THE EFFECTS OF WARMING, NITROGEN ADDITION AND BARK HERBIVORY ON ABOVE- AND BELOW-GROUND VOLATILE ORGANIC COMPOUNDS OF SCOTS PINE (*Pinus sylvestris*) SEEDLINGS

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ABSTRACT

Climate warming is associated with increasing nitrogen load and higher herbivory pressure in the future. As such, the emissions of volatile organic compounds (VOCs) from the shoots and rhizosphere of Scots pines are predicted to change. Changes in VOC emission pattern would affect the boreal ecosystem as VOCs influence plant defense and interactions as well as atmospheric feedbacks. Scots pine (*Pinus sylvestris*) seedlings grown in chamber conditions were exposed to warming (+2 °C increase to spring, summer and early autumn ambient temperature levels simulating the May-September ambient day and night temperatures), nitrogen addition (30 kg N ha^{-1} a^{-1} increase) and bark herbivory in growth chambers from May to September in 2014. The shoots emitted mainly monoterpenes (MNTs) while rhizosphere emitted mainly other VOCs (OVOCs). In general, more VOC emissions occurred during the first month of the experiment (May), but later the emissions of MNTs, sesquiterpenes (SQTs) and OVOCs were reduced. Nitrogen addition enhanced the effects of warming and herbivory and resulted in increased SQT and MNT emissions from the shoots of Scots pine. Herbivory also caused increased emissions of OVOC from the rhizosphere of Scots pine. The emission of volatile organic compounds increased as gas exchange declined suggesting that Scots pine can emit VOCs passively.

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ABBREVIATIONS

BVOC	Biogenic Volatile Organic Compounds
DMAPP	Dimethylallyl Pyrophosphate
FAC	Fatty-acid-Amino-acid Conjugates
GC-MS	Gas Chromatography-Mass Spectrometry
GLV	Green leaf Volatile
Gs	Stomatal conductance
HPL	hydroperoxide lyase
IPCC	Intergovernmental Panel on Climate Change
IPP	Isopentenyl diphosphate
MEP	Methylerythritol Phosphate
MNT	Monoterpene
MVA	Mevalonic Acid
NH_3	Ammonia
NO	Nitric oxide
NO_2	Nitrogen dioxide
OVOC	Other Volatile Organic Compound
Pn	Photosynthesis
SQT	Sesquiterpenes
VOC	Volatile Organic Compound

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INTRODUCTION

Plants live in very complex environments and as such they come in interaction with quite a number of stressors (Ponzio et al. 2014). The plant-environment interactions and communications are shaped and mediated by volatile organic compounds (VOCs) which the plants emit (Laothawornkitkul et al. 2009). The different stress factors present in the plant environment can affect VOC emissions (Loreto et al. 2014). Hence, it is important to understand how multiple stressors affect the emission of volatile compounds as this could help in predicting the effects of global climate change.

Volatile organic compounds (VOCs) are important in–atmospheric chemistry, as they are known to react with nitrogen oxides (NO_x), hydroxyl radicals (OH), and ozone (O_3), in the troposphere, affect the oxidation capacity of the atmosphere, and can in combination with sufficient concentration of nitrogen oxide lead to the production of ozone and other photo-oxidants thus significantly contributing to the formation of secondary aerosol (Pinto et al. 2010). According to Guenther et al. (2012), the quantity of biogenic volatile organic compounds (excluding methane) emitted by plants to the atmosphere amounts to 1 Pg C per year, the bulk of which is produced by trees largely due to their big size and lengthy life span (Holopainen 2011). The type and quantity of VOCs produced by plants depends largely on environmental conditions such as changes in temperature, light and humidity (Tarvainen et al. 2005, Yuan et al. 2009) and stress such as herbivore attacks (Loreto et al. 2000) or changes in nutrient supply (Kivimäenpää et al. manuscript).

The soil and rhizosphere are complex systems where soil microbial community interacts with each other and with organic nutrients in the soil, and many biotic processes (e.g. nutrient cycling, decomposition) also take place in the soil. It is also a highly heterogeneous reservoir of biological diversity, abundant in fungal and bacterial cells (Egamberdieva et al. 2008), plant tissues, decomposing litter and dead organic materials (Penuelas et al. 2014), all of which contribute to the emissions of VOC from the soil via microbial degradation (Kögel-Knabner 2002; Penuelas et al. 2014). Data from recent studies conducted in temperate, boreal and Mediterranean ecosystems show that soil VOC emissions are between zero to three order of magnitude lower than those emitted from the shoots (Penuelas et al. 2014). Plants also produce and release secondary metabolites into the rhizosphere, thus making the below-

ground system as much an important source (and/or sink) of VOCs as the above-ground parts of plants (Cesco et al. 2012).

The past decade spanning from 2003 to 2012 has seen the global mean temperature increased by an average value of 0.78ºC from 1880 to 2012, and this increment is predicted to continue to increase to an approximate value of 2 ºC by the end of this century (IPCC 2013). This warming is expected to also increase soil temperature, and soil warming has been shown to increase the rate of photosynthesis (Pumpanen et al. 2012). Nitrogen exists in the air in the forms of nitric oxide (NO) and nitrogen dioxide (NO_2) which are pollutants, and these different forms of nitrogen are deposited on many ecosystems (Manning et al. 2006). Once deposited, soils rapidly accumulate nitrogen (Raciti et al. 2011), and according to Korhonen et al. (2013), N is accumulating in Southern Finland Scots pine forests at a rate of 7 kg N ha^{-1} yr^{-1}, while the annual deposition rate in European boreal forests is estimated to be at 10 kg N ha^{-1} yr^{-1} (Flechard et al, 2011; Mustajärvi et al. 2008). This increasing temperature and accumulating nitrogen can affect natural ecosystems, Kivimäenpää et al. (manuscript) found that N addition enhanced the effect of warming and increased the emission of MNT from Scots pine by as much as 4 fold. Previous studies have also linked increases in the emission of VOCs to the increasing temperature (Holopainen and Gershenzon 2010).

One of the effects of climate change is the redistribution of insects (Ammunet et al. 2012; Logan et al. 2003), which in turn could mean more frequent herbivore attacks in forests and ultimately lead into increased VOC emissions. Even relatively small amounts of insect/herbivore attack on plants can significantly impact VOC emissions at the plant level (Heijari et al. 2011). Pine weevils (*Hylobius abietis L.*) are known to feed and cause damage on the bark of Scots pine seedlings, increasing the quantity of BVOCs emitted by the seedlings (Heijari et al. 2011). Plants also can release more volatiles to protect themselves against further herbivore attack (Blande et al. 2014; Furstenberg-Hägg et al. 2013).

In this work the above- and below-ground VOC emissions of the common conifer in the boreal zone, Scots pine (*Pinus sylvestris L.)*, were measured while the trees were exposed to experimental warming, moderate N addition and bark herbivory alone and in combination during year 2014. Experiment was performed in growth chambers for over 5 months (from early April until end of September), the growing conditions in the chambers simulated the growing season conditions from May to September of Central Finland, while herbivores (pine

weevils, *Hylobius abietis*) were allowed to feed on bark for 24 hours in early July 2014. The main aim was to assess if the single effects of warming, N addition and bark herbivory were similar or different from the interaction effects of these factors, as there is still relatively little knowledge about the above interactions.

2. SCOTS PINE AND VOCs IN THE CHANGING CLIMATE

2.1. SCOTS PINE AS A VOC EMITTER

The boreal ecosystem (taiga) is the largest ecosystem on earth, and in Europe this ecosystem is dominated by coniferous trees which are ever-green and active in cool weather (Aaltonen et al. 2013). Scots pine (*Pinus sylvestris* L.) is a widely spread and ever-green coniferous tree species native to northern Europe and very common in the European boreal forest. With over one hundred subspecies, varieties, and forms of Scots pine described, it spans from the Scandinavia to the coastal area of eastern Siberia of Russia where it is an important tree used as pulp and timber products (Hämet-Ahti et al. 1992).

In Finland, conifer forests are dominated by Scots pine (50% of trees) (Finnish Forest Research Institute 2013). Scots pine is known to emit monoterpenes, δ-3-carene and alpha-pinene being the most abundant, accounting for between 60 – 70 % of total VOC emissions (Rinne et al. 2000; Tarvainen et al. 2005). Heijari et al. (2011) found that sesquiterpenes accounted for only 1.2 % of emissions from Scots pine, unlike other boreal species, Norway spruce (*Picea abies* L.) and downy birch (*Betula pubescens* L.), which emit sesquiterpenes in higher amounts especially in the summer months (Hakola et al. 2003).

2.2. BIOSYNTHESIS OF PLANT VOLATILES

Plant volatiles are classified into three major groups based on their derivatives: terpenoids (or isoprenoids), phenylpropanoids/benzenoids, and fatty acid derivatives (Loreto *et al.* 2001). The biochemical pathways by which these plant volatiles are synthesized entails series of enzymatic modifications such as hydroxylations, acetylations, and methylations (Pichersky *et al.* 2006). It is this series of conversions that increases the diversity of the volatiles emitted by the plants and the large variety of terpenoids emitted by Scots pine.

Terpenoids (isoprenoids) are the largest class of plant secondary metabolites, they include many volatile compounds (see Table 1). Terpenoids have a high vapor pressure which allows them to be released into the atmosphere. Terpenoids being lipophilic liquids and having high vapor pressures easily cross membranes and are then released into the atmosphere or soil (Pichersky *et al.* 2006). Terpenoids are synthesized from the methyl-erythritol-phosphate/mevalonic acid (MEP/MVA) biochemical pathway and originate from the

universal five carbon precursors, isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate (DMAPP), which are derived from two alternative pathways (Rodriguez-Concepcion and Boronat, 2002). Terpenoids in conifers are synthesized in the resin cells or ducts within the tissues of the stem and needles (Miller *et al.* 2005) and can then be released once the plant is attacked by herbivore or it suffers a mechanical damage.

Table 1. Classification of terpenoids based on their number of carbon units (Engelberth, 2006)

Class	**Carbon units**
Isoprene	C5
Monoterpenes	C10
Sesquiterpenes	C15
Homoterpenes	C11 and C16
Diterpenes	C20
Sesterterpenes	C25
Triterpenes	C30
Sesquaterpenes	C35
Tetraterpenes	C40
Polyterpenes	>C40

Mevalonate pathway (produces Mevalonic acid MVA) and non-mevalonate pathway (produces methylerythritol phosphate MEP) are two independent and separate pathways that form the C5-isoprene building units. The sesquiterpenes (C15) are formed by the MVA pathway, while the MEP pathway produces volatile hemiterpenes (C5), monoterpenes (C10) and diterpenes (C20). The mechanism of transport of these volatiles from their site of biosynthesis to the point of emission into the atmosphere is still a subject of research; however, a review by Jetter (2006) proposed that it involves four steps:

i) intra-cell trafficking
ii) transport across the plasma membrane and cell wall
iii) transfer through the cuticle, and
iv) evaporation at the surface of the cuticle

The biosynthetic route of homoterpenes (C11 and C16) is still unclear, but Degenhardt and Gershenzon (2000) suggest that they are derived by oxidative degradation of geranyl-linalool (C20) and (3*S*)-(*E*)-nerolidol (C15). Asides homoterpenes, plants produce other volatile compounds that are derived from carotenoids and have 8 to 18 carbon skeletons or isoprene units. These plant volatiles are also synthesized de novo with their production being temporally and spatially regulated (Rodriguez-Concepcion and Boronat, 2002). Plants can also emit other reactive volatile organic compounds (OVOC) which accounts for approximately 22.5% of VOCs emitted annually (Guenther et al. 1995). Kesselmeier and Staudt (1999) also noted that plants emit alkanes, alkenes, alcohols, esters, carbonyls, and acids from biogenic sources. Plants also emit trace amount of Green leaf volatiles (GLVs). GLVs are C6–aldehydes, C6-alcohols, and their acetates emitted by green plants. They are biosynthesized via the lipoxygenase/hydroperoxide lyase (HPL) pathway. However, plants rapidly produce and emit greater quantity of GLVs after wounding (Matsui 2006)

2.3. THE ROLE OF VOCs

In nature, plants live in a complex and diverse environment where they are involved in many forms of interactions (Loreto et al. 2014; Ponzio et al. 2014), and are exposed to a wide range of biotic and abiotic stressors. To communicate and interact with the environment, plants emit VOCs. According to Dudareva et al. (2006), over 1700 volatile compounds have been identified from more than 90 plant families. The VOCs serve a host with several functions as they can aid seed dispersion by attracting pollinators, and they also help in protecting plants from pathogens, herbivores, florivores and parasites.

VOCs released by herbivore-infested plants help plant to survive the herbivore attack (Dudareva et al. 2013). VOCs serve as semiochemicals, mediating plant-plant interactions and act as a form of an alarm signal, inducing the expression of defense genes and causing the emission of volatile compounds (Furstenberg-Hägg et al. 2013). The volatile compounds emitted function either in form of direct defense or an indirect effect. Direct effect means that the emitted VOC repel or intoxicate microbes and animals/herbivores immediately after release (De Moraes *et al.* 2001; Kessler and Baldwin, 2001). The indirect effect means that VOC emission increases the attractiveness of the leaves to the natural enemies (carnivores) of the damaging herbivore thereby decreasing the susceptibility of the plant to the damaging

herbivores (Rasmann *et al.* 2005; Blande et al. 2014). Attacked plants are also able to communicate with neighbouring plants through the VOCs they emit and alert them of a potential future attack (Furstenberg-Hägg et al. 2013), thereby making the alerted plants better prepared for a stronger response once attacked (Vancanneyt *et al.* 2001; Junker et al. 2011). Therefore, VOCs help in driving plant biodiversity and reproductive advantage by ensuring their reproduction, greater plant productivity and continued existence.

Plant growth and development is an energy-demanding process that also requires good nutrient availability (Newingham et al. 2007). Therefore, plants use VOCs to help regulate their defense according to need, thereby helping to conserve valuable resources necessary for their growth and development, by reallocating some of the resources upon attack. In this way, the plant can sustain the high photosynthetic activity needed for compensatory growth (Newingham et al. 2007). While feeding on plants, herbivores release elicitors such as lytic enzymes, or fatty-acid-amino-acid conjugates (FAC) from their oral secretions into plants (Musser *et al.* 2002). These elicitors make the plant to emit a greater variety and quantity of volatile compounds (Maffei *et al.* 2004). These VOCs are not only emitted from the sites of the plants attacked by the herbivore but also from the other parts of the plant. Musser et al. (2002) suggested that the VOCs emitted from injured plant show some level of specificity that corresponds to the type of the attacking herbivore thus acting as direct repellents of such herbivores. This specificity of the VOCs emitted by the plants upon attack by herbivores could be a potentially important property that can be used in agriculture, forestry and pest control.

Different VOCs have different fates once released into the atmosphere, depending on their reactivity and degree of volatility. VOCs undergo photochemical reactions in the atmosphere which can lead to the formation of secondary organic aerosols (Koch et al. 2000; Pinto et al. 2010). VOCs are O_3 precursors, but they are also easily oxidized by O_3. VOCs and oxides of nitrogen (NOx) react in the presence of solar UV-radiation resulting in the production of ozone (O_3) in the troposphere. VOCs react with O_3, OH and NO_3 radicals and transform to less volatile organic compounds. These VOCs then in turn condense as secondary organic aerosols (SOA) and in a positive feedback process, leads to the formation of more O_3 (Llusia et al. 2002; Pinto et al. 2010, Kulmala et al. 2013).SOA particles warm the climate locally, reflect solar radiation back to space, and have regional or continental effects (Tunved et al. 2006).

The presence of ozone can disrupt the activation of defense genes (Heath 2008), decrease photosynthesis (Long and Naidu 2002), and alter the emissions of VOCs thereby affecting plant interactions (Pinto et al. 2007). Thus, VOCs indirectly play an important role in the radiation balance of the atmosphere and can cause unpredictable damages or effects on the atmosphere-biosphere chemistry and climate as a whole (Penuelas et al. 2009) especially in or around urban areas where air quality is often poor due to natural and anthropogenic air pollutions.

Roots provide anchorage, mechanical support and serve as a means of nutrient and water uptake for plants (Fitter 1991). However, recent findings reveal that plants also emit diverse VOCs from their roots (Loreto et al. 2001; Robert et al. 2012) and that soil and roots can be both a source and sink for VOCs (Guenther 1999; Rasmann et al. 2005; Smolander et al. 2006; Leff and Fierer 2008). Roots also carry out other specialized roles such as regulating the microbial activity and nutrient availability in their immediate surroundings by synthesizing, accumulating and secreting a wide range of organic compounds to the rhizosphere around them (Lin et al. 2007). They also mediate interactions among bacteria, fungi and plants, alter rates of nutrient cycling (Smolander et al. 2006), as well as serve as a source of carbon and energy for microbes present in the soil (Lin 2007). Root volatiles contribute to the rhizosphere defense system by acting as antimicrobial or antiherbivore substances or by attracting enemies of root-feeding herbivores (Robert et al. 2012). They can also inhibit or reduce the seed germination and growth of competitive neighboring plants (i.e. have allolopathic effects), encourage symbioses as well as alter the soil physical and chemical properties (Nardi et al. 2000).

2.4. WARMING AND VOC EMISSIONS IN BOREAL REGION

The mean global temperature increased by 0.78 ^{o}C in the last century, and with a further increase of 2 ^{o}C is expected to occur by the end of this century (IPCC 2013). Boreal forests are dominated by conifer trees, which are known emitters of significant quantities of monoterpenes and sesquiterpenes (Rinne et al. 2009), and Finland is predominantly covered by boreal forests (Blanch et al. 2007). Warmer climate means an elongated growing season and longer period of plant activity, a long term change in land cover and species composition. Due to the warmer temperatures in some ecosystem, more plants are able to grow there and

produce also more litter, which translates to higher fertilization of forests and increased quantity of volatile compounds emitted (Blanch et al. 2007).

The quantity of BVOCs emitted is expected to increase with increasing temperature (Penuelas and Staudt 2010). Simulation studies by Briceno-Elizendo et al. (2006) also showed that warming and increased precipitation could enhance growth in boreal forests by as much as 50%. Ge et al. (2013) have predicted increased productivity and dominance of conifers in Finnish forests as global warming increases, this increased dominance is predicted to be stronger in the northern part of Finland than in the south and would cause a significant increment in the quantity of VOCs emitted.

2.5. NITROGEN ADDITION EFFECTS ON VOC EMISSIONS

One of the negative effects of urbanization is the emissions of high concentrations of air pollutants from several anthropogenic sources such as traffic, combustion and energy production. Increasing human population has also placed increased demands on agricultural outputs (Erisman et al. 2003; Bell et al. 2011). N fertilization tends to increase the productivity of boreal forests (Saarsalmi and Mälkönen, 2001). Therefore the use of nitrogen fertilizers has increased (Mustajärvi et al. 2008). According to Korhonen et al. (2013), atmospheric deposition from industries, traffic and agriculture is the largest external N input into forests. The nitrogen released into the atmosphere can be deposited in various forms; wet and dry, oxidized and reduced. The nitrogen from combustion processes are in the NOx form, while those from agricultural fertilization are in the NH_3 form. When deposited nitrogen travels from the atmosphere through forest canopies and vegetation to the soil, it undergoes different series of transformation reactions (such as N fixation, mineralization, nitrification, leaching, plant assimilation, ammonia volatilization, denitrification and immobilization) before the compounds are then taken up by plants through their roots or foliage (Hyvönen et al. 2007).

Nitrogen oxides (NOx) which include nitric oxide (NO) and nitrogen dioxide (NO_2) are major components of anthropogenic N emissions. These nitrogen oxides are capable of reacting with hydroxyl radicals in the troposphere and cause environmental effects such as formation of secondary organic aerosols (Pinto et al. 2010). Study by Bell et al. (2011) showed that exposure to NO_2 and NO fumigation can have varying effects on plant growth: herbaceous plants showed species-specific responses such as increasing or decreasing rates of

photosynthesis and stomatal conductance to different levels of NO_2 and NO both singly and in combination.

Ruoho-Airola et al. (2015) suggested that at current emission levels (~7 kg N $ha^{-1}yr^{-1}$), nitrogen deposition poses no hazard but could even increase forest growth in Finland/Scandinavia. Flechard et al. (2011) estimated that between approximately 10 kg N $ha^{-1}yr^{-1}$ is deposited in European boreal forest. However, the deposition level is strongly dependent on weather conditions, especially precipitation amount (Ruoho-Airola et al. 2015). Studies by Kivimäenpää et al. (manuscript) found that nitrogen availability can enhance the effect of warming in increasing BVOC emissions from Scots pine. In this open field exposure experiment, warming alone increased, and N addition in combination further increased VOC emissions.

2.6. HERBIVORY AND VOC EMISSIONS

One of the effects of climate change is an expected increase in herbivore outbreak especially in boreal regions (Jepsen et al. 2008), and an increase in VOC emissions, as plants are known to emit VOCs when attacked by herbivores (Arimura et al. 2004). Previous laboratory studies have shown that bark herbivory by pine weevil increases monoterpene emissions from Scots pine (Faiola et al. 2015). Pine weevil, *Hylobius abietis,* (Fig. 1) is one of the most prominent and commercially important herbivore in European forests. It feeds on the bark of young seedlings and on freshly cut Scots pine (Heijari et al. 2011) and can deform the stem, affect tree growth and lead to increased mortality of forest trees which are of extensive environmental, commercial and aesthetic importance. Pine weevils are often attracted by volatile compounds, especially α-pinene and ethanol, and they feed optimally at temperatures between 19 and 28 °C (Brixey, 1997).

Figure 1. Adult pine weevil (*Hylobius abietis L.*) feeding on Scots pine. Photo by: Jarmo Holopainen

Pine weevil feeding has also been shown to induce the emission of MNT and SQT from both the damaged bark and intact shoots of Scots pine (Heijari et al. 2011) and Norway spruce (Blande et al. 2009). The study by Blande et al. (2009) showed that herbivory feeding by pine weevil increased MNT emissions by as much as 8 folds, SQT emissions by about 45 folds and GLV emissions by almost 4 folds from Norway spruce.

Heijari et al. (2011) found that pine weevil feeding on the bark of Scots pine nearly quadruples the emission of monoterpenes while it increases the emission of SQTs by as much as seven fold. The mode of attack of herbivores (i.e. chewing, sucking or piercing) also affects the kind of volatile compounds plants emit after attack as it determines the pathway and mechanism of defense pathway that would be activated (Loreto *et al.* 2001; Ponzio et al. 2013; Loreto et al. 2014).

3. AIMS OF THE RESEARCH

The main aim of this research was to measure the quantity of volatile organic compounds emitted by Scots pine seedlings exposed to warming (+2 ^{o}C temperature elevation), nitrogen addition (dose of 30 kg N/ha/a) and bark herbivory alone and in combination. Experiment was conducted in controlled chambers for over a five-month period (May-September) in 2014. The research questions were as follow:

1. Does nitrogen addition, attack by herbivore and warming singly or in any combination have an effect on the emission dynamics of the shoots of Scots pine seedlings?
2. Does nitrogen addition, attack by herbivore and warming singly or in any combination have an effect on the emission dynamics of the rhizosphere of Scots pine seedlings?
3. Are single exposure effects on VOCs similar or different from those of interaction effects?

4. MATERIALS AND METHODS

4.1 CHAMBER EXPERIMENT: Experimental design, plant and animal material

Scots pine seedlings originating from Southern Finland (seed origin: Imatra, N 61^{o} 9' E 28^{o} 47', 60 m) were grown in the nursery of the Natural Resources Institute Finland (previously known as Finnish Forest Research Institute), Suonenjoki, Finland. The seedlings were kept in an open-field nursery at the Research Garden, University of Eastern Finland, Kuopio campus, over winter 2013-2014. One month before the start of the chamber experiment, the Scots pine seedlings were moved indoors to a cold room (+4 ^{o}C) on 31.3.2014, where they were for three days and were then transferred on 3.4.2014 to +6 ^{o}C for two days and at that time they were also sprayed with water. On 5.4.2014 they were moved to a room with +10 - 14 ^{o}C for one day and sprayed with water before they were finally moved to greenhouse conditions (c. +20 ^{o}C). Seedlings were planted in pots on 8.4.2014.

In total, 48 randomly selected Scots pine seedlings were planted into 5 L pots (20 x 20 x 20 cm, Orthex Eden, Oy Orthex Finland Ab, Finland) filled with forest soil which was collected from a Scots pine stand located near the Natural Resource Institute Finland, Suonenjoki Research station. Before placing the soil into the pots, it was sieved through 0.5 cm mesh to remove stones and debris. In the bottom of each pot, there was a 2 cm layer of LECA to prevent sand and finer particles to block holes in the bottom of pots (holes allowed excess water to drip out). Experimental seedlings were planted on 8th of April 2014, and at that time their initial stem height and base diameter were also measured. Seedlings were one-year-old in the beginning of the chamber experiment.

On the planting day, half of the pine seedlings were randomly selected to nitrogen (N) addition treatments, and they received a dose of 30 kg N/ha/a (Peatcare Slow Release 1 fertilizer, N:P:K, 9:3.5:5, Yara), whereas the rest of the seedlings were not fertilized and served as N treatment controls. On 9th of April 2014, potted seedlings were transferred from the Research Garden to the Department of Environmental Science, University of Eastern Finland, Kuopio, where chamber experiment was initiated in six Weiss growth chambers (Weiss Bio 1300, Weiss Umwelttechnik Gmbh, Germany). Seedlings were divided evenly between the six chambers so that each chamber contained in total 8 seedlings (6 chambers x 8 seedlings = 48). Three of the chambers were maintained at ambient temperature conditions and three chambers were in the warming treatment (+ 2 ^{o}C increase to spring, summer and

early autumn ambient temperature levels simulating the May-September ambient day and night temperatures), thereby giving a replicate unit of 3. In each chamber half of the seedlings were N-fertilized (N+) and half of the pines were growing in the prevailing soil N level (N- seedlings).

Temperature programs in chambers were based on the long-term weather data collected from Ruohoniemi experimental field, UEF, Kuopio. The maximum light intensity was 250 µmol m^{-2} s^{-2} (PAR), the photoperiod ranged from 17:7 h to 20:4 h (light:dark h). Other chamber details are shown in Table 2. The trees were watered in the chambers with tap water as needed, and extra care was taken to prevent them from being water logged or too dry. In addition to seedling pots, there was one empty pot (no soil + no seedling) and one pot containing only forest soil serving as blank controls for soil+rhizosphere VOCs. The pots positions in the chambers were mixed so that they were not all the time in the same position during the duration of the experiment.

Pine weevils (*Hylobius abietis*) for the herbivory experiment were trapped in two pine dominated and recently (one year ago) clear cut state forest sites in Pieksämäki region in Central Finland (Silmutsuo: N 62º24'46" E 26º49'48" and Tervanen: N 62º28'12" E 27º2'48"). The used pitfall traps were lidded plastic buckets (3 L) with eight (2 cm Ø) holes drilled around the top edge. Turpentine and 96% ethanol were used as bait in the traps; separate test tubes attached inside the trap contained about 15 ml of each bait. The tubes were covered with perforated parafilm to allow slow evaporation of the baits. 30 and 28 traps were buried in the ground in Silmutsuo in Tervanen, respectively, so that the holes were at the ground surface level allowing walking weevils easily enter the traps (Figure 2a).

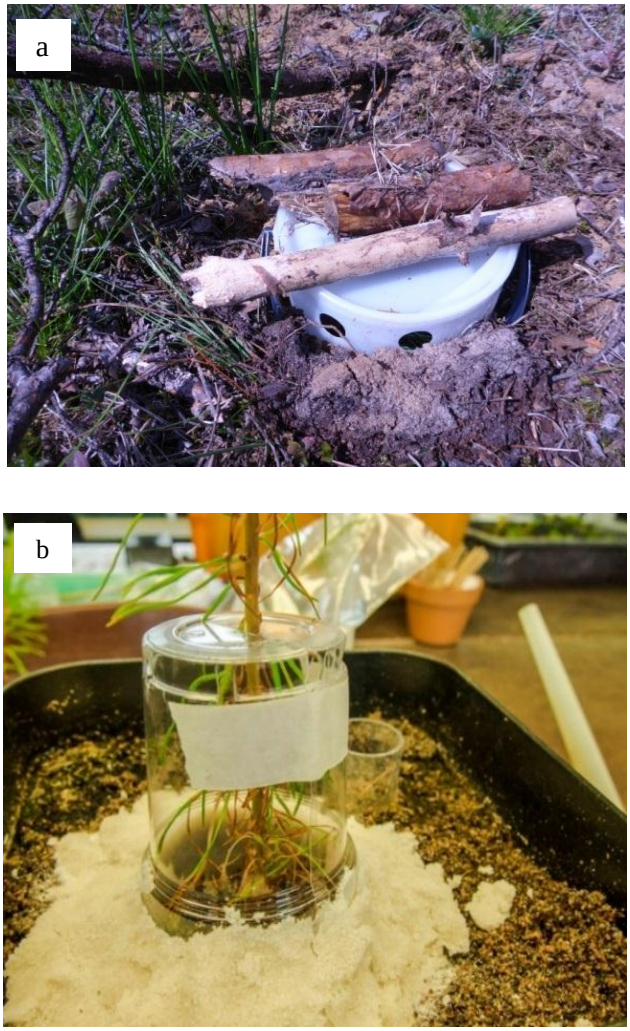


Figure 2a-b. a) Pitfall placed in the ground. The baits (turpentine and 96% ethanol) were in 15 ml tubes attached to the interior wall of the trap. Bait tubes were covered so that the animal did not drop in them and drowned into the bait liquids. Traps were covered with lid (rain cover). Animal entered the trap by walking through the holes carved 2 cm below the lid of the trap. Photo by Gideon Olaleye. b) The restriction of the herbivore feeding to a part of the Scots pine seedlings covered by the plastic cup. Nilsiä quartz sand prevents the herbivore from escaping into the soil while the cup, tapered and plugged with wool, restricts its movement to other part of the plant. Photo by Anne Kasurinen.

Table 2. Growing conditions of the Scots pine trees in the chambers during the six months of the experiment. AT = ambient temperature chamber, E = elevated temperature chamber (n = 3 per temperature treatment). May program ran from 9.4.2014 to 3.6.2014, June program ran from 3.6.2014 to 4.7.2014, July program ran from 4.7.2014 to 4.8.2014, August program ran from 4.8.2014 to 1.9.2014, and September program ran from 1.9.2014 to 2.10.2014. Night time is defined as zero light level, and maximum PAR level was 250 µmol m^{-2} s^{-1}. Values in table are averages of three chambers per temperature level and minimum and maximum temperatures and relative humidity's (ranges for three chambers per temperature level) for each month. RH% is relative humidity %.

		Temperature ºC		**min-max ºC**		**RH%**		**min-maxRH**		**Hours**		**Time**		**max PAR hours**
		Day	Night	Day	Night	Day	Night	Day	Night	Day	Night	Day	Night	Day
May	AT	12.1	8.8	7.6-15.9	8.2-9.4	65.6	77.1	54.0-85.1	74.6-79.5	20h	4h	04-24	24-04	3h
	ET	14.1	10.8	9.6-17.9	10.2-11.3	65.6	77	52.4-81.7	74.3-79.7	20h	4h	04-24	24-04	3h
June	AT	14.8	10.9	10.1-18.2	10.2-11.8	70.3	84.9	54.2-88.6	81.8-88.2	20h	4h	04-24	24-04	10h
	ET	16.8	12.9	12.1-20.1	12.2-13.8	70.4	84.9	49.7-88.3	81.8-87.9	20h	4h	04-24	24-04	10h
July	AT	19.7	15.4	15.1-22.7	15-16.1	76.8	93.1	63.6-97.4	89.4-95.6	20h	4h	04-24	24-04	9h
	ET	21.7	17.4	17.0-24.4	17.0-18.1	76.7	93.1	63.8-96.2	89.9-95.5	20h	4h	04-24	24-04	9h
August	AT	16.6	13.1	12.1-19.6	12.2-14.3	78.8	91.6	66-98	85.8-96.2	17h	7h	05-23	22-05	5h
	ET	18.6	15.1	14.1-21.3	14.2-16.3	78.8	91.7	65.5-97.2	85.8-96.4	17h	7h	05-22	22-05	5h
September	AT	11.5	9.4	9.5-14.2	9-10	82.5	93	69.4-96.7	90.8-95.3	17h	7h	07-24	24-08	5h
	ET	13.5	11.4	11.4-16.1	11.0-11.9	82.5	93	73.8-94.6	90.3-95.4	17h	7h	07-24	24-07	5h

The traps were set on 30 May, 2014 and the animals were collected on 5, 9 and 16 June of 2014. Altogether 58 weevils were caught during the trapping period. The weevils were kept in plastic containers in refrigerator at +4 – +5 ^{o}C and fed with fresh pine twigs before the experiment.

The herbivory experiment was performed on the 30.6.-1.7.2014. Prior to the experiment, the weevils were kept at room temperature to make them more active and their weight was taken (initial weight of the animals ranged between 59,85 – 133,70 mg). Then weevils were placed in petri dishes and starved for 24 hours prior to the experiment in order to induce feeding. After 24 h animal weights were taken again (now they ranged from 59,40 – 128,45 mg). Two weevils was introduced to 12 Scots pine seedlings used for the herbivory experiment, i.e., one N- and one N+ seedling from each chamber were used for the herbivory feeding (H+). The weevils were introduced to the seedlings in a localized way (Figure 2b). Plastic cups (250 ml) were cut from one side open and carefully placed around the basal stem on top of the soil. Animals (2 weevils per cage) were placed inside the cups and then cup was sealed with tape along the cut part and cotton wool covered the cup part touching the stem. Wet Nilsiä quartz sand (particle size 0.5-1 mm, producer: SP Minerals Oy Ab, Finland) was used to seal the cup part touching the soil so as to prevent the weevils from escaping to the surrounding pots and seedlings during the feeding period. Animals were allowed to feed for 24 hours after which they were taken out and their weight were taken again immediately after the feeding experiment to check for weight gain. After the feeding experiment, the animal weights ranged from 60,5 – 132,75 mg, indicating a net weight gain in the weevils. The cup effect on VOCs was tested by placing a similar cup cage around the stem but without animals (12 seedlings, one N+ and N- seedling per chamber), but since these seedlings did not have clear difference in their VOC emissions to the cupless seedlings, all the remaining seedlings without animals were combined together to represent the H- treatment. Thus, in total there were eight treatments in the chambers: 1) ambient temperature + no N addition + no animals = control (C); 2) ambient temperature + N addition + no herbivory = N; 3) ambient temperature + no N addition + herbivory = herbivory (H); 4) ambient temperature + N addition + herbivory = NH; 5) elevated temperature + no N addition + no herbivory = T; 6) elevated temperature + N addition + no herbivory = TN; 7) elevated temperature + no N addition + herbivory = TH and 8) elevated temperature + N addition + herbivory = TNH (n = 3 for each treatment).

4.2. GAS EXCHANGE AND NEEDLE GROWTH MEASUREMENTS

Gas exchange parameters (photosynthesis and stomatal conductance) were measured with infrared gas analyzer (Licor 6400XT, Licor, Lincoln, Nebraska, USA) attached to opaque conifer chamber (Licor 6400-22, Licor, Lincoln, Nebraska, USA) once a month from 29.4.2014 onwards during the whole chamber experiment. Measurements were performed from old shoot in the first measurement (May), and from old and new shoots in the latter measurements (June-September) from two seedlings per chamber making a total of 12 Scots pine seedlings used for the gas exchange measurements. The light saturation point of photosynthesis in order to find out the PAR to be used in the gas exchange measurements was determined from a light curve (fig 3), 1500 CO_2 µmol m^{-2} s^{-1} was used as the light level in the measurements.

For these measurements, c. 7.5 cm of old main shoot were placed inside the conifer chamber, whereas in new shoot measurements the length of the measured shoot in the conifer chamber varied between 1,3 – 8,2 cm. Licor uses certain fixed needle area in Pn and Gs data calculations, therefore the gas exchange data needed a needle area correction. For the needle area correction, the total number of needles per 2 cm length of old shoot from one side of the stem was counted and multiplied by two to get the needle number on both side of the 2 cm length of the stem, and then this was extrapolated to 7,5 cm to get the needle number of the whole old shoot length inside the conifer chamber. For the new main shoot, all the needles along the whole length of one side of new shoot was counted as the whole new shoot was placed inside the conifer chamber. In addition, needle length from five needles per old shoot measurement site (2 cm-long area in old shoot) and from new main shoot were measured. For the needle area correction, new main shoot total length was also measured. Then formula: Needle Area = ((4.2235 x needle length) – 15.6835) x needle number in cuvette (Flower-Ellis and Olsson 1993) was used to correct the needle area.

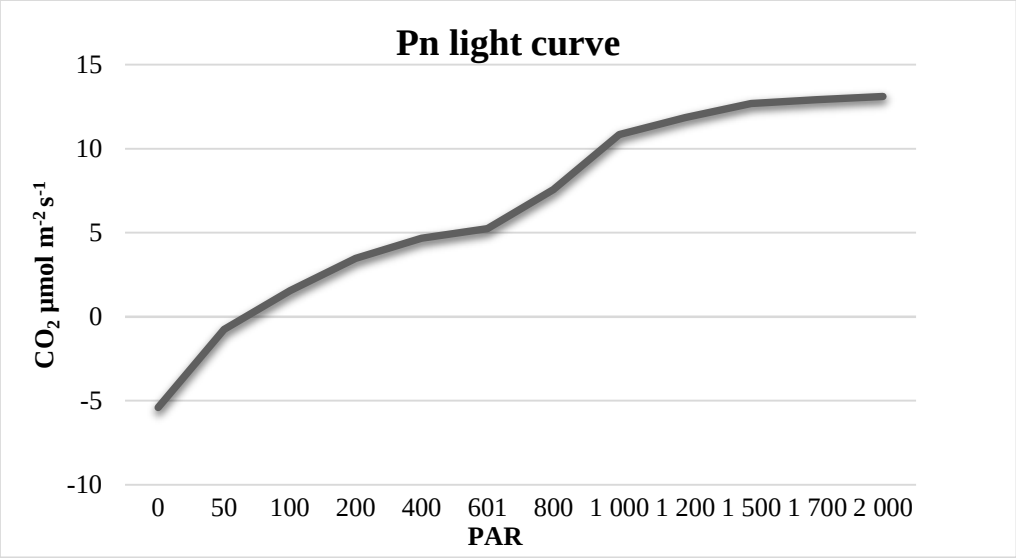


Figure 3. Light intensity curve showing the light saturation point at which gas exchange measurements were done. 1500 µmol m^{-2} s^{-1} was the light level used.

4.3. VOC SAMPLING and ANALYSIS

VOC samples were collected from the shoots and rhizosphere of all 48 Scots pine seedlings. Sampling was performed once a month during the experiment under May to September conditions (altogether five times). For the shoots, the sampling was carried out on April 29-30, June 17-18, July 21-22, August 26, and September 29, 2014, while the rhizosphere sampling took place on May 5-7, June 25-27, August 4-6, September 30 to October 2, 2014, respectively. Sampling was done using the headspace collection technique with polyethylene terephthalate (PET) cooking bags (25 x 38 cm, Rainbow) for shoot and rhizosphere soil. Samples from empty PET bag were also collected to serve as a blank sample for shoot VOC data, whereas pot containing soil only and an empty pot (no soil + no seedling in it) served as blank samples for rhizosphere VOC data.

Pre-heated PET bags (pre-heating at 120 ^{o}C for 1 hour in order to remove impurities) were used for the sampling. The shoots or the tube opening (28 mm in diameter) to the rhizosphere were covered with the PET bags, and air filtered through charcoal and MnO_2 scrubber was passed into the input line at a flow rate of 400 ml/min (as calibrated with a Buck soap bubble calibrator)

through an opening in the bag that was fastened and tightened to prevent introduction of impurities. The purified air flowed for 10 minutes before each sampling to allow for flushing of the sampling bag. After flushing, VOCs were collected into sampling tubes (filled with Tenax TA and Carbopack B adsorbents) via an input line at a flow rate of 200 ml/min. Sampling time for shoots lasted 10 minutes and 1 hour for the rhizosphere soil. The sampling was done without removing the plants or pots from the chambers. Air temperature and humidity inside the bag was monitored during the sampling with button-size data loggers. The sampling tubes were sealed and stored at +4 ^{o} C in a refrigerator until GC-MS analysis.

The sampling tubes containing the VOC samples were analyzed with a gas chromatography – mass spectrophotometry (GC – MS) (Hewlett-Packard GC 6890, MSD 5973). The compounds were desorbed into a thermal desorption unit (Perkin-Elmer ATD 400 Automatic Thermal Desorption system) for 10 minutes at 250 ^{o}C and injected into a HP-5 capillary column (50 m long x 0.2 mm internal diameter x 0.33 µm film thickness), with helium used as the carrier gas. The temperature program sequence was 50 ^{o}C for 1 min, followed by increases of 5 ^{o}C per minute to 210 ^{o}C and 20 ^{o}C per minute to 250 ^{o}C with a final hold time of 250 ^{o}C for 1.5 min.

The fragmented compounds were ionized in the ionization chamber, and the resulting VOCs identified by comparing their mass spectra against those in the Wiley library. The compounds were then quantified by comparing their peak areas and retention time with the available reference compounds used as standards. A set of Terpenoid and GLV (Green leaf volatile) compounds was used as standard and analyzed similarly with the samples. The data from the GC-MS analysis were then run on a macro in Excel to ease the analysis. Volatile organic compounds with identification certainty of 70% or more and that occurred in at least 5 samples per one sampling (i.e. 48 samples) were taken into account in further data analysis.

Alpha pinene (from the Terpenoid standard) was used as reference to calculate the concentrations of terpenes while cis-3-hexen-1-ol (from the GLV standard) was used as reference for other compounds. This is based on the assumption that all compounds have similar response to the standard compounds. The emission rates were then related to the needle area/soil surface area using the formula:

$$E = \frac{(C_{out} - C_{in})F}{needle\ or\ soil\ surface\ area}\ x\ 60\ min$$

E = emission rate

C_{out} = Concentration of VOC in the sampled air per liter (total sampled volume was 2 L for shoots and 12 L for rhizosphere)

C_{in} = Concentration of VOCs in incoming air (assumed 0)

F = Inflow of purified air, 0.4 L/min

Shoot lengths and needle number and length were measured at the end of each sampling and used in calculating the VOC emission per needle surface area. The needle area was then calculated for old and new shoot using the needle length and needle number and using Flower Ellis and Olsson (1993) formula (see above Pn needle area calculation), but now for whole shoot length enclosed in the bag.

The compounds found from blank samples were analyzed and quantified similarly as those in actual samples. If any of the compounds identified in the empty bag or pot appeared in less than three samples, the quantity emitted from it was then subtracted from that of the particular compound emitted in any of the other samples. The rhizosphere emission rate was corrected using the area of the soil collars (615,83 mm^2).

4.4. STATISTICAL TESTING

IBM SPSS Statistics 21 was used for the statistical analysis of the data. The linear mixed model ANOVA (LMM ANOVA) analysis was used to test for the main and interaction effects of warming and N addition on the gas exchange and warming, N addition and herbivory effects on the above- and below-ground VOC emissions of the Scots pine seedlings. In gas exchange design, tree identity nested within chamber was used as a random factor, whereas in VOC design, only chamber identity was used as a random factor. Though herbivory treatment was not performed until in June-July 2014, May 2014 data was used as an initial VOC emission level data for both H+ and H- seedlings, and therefore May measurement was included in the linear mixed model analysis of variance with the other dates. Spearman's non-parametric correlation test was then used to check the relationships between gas exchange and quantity of VOCs emitted from the shoots and rhizosphere. For the correlation test, Pn, Gs, and all VOC (shoot and

rhizosphere VOCs) data from the same measurement dates were tested together to get the overall correlation pattern, and then split across time (i.e. May, July and September) to check for correlations at different time points. Results were considered statistically significant when $p \leq 0.05$, and marginally statistically significant when $p \leq 0.1$.

5. RESULTS

5.1 ABOVE-GROUND VOCs

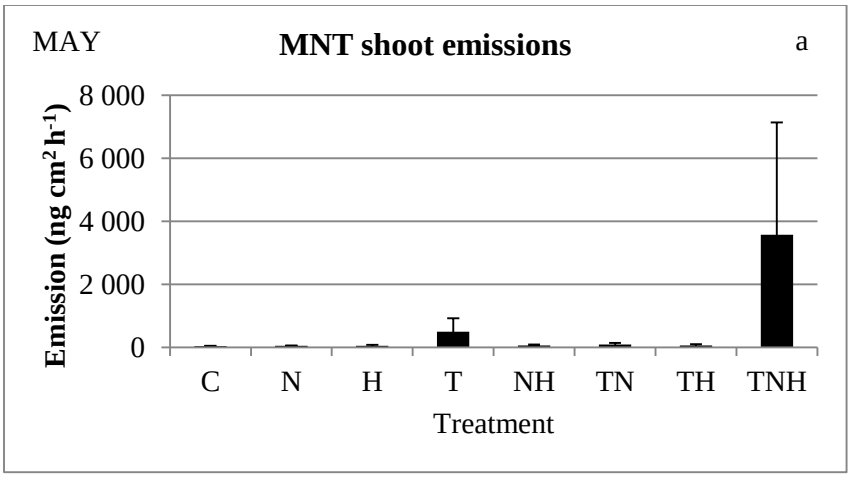
Twenty-six (26) compounds were emitted from the shoots, 14 of these being monoterpenes (MNTs) and 11 other volatile organic compounds (OVOCs), while there was only one sesquiterpene (SQT) (Tables 3 and 5). Compounds such as α-pinene, δ-3-carene, limonene, camphene and myrcene were the most abundant of the MNTs emitted. α-pinene accounted for almost 80 % of the MNTs emitted from the shoots. Nonanal, styrene, benzenemethyl, n-decanal and acetic acid were the most abundant of the OVOCs emitted from the shoots, accounting for over 90 % of the OVOCs. The emissions of MNTs, SQTs and OVOCs generally and significantly reduced (Table 4) as the experiment progressed, as they were highest in May and lowest in September (Figs 4-6).

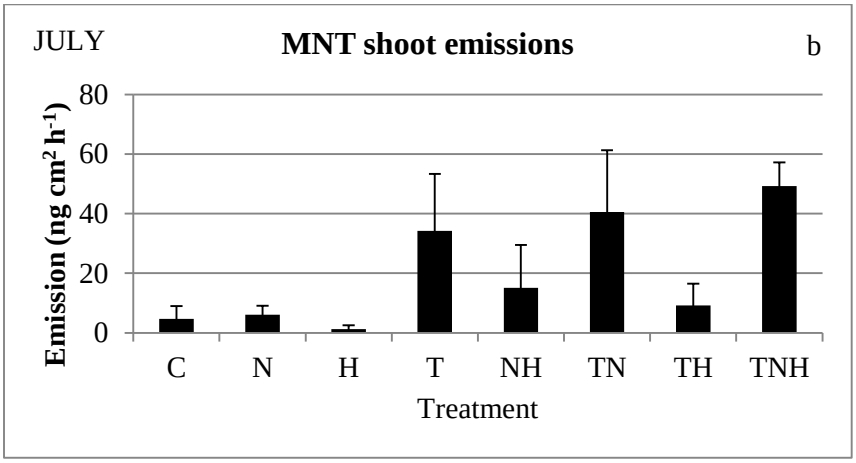
Table 3. Individual MNT and SQT compound emissions (ng cm^{-2} h^{-1}) measured from the shoots of Scots pine seedlings grown in different treatments (data not statistically tested). C = Control (i.e. ambient temperature, no N addition and no herbivory), N = Nitrogen treatment, H = Herbivory treatment, T = Temperature treatment, NH = Nitrogen + Herbivory treatment, TN = Temperature + Nitrogen treatment, TH = Temperature + Herbivory treatment, TNH = Temperature + Nitrogen + Herbivory treatment. Values are means±SEs of compounds averaged over three measurement occasions (May, July and September 2014). All other compounds are MNTs except b-caryophyllene.

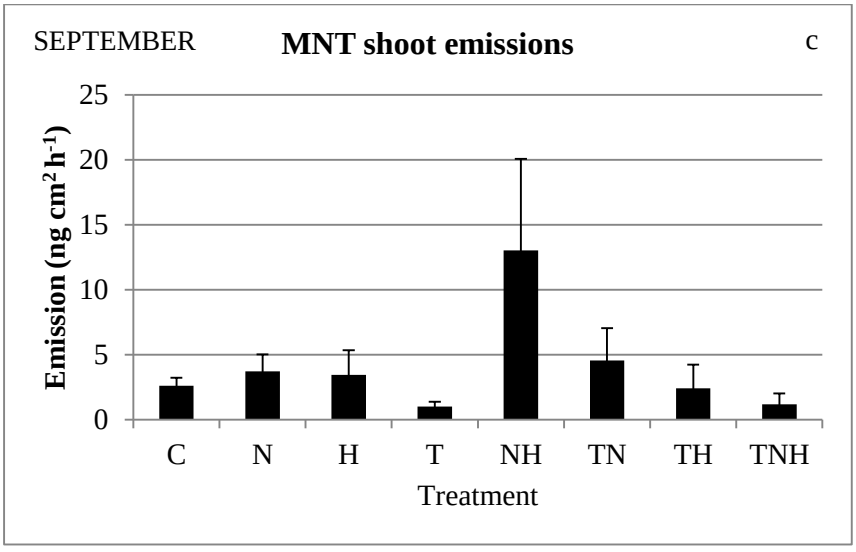
VOC emissions (ng cm^2 h^{-1})	C	N	H	T	NH	TN	TH	TNH
α - pinene	10.39±4,76	12.46±3,62	5.93±4,21	101.76±79,43	20.53±10,84	35.95±17,26	7.00±2,71	48.41±33,95
β-pinene	0.02±0,02	0.02±0,02	0.01±0,01	0.47±0,36	0.48±0,48	0.59±0,44	0.05±0,05	0.61±0,38
δ-3-Carene	1.50±0,97	3.32±1,56	8.05±6,89	62.69±58,70	2.91±2,52	2.78±1,30	0.87±0,54	1016.96±1016,30
Camphene	0.29±0,11	0.53±0,19	0.31±0,12	2.56±1,15	1.18±0,56	2.29±1,07	1.69±1,50	10.78±9,03
Sabinene	0.12±0,07	0.20±0,06	0.22±0,12	1.07±0,71	0.21±0,11	0.32±0,16	1.14±1,13	51.08±50,40
Tricyclene	0.00±0,00	0.06±0,05	0.03±0,03	0.27±0,20	0.16±0,14	0.37±0,27	0.51±0,49	0.11±0,11
Myrcene	0.25±0,11	0.27±0,10	0.39±0,30	3.23±2,51	0.30±0,16	0.57±0,28	3.43±3,37	56.70±56,69
Limonene	0.46±0,26	0.23±0,08	0.32±0,17	1.57±0,77	1.30±0,77	0.59±0,20	1.86±1,48	0.20±0,13
Trans-β-ocimene	0.04±0,03	0.04±0,02	0.22±0,22	0.40±0,13	0.33±0,27	0.92±0,57	0.00±0,00	12.00±11,64
ϑ-terpinene	0.02±0,01	0.03±0,01	0.11±0,09	0.59±0,51	0.18±0,08	0.09±0,03	0.68±0,57	9.39±9,16
Terpinolene	0.01±0,01	0.02±0,01	0.03±0,02	0.16±0,13	0.05±0,03	0.15±0,11	0.00±0,00	0.37±0,23
1,8-Cineole	0.00±0,00	0.00±0,00	0.00±0,00	0.02±0,01	0.00±0,00	0.00±0,00	0.00±0,00	0.03±0,02
α-Terpinolene	0.13±0,10	0.28±0,14	0.79±0,70	0.49±0,26	0.15±0,15	0.27±0,14	4.34±4,34	0.18±0,18
α-phellandrene	0.00±0,00	0.00±0,00	0.02±0,02	0.20±0,17	0.05±0,05	0.05±0,03	0.23±0,23	1.11±1,05
Total MNT	13.23±5,36	17.46±5,26	16.43±12,67	175.49±143,62	27.82±12,59	44.93±19,17	21.80±14,66	1207.92±1187,70
β-caryophyllene	0.0002±0,0002	0.003±0,002	0.020±0,020	0.091±0,060	0.090±0,084	0.162±0,068	0.000±0,000	0.609±0,044

In May, there was no clear N or T effects, only TNH showed a huge variation (fig. 4a), but in general other H+ trees did not differ from each other clearly. Warming increased the emissions of MNT from the shoots in July especially, alone and in combination with N addition, whereas in September these effects were reduced. Herbivory reduced the warming effect in July without N addition (as seen in the TH column in fig 4b), but N addition effect was clearer (N caused an increase in MNTs) under herbivory. Since TNH treatment had again the highest bar in July after the herbivory experiment, it seems that herbivory did not modify this interaction, but these trees just had a similar response before and after the herbivory experiment. In September, the nitrogen addition and herbivory in combination (NH trees) increased the MNT emissions.

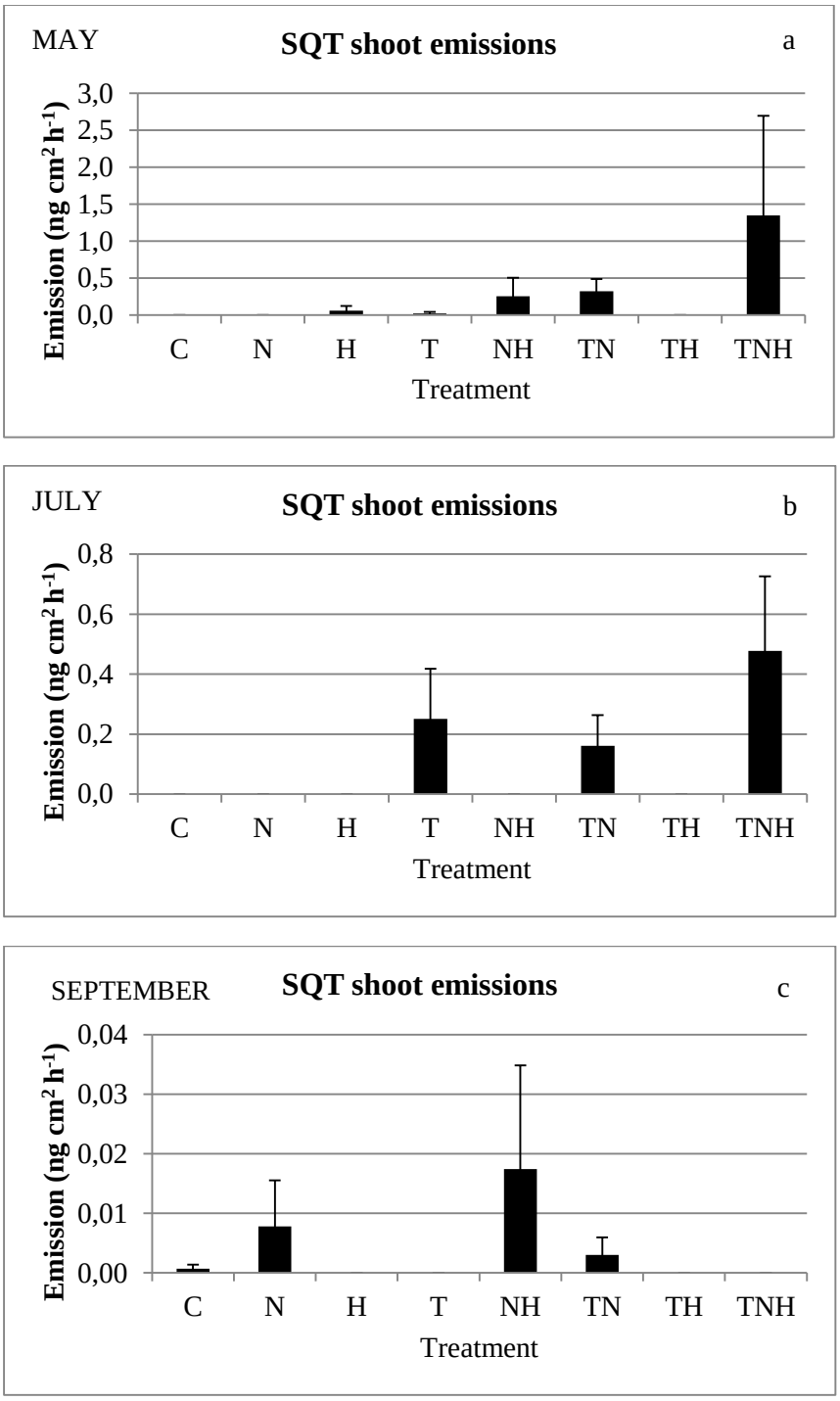
There was a marginally significant time x N interaction in shoot SQTs (Table 4). Thus, N effects on SQTS became clearer in September, as nitrogen addition either singly or in combination with herbivory and warming increased SQT emissions from the shoots (fig 5c).







Figures 4a-c. Monoterpene (MNT) emissions from the shoot of Scots pine seedlings based on the administered treatments, control (C), nitrogen addition (N), herbivory (H), elevated temperature (T), nitrogen and herbivory (NH), elevated temperature and nitrogen addition (TN), elevated temperature and herbivory (TH), and a combined treatment of elevated temperature, nitrogen and herbivory (TNH) in May, July and September 2014. Values are means±SEs (n = 3 per treatment).



Figures 5a-c. Sesquiterpene (SQT) emissions from the shoot of Scots pine seedlings based on the administered treatments, control (C), nitrogen addition (N), herbivory (H), elevated temperature (T), nitrogen and herbivory (NH), elevated temperature and nitrogen addition (TN), elevated temperature and herbivory (TH), and a combined treatment of elevated temperature, nitrogen and herbivory (TNH) in May, July and September 2014. Values are means±SEs (n = 3 per treatment).

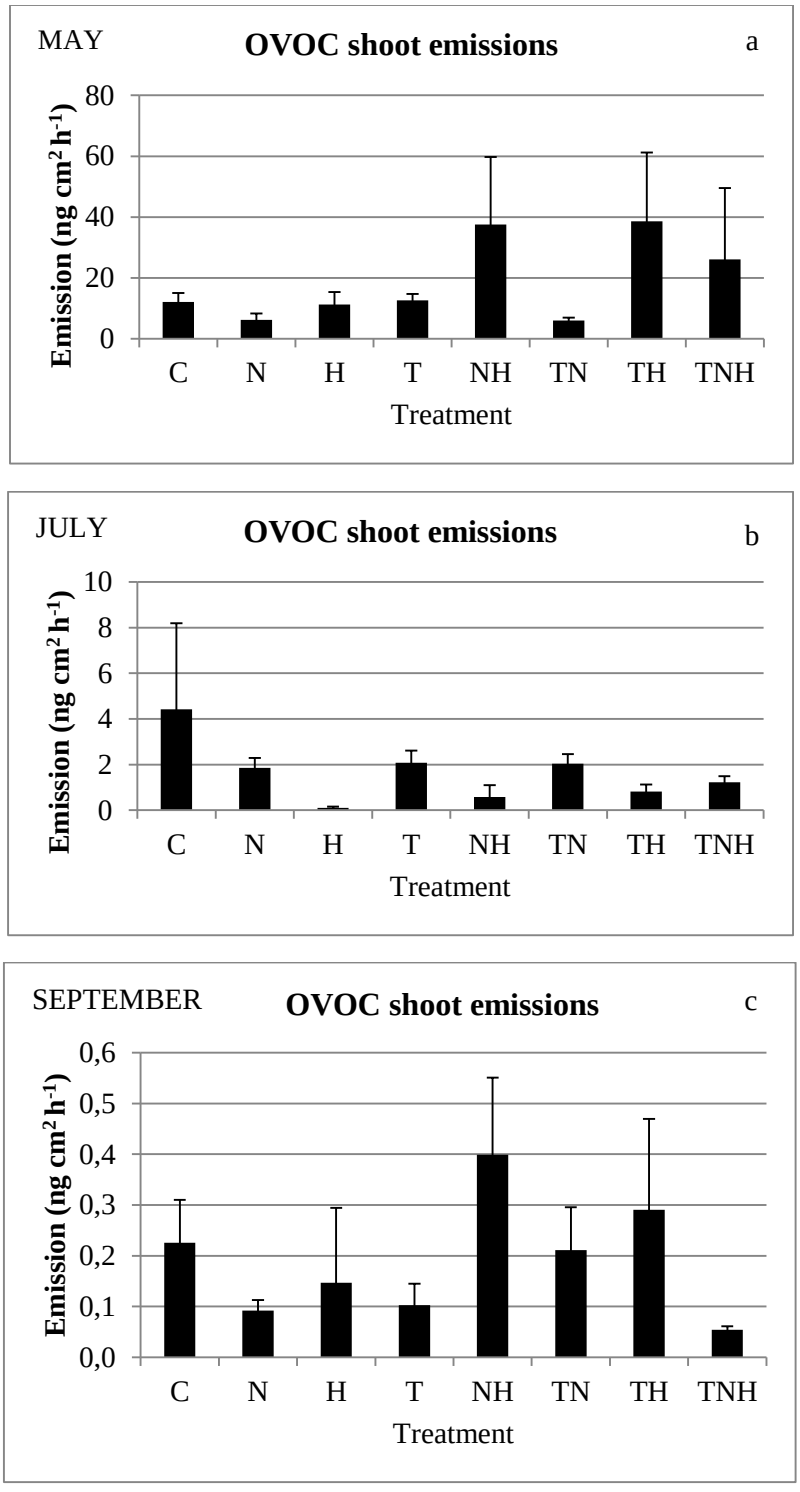


Figure 6a-c. OVOC emissions from the shoots of Scots pine seedlings based on the administered treatments, control (C), nitrogen addition (N), herbivory (H), elevated temperature (T), nitrogen and herbivory (NH), elevated temperature and nitrogen addition (TN), elevated temperature and herbivory (TH), and a combined treatment of elevated temperature, nitrogen and herbivory (TNH) in May, July and September 2014. Values are means±SEs (n = 3 per treatment).

Table 4. The p-values for main effects and their interactions used in mixed linear model ANOVA analyses. Data is for VOCs (MNTs and OVOCs) emitted by the shoot and rhizosphere of Scots pine seedlings. The effect is considered statistically significant when $p \leq 0.05$ and marginally statistically significant when $p \leq 0.1$ (shown in bold).

Factors	Shoots			Rhizosphere	
	Total MNT	Total SQT	Total OVOC	Total MNT	Total OVOC
T	.236	**.026**	.578	.390	.849
N	.181	**.025**	.981	.421	.906
H	.201	.163	**.013**	.238	.375
T x N	.194	**.069**	.175	.465	.515
T x H	.213	.453	.489	.348	.302
N x H	**.099**	**.070**	.276	.371	.175
T x N x H	.103	.157	.133	.513	.678
Time	**.044**	**.054**	**.000**	**.011**	**.000**
Time x T	**.066**	.218	.687	.713	.565
Time x N	.189	**.065**	.992	.633	.354
Time x H	.195	.191	**.000**	.700	**.033**
Time x T x N	.193	.174	.114	.669	.783
Time x T x H	.202	.635	.759	.747	.751
Time x N x H	**.075**	.315	.442	.712	.188
Time x T x N x H	**.073**	.550	.173	.575	.541

Herbivory without N addition reduced MNT and SQT emissions (figures 7a-b), but since this data also included the May measurements where herbivory was not actually yet affecting, the positive response to N and H in combination is probably due TNH tree response (highest MNT and SQT emissions occurring from these seedlings in May and July). A marginally statistically significant T x N interaction on SQTs, however, showed clearly that though both warming and N addition alone increased the SQT emissions, the effect was most pronounced when seedlings were exposed to both warming and N addition in combination (fig 8).

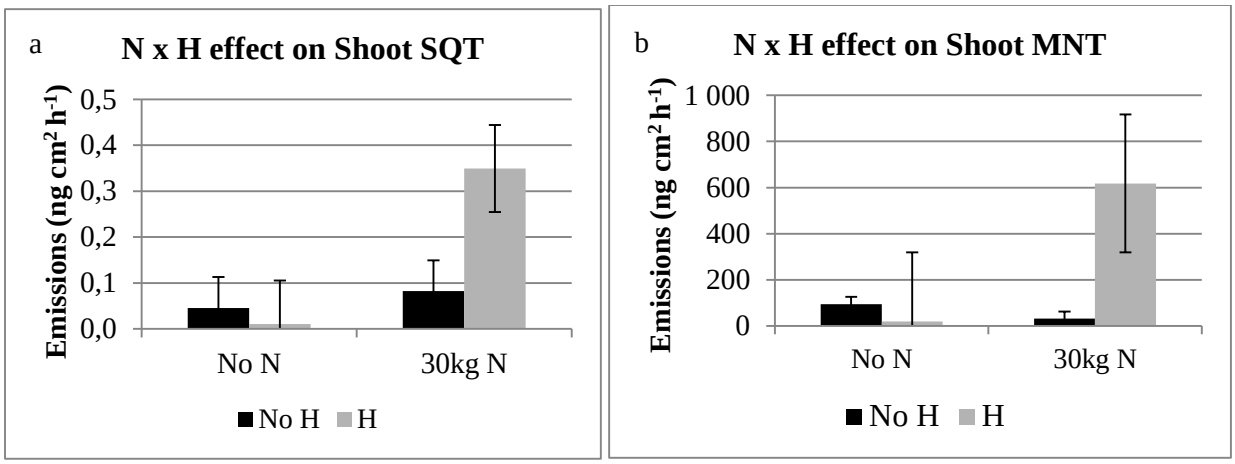


Figure 7a-b. Effect of herbivory and N addition interaction on the emissions of (a) SQT and (b) MNT from the shoots of Scots pine. Values are means ±SEs of three measurement occasions, n = 3 per treatment for each occasion.

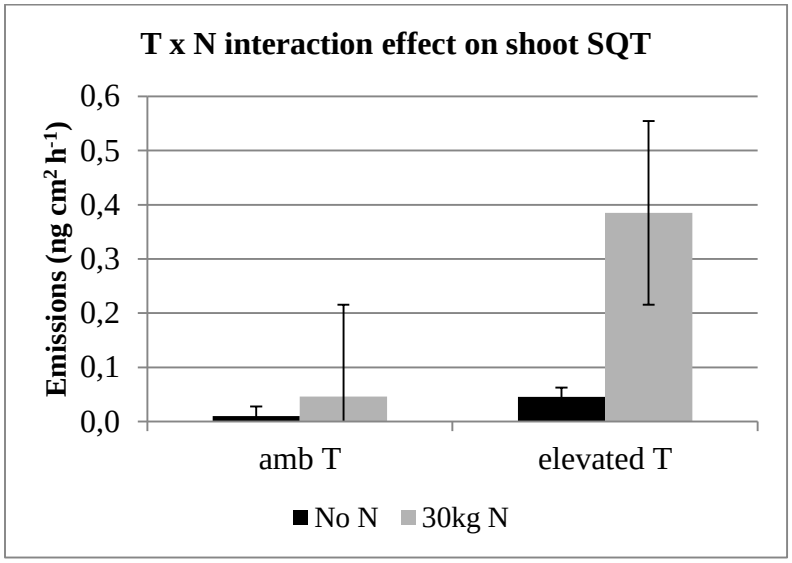


Figure 8. Effect of warming and nitrogen addition on SQT emissions from the shoots of Scots pine. Values are means ±SEs of three measurement occasions, n = 3 per treatment for each.

Table 5. Warming, N addition and herbivory effects on OVOC emissions (ng cm^2 h^{-1}) measured from the shoots of Scots pine seedlings. C = Control (i.e. ambient temperature, no N addition and no herbivory), N = Nitrogen treatment, H = Herbivory treatment, T = Temperature treatment, NH = Nitrogen + Herbivory treatment, TN = Temperature + Nitrogen treatment, TH = Temperature + Herbivory treatment, TNH = Temperature + Nitrogen + Herbivory treatment. Values are means±SEs of compounds averaged over three measurement occasions (May, July and September 2014).

VOC emissions (ng cm^2 h^{-1})	C	N	H	T	NH	TN	TH	TNH
Nonanal	2.60±0,94	1.57±0,63	1.42±0,91	2.61±0,79	1.35±1,10	1.34±0,45	3.62±2,55	3.69±3,69
Styrene	1.53±0,92	0.16±0,06	2.09±1,99	0.88±0,41	1.79±1,69	0.42±0,24	1.25±0,79	0.13±0,13
Benzenemethyl	0.47±0,43	0.07±0,02	0.00±0,00	0.05±0,02	0.04±0,03	0.08±0,02	0.08±0,06	0.23±0,17
N-Decanal	0.29±0,10	0.20±0,08	0.07±0,07	0.36±0,14	0.20±0,17	0.22±0,09	0.32±0,18	0.43±0,19
Acetic acid	0.17±0,09	0.43±0,27	0.05±0,02	0.40±0,29	0.05±0,02	0.34±0,22	0.16±0,07	0.30±0,12
Benzene	0.16±0,05	0.13±0,04	0.05±0,03	0.16±0,05	0.10±0,05	0.12±0,04	0.12±0,06	0.05±0,03
Hexanoicacid-2-ethyl	0.12±0,07	0.07±0,03	0.00±0,00	0.09±0,04	0.00±0,00	0.09±0,03	0.07±0,04	0.09±0,05
Benzene-1-methyl-4,1-methylethyl	0.12±0,09	0.02±0,02	0.05±0,02	0.29±0,22	0.04±0,04	0.05±0,02	0.36±0,25	4.08±3,99
Hexanal	0.08±0,04	0.01±0,01	0.10±0,05	0.06±0,03	0.03±0,03	0.04±0,02	0.02±0,02	0.05±0,05
Benzene-1,2-dimethyl	0.05±0,03	0.02±0,01	0.01±0,01	0.04±0,03	0.00±0,00	0.04±0,02	0.03±0,03	0.06±0,06
Nethyl-1,3-dithioisoindoline	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00	9.22±7,33	0.00±0,00	7.20±7,20	0.01±0,01
Total OVOC	5.59±1,92	2.70±0,93	3.82±2,20	4.94±1,49	12.83±8,90	2.73±0,66	13.25±9,11	9.11±7,99

Herbivory seedlings (H+ seedlings) had clearly highest OVOC emissions from the shoots of Scots pine when compared to other seedlings (Table 4). In May, before actual herbivory treatment, H+ seedlings had more than doubled OVOC emissions (fig 9a), but after herbivory in July and September, this trend was changed to opposite, and herbivory thus reduced the OVOC emissions when compared to seedlings without herbivory (fig 9b).

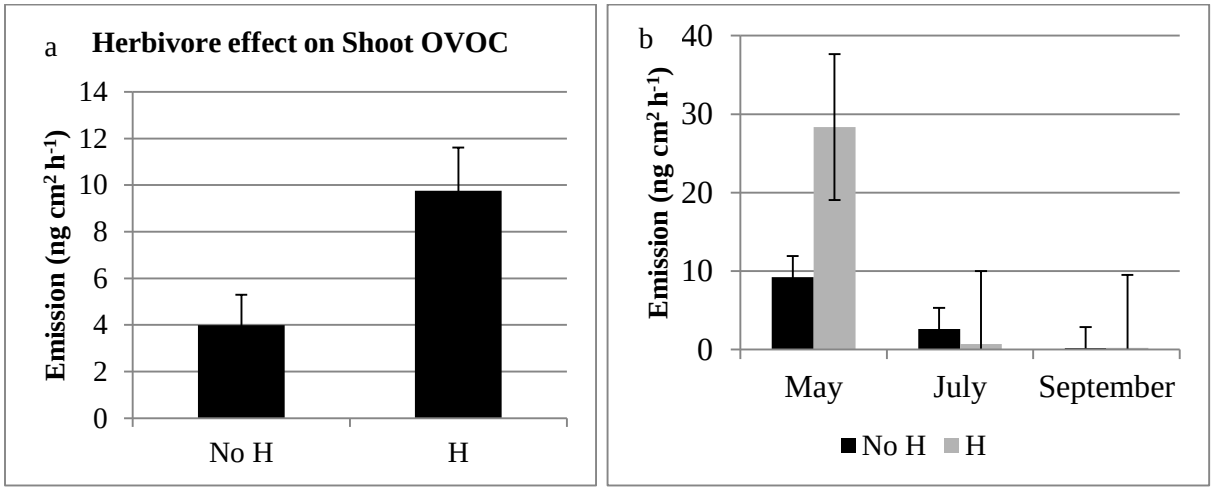


Figure 9a, b: Effect of (a) herbivory feeding (all months combined) and (b) herbivory feeding, months separated, on emissions of OVOCs from the shoots of Scots pine.

5.2. BELOW-GROUND VOC EMISSIONS

From the rhizosphere, thirty-seven (37) compounds were identified after VOC analysis (Tables 8 and 9), 4 monoterpenes (MNT) and 33 other volatile compounds (OVOC). α-Pinene, δ-3-Carene, limonene, and 8-terpinene were the monoterpenes identified, while nonanal, decanal, acetic acid, benzene, propanoic acid, and 1-hexanol-2-ethyl were the most abundant of the other VOCs emitted, jointly accounting for about 73% of the total OVOCs emitted. The number and quantity of OVOCs emitted was more than that of the MNTs (Tables 6 and 7), more volatile compounds being emitted in the first month than in other times (fig 10). The quantity of the OVOCs emitted significantly reduced with time (Table 4) while the quantity of MNT significantly reduced in July but increased again in September (fig 10).

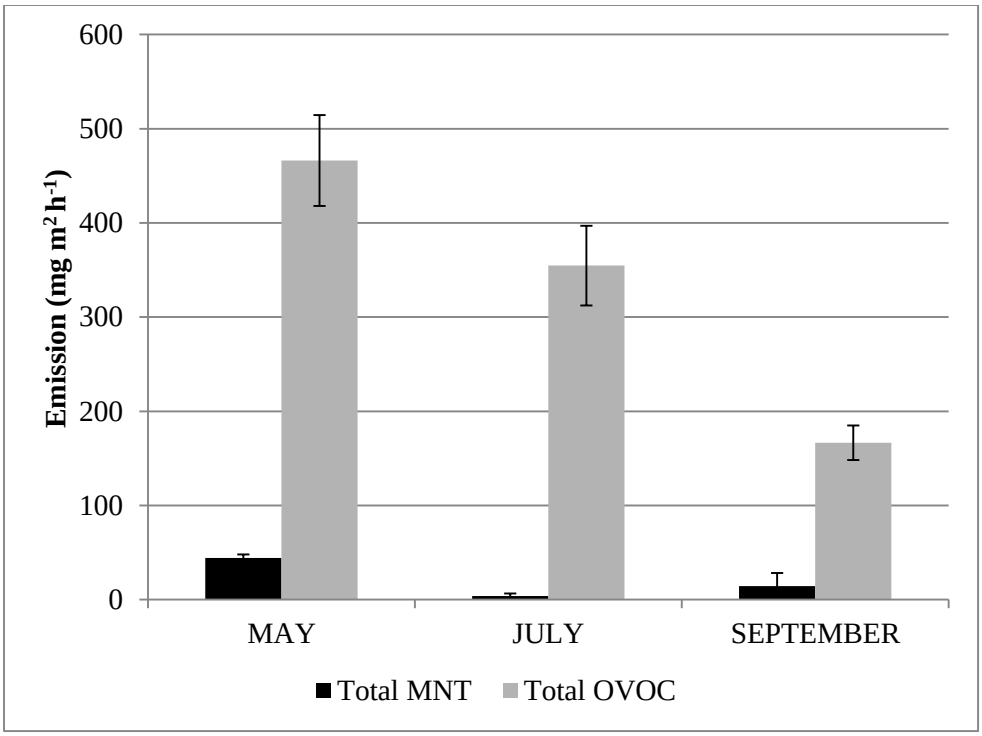
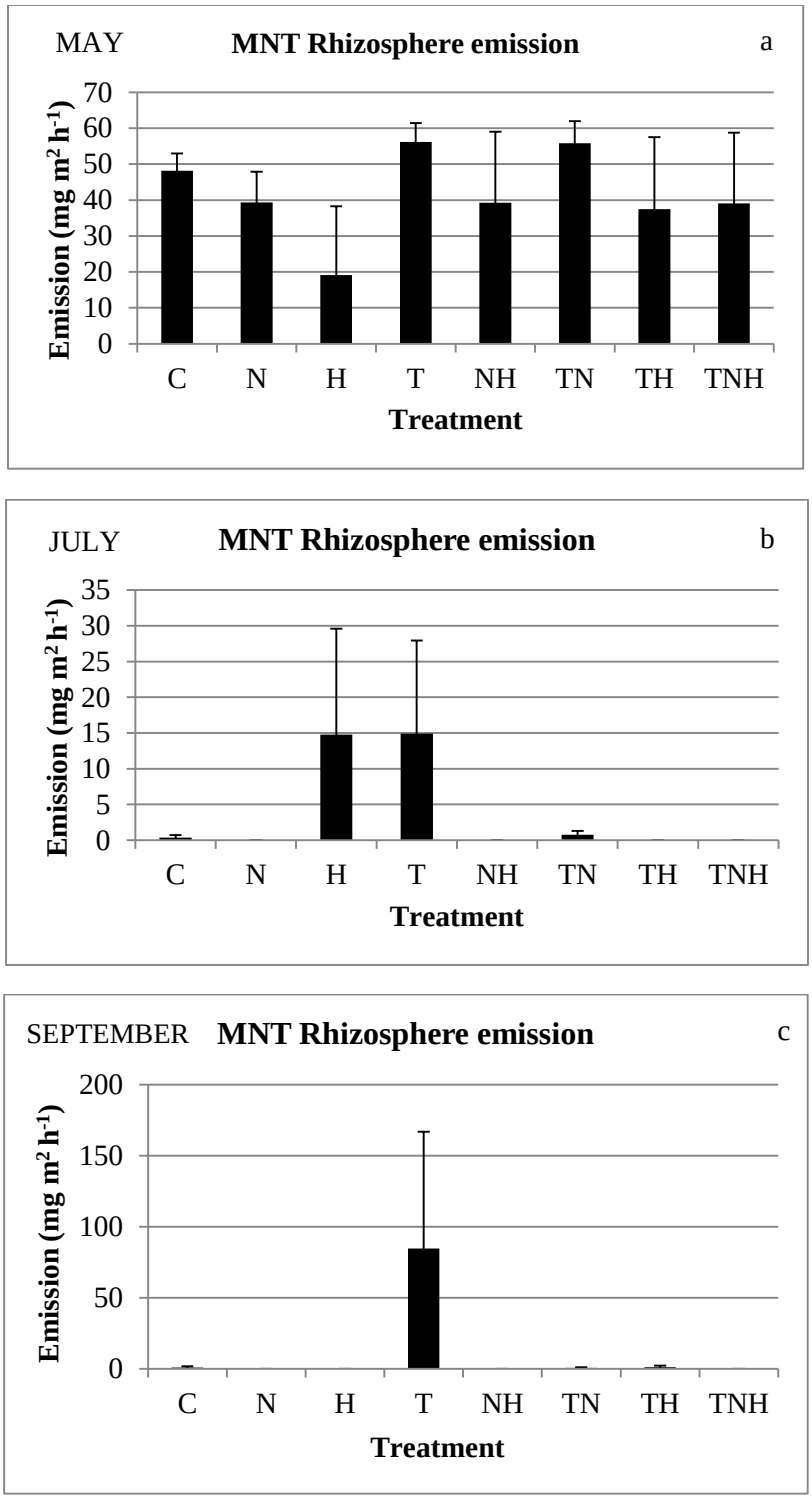


Figure 10. Total amount of MNTs and OVOCs emitted from the rhizosphere of Scots pine seedlings in May, July and September 2014.

There was only a statistically significant time and time x herbivory interaction on rhizosphere OVOCs in this study (Table 4). Herbivory seedlings had originally lower OVOC emissions in May, but after herbivory experiment OVOC emissions were increased significantly from the rhizosphere by about 85 mg m^2 h^{-1} (~21 %) in July, the month of the herbivory experiment and also slightly increased OVOC emissions in September (fig 13). In MNT emissions no clear warming, N addition or herbivory effects were seen (Table 4, Figure 11a-c).

Table 6. Warming, N addition and herbivory effects on MNT emissions (mg cm^{-2} h^{-1}) measured from the rhizosphere of Scots pine seedlings. C = Control (i.e. ambient temperature, no N addition and no herbivory), N = Nitrogen treatment, H = Herbivory treatment, T = Temperature treatment, NH = Nitrogen + Herbivory treatment, TN = Temperature + Nitrogen treatment, TH = Temperature + Herbivory treatment, TNH = Temperature + Nitrogen + Herbivory treatment. Values are means±SEs of compounds averaged over three measurement occasions (May, July and September 2014).

VOCs Emissions (mg m^2 h^{-1})	C	N	H	T	NH	TN	TH	TNH
α - pinene	12.31±4,15	9.68±3,88	5.40±4,20	46.14±28,60	8.73±5,80	14.31±4,81	10.11±6,60	8.92±5,93
Limonene	3.27±1,20	2.64±1,11	0.00±0,00	3.13±1,42	0.00±0,00	2.35±1,12	2.75±1,85	2.76±1,83
δ-3-Carene	0.58±0,33	0.46±0,26	5.91±4,07	1.40±0,81	2.74±2,13	1.41±0,86	0.00±0,00	1.34±0,88
ϑ-terpinene	0.31±0,31	0.32±0,32	0.00±0,00	1.03±0,73	1.61±1,61	1.04±0,76	0.00±0,00	0.00±0,00
Total MNT	16.47±5,65	13.11±5,24	11.31±7,56	51.70±28,64	13.08±8,68	19.11±6,59	12.86±8,45	13.02±8,64



Figures 11a-c: Mean (±SE, n = 3) of monoterpene (MNT) May, July and September emissions from the rhizosphere of Scots pine seedlings based on the administered treatments, control (C), nitrogen addition (N), herbivory (H), elevated temperature (T), nitrogen and herbivory (NH), elevated temperature and nitrogen addition (TN), elevated temperature and herbivory (TH), and a combined treatment of elevated temperature, nitrogen and herbivory (TNH) in May, July and September 2014. Values are means±SEs (n = 3 per treatment).

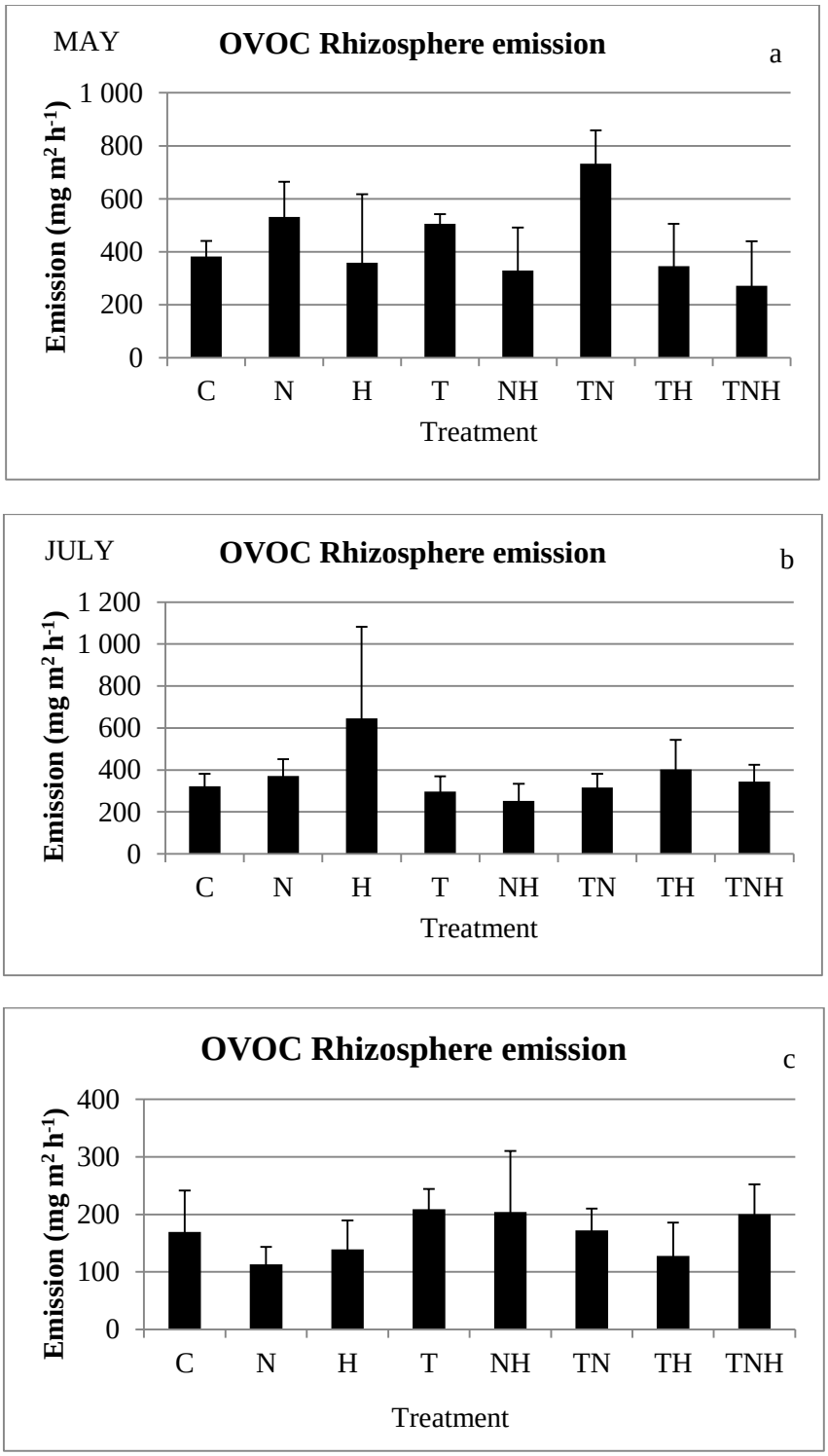


Figure 12a-c. Mean (±SE, n = 3) May, July and September OVOC emissions from the rhizosphere of Scots pine seedlings based on the administered treatments, control (C), nitrogen addition (N), herbivory (H), elevated temperature (T), nitrogen and herbivory (NH), elevated temperature and nitrogen addition (TN), elevated temperature and herbivory (TH), and a combined treatment of elevated temperature, nitrogen and herbivory (TNH) over the three samplings.

Table 7. OVOC emissions (mg cm^{-2} h^{-1}) measured from the rhizosphere of Scots pine seedlings grown in different treatments (data not statistically tested). C = Control (i.e. ambient temperature, no N addition and no herbivory), N = Nitrogen treatment, H = Herbivory treatment, T = Temperature treatment, NH = Nitrogen + Herbivory treatment, TN = Temperature + Nitrogen treatment, TH = Temperature + Herbivory treatment, TNH = Temperature + Nitrogen + Herbivory treatment. Values are means±SEs of compounds averaged over three measurement occasions (May, July and September 2014).

VOCs Emissions (mgm^2h^{-1})	C	N	H	T	NH	TN	TH	TNH
Nonanal	134.03±26,3	156.56±41,1	232.78±112,1	141.95±26,7	128.15±44,3	234.04±48,4	163.36±44,1	116.94±38,5
Decanal	22.14±3,82	28.21±5,65	38.03±19,53	26.42±3,11	23.74±7,15	31.84±5,74	21.40±6,38	29.76±6,39
Styrene	18.65±6,06	29.69±16,32	13.22±11,31	18.01±5,92	15.35±10,21	5.59±1,86	14.24±8,75	5.82±3,23
Acetic acid	13.03±4,66	10.32±3,15	15.19±7,11	8.59±2,93	14.79±5,47	20.10±4,74	9.11±6,54	12.55±7,10
1,2-benzenedicarboxylicaciddiethylester	12.61±5,38	5.14±2,51	8.26±7,40	10.61±3,75	0.98±0,98	8.58±3,90	7.34±7,34	18.00±11,96
1-hexanol-2-ethyl	8.48±1,82	5.98±1,54	12.60±10,70	14.18±2,52	1.45±0,96	8.41±2,76	9.12±4,23	6.22±2,87
Benzene	8.39±1,59	6.20±1,84	5.55±2,02	6.69±2,05	5.61±2,29	7.82±2,19	2.63±1,08	12.75±3,75
Propanoicacid-2-methyl-3-hydroxy-2,4,4-trimethylpentylester	7.11±3,01	4.67±2,24	1.91±1,91	6.85±2,29	6.20±2,64	5.42±2,60	11.97±7,48	1.07±1,07
Benzenemethyl	5.86±1,89	8.47±2,20	5.90±3,50	13.64±4,41	6.39±2,57	8.59±2,67	7.29±3,96	8.79±4,66
Benzene-1,3-dimethyl	5.73±1,72	4.79±1,51	3.36±2,74	6.48±2,02	5.46±3,62	3.67±1,56	0.99±0,99	7.33±3,98
ethane-1,1,1-trichloro	5.32±2,83	13.81±6,37	3.47±2,01	10.80±4,28	14.85±10,89	10.52±4,39	14.52±13,14	11.14±6,55
1-Propanol-2-methyl	5.26±3,13	6.84±3,46	1.33±1,33	6.65±3,88	3.19±3,19	5.72±2,59	2.07±2,07	5.19±3,45
2,5-cyclohexadiene-1,4-dione-2,6-bis-1,1-dimethylethyl	5.17±1,58	5.98±1,42	2.88±1,99	8.17±2,25	5.70±2,26	5.19±1,54	3.63±1,84	6.29±3,92
Benzaldehyde	4.99±1,71	4.82±2,09	9.85±5,63	7.25±2,28	2.46±1,72	5.14±1,87	1.92±1,92	5.05±3,34

VOCs Emissions (mgm^2h^{-1})	C	N	H	T	NH	TN	TH	TNH
Isobutylalcohol	4.55±3,72	6.39±5,48	0.00±0,00	0.58±0,58	0.00±0,00	1.34±0,92	0.00±0,00	0.00±0,00
Hexanal	4.18±1,23	4.98±1,42	3.93±3,00	5.04±1,64	2.97±1,63	5.93±1,89	1.71±1,71	3.50±1,99
Phenol	4.15±1,19	4.04±1,30	3.52±2,48	5.69±1,16	2.27±1,78	3.30±1,29	3.94±2,13	3.01±1,96
Tridecane	3.85±1,78	2.94±1,21	0.96±0,96	2.79±1,35	1.33±1,01	4.93±1,85	1.35±1,35	1.02±1,02
Benzeneethyl	3.68±1,01	4.38±2,17	0.69±0,69	4.70±1,13	3.10±1,79	1.54±0,58	2.73±1,31	2.26±1,24
Octanal	3.64±1,17	5.47±1,47	4.75±3,92	3.51±1,67	4.78±2,00	5.38±2,34	5.44±2,40	1.80±1,21
Benzene-1,4-dimethyl	2.72±1,11	5.79±2,86	2.60±1,92	4.25±1,43	7.88±7,21	6.08±2,09	1.88±1,44	2.19±1,68
Quinoline-1,2-dihydro-2,2,4-trimethyl	2.07±0,94	1.93±0,75	1.34±0,89	2.12±0,84	0.93±0,93	2.02±0,80	1.14±0,88	1.73±1,33
Benzene-1,2-dimethyl	0.96±0,41	0.83±0,49	1.24±1,24	3.08±1,28	1.61±1,61	3.34±1,10	2.66±2,02	1.00±0,67
3-heptanone	0.79±0,48	0.57±0,44	0.68±0,68	0.32±0,32	0.00±0,00	1.07±0,44	0.00±0,00	0.60±0,60
Naphthalene-1,3-dimethyl	0.76±0,76	0.09±0,09	0.09±0,09	0.57±0,39	0.00±0,00	0.66±0,66	0.00±0,00	0.00±0,00
1-butanol	0.62±0,46	1.43±0,69	0.07±0,07	1.69±0,80	0.00±0,00	0.80±0,55	0.91±0,91	0.00±0,00
Piperidine	0.61±0,42	0.43±0,43	0.00±0,00	0.36±0,36	0.00±0,00	1.51±0,92	0.00±0,00	1.70±1,70
1,3,5,7-cyclooctatetraene	0.50±0,37	3.55±1,66	4.17±2,32	2.77±1,02	1.44±1,03	2.48±1,42	0.00±0,00	1.82±1,82
2-pentanone-4-methyl	0.40±0,40	0.90±0,59	0.65±0,65	0.85±0,59	1.20±0,81	0.95±0,53	0.00±0,00	1.55±1,03
Propanoicacid-2-methyl-2-ethyl-3-hydroxyhexylester	0.32±0,28	1.06±0,60	0.00±0,00	0.72±0,52	0.00±0,00	0.37±0,37	0.33±0,33	1.17±1,17
5,9-undecadien-2-one-6,10-dimethyl	0.31±0,31	1.92±1,16	0.76±0,76	1.23±0,85	0.00±0,00	1.56±0,90	0.00±0,00	1.04±1,04
Heptanal	0.15±0,15	0.20±0,20	1.63±1,11	0.95±0,52	0.00±0,00	1.76±0,85	0.00±0,00	0.00±0,00
1-nonene	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00	1.30±0,71	0.00±0,00	0.93±0,93
Total OVOC	291.02±40,9	338.40±64,8	381.42±164,3	327.50±41,6	261.83±63,3	406.95±73,7	291.69±76,4	272.22±59,5

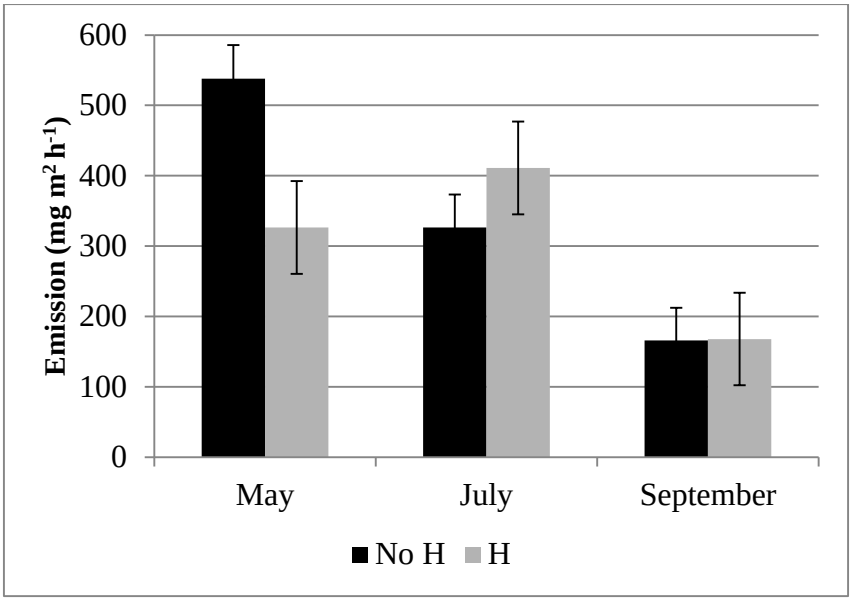


Figure 13. Time x herbivory feeding interaction effect on the emission of OVOCs from the rhizosphere of Scots pine. Values are means±SEs (n = 9).

5.3. GAS EXCHANGE

5.3.1. Photosynthesis

In general, the old shoots photosynthesized more than the new shoots (fig 14). The photosynthesis rate in both the old and new shoots increased gradually with time until August and dropped again in September (fig 15). However, nitrogen addition significantly (time x N interaction, p = 0.005, Table 8) increased photosynthesis rates of the old shoots in the early stages of the experiment (May and June) but then its effect diminished in July, and ultimately in August and September N addition decreased Pn rates in old shoots (fig 16). In contrast to old shoots, warming alone and in combination with N addition (T and TN) increased photosynthesis in new shoots when compared to C and N treatments (p = 0.017 for warming main effect, Table 7), treatment effects on new shoot Pn being similar over time (Table 8; Pn range 1,33 – 2,18 CO_2 µmol m^{-2} s^{-1} in May – September 2014 period).

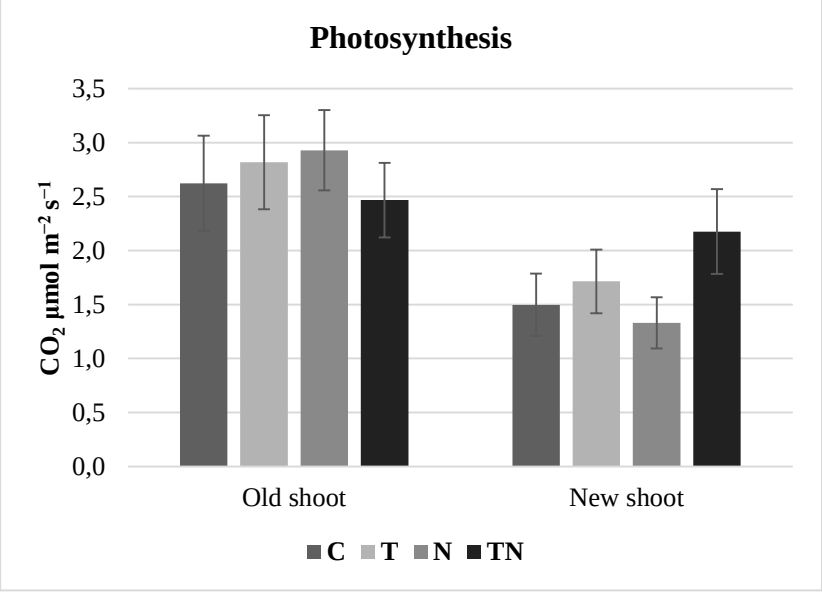


Figure 14. Overall photosynthesis rates of the shoots of Scots pine in different warming and N levels. Treatments: control (C), nitrogen addition (N), elevated temperature (T), elevated temperature and nitrogen addition (TN). Values are means ±SEs of five measurement occasions, n = 3 per treatment for each occasion (n = 15 per treatment in total).

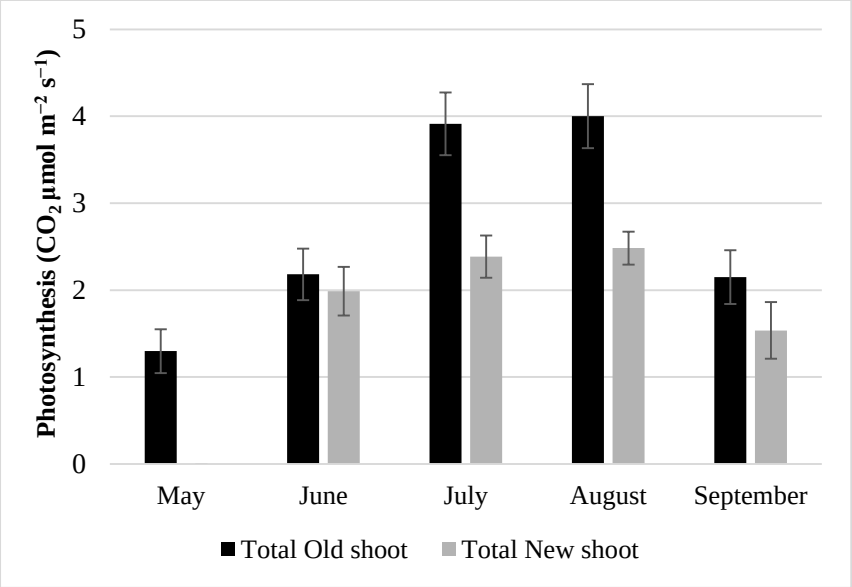


Figure 15. Overall photosynthesis rate of the old and new shoot of Scots pine seedlings over time. Values are means ±SEs of five measurement occasions, n = 3 per treatment for each occasion (n = 15 per treatment in total)

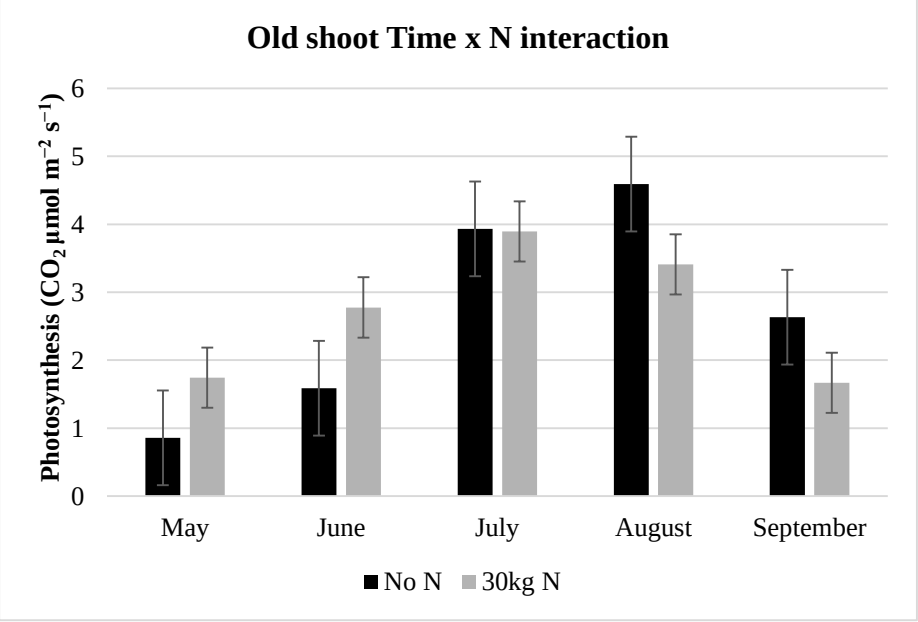


Figure 16. Effects of nitrogen addition over time on the photosynthesis rate of old shoot of Scots pine seedlings. Values are means ±SEs when C and T trees are combined (no N) and when N and TN trees are combined (30 kg N ha^{-1} a^{-1} added), n = 6 at each N level.

Table 8. P-values of different treatment levels on gas exchange, photosynthesis (Pn) and stomatal conductance (Gs) from the new and old shoots of Scots pine seedlings. P-value is considered to be statistically significant when p≤0.05 and marginally statistically significant when p≤0.1.

	p-value			
Factors	**Old shoot**		**New shoot**	
	Pn	Gs	Pn	Gs
T	0,782	0,305	**0,017**	**0,023**
N	0,963	0,913	0,495	**0,021**
T x N	0,503	0,689	0,149	**0,031**
Time	**0,000**	**0,000**	**0,000**	**0,002**
Time x T	0,774	**0,027**	0,639	0,242
Time x N	**0,005**	0,221	0,953	0,175
Time xT x N	0,796	0,521	0,775	0,214

5.3.2. Stomatal conductance

Generally, just as with the photosynthesis, the old shoot had higher stomatal conductance rate than the new shoot (fig 17). The effect of warming on the rate of stomatal conductance in the old shoot varied over time significantly (time x T, p = 0,027). Thus, in May, June and September, stomatal conductance was similar between the warming and non-warmed condition, but in July and August, stomatal conductance was higher in the Scots pine seedlings under the ambient temperature exposure than in warming treatment (fig 18).

In the new shoot, warming and nitrogen addition increased the stomatal conductance rate (Table 8, Figs 17), however, the combined TN treatment had a synergy effect, as increase in the rate of stomatal conductance was more than the sum of the individual treatment effect.

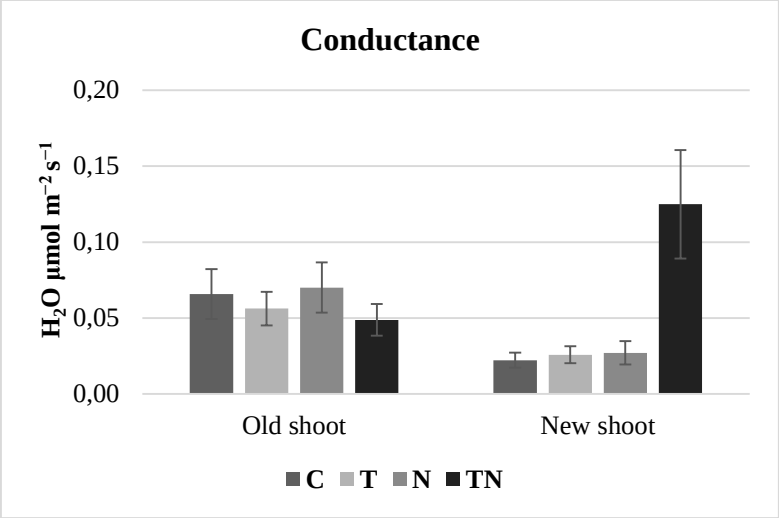
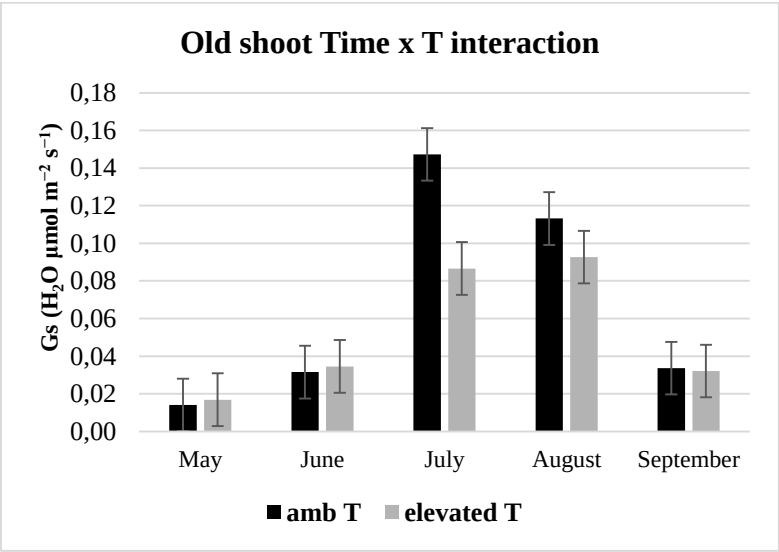


Figure 17. Effect of warming and N addition on the stomatal conductance of the shoots of Scots pine. Treatments: control (C), nitrogen addition (N), elevated temperature (T), elevated temperature and nitrogen addition (TN). Values are means ±SEs of five measurement occasions, n = 3 per treatment for each occasion (n = 15 per treatment in total).



Figures 18. The temperature effect over time on the stomatal conductance rate of the old shoot of Scots pine seedlings over time.

5.4. GAS EXCHANGE AND VOC EMISSION CORRELATION

The non-parametric correlation test between the whole gas exchange and the emissions of VOC (Tables 9 and 10) revealed a negative correlation between gas exchange parameters and the

quantity of OVOCs emitted from the old shoot. In the new shoot, there was also a negative correlation between the photosynthesis rate and the quantity of MNT emitted from the shoot (Table 9). There was also a negative correlation between the new shoot photosynthesis and stomatal conductance rate and the quantity of MNT emitted from the shoots and rhizosphere soil (Table 9). All of these negative correlations means that the quantities of MNT and OVOCs emitted increased as the photosynthesis and stomatal conductance reduced and vice versa.

At the time point level, correlation test between the gas exchange and VOC emission showed a negative correlation between the new shoot stomatal conductance and shoot OVOC in September (Table 10) meaning that the emissions of OVOC from the shoots increased as the stomatal conductance in the new shoot reduced and vice versa. A positive correlation was instead found between the new shoot stomatal conductance and the quantity of SQT emitted in July (Table 10) meaning that the emission of SQT from the shoot increased as the stomatal conductance in the new shoot increased.

Table 9. Non-parametric correlation between gas exchange and quantity of emission of volatile organic compounds for the whole data (3 measurement times combined). **. Correlation is significant at the 0.01 level, * correlation is significant at the 0.05 level

	Shoot MNT	Shoot OVOC	Shoot SQT	Rhizosphere MNT	Rhizosphere OVOC
Old Shoot Pn	-.180	**-.441****	-.068	-.252	.103
Old Shoot Gs	-.189	**-.460****	-.021	-.283	.123
New Shoot Pn	**-.380***	**-.577****	-.052	**-.522****	.050
New shoot Gs	**-.379***	**-.625****	-.045	**-.554****	.024

Table 10. Non-parametric correlation between gas exchange and quantity of emission of volatile organic compounds for the data at three different measurement times. **. Correlation is significant at the 0.01 level, *. Correlation is significant at the 0.05 level. There were no new shoot measurements done in May.

Time		Shoot MNT	Shoot OVOC	Shoot SQT	Rhizosphere MNT	Rhizosphere OVOC
May	Old Shoot Pn	.497	.559	.477	.218	-.147
	Old Shoot Gs	.503	.503	.450	.276	-.126
July	Old Shoot Pn	-.387	-.322	-.430	.306	.133
	Old Shoot Gs	-.556	-.441	-.570	.393	.063
	New Shoot Pn	.028	.301	.360	-.480	.126
	New shoot Gs	.261	.441	**.640***	-.393	-.084
September	Old Shoot Pn	.084	-.417	-.306	-.131	-.231
	Old Shoot Gs	.203	-.462	-.131	-.306	-.217
	New Shoot Pn	-.161	-.137	.044	.306	-.070
	New shoot Gs	-.371	**-.585***	-.306	-.393	-.161

6. DISCUSSION

6.1. VOC EMISSIONS FROM SCOTS PINE SHOOTS

The blend of MNTs emitted from the shoots in this study is also consistent with the findings of Tarvainen et al. (2005), Räisänen et al. (2009) and Aaltonen et al. (2010) who found that MNTs made up to 85 % of the total VOCs emitted from Scots pine grown in forests. Shao et al. (2001) also suggested that monoterpene emissions from Scots pine (*Pinus sylvestris)* can occur from storage pools as well as from processes that are linked to monoterpene biosynthesis, and that monoterpene emissions are influenced by photosynthetically active radiation. In this study, MNT made up 81 % of the total VOCs emitted from the shoot. The quantities of both the MNT and OVOC emitted significantly reduced with time, being highest in the first month, and gradually decreasing as the experiment progressed.

Tarvainen et al. (2005) observed a seasonal cycle for monoterpene emissions of Scots pine grown in a boreal forest. This cycle shows temperature dependence: emission rates were very high in spring, dropped slightly lower in summer and then followed with a gradual decrease into autumn. This probably explains the considerable difference in the quantity of MNT and OVOCs emitted from the shoot, as MNTs can also be emitted from storage pools while the OVOCs are not.

N addition enhanced the effect of herbivory feeding, increasing the MNTs emitted from the shoots. N addition also enhanced the effect of warming, forming a synergistic relationship and increasing the quantity of SQT emitted. This is consistent with the findings of Kivimäenpää et al. (manuscript) who observed that N addition enhanced the effect of warming and as much as quadrupled the quantity of VOCs emitted. The increment in the emissions of OVOCs caused by herbivory suggests the possibility of most of the OVOCs being emitted in high quantities as an allelopathy response to the ‘shock’ of the herbivore attack (Holopainen and Gershenzon 2010). This suggests the possibility of an instantaneous emission of volatile compounds in response to environmental stressors.

6.2. VOC EMISSIONS FROM SCOTS PINE RHIZOSPHERE

The number and quantity of MNTs emitted from the rhizosphere is very low when compared to those emitted from the shoots. This agrees with the findings of Asensio et al. (2008) where soil monoterpene emissions were very low when compared with foliar VOC emission rates. α-pinene was the most abundant MNT volatile compound from the shoot and the second most abundant from the rhizosphere, accounting for up to 69% of the monoterpenes emitted from the shoot and up to 56% of those from the rhizosphere. This comes close to the findings of Smolander et al. (2006), where α-pinene accounted for 65% of the total monoterpene concentration in pine and soils, while δ-3-carene and myrcene accounted for 12% and 9% of the monoterpenes respectively. In this study, δ-3-carene made up ~ 6% of the MNT emitted and no myrcene was found.

In this study, the number and quantity of OVOCs emitted were higher in the first month than in other months. A possible explanation for the large number of volatiles found in the rhizosphere may be that roots are known to produce different VOCs than leaves (Erb et al. 2010) mainly because they respond differently to elicitors (Schmelz et al. 2009). Another possible reason for the high number of OVOCs emitted from the rhizosphere may be related to the role of soils as a sink and emitter of VOCs (Asensio et al. 2008). The numerous interactions present in soils, ranging from microbial activities, organic matter decomposition (Leff and Fierer 2008) and even root exudates (Seawald et al. 2010) can influence the type and quantity of VOCs emitted from the rhizosphere.

Herbivory increased the quantity of OVOC emitted from the rhizosphere in July (the month of the herbivory experiment) suggesting that above-ground herbivore attack can induce the emissions of volatile compounds, even from the roots, to help protect plants from the herbivores. The mechanism behind this may, however, be complex and is not studied in this work in detail, given that the rhizosphere is a complex environment where several interactions occur. The qualitative changes of VOCs emitted from the rhizosphere after herbivory may also be supported by the findings of Musser et al. (2002) which suggested a relationship between the VOCs emitted from the injured plants and the attacking herbivore. The increment in OVOC emissions brought about by herbivory in July, but little effect on VOC emissions in September, suggests that the

plants have possibly re-adjusted their physiological processes to address the damage caused by the herbivore attack, thus increasing the variety and quantity of VOCs in that time and not much further than the period of attack.

6.3. GAS EXCHANGE RESPONSES

Generally, over time, photosynthesis and stomatal conductance rate increased until August and decreased in September, and the old shoots had a higher photosynthesis and stomatal conductance rate than new shoots. Warming (T) increased photosynthesis rate in the new shoots until July, which follows the findings of Pumpanen et al. (2012) that warming increased photosynthesis in Scots pine and other conifers. However, this increment in the rate of photosynthesis caused by warming gradually reduced beginning from August suggesting that it follows a seasonal cycle.

Emission of most MNTs is known to be light dependent (Yuan et al. 2009), and according to Komenda et al. (2003), photosynthetic light absorption rate significantly affects the emission of monoterpenes, especially α-pinene. However, the mechanism of interaction of PAR with VOC emission rates is yet to be fully understood. In this study, June and July had the longest PAR exposure hours and although the first month, May, had the highest emission rate of VOCs both from the rhizosphere and shoots, possibly due to the initial adjustment of the Scots pine saplings to the stressors/treatments applied, the next highest emissions came in July the month with the second longest PAR duration, corroborating the likelihood of a relationship between PAR and the emission rate of volatile compounds.

The effects of nitrogen addition on the photosynthesis rate in the Scots pine needles followed a time pattern in old shoots; its effect was prominent in the early stages of the experiment, increasing photosynthesis rate in the old shoot till July and then waned with time. This can be explained by the reallocation of the nitrogen to meet the nutritional requirement of nitrogen especially by the old shoot. The variations in the effect of nitrogen addition on photosynthesis was also reported by Bell et al. (2011) who noted that plants show different responses such as increasing and decreasing rates of photosynthesis and stomatal conductance to different levels of nitrogen addition both singly and in combination with other treatments, and that such effect has

some level of dependence on the form in which the nitrogen is taken up from the soil to the plant. The synergistic interaction between warming and N addition in the new shoots, which also increased stomatal conductance, suggests that the Scots pine needles become more active when N is well available at warm temperatures.

This study also found a negative correlation between gas exchange and VOC emission, with VOC emissions increasing as the photosynthesis/stomatal conductance reduces. A possible explanation for this could be that plants emit VOCs passively even when the stomata are closed and Pn is low.

7. CONCLUSION

According to the present study pine weevil herbivory on Scots pine bark can elicit the emissions of OVOCs from the rhizosphere as seen in the increased quantity of OVOC emitted from the rhizosphere in July, the month of the herbivory experiment. Furthermore, nitrogen addition can enhance the effects of warming on shoot SQT, and vice versa, but moderate warming (+2 °C increase) effects on shoot MNTs or OVOCs were not seen and also N effects on MNTs was variable over time. The emission of volatile organic compounds from shoots and rhizosphere soil increased while foliar gas exchange reduced suggesting the possibility of passive emissions of VOCs. Current results nonetheless indicate that some warming and N interactions can occur in VOC emissions, and that also herbivory pressure can modify N response of VOCs in conditions where N availability is increased.

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