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INVESTIGATING SHORT-TERM NITROGEN POLLUTION ON THE PHYSIOLOGY AND BIOGENIC VOLATILE ORGANIC COMPOUND (BVOC) EMISSIONS FROM *PINUS SYLVESTRIS* IN SCOTLAND

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ABSTRACT OF THESIS

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Title of Thesis **Investigating short-term N pollution on the physiology and biogenic volatile organic compound (BVOC) emissions from *Pinus sylvestris* in Scotland**

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Biogenic volatile organic compounds (BVOCs), produced by plants, such as isoprene and monoterpenes, can influence regional and global atmospheric chemistry. Although certain factors controlling the emission rates of BVOCs from plants are reasonably well understood, the influence of pollutants, such as ammonia (NH_3) deposition, is yet unclear. Monoterpene emission rates were measured from *Pinus sylvestris* (Scots pine) trees (7 years old) originating from four distinct locations in Scotland, and grown under ambient conditions at Centre for Ecology and Hydrology (CEH), Edinburgh.

Nine monoterpene compounds were emitted from *Pinus sylvestris*, with α-pinene and δ^3-carene being the most abundant emitted compounds. The mean total monoterpene emission rate was 1.39±1.92 μg g^{-1} h^{-1} (based on a dry weight of needles). Monoterpene emissions of *Pinus sylvestris* were found to be independent of genetic factors, photosynthetic rates, and a narrow range of instantaneous temperature changes. A tendency of increasing in emission rates, over time, was associated with new needle growth, and with historical accumulated temperature and PAR. However, the significance of these relationships needs further investigation.

NH_3 treatments were applied to selected pine shoots using a PET bag-enclosure method. The effects of short-term dry NH_3 deposition (up to 168.5 μg m^{-3}) tended to decrease monoterpene emission rates of young *Pinus sylvestris*, particularly α-pinene and δ^3-carene. Although no statistical evidence was found for the effects of NH_3 treatment on emission rates, these results nonetheless provide a first valuable, comparative feasibility study that can be used as a grounds for investigating the effects of N-treatment on BVOC emissions.

KEYWORDS: biogenic volatile organic compounds, BVOC, monoterpenes, α-pinene, δ^3-carene, Scots pine, *Pinus sylvestris*, ammonia, NH_3, dry deposition

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CHAPTER 1

PROJECT BACKGROUND AND LITERATURE REVIEW

In this chapter, a review of relevant literature will be provided, in order to form a contextual background for the study, and to identify the gap in research that this project is attempting to address. Specific technical details of the methodology, developed and used, are given in Chapter 2. Results relating to monoterpene emissions will be clearly presented, and the effects of dry ammonia deposition on emissions will be discussed in Chapter 3. Chapter 4 will conclude this study and outline future research opportunities.

1.1 Introduction

Volatile organic compounds (VOCs) are an important group of air pollutants that are emitted from numerous types of sources, both natural and anthropogenic (Hewitt, 1999; de Gouw & Warneke, 2007). Most anthropogenic VOCs are ubiquitous in industrial settings and occur at street level in urbanised areas (Hester & Harrison, 1995), while biogenic organic compounds (BVOCs) are derived from various natural sources. BVOC emissions, such as isoprene and monoterpenes, are plant-species specific. They are largely controlled by temperature, but the emissions of some compounds are also controlled by light (Guenther, 1997). As the largest source of non-methane VOCs, BVOCs play a crucial role in regional and global atmospheric chemistry

(Laothawornkitkul *et al*., 2009; Peñuelas & Llusià, 2003). Many BVOCs (*e.g.* isoprene and monoterpenes) are photochemically reactive, and strongly affect the concentrations of OH and O_3 in the atmosphere (Arneth *et al.*, 2011). They can also increase the formation of secondary organic aerosols (SOAs) (Mentel *et al*., 2013; Ormeño & Fernandez, 2012). BVOCs also play an important role in the ecological functions of the biosphere (Lerdau, 2007). While extensive studies on BVOC emissions and their biotic and abiotic controls have been undertaken in the last two decades, there is still a lack of detailed information on BVOCs from many important plant species (Arneth *et al*., 2008; Lerdau & Slobodkin, 2002), and little work on the effect of pollution on these emissions.

The deposition of global reactive nitrogen (N), as a result of anthropogenic activities like industrialisation, agricultural intensification and fossil fuel combustion, has increased dramatically since the 19th century (Fowler *et al*., 2004). Emitted N-compounds re-enter the ecosystem via precipitation (wet deposition) or via gas or aerosol (dry) deposition. This increased level of N deposition can seriously affect the normal functioning and sustainability of semi-natural terrestrial ecosystems (Bertills & Nasholm, 2000; Southon *et al.*, 2013). Measurements taken in various semi-natural vegetation habitats in the UK showed high levels of background ammonia (NH_3) (> 10 μg m^{-3}) (Leith *et al*., 2005), and increasing levels of atmospheric NH_3 deposition resulting from agricultural sources (Sutton *et al*., 2001). About 85.9 % of NH_3 emissions in Scotland are a direct result of agriculture (Thistlethewaite *et al*., 2013), particularly livestock units (Sutton *et al*., 2004).

Scots pine (*Pinus sylvestris*) is an important species in Scotland, and is a known monoterpene emitter (Simpson *et al.*, 1999; Komenda & Koppmann, 2002). It is also an important species in boreal forests, which constitute about one-third of global forest cover (Spracklen *et al*., 2008). Boreal forests are affected by climatic and anthropogenic disturbances, such as increasing temperature and atmospheric nitrogen deposition (Gaige *et al.*, 2007; Magnani *et al*., 2007; Gundale *et al.*, 2013), which in turn are likely to affect BVOC emissions from forest trees. Until now, there have been no studies investigating the effects of increased NH_3 deposition on the BVOC emissions of Scots pine. Previous studies have shown that N deposition can result in increased above-ground biomass for some upland species (Leith *et al*., 2001; Leith *et al*., 1999). In addition, Lerdau *et al.* (1995) showed that higher nitrogen availability resulted in higher leaf monoterpene concentrations in Douglas fir, and therefore greater resultant fluxes. It is therefore reasonable to expect that there may be some resultant effects of N deposition on BVOC emissions of Scots pine.

1.2 Biogenic Volatile Organic Compound (BVOC) Emissions

1.2.1 What are BVOCs?

The biogenic organic compounds (BVOCs) are carbon-containing compounds emitted naturally into the atmosphere (Niinemets *et al*., 2004). They are emitted from natural sources, including plants, animals and anaerobic processes in bogs (Hester & Harrison, 1995). The majority of BVOC emissions occur due to production in various plant tissues and physiological processes, and are plant species specific. BVOCs are released from plant organs both above and below ground. Generally, the widest variety of BVOCs is

released by flowers and fruits, with emission rates peaking on maturation, while leaves have the highest level of mass emission rates (Laothawornkitkul *et al*., 2009). Grass species emit relatively large amounts of oxygenated BVOCs and some monoterpenes (Fukui & Doskey, 2000), whilst the vegetative parts of woody plants are more likely to release a diverse mixture of terpenoids, such as isoprene, monoterpenes and sesquiterpenes (Owen *et al*., 2001). In addition, damaged plants may emit increased amounts of these compounds (Laothawornkitkul *et al*., 2009). BVOC emissions are controlled or affected by many biotic and abiotic factors, for example temperature, photosynthetically active radiation (PAR) for some compounds, CO_2 concentration, drought, herbivory, oxidative stresses, availability of biochemical substrate and the synthase enzymes activity (Owen & Peñuelas, 2005).

Global BVOC emissions are estimated to be of the order of 1000 Tg Carbon annually, composed of 50 % isoprene, 15 % monoterpenes and 35 % other VOCs (Guenther *et al.*, 2012; Peñuelas & Llusià, 2003). However, wide variations are found at regional and local scales, and they are primarily associated with the location of forested areas (Simpson *et al*., 1999; Guenther, 1997). National emissions depend on the land use characteristics of different countries (Lindfors & Laurila, 2000). The main BVOC emitting regions in the UK are coniferous forests in Scotland (mainly isoprene and monoterpenes) and *Populus* (poplar) rich areas in eastern England (mainly isoprene) (Stewart *et al*., 2003). The annual biogenic monoterpenes and isoprene fluxes in Great Britain were estimated as 83 kt a^{-1} and 8 kt a^{-1} respectively for the model year 1998 (Stewart *et al*., 2003). Estimates of BVOC emissions are based on models considering biomass distribution, plant-specific emission factors, and algorithms describing these

emissions as a function of temperature, photosynthetic active radiation (PAR), and moisture (Guenther *et al*., 2012; Komenda *et al*., 2003).

BVOCs are diverse and include terpenoids, alkenes, alkanes, alcohols, esters, carbonyls and acids (Peñuelas & Llusià, 2003). An estimate of about 40 BVOCs emitted from vegetation can influence the atmospheric chemistry due to their high emission rates (Guenther *et al*., 2000). Table 1-1 shows the main classes of BVOCs, the major groups of BVOC-emitting plants and the estimates of current fluxes into the atmosphere.

Table 1-1: The major classes of BVOCs, major groups of BVOC-emitting plants and estimates of current fluxes into the atmosphere (Laothawornkitkul *et al*., 2009).

BVOC Species	Present estimated annual global emission (10^{12} g C)	Atmospheric Lifetime (day)	Example	Main emitting plants
Isoprene	412-601	0.2		*Populus, Salix, Platanus, Elaeis, Casuarina, Picea* and *Eucalyptus*
Monoterpene	33-480	0.1-0.2	α-pinene, β-pinene, δ^3-carene, α-phellandrene	*Lycopersicon, Quercus, Cistus, Malus, Pinus* and *Trichostema*
Other reactive BVOCs	-260	<1	Acetaldehyde, 2-methyl-3-buten-2-ol (MBO) and hexenal family	Grassland (mix of C3 plants), *Vitis*, *Brassica*, *Secale* and *Betula*
Other less reactive BVOCs	-260	>1	Methanol, ethanol, formic acid, acetic acid and acetone	Grassland (mix of C3 plants), *Vitis*, *Brassica*, *Secale* and *Betula*
Total	700-1000	0.2		

1.2.2 Why do plants produce and emit BVOCs?

The mechanisms of BVOCs emissions from plants have been studied for over two decades. Although the ecological reasons for plant BVOC emissions are still unclear, there is now a greater understanding of the fact that BVOCs synthesised within plant tissues play an important role in signalling, and form part of a plant's defensive and protective systems, in response to internal (genetic and biochemical) and external (ecological) factors, both abiotic and biotic (Hewitt *et al*., 2011). For example, isoprene, which is emitted immediately after production in the plant leaf, can protect the plants' photosynthetic apparatus from transient high-temperature damages, serving as an antioxidant in leaves (Sharkey *et al*., 2008). BVOCs can also attract pollinators and contribute to plant-plant interactions (Hansen *et al.*, 1999; Sharkey & Yeh, 2001). Monoterpenes are usually accumulated in specialised organs in leaves and stems, although in some species monoterpene emissions are also light dependent and are emitted instantaneously in the same way as isoprene (Staudt & Seufert, 1995; Street *et al*., 1997). Monoterpenes play a similar role to isoprene, as they are part of a plant's defence strategies against stress reactions, with additional functions in anti-herbivory and anti-pathogens, allelopathy and plant wound healing (Holopainen *et al*., 2013; Pichersky & Gershenzon, 2002).

1.2.3 What are the effects of BVOCs on the atmosphere?

BVOCs have been extensively studied over the last 30 years, not only because of their importance in plant physiology (Sharkey & Yeh, 2001), but also because they get involved in atmospheric gas-phase chemistry and particle formation (Janson *et al.*, 1999).

When emitted BVOCs enter into the atmosphere, they are subject to photochemical oxidation reactions, which influence the tropospheric concentration of hydroxyl radicals (Arneth *et al.*, 2011), therefore affecting the capacity of oxidation in the troposphere and the lifetime of methane (Atkinson, 2000, Figure 1-1; Laothawornkitkul *et al*., 2009). In combination with sufficient levels of concentrations of nitrogen oxide, BVOCs play a role in the chemistry of the production of tropospheric ozone and other photooxidants (Fehsenfeld *et al*., 1992), thus having an impact on regional ozone pollution (Komenda *et al*., 2003; Rinne *et al*., 2007).

BVOCs, particularly monoterpenes, also have the potential to contribute to the chemistry of the formation and growth of atmospheric aerosol particles (Kulmala *et al*., 2004; Tunved *et al*., 2006; Spracklen *et al*., 2008), by increasing the number and size of aerosol particles (Mentel *et al*., 2013). Aerosols both directly and indirectly influence on the radiation budget of the Earth via scattering, absorption of sunlight and through alteration of cloud properties (Ormeño & Fernandez, 2012; Rinne *et al*., 2007).

In addition, BVOC emissions may also have a significant impact on the carbon balance of the ecosystem, at an amount of 0.2–10 % of the assimilated carbon being re-emitted to the atmosphere, depending on, for example, the time of year, temperature or water availability (Guenther 2002, Sharkey *et al*, 1996). As a result of the significant source strength of BVOCs and the wide range of reactions that they cause in the troposphere, BVOCs have important effects on the atmospheric concentrations of many compounds, with implications not only for the climate, but also for our health.

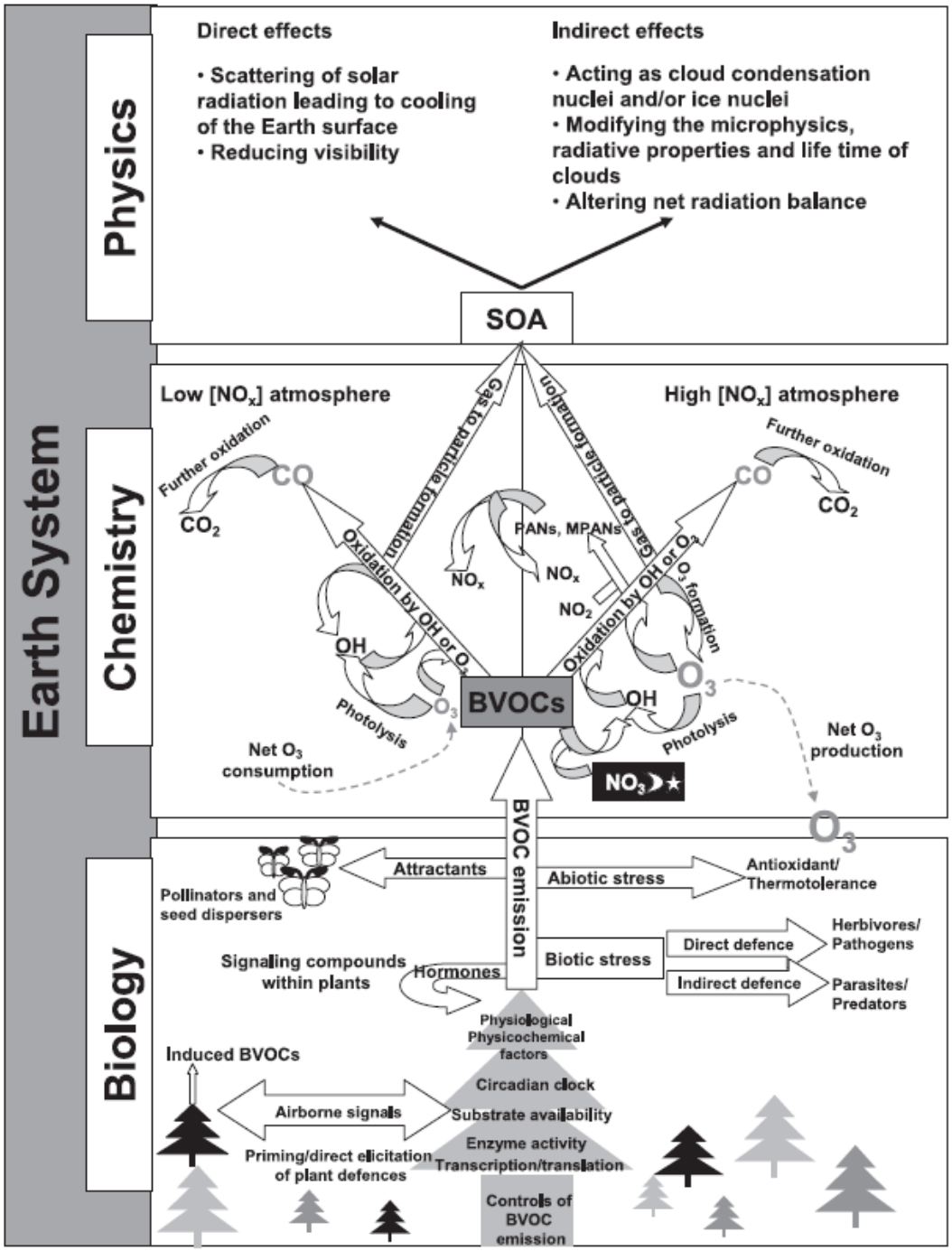

Figure 1-1: Schematic diagram summarising the current understanding of the roles of BVOCs in the Earth system. BVOCs exert their roles in the biological, chemical and physical components of the Earth system, providingW a connection between the biosphere and the atmosphere. The three compartments labelled biology, chemistry and physical processes occur in the atmosphere. (SOA = Secondary organic aerosol; = night time) (Laothawornkitkul *et al.*, 2009).

1.3 Nitrogen Deposition

Nitrogen pollution resulting from industrial and intensive agricultural practices have increased rapidly since the 19th century (Fowler *et al*., 2004), with global anthropogenic sources now almost double the natural sources (Galloway, 1998). NH_3 emissions are estimated to have at least doubled over the last century across Europe (DEFRA, 2002), concomitant with intensification of agriculture, and an increase in the use of nitrogen fertilisers. The dry and wet deposition of nitrogen takes two main forms, reduced (NH_x – ammonia and ammonium) and oxidised (NO_y – nitrogen oxides, nitric acid and particulate nitrate) (Stevens *et al*., 2011). Due to an increased demand for food, Galloway *et al*. (2004) predicted that by 2050, terrestrial NH_x deposits will have increased by 133 % on 1990s levels, while NO_y deposits will have increased by up to 70 % over the same period.

Ammonia is one of the major atmospheric pollutants that potentially deteriorates the ecosystems and contributes to health problems in humans. Ammonia is a colourless gas composed of nitrogen (N) and hydrogen (H), represented by thechemical symbol NH_3. This gas is released mainly as a result of naturally occurring processes, *i.e.* the breakdown of the urea excreted by farm livestock and other mammals, or the uric acid excreted by birds. Ammonia is highly soluble in water and readily reacts with other substances in the atmosphere to form ammonium (NH_4^+) compounds such as ammonium sulphate and ammonium nitrate. The life-time of ammonia in the atmosphere is relatively short (a few days), due to its active reaction with acidic gases to form particulates or is deposited back to ecosystems as either wet (via precipitation) or dry (as a gas or aerosol) deposition (Figure 1-2) (Stevens *et al*., 2011). Almost half

of all nitrogen deposition is dry deposition, which involves direct nitrogen particulate deposition and nitrogen gas assimilation by plants and soils (Asman *et al.*, 1998).

Because of the high reactivity of ammonia gas, it will mostly be deposited in close proximity to its origin of emission. However, atmospheric levels of ammonia may be greater in some areas, and they are known to travel long distances by wind before being deposited by rainfall (Krupa, 2003). Besides, ammonia also reacts easily with SO_2 and NO_x in the atmosphere to produce sulphuric acid and nitric acid within particles in the atmosphere (Figure 1-2). These particles can travel for hundreds of miles, thus ammonia emissions from one country may cause damage in another. This is the true in the case of acid rain, a natural phenomenon with problems that were first identified in the middle of the last century (*e.g.* Likens *et al*., 1972).

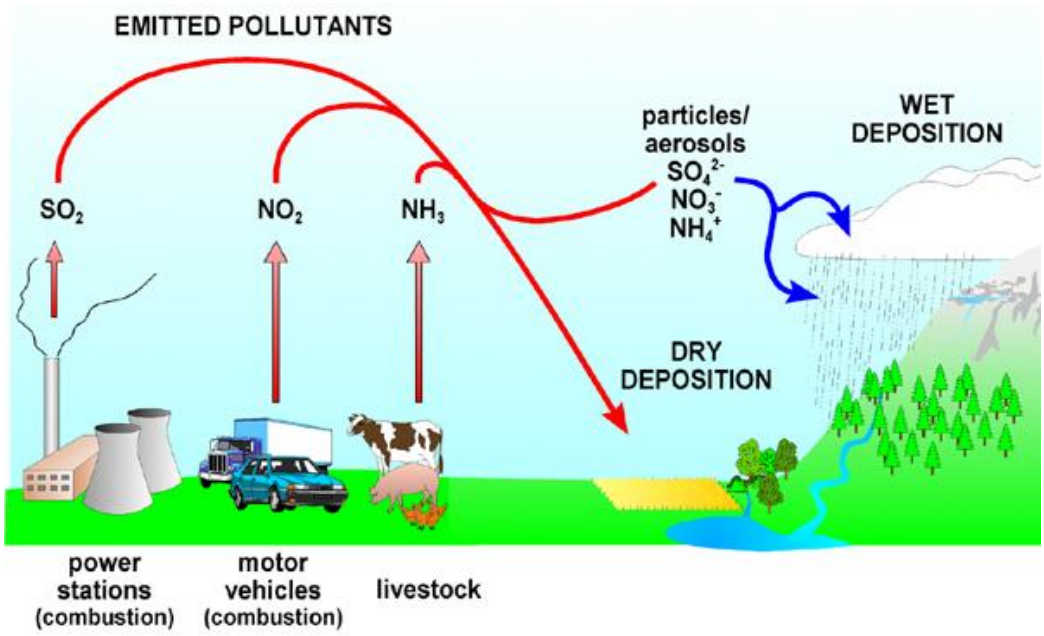

Figure 1-2: Schematic of the major sources and pathways of NH_3 and NO_x in the atmosphere (Sutton *et al.*, 2004). SO_2 and NO_x emissions result primarily from combustion sources, most NH_3 emissions are from agricultural activities.

1.3.1 Effects of N deposition

It is now recognised that nitrogen deposition can have profound effects on terrestrial ecosystems. The uptake of NH_3 by vegetation is mainly through the stomata, and is for most plant species, linearly related with the atmospheric NH_3 concentrations up to high exposure levels (Van der Eerden & Perez-Soba, 1992). In high concentrations (*e.g.* 600 μg m^{-3} for 24 hours), NH_3 can cause direct injury to sensitive plant species, disturb the acid-base regulation in plant cells, and inhibit photosynthesis (Van der Eerden & Perez-Soba, 1992). However, under most conditions, it can be converted by the plant through the glutamine/glutamate cycle and stored in the form of amino acids, or used to directly contribute to biomass production (Van der Eerden & Perez-Soba, 1992).

The deposition of ammonia from the atmosphere may damage plant communities that have evolved on nutrient-poor habitats, such as upland peat bogs and heathlands, by increasing the amount of NH_3 in the soil (Krupa, 2003). While this extra NH_3 may increase the growth of plants adapted to a limited NH_3 supply (such as heathers), other plants (such as rough grasses) can use the N more effectively (Jones *et al*., 2014). For example, in a detailed study conducted in close proximity to a poultry farm in southern Scotland, the number of plants adapted to low N (such as wood sorrel and many moss species) decreased close to the farm, while the number of species adapted to high NH_3 (such as rose bay willowherb and certain grasses) increased (Pitcairn *et al*., 1998). This enrichment of NH_3, also known as 'eutrophication', can overwhelm existing species that are not adapted to cope with an overabundance of NH_3, which means that they get replaced by fast-growing grass species and other species. This may have implications for biodiversity and the degradation of ecosystems.

It is also thought that ammonia may cause soil to become more acidic. Once deposited on soil, ammonia can be oxidised to nitrate by a chemical process that increases soil acidity (Krupa, 2003; Pearson *et al*., 1993). Some soils can neutralise limited amounts of acid, but they then reach a point where they are not able to cope with the increasing amount of acid deposited (Krupa, 2003). Once this level has been reached and soil acidity continues to increase, toxic elements such as aluminium will become more available to plants, whilst other elements vital for growth will become less available (Pearson & Stewart, 1993). Toxic elements may then be leached into surface waters, where they poison fish and other aquatic life. While acidification is largely caused by sulphur dioxide emitted from industry, N can also play a role. Nitrogen deposition is thought to be delaying the recovery of habitats, now that sulphur dioxide emissions have been reduced (Krupa, 2003).

Increased N deposition can also affect terrestrial ecosystems by increasing their net primary productivity (LeBauer & Treseder, 2008). Some experimental and observational studies have shown that the richness of plant species can be reduced by increasing N deposition in lichens, bryophytes and legumes (Bobbink & Lamers 2002). Nitrogen-induced changes in biomass production often result in changes in competitive ability (Van der Eerden & Perez-Soba, 1992). For example in boreal forests, increased N deposition affects boreal plant communities, by increasing the abundance of nitrophilous plants and decreasing the abundance of characteristic ground cover species (Allen *et al*., 2004). According to various experiments, such floristic changes can occur with nitrogen additions as low as 5 kg N ha^{-1} yr^{-1} (Allen *et al*., 2004). In Scotland, a survey over four wooded areas (including Scots pine plantation) near four

livestock farms (*i.e.* significant point source of nitrogen) showed a decrease in species diversity of ground flora within 300 m of the emission source (Pitcairn *et al*., 1998).

1.3.2 Wet and dry deposition in Scotland

1.3.2.1 Dry deposition

Dry deposition of N involves the direct input of atmospheric N gasses and aerosols into plants and soils via both the wind and gravity. It accounts for 40–50 % of total N deposition (Allen *et al*., 2004). Dry deposition of NH_3 shows high spatial variables, with the highest deposition rates occurring close to source of emissions, and decreasing rapidly with distance (Stevens *et al*., 2011). Gaseous NH_3 concentrations vary spatially, according to distance from the source, strength, type of source (*e.g.* poultry, pigs or cattle), meteorological conditions, wind direction and temperature, but it is highest in areas of intensive agriculture (Sheppard *et al*., 2011). Much larger concentrations have been reported immediately downwind of animal units; for example, average annual concentrations of 30-60 μg m^{-3} were measured about 15 m from chicken farms in southern Scotland. Therefore, the toxic effects of ammonia on vegetation in Scotland are likely to be of more concerns, close to large sources (Pitcairn *et al*., 1998).

Of the total UK NH_3 emissions of 290.3 kt in 2011, approximately 36 kt occurred in Scotland (Thistlethewaite *et al*., 2013). Large areas used for intensive agricultural activities showed significant emissions, air concentrations, and deposition of NH_3. As shown in Figure 1-3, around 86 % of ammonia emissions in Scotland are derived from agricultural activities, in particular from cattle and poultry industries (Sutton *et al*.,

2004). These emissions derive mainly from the decomposition of urea in animal wastes and uric acid in poultry wastes (Thistlethewaite *et al.*, 2013).

The largest source of NH_3 in the atmosphere in Scotland is animal husbandry, especially the decomposition and volatilisation of animal wastes (Figure 1-3; Sutton *et al.*, 2004). Other sources in Scotland include the application of NH_3-based fertiliser (particularly urea), and a wide range of non-agricultural sources such as sewage, vehicular emissions, volatilisation from soils and oceans, and industrial processes (about 15 %) (Sutton *et al.*, 2004).

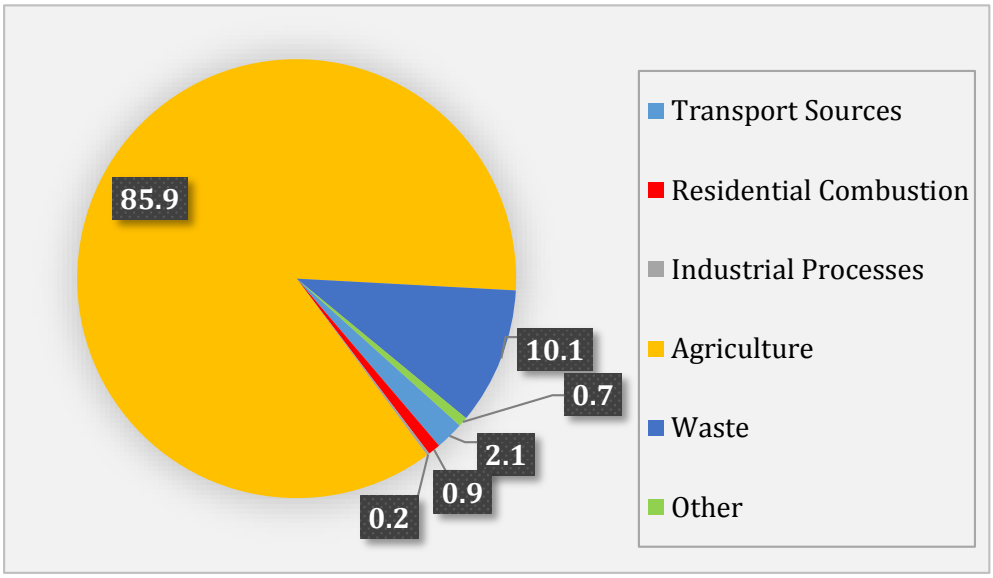

Figure 1-3. Sources of ammonia emissions in the Scotland in 2011 (Thistlethewaite *et al.*, 2013). 'Other' for ammonia includes emissions from energy industries, industrial combustion, fugitive and solvent processes. Ammonia emissions in Scotland have declined by 23 % since 1990 and account for 13 % of the UK total in 2011. Manure management represents 65 % of total ammonia emissions in 2011, which has declined by 11 % since 1990. Ammonia emissions in Scotland have increased in recent years, with a 7 % increase between 2008 and 2011 driven primarily by increasing emissions from composting and biogas production via anaerobic digestion (Thistlethewaite *et al.*, 2013).

1.3.2.2 Wet deposition

Wet deposition is the atmospheric input of dissolved nitrogen species in precipitation, primarily nitrate (NO_3^-) and ammonium (NH_4^+) which have transformed from emissions of nitrogen oxides and ammonia (Allen *et al*., 2004; Hornung *et al*., 1995). The cool, wet and windy climate of Scotland is ideal for the efficient removal of nitrogen pollutants from the atmosphere via rain. However, relative amounts of nitrate and ammonium in atmospheric N deposition vary according to local sources of pollution. Wet deposition of both NH_x and NO_y showed less spatial variability than dry deposition of NH_3, as a result of the diffuse and point source origins of these two forms of pollutant (Stevens *et al*., 2011).

1.4 Nitrogen Deposition Effects on BVOC Emissions

1.4.1 BVOCs from *Pinus sylvestris*

Generally, isoprene is emitted mainly by deciduous trees, and coniferous trees are mainly monoterpene emitters, although some plants are both isoprene and monoterpene emitters (*e.g.* Stika spruce), or do not emit at all (Hester & Harrison, 1995). In Scotland, isoprene is by far the most thoroughly investigated BVOCs due to its high emission rates from many different plant species, its predominance in many non-urban air masses, and its high reactivity (Wiedinmyer *et al*., 2004).

In Scotland, the most important and abundant conifers are Scots pine (*Pinus sylvestris*). It is a native species in Scotland, and is also known to be a monoterpene emitter (Jason, 1993; Kivimaenpaa *et al*., 2012). It is also an important species in boreal forests. Because of the major contribution of coniferous trees (including Scots pine) to boreal forest (Figure 1-4; Prentice & Leeman, 1990; Kellomaki *et al*., 2001), the most important BVOCs emitted by these forests are monoterpenes ($C_{10}H_{16}$), with the strength of emission depending on the tree species and varying according to temperature and light among other variables.

Figure 1-4: Boreal forests (dark grey) are an extensive ecosystem covering over 15 million km² of northern Siberia, North America and northern Europe and constituting about one-third of global forest cover (Spracklen *et al*., 2008).

1.4.1.1 Characteristics of monoterpenes

Monoterpenes are an important group of BVOCs, because of their abundance and frequent appearance in nature (Holopainen *et al*., 2013). The monoterpene family consists of a wide range of different $C_{10}H_{16}$ substances that are secondary products

emitted by a wide range of plants. Major monoterpenes include α-pinene, β-pinene, δ^3-carnene, limonene, camphene, *etc.* (Zhao *et al*., 2012). Figure 1-.5 shows the molecular structure of some of the major monoterpene compounds.

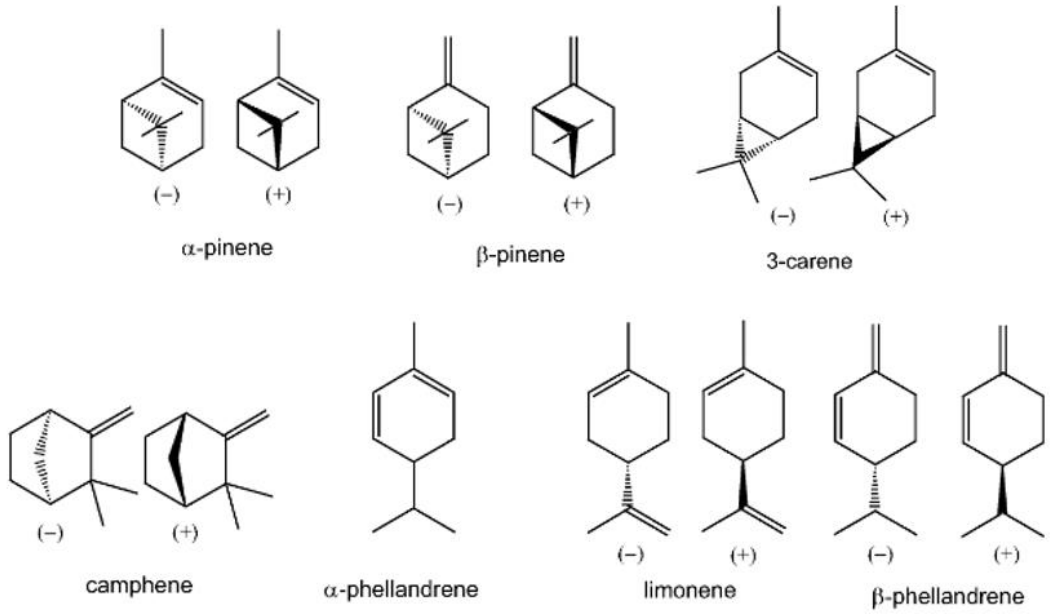

Figure 1-5: The molecular structures of some important monoterpenes (Persson, 2003).

Different monoterpene compounds have different volatilities, depending on vapour pressure within the tissue where the compound is synthesised or stored, which in turn is controlled by temperature and the compound concentration within the tissues (Lerdau *et al*., 1997).

1.4.1.2 Monoterpenes emissions from *Pinus sylvestris*

Monoterpene emissions from Scots pine are mainly from needles (Kivimaenpaa *et al*., 2012), although they also originate from branches, in which the xylem or phloem contains higher concentrations of stored monoterpenes than needles (Sallas *et al*., 2003). However, such emissions are mainly associated with physical damage of plant organs. Monoterpene emission of conifers has been found to increase after wounding, which breaks the intact storage structures within the tissue (Loreto *et al*., 2000). Moreover, monoterpene emissions can also be induced by herbivory. For example, *α*-pinene released by a wounded *Pinus sylvestris* acts as an attractant for large pine weevil (*Hylobius abietis*), so damage to a conifer can increase herbivory damage (Kask *et al*., 2013). In addition, other abiotic stresses can influence monoterpene emissions. According to Heiden *et al*. (1999), long-term low level (50 ppb) ozone exposure increases the emission of monoterpenes in Scots pine.

A very strong temperature-dependence of the monoterpene emissions from Scots pine was reported by Komenda & Koppmann (2002) and Tarvainen *et al*. (2005). The rate of monoterpene emissions was found to increase exponentially with temperature (Komenda & Koppmann, 2002), and the highest emissions were found in spring and early summer, the lowest in late summer and higher again towards autumn (Jason, 1993; Tarvainen *et al*., 2005). Hakola *et al*. (2006) found a similar pattern, and attributes the increase of emissions in autumn to the growth of new needles.

The monoterpene emission of conifers is generally regarded as being light-independent (Kesselmeier & Staudt, 1999), as it is stored, after synthesis, in in large pools located in resin ducts or glands (Lerdau *et al*., 1997). Thus, these emissions are associated with vapour pressure and transport resistance along the diffusion path (Simon *et al*., 1994; Kesselmeier & Staudt, 1999). However, several studies have shown that emitted monoterpenes may also originate from PAR-dependent biosynthesis in many plants, including Scots pine, which suggests that monoterpene emissions are a combination of stored and recently synthesised compounds (Staudt *et al*., 1997; Shao *et al*., 2001). In addition, Hester & Harrison (1995) showed that Stika spruce was found to slightly increase its monoterpene emissions alongside an increase in light intensity between 400 and 1000 μmol m^{-2} s^{-1}, whilst the average emission rates demonstrated by Simon *et al*. (1994) were found to be a linear function of light intensity for maritime pine (*Pinus pinaster*). These findings are supported by the positive light-dependency of *α*-pinene emissions from Norwegian spruce (*Picea abies*) (Steinbrecher, 1989).

Monoterpenes emissions of *Pinus sylvestris* have been observed in many studies, as is demonstrated in Table 1-2 (Hewitt & Street, 1992; Lindfors & Laurila, 2000; Schurgers *et al*., 2009; *etc*). In Finland, observed that BVOC emissions from Scots pines vary between 21 and 1670 ng g (leaf biomass)$^{-1}$ h^{-1} (about 40 % are monoterpene emissions) (Tarvainen *et al*., 2005). The major emission species from Scots pine were reported to be *α*-pinene and δ^3-carene (Figure 1-6) (Komenda & Koppmann, 2002; Rinne *et al*., 2000).

Table 1-2: Comparison of monoterpene emission rates from *Pinus sylvestris*.

Emission Rate, µg g(dw)$^{-1}$ h^{-1}	Reference
12.1[b] (7.7)[a]	*Isidorov et al.* [1985]
0.8[c] (1.3)[a]	*Jason* [1993]
6[b] (3.8)[a]	*Staudt* [1997]
0.06 – 0.64[d] (young pines)	*Komenda & Koppmann* [2002]
0.24 – 3.7[d] (mature pines)	*Komenda & Koppmann* [2002]
1.16[d]	*Tarvainen et al.* [2005]

[a] Numbers in parenthese were calculated for a temperature of 25°C.
[b] Values are normalised to 30°C.
[c] Values are normalised to 20°C.
[d] Values are normalised to 25°C.

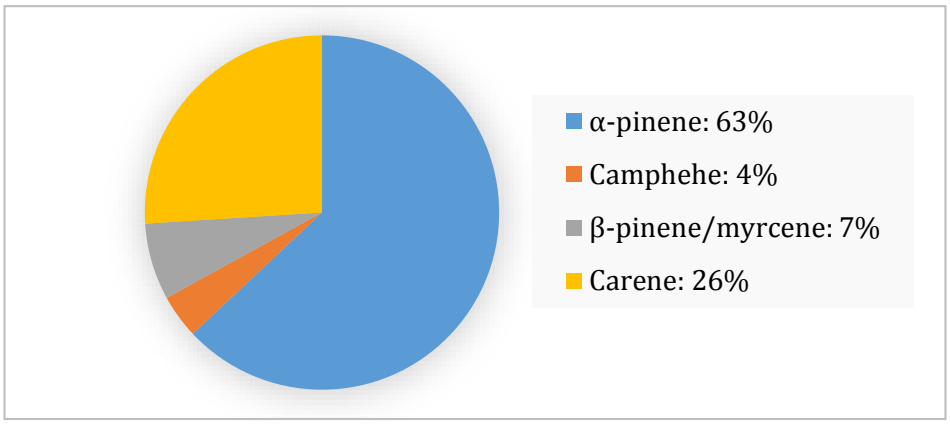

Figure 1-6: Species profiles of monoterpene fluxes (Rinne *et al.*, 2000). α-pinene and δ^3-carene were the most important species in the total monoterpene flux in Finland.

1.4.1.3 Other BVOC emissions from *Pinus sylvestris*

Isoprene emissions were detected from Scots pine in central and northern Finland, but they were too low to be quantified (Steinbrecher *et al.*, 1999). Jason & DeServes (1999) also observed isoprene emissions in Finland and Sweden, but they were lower than 25 ng (C) g^{-1} h^{-1}.

Sesquiterpenes, the largest and most diverse class of terpenoids (Holopainen *et al*., 2013), have been detected in the emissions of *Pinus sylvestris*. About 0.01–6.9 % of total BVOCs emitted from *Pinus sylvestris* are sesquiterpenes (Bäck *et al*., 2012). Sesquiterpenes are emitted from storage pools and can be important sinks for oxidants and precursors to aerosols in rural regions (Wiedinmyer *et al*., 2004). Because sesquiterpenes have atmospheric lifetimes of only a few minutes, due to rapid reaction with O_3 (Tarvainen *et al*., 2005), they are difficult to study, and little is known about their emission rates (Wiedinmyer *et al*., 2004). Hakola *et al*. (2006) investigated sesquiterpene emissions from *Pinus sylvestris* in Finland, and shows that they occur mainly in the middle of summer, with the most abundant sesquiterpene being *β*-caryophyllene. The study hinted at the defensive role of sesquiterpene emissions, due to a high correlation between maximum emissions and the maximum concentration of pathogen spores.

Sesquiterpenes react readily with atmospheric ozone, and they have a high potential to form secondary organic aerosols (SOAs) (Tarvainen *et al*., 2005). This reactivity with ozone could lead to the formation of new atmospheric particles (Bonn & Moortgat, 2003). The growth of these particles is affected by the oxidation products of sesquiterpenes (Kulmala *et al*., 2004).

1.4.2 Effects of NH_3 deposition

1.4.2.1 Effects of N deposition on BVOC Emissions

Enhanced NH_3 deposition has been shown to have an impact on BVOC emissions from vegetations. Wang *et al*. (2012) showed that the addition of nitrogen significantly decreases the quantity of monoterpene emissions in semi-arid grasslands in China, due to reduced coverage of *Artemisia frigida.* However, very few studies have investigated the effects of NH_3 on BVOC emissions from *Pinus sylvestris.* In a field study that aimed to identify the source of new particle formation, Jason *et al*. (2001) found no effects of increased NH_3 concentration during nucleation events on emissions from *Pinus sylvestris*. Judzentiene *et al*. (2007) found evidence of change in the monoterpene content of *Pinus sylvestris* needles in nitrogen pollution gradients, but how this might affect emissions is as yet unknown. Although no studies investigate the effect of increased NH_3 deposition on BVOC emissions for *Pinus sylvestris*, previous studies have shown that N deposition (dry NH_3, wet NH_4Cl and wet NH_4NO_3) onto upland species can result in increased above-ground biomass for some herb and grass species, such as *Eriophorum vaginatun, Erica cinerea* and *Nardus stricta* (Leith *et al*., 1999; Leith *et al*., 2001). In addition, Lerdau *et al*. (1995) showed that higher nitrogen treatment can result in higher leaf monoterpene concentrations on Douglas fir, and greater resultant fluxes have been found, although this is a study on the effect of N availability (and not pollutant N) on BVOC emissions. It is therefore reasonable to expect there may be some effects of N deposition on the BVOC emissions of *Pinus sylvestris*.

1.4.2.2 Research gaps addressed in this study

To date, there are no studies on the effect of dry NH_3 deposition on BVOC emissions from Scots pine. This study will focus on monoterpene emissions from *Pinus sylvestris*, and will concentrate on short-term monoterpene emissions, even though they show large seasonal variations (Tarvainen *et al*., 2005). This study aims to investigate the effects of dry NH_3 deposition treatments on the exchange of BVOCs between *Pinus sylvestris* and the atmosphere. Furthermore, the physiology and carbon exchanges of *Pinus sylvestris* will be examined. The measurement of the emissions BVOC from *Pinus sylvestris* shoots, leaf temperature and photosynthetic rates, both before and after NH_3 treatments, will be taken in the laboratory at CEH. The rate of photosynthesis and BVOC emissions for *Pinus sylvestris* are hypothesised to be reduced in line with high NH_3 pollution treatments. The questions explored include:

(a). Are there differences in the monoterpene emissions and growth rates of Scots pine trees from different genetic stock?

(b). Do short-term simulations of dry NH_3 deposition affect photosynthesis and monoterpene emissions from Scots pine?

(c). Does historic ambient temperature affect emissions?

CHAPTER 2

METHODOLOGY APPROACH

To investigate the effects of dry NH_3 deposition on BVOC emissions from *Pinus sylvestris* (Scots pine), four distinct genotypes were selected. The BVOC flux and biomass measurements, NH_3 treatment method development and NH_3 measurement, and analysis of BVOC emissions from Scots pine (*Pinus sylvestris*) are described and evaluated in this chapter. Sampling and analyses of BVOCs emitted by Scots pine and NH_3 treatment experiments were conducted in the laboratory at Centre for Ecology and Hydrology (CEH) in Edinburgh. Leaf-level measurements and BVOC samples were taken from 7th May 2014 to 11th July 2014. Analysis of BVOC samples taken from Scots pine (*Pinus sylvestris*) was undertaken using a gas chromatography mass spectrometry (GC-MS) with automated thermal desorption (ATD) as the injection system.

2.1. Study Species - *Pinus sylvestris*

A BVOC emission screening study was conducted on four native populations of *Pinus sylvestris*. The tree saplings had been grown from seed collected from four locations in Scotland (see map in Figure 2-1 and Table 2-1; Salmela *et al*., 2013), which were geographically distinct (North, South, East, West: upland and lowland). Preceding this study, pine cones had been collected, and the extracted open-pollinated seeds were established in a glasshouse-based common-garden at CEH in 2007. After germination,

the seedlings were transferred to pots and preserved in natural light conditions with applied watering. The tree saplings were 7 years old at the time of this experiment. The trees selected for this investigation (Table 2-2) were kept in natural ambient conditions in the grounds of CEH. Four months before the experiment started, the saplings were re-potted and left to acclimatise to their new pots. Before and between the individual experiments the plants remained outside under ambient conditions.

Figure 2-1: Map of sampled native *Pinus sylvestris* populations (Salmela *et al.*, 2013) and the location of CEH (55.86° N, 3.21° W) in Scotland.

Table 2-1: Site details for the populations of *Pinus sylvestris* in Scotland included in this study (Salmela *et al.,* 2013). Their latitude (Lat.), longitude (Long.), altitudinal range sampled (Alt.), core pinewood area and mean (1961–2000) calculated climate features: growing season length (GSL; days), growing degree days (GDD: day degrees), and February and July mean temperatures (FMT and JMT).

Site	Lat.	Long.	Alt. (m)	Area (ha)	GSL (d)	GDD (d)	FMT (˚C)	JMT (˚C)
Abernethy (AB) - E	57.21	3.61	311-370	2452	211	990	1.15	12.73
Cona Glen (CG) - W	56.79	5.33	89-193	189	246	887	2.20	11.73
Crannach (CR) - S	56.58	4.68	258-338	70	231	1019	1.81	12.62
Glen Einig (GE) - N	57.96	4.76	45-92	27	242	1089	2.19	13.15

Table 2-2: Populations of *Pinus sylvestris* in Scotland included in this study. Site name, cone code and number of trees (45 trees in total). Cone code is the code of the cone that the tree had been grown from, and they were used to identify the origin and number of each tree sapling. They were derived from Salmela *et al.* (2013), who described the first work on these trees.

Site	Cone Code	Number of trees
Abernethy (AB) East	AB1	1
	AB2	1
	AB3	2
	AB4	1
	AB5	2
	AB6	2
	AB7	1
	AB10	2
Cona Glen (CG) West	CG1	1
	CG3	3
	CG4	1
	CG6	3
	CG8	1
	CG13	2
	CG14	1
Crannach (CR) South	CR1	1
	CR2	2
	CR3	1
	CR4	1
	CR6	1
	CR7	2
	CR8	2
	CR10	1
Glen Einig (GE) North	GE1	1
	GE2	1
	GE3	1
	GE6	1
	GE7	2
	GE8	3
	GE9	1

Two shoots from each tree were selected according to the dimension of the Plant Leaf Chamber (PLC) (*i.e.* approx. 7 cm Length x 3 cm Width). Each shoot was labelled using white Teflon tape, and each shoot was marked with either a black or blue colour on the Teflon tape, as shown in Figure 2-2. Black-marking shoots were later used as control shoots in the NH_3-treatment experiment, while blue-marking shoots were used as NH_3 treatment shoots.

Figure 2-2: Pictures of labelling and marking tree shoots. Left: black-marking shoot; Right: blue-marking shoot.

2.2. Ammonia Treatment

Carfrae *et al*. (2004) suggested that very high concentrations of NH_3 might have biological significance, and that it is unclear whether damage of vegetation is caused by high concentrations for a small amount of time or by accumulated NH_3 deposition. The NH_3 treatment experiment here aimed to investigate whether the short-term

simulations of dry NH_3 deposition can affect photosynthesis and monoterpene emissions from Scots pine.

We aimed to achieve a low-dosing and a high-dosing strategy, based on information from the literature: (1) Leith *et al*. (2005) and Skiba *et al*. (2006), who reported concentrations of NH_3 from intensive livestock units of ~100 μg m^{-3}; and (2) Health Protection Agency (2011), which suggested a long-term exposure limit for NH_3 in the UK is 17382 μg m^{-3}. However, treatment trials (described below) showed it was impossible to achieve and quantify a precise NH_3 dose within the enclosure bags. We therefore aimed for a binary experiment: 'NH_3 treatment' and 'No NH_3 treatment'.

2.2.1 Conditioning materials used for NH_3 treatment

Ammonia is an extremely reactive gas, and significant losses of NH_3 can be expected to the walls of Tedlar® bags that were used to collect NH_3 (Huebner *et al.*, 2004), the syringe that was used to inject the NH_3 into the PET bags, and the PET (*Polyethylene terephthalate*) enclosure bags that were used to enclose and treat the shoots. Therefore, before the treatment was undertaken, a conditioning process was devised for the Tedlar® bags, for the PET bags, and for the syringe. This was an attempt to saturate the materials of the bags and syringe with NH_3 and therefore reduce losses when NH_3 treatment was applied.

A Tedlar® bag was first conditioned by repeatedly filling and discharging with NH_3, followed by overnight storage filled with NH_3. This was to saturate the Tedlar® bag walls with NH_3. The Tedlar® bag was then re-filled with NH_3 gas from the gas cylinder and the syringe to be used for administering the NH_3 treatment was filled and emptied several times from the bag. The syringe was then filled with NH_3 and left overnight in the fume cupboard to saturate the NH_3-receptor sites on the syringe walls. Finally, three PET bags to be used during treatment were filled with NH_3 from the gas cylinder, sealed with wire, and left in the fume cupboard overnight to saturate the PET bag walls with NH_3.

2.2.2 ALPHA sampler

The CEH ALPHA (Adapted Low-cost Passive High Absorption) passive sampler was used to measure NH_3 concentration in the PET bags. A detailed description of ALPHA sampler set-up and its performance is given in Tang *et al*. (2001). In short, an ALPHA sampler consists of a polyethylene vial, 26 mm high and with an outer diameter of 27 mm. One end contains a 27 mm diameter PTFE membrane, through which gaseous NH_3 diffuses and is adsorbed onto a citric acid-coated collection filter located at the other end of the diffusion path (Figure 2-3). The membrane prevents particle collection and thus the NH_3 concentration is not biased high by collection of ammonium aerosol (Puchalski *et al*., 2011). The membrane also serves to establish a turbulence-free diffusion path between the membrane and the collection filter, thus avoiding 'wind-shortening' of the diffusion path (Puchalski *et al*., 2011).

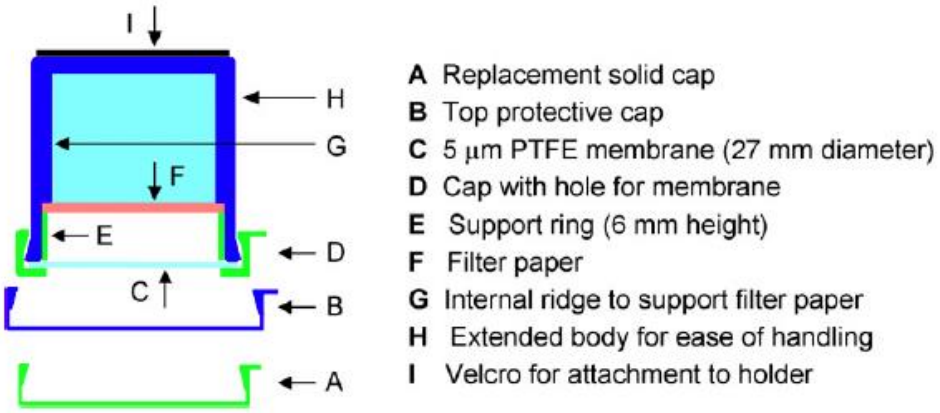

Figure 2-3: Outline diagram of a single CEH ALPHA sampler (Tang *et al.*, 2001).

The ALPHA sampler is sensitive enough to resolve low concentrations (<1 μg m^{-3} NH_3) in background areas (Tang *et al*., 2001). However, Tang *et al*. (2001) recognised that errors occurring in passive sampling of NH_3 are often due to contamination artefacts, which can be minimised by careful handling of the samplers.

2.2.3 AMFIA analysis of ALPHA samplers

AMFIA (AMmonia Flow Injection Analysis) system was used to analyse ALPHA samplers. This analysis system is based on selective dialysis of ammonium across a membrane at high pH with subsequent analysis of conductivity. Calibration standard solutions (*i.e*. 0.1 ppm, 1 ppm, and 10 ppm) and QC solutions (*i.e*. 0.2 ppm, 0.9 ppm, 2 ppm, and 9 ppm) were made for a calibration run in advance of the sampler run. For the sample run, the acid coated filter papers inside the ALPHA samplers were taken out and placed over a 20 ml polystyrene pot. 3 ml of deionised water was added to the filter paper in the pot, and the pot was capped with a clean cap and labelled. The ammonium was extracted after one hour, and the extract for each sample was analysed by AMFIA

system. The ALPHA sampler uptakes NH_3 at a rate of 4.34 x 10^{-3} m^3 h^{-1} (Tang *et al*., 2001). The air concentration of NH_3 was calculated by equation (a):

$$\text{Air concentration of } NH_3 = \frac{(m_e\text{-}m_b)}{(R \times t)} \qquad \text{(a)}$$

Where m_e is the amount of a pollutant collected on an exposed sample (μg), m_b is the amount of a pollutant in the blank sample (μg), R is the uptake rate of the ALPHA sampler (4.34 x 10^{-3} m^3 h^{-1}), and t is the exposure duration (h).

2.2.4 Method development of NH_3 treatment

A treatment dosing test trial was carried out before the actual dosing experiment. An NH_3-conditioned Tedlar® bags was filled with NH_3 gas from the gas cylinder, and three NH_3-conditioned PET bags (Koziel *et al*, 2005) were injected with NH_3 gas from the Tedlar® bag using the NH_3-conditioned syringe. Two ALPHA samplers were placed in each PET bag to test the reliability and efficacy of the method. Each bag's samplers were removed, stored and replaced in the bag each day. Two ALPHA samplers were placed in bag 1 which contained NH_3 for 2 hours each day for four consecutive days. A further two ALPHA samplers were placed in bag 2 which contained NH_3 for 8 hours each day for four consecutive days, and two ALPHA samplers were placed in Bag 3 which was used to test for 24 hours for four consecutive days. The ALPHA samplers were removed between consecutive days' dosing, and kept in clean sealed plastic bags until the next day's dosing. Thus the samplers were measuring the cumulative dose over four days. The resulting NH_3 concentrations detected by the samplers (Table 2-3) were calculated using mean value of two ALPHA samplers placed in each bag. Bag 1 showed a high

concentration of NH_3 could be due to contaminations (*i.e.* not wearing gloves when the ALPHA samplers were put into the bag 1 on the first day). Each of the two samplers in each bag showed similar results, indicating that the method using the ALPHA samplers was reliable.

Table 2-3: Results of the NH_3 dosing trial.

Bag number	Date out	Time out	Date in	Time in	Exposure Time (h)	NH_3 ($\mu g\ m^{-3}$)
1	12/06/14	12:15	15/06/14	09:46	8.8	33.27
2	12/06/14	12:15	15/06/14	10:05	39.1	8.30
3	12/06/14	12:15	15/06/14	10:05	97.8	9.05

Due to time constraints, only two of the four Scots pine tree groups were selected for the NH_3 dosing experiment. Trees from Abernethy (AB) and Cona Glen (CG) provenances were chosen to carry out the NH_3 treatment experiment. Three trees from the AB group and three trees from the CG group were selected for the initial two weeks' low dosing experiment. Another three trees from the AB group and three trees from the CG group were selected for the subsequent high dosing experiment.

NH_3 treatment experiments were carried out using a PET bag-enclosure method (shown in Figure 2-4). The PET bags provided approximately a volume of 2.5 l to enclose the treatment (blue-marking) shoot, which was accompanied by an ALPHA sampler placed inside the bag in order to measure NH_3 concentration. Another 2.5 l

PET bag without NH_3-conditioning enclosed the control (black-marking) shoot, also accompanied by an ALPHA sampler placed inside.

From the trials, we assumed that a treatment regime of a 25 ml injection of 14601 µg m^{-3} into a PET treatment bag for two hours on four consecutive days would suffice for the low-dose treatment, and 25 ml of ambient air (from the lab) was injected into the control bags using a control syringe.

For the high dosing experiment, three NH_3-conditioned PET bags (5 l) were filled with 14601 µg m^{-3} NH3 gas straight from the gas cylinder. In the laboratory, the NH_3-filled PET bags were used to enclose on the treatment (blue-marking) shoots with an ALPHA sampler in each bag. To attempt to compensate for the loss of NH_3 from the PET bags when enclosing the sample shoot, once the shoot had been installed, 3 x 60 ml of NH_3 gas were injected into the treatment bags using an NH_3-conditioned syringe. For the control shoots, the same method was used as for the low-dosing treatment. Black-marked control shoots were enclosed in a PET bag inflated with ambient air, and 3 x 60 ml of ambient laboratory air was injected. Both the control and the treatment enclosure were left undisturbed for two hours in the laboratory.

The shoots were then removed from the PET bags, and the ALPHA samplers were placed in separate clean bags to be used again for the next day's treatment process. Both NH_3 treatment and control treatment were applied to the same shoots, in the same manner for four consecutive days. BVOC sampling was undertaken one day after each treatment, for four consecutive days.

Figure 2-4: Control and treatment enclosure using PET bags.

2.3. BVOC Sampling

To investigate possible variances between genetic provenances, each tree listed in Table 2-2 was sampled for BVOC emissions, and CO_2 exchange was also measured. To do this, an ADC LCpro (ADC Bioscientific Ltd., UK) leaf gas exchange cuvette was used. The ADC LCpro leaf cuvette is a portable photosynthesis system, which can control and measure water vapour (H_2O) from the leaf surfaces by two high quality humidity sensors and carbon dioxide (CO_2) exchange from the leaf surfaces by an infrared gas analyser (IRGA). The system (Figure 2-5, a & b) also measures leaf and chamber air temperature, PAR (Photosynthetically Active Radiation), atmospheric pressure and the rate of airflow. It was modified for sampling BVOC emissions by introducing a sampling

port into the gas outlet line projecting from the cuvette. The LCpro is a dynamic system with a continuous flow of inflow air via the cuvette head. It operates as an 'Open System'. This system allows precise leaf-level gas exchange of CO_2 & H_2O to be measured and is also suitable for BVOC emission screening of plant species under controlled conditions.

The leaf cuvette had been serviced and fine-tuned before the measurement campaign, to ensure that it was in optimal working order. Ambient air from outside the building was pumped through the leaf cuvette at a flow rate of 300 µmol s^{-1} so that ambient concentrations of H_2O and CO_2 entering the leaf chamber reached a stable equilibrium with the gas exchange of the enclosed plant material within the chamber. The ADC system was fitted with a charcoal filter (Figure 2-5, c) on the inlet line to expunge ambient BVOCs and ozone (O_3) from the ambient inflow air. This had the effect of elevating the ambient CO_2 concentrations by about 50 ppm on average in the LCpro, but this was fairly consistent for all emission samples (Misztal *el al.*, 2010).

The leaf cuvette was installed on a tree shoot and left to equilibrate for a minimum of 30 minutes, at a temperature of 25˚C and a light level (PAR) of 1000 µmol m^{-2} s^{-1}. Thus, all emissions were measured at a standard temperature and PAR (Guenther *et al.*, 1995). Thirty minutes is generally considered sufficient time to allow the BVOC emissions to equilibrate after the installation of the leaf cuvette (Greenberg *et al.*, 2003), and even if equilibration was not achieved, all shoots were treated in the same way. Setting the cuvette at a temperature more representative of the natural Scottish environment, *i.e.* lower than 25 ˚C, would not always result in the temperature reaching the target value, as the cooling capacity of the ADC is very limited. CO_2 and humidity

were both set to ambient conditions during the equilibration. An absorbent tube filled with 200 g Tenax and 100 mg Carbotrap was fitted to the cuvette outlet port (Figure 2-5, e), and cuvette air containing BVOC gas emissions were drawn through the tube at a flow rate of 200ml min^{-1} using a 'Pocket Pump 210-1002MTX' air sampling pump (Figure 2-5, f). BVOC samples were taken for 12.5 minutes, yielding samples of 2.5 l in the adsorbent tube. In addition, a 'Blank' sample was also obtained at the beginning of most sampling days from an empty leaf cuvette (*i.e.* without shoot in the leaf chamber). This was undertaken in exactly the same manner used in leaf emission sampling, to give background blank values, which were subsequently subtracted from emission samples. After BVOC sampling, the adsorbent tubes were stored in a cold room at a temperature of 5˚C until the GC-MS analysis was carried out. No significant deterioration of samples occurs in sample tubes stored in this way over a period of four to six weeks (Schrader *et al*., 2001).

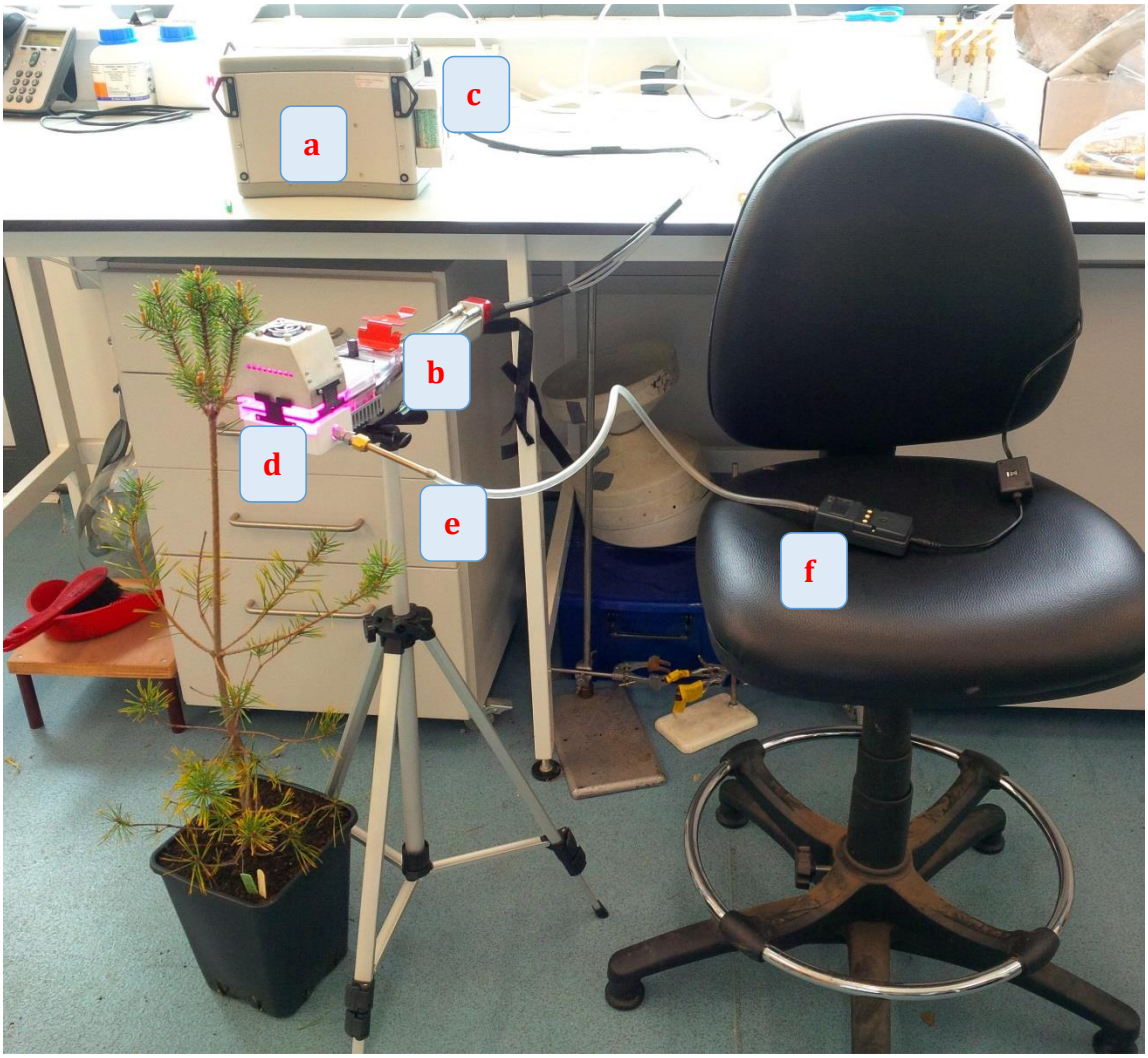

Figure 2-5: Experimental set-up of BVOCs sampling by ADC LCpro leaf cuvette. (a). ADC; (b). Leaf cuvette; (c). Charcoal filter; (d). Pine shoot enclosed in the chamber of leaf cuvette; (e). Adsorbent tube; (f). Air sampling pump.

2.4. GC-MS Analysis

BVOC samples were analysed using a Perkin Elmer automatic thermodesorption device, Turbomatrix™ ATD (Automated Thermal Desorption), coupled with a gas chromatograph-mass spectrometer (Perkin Elmer GC-MS). The carrier gas used was Helium (He). Compounds were desorbed at 280˚C for 6 minutes onto a Tenax-TA cold trap, which was maintained at -30 ˚C for 5 minutes. Secondary desorption was at 280˚C

for 5 minutes onto the GC column. The GC column was held at 35 ˚C for 2 minutes, then heated to 160 ˚C at 4 ˚C min^{-1}, followed by a final heating to 300 ˚C at 45 ˚C min^{-1}. The temperature was held at 300 ˚C for 10 minutes. The total run-time was 85 minutes for one sample. The level of analytical precision was around 5 % for monoterpenes (Misztal *et al*., 2010). The GC-MS instrument was tuned before analysing a sample to assure the quality of detection.

Liquid standards were injected into clean sample tubes in a flow of helium. Monoterpene quantification was achieved by injecting and analysing 3 µl of 10 ng $µl^{-1}$ or 5 µl of 5 ng $µl^{-1}$ mixed monoterpene liquid standards in methanol onto adsorbent tubes. The injected monoterpene standards were purged with helium gas for 2-3 minutes to evaporate off the methanol.

A standard calibration confirmed linear response across the expected range of sample concentrations. Table 2-4 summarises the calibration of response factors across the entire period of experiment. In each analysis, two standards were run at the start of each batch of analysis, and one or two standards were included every five samples for quantification.

Table 2-4: Summary of calibration and response factors for three monoterpene compounds.

	Date of Calibration	Method of Response Factor (RF) Value	α-pinene RF (ng area^{-1})	β-pinene RF (ng area$_{-1}$)	δ^3-carene RF (ng area^{-1})
Pre-treatment Calibration	2/6/14	slope of calibration graph	76.34	83.33	76.92
	6/6/14	slope of calibration graph	68.69	62.81	69.21
	6/6/14	slope of calibration graph	9.97	14.88	8.98
	12/6/14	slope of calibration graph	70.92	144.93	97.09
	13/6/14	mean of 6 5 µl injections	74.90	93.60	89.02
	16/6/14	slope of calibration graph	153.00	96.00	126.00
	23/6/14	slope of calibration graph	82.50	93.40	85.02
	24/6/14	mean of 10 5 µl injections	80.75	97.20	94.94
	26/6/14	mean of 10 5 µl injections	74.70	83.00	84.81
	27/6/14	mean of 10 5 µl injections	87.99	97.95	101.67
Post-treatment Calibration	4/7/14	mean of 6 5 µl injections	90.49	99.88	106.88
	7/7/14	mean of 10 5 µl injections	126.71	141.17	148.25
	9/7/14	mean of 10 5 µl injections	142.31	156.77	165.21
	11/7/14	mean of 8 5 µl injections	134.78	148.56	159.28

The determination of BVOC emitted from vegetation relies on the accuracy and precision of the analytical methods employed for this purpose (Larsen *et al*., 1997). During the analyses, the GC-MS-ATD transfer line fractured. Until the problem was identified and repaired, the response factors from monoterpene standards showed a high variability over several days' calibration trials. Repeated calibration and running a QA (quality assurance) standard for every five samples minimised the effect of these problems on analytical results, however uncertainty in the subsequent emissions calculations was higher than usual.

The identification of the compounds in the chromatogram was achieved by comparing relative retention times (Figure 2-6, Table 2-5) and mass spectra (Figure 2-7) of samples with the existing mass spectral library. Retention time (time elapsed between

injection and elution) is the amount of time that a compound is retained in the GC column. It aids in differentiating between compounds. Peak areas for base ions, m/e = 93 ion (where m = mass, and e = ion charge), were used for the quantification of monoterpene compounds. The average response of the 93 ion for α-pinene, β-pinene and δ^3-carene in monoterpene standards was used to quantify the peaks in each sample.

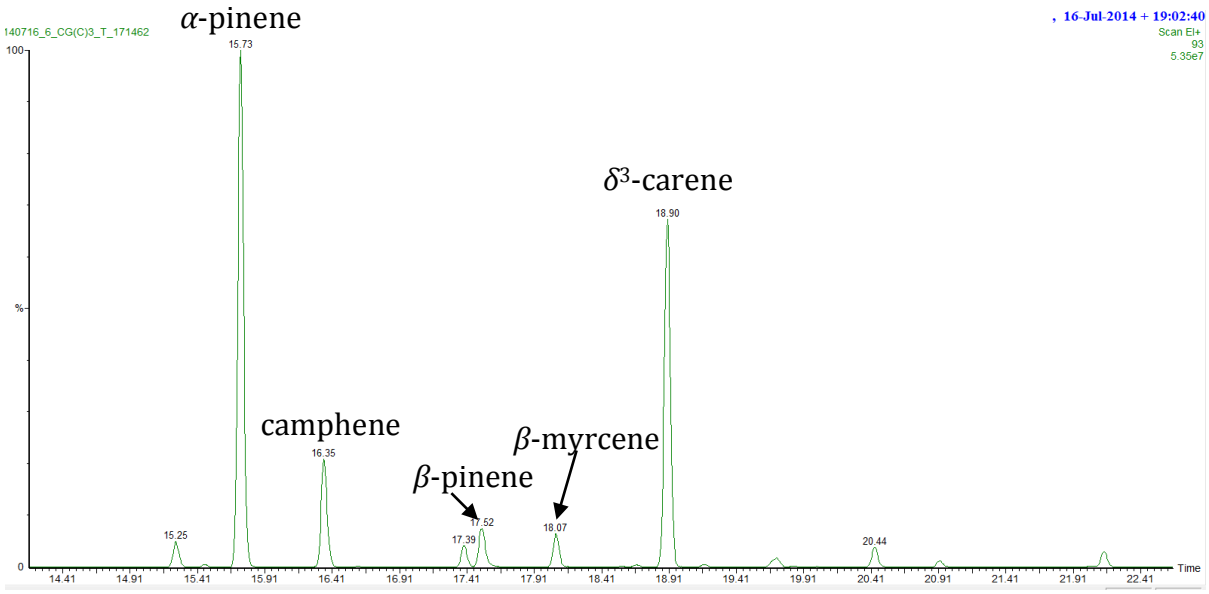

Figure 2-6: A chromatogram of peak m/e = 93 from an air sample taken by leaf cuvette from a pine shoot, showing the retention time for each compound identified by GC-MS.

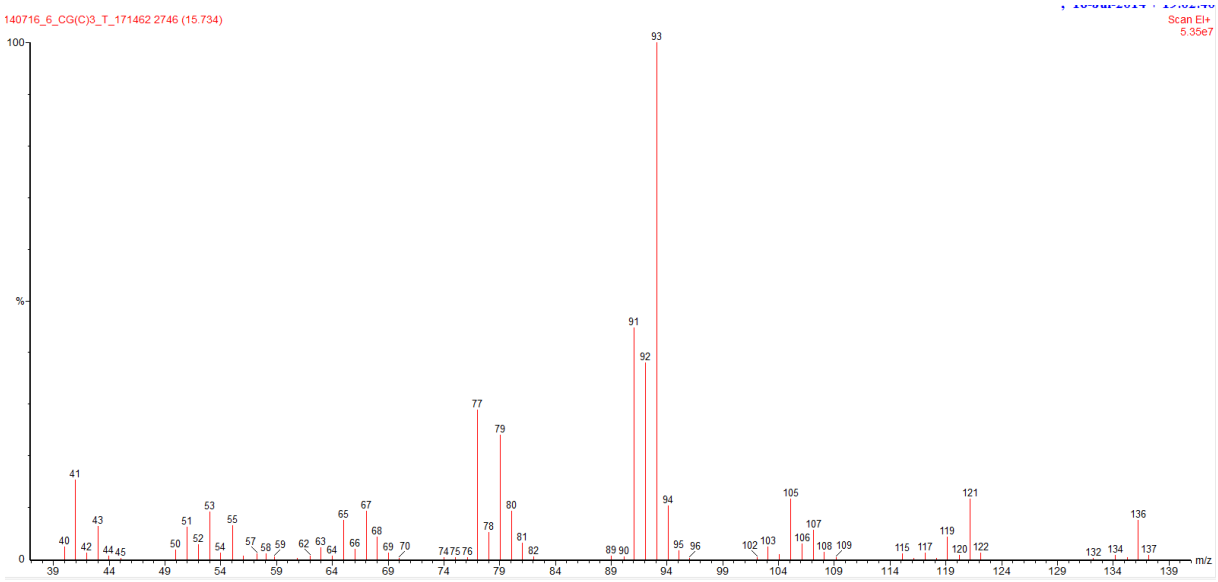

Figure 2-7: Example of mass spectra graph for the compound *α*-pinene. Main ions for *α*-pinene are 93, 91, 92, 77 and 79.

Table 2-5: Retention time (min) for each compound.

Compound	Retention Time (min)
***α*-pinene**	15.72
camphene	16.25
***β*-pinene**	17.51
***β*-mycrene**	18.05
***α*-phellandrene**	18.66
***δ*^{3}-carene**	18.88
Limonene	19.67
***β*-phellandrene**	19.68
Eucalyptol	19.81

2.5. Calculations

2.5.1 Shoot growth and biomass

Three measurements of shoot length were recorded throughout the experiment. Initial measurements were taken on the day of the first BVOC sampling (on 7th May 2014); the second set of measurements were noted on 30th May 2014; and the third set of measurements were recorded on 23rd Jun 2014 from 12 trees that were selected to experiment NH_3 treatment, and on 26th Jun 2014 for other non-treatment tree shoots. At the start of growth measurements, new growth was observed as a bud on all shoots (as shown in Figure 2-2).From photographic records, new growth commenced from the buds after 20th May 2014. Growth rate was calculated by fitting a regression line of shoot length against date recorded.

After the last post-treatment BVOC sampling was taken, the pine shoots (both controls and treatments) were harvested. Newly grown shoots (new growth started from the end of the third week of pre-treatment sampling) and old shoots (old growth from the previous year) were separated (Figure 2-8), and the stems and needles were weighed separately. The dry weight of the biomass was determined by drying the needles and stems at 70 ˚C for 72 hours. Total dry mass used in the calculation was calculated by accumulating the dry masses of the newly grown needles and stems and the original needles and stems. Dry mass of needles used in the calculation included only the newly grown needles and the original needles.

Figure 2-8: Left: a picture of harvested pine shoot; Right: a picture of new and old needles and stems that were separated before weighing.

2.5.2 Emission rate calculation

The size of a spectral peak is proportional to the quantity of the substance that reaches the detector in the GC instrument. Quantification was based on the response of ion 93. Response factors for all standards were calculated using the known quantity of the substance injected, divided by the peak area of the resulting chromatographic peak for ion 93. The response factors for samples were then obtained by averaging the response factors of the standards, which were analysed immediately prior to and after the samples.

The compounds detected in the blank samples were assumed to be impurities or artefacts originating from the sampling environment or degradation of the adsorbent. Their peak areas were subtracted from the corresponding peaks of the samples that were taken on the same day.

BVOC emission rates per unit of dry weight biomass (μg g^{-1} h^{-1}) were calculated by using the following equation (b):

$$\text{Emission rate} = \frac{[\,(\text{Mass}_s - \text{Mass}_b) \times \text{Vol}_T\,]}{(\text{Vol}_s \times t \times \text{Mass}_l)} \qquad \text{(b)}$$

Where Mass_s is the mass of compound in the sample (ng; which was calculated by multiplying peak area of sample and response factor), Mass_b is the mass of compound in the blank (ng; which was calculated by multiplying peak area of cuvette blank and response factor), t is the sample duration (h), Vol_T is total volume of air (ml) passing through the cuvette (calculated by multiplying cuvette flow rate and sample duration), Vol_s is the volume of sample (ml; which was calculated by multiplying the sample flow rate and sample duration), and Mass_l is the dry weight biomass of plant material (g).

2.6. Statistical Analyses

Data was analysed using One-way ANOVA. Data were checked for normality before the analysis. Where data were not normally distributed, or where the variance of the samples were not equal, data was either transformed using Log_{10} transformation, or analysed using Non-parametric analysis of Mann-Whitney Test. Tukey Multiple Comparison Test was used to analyse data of shoot growth. Statistical tests were considered significant at $P < 0.05$. All the statistical analyses were performed using Minitab 16 Statistical Software.

To investigate how the emission rates varied in terms of temperature conditions, meteorological data from Easter Bush Meteorological Station (100 m away from CEH) were used to create climatic profiles over the BVOC sampling period. Temperature and PAR data for each sampling day used 24-hour mean preceding the emissions and 7-day mean preceding the emissions.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Speciation and Variation of BVOC Emissions

In this report, the term 'pre-treatment' refers to samples collected between 7th May 2014 and 30th May 2014, whilst 'post-treatment' refers to samples collected between 18th June 2014 and 11th July 2014 when experimental shoots had been sampled after enclosure in the PET bags, with one shoot on each experimental tree subjected to ammonia (NH_3) treatment, and a second 'control' shoot had been subject to 'control' ambient air treatment (see Chapter 2).

BVOC emissions were detected from all measured tree shoots. Among all the samples analysed by GC-MS, nine compounds were identified as monoterpenes (Table 3-1). Monoterpenes are characterised by ion 93, 92 and 91. Some compounds were not dominated by ion 93, for example, β-mycrene was sometimes dominated by ion 93, but other times dominated by ion 41, and limonene was characterised by both ion 68 and ion 67. A good match with the spectral library was shown as a higher matching quality, which gave a score of up to 1000. Scores over 900 were generally accepted as good matching qualities. Smaller peaks of compound were hard to identify with high quality matches (*i.e.* matching qualities were less than 900), so the identification of unknown compounds was carried out by comparing their retention times with standards in addition to checking their ion spectra. Pre-treatment samples and post-treatment

samples showed a similar diversity of emitted compounds, with eight compounds and nine compounds, respectively. Eucalyptol was emitted from one shoot in post-treatment samples, but its peak area was very small.

Table 3-1: Compounds that were identified by GC-MS from pre-treatment and post-treatment samples, main ions and matching quality range.

Compound	Main ions	Matching quality range
α-pinene	93 91 92 77 79	894 – 995
β-pinene	93 41 69 91 79	624 – 993
δ^3-carene	93 91 77 79 92	696 – 987
camphene	93 121 79 91 67	795 – 990
β-mycrene	41 93 69 91 79	706 – 985
β-phellandrene	93 91 77 79 41	828 – 978
α-phellandrene	93 91 77 92 136	848 – 911
Limonene	68 67 93 79 94	719 – 974
Eucalyptol	43 81 93 71 108	936

Although the diversity of compounds in pre-treatment and post-treatment samples was similar, each sample shoot emitted different compounds. As shown in Figure 3-1, the most frequently emitted compounds in pre-treatment samples were α-pinene (96–100 % of samples), β-pinene (88–100 % of samples) and δ^3-carene (75–95 % of samples), whilst all post-treatment samples emitted α-pinene, camphene and β-myrcene. The BVOCs least frequently emitted were β-phellandrene (only 3 out of 87 samples) and β-myrcene (only 5 out of 87 samples) in pre-treatment samples. Post-treatment pine shoots also emitted α-phellandrene and eucalyptol, but these compounds only appeared in one or two samples. Generally, post-treatment samples emitted more compounds than pre-treatment samples.

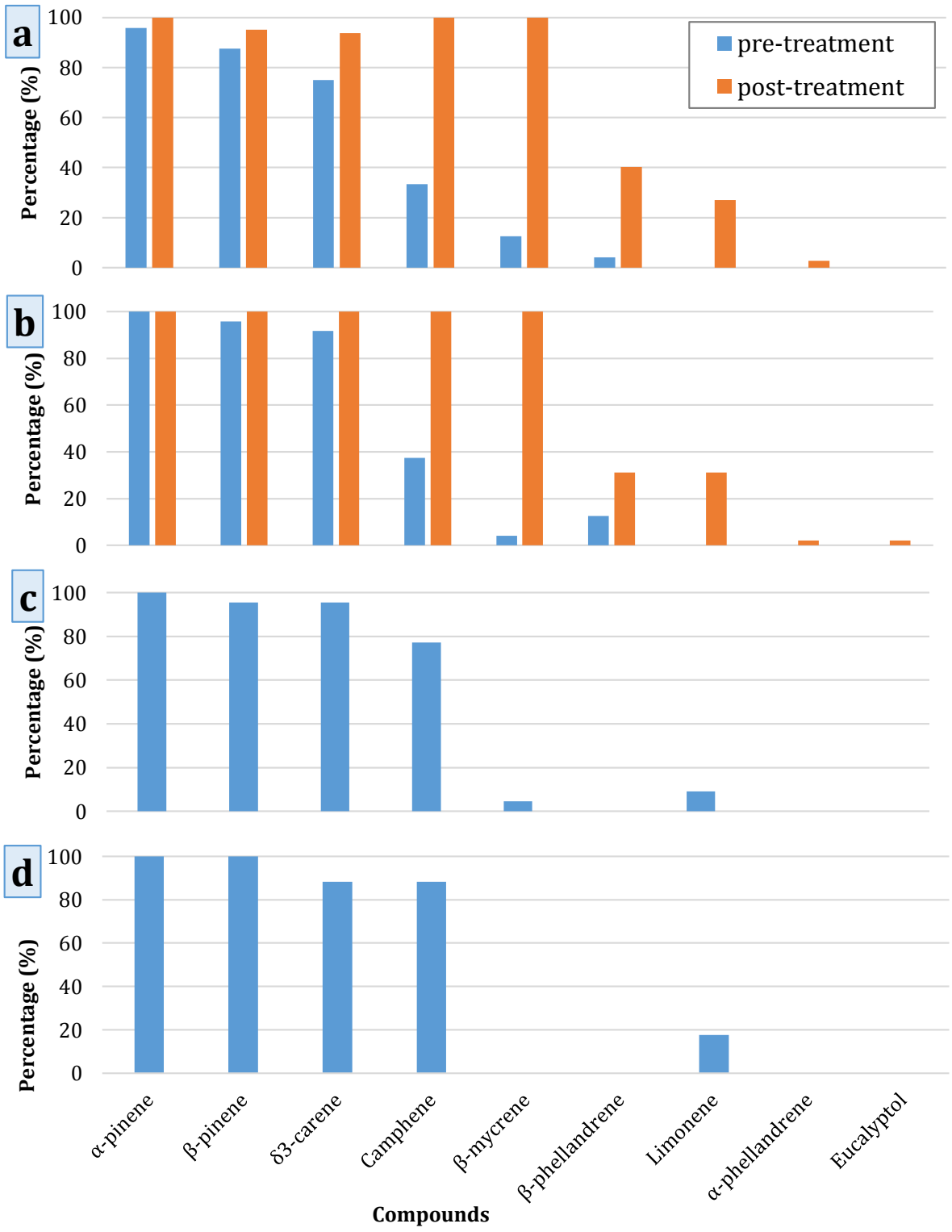

Figure 3-1: The percentage of pre-treatment samples and post-treatment samples for each compound identification in each provenance. (a). AB: pre-treatment n = 24, post-treatment n = 6; (b). CG: pre-treatment n = 24, post-treatment n =6; (c). CR: pre-treatment n = 22, no post-treatment samples taken; (d). GE: pre-treatment n = 17, no post-treatment samples taken; where n = number of measurements.

There was a sharp increase in the percentage of samples that emitted *β*-myrcene and camphene, between pre-treatment and post-treatment samples, in both the AB and CG groups. In addition, the diversity of emitted compounds also increased from six compounds to eight compounds in the AB group, and from six compounds to nine compounds in the CG group.

Camphene, *β*-myrcene, *β*-phellandrene and limonene were detected in many of the pre-treatment and post-treatment samples. There were no standards available to quantify these compounds, though the response of the 93 ion and the relative amount of 93 ion in the non-standard compound can be used to perform the quantification. However, due to time constraints, only *α*-pinene, *β*-pinene and *δ*³-carene were quantified and used in the subsequent results and discussion in the work presented here.

There was a notable range of emission rates in pre-treatment samples (Figure 3-2, blue), particularly for *α*-pinene (0.27–26.32 μg g^{-1} h^{-1}, total dry mass basis) and *δ*³-carene (0.95–31.56 μg g^{-1} h^{-1}, total dry mass basis). This variation would be even larger, if emission rates were calculated using only the dry mass of needles (Figure 3-2, red).

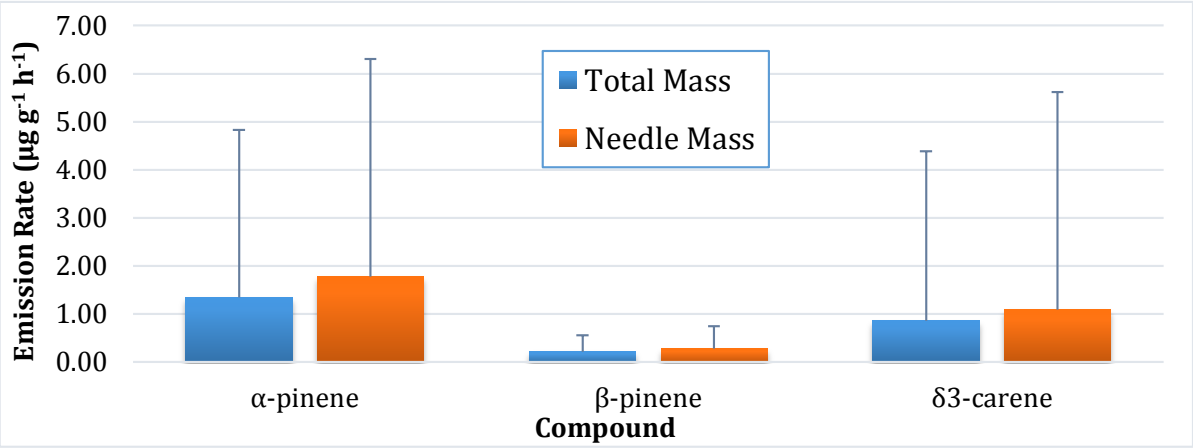

Figure 3-2: Mean emission rates (μg g^{-1} h^{-1}) of all the pre-treatment pine shoots, based on total dry mass (blue) and dry needle mass (red). Error bars are 1 standard error (n = 87); where n = number of measurements.

The high level of standard errors derives from several samples with extremely high emission rates, and post-treatment measurements also show several unusually high emissions (data not shown). These emission rates could be caused by small wounds. Wounded needles release large amounts of monoterpenes, usually contained in the resin ducts of *Pinus sylvestris* (Loreto *et al*., 2000; Sampedro *et al*., 2010). Thus, a large amount of monoterpenes may have been emitted due to shoot damage caused by installing the shoots or bending the elongated new shoots in the leaf chamber during sampling. For example, in the post treatment measurements, emission rates from both shoots of tree AB(B)10 were extremely high for α-pinene and δ^3-carene for three consecutive days. Their new shoots were both 7.5 cm at the time of BOVCs sampling, which were 3.2 and 3.5 cm longer than the average new growth of the group AB shoots. Previous studies (Hakola *et al*., 2006; Bäck *et al*., 2012) show that high emissions induced by physical damages are usually sustained for a few days. Therefore, these high emission rates (> 5 μg g^{-1} h^{-1}) were excluded from further estimates of mean emission rates. Figure 3-3 shows the mean emission rates of all pre-treatment samples, excluding emission rates that were recorded as being higher than 5 μg g^{-1} h^{-1}.

The total mean monoterpene emission rate was found to be 1.11±1.67 μg g^{-1} h^{-1} when using total dry mass, whilst the needle-mass based average emission rate was 1.39±1.92 μg g^{-1} h^{-1}. The highest average emission rate of pre-treatment samples was from α-pinene (0.57±0.76 μg g^{-1} h^{-1}), followed by δ^3-carene (0.33±0.57 μg g^{-1} h^{-1}) and β-pinene (0.21±0.35 μg g^{-1} h^{-1}) (±SE) (Figure 3-3). These emission rates were based on a total dry mass of shoot samples. Mean emission rates based on the dry mass of needles demonstrated a similar pattern, but emission rates were generally 20–24 % higher than those based on total mass (data not shown). Therefore, the leaf-level measurements of monoterpenes showed that the most abundant compounds emitted

(in terms of emission rate) were *α*-pinene and *δ*³-carene in this study, which is consistent with results found in the literature (Rinne *et al.*, 1999; Komenda & Koppmann, 2002; Simpson *et al.*, 1999). However, the main compounds emitted in southern Finland were *δ*³-carene only, and in northern Finland they were *α*-pinene and *β*-pinene (Tarvainen *et al.,* 2005). These differences in emission proportion could be due to genetic factors, as this dissertation will discuss later.

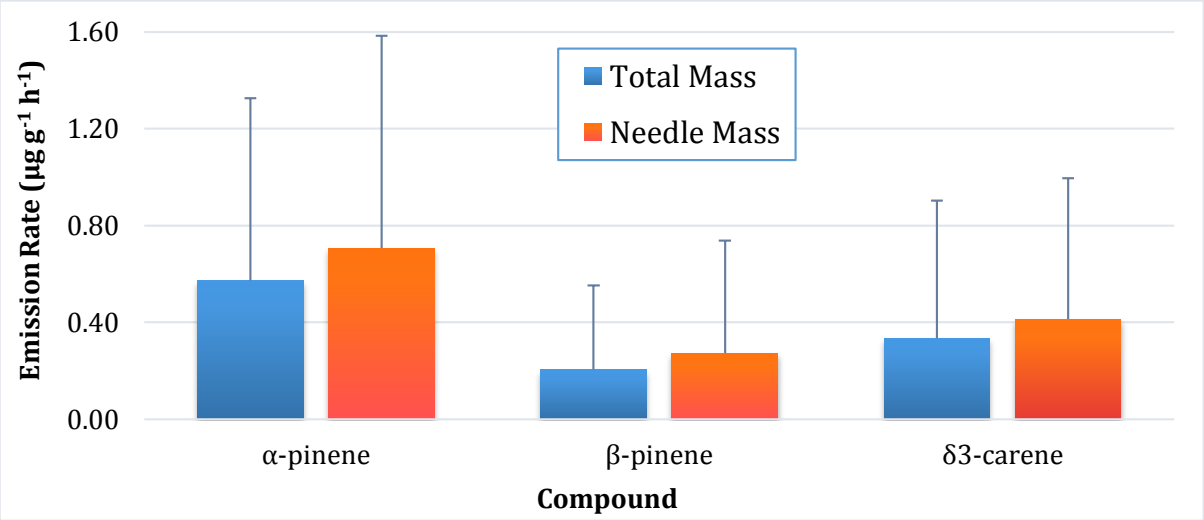

Figure 3-3: Mean emission rates (µg g^{-1} h^{-1}) of all the pre-treatment pine shoots, based on total dry mass (blue) and dry needle mass (red). High emission rates (> 5 µg g^{-1} h^{-1}) were excluded from mean emission rates in the graph. Error bars are 1 standard error (n = 87); where n = number of measurements.

It has been suggested that most monoterpene emissions from Scots pine are volatised from the needle storage pool (Kivimaenpaa *et al.*, 2012; Steinbrecher and Ziegler, 1997). It is true that monoterpenes can also be emitted from the stem, but they are mainly associated with small wounds (Loreto *et al.*, 2000). Therefore in this study, needle mass emission rates are used for the purposes of this study and subsequent discussions.

According to Rinne *et al*. (2000) and Bäck *et al*. (2012), the share of α-pinene, *β*-pinene and *δ*[3]-carene was over 40–97 % of the total monoterpene emissions from *Pinus sylvestris*, and all of the other compounds (*e.g.* limonene, camphene, *etc.*) were less than 10 % (Bäck *et al*., 2012) . Our results were measured at 25 °C (they only included three main compounds), and are therefore in accordance with other measurements relating to *Pinus sylvestris* (Table 3-2).

Table 3-2: Comparison of monoterpene emission rates from *Pinus sylvestris*.

Emission Rate, µg g(dw)$^{-1}$ h^{-1}	Reference
12.1[b] (7.7)[a]	*Isidorov et al.* [1985]
0.8[c] (1.3)[a]	*Jason* [1993]
6[b] (3.8)[a]	*Staudt* [1997]
0.06 – 0.64[d] (young pines)	*Komenda & Koppmann* [2002]
0.24 – 3.7[d] (mature pines)	*Komenda & Koppmann* [2002]
1.16[d]	*Tarvainen et al.* [2005]

[a] Numbers in parenthese were calculated for a temperature of 25°C.
[b] Values are normalised to 30°C.
[c] Values are normalised to 20°C.
[d] Values are normalised to 25°C.

However, it should be noted that the reliability and comparability of reported emission factors could be biased by the diverse experimental conditions used in these studies. The monoterpene emission rates reported by a wide variety of different authors, for the same plant species, may include a different number of identified compounds, though usually consisting of the quantitatively most important ones (Kesselmeier & Staudt, 1999). In addition, such emissions may be recorded under conditions other than standard PAR and temperature, and standardised to 25°C afterwards. This normalisation procedure may yield emission rates with an error comparable to the deviation of the actual light and temperature influences of the regarded emissions from

applied algorithms (Kesselmeier & Staudt, 1999). Moreover, the emission measurements in this study were recorded under carefully controlled light and temperature conditions in the laboratory, on young potted pine trees. The emission measurements in Table 3-2 were recorded under ambient conditions and performed in the field, on mature plants of natural stands. Komenda and Koppmann (2002) measured emission rates of 0.06–0.64 μg g^{-1} h^{-1} from young pines (3-4 years old), their samples were younger than the trees measured for the data presented here (7 years old).

3.2. Genetic Provenance Effects

The *Pinus sylvestris* trees used in this study originated from four geographically distinct locations (AB: east, CG: west: CR: south, GE: north) in Scotland. In all of these provenances, mean emission rates in *α*-pinene were amongst the highest of all emitted compounds (Figure 3-4). In each provenance, emission rates for each compound showed a similar trend: the highest emission rates were *α*-pinene, followed by *β*-pinene and *δ*³-carene (Figure 3-4). The most notable difference in emission rates between compounds were found in the GE group (0.67±0.79, 0.19±0.31 and 0.18±0.16 μg g^{-1} h^{-1} (±SE) for *α*-pinene, *β*-pinene and *δ*³-carenes, respectively). Groups GE and CG tended to have higher emission rates of *α*-pinene than the other two provenances (Figure 3-4), although the differences were not statistically significant for compound emissions (ANOVA: $P > 0.05$). Pine shoots from the GE and CG groups had a higher mean emission rate than those in the other two provenances, and *β*-pinene- and *δ*³-carene-emitters were mainly from the CR group, although they were also associated with high standard errors.

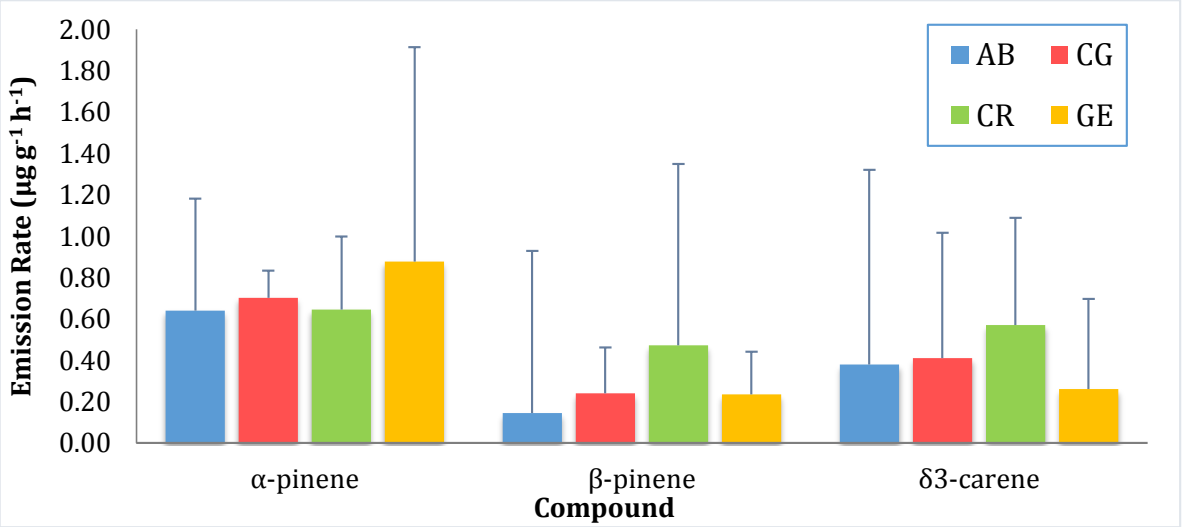

Figure 3-4: Mean emission rate (µg g^{-1} h^{-1}) in each provenance of trees for each compound (dry needle mass based; pre-treatment measurements only). Error bars are 1 standard error (Tree samples from AB: n = 24; Tree samples from CG: n = 24; Tree samples from CR: n = 22; Tree samples from GE: n = 17; where n = number of measurements).

Clear inter-population differences were found in the contribution of each compound to total monoterpene emissions (considering only the three main compounds) from individual trees (Figure 3-5). Differences in compositions of monoterpene emissions between provenances could be attributed to genetic differences (Komenda & Koppmann, 2002). Genetic factors were found to be more important than environmental factors in controlling monoterpene compositions (Tobolski & Hanover, 1971). Different genotype of species will have different compositions of volatile organics, and consequently, emission patterns will be distinctive (Tobolski & Hanover, 1971).

Of 87 samples, one sample was anomalous and showed no emissions at all, and 52 had 50–100 % of α-pinene emissions. Among these, 14 samples belonged to the CG group, 14 were from the CR group, 12 were from the AB group and 12 were from the GE group. Samples from the CG group demonstrated a higher α-pinene emission contribution than samples in the other groups. About 20 % of the all the trees emitted mainly α-pinene and almost no δ^3-carene at all or at very low emission rates. On the other hand, δ^3-carene made up over half of overall monoterpene emissions in only 19 samples. Of these δ^3-carene-emitters, the highest emission contributions of δ^3-carene occurred mostly in shoots from the AB group. However, in this study, variability was higher within populations rather than between populations. Each provenance appears to include trees with emission compositions across the entire spectrum of observed ratios. It is clear that α-pinene is the compound that makes the most difference when it comes to emission blends for individual Scots pine shoots. δ^3-carene and β-pinene were present in most of the samples, although in some cases in relatively low proportions. Overall, it appears that lower α-pinene emissions are usually accompanied by higher δ^3-carene emissions.

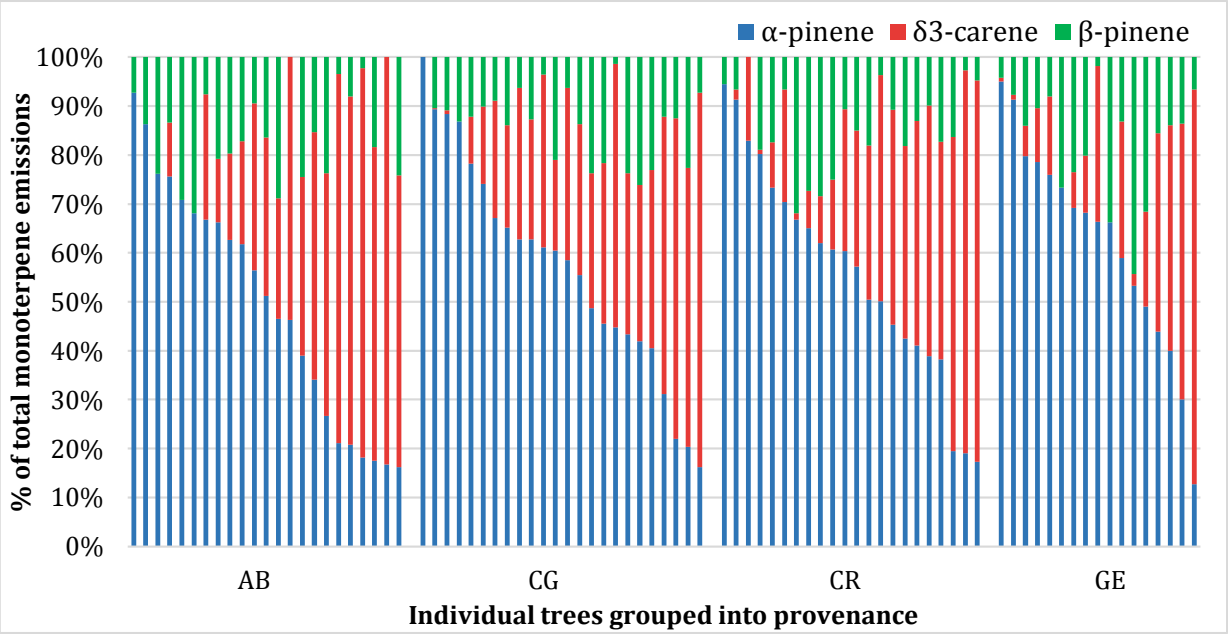

Figure 3-5: The percentage contribution of each emitted compound to the total monoterpene emissions of Scots pine for each provenance. (Pre-treatment tree samples from the AB group: n = 24; Tree samples from the CG group: n = 24; Tree samples from the CR group: n = 22; Tree samples from the GE group: n = 17; where n = number of measurements).

Emission rates of monoterpenes could potentially be related to tissue concentrations, because plants store monoterpenes in specialised compartments that contain complex solutions of monoterpenes (Lerdau *et al*., 1994). Monoterpene concentrations within the tissue control vapour pressure where the compound is synthesised or stored, and this controls the emission rates of different monoterpenes (Lerdau *et al*., 1997). Nerg *et al*. (1994) observed that concentrations of monoterpenes, especially δ^3-carene, α-pinene, β-pinene and myrcene were highest in plants from areas with a lower temperature and a longer dormancy (Nerg *et al*., 1994). Therefore, it was expected that trees from colder origins would have higher monoterpene concentrations, thus higher monoterpene emission rates. However, this is not the case in this study, where the AB

group originated from the region with lower average annual temperature, but showed the lowest absolute monoterpene emissions out of all four provenances (Figure 3-6).

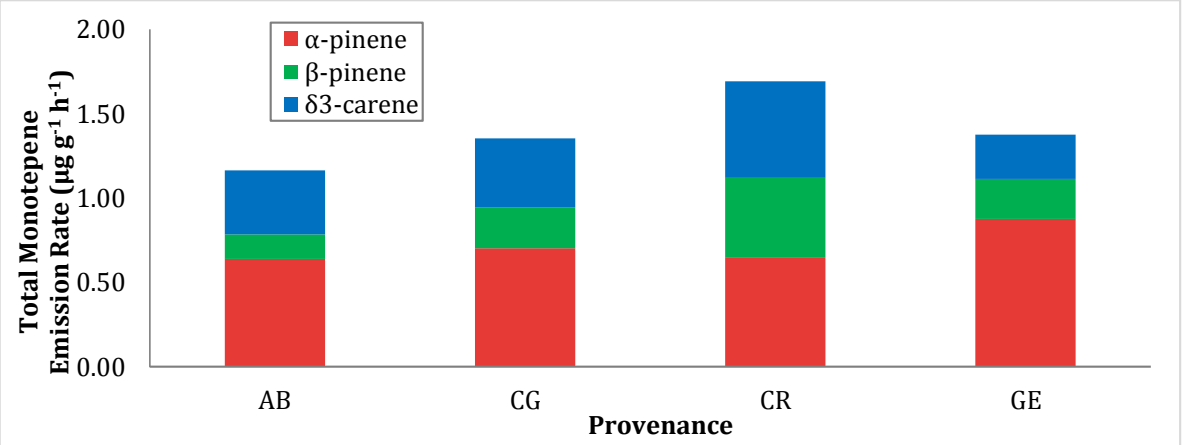

Figure 3-6: Mean total monoterpene emission rates for each provenance, shown mean emission rate of each compound separately. Pre-treatment tree samples from AB: n = 24; Tree samples from CG: n = 24; Tree samples from CR: n = 22; Tree samples from GE: n = 17; where n = number of measurements. Differences are not significant, ANOVA: $P > 0.05$.

The trees used in this study originated from four different locations across Scotland, where steep gradients in temperature, between west and east, can commonly be found. Salmela *et al*. (2013) investigated these same populations of Scots pine at CEH, Edinburgh, and found environmentally driven genetic differentiation, especially the timing of bud flush, with those from cooler origins generally flushing earlier, but there were some variations within populations. However, with no statistical evidence, the genotype of these trees cannot explain the trend of BVOCs emissions that were observed, and further investigation is needed (see Chapter 4).

3.3. Effects of NH_3-Treatment on BVOC Emissions

This study looked at 'post-treatment' monoterpene emission rates from shoots under ambient air conditions (control) and under NH_3 dosing conditions (treatment). Control emissions (*i.e.* emissions from shoots which received ambient air treatment) of *α*-pinene averaged 1.52±1.28 µg g^{-1} h^{-1} (±SE) while NH_3 treatment emissions of α-pinene averaged 1.19±1.08 µg g^{-1} h^{-1} (±SE). Mean emission rates of *α*-pinene and δ^3-carene were generally higher in all control samples (4 weeks measurements) compared to treatment shoots, and negligible differences were observed between treatment and non-treatment samples for *β*-pinene emission rates (Figure 3-7). Although a trend of reduction was observed in monoterpene emission rates by NH_3 dosing, this was not statistically significant (ANOVA*: P* > 0.05).

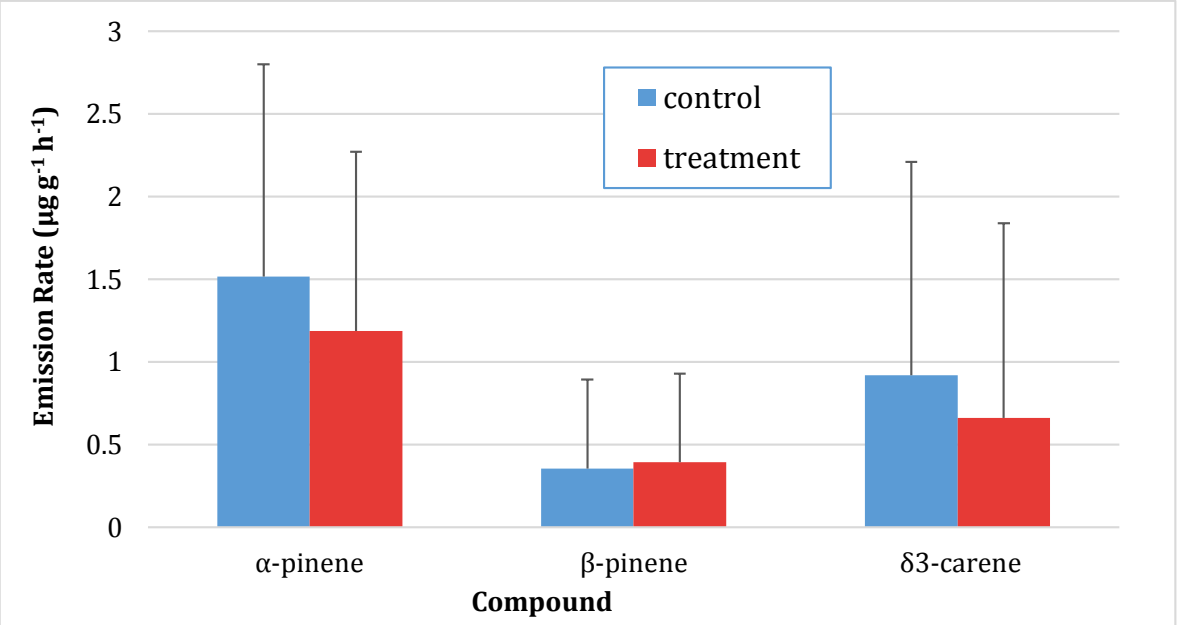

Figure 3-7: Mean emission rates (µg g^{-1} h^{-1}) (dry needle mass basis) of all the post-treatment pine shoots (provenance AB and CG considered together; control shoots: blue; treatment shoots: red). High emission rates (> 5 µg g^{-1} h^{-1}) were removed from mean emission rates in the graph. Error bars are 1 standard error. Control: n(α-pinene)=40, n(*β*-pinene)=44, n(δ^3-carene)=41; Treatment: n(α-pinene)=33, n(*β*-pinene)=45, n(δ^3-carene)=41; where n = number of measurements.

Both low and high NH_3 dosing strategies were applied in the treatment campaign. In low-dosing treatments, the NH_3 concentration (measured using the ALPHA samplers) for controls was 1.69–5.47 μg m^{-3}, and 5.45–24.8 μg m^{-3} for treatments; in high dosing treatments, the concentration for controls varied between 2.2 and 6.37 μg m^{-3}, whilst the treatment concentration reached 84.9–168.5 μg m^{-3} (data shown in Appendix). However, no significant changes in emission rates were identified between controls and treatment shoots in either low- or high-dosing treatments (Table 3-3; ANOVA*: P* > 0.05).

Table 3-3: Weekly means and standard deviations (SD) of control and treatment emission rates. AB week 1 and CG week 1: low NH_3 dosing; AB week 2 and CG week 2: high NH_3 dosing.

		***α*-pinene**		***β*-pinene**		***δ*³-carene**	
		Mean	SD	Mean	SD	Mean	SD
Control:	AB wk 1	1.14	1.32	0.32	0.71	0.74	0.92
	CG wk 1	1.50	1.16	0.30	0.43	0.73	1.05
	AB wk 2	1.44	1.48	0.49	0.75	1.26	1.55
	CG wk 2	1.91	1.31	0.32	0.21	0.99	1.63
Treatment:	AB wk 1	0.84	0.64	0.10	0.09	0.30	0.23
	CG wk 1	0.98	0.69	0.26	0.19	0.55	0.42
	AB wk 2	1.51	1.66	0.74	0.87	0.84	1.41
	CG wk 2	1.69	1.40	0.40	0.35	0.94	1.85

There are very few previous studies investigating the effects of NH_3 on BVOCs emissions from *Pinus sylvestris*. In a field study that aimed to identify the source of new particle formation episodes, Jason *et al*. (2001) found no clear effects of increased NH_3 concentrations during nucleation events on emissions from *Pinus sylvestris*. Judzentiene *et al*. (2007) found evidence of change in the monoterpene content of *Pinus sylvestris* needles in nitrogen pollution gradients, but how this might affect emissions

is not known. Effects of other air pollutants on physiology and monoterpene emissions from pine species have been extensively explored in the relevant literature. For example, long-term ozone effects on monoterpene emissions of 5-year-old pine trees were investigated, and it was found that emission rates for the ozone-treated pines were 40 % higher than control emission rates, but with no visible damage to the needles (Heiden *et al*., 1999).

Most NH_3 emissions come from intensive livestock units, especially housing and manure storage (Groot Koerkamp *et al*., 1998), 2-hour NH_3 exposure each day in this study is thought to be similar to the dose that an ecosystem might receive from agricultural sources (*e.g.* when farmers clear manure out of barns, *etc*.). Under three/four-consecutive-day treatment bursts (see Chapter 2), we observed a general tendency of decrease in monoterpene emission rates with NH_3 treatment. We therefore suggest that short-term NH_3 concentrations (up to 168.5 μg m^{-3}) may include a small decrease in monoterpene emission rates from young (7 year-old) *Pinus sylvestris*, but the effect was not statistically significant.

3.4. Photosynthesis Rates and Monoterpene Emissions

For emissions that are light-dependent, and occur immediately after synthesis, it is sometimes possible to observe a correlation between the instantaneous photosynthesis rate and the rate of emissions. In this case, there appeared to be variable relationships between monoterpene emissions and photosynthetic rates. In the pre-treatment measurements, a negative correlation of photosynthetic activity and

monoterpene emissions was shown in each provenance except GE, which showed a positive correlation. These relationships were more variable in the post-treatment measurements. Under low dosing treatment, a tendency towards a positive correlation was observed for the AB group and the CG group showed a tendency towards a negative correlation. In contrast, the AB group in high dosing treatment measurements showed a tendency of very weak negative correlation, whereas the CG group showed a tendency of positive correlation.

With no statistical significances shown ($P > 0.05$), the low R^2 values of these correlations indicated that the photosynthetic rate only accounted for a very small amount of the variability within this emission data. This variable relationship seems to indicate that instantaneous photosynthesis is not responsible for providing substrates for terpenoid synthesis and instantaneous emissions at the times when emissions were measured (Bäck *et al*., 2005; Llusià & Peñuelas, 2000). It is likely that most of the emissions observed were from stored pools. In addition to this, measurements of emissions in the dark would indicate whether or not there was a contribution of light-dependent emissions in the total emissions observed, but this was outside the scope of this study. We conclude that the monoterpene emissions of *Pinus sylvestris* were independent of leaf photosynthetic rates.

3.5. Effects of Environmental Factors on BVOC Emissions

The rates of monoterpene emissions can be influenced by a diverse range of environmental factors. Measurements were made in the controlled environment leaf cuvette within a very narrow range of leaf temperatures (26.5 – 28 °C), and there was no apparent effect of leaf temperature on emission rates. The observed weak/no dependency of leaf-level emission rates, with the measured environmental variables, was unexpected since earlier studies have shown the dependency of monoterpene emissions on temperature (Kesselmeier & Staudt, 1999; Lerdau *et al*., 1997) and on PAR (Shao *et al*., 2001). However, most of the studies relating to the dependencies on environmental variables involved a wide range of temperatures (and PAR) values, on the basis of branch or canopy emission measurements. Tarvainen *et al*. (2005) suggested that temperature could explain most of Scots pine monoterpene emission rates during the summer, but not during spring. In this particular study, there were no effects of leaf temperature on emission rates during spring within a narrow range (within 2 °C) of temperature values.

3.6. Changes in BVOC Emissions over Time

It has been recognised that rates of monoterpene emissions from *Pinus sylvestris* change significantly over time and across the seasons (Bäck *et al*., 2012). In this study, the sampling time lasted only one month. Therefore, it is not known, whether the rates of monoterpene emissions were higher in spring and summer than they were in autumn or winter.

Monoterpene emission rates are usually higher from buds and growing needles (Aalto *et al*., 2014). All the selected shoots in this study had small buds at the top of their shoots, at the beginning of the BVOC measurements. New needle growth commenced during the later pre-treatment BVOC measurements on the CR and GE groups. Therefore, higher pre-treatment emission rates were expected from the CR and GE groups. This was the case (Figure 3-6), with the highest total monoterpene emission rates occurring in the CR group, followed by the GE group.

The biomass development of shoots is a significant source of monoterpenes during springtime (Hakola *et al*., 2006). Aalto *et al*. (2014) found that the emission of monoterpenes from growing needles were 3.5 times higher than that of mature (1-year-old) needles, during the most intensive needle growth period. The tree shoots selected for use in this study were growing during the period of VOC sampling (May to July), which was the active growing season for pine trees. All the shoots were originally ~7 cm long, but began to exhibit new growth at the beginning of the pre-treatment BVOC measurements. There was no significant difference in old-growth dry needle mass between the four provenances (ANOVA*: P* > 0.05). However, the amount of new shoot growth (length) recorded was significantly different between tree origins (ANOVA: F = 5.38, d.f. = 3, 90, *P* = 0.002). Shoot length growth in the AB group was significantly less than in the other three groups (Tukey multiple comparison test, *P* < 0.05), with the biggest difference occurred between the AB and CG groups (Table 3-4). Although emission rates between the AB and CG groups in the post-treatment samples were not significantly different, we did observe a consistent increase in emissions (expressed on a dry needle biomass basis) over time (Figure 3-8, a & c).

Table 3-4: Mean new-grown shoot length and standard deviations (SD) of the four provenances. Means followed by the same letter do not differ significantly from each other (Tukey test, $P > 0.05$).

Provenance	Mean grown length (cm)	SD
AB	4.208 [a]	0.484
CG	5.979 [b]	0.319
CR	5.932 [b]	0.293
GE	5.667 [b]	0.311

The trees were grown under ambient conditions, from seedlings that were raised at CEH, in Edinburgh, and monoterpene emissions are likely to have been affected by ambient temperatures (and possibly PAR) during their growth (Blanch *et al*., 2011). There was a trend of increase in monoterpene emission rates over time in both the AB and CG groups, and the historical mean temperature and PAR of 24 hours and 7 days preceding the emissions also showed an increase over time (Figure 3-8). These findings agreed with reports that demonstrated the correlation between continuous BVOC measurements and temperature and PAR over time (Blanch *et al*., 2011). However, as discussed previously, the increase in emission rates over time was largely due to growing shoots. Therefore, whether the historical temperature and PAR had an effect on monoterpene emission rates over time needs further investigations (see Chapter 4).

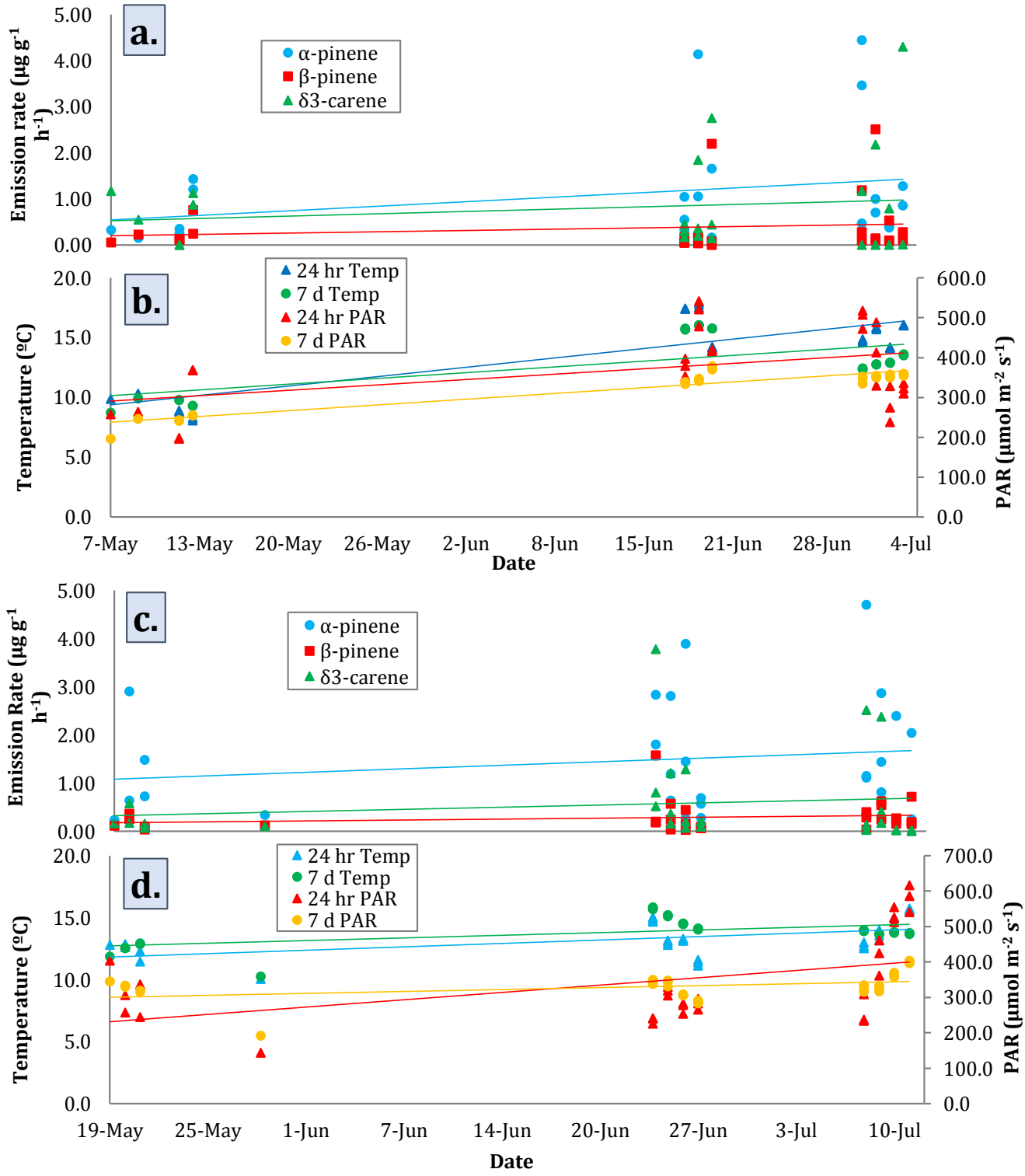

Figure 3-8: Emission rates of monoterpenes (*α*-pinene, *β*-pinene and *δ*³-carene) for control shoots that had both pre- and post-treatment measurements, temperature and PAR over time. Temperature and PAR values were expressed as mean of 24 hours data and 7 days data preceding the emission. (a.): AB trees, n = 12, where n = number of measurements; (b.) 24-hour and 7-day mean temperature and PAR preceding the emission of the AB shoots; (c.) CG trees, n = 12, where n = number of measurements; (d.): 24-hour and 7-day mean temperature and PAR preceding the emission of the CG shoots.

3.7. Uncertainties and Limitations

3.7.1 Uncertainties of emission measurements

The analysis of blank samples indicated possible contaminants that may have originated from carryovers in the leaf chamber, unclean ambient air (*i.e.* hydrocarbons that were not removed by charcoal filter), adsorbent tubes, or other laboratory contaminations. This may have had an effect on calculated emission rates. Zero emission rates in some samples were a result of higher contaminated BVOCs in the blank sample on that particular sampling day. This could be improved, in future, by cleaning the inside of the leaf chamber, cleaning desorbed tubes before sampling and changing charcoal in the filter more frequently (*i.e.* change every day), *etc*..

There are uncertainties associated with BVOC measurements, which were not accounted for in the calculations of emission rates. Owen (1997) measured an overall uncertainty of 26 % associated with shoot-enclosure emission rate measurements. To be specific, uncertainties were associated with each component of measurement, such as the mass of compounds in the samples and the blanks detected by GC-MS, sample duration time and the biomass of sampled shoots, with the largest variation coming from the measurements of air flow rate (Owen, 1997; Owen *et al*., 2001). Because of the problem with the ATD transfer line, uncertainties in reported measurements are higher than usual (see Chapter 2).

3.7.2 Limitations of methodology

To our knowledge, this is the first study to directly investigate the effects of N deposition on BVOC emissions of *Pinus sylvestris*. However, due to time constrains, the development of the treatment methods does contain many problems.

The NH_3 treatment experiments were undertaken using a PET bag-enclosure method. Due to the high reactivity of NH_3 gas, there were losses of NH_3 by walls of PET bags, although they were conditioned. During the high-dosing experiments, NH_3-filled PET bags were used to enclose treatment shoots. However, vast amounts of NH_3 were leaked away when inserting shoots into the bag, although extra 3 x 60ml of NH_3 were injected into the enclosure bag in order to compensate for these losses. These were the main reasons why both the low-dosing and high-dosing of NH_3 did not achieve the expected concentrations of 100 μg m^{-3} and 17382 μg m^{-3}, respectively. In addition, there may have been some 'pockets' of concentrations of NH_3 within the bags, due to the fact that there was no fan in the bag to evenly spread out injected NH_3.

This experiment could be improved by using a higher concentration of ammonia standard, or by enclosing the whole plant within an environment-controlled chamber, and fumigated with NH_3. Despite the limitations associated with this NH_3 treatment method, this study forms a springboard for further development and research in this area.

CHAPTER 4

CONCLUSION AND FUTURE WORK

4.1. Conclusion

We conclude that on the basis of the GC-MS analysis of measured monoterpene emissions, nine monoterpene compounds released by *Pinus sylvestris* were identified: α-pinene, β-pinene, δ^3-carene, camphene, β-mycrene, β-phellandrene, α-phellandrene, limonene and eucalyptol. Post-treatment samples emitted more compounds (*i.e.* α-phellandrene, β-phellandrene and eucalyptol) than pre-treatment samples.

Pinus sylvestris emitted α-pinene and δ^3-carene as their dominant monoterpenes. The total mean monoterpene emission rate was 1.39±1.92 μg g^{-1} h^{-1} (based on a dry weight of needles) (±SE) when only considering α-pinene, β-pinene and δ^3-carene. Mean emission rates of α-pinene, β-pinene and δ^3-carene from pre-treatment samples were 0.71±0.88, 0.27±0.47 and 0.41±0.58 μg g^{-1} h^{-1} (based on a dry weight of needles) (±SE), respectively. The pattern of emission rates observed between four provenances could not be explained by genetic factors. However, higher emission rates were found to be related to the rapid growth of new shoots.

Monoterpene emissions of *Pinus sylvestris* were independent of leaf photosynthetic rates, a finding which is supported by most other studies. No effects of instantaneous foliar temperature on emission rates during spring within a narrow range of temperature values (within 2 °C) were found in this study. Monoterpene emissions, over time, tended to follow the trend of accumulated previous day's and previous week's temperature and PAR. However, considering it was found to be the growth and elongation of new shoots that had a major impact on monoterpene emission rates, we would need to further investigate to find out whether historical temperature and PAR had an effect on monoterpene emission rates over time.

Short-term dry NH_3 treatment (up to 168.5 μg m^{-3}) tended to decrease the monoterpene emission rates of young *Pinus sylvestris*, particularly *α*-pinene and *δ*^{3}-carene, but the effect was not statistically significant. Although there were some limitations and uncertainties associated with the NH_3 treatment method, and no evidence that monoterpene fluxes were affected by short-term ammonia treatment, these results nonetheless provide a first valuable, comparative feasibility study with which to further investigate the effects of N-treatment on BVOC emissions. This further understanding of the changes associated with BVOC emissions is important, especially given the predicted rise in atmospheric N concentrations and the sensitivity of coniferous forests to atmospheric composition and deposition.

4.2. Further work

Further work could focus on the following aspects:

(1) **More genotype measurements**: Measurements from other genotypes are needed. In this study, measurements were taken on only four of 21 genotypes that originally investigated by Salmela *et al.* (2013) due to time constraints.

(2) **Methodology improvements**: Acclimatising the trees in a constant environment before and during the measurement might help reduce the variance in the data. Increasing replication of measurements could reduce the uncertainties associated with measurements.

(3) **Genotype investigations:** As shown in other studies, genotype of *Pinus sylvestris* affects the concentration and composition of monoterpenes within the leaf tissue (Komenda & Koppmann, 2002; Tobolski & Hanover, 1971). Therefore, concurrent measurements of monoterpene emissions and monoterpene content from the genotypes that were found to differ would provide further information about differences in emissions between genotypes and also the relationship between content and emissions of monoterpenes.

(4) **Effects of historical temperature and PAR**: To further investigate the effects of historical temperature and PAR on monoterpene emissions over time, experiments could be done in environment-controlled chambers. For example, two laboratory experiments controlled by different environmental conditions could be conducted simultaneously, with trees of similar size and origin.

(5) **Effects of N deposition**: The effects of N deposition on BVOC emissions from *Pinus sylvestris* could be further investigated by increasing the treatment duration by several weeks, months, and years, and taking into account seasonal variations of emission rates. The concentration of the ammonia standard used could also be increased. Ideally, fumigation chambers with programmable dosing of NH_3 would make it possible to dose whole trees (as would occur in the normal environment).

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Appendix

		Treatment	Tree name	Exposure Time (h)	NH_3 ($\mu g\ m^{-3}$)	NH_3 (ppb)
Low-dosing of NH_3	**week 1**	Control	AB(C)1	6.15	5.47	7.87
		NH_3	AB(C)1	6.18	24.81	35.68
		Control	AB(A)4	6.15	2.77	3.99
		NH_3	AB(A)4	6.15	16.56	23.82
		Control	AB(D)6	6.17	5.17	7.44
		NH_3	AB(D)6	6.10	10.96	15.77
	week 2	Control	CG(C)1	8.20	4.64	6.67
		NH_3	CG(C)1	8.25	7.47	10.74
		Control	CG(A)8	8.23	5.04	7.25
		NH_3	CG(A)8	8.22	10.90	15.68
		Control	CG(A)6	8.28	1.69	2.43
		NH_3	CG(A)6	8.18	5.49	7.84
High-dosing of NH_3	**week 3**	Control	AB(C)6	8.10	6.37	9.16
		NH_3	AB(C)6	8.25	168.47	242.30
		Control	AB(B)7	8.08	4.22	6.07
		NH_3	AB(B)7	8.20	84.86	122.04
		Control	AB(B)10	8.03	3.92	5.63
		NH_3	AB(B)10	7.85	100.22	144.14
	week 4	Control	CG(C)3	9.32	4.03	5.79
		NH_3	CG(C)3	9.15	162.19	233.27
		Control	CG(B)6	9.05	2.81	4.04
		NH_3	CG(B)6	9.10	99.36	142.90
		Control	CG(A)13	8.58	2.20	3.17
		NH_3	CG(A)13	8.42	109.36	157.29