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ALKYL AMINES IN BOREAL FOREST AND URBAN AREA

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Academic dissertation

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Antti-Jussi Sakari Kieloaho
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Abstract

Low-molecular-weight alkyl amines are reactive organic nitrogen compounds that are important precursors in secondary aerosol formation. Atmospheric aerosols have direct and indirect effects on Earth's climate system. Alkyl amines are emitted from marine and terrestrial ecosystems, agricultural activities and other anthropogenic sources. In terrestrial ecosystems, the quantities in the different parts of an ecosystem and formation processes are not well understood.

Alkyl amine soil concentration and biosphere–atmosphere exchange measurements are scarce. The main focus of this thesis is to determine concentrations of alkyl amines in ambient air in boreal forest and urban area, and further identify possible sources and reservoirs of alkyl amines in boreal forest. The main results presented in the thesis consist of a timeseries of gas-phase concentrations of alkyl amines measured over several months, concentrations of alkyl amines in the soil and fungal biomass, and an emission estimation based on the measured concentrations.

Alkyl amines were studied in two northern latitude environments: in a boreal Scots pine (*Pinus sylvestris* L.) forest at the SMEAR II station in Hyytiälä and in an urban background area at the SMEAR III station in Helsinki. To quantify ambient air concentrations of alkyl amines in these environments, sample collection and analytical methods were developed. Ambient air concentrations of alkyl amines were measured from May to October 2011 in the forest site and from May to August 2011 in the urban site. The effect of the measured ambient air concentrations of alkyl amines on the local air chemistry was also assessed together with aromatic hydrocarbons and terpenoids.

To assess boreal forest soil as a source of alkyl amines, a pot-scale experiment was set up. In the experiment Scots pine seedlings were grown on humus soil collected from the forest site, and the effects of Scots pine and soil organic matter (SOM) degrading enzymes on alkyl amine soil concentrations were studied. In addition, fungal strains common in boreal forest soils were cultured, and the alkyl amine concentrations in the grown fungal biomass were quantified. The role of boreal forest soil as a source or as a sink of atmospheric alkyl amines was studied using a gradient-diffusion approach. In the approach, the soil–atmosphere exchange of selected alkyl amines was estimated. This was done by describing dissolution/volatilisation on water and transport processes, and utilizing the quantified soil and ambient air gas-phase concentrations of the selected alkyl amines found in the studied boreal forest.

The gas-phase concentrations of alkyl amines in ambient air were found to be higher in the forest site than in the urban site. In the forest site, the atmospheric concentrations appeared to be linked to soil and vegetation activity based on the seasonal course of the measured alkyl amines. Litterfall, a phenological event, coincides with the concentration maxima of some of the measured alkyl amines. In the pot-scale experiment, the SOM degrading enzymes were found to have no effect on the soil concentrations of alkyl amine while the presence of Scots pine was found to have an effect on the concentrations of some of the measured alkyl amines. The soil concentrations of alkyl amines were found to be lower than those measured from the fungal biomass. The most abundant fungal groups (ectomycorrhizal and saprotrhopic fungi) in the forest soil contained the highest quantities of alkyl amines revealing that fungal biomass may be an important reservoir of alkyl amines in boreal forest soil. Based on the flux estimate, the boreal forest soil was found to act as both a source and a sink of alkyl amines. The direction of the flux was dependent on the studied alkyl amines and environmental conditions in the forest site. Soil pH was found to be one of the most critical factors determining the direction of the flux between the soil and the atmosphere.

Keywords: alkyl amine, boreal forest, forest soil, urban, air chemistry

Contents

List of publications

This thesis consists of an introductory review, followed by four research articles. In the introductory part, these papers are cited according to their roman numerals. **Paper I**, **II**, and **III** are reproduced with the kind permission of Elsevier. **Paper IV** is reproduced under the Creative Commons Attribution 3.0 License.

I Kieloaho, A.-J., Héllen, H., Hakola, H., Manninen, H.E., Nieminen, T., Kulmala, M., Pihlatie, M. (2013). Gas-phase alkylamines in a boreal Scots pine forest air, *Atmos. Environ.*, 80:369–377.

II Héllen, H., Kieloaho, A.-J., Hakola, H. (2014). Gas-phase alkyl amines in urban air; comparison with a boreal forest site and importance for local atmospheric chemistry, *Atmos. Environ.*, 94:192–197.

III Kieloaho, A.-J., Pihlatie, M., Dominquez Carrasco, M., Kanerva, S., Parshintsev, J., Riekkola, M.-L., Pumpanen, J., Heinonsalo, J. (2016). Stimulation of soil organic nitrogen pool: the effect of plant and soil organic matter degrading enzymes, *Soil Biol. Biochem.* 96:97–106.

IV Kieloaho, A.-J., Pihlatie, M., Launiainen, S., Kulmala, M., Riekkola, M.-L., Parshintsev, J., Mammarella, I., Vesala, T., Heinonsalo, J. (2016) Soil concentrations and soil-atmosphere exchange of alkylamines in a boreal Scots pine forest, *Biogeosciences Discuss.* doi:10.5194/bg-2016-363, in review.

1 Introduction

Organic nitrogen compounds in the atmosphere and in soils are a ubiquitous but poorly understood part of the global nitrogen cycle (Vranova et al., 2011; Jickells et al., 2013). Low-weight alkyl amines are organic compounds with an amino group and with one to six carbon atom chains bound to a nitrogen atom, hereafter simply referred to as alkyl amines. Even though alkyl amines are present in small quantities in the atmosphere they may have a considerable impact on atmospheric processes due to their reactive nature.

It has recently been observed that alkyl amines enhance atmospheric aerosol formation and growth, and hence have an indirect impact on the climate system (Almeida et al., 2013; Bergman et al., 2015; Lehtipalo et al., 2016). Aerosols have both direct and indirect effects on the climate system (IPCC, 2007). The direct effects of aerosols on the climate system are caused by the scattering and absorption of sunlight. Depending on the chemical and optical properties of the aerosols the effect may be cooling or warming. Aerosols have indirect effects on climate through cloud formation because they act as cloud condensation nuclei affecting the lifetime and properties of clouds, and thus, indirectly affect global albedo (IPCC, 2007). The climatic implications related to aerosols are not well known due to their complex indirect effects on the climate system (IPCC, 2007).

Alkyl amines have been taken into use recently as a climate change mitigation. They are used in industrial applications e.g. in commercial carbon capture to bind carbon dioxide from pre- and post-combustion processes (Mumford et al., 2015). Large-scale industrial production and applications of alkyl amines due to commercial carbon capture may increase anthropogenic emissions into the atmosphere and may further increase risks related to the use of alkyl amines (Reynolds et al., 2012; Mazari et al., 2015). Even though alkyl amines have not been found to be toxic (Ge et al., 2011), photo-oxidation products of alkyl amines (e.g. nitrosamines) are toxic, and may raise public health issues (Ravnum et al., 2014) and environmental concerns (Poste et al., 2014).

Despite the recent interest towards alkyl amines, little is known about their natural formation, biogenic and anthropogenic emissions, or atmospheric abundance, making it difficult to assess their environmental impacts (Poste et al., 2014; Ravnum et al., 2014; Kanakidou et al., 2016). To address their importance, alkyl amine emissions and atmospheric reactions have already been implemented in three global models study-

ing atmospheric aerosol formation (Myriokefalitakis et al., 2010; Yu and Luo, 2014; Bergman et al., 2015). These models have high uncertainties because the emission inventories of alkyl amines of terrestrial origin are based only on fixed ammonia emission rates, this is due to a lack of gas-exchange and atmospheric concentration measurements of alkyl amines (Sintermann and Neftel, 2015).

1.1 Objectives

The main focus of this thesis is to determine concentrations of alkyl amines in ambient air in a boreal forest and an urban area, and further identify possible sources and reservoirs of alkyl amines in the boreal forest. The concentration measurements and the estimation scheme provide the atmospheric modelling community with results and tools with which they can better describe the seasonal variability of alkyl amines, especially in boreal forest environments on a local or regional scale. The main aims of the thesis are shown in Figure 1, which links aims of the thesis together and to the topics discussed in the thesis.

The first aim is to develop a method for atmospheric amine measurements (**Paper I**) and to utilize this method in two environments, in a Scots pine forest and in an urban environment (**Paper I** and **Paper II**), and to assess the impacts of alkyl amines on local air chemistry (**Paper II** and **Paper IV**). **The second aim** is to quantify alkyl amine concentrations in a boreal forest soil (**Paper III**) and in fungal species present in a boreal forest soil (**Paper IV**) in order to estimate soil as a potential source of alkyl amines. **The third aim** is to take the first steps towards a more comprehensive understanding of the cycling of alkyl amines on an ecosystem scale (**Paper IV**).

The first aim was met by measuring ambient air concentrations in a boreal forest site and in an urban measurement station in Southern Finland. The second aim was met by conducting a pot-scale experiment to study further the possible source processes of alkyl amines and the effects of plants on alkyl amines in the soil (**Paper III**), and by quantifying alkyl amine concentrations in pure cultured fungal biomass (**Paper IV**). The third aim was met by estimating soil–atmosphere exchange of selected alkyl amines by describing their phase transitions and transport processes taking into account the quantified soil and ambient air concentrations of selected alkyl amines found in boreal forest ecosystem (**Paper IV**).

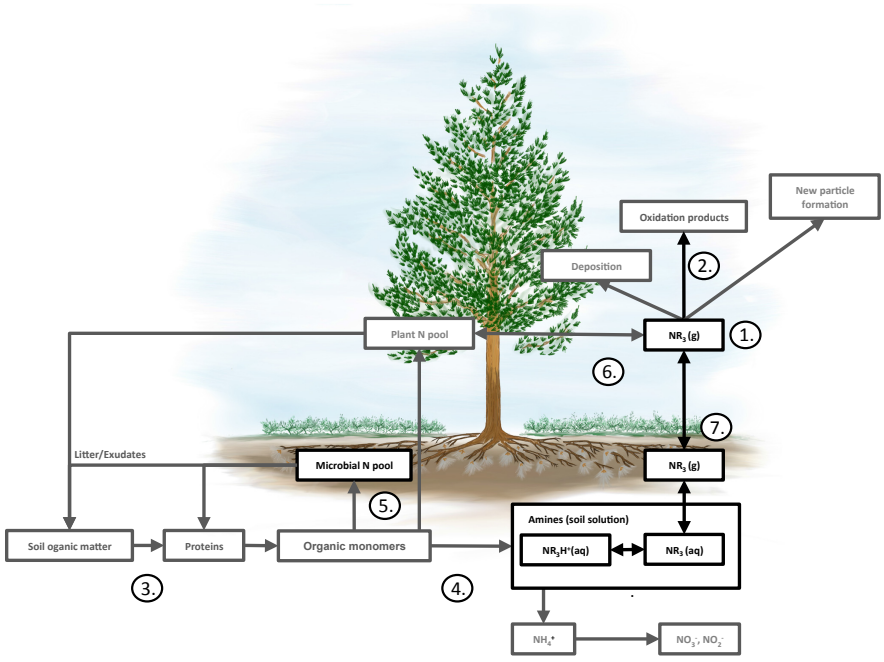


Figure 1: A simplified scheme of alkyl amines (NR_3) as part of the nitrogen cycle in a boreal Scots pine forest. The scheme shows how alkyl amines are linked to different biospheric and atmospheric processes and pools in a forest ecosystem. The numbers denote the topics dealt with in Section 2. The topics are as follows: 1. atmospheric concentrations, 2. atmospheric removal processes, 3. soil decomposition, 4. alkyl amine formation processes in soil, 5. plant uptake and microbial immobilization of alkyl amines, 6. emissions of alkyl amines, 7. the gradient-diffusion approximation to estimate emissions. The boxes denote pools of nitrogen, and the arrows show the main transformation and transport processes. The pools and processes studied in this thesis are shown in black.

2 Background

2.1 Amines

An amine is an organic molecule with an amino group. Primary, secondary and tertiary amines have the general formula RNH_2, R_2NH or R_3N, respectively, where R can be an alkyl or aryl group. Those alkyl amines relevant to the thesis have one to four carbon

atom chains bound to a nitrogen atom, and they are shown in Figure 2.

Amines have a lone electron pair in a nitrogen atom that can bind a proton (H^+) and act as a base (Eq. 1) in same way as ammonia NH_3. Therefore, alkyl amines share similar chemical behaviour with NH_3.

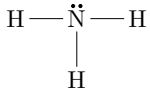
$$R_3N\!: + H_2O \rightleftharpoons R_3NH^+ + OH^- \quad (1)$$

The basicity of alkyl amines depends on the number and length of organic chains. The capability of a base to bind a H^+ is described by the base dissociation coefficient (pK_b). Inorganic NH_3 is considered to be a relatively strong base with a pK_b of 4.74. Monomethylamine, dimethylamine, and trimethylamine have pK_b values of 3.34, 3.27. and 4.19, respectively. Alkyl amines are water soluble and have high vapour pressures (e.g. dimethylamine and trimethylamine vapour pressures are 203 kPa and 215 kPa at 298.15 K, respectively) meaning that they evaporate easily at room temperature.

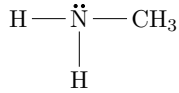
Low alkyl amine concentrations in environmental matrices, especially in the atmosphere, and high reactivity make it challenging to measure alkyl amines. Various analytical methods and sample treatment procedures have been developed for measuring alkyl amines in environmental matrices (Fekete et al., 2010; Szulejko and Kim, 2014). Nowadays, gas, liquid or ion chromatography is used to separate target compounds, and alkyl amines are detected with a range of detectors (e.g. ultraviolet-visible spectroscopy, nitrogen-phosphorus detector, or mass spectrometer) with or without pre-analytical derivatisation procedures or pre-concentration by cryotraps or solid sorbents (Katoaka, 1996; Fekete et al., 2010; Szulejko and Kim, 2014). Due to low ambient air concentrations of alkyl amines, a pre-concentration or large sample volumes are needed, and the most common methods used are reviewed by Szulejko and Kim (2014). Recently, also a direct chemical ionisation mass spectrometry method from ambient air has been successfully developed (Sellegri et al., 2005; Sipilä et al., 2015).

2.2 Amines in the Atmosphere

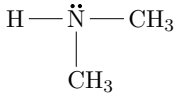
Typical ambient air concentrations of alkyl amines (containing one to four carbon atoms) are lower than a few tens of parts-per-trillion (volume fraction, ppt_V) having occasional high concentrations due to nearby point sources (Table 1). Based on the



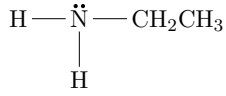
Ammonia
17.03 g mol^{-1}



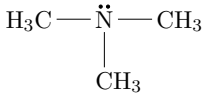
Monomethylamine
(MMA)
31.06 g mol^{-1}



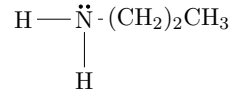
Dimethylamine
(DMA)
45.08 g mol^{-1}



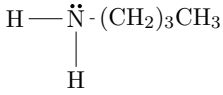
Ethylamine
(EA)
45.08 g mol^{-1}



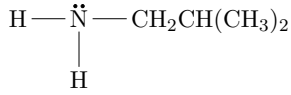
Trimethylamine
(TMA)
59.01 g mol^{-1}



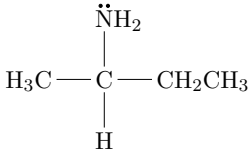
Propylamine
(PA)
59.01 g mol^{-1}



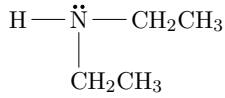
Butylamine
(BA)
73.14 g mol^{-1}



Isobutylamine
(iso-BA)
73.14 g mol^{-1}



sec-Butylamine
(*sec*-BA)
73.14 g mol^{-1}



Diethylamine
(DEA)
73.14 g mol^{-1}

Figure 2: Ammonia (NH_3) and low molecular weight alkyl amines with their abbreviations and their molecular mass relevant to the thesis.

measured alkyl amine concentrations in rainwater Cornell et al. (2003) estimated atmospheric alkyl amine concentrations to range from 0.3 to 4 ppt_V. In comparison, the typical NH_3 concentrations in continental air is around 25 ppb_V (Cornell et al., 2003) with high variability in atmospheric concentrations depending on the location of nearby sources, e.g. agricultural activity. In Finland, the ambient air concentration of NH_3 ranged from 20 ppt_V to 830 ppt_V in the forest site (Makkonen et al., 2014) and in the urban station from below 450 ppt_V to 3000 ppt_V (Makkonen et al., 2012). Despite the lower atmospheric concentrations of alkyl amines as compared to NH_3, dimethylamine concentration of 5 ppt_V has been shown to enhance atmospheric aerosol formation rate by more than a 1000-fold compared to an NH_3 concentration of 250 ppt_V (Almeida et al., 2013).

Ambient air concentrations of alkyl amines vary greatly depending on the vicinity of the sources and the physical and chemical conditions in the atmosphere. The ambient air concentrations of alkyl amines containing one to four carbon atoms range in rural areas from negligible to 42 ($\pm$51) ppt_V (Table 1), and in urban areas from negligible to 500 ppt_V (Table 1). As most measurements are conducted in urban areas or in the vicinity of agricultural activities the measured ranges vary greatly. Ambient air concentrations of alkyl amines are typically measured in short-term campaigns. In two modelling studies the global gas-phase methylamine concentrations have been estimated to be around 2 ppt_V being higher in the continental surface air than in the marine atmosphere (Yu and Luo, 2014; Bergman et al., 2015). The modelling results should naturally be taken with caution due to a lack of measurements of alkyl amine fluxes and a small number of available air concentration measurements.

2.2.1 Emissions into the Atmosphere

Amines can be emitted from various anthropogenic and natural sources into the atmosphere. The main anthropogenic sources of amines are animal husbandry, industry, and combustion processes (Ge et al., 2011; Schade and Crutzen, 1995; Sintermann et al., 2014). Natural sources have been identified in marine environments (Ge et al., 2011; Carpenter et al., 2012) and in terrestrial vegetation and soils (Sintermann and Neftel, 2015). However, in terrestrial environments the origin of amines is not extensively documented.

The global emissions into the atmosphere have been estimated using monomethylamine,

Table 1: The measured atmospheric methylamine concentrations in ppt_V grouped by the number of carbon atoms in a alkyl amine molecule.

Study	C_1	C_2	C_3	C_4	Environment
Grönberg et al. (1992)	10±3	1.8±0.6	41±14	1.7±0.4	Rural
VandenBoer et al. (2012)		6.5±2.1		<1.0 (+C_3)	Rural
Sellegri et al. (2005)		<32	34-80		Rural
You et al. (2014)	<0.1-4	<0.5	1-10	<3.3-50	Rural
Grönberg et al. (1992)	16±5	0.5±0.3	5.2±2	1.4±0.3	Urban
Chang et al. (2003)	1.9-21	1.9-34	3-34		Urban
Hanson et al. (2011)	<0.2	0.5-2	4-15	<2	Urban
VandenBoer et al. (2011)		<2.7		<2.7 (+C_3)	Urban
Yu and Lee (2012)		8±3	16±7		Urban
Zheng et al. (2015)	<29	<42	<14		Urban
Freshour et al. (2014)	<3	20	30	1-500	Urban
Van Neste et al. (1987)	0.3-1.3	2.3-5.9	0.7-2.5		Marine
Gibb and Mantoura (1999)	0.9-5.9	0.4-21	<0.3		Marine
Freshour et al. (2014)	20	100	10-15	<5.0	Marine

dimethylamine, and trimethylamine called methylamines (Schade and Crutzen, 1995; Yu and Luo, 2014; Bergman et al., 2015). Global emission estimations of methylamines are based on emission inventories of NH_3. Estimated methylamine fluxes are assumed to have a constant ratio with an NH_3 flux (Schade and Crutzen, 1995; Yu and Luo, 2014). Schade and Crutzen (1995) estimated global emissions of methylamines to be 285 ± 78 Gg N yr^{-1}. The emission inventory included emissions from animal husbandry, marine environment, and biomass burning. Based on inventories, the main methylamine emitted was trimethylamine (169 ± 33 Gg N yr^{-1}) followed by dimethylamine and monomethylamine (83 ± 26 Gg N yr^{-1} and 33 ± 19 Gg N yr^{-1}, respectively).

The estimated global emissions of methylamines by animal husbandry into the atmosphere is about 0.7% of animal husbandry NH_3 emissions (23.3 Tg N yr^{-1}) (Schade and Crutzen, 1995). In the case of trimethylamine, Kuhn et al. (2011) and Sintermann et al. (2014) have revised the emission estimate for animal husbandry to fall in a range from 3 Gg N yr^{-1} to 93 Gg N yr^{-1}, the estimates are smaller than reported by Schade and Crutzen (1995) (108 ± 26 Gg N yr^{-1}). The difference is due to changes in agricultural practices and depends on the used ratio between trimethylamine and NH_3.

Kuhn et al. (2011) have proposed an additional agricultural emission source, that from hay and silage, based on their measurements using a similar upscaling scheme as Schade and Crutzen (1995). Kuhn et al. (2011) estimated trimethylamine emissions from global vegetation to be 25 Gg N yr^{-1}. Cornell et al. (2003) have estimated atmospheric emissions to be 200 Tg N yr^{-1} in the continental boundary layer having the same order of magnitude than the total methylamine emissions reported by Schade and Crutzen (1995). In a more recent study by Yu and Luo (2014), the global emission estimate for methylamines was 331 ± 26 Gg N yr^{-1} and for NH_3 the emission estimate was 58 Tg N yr^{-1} and in the study by Bergman et al. (2015) it was estimated that global emissions were 326 Gg N yr^{-1}, in which terrestrial emissions accounted for 72%.

The estimated marine source of alkyl amines is significant (Schade and Crutzen, 1995). In an early marine emission estimate Schade and Crutzen (1995) estimated methylamine emissions at 80 Gg N yr^{-1} from coastal waters. In more recent articles by Bergman et al. (2015) and Yu and Luo (2014), marine sources were estimated to be in a range from 80 Gg N yr^{-1} to 330 Gg N yr^{-1} based on surface water chlorophyll A concentrations. The range was smaller than emissions estimated by Myriokefalitakis

et al. (2010) (800 Gg N yr^{-1}) based on a constant ratio with NH_3.

Biomass burning has been estimated to release methylamine in the amount of 60 ± 28 Gg N yr^{-1} (Schade and Crutzen, 1995). In recent years, amines have been employed in carbon dioxide removal technologies. Laboratory simulations of the capture process have shown a release of alkyl amines (Reynolds et al., 2012). In emission inventories of active carbon capture facilities emissions of only ethanolamine into the atmosphere have been reported so far (Reynolds et al., 2012; Mazari et al., 2015).

2.2.2 Atmospheric Removal Processes

In the atmosphere, the most important removal mechanisms of amines are gas-phase reactions, heterogeneous uptake by atmospheric particles and wet and dry deposition (Ge et al., 2011). Gas-phase reactions of amines include reactions with atmospheric oxidants and acid–base reactions (Ge et al., 2011). Heterogeneous uptake refers to reactions of gas-phase alkyl amines with an atmospheric particle phase (Wang et al., 2010).

Alkyl amines are readily oxidized by hydroxyl (OH) radical and to a lesser extent by nitrate (NO_3) radical and ozone (O_3) (Murphy et al., 2007; Nielsen et al., 2012). The typical lifetime of alkyl amines against OH radicals is a few hours (Lee and Wexler, 2013). In atmospheric photo-oxidation reactions of amines, amides ($R_1C(O)NR_2$), aldehydes (RCHO), imines ($R_1N{=}CR_2R_3$), nitrosamines ($R_1(R_2)NN{=}O$), and nitroamines ($R_1(R_2)NNO_2$) (Nielsen et al., 2012; Lee and Wexler, 2013) are formed (Figure 3). The photo-oxidation reaction products of amines are known to be carcinogenic (Lee and Wexler, 2013).

Alkyl amines may undergo acid–base reactions with inorganic (e.g. sulphuric acid, H_2SO_4, nitric acid HNO_3, chloride acid, HCl) and organic acids (Murphy et al., 2007; Liu et al., 2012). In the gas phase, acid–base reactions of alkyl amines and H_2SO_4 form aminium sulphate molecular clusters (Almeida et al., 2013). The formed aminium sulphate clusters do not evaporate as easily as those of ammonium sulphate (Kürten et al., 2014). Therefore alkyl amines can act as strong stabilizing agents in colliding molecular clusters enhancing the growth of the clusters to atmospheric secondary aerosols, and further increase the number of cloud condensation nuclei in the atmosphere (Lehtipalo et al., 2016). Gas-phase cluster formation diminishes the difference between gases and

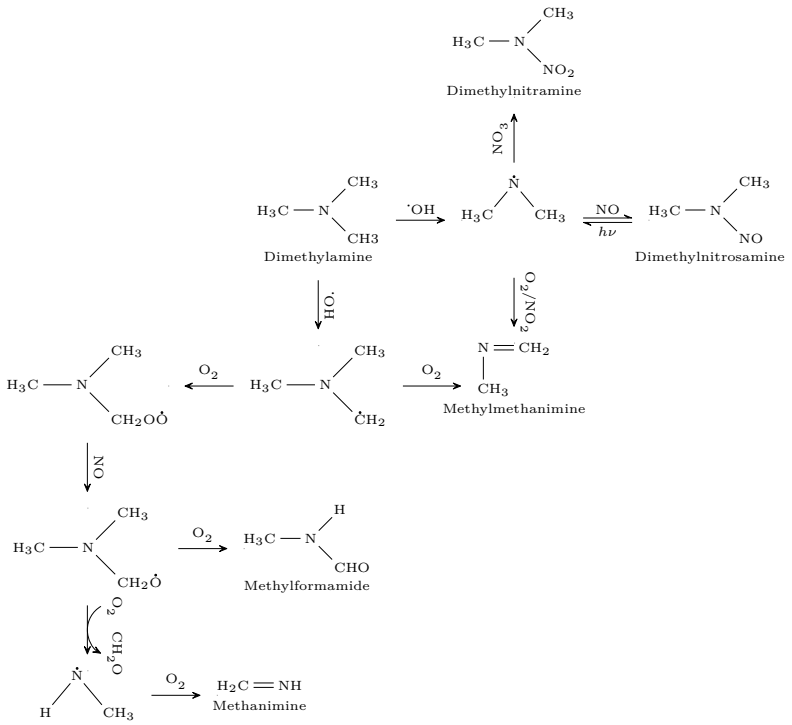


Figure 3: An example of photo-oxidation reactions of alkyl amines in the athmosphere, reaction scheme of major photo-oxidation routes of dimethylamine. Reaction scheme adapted from Nielsen et al. (2011, 2012).

aerosols in the atmosphere.

Yu and Luo (2014) identified that the most important removal processes of methylamines in the atmosphere are oxidation by OH radicals, dry an wet deposition and heterogeneous uptake by atmospheric particles (Figure 1). Removal of the methylamines by OH radicals vary from -80 Gg N yr^{-1} to -221 Gg N yr^{-1} depending on the used oxidation rate, this being the most effective removal mechanism. Heterogeneous uptake by atmospheric particles is highly dependent on the chemical composition of the particles. Assuming only sulphuric acid particles, methylamine removal from the atmosphere ranged from negligible to -210 Gg N yr^{-1} while the removal by atmospheric wet and dry deposition ranged from -41 Gg N yr^{-1} to -100 Gg N yr^{-1}.

2.3 Alkyl Amines in Terrestrial Environments

2.3.1 Soil and Vegetation as a Source of Alkyl Amines

Soil is a heterogeneous habitat which consists of mineral material released from weathered rock, organic material, and is void of continuous pore space filled with either water or air (Figure 4). Soil organic material (or matter) is a blend of fresh and transformed dead parts of organisms (plants, animals, and microbes) living above or beneath the soil surface. In the decomposing process, soil animals shred plant litter while feeding on it, and soil microbes transform soil organic matter to utilize it as a source of nutrient, energy and carbon.

Decomposition processes are typically driven by fungal communities in boreal forest soils (Lindahl et al., 2002). The most important fungal groups are saprothropic and mycorhizal fungi (Santalahti et al., 2016). Saprothrops are free living soil organic matter and litter decomposing fungi (Lindahl et al., 2002). In boreal forests, mycorrhizal fungi form symbiosis either with trees (ectomycorrhizal fungi) or with shrubs (ericoid mycorrhizal fungi) (Smith and Read, 2008). In the symbiosis between autotrophic plants and heterotrophic fungi, autotrophs provide sugars from photosynthesis to fungi and heterotrophic fungi improve availability of water and decompose soil organic matter to release nutrients (Smith and Read, 2008). Unlike mycorrhizal fungi, endophytic fungi grow entirely within plant tissue in a symbiotic relationship with the host plant or their functional role is unclear (Rodriguez et al., 2009).

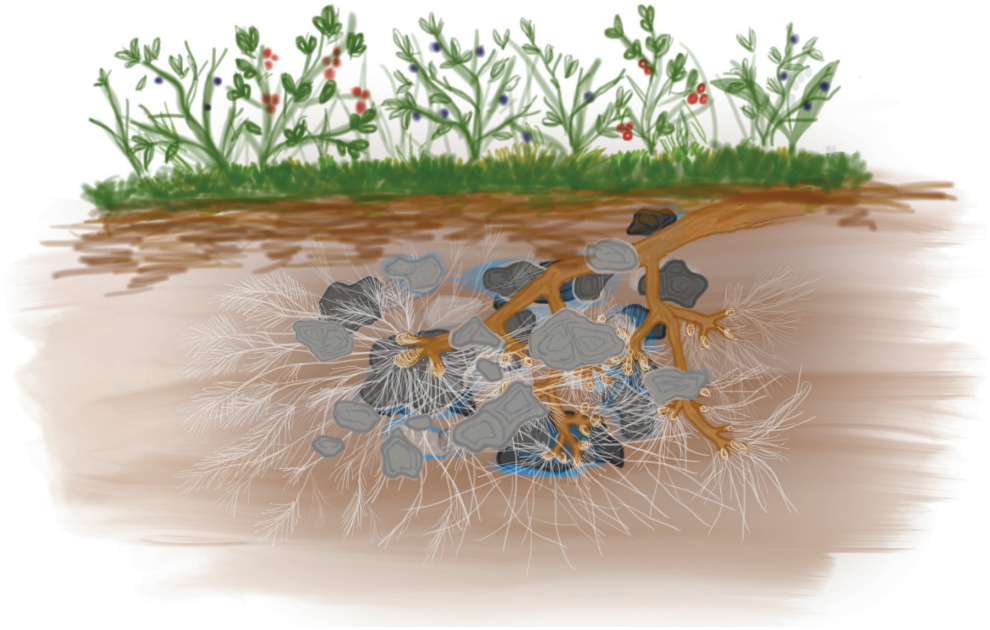


Figure 4: The simplified scheme of structure of soil in boreal forest. In general, soil consists mineral material with various particle sizes (from clays to rocks), organic material (e.g. roots, fungal hyphae, and litter), and maze of pore space filled with either water or air.

In a comprehensive review, Sintermann and Neftel (2015) stated that soil contains precursor substances of amines and that degradation of soil organic matter may produce amines (Figure 1). Based on studies made on spoiled food (Santos, 1996), Yan et al. (1996) and Xu et al. (2006) suggested that amines are produced during degradation of organic nitrogen compounds, especially in decarboxylation of amino acids. Dippold and Kuzyakov (2013) stated that direct microbial amino acid decarboxylation is unlikely to occur due to more favourable metabolic pathways (e.g. amino acid deamination).

Rappert and Müller (2005) have reviewed that alkyl amines are produced from quaternary ammonium compounds (e.g. carnitine, choline and betaine) through microbial activity. Quaternary ammonium compounds can be found in soil solutions in various environments (Warren, 2013b, 2014b) and can act as osmolytes in soils (Warren, 2013a, 2014a). Kim et al. (2001) introduced a process in which trimethylamine is formed from quaternary ammonium compounds. In the process, trimethylamine can

be degraded further to dimethylamine, monomethylamine, and ammonia by removing methyl groups in aerobic and anaerobic conditions. Microbes (archaea and bacteria) utilizing methylated compounds are ubiquitous in various environments and have been found to utilize methylamines (Rappert and Müller, 2005; Chistoserdova, 2015).

Fungi and plants can take up amino acids present in soil solution and use organic nitrogen as a nitrogen source (Näsholm et al., 2009). Plants and ectomycorrhizae are known to take up monomethylamine from soils (Kielland, 1994; Wallende and Read, 1999; Javelle et al., 1999) as it has been used as ammonium and nitrate transport analogue in studies to determine plant uptake of organic nitrogen. Methylamines are known to be degraded in several plant metabolic pathways (Suzuki, 1972; Boraphech and Thiravetyan, 2015), but relevance of amines as a nitrogen source to plants have remained unknown (Shiraishi et al., 2002; Vranova et al., 2011). In soils, trimethylamine have been found to act as a fungicide produced by soil microbes other than fungi (Xu et al., 2004). Methylamines can also be utilized in methanogenesis in thawing permafrost soil (Coolen and Orsi, 2015). Methylamine soil concentration measurements are scarce, Yu et al. (2002) reported soil litter leachates contain monomethylamine from 97±58 µmol L to 766±154 µmol L in Bishop pine (*Pinus muricata* D.Don.) and Mendocino cypress (*Cupressus pigmaea* (Lemmon) Sarg.) forests.

2.3.2 Exchange Processes of Alkyl Amines in Soil–Plant–Atmosphere Interfaces

Flowering plants in springtime, non-flowering vegetation during the growing season, and fungal sporocarps and decomposing litter towards autumn have been noted to release amines into the atmosphere (Sintermann and Neftel, 2015). Sintermann and Neftel (2015) measured trimethylamine emissions of Midland hawthorn (*Crataegus laevigata* (Poir.) DC) ranging from 0.019 to 0.038 nmol s^{-1} (45 flowers) and common dogwood (*Cornus sanguinea* L.) emission rates from 0.011 to 0.022 nmol s^{-1} (five umbels) in a dynamic chamber system. Farm animal excrement has previously been considered to be a strong trimethylamine source (10 nmol s^{-1}) (Sintermann et al., 2014). In a comparison, Sintermann and Neftel (2015) pointed out that a small number of measured flowers growing in a field can emit more trimethylamine than mixed farm animal excrement spread over one m^2. The only reported ecosystem scale alkyl amine flux measurement has been conducted over June to July 2009 in a Douglas fir (*Pseudotsuga*

menziesii (Mirb.) Franco) forest in the Netherlands (Copeland et al., 2014). The measured alkyl amine was trimethylamine and during the one-month measurement period trimethylamine flux ranged from -8 to 8 μmol m^{-2} h^{-1} and showed a net deposition during the measurement period.

Plants may take up atmospheric trimethylamine as demonstrated with tropical *Pterocarpus indicus* Willd. and *Sansevieria trifasciata* Prain (Boraphech and Thiravetyan, 2015). Boraphech and Thiravetyan (2015) found that gas-phase trimethylamine can be taken up by stomatal uptake or it can be deposited on the epicuticular wax. In general, the deposition and uptake processes are not well known (Hertel et al., 2011). However, there are indications that deposition and temperature dependent evaporation of methylamines and NH_3 are similar (You et al., 2014).

Assuming analogous behaviour to NH_3 based on You et al. (2014) and Sintermann and Neftel (2015), alkyl amines may have sink and source processes within vegetation canopies and in the soil. In the case of NH_3, the exchange processes are bi-directional in nature, and as a result the exchange depends on the concentrations and acidity of a solution (H^+ concentration) (Sutton et al., 1998). In plants, leaf apoplastic concentrations of NH_4^+ and H^+ affect the stomatal concentration of NH_3 (Farquhar et al., 1980). Similarly, in soils the gas-phase concentration of NH_3 in the soil air space depends on ammonium NH_4^+ and H^+ concentrations in soil solution (Nemitz et al., 2001). The concentration of soil air above soil solution can be estimated by assuming equilibrium between the aqueous solution and the gas phase (Farquhar et al., 1980; Nemitz et al., 2000).

The soil–atmosphere exchange process is driven by the concentration gradient between reservoirs, e.g. soil, vegetation and the atmosphere (Figure 1). Mass exchange between soil–plant–atmosphere interfaces can be estimated by using a gradient-diffusion approximation presented as an electrical circuit analogy (Hicks et al., 1987; Seinfield and Pandis, 1998; Sutton et al., 1998). In the analogy, the transport path from a source/sink to a measurement point is divided into distinctive layers. Within each layer the transport process is described as a resistance. In free air, resistance is characterized by turbulent mixing. In air close to surfaces, transport is due to compound specific diffusion but is affected by atmospheric turbulence. In air-filled soil spores, transport is characterized by the elongated diffusion pathway due to molecular diffusion through porous media.

3 Methodology

3.1 Measurement Sites

Alkyl amines were studied in two northern latitude measurement sites in southern Finland, one in a boreal forest (Hari and Kulmala, 2005) and another in an urban environment (Järvi et al, 2009). At the measurement stations various environmental parameters were monitored routinely (Hari and Kulmala, 2005; Järvi et al, 2009; Junninen et al., 2009) and the collected data was used in **Paper I**, **II**, **IV**. In addition, the ambient air concentrations of volatile organic compounds (VOC) were measured in two campaigns with a thermodesorption instrument connected to a gas-chromatography mass spectrometer (TD-GS-MS). The VOC measurements are described in more detail by Hakola et al. (2012) for the forest site and by Héllen et al. (2012) for the urban site.

3.1.1 Forest Site

The forest site is a Scots pine dominated forest located at the SMEAR II station (Station for Measuring Ecosystem–Atmosphere Relations) at Hyytiälä (61°51'N, 24°17'E, 180 m a.s.l) in southern Finland (Hari and Kulmala, 2005). The stand is about 50 years old in 2011 and dominated by Scots pine (*Pinus sylvestris* L.) with an average height of 18 m in 2011. The forest canopy is open with an average tree density of about 1370 stems per hectare (Ilvesniemi et al., 2009). In the understory, an occasional Norway spruce (*Picea abies* (L.) H. Karst.), birch (*Betula* L. spp.), and European aspen (*Populus tremula* L.) are present.

At the ground level, the predominant plant species are lingonberry (*Vaccinium vitis-idaea* L.), billberry (*Vaccinium myrtillus* L.), wavy hairgrass (*Deschampsia flexuosa* (L.) Trin.), and heather (*Calluna vulgaris* (L.) Hull). The most common mosses are Schreber's big red stem moss (*Pleurozium schreberi* (Brid.) Mitt.) and a dicranum moss (*Dicranum* Hedw. sp.) (Ilvesniemi et al., 2009). The soil at the site is Haplic podzol on glacial till, with an average depth of 0.5 m to 0.7 m. The mean pH_{H_2O} at 2 cm depth in mineral soil is 5.3 (**Paper IV**).

3.1.2 Urban Site

The urban measurement station SMEAR III is located about 5 km northeast of the Helsinki city center (60°12'N, 24°58'E, 28 m a.s.l) in southern Finland (Järvi et al, 2009). In the vicinity of the SMEAR III station there are buildings, parking lots, roads, patchy forest, low vegetation, and gardens. The surroundings can be divided into three different land-use areas; to the north, buildings and parking lots, to the west, gardens and parks, and to the east, main roads and buildings. The Baltic sea front is about one kilometre to the east from the station. The total population of the Helsinki metropolitan area in year 2011 was approximately 1 million and the coverage of the commuting area was 764 km^2.

3.2 Measurement Methods of Alkyl Amines in Environmental Samples

Alkyl amines were measured from three different environmental sample matrices: soil extracts, fungal hyphal biomass, and air samples collected onto filters in the forest and urban sites. The measured alkyl amines from different samples are listed in Table 2. Hereafter, the abbreviations presented in Table 2 are used for the measured alkyl amines.

Table 2: List of alkyl amines measured in different environmental sample matrices. The terms used in the sample matrix are as follows: air is ambient air, soil is boreal forest soil, and fungal is fungal hyphal biomass.

Alkyl amine	Abbreviation	Sample matrix	Paper
Monomethylamine	MMA	soil, fungal	III, IV
Dimethylamine	DMA	air, soil, fungal	I, II, III, IV
Ethylamine	EA	air	I, II
Trimethylamine	TMA	air	I, II
Propylamine	PA	air	I, II
Diethylamine	DEA	air, soil, fungal	I, II, III, IV
Butylamine	BA	air	I, II
Isobutylamine	iso-BA	soil, fungal	III, IV
sec-Butylamine	sec-BA	soil, fungal	III, IV

3.2.1 Sample Collection and Analysis of Alkyl Amines in Ambient Air

The sample collection and analytical methods used to determine ambient air concentrations of alkyl amines were developed based on the methods introduced by Rampfl et al. (2008). The sample collection method is based on air drawn through a stack of filters. The used methods were robust, cheap to implement and maintain in various environments if compared to modern on-line techniques (Szulejko and Kim, 2014; Sipilä et al., 2015; Hemmilä et al., 2014). As a drawback, due to low ambient air concentrations of alkyl amines large sample volumes are needed and samples have to be collected over several days to achieve detectable quantities of alkyl amines.

Samples were collected by pumping ambient air through a stack of filters at a flow rate of 16 L min^{-1}. The sample collection time was 24 h during the weekdays and 72 h over the weekends. Later, the collected samples were pooled as weekly samples. The stack of filters consisted of a polytetrafluoroethylene (PTFE) membrane filter (3.0 µm FS, Millipore: Fluoropore®; EMD Millipore Corp., Billerca, MA, USA) to remove particles from the sample flow, and a phosphorus-acid-impregnated fibreglass filter to collect alkyl amines as aminium phosphate ($R_3NH^+H_2PO_4^-$) salts. The fibreglass filters were impregnated with phosphorus acid according to the procedure introduced in Rampfl et al. (2008). The sufficient collection capacity of the impregnated filters was tested. In the tests, alkyl amines were injected into zero air flow and collected onto the impregnated filters (**Paper I**). Alkyl amines were not observed to pass through the impregnated filters.

Aminium ions were extracted from the filters with ultrapure water in an ultrasonic bath and were analysed by high-performance liquid chromatography electrospray ionization – ion trap mass spectrometer (HPLC-ESI-ITMS) (Agilent 1100 series LC/MSD; Agilent Technologies Santa Clara, CA, USA). Deuterated diethyl-d_{10}-amine (Sigma–Aldrich: Isotec™; Sigma–Aldrich, St. Louis, MO, USA) was used as an internal standard and a five-point external standard was used for all measured alkyl amines. In the LC system, a Discovery® HS F5 HPLC (Supelco Analytical; Supelco Inc., Bellefonte, PA, USA) was used as an analytical column and an HPLC SecurityGuard™ cartridge (Phenomenex®; Phenomenex, Torrence, CA, USA) was used as a precolumn. Water and acetonitrile with formic acid (0.02%) as a buffer were used as eluents.

The measured compounds were EA, DMA, TMA, PA, DEA, BA, and triethylamine. The column was unable to resolve DMA and EA or TMA and PA, and their results

are given as a sum (hereafter DMA+EA and TMA+PA, respectively). Uncertainty of the analyses is reported as standard deviation between four parallel analysis results and shown as error bars in Figure 7. The limit of detection was determined to be three times the standard deviation of the field blank levels. The detection limits are shown separately for the forest and urban background sites in Table 4. Ambient air concentrations were measured parallel at the forest stand in Hyytiälä and at the urban background site in Helsinki from May to August 2011 and measurements were continued in Hyytiälä till the end of October 2011. A more comprehensive description of the sample collection and analytical method can be found in **Paper I**.

3.2.2 Determination of Amines in Soil and Fungal Biomass

In order to determine amine concentrations from the soil, fresh soil samples were extracted with using 1 M potassium chloride (1:4). Amine contents in fungal functional groups (ectomychorrhiza, ericoid mychorrhiza, endophytes, saprotrophic fungi) found in the boreal forest soil were determined by growing 19 fungal strains in axenic liquid cultures (Bäck et al., 2010). From the pure fungal culture the biomass was collected after six weeks and extracted and analysed for amines. A hyphal biomass was extracted with 1% acetic acid–acetonitrile (1:1) solution by using an assisted dynamic sonication method.

The analytical method to quantify amine from the soil and fungal biomass extractions is introduced by Ruiz-Jiminez et al. (2012). The development of the analytical method continued along with the measurements. The extracts were first dansylated, and analysed by high-performance liquid chromatography electrospray ionization – triple quadrupole mass spectrometry (Agilent 1260 Infinity and Agilent 6420, respectively; Agilent Technologies, Santa Clara, CA, USA). In the LC system, a Hibar® HR RP-18 endcapped (Purosphere®; Merck KGaA, Darmstad, Germany) was used as an analytical column. Water and acetonitrile with acetic acid (1%) as a buffer were used as eluents.

A sample extraction and analytical method to measure amine concentrations in soil and fungal hyphae are described in more detail in **Paper III** and **Paper IV** and the measured amines are presented in Table 2.

3.3 Pot-Scale Experiment to Study Alkyl Amines in Soil

The effect of plant and soil organic matter degrading enzymes on amine synthesis taking place in soil were studied in a pot-scale experiment. The experiment was carried out in a greenhouse from May to October under natural light and in a temperature ranging from 15 °C to 20 °. The experiment consisted of six treatments altogether (Figure 5). Three of the treatments were planted with one-year-old Scots pine seedlings and three were non-planted. Planted and non-planted treatments were divided further into three enzymatic treatments to stimulate soil organic matter degradation; soil amended with BSA (Bovine Serum Albumin), proteases, and laccase, manganese peroxidase and protease. The BSA amended soil was regarded as the control treatment.

The soil used in the experiment was organic (mixed F and H horizons) and it was collected in the vicinity of the SMEAR II station at Hyytälä. The experiment had three phases, a 14-week stabilizing period after the set-up to let the soil stabilize and the seedlings grow, a six-week enzyme treatment period and a four-week post-treatment period to let the soil stabilize and the seedlings grow. Approximately six months after the set-up, the experiment was harvested. For a more detailed description of the experiment and chemical analysis conducted see **Paper III**.

3.4 Transport of Alkyl Amines and Atmospheric Reactivity

To estimate the soil–atmosphere mass transport of selected alkyl amines (DMA and DEA) a gradient-diffusion approximation was used. The approximation is presented as an electrical resistance analogy (Hicks et al., 1987; Seinfield and Pandis, 1998; Sutton et al., 1998). In the resistance analogy, the transport path is divided into layers and each layer is described as a resistance (Figure 6). Soil air concentrations were calculated by using soil concentrations of DMA and DEA measured in the pot-scale experiment by assuming equilibrium between the aqueous soil solution and the soil air (Farquhar et al., 1980; Nemitz et al., 2000). A more detailed description of the estimation methods are presented in **Paper IV**. In the calculations, analogous behaviour of alkyl amines to NH_3 was assumed.

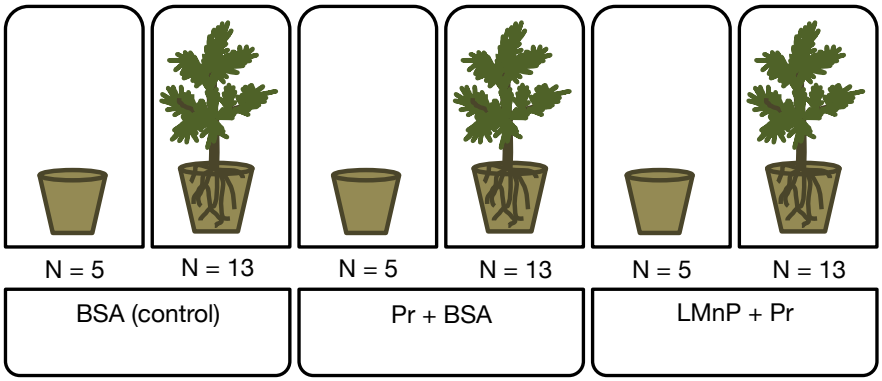


Figure 5: The set-up of an experiment to study effect of a Scots pine and soil organic matter degrading enzymes on organic nitrogen pools in boreal forest soil in **Paper III**. The experiment consists of three different enzymatic treatments with or without Scots pine seedlings. In the control treatment soil was amanded with bovine serum albumin (BSA), Pr+BSA treatments were amanded with proteases (Pr) and with BSA, and LMnP+Pr treatment was amanded with Laccase, Manganese peroxidase (LMnP) and with Pr.

3.4.1 Estimation of Soil Air Concentrations of Alkyl Amines

In an aqueous solution, the concentration of alkyl amine is the sum of the non-dissociated (R_3N) and the dissociated (R_3NH^+) species. The non-dissociated fraction (f_{R_3N}) of the total alkyl amine concentration can be estimated by using the equilibrium thermodynamic principle (Montes et al., 2009), when the activity of R_3N and R_3NH^+ are assumed to be equal (Eq. 2)

$$f_{R_3N} = \frac{[R_3N]}{[R_3N] + [R_3NH^+]} = \frac{1}{1 + \frac{[H^+]}{K_{a,T}}}. \quad (2)$$

The f_{R_3N} is dependent on the soil H^+ concentration and temperature corrected acid dissociation constant ($K_{a,T}$). The negative logarithms of acid dissociation constants (pK_a) of DMA and DEA are 10.3 and 10.5, respectively. The temperature dependency was taken into account as presented in **Paper IV**. The soil solution concentrations of DMA and DEA are based on the measured 1M KCl extractions presented in **Paper III**. The soil solution concentrations of DMA and DEA were 92.3 µmol L^{-1} and 0.296 µmol L^{-1}, respectively.

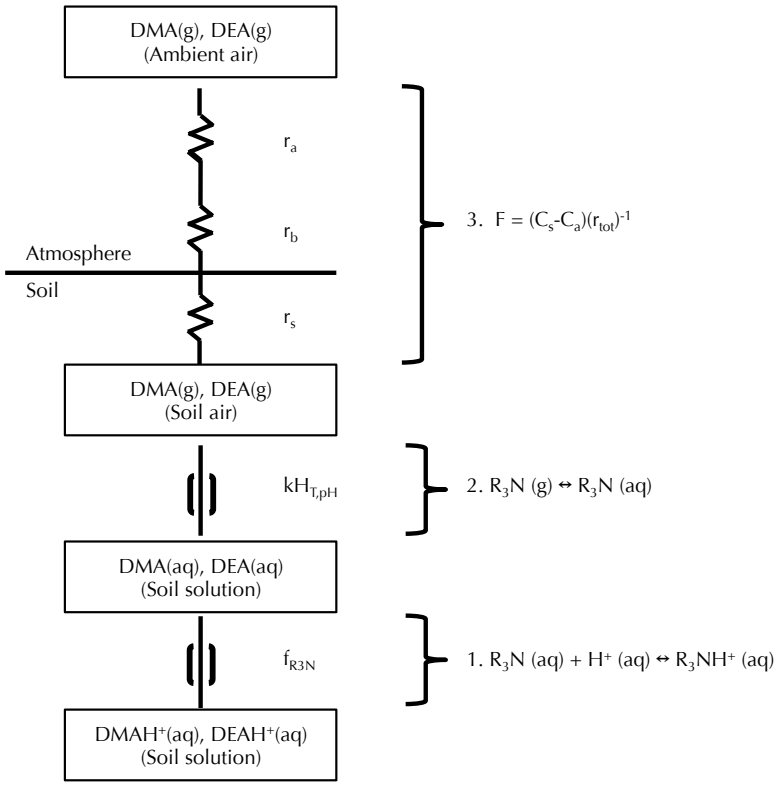


Figure 6: The scheme used for soil flux estimation of dimethylamine (DMA) and diethylamine (DEA) from reactions occurring in soil solution to transfer from soil air to ambient air. The boxes denote DMA and DEA concentrations in soil solution, soil air and ambient air. The numbers denote the steps in the flux estimation. Step 1: acid-base reaction and protonation of alkylamine; step 2: the partitioning of non-protonated DMA and DEA between the aqueous and gas phases; step 3: the flux of DMA and DEA between the soil and ambient air in which the flux is determined by dividing the concentration gradient by the sum of resistances (soil resistance, r_g; quasi-laminar layer resistance, r_b; aerodynamic resistance, r_a).

The gas-phase concentration in soil pore space can be determined according to Henry's law (Eq. 3), which states that the solubility of non-dissociated gas in a solution is directly proportional to the gas-phase concentration of alkyl amine above the solution.

$$k_H = \frac{C_{aq}}{C_s} \tag{3}$$

where k_H is the temperature and pH dependent Henry's law coefficient, C_{aq} is the non-dissociated aqueous phase concentration, and C_s is the gas-phase concentration in soil air. The temperature dependency was taken into an account as presented in **Paper IV**. To take into an account pH dependence of k_H, the effective Henry's law coefficients $k_{H,ef}$ were calculated as presented in Seinfield and Pandis (1998)

$$k_{H,ef} = k_H \frac{1 + [\mathrm{H}^+]}{K_{a,T}} \tag{4}$$

where $k_{H,ef}$ is the effective Henry's law coefficient taking into account the dissociation reaction in aqueous phase, $[\mathrm{H}^+]$ is the proton concentration in a solution and $K_{a,T}$ is the temperature corrected acid dissociation coefficient.

3.4.2 Estimation of Soil–Atmosphre Exchange of Alkyl Amines

To estimate the flux between the soil–atmosphere boundary a flux-gradient relationship was used

$$F = \frac{C_s - C_a}{r_{tot}}, \tag{5}$$

where C_s and C_a are the concentrations (nmol m^{-3}) of DMA or DEA in soil air space and the ambient air concentration in below canopy air space, respectively. C_a is the measured ambient air concentration presented in **Paper I**. In the flux-gradient relationship, r_{tot} is the sum of soil resistance (r_g), quasi-laminar flow resistance (r_b), and aerodynamic resistance (r_a).

The soil resistance (r_g, $\mathrm{m\ s}^{-1}$) describes molecular diffusion of gas through a porous soil matrix whose cross-sectional area is reduced and diffusion pathway length is increased relative to free air. The r_g in depth Δz in organic soil is estimated as

$$r_g = \frac{\Delta z_s}{D_p} = \frac{\Delta z_s}{D_o \theta_a^b}, \tag{6}$$

where molecular diffusivity D_p in soil is computed from the molecular diffusivity in free air (D_o) and air filled porosity (θ_a). b (1.1) is an empirical parameter to take into account the reduced cross-sectional area and the increased diffusion pathway length in porous media as reported for the humus layer in Glinski and Stepniewski (1985) .

At the soil surface, a quasi-laminar boundary layer is formed, in which movement of gas is due to molecular diffusion, and the thickness of the layer is dependent on the turbulence above the layer. The transport through the layer is described as quasi-laminar layer resistance (r_b, m s^{-1}) according to Schuepp (1977)

$$r_b = \frac{Sc - \ln {}^{\delta_o}/_{z_1}}{k_v u_{*g} z_1}, \tag{7}$$

where Sc is the Schmidt number, k_v the von Karman constant (0.41), u_{*g} the near-ground friction velocity, $\delta_o = {}^{D_o}/_{k_v u_{*g}}$ is the height above ground where molecular diffusivity and turbulent transport efficiency are equal, z_1 the height below which the wind profile is assumed logarithmic.

The mass transport between the soil surface and the concentration measurement point (z_m) in free air is due to atmospheric turbulence. The turbulent transport of gas is described as an aerodynamic resistance (r_a). The r_a is calculated by integrating the inverse of eddy diffusivity K_s over the layer as introduced by Baldocchi (1988)

$$r_a = \int_0^{z_m} \frac{1}{K_s(z)} \mathrm{d}z \tag{8}$$

The $K_s(z)$ profile and u_{*g} needed to calculate r_a and r_b, respectively, are obtained from a first-order closure model for momentum exchange within the canopy by Launiainen et al. (2013, 2015).

3.4.3 Sensitivity analysis of Soil Air Concentrations and Soil–Atmosphre Exchange Estimates

To assess the effect of environmental variables on estimated soil air concentrations and fluxes, a sensitivity analysis was performed. The measured ranges were used to assess the sensitivity of calculations to variations in temperature, soil water content, friction velocity and pH. The range of temperature used in the sensitivity analysis was from 0 to 20 °C. Soil water content ranged from 0.1 to 0.4 m^3 m^{-3}, and friction velocity from 0.1 to 1.5 m s^{-1}. The 10 and 90 percentile values were calculated for pH from long-term suction cup lysimeter measurements, and used as the lower (4.5) and upper (6.0) limit for the range. As the soil solution concentrations were assumed to be constant during the study period from May to October 2011, the soil solution concentrations were based on a 1M KCl extraction, giving the upper limit for the range. The effect of soil solution concentrations was assessed using a range from 0 to 100 µmol L^{-1}. In the flux estimation, the effect of soil depth was assessed using a range from 0 to 0.15 m.

3.4.4 Atmospheric Reactivity of Alkyl Amines

The relative role of alkyl amines in the local chemistry at the measurement sites was assessed by scaling the measured alkyl amine concentrations against hydroxyl radical (OH) reactivity and compared with those of volatile organic compounds (VOCs, 13 aromatic hydrocarbons and 8 terpenes) measured at the forest site (SMEAR II) and at the urban site (SMEAR III) (**Paper II**). The OH reactivity (s^{-1}) was calculated from the reaction rate constant ($k_{X,OH}$, Table 3) between a compound and the OH radical and from the atmospheric concentration ([X], volumetric mixing ratio) of a compound (Eq. 9)

$$\text{OH reactivity} = k_{X,OH}[\text{X}]. \tag{9}$$

As DMA and DEA are reactive compounds in the atmosphere, the importance of the losses due to chemical reactions occurring during the transport process in free air was evaluated by calculating the Damköhler number (DA)

$$DA = \frac{\tau_{tr}}{\tau_{ch}}. \tag{10}$$

DA is the ratio of characteristic turbulent timescale τ_{tr} to the chemical lifetime τ_{ch}. $\tau_{tr} = {}^{r_a}/_{z_{mea}}$ is the transport time between soil surface and the concentration measurement point within the canopy. In the ambient air DMA and DEA are mainly reacting with OH radicals (Ge et al., 2011) and their τ_{ch} are 3.2 h and 2.6 h, respectively (**Paper II**). A DA smaller than unity indicates that chemical reactions are not disturbing the linkage between the measurement point and the source or sink. If a DA value is smaller than 0.1, flux divergence due to chemical reactions can be neglected from flux estimates (Rinne et al., 2012).

Table 3: The reaction rates constants (k(OH)) of the alkyl amines with atmospheric OH radical. The reaction rates of α-pinene (monoterpene) and toluene (aromatic hydrocarbon) are given for a comparison.

Alkyl amine	k(OH) [cm^3 molec^{-1} s-1]	Reference
Dimethylamine	6.62E-11	Manion et al. (2015)
Trimethylamine	6.11E-11	Manion et al. (2015)
Ethylamine	2.77E-11	Manion et al. (2015)
Diethylamine	8.07E-11	Chemfinder (2013)
Butylamine	3.4E-11	Chemfinder (2013)
α-pinene	5.22E-11	Manion et al. (2015)
toluene	6.16E-12	Manion et al. (2015)

4 Results and Discussions

4.1 Alkyl Amines in Ambient Air

In both the forest and urban measurement sites, DMA+EA was the most abundant alkyl amine measured, with an average ambient air concentration of 42.2 ppt_V in the forest site and 23.6 ppt_V in the urban site (Table 4). The second most abundant measured alkyl amine was TMA+PA, with an average ambient air concentration of 21.1 ppt_V and 8.4 ppt_V in the forest and urban sites, respectively. The weekly course of the measured alkyl amine concentrations in both measurement sites is shown in Figure 7.

Table 4: The mean ambient air concentrations (ppt_V), measured range and detection limits of alkyl amines at the forest site (SMEAR II) in Hyytiälä (**Paper I**) and at the urban site (SMEAR III) in Helsinki (**Paper II**), Finland. The measurement period was from May to October 2011 in the forest site and from May to August 2011 in the urban site. Alkyl amines included in the thesis were dimethylamine+ethylamine (DMA+EA), trimethylamine+propylamine (TMA+PA), diethylamine (DEA), and butylamine (BA). The detection limit (DL) was three times the standard deviation of the field blank levels.

Alkyl amine	Forest [ppt_V]	DL (forest)	Urban [ppt_V]	DL (urban)
DMA+EA	42.2 (20.1-157)	0.2	23.6 (<DL-54.9)	9.5
TMA+PA	21.1 (4.6-103)	0.4	8.4 (3.9-26.9)	2.4
DEA	8.1 (<DL-15.5)	6.7	0.3 (<DL-1.3)	0.08
BA	2.4 (<DL)	8.9	0.3 (0.1-0.56)	0.06

The highest EA+DMA (157 $\pm$ 20 ppt_V) and TMA+PA (102 $\pm$ 61 ppt_V) concentrations were measured from September to October in the forest site. In the urban site, the highest concentration of DMA+EA (55 ppt_V) was measured in early August and the highest concentration of TMA+PA (8.4 ppt_V) was measured in mid-June. In the forest site, DEA had a different seasonal course compared to EA+DMA and TMA+PA, having the highest concentrations (7.4 ppt $_V$) in June to July (Figure 7). In the urban background site, other observed alkyl amines (DEA and BA) were lower than 1 ppt_V during the measurement period, and in the forest site, the measured concentration of BA was lower than the detection limit (8.9 ppt_V) during the measurement period.

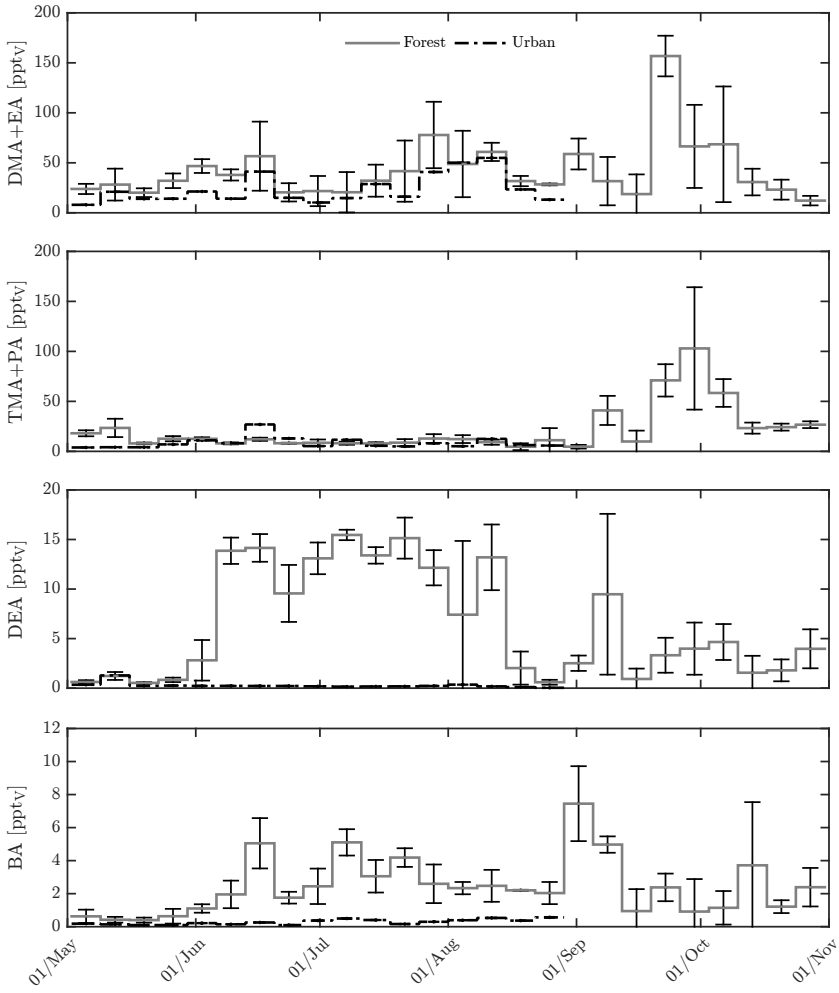


Figure 7: The ambient air concentrations (ppt_V) of the measured alkyl amines and their standard deviations at the SMEAR II forest site in Hyytiälä from May to October 2011 and at the SMEAR III urban site in Helsinki from May to August 2011 (**Paper I** and **Paper II**). DMA+EA stands for dimethylamine+ethylamine, TMA+PA trimethylamine+propylamine, DEA diethylamine, and BA butylamine. The detection limits (forest/urban) in ppt_V: DMA+EA 0.2/9.5, TMA+PA 0.4/2.4, DEA 6.7/0.08, and BA 8.9/0.06.

When the measured concentrations from both sites were compared, the ambient air concentrations of alkyl amines were higher in the forest site than in the urban site. The concentration differences between the sites were more prominent for DEA and BA than for DMA+EA and TMA+PA. At the forest site, the occurrence of the concentration maxima was different between the measured amines. The highest concentrations of EA+DMA and TMA+PA were observed in September and October. In the case of DEA, the highest concentrations were observed during July and August indicating different sources as in the case of EA+DMA and TMA+PA.

To achieve detectable quantities of alkyl amines, samples were collected for several days. During the collection time, it is possible that alkyl amines evaporated from particles on the PTFE filter causing an artefact in the gas-phase measurements. The large variation around the measurement point is due to the fact that samples had quantities of alkyl amines close to the resolving capability of the analytical device (HPLC-ESI-MS). Repeated analyses of samples were conducted to determine the concentration and its variation. The differences in the detection limits between the forest and urban sites are due to the method used. The detection limits were based on variation in concentrations in the field blanks. The variation in the detection limits are caused by differences in handling, storage and shipment of the samples between the measurement sites.

The ambient air concentrations measured in the forest site and in the urban site are in line with the other alkyl amine measurements conducted in various environments (rural, forest, urban and marine environments) in different parts of the world (Table 1). Unexpectedly, the ambient air concentrations were higher in the forest environment than in the urban background, despite agricultural activity in the vicinity of the urban site (Makkonen et al., 2012). By comparison, the ambient air concentrations of ammonia (NH_3) were orders of magnitude higher than those of alkyl amines (Makkonen et al., 2012, 2014). In the forest site, the concentrations of NH_3 ranged from 20 ppt_V to 830 ppt_V (Makkonen et al., 2014), and in the urban site from below 450 ppt_V to 3000 ppt_V (Makkonen et al., 2012). Unlike in the case of alkyl amines, the ambient air concentrations of NH_3 were higher in the urban site. In the both sites, concentrations of NH_3 had the lowest values in the winter, and the highest concentrations were measured in the spring at the urban site (Makkonen et al., 2012) and in the summer at the forest site (Makkonen et al., 2014). The higher concentrations of alkyl amines measured in the forest station imply a strong source within or in the vicinity of the

studied forest.

There were two other ambient air concentration measurements of alkyl amines conducted in the forest site. The most recent study (Sipilä et al., 2015) measured alkyl amines by using a chemical ionization atmospheric pressure interface time-of-flight mass spectrometer (CI-API-ToF). The ambient air concentrations of DMA measured by Sipilä et al. (2015) were much lower than those presented in the **Paper I**. In the measurements of Sipilä et al. (2015), the ambient air concentrations of DMA ranged from 0.01 ppt_V to 1.5 ppt_V, from May to June 2013. Sipilä et al. (2015) advice to take these results with caution due to problems in the measurement system and due to the fact that they did not calibrate the instrument against known alkyl amines. The preliminary results of Hemmilä et al. (2014) from June to July 2014 measured by an online ion chromatography connected with a quadrupole mass spectrometer, the ambient air concentrations of DMA, EA and DEA were in the same range as the concentrations of DMA+EA and DEA presented in **Paper I**.

The ambient air concentrations of the measured alkyl amines were compared with environmental parameters continuously measured at the measurement sites and VOC measurements conducted at the sites during the measurement period. Pearson's correlation analysis was performed on the measured alkyl amines **Paper I**. It has to be noted that the one-week sampling time of the ambient air coupled with atmospheric mixing, chemical reactions and gas-to-particle conversion occurring in the atmosphere, deposition of alkyl amines onto surfaces, and temperature dependent evaporation from the surfaces affect the measured concentrations (Seinfield and Pandis, 1998; Ge et al., 2011) and makes it difficult to assess sources and sinks of alkyl amines in the sites.

In the forest site, the measured alkyl amine concentrations of DMA+EA and TMA+PA had a positive correlation ($r=0.47$ for DMA+EA and $r=0.47$ for TMA+EA, $p<0.05$) with the litterfall. In addition, TMA+PA had a positive correlation ($r=0.57$, $p<0.05$) with the soil water content. This may be due to the TMA+PA peak measured in late autumn and the low concentrations measured during the summer. This can be seen as a negative correlation ($r=-0.43$, $p<0.05$) with the air temperature. The measured ambient air concentrations of DEA had a statistically significant ($p<0.05$) relationship with both the soil and air temperatures ($r=0.67$ and $r=0.63$, respectively). This may explain the resemblance between the course of ambient air concentrations of DEA and those of VOCs. When comparing the measured ambient air concentrations of alkyl amines with atmospheric trace gases (nitric oxide, nitric oxide and nitrogen dioxide,

ozone, and carbon monoxide), only in the case of DMA+EA a correlation was found. DMA+EA had a negative correlation with carbon monoxide (r=-0.50, $p<0.05$). At the urban site, DEA peaked with sulphur dioxide and nitric oxide. Elevated concentrations of DEA, sulphur dioxide and nitric oxide coincide with wind from the south or southwest where a seafront, a harbour and a power plant are located.

The measured ambient air concentrations of DEA have a similar seasonal pattern to NH_3 as previously measured by Makkonen et al. (2012). Makkonen et al. (2012) suggest that the highest concentrations of NH_3 in the forest site may be emitted from the vegetation based on temperature dependency of NH_3, differences in diurnal cycle between gas-phase NH_3 and particulate NH_4^+, and similarity of the ambient air concentrations of NH_3 to ambient air concentrations of monoterpenes. The ambient air concentrations of DEA and monoterpenes (camphene, β-pinene, α-pinene, and Δ^3-carene) were found to be similar during the measurement period from May to October 2011, and as in the case of NH_3, it is possible that the source of the DEA lies in the vegetation. A similar temperature dependency has been found for NH_3 and alkyl amines with three carbon atoms (e.g. TMA) in measurements in a deciduous forest in Alabama (US) (You et al., 2014).

In late autumn (from September to October), Scots pines shed old needles. It has been shown that soil emissions of nitrous oxide (N_2O) and monoterpenes are increased during the litterfall (Pihlatie et al., 2007; Aaltonen et al., 2011; Mäki et al., 2016). Fresh needle litter provides easily decomposable nitrogen containing material for soil decomposers, and nitrogen rich leachate from fresh needle litter has an even more immediate effect on soil nitrogen concentrations (Pihlatie et al., 2007; Starr et al., 2014). The litterfall and the fungal community change (Santalahti et al., 2016) coincide with the measured ambient air concentration peaks of DMA+EA and TMA+PA in the boreal forest site in autumn.

4.2 Alkyl Amines in Fungal Biomass

The sum of all of the observed amines (including ethanolamine and histamine) ranged from 19 to 5400 μg g^{-1} d.w. in the fungal pure culture strains (**Paper IV**). Amine concentrations were highly variable between the fungal strains, the most abundant amines were DMA (93 μg g^{-1} d.w.) and MMA (6.3 μg g^{-1} d.w.), based on the median values. The measured mean amine concentrations in the fungal functional groups are

presented in Table 5. The highest amine concentrations were observed in the saprotrophic and ectomycorrhizal fungi (1005 ± 878 μg g^{-1} d.w. and 717 ± 390 μg g^{-1} d.w., respectively). DMA had the highest concentration from all the measured amines across the functional groups ranging from 49 (± 11) to 856 (± 791) μg g^{-1} d.w.. Other measured alkyl amines (MMA, DEA, iso-BA, and sec-BA) ranged from 0.4 (± 0.2) to 42 (± 35) μg g^{-1} d.w. (Table 5). The concentration of DEA in fungal hyphal biomass separated from the boreal forest soil was found to be in the same range as in the pure cultured ectomycorrhizal fungal biomass (**Paper IV**).

Ectomycorrhizal and saprotrophic fungi have been found to be the most abundant fungal groups in the Scots pine forest (Santalahti et al., 2016). Wallander et al. (2001) have estimated that ectomycorrhizal fungal biomass ranges from 700 to 900 kg ha^{-1} in Swedish boreal Norway spruce forest. In an extensive review by Sintermann and Neftel (2015), MMA and DMA have previously been found in fungal sporocarps. Unlike fungal hyphae, fungal sporocarps occur seasonally and periodically, whereas fungal hyphae is present in forest soil throughout the year (Santalahti et al., 2016). The high quantities of alkyl amines measured in the fungal hyphal biomass imply that fungal biomass is an important reservoir of alkyl amines in boreal forest soil.

According to Santalahti et al. (2016), the soil fungal community of the boreal forest soil went through two clear seasonal changes, one in early spring and another in late autumn. From early spring to late autumn, the fungal community is dominated by ectomycorrhizal fungi. In late autumn ectomycorrhizal fungi disappears and saprotrophic fungi dominates the fungal community during winter. As the fungal hyphae of ectomycorrhizal fungi contained high quantities of alkyl amines, it may have been released into the soil while ectomycorrhizal fungi disappeared in late autumn, and subsequently into the atmosphere.

4.3 Alkyl Amines in Soil

Alkyl amine concentrations in the soil were measured in the pot experiment presented in **Paper III**. In the experiment, the effects of a Scots pine and soil organic matter degrading enzymes on soil organic nitrogen were studied. Interestingly, an addition of protease or organic matter degrading enzymes did not have an effect on the alkyl amine nitrogen contents in the soil implying that released amino acids are not necessarily precursors for alkyl amines in soil, in line with Dippold and Kuzyakov (2013).

Table 5: The mean of alkyl amine concentrations in the fungal functional groups and in the agar growth media, and their standard errors. The number of cultured fungal strains in the functional groups is denoted separately. The sum of amines includes also ethanolamine and histamine

Alkyl amine	Ectomychorrhiza [μg g^{-1} d.w.]	Ericoid mycorrhiza [μg g^{-1} d.w.]	Endophytic fungi [μg g^{-1} d.w.]	Saprotrophic fungi [μg g^{-1} d.w.]	Agar media [μg g^{-1} d.w.]
Monomethylamine	29 (± 16)	10 (± 1.9)	2.4 (± 0.4)	42 (± 35)	1.2
Dimethylamine	622 (± 333)	236 (± 42)	49 (± 11)	856 (± 791)	28
Diethylamine	16 (± 9.9)	5.4 (± 0.1)	1.1 (± 0.2)	16 (± 15)	0.9
Isobutylamine	4.7 (± 2.5)	2.2 (± 0.1)	0.4 (± 0.2)	4.6 (± 3.9)	0.3
sec-Butylamine	11 (± 9.7)	0.9 (± 0.9)	4.1 (± 0.9)	40 (± 19)	n.d.
Sum of amines	717 (± 390)	266 (± 46)	59 (± 12)	1005 (± 878)	32
N	7	3	7	2	

In general, the sum of nitrogen content in the total measured amines (including cadaverine, spermidine, ethanolamine and histamine) and nitrogen content in form of nitrate (NO_3^-) were found to be in the same range. The sum of alkyl amine nitrogen content in the soil was not statistically different ($p< 0.05$) between the planted and non-planted treatments (15 ± 1.5 μg-N g^{-1} SOM without plant and 12 ± 1.0 μg-N g^{-1} SOM with plant). However, there were a few measured alkyl amines in the soil that were affected by the presence of a pine seedling (Table 6). The nitrogen content of DMA was higher in the non-planted treatments. In cases of iso-BA and sec-BA nitrogen the contents were higher in the presence of a Scots pine. The nitrogen contents of MMA and DEA did not differ between planted and non-planted treatments. DMA was the most abundant alkyl amine found in the soil followed by MMA (Table 6). From the studied alkyl amines, DEA had the lowest soil concentrations (Table 6).

The concentrations of DMA was found to be lower in presence of a plant which may be due to seedlings taking up DMA. The concentration of MMA did not differ between the planted and non-planted treatments, even though plants are known to take up MMA when used as reference compound for ammonium NH_4^+ (Kielland, 1994; Wallende and Read, 1999; Javelle et al., 1999). The roles of DMA and MMA as a nitrogen source have remained unclear. The reason for the higher concentrations of iso-BA and sec-BA in the planted treatments remains unexplained. It is plausible that these are secondary metabolites as in the case of TMA which has been found to act as a fungicide (Xu et al., 2004).

Table 6: The alkyl amine concentrations and their standard errors in boreal forest soil planted with (n=39) or without (n=15) a Scots pine as presented in **Paper III**. The different letters (a and b) indicate a statistically significant ($P < 0.05$) difference between the planted and non-planted treatments. Note that the sum of amines is nitrogen content in the soil (μg-N g^{-1} d.w.) and the rest are concentrations (μg g^{-1} d.w.) in the soil. The sum of amines includes cadaverine, spermidine, ethanolamine and histamine.

Alkyl amine	Planted	Non-planted	
Monomethylamine	3.3 (± 0.64)^a	4.2 (± 0.44)^a	[μg g^{-1} d.w.]
Dimethylamine	17 (± 2.6)^a	32 (± 3.9)^b	[μg g^{-1} d.w.]
Diethylamine	0.05 (± 0.00)^a	0.10 (± 0.00)^a	[μg g^{-1} d.w.]
Isobutylamine	1.5 (± 0.26)^b	0.78 (± 0.10)^a	[μg g^{-1} d.w.]
sec-Butylamine	2.3 (± 0.21)^b	1.5 (± 0.10)^a	[μg g^{-1} d.w.]
Sum of amines	12 (± 1.0)^a	15 (± 1.5)^a	[μg-N g^{-1} d.w.]

The fact that we did not find any treatment effects of soil organic matter degrading enzymes on the soil concentrations may be due to the design of the experiment. The experiment was specifically designed to study nitrogen bound to soil organic matter, and enzymatic release of nitrogen from soil organic matter. In the experiment, dissolved nitrogen could already have been volatilized or immobilized by plants and microbes, or leached from the pots before quantification that took place one month after the last enzyme addition. As the measured soil concentrations are based on the pot-scale experiment conducted in a greenhouse, the results obtained from the experiment do not fully represent soil concentrations in the forest soil, for example, the nitrogen content of NH_4^+ was higher in the experiment than the concentrations based on the field measurements (Korhonen et al., 2013). The soil concentration of MMA (50 μmol L^{-1}) in the experiment was one order of magnitude smaller than those MMA concentrations (480 μmol L^{-1}) in soil litter leachates reported by Yu et al. (2002).

It has been suggested that MMA, DMA, TMA, and NH_3^+ are formed in a sequential degradation process of quaternary ammonium compounds (Kim et al., 2001; Rappert and Müller, 2005). Quaternary ammonium compounds have been measured from soil in various environments (Warren, 2013b, 2014b). Soil concentrations of quaternary ammonium concentrations in boreal forests have not been measured or reported. Assuming that alkyl amines share similar formation and consumption processes with NH_4^+ in boreal forest soil would mean that soil solution concentrations are expected to have two maxima during a year (in early spring and in late autumn) (Pajuste and Frey, 2003). Pajuste and Frey (2003) suggested that the two maxima of NH_4^+ in soil are due to the change in balance between uptake and release rates during the growing season. The release is temperature dependent microbial decomposition and mineralisation and the uptake is nutrient consumption of plants or microbes. In spring, NH_4^+ is released at a faster rate than plants are taking up NH_4^+, but the uptake rate increases towards late summer exceeding the release rate of NH_4^+, leading to a concentration minimum in soil during summer. Towards late autumn the release rate decreases at a lower pace than the uptake rate, leading to a slight increase in soil NH_4^+ concentration.

4.4 Estimation of Soil Air Concentrations and Soil–Atmosphere Exchange

During the study period from May to October 2011 the mean (± standard deviation) soil air concentration for DMA was 27 (±5.1) nmol m^{-3} and for DEA 0.032 (±0.006) nmol m^{-3} (Figure 8, panel A). Due to the constant values used for the soil solution concentrations and pH, the seasonal trend of the soil air concentrations followed the measured soil temperature (Figure 8, panel C). When the long-term mean soil pH (5.3) and the soil solution concentration obtained from the experiment were used, the soil air concentrations for DMA were higher than the measured mean ambient air concentration (1.7 nmol m^{-3}), and in the case of DEA, the measured mean ambient air concentration (0.26 nmol m^{-3}) was higher than the estimated soil air concentration.

From May to October the mean estimated soil flux for DMA was 170 (±51) nmol m^2 d^{-1} and for DEA -1.2 (±1.2) nmol m^2 d^{-1} (Figure 8, panel B). The estimated flux of DMA had increased towards July reaching the highest values at the end of July and had decreased towards autumn. Unlike the measured ambient air concentration of DMA, the flux did not peak in September. The estimated DEA flux was negative in summer from June to August, and otherwise DEA flux remained near zero, having slightly negative fluxes.

Exchange processes of alkyl amines are mainly studied in rural environments, and the studied emissions are related to agricultural activities (Schade and Crutzen, 1995; Kuhn et al., 2011). TMA exchange have previously been measured on an ecosystem scale above a Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) forest in the Netherlands from June to July (Copeland et al., 2014). The mean TMA flux ranged from -192 to 192 µmol m^{-2} d^{-1} being over an order of magnitude higher than the estimated DMA flux (170 nmol m^{-2} d^{-1}) presented in the thesis. Copeland et al. (2014) explained that the high fluxes were due to nearby agricultural activities. As in the case of alkyl amines, NH_3 measurements in forest environments are scarce. In a Danish deciduous forest, Hansen et al. (2013) observed NH_3 emissions after leaf fall in autumn.

It is good to note that the used estimation method is a straightforward representation of the system. The estimation method does not take into account possible fluctuation of soil solution concentrations of DMA and DEA due to changes in soil microbial community (Santalahti et al., 2016) nor does it describe the uptake–release dynamics

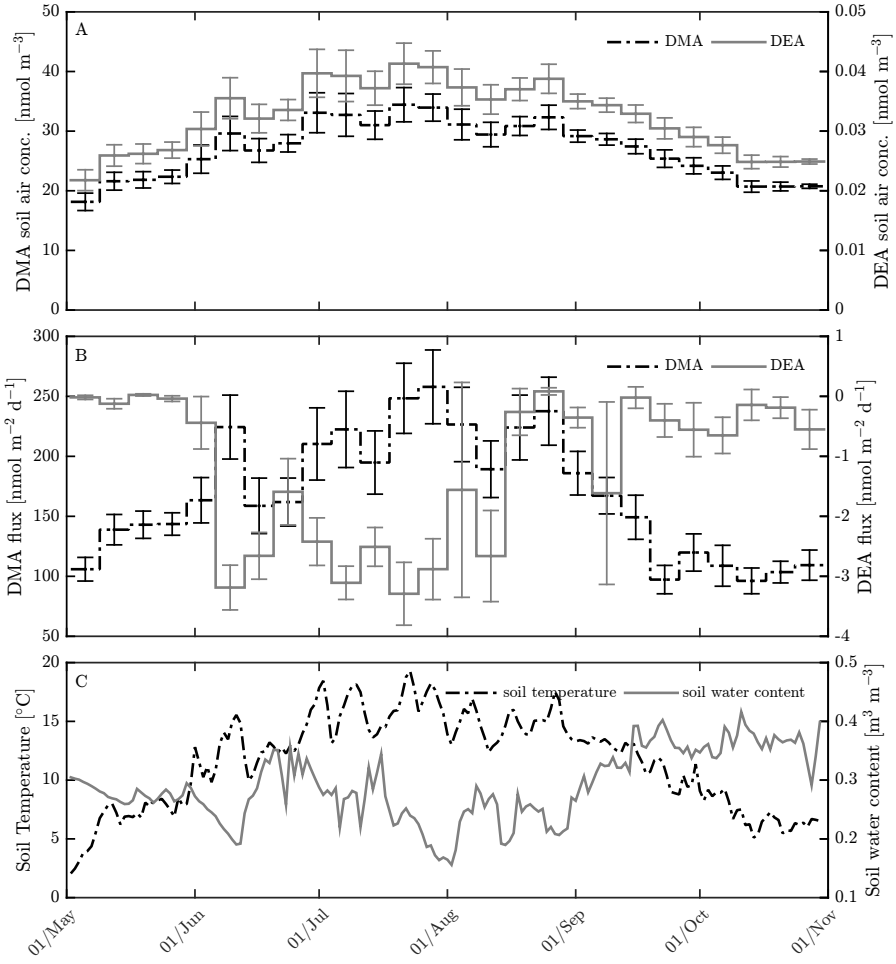


Figure 8: The estimated soil air concentrations (panel A) and soil fluxes (panel B) of dimethylamine (DMA) and diethylamine (DEA) with their standard deviations. In the estimations a long-term mean soil solution pH 5.3, an average soil depth 0.05 m, and a constant soil solution concentrations of DMA and DEA (92.3 μ mol L^{-1} and 0.296 μ mol L^{-1}, respectively) were used. In panel C, soil temperature and soil water content in the studied boreal forest site. Thr study period was from May 2011 to October 2011.

of nutrients in soil (Pajuste and Frey, 2003). As the estimated soil fluxes are based on soil air concentration estimates, the fluxes do not explain the elevated ambient air concentrations (especially those of DMA) in autumn. The missing autumn peak in the estimated soil air concentrations and the estimated soil fluxes may be the result of the use of the constant values for soil solution concentrations and soil pH in the estimation method. In the soil air estimation, the level of the estimated soil air concentrations was based on the measured soil solution concentrations in the experiment, while the variability of the estimated soil air concentration was the result of changes in soil temperature during the study period. The constant values together with other environmental variables make it possible to study environmental conditions affecting the direction of the flux of alkyl amines between soil and the atmosphere. A sensitivity analysis was done for each environmental parameter used in the estimation to find the environmental conditions where boreal forest soil can act as a sink or as a source for the studied alkyl amines. The ranges of the assessed parameters in the sensitivity analysis were chosen to represent the measured variability in the studied forest environment.

The sensitivity of the estimated soil air concentrations to soil solution concentration, temperature, and pH were studied. The soil air concentration increased linearly with the soil solution concentration in the assessed range, by 29 nmol m^{-3} for DMA and by 11 nmol m^{-3} for DEA. The soil air concentration had a non-linear relationship with the soil temperature. In the assessed temperature range, the soil air concentration increased by 24 nmol m^{-3} for DMA and by 0.03 nmol m^{-3} for DEA. The soil air concentration increased in a non-linear manner with the increasing soil pH. In the assessed pH range, the increase of the soil air concentration was 680 nmol m^{-3} for DMA and 0.81 nmol m^{-3} for DEA. The non-linear increase with the soil pH was the result of Eq. 1 and Eq. 4, which describe the dissociation of an alkyl amine in soil solution and the pH-dependency of partition of an alkyl amine between soil solution and soil air.

The effect of the environmental variables (soil solution concentration, pH, temperature, soil water content, soil depth, and friction velocity) on the estimated fluxes are shown in Figure 9. In the soil fluxes there was a linear increase with increasing soil solution concentrations in the studied range. The flux estimate had a strong exponential relationship with the soil pH. The strong effect of pH on the flux estimation follows from Eq. 1 and Eq. 4, which describe the dissociation of an alkyl amine in soil solution and the pH-dependency of partition of an alkyl amine between soil solution and soil

air. In the studied pH range, the estimated DMA flux was positive after pH 4.7 and DEA flux was positive after pH 5.7. The estimated DMA flux varied in the assessed pH range from -0.67 to 4500 nmol m^2 d^{-1} and the flux of DEA varied from -1.4 to 2.7 nmol m^2 d^{-1}.

In the assessed temperature range, increases in temperature increased fluxes from the soil to the atmosphere near-linearly from 81 nmol m^2 d^{-1} to 255 nmol m^2 d^{-1} for DMA, and for DEA from -1.1 nmol m^2 d^{-1} to 1.3 nmol m^2 d^{-1} (Figure 9). Increases in soil water content decreased fluxes in a near-linear manner. This is due to the non-linear decrease of r_g with increasing soil water content (Eq. 6). In the studied soil water content range, the DMA flux changed from 241 nmol m^2 d^{-1} to 122 nmol m^2 d^{-1} and the DEA flux changed from -1.7 nmol m^2 d^{-1} to 0.84 nmol m^2 d^{-1} (Figure 9).

The Estimated soil fluxes were dependent on the assumed depth of sources and sinks of alkyl amines in the soil. This is due to the dominating role of r_g in the flux estimation. The soil flux decreased with increasing soil depths, and the sensitivity was at its strongest when the soil depth was below 0.03 m. Friction velocity had a minor role in the flux estimation. The strongest effect on flux estimation occurred with friction velocity values smaller than 0.2 m s^{-1}. In general, increasing friction velocity increases fluxes from soil to the atmosphere mostly due to an increase in r_b. It should be noted that if a source or sink is located close to a soil surface and the atmosphere is almost still, friction velocity may become important affecting soil–atmosphere fluxes. Under those conditions, the dominating effect of r_g diminishes and both r_b and r_a increase as the result of the low-turbulent conditions being of the same order of magnitude.

In the sensitivity analysis of the soil air concentration and soil flux estimates, it was found that both were highly sensitive to changes in soil pH. In the assessed pH range (from 4.5 to 6.0) both DMA and DEA had a pH value where the mean flux estimate was zero. With the zero mean the soil pH was 4.7 for DMA and 5.7 for DEA. Below these pH values soil acted as a sink of DMA and DEA. According to the sensitivity analysis, it seems that even the slightest change in soil pH or in soil solution concentration affects the direction of the alkyl amine flux in the boreal forest soil. Based on the estimation at the typical soil pH (5.3) together with the high quantities of DMA found in the fungal hyphae and in the soil extracts, the boreal forest soil could have been a source of DMA. In the case of DEA, the soil acted as a sink, but it is likely that DEA may have been emitted from the vegetation in a similar manner to what has been proposed for NH_3 (Makkonen et al., 2014).

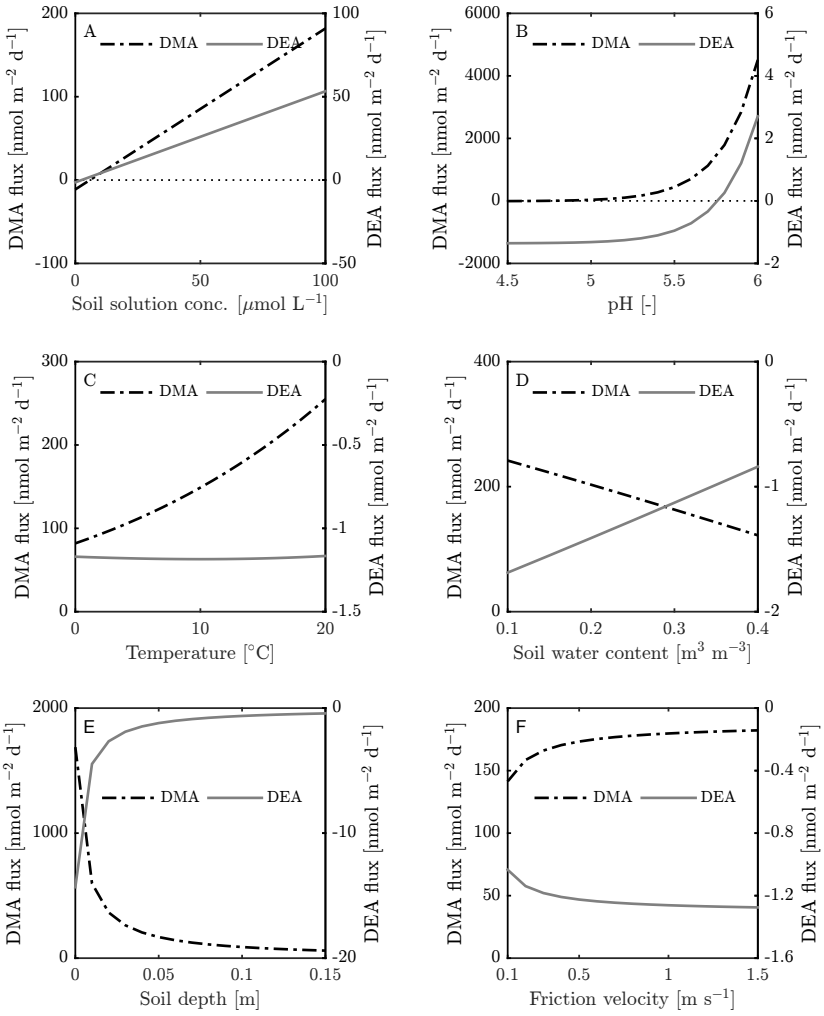


Figure 9: The effect of soil solution concentration (panel A), soil pH (panel B), temperature (panel C), soil water content (panel D), soil depth (panel E), and above-canopy friction velocity (panel E) on the estimated soil fluxes of dimethylamine (DMA) and diethylamine (DEA) in the boreal forest.

4.5 Atmospheric Reactivity of Alkyl Amines

Relative contributions of atmospheric compounds (terpenes, aromatic hydrocarbons and alkyl amines) to OH reactivity were assessed in the forest site and in the urban site. In the forest site from June to August, terpenes had a major contribution to OH reactivity and in May, September and October alkyl amines had an almost equal contribution with terpenes (Figure 10b), ranging from 10% in August to 44% in October. During the summer months (June, July and August), terpene concentrations in the ambient air were high due to increased emissions of biogenic VOCs that decreased alkyl amine contribution. In the case of the urban site, relative OH reactivity was assessed only in May and June, and the contribution of alkyl amines to OH reactivity in the urban site remained minor (14% in May and 6% in June) compared to the aromatic hydrocarbons and terpenes (Figure 10a). The sum of OH reactivity of the measured alkyl amines in July 2011 was around 10% of the unknown OH reactivity measured by Noelscher et al. (2012) in July 2010. Sinha et al. (2010) and Noelscher et al. (2012) have measured total OH reactivities by using the comparative reactivity method with a proton transfer reaction-mass spectrometer at the forest site. Unknown OH reactivity not due to terpenes or aromatic hydrocarbons was measured at 50% and 68% in the summers of 2008 and 2010, respectively (Sinha et al., 2010; Noelscher et al., 2012). It can be stated that alkyl amines may explain part of the unknown OH reactivity measured at the forest site. At the forest site, the contribution of alkyl amines to OH reactivity was at its highest in spring and autumn when terpene concentrations in the ambient air were low.

For DMA and DEA the Damköhler numbers (DA) were calculated from May to October, and the DA ranged from 0.013 to 0.026 for DMA and from 0.017 to 0.033 for DEA. Due to DA values lower than 0.1 the removal of DMA and DEA by chemical reactions in the ambient air can be neglected for the flux estimates (Rinne et al., 2012). Even though alkyl amines are reactive in the atmosphere, their lifetime against the main atmospheric oxidant (OH radical) remains longer than the timescale of turbulent transport. At least DMA and DEA are effectively transported within the forest canopy without major losses due to chemical reactions occurring in the ambient air.

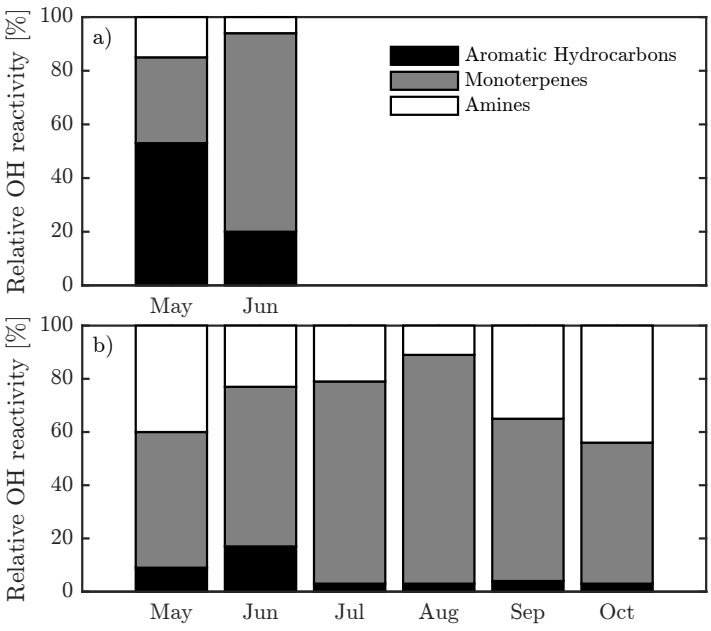


Figure 10: Monthly relative contributions of the measured alkyl amines, aromatic hydrocarbons and terpenes (isoprene and monoterpenes) concentrations to OH reactivity at the urban site (panel a) in May and June 2011 and in the forest site (panel b) from May to October 2011 (**Paper II**).

5 Conclusions

A method for measuring gas-phase alkyl amines was adopted (Rampfl et al., 2008) and developed further. The method was used in two environments, in a boreal Scots pine forest and in an urban site. For the forest site, a unique dataset covered six months of gas-phase concentrations of alkyl amines in ambient air. The dataset showed that alkyl amines have clear seasonal variation. The study concluded that ambient air concentrations of alkyl amines were higher in the forest site than in the urban site. In both sites, DMA+EA had the highest concentrations. Alkyl amines react in the atmosphere with hydroxyl (OH) radicals and ambient air concentrations of alkyl amines have an influence on local atmospheric chemistry. It was shown that alkyl amines and

terpenes have an almost equal contribution to OH reactivity in the forest site. In the forest site, the measured concentration maxima were dependent on the measured alkyl amine, for DEA the maxima occurred in the summer and for DMA+EA and TMA+PA in the autumn. The summer maxima of DEA were similar to the concentration maxima observed for monoterpenes and ammonia in the forest site, indicating a possible source in the forest vegetation. The autumn maxima of DMA+EA and TMA+PA coincided with the litterfall and with the change in the microbial community in the soil. Based on the measurements in the forest site, ambient air concentrations of alkyl amines seem to be linked to soil activity, vegetation and litterfall.

The soil fungal hyphae and boreal forest soil were shown to contain high quantities of alkyl amines, especially MMA and DMA. Based on this, it seems that fungal hyphae form a large pool of alkyl amines in boreal forest soil. The nitrogen content of alkyl amines in soil solution can be relatively high, in the same order of magnitude with nitrate nitrogen (NO_3^--N). When the hyphal concentrations were compared with the concentrations measured in the soil extracts, the alkyl amine concentrations were lower in the soil extracts than in the fungal hyphal biomass. There is a need to study further the interactions of plants and alkyl amines found in soil, and especially, to study source processes of alkyl amines in soil, e.g. the effects of renewal of soil microbial community on soil concentrations of alkyl amines, and alkyl amine formation from the degradation process of quaternary ammonium compounds in boreal forest soil.

In the estimation of soil–atmosphere exchange, it can be stated that alkyl amines can be released from soil into the atmosphere under favourable environmental conditions. Under the typical environmental conditions the soil was a source of DMA and a sink of DEA. The direction of the estimated flux was dependent on soil temperature, soil water content and soil pH, but also on soil solution concentration of alkyl amine. The concentration measurements and the estimation provide the atmospheric modelling community with results and tools that help take into account alkyl amines in models describing soil–atmosphere interactions in northern latitude terrestrial forest environments.

It is self-evident that to improve the estimation of alkyl amine exchange, we need continuous measurements of alkyl amines in soil solution and in soil air, but we also need to improve our knowledge of the ecosystem processes that affect the formation or consumption of alkyl amines in soil. For a better understanding, we need more knowledge of phenological events, like litterfall, occurring in the forest ecosystem. These

events affect the physical and chemical conditions of soil, e.g. its pH and substrate availability, and nitrogen processes. To improve flux estimation of alkyl amines at the ecosystem level, sink and source processes in vegetation should be studied and included in the flux estimation. In addition, condensation and evaporation of alkyl amines in and from atmospheric particles should be described in the estimation as a sink or a source process of alkyl amines.

6 Review of Papers and Author's Contributions

Paper I presents the sample collection and analysis method for measuring ambient air concentrations of alkyl amines. In addition, **Paper I** reports seasonal variation in gas-phase concentration of alkyl amines in ambient air in a boreal Scots pine forest from May to October 2011. The observed concentrations of alkyl amines were compared with aerosol formation events and biophysical measurements at the site. The author led the planning and writing of the article and was responsible for the measurements, chemical and data analysis done for the study.

Paper II reports ambient air concentrations of alkyl amines measured in ambient urban air from May to August 2011. The influence of alkyl amines on the total OH reactivity was assessed in the urban and in the forest sites. The author was responsible for the alkyl amine measurements and chemical analysis. The author's contribution was to assist the lead author Dr. Heidi Héllen in writing the article and to provide the necessary data.

Paper III investigates the effect of soil organic matter degrading enzymes and trees on soil organic nitrogen storage and cycling. Additionally, **Paper III** presents the soil concentrations of alkyl amines in the boreal forest soil. The author make a major contribution to the writing process. In addition, the author was responsible for a significant part of the chemical analysis, data processing and statistical analysis conducted.

Paper IV studies the influence of soil conditions and soil concentrations on the measured ambient air concentrations of alkyl amines and presents an approach for estimating soil fluxes of amines in a boreal Scots pine forest. The author's contribution was to lead the planning and writing the article. The author was responsible for the data analysis and calculations with support from Doc. Samuli Launiainen.

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Gas-phase alkylamines in a boreal Scots pine forest air

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HIGHLIGHTS

- Alkylamines (EA + DMA, TMA + PA, and DEA) were observed in a boreal forest air.
- DEA showed a summer maximum, whereas EA + DMA and TMA + PA showed autumn maxima.
- Amines seem to be linked with vegetation, soil activity, and litterfall.
- In autumn, weeks with most new particle formation days had the high concentration of alkylamines.

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ABSTRACT

Alkylamines are highly reactive volatile nitrogen compounds that may take part in aerosol formation and growth in the atmosphere in boreal forests. We measured alkylamine concentrations from May to October 2011 in a boreal forest at the SMEAR II station in Hyytiälä, southern Finland. The weekly air samples were collected in phosphoric acid-impregnated fiberglass filters through a polytetrafluoroethylene (PTFE) filter and analyzed with a high performance liquid chromatography electrospray ionization ion-trap mass spectrometer.

Ethylamine (EA) and dimethylamine (DMA), propylamine (PA) and trimethylamine (TMA), and diethylamine (DEA) were observed on levels above the detection limits, while butylamine and triethylamine were under the detection limits. The highest concentrations for EA + DMA (157 ± 20 ppt_V) and PA + TMA (102 ± 61 ppt_V) were observed from September to October. For DEA the seasonal course was different and the highest concentrations were measured during the summer (max 15.5 ± 0.5 ppt_V, early July).

The amine concentrations were compared with those of ambient air ions, other trace gases (O_3, NO, NO_x and CO and monoterpenes), and with soil and air temperatures and litterfall. Positive and negative cluster ions did not correlate with the amine concentration measured. However, peaks in the positive and negative intermediate ions showing similar concentrations occurred simultaneously with peaks in EA + DMA and DEA during the summer. The number of new particle formation days followed the sum of the alkylamine concentrations observed during the autumn. The autumnal monoterpene emissions from the forest floor and litterfall maxima coincided with the elevated or peaked EA + DMA and PA + TMA concentrations. Similar to the monoterpene concentrations in the forest air, amine concentrations seem to be linked with vegetation, soil activity, and litterfall, and rather than with other trace gases in the atmosphere.

1. Introduction

Reactive nitrogen in the atmosphere, especially the concentrations and reactions of ammonia (NH_3), nitric acid (HNO_3), nitrous acid (HNO_2) and organic nitrogen compounds, such as alkylamines, have stimulated widespread interest during the last decade (Neff et al., 2002; Cornell et al., 2003; Ge et al., 2011).

An amine has the general formula R_3N, R_2NH or RNH_2, where R can be an alkyl or an aryl group. In the atmosphere, amines act as bases and can be protonated in a manner similar to that of NH_3. Amines are reactive and have lifetimes of several hours against OH-radicals (Atkinson et al., 1978), therefore they are not transported long distances in the atmosphere (Ge et al., 2011).

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Alkylamines can be emitted from a variety of anthropogenic and natural sources. Globally, animal husbandry, industry, and combustion processes are the main anthropogenic sources, and oceans, vegetation, biomass burning, and soils are the main natural sources (Ge et al., 2011).

Due to their high reactivity, alkylamine concentration measurements in the atmosphere are scarce and mostly concentrated within short time periods. Various analytical techniques and procedures have been developed for determining amines from air and various other environmental matrices (Fekete et al., 2010 and references therein); however, measuring low ambient concentrations of these compounds remains challenging.

Alkylamines participate in atmospheric aerosol formation and growth (Angelino et al., 2001; Silva et al., 2008; Kurtén et al., 2008; Smith et al., 2009; Yu et al., 2012). This process of new particle formation (NPF) constitutes a globally important source of cloud condensation nuclei, with potentially large implications for Earth's climate system (Andreae and Rosenfeld, 2008; Merikanto et al., 2009; Wang and Penner, 2009).

In the atmosphere, both sulfuric acid and low-volatility organic vapors, in addition to NH_3 and possibly alkylamines, are likely to participate in NPF (Kerminen et al., 2010; Riipinen et al., 2012). Thus, the growth of the cluster ions in the intermediate size ranges is dependent on organic vapors (e.g. monoterpenes, $C_{10}H_{16}$) that condense in the particle phase from the gas phase in the atmosphere. Alkylamines and especially dimethylamine has been shown to participate in formation and growth of sub-3-nm particles (Bzdek et al., 2011; Yu et al., 2012). Luts et al. (2011) showed diethylamine to increase life time of negative cluster-sized ions.

Due to NPF occurring in the atmosphere at the Hyytiälä SMEAR II site (Hirsikko et al., 2005), intermediate-sized (1.7–3 nm) ions typically showed two clear concentration maxima during the year: in early spring and in autumn (Manninen et al., 2009). The sulfuric acid, high ozone and low water vapor concentrations are mainly responsible for NPF in the spring and the contribution of organic vapors (e.g. monoterpenes and their oxidation products) increases from late spring onwards; however, the autumnal maxima in NPF could not be fully explained by the same parameters as those in the spring (Bonn et al., 2009) and autumnal NPF can be explained by elevated concentrations organic vapors in the atmosphere.

In this study, a sample collection and analytical method for measuring ambient air concentrations of low-molecular-weight aliphatic amines was developed and tested. The concentrations of dimethylamine (DMA, $(CH_3)_2NH$), ethylamine (EA, $(C_2H_5)NH_2$), trimethylamine (TMA, $(CH_3)_3N$), propylamine (PA, $(C_3H_7)NH_2$), diethylamine (DEA, $(C_2H_5)_2NH$), butylamine (BA, $(C_4H_9)NH_2$), and triethylamine (TEA, $(C_2H_5)_3N$) in ambient air were measured in a boreal Scots pine (*Pinus sylvestris* L.) forest from May to October 2011. Furthermore, the amine concentrations were compared with aerosol formation events, other trace gases, and meteorological and biophysical measurements at the site.

2. Materials and methods

2.1. Measurement site

The measurements were conducted in a Scots pine forest at the SMEAR II station (Station for Measuring Forest Ecosystem–Atmosphere Relations) at Hyytiälä (61°51′N, 24°17′E, 180 m a.s.l.) in southern Finland (Hari and Kulmala, 2005).

The forest stand at the SMEAR II station is 48 years old and dominated by Scots pine and occasional Norway spruce (*Picea abies* (L.) H. Karst.), birch (*Betula* L. spp.), and European aspen (*Populus tremula* L.), mainly in the understory. The stand height is about 18 m and the canopy is open, with an average tree density of about 1370 stems per hectare (Ilvesniemi et al., 2009). At the ground level, the predominant plant species are lingonberry (*Vaccinium vitis-idaea* L.) and bilberry (*Vaccinium myrtillus* L.), wavy hairgrass (*Deschampsia flexuosa* (L.) Trin.), and heather (*Calluna vulgaris* (L.) Hull). The most common mosses are Schreber's big red stem moss (*Pleurozium schreberi* (Brid.) Mitt.) and a dicranum moss (*Dicranum* Hedw. sp.) (Ilvesniemi et al., 2009) The soil at the site is Haplic podzol on glacial till, with an average depth of 0.5–0.7 m.

The 30 year average annual precipitation at Hyytiälä is 711 mm and the annual mean temperature 3.5 °C (Pirinen et al., 2012). During the measurement period from May to October 2011, the cumulative precipitation was 535 mm and the mean air temperature was 11.4 °C. The 30 year mean cumulative precipitation and temperature of May to October in Hyytiälä are 433 mm and 6.8 °C, respectively.

2.2. Sample collection

The samples were collected from early May to late October 2011 at 2.5 m above ground level in the trunk space of the forest. The air samples were collected in filters that were shielded against direct sunlight and rain. The ambient air was pumped through a stack of filters with a flow rate of 16 L min^{-1}.

The stack of filters consisted of a polytetrafluoroethylene (PTFE) membrane filter (Millipore: Fluoropore® 3.0 μm FS; EMD Millipore Corp., Billerica, MA, USA) and an acid-impregnated fiberglass filter. The PTFE filter was used to collect the particles before the impregnated filter. The impregnated filter was used for trapping gas-phase amines as salts onto the fiberglass filter. In the reaction with phosphoric acid, the amines form aminium phosphate (R_3NH^+ $H_2PO_4^-$). For the acid-impregnated filters, the fiberglass filters were treated with phosphoric acid in methanol, according to the procedure introduced by Rampfl et al. (2008), in which fiberglass filters were soaked in a 5% phosphoric acid-methanol solution and dried in an oven at 40 °C.

The duration of sample collection was 24 h on weekdays and 72 h over the weekends. After the sample collection, the filters were extracted as described below. To reach the detection limit, the 24-h and 72-h samples from the same week had to be pooled together into a weekly sample. Prior to the field measurements, the filter's capacity to trap amines was tested in the laboratory. Known concentrations of amines in solution were injected into a stream of amine-free air, using a syringe pump. The amines were collected onto the stack of filters with a sampling flow of 16 L min^{-1}. The filters were analyzed through the normal analytical procedure, as described below. The amount of amines injected was found on the first impregnated filter, and no breakthrough of the filter was detected even after an injection of 10-fold ambient air amine concentrations during the 24-h sampling time.

2.3. Analytical methods

Aminium ions were extracted from the acid-impregnated filters with ultrapure water in an ultrasonic bath for 1 h. Prior to the extraction, an internal standard, deuterated diethyl-d_{10}-amine (Sigma–Aldrich: Isotec™; Sigma–Aldrich Corp., st. Louis, MO, USA), was injected onto the filters. The analytical method for gas-phase amines is based on methods introduced by Rampfl et al. (2008). The extract was analyzed by high performance liquid chromatography electrospray ionization ion trap mass spectrometer (Agilent 1100 series LC/MSD trap; Agilent Technologies, Santa Clara, CA, USA). For quantitative analysis, 5-point external standards for all the aliphatic amines measured were used.

In the LC system, a Discovery® HS F5 HPLC (Supelco Analytical, Bellefonte, PA, USA) was used as an analytical column, and HPLC

SecurityGuard™ Cartridge (Phenomenex® Inc., Torrance, CA, USA) as a pre- column, both at 40 °C. During the 30-min analysis, a constant flow (250 μL min^{-1}) of solvents, water, and acetonitrile with 0.02% formic acid as an ion-exchange reagent was used. As the analysis proceeded, the proportion of acetonitrile was increased gradually. After 5 min, the acetonitrile was increased from 5% to 25% during 7 min, later 50%, and finally after 25 min decreased back to 5%. A chromatographic separation was divided into segments for each target compound by its retention time. In each segment, a target compound-specific mass range was followed by a mass spectrometric analysis for achieving higher analytical precision and for dampening the background noise. Analysis was conducted four times for each sample. The uncertainty of the analyses was reported as a standard deviation between the parallel analyses results. The detection limits were three times the standard deviations of the field blanks. The column used was unable to separate all of the compounds and therefore DMA and EA, and TMA and PA were handled as pairs, respectively. The detection limits for the amines measured were 0.2 ppt_V for EA + DMA, 0.4 ppt_V for TMA + PA, 6.7 ppt_V for DEA, 3.2 ppt_V for TEA, and 8.9 ppt_V for BA.

2.4. Supplementary measurements

Measurements of other trace gas concentrations, and number-size distribution of the cluster ions and aerosol particles, as well as air temperature, precipitation, soil temperature and moisture, and the amount of litterfall at the SMEAR II station were used to link the ambient air amine concentrations with biophysical processes in the atmosphere, vegetation and soil. The air temperature at 4.2 m above the ground and the humus layer temperature were measured using PT-100 resistance thermometers. The precipitation and precipitation intensity were measured with a weather sensor (FD12P, Vaisala Oyj, Vantaa, Finland), and the soil water content in the humus layer was measured with a time-domain reflectometer (TDR 100; Campbell Scientific Inc., Logan, UT, USA).

Litterfall was collected monthly (usually on the 5th day of each month) from 20 aboveground litter collectors. The litterfall samples were dried at 60 °C for 24 h and separated into six fractions (needle, bark, twig, cone, leaf, and other) and weighed. In the present study, only the needle and leaf fractions were used.

The positive and negative cluster ions and charged particles were measured with a neutral cluster and air ion spectrometer (NAIS; Kulmala et al., 2012; Mirme and Mirme, 2013) in the size range 0.8–42 nm. The NAIS measures ion and particle-size distributions in 28 size fractions with a 5-min time resolution. It measures the number-size distributions of the charged aerosol particles and cluster ions down to molecular sizes. To compare with the amine concentrations, the ion-size classification of 0.8–1.7 nm for cluster ions, and 1.7–3 nm for intermediate ions were used for both the positive and negative ion concentrations following classification scheme introduced by Hirsikko et al. (2007).

The monoterpene concentrations were measured in the forest air with an on-line thermal desorpter connected to a gas chromatograph coupled with a mass-selective detector (TD-GC-MS), described in detail by Hakola et al. (2012). The monoterpenes included α-pinene, β-pinene, Δ^3-carene, camphene, terpinole, p-cymene, limonene and 1,8-cineol.

2.5. Data analysis

Weekly averages of the atmospheric trace gas concentrations, cluster ions and aerosols, monoterpenes, soil and air temperatures, soil moisture, and precipitation were calculated to compare them with the measured amine air concentrations. Pearson's correlation analyses between the weekly variables were performed and

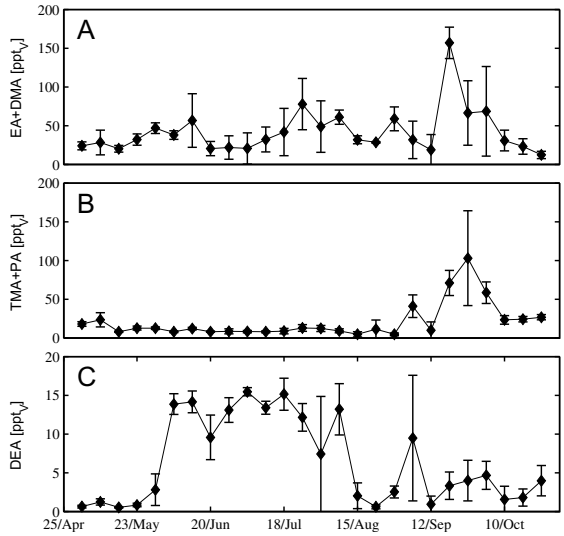

Fig. 1. Ambient air concentrations (ppt_V) and their standard deviations during the measurement period from early May to late October in 2011 at the Hyytiälä SMEAR II station. A) ethylamine + dimethylamine (EA + DMA), B) trimethylamine + propylamine (TMA + PA), C) diethylamine (DEA).

significant correlations were accepted with a confidence level of $p < 0.05$. Correlations between the amine concentrations and litterfall were calculated between the monthly cumulative litterfall and those amine concentrations measured closest to the litterfall collection date.

3. Results

3.1. Alkylamine concentrations

EA + DMA, PA + TMA, and DEA were observed at levels above the detection limits. The sampling date refers to the first day of the sampling week.

The highest concentrations were observed from September to October for EA + DMA (157 ± 20 ppt_V) and for PA + TMA (102 ± 61 ppt_V). The concentrations of EA + DMA also peaked on June 13, July 25, and August 29, and TMA + PA on September 5; however, these peaks were approximately half of those observed in the autumn maximum (Fig. 1). The DEA seasonal course was different from that of the other amines. Instead of the autumn peaks, the highest concentrations were measured during the summer, with the highest value of 15.5 ± 0.5 ppt_V on July 4. In the autumn, the concentration of DEA was slightly higher than in early spring, about half of that measured in the summer.

3.2. Soil parameters and litterfall

The seasonal variations in soil temperature, water content, and litterfall are shown in Fig. 2. The lowest soil temperature (3.6 °C) in the humus layer was observed at the beginning of the measurement period in early May, and the highest humus layer temperatures (17.1 °C) were observed in late of July. From the maximum in July, soil temperatures decreased towards the end of the measurement period, remaining relatively higher (6.3 °C) in October compared with the beginning of the measurement period. The lowest soil-water content (0.19 m^3 m^{-3}) in the humus layer was

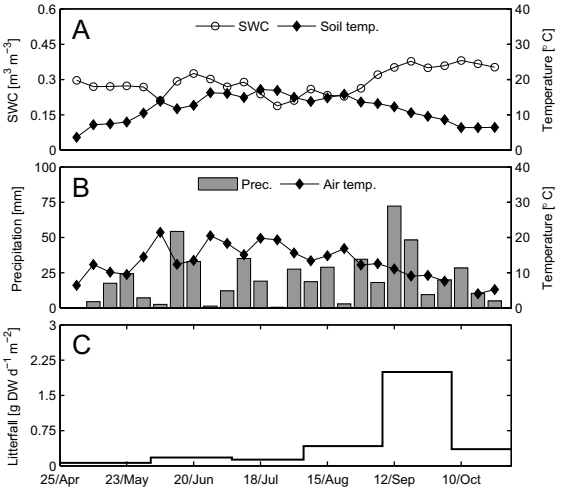

Fig. 2. A) Soil-water content (SWC, $m^3\ m^{-3}$) and soil temperature (°C) in the O horizon, B) precipitation (mm) and air temperature (°C) at 4.7 m, and C) needle and leaf litterfall (g DW $d^{-1}\ m^{-2}$) per length of the measurement period during May to October 2011 at the SMEAR II station.

observed in early August, while the highest (0.38 $m^3\ m^{-3}$) values were measured in September and October (Fig. 2). The monthly mean air temperature, precipitation, soil-water content, and soil temperature in the O horizon are presented in Table 1.

The litterfall was fairly constant from May to early September (0.2 g dry weight $m^{-2}\ d^{-1}$; Fig. 2). During September and October, the litterfall increased 10-fold (2 g DW $m^{-2}\ d^{-1}$) compared with the values measured during May to early September. The litterfall, EA + DMA and TMA + PA correlated positively ($r = 0.5$ and $r = 0.7$, respectively, $p < 0.05$, Table 2).

In the case of DEA, we found no strong autumn peak and no correlation with the litterfall and therefore we suspect that DEA has different sources than EA + DMA and PA + TMA. However, the DEA air concentration correlated positively with the air and soil temperatures ($r = 0.6$, $p < 0.05$). In addition, the soil humus layer water content showed weak positive correlation with TMA + PA ($r = 0.6$, $p < 0.05$), but no relation with EA + DMA or with DEA was found.

3.3. Meteorology and trace gases

We found no correlation between the amine concentrations and rain events. There were also no clear drops in the EA + DMA and TMA + PA concentrations. The rain events seemed to have a more pronounced effect on the DEA concentrations. After some rainy episodes, the concentration of DEA showed a rain-induced decrease; however, this could not be observed after every rain event. The air temperature correlated with the DEA concentrations and the EA + DMA concentrations during the summer period, but similar correlations were not observed in the last months of the measurement period. The TMA + PA concentrations during the summer months were close to the detection limit, and therefore the possible concentration decrease by rain events, or the effect of air temperature on the TMA + PA concentrations could not be seen.

The amine concentrations were also examined in relation to other trace gases i.e. nitric oxide (NO), nitrogen oxides (NO_x), carbon monoxide (CO) and ozone (O_3) and atmospheric ions. The highest NO and NO_x mixing ratios were measured in late May and during the autumn, respectively (Table 1). NO_x showed a clear minimum in late July, while O_3 and CO showed minima during September. For O_3 the highest concentrations were observed in May and for CO at the end of the measurement period. The concentration fluctuations were smaller for CO and O_3 than those for NO and NO_x; however, the CO concentrations were highly variable during the summer months of June, July, and August. The monthly mean concentrations and their standard deviations during the measurement period are shown in Table 1. No strong correlations were found when the amine concentrations measured were compared with the atmospheric trace gases. The best correlation observed was between CO and EA + DMA ($r = -0.5$ and $p < 0.05$).

3.4. Alkylamines and air ions

The positive cluster ions (0.8–1.7 nm) had the highest weekly median concentration of 644 ions per cm^3 on the first week of the measurement period. The negative cluster ions had the highest weekly median concentrations on July 4 and August 8, with 558 and 536 ions per cm^3, respectively. The median positive and negative cluster ion concentrations throughout the period (from May to October) were 449 and 418 ions per cm^3, respectively. The positive and negative cluster ions concentrations did not have any decreasing or increasing trends during the measurement period. Our amine measurement began late (May) with respect to the ion concentration maxima during the spring (March–April), and therefore the measurement period for amines did not cover all the spring maxima in the ion number concentrations. There was no clear autumn maximum in the positive ion clusters, but in the 1.7–3-nm negative intermediate ions a small autumn maximum was observed from early September to late October (Fig. 3). This peak occurred at the same time as the autumn peaks in EA + DMA and PA + TMA, and smaller autumn peaks of DEA. However, none of the correlations of cluster ion concentrations with the amine concentrations were statistically significant.

The positive intermediate (1.7–3 nm) ions had the highest weekly median concentrations (41 cm^{-3}) on June 1, and thereafter the concentrations decreased towards the end of the measurement period, as shown in Fig. 3. The positive intermediate ions also peaked on June 20, June 27, and August 1. The negative intermediate ions showed the highest median concentration (26 cm^{-3}) on June 6 (Fig. 3) and thereafter the concentrations peaked on June 20, June 27, and August 1. The positive and negative intermediate ion concentrations followed each other's concentration development in the atmosphere. The median positive and negative intermediate ion number concentrations throughout the period were 16 and 17 cm^{-3}, respectively. The negative intermediate ion concentrations decreased less during autumn compared to the positive ion concentrations in the same size range. The positive and negative 1.7–3-nm ions peaked in the same weeks during the summer months, and the negative ions increased in concentration in September and October.

During the summer period, the positive and negative intermediate ions peaked together with EA + DMA and DEA concentrations in early June, July, and August, as shown in Fig. 3. The positive and negative 1.7–3-nm ions showed the most significant correlation ($r = 0.5$ and $p < 0.05$; $r = 0.4$ and $p < 0.05$, respectively) with the DEA gas-phase concentration.

To test for a connection between NPF and the amines, all the days during May–October were divided into NPF event (NPF occurs) or non event (NPF did not occur) days, following the classification summarized by Kulmala et al. (2012). The event days formed three short periods (May 2 - May 9, May 23 - June 6 and July 25 - August 8), during which the events occurred more frequently per week and the weekly median nucleation mode particle concentrations were higher than during the rest of the period (Fig. 4).

Table 1
Monthly mean trace gas concentrations, mean air and soil (humus layer) temperatures and mean soil-water content and their standard deviations. Precipitation is presented as monthly sum in mm of rain.

		May	June	July	August	September	October
NO	ppb	0.06 ± 0.05	0.05 ± 0.03	0.04 ± 0.05	0.03 ± 0.07	0.03 ± 0.10	0.03 ± 0.05
NO_x	ppb	1.07 ± 0.61	0.69 ± 0.59	0.37 ± 0.33	0.43 ± 0.50	0.58 ± 0.88	1.05 ± 1.01
O_3	ppb	37.7 ± 7.8	32.0 ± 11.09	28.5 ± 9.68	22.8 ± 9.36	18.3 ± 6.59	21.7 ± 5.45
CO	ppb	139.3 ± 11.3	125.7 ± 14.3	125.8 ± 25.5	109.2 ± 20.7	100.7 ± 11.7	118.4 ± 29.5
Air temperature	°C	9.6 ± 4.9	16.2 ± 5.5	18.4 ± 4.3	14.9 ± 3.5	10.9 ± 2.7	5.6 ± 2.3
Soil temperature	°C	5.21 ± 2.3	12.80 ± 2.2	16.37 ± 2.1	14.75 ± 1.6	11.83 ± 1.6	7.31 ± 1.3
Soil-water content	$m^3\ m^{-3}$	0.28 ± 0.02	0.28 ± 0.05	0.25 ± 0.05	0.23 ± 0.04	0.34 ± 0.05	0.36 ± 0.03
Precipitation	mm	52	91	68	96	164	65

In May, the concentration sum of the alkylamines observed did not increase, and on June 6 the event days peaked earlier than the alkylamines. In the summer, the event days peaked with the alkylamine concentrations from July 25 to August 8.

To test for a correlation between NPF and the amines, the concentrations of nucleation mode particles 3–25 nm in diameter were compared with the amine concentrations. The weekly medians of the nucleation mode particle concentrations are shown in Fig. 4. The nucleation mode particle concentrations were highest at the beginning of the measurement period in May, reflecting the spring-time maximum in NPF event frequency in Hyytiälä. In the summer (June–August), the NPF events occurred less frequently, and this is seen in the lower nucleation mode concentrations. The summertime nucleation events also began typically from larger particle sizes than during other times of the year. Towards the autumn, the weekly medians of the nucleation mode concentrations increased again.

Compared with the total alkylamine concentrations, this autumn peak in nucleation mode concentrations in late September indicated that similarity, as in the intermediate ion concentrations, showed no clear association between the NPF events and amine concentrations in Hyytiälä. The weekly number of NPF event days also showed no correlation with the amine concentrations. This could have been due to the amine concentrations that, throughout the measurement period, were high enough so as not to limit the occurrence or magnitude of NPF.

3.5. Monoterpenes

Three monoterpenes, α-pinene, Δ^3-carene, and camphene, also clearly increased in concentration during September and October and their concentrations acted in a manner similar to that of the amine concentrations of EA + DMA and TMA + PA measured in the autumn (Figs. 5 and 6). The concentration developments of DEA, camphene, β-pinene, α-pinene and Δ^3-carene were similar throughout the measurement period (Fig. 5). The concentrations were high throughout the summer months but decreased after August towards the end of the measurement period.

4. Discussion

In comparison with earlier studies, in the present study measurement period lasted six months in a boreal forest as the most of existing measurements of gas-phase alkylamine concentrations were conducted in urban or rural areas in short-term measurement campaigns (Grönberg et al., 1992; Hanson et al., 2011; VandenBoer et al., 2012) (Table 3).

The gas-phase alkylamine concentrations in rural background air have mostly been in the same concentration range as those in the present study. However, the measurements of VandenBoer et al. (2012, 2011) and Hanson et al. (2011) were obtained from online measurement systems (ion chromatography and proton-transfer mass spectrometry, respectively). Ambient air alkylamines concentrations in urban or rural atmospheres in southern Sweden (Grönberg et al., 1992) were similar to those in our study, and the measurements were conducted by a method similar in principle (acid trap) to that in the present study.

Sellegri et al. (2005) measured ambient air DMA and TMA concentrations at the SMEAR II forest site during a campaign by using a chemical-ionization mass spectrometer. In their study the TMA concentrations were similar to those of the TMA + PA concentrations measured in our study. For DMA, their detection limit was relatively high (32 ppt_V) and the concentrations measured were below the detection limit. Most of these studies were also conducted in short periods of time (within a few days), thus not revealing annual or seasonal variability.

Table 2
Pearson's correlation coefficients for supplementary measurements with EA + DMA, TMA + PA, DEA, and sum of detected amines.

	Air temperature	Precipitation	Precipitation intensity	Pos. cluster ions, 0.8–1.7 nm	Neg. cluster ions, 0.8–1.7 nm	Pos. intermediate ions, 1.7–3.0 nm	Neg. intermediate ions, 1.7–3.0 nm	NO	NO_x
	(°C)	(mm)	(mm h^{-1})	(# cm^{-3})	(# cm^{-3})	(# cm^{-3})	(# cm^{-3})	ppb_V	ppb_V
EA + DMA	−0.01	0.25	0.26	0.03	0.29	−0.20	0.01	−0.04	−0.12
TMA + PA	−0.43*	−0.02	0.01	−0.14	0.15	−0.41*	−0.24	−0.01	0.26
DEA	0.63*	−0.05	−0.09	0.26	0.33**	0.24	0.12	0.24	−0.35**
Sum of detected amines	−0.13	0.15	0.16	0.31	−0.02	−0.31	−0.10	0.00	−0.01

	O_3	CO	Soil water content	Soil temperature	Litterfall
	ppb_V	ppb_V	($m^3\ m^{-3}$)	(°C)	(g $m^{-2}\ d^{-1}$)
EA + DMA	−0.29	−0.5*	0.01	0.11	0.47*
TMA + PA	−0.30	−0.36**	0.57*	−0.33**	0.74*
DEA	0.05	−0.01	−0.33**	0.67*	−0.23
Sum of detected amines	−0.34**	−0.50*	0.30	0.00	0.63*

* $p > 0.05$, ** $p > 0.10$.

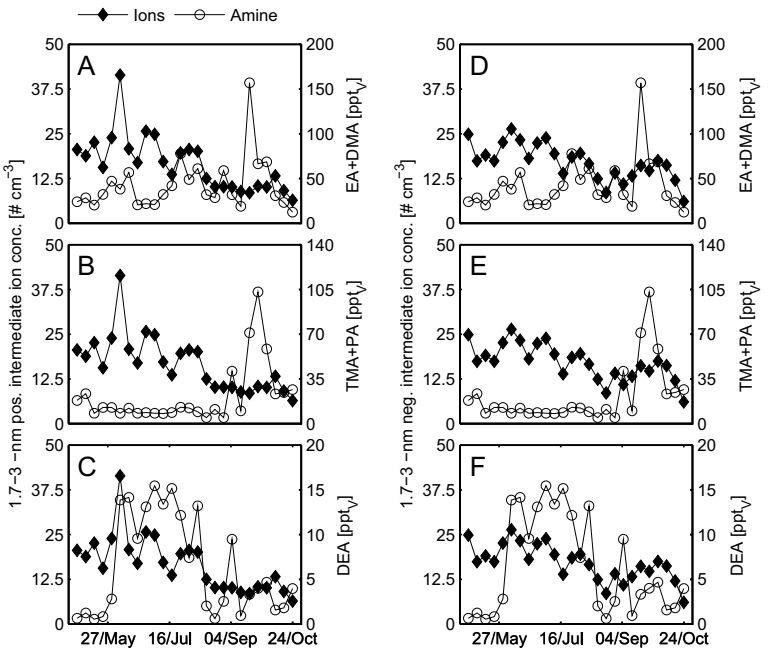

Fig. 3. Median positive (A, B, and C) and negative (D, E, and F) intermediate ion concentrations (cm^{-3}) for size ranges of 1.7–3 nm, and amine concentrations (ppt_V) of EA + DMA (A and D), TMA + PA (B and E), and DEA (C and F) during May–October 2011 at the SMEAR II station.

In this study, litterfall showed strong autumnal peaks at the same time as the peaks observed for the EA + DMA and PA + TMA concentrations. Similar autumnal peaks have also been observed in soil monoterpene and greenhouse gas nitrous oxide (N_2O) emissions from the SMEAR II site (Pihlatie et al., 2007; Aaltonen et al., 2011). Pihlatie et al. (2007) found that the N_2O was produced in the organic top soil and suggested that in this nitrogen-limited forest ecosystem, the new nitrogen input in the form of litter stimulated the N_2O formation in the top soil.

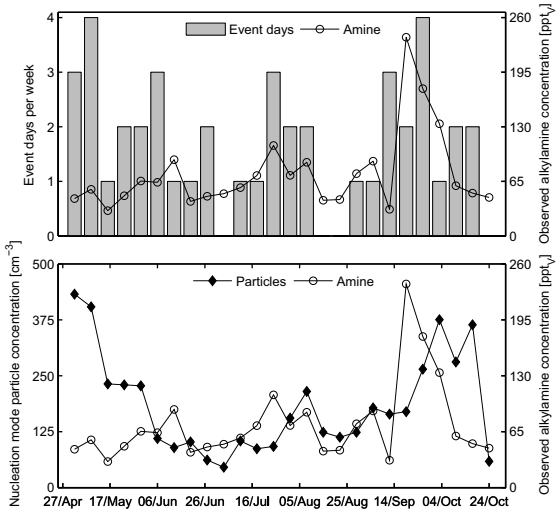

Fig. 4. In the upper panel, new particle formation days during each week. In the lower panel, medians of nucleation mode particle concentrations (cm^{-3}) during each week. Alkylamine concentrations (ppt_V) are the sum of the observed concentrations in the gas phase during the measurement period.

The autumnal monoterpene emissions from the forest floor coincided with the elevated or peaked EA + DMA and PA + TMA concentrations, indicating that the sources of these amines may be similar to those of the monoterpenes from the forest floor (Aaltonen et al., 2011, 2012). Most of the monoterpenes measured in boreal forest ecosystems originate from tree foliage (Rinne et al., 2009); however, the contribution of the forest floor and soil to the emission increases in the autumn (Aaltonen et al., 2012).

Autumnal monoterpene emissions have been associated with litterfall and decomposition processes in the soil after addition of fresh decomposable material in the form of needles and leaves (Hakola et al., 2003; Aaltonen et al., 2011). Monoterpene air concentrations were maximal either in June and July (α-pinene and Δ^3-carene) or in late August or early September (β-pinene, 1,8-cineol), depending on the species emitted (Hakola et al., 2012). In general, monoterpene emission potentials for Scots pine are highest in June and for Norway spruce in May (Hakola et al., 2006, 2003). Scots pine also emits monoterpenes in late autumn; however, due to lower emission potentials and colder weather, these emissions from tree canopies are usually low (Hakola et al., 2006). The possible processes linked to amine formation in the forest floor are not known; however, amines may be formed in amino acid carboxylation in the soil (Yan et al., 1996a, 1996b) and recycling of soil dissolved organic nitrogen (Yu et al., 2002). The positive correlation suggests that amine formation may occur in the forest floor (soil and ground vegetation) during the autumn.

A correlation between the intermediate ion and amine concentrations would indicate that gaseous amines may take part in particle formation and growth. In the autumn, the high occurrence

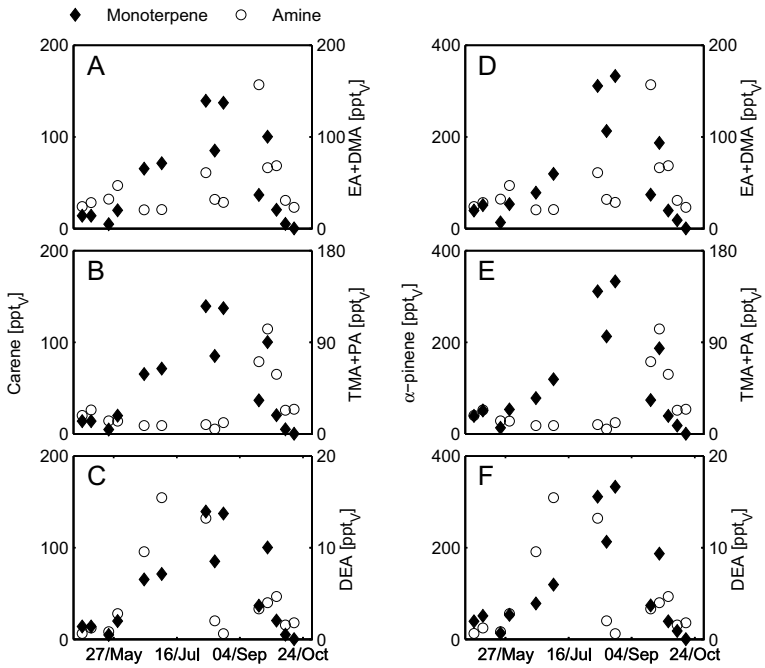

Fig. 5. Δ^3-carene (A, B, and C) and α-pinene (D, E, and F) concentrations (ppt$_V$) during the measurement period along with measured amine concentrations (ppt$_V$) of EA + DMA (A and D), TMA + PA (B and E), and DEA (C and F) for same measurement periods as for Δ^3-carene and α-pinene.

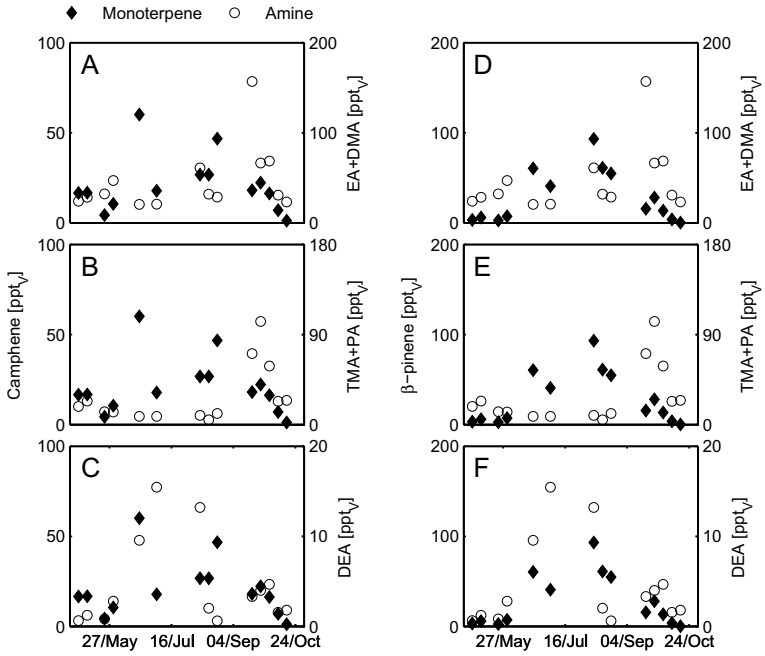

Fig. 6. Camphene (A, B, and C) and β-pinene (D, E, and F) concentrations (ppt$_V$) during the measurement period along with measured amine concentrations (ppt$_V$) of EA + DMA (A and D), TMA + PA (B and E), and DEA (C and F) for same measurement periods as for camphene and β-pinene.

Table 3
Comparison of previously and in this study measured alkylamine concentrations (ppt_v) and their standard deviations in rural and urban atmospheres. Units are in ppt_v.

Grönberg et al. (1992)	DMA	0.5 ± 0.3	TMA	5.2 ± 0.3	DEA	1.4 ± 03	TEA	<0.5	Urban
Sellegri et al. (2005)	DMA	<32	TMA	34–80					Urban
Hanson et al. (2011)	DMA	0.5–2.0	TMA	4.0–15	DEA	10–30	TEA	3.0–25	Urban
VandenBoer et al. (2012)	DMA	6.5 ± 2.1	TMA + DEA	1.0–10					Urban
Van Neste et al. (1987)	DMA	<0.3	TMA	0.8 ± 0.4					Rural
Grönberg et al. (1992)	DMA	1.8 ± 0.6	TMA	41 ± 14	DEA	1.7 ± 0.4	TEA	0.7 ± 0.5	Rural
VandenBoer et al. (2011)	DMA	<2.7	TMA + DEA	<2.7					Rural
This study[a]	EA + DMA	42 ± 30	PA + TMA	21 ± 23	DEA	6.5 ± 5.6	TEA	<3.2	Rural

[a] Mean of throughout the measurement period and its standard deviation.

of the event days and the high amine concentrations coincide, which indicates that during this period amines (EA + DMA and TMA + PA) may take part in NPF. In previous studies conducted at the SMEAR II forest site, Ruiz-Jimenez et al. (2011) measured the DEA during NPF (in 50-nm ultrafine particles) in the summer but not in the autumn, which is consistent with our measurements of elevated DEA during the summer.

Atmospheric NPF is dependent on several factors such as relative humidity and sulfuric acid concentrations, rather than only on the atmospheric concentration of alkylamines. Paasonen et al. (2012) modeled cluster formation of sulfuric acid, DMA, and TMA under different meteorological conditions and found that in the concentration range from 10^5 to 10^7 amine molecules per cm^3 (in the present study the sum of amines ranged from 10^6 to 10^9 molecules per cm^{-3}), the formation rate of aerosol particles in the atmosphere was dependent on the concentration of sulfuric acid and relative humidity.

The lack of correlation between the alkylamine concentrations and the intermediate ion as well as nucleation mode particle concentrations in our study may have resulted from different time scales of the measurements. The weekly averaging in the alkylamine measurements resulted in estimates of the source behavior on a seasonal scale, but the 1-week-long sampling time may have masked the short-term variations in the atmospheric concentrations. However, the amine concentrations and atmospheric intermediate ion concentrations showed similarities during the measurement period, but based on the measurements we could not distinguish whether the changes in the concentrations of amines and atmospheric intermediate ions were related.

5. Conclusions

Our measurements covered a long time series during which amines showed pronounced seasonal variability; DEA showed a summer maximum, whereas EA + DMA and TMA + PA showed maxima during the autumn. Similar to the monoterpene concentrations in the forest air, amine concentrations seem to be linked with vegetation, soil activity, and litterfall, rather than to other trace gases in the atmosphere. Atmospheric monoterpene concentrations in autumn especially those of α-pinene, Δ^3-carene, and camphene, had elevated concentrations similar to that of the measured amine concentrations of EA + DMA and TMA + PA. DEA and β-pinene air concentrations were similar during summer. Peaks in the positive and negative intermediate ions showing similar concentrations occurred simultaneously with peaks in EA + DMA and DEA during the summer. The number of new particle formation days peaked with sum of the alkylamine concentrations observed during the autumn.

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Paper II

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Gas-phase alkyl amines in urban air; comparison with a boreal forest site and importance for local atmospheric chemistry

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HIGHLIGHTS

- Several alkyl amines were observed in urban background air.
- The concentrations of alkyl amines were higher in boreal forest air than in urban background air.
- Amines strongly impacted local atmospheric chemistry.

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ABSTRACT

Low-molecular-weight aliphatic amines were measured in the ambient urban background air at the SMEAR III station (Station for Measuring Forest Ecosystem–Atmosphere Relations III) in Helsinki, Finland, from May until late August 2011. The alkyl amines measured were dimethylamine (DMA), ethylamine (EA), trimethylamine (TMA), propylamine (PA), diethylamine (DEA), butylamine (BA) and triethylamine (TEA).

Of these amines, DMA + EA and TMA + PA were the most abundant, with average concentrations of 24 and 8 ppt. The ranges of weekly mean concentrations of DMA + EA and TMA + PA were <DL (9.5 ppt) – 55 ppt and 4–27 ppt. The concentrations of all amines in urban background air in Helsinki were lower than at a boreal forest site (SMEAR II), indicating the presence at the latter site of some additional sources. Amine lifetimes are short, varying from 2.3 h to 7.6 h against hydroxyl (OH) radicals. The amine concentrations were scaled against OH reactivity and compared with the OH reactivities of aromatic hydrocarbons and terpenes. The results showed that amines strongly influenced the total OH reactivity, especially at the boreal forest site in May, September and October, showing contributions almost as high as those of monoterpenes.

1. Introduction

The most common and abundant amines in the atmosphere are low-molecular-weight aliphatic amines with carbon numbers of 1–6, such as methylamine (MA, $(CH_3)NH_2$), dimethylamine (DMA, $(CH_3)_2NH$), trimethylamine (TMA, $(CH_3)_3N$), ethylamine (EA, $(C_2H_5)NH_2$), diethylamine (DEA, $(C_2H_5)_2NH$) and triethylamine (TEA, $(C_2H_5)_3N$) (Ge et al., 2011a). These amines have sources that are both anthropogenic (e.g. combustion, biomass burning and animal husbandry) and biogenic (oceans, soil and vegetation) (Ge et al., 2011a).

The major removal processes for gas-phase alkyl amines are reactions with atmospheric oxidants, such as hydroxyl (OH) radicals and ozone (O_3) (Finlayson-Pitts and Pitts, 2000). Amines may react also with nitrate (NO_3) radicals (Murphy et al., 2007), but the reaction is only known for TMA. As in ammonia, amines can also be partitioned into aqueous aerosols. Reactions with atmospheric acids, such as HNO_3, H_2SO_4 and HCl, to form solid salts are also possible (Ge et al., 2011b; Murphy et al., 2007). The partitioning of amines into aqueous aerosols is strongly dependent upon pH, being greatest for acidic aerosols. For several common amines, the tendency to partition into the particle phase is similar to or greater than that of ammonia.

Lately, amines have become of interest, since they are believed to participate in the formation of new particles, as observed in several environments throughout the world. There is evidence, both from laboratory experiments (Almeida et al., 2013; Erupe

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et al., 2011) and field measurements (Smith et al., 2010), that amines play an important role in atmospheric nucleation. This process of new particle formation is a globally important source of cloud condensation nuclei, having a net cooling effect on the climate (Merikanto et al., 2009; Wang and Penner, 2009).

Studies of the atmospheric concentrations of gas-phase amines are scarce, and most of them are based only on short campaigns. Several techniques have been used. VandenBoer et al. (2011) used an online ion chromatograph (ambient ion monitor-ion chromatograph, AIM-IC) for detecting amines in the gas phase and in fine particulate matter in a 10-day campaign in Toronto. Hanson et al. (2011) developed a method using ambient pressure proton transfer mass spectrometry (AmPMS) for detecting both ammonia and amines. Sellegri et al. (2005) used a chemical ionization mass spectrometer (CIMS) for measuring DMA and TMA in a boreal forest, but the DMA mixing ratios remained below the detection limit (DL; 32 parts per trillion, ppt) throughout the 12-day campaign. Akyuz (2008) trapped amines as their hydrochloric salts in an aqueous acidic solution and, after several sample preparation steps, analysed them with a gas chromatograph–mass spectrometer (GC–MS). Gronberg et al. (1992) studied amines in Sweden by sampling in dilute H_2SO_4 and measuring by gas enrichment in a liquid flow system followed by a GC. In spite of these several techniques, measuring amines quantitatively at atmospherically relevant concentrations is still a challenge.

In this study, the concentrations of DMA, EA, TMA, propylamine (PA, $(C_3H_7)NH_2$), DEA, butylamine (BA, $(C_4H_9)NH_2$) and TEA were measured in urban air in Helsinki, Finland, using acid-impregnated filters and the liquid chromatography–mass spectrometry (LC–MS) method. The results were compared with those obtained in a boreal Scots pine *Pinus sylvestris* L. forest, and the importance of amines for local atmospheric chemistry was estimated.

2. Experimental

2.1. Measurement sites

Measurements were conducted at the urban background station SMEAR III (Station for Measuring Ecosystem–Atmosphere Relations III) in Helsinki, southern Finland (60°12′N, 24°58′E, 26 m above sea level, a.s.l.). The station is located about 5 km northeast of the centre of Helsinki. In 2011, the total population of the Helsinki metropolitan area was approximately 1 million, and the area of conurbation 764 km^2. The Baltic Sea is located to the south of Helsinki. The surroundings of the site are very heterogeneous, consisting of buildings, parking lots, roads, patchy forest and low vegetation. A more detailed description of the site can be found in Järvi et al. (2009). The measurements at SMEAR III were compared with those from the SMEAR II station in a Scots pine forest at Hyytiälä (61°510′N, 24°170′E, 180 m a.s.l.) in southern Finland (Hari and Kulmala, 2005).

2.2. Measurement methods

The measurements were conducted from early May until late August in 2011 on the roof of the station building at a height of 3 m. The amines were collected as salts, using phosphoric-acid-impregnated fibreglass filters. A polytetrafluoroethylene (PTFE) membrane filter (Millipore: Fluoropore®; EMD Millipore Corp., Billerica, MA, USA, 3.0 μm FS) was used in front of the impregnated filters to remove particles. The impregnated filters were prepared according to the procedure introduced by Rampel et al. (2008) and Kieloaho et al. (2013). The sampling flow through the stack of filters was 16 l min^{-1}, the sampling time being 24 h on weekdays and 72 h over the weekends. For the analysis, the filters were pooled and analysed as weekly samples.

Aminium ions were extracted from the filters with ultrapure water in an ultrasonic bath for 1 h, after which the extract was analysed by a high-performance liquid chromatography-electrospray ionization-ion trap mass spectrometer (HPLC-ESI-ITMS) (Agilent 1100 series LC/MSD trap; Agilent Technologies, Santa Clara, CA, USA). As an internal standard, deuterated diethyl-d_{10}-amine (Sigma–Aldrich: Isotec™; Sigma–Aldrich, St. Louis, MO, USA) and a 5-point external standard for all measured alkyl amines was used. In the LC system, a Discovery® HS F5 HPLC (Supelco Analytical; Supelco Inc., Bellefonte, PA, USA) was used as the analytical column and an HPLC SecurityGuard™ cartridge (Phenomenex®; Phenomenex, Torrance, CA, USA) as a precolumn. Water and acetonitrile with 0.02% formic acid as a buffer were used as eluents. The compounds measured were EA, DMA, TMA, PA, DEA, TEA and BA. The column was unable to separate DMA and EA or TMA and PA, so their results are given as a sum (i.e., DMA + EA and TMA + PA). The limits of detection calculated as three times the standard deviation of the field blank levels are listed in Table 1. The uncertainty of the analyses is reported as a standard deviation between the four parallel analysis results and shown as error bars in Fig. 1. A more detailed description of the sampling and analysis method, as well as of the measurements at the SMEAR II site, can be found in Kieloaho et al. (2013).

Filter sampling suffers from both positive and negative artifacts. Particulate amines may be volatilized during collection and result in positive artifacts at gas-phase concentrations. However, the solid/gas equilibrium dissociation constants for most amines are much smaller than for ammonia and are not volatilized as easily (Ge et al., 2011b) For ammonia, results with Comparative Programme for Monitoring and Evaluation of Long-Range Transmission of Air Pollutants in Europe (EMEP) filter pack sampling have been in good agreement with denuder-based Monitor for Aerosols and Gases in Ambient Air (MARGA; Metrohm Applikon B.V., Schiedam, The Netherlands) measurements and no positive artifacts due to volatilization from the front filter were found in measurements conducted at the SMEAR II site (Makkonen et al., in press). On the other hand, some of the gaseous amines may be retained by the front filter and breakthrough or reactions at the impregnated filter can result in negative artifacts. Breakthrough tests were conducted by injecting amines into the zero air flow and collecting onto the impregnated filters for 24–72 h with the flows used for ambient air sampling. No breakthrough was observed. For minimization of the artifacts, the samples were collected as 24-h samples during weekdays and 72-h samples during weekends and pooled later as weekly samples. Having the sampling system at the sampling temperature also decreased the interferences.

2.3. Supplementary measurements

In addition to the amine measurements, trace gas and volatile organic compound (VOC) concentrations, as well as meteorological parameters, were also measured at the SMEAR III station. Nitrogen oxides (NO_x) were measured with a chemiluminescence analyser (TEI42S, Thermo Fisher Scientific, and Waltham, MA, USA) and sulphur dioxide (SO_2) with an ultraviolet (UV) fluorescence analyser (Horiba APSA 360; Horiba Ltd., Kyoto, Japan). VOCs comprising 8 monoterpenes and 13 aromatic hydrocarbons (HCs) were measured, using an in situ thermal desorpter connected to a GC coupled with an MS (TD–GC–MS). The VOC measurements at the SMEAR III and II stations are described in detail by Hellén et al. (2012) and Hakola et al. (2012).

Table 1
Mean values and range of concentrations of alkyl amines at the urban background site (SMEAR III) in Helsinki and at the forest site (SMEAR II) in Hyytiälä, Finland, in May–August 2011 together with OH reactivities (s^{-1}).

	DL	Urban (pptv)	Forest[a] (pptv)	OH reactivity urban (s^{-1})	OH reactivity forest (s^{-1})
DMA + EA	9.5	23.6 (<DL–54.9)	39.1 (20.1–77.8)	0.277 (<DL–0.634)	0.451 (0.232–0.898)
TMA + PA	2.4	8.4 (3.9–26.9)	10.2 (4.6–23.4)	0.126 (0.059–0.404)	0.153 (0.069–0.352)
BA	0.06	0.3 (0.1–0.56)	2.7 (0.4–7.4)	0.003 (0.001–0.005)	0.022 (0.003–0.062)
DEA	0.08	0.3 (<DL–1.3)	8.1 (0.5–15.5)	0.006 (<DL–0.026)	0.161 (0.010–0.308)
TEA	0.01	0.1 (<DL–0.16)	1.6 (0.7–2.9)	0.002 (<DL–0.004)	0.036 (0.016–0.066)

[a] Adapted from Kieloaho et al., 2013.

2.4. Calculation of OH reactivities of amines

The OH reactivities (s^{-1}) of the various compounds were calculated, using Eq. (1).

$$\text{OH reactivity} = k_{X,OH}[X] \quad (1)$$

where $k_{X,OH}$ is the reaction rate constant between X and OH and [X] is the VOC mixing ratio. For DMA + EA eluting together, the average reaction rate constant was used. For TMA + PA, the (rate constant of TMA) $k_{TMA,OH}$ was used, since the (reaction rate constant for PA) $k_{PA,OH}$ was not known. The OH reactivity determines in an approximate manner the compound's relative role in OH chemistry (as a possible O_3 or secondary organic aerosol (SOA) precursor) in the atmosphere.

3. Results

3.1. Alkylamine concentrations

Of the measured amines DMA + EA was the most abundant, with an average concentration of 24 parts per trillion by volume (pptv) (Table 1). TMA + PA also showed a somewhat higher concentration (on average 8 pptv), but for the other amines the concentrations were below 1 ppt. The DMA + EA weekly average concentrations varied from below the DL (9.5 ppt) to 55 pptv (Fig. 1). The highest values were measured in late July and early August. The variability in concentrations was not consistent for all species (Fig. 1), indicating that different sources may exist for each. Industrial and combustion processes are globally the main anthropogenic sources of amines (Ge et al., 2011a) and are also most likely the main local sources in Helsinki. During week 19 (9–15 May, 2011), the DEA concentration peaked together with those of nitrogen dioxide (NO_2) and SO_2 (Figs. 1 and 2). Automobile traffic is no longer a source of SO_2 in Finland, but ship traffic or power plant emissions could explain the elevated concentrations of both SO_2 and NO_2 and possibly also of DEA. Further detailed inspection of SO_2 showed that during week 19, the concentrations peaked in the afternoon of 9 May. Wind direction during the peak was from the south or southwest, which is in the direction of harbours and power plants. No correlations of other amines with NO_2 or SO_2 were found, and no correlations with meteorological parameters were found for any of the amines. This was expected, since with weekly averages much information is lost.

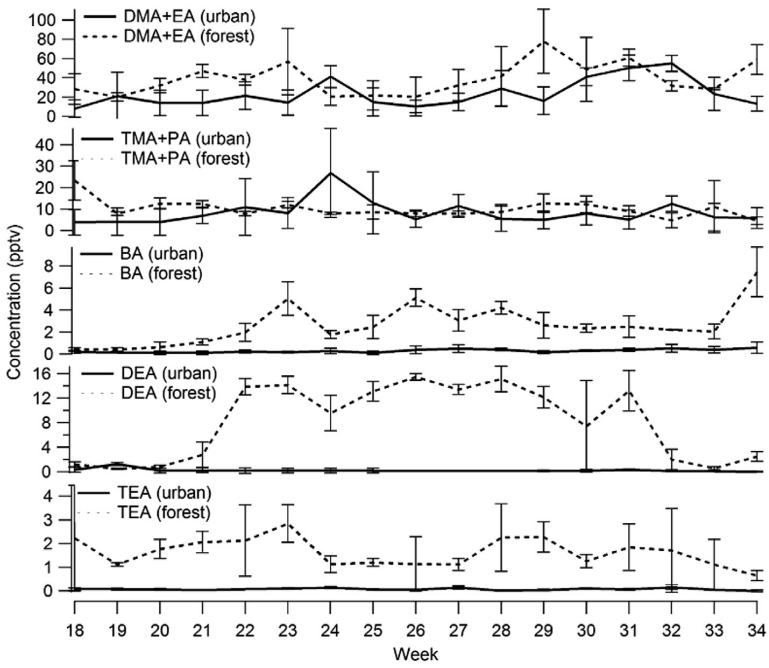

Fig. 1. Concentrations of various alkyl amines at the SMEAR III urban background station in Helsinki and at the SMEAR II boreal forest site in Hyytiälä (Kieloaho et al., 2013), Finland in May–Aug 2011.

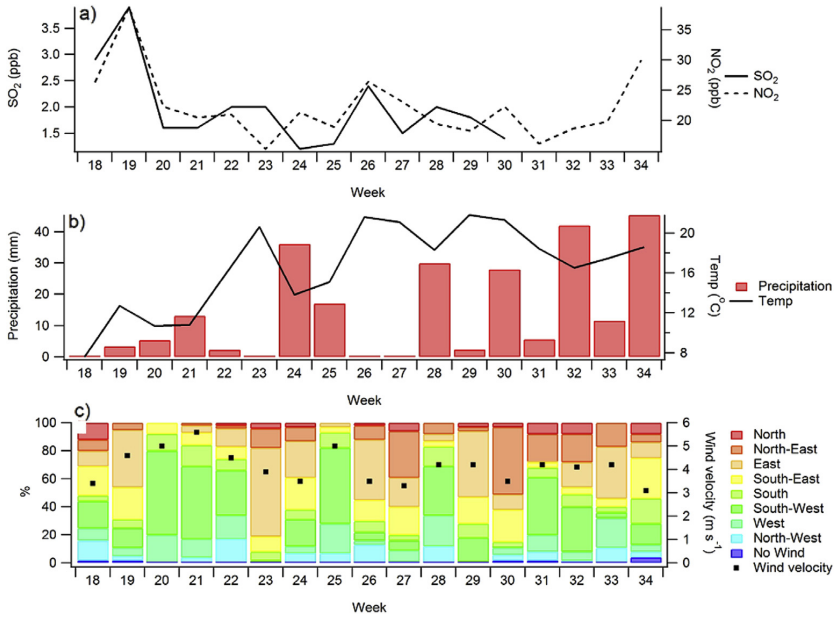

Fig. 2. a) SO_2 and NO_2 concentrations, b) weekly precipitation (mm) and average temperature (°C) and c) weekly distribution (%) of wind directions and average wind velocity ($m\ s^{-1}$) in Helsinki at the SMEAR III site in May–Aug 2011.

The results from the urban SMEAR III site were compared with those measured at the same time in a boreal forest (SMEAR II). The amine measurements at SMEAR II are presented in Kieloaho et al. (2013). At the SMEAR II site, DMA + EA and TMA + PA were also the most abundant alkyl amines. For all the amines, the average concentrations were higher at the boreal forest site than at the urban background site. The DMA + EA and TMA + PA concentrations were slightly higher, but the other amines were clearly higher (Fig. 1). Since the lifetimes of amines are short and they are not transported over long distances, these results indicate that there were some significant amine sources in the boreal forest or nearby that were not found at the urban site. The lower concentrations at the urban site could also have been due to more rapid oxidation. However, oxidation due to OH radicals and O_3 was similar at both sites. The mean O_3 concentrations at SMEAR III and II were 30 and 34 parts per billion (ppb), respectively. OH radicals were produced at the same rate at both sites and the OH reactivity-scaled concentrations of VOCs (i.e., OH sinks) were similar (Hellén et al., 2012; Hakola et al., 2012). Faster NO_3 radical reactions could explain the lower concentrations. The reaction rate constant was found only for TMA, and, based on it, NO_3 radical reactions are much slower than OH radical or O_3 reactions and are not able to explain the lower concentrations.

Vegetation and soils were probably the main sources of amines at the SMEAR II (forest) site. Kieloaho et al. (2013) showed that amine concentrations were linked with vegetation, soil activity and litterfall. Other possible sources for amines at this site could be agriculturally based (Kuhn et al., 2011). However, there was no agricultural activity on the 5-km radius plot. Williams et al. (2011) showed some evidence for agricultural land use, but this was only naturally growing vegetation. Therefore, agricultural activity was probably not a significant source at this site.

At the SMEAR II site, the seasonal course of DEA was different from that of the other amines (Kieloaho et al., 2013), having its highest concentrations in summer (June/July). This was not seen at the urban SMEAR III site, where DEA peaked in May. The BA concentrations showed some positive correlation ($r = 0.6$) between the sites, but this did not hold for any of the other compounds.

Table 2
Reaction rates (k) and tropospheric lifetimes (τ) of the studied amines in hours (h) with respect to OH radical concentrations typical of Finland in May–Aug (1.32E+6 molec cm^{-3}), O_3 concentrations (30 ppb) concurrently measured at the SMEAR III site in May–Aug 2011 and NO_3 radical concentrations (<100 ppt) typically measured in polluted air masses (Platt and Janssen, 1995).

	DMA	TMA	EA	DEA	TEA	BA
k(OH) (cm^3 $molec^{-1}$ s^{-1})	6.62E-11[(1)]	6.11E-11[(1)]	2.77E-11[(1)]	8.07E-11[(2)]	9.26E-11[(2)]	3.4E-11[(2)]
k(O_3) (cm^3 $molec^{-1}$ s^{-1})	1.66E-18[(1)]	7.84E-18[(1)]		1.33E-17[(3)]	8.20E.17[(3)]	
k(NO_3) (cm^3 $molec^{-1}$ s^{-1})		4.4E-16[(4)]				
τ(OH) (h)	3.2	3.4	7.6	2.6	2.3	6.2
τ(O_3) (h)	226	48		28	4.6	
τ(NO_3) (h)		>257				

Note: The monthly means of the daytime OH concentration used can be found in Hakola et al. (2003). The reaction rates at 298 ± 5 K were obtained from the [(1)] NIST Chemical Kinetics Database, [(2)] Chemfinder, The predicted data were generated, using the US Environmental Protection Agency's EPISuite™; for more information see their website (http://www.epa.gov/opptintr/exposure/pubs/episuite.htm, accessed 26.11.2013), [(3)] Gai et al. (2010), [(4)] Silva et al. (2008).

3.2. Reactivity of amines

The lifetimes of amines (Table 2) against OH radicals are short, varying from 2.3 h to 7.6 h. They exhibit reactions as fast or faster than those of most monoterpenes or aromatic HCs (Hellén et al., 2012). O_3 reactions are also important, with lifetimes from 4.6 h to 226 h (Table 2). The reaction rate with NO_3 radicals was found only for TMA, but the lifetime was longer than those against OH radicals and O_3. In addition to these reactions, amines are removed from the atmosphere through gas-particle partitioning and wet deposition (Ge et al., 2011b). The reaction rate of PA is not known.

The amine concentrations were scaled against OH reactivity and compared with the reactivities of the VOCs. Since direct OH reactivity measurements were not available, the amine reactivities were compared with the sum of these individually measured VOC reactivities. This comparison could determine the relative roles played by amines in local chemistry at the sites. Due to instrumental problems, the VOC measurements were conducted at the SMEAR III site only in May and July 2011. A more detailed description of the measurements can be found in Hellén et al. (2012). Terpenes are mainly emitted from biogenic sources and aromatic HCs from anthropogenic sources. Compared with the total OH reactivity of the VOCs, the alkyl amines measured contributed 14% in May and 6% in July (Fig. 3). In July, the concentrations of terpenes (isoprene and monoterpenes) were high, due to biogenic emissions, and therefore the contribution of amines decreased (Hellén et al., 2012; Hakola et al., 2012; Yassaa et al., 2012). In May, DMA + EA had as high reactivity-scaled concentrations as those of α-pinene (monoterpene) or toluene (aromatic HC).

At the SMEAR II boreal forest site, alkyl amines showed almost as high an influence on OH reactivity as did monoterpenes in May, September and October 2011, but during the summer months the monoterpenes were predominant (Fig. 3). Emissions of monoterpenes are known to be high in summer (Tarvainen et al., 2007), which explains their large contribution. However, the contributions of the alkyl amines in June, July and August were still relatively high at 23%, 21% and 10%, respectively. Isoprene was not measured, due to analytical problems. However, the forest is mainly composed of Scots pines, which are not high isoprene emitters (Hakola et al., 2006), and isoprene concentrations in previous studies have been low (Hakola et al., 2003).

Sinha et al. (2010) and Noelscher et al. (2012) used the comparative reactivity method (CRM) with a proton transfer reaction-mass spectrometer (PTR-MS) at the SMEAR II station and found abundant unknown OH reactivity that was not explained by traditionally measured VOCs and trace gases. The contribution of the unknown reactivity was 50% and 68% in summers 2008 and 2010, respectively. The total directly measured OH reactivity at the SMEAR II site in summer 2010 was 12.4 s^{-1}. The sum of the reactivities of the amines in our study in July 2011 was 0.89 s^{-1}, which is 10% of the unknown reactivity found by Noelscher et al. (2012). The highest OH reactivities (1.65 s^{-1}) of the amines were measured in September. Based on this, the alkyl amines could explain a significant part of the unknown OH reactivity found at the SMEAR II site.

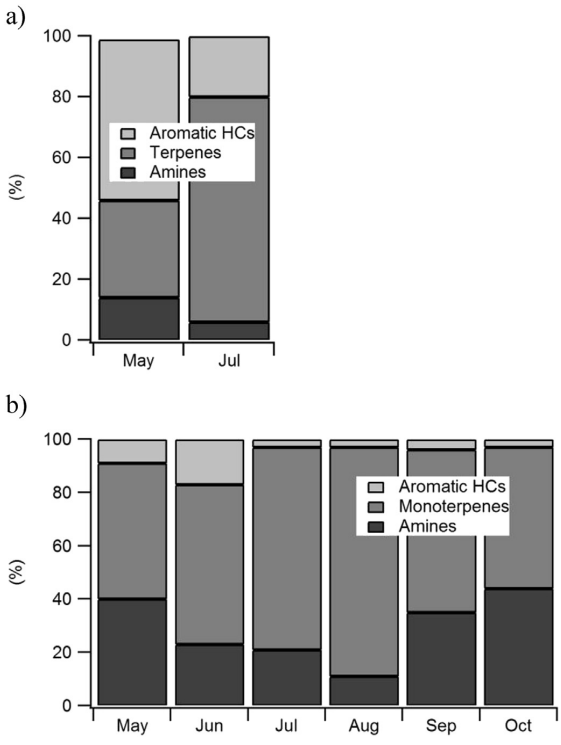

Fig. 3. Relative contributions of amines, aromatic hydrocarbons (HCs) and terpenes (isoprene + monoterpenes) to OH reactivity during various months a) at the urban background site (SMEAR III) in Helsinki in 2011 (this study) and b) at the boreal forest site (SMEAR II) in Hyytiälä, Finland in 2011.

4. Conclusions

The concentrations of gas-phase alkyl amines were studied at an urban background site in Helsinki, Finland, and the results were compared with those from a boreal forest site. Of the alkyl amines measured, DMA + EA and TMA + PA were the most abundant at both sites. The concentrations of all the amines were higher in the boreal forest, which indicated the presence of some amine sources that are not found in urban air. The higher concentrations could also have been due to slower oxidation with NO_3 radicals, but based on the reaction rate of TMA this is probably not important. In Helsinki, the DEA concentration peaked together with that of NO_2 and SO_2, indicating ship traffic or power plant emissions as the source.

Alkyl amines are very reactive and make important contributions to local atmospheric chemistry both in urban and boreal forest air. They greatly impacted OH reactivity, especially at the boreal forest site, where they explained a significant part of the unknown OH reactivity found in previous studies (Noelscher et al., 2012; Sinha et al., 2010).

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Stimulation of soil organic nitrogen pool: The effect of plant and soil organic matter degrading enzymes

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ABSTRACT

The majority of nitrogen (N) in boreal forest soils is bound to soil organic matter (SOM) in forms not readily available to plants. Northern boreal forest ecosystems are often N limited, despite atmospheric N deposition, and the utilization of organic N from SOM is of crucial importance to the site productivity. The effect of microbial produced oxidative SOM degrading enzymes (laccase and manganese peroxidases) and proteases on soil N forms and availability was studied in a pot experiment with or without a Scots pine (*Pinus sylvestris* L.) seedling. The combination of SOM degrading enzymes and proteases decreased the total soil N content and increased the N losses significantly in the absence of the Scots pine seedlings. The total soil N content also decreased in the presence of the Scots pine seedlings, irrespective of the enzyme treatment. Most of the other N parameters studied were not sensitive to enzyme additions, and differed only between planted and non-planted treatments. Our results show that the alkylamine content of boreal forest soil are at the same levels as nitrate. We showed that SOM decomposition, stimulated by oxidative enzyme additions, is a key step in soil organic N utilization, while proteases alone do not increase N use from SOM. Plants stimulate N losses from SOM highlighting the importance of rhizosphere processes in soil C and N cycling.

1. Introduction

A large part of total soil nitrogen (N) of boreal forest soil is in organic forms (Korhonen et al., 2013). The total N quantities in such ecosystems can be surprisingly high, as was shown by Korhonen et al. (2013) who quantified the N balance of an entire Scots pine (*Pinus sylvestris* L.) forest stand. They found that the total soil N pool was 2070 kg N ha^{-1} and the annual uptake by trees amounted to 50 kg N ha^{-1}. Mineral N concentrations are usually low, despite the large total N pool, compared to the plant uptake and the ecosystem productivity is limited by the availability of N (Korhonen et al., 2013) indicating poor N availability of organic N for plants and microbes (Schulten and Schnitzer, 1998; Knicker, 2011; Korhonen et al., 2013). Nitrogen is integrated to SOM via stabilization processes that form either chemically recalcitrant N, or protect N physically from microbial utilization (Schulten and Schnitzer, 1998; Rillig et al., 2007). The majority of soil organic N is proteinaceous material (ca. 40%) or heterocyclic N including purines and pyrimidines (cs. 35%) (Schulten and Schnitzer, 1998). Decomposition of SOM and SON is regulated by many processes and factors including molecular structure, condensation reactions, fire residues, rhizosphere inputs, physical disconnection, soil depth, freezing-thawing and microbial products, as highlighted by Schmidt et al. (2011). In all, multiple processes may preserve but also release N from SOM and significantly affect soil C and N cycling.

The release of the chemically recalcitrant N from SOM and the formation of more readily available forms of N are of crucial importance for ecosystem productivity in N limited forest ecosystems (Schimel and Bennet, 2004; Knicker, 2011). The release of N from the large recalcitrant organic N pool in boreal forest soil SOM is enhanced by the presence of degrading enzymes such as laccase

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and manganese peroxidases (MnP) (Von Lützow et al., 2006; Kleber, 2010; Dungait et al., 2012). Additional biochemical processes are responsible for transformation of larger macromolecules to dissolved organic nitrogen (DON), including peptides or amino acids, which can be taken up by plants or microbes (Schimel and Bennet, 2004). Finally, peptides and amino acids are mineralized via microbial ammonification and nitrification–denitrification processes to inorganic nitrogen, ammonium (NH_4^+) and nitrate (NO_3^-) (Schimel and Bennet, 2004; Fig. 1). The depolymerization of proteinaceous material and the release of monomers and oligopeptides (e.g. amino acids) has been proposed to be the key step in regulating N uptake by plants and microbes in boreal forest ecosystems (Schimel and Bennet, 2004).

The formation of a symbiotic relationship between trees and ectomycorrhizal (ECM) fungi is widespread in boreal ecosystems. The fungal symbionts improve nutrient uptake of the host plant, especially from organic sources (Smith and Read, 2008). In exchange, plants allocate a significant portion of the C assimilated in photosynthesis to the ECM fungi and associated microflora (Smith and Read, 2008). Fungal symbionts increase N availability by stimulating SOM decomposition via root exudation and the excretion of extracellular enzymes (Talbot and Treseder, 2010; Drake et al., 2011; Phillips et al., 2011; Kaiser et al., 2011; Lindén et al., 2014). Recently it has been shown that manganese peroxidase (MnP)-like peroxidases, enzymes essential in the degradation of lignin-compounds, may be produced by the common ECM genus *Cortinarius* (Bödeker et al., 2009). Furthermore, class II peroxidase-encoding genes have been identified in a wide range of ECM fungi (Bödeker et al., 2009). ECM fungi are also known to degrade SOM using a Fenton-reaction based mechanism in a similar way as brown-rot fungi (Rineau et al., 2012). A non-specific oxidative enzyme, laccase, is produced by many fungi, including ECM fungi (Chen et al., 2003; Luis et al., 2004; Heinonsalo et al., 2012). Several laccase genes are encoded in the genome of the common ECM genus *Piloderma* sp. (Kohler et al., 2015) and laccase gene expression was related to N availability (Chen et al., 2003). Talbot and Treseder (2010) suggested that the symbiotic relationship between trees and their ECM fungi is the key factor in stimulating SOM decomposition and the subsequent release of N for use by plants and microbes.

The microbial nitrogen turnover processes may also result in a small fraction of nitrogen being lost to the atmosphere in the form of nitrogen monoxide (NO), nitrous oxide (N_2O), molecular nitrogen (N_2), or volatile organic nitrogen (e.g. amines) (Fig. 1). Very little information is available on the formation, production rates, or function of the volatile organic nitrogen compounds, such as amines, in boreal forest soil. However, as amines have been suggested to be formed via aminification processes (Yan et al., 1996; Yu et al., 2002), such as the decarboxylation of amino acids (Dudareva et al., 2013), it is possible that during depolymerization of SON amine formation is also induced. Amines are reactive compounds in the atmosphere and even in very low concentrations they participate in chemical reactions leading to formation and growth of aerosols in the atmosphere (Kurten et al., 2008; Almeida et al., 2013; Kurten et al., 2014). In addition to their importance to the atmospheric chemistry, amines may also have important and currently unknown functions in the cycling of N in the soil (Schulten and Schnitzer, 1998; Vranova et al., 2011; Warren, 2013). However, due to technical challenges in measuring amines in the atmosphere and in soils, there are few studies reporting amine concentrations in the atmosphere (Sellegri et al., 2005; VandenBoer et al., 2012; Kieloaho et al., 2013; You et al., 2014) and especially in soils (Yu et al., 2002).

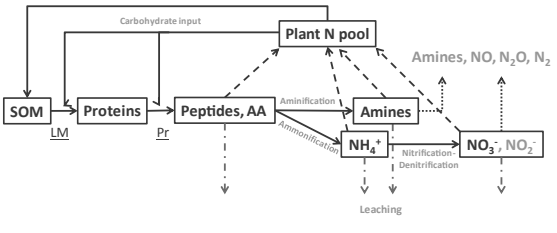

Fig. 1. A schematic representation of N cycling in boreal forest ecosystems, modified from Schimel and Bennet (2004). Boxes: compounds in the soil or plant, solid arrows: microbial and plant-driven processes, dashed arrows: plant N uptake or N loss from the soil via leaching or volatilization. Black color: quantities measured in the experiment, gray color: quantities not measured but are part of N cycle. Underscored LM and Pr refers to reactions stimulated by enzyme treatments in this study: LM = laccase and manganese peroxidase, and Pr = protease additions, respectively.

The aim of the experiment was to study the effect of SOM degrading enzymes laccase (L) and manganese peroxidase (M) and protein degrading enzymes (proteases, Pr) on soil N cycling with the presence or absence of a Scots pine seedling (*P. sylvestris* L.). The concentrations of NO_3^-, NH_4^+, amino acids, alkylamines, total N, recalcitrant N, degradable and proteinaceous N, plant biomass, enzyme activities and ECM fungal root tip numbers were quantified after the enzyme treatments. The schematic presentation of hypothetical boreal forest soil N cycling, effective points of enzyme treatments and the measured N forms are shown in Fig. 1. We hypothesize that 1) the addition of SOM and protein degrading enzymes increase SOM decomposition and the proportion of N forms which are available to the plant, 2) the presence of a plant stimulates SOM decomposition and N availability in the soil, 3) increased protein degradation in protease treatments (Pr) induces amine synthesis and amine concentration in the soil.

2. Materials and methods

2.1. Experimental setup

The boreal forest soil used in the experiment was collected in May 2011 in the vicinity of SMEAR II station of Helsinki university at Hyytiälä (61°51′N, 24°17′E) in Southern Finland (Hari and Kulmala, 2005). The soil at the site is Haplic podzol and the stand is dominated by Scots pine (*P. sylvestris* L.) and occasional Norway spruce (*Picea abies* (L.) H. Karst.), Silver birch (*Betula* spp. L.), or European aspen (*Populus tremula* L.) could be found mainly in the understory. A detailed site description can be found in Ilvesniemi et al. (2009). Only the organic soil layer (mixed F/H horizon, later called 'soil') was used in the experiment. Large roots were removed, the fresh soil was homogenized and sieved through a 4 mm mesh, and stored at +4 °C for one month until the experiment was established. The total N content of the soil was 6.9 mg-N g^{-1} DW.

The experimental setup is shown in Fig. 2. The experiment consisted of six treatments. Three of the treatments were planted with one-year-old nursery grown Scots pine seedlings (N = 13) and three were non-planted treatments (N = 5). Altogether the experiment consisted of 54 pots, 39 with and 15 without Scots pine seedlings. Within the planted and non-planted treatments, three enzymatic treatments were created: 1) soil amended with BSA protein only (Bovine Serum Albumin, BSAS 0.01, Bovogen, Australia; in total 205.6 mg) was regarded as control treatment (Con). 2) Protease treatment (Pr) was created by adding two kinds of proteases: 2.16 mg protease 1 (*Streptomyces griseus*, Sigma–Aldrich, P5147, 0.84 activity units per mg), and 112.5 mg protease 2 (*Rhizopus* sp., Sigma–Aldrich P0107, 0.2 activity units per mg). 3) Laccase, manganese peroxidase and protease (LMPr) treatment was created by adding a mixture of enzymes including 90 mg laccase

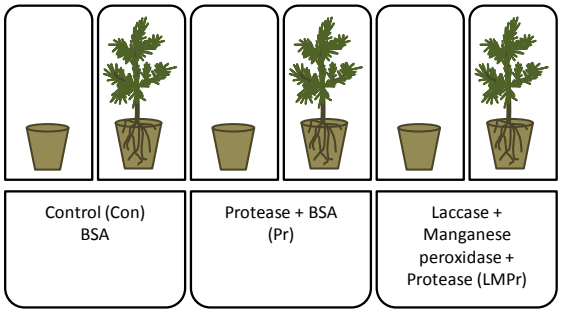

Fig. 2. Experimental design showing control protein (Bovine serum albumin, BSA) and enzyme (proteases and BSA in Pr, laccase, manganese peroxidase and proteases in LMPr) additions to the non-planted and planted treatments. All treatments received equal amount of proteins (BSA or enzymes) and had therefore same nutritional effect in soil.

(*Trametes versicolor*, Sigma–Aldrich 38429, activity 0.5 units per mg), 0.9 mg manganese peroxidase (*Nematoloma frowardii*, Sigma–Aldrich 41563, 4.2 activity units per mg), and same quantities of proteases as described above. In the protease treatment (Pr), 90.9 mg of BSA was also added to obtain an equal quantity of protein (205.6 mg) and the same nutritional effect of protein additions in all the treatments. The added protein N in each treatment corresponded to 12.5% of the original total N content of the soil (N content of the proteins assumed to be 16%). An activity unit corresponds to the amount of enzyme, which converts 1 μmol of substrate per minute in controlled conditions. Enzymes were selected based on the following criteria: commercial availability and the source organisms are relevant for soil environment. BSA was chosen for nutritional protein component for the experiment because it is a non-functional protein in soil environment and commercially available.

The experiment was established by inserting 0.2 L (38 g DW) of soil or soil and a plant into pots and letting them stabilize and grow for 14 weeks, after which the enzyme and protein additions were started. Enzymes were dissolved in 1 ml of deionized water and spread evenly on the soil surface. The enzyme additions took always place one day after the regular irrigation. During the six-week enzyme treatment period, proteins and enzymes were added nine times (quantity of protein added was in total 205.6 mg). After this treatment period, the pots were allowed to stabilize and seedlings grow for another four weeks, after which they were harvested. The experiment lasted for approximately six months and was carried out under natural daylight in a greenhouse between May and October with temperature ranging between 15 °C and 20 °C. During the experiment, pots were irrigated three times per week with distilled water and the excess irrigation water was allowed to drain from the pots. The average soil water content was 0.21 m^3 m^{-3} at the time of harvest.

2.2. Biomass harvest and soil sampling

At harvest, the shoot was divided into stem and needles, dried and weighed. The roots were separated from the soil and fresh and dry weight measured, and the mycorrhizal and non-mycorrhizal root tips were counted under a stereo microscope (Wild M5-63071, Heerbrugg, Switzerland). Rhizomorph-forming mycorrhiza (Agerer, 2001) were counted separately (Heinonsalo et al., 2010).

Soil was sieved using a 4 mm mesh, homogenized manually and separated into the following compartments: 0.5 g FW for enzyme analysis and 5 g FW for KCl extraction (to analyze dissolved N, NO_3^-, NH_4^+, total amino acid, Bradford protein, and amine contents) that were done immediately after harvest. The rest of the soil was divided into smaller units and kept frozen at −20 °C until dried at 50 °C and ground using a ball-grinder (2000-230 Geno/Grinder, SpexSample Pred, US) for other chemical analysis.

2.3. Chemical analysis

A schematic representation of all chemical analysis performed is shown in Table 1. The conducted analyses are described in detail below.

2.3.1. Total C and N contents and pH

Total nitrogen and carbon content were determined from dried and ground soil samples and biomass by a Vario MAX CN elemental analyzer (ELEMENTAR Analysensysteme, Hanau, Germany). The ash content and the amount of SOM were determined by loss on ignition at 550 °C. The material lost on ignition was regarded as SOM.

Soil pH was measured from mixed water-soil suspension (1:2.5) from fresh soil samples using a pH meter (Orion research SA720 pH/ISE, pH electrode Orion 8102BN). Soil suspensions were let to stand overnight at 4 °C before measurement.

2.3.2. Recalcitrant and degradable N contents using acid hydrolysis

Acid hydrolysis was used to separate soil N into two pools: *Hydrolysable N*, hereafter referred as '*degradable N*' pool, was released by acid hydrolysis and quantified in dissolved forms. This pool is hypothesized to be more readily accessible for soil organisms and plants. The *non-hydrolysable N* pool, hereafter referred as '*recalcitrant N*' was the N pool that was not removed from the soil sample during acid hydrolysis. This fraction of soil N is hypothesized not to be directly utilizable by organisms.

Acid hydrolyzation was done in an autoclave by using 4 M methyl sulfonic acid (MSA) following the method introduced by Martens and Loeffelmann (2003) and modified by Olk et al. (2007). Soil samples were dried 48 h at 50 °C, and then pulverized by a mechanical ball-grinder (2000-230 Geno/Grinder, SpexSample Pred, US) at 28 rpm, for 25 s. Three replicate pulverized soil samples were weighed (250 mg each), and 2 ml of 4 M MSA with 2 mg ml^{-1} of tryptamine was added. Tryptamine was used to prevent amino acid breakdown during the hydrolysis procedure. The soil samples with MSA addition were autoclaved at 136 °C and 134 kPa for 90 min. The samples were mixed in a vortex after the autoclave treatment, transferred to 15 ml falcon tubes and centrifuged 5 min at 4000 rpm (Centrifuge 5810R, Eppendorf). The supernatant was moved to another test tube, and 2 ml of distilled water was added to the remaining pellet to wash the remaining soluble N from the pellet. This washing water was mixed with the supernatant, and the combined extract was neutralized with 5 M KOH solution and stored at −20 °C until subsequent analysis. Extracts from acid hydrolysis were diluted to 1:20 and analyzed for total dissolved N content (TOC-Vcph/cpn TNM-1, Shimadzu Corporation, Kyoto, Japan), forming the degradable N pool. Recalcitrant N was calculated by subtracting degradable N content from the total soil N content.

2.3.3. KCl-dissolved nitrogen forms

In order to determine soil dissolved organic and inorganic nitrogen concentrations, fresh soil samples (5 g FW) were extracted (1:4) using 1 M potassium chloride (KCl) and shaken in a reciprocal shaker (120 rpm) for 80 min. Soil suspensions were first centrifuged (1500 rpm) and then filtered through 0.45 μm cellulose acetate filters (Pall Life Sciences, Ann Arbor, Michigan, US) and frozen at −20 °C until subsequent analysis. Dissolved total nitrogen concentrations were determined by a total organic carbon analyzer

Table 1
Soil chemical analysis performed in the experiment.

Sample material	Pre-treatment	Analysis	N form analyzed	Notes
Fresh soil	KCl-extraction	Indophenol and Griess reaction	NH_4^+, NO_3^-	
	KCl-extraction	OPAME	Primary amines	Hereafter, 'total amino acids'
	KCl-extraction	HPLC-MS	Alkylamines	
	KCl-extraction	Bradford-assay	Proteinaceous material	
	None	pH		In water
Dried and ground soil	P-buffer extraction	TOC-TN	Total dissolved N	
	P-buffer extraction	Phenol precipitation	Proteinaceous material	
	MSA extraction	TOC-TN and C/N	Hydrolysable N	Hereafter, 'degradable N'
	MSA extraction	TOC-TN and C/N	Non-hydrolysable N	Hereafter, 'recalcitrant N'
	None	C/N analysis	Total N	
	None	Oven +550 °C		Ash content

equipped with total nitrogen unit (TOC-Vcph/cpn TNM-1, Shimadzu Corporation, Kyoto, Japan).

2.4. Extractable proteinaceous material

Proteinaceous material was determined from KCl-extract using Bradford-assay where protein content in soil solution is quantified by dyeing dissolved proteins with Coomassie brilliant blue (Bradford, 1976) using a Bradford reagent kit (Sigma–Aldrich Co., US, Missouri, St. Louis). The lower size limit for detectable proteins in the Bradford assay was 3000–5000 Da, as reported by manufacturer (Sigma–Aldrich Co., US, Missouri, St. Louis). The humic acids of soil solutions may interfere with the Coomassie brilliant blue dye (Roberts and Jones, 2008). This interference can be taken into account by measuring the humic acid content in the soil extracts and correcting for the interference (Jones and Kielland, 2012). In our case, color of the soil extracts was compared with humic acid standards (MW >300, Sigma–Aldrich Co., US, Missouri, St. Louis). The formation of the Bradford reagent chromophore with humic acid standards was measured and the interference due to the presence of humic acids was subtracted from the samples accordingly. Bradford protein content of the samples was calculated to mg of N based on the estimated N content (16%) of control protein BSA.

2.5. NO_3^-, NH_4^+ and total amino acid contents

We used colorimetric microplate assay methods introduced by Hood-Nowotny et al. (2008) to determine inorganic nitrogen (NO_3^- and NH_4^+) concentrations in 1 M KCl soil extracts. The colorimetric assay for NH_4^+ was a modified indophenol method based on the Barthelot reaction (Kandeler and Gerber, 1988), and a modified acidic Griess reaction was used for NO_3^- (Miranda et al., 2001). A fluorescent microplate method was used to determine soil total primary amines (hereafter, total amino acid concentration) as introduced by Jones et al. (2002) and modified by Darrouzet-Nardi et al. (2013). The method is based on the formation of fluorescent thio-substituted isoindole in reaction of *o*-phthaldialdehyde with β-mercaptoethanol (OPAME) and the amine groups of amino acids. Absorbance and fluorescence values were measured with a microplate reader (Infinite M200, Tecan Group Ltd., Switzerland, Männedorf).

2.6. Dissolved alkylamine content

Alkylamine concentrations were determined from 1 M KCl soil extracts using the analytical method introduced by Ruiz-Jiminez et al. (2012). Soil extracts were first dansylated, and analyzed by high performance liquid chromatography – triple quadrupole mass spectrometry with electrospray as the ionization method. C18 columns were used for the separation of dansylated amines. 3-phenyl-propylamine was used as an internal standard. The compounds detected were methylamine (CH_3NH_2), dimethylamine ($(CH_3)_2NH_2$), diethylamine ($(C_2H_5)_2NH$), sec-butylamine ($C_2H_5CH_3CHNH_2$), isobutylamine ($(CH_3)_2CHCH_2NH_2$), cadaverine ($NH_2(CH_2)_5NH_2$), spermidine ($NH_2(CH_2)_4$ $NH(CH_2)_4NH_2$), ethanolamine ($NH_2C_2H_4OH$) and histamine ($N_2H_2C_3H_2C_2H_4NH_2$).

2.7. P-buffer–dissolved nitrogen forms

2.7.1. Dissolved total N

Dissolved total nitrogen content extracted by phosphate-buffer (P-buffer) was determined from vacuum dried and ground soil. From each sample, three 500 mg replicates were weighed and added to 10 ml 67 mM P-buffer (pH 6). Samples were shaken for 3 h in a reciprocal shaker (400 rpm) at room temperature, and after shaking raw extracts were centrifuged at 4700 *g* at 4 °C. The supernatant was collected and filtered through a 0.45 μm syringe membrane filter (PALL IC Acrodisc, Supor (PES) Memebrane). Dissolved total nitrogen concentrations were determined by a total organic carbon analyzer equipped with total nitrogen unit (TOC-Vcph/cpn TNM-1, Shimadzu Corporation, Kyoto, Japan).

2.7.2. Extractable proteinaceous material

Proteinaceous N was determined from P-buffer extract using phenol extraction and methanol-ammonium-acetate (MeOH–HN_4^+-acetate) precipitation as introduced by Kanerva et al. (2013). To separate proteinaceous material from the filtered P-buffer extract, 3.75 ml of filtrate was mixed with 1.25 ml saturated buffer phenol (pH 7.9). The phenol phase was separated and proteinaceous material was precipitated with 1.3 ml ice cold MeOH–NH_4^+-acetate. The resulting precipitate was washed with acetone to purify it from added N originated from reagents. The precipitate was dissolved in 200 μL of 1 M NaOH. The total nitrogen content of the dissolved precipitates was determined by a total organic carbon analyzer equipped with total nitrogen unit (TOC-Vcph/cpn TNM-1, Shimadzu Corporation, Kyoto, Japan).

2.8. Enzyme activity measurements

From freshly taken soil samples, soil solution was collected from 500 μl of soil using Costar® Spin-X® CLS8162 (Corning Inc., NY, USA) centrifuge tube filter containing a cellulose acetate membrane (pore size 0.45 μm) and centrifuged at +4 °C and 15 700 *g* for 30 min (see detailed protocol in Heinonsalo et al., 2012). Protease production was measured from the soil solution using a PF0100 Protease Fluorescent Detection Kit (Sigma–Aldrich, St. Louis, MO, USA) according to manufacturer's recommendations (see also Lindén et al., 2014). To assess the activity of laccase and peroxidase from the soil solution, a coupled assay using 2,2′-azino-bis-(3-ethylthiazoline-6-sulfonate) (ABTS) as substrate and hydrogen

peroxide (H_2O_2) was used and the resulting color change was measured spectrophotometrically at a wavelength of 420 nm (Hofrichter et al., 1998; Heinonsalo et al., 2012). The activities were measured to verify whether the added enzymes could still be detected after one month after the last artificial enzyme addition.

2.9. Losses of nitrogen

The loss of nitrogen during the experiment was estimated by comparing the total N in the soil (mg-N g^{-1} SOM) at the harvest to the N content of the original soil before the experiment.

2.10. Statistical testing

Treatment effects were tested with two-way univariate analysis of variance (ANOVA) followed by least significant differences (LSD) post hoc test. Differences between planted and non-planted treatments were tested with Student's t-test. When assumptions of normality or equality of variances were not met, the non-parametric Mann–Whitney or Kruskal–Wallis tests were used. Pearson's test for correlation was used to test linear relationships between measured variables. Statistical testing was performed by IBM® SPSS® Statistics for Windows® version 21 (IBM Corp. Released 2012. Armonk, New York, United States).

3. Results

3.1. Total soil N, degradable and recalcitrant N

In this study, the most pronounced treatment effects were found in the largest N pools (total soil N, recalcitrant N) and in the lost N fraction. There was a trend of lower total soil N content in non-planted treatments if both organic matter oxidizing and protease enzymes were added (LMPr treatment) compared to the control (Con) and protease (Pr) treatments (Table 2). In the planted treatments, the enzyme additions did not decrease the total soil N content (Table 2). However, the presence of a plant decreased the total soil N content similarly as the LMPr treatment in non-planted soil (Table 2). The loss of N from the soil showed the same pattern: non-planted LMPr treatment had lost more N than non-planted Con and Pr treatments, and the loss from non-planted LMPr treatment was statistically the same as in all planted treatments, irrespective the enzyme additions (Table 2). Similar difference between treatments was also observed in SOM content and C percentage, which were decreased in all planted treatments and in non-planted LMPr treatment (Table 3).

The same trend was seen in recalcitrant N contents between treatments: non-planted LMPr treatment had lower recalcitrant N content than non-planted Con and Pr treatments (Table 2). In the case of degradable N, no enzyme-induced statistically significant differences were found between planted and non-planted treatments (Table 2).

When the mean of all planted treatments was compared to the mean of all non-planted treatments, the total soil N content was significantly ($P < 0.05$) lower in the planted treatments, and more N was lost from the planted treatments compared to non-planted soil (Table 4). No statistically significant differences between planted and non-planted treatments in recalcitrant N or in degradable N contents were found, although the mean values were lower in planted treatments (Table 4).

3.2. Dissolved N pools

No statistically significant enzyme effect between planted and non-planted treatments was found in the total dissolved N pools after either KCl- or P-buffer extraction (Table 2). Also, no enzyme-induced statistically significant differences within planted or non-planted treatments were found in phenol extracted proteinaceous N, Bradford protein N, total amino acid N, NH_4^+–N or NO_3^-–N contents or in the sum of amines (Table 5). Dissolved N from KCl- and P-buffer extraction correlated positively but not significantly with each other (Table S1), whereas no correlation was found between the phenol extracted protein material and Bradford protein contents (Table S1).

However, statistically significant ($P < 0.05$) differences were found when the mean of all planted treatments were compared to the mean of all non-planted treatments. Dissolved total N (P-buffer and KCl-extracted) and NH_4^+–N concentrations were lower, but total amino acid N content was higher in planted treatments (Table 4), whereas no differences between planted and non-planted treatments were found in proteinaceous N (phenol extracted or Bradford proteins) or soil NO_3^-–N concentrations.

The presence of a seedling also had a statistically significant ($P < 0.05$) effect on many measured alkylamines. Dimethylamine and histamine N content were significantly ($P < 0.05$) higher in the non-planted treatments compared to the planted treatments. By contrast, mean N contents of spermidine, ethanolamine, sec-butylamine, iso-butylamine and cadaverine were significantly lower ($P < 0.05$) in non-planted compared to planted treatments (Table 6). Only methylamine and diethylamine N content did not differ between the planted and non-planted treatments. Despite the clear compound-specific differences, the presence of a seedling did not have a significant effect on the total sum of alkylamine N found in the soil (Table 6). Interestingly, the sum of N in the measured amines was found to be in the same range as the NO_3^-–N content of the soil (Table 5 and Table S2).

We hypothesized that increased amino acid concentrations would increase amine synthesis. However, no correlation between total amino acid content and sum of alkylamines was found (Table S1). Instead the total sum of alkylamines correlated

Table 2
Mean soil total nitrogen (N), recalcitrant N, degradable N, the amount of dissolved N in phosphate buffer and in 1 M KCl, and the total N lost from the soil over the experiment in non-planted (soil, n = 5) and planted (seedling, n = 13) treatments. The mean values are followed by standard error.

		Total soil N		Recalcitrant N	Degradable N	Dissolved N (phos. buffer)	Dissolved N (1 M KCl)	Lost N
		[mg-N g^{-1} SOM]	[mg-N g^{-1} DW]	[mg-N g^{-1} SOM]	[mg-N g^{-1} SOM]	[mg-N g^{-1} SOM]	[mg-N g^{-1} SOM]	[mg g^{-1} SOM]
Soil	Con	16.3 (±0.9)^{a}	6.0 (±0.2)^{a}	11.8 (±1.1)^{a}	4.4 (±0.6)^{a}	1.2 (±0.1)^{a}	1.2 (±0.3)^{a}	2.3 (±0.5)^{a}
	Pr	16.4 (±0.5)^{a}	6.2 (±0.3)^{a}	13.0 (±0.9)^{a}	3.5 (±0.5)^{a,b}	1.1 (±0.1)^{a}	0.9 (±0.2)^{a}	2.0 (±0.9)^{a}
	LMPr	14.5 (±0.6)^{a,b}	5.0 (±0.4)^{b}	10.9 (±0.6)^{a,b}	3.6 (±1.1)^{a,b}	1.0 (±0.1)^{a,b}	0.8 (±0.2)^{a}	6.6 (±1.9)^{b}
Seedling	Con	14.3 (±0.5)^{b}	4.6 (±0.2)^{b}	11.3 (±0.4)^{a,b}	3.0 (±0.4)^{b}	0.8 (±0.1)^{b,c}	0.7 (±0.1)^{a}	7.4 (±0.5)^{b}
	Pr	13.9 (±0.4)^{b}	4.4 (±0.1)^{b}	11.5 (±0.5)^{a}	2.4 (±0.4)^{b}	0.8 (±0.1)^{b,c}	0.8 (±0.1)^{a}	7.8 (±0.5)^{b}
	LMPr	13.9 (±0.4)^{b}	4.5 (±0.2)^{b}	10.2 (±0.4)^{b}	3.7 (±0.3)^{a,b}	0.7 (±0.1)^{c}	0.6 (±0.1)^{a}	7.3 (±0.5)^{b}

Con, control; Pr, protease addition; LMPr, laccase, manganese peroxide and protease addition.
Different letters indicate significant ($P < 0.05$) difference.

Table 3
Soil CN-ratio, ash content, soil organic matter (SOM) and C content and pH in non-planted (soil, n = 5) and planted (seedling, n = 13) treatments. The mean values are followed by standard error.

		CN-ratio	Ash content	SOM content	C%	pH_{H_2O}
		Ratio	[g g^{-1} DW]	[g g^{-1} DW]		
Soil	Con	30 (±1)^{a}	0.63 (±0.01)^{b}	0.37 (±0.01)^{a}	18 (±1)^{a}	4.8 (±0.06)^{b}
	Pr	30 (±1)^{a}	0.62 (±0.01)^{b}	0.38 (±0.01)^{a}	18 (±1)^{a}	4.6 (±0.14)^{ab}
	LMPr	30 (±1)^{a}	0.67 (±0.02)^{a}	0.33 (±0.02)^{b}	15 (±1)^{b}	4.6 (±0.05)^{ab}
Seedling	Con	33 (±1)^{a}	0.68 (±0.01)^{a}	0.32 (±0.01)^{b}	15 (±1)^{b}	4.1 (±0.05)^{a}
	Pr	33 (±1)^{a}	0.68 (±0.01)^{a}	0.32 (±0.01)^{b}	14 (±1)^{b}	4.2 (±0.08)^{a}
	LMPr	33 (±1)^{a}	0.68 (±0.01)^{a}	0.33 (±0.01)^{b}	15 (±1)^{b}	4.3 (±0.11)^{ab}

Con, control; Pr, protease addition; LMPr, laccase, manganese peroxide and protease addition.
Different letters indicate significant (P < 0.05) difference.

Table 4
Mean soil N concentrations, ash and SOM content and pH in the non-planted (soil, n = 15) and planted (seedling, n = 39) treatments and their standard error.

	Soil	Seedling	Units	df
Total soil N	15.8 (±0.4)^{a}	14.0 (±0.3)^{b}	[mg-N g^{-1} SOM]	51
	5.7 (±0.2)^{a}	4.5 (±0.1)^{b}	[mg-N g^{-1} DW]	51
Recalcitrant N	12.0 (±0.5)^{a}	10.9 (±0.3)^{a}	[mg-N g^{-1} SOM]	51
Degradable N	3.9 (±0.4)^{a}	3.0 (±0.2)^{a}	[mg-N g^{-1} SOM]	51
Dissolved N (phos. buffer)	1.1 (±0.1)^{a}	0.79 (±0.03)^{b}	[mg-N g^{-1} SOM]	46
Dissolved N (1 M KCl)	1.0 (±0.1)^{a}	0.72 (±0.06)^{b}	[mg-N g^{-1} SOM]	51
Proteinaceous N	0.49 (±0.03)^{a}	0.44 (±0.02)^{a}	[mg-N g^{-1} SOM]	42
Bradford protein N	8.1 (±1.4)^{a}	11 (±1)^{a}	[μg-N g^{-1} SOM]	50
Total amino acid N	68 (±5.0)^{b}	100 (±7)^{a}	[μg-N g^{-1} SOM]	49
NH_4^+−N	290 (±48)^{a}	51 (±3)^{b}	[μg-N g^{-1} SOM]	13
NO_3^-−N	22 (±9)^{a}	13 (±7)^{a}	[μg-N g^{-1} SOM]	51
CN-ratio	30 (±1)^{b}	33 (±1)^{a}	Ratio	45
Ash content	0.64 (±0.01)^{b}	0.68 (±0.00)^{a}	[g g^{-1} DW]	15
SOM content	0.36 (±0.01)^{a}	0.32 (±0.00)^{b}	[g g^{-1} DW]	16
Lost N	3.43 (±0.82)^{b}	7.49 (±0.29)^{a}	[mg g^{-1} SOM]	51
pH_{H_2O}	4.7 (±0.2)^{a}	4.2 (±0.3)^{b}		51

Different letters indicate significant (P < 0.05) difference.

Table 5
Mean phenol extracted proteinaceous N in P buffer soil extract, Bradford-protein-, amino acid-, NH_4^+−, NO_3^-−N concentrations, sum of all amines in 1 M KCl soil extract in non-planted (soil, n = 5) and planted (seedling, n = 13) treatments. The mean values are followed by standard error.

		Proteinaceous N	Bradford protein N	Total amino acid N	NH_4^+−N	NO_3^-−N	Sum of amines
		[mg-N g^{-1} SOM]	[μg-N g^{-1} SOM]	[μg-N g^{-1} SOM]	[μg-N g^{-1} SOM]	[μg-N g^{-1} SOM]	[μg-N g^{-1} SOM]
Soil	Con	0.4 (±0.1)^{a}	9.9 (±2.4)^{a}	67 (±7.7)^{a}	410 (±109)^{a}	35 (±25)^{a}	14 (±2.6)^{a}
	Pr	0.5 (±0.1)^{a}	5.8 (±2.2)^{a}	63 (±4.1)^{a}	260 (±53)^{a}	19 (±9.1)^{a}	16 (±3.2)^{a}
	LMPr	0.5 (±0.0)^{a}	9.1 (±3.6)^{a}	76 (±16)^{a}	179 (±50)^{a,b}	9.2 (±4.4)^{a}	16 (±2.2)^{a}
Seedling	Con	0.5 (±0.0)^{a}	8.5 (±1.3)^{a}	102 (±11)^{a}	51 (±4.2)^{b}	0.3 (±0.0)^{a}	11 (±2.6)^{a}
	Pr	0.4 (±0.0)^{a}	11 (±1.3)^{a}	117 (±14)^{a}	55 (±6.6)^{b}	17 (±16)^{a}	13 (±1.1)^{a}
	LMPr	0.5 (±0.1)^{a}	12 (±1.4)^{a}	83 (±12)^{a}	49 (±4.9)^{b}	20 (±14)^{a}	13 (±1.3)^{a}

Con, control; Pr, protease addition; LMPr, laccase, manganese peroxide and protease addition.
Different letter indicate significant (P < 0.05) difference.

Table 6
Mean amine concentrations and their standard error of the mean in 1 M KCl soil extracts in non-planted (soil, n = 15) and planted (seedling, n = 39) treatments. The mean values are followed by standard error.

	Soil	Seedling	df
	[mg-N g^{-1} SOM]	[mg-N g^{-1} SOM]	
Dimethylamine	10 (±1.2)^{a}	5.2 (±0.80)^{b}	50
Spermidine	1.9 (±0.15)^{b}	4.3 (±0.35)^{a}	48
Methylamine	1.9 (±0.20)^{a}	1.5 (±0.29)^{a}	50
Ethanolamine	0.36 (±0.03)^{b}	0.60 (±0.03)^{a}	50
sec-Butylamine	0.29 (±0.02)^{b}	0.44 (±0.04)^{a}	45
iso-Butylamine	0.15 (±0.02)^{b}	0.28 (±0.05)^{a}	44
Histamine	0.14 (±0.08)^{a}	0.08 (±0.01)^{b}	50
Cadaverine	0.01 (±0.01)^{b}	0.03 (±0.01)^{a}	39
Diethylamine	0.02 (±0.00)^{a}	0.01 (±0.00)^{a}	50
Sum of amines	15 (±1.5)^{a}	12 (±1.0)^{a}	50

Different letters indicate significant (P < 0.05) difference.

positively with total soil N content and degradable N, and negatively with lost N content (Table S1). In the treatments Con, Pr and LMPr, no clear enzyme effect neither on specific amine compounds nor total sum of alkylmines was observed (Table S2).

3.3. Enzyme activities

At harvest, one month after the last enzyme addition, no detectable increase in protease, laccase or peroxidase activities could be measured indicating that no differences between enzyme treatments or controls were found (data not shown).

3.4. Plant biomass

In the treatments with Scots pine, there were no enzyme-related differences in the total biomass of seedling, total needle

biomass or in needle nitrogen content (Table 7). However, root biomass was significantly ($P < 0.05$) higher in Con and Pr treatments (1.5 g DW) than in LMPr treatment (0.9 g DW), and this difference was observed also in the root-shoot ratio. There were no significant differences in the total number of root tips, rhizomorphic root tips or mean ectomycorrhizal colonization rate between Con, Pr and LMPr treatments.

4. Discussion

Our results show that the presence of Scots pine accelerates N release from soil organic matter (SOM) in a similar way as the addition of oxidative enzyme and protease in non-planted soil. We observed significantly higher N losses and lower N content from the bare soil that was treated with SOM oxidizing and protease enzymes (LMPr treatment) compared to control (Con) and protease (Pr) treated soils. Similar N losses were observed in all planted treatments. The addition of proteases alone to bare soil did not induce similar effects indicating that oxidative enzymes, rather than proteases are the key enzymes in regulating the release of N from SOM. Surprisingly, our analysis of organic and inorganic N compounds in the soil revealed that the sum of all measured alkylamines is quantitatively in the same range as the amount of nitrate (NO_3^-) in the soil. This observation raises questions about the functional role of amines in soil N cycling.

In the soil N-cycling model (Fig. 1) modified from Schimel and Bennet (2004), the SOM needs to be disintegrated resulting in the release and degradation of N-containing polymers to monomers in order to facilitate further N cycling and plant N uptake processes. SOM has been shown to be stabile as a result of protection through interactions with mineral particles (Torn et al., 2007). Furthermore, a portion of the SOM may also be stabile due to chemical recalcitrance (Schmidt et al., 2011) or due to e.g. location in deeper layers of soil or physical disconnection between decomposers and SOM (Schmidt et al., 2011). As it has been shown that the decomposition of recalcitrant, often older SOM, is connected to the belowground carbon allocation of the plants through the so-called priming effect (Kuzyakov et al., 2000; Fontaine et al., 2007; Lindén et al., 2014), there seems to be an intimate link between plants, SOM decomposition and N uptake from SOM (Phillips et al., 2011). However, it remains unclear how much a plant affects soil C balance through stabilization or priming-related processes. This question was recently addressed by Sulman et al. (2014), who developed a soil organic carbon (SOC) model CORPSE (Carbon, Organisms, Rhizosphere, and Protection in the Soil Environment) that was used to estimate the effect of rising CO_2 levels on SOC transformation and decomposition. They showed that in ecosystems with recalcitrant litter and low clay content, as most boreal forest soils are, SOC losses were increased through observed N uptake and plant-dependent priming. Our experimental results suggest that the presence of a plant induces N release from the recalcitrant organic matter in boreal forest soils. The lower total soil N in planted treatments was interpreted to result from an increased decomposition of the SOM and a formation and a release of dissolved N, which was then either taken up by the plants or lost from the soil. It is noteworthy that in all treatments the total soil N content was lower in the end of the experiment than in the beginning despite the fact that extra N was added to the soil together with the added enzymes and BSA protein. Therefore, the decrease in soil N content indicates that also the original SON was affected by the treatments.

Interestingly, the presence of a plant over-ruled the effect of the enzyme treatments, and the presence of a plant led to a lower total soil N content and a greater N loss, similar as in LMPr treatment without a plant. The enzymes in LMPr treatment resembled rhizospheric priming effect of a pine seedling, which has been suggested to enhance the release of N through decomposition of SOM in N limited systems (Dijkstra et al., 2013). Brzostek and Finzi (2011) showed in a field experiment that the absence of ectomycorrhizal fine roots reduced the activity of proteases and chitinolytic and ligninolytic enzymes. Ectomycorrhizal fungi has been shown to be potential organic matter decomposers (Lindahl and Tunlid, 2015), and based on the fungal genome analysis, it has been shown that mycorrhizal fungi have retained a unique array of genes to decompose lignocellulose (Kohler et al., 2015). Our results support the hypothesis that the plant-induced mechanism of SOM decomposition could be enzymatic, as was seen in non-planted treatments in our study. As the soil in planted LMPr treatment was supplemented with enzymes needed to obtain nutrients from SOM, the plants may not have needed to invest as much resources to gain N from SOM, which could be seen in a lower root biomass and lower root-shoot ratio in LMPr treated seedlings. Scots pine is known to optimize shoot-root-ratio according to the availability of nutrients, leading to an increased amount of fine roots in low nutrient conditions (Helmisaari et al., 2007).

It could be assumed that the organic N in SOM that is not delivered in acid hydrolysis (recalcitrant N) would be that fraction of soil N that is not sensitive to either enzyme treatments or presence of plant. Any changes in soil N pools would logically occur in the N pool that can be released from SOM with strong acids. Our finding that the ratio of recalcitrant N to degradable N remained largely the same in enzyme treatments, and with or without a plant, indicates that the plant and the plant-associated microbes are equally able to utilize both the recalcitrant N and potentially degradable pools of N, a result that also Whiteside et al. (2012) found with arbuscular mycorrhizal fungi. Our finding supports the hypothesis of Dungait et al. (2012) that chemical recalcitrance is not the major limiting factor in SOM decomposition.

After initial SOM decomposition, the released organic N forms including proteinaceous material need to be degraded into smaller units that can be utilizable by the majority of soil organisms and plants. Enzyme treatments did not have a significant effect on proteinaceous material content measured from soil, even though

Table 7
Total pine seedling biomass, needle and root biomass, needle N content, root-shoot ratio, total root tip number, proportion of rhizomorphic root tips and ectomycorrhizal colonization of roots. The mean values are followed by standard error (n = 13).

		Biomass	Needle biomass	Root biomass	Needle N content	Root-shoot ratio	Total root tips	Rhizomorphic root tips	Ectomycorrhizal colonization
		[g DW]	[g DW]	[g DW]	[mg-N g^{-1} DW]		g^{-1} DW	%	%
Seedling	Con	3.8 (±0.2)[a]	1.7 (±0.2)[a]	1.5 (±0.2)[a]	27 (±3.5)[a]	0.40 (±0.07)[a]	1930 (±156)[a]	29 (±6.3)[a]	98 (±0.7)[a]
	Pr	4.1 (±0.3)[a]	2.0 (±0.2)[a]	1.5 (±0.1)[a]	29 (±2.8)[a]	0.35 (±0.03)[a]	1900 (±196)[a]	26 (±6.3)[a]	98 (±0.4)[a]
	LMPr	3.6 (±0.2)[b]	2.1 (±0.2)[a]	0.9 (±0.1)[b]	24 (±2.3)[a]	0.21 (±0.03)[b]	2110 (±159)[a]	23 (±6.7)[a]	97 (±1.0)[a]
df		2	2	2	2	2	2	2	2

Con, control; Pr, protease addition; LMPr, laccase, manganese peroxide and protease addition.
Different letters indicate significant ($P < 0.05$) difference.

SOM degrading enzymes were hypothesized to increase the amount of available proteinaceous N in soil. It may be that the depolymerization rate of proteins is faster than the release of proteinaceous material from SOM and the released proteinaceous material may be utilized efficiently by soil microbes or leached out from the system, hence leaving the system before being quantified. The same result was found for total dissolved N (KCl and P-buffer extraction), organic monomers (amino acids), low molecular weight (LMW) compounds (alkylamines) and inorganic N forms (NH_4^+, NO_3^-): enzyme treatments did not cause any significant differences in their quantities in the soil. Based on our results, it seems evident that the N found in dissolved forms in the soil is accessible to plants and soil organisms and the presence or absence of protease enzyme is not limiting the use of proteinaceous material in soil. However, the presence of a plant affects the quantities of most N forms in the soil, and therefore the influence of plant roots and rhizosphere microflora is of key importance when soil C and N cycling are studied. Lower levels of dissolved N, NH_4^+–N and NO_3–N as well as lower pH in the planted treatments compared to non-planted treatments seem to be a result of plant nutrient uptake, while the reason for higher levels of amino acids remains unclear. Several processes, e.g. plant nutrient uptake, decomposition and nitrogen transformation processes, are known to decrease soil pH (Clarholm and Skyllberg, 2013), as observed in our results. When interpreting and comparing the results of different N forms in soil, it is important to consider their different turnover times in soil.

The presence of a plant had significant effects on most alkylamines found in the soil. Soil concentrations of spermidine, ethanolamine, sec-butylamine, iso-butylamine and cadaverine were markedly higher in planted treatments, while those of dimethylamine and histamine were lower in planted treatments compared to the non-planted soils. This pattern indicates that the amines have different roles in plant-soil systems, e.g. with respect to being released from SOM or taken up by the plant. It is well known that plants and mycorrhizal fungi in N poor ecosystems can utilize organic nitrogen in the form of amino acids (Kielland et al., 2006, 2007; Talbot and Treseder, 2010). Recently, Sintermann and Neftel (2015) reviewed that a wide range of plant species contain high concentrations of amines, and they demonstrated amine (trimethylamine) emissions from flowering plants. However, there is no information available whether amines present in soil could be taken up by plants, or whether amines within the plants could be excreted through the roots to the soil. The impact of plant's amino acid uptake on plant or soil amine concentrations is not known.

Yan et al. (1996) and Xu et al. (2006) have suggested that during the degradation of organic N compounds low-weight alkylamines may be produced. Kim et al. (2001) and Rappert and Müller (2005) have showed that microbial degradation of quaternary ammonium compounds (e.g. carnitine, choline and betaine) that have often reported from soil solutions (Warren, 2013; Warren, 2014), produces low-weight alkylamines (trimethylamine, dimethylamine and monomethylamine) in both aerobic and anaerobic soil conditions. Therefore, as the soil microbial communities are critically important in soil N turnover processes, and are affected by the plant due to e.g. rhizodeposition, the role of microbes in the turnover of amines in the soil clearly needs further investigation.

Although we cannot specify the role of different alkylamines in soil N turnover processes, it seems clear that organic layer in boreal forest soil contains large quantities of different alkylamines, and that plant-induced processes affect the levels of these compounds. The fact that the sum of measured amines was in the same range as that of NO_3^-–N content in the soil demonstrates that alkylamines may also be an important group of compounds in N cycling in soil-plant systems. The alkylamines produced in the soil are present in significant concentrations and may be the source of the amines detected in the atmosphere of a boreal forest as suggested by Kieloaho et al. (2013).

Our study allow us to evaluate different extraction and analysis methods as we used several methods to estimate protein content in soil extracts. It is known that protein extraction is extremely challenging in organic boreal forest soils (Keiblinger et al., 2012; Kanerva et al., 2013). As proteins or their residuals and short peptide chains are an integral part of the total soil N pool (Korhonen et al., 2013), it is obvious that their extraction depends on the buffers used and the rate of disintegration of SOM structures: the more the SOM is degraded in the extraction process, the more organic N forms are released. We found low correlation between the two methods used to estimate proteinaceous material in soil. This can be explained by differences in sample preparations and by the range of molecular size of proteinaceous material found from soil. Bradford protein N content in our study was on same range as those measured from L- and O-horizons of a Black spruce forest in Alaska (US) by Jones and Kielland (2012). However, in the case of Bradford protein content the lower limit of detection is 3000–5000 Da meaning that some of the smaller proteins cannot be detected by this method. Jones and Kielland (2012) and Smolander and Kitunen (2002) suggested that most of the soil proteinaceous material is found in size class smaller than 5000 Da in the form of peptides which are not detected by using Bradford-assay. Dissolved total N measured by using two extraction methods (P-buffer and KCl extraction) correlated positively but not significantly. This finding supports earlier observations that extraction method and sample pre-treatments affect yield of dissolved total N or individual components found in dissolved fraction (Jones and Willet, 2006).

The tight connection between plant and soil processes in forest ecosystems can be seen in many field and laboratory experiments (e.g. Brzostek and Finzi, 2011; Lindén et al., 2014). In forest ecosystems, the belowground carbon allocation in the form of fresh photosynthates largely controls the seasonal dynamics of microbial N cycling, fungal abundance and soil N availability (Högberg et al., 2001; Kaiser et al., 2011). When soil-tree continuum was interrupted due to a girdling experiment, the soil N cycling dynamics were disturbed, fungal abundance, fine root biomass and microbial N uptake were reduced, and the dissolved inorganic N concentration in the soil increased due to the slowed down N uptake by the trees (Kaiser et al., 2011). All these results support the general understanding of the plant-related stimulation of SOM decomposition as well as the release of organic N in the presence of plants and their associated mycorrhizal fungi (Talbot and Treseder, 2010; Drake et al., 2011; Lindén et al., 2014). Talbot and Treseder (2010) suggested that the availability of carbon resources for the mycorrhizal fungus influences the capacity of the fungi to produce extracellular enzymes that catalyze the breakdown of polymeric organic N. In this experiment, the effect of plants on soil N availability was reflected in the decreased total amount of N and increased N losses from soil. The plant-induced stimulation of SOM decomposition and the release of N was shown to apply both to the recalcitrant and potentially degradable forms of N.

5. Conclusions

In our study we demonstrate that the recalcitrant N pool in the organic layers of boreal forest soil can be mobilized if organic matter oxidizing enzymes are present. We show experimentally the stimulating influence of enzymes and plant on mobilization of recalcitrant N pools in the soil, a large N reservoir that often has been considered inactive. This recalcitrant N pool is also an

important N source for forest productivity in N limited ecosystems, such as boreal forests. Our study demonstrates the intimate link between forest trees and SOM degrading processes, and highlights the importance of including plant-induced SOM decomposition in global carbon models. Concerning the atmospheric relevant alkylamines, we showed that the sum of all measured alkylamines is quantitatively in the same range as the amount of nitrate (NO_3^-) in the soil indicating their potential importance in soil N cycling, and showing that boreal forest soils may act as a source of alkylamines to the atmosphere.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.01.013.

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Paper IV

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Soil concentrations and soil-atmosphere exchange of alkylamines in a boreal Scots pine forest

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Abstract

Alkylamines are important precursors in secondary aerosol formation in the boreal forest atmosphere. To better understand the behaviour and sources of two alkylamines, dimethylamine (DMA) and diethylamine (DEA), we estimated the magnitudes of soil-atmosphere fluxes of DMA and DEA using a gradient-diffusion approximation based on measured concentrations in soil solution and in the canopy air space. To compute the amine fluxes, we first estimated the soil air space concentration from the measured soil solution amine concentration using soil physical (temperature, soil water content) and chemical (pH) state variables. Then, we used the resistance analogy to account for gas transport mechanisms in the soil, in soil boundary layer and in the canopy air space. The resulting flux estimates revealed that the boreal forest soil with a typical long-term mean pH 5.3 is a possible source of DMA (170 ±51 nmol m^{-2} d^{-1}) and a sink of DEA (-1.2 ±1.2 nmol m^{-2} d^{-1}). We also investigated the potential role of fungi as a reservoir for alkylamines in boreal forest soil. We

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found high DMA and DEA concentrations both in fungal hyphae collected from field humus samples and in fungal pure cultures. The highest DMA and DEA concentrations were found in fungal strains belonging to decay and ectomycorrhizal fungal groups, indicating that boreal forest soil, and in particular, fungal biomass may be an important reservoir for these alkylamines.

1 Introduction

Aerosols are important in cooling the atmosphere through increasing the scattering of sunlight and increasing albedo through cloud formation. In boreal forests, volatile organic compounds emitted from the biosphere largely drive aerosol formation, and aerosol growth to cloud condensation nuclei (Kulmala et al., 1998; Kerminen et al., 2010; Riipinen et al., 2012). Amines have been suggested to be one of the key compounds in the aerosol formation process (Angelino et al., 2001; Silva et al., 2008; Kurtén et al., 2008; Smith et al., 2009; Yu et al., 2012; Almeida et al., 2013).

Amines are nitrogenous organic molecules in the form of NR_3, where R denotes hydrogen or alkyl or aryl group. Low-weight alkylamines, which have one to six atom carbon chains bound to a nitrogen atom, are known to be degradation products of amino-acid-rich substrates, such as dairy or fish (Ge et al., 2011a). However, the origin of these amine compounds in natural environments is poorly understood. Sintermann and Neftel (2015) concluded that flowering of vegetation especially in springtime, and non-flowering vegetation during growing season are potential sources of alkylamines. Sintermann and Neftel (2015) suggested that the contribution of fungal sporocarps and decomposing organic matter as amine sources increases towards the autumn.

Low-weight alkylamines may be produced in soils during the degradation of organic N compounds, especially amino acid decarboxylation (Yan et al., 1996; Xu et al., 2006). Kim et al. (2001) and Rappert and Müller (2005) showed that quaternary ammonium compounds (e.g. carnitine, choline and betaine), often present in soil solution (Warren et al., 2013; Warren, 2014), could be degraded to alkylamines (trimethylamine, dimethylamine and monomethylamine) by the soil microbial community using both aerobic and anaerobic pathways. Sintermann and Neftel (2015) stated that decaying organic matter contains elevated levels of precursor substances for alkylamine production, hence indicating that decaying organic matter may be a source of alkylamines.

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Concentrations of alkylamines in atmospheric particles and in gas phase are rarely reported from boreal ecosystems, despite the importance of amines in aerosol formation processes (Mäkelä et al., 2001; Smith et al., 2009; Kieloaho et al., 2013), mostly due to challenges in detecting these compounds. Mäkelä et al. (2001) reported elevated concentrations of dimethylaminium (protonated dimethylamine) during particle formation periods in boreal forest. In our previous study (Kieloaho et al., 2013), we found the gas-phase alkylamines in boreal forest air, and we concluded that the seasonal variations in the atmospheric amine concentrations is linked to vegetation dynamics and soil activity.

Direct flux measurements of alkylamines are difficult to perform and are very rarely made (Sintermann and Neftel, 2015) due to the high reactivity of amines and lack of suitable measurement techniques and instrumentation. However, the magnitude of fluxes can be indirectly estimated if the concentrations of the target compounds in different reservoirs (e.g. vegetation, soil and atmosphere) are known. In general, the fluxes are driven by a concentration gradient between the reservoirs, such as ambient air and an aqueous solution. As follows, gas-phase concentration in soil air can be calculated by assuming equilibrium between the aqueous solution and the gas-phase above the solution (Farquhar et al., 1980; Nemitz et al., 2000). Furthermore, the fluxes through a soil-atmosphere boundary can be estimated using a gradient-diffusion approximation, often presented by an electrical resistance analogy (Hicks et al., 1987; Seinfield and Pandis, 1998; Sutton et al., 1998).

In this study, we used three layers to estimate the potential exchange of two alkylamines, dimethylamine (DMA) and diethylamine (DEA), between soil and the atmosphere (Figure 1). Amine concentrations in boreal forest soil and in fungal hyphae were measured, and used to estimate potential fluxes of the selected alkylamines from a boreal Scots pine forest soil to the atmosphre. We hypothesize that by using soil amine concentration data and the resistance analogy, it is possible to estimate the potential sources and sinks of alkylamines in the soil.

2 Materials and methods

2.1 Study site and supplementary measurements

Study site is a Scots pine forest at the SMEARII station (Station for Measuring Forest Ecosystem – Atmosphere Relations) at Hyytiälä (61°84′N, 24°26′E, 180 m a.s.l.) in southern Finland (Hari and Kulmala, 2005). The forest stand at the SMEARII station is approximately

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50 years old and dominated by Scots pine (*Pinus sylvestris* L.) with Norway spruce (*Picea abies* (L.) H. Karst.), birch (*Betula* L. spp.), and European aspen (*Populus tremula* L.), found occasionally in the understory. The most common plant species at the ground level are bilberry (*Vaccinium myrtillus* L.), lingonberry (*Vaccinium vitis-idaea* L.), wavy hairgrass (*Deschampsia flexuosa* (L.) Trin.), and heather (*Calluna vulgaris* (L.) Hull.). The most common mosses are Schreber's big red stem moss (*Pleurozium schreberi* (Brid.) Mitt.), and a dicranum moss (*Dicranum* Hedw. sp.) (Ilvesniemi et al., 2009). The soil at the site is Haplic podzol on glacial till, with an average depth of 0.5-0.7 m.

A half hour average of soil water content (at 0.05 m), soil temperature (at 0.05 m) and above canopy (at 23 m) friction velocity was used in the calculations of DMA and DEA equilibrium gas-phase concentrations in soil air, and to calculate DMA and DEA soil-atmosphere exchange. Soil temperature was measured using PT-100 resistance thermometers, and soil water content was measured with a time-domain reflectometer (TDR 100; Campbell Scientific Inc., Logan, UT, USA). A mean pH-value of 5.3 measured over 14-years, and sampled once per month during snow free period from three replicate suction cup lysimeters at 2 cm depth in the mineral soil was used. The 10 and 90 percentiles of the soil pH were 4.5 and 6.0, respectively.

The ambient air concentrations of DMA and ethylamine (EA), and DEA were measured at 2 m, below the overstory canopy (Kieloaho et al. 2013) and used in the flux estimation. The analytical procedure was incapable to resolve DMA and EA, and therefore only the sum of these compounds is reported, and later referred as DMA concentration. The DMA+EA and DEA air concentration measurements were conducted from May 2011 to October 2011 by collecting weekly air samples into phosphoric acid impregnated glass fiber filters described in detail in Kieloaho et al. (2013). Measured ambient air concentrations of DMA+EA varied from 0.49 to 6.4 nmol m^{-3}, and the mean observed air concentration with standard deviation was 1.7±1.2 nmol m^{-3} (Kieloaho et al., 2013). The highest concentration of DMA+EA (6.4±0.83 nmol m^{-3}) was measured in October. Ambient air concentration of DEA varied from 0.02 to 0.63 nmol m^{-3} the mean being 0.26 (±0.22) nmol m^{-3} (Kieloaho et al., 2013).

2.2 Soil and fungal hyphae samples

Soil samples were collected at same time in May 2011. The first soil samples were used to screen the concentrations of amines in the humus layer, mineral soil and visible fungal

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hyphae. A 10-liter sample of the humus layer (F/H-horizon) and a 5- liter sample from the underlying mineral B-horizon were collected. The soil was homogenized and stored at +4°C (for about day) until three 2 mL samples of mineral soil, humus layer, and visible fungal rhizomorphic hyphae were collected.

The second soil samples were stored at +4°C until used in the greenhouse experiment where the effects of soil organic matter decomposing enzymes on nitrogen turnover processes were studied (Kieloaho et al., 2016). The soil samples were extracted with 1 M KCl, and analyzed for low molecular weight amines as described in chapter 2.3.

In total 19 different fungal strains, representing 14 different Ascomycete and Basidiomycete fungal species were grown one by one for six weeks in LN-AS media containing axenic liquid cultures (Bäck et al. 2010). The strains were divided into four functionally distinct groups: ectomycorrhiza, ericoid mycorrhiza, endophytes and decay fungi based on their sequence identification. Individual strains used in this study are listed in Table C1.

Fungal biomass was collected from the liquid cultures using a Miracloth filter, rinsed with distilled water and stored at -20°C until extracted and analyzed for amines. Agar plugs and the growth media, used for fungal inoculation in flask cultures, were analyzed separately for amine concentrations as negative controls.

2.3 Low molecular weight amine analysis

Fungal biomass samples and the first set of soil samples were extracted by dynamic sonication assisted extraction for 20 minutes with flow rate of 0.5 mL min^{-1} (1% aqueous acetic acid – acetonitrile, 1:1). Samples inserted in extraction chambers made of polyether ether ketone (PEEK, 5 cm length, i.d. 7.5 mm) equipped with screw caps. After extraction, samples were filtered through the 0.45-µm syringe filters. Extraction solvent was pumped through the extraction chambers, which were immersed in ultrasonic bath (Branson Sonifier S-250 A, Branson, Danbury, CT, USA) using Jasco PU-980 HPLC pumps (Jasco Corp., Easton, MD, USA).

The samples were statically extracted for 30 minutes. Mineral and humus soil samples, 21.8 g and 16.2 g of fresh weight, respectively, were extracted by sonication with 40 mL dichloromethane-methanol (1:1) together with 1 mL 1M HCl for 30 minutes. Also, 700 mg fresh weight of fungal hyphae samples was weighed and extracted with 10 mL of the extraction solvent with addition of 100 µL 1M HCl. After the extraction, the fungal and

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mineral soil samples were evaporated to 5 mL and humus soil sample to 15 mL, and then filtered through 0.45 μm acetyl cellulose syringe filters.

Low molecular weight alkylamines in extracts from soil, soil fungal hyphae and fungal pure cultures were analyzed with the analytical method introduced by Ruiz-Jiminez et al (2012). Soil extracts and cultured fungal biomass extracts were first dansylated. Since dansylated amines are relatively unstable, derivatized samples were analyzed immediately or within 24 hours. Acetaminophen was used as an internal standard (the final concentration of the standard was 1 ng at the detector). The derivatization procedure tends to overestimate amine concentrations but the estimations of the relative amounts to the internal standard of amines are presumed to be accurate (Ruiz-Jiminez et al (2012).

Analysis of the samples was performed with an Agilent 1260 Infinity liquid chromatograph coupled via electrospray ionization to an Agilent 6420 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The initial mobile phase was a mixture of 50% A (water acidified with 1% acetic acid) and 50% B (acetonitrile). Sample volume of 20 μL was injected and a linear gradient to 100% B in 10 minutes was applied. After 7 minutes in 100% B, mobile phase was decreased to 50% B in one minute. The column was let to equilibrate before the next injection for 7 minutes in 50% of B. A Hibar HR column (Purosphere, RP-18, endcapped, 2 μm, 50 mm x 2.1 mm, Merck, Darmstadt, Germany) was used and the temperature was kept at 40 °C. Ionization parameters were as follows: drying gas (nitrogen) temperature 300 °C, gas flow 7.5 L min^{-1} and nebulizer (nitrogen) 35 psi. MS1 and MS2 heaters were kept at 100 °C. The dynamic multiple reaction monitoring acquisition method was applied. MassHunter Quantitative Analysis software B.04.00 was used for data processing.

To identify amines in the samples the following external standards were used: isopropylaniline, tripropylamine, 2-amino-1-butanol, DL-2-aminobutyric acid and diethylamine for the first field soil samples and the soil fungal hyphae, and methylamine, dimethylamine, ethanolamine, diethylamine, dibutylamine, and *sec*-butylamine for the second soil samples (Kieloaho et al., 2016) and for pure fungal culture strains.

2.4 Concentrations of DMA and DEA in soil air

The concentrations of DMA and DEA in soil solution (aq.) are obtained from the measurements in the greenhouse experiment on boreal forest soil (Kieloaho et al., 2016), and

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assumed to be constant during the whole study period. The DMA and DEA concentrations in soil solution were 92.3 μmol L^{-1} and 0.296 μmol L^{-1}, respectively.

The concentrations of non-dissociated DMA and DEA are calculated from the measured soil solution concentrations based on reversible acid-base reaction

$$R_3N\ (aq) + H^+\ (aq) \leftrightarrow R_3NH^+\ (aq), \quad (1)$$

where R_3N is non-dissociated amine molecule and R denotes either methyl or ethyl organic side group or hydrogen atom. The dissociation reaction reaches a temperature dependent equilibrium, which is independent of reactant and reaction product concentrations.

A concentration in soil solution is a sum of non-dissociated (R_3N) and dissociated (R_3NH^+) forms of amines. In the first step, using equilibrium thermodynamic principles, the fraction (f_{R3N}) of total amine concentration present as non-dissociated form can be estimated (Montes et al., 2009), when the activity of R_3N and R_3NH^+ are assumed to be equal. The activity of protons [H^+] in soil solution is based on the measured pH values. Equilibrium dissociation coefficients (pK_a) for DMA and DEA are 10.3 and 10.5, respectively, and K_a is a negative logarithm of pK_a,

$$f_{R3N} = \frac{[R_3N]}{[R_3N]+[R_3NH^+]} = \frac{1}{1+\frac{[H^+]}{Ka}}. \quad (2)$$

In the second step, the non-dissociated DMA and DEA are partitioned between aqueous phases and soil air,

$$R_3N\ (aq) \leftrightarrow R_3N\ (g). \quad (3)$$

According to Henry's law, the solubility of non-dissociated gas in a solution is directly proportional to the partial pressure of the gas above the solution

$$k_H = \frac{c_{R3N}}{p_{soil}}, \quad (4)$$

where k_H is Henry's law coefficient, c_{R3N} is non-dissociated aqueous phase concentration and p_{soil} is a partial pressure of alkylamines in soil gas phase. Due to temperature dependence, acid dissociation (K_a) and Henry's law coefficients were corrected for temperature by Van t'Hoff equation

$$k_{(T)} = k_1 e^{\frac{+\delta H^\circ}{R(T_2^{+1}+T_1^{+1})}}, \quad (5)$$

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where $k_{(T)}$ is the temperature corrected coefficient, k_1 is the coefficient to be corrected, δH^o is the enthalpy change in reaction or phase transition, R is the molar gas constant, and T_1 and T_2 are temperatures in Kelvins. To take into an account the effect of acid dissociation on the partitioning of DMA or DEA between the aqueous and gas phases, a temperature corrected acid dissociation coefficient was used to calculate the effective Henry's law coefficients according to Seinfield and Pandis (2006)

$$k_{H(T,pH)} = k_{H(T)} \left(\frac{1+[H^+]}{K_{a(T)}}\right), \quad (6)$$

where $k_{H(T)}$ is the temperature corrected Henry's law coefficient, $[H^+]$ is measured proton concentration of aqueous phase and $K_{a(T)}$ is the temperature corrected acid dissociation coefficient.

Henry's law coefficient, the acid dissociation coefficient, the acid dissociation reaction and phase change energies were retrieved for DMA and DEA from National Institute of Standards and Technology Chemistry WebBook (Linstrom and Mallard, 2014).

2.5 Estimation of soil-air fluxes of DMA and DEA

The soil-air fluxes (F, nmol m^{-2} d^{-1}) of DMA and DEA were estimated using flux-gradient relationship (Figure 1) as

$$F = \frac{C_s + C_a}{r_{tot}}, \quad (7)$$

where C_s and C_a are concentrations (nmol m^{-3}) in the soil air space and in the atmosphere at 2.0 m above the forest floor, respectively, and r_{tot} (s m^{-1}) is the total gas transport resistance, which includes soil resistance (r_g), quasi-laminar boundary layer resistance (r_b) and aerodynamic resistance (r_a) in series.

In soil, the gas transport is dominated by molecular diffusion though the air-filled part of soil matrix. The soil resistance (r_g, s m^{-1}) in the organic soil layer of depth Δz_s (here 0.05 m) is estimated as

$$r_g = \frac{\Delta z_s}{D_p} = \frac{\Delta z_s}{D_o {\theta_a}^b}, \quad (8)$$

where the molecular diffusivity in soil D_p is computed from the molecular diffusivity in free air (D_o), using air-filled porosity (θ_a) to account for the reduced cross-sectional area and

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increased path length in the soil relative to free air. The parameter b = 1.1 as reported for humus layer in Glinski and Stepnieswski (1985).

The transport through the quasi-laminar boundary layer at the soil surface is described by the soil boundary-layer resistance (r_b, s m^{-1}) following Schuepp (1977)

$$r_b = \frac{Sc+\ln(\delta_o/z_1)}{k_v u_{*g} z_1}, \quad (9)$$

where Sc is the Schmidt number, k_v (~0.41) is the von Kárman constant, u_{*g} is the near-ground friction velocity, the height above the ground, where the molecular diffusivity and turbulent transport efficiency equal, is $\delta_o = D_o/k_v u_{*g}$, and z_1 is the height below which the wind profile is assumed logarithmic. The model for r_b applied here is identical to that used to compute gas-transfer e.g. in Baldocchi (1988), Nemitz et al. (2001) and Launiainen et al. (2013).

The aerodynamic resistance (r_a) accounts for the turbulent gas transport between the soil surface and concentration measurement height (z_m) in the canopy air space. The r_a is calculated by integrating the inverse of eddy diffusivity (K_s, m^2 s^{-1}) over the layer as in Baldocchi (1988)

$$r_a = \int_0^{z_m} \frac{1}{K_s(z)} dz. \quad (10)$$

The profile of $K_s(z)$ within the canopy and the value of u_{*g} needed for computing r_a and r_b, are provided by a first-order closure model for momentum exchange within the canopy as in Launiainen et al. (2013, 2015). As shown in Supplement B, the model computes mean velocity, momentum flux ($\overline{u'w'}$) and K_s profiles from local balance of momentum absorption and canopy drag neglecting the effects of atmospheric stability. The latter have been shown modest for below-canopy flow statistics at the SMEAR II –site (Launiainen et al., 2007).

For DMA and DEA flux estimates, the measured weekly mean ambient air concentrations and their standard deviations (Kieloaho et al., 2013) were used. Soil air concentrations and total resistances were obtained from the calculated half-an-hour values and averaged to weekly means and their weekly standard deviations. Gaussian error propagation was used to estimate the error of flux estimate with an assumption that errors of concentration gradient ($C_{gr} = C_s - C_a$) and total resistance (r_{tot}) are independent from each other. The error, expressed as

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standard deviation of soil flux (F_{std}), was calculated from normalized standard deviations of C_{gr} and r_{tot}

$$F_{std} = F\sqrt{\left(\frac{C_{gr,std}}{C_{gr}}\right)^2 + \left(\frac{r_{tot,std}}{r_{tot}}\right)^2}. \quad (11)$$

2.6 Chemical reaction and turbulent transport timescales

Ratio between turbulent transport timescale and chemical reaction timescale (Damköhler number, DA) is a measure of flux divergence due to chemical reactions occurring in the ambient air. As DMA and DEA are reactive gases, their respective

$$DA = \frac{\tau_{tr}}{\tau_{ch}} \quad (12)$$

were calculated to compare their atmospheric lifetimes (τ_{ch}) to characteristic turbulent timescale $\tau_{tr} = r_a/z_m$ which are associated to transport between the soil and the atmosphere, in this case the within-canopy measurement height. DMA and DEA mainly react in the atmosphere with hydroxyl (OH) radicals, and the chemical timescales τ_{ch} for DMA and DEA are 3.2 h and 2.6 h, respectively (Héllen et al., 2014). DA smaller than unity indicates that chemical reactions play a minor role in linking measured flux at a given height to sinks/sources below the measurement height (Rinne et al., 2012). When DA is smaller than 0.1, the role of chemical reactions is typically neglected in flux estimates (Rinne et al., 2012).

2.7 Sensitivity analysis

The sensitivities of the calculated resistances and estimated soil air concentrations and soil fluxes were assessed by one-at-a-time method by studying the effect of the measured variable on the calculated variable. In case of soil air concentrations, the studied variables were pH (from 4.0 to 6.0), temperature (from 0 to 20 °C) and soil solution concentration (from 0 to 100 μmol L^{-1}), as these variable have an effect on dissociation and separation between gas and aqueous phases of DMA and DEA. The measured soil solution concentrations were based on 1 M KCl extractions. The soil solution concentration of DMA was used as the upper limit for the soil solution concentration range.

The effects of environmental variables on resistances were assessed separately for r_g, r_b, and r_a. In case of the r_g, effect of soil water content (from 0.1 to 0.45 m^3 m^{-3}) was assessed due to its effect on soil spore space continuum. In addition, soil temperature (from 0 to 25 °C) and

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soil depth (from 0 to 0.15 m) were studied as they affect diffusion and the length of the diffusion pathway. For r_b, the effects of temperature (from 0 to 25 °C) and friction velocity (from 0.1 to 0.15 m s^{-1}) were assessed as they have effects on diffusion and thickness of quasi-laminar layer, respectively. In case of r_a, the effect of friction velocity (from 0.1 to 0.15 m s^{-1}) was studied as it determines the effectiveness of turbulent transport.

3 Results

3.1 Amine contents in soil, soil-derived fungal hyphae, and pure fungal cultures

Concentrations of DEA in humus soil and in fungal hyphae restricted from the humus were 0.3 μg g^{-1} FW and 2.9 μg g^{-1} FW (Table 1), respectively. Amine concentrations in the mineral soil were below the detection limit of 0.01 μg g^{-1} FW. DMA was not measured from field samples, as it was not included in standards used for the first soil samples. The results for other amine compounds (2-amino-1-butanol and DL-2-aminobutyric acid) analyzed from field samples are presented in the supplementary material (Table A1).

The highest DMA and DEA concentrations in the fungal pure cultures were measured in the decay fungi (Table 1). DMA concentrations were much higher than those of DEA throughout the all functional groups, and concentration of DMA varied from 25 μg g^{-1} FW in endophytic fungi to 360 μg g^{-1} FW in decay fungi. Three out of four most amine containing fungal strains belonged to ectomycorrhiza. DEA concentrations in soil fungal hyphae (2.9 μg g^{-1} FW), ectomychorrhiza (2.5 μg g^{-1} FW) and ericoid mycorrhiza (1.9 μg g^{-1} FW) were in similar range, while the concentrations in humus and mineral soil were markedly lower (Table 1). Amine concentrations of DMA and DEA and other measured amines (methylamine, ethanolamine, *sec*-butylamine, and dibutylamine) of individual strains, as well as the mean amine concentrations of ecological fungal groups, are shown in supplementary material (Table C1 and Table C2, respectively).

3.2 Estimated soil air concentrations

Over the study period, the estimated mean soil air concentrations of DMA and DEA with standard deviation, at mean soil pH (5.3), were 27±5.1 nmol m^{-3} and 0.032±0.006 nmol m^{-3}, respectively. The effect of soil temperature, soil pH and soil solution concentration on amine

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concentrations in soil air are shown in Figure 3. The soil air concentration follows the seasonal trend in soil temperature (Figure 2). For DMA, the mean soil air concentration was higher than the measured mean ambient air concentration (1.7 nmol m^{-3}) during the study period. For DEA, the mean soil air concentration was lower than the measured ambient air concentration (0.26 nmol m^{-3}).

Sensitivity of estimated soil air concentration to soil solution concentration was assessed using a soil solution concentration range from 0 to 100 μmol L^{-1}. Soil air concentration changed linearly in the studied range 29 nmol m^{-3} for DMA and 11 nmol m^{-3} for DEA (Figure 3A).

Soil air concentrations of DMA and DEA are highly sensitive to soil pH. The non-linear relationship is caused by pH-dependency of dissociation of an alkylamine in soil solution (Eq. 2), and partition of an alkylamine between aqueous solution and gas-phase (Eq. 6).

In the measured range soil air concentration change was 680 nmol m^{-3} for DMA and 0.81 for DEA (Figure 3C). Soil air concentrations in pH 4.0 were 0.07 nmol m^{-3} for DMA and less than 0.01 nmol m^{-3} for DEA. In pH 5.1 soil air concentrations for the both compounds starts to increase rapidly from 10 nmol m^{-3} for DMA and from 0.01 nmol m^{-3} for DEA to soil air concentrations in pH 6.0, 680 nmol m^{-3} for DMA and 0.81 nmol m^{-3} for DEA.

Soil temperature had a minor effect on soil air concentrations than pH in assessed ranges. The concentration change in the temperature range was 24 nmol m^{-3} for DMA and 0.03 nmol m^{-3} for DEA (Figure 3B). Sensitivity of soil air concentration was not assessed for soil water content because it has an effect only to the transport of DMA and DEA through the soil.

Estimated soil air concentration did not correlate with measured ambient air concentration in case of DMA ($r=0.09$, $p=0.68$), but it correlated in case of DEA ($r=0.67$, $p<0.01$) (Figure 7A and 7B, respectively).

3.3 Resistances and chemical reaction timescale

The mean total resistance for soil-air pathway (r_{tot}) was 13 500 (±2300) s m^{-1} for DMA and 18 500 (±3200) s m^{-1} for DEA The r_{tot} was dominated, i.e. the transfer of the studied amines mostly limited, by slow diffusion of through the soil matrix (soil resistance, r_g). The mean soil resistance of both gases was ~14 000 s m^{-1} (Figure 4B) hence being 1 and 2 orders of

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magnitude larger than quasi-laminar resistance (r_b, 1200 s m^{-1}) and aerodynamic resistance (r_a, 110 s m^{-1}), respectively (Figure 4C).

Sensitivity of each resistance component to environmental variables (soil water content, temperature and friction velocities and in case of r_g organic soil depth) was assessed separately (Figure D1). In short, r_g increases linearly with length of the diffusion pathway (Δz_s) and non-linearly with increasing soil water content (eq. 8). The temperature sensitivities of r_g and r_b are weak in the studied temperature range, and caused by weak decrease of molecular diffusivity with temperature. The r_b (eq. 9) and r_a both decrease nearly order of magnitude when the above-canopy friction velocity increases from 0.1 to 1.5 m s^{-1}, while the r_b to r_a -ratio is quasi-conserved. Most of the non-linear decrease of r_b and r_a occurs at u_* below 0.5 m s^{-1} (Figure D1).

For DMA, Damköhler number (DA) ranged from 0.013 to 0.026 and having a mean of 0.019 (±0.004). For DEA, DA ranged from 0.017 to 0.033 with a mean of 0.023 (±0.005). Due to DA numbers lower than 0.1 the removal of DMA and DEA by chemical reactions in the canopy air space can be considered negligible for the flux estimates.

3.4 Estimated soil fluxes

The mean soil-atmosphere fluxes of DMA and DEA over May to November 2011 measurement period were 170 (±51) nmol m^{-2} d^{-1} and -1.2 (±1.2) nmol m^{-2} d^{-1}, respectively (Table 2). The DMA flux increased from the spring to summer, and then decreased in the autumn. Unlike in the ambient air concentrations (Figure 2B), there was no autumnal peak in the estimated DMA fluxes (Figure 5). The seasonal pattern in DEA flux did not follow the changes in soil temperature or moisture, and the fluxes were negative most of the measurement period. Several strong and distinct DEA uptake periods were estimated in June, August and October (Figure 5).

Effects of environmental variables (pH, temperature, soil water content, soil depth, and friction velocity) on estimated soil fluxes are shown in Figure 6. A linear increase in soil solution concentration would increase flux from soil to the atmosphere linearly. (Figure 6A). The pH has strong effect in the partitioning of DMA and DEA between aqueous and gas phases (Figure 3C), and thus also in the flux estimates (Figure 6B). The fluxes computed for 10 and 90 percentiles of measured soil pH (4.5 and 6.0, respectively) were -0.67 (±0.68) nmol

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m^{-2} d^{-1} and 4500 (±1300) nmol m^{-2} d^{-1} for DMA, and -1.4 (±1.2) nmol m^{-2} d^{-1} and 2.7 (±1.0) nmol m^{-2} d^{-1} for DEA, respectively (Table 2).

According to the sensitivity analysis, both amines reach a zero flux point below which the emission from the soil will turn into an uptake to the soil in the measured pH range from 4.5 to 6.0. This turning point (compensation point with respect to pH) occurred at pH 5.7 for DEA and at pH 4.7 for DMA was (Figure 6B). A 10% decrease in soil solution concentration of DMA increased the turning point pH by 0.1 and similarly an increase in soil solution concentration of DMA decreased the turning point by 0.1 pH unit. The turning point of DEA was less affected by the soil solution concentration. A change of 10% in DEA soil solution concentrations lead to a change in turning point pH of ±0.06. Decrease in pH decreased the available DMA and DEA concentrations and affected partitioning between soil water and soil air, but the proton concentration had no influence on the transport processes.

Soil temperature increase from 0 to 20 °C increased DMA fluxes from 81 nmol m^{-2} d^{-1} to 255 nmol m^{-2} d^{-1} near-linear manner, and DEA fluxes from -1.1 nmol m^{-2} d^{-1} to 1.3 nmol m^{-2} d^{-1} (Figure 6C) near-linearly. Fluxes decrease near-linearly with increasing soil water content (Figure 6D). This is due to non-linear increase of r_g with increasing soil water content (Figure D1). In assessed soil water content range DMA flux changed from 241 nmol m^{-2} d^{-1} to 122 nmol m^{-2} d^{-1} and DEA flux from -1.7 nmol m^{-2} d^{-1} to -0.84 nmol m^{-2} d^{-1} (Figure 6D).

The estimated soil-atmosphere fluxes are sensitive to the assumed depth of amine sources/sinks in the soil. Because of the dominating role of soil resistance, the absolute value of flux decrease with soil depth, and the sensitivity is strongest when soil depth is under 0.03 m (Figure 6C) Increasing friction velocity decreases soil boundary layer and aerodynamic resistances and modestly affect the flux estimates (Figure 6F). The strongest impact occurs friction velocity values smaller than 0.2 m s^{-1}, and is mostly due to r_b (Figure D1). It should be noted that the friction velocity may become an important factor affecting the flux estimates in calm conditions if the amine sources or sinks are located very close to the surface leading r_g and r_b being of same order of magnitude.

4 Discussion

The results of this study shows that soil is an important reservoir of alkylamines, and our results suggest that this may be due to high amine concentrations in fungal hyphae in the

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boreal forest soil. Furthermore, we show in the flux estimation that these compounds can be released from the soil into the atmosphere under favorable environmental conditions. The source-sink behavior was dependent on soil conditions including temperature, soil water content and pH. Soil was shown to act as a source of DMA and a sink of DEA. The fact that both the DMA and DEA concentrations were much higher in the fungal hyphae and in fungal pure cultures as compared to the humus or mineral soil, indicate that the fungal community may be the primary source of these alkylamines in boreal forest soils.

Both the concentrations of DMA and DEA in humus samples from the greenhouse experiment (Kieloaho et al., 2016) were lower than those of the fungal pure cultures (Table 1). The DMA concentrations were higher than DEA concentrations in the humus samples and in pure fungal cultures. Overall, the DEA concentration in the humus samples of the greenhouse experiment were lower than those measured from the field humus samples (Table 1).

In both sample types, field collected hyphae and pure fungal cultures, DEA were found in the same range strongly supporting each other, and show that fungi are a reservoir of DEA. DEA concentrations found in the humus soil may reflect concentrations found in fungal biomass and may be of fungal origin. In the pure fungal culture biomass, DMA concentrations were 50 times higher than those measured for DEA. DMA concentrations were also higher than DEA concentrations in the soil used in the greenhouse experiment.

Fungal sporocarps were shown to contain of monomethylamine, dimethylamine and trimethylamine (Sintermann and Neftel, 2015). However, these measurements were based on fungal sporocarps and not on fungal hyphae, which is the only one form of fungi present in forest soils. Fungal sporocarps occur seasonally and sporadically mainly in autumn, whereas fungal hyphae are found throughout the year in forest soil (Santalahti et al., 2016). Therefore, the sporocarp data does not necessarily reflect the most important fungal contribution as a source of alkylamines in boreal forest ecosystems.

The fungal community of boreal forest soil undergoes seasonal variation. Santalahti et al. (2016) observed a clear soil fungal community shift in which the ectomycorrhizal fungi seem to disappear in late autumn while saprotrophic community dominates in the winter. In this study we show that ectomycorrhizal fungi contain high quantities of DMA and DEA, which could be released into the soil solution, and subsequently to the atmosphere during their disappearance in late autumn. In boreal Norway spruce forest in Sweden, Wallander et al.

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(2001) estimated that humus contains 700-900 kg ha^{-1} ectomycorrhizal hyphae, which is equal to the amount of fine roots found in humus.

The estimated soil air concentrations correlated positively with the measured ambient air concentrations of DEA, but not with DMA. Kieloaho et al. (2011) found strong correlation between ambient air concentration of DEA and ambient air monoterpene concentration, and suggested that the source of DEA might be in vegetation as has been suggested for monoterpenes (Hakola et al., 2006). In this study, the estimated soil air concentrations of DEA were smaller than the measured ambient air concentrations, which suggest that the soil is not necessarily a source of atmospheric DEA. The soil air concentrations are based on limited data of soil solution concentrations, and the results serve as the first estimates for both soil air concentrations and soil fluxes for DMA and DEA. DMA and DEA were assumed to have similar exchange processes with NH_3, having both sink and source behavior between the soil and the atmosphere

At the end of September and in October, the flux estimates of DMA and DEA did not explain the elevated atmospheric concentrations of DMA and DEA (Figure 2B). This missing autumnal peak in the fluxes might be due to a rapid change in soil DMA concentration, which could not be taken into account in the soil air concentration estimates due to the lack of continuous soil solution concentration measurements. During the autumn (from September to October), litterfall provides an input of fresh decomposable material into the soil, which also has an immediate effect on soil nitrogen concentrations due to the nitrogen rich leachate from the needle litter (Pihlatie et al., 2007; Starr et al., 2014). It was also recently shown that a common ectomycorrhizal fungal genus *Piloderma* sp., which also contained the highest quantities of alkylamines in our study, has a clear seasonal pattern, and it seems to disappear from the soil in late autumn (Heinonsalo et al. 2015). *Piloderma* sp. was shown to be active in protease production, protease is an enzyme that facilitates the decomposition of proteins, possibly due to the protease activity *Piloderma* sp. was also found to be able to obtain N from organic sources and deliver proteinaceous N to the host plant Scots pine. This involvement of ectomycorrhizal fungi in soil organic N cycling may make them 'nitrogen hotspots' that release also alkylamines into soil solution after their death (Heinonsalo et al. 2015).

Flux estimates were found to be sensitive to soil temperature, soil pH and soil water content, and soil resistance had a major effect on transport, while aerodynamic and quasi-laminar resistances had only minor effects on the fluxes of DMA and DEA. We found that DMA and

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DEA flux estimates were especially sensitive to change in soil pH. Flux estimates were calculated based on three pH values, mean pH (5.3) and 10 and 90 percentiles (4.5 and 6.0, respectively). The pH, in which the mean flux estimate is zero, is a compensation point with respect to soil pH. Below the compensation point pH, direction of alkylamine flux is into the soil and soil is a sink of alkylamines. The compensation point pH occurred for DMA at pH 4.7, which is lower than the mean measured pH from suction lysimeters, indicating that boreal forest soil can act as a DMA source at least occasionally. In contrary, for DEA the compensation point with respect to pH was 5.7, which is close to the 90 percentile (pH 6.0), indicating that soil is a sink of DEA. The compensation point pH is dependent on soil solution concentration of the amine. Hence, it is clear that even a slight change in soil pH or alkylamine concentration in soil solution could determine the capability of boreal forest soil to act as a source or a sink of alkylamines.

Current understanding of the atmospheric alkylamine sources is mainly from rural areas where the alkylamine emissions are related to agricultural activities (Schade and Crutzen, 1995; Kuhn et al., 2011). Schade and Crutzen (1995) have suggested using a constant ratio between trimethylamine (TMA) and NH_3 in total agricultural emissions as a proxy for agricultural alkylamine emissions. TMA emissions were 0.3% from NH_3 emissions from livestock farming and it can be partly explained by the same formation pathway of alkylamines and NH_3 (Kim et al., 2001; Rappert and Müller, 2005). The proxy was, however, revised by Kuhn et al. (2011), who suggested that TMA emissions are 0.1% from NH_3 emissions for both livestock farming and vegetation. Mineral soils have been found to be a sink for atmospheric NH_3 while litter of organic layer may act as a source of NH_3 (Neftel et al., 1998; Schjoerring et al., 1998; Nemitz et al., 2000).

It has been proposed that NH_4^+ is adsorbed onto soil particles in mineral soil, and hence is not available for gas exchange between soil solution and gas phase (Neftel et al., 1998). On the other hand, peat soil and litter layer have been shown to be periodically sources of atmospheric NH_3 in the laboratory (Schjoerring et al., 1998) and in the field (Nemitz et al., 2000). Previously Hansen et al. (2013) observed NH_3 emissions after a litterfall in a deciduous forest in Denmark, indicating that changes in nitrogen inputs may influence NH_3 dynamics. The ambient air measurements of NH_3 in boreal forest air indicate that NH_3 may be emitted from the ecosystem in the summer and in autumn as the concentrations of NH_3 in boreal forest air peak during this period, and remain lower in the spring and in winter months

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(Makkonen et al., 2014). To our knowledge, the only measured alkylamine fluxes from forested areas are TMA fluxes measured above a Douglas fir forest from June to July in Netherlands (Copeland et al., 2014). The mean TMA flux during this one-month measurement period was around zero showing occasional uptake and emission from -192 to 192 μmol m^{-2} d^{-1}, which is one order of magnitude higher than the DMA flux estimate (170 nmol m^{-2} d^{-1}) in this study.

At the moment, ambient air concentration measurements of alkylamines from remote forested areas are scarce. Recently, there have been several efforts to measure ambient air amine concentrations using online ion chromatograph connected with quadruple mass spectrometer (Hemmilä et al., 2014) and CI-API-ToF (Kulmala et al., 2013; Sipilä et al., 2015). However, they are so far only the first steps in characterizing the amine concentrations and no continuous datasets are yet available. Flux estimation presented in this study was based on ambient air concentration measurements conducted by Kieloaho et al. (2013). More recently, Sipilä et al. (2015) suggested that measured maximum ambient air concentrations of DMA is 0.06 nmol m^{-3} in spring and early summer (from May to June 2013), but due to problems in measurement system, and lack of calibration they advised to take these numbers by caution. This implies that if the forest soil is a reservoir of DMA, the real fluxes may be higher than those presented in this study if the atmospheric concentrations of DMA are as low as those presented by Sipilä et al., (2015). On the other hand, Hemmilä et al. (2014) reported preliminary results of ambient air concentrations of DMA and DEA in summer-time (June-July) at Hyytiälä Scots pine forest to be 0.4 nmol m^{-3} and 0.08 nmol m^{-3} for DMA and DEA, respectively. These results from June to July indicate that the ambient air measurements by Kieloaho et al. (2013) are in the correct range. The week long sampling time of ambient air DMA+EA and DEA concentrations (Kieloaho et al., 2013) coupled with the mixing of air, atmospheric sink processes and deposition of alkylamines onto the surfaces affect the measured concentrations, and diminish the relationship between source and ambient air concentrations. Hence, the flux estimates for DMA and DEA in this study can be used as the first attempts to estimate potential soil-atmosphere exchange in forests.

The concentration of ammonium in soil water is expected to change with substrate availability, environmental conditions, microbial activity, and due to assimilation of nutrients by either soil microbes or vegetation (Pajuste and Frey, 2003). Assuming that DMA and DEA share similar formation and consumption processes with ammonium in the soil, as suggested

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by Kim et al. (2001) and Rappert and Müller (2005), DMA and DEA concentrations in boreal forest soil may have two maxima during a year, in early spring and in late autumn (Pajuste and Frey, 2003). The two maxima are due to the combination of supply and demand of ammonium from temperature dependent ammonium releasing soil processes (decomposition and mineralization), and plant and microbial uptake rates. In the spring, the decomposition produces ammonium while the plant-uptake still remain rather low, whereas towards the late summer, plant uptake exceeds the mineralization rate leading to minimum concentrations in the soil. In late autumn, plant uptake decreases faster than the mineralization rate leading to a slight increase in ammonium concentration in soil (Pajuste and Frey, 2003).

5 Conclusion

We have shown that boreal forest soil and fungal hyphaea in the soil contain alkylamines, which can be released to the atmosphere in favourable conditions. We hypothesize that the soil-atmosphere exchange of the studied alkyamines (DMA and DEA) can be estimated based on soil temperature, soil water content and especially soil pH. Soil was shown to be a source of DMA, and a sink of DEA at typical soil pH (5.3) levels. The flux estimation method presented here is a first attempt to quantify the sources and sinks of alkylamines and other similar compounds that are difficult to measure directly in forest ecosystems. In boreal forest soil, fungal hyphae seem to form a large pool of low molecular weight amines like DMA and DEA. Therefore, we propose that fungi are the origin of alkylamines in boreal forest soils. The functional role of boreal forest soil as a source of low molecular weight amines, and their potential emissions needs to be further investigated in relation to air chemistry and atmospheric aerosol formation processes. In parallel, more measurements on atmospheric and soil air amine concentrations are needed to confirm the flux estimates provided in this study.

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Table 1. The mean dimethylamine and diethylamine concentrations (μg g^{-1} fresh weight) and their standard deviations in the field samples, different fungal functional groups and in the humus soil in a greenhouse experiment.

	Dimethylamine (DMA)	Diethylamine (DEA)
Field samples	μg g^{-1} FW	μg g^{-1} FW
Soil fungal hyphae	n.m.	2.9
Humus soil	n.m.	0.3
Mineral soil	n.m.	<0.01
Pure culture samples		
Ectomycorrhiza	116 (±34)	2.5 (±0.9)
Ericoid mycorrhiza	80 (±18)	1.9 (±0.5)
Endophyta	25 (±12)	0.49 (±0.23)
Decay fungi	360 (±320)	6.8 (±5.9)
Control agar media	4.3	0.13
Experimental samples[a]		
Humus soil (with plant)	4.3 (±3.9)	0.03 (±0.02)
Humus soil (without plant)	6.7 (±2.2)	0.02 (±0.01)
Mean of humus soil	4.6 (±3.2)	0.02 (±0.02)

[a] Kieloaho et al. (2016)

n.m.: not measured

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Table 2. Mean soil air concentrations and flux estimates for dimethylamine and diethylamine followed by their standard deviations in different soil pH values measured in lysimeter waters at 2 cm depth at SMEAR II station.

	Dimethylamine (DMA)			Diethylamine (DEA)		
soil pH	4.5	5.3	6.0	4.5	5.3	6.0
soil air conc. [nmol m^{-3}]	0.68±0.13	27±5.0	680±130	8.1×10^{-4}	0.03±0.01	0.81±0.15
flux/ measured air conc. [nmol m^{-2} d^{-1}]	-0.67±0.68	170±51	4500±1300	-0.14±0.12	-1.2±1.2	2.7±1.0

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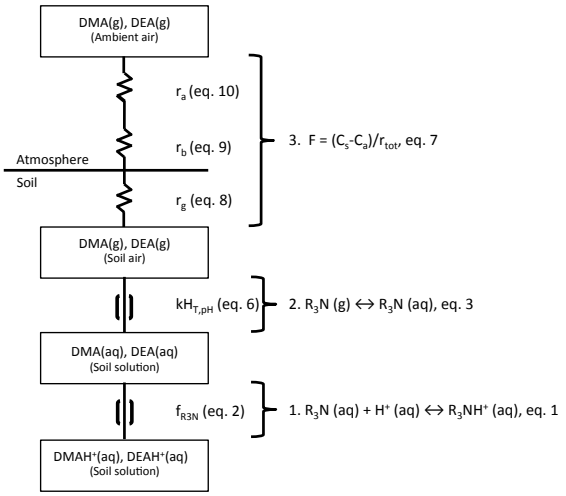

Figure 1. Scheme used for soil-atmosphere flux estimation of dimethylamine (DMA) and diethylamine (DEA) from reactions occurring in soil solution to transfer from soil air to ambient air. Boxes denote DMA and DEA concentrations in soil solution, soil air and ambient air. Numbers denote for steps in the flux estimation. Step 1: acid-base reaction and protonation of alkylamine; step 2: partitioning of non-protonated DMA and DEA between aqueous and gas phases; step 3: flux of DMA and DEA between soil and ambient air in which flux is determined by dividing concentration gradient by sum of component resistances (soil resistance, r_g; quasi-laminar boundary-layer resistance, r_b; aerodynamic resistance, r_a).

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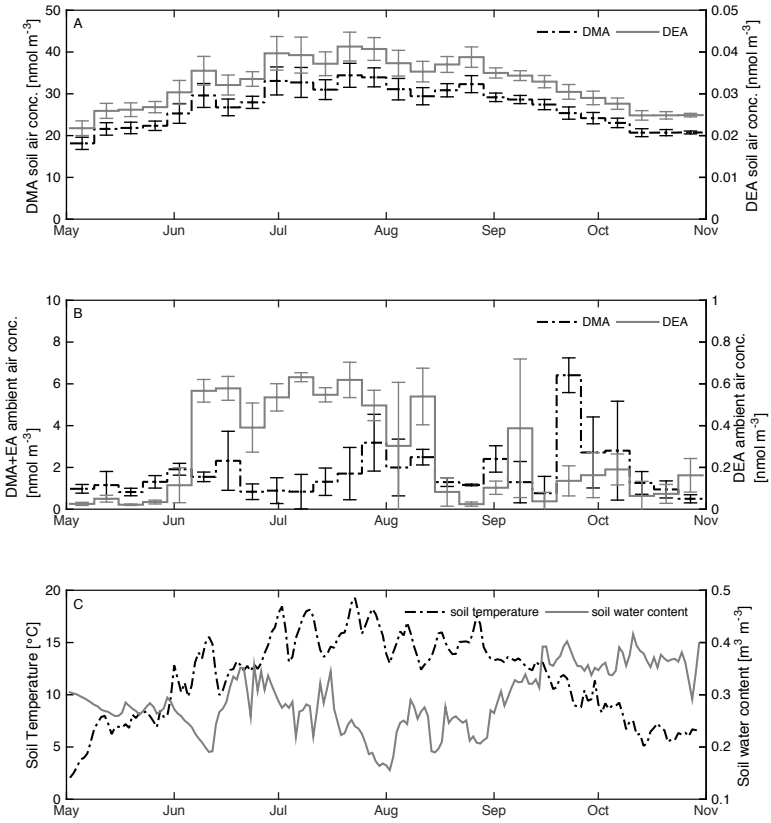

Figure 2. Estimated soil air concentrations of dimethylamine (DMA) and diethylamine (DEA) with their standard deviations at mean soil water pH of 5.3 (panel A), measured ambient air concentration of DMA + ethylamine and DEA (panel B) with their standard deviations redrawn from Kieloaho et al. (2013). In panel C, measured soil temperature and soil water content from May 2011 to October 2011 are shown.

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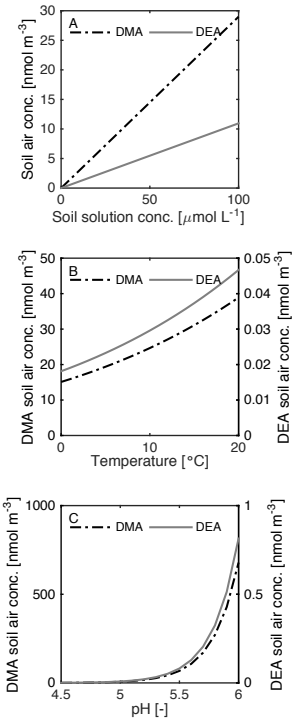

Figure 3. Effects of soil solution concentration (panel A), soil temperature (panel B) and soil solution pH (panel C) on estimated soil air concentrations of dimethylamine (DMA) and diethylamine (DEA).

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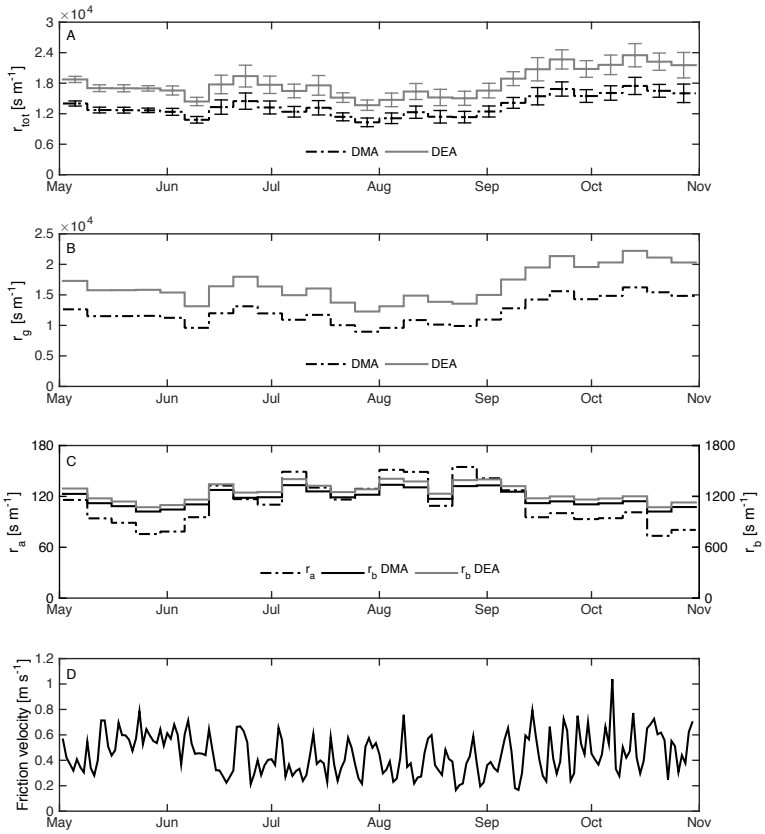

Figure 4. Total resistance (r_{tot}, s m^{-1}) of dimethylamine (DMA) and diethylamine (DEA) and its components: soil resistance (r_g, panel B) aerodynamic resistance (r_a, s m^{-1}, panel C), quasi-laminar resistance (r_b, s m^{-1}, panel B). Soil resistance (r_g, s m^{-1}) is calculated using 0.05 m soil depth. In panel D the daily mean of above canopy friction velocity (m s^{-1}) from May 2011 to October 2011 is shown. The errorbars in panel A show ± one standard deviation.

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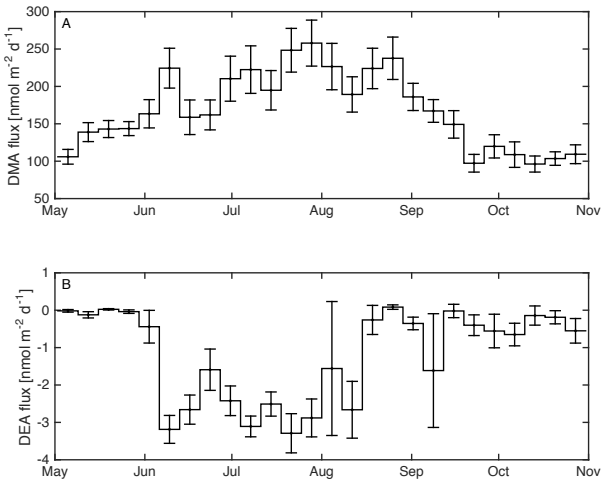

Figure 5. Weekly averages of estimated fluxes of dimethylamine (DMA, panel A) and diethylamine (DEA, panel B) and their standard deviations from May 2011 to October 2011. In emission estimation constant soil solution pH 5.3, average soil depth of 0.05 m and constant soil solution concentrations of DMA and DEA (92.3 µmol L^{-1} and 0.296 µmol L^{-1}, respectively) were used.

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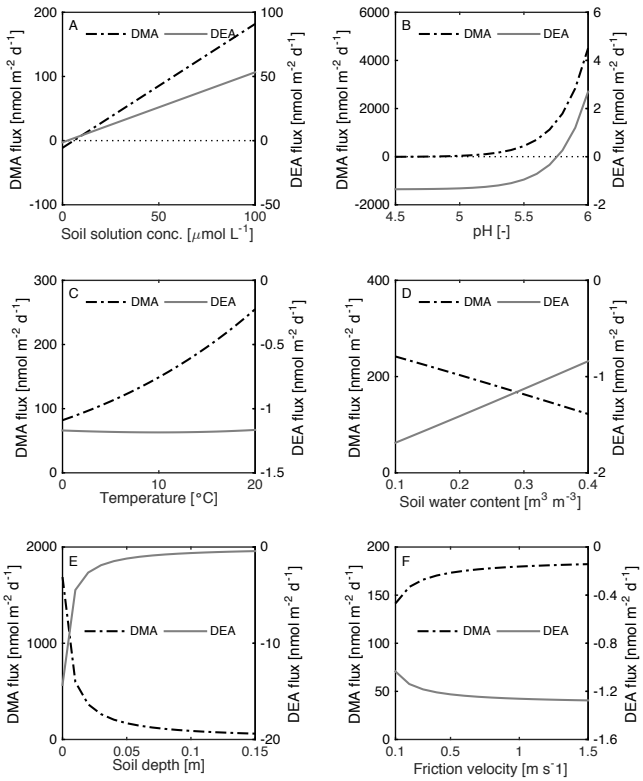

Figure 6. Effect of soil solution concentration (panel A), soil pH (panel B), temperature (panel C), soil water content (panel D), organic soil depth (panel E), and above-canopy friction velocity (panel F) on soil flux estimates of dimethylamine (DMA) and diethylamine (DEA).

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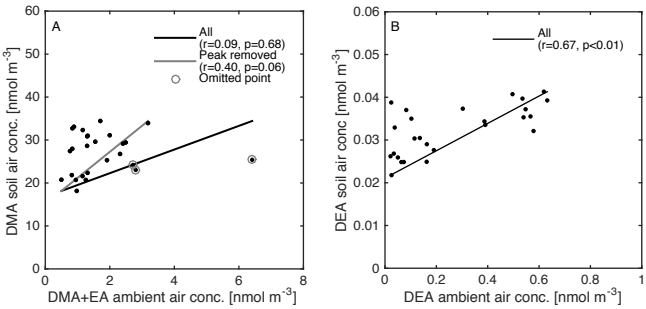

Figure 7. The comparison of measured ambient air concentrations and estimated soil air concentrations of dimethylamine DMA and diethylamine DEA (panel A and B, respectively) with linear least square fits. In case of DMA, three data points from autumn have been omitted (see Sect. 3.3); while also the least square fits without removed points are shown for comparison.