood of a 20-m-tall Scots pine may comprise about 3.5 kg of resin and 1 kg of monoterpenes. The resin amount in the needles of a similar-sized tree, however, may be only 25 g, given that the monoterpene content is 0.5% of the needle dry weight (mean of 115 samples in study V). Using pine biomass distributions (stems, branches, needles, roots) from the SMEAR II measurement site (Ilvesniemi et al. 2009) and the monoterpene contents of 0.6% and 0.5% for woody tissues and needles, respectively, the monoterpene storage pool on an ecosystem level would be 580 kg ha^{-1}. The majority of the storage pool (370 kg ha^{-1}) is in the stem tissues.

2.4 Climate change reflected in VOC fluxes

Since the 1950s, many of the worldwide changes observed in the climate have been unparalleled over decades to millennia (IPCC 2013). For instance, the concentrations of carbon dioxide, methane and nitrous oxide have risen to levels unprecedented in at least the last 800 000 years (IPCC 2013). This has put the biosphere in a challenging situation, where organisms need to adapt to the rapidly changing environment. Global change is a wider concept than climate change: it includes alterations in land use, environmental pollution, eutrophication and alien species invasions, for example. Both global and climate change have drastic impacts on vegetation, and they are reflected in VOC fluxes.

In VOC models, increasing the temperature exponentially increases most VOC emissions in the short term. This is because a temperature increase accelerates enzymatic processes, i.e. VOC synthesis, raises the vapour pressures of VOCs, and decreases the resistance of the VOC diffusion pathway from tissues to the atmosphere (Tingey et al. 1991). However, although the effects of rising temperatures are rather well known in short time scales, the longer-term effects are much more poorly identified (Peñuelas and Staudt 2010). In the long term, the indirect effects of rising temperatures will become emphasized, e.g. in terms of lengthening of the growing season.

Global and climate change indirectly affect the global vegetation cover, land use, seasonality, phenology, water and nutrient availability and many more aspects (Peñuelas and Staudt 2010, Rosenkranz et al. 2015). The constitutive and induced VOC emissions

may respond differently to the stresses brought by climate change (e.g. Zhao et al. 2017), and thus their effects on the complex climate system (Fig. 4) may be unforeseeable. However, a recent modelling study by Hantson et al. (2017) concluded that the decreasing trend in monoterpene emissions globally will probably continue and that a global increase in BVOC emissions is improbable by the year 2100.

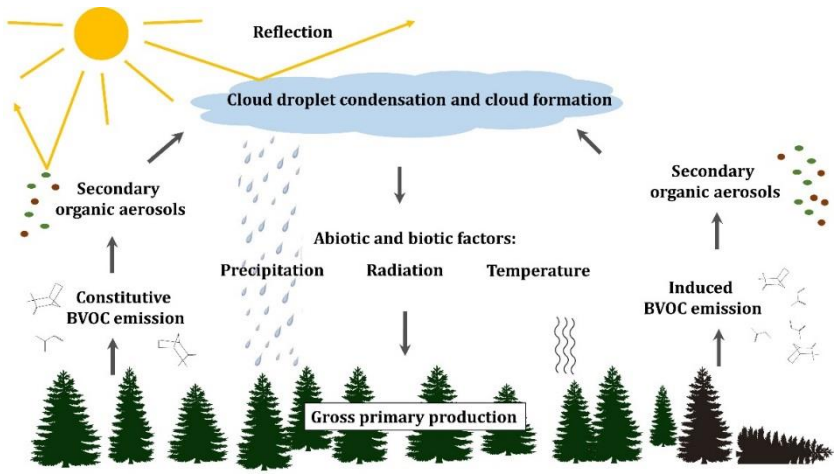

Figure 4. A schematic figure illustrating the feedbacks within the plant–BVOC–SOA–climate loop, modified from Zhao et al. (2017) and Kulmala et al. (2014).

3 OBJECTIVES

This study aimed to improve the quantification of boreal coniferous forest terpenoid production, in particular concerning the so far very little studied woody tissues. The aboveground stem and branch biomass (heartwood, sapwood, inner and outer bark) clearly dominates in boreal forest ecosystems. For example, in a Scots pine stand in the sapling stage, the mentioned components account for 88% of the total aboveground biomass (Helmisaari et al., 2002). In a mature stand, the share is even higher, being 95% (Helmisaari et al., 2002). However, knowledge of the VOC fluxes of living woody biomass is very scarce. Thus, this study focused on the variability in emissions from woody compartments, as well as their environmental, physico-chemical and physiological drivers.

Additionally, the aim was to increase knowledge of the previously poorly known relationships between terpenoid synthesis, storage and emissions, and how they vary with respect to environmental stimuli *in situ*. The specific aims were (in parentheses, the papers where they were considered):

to quantify the VOC fluxes of woody tissues with continuous measurements (I);

to analyse the seasonal patterns of tree stem VOC emissions (I, II);

to examine the links between monoterpene synthesis, storage and emission *in situ* (III);

to link the dynamics of stem monoterpene emissions and resin pressure to the physiological processes of a tree (IV);

to provide understanding on within- and between-tree variations in monoterpene emissions and their linkage to ambient air concentrations (V).

To separate the VOC emissions from woody sources or shoots from other VOC sources in the ecosystem, enclosure measurement techniques are appropriate. The advantages and disadvantages of the enclosure techniques are discussed in the methods section.

4 METHODS

4.1 Measurement site SMEAR II

All the samples were collected and all the online analyses were performed at the SMEAR II measurement station (Station for Measuring Ecosystem-Atmosphere Relations) at the Hyytiälä Forestry Field Station in Juupajoki, southern Finland (61°51'N, 24°17'E, 181 m a.s.l.) in the southern boreal vegetation zone. The site is a ca. 55-year-old managed forest dominated by Scots pine. The forest was regenerated by seeding after a prescribed burning in 1962. The site has been thinned once in 2002, its density is 1,075 trees ha^{-1} and the leaf area index is 3.5 m^2 m^{-2} (Ilvesniemi et al. 2009). The dominant height of the trees is about 18 m, with a breast height diameter of about 20 cm. The typical annual tree stem growth rate is 8 m^3 ha^{-1} (Vesala et al. 2005). Several tree canopies are accessible via scaffolding towers at the site.

The soil is classified as haplic podzol formed from glacial till. The mineral soil layer on bedrock is fairly thin, on average only 0.5–0.7 m. The ground is fully covered with dwarf shrubs of bilberry (*Vaccinium myrtillus* L.) and lingonberry (*Vaccinium vitis-idaea* L.), and mosses such as Schreber's big red stem moss (*Pleurozium schreberi* (Brid.) Mitt.) and *Dicranum* Hedw. spp. Some Norway spruces, European aspens (*Populus tremula* L.) and birches (*Betula* spp.) grow as a mixture in the stand. The site is very characteristic from both a local and country-wide perspective. It is also representative of the northern hemisphere, as the natural distribution of Scots pine extends from Spain and Scandinavia in the west almost to the coast of the Pacific Ocean in the east, and from the northern timberline to the Mediterranean and the mountains of the Middle East in the south.

The climate at the site is typical for boreal regions, with cold and snowy winters and mild and humid summers. The mean annual temperature during the period 1981–2010 was 3.5 °C, being –7.2 °C in January and 16.0 °C in July on average (Pirinen et al. 2012). The annual precipitation is 711 mm, being distributed over 202 days on average (Pirinen et al. 2012). The growing season (daily mean temperatures constantly over 5 °C) usually covers a period of 165–175 days.

The site has been comprehensively studied in recent decades, and several long time series are available, for example on micrometeorological and atmospheric compositional parameters, most of them via the online tool SmartSMEAR (http://avaa.tdata.fi/web/smart/smear). Many of these have been utilised as auxiliary data in this thesis.

4.2 Gas exchange measurement techniques

A large part of this study consisted of flux measurements, mainly conducted on a small scale with enclosures on different tree compartments (Table 1). At the ecosystem scale, gas fluxes can be measured with various micrometeorological methods such as eddy covariance or gradient methods. The selection of an appropriate measurement method depends on the data needs. For example, the requirements of temporal and spatial resolution often limit the available measurement techniques. Both small-scale enclosure methods and large-scale micrometeorological methods are important in gaining a

complete picture of the total fluxes over biomes. Different methods complement each other, and, in the best case, their simultaneous use provides the full picture of the studied processes behind the observed fluxes. Moreover, measurements in both controlled laboratory conditions and naturally variable conditions *in situ* offer divergent insights into the studied processes.

4.2.1 Enclosure measurements

The matter fluxes from varying sources, e.g. plants, can be measured in many ways. One of the most established ways is the enclosure method, in which the study object, such as a tree branch, is enclosed in a chamber, in which the gas concentrations can be monitored. The object's gas exchange can be measured either directly in the chamber (headspace sampling) or from ingoing and outgoing air. For practical reasons, the enclosures are usually rather small (<100 l in volume) (e.g. Ortega and Helmig 2008, Niinemets et al. 2011), but some that enclose whole trees exist (e.g. Pier 1995, Barton et al. 2010). The smallest enclosures, usually with rigid structures, are often called cuvettes.

No measurement technique is perfect in all aspects, and this also applies to enclosures. In the case of appropriate VOC enclosure measurements, the requirements are even tighter than, for instance, in CO_2 measurements. Gas exchange measurements with enclosures generally imply that only negligible air chemistry takes place in the enclosed air. In the case of usually rather reactive VOCs, this is even more critical than in the case

Table 1. A summary of the applied measurement objects and methods of this thesis.

	Article				
	I	II	III	IV	V
Tree part					
Stem	x	x		x	
Shoot			x		x
VOC process					
Synthesis			x		
Storage			x	x	
Emission	x	x	x	x	x
Analysis technique					
GC-MS	x		x		x
PTR-MS	x	x		x	
Studied variability					
Intra-tree	x				
Between-tree	x		x		x
Seasonal	x	x	x	x	

of CO_2. This is why the materials of the enclosure system play an important role. By using materials that are as inert as possible and removing reactive pollutants from the air supplied to the enclosure, the air chemistry as well as the absorption and desorption processes of the surfaces can be considerably supressed. In addition, gentle handling of the study object and careful enclosure installation are key steps in acquiring unbiased flux estimates. Niinemets et al. (2011) have provided a comprehensive review of the good practices in VOC enclosure measurements.

4.2.1.1 Steady-state stem enclosures

In the studies of this thesis, gas exchange was measured with a variety of enclosures. The stem enclosures utilized in studies I, II and IV were continuously closed. Their fluxes were calculated based on steady-state calculation methods (Kolari et al. 2009), where the flux is proportional to the concentration difference between the air entering and leaving the enclosure. The fluxes in the shoot chambers were proportional to the rate of concentration change during the chamber closure. This type of chamber measurement is also called dynamic or non-steady-state measurement.

Before 2012, the CO_2 fluxes of pine stems at SMEAR II were studied with small stem enclosures. However, their material and size were not ideal for sampling VOCs. Prior to the start of VOC emission measurements from the stems, appropriate enclosures needed to be developed. The aim was to have enclosures that would be a) adjustable to different stem sizes, b) suitable for use as a part of the existing gas exchange measurement system, and c) functional year-round in variable boreal conditions. The outcome was a wrap-around steady-state enclosure (Fig. 5a), which was included in the SMEAR II gas exchange measurements in early spring 2012. In the enclosure, a tube spiral made of polyethylene-coated aluminium (Synflex, Eaton, USA) was wrapped around the stem to retain an air space between the foil and the bark. Moreover, an FEP tape-covered (Fluorplast, Maalahti, Finland) aluminium brace for inlet and outlet connectors was placed between the spiral and the foil. The surface of the enclosures was made of transparent, UV-permeable, 0.05-mm-thick FEP foil (Fluorplast, Maalahti, Finland). Once the foil had been wrapped two to three times around the stem, the vertical joint was sealed with FEP tape. The enclosure was closed from the both ends with slightly elastic binds or cable ties. The inlet and outlet connectors were screwed on the aluminium brace through small holes in the foil. The air tubes to and from the enclosures were made of FEP and PTFE. Above the enclosure, rainwater flow along the stem was blocked with a collar-type rain cover. The enclosure was mounted without damaging the bark to avoid induced emissions from resinous wounds. The bark surface was so smooth that no levelling with a knife was needed to attain adequate airtightness. The enclosure height was optimised so that the enclosure fitted between branch whorls. One enclosure of a similar structure was installed on a needle-free section of a branch (Fig. 5b).

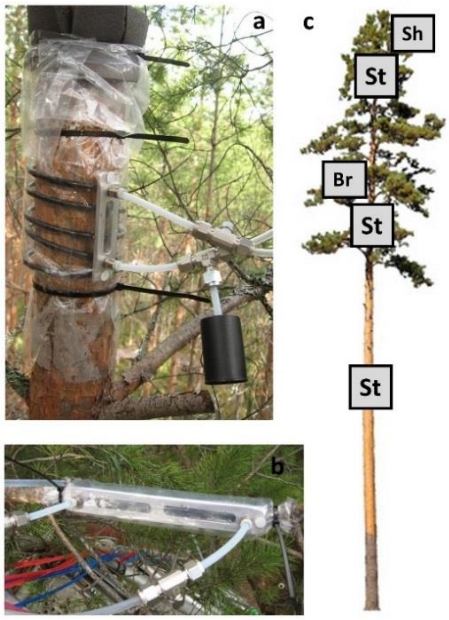

Figure 5. Dynamic enclosures installed on the stem within the living canopy (a) and on a living branch (b). Figure c schematically illustrates the positions of stem (St), branch (Br) and shoot (Sh) enclosures on a pine.

The enclosure heights, dimensions and positions on the trees, as well as the tree parameters, are presented in Tables 2 and 3. Currently, these enclosures have been replaced with more advanced versions, which have opening lids to enable better ventilation. However, this thesis includes no data from these newer enclosures.

The measurements are based on the steady state in the stem enclosure: the flow rate through the enclosure in the steady state was about 1 l min^{-1}. The sampling time for flux calculations was 2 min 45 s, and samples were taken about 24 times per day, depending on the season. To avoid the accumulation of gases inside the enclosure and sample tubing, they were flushed with ambient air at a rate of about 0.4 l min^{-1} between the samplings. The flows were controlled via magnetic valves and mass flow controllers. Sample tubes were heated a few degrees above the ambient air temperature to keep the sample air temperature above the ambient dew point.

In addition to the VOC fluxes, the enclosures were utilized to quantify the stem respiration and transpiration. Up until the end of April 2013, the H_2O and CO_2 exchanges were quantified with URAS 4 infrared light absorption gas analysers (Hartman and Braun, Frankfurt am Main, Germany), and from May 2013 onwards with a Li-840A analyser (Li-Cor, Lincoln, NE, USA). The temperature inside some of the enclosures was followed with copper–constantan thermocouples.

All the fluxes from the stem are expressed per m^2 of bark area. The stem bark area was defined simply as a smooth cylinder surface ignoring the fissures (cracks) in the bark. The whole-tree bark areas were estimated with detailed tree structure measurements at the same site (Kourosh Kabiri, unpublished data) and the LIGNUM tree architecture model (first introduced by Perttunen et al. 1996).

Table 2. Compendium of the enclosures utilised in this thesis.

Enclosure	Tree #	Height from the ground, m	Stem diameter at measurement height, cm	Period when enclosure in use*	Number of VOC measurement days
Stem enclosure 1	1	7	11.6	May 2012–Feb 2014	201
Stem enclosure 2	1	12	8.4	Mar 2012–Jun 2014	460
Stem enclosure 3	1	16.5	3.5	May 2012–Feb 2014	149
Branch enclosure	1	14	1.7^{+}	Mar 2012–May 2014	444
Stem enclosure 4	1	12	9.1	Mar 2015–Jun 2015	73
Stem enclosure 5	2	12.5	10.7	Feb 2014–Aug 2015	232
Stem enclosure 6	3	12.5	9.4	Feb 2014–Aug 2015	196

*Not in use all the time, $^{+}$branch diameter

Table 3. The key parameters of the study trees, measured once each tree was included in the measurements. The tree numbers refer to those in Table 1.

Tree#	Tree height, m	Lower limit of living canopy, m	Diameter at 1.3 m, cm
1	18.6	10	20.4
2	18.6	10.5	21.6
3	17.2	7	22.9

4.2.1.2 Dynamic shoot enclosures

The shoot enclosures to quantify Scots pine foliage VOCs online (e.g. Aalto et al. 2014, 2015) were connected to the same gas exchange system as the stem enclosures. A thorough description of the system and evaluation of its accuracy in VOC measurements is available in Kolari et al. (2012).

In addition to the online shoot enclosure, dynamic flow-through shoot enclosures (Hakola et al. 2006) were employed in studies I, III and V. The cylindrical enclosures, about 4.5 l in volume, consisted of PTFE end plates connected to each other with four FEP-covered rods and transparent FEP foil pulled around the frame. The foil was airtightly closed with rubber bands around the end plates, and its joint was sealed with

FEP tape. The flow through the enclosure was about 4 l min^{-1} during sampling. If the same shoot was sampled repeatedly, the enclosure was kept in place, but the middle part of the end plate was opened and/or the foil was taken off for ventilation.

The air entering the enclosures passed through an ozone-removing scrubber (a cartridge with a MnO_2-coated copper net) to avoid oxidation of the sample volatiles. The air also passed through an active charcoal filter removing organics. The air temperature inside the enclosure was followed with a thermocouple and the radiation levels just above the enclosure with a PAR sensor (LI-190SZ, Li-Cor, Lincoln, NE, USA). The shoot was allowed to equilibrate in the closed, ventilated enclosure before the start of the VOC sampling.

4.2.2 VOC analysis with mass spectrometry

Mass spectrometry is a widely employed measurement technique to study VOCs. Mass-spectrometric techniques were applied in all studies, I–V. The long-term monitoring of stem VOC emissions was performed with a trace gas analyser, a proton transfer reaction quadrupole mass spectrometer (PTR-Q-MS, Ionicon, Innsbruck, Austria; Hansel et al., 1995) connected to the extensive gas exchange measurement system at SMEAR II.

In the analyser, the compound detection is based on ionisation. In the ion source of a PTR-MS, hydronium reagent ions, H_3O^+, form from pure water, H_2O. In the drift tube, these ions transfer protons to the compounds (R) in the sampled air:

$H_3O^+ + R \rightarrow RH^+ + H_2O$

In addition to the direct proton transfer, some other minor reactions such as ligand switching may occur in the ion source depending on the ionization parameters. To enable proton transfer, the compounds (R) must have a higher proton affinity than water. This is why, for example, methane and carbon dioxide cannot be analysed with this instrument. However, most hydrocarbons and some inorganic compounds such as nitrous acid (HNO_2), nitric acid (HNO_3) and ammonia (NH_3) are detectable based on their proton affinities. Ionization by proton transfer is considered as a rather non-destructive and soft method compared to other ionization techniques, i.e. most of the compounds do not fragment in the process.

Next, the protonated ions move to the quadrupole analyser. Basically, a quadrupole analyser works as a filter, which allows ions through based on their mass-to-charge (m/z) ratios. The unitless parameter m/z describes the ratio of the mass of an ion (m) to its charge number (z). If the number of charges equals one, the m/z ratio equals the ion mass. Finally, the ions hit the detector, where their number is calculated as counts per second (cps). The number of ions is usually multiplied: in the analyser in question, this is done with a secondary electron multiplier (SEM). In this thesis, m/z 33 was used as a proxy for methanol and m/z 137 as a proxy for monoterpenes in the sample air.

The counts per second were recorded every 10 seconds. The inlet flow to the instrument was about 70–90 ml min^{-1}. The pressure of the reaction chamber varied between 1.9 and 2.1 mbar, whereas the detection chamber pressure remained very constantly at $1.1*10^{-5}$ mbar.

4.2.3 VOC analysis with gas chromatography

Gas chromatographic techniques were the main methods for analysing VOCs a few decades ago. They still warrant a place in VOC analysis due their supreme ability to separate volatile compounds from each other. However, they often require sample pre-treatment and thus are an offline analysis, which is reflected in the poor time resolution of the analysis. In recent years, gas chromatographic methods have made progress, and their time resolution has improved. Now, plant enclosures can be coupled with an online GC-MS, as presented in Hakola et al. (2017).

The gas chromatographic technique was applied in studies I, III and V, whereas both gas and liquid chromatographic techniques were applied in study III. Generally, the online PTR-MS measurements provided long-term data with a high temporal resolution, and these were complemented with occasional offline GC-MS measurements. The advantage of GC-MS measurements was that they enabled the compound-specific determination of monoterpenes, while PTR-MS only provided the sum of monoterpenes.

For the gas chromatographic analyses in this thesis, VOC samples from air were collected into stainless-steel adsorbent cartridges, known as Tenax tubes (Supelco, Bellefonte, USA), filled with the porous organic polymer Tenax-TA and Carbopack-B (Supelco, Bellefonte, USA) (Harper 2000). The adsorbent tubes were stored in a cooler before and after the sampling. The samples were analysed in the organic chemistry laboratory of the Finnish Meteorological Institute. They were first concentrated with a thermodesorption device (PerkinElmer TurboMatrix 650), after which the compounds were separated from each other with a gas chromatograph (PerkinElmer Clarus 600), and subsequently quantified with a mass spectrometer (PerkinElmer Clarus 600T).

The monoterpene storage pools in study III were defined as in Fischbach et al. (2000, 2002) and Ghirardo et al. (2010) with some minor modifications, as described in the paper. The *in vitro* monoterpene synthase activities, in turn, were analysed similarly as in Ghirardo et al. (2012).

In study V, shoot emissions were analysed with a SPME-GC-MSD (solid-phase microextraction gas chromatograph mass selective detection) and the ambient air monitoring was conducted with an online TD-GC-MSD (thermal desorption gas chromatograph-mass selective detector). SPME sampling was performed by allowing the air flow from the enclosure into a glass compartment and exposing SPME fibre (65 µm DVB-PDMS fibre coating, Sigma-Aldrich, Supelco, Germany) to it. Immediately after the sample collection in the fibre, the sample was analysed gas-chromatographically in a way that enabled enantiomeric separation. A more detailed description of the applied procedures can be found in paper V.

4.3 Other stem measurements

To complement the picture of stem VOC fluxes and their dynamics, the flux data were juxtaposed with other data from the same or neighbouring trees in the same stand. The stem diameter and sap flow measurements were of particular importance in this case.

Tree stem diameters show reversible and irreversible changes. The irreversible ones are caused by cambial growth and typically take place for a fixed period in midsummer. The reversible ones are seen as minor diel diameter changes. Xylem diameter changes indicate fluctuations in the xylem water potential (Irvine and Grace 1997), whereas whole

stem diameter changes indicate fluctuations mainly in the water content and pressure of inner bark. Generally, in summer, xylem shrinks in the daytime during ample transpiration through the foliage, while in the night, xylem swells as water uptake from soil is greater than water loss by transpiration. Laboratory tests have shown that only sapwood affects the changes in xylem diameter (Irvine and Grace 1997). The changes in xylem dimensions can be followed with linear variable displacement transducers (Irvine and Grace 1997), also known as point dendrometers. The method is non-invasive and well suited to continuous long-term measurements. However, transducer measurement values correlate with the water potential only if xylem is unfrozen. A comprehensive description of the dendrometer measurements at SMEAR II is provided in Sevanto et al. (2005). Based on the diameter measurements, the daily radial growth was modelled as in Chan et al. (2016).

Xylem sap flow moves water and dissolved solutes between different parts of trees, and up to tens of metres from the roots to the top of the canopy. Sap flow measurements at SMEAR II are conducted with Granier-type heat dissipation methods (e.g. Granier 1987, paper II), where two probes are inserted into the sapwood.

The resin pressures of Scots pine stems were measured with pressure gauges (Swagelok 316SS and WIKA111.16.40.16). The gauges were installed in drilled holes (4 cm deep) through glycerine-filled brass tubes (10 cm in length and 3.17 mm in outer diameter) and sealed with silicone. The holes were positioned slightly tangentially to puncture more resin ducts. Pressure values were recorded every 0.5 h starting about 24 h after the installation. Due to gradual resin crystallization in the tube, the diurnal variation in pressure continuously diminished and eventually reached the limit of detection, when measurement was stopped (about three weeks after installation). The measurement protocol is described in more detail in paper IV.

5 RESULTS & DISCUSSION

5.1 Scots pine stem VOC emissions

In study I, the monoterpene and methanol emission rates were followed for several consecutive years. The enclosures at different heights of a tree revealed that the emission rates per unit bark area were highest close to the tree top (Fig. 6). Emission rates followed a clear diurnal cycle for most of the year: minimum emission rates were observed during the night and maximum rates usually in the afternoon. Emission rates also displayed a seasonal cycle, which largely followed the changes in ambient air temperatures. However, the monoterpene emission rates were observed to be de-coupled from air temperatures in early spring, as shown in the next section.

The VOC emission rates from living non-damaged woody parts of trees were generally low. Per unit bark or leaf area, they fall below the corresponding rates from the photosynthesising leaf tissues. At the ecosystem level and over the seasonal cycle, the proportion of monoterpene emissions from intact woody conifer compartments (stems, branches) may comprise a few per cent. This share is considerably smaller than that of foliage (roughly about 80–90%) and the forest floor (roughly about 10%), estimated based on the shoot, forest floor and ecosystem-scale measurements at the same site (currently unpublished comparison data).

Figure 6. The Scots pine stem surface is covered by various types of bark, which undergo dynamic changes as the tree ages. This variability and dynamic change is also visible in the volatile emissions from the stem tissues. Examples of pine bark variation in relation to tree height: a–d, pine stem bark from the top towards the stem base; e, branch bark inside a living canopy; f, a grafted pine stem close to the SMEAR II site.

The monoterpene spectra of stem emissions varied between trees and sampling dates. The spectra of the same sampling point were sometimes extremely different, even on two consecutive days. Moreover, the results suggest that there may be qualitative differences in the monoterpene emission blend within a tree.

The pine stems were observed to emit methanol, and the emission rates correlated positively with the xylem sap flow rate and shoot transpiration rate. This suggests that dissolved methanol is substantially moved along the stem transpiration flow. Thus, the majority of the observed methanol was unlikely to have originated from the enclosed section of stem. Moreover, the observed emissions do not all necessarily originate only from the Scots pine stem tissues. Although no lichen thallus was visible in any of the enclosures, it cannot be ruled out that there were lichens, bacteria, yeasts or other epi- or endophytes growing on the bark surface (Lindow and Brandl 2003, Vorholt 2012). Earlier studies have shown that lichens may both emit and absorb an array of volatiles (Ott and Zwoch 1992, Gries et al. 1994, Schieleit and Ott 1997, Kuhn and Kesselmeier 1996, Wilske and Kesselmeier 1999).

Methanol emission measurements were largely impacted by the tendency of methanol to dissolve in water (Laffineur et al. 2012). Emission data measured at higher than 70% relative humidity showed considerable deposition and release periods related to the wet inner surfaces of the enclosure system. Thus, the methanol data utilized in the further analyses were limited to the occasions with relative humidity below 70%. To decrease the impact of water-soluble compound deposition on the enclosure system surfaces, new better-ventilated stem enclosures are currently in use at the SMEAR II measurement site. They are open between the samplings, which reduces the amount of condensed water on the inner surfaces.

5.2 The effects of spring recovery on stem monoterpene emissions

An exceptional springtime coupling of monoterpene emission rates and tree water relations was described in study II. A rapid (lasting several hours) but large (up to tens of ng m^{-2} s^{-1}) monoterpene emission burst from stem coincided with the recovery of stem water transport. The burst occurred shortly after the last freezing period of the spring in both 2012 and 2013. In tandem with the high monoterpene emissions, the stem radius showed an irregular behaviour that differed from both the regular pattern observed in summer conditions and the patterns related to frozen stems. Moreover, exceptional nighttime sap flow was observed. The emission burst took place in the early spring, when the ground was still covered with snow and the growing season was about to start.

Several counteracting and overlapping physical and chemical processes may have caused the observed phenomena. However, the observed anomalies were most likely related to the refilling of embolized xylem tracheids in spring. They indicated the spring recovery of tree tissues, i.e. a phase change in stem water transport capacity, which preceded the active period of intensive photosynthesis and growth. However, a recent study by Lim et al. (2016) has shown that resin acid (diterpene) synthesis in Scots pine sapwood peaks strongly in early spring (April). Further transcriptomic studies could reveal whether monoterpene synthesis in sapwood is also intensive at the same time, and if this could explain the observed emission peaks.

5.3 Relationships between monoterpene synthesis, storage and emission

Study III concerned the VOC synthesis–storage–emission continuum. The study concentrated on monoterpenes, the main volatile constituent of Scots pine oleoresin. Monoterpene synthase activity, as used in study III, refers to the maximum potential of the needles to produce monoterpenes in favourable conditions. Thus, the real synthesis rate may be significantly lower than the maximum potential due to limiting environmental factors such as low temperatures or restricted substrate availability. Seasonality and needle aging were found to be the main factors causing the variation in monoterpene synthase activity, storage pools and emissions. In principal component analysis, they together comprised more than half of the total variation.

Monoterpene synthase activities in needle tissues were shown to be highest during the first year after needle formation, after which the activity stabilized at a lower level for the rest of the needles' lifetime. The needle monoterpene storage pool, on the other hand, remained rather constant both quantitatively and qualitatively as needles aged (Fig. 7).

There was a notable qualitative difference between synthesised, stored and emitted monoterpenes (Fig. 7). For example, in tree #3 of this study, the relative proportion of δ-3-carene was constantly very low in the monoterpene synthase assay, higher in the stored monoterpenes, but dominant in the emission profile. This indicates a compound-specific temporal decoupling between biosynthesis, storage and emission of Scots pine needle monoterpenes. Similar de-coupling between needle monoterpene contents and shoot monoterpene emissions has been observed in Douglas fir (*Pseudotsuga menziesii*) by

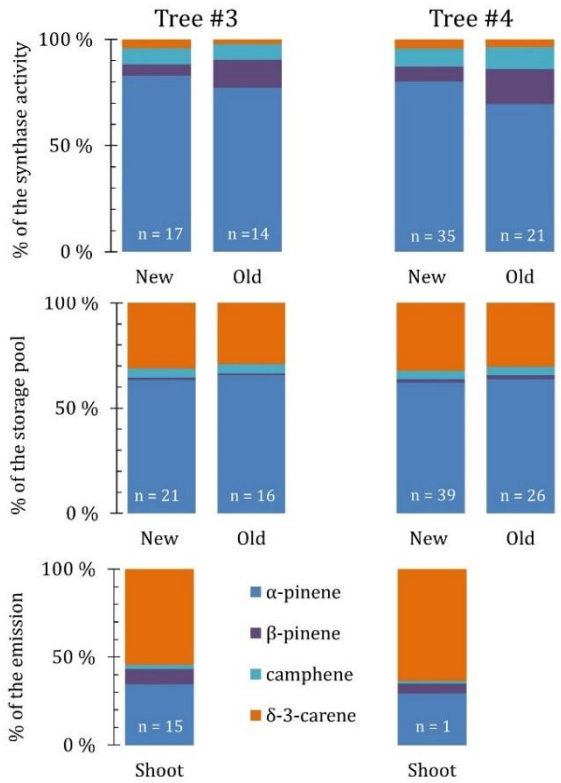

Figure 7. The relative proportions of different monoterpenes in the synthase activities and storage pools of needles and in the emissions from shoots. The proportions are averages of samples from Scots pine #3 (left) and #4 (right) of study III. *New* refers to the needles in their first year (until the end of June in the year following their maturation), and *old* to the needles older than that. Sampled emissions originated from both new and old needles and a twig.

Schindler and Kotzias (1989). In some exposure studies, δ-3-carene has been considered as the least toxic monoterpene to many herbivores and fungi (Raffa 2014, Reid et al. 2017). This is an interesting point, which may open many insights into the complex relationships between Scots pines and other organisms.

5.4 The effects of resin pressure on monoterpene emissions

Resin is under positive pressure in the resin duct system. In study IV, the changes in Scots pine stem resin pressure were positively correlated with the ambient temperature and shoot transpiration rate. The highest pressures were recorded in the afternoon, whereas the lowest ones occurred in the early morning hours before sunrise. The daily pressure range was 0.5–1.5 bar, and the highest recorded pressure was about 9 bar. In each measurement period, the resin pressure followed a similar diurnal pattern, although the actual pressure recordings continuously decreased due to gradual crystallization of the resin in the bored hole and pressure gauge tube. The observed diel pattern was the opposite of the pressure observations from American pine species in earlier studies (e.g. Schopmeyer et al. 1952, Bourdeau and Schopmeyer 1958, Vité 1961, Lorio and Hodges 1968ab, Helseth and Brown 1970). The reason behind the differences may be the climatic differences between the measurement sites and resulting differences in the xylem water potential.

The lowest resin pressures were recorded in the upper part of stem, where the lowest water potentials also occurred. The stem monoterpene emission rates were observed to be related to both temperature and resin pressure, but as temperature also affects resin pressure, part of the temperature effect comes through resin pressure. Moreover, there might be a lag between the resin pressure changes and monoterpene emissions, which could not, however, be defined in this study due to differences in measurement intervals. The results indicate that the effect of temperature overrides the effects of tree water status on resin pressure. The dominance of temperature is also illustrated in the fact that no resin pressure could be measured in the spring before May, because viscous resin did not flow to the measurement tube.

5.5. The diel cycles and chirality of VOC emissions and their effects on ambient air

Some monoterpenes are chiral, i.e. they exist in nature in two mirror image forms (cis and trans). For example, α-pinene has two chiral forms, which are both present in Scots pine emissions: (+)-α-pinene and (–)-α-pinene. The different forms are also known as enantiomers. They are crucial in many ecological interactions, as, for example, many herbivores are only able to perceive either the (+) or (–) enantiomer emitted by their host plant. Earlier studies (e.g. Persson et al. 1993, Valterova et al. 1995, Sjödin et al. 1996) have shown that marked within-tree and between-tree variation occurs in the enantiomeric composition of conifer resin. However, the enantiomeric analysis of VOCs was only conducted in study V due to technical restrictions.

Paper V was the first study on the enantiomeric ratios of boreal tree VOC emissions and simultaneous ambient air concentrations covering full diurnal cycles. The results demonstrate that not only were the emission blends of the studied four Scots pines different, but also the stereochemistry of the emitted compounds. An interesting feature

in the results was that in α-pinene emissions from Norway spruce, the (–) form was more abundant and followed the diurnal cycle of light intensity (PAR) with a lag, but the (+) form remained rather stable around the clock. This suggests that the latter form mainly originates from the storage pools, whereas the former originates from *de novo* synthesis. Without chiral analysis, it would have remained unclear that a part of the α-pinene emissions were close to constant around the clock.

Once the compounds have volatilized out of the plant tissues, they form part of the ambient air. Their average lifetimes in the atmosphere vary from a few seconds to several years, largely depending on the compound and the prevailing conditions (e.g. Kesselmeier and Staudt 1999, Atkinson 2000). Thus, the ambient air concentrations observed in study V represent the outcome of ecosystem emission and deposition, possible long-range transport in air masses, and the reactivity of the emitted compounds with atmospheric oxidants.

Monoterpene concentrations in ambient forest air are highest during the night, although the monoterpene emissions are lowest at that time (V). At night, the atmospheric boundary layer is low, and the reactions of monoterpenes with O_3 and OH radicals are suppressed. In the day, the high boundary layer and ample reactions with O_3 and OH keep the monoterpene concentrations low, although emissions are highest. The chiral analysis revealed that the ambient air concentration of α-pinene was dominated by the (+) form throughout the campaign period. This suggests that the Scots pines of the carene chemotype dominate in the surroundings of SMEAR II, because carene chemotypes of Scots pine mainly emit the (+) form of α-pinene.

Two diurnal isoprene mixing ratio peaks have earlier been observed (Dreyfus et al. 2002), but here, three peaks were observed: in the morning at about 7 AM, around noon, and in the evening at 7 PM. However, the highest isoprene mixing ratios were observed around 7 PM. This is probably due to the low boundary layer and continuing isoprene emissions (originating from light-dependent *de novo* synthesis) from Norway spruce and European aspen in high light and temperature conditions.

6 CONCLUSIONS

Scots pine is not a homogenous source of volatiles, but a combination of emission sources with different properties and dynamics. Monoterpene emissions from foliage are dependent on the incident temperature and partly also compound specifically on the light intensity (e.g. Tarvainen et al. 2005), as well as on the seasonal cycle of needles, including growth and senescence periods (Aalto et al. 2014, 2015). Volatile emissions from foliage are partly controlled by stomata (e.g. Niinemets and Reichstein 2003), but this control mechanism is absent for emissions originating from woody tissues. Thus, monoterpene emissions from woody tissues are more strongly controlled by temperature, as well as by the physiology of the whole tree. Emissions from pine stems are connected to the stem water relations, at least during the spring recovery period (paper II) and over the growing period in the case of water-soluble compounds (I). Moreover, intra- and inter-tree variation in the diffusion barriers of stem tissues (e.g. properties of early- and latewood, bark properties) affect the VOC fluxes (Wildhalm et al. 2015).

The majority of research has focused on foliar VOC release. VOC release from woody compartments follows the same regularities as in the case of any other plant tissue, but there are some differences as well, such as the mean distance between synthesis and emission sites usually being larger than in the foliage.

To date, the role of woody tissues in forest BVOC fluxes has largely been unknown. In paper I, it was demonstrated that woody tree tissues have a minor contribution to the VOC fluxes at the forest ecosystem level under normal, non-stressed conditions. The contribution may, however, increase considerably if a forest stand is exposed to major stress stimuli. In natural conditions, such stimuli may be caused by a herbivore attack or storm. In commercial forests, recurrent clearances, thinnings and final harvests are the most common sources of stress. It is obvious that the contribution of BVOC fluxes is different in the case of different coniferous and deciduous tree species. Thus, future studies should continue to measure the emissions from woody tissues of other tree species than Scots pine.

The linkages between VOC synthesis, storage and emissions were examined in study III. The linkages appeared even more complex than expected. The composition of the monoterpene pool in needles does not reflect the composition of monoterpene emissions released from the shoots. This means that emission estimates based on the storage pool composition or storage pool estimates based on emission spectra are not reasonable and should be avoided. Besides, the linkages are highly compound-specific, as demonstrated, for example, by the above-discussed case of δ-3-carene. Thus, generalization over, for instance, all monoterpenes or other compound groups should be avoided. Conducting a similar comparison but with woody tissue monoterpene synthase activities, storage pools and emissions would be fascinating and would provide insights into the differences in foliage and woody tissue VOC dynamics.

The chiral properties of volatile compounds were only investigated in paper V due to limited technical facilities. However, chirality would have been interesting and may also have opened new insights in the other studies of this thesis: Some physiological changes in a plant may only be reflected in the chirality of a compound, while the emission rate of the same compound may remain very much the same. The chiral properties of volatiles should be further investigated in future VOC studies, especially related to plant stress reactions and herbivory.

Peñuelas and Staudt (2010) concluded in their extensive review on BVOCs and global climate change that BVOC emissions will most likely globally increase as a consequence of ongoing global change. Gauthier et al. (2015), on the other hand, have concluded that the health of boreal forests is under threat due to global change. Thus, it appears evident that the BVOC fluxes of boreal forests will change in the coming decades, but the details (magnitude, timing etc.) are uncertain. In the summer, increased temperatures will probably lead to increased volatilization, but higher temperatures will also accelerate synthesis if other factors such as water availability do not limit it. Thus, the net effect on resin reservoirs, for example, remains uncertain. The monoterpene loss from storage pools may increase in the dormant period if ambient temperatures rise and the vaporization correspondingly increases. If the net primary productivity and the biomass of forest ecosystems increase as expected, the amount of volatiles released into the atmosphere will also increase. However, many factors such as land use and the resource availability of plants considerably affect the net emissions. One factor that climate change will not affect to a great extent is light (PAR) availability. It will continue to limit winter photosynthesis in the boreal region in the future and will be reflected in VOC synthesis and especially in *de novo* emissions in winter.

Boreal tree species have a high adaptive capacity, which assists their survival under recurrent disturbances and in a wide climatic range (Gauthier et al. 2015). Scots pine shares the same traits underlying this capacity as many other boreal conifers: it has wide-ranging population through the northern hemisphere, a large tolerance for an array of environmental constrains, and wide within-population genetic diversity (Gauthier et al. 2015). This is reflected in the VOC dynamics of Scots pine: trees show considerable within-population variability in their terpene emission spectrum and strength (I, III, Bäck et al. 2012). Terpenes are a part of the effective resin-based defence system against herbivores and pathogens, which Scots pine has developed in the course of tree generations. The resin-based defence system has clearly been very successful, as Scots pine is the dominant tree species over large areas.

The studies of this thesis have offered the first insights into VOC emissions from the woody compartments of Scots pine, but the volatile emissions from the woody compartments of 60 065 other tree species (Beech et al. 2017) remain to be studied. However, the VOC dynamics of Scots pine still offer many open research questions. For example, the VOC synthesis–storage–emission continuum in stem tissues remains unstudied. In addition, the connections of stem VOC dynamics and whole-tree physiological processes warrant further research, including studies on resin dynamics and properties *in situ*. Knowledge of qualitative and quantitative tree-to-tree variation has increased in recent years, but the larger picture is still incomplete.

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