**Dissertationes Forestales 72**

# Impacts of climate change and forest management on monoterpene emission and needle secondary compounds of Boreal Scots pine (*Pinus sylvestris* L.)

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Academic dissertation

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*Title of dissertation:* Impacts of climate change and forest management on monoterpene emission and needle secondary compounds of Boreal Scots pine (*Pinus sylvestris* L.)

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The European boreal forests emit significant amounts of biogenic volatile organic compounds, including monoterpenes. Besides their various ecological roles in plants, emitted monoterpenes have an impact on the physical and chemical properties of the lower troposphere. Changes in the emission potential of these forests are very important, and it is essential to evaluate the effects of anthropogenic disturbances and the predicted climate change on the emissions, and the factors affecting the emissions, including the accumulation of secondary compounds in trees. Hence, the objectives of this study were to quantify the monoterpene flux of a Scots pine forest stand throughout one growing period, including the contribution of the tree canopy to the total ecosystem flux, to evaluate the effects of timber felling on the ambient monoterpene concentration of Scots pine forest air, and to evaluate the long-term effects of elevated $CO_2$ and temperature on monoterpene emission and needle secondary compounds (monoterpenes, HPLC-phenolics and condensed tannins) of Scots pine saplings grown in closed-top environmental chambers.

Timber felling significantly increased the ambient monoterpene concentration of a Scots pine forest. Logging residue was the most important factor explaining the increment of the aerial concentration of monoterpenes. The amount of monoterpenes released from the residue appeared to depend on its temperature, which, in turn, is dependent on the microclimatical conditions of the managed site. Therefore, the shading of the remaining canopy cover can indirectly affect the monoterpene release from the emitting biomass. The tree canopy was found to contribute most of the ecosystem scale monoterpene flux. Hence, partial or total removal of the tree canopy will have a decreasing effect on the total emission of managed sites due to the reduction of the monoterpene emitting foliage. The significant increase in the concentration induced by the felling implies that there is a great potential impact of forest management on local or even regional atmospheric chemistry.

Increased carbon availability with increased temperature induced changes in the accumulation and emission of secondary compounds of Scots pine. The monoterpene emission was increased substantially due to elevation of $CO_2$ and temperature, whereas the needle monoterpenes were reduced. The responses of the needle secondary compounds to the elevation of $CO_2$ and temperature were variable and dependent on the stability of the compound (metabolite – end product), phase of growth and the needle age. Predicted changes in the tree species distribution in the Boreal zone will likely affect the monoterpene emissions of Scots pine on a larger scale. In the Finnish forests, the proportion of Scots pine is predicted to reduce with increased dominance of birches and Norway spruce. Nevertheless, the increased emission capacity of individual trees due to the predicted climate change will compensate a great part of the loss of emitting biomass of Scots pine.

**Keywords:** BVOC, boreal forest, logging, silviculture, elevated $CO_2$ and temperature, secondary metabolism

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Joensuu, September 2008

Tommi Räisänen

## LIST OF ORIGINAL ARTICLES

This thesis is a summary of the following articles, which are referred to in the text by their Roman numerals (I-IV):

I Räisänen, T., Ryyppö, A. & Kellomäki S. Monoterpene emission of a boreal Scots pine (*Pinus sylvestris* L.) forest (Manuscript).

II Räisänen, T., Ryyppö, A. & Kellomäki S., 2008. Impact of timber felling on the ambient monoterpene concentration of a Scots pine (*Pinus sylvestris* L.) forest. Atmospheric Environment 42:6759-6766. doi:10.1016/j.atmosenv.2008.05.035

III Räisänen, T., Ryyppö, A. & Kellomäki S., 2008. Effects of elevated $CO_2$ and temperature on monoterpene emission of Scots pine (*Pinus sylvestris* L.). Atmospheric Environment 42:4160–4171. doi:10.1016/j.atmosenv.2008.01.023

IV Räisänen, T., Ryyppö, A., Julkunen-Tiitto, R. & Kellomäki, S., 2008. Effects of elevated $CO_2$ and temperature on secondary compounds in the needles of Scots pine (*Pinus sylvestris* L.). Trees 22:121–135. doi:10.1007/s00468-007-0175-6

T. Räisänen participated in research planning, was responsible for sample collection and conducted most of the laboratory analyses for articles I and II. Räisänen performed all of the data analyses and was the main author of articles I-IV.

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# INTRODUCTION

## Secondary compounds

Secondary compounds are a diverse group of organic compounds which are not directly involved in plant growth or development. Most of these compounds have significant ecological importance in the interactions between plant and its environment, since they function in protection against herbivores, bacteria and fungi, or as attractants for pollinators, or as allelopathic agents (e.g. Croteau et al. 2000). Plant secondary compounds can be divided into three groups on the basis of the different biosynthetic pathways they originate: alkaloids, phenolics and terpenoids. However, division of compounds between primary and secondary metabolites is not that unequivocal, since some compounds within these groups have significant functional roles in plant primary metabolism, for instance as hormones, electron carriers, or structural components of membranes (Kozlowski and Pallardy 1997).

Alkaloids are mainly synthesized from amino acids and therefore contain one or more nitrogen atoms, and are most common in herbaceous plants (Croteau et al. 2000). Most plant phenolic compounds are products of phenylpropanoid metabolism and they have a very broad range of physiological roles (Hahlbrock and Scheel 1989). For instance, flavonoids serve as defences against herbivores and pathogens, and also many flavonoids protect plants against the harmful UV-B radiation (e.g. Schnitzler et al. 1997). Condensed tannins (proanthocyanidins), polymers of flavan-3-ol units, are synthesized by the phenylpropanoid-acetate pathway. Condensed tannins are frequent constituents of woody plants, and are generally considered as substances that significantly reduce the growth and survival of many herbivore species due to their protein precipitation ability (Croteau et al. 2000). Terpenoids are hydrocarbons formed of varying numbers of isoprene units ($C_5H_8$). The metabolical precursors of terpenoids are isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) (McGarvey and Croteau 1995). IPP is synthesized by the mevalonic acid (MVA) pathway in the cytosol and the methylerythritol (MEP) pathway in plastids. Monoterpenes ($C_{10}H_{16}$) are dominantly synthesized by the MEP pathway, although the pathways may not be completely independent (Schuhr et al. 2003). Monoterpenes are probably best known for their role in volatile essences of flowers and the essential oils of herbs and spices, but monoterpenes also function in plant defence as constitutive substance and phytoalexins against herbivores (Seybold et al. 2006) and microbes (Steinbrecher and Ziegler 1997). Additionally, monoterpenes serve as solvents for resin acids in oleoresin (Gershenzon and Dudareva 2007) which is accumulated in multicellular secretory structures in conifer tissues (e.g. Turtola et al. 2002) (Figure 1).

## Biogenic volatile organic compounds

### *Global and regional emissions of BVOCs*

Terrestrial vegetation emits large quantities of biogenic volatile organic compounds (BVOCs) into the atmosphere. A wide variety of compounds are included in BVOCs, such as many terpenoids, carbonyls, alcohols, esters, ethers, and acids (Kesselmeier and Staudt 1999). Excluding methane, (the most abundant VOC, but usually not included in the BVOCs), isoprene and monoterpenes are the dominant BVOCs emitted by vegetation on a

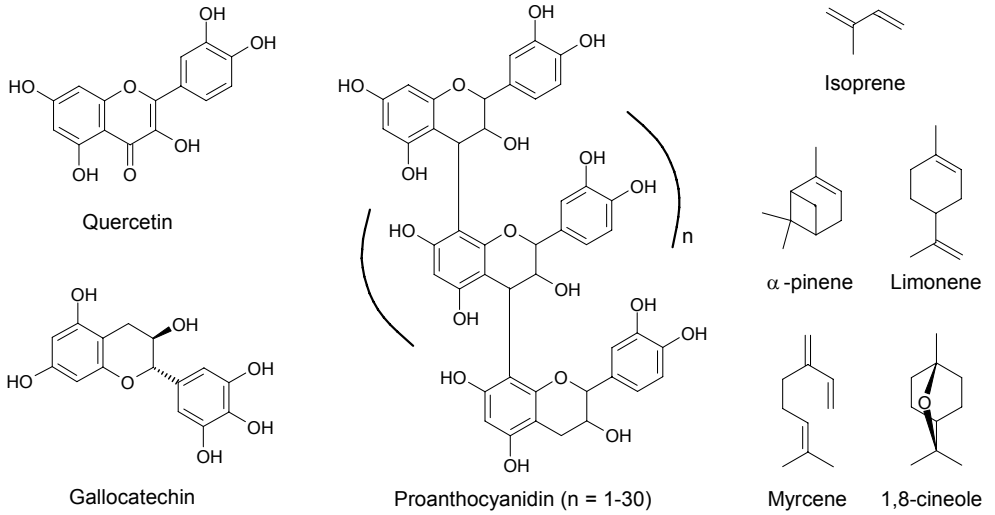


**Figure 1.** Molecular structures of selected phenolic compounds (quercetin (a flavonol), gallocatechin (a flavan-3-ol), and proanthocyanidin (condensed tannin)) and monoterpenes (α-pinene, limonene and myrcene), an oxygenated monoterpene (1,8-cineole) and the hemiterpene isoprene.

global scale (Guenther et al. 1995). Large geographical variation exists in the BVOC emission, largely following the distribution of forested areas (Simpson et al. 1999). The boreal vegetation zone is one of the major sources of BVOC (mainly isoprene and monoterpenes) emission (Guenther et al. 1995) due to the large emission potential of the boreal tree species (Kesselmeier and Staudt 1999). Globally, the emission of BVOCs have been estimated to exceed the VOC emissions from anthropogenic sources (Fehsenfeld et al 1992, Guenther et al. 1995). However, variation in the regional contribution of anthropogenic and biogenic emissions is great, depending on the land-use characteristics and population density in relation to the natural environment of different countries (Simpson et al. 1999). In Finland, the biogenic emissions are estimated to be almost two-fold compared to the anthropogenic emissions (Lindfors and Laurila 2000). The BVOC emissions from the Finnish forests are dominated by monoterpenes (45% of the total annual emissions of 319 kilotonnes i.e. 0.71 tonnes of monoterpenes per $km^2$ forest land), whereas the isoprene emission is only about 7% of the total (Lindfors and Laurila 2000). Monoterpenes are emitted by all main tree species found in the forests of Finland (Kesselmeier and Staudt 1999).

*Monoterpene emission*

The group of monoterpenes consists of acyclic, mono-, bi- and tricyclic compounds (Figure 1) with differing chemical properties, but the mutual characteristic for all monoterpenes is that they are highly volatile (Steinbrecher and Ziegler 1997). The volatility of a particular compound is dependent on its vapor pressure within the tissue where it is synthesized or stored, which in turn is controlled by temperature and the compound concentration within the tissues (Lerdau et al. 1997). Besides temperature, the physicochemical constraints of

plant VOC emission are related to leaf structure and stomatal openness (Niinemets and Reichstein 2003). In the case of monoterpenes, reduction in stomatal conductance does not appear to affect the emission rate due to the rapid upsurge of compound partial pressure within the leaf, so that the decrease in diffusion conductance is compensated by increased diffusion gradient from the within-leaf air space to the surrounding air (Niinemets et al. 2004). The monoterpene emission of conifers is generally regarded as light-independent because it mainly originates from compounds stored after synthesis in special organs, such as resin ducts in *Pinus* species (Monson et al. 1995, Lerdau et al. 1997). However, several studies have shown that the emitted monoterpenes originate also from light-dependent biosynthesis, indicating that monoterpene emission from many plants, including Scots pine, is a combination of stored and recently synthesized compounds (Kesselmeier et al. 1997, Staudt et al. 1997, Shao et al. 2001). Therefore, the emission of monoterpenes from plant foliage is also physiologically controlled by the rate of synthesis via intermediate availability and enzyme activity (Niinemets et al. 2004).

The reason for plants to emit monoterpenes or the function of the emission is not completely clear. It has been shown that monoterpenes can make the photosynthetic apparatus more resistant to high temperatures, thus protecting it against heat stress (Loreto et al. 1998, Delfine et al. 2000). Volatilized monoterpenes also have a potential to react with various oxidizing agents present in the atmosphere (Calvert et al. 2000) and thus could protect plants against internal oxidative damage (Loreto et al. 2004). Herbivory has been found to induce monoterpene emission, which can attract the natural enemies of the herbivores (Paré and Tumlinson 1999, Kessler and Baldwin 2001). Furthermore, not only the monoterpenes inside plant organs are detrimental to herbivores (reviewed by Seybold et al. 2006), but also the volatilized vapor itself has been found to be toxic (Raffa et al. 1985). Excluding these protective functions, the emission occurring in "normal" or undisturbed conditions could simply be a result of the high volatility of these compounds.

*Transformation processes of BVOCs*

Once released into the atmosphere, BVOCs have a significant role in the chemistry of the lower troposphere. Transformation processes of these compounds include reactions with the hydroxyl radical (OH), nitrate radical ($NO_3$) and ozone ($O_3$) (Yu et al. 1999, Atkinson 2000, Larsen et al. 2001, Atkinson and Arey 2003). For the majority of BVOCs, wet and dry deposition is thought to be of minor importance in the tropospheric losses of these compounds (Atkinson 2000). Tropospheric $O_3$ is produced by reactions of BVOCs and oxides of nitrogen ($NO_x$) in the presence of sunlight, resulting in the existence of $O_3$ throughout the troposphere (Calvert et al. 2000). These reactions are particularly important in urban, polluted areas where air quality standards are often violated (Bell et al. 2004). The reaction processes can affect the oxidizing potential of the atmosphere, thus indirectly controlling the accumulation of methane and other greenhouse gases in the atmosphere by increasing their atmospheric lifetimes due to the consumption of the oxidizing agents (Lelieveld et al. 1998). A different outcome of the reactions of BVOCs in the atmosphere is the formation of secondary organic aerosols (SOA). For instance, volatilized monoterpenes and sesquiterpenes contribute to SOA formation through gas/particle partitioning of their tropospheric reaction products (Hoffmann et al. 1997, Christoffersen et al. 1998, Kamens et al. 1999, Koch et al. 2000). The importance of SOAs is in their influence on the radiative balance of the atmosphere, which is also associated with climate change (Pilinis et al. 1995,

IPCC 2007). The European boreal forest region is found to be a substantial source of atmospheric aerosols due to the ample emission of monoterpenes (Tunved et al. 2006).

All gaseous monoterpenes react with the oxidizing agents in the atmosphere (OH, $O_3$, $NO_3$) (Calvert et al. 2000). The atmospheric lifetimes of individual monoterpenes are variable, mainly depending on the chemical structure of each compound, but also strongly depending on the concentrations of the oxidizing agents in the atmosphere (Atkinson and Arey 2003). For instance, the lifetime of $\Delta^3$-carene for reaction with OH is almost half of that of α-pinene (if the OH radical concentration is assumed to be $2.0 \times 10^6$ molecules $cm^{-3}$), but then again the lifetime of $\Delta^3$-carene for reaction with $O_3$ (assumed concentration of $7.0 \times 10^{11}$ molecules $cm^{-3}$) is more than double compared to α-pinene (Calvert et al. 2000). α-pinene, one of the most abundant monoterpenes found in the air above the Finnish forests (e.g. Rinne et al. 2000), has an estimated average lifetime of 2.6 h for reaction with OH, 4.6 h for $O_3$ and 11 min for $NO_3$ (with same concentrations of the oxidizing agents as described above) (Calvert et al. 2000). However, the lifetimes vary with season and time of day (Kesselmeier and Staudt 1999) together with varying concentrations of the oxidizing agents (Tsigaridis and Kanakidou 2002).

**Climate change – impacts on secondary metabolites and BVOC emissions**

Today's atmospheric $CO_2$ concentration is estimated to be doubled by the end of this century. Along with the increased levels of other greenhouse gases this is predicted to increase the mean surface temperature by 1.8-4.0°C (best estimates) on a global scale (IPCC 2007). Coupled with rising temperature the increasing $CO_2$ levels in the atmosphere will likely have diverse effects on the primary plant metabolism (Morison and Lawlor 1999), which has been the center of the intensive research focus for over 20 years. However, the predicted climate change may also affect the secondary metabolism and induce changes in the accumulation of secondary compounds (e.g. Saxe et al. 1998), which in turn, can affect the ability of trees to adapt to their biotic and abiotic environment.

In terms of plant metabolism, the key issue of the changes in the atmospheric $CO_2$ concentration is the increase of carbon available for the fundamental physiological processes of plants. Since the production of secondary compounds can be considered as a trade-off between the allocation of carbon to growth and production of carbon-based defences, it is possible that the excess carbon that is not used for growth is directed to the production of secondary compounds (Bryant et al. 1983, Drake and Gonzàles-Meler 1997, Fritz et al. 2006). However, the effects of increased $CO_2$ and concomitant increase in temperature on carbon-based secondary metabolites are quite variable depending on the studied plant species, age and the duration of the exposure (reviewed by Peñuelas and Estiarte 1998).

As described earlier, in terpene-storing species (such as Scots pine), the storage pool is considered to be the major source of terpene emission (Kesselmeier and Staudt 1999), but also the rate of immediate terpene synthesis can affect the overall emission (Shao et al. 2001). Elevation in atmospheric $CO_2$ concentration is not thought to affect the pool emission directly, but changes in the pool size within tissues can occur, although the previously reported findings are not very consistent. Monoterpene levels in the needles of Douglas fir (Litvak et al. 2002, Snow et al. 2003) and Scots pine (Sallas et al. 2003) have been reported to reduce due to elevated $CO_2$, but then again no significant effect has been found in other studies with Douglas fir, Ponderosa pine (Constable et al. 1999) or Scots

pine (Kainulainen et al. 1998). The effect of elevated temperature on the monoterpene pool size is equally unclear. Elevation of growth temperature has been found to decrease (Snow et al. 2003) or have no effect (Constable et al. 1999) on the monoterpene concentration in needles of Douglas fir but increase the concentration in Scots pine (Sallas et al. 2003). Similarly, the effects of $CO_2$ elevation on BVOC emission reported in literature are not consistent at all, and vary greatly due to the different experimental approaches. For instance, elevated $CO_2$ has been reported to increase the monoterpene emission of non-storing species as *Quercus ilex* (Staudt et al. 2001) or *Betula pendula* (Vuorinen et al. 2005), but no significant effect on monoterpene emission has been found in monoterpene storing species as *Pseudotsuga menziesii* or *Pinus ponderosa* (Constable et al. 1999). Short-term elevation of $CO_2$ has generally been found to decrease the BVOC emission, particularly the purely synthesis-originated emissions, since the terpenoid emission is uncoupled from photosynthesis by reductions in the availability of the terpenoid precursors (Loreto et al. 2001, Rosenstiel et al. 2003).

**Assessing the BVOC emissions**

Efforts to make inventories of the BVOC emissions of forests are often based on the assumption that the emission potential of a certain area is static (e.g. Lindfors and Laurila, 2000) or that the emission capacity is controlled only by meteorological factors or the seasonal changes of the emission (e.g. Tarvainen et al. 2007). Additionally, the emission potential itself in an area or ecosystem can be slightly ambiguous. In some previous studies the measured total ecosystem flux has been interpreted to represent the emission of the tree canopy (Rinne et al. 2000) or respectively the canopy scale emissions are thought to represent the total flux of the ecosystem (Lindfors and Laurila 2000, Komenda and Koppman 2002). However, besides the canopy layer, emission originating from the forest floor (litter, root system, ground layer of plants) contribute to the total ecosystem flux (Janson 1993, Hellén et al. 2006). Generally, the naturally occurring emission is very sensitive to any kind of disturbance. The emission has been reported to increase dramatically due to several stressors to the emitting plants (e.g. Litvak et al. 1999, Loreto et al. 2000). It can be of major importance to assess the potential impact of biotic or abiotic disturbances, such as herbivory (Litvak et al. 1999) or forest management (Schade and Goldstein 2003) on the BVOC emissions of forests in order to obtain a comprehensive understanding of the emissions at a regional level.

Forestry land covers 86% of the land area of Finland. The total growing stock in the forests is 2176 million $m^3$ with an annual increment of 97 million $m^3$ of wood. Half of the growing stock consists of Scots pine (Peltola 2006). During the years 2001-2005, on average 56 million $m^3$ of wood was felled annually as a result of wood procurement of the forest industry (Nuutinen and Hirvelä 2006). Hence, forest management is the dominant disturbance in the forests of Finland. Annually, 4000-5500 $km^2$ of forests are subjected to commercial thinnings (61%), clear-cuts (29%) and other cuttings, which corresponds to 2-3% of the total forest land (Peltola 2006).

Damaging plant organs has been found to increase the terpene emissions of conifers, because wounding breaks the intact storage structures within the tissues (Litvak and Monson 1998, Litvak et al. 1999, Loreto et al. 2000). Therefore, cutting and pruning of trees can be expected to increase monoterpene emissions from silviculturally managed sites mostly as a result of volatilization from the decaying logging residue. Studies on the effects

of cuttings on the BVOC emissions of forests are very rare. Schade and Goldstein (2003) studied the effects of thinning on the mixing ratios and emission rates of monoterpenes in a ponderosa pine (*Pinus ponderosa*) plantation. During the thinning, monoterpene fluxes increased by a factor of forty, and further model estimation showed that the annual emission was increased by a factor of five due to higher basal emission rates. Considering this, in areas with a high proportion of intensely managed forests, such as Finland, the BVOC flux increment is likely too significant to be ignored in regional emission inventories.

## Objectives

The European boreal forest is a significant contributor to the global emissions of BVOCs, especially monoterpenes, hence it is important to assess the actual release of these compounds in order to better understand their significance in the biosphere-atmosphere interactions (Figure 2). Accordingly, changes in the emission potential of these forests are very important, and therefore it is essential to evaluate the effects of anthropogenic disturbances, and furthermore the effects of the predicted climate change on the emissions, and the factors affecting the emissions. Therefore, the specific objectives of this study were to

(1) quantify the monoterpene flux of a typical Scots pine forest stand in Eastern Finland throughout one growing period, including determination of the contribution of the tree canopy to the total ecosystem flux (I),

(2) evaluate the effects of felling of Scots pine timber on the ambient monoterpene concentration of forest air below the canopy layer (II),

(3) evaluate the long-term effects of elevated $CO_2$ concentration and temperature on monoterpene emissions of Scots pine saplings grown in closed-top environmental chambers (III), and

(4) evaluate the long-term effects of elevated $CO_2$ and temperature on secondary compounds, in particular monoterpenes, low molecular weight phenolics and condensed tannins in the needles of Scots pine saplings grown in closed-top environmental chambers (IV).

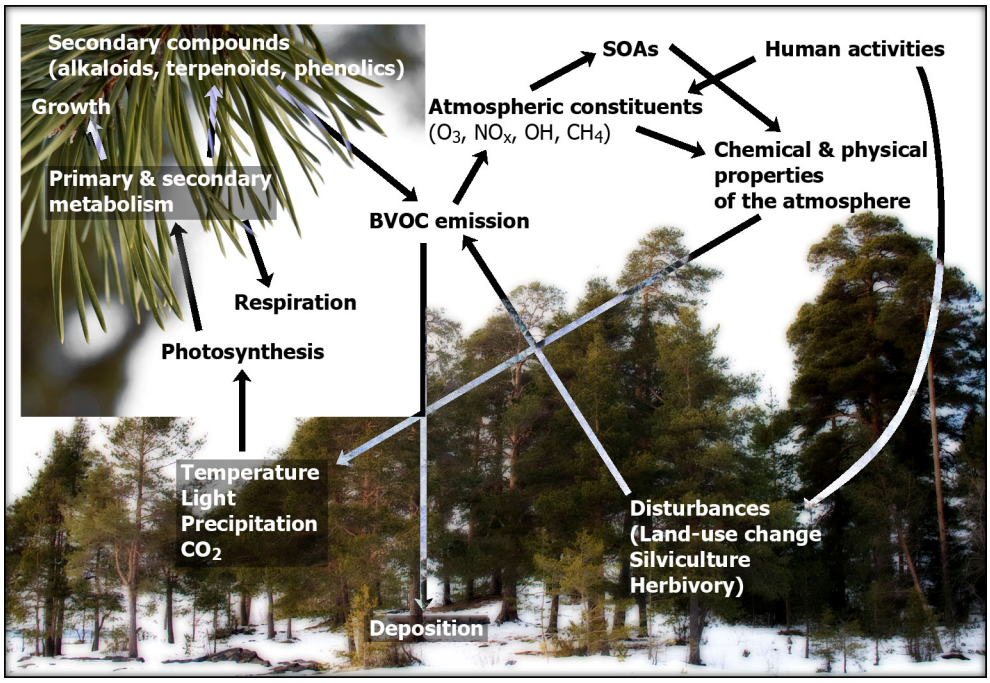


**Figure 2.** Biosphere-atmosphere interactions of BVOCs.

# MATERIALS AND METHODS

## Experimental arrangements and measurements

*Study areas*

Ecosystem scale monoterpene fluxes (I) were measured in a 50-year-old pure Scots pine (*Pinus sylvestris* L.) forest stand located at Huhus, Ilomantsi, in eastern Finland (62°52´N, 30°49´E, 145 m a.s.l.). The terrain has a slope of 2-5% and the Scots pine stand extends uniformly for at least 2 km around the measurement location. The mean stand density is 1176 stems $ha^{-1}$, with a mean height of 13.8 m and a mean diameter at breast height of 14.2 cm (in the year 2003). The site has a homogenous overstorey and understorey vegetation. The soil surface is covered with small patches of needle litter (30%) or lichen (65%). The understorey is mainly mosses (*Dicranum* spp, *Pleurozium schreberi*) and dwarf shrubs (*Vaccinium vitis-idea*, *Calluna vulgaris*). The soil is a sandy podzol and the depth of the soil organic layer is about 15-20 cm. The climate at the site is characterized by cold winters with persistent snow cover. The monthly mean temperature is -10.4°C in January and 15.8°C in July. The mean annual precipitation (1961-2000) is 724 mm, of which about 38% is received as snow.

The experiment to study the effects of timber felling on the ambient monoterpene concentration of a Scots pine forest (II) was conducted in the vicinity of Mekrijärvi Research Station (62°47'N, 30°58'E, 145 m a.s.l.) in Eastern Finland, in an even aged

Scots pine stand (ca. 40 yrs). The soil at the site is a podsolized sandy loam, and the average depth of the organic layer is 3 cm (including litter). The ground cover of this *Calluna*-type stand is characterized by dwarf shrubs (*Vaccinium vitis-idea*, *V. myrtillus*), mosses (*Pleurozium schreberi*, *Polytrichum commune*, *Dicranum polysetum*), and lichens (*Cetraria islandica*, *Cladina* spp., *Cladonia* spp.) (Niinistö et al. 2004). A few bushes of juniper (*Juniperus communis*) (<2m tall) and a few stems of birch (*Betula pubescens*) (<5cm in diameter) were present. Since the site is only 13 km away from the Huhus experimental site, the climatic variables are practically the same as at the Huhus site.

Controlled environmental chambers with different treatments of elevated atmospheric $CO_2$ concentration and temperature were used to study the effects of the predicted climate change on the monoterpene emission of Scots pine (III) and the needle concentration and content of secondary metabolites (IV). Measurements were made in closed-top chambers built on a naturally seeded stand of Scots pine located near the Mekrijärvi Research Station. The soil properties and climatic variables are very similar to those in the experimental area used in the timber felling experiment (II).

*Measuring ecosystem monoterpene flux*

In the Huhus experimental site (I), volatilized monoterpenes were collected from the top of two measurement towers (34 m and 18 m high) established at the centre of the site ca. 5 m apart from each other. The air was sucked simultaneously from the top of both towers through teflon tubing into adsorbent tubes (Perkin-Elmer) containing Carbotrap/Carbopack X in automatic sampling devices (Perkin-Elmer STS 25 including Amtec Alpha-2 flow controlled pumps calibrated each time with a bubble flow meter). The sampling time was 120 min and the flow rate through the sample tubes was about 60 ml $min^{-1}$. Due to limited amount of the adsorbent tubes, sampling was performed in campaigns of 4 days at the maximum throughout the growing period of 2003 (June-September). Eddy covariance measurements with an averaging time of 30 min were conducted at the top of the 34 m tower. The eddy covariance measurement system was used to obtain the vertical fluxes of momentum and sensible heat needed for calculations of friction velocity and stability functions, that in turn were used in the calculation of the monoterpene flux, as will be explained later. For a detailed description of the eddy covariance measurement system and data processing, see Wang et al. (2004). In addition to the eddy covariance measurements, several meteorological variables were measured at the site by a Vaisala weather station (I).

In May 2003 a 12 m tall scaffolding was built at the center of the site, about 30 m from the measurement towers. Six representative Scots pine trees around the scaffolding were chosen for branch-level measurements of monoterpene emission. One branch per each sample tree was selected and two needle age classes were included in the emission measurements (current-year and one-year old needles). The emitted monoterpenes were sampled using a portable gas analyser (LI-COR LI-6400) with a cylindrical branch cuvette (LI-COR 6400-05). The selected section of the branch was enclosed in the cuvette and the air inside the cuvette was sucked with a pump (Amtec Alpha-2) through a teflon tube into an adsorbent tube (Perkin-Elmer) containing Carbotrap/Carbopack X. During the sampling, sensors of the gas analyser was used to measure the air temperature and photosynthetically active radiation (PAR) inside the cuvette. The branch-level measurements of monoterpene emission were performed during daytime between June 26 and September 12 (Figure 3).

**Figure 3.** The scaffolding used in the branch-level measurements of monoterpene emission in Huhus (left), and the actual sampling being conducted on top of the scaffolding using the gas-analyser (right) (I).

As the foliage is considered to be the major source of monoterpene emission of Scots pine, needle samples were collected from the sample trees described above, to determine the needle area, fresh weight and dry weight (60°C for 48 h) of the needles, and additionally the monoterpene concentration of the needles (i.e. the monoterpene pool size). Total foliage area of the site was estimated earlier using measurements of harvested trees (Wang et al. 2004). During the measurement period, the total needle area of the stand ranged between 1.97 – 2.12 $m^2$ per $m^2$ ground surface area.

Occurrence of European pine sawfly (*Neodiprion sertifer*) was noticed in early July at the research site. The larvae of this herbivore consume the older needles of Scots pine usually between June and mid-July, leaving the current-year needles intact. The damages occur mostly on dry and infertile soils, such as the measurement site, with regional mass occurrences at 5-6 year intervals in Finland (Hanski 1987, Virtanen et al. 1996). The occurrence in the measurement year 2003 was not severe and only minor defoliation of trees was detected. Some of the sample trees used in the branch-level measurements were also infested with the larvae, but no larvae were found feeding on the measurement branches. In the end of July the larvae left the canopy to pupate.

*Timber felling experiment*

In summer 2004, four experimental plots (diameter 25 m) were established in the experimental area near the Mekrijärvi Research station (II). Three of the plots were felled manually using chain saws during June 8-9 2004 with different intensities: one plot was clear-cut and two were thinned with removal of 30% and 60% of the tree basal area. The fourth plot was left untouched as a control plot. The felled stem wood was cut to a length of ca. 3 m and piled in small stacks. A minimum of 20 m buffer of untouched forest was left between the managed plots. All of the felled biomass was left at the plots evenly distributed for the measurement period of June – September (Table 1 in article II).

**Figure 4.** Manual timber felling underway in Mekrijärvi (left), and the measurement equipment installed at the centre of the clear-cut plot after the felling (right) (II).

After the felling, volatilized monoterpenes were collected from each plot in four day periods using the same automated samplers used in Huhus. The samplers were placed inside hooded stands at the centre of each plot at a sampling height of two meters, and the monoterpenes were sucked into the adsorbent tubes. Sampling flow rate was 60 ml $min^{-1}$ for 120 min. Weather stations (a-Weather, a-Lab Ltd) were installed at the centre of each plot, within 1 m of the monoterpene sampler. During the monoterpene sampling, these stations were used to measure the microclimatic conditions of the plots, including air temperature, soil temperature (5cm depth), solar radiation, wind speed and direction, air pressure, humidity and precipitation (II). Excluding soil temperature, all variables were measured at a height of ca. 1 meter (Figure 4).

The mass of the logging residue left at the site was measured throughout the measurement period. Samples of logging residue (ca. 2 kg of cut branches, diameter = 10-30 mm) were placed in plastic baskets (3 per plot) before the measurements and the mass change was monitored five times during the measurement period. Respectively, mass change of the needles in the cut canopies was measured, as well as the mass change of needles separated from the branches.

*Environmental chambers*

In summer 1996, sixteen chambers (see the detailed information of the chambers from Kellomäki et al. (2000)) were built around individual Scots pine saplings of similar age (ca. 14 years), diameter (3.8 ± 0.5 cm) and height (3.0 ± 0.3 m) (Figure 5). To reduce shading, all trees within 2 m of the chambers were cut down one year before the experiment. Chamber treatments utilized two primary factors ($CO_2$ and temperature) and included (1) ambient temperature and $CO_2$ concentration (AT+AC), (2) ambient temperature and elevated $CO_2$ concentration (AT+EC), (3) elevated temperature and ambient $CO_2$ concentration (ET+AC), and (4) elevated temperature and $CO_2$ concentration (ET+EC). Thus, the experiment followed a factorial design, with one tree in a chamber as the basic

**Figure 5.** Controlled environmental chambers at Mekrijärvi (III and IV).

treatment unit, replicated four times. The enriched $CO_2$ concentration was ca. 700 µmol $mol^{-1}$ throughout the year. The warming treatments were designed to correspond to the climatic scenario predicted for the site after a doubling of the atmospheric $CO_2$ concentration; i.e. temperature inside the chambers automatically followed the seasonal pattern of outside temperature being 6°C above ambient in winter (December – February), 4°C above ambient in spring and autumn (March – May and September – November), and 2°C above ambient in summer (June – August).

The monoterpenes emitted by the sample trees grown in the chambers were collected between June and September in 2001 and between April and September in 2002 (at the time of sampling tree age was ca. 20 years) (III). The method was to use the closed-top chambers as open cuvette systems during their normal operation. Unfiltered air was pumped through a duct 3.5 m above ground. Due to slight overpressure, air left the chambers through a louvered shutter located about 0.3 m above the ground creating a downward vertical flux inside the chambers. The emitted monoterpenes were collected into adsorbent tubes with automatic sampling devices located at the height of two meters inside the chambers. As the mixed air mass moved from top towards bottom in the chambers and through the tree crown to the automatic sampler, the monoterpene samples collected represented the total emission of the tree crown. Furthermore, the emissions from the chamber floor were excluded from the sampling by the downward vertical flux of the air. The emissions through the intact trunk bark of the trees were considered negligible compared to the needle emissions. Accordingly sampled forest air (at the height of 2 m) was used as a background.

In order to study the emission at different stages of growing period, in this case emission during and after shoot growth, elaborate measurements of the growth (particularly initiation and cessation of shoot growth) of the trees were performed. Respectively, dry and fresh mass and area of the needles were determined. The development of total needle dry mass of the sample trees during the two measurement periods was calculated using five measurements of needle mass per every tree. Relative needle growth rate during the growing season was estimated using these measurements. Needle shedding was taken into account by using the total needle dry mass after needle growth and the mass at the end of

the growing season. The current total needle dry mass was calculated for every emission measurement date.

For the analyses of the needle secondary compounds (IV), needle samples were collected between June and August in 2000 and 2001. In 2000 one-year-old needles (C+1) were collected as three replicates/chamber/time in weeks 24 (during shoot elongation) and 26 (during needle elongation), and both current year (C) and C+1 needles in week 34 (full-grown shoots and needles) from the upper third of the canopy and accordingly from four trees growing in open air (controls for chamber effect) in the area. Respectively, in the year 2001 the needle samples were collected in weeks 23, 26 and 35. The C+1 needles collected in the year 2001 corresponded to the C needles in the year 2000. The 10 pairs of needles for the monoterpene analysis were collected into cryo tubes and immediately frozen and stored in liquid nitrogen until the transfer to deep freezer (-85 °C). At the last sampling weeks in both years respective needles for the analysis of chlorophyll and nutrients were collected in plastic bags on ice and stored at -75 °C until the analysis. The needles for the measurement of needle area, fresh weight and dry weight (60 °C for 48 h) were also collected on ice and the area and fresh weight were measured during the day of sampling. The dried needles were used for nutrient analysis.

## Chemical analyses

*Volatilized monoterpenes*

The absorbent tubes used in the sampling of volatilized monoterpenes (I, II, III) were stored at 5°C in air-tight boxes before and after the sampling. The samples were first preconcentrated with a thermal desorber (Perkin-Elmer ATD Turbomatrix) connected via transfer line (GC column heated to 200°C) to gas chromatograph (Agilent 6890) with a 1701 column (Perkin-Elmer Elite, 30m, i.d. 0.25 mm) and mass selective detector (Agilent 5973). The absorbent tubes were flushed with helium (25 ml $min^{-1}$) at room temperature for 10 min to reduce possible humidity. Desorption was 20 min at 280°C, with prefocusing of the sample in a low-flow cold trap packed with Carbotrap/Carbopack X, at 5°C. For the beginning of the separation the cold trap was heated at a rate of 40°C $s^{-1}$ to 280°C. The initial temperature in the oven was 35°C for 1 min. After that the temperature was increased at a rate of 4°C $min^{-1}$ to 140°C and then at a rate of 45°C $min^{-1}$ to 200°C which was kept for 5 min. The flow of helium in the column was 1.0 ml $min^{-1}$. The entire sampling and analysing system was tested for sample losses or rearrangements regularly during the analyzing periods.

Monoterpenes were identified by comparing the mass spectra with those of Wiley library and pure standards. The gaseous terpene standards (ng-level) of the main emitted monoterpenes were produced with a thermostated permeation device (Staudt et al. 1995) supplied with commercially available terpene standards (camphene, 1,8-cineole, myrcene, sabinene, α-pinene, β-pinene, $\Delta^3$-carene, limonene, *p*-cymene, ocimene; pure standards from Fluka, Sigma). Quantification was generally achieved by comparison of the response for the *m/z* 93 ion with the exception of limonene, *p*-cymene and 1,8-cineole (*m/z* 68, *m/z* 119 and *m/z* 154 ion, respectively).

*Needle secondary compounds and nutrients*

The monoterpenes in the needles collected from the sample trees (I, III and IV) and logging residue (II) were extracted with the method given by Llusià and Peñuelas (2000) (IV). An internal standard ($\Delta^2$-carene) was used to estimate the possible dilution or concentration of the studied compounds in the samples during the analysis. The extracted compounds were quantified using gas chromatography with mass selective detector and identified similarly as in the case of the analysis of the volatilized monoterpenes. Soluble HPLC-phenolics (low molecular weight phenolics) and condensed tannins were analysed from the same samples as the monoterpenes (IV). The soluble phenolics were extracted according to Julkunen-Tiitto (1996) (IV) and analysed with high-performance liquid chromatography (HPLC). For the HPLC analyses, the dried samples were dissolved in 400 µl of methanol:water (1:1) and 20 µl were injected to HPLC (HP Series 1100 LC/DAD). The identification of the compounds was based on retention time and UV-spectra at 220 and/or 320 nm, and the quantification on reference factors of standards ((+)-catechin, myricetin 3-rhamnoside, quercetin 3-galactoside, kaempferol 3-glucoside, apigenin 7-glucoside, taxifolin). Condensed tannins were analysed from the dried pellets used for monoterpene extraction by an acid butanol assay described by Porter et al. (1986). In addition to the secondary compounds, chlorophyll was extracted from fresh needles (same sample as used for monoterpene analysis) in buffered 80 % aqueous acetone and analysed spectrophotometrically according to the method of Porra (Porra et al. 1989). Nitrogen was analysed by the method of Kjeldahl (Halonen et al. 1983). Concentrations of K, Ca, Mg and Mn were determined by atomic absorption spectrometry of dry ash digested in HCl. P was measured photometrically/colorimetrically in a HCl extract by the molybdate-hydrazine method (Halonen et al. 1983).

## Data analyses

*Gradient method*

In the atmospheric boundary layer, a hydrocarbon flux can be described using a concentration gradient ($\partial\chi/\partial z$) defined between two levels above a source level and a turbulent diffusion coefficient ($K_\chi$) of the hydrocarbon constituent (Eq. 1).

$$F_\chi = -K_\chi \frac{\partial \chi}{\partial z} \tag{1}$$

The monoterpene flux above a Scots pine forest (I) was quantified using this micrometeorological gradient method which is based on the Monin-Obukhov similarity theory (Monin and Obukhov 1954). Eddy covariance measurements of momentum and sensible heat fluxes were used to obtain the turbulent exchange coefficients and the samples of monoterpene concentration from the two towers to obtain a measured gradient of monoterpenes. The monoterpene flux of the ecosystem was ultimately calculated using Eq 1. in article I. Although similarity theories can fall apart near uneven surfaces (e.g. Garrat 1978), the roughness sublayer (RSL) correction was not used since the studied forest is quite homogenous and dense and the effect of the RSL at the site has been estimated to be relatively small (Rinne et al. 2000). Additionally, the timescale of the vertical mixing ($\tau =$

$z/u_*$, where $z$ is the measurement height and $u_*$ is the friction velocity) is much shorter than the rate of chemical degradation of the studied compounds (Calvert et al. 2000). Therefore, the chemical losses during the vertical transport along the concentration gradient were considered to be of minor importance.

*Emission modelling*

The classic approach for describing the emission of monoterpenes of conifers is to consider only the temperature dependency of the emission (Eq. 2 in article I). This simple emission dependency was utilized to get an estimate of the contribution of canopy emission to the total monoterpene flux of the forest measured in article I by calculating the emission potential of the total ecosystem as well as the emission potential of the canopy using the above-mentioned ecosystem flux and branch emission measurements together with the concomitant temperature measurements. In order to obtain a quantity estimate of the monoterpenes emitted from trees grown in different treatments of elevated $CO_2$ and temperature (I), algorithms described by Guenther (1997) were used to model the monoterpene emissions throughout a reference period of June-September 2002. In addition to the emission originating from the storage pool, the emissions derived from biosynthesis can be described by considering leaf temperature and photosynthetically active radiation (PAR) as descriptive variables (Eq. 3 in article III). The total emission of monoterpenes from storage and biosynthesis can be described by equation (2):

$$E = EF_{pool} \cdot \exp\left[\beta\left(T - T_s\right)\right] + EF_{synthesis} \cdot C_L \cdot C_T \qquad (2)$$

where $EF_{\mathrm{pool}}$ and $EF_{\mathrm{synthesis}}$ are emission factors (i.e. emission potentials at standard conditions) describing the emissions from the storage pool and biosynthesis, respectively; $\beta$ describes the temperature dependency of the storage-originated emission, and $T$ is the measured temperature and $T_{\mathrm{s}}$ the used standard temperature (25°C in article III, 30°C in article I); $C_{\mathrm{L}}$ and $C_{\mathrm{T}}$ are correction terms for PAR and temperature describing the synthesis-originated emission. Equation (2) is a synthesis of equations (2) – (6) in article III.

*Emission normalization*

Due to the variable environmental conditions (different temperature treatments and variability in received PAR) for individual trees studied in article III, the emission data would not be fully comparable without a normalization procedure. Normalization also reveals the possible acclimation of trees to the surrounding environmental conditions. Normalized emission rates in standard temperature (25°C) and standard PAR (1000 μmol $m^{-2}$ $s^{-1}$) were derived for each measurement by dividing each emission measurement with the temperature and correction terms in equation (2) (Eq. 7 in article III). Additionally, for some statistical analyses described later, the emissions measured from the branch level (LI-COR measurements) in article I were normalized using only the correction term of the pool algorithm (Eq. 2 in article I).

*Graphical vector analysis*

Since the concentration data alone can be misinterpreted, graphical vector analysis (GVA) was used to evaluate the changes in the relationships between absolute compound content

and needle mass caused by the environmental treatments used in article IV (Koricheva 1999). The method required three-dimensional plotting of the compound concentrations (Y-axis), total needle content (X-axis) and total needle dry mass (Z-axis). The data were scaled to relative values using the values in the treatment AT+AC as the standard. The aim of the GVA was to evaluate the treatment effects on relative carbon partitioning to the measured secondary compounds taking into account the changes in needle mass. The direction of the plotted vectors could therefore indicate whether the treatments caused the compounds to dilute or concentrate due to the increased or decreased needle mass.

*Statistical analyses*

The effects of the environmental treatments used in articles III and IV were tested with one-way analysis of variance (ANOVA). Two-way ANOVA (general linear model (GLM) procedure in article III) was used to determine the factor effects (factors: $CO_2$ and temperature). Since the C+1 needles were collected at three different times of the growing period in article IV, linear mixed models procedure was used for the repeated measurements. In the case of the C needles GLM procedure was used because of only one sampling time. Also the analysis of chlorophyll and nutrients of C+1 needles was made by the GLM procedure since the needle sampling was done only in the last sampling time for those substances. The chamber effect for each studied variable in article IV was analysed with independent samples t-test by comparing the values of outdoor grown trees with the trees grown in AT+AC. In addition, Pearson correlation coefficients between the concentrations of the studied compounds and needle characteristics (area, dry weight, SLW) were calculated.

Normalized emission rates obtained from the two age classes of the branch level emission measurements in article I were compared with two-way ANOVA. The measured aerial monoterpene concentration data obtained from the felling experiment (II) was divided into six blocks for statistical comparisons between the treatments. Each block corresponded to a time period of continuous concentration measurement (2-4 days), and only data with simultaneous observations from every plot was included in the analysis. The significance of the differences between the treatments within each block (time period) was tested with one-way ANOVA.

# RESULTS

### Monoterpene concentrations

The identified and quantified monoterpenes included in the analyses of all articles were α-pinene, camphene, β-pinene, sabinene, myrcene, $\Delta^3$-carene, limonene, *p*-cymene and 1,8-cineole. In addition to these compounds, tricyclene and β-phellandrene were quantified for the analyses in articles I and II. Limonene was excluded from the data analysis of article I due to an inconsistent concentration pattern which was likely caused by some external disturbance during sampling or sample analyses.

In the ecosystem flux measurements (I), α-pinene and $\Delta^3$-carene contributed 66-79% of the total aerial monoterpene concentration (Table 1, I). The share of α-pinene increased substantially in September but otherwise the proportions of $\Delta^3$-carene and α-pinene

remained stable through the measurement period. α-pinene and $\Delta^3$-carene were also found to be the most abundant compounds in the branch-level emission (Table 2, I). The concentration of total monoterpenes inside the needles varied between the sample trees in the range of 3.0-5.5 mg $g_{dw}^{-1}$. α-pinene was clearly the dominant compound contributing 47-55% of the total monoterpene concentration in the needles (Table 3, I). In the needles of only one of the six sample trees $\Delta^3$-carene exceeded the share of α-pinene.

Measured from the air above the experimental plots of the timber felling experiment (II), α-pinene and $\Delta^3$-carene contributed roughly 70-80% of the total monoterpenes (Figure 2, II). The proportion of α-pinene remained relatively stable in the air of the harvested plots, but in the unmanaged control plot α-pinene gradually increased in proportion from a share of 45% to 55% at the end of the measurement period. The proportion of $\Delta^3$-carene was similar in the air of every plot, ranging between 15-20%. $\Delta^3$-carene tended to reduce in proportion during the measurement period. There was no consistent pattern of any certain compound compensating the lower proportion of α-pinene in the control plot, but instead the proportions of all the other compounds were accordingly elevated.

The emitted monoterpenes of the trees grown in the environmental chambers (III) were also dominated by α-pinene (50-58 % of the total emission) and $\Delta^3$-carene (13-31 %) (Table 1, III). The proportions of the monoterpene species varied between individual trees, but no treatment effect on the composition of monoterpene emission was observed. Respectively, the proportions of individual monoterpenes within the needles (IV) varied substantially between the trees with no apparent relation to the environmental treatments. However, in every tree the most abundant compounds within needles were α–pinene with a proportion of 45-75% and $\Delta^3$-carene with a proportion of 9-41%( Table 1, IV). A lower proportion of α-pinene was compensated by an increased proportion of $\Delta^3$-carene and vice versa. Furthermore, the total amount of monoterpenes was not found to depend on the proportions of these compounds.

**Canopy and ecosystem scale monoterpene emission**

At the Huhus site (I), highest emission rates from C+1 needles of the sample trees were measured in late June – early July. The emission rate decreased after mid-July towards the end of the growing season although the temperature at the site remained high until mid-August (27-34°C, measured from the branch cuvette) (Figure 3, I). The normalised needle weight based emissions from C needles were significantly higher than emissions from C+1 needles, but the needle area based emissions of C and C+1 needles did not differ significantly. An exponential dependency of monoterpene emission rate and temperature was observed (Figure 4, I). Emission potential of total monoterpenes at standard temperature of 30°C was calculated using equation (2) in article I. The obtained value for C+1 needles was 94 ± 7 ng $m^{-2}$ $s^{-1}$ and for C needles 112 ± 12 ng $m^{-2}$ $s^{-1}$ (1125 ± 96 ng $g_{dw}^{-1}$ $h^{-1}$ and 2270 ± 364 ng $g_{dw}^{-1}$ $h^{-1}$, respectively).

Most of the monoterpene fluxes measured by the gradient method were in the range of 50 to 500 ng $m^{-2}$ $s^{-1}$ (Figure 5, I). The monoterpene flux tended to be lowest in the morning. After midday the flux started to increase reaching a maximum typically in the late afternoon and then decreased again during the evening (Figure 6, I). Very stable atmospheric conditions that occurred during the night led to an accumulation of emitted monoterpenes in the air and therefore caused high flux observations in the early morning as the air started moving. Most of the night-time flux observations were excluded due to weak

turbulence. Highest fluxes of total monoterpenes were measured in the first half of July (Figure 5, I). The measured fluxes did not correlate well with any meteorological parameter although a slight exponential dependency on air temperature and infra-red temperature of the canopy was found. The temperature dependency was reduced by the high flux observations in late June and early July. Emission potential at standard temperature of 30°C was calculated using the measured total fluxes and air temperature data (Eq. 2, I). The obtained value for the total monoterpene flux potential of the ecosystem was 290 ± 44 ng $m^{-2}$ $s^{-1}$.

Total ecosystem monoterpene flux and canopy emission were calculated for the measurement period of June – September using eq. (2) (I) and the measured temperature data (Figure 8, I). Needle area based emission was upscaled by multiplying it with the current total needle area of the stand ($m^2$ $m^{-2}$ ground). Total ecosystem flux from June 1 to September 30 was 502 mg $m^{-2}$ and total canopy emission in the same time period was 373 mg $m^{-2}$. Hence, on the basis of the calculation, the canopy emission was 74% of the ecosystem flux.

**Impacts of felling on ambient monoterpene concentration**

The aerial monoterpene concentrations differed most between the differently managed plots during the first seven weeks after the felling and the differences were diminished in mid-August (68 days after the felling) (Figure 3 and Table 2, II). Compared to the unmanaged control plot, the aerial monoterpene concentration was on average 3 times higher in June during the first measurement campaign (13-14 days after felling), and during the first seven weeks the concentration remained 2-3 times higher above the clear-cut plot compared to the control. The highest aerial concentrations in every plot were observed in late July. The last concentration observations in late-September (105-107 days after felling) showed only minor differences between the plots. The aerial concentrations measured from the two thinned plots did not differ significantly, although the amount of felled biomass was over two times greater in the more intensely thinned plot (Table 1, II).

Differences in the microclimate of the plots were found. Removal of the tree canopy clearly affected the amount of received solar radiation on the soil surface, which in turn increased soil temperature in the managed plots (Figure 1, II). The aerial monoterpene concentration was found to correlate best with soil temperature, but correlations with other meteorological parameters were not as clear, although the correlation with air temperature was almost as strong. Additionally, an exponential dependency of concentration on soil temperature was observed, but the variation was great since the aerial monoterpene concentration is affected by many other factors as well (Figure 5, II). The observed differences in the aerial monoterpene concentrations between the plots were largest simultaneously with the largest differences in soil temperature.

The mean total monoterpene concentration in the needles collected from the logging residue at the harvested plots was 1.8 ± 0.9 mg $gdw^{-1}$. The needle samples were collected randomly from the logging residue of each plot throughout the measurement period, and therefore no real comparison of needle concentration between the plots could be made. However, all needle samples contained a higher proportion of α-pinene (75 ± 4%) than was measured in the air. The mass reduction of logging residue (branches) left at the site was fastest in the clear-cut plot. Three weeks after the felling, the mass of the residue had reduced by 30% resulting in a total loss of 35% in the end of the monitoring period (Figure

6, II). In the thinned plots, the mass reduction was not as great (27-28% in the end of the monitoring period). Respectively, no clear differences in the changes of needle mass could be observed between the felled plots (Figure 7, II).

### Effects of elevated $CO_2$ and temperature

*Monoterpene emission*

When compared to the controls (ambient $CO_2$ and temperature) the combination of elevated $CO_2$ and temperature significantly increased normalized monoterpene emission rate for the whole growing period (23%), whereas elevated $CO_2$ had no significant effect (-4%), and elevated temperature even decreased (-41%) the emission rate (Table 2, III). The increasing effect of the combination of elevated $CO_2$ and temperature was strongest during shoot growth (54%) when elevated $CO_2$ alone also had an increasing effect on the total emission (Table 2, III). After shoot growth, no significant differences in emission rate were found among the treatments. In treatments other than AT+AC the normalized emission rate tended to be higher during shoot growth than after shoot growth (15% higher in AT+EC, 11% in ET+AC and 33% in ET+EC) (Table 2, III), but the differences between the two time periods were not statistically significant in any treatment.

Emission modelling showed that the total amount of monoterpenes emitted from May 1 to September 30 in elevated $CO_2$ was 5% greater and in elevated temperature 9% lesser than in ambient conditions (Table 3, III). The combination of elevated $CO_2$ and temperature increased the amount of emitted monoterpenes over the growing period by 126% compared to the total emission in ambient conditions. The modelled total emission of monoterpenes was highest in June in AT+AC and AT+EC. In contrast, when temperature was elevated (ET+AC and ET+EC) the monthly emission was highest in July (Figure 4, Table 3, III).

*Secondary compounds and nutrients within needles*

Elevated $CO_2$ significantly reduced the concentration of total needle monoterpenes (Table 1, IV), but the effect of elevated temperature was not equally clear. Compared to AT+AC, the monoterpene concentration in ET+AC was slightly lower or higher depending on the study year and needle age. The combination of elevated $CO_2$ and temperature tended to have the greatest effect on the monoterpene concentration although the factor ($CO_2 \times T$) effect was found insignificant. In ET+EC the concentration was approximately only half of those in AT+AC in both study years and needle ages. The vector analysis showed that the monoterpenes in AT+EC were slightly concentrated due to a small reduction in the mass of C needles (Figure 3, IV). The opposite effect occurred in ET+EC when the monoterpenes were slightly diluted due to enhaced needle growth. The total monoterpene needle concentration was higher in the year 2000 than in 2001 in every treatment and in both needle ages (Table 1, IV) except in the treatment ET+AC in C+1 needles, which had almost the same monoterpene concentration in both years.

Twenty different HPLC-phenolic compounds were identified and quantified in the needles, and grouped into seven subsets for further analysis according to the biosynthetic pathways of the compounds. These subsets were 1) cinnamic acid, 2) flavan-3-ols, 3) taxifolin, 4) non-acylated flavonols, 5) flavones, 6) flavonols, and 7) acylated flavonols (IV). The concentration of taxifolin was extremely high in the needles of three trees

doubling the concentration of total HPLC-phenolics in these trees. The higher taxifolin concentration was not related to the treatments and the higher taxifolin concentration did not affect the concentrations of any of the other phenolic compounds. Therefore, taxifolin was excluded from further analyses. The abundaces of the different HPLC-phenolics differed between the two studied needle age classes (Figure 4, IV). In general, the environmental treatments had no effect on the concentrations of the individual phenolic groups or on the total concentration of HPLC-phenolics (Table 2, IV). Overall, the concentrations of total HPLC-phenolics were slightly higher in the year 2001 than in 2000 in both needle ages and every treatment.

The effects of the environmental treatments on the concentration of condensed tannins were somewhat different in the two needle ages (Table 3, IV). The tannin concentrations were lowest at the first sampling time in C+1 needles and were increased substantially by the time of the second sampling in every treatment. Elevated temperature decreased the tannin concentration significantly in C+1 needles in both years. Compared to AT+AC, condensed tannins were slightly concentrated in AT+EC (Figure 6, IV). Due to increased needle mass the tannins were diluted in ET+EC. The same indications of dilution were partly seen in ET+AC.

The analysed nutrients in the needles were N, Ca, K, Mg, Mn and P. No significant differences were observed in the concentrations of these nutrients between the two study years and needle ages, although generally the concentrations tended to be slightly lower in C+1 needles compared to C needles in every treatment (Figure 7, IV). The effect of the treatments on the concentrations of nutrients other than N was unsubstantial. N concentration was significantly reduced by the factor $CO_2$ in C+1 needles in both years and in C needles in the year 2000. Also the interaction of $CO_2$ and temperature decreased the concentration of N in both needle ages and years.

## DISCUSSION

### Monoterpene emission of a Scots pine forest

The differences in the proportions of individual monoterpenes measured in Huhus (I) from the towers and canopy indicates that a part of the emission could have originated from sources other than the canopy itself and that the compound composition of the additional emissions was more emphasized on α-pinene. This is further supported by the differences between the canopy and ecosystem scale emission modelling over the reference period of June-September (I), although some uncertainty is included in the emission calculations due to the relatively weak temperature dependency of the stand-level monoterpene flux. Monoterpene emission of a Scots pine forest floor (ground vegetation, litter and root system) has also earlier been estimated to contribute to the ecosystem emission by as much as 30% (Janson 1993). Hellén et al. (2006) found that the monoterpene emission of a Scots pine forest floor contributed significantly to the ecosystem scale monoterpene flux especially in spring and autumn, whereas summer emission was not as significant. On the other hand, considering the differing atmospheric lifetimes of individual monoterpenes, it is possible that the observed difference in the compound composition between canopy emission and total ecosystem flux is partly a consequence of the different rates of chemical degradation of individual compounds. Changes in the concentrations of the oxidizing agents in the

atmosphere could have affected the proportional changes in the measured aerial concentrations in Huhus (I), but also in the timber felling experiment (II). Some differences were also found between the compound composition of monoterpenes within the needles and the emitted monoterpenes (I). These variations are likely due to the divergent physico-chemical characteristics affecting the volatility of each compound (Niinemets and Reichstein 2003). The observed large difference in needle mass based emission rates between the measured needle age classes can partly be explained by differences in the dry matter density (mass/area-ratio) between the age classes (C+1 needles: 302 $g_{dw}$ $m^{-2}$, C needles 165 $g_{dw}$ $m^{-2}$). This variability could introduce a potential error when upscaling needle mass based emissions, especially if changes in the proportions of the needle age classes are not considered accordingly.

Hakola et al. (2006) found the emission potential of Scots pine to vary within a measurement period of April to October. Based on their branch-level studies the emission potential was highest in June and lowest in September. Due to the limited sample size, no clear seasonality of the branch-level emission or the ecosystem flux could be detected in this study, although the flux was greatly reduced in the end of the growing period. Earlier studies of the monoterpene emissions of Scots pine have shown broad variability of emission rate depending mostly on tree age and provenance (Kesselmeier and Staudt 1999). The previously reported temperature standardized (30°C) emission rates for Scots pine are in the range of 0.38 – 12.1 µg $g_{dw}^{-1}$ $h^{-1}$ (Isidorov et al. 1985, Janson 1993, Komenda and Koppmann 2002, Ruuskanen et al. 2005, Rinne et al. 2007). The standard emission rates obtained from the branch cuvette measurements of our study (1.1 µg $g_{dw}^{-1}$ $h^{-1}$ for C+1 needles, 2.3 µg $g_{dw}^{-1}$ $h^{-1}$ for C needles) fall on the lower part of that range.

The highest ecosystem monoterpene fluxes were simultaneous with the time period of the sawfly occurrence (I). This implies that the ecosystem flux could have been increased by the needle damages caused by the herbivores. Previously, herbivory has shown to increase monoterpene emissions by eliminating the diffusive resistance of intact storage structures due to the damage, and also by directly enhancing the monoterpene synthesis (Litvak and Monson 1998, Litvak et al. 1999). The consumption of needles by herbivores has been found to increase the emission more than just mechanical wounding (Litvak and Monson 1998). Wounding of the needles of *Pinus pinea* massively increased the emission of monoterpenes (mainly limonene and α-pinene) of resin ducts (Loreto et al. 2000). The monoterpene release of *P. Pinea* decreased quite rapidly and the wounding did not cause a long-term increment in the emission. In our study, the increased emission observed in July was not correlated to the needle monoterpene concentration. However, only unwounded needles were included in the concentration analyses, thus it is impossible to say whether the larvae feeding had an effect on the monoterpene concentration of the wounded needles. Furthermore, no clear effect of the feeding on the branch-level emissions was found in our study, since the measurement branches remained untouched by the larvae during the infestation. The poor temperature dependency of the flux (Figure 7, I) could at least partly be explained by this flux increasing factor since the herbivore-induced effect on the emission is less dependent from environmental factors.

The calculated total ecosystem monoterpene flux during the four month measurement period of June - September (502 mg $m^{-2}$ or 502 kg $km^{-2}$) corresponds to the annual monoterpene emission flux value of 567 kg $km^{-2}$ estimated by Tarvainen et al. (2007) for the middle boreal zone of Finland. Since the flux calculation is based on ambient temperature, combined fluxes outside this four month period will not be very high due to the low temperatures. The difference between canopy emission and ecosystem flux

estimations (Figure 8, I) could represent the contribution of forest floor emission and herbivore induced emission increment to the total flux. However, large uncertainties included in the emission potential calculations reduce the reliability of this estimation.

**Effect of felling on the ambient monoterpene concentration of a Scots pine forest**

In a previous experiment, monoterpene mixing ratio above a young ponderosa pine plantation canopy was found to increase dramatically during thinning (Schade and Goldstein 2003) and the monoterpene flux of the site was enhanced 40 times during the thinning period of 34 days. Due to practical reasons, the aerial monoterpene concentration in our study could not be measured during the actual harvesting (2 days) and therefore the magnitude of the immediate monoterpene release from the cut tree material remains unclear.

The measured monoterpene concentration at the site typically tended to be highest during night-time (Figure 4, II). Similar diurnal trend has also been observed by Janson (1992) in a mature Scots pine forest and Hakola et al. (2000) in an open field surrounded by Scots pine dominated forest. The observed diurnal variation of the aerial concentration is contrary to the diurnal variation of monoterpene emission (Steinbrecher et al. 1999) which mostly depends on the current temperature and radiation conditions (Shao et al. 2001, Tarvainen et al. 2005). Similar effect was observed in the ecosystem flux measurement (I) at the Huhus site, where the monoterpene concentration tended to rise during night-time, but the monoterpene flux peaked usually in the afternoon. The monoterpene concentration above the plots is not only dependent on the current emission rate of monoterpenes but also on the vertical mixing of the air above ground. Hence, during nocturnal stationary conditions, the monoterpenes emitted from the ground and logging residue accumulated in the air resulting in the observed high concentrations.

The observed differences in the aerial monoterpene concentrations between the plots were concomitant with the differences in soil temperature. In addition, the mass reduction of logging residue (branches) left at the site was fastest in the clear-cut plot where the change in the temperature and radiation conditions were greatest, and respectively in the thinned plots the mass reduction was not as great. Palviainen et al. (2004) observed about 20% reduction in air dry branch mass and about 30% reduction in needle mass of Scots pine logging residue one year after a clear-cut. The loss of mass of needles and branches observed in our study and in the work of Palviainen et al. (2004) was probably mostly a result of drying, since the material used in the beginning of the measurements was still partly fresh. The mass reduction of waterless biomass is significantly smaller. The barkless dry mass of Scots pine branches left at a clear-cut area has been found to change less than 10% during the first year of decomposition (Hyvönen et al. 2000). However, the differences in mass reductions observed in our study can indicate the effects of different local microclimatic conditions of the plots.

If soil temperature is presumed to indicate the temperature of the logging residue, the increased aerial concentration observed in the managed plots is likely a result of increased monoterpene volatilization from the residue, since the storage-based monoterpene emission is strongly controlled by temperature (Kesselmeier and Staudt 1999). This would particularly apply to the needles of the logging residue. Since monoterpenes are easily volatilized and in general have poor solubility in water (Weidenheimer et al. 1993), they are more likely to exit needle litter via volatilization than leaching. The monoterpene concentration of shed needle litter of Scots pine has been found to decrease linearly during

the first year of litter decomposition and only a few percent of the original concentration remains after the first year (Kainulainen and Holopainen 2002, Kainulainen et al. 2003). Accordingly, stemwood contains monoterpenes as well. α-pinene and $\Delta^3$-carene have been found to be the most abundant monoterpenes in the stemwood of Scots pine, and the stemwood emission is dominated by these compounds (Manninen et al. 2002, Turtola et al. 2002).

**Effects of elevated $CO_2$ and temperature on monoterpene emission and needle secondary compounds of Scots pine**

The environmental treatments had no effect on the composition of the monoterpene emission (III), since the proportions of individual monoterpenes in the total emission varied independently of the treatments from tree to tree. The composition of monoterpene emission is typical for an individual tree but not for the whole species (Komenda and Koppmann, 2002), and accordingly the differences in major monoterpene compounds in Scots pine needles are controlled primarily by the genotype of a tree (Hiltunen et al. 1975, Hiltunen 1976, Thoss et al. 2007). Furthermore, large differences in the concentrations of total and individual monoterpenes in needles of Scots pine were found (Kainulainen et al. 1998) between trees from the same stand that was used in this study. Also in this study, the concentrations of α-pinene and $\Delta^3$-carene within needles (IV) varied from tree to tree. The differences remained the same from year to year and were not affected by the treatments, and thus were determined by the high-$\Delta^3$-carene or low-$\Delta^3$-carene chemotype of a single tree (Hiltunen et al. 1975, Hiltunen 1976).

Elevated $CO_2$ concentration increased the rate of photosynthesis (Laitinen 1998) and decreased the rate of respiration (Kellomäki and Wang 1998, Zha et al. 2001) of the Scots pines in this chamber experiment, which could have influenced the allocation of carbon into monoterpenes (Heyworth et al. 1998, Sallas et al. 2001). At ambient temperature the increasing effect of elevated $CO_2$ concentration on the monoterpene emission rate was not clearly seen in this study. Elevated $CO_2$ has been reported to increase the monoterpene emission of non-storing species (Staudt et al. 2001, Vuorinen et al. 2005), but no significant effect on monoterpene emission has been found in monoterpene storing species (Constable et al. 1999). Instead, our results showed that the combination of elevated $CO_2$ and temperature increased the emission rate when the effect of elevated $CO_2$ exceeded the inhibiting effects of elevated temperature. One explanation for the emission increment in elevated $CO_2$ and temperature could be the increasing effect of isoprenoids on thermotolerance of trees (Delfine et al. 2000).

Elevated $CO_2$ significantly decreased the total monoterpene concentration in C and C+1 needles at each sampling time and both years. The lower concentration was not a consequence of dilution and in fact monoterpenes were slightly concentrated in C needles in both study years. Also in previous experiments with conifers, needle monoterpene concentration has been found to decrease due to elevated $CO_2$ (Williams et al. 1994, Litvak et al. 2002, Sallas et al. 2003, Snow et al. 2003). On the other hand, Heyworth et al. (1998) and Sallas et al. (2001) observed an increase in the concentration of α-pinene in Scots pine seedlings exposed to elevated $CO_2$. And furthermore, at ultra-high $CO_2$ enrichments, α-pinene and β-pinene concentrations were increased in Loblolly pine seedlings (Tisserat and Vaughn 2003). The reduced N concentration of the needles can partially explain the reduction in needle monoterpene concentration at elevated $CO_2$. Increased N availability

increases the amount of resin ducts, as well as the concentration of oleoresin, in the needles of Scots pine (Kainulainen et al. 1996). The formation of resin-producing structures in pine species is dependent on N (Björkman et al. 1991) which suggests that the monoterpene storage was in fact limited by N even though the availability of carbon was enhanced due to the $CO_2$ enrichment.

Elevated temperature decreased the needle concentration of monoterpenes, but only in C+1 needles. Previously, the elevation of temperature has been oberved to increase the total monoterpene concentration in the needles of Scots pine and Norway spruce seedlings which was suggested to be a response to increased emission of these compounds at higher temperatures (Sallas et al. 2003). On the basis of the emission measurements (III) this clearly is not the case, since the emission was in fact found to decrease as a result of temperature elevation. Elevated temperature has been found to inhibit photosynthesis and additionally increase the metabolic rates resulting in increased consumption of assimilates via respiration (Farrar and Williams 1991, Rowland-Bamford et al. 1996, Zha et al. 2001). This may result in a smaller allocation of carbon into secondary compounds. In fact, the concentration of monoterpenes tended to be lower in the warmer growing season 2001 in both needle ages compared to the cooler year 2000 showing the inverse relationship between temperature and monoterpene concentration.

The interaction of elevated $CO_2$ and temperature tended to reduce the monoterpene concentration in C and C+1 needles substantially although the effect was not statistically significant. Similar effect has been reported in Douglas fir (Litvak et al. 2002, Snow et al. 2003). Sallas et al. (2003) found that the decrease in monoterpene concentration caused by elevated $CO_2$ and the increase caused by elevated temperature counteracted each other and as a result the interaction had no effect on the monoterpene concentration of Scots pine needles. In this study, elevated temperature and $CO_2$ had a decreasing effect on the concentration. In contrast, the total monoterpene emission was significantly increased by elevation of $CO_2$ and temperature (III). Although the constitutive monoterpene pool within needles is a major source of emission, the size of the pool appears to have remained high enough not to inhibit the emission (Lerdau et al. 1997).

The increasing effect of elevated $CO_2$ and temperature and the effect of elevated $CO_2$ alone on the emission potential was clear during tree shoot growth. Significantly higher rates of emission during early summer is typical for conifers (Sabillón and Cremades 2001, Kim et al. 2005), as Scots pine, which appears to have a multifold emission potential of monoterpenes in early growing season compared to late growing season (Komenda et al. 2003). Similarly the normalized emission rate of Scots pine is significantly higher in spring compared to the latter part of growing period (Janson 1993, Komenda and Koppmann 2002). In addition, Janson (1993) found the summer maximum of normalized monoterpene emission rate to occur in June – July in Scots pine and Norway spruce forests in southern and central Sweden. Since most of the terpene synthesis for storage occurs in the resin duct epithelium of needles which is most active during needle elongation (Staudt et al. 1997), the higher emissions during early summer could be explained by the developmental and physiological state of plant organs (Steinbrecher and Ziegler 1997). The trees grown under ambient $CO_2$ and temperature had no apparent difference of the normalized emission rates between early and late growing period. Therefore it seems that the influences of elevated $CO_2$ and temperature were concentrated on the early phase of the growing period and that the effects diminished towards the end of the growing period.

Although the environmental treatments had only minor effects on needle HPLC-phenolics, the concentrations changed with needle age. The changes in the concentrations

of the different groups of phenolics according to needle age were due to the different characteristics of the compounds i.e. the more stable metabolites tended to accumulate in the needles whereas the more dynamic ones could decrease in concentration through time. The concentration of condensed tannins was increased substantially by elevated $CO_2$. These stable end products that accumulate with needle age have consistently been observed to increase due to both long-term and short-term $CO_2$ enrichment in several tree species (Lavola and Julkunen-Tiitto 1994, Hättenschwiler and Schafellner 1999, Kinney et al. 1997, Kuokkanen et al. 2001). Nevertheless, Heyworth et al. (1998) reported no significant effect of elevated $CO_2$ on the concentration of condensed tannins in Scots pine needles. Condensed tannins were also reduced by temperature elevation, and the effect was more clearly seen in C+1 needles. The same trend has been found in the leaves of *B. pendula* (Kuokkanen et al. 2001). The concentration of condensed tannins was higher in C+1 needles than in current-year needles in every treatment at the last sampling time of both years. This was expected as condensed tannins tend to accumulate in the needles with needle age (Kozlowski and Pallardy 1997).

The reversed monoterpene and phenolic concentration changes between the two experimental years are likely to be a cause of the competitive trade-off between different biosynthetic pathways of secondary compounds and growth of the pines. The biosynthesis of most phenolics and derived compounds (such as condensed tannins) compete with the precursor (phenylalanine) of protein synthesis and thus with plant growth (Jones and Hartley 1999). The biosynthesis of monoterpenes occurs without direct competition with protein synthesis, although the metabolic costs for their production are high (Haukioja et al. 1998). Therefore, any change in primary production could affect the amount of phenolics and their derivatives, whereas the amount of monoterpenes would be more insensitive to such changes. The studied trees did in fact grow more in the year 2000 compared to the year 2001 in terms of radial growth (Kilpeläinen et al. 2005), which would partly explain the higher concentrations of HPLC-phenolics and condensed tannins in the year 2001.

The model-based emission estimates for the five month period calculated in article III are extremely high compared to the monoterpene concentrations within the needles (IV). In AT+AC, the amount of calculated emission for the five month period in the year 2002 was 2.38 mg $g_{dw}^{-1}$, when the needle monoterpene pool was 3.45-3.89 mg $g_{dw}^{-1}$ (varying between sampling times) in the year 2001. In ET+EC the pool was significantly smaller (1.64-1.97 mg $g_{dw}^{-1}$), whereas the calculated emission for the five month period (5.37 mg $g_{dw}^{-1}$) clearly exceeded the volume of the pool. This could indicate that the emission potential values obtained from the chamber measurements are too high, and unless the excess emission is not originating directly from biosynthesis, this difference cannot be explained. However, the emission modelling was done only for the year 2002, whereas the needle concentrations were studied in the earlier two years. It is possible that the amount of needle storages in 2002 differed from the earlier years, although this is not likely, since the concentrations measured in the C needles in 2001 were similar to those of the older needles. Furthermore, the high amount of total emission is partly explained by overestimation of the model in the low temperature range, which is prevalent during spring and autumn. Also the use of a single emission potential value to describe the emissions of longer time periods can be problematic and introduce potential errors in the emission estimates, especially since the emission potential has been found to change within a growing period (Hakola et al. 2006). Additionally, even small changes in the emission potential values used in the modelling result in relatively large changes in the calculated total emission due to the non-linear relationship between the emission and the determining environmental variables

(temperature and PAR). Overall, the amount of total emission calculated for the reference period of five months should not be taken as gospel truth, but instead the relative differences between the treatments, which are easily comparable, should be considered.

## CONCLUSIONS

At the Huhus site, it has been estimated that the net ecosystem production (NEP) i.e. the net carbon accumulation of the ecosystem was 229 g C $m^{-2}$ in the year 2003 (Zha et al. 2007). In proportion to the total carbon fluxes of the ecosystem, the quantity of emitted carbon in the form of monoterpenes is nearly negligible. However, the significance of these emissions is in the atmospheric impacts of these compounds due to their high photochemical reactivity. Biotic disturbances, such as herbivory, could strongly increase the total amount of monoterpenes emitted into the atmosphere. Since winter temperature minimum is a significant factor explaining the outbreak patterns of several herbivores, such as the European sawfly (Virtanen et al. 1996), it can be expected that the predicted climate change can increase the frequency of outbreaks and furthermore has a potential to significantly increase the herbivore-induced emission of monoterpenes.

On the basis of this study, human-induced disturbance, i.e. timber felling significantly increases the ambient monoterpene concentration of a Scots pine forest. In addition to the stumps of the cut trees, the logging residue is the most important factor explaining the increment of the aerial concentration of monoterpenes. The amount of monoterpenes released from the residue depends on its temperature which is dependent on the microclimatical conditions of the managed site. Therefore, the remaining canopy cover can indirectly affect the monoterpene release from the emitting biomass. Although there was an observed difference in the rate of decay of the logging residue between the differently managed plots, there is no reason to presume that the long-term mass-based amount of monoterpenes released from the cut biomass would differ between the managed areas. Since the aerial monoterpene concentration was found to depend on soil temperature, the monoterpene release from the residue could be accelerated due to the changed microclimate of the clear-cut. The greatest difference between the treatments would therefore be in the temporal scale of the concentration increment.

In Scots pine dominated forest, the tree canopy was found to contribute most of the ecosystem scale monoterpene flux (74%). Therefore, partial or total removal of the tree canopy will have an effect on the total emission of managed sites due to the reduction of the monoterpene emitting foliage. On the basis of this study, felling increases the ambient monoterpene concentration in a relatively short time-scale (at least seven weeks after the felling). However, after clear-cut, the complete absence of the growing tree biomass will likely reduce the long-term basal emission of the managed area. Furthermore, in a time scale of several years, the species composition and the emission spectrum of the next generation of trees will also determine the long-term impacts of felling on the emission. The actual increment of emitted monoterpenes as a result of the fellings is unclear, since the aerial monoterpene concentration cannot directly be used to describe the monoterpene flux of the site. However, the significant increase in the concentration induced by the felling implies that there is a great potential impact on local or even regional atmospheric chemistry. Therefore, actual flux measurements above a felled forest stand are needed to fully understand the magnitude of emission increment caused by fellings.

On the basis of the chamber experiments, the amount of monoterpenes directly released by Scots pines into the atmosphere during a growing season will increase substantially in the predicted future climate. Increased carbon availability with increased temperature cause changes in the allocation of carbon between growth and secondary compounds. Although the combined effects of elevated $CO_2$ and temperature together on the emission were strongest during shoot elongation (May – June), higher temperatures in the latter part of the growing period causes the absolute enhancement of the emission to be greater in the late growing period. The responses of the needle secondary compounds to the elevation of $CO_2$ and temperature are variable and depend strongly on the properties and characteristics of each compound as well as on the interrelation between secondary and primary production of the trees. In general, the increased monoterpene emission due to the environmental treatments appeared to consume the needle pool, particularly when both $CO_2$ and temperature were elevated. Besides the direct influence of the changed growing conditions on individual trees, changes in the species distribution will likely affect the emissions on a larger, regional scale. In southern Finland, the proportion of Scots pine is predicted to reduce from the current 45% to 5% (% of volume) with increased dominance of birches (*Betula pendula* and *Betula pubescens*), and in northern Finland from 63% to 40% with increased share of Norway spruce and the birches (Kellomäki et al. 2001). These distributional changes will constrain the decreasing effect on the total amount of monoterpenes emitted into the atmosphere by Scots pine forests. Nevertheless, the increased emission capacity of individual trees due to the predicted climate change will compensate a great part of the loss of emitting biomass of Scots pine.

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