The role of terpenes in carbon and nitrogen cycling in boreal forest soils

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Academic dissertation

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ABSTRACT

Cycling of carbon (C) and nutrients plays pivotal role for functioning of every ecosystem. Biogeochemical cycles of carbon and nitrogen (N) are balanced by a network of inter-actions between plants, litter and soil chemistry, microbial communities, enzyme machinery and climate conditions. This thesis focuses on the role of terpenes in C and N transformations in boreal forest soils. Terpenes are abundant plant secondary compounds. The focus was on certain mono-, di-, and triterpenes.

Soil incubation experiments revealed that terpenes increased the mineralization of carbon but decreased net nitrogen mineralization and net nitrification. Additionally they increased the amounts of carbon and nitrogen in the microbial biomass through enhancement of bacterial growth; however, they inhibited fungal growth. This study suggests that terpenes can act as a C source for some microbial communities. Moreover, terpenes showed inhibitory potential against enzymes, which are involved in C, N, P, S cycling. The mechanism of inhibition seems to be based at least partially on ability of terpenes to bind enzymes.

The field experiment presented the effect of logging residues and wood ash on composition of terpenes and C and N cycling in soil five years after clear-cutting a Norway spruce stand. Logging residue treatment increased the concentrations of certain terpenes in the organic layer. Both, logging residue and wood ash treatments increased net N mineralization and net nitrification. Some changes in terpene concentrations correlated with C and N cycling processes, but the relationship between terpene concentration and C and N cycling processes remained still unclear in the field conditions.

In conclusion, terpenes can affect C and N transformations in boreal forest soil. It is probable that terpenes change N cycling retaining more N in organic forms and potentially decrease nitrogen losses from forest ecosystem.

To my beloved family:
My dear husband Bartosz
and son Sebastian

ACKNOWLEDGEMENTS

Vantaa, September 2016   Sylwia Adamczyk

LIST OF ORIGINAL ARTICLES

The thesis is based on the following articles, which are referred to in the text by the Roman numerals I–VI. The articles I–III are reproduced with the kind permission of publishers, while the IV is the author's version of the submitted manuscript:

I   Adamczyk S., Adamczyk B., Kitunen V., Smolander A. (2011). Influence of diterpenes (colophony and abietic acid) and a triterpene (beta-sitosterol) on N and C transformations in birch (*Betula pendula*) soil. Biology and Fertility of Soils 47: 715-720.
      http://link.springer.com/article/10.1007%2Fs00374-010-0529-x

II   Adamczyk S., Kiikkilä O., Kitunen V., Smolander A. (2013). Potential response of soil processes to diterpenes, triterpenes and tannins: Nitrification, growth of microorganisms and precipitation of proteins. Applied Soil Ecology 67: 47-52.
      http://www.sciencedirect.com/science/article/pii/S0929139313000632

III Adamczyk S., Adamczyk B., Kitunen V., Smolander A. (2015). Monoterpenes and higher terpenes may inhibit enzyme activities in boreal forest soil. Soil Biology and Biochemistry 87: 59-66.
      http://www.sciencedirect.com/science/article/pii/S0038071715001480

IV   Adamczyk **S.,** Kitunen V., Lindroos A-J., Adamczyk B., Smolander, A. Soil carbon and nitrogen cycling processes and composition of terpenes five years after clear-cutting a Norway spruce stand: Effects of logging residues. Manuscript.

THE AUTHOR'S CONTRIBUTION

Sylwia Adamczyk was responsible for the summary part of this thesis as well as she was the main author in papers I, II, III, IV. She performed most of the experimental work, calculated and interpreted the results.

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1. INTRODUCTION

The boreal forest ecosystem is characterized by low soil pH, high production of plant secondary compounds, high soil organic matter (SOM) content, and nitrogen (N)-limitation (Prescott et al. 2000; DeLuca and Boisvenue 2012). Despite that boreal forest soil contains a large nitrogen pool, most of it is in various organic forms, which are bound or complexed with soil compounds that are physically protected from decomposition (Schmidt et al. 2011). Thus, soil N needs to be released from these boundaries, depolymerized and mineralized into inorganic N before uptake by plants (Schimel and Bennett 2004). A short-cut for this pathway, in which plants can directly take up small organic N forms has also been shown (e.g. Schimel and Bennett 2004; Näsholm et al. 2009), but the ecological significance of this phenomenon is currently not well defined (Gärdenäs et al. 2011).

It has been suggested that plant secondary compounds have potential to play significant roles in the N cycling and nitrogen availability of boreal forest soils (Lodhi and Kilingberg 1980, Kraus et al. 2003, Smolander et al. 2012). Some work has been performed on tannins and monoterpenes, but the role of higher terpenes still remains unclear. My thesis concentrates on the various effects of terpenes on carbon (C) and N cycling processes in boreal forest soil, paying special attention to higher terpenes. The applied part of my study focuses on terpenes and soil C and N cycling beneath logging residues.

1.1. Plant secondary compounds

Plants produce a wide variety of compounds to sustain growth, development and reproduction. In addition to primary metabolites, which play a clear role in the above-mentioned processes, plants also produce vast amounts of compounds called secondary compounds (=secondary metabolites), which are basically not needed for plant growth.

The main role of plant secondary compounds is ascribed to defence against pathogens and herbivores (e.g. Harborne 1997; Agrawal and Weber 2015). Plant secondary compounds may act as attractants for seed-dispersing animals and as allelopatic agents (Croteau et al. 2000). They can act as antioxidants protecting leaves from UV radiation and excess of light (Close and McArthur 2002).

Extractable plant secondary compounds can comprise even up to 30% of the dry weight of terrestrial plants, especially in forest ecosystems (Horwath 2015). However, the concentrations of plant secondary compounds are species- and organ- specific. For instance, estimated concentration of plant secondary compounds in pine and spruce needles and birch leaves accounts for 52, 140, 78 g/kg d.m. (dry matter), respectively (Kanerva et al. 2008). In addition, environmental biotic and abiotic factors like e.g. pathogen attack, vertebrate and invertebrate herbivory, soil nutrient deficiency, light, temperature, elevated CO_2 and drought affect concentration of plant secondary compounds (Obst 1998, Tharayil et al. 2011, Väisänen et al. 2013). Moreover, difficulties in extraction and quantification methods affect the results of plant secondary compounds (Kögel-Knabner, 2002).

Plant secondary compounds consist of three major groups: terpenes, phenolic compounds (including tannins) and alkaloids (not included in this study).

1.1.1. Terpenes

Terpenes are the largest and the most diverse group of plant secondary compounds. Their concentrations in leaves usually range from 1% to 2% of the dry weight (Langenheim 1994). In plant cells terpenes are formed in the cytosol, plastids and mitochondria (Bramley 1997). Terpenes occur in resin, a heterogeneous mixture of fats and fatty acids, steryl esters, sterols and waxes. Resin is synthetized by coniferous and deciduous trees, however conifer trees produce larger amount of resin (Back and Ekman 2000). Terpenes are also produced by microbes, including soil microbes (Stahl and Parkin 1996; Bäck et al. 2010), some marine organisms (Jansen and De Groot 2004), and insects (Laurent et al. 2003).

Chemically, terpenes are derived from units of isoprene (C_5H_8). Their structure can be acyclic, monocyclic, bicyclic or polycyclic (Fig.1). Monoterpenes have two isoprene units ($C_{10}H_{16}$), sesquiterpenes have three isoprene units ($C_{15}H_{24}$). Higher terpenes such as diterpenes, have four isoprene units ($C_{20}H_{32}$), triterpenes have six ($C_{30}H_{48}$), tetraterpenes have eight ($C_{40}H_{64}$), while terpenes having >C40 are called polyterpenes (Langenheim 1994).
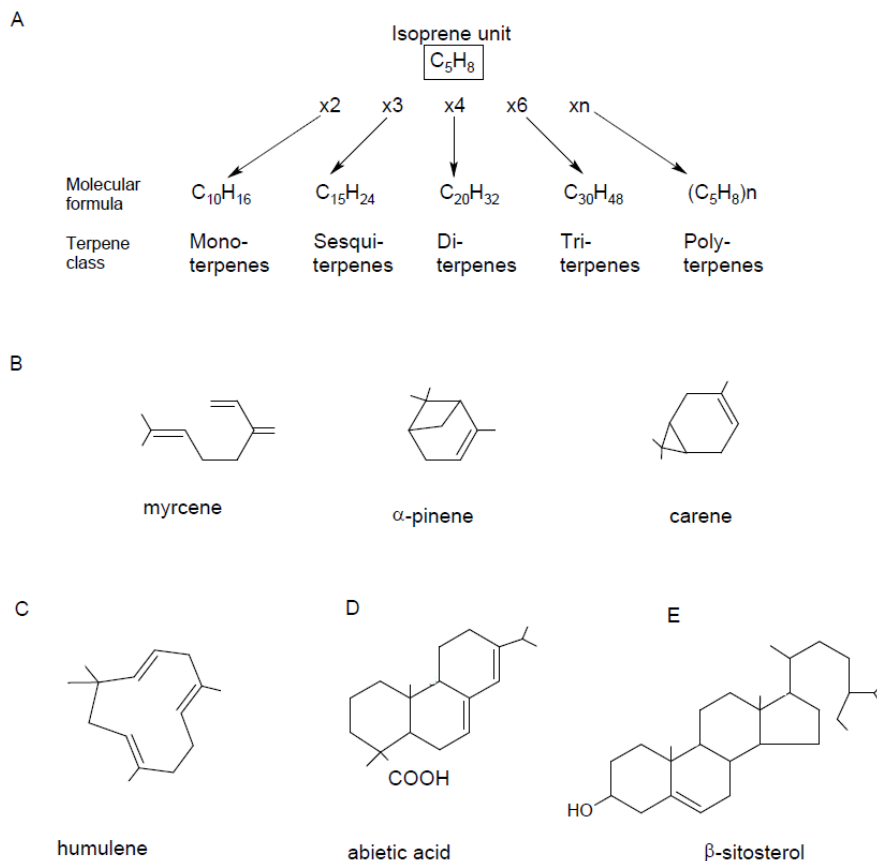

Fig. 1. Terpene: classification A) and examples of monoterpenes B), sesquiterpenes C), diterpenes D), triterpenes E).

Terpene production depends on plant species and on environmental conditions (Munné-Bosh et al. 2000; Smolander et al. 2012). For example, diterpenes occur in low concentration in birch, but they are abundant in conifers (Kanerva et al. 2008).

Monoterpenes are the main components of plant volatile essential oils, and especially high concentrations are found in conifers (Amaral 1998). The most abundant monoterpenes in Norway spruce (*Picea abies* L.) and Scots pine (*Pinus sylvestris* L) resin are α-pinene and β-pinene. Amount of carene can be significant in Scots pine resin, but it depends on region and genetic varieties. Carene is a common monoterpene in Scots pine needles and along with α-pinene composes 80% of the total amount of monoterpenes (Hiltunen 1975, Tarvainen et al. 2005). Monoterpenes accumulate in the resin ducts and are used by plants as toxins or deterrents against herbivores (Steinbrecher and Ziegler 1997). With other volatile organic compounds (VOC), monoterpenes are emitted to the atmosphere where they play important role in atmospheric chemistry and physics (Peñuelas and Llusia 2003).

Sesquiterpenes act as pheromones and juvenile hormones. In the boreal forest, Scots pine and Norway spruce needles contain larger amounts of sesquiterpenes than the leaves of silver birch (*Betula pendula* Roth.) (Kanerva et al. 2008).

Diterpenes are found in resin. Secretion of resin acts as a part of the resistance mechanism that conifers employ against bark beetles and their associated pathogenic fungi. Physiologically active diterpene groups include for example vitamin A (retinol), phytohormones that regulate plant growth and germination, gibberellins, fungal hormones that stimulate the switch from asexual to sexual reproduction, trisporic acid and disease resistance agents (phytoalexins) (Cooper-Driver and Le Quesne 1987). Dehydroabietic acids is the most abundant compound present in resin, and it constitutes the solid fraction of the oleoresin in coniferous trees.

Triterpenes are produced by plants; but they are also synthetized by some bacteria and fungi. In plants, triterpenes often accumulate as conjugates with carbohydrates and other macromolecules, most notably as triterpene glycosides (saponins), providing protection against pathogens and pests (Thimmappa et al. 2014). A common triterpene, β-sitosterol, is more abundant in the deciduous leaves (birch) than in the conifer tree needles (spruce, pine) (Kanerva et al. 2008).

1.1.2. Tannins

Tannins are a widespread group of plant secondary compounds. Chemically, they are polyphenols usually divided into hydrolysable tannins (HT) and condensed tannins (CT) (Fig. 2). Hydrolysable tannins are grouped into gallotannins and ellagitannins, which are made up of gallic acid or hexahydroxydiphenic acid esters, respectively, linked to a sugar moiety. Condensed tannins (proanthocyanidins) are polymers of three-ring flavonols joined by C–C bonds (Haslam 1989). Monomers of CT can be divided into procyanidins and prodelfinidins. Pines and spruces produce only CT; birches contain CT as well as HT (Kraus et al. 2003).

Tannins are the fourth most abundant compounds in vascular plant tissue after cellulose, hemicelluloses and lignin (Kraus et al. 2003). The concentration of tannins in plants has been estimated to be up to 20% of the dry weight (Kraus et al. 2003). Condensed tannins are produced by chloroplast-derived organelle called tannosome and CTs are stored mainly in vacuoles (Brillouet et al. 2013). Tannin concentration in plants can change in response to environmental conditions (Kraus et. al 2003). Many studies have shown that high tannin

concentration can be found in plants living in conditions of low soil fertility and low pH (i.e. Northup et al. 1998).

The effect of tannins on living organisms is very broad: from their important role in plant defence against herbivores and pathogens (Herms and Mattson 1992) and allelopathy (Rawat et al. 1998) to their influence on humus formation, degradation of soil organic matter and nitrogen cycling (i.e. Chapin 1995, Fierer et al. 2001, Kraus et al. 2003; Kanerva et al. 2006).
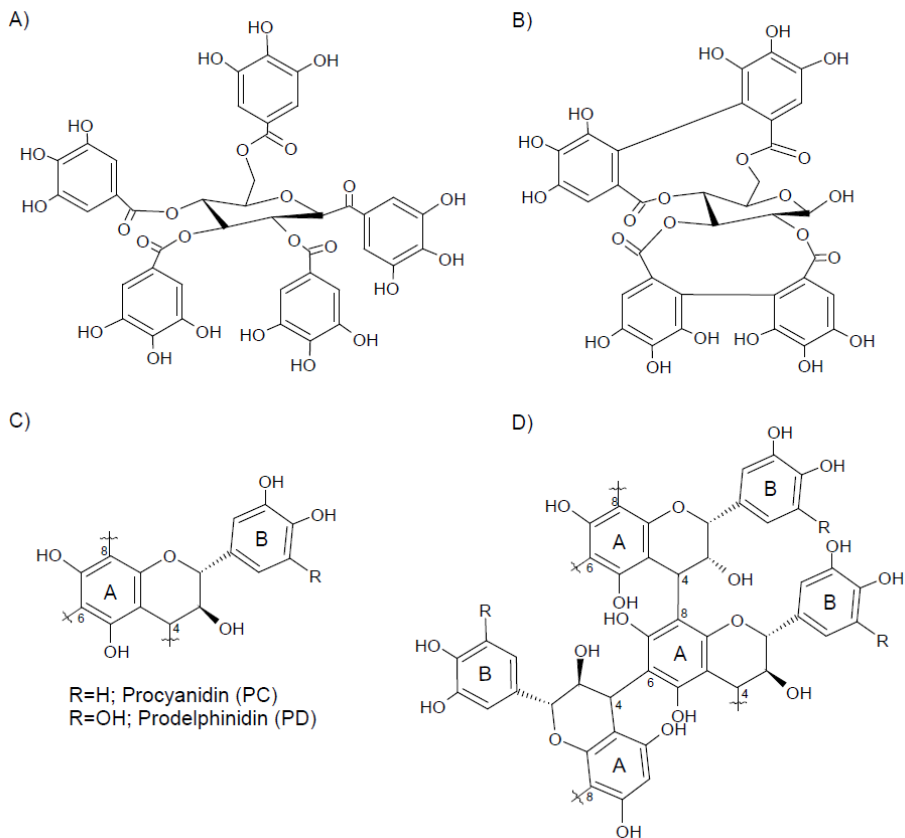
Fig 2. Molecular structures of tannins: hydrolysable tannins: A) a simple gallotannin, B) a simple ellagitannin, and condensed tannins: C) monomer, D) trimer (modified from Kraus et al. 2003).

1.2. C and N cycling in boreal forest soil - potential role of plant secondary compounds

The boreal forest is characterized by low rate of decomposition, corresponding to slow nutrient cycles and the accumulation of soil organic matter (SOM) during forest growth (Lupi et al. 2013). Such slow decomposition emerges from low temperature, litter rich in lignin and polyphenols (mainly tannins) and N-limitation (Prescott et al. 2000). Moreover, it has been emphasized that SOM decomposition is controlled not only by its chemistry but rather physiological processes, such as microbial responses to environment conditions and social dynamics among microbes are the main drivers of decay rates (Schmidt et al. 2011, Kaiser et al. 2015).

C cycling rate is linked to availability of nitrogen. The major pathways of nitrogen input to the ecosystem are biological N_2 fixation and atmospheric N deposition, albeit in Finland N deposition is usually low with some exceptions in southern Finland (Poikolainen et al. 2009). In soil, nitrogen exists in different forms, from plant-available inorganic and small organic forms to unavailable polymers. N forms are transformed during nitrogen cycling, consisting of microbial-driven processes: depolymerisation of high-molecular mass organic N, mineralization (ammonification), nitrification, immobilization, and denitrification. Disturbances, e.g. harvesting, N fertilization and N deposition affect N cycling and increase the risk for N losses. Stimulated nitrification and denitrification can lead to N losses from the forest ecosystem via leaching of NO_3 (nitrate) and the creation of gaseous N-forms (N_2, N_2O).

There are different paradigms of soil N cycling. The classical paradigm is based on N mineralization as the most critical step during the transformation of organic N forms into plant-available mineral forms. The rate of N mineralization is low in boreal coniferous forest soil and was annually estimated to account for 0.5-3% of the total amount of nitrogen (Persson and Wiren 1995). Recently it has been recognized that the depolymerisation of N-containing components into plant-available organic N forms is a main point in the N-cycling particularly in N-limited environments and this emphasizes the role of extracellular enzymes in the decomposition process (Schimel and Bennet 2004, Kieloaho et al. 2016).

The positive feedback mechanisms between mycorrhizal fungi and plants may drive the ecosystem to stronger N limitation. According to some studies, higher carbon allocation from trees to mycorrhizal fungi boosts nitrogen retention in soil mycelium at low N supply (Näsholm et al. 2013, Franklin et al. 2014). In boreal forest soil, tannins can shift N cycling from mineral- to organic-dominated pathways, decreasing N losses from the ecosystem (Northup et al. 1995, 1998).

The soil concentrations of plant secondary compounds, including terpenes and tannins, depend on input of these compounds to the soil and on their degradation rate. Several factors affect their concentrations in soil such as plant species, environmental conditions and microbial community structure (Kanerva et al. 2008, Adamczyk et al. 2009, Smolander et al. 2012). Additionally, forest management may change the input of plant secondary compounds to soil and also can change soil chemical and physical properties. During traditional harvesting, so-called "stem-only-harvest (SOH)" logging residues, consisting of branches and stem tops with needles are left on the site providing a large input of C and nutrients to soil and also plant secondary compounds (Obst 1998). On the contrary, whole-tree-harvest (WTH) includes harvesting of logging residues for bioenergy purposes.

1.2.1. The role of terpenes in C and N cycling in soil

In general, there is not much data available on the concentrations of different terpenes in boreal soils but some studies from Finnish forests are accessible. The highest terpene concentrations were observed in litter layer, lower in organic layer of the soil. Moreover, also the composition of terpenes changes from litter to organic layer (Kanerva et al. 2008, Stark et al. 2012). In the litter layer in different conifers, amounts of monoterpenes were 1-5 g/kg o.m. (organic matter) (Smolander et al. 2012) and the sum of concentration sesqui-, di-, and triterpenes range from 1.5 to 21 g/kg o.m (Kanerva et al. 2008). In the organic layer, the concentration of monoterpenes range from 0.1 to 0.2 g/kg o.m. under the Scots pine and Norway spruce and the sum of concentration of sesqui-, di-, and triterpene were from 0.5 to 5 g/kg o.m. (Smolander et al. 2012). The amount of terpenes in soil depends on the tree species and is highest under pine and lowest under birch (Smolander et al. 2005, 2008, 2010, Kanerva et al. 2008). Organic layer under birch contained less sesquiterpenes and diterpenes than the soils under pine and spruce (Kanerva et al. 2008).

There is evidence available that monoterpenes can affect C and N cycling in soil. Some studies have shown that monoterpenes (e.g. α-pinene, β-pinene, carene and myrcene) inhibit the net N mineralization and net nitrification (White 1986, 1991, 1994, Paavolainen and Smolander 1998, Smolander et al. 2006, Uusitalo et al. 2008). Mechanically, nitrification can be decreased by the direct effect of monoterpenes on ammonia monooxygenase through non-competitive inhibition (White 1991, 1994, Ward 1997). Monoterpenes have been shown to increase C mineralization (CO_2 production) in forest soil (Paavolainen and Smolander 1998, Smolander et al. 2006), and to decrease the amount of C and N in the soil microbial biomass (Smolander et al. 2006). These results suggest that monoterpenes can act as a C source for some microbial communities while being toxic to others. Moreover, Ludley et al. (2009) suggested that vapours of α-pinene and β-pinene may increase colonization of tree roots by the ectomycorrhizal fungus, and decrease respiration rate of some saprotrophic fungi.

Some studies show that certain triterpenes possess antibacterial and antifungal potential (Aderiye et al. 1989; Smania et al. 2003; Popova et al. 2009); however, these studies were not conducted under soil conditions. In a study with sandy arable soils, β-sitosterol negatively correlated with the mineralization of soil organic N (Heumann et al. 2011). Except that, nothing is known about the effects of higher terpenes on soil processes.

1.2.2 The impact of tannins on C and N cycling in soil

Effects of tannins on soil processes depend on tannin structure. Condensed tannins (CT) decreased net N mineralization in the soil or litter under several tree species like balsam poplar, Scots pine and Norway spruce (Schimel et al. 1996, 1998, Kanerva et al. 2006, Kanerva and Smolander 2007). Condensed tannins decreased also C mineralization and microbial biomass C and N (Fierer et al. 2001, Kanerva et al. 2006, Nierop et al. 2006; Kanerva and Smolander 2007). Likewise hydrolysable tannin (HT), a tannic acid, can decrease net N mineralization (Kanerva et al. 2006, Nierop and Verstraten 2006, Kanerva and Smolander 2007), however it acts as a C source because of rapid increase in CO_2 production; this indicates that decreased net N mineralization is due to immobilization.

Soil HT concentrations are very low (Adamczyk et al. 2008, 2009), which could emerge from generally low concentrations in plant litter or fast decomposition. On the contrary, CT

concentrations are much higher, up to a few g per kg SOM (Preston et al. 2006, Smolander et al. 2005, 2008, Kanerva et al. 2008, Adamczyk et al. 2009).

The effect of tannins on nitrification is not clear (Kraus et al. 2003), however, some experiments suggest that CT inhibit nitrification (Nierop et al. 2006, Kraal et al. 2009). The mechanism of such inhibition has so far not been clarified, but this process is probably dependent on tannin concentration and structure. According to Bardon et al. (2014, 2016), procyanidins (a group of condensed tannins) inhibit denitrification through decreasing NO_3^- reductase activity; mechanically procyanidins change conformation of this enzyme.

The high tannin concentration in boreal forest soil may partially account for the phenomenon of soil organic N recalcitrance. Tannins affect C and N transformation in soil by creating complexes with proteins (Hagerman and Butler 1981, Fierer et al. 2001, Kraus et al. 2003) and some other organic N compounds (Adamczyk et al. 2011). Tannin-protein complexes are relatively recalcitrant to decomposition but may act as a source of N which is recovered by the mycorrhizal symbionts of certain plant species (Northup et al. 1995, 1998). As tannins also interfere with enzymes (e.g. Adamczyk et al. 2009, Triebwasser et al. 2012), their influence on plant N uptake and SOM decomposition appears highly complicated.

2.   AIMS OF THE STUDY

A gap of knowledge concerning the effect of terpenes on soil processes supports the need for this study. Certain studies have shown the inhibition of N cycling processes by monoterpenes, but almost nothing is known about the possible effects of higher terpenes on soil processes.

The aim of this thesis was to explore the role of terpenes in C and N cycling processes, and the influence of logging residues on these processes and terpene concentrations in boreal forest soil. Effects of terpenes were studied in soil incubation experiments (I, II, III) by exposing soils to different terpenes and monitoring C and N transformations. The mechanisms behind these changes were examined in more detailed studies, e.g. through studies of enzyme activities. The last section of my thesis evaluates the role of terpenes in connection to energy tree harvest in the field experiment (IV). Logging residue harvest can change the amount of terpenes in soil, thus affecting their role.

Hypotheses:

1. Terpenes inhibit microbial processes related to N cycling and stimulate C cycling in boreal forest soils

2. Logging residues increase amount of terpenes in soil and this inhibits microbial processes in N cycling in forest soils.
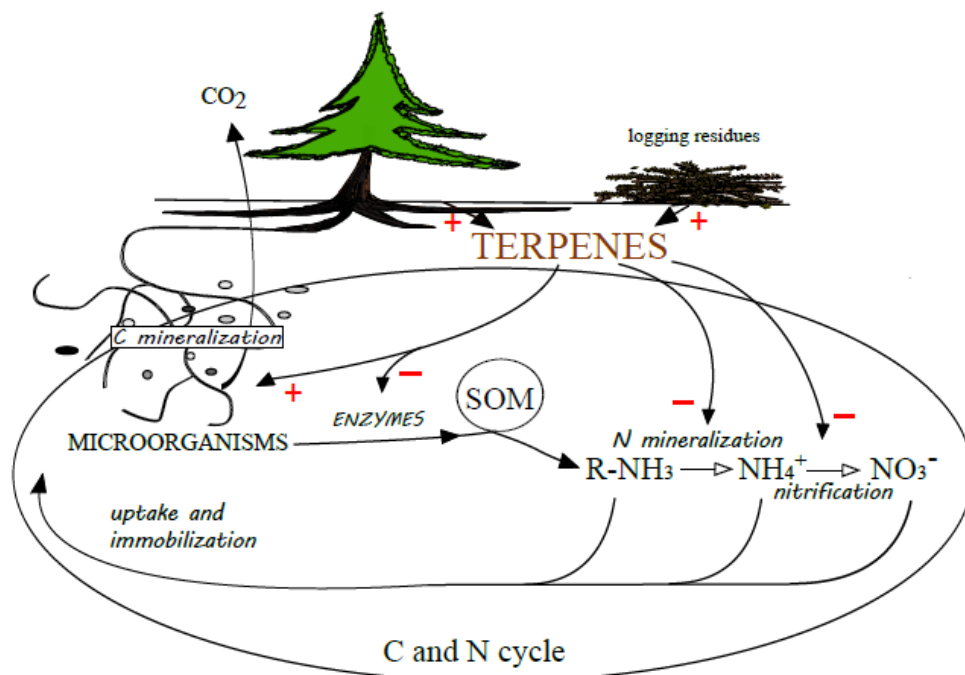
Scheme 1. Potential effect of terpenes on C and N cycle processes in boreal forest soil. SOM – soil organic matter, R – monomer of organic compound, - - inhibition, + - stimulation

3. MATERIALS AND METHODS

The methods applied here are described in details in I, II, III, IV.

3.1. Study sites and soil sampling

To study the changes in soil C and N cycling due to exposure to terpenes (soil incubation experiments, I, II, III), soil samples were taken from two sites, first located in Kivalo in Northern Finland (66°20´N, 26°40´E) and the second one in Kerimäki in Southeastern Finland (61°51´N, 29°22´E). The Kivalo site was a tree species experiment including silver birch plots (*Betula pendula* L.) (Smolander et al. 2002). Originally, this study site had been a homogeneous Norway spruce stand, which had been clear-cut and burned in 1926. The Kerimäki site was a fertilization experiment (Smolander et al. 2000). It had originally been a Norway spruce stand, which was harvested by clear-cutting in 1993 and planted with silver birch seedlings in 1994. This site was N-fertilized 35 and 12 years before the clear-cutting. Kerimäki, *Oxalis-Myrtillus* site type, was more fertile than Kivalo, *Hylocomium-Myrtillus* site type (Cajander 1949). On both study sites the soil type was Podzol and humus type was mor. From both study sites representative samples of organic layer (*Ofh*) were taken; Kivalo soil was collected in 2008, Kerimäki in 2009 from previously N-fertilized plots.

In this study, soils differing in characteristics were used to compare terpene effects on C and N cycle. Organic layer of birch stands was selected since it was expected to contain less terpenes than organic layer under corresponding coniferous stands. Kerimäki soil was more fertile than SOM-rich Kivalo (C/N ratio: 19.5 in Kerimäki, 30 in Kivalo; Table 1).

Soil samples were sieved and water holding capacity (WHC), dry and organic matter contents, pH and total C and N were measured (part of the data in Table 1).

Table 1. Basic soil characteristics and concentration of terpenes and tannins in organic layer. Results are presented as mean values (I, II, III).

	Kivalo soil (N-poor soil)	Kerimäki (N-rich soil)
pH	4.2	5.0
Organic matter (%)	81	30
Total C (g/kg o.m.)	600	532
Total N (g/kg o.m.)	20	27
C/N ratio	30	19
Sesquiterpenes (g/kg o.m.)	0.09[#]	-
Diterpenes (g/kg o.m.)	0.26	0.85
Triterpenes (g/kg o.m.)	0.24	0.12
Condensed tannins (g/kg o.m.)	2.50[¤]	0.20

[#] data taken from Kanerva et al. (2008); [¤] data taken from Adamczyk et al. (2013)

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To study the effect of logging residues and wood ash on terpenes and C and N transformations, soil samples were taken from site located in Anjalankoski, Southern Finland (60˚N, 26˚E) five years after clear-cutting (IV, Table 3). Before clear-cutting, the main tree species was Norway spruce and the study site was Myrtillus site type according to the Finnish forest-site type classification (Cajander 1949). At the beginning of this experiment, after clear-cutting, four treatments were established; three logging residues levels (0, 10, 40 kg/m^2) (Photo 1) and commercial wood ash (0.3 kg/m^2). There were four replicates per treatment. Soil cores from each plot were divided into organic layer (Ofh) and the uppermost part of mineral soil.

All these experiments were established by Natural Resources Institute Finland (Luke) (former Finnish Forest Research Institute - Metla).

Photo 1. Field experiment at the beginning; logging residues piles. Photo: Erkki Oksanen, Metla.

3.2. Analysis of terpenes and tannins

To determine sesqui-, di- and triterpenes, sieved and dried samples of soil and plants were extracted with acetone and extract was evaporated to dryness. For sesquiterpenes analysis dried extracts were re-dissolved in chloroform. For di- and triterpenes analysis dried extracts were dissolved in pyridine + N,O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA). To determine monoterpenes, fresh soil and plant samples were sieved and crushed in a mortar with liquid nitrogen and extracted with pentane spiked with internal standard (chlorodecane). Terpene concentrations were determined with gas chromatography-mass spectrometry (GC-MS) (Smolander et al. 2008). Identification of terpenes was based on reference compounds, mass spectrometric data and on the literature (I, IV, Pohjola 1993).

Concentration of condensed tannins was determined from air dried soil samples using modified acid-butanol assay (Terrill et al. 1992, Waterman and Mole 1994, Ossipova et al. 2001). Condensed tannins extracted and purified from Norway spruce needles were used as a standard (II, IV).

3.3. Measurements of soil C and N transformations

Changes in C and N cycling were studied after exposing the soil to terpenes at constant moisture and temperature in glass bottles (I, II, III). We used different amounts of higher terpenes, namely 10mg, 50mg (and 20mg in III), where the lowest amount of terpenes corresponded to natural terpene amounts in coniferous soil (Table 1). We used commercial terpenes: monoterpenes (α-pinene, carene, myrcene) (III), diterpenes (abietic acid, colophony) (I, II, III), triterpenes (β-sitosterol) (I, II, III). Colophony is a mixture of diterpenes which consisted of abietic acid (37.7 %), palustric acid (22.2 %), neoabietic acid (18.4 %), pimaric acid (8.4 %), dehydroabietic acid (7.6 %), and isopimaric acid (5.7 %), according to GC-MS analysis (I).

In the field experiment (IV) soil samples from different field treatments were incubated for 4 weeks at constant moisture and temperature in glass bottles.

In all studies net nitrogen mineralization and net nitrification were determined after extraction with KCl; net N mineralization was estimated as the accumulation of NH_4-N and (NO_2+NO_3)-N during incubation and net nitrification as the accumulation of (NO_2+NO_3)-N during incubation. NH_4-N, NO_3-N and NO_2- N (nitrite) were measured with a flow injection analyser (FIA) (I, II, IV).

Potential nitrification was studied in a soil-suspension experiment with excess of NH_4-N at constant shaking (II, IV). Soil samples were incubated with mineral solution at dark at constant temperature. Every day the pH was adjusted (to 5.5 in II and to both, natural soil pH and to pH 6 in IV). NH_4-N and NO_2+NO_3-N were measured using FIA. In this study we used terpenes and tannins. As tannins we used condensed tannins extracted from Norway spruce needles and as hydrolysable tannin, commercial tannic acid (II, characterized in Adamczyk et al. 2012). Tannic acid contained simple galloylglucoses (tri-, tetra- and pentagalloylglucoses), gallotannins (hexa- to tridecagalloylglucoses), but also gallic acid and digallic acid (see Introduction part 1.1.2). The average molecular mass was 1000 Da.

Aerobic C mineralization was measured as CO_2 evolution by sampling soil bottles headspace and analysing the amount of CO_2 by gas chromatograph (I, III, IV).

3.4. Analysis of microbial biomass and bacterial and fungal growth rates

Microbial biomass C and N were determined using the fumigation-extraction method (I, IV). Soil samples were fumigated with ethanol-free chloroform vapour. C and N flushes from the microbial biomass were calculated by subtracting K_2SO_4- extractable organic C and N in unfumigated control samples from those in fumigated samples. C and N flushes were converted to microbial biomass with the formulas of Martikainen and Palojärvi (1990).

To assess the relative rate of bacterial growth the ^{3}H-thymidine incorporation technique was used, where ^{3}H-thymidine was incorporated into bacterial cells (II). The radioactivity was used as a measure of the rate of bacterial growth (Bååth et al., 2001). The growth rate of fungi was estimated as ^{14}C-acetate incorporation into ergosterol. Acetate is a precursor of ergosterol, a molecule specific to fungi, and the amount of radioactivity detected in ergosterol can be used as an indicator of fungal activity (Bååth 2001).

3.5. Enzyme activity measurements *in vitro* and in soil

We used following enzymes involved in C, N, S, P transformations: beta-glucosidase (from *Aspergillus niger*), chitinase (from *Trichoderma viridae*), protease (from *Aspergillus saitoi*), arylsulfatase (from *Trichoderma viridae*), acid phosphatase (from wheat germ). Enzyme activities were studied with the following commercial substrates: p-nitrophenyl β-D-glucopyranoside, p-nitrophenyl N-acetyl-β-D-glucosamidine, hemoglobin, p-nitrophenyl sulfate, and p-nitrophenyl phosphate, respectively.

In *in vitro* experiment (III), different amount of higher terpenes (colophony, abietic acid, β-sitosterol), separately, were incubated with enzyme in acetate buffer. After the incubation, enzyme substrate was added. Enzymatic reaction was stopped with trichloroacetic acid (TCA) for proteolytic activity or TRIS-NaOH for arylsulfatase, beta-glucosidase, chitinase, and acid phosphatase activities. Monoterpenes (α-pinene, myrcene and carene) or water (control) were added to the glass bottles, covered with gas-tight septa and warmed up to increase evaporation. Then, air from head space was taken and injected into the buffer containing enzyme. Liquids containing buffer, enzyme and monoterpene/water evaporates were mixed with substrate, incubated and reaction was stopped; finally, absorbance was measured. The results are presented as a residual activity of control (buffer without terpenes). Concentrations of monoterpenes used here were measured with GC-MS.

In soil study (III), higher terpenes, colophony, abietic acid, β-sitosterol, were added to soil samples. Monoterpenes (α-pinene, myrcene and carene) were added to small glass bottles and hanged from the septa inside of soil bottles. After incubation with terpenes or terpene vapours, soil samples were mixed with acetate buffer and with substrates (section 3.3). The reaction was stopped with TRIS-NaOH, samples were centrifuged and the absorbance of supernatants was measured. The results were presented as residual activity of the control (soil samples without terpenes). Acid phosphatase and arylsulfatase activities were not studied, because P and S are not the limiting nutrients in the soil (Finér et al. 2005).

3.6. Protein precipitation studies

Here we studied precipitation of bovine serum albumin (BSA) (II) and enzymes (arylsulfatase, β-glucosidase, phosphatase, and chitinase) (III) by higher terpenes. Higher terpenes, colophony, abietic acid, β-sitosterol, were hydrated with acetate buffer and then protein was added. After shaking and filtration of samples Bradford reagent was added and absorbance was read on spectrophotometer. Bradford reagent reacted with proteins in the conditions used here; it did not react with terpenes. Protease was omitted here due to lack of reaction with Bradford reagent. The results are shown as percentage loss of the initial amount of protein.

3.7. Statistical analysis

Differences between treatments were determined by analysis of variance (ANOVA), followed by Tukey's (I, II, IV) or Dunnett's (II, III) test using the significance level of $P<0.05$. When needed, transformations were made to fulfil the assumptions of the ANOVA. The assumption of normality was assessed using Kolmogorov-Smirnov and Shapiro-Wilk tests and homogeneity of variances using Levene's test. To describe the relationships between terpenes and proteins, enzymes (III) and soil processes (IV), Pearson (III) or Spearman (IV) coefficients were calculated.

4. RESULTS AND DISCUSSION

The role of plant secondary compounds in the regulation of N and C transformations in forest soil is poorly understood, and there is lack of knowledge concerning the effect of higher terpenes on these processes. Some studies indicate that monoterpenes can influence C and N mineralization, nitrification and microbial biomass (White 1986, 1991, 1994, Ward et al. 1997, Paavolainen and Smolander 1998, Smolander et al. 2006; Uusitalo et al. 2008). Monoterpenes, as also some other volatile organic compounds (VOC), may trigger strong changes in the composition and functioning of soil microbial communities (Asensio et al. 2012). There is lots of evidence that tannins can affect C and N transformations in soil by decreasing N mineralization (Schimel et al. 1996, 1998, Kanerva et al. 2006), increasing or decreasing C mineralization (Kraus et al. 2004, Kanerva and Smolander 2007), creating complexes with proteins (Hagerman and Butler 1981, Fierer et al. 2001, Kraus et al. 2003) and decreasing enzyme activities (Kraus et al. 2003).

This thesis adds knowledge about the potential role of terpenes, in particular of higher terpenes in boreal forest soil. Results showed that terpenes, including higher terpenes, can affect C and N transformations and microbial biomass. Here for the first time we proved that some terpenes also inhibit soil enzyme activity. Moreover, logging residues can have an impact on terpene concentration as well as C and N cycling and the microbial biomass in boreal forest in soil.

Table 2 presents observed trends for the effects of different treatments on C and N cycling processes in soil incubation experiments and in the field experiment.

Table 2. Observed trends for the effects of different treatments; + -stimulation, - -inhibition, O -no effect, nd -not determined.

		Soil incubation experiments (I, II, III)					Field experiment (IV)		
		Diterpenes		Triterpenes	Monoterpenes	Tannins	Logging residues		Wood ash
		Abietic acid	Colophony	β-sitosterol	α-pinene		10 kg	40 kg	
C mineralization		+	+	+			+	+	0
Microbial biomass	C	+	+	+			+	+	0
	N	+	+	+	nd	nd	0	0	0
Growth rate	bacterial	+	+	+			nd		
	fungal	-	-	-					
Net N mineralization		-	-	-			+	+	+
Net nitrification		-	-	-		-	+	+	+
β-glucosidase activity	*in vitro*	-	-	-	-				
	in soil	-	-	-	-				
Chitinase activity	*in vitro*	-	-	-	-				
	in soil	-	-	-	-				
Acid phosphatase	*in vitro*	-	-	-	-	nd	nd		
	in soil	nd	nd	nd	nd				
Arylsulfatase activity	*in vitro*	-	-	-	-				
	in soil	nd	nd	nd	nd				
Protease activity	*in vitro*	-	-	-	-				
	in soil	nd	nd	nd	nd				
Protein precipitation:									
-bovine serum albumin		+	+	+					
-β-glucosidase		+	+	0	nd	nd	nd		
-Chitinase		+	+	+					
-Acid phosphatase		+	+	+					
-Arylsulfatase		+	+	+					

4.1. How terpenes can affect microbial biomass and C and N cycling processes in boreal forest soil?

During decomposition of litter terpenes and other plant secondary compounds are released to the soil and may affect decomposition processes. In this study, soil organic layer contained di- and triterpenes in the concentration range from 0.5 g/kg o.m. in N-poor Kivalo soil to 1 g/kg o.m. in N-rich Kerimäki soil (I, II, III, Table 1).

The addition of terpenes, in amounts which may occur in boreal forest soil, in the soil incubation experiment, showed that colophony, abietic acid and β-sitosterol affected microbial biomass C and N and mineralization of C in both soils (I). According to hypothesis 1, C mineralization (CO_2 evolution) was enhanced especially for higher amount of colophony. Terpenes increased microbial biomass C and N in the N-rich Kerimäki soil and showed tendency to increase microbial biomass C and N in the N-poor Kivalo soil (I). Higher terpenes increased bacterial growth, but they decreased fungal growth (II). The results indicate that increase in overall microbial biomass can be explained rather by bacterial growth and not fungal. Taking into account that higher terpenes contain substantial amounts of carbon (e.g. 10 mg of colophony contains 7.9 mg C) terpenes can act as a C source for some microbial communities. This is supported by other study, in which abietic acid acted as the sole carbon source for aerobic bacteria, *Alcaligenes* isolated from the soil (Cross and Myers 1968). In our study in soil conditions, it was also possible that microbial growth increased due to microbial use of debris produced by microorganisms for which terpenes are toxic. In the future study, uptake of C from microbes which used terpenes as a C source could be studied with labelled terpenes. It has been shown that certain triterpenes can have antibacterial and antifungal effects (Uribe et al., 1985; Aderiye et al. 1989, Smania et al. 2003, Popova et al. 2009). According to the literature, monoterpenes can decrease the amount of C and N in the soil microbial biomass (Smolander et al. 2006). However, it was also shown that monoterpenes can increase C mineralization (CO_2 production) in forest soil (Paavolainen and Smolander 1998, Smolander et al. 2006, Uusitalo et al. 2008) and that monoterpenes can be biodegraded by mixed culture derived from soil (Misra et al. 1996). All in all, monoterpenes can affect stronger C mineralization than higher terpenes.

Hypothesis 1 is supported by our findings that higher terpenes decreased net N mineralization in both N-rich and N-poor soils (I), however, in the N-rich soil higher amounts of terpenes were needed to observe this effect. It was indicated earlier that higher terpenes can act as a source of carbon for microbes; hence, the decrease of net N mineralization may be partially caused by microbial N immobilization, although toxic effects cannot be excluded. Studies on internal N cycling would reveal gross rates of mineralization and immobilization, but these studies were not made here. An experiment conducted by Uusitalo et al. (2008) showed that also monoterpenes, such as α-pinene, strongly inhibited N net mineralization in soils from the same sites, which is in accordance with previous studies (White 1986, 1991, 1994, Paavolainen and Smolander 1998, Smolander et al. 2006).

Net nitrification in undisturbed boreal forest soils is usually low and depends on ammonium (NH_4-N) availability, pH, the amount of nitrifying bacteria, and soil composition. In the N-poor Kivalo soil, net nitrification was negligible (I). In the N-rich Kerimäki soil, net nitrification was partially inhibited by higher amounts of higher terpenes, most clearly by colophony. The exact mechanism of net nitrification decrease is unknown, however, inhibition can be indirect, as the substrate was decreased (decreased

mineralization of N), or higher terpenes may directly affect nitrifying bacteria. In this study, ammonium was always found in soil samples at the end of all incubations, so the presence of the substrate did not restrict nitrification process.

Soil suspension experiment with an excess of NH_4-N showed potential nitrification in soils incubated with terpenes and tannins (II). In this experiment excess of substrate for nitrification and continuous shaking, which prevented the loss of NO_3 by denitrification, created optimal conditions to nitrification process. This study confirmed that higher terpenes and tannins could inhibit net nitrification (II).

As compared to monoterpenes the inhibition of net nitrification by higher terpenes was not so dramatic. Monoterpenes can completely stop net nitrification (White 1986, 1991, 1994, Ward et al. 1997, Paavolainen and Smolander 1998, Uusitalo et al. 2008) by direct effect on ammonia monooxygenase (White 1991, 1994). Moreover, also other plant secondary compounds, tannins, can inhibit net nitrification slightly (Nierop et al. 2006); although the mechanism is not clear.

4.2. Influence of terpenes on enzyme activities and protein precipitation

Plant secondary compounds are abundant in litter and they may modify N and C cycling in soil by altering microbial processes and populations (Smolander et al. 2012). As decomposition is driven by multiple sets of enzymes, a possible influence of litter compounds on enzyme activity appears to have a significant role in controlling this process. For example, reaction between tannins and enzymes may lead to inhibition of enzyme activity (Kraus et al. 2003, Schimel et al. 1998, Adamczyk et al. 2009, Triebwasser et al. 2012; Bardon et al. 2014, 2016), although sometimes the decrease was not observed (Juntheikki and Julkunen-Tiitto 2000).

Our results revealed, accordingly to hypothesis 1, that mono- and higher terpenes decreased enzyme activity both in *in vitro* and in soil studies (III). Studies *in vitro* included enzymes involved in C (beta-glucosidase), N (protease, chitinase), S (arylsulfatase), and P (acid phosphatase) transformations. The inhibition level was dependent on enzyme type, terpene amount and their molecular structure. Monoterpenes showed low inhibition (e.g. up to 74% of residual activity after incubation with α-pinene), while higher terpenes had stronger inhibitory effect, e.g. 20 mg of colophony decreased proteolytic activity to only 18% of the control (III).

Mechanically, a decrease in enzyme activity might emerge from the ability of terpenes to bind proteins (II), mechanism suggested for tannins (Kraus et al. 2003). Colophony and abietic acid showed a positive correlation between the decrease in activity and the precipitation of an enzyme (III). According to the *in vitro* experiment (II and III), about 20% of the protein, bovine serum albumin, was precipitated by diterpenes (abietic acid and colophony) and triterpenes (β-sitosterol), moreover, colophony precipitated up to 29% of acid phosphatase. Due to the low water solubility of terpenes, proteins may be precipitated via adsorption, or through terpene functional groups, although their number is small. Some abilities of terpenes to precipitate proteins suggest that these plant secondary compounds may also play a role in stabilizing of proteins in the soil.

Studies in soil conditions confirmed the ability of terpenes to decrease beta-glucosidase and chitinase activity (III) in both Kivalo and the Kerimäki soils. More profound effects in decreases of enzyme activity were observed in the less fertile, highly organic Kivalo soil. The volatile monoterpenes, α-pinene, carene and myrcene, and higher terpenes, abietic acid,

colophony and β-sitosterol, all decreased more beta-glucosidase activity than chitinase activity, although pattern of inhibition is not clear (III).

In highly heterogenic soil conditions changes in activity were less clear than in *in vitro* studies, as soil is composed of numerous compounds potentially affecting the reactions of terpenes with enzymes. Additionally, it was possible that some of soil microbial populations could use added terpenes as a source of carbon, which is well-supported by increase in CO_2 production (III). As we used terpenes in ranges of concentrations typical for boreal forest soil, in natural conditions modification of enzyme activity may be also observed, however, numerous other factors, like moisture and temperature can modify the effect of terpenes on enzymes (Asensio et al. 2012). On the other hand, interaction of enzymes with terpenes may lead to enzyme stabilization in soil. These enzymes, nevertheless of decreased activity, may represent important reservoir of the potential activity and may also be a source of substrate turnover during periods when microbial biomass is low (Stursova and Sinsabaugh 2008). All in all, our studies indicate that terpenes influence on enzyme machinery in boreal forest soil.

4.3. Effect of logging residues on terpenes and C and N transformations in soil – field experiment

Some long-term decreases in concentration of sesqui-, di-, and triterpenes in the organic layer have been detected in coniferous thinning stands due to whole-tree harvest (Smolander et al. 2010, 2013). In the field experiment at the clear-cut site (IV), the concentrations of terpenes (except of monoterpenes) and tannins were higher in soil under logging residues than in the control with no logging residues, five years after establishment of treatments (see Table 3). For example, the amounts of di- and triterpenes in treatment with 40 kg/m^2 of logging residues were about 30% and 20 % higher than in treatment without residues, respectively. Additionally, logging residues increased pH of the organic layer (Table 3). These results are in accordance with hypothesis 2 that logging residues increase concentration of soil terpenes.

Table 3. Soil characteristics and concentration of terpenes and condensed tannins in organic layer in Anjalankoski field experiment, five years after clear-cutting (IV).

	Control	Logging residues		Wood ash
		10kg	40kg	
pH	3.92	4.07	4.22	3.86
Organic matter (%)	44.5	43.4	53.1	43.8
Total C (g/kg o.m.)	505	481	510	484
Total N (g/kg o.m.)	15.0	17.0	18.1	16.0
C/N ratio	28.4	27.8	28.1	29.3
Monoterpenes (g/kg o.m.)	0.190	0.170	0.210	0.110
Sesquiterpenes (g/kg o.m.)	0.067	0.093	0.139	0.078
Diterpenes (g/kg o.m.)	0.493	0.527	0.630	0.388
Triterpenes (g/kg o.m)	0.405	0.445	0.477	0.242
Condensed tannins (g/kg o.m.)	0.134	0.525	0.792	0.028

To counteract the loss of nutrients due to tree harvesting, wood ash can be added to the soil. It contains all major mineral nutrients present in the plant except N, thus, wood ash can be a potential fertilizer. Concentration of terpenes and tannins were lower in the soil amended with ash than in control (IV, Table 3). Wood ash addition resulted in reduction of tannin level in Acacia litter (Ben Salem et al. 2005). However, we do not know whether changes in litter quality or in soil conditions were more important in our study.

Many studies have so far suggested that forest bioenergy harvesting affects soil properties and tree growth. It was shown that WTH causes long term decrease in tree growth in Norway spruce and Scots pine thinning stands (Helmisaari et al., 2011), slight changes in soil nutrients, such as decreases in the amounts of exchangeable base cations (Tamminen et al. 2012), and changes in microbial processes and soil organic matter composition (Smolander et al. 2008, 2010, 2013).

In long term experiments in Norway spruce thinning stands in Finland, the rates of net N and C mineralization tended to be lower in WTH than SOH treatment (Smolander et al. 2008, 2010). According to Olsson et al. (1996), logging residue removal also on a clear-cutting area can decrease N mineralization. Smolander et al. (2015) observed that with regard to soil microbial activities and nutrient status, ten years after clear-cutting, WTH and SOH were clearly different in only one site from 5 studied sites. Moreover, retaining logging residue on the site may increase enzyme activities in the soil organic layer. In pine stands enzymes involved in C, N and P cycling, namely beta-glucosidase, beta-glucosaminidase, protease, and acid phosphatase raised the activity in response to increasing amounts of logging residue (Adamczyk et al. 2015) probably because of increased organic matter input. The response of soil characteristics to harvest of logging residues seems to depend on the site, the amount of residues and passage of time since harvest (Smolander et al. 2013). To summarize, no coherent effects of logging residue harvesting have been observed in boreal forest soils on soil productivity, however, if the effects are observed they indicate positive role of logging residues on soil nutrient status and microbial activities (Thiffault et al. 2011, Achat et al. 2015).

The field experiment (IV) showed only slight increases of C mineralization and microbial biomass C in organic layer under logging residue treatments. The mineral layer appears to be less sensitive to variations in C and N stock caused by forest management practices (Piirainen et al. 2015), and thus no effect was observed in this layer. Smolander et al. (2013, 2015) reported that in thinning stands and clear-cutting experiments leaving logging residue at the site did not increase significantly C mineralization in humus layer or only a small effect was observed (Smolander et al. 2008, 2010).

Nitrification is a harmful process because it increases the risk for N-loss through leaching or denitrification (Paavolainen and Smolander 1998). Net nitrification is usually negligible in undisturbed boreal forest soil but certain forest management treatments, such as clear-cutting and fertilization, may increase net nitrification (Smolander et al. 2000). On the contrary to hypothesis 2, in the field experiment five years after clear-cutting, logging residues increased net N mineralization and, in particular, net nitrification in the organic layer (IV). The reasons behind this include the leaching of dissolved organic N from the residues (Qualls et al. 2000, Robertson et al., 2000), and an increase in pH or changes in physical conditions like temperature and moisture (Smolander et al. 2013). These results are supported by other experiments, in which the highest NO_3-N concentrations were observed in percolation water under the highest amount of logging residues (Rosen and Lundmark-Thelin 1987, Wall, 2008, Lindroos et al., 2016). Wood ash also increased net N net mineralization and net nitrification. Surprisingly, wood ash did not cause any increase

of soil pH, perhaps due to a previous intensification of nitrification (Pietikäinen and Fritze 1995). The soil suspension experiment affirmed that logging residue and wood ash raised net nitrification (IV) and that net nitrification is pH-dependent; in native pH of soil (3.5-4), net nitrification was negligible; in pH 6, net nitrification was intensive in logging residue and in wood ash treatments. As both, logging residues and wood ash stimulated net N mineralization, our results seem to support the idea of wood ash addition in order to counteract the effects of decreased amounts of nutrients due to logging residues harvest. However, wood ash also stimulated net nitrification which is not desired since it can increase the risk for N losses.

Some correlations between the concentrations of terpenes and C and N mineralization were observed (IV). C mineralization correlated negatively with the amount of triterpenes and β-sitosterol. Net N mineralization and net nitrification correlated positively with sesqui- and diterpenes, so the inhibitory effect of terpenes observed in soil incubation experiments was not seen. The amount of monoterpenes did not correlate with C and N mineralization. Time scale could affect the results significantly. As 5 years had elapsed from the treatment, maybe the effects of logging residue on monoterpenes were not visible anymore due to their volatility (Haapanala et al. 2012). As shown by Strömvall and Petersson (1991) already during harvesting of Scots pine as well as Norway spruce significant amounts of monoterpenes were volatilized. On the other hand, higher terpenes due to their low solubility enter the soil with decaying tree residues and their effect may be observed after longer time than the effect of monoterpenes.

4.4. Criticism and applications of findings

Results in this thesis highlight the role of terpenes and, in particularly, higher terpenes, which have not been studied before in boreal forest soil.

Our soil incubation experiments show that terpenes, in natural soil concentrations, inhibit microbial processes in N cycling. However, terpenes also provide energy source for some microorganisms as evidenced by increased CO_2 evolution after terpene additions. The soil incubation experiments present how terpenes affect the soil, without the effects of plants. To get information of internal N cycling, gross rates with ^{15}N labelling should be used, including amino acid pool dilution method.

Our field study did not show the effect of terpenes clearly. Although, as suggested by second hypothesis, amounts of terpenes and tannins in soil under logging residue increased but net N mineralization and especially net nitrification also increased.

Dissimilarities in results from experiments emerge from the fact that in soil incubation experiments we added only plant secondary compounds. In the field experiment, logging residue, although rich in tannins and terpenes, provided to soil also plenty of easy-to-decompose compounds, including C and N compounds, and probably improved physical conditions in soil, which both triggered enhanced mineralization and nitrification. Hence, these counteracting forces could hide possible inhibitory effects of plant secondary compounds.

This work helps to understand the role of terpenes in natural conditions. Since terpenes have the capacity to inhibit net N mineralization, they, as well as tannins, can play a role in retaining N in organic forms. Based on results from soil incubation experiments, higher ratio of total N to mineral N under terpene treatments in comparison to control may indicate that terpenes change N cycling retaining more N in organic forms which potentially

decreases N losses from forest ecosystem. Inhibition of net nitrification by plant secondary compounds additionally decreases the risk of N leaching in boreal forest soils. Future studies should focus on monitoring the effects of different terpene additions in time in different types of soils. Moreover, other effects such as possible changes in microbial community structure should be studied as well as gross rates of N transformations.

5. CONCLUSIONS

Results presented in this thesis add knowledge concerning the effects of terpenes on C and N cycling in boreal forest soil. The results from the soil incubation experiments revealed that terpenes can affect C and N transformations in the organic layer of boreal forest soil in several ways:

- Terpenes increased C mineralization, microbial biomass C and N and they regulated soil microbial populations. This indicates that some of microbial communities were able to use terpenes as a carbon source but for other microbial communities, terpenes acted as inhibitors.
- Terpenes decreased net N mineralization and net nitrification; also tannins were able to decrease net nitrification.
- Terpenes decreased enzyme activities, both in vitro and in soil conditions. The mechanism of inhibition seems to be partially dependent on the precipitation of enzymes by higher terpenes. Terpenes can to some extent precipitate proteins, mainly at low pH, and may therefore play a role in stabilizing proteins, including enzymes.

Additionally, the field experiment showed that logging residues and wood ash appear to stimulate N cycling processes in boreal forest soil.

- Five years after clear-cutting, logging residues and wood ash increased net N mineralization and particularly net nitrification.
- Logging residues increased the concentrations of certain terpenes.
- Although some correlations were observed, the relationship between terpene concentration and C and N cycling processes remained unclear in the field conditions. Logging residue provides not only tannins and terpenes but also easy-to-decompose C and N and possibly changes soil physical conditions, which can trigger enhanced mineralization and nitrification. Therefore, soil incubation and field experiments did not show the same pattern.

In conclusion, terpenes can affect C and N transformations in boreal forest soils. However, more studies are needed to profoundly investigate the effect of different terpenes on various processes and in wide range of soils. Additionally, future research should combine proteomic and metagenomic tools to study directly microbial communities with thorough understanding of the chemical and biological effects of different terpene classes.

6. REFERENCES

Achat, D.L., Deleuze, C., Landman, G., Pousse, N., Ranger, J., Augusto, L., 2015. Quantifying consequences of removing harvesting residues on forest soils and tree growth – meta-analysis. Forest Ecology and Management 348: 124-141.
http://dx.doi.org/10.1016/j.foreco.2015.03.042

Adamczyk, B., Adamczyk, S., Kukkola, M., Tamminen, P., Smolander, A., 2015. Logging residue harvest may decrease enzymatic activity of boreal forest soils. Soil Biology and Biochemistry 82: 74-80.
http://dx.doi.org/10.1016/j.soilbio.2014.12.017

Adamczyk, B., Adamczyk, S., Smolander, S., Kitunen, V., 2011. Tannic acid and Norway spruce condensed tannins can precipitate various organic nitrogen compounds. Soil Biology Biochemistry 43: 628-637.
http://dx.doi.org/10.1016/j.soilbio.2010.11.034

Adamczyk, B., Kitunen, V., Smolander, A., 2008. Protein precipitation by tannins in soil organic horizon and vegetation in relation to tree species. Biology and Fertility of Soils 45: 55–64.
http://dx.doi.org/10.1007/s00374-008-0308-0

Adamczyk, B., Kitunen, V., Smolander, A., 2009. Polyphenol oxidase, tannase and proteolytic activity in relation to tannin concentration in the soil organic horizon under silver birch and Norway spruce. Soil Biology and Biochemistry 41: 2085-2093.
http://dx.doi.org/10.1016/j.soilbio.2009.07.018

Adamczyk, B., Kitunen, V., Smolander, A., 2013. Response of soil C and N transformations to condensed tannins and different organic N-condensed tannin complexes. Applied Soil Ecology 64: 163-170.
http://dx.doi.org/10.1016/j.apsoil.2012.12.003

Adamczyk, B., Salminen, J-P., Kitunen, V., Smolander, A., 2012. Precipitation of proteins by tannins: effects of concentration, protein-tannin ratio and pH. International Journal of Food Science and Technology 47: 875-878.
http://dx.doi.org/10.1111/j.1365-2621.2011.02911.x

Aderiye, B.I., Ogundana, S.K., Adesanya, S.A., Roberts, M.F., 1989. The effects of β-sitosterol on spore germination and germ-tube elongation of *Aspergillus niger* and *Botryodiplodia theobromae*. International Journal of Food Microbiology 8: 73–78.
http://dx.doi.org/10.1016/0168-1605(89)90082-2

Agrawal, A.A., Weber, M., 2015. On the study of plant defence and herbivory using comparative approaches: how important are secondary plant compounds. Ecology Letters 18: 985-991.
http://dx.doi.org/10.1111/ele.12482

Amaral, J.A., Ekins, A., Richards, S.R., Knowles, R., 1998. Effect of selected monoterpenes on methane oxidation, denitrification, and aerobic metabolism by bacteria in pure culture. Applied and Environmental Microbiology 64: 520-5. http://dx.doi.org/ 0099-2240/98/$04.00

Asensio, D., Rapparini, F., Peñuelas, J., 2012. AM fungi root colonization increases the production of essential isoprenoids vs. nonessential isoprenoids especially under drought stress conditions or after jasmonic acid application. Phytochemistry 77: 149-161. http://dx.doi.org/10.1016/j.phytochem.2011.12.012

Bååth, E., 2001. Estimation of fungal growth rates in soil using C-14-acetate incorporation into ergosterol. Soil Biology and Biochemistry 33: 2011-2018. http://dx.doi.org/10.1016/S0038-0717(01)00137-7

Bååth, E., Pettersson, M., Söderberg, K.H., 2001. Adaptation of a rapid and economical microcentrifugation method to measure thymidine and leucine incorporation by soil bacteria. Soil Biology and Biochemistry 33: 1571-1574. http://dx.doi:10.1016/S0038-0717(01)00073-6

Back, E.L., Ekman, R., 2000. Definitions of wood resin and its components. In: Back EL, Allen LH (eds) Pitch control, wood resin and deresination. TAPPI, Atlanta, pp. vii – x.

Bäck, J., Aaltonen, H., Hellén, H., Kajos, M.K., Patokoski, J., Taipale, R., Pumpanen, J., Heinonsalo, J., 2010. Variable emissions of microbial volatile organic compounds (MVOCs) from root-associated fungi isolated from Scots pine. Atmospheric Environment 44: 3651–3659. http://dx.doi.org/10.1016/j.atmosenv.2010.06.042

Bardon, C., Piola, F., Bellvert, F., Haichar, F.Z., Comte, G., Meiffren, G., Pommier, T., Puijalon, S., Tsafack, N., Poly, F., 2014. Evidence for biological denitrification inhibition (BDI) by plant secondary metabolites. New Phytologist 204: 620-30. http://dx.doi.org/10.1111/nph.12944

Bardon, C., Poly, F., Piola, F., Pancton, M., Comte, G., Meiffren, G., Haichar, F.Z., 2016. Mechanism of biological denitrification inhibition (BDI): procyanidins induce an allosteric transition of the membrane-bound NO3-reductase through membrane alteration. FEMS Microbiology Ecology. http://dx.doi.org/10.1093/femsec/fiw034

Ben Salem, H., Sourour Abidi., Makkar, H.P.S., Nefzaoui, A., 2005. Wood ash treatment, a cost-effective way to deactivate tannins in *Acacia cyanophylla* Lindl. Foliage and to improve digestion by Barbarine sheep. Animal feed science and technology 122, 93-108. http://dx.doi.org/10.2016/j.anifeedsci.2005.04.013

Bramley, P.M., 1997. Isoprenoid methabolism. In: Dey, P.M. and Harborne, J.B. (eds.). Plant Biochemistry. Academic Press, London, pp. 417-437.

Brillouet, J-M., Romieu, C., Schoefs, B, Solymosi, K., Cheynier, V., Fulcrand, H., Verdeil, J-L., Conéjéro, G., 2013. The tannosome is an organelle forming condensed tannins in the chlorophyllous organs of *Tracheophyta*. Annals of Botany 112: 1003-1014. http://dx.doi.org/10.1093/aob/mct168

Cajander, A.K., 1949. Forest types and their significance. Acta Forestalia Fennica 56: 1-71.

Chapin, F.S.III, Shaver, G.R., Giblin, A.E, Nadelhoffer, K.J., Laundre, J.A., 1995. Responces of arctic tundra to experimental and observes changes in climate. Ecology 76: 694-711. http://dx.doi.org/10.2307/1939337

Close, D.C., McArthur, C., 2002. Rethinking the role of many plant phenolics - protection from photodamage not herbivores? Oikos 99:166–172 http://dx.doi.org/10.1034/j.1600-0706.2002.990117.x

Cooper-Driver, G.A., Le Quesne, P.W., 1987. Diterpenoids as insect antifeedants and growth inhibitors: role in Solidago species. In: Allochemical: Role in Agriculture and Forestry, pp. 534-550.

Cross, B. E., Myers, P.L., 1968. The bacterial transformation of abietic acid. Biochemical Journal 108: 303-310.

Croteau, R., Kutchan, TM, Lewis, NG., 2000. Natural products (secondary metabolites) In: Buchanan B., Gruissem W, Jones R (Eds) Biochemistry and molecular biology of plants. American Society of Plant Physiologists, Rockville, pp. 1250-1318.

DeLuca, T., Boisvenue, C., 2012. Boreal forest soil carbon: distribution, function and modelling. Forestry 85: 161-184. http://dx.doi.org/10.1093/forestry/cps003.

Fierer, J.A., Schmiel, J.P., Cates, R.G., Zou, Z., 2001. The influence of balsam poplar tannin fractions on carbon and nitrogen dynamics in Alaskan taiga floodplain soils. Soil Biology and Biochemistry 33: 1827-1839. http://dx.doi.org/10.1016/S0038-0717(01)00111-0

Finér, L., Mannerkoski, H., Piiranen, S., Lauren, A., Koivusalo, H., Kokkonen, T., Penttinen, S., 2005. Nutrient fluxes in managed boreal forests. In: Jalkanen, Anneli, Nygren, Pekka (Eds.), Sustainable Use of Renewable Natural Resources-from Principles to Practices. University of Helsinki Department of Forest Ecology Publications 34.

Franklin, O., Näsholm, T., Högberg, P., Högberg, M.N., 2014. Forests trapped in nitrogen limitation – an ecological market perspective on ectomycorrhizal symbiosis. New Phytologist 203: 657–666. http://dx.doi.org/10.1111/nph.1284

Gärdenäs, A.I, Ågren, G.I, Bird, J.A, Clarholm, M., Hallin, S., Ineson, P., Kätterer, T., Knicker, H., Ingvar Nilsson, S., Näsholm, T., Ogle, S., Paustian, K., Persson, T., Stendahl, J., 2011. Knowledge gaps in soil carbon and nitrogen interactions – From molecular to global scale. Soil Biology and Biochemistry 43: 702–717. http://dx.doi.org/10.1016/j.soilbio.2010.04.006

Haapanala, S., Hakola, H, Hellen, H, Vestenius, M, Levula, J, Rinne, J, 2012. Is forest management a significant source of monoterpenes into the boreal atmosphere? Biogeosciences 9: 1291–1300. http://dx.doi.org/10.5194/bgd-8-8067-2011

Hagerman, A.E., Butler, L.G., 1981. The specificity of proanthocyanidin-protein interactions. Journal of Biological Chemistry 256: 4494-4497.

Harborne, J.B., 1997. Role of phenolic secondary metabolites in plants and their degradation in nature. In: Cadish G., and Giller K.E. (eds). Driven by nature: Plant litter quality and decomposition. Wallingford : CAB International, UK, pp. 67-74.

Haslam, E., 1989. Plant polyphenols - vegetable tannins revisited. In: Phillipson JD, Ayres DC, Baxter H (eds) Chemistry and pharmacology of natural products. Cambridge University Press, Cambridge, pp. 230.

Helmisaari, H-S., Hansen, K.H., Jacobson, S., Kukkola, M., Luiro, J., Saarsalmi, A., Tamminen, P., Tveite, B., 2011. Logging residue removal after thinning in Nordic boreal forests: long-term impact on tree growth. Forest Ecology and Management 261: 1919-1927. http://dx.doi.org/10.1016/j.foreco.2011.02.015

Herms, D.A., Mattson, W.J., 1992. The dilemma of plant: to grow or defend. The Quarterly Review of Biology 67: 283 – 335. http://dx.doi.org/10.1086/417659

Heumann, S., Schlichting, A., Böttcher, J., Leinweber, P., 2011. Sterols in soil organic matter in relation to nitrogen mineralization in sandy arable soils. Journal of Plant Nutrition and Soil Science 174: 567-586. doi: 10.1002/jpln.200900273

Hiltunen, R., Juvonen, S., Tigersted, P.M.A., 1975. Geographical variation in some monoterpenes in Scots pine (*Pinus sylvestris* L.) in Finland. Farmaseuttinen Aikakauslehti 84: 73-82.

Horwath, W., 2015. Carbon cycling: The dynamics and formation of organic matter. In: E.A. Paul (eds), Soil Microbiology, Ecology and Biochemistry, 4th Edition. Academic Press, Elsevier, pp. 339-382.

Jansen, B.J.M., deGroot, A., 2004. Occurrence, biological activity and synthesis of drimane sesquiterpenes. Natural Product Reports 21: 449–477. http://dx.doi.org/10.1039/B311170A

Juntheikki, M., Julkunen-Tiitto, R., 2000. Inhibition of beta-glucosidase and esterase by tannins from *Betula salix* and *Pinus species*. Journal of Chemical Ecology 26: 1151–1165. http://dx.doi.org/10.1023/A:1005427809812

Kaiser, C., Kilburn, M.R., Clode, P.L., Fuchslueger, L., Koranda, M., Cliff, J.B., Solaiman, Z.M., Murphy, D.V., 2015. Exploring the transfer of recent plant photosynthates to soil microbes: mycorrhizal pathway *vs* direct root exudation. New Phytologist 205: 1537–1551. http://dx.doi.org/10.1111/nph.13138

Kanerva, S., Kitunen, V., Kiikkilä, O., Loponen, J., Smolander, A., 2006. Response of soil C and N transformations to tannin fractions extracted from Norway spruce and Scots pine needles. Soil Biology and Biochemistry 38: 1364–1374. http://dx.doi.org/10.1016/j.soilbio.2005.10.013

Kanerva, S., Kitunen, V., Loponen, J., Smolander, A., 2008. Phenolic compounds and terpenes in soil organic horizon layers under silver birch, Norway spruce and Scots pine. Biology and Fertility of Soils 44: 547 –556. http://dx.doi.org/10.1007/s00374-007-0234-6

Kanerva, S., Smolander, A., 2007. Microbial activities in forest floor layers under silver birch, Norway spruce and Scots pine. Soil Biology and Biochemistry 39: 1459-1467. http://dx.doi.org/10.1016/j.soilbio.2007.01.002

Kanerva, S., Smolander, A., 2008. How do coniferous needle tannins influence C and N transformations in birch soil. European Journal of Soil Biology 44: 1-9. http://dx.doi.org/10.1016/j.ejsobi.2007.08.001

Kieloaho, A.J., Pihlatie, M., Dominguez-Carrasco, M., Kanerva, S., Parschintsev, J., Riekkola, M-L., Pumpanen, J., Heinonsalo, J., 2016. Stimulation of soil organic N pool: the effect of plant and soil organic matter degrading enzymes. Soil Biology and Biochemistry 96: 97-106. http://dx.doi.org/10.1016/j.soilbio.2016.01.013

Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biology and Biochemistry 34: 139-162. http://dx.doi.org/10.1016/S0038-0717(01)00158-4

Kraal, P., Nierop, K.G.J., Kaal, J., Tietema, A., 2009. Carbon respiration and nitrogen dynamics in Corsican pine litter amended with aluminium and tannins. Soil Biology and Biochemistry 41: 2318-2327. http://dx.doi.org/10.1016/j.soilbio.2009.08.017

Kraus, T.E.C., Dahlgren, R.A., Zasoski, R.J., 2003. Tannins in nutrient dynamics of forest ecosystems - a review. Plant and Soil 256: 41-66. http://dx.doi.org/10.1023/A:1026206511084

Kraus, T.E.C., Zasoski, R.J., Dahlgren, R.A., Horwath, W.R., Preston, C.M., 2004. Carbon and nitrogen dynamics in a forest soil amended with purified tannins from different

plant species. Soil Biol Biochem 36: 309-321. http://dx.doi.org/10.1016/j.soilbio.2003.10.006

Langenheim, J.H., 1994. Higher plant terpenoids: a phytocentric overview of their ecologcal roles. Journal of Chemical Ecology 20: 1223-1280. http://dx.doi.org/10.1007/BF02059809.

Laurent, P., Braekman, J-C., Daloze, D., Pasteels, J., 2003. Biosynthesis of defensive compounds from beetles and ants. European Journal of Organic Chemistry 2003: 2733–2743. http://dx.doi.org/10.1002/ejoc.200300008

Lindroos, A-J., Tamminen, P., Heikkinen , J., Ilvesniemi, H., 2016. Effect of clear-cutting and the amount of logging residue on chemical composition of percolation water in spruce stands on glaciofluvial sandy soils in Southern Finland. Boreal Environment Research 21: 134-148.

Lodhi, M.A.K., Killingbeck, K.T., 1980. Allelopathic inhibition of nitrification and nitrifying bacteria in a Ponderosa pine (*Pinus ponderosa* Dougl.) community. American Journal of Botany 67: 1423–1429.
http://www.jstor.org/stable/2442870

Ludley, K.E., Robinson, C.H., Jickells, S., Chamberlain, P.M., Whitaker, J., 2009. Potential for monoterpenes to affect ectomycorrhizal and saprotrophic fungal activity in coniferous forests is revealed by novel experimental system. Soil Biology and Biochemistry 41: 117–124.
http://dx.doi.org/10.1016/j.soilbio.2008.10.005

Lupi, C., Morin, H., Deslauriers, A., Rossi, S., Houle, D., 2013. Role of Soil Nitrogen for the Conifers of the Boreal Forest: A Critical Review. International Journal of Plant and Soil Science 2: 155-189.
http://dx.doi.org/10.9734/IJPSS/2013/4233

Martikainen, P.J., Palojärvi, A., 1990. Evaluation of the fumigation-extraction method for the determination of microbial C and N in a range of forest soils. Soil Biology and Biochemistry 22: 797-802.
http://dx.doi.org/10.1016/0038-0717(90)90159-W

Misra, G., Pavlostathis, S., Perdue, E.M., Araujo, R., 1996. Aerobic biodegradation of selected monoterpenes. Applied Microbiology and Biotechnology 45: 831-838.
http://dx.doi.org/10.1007/s002530050770

Munné-Bosch, S., Alegre, L., Schwarz, K., 2000. The formation of phenolic diterpenes in *Rosmarinus officinalis* L. under Mediterranean climate. European Food Research and Technology 210: 263-267.
http://dx.doi.org/10.1007/s002179900108

Näsholm, T., Högberg, P., Franklin, O., Metcalfe, D., Keel, S.G., Campbell, C., Hurry, V., Linderand, S., Högberg, M.N., 2013. Are ectomycorrhizal fungi alleviating or

aggravating nitrogen limitation of tree growth in boreal forests? New Phytologist 198: 214-221.
http://dx.doi.org/10.1111/nph.12139.

Näsholm, T., Kielland, K., Ganeteg, U., 2009. Uptake of organic nitrogen by plants. New Phytologist 182: 31-48
http://dx.doi.org/10.1111/j.1469-8137.2008.02751.x

Nierop, K.G.J., Preston, C.M., Verstraten, J.M., 2006. Linking the B ring hydroxylation pattern of condensed tannins to C, N and P mineralization. A case study using four tannins. Soil Biology and Biochemistry 38: 2794-2802. http://dx.doi.org/10.1016/j.soilbio.2006.04.049

Nierop, K.G.J., Verstraten, J.M., 2006. Fate of tannins in Corsican pine litter. Journal of Chemical Ecology 32: 2709–2719.
http://dx.doi.org/10.1007/s10886-006-9194-9

Northup, R.R., Dahlgren, R.A., McColl, J.G., 1998. Polyphenols as regulators of plant-litter-soil interactions in Northern California's pygmy forest: a positive feedback? Biogeochemistry 42: 189 – 220.
http://dx.doi.org/10.1023/A:1005991908504

Northup, R.R., Yu, Z., Dahlgren, R.A., Voigt, K.A., 1995. Polyphenol control of nitrogen release from pine litter. Nature 377: 227-229.
http://dx.doi.org/10.1038/377227a0

Obst, J.R., 1998. Special (secondary) metabolites from wood. In: Bruce, A., Palferyman, J.W. (eds.), Forest Products Biotechnology. Taylor and Francis, pp. 151-165.

Olsson, B. A., Staaf, H., Lundkvist, H., Bengtsson, J., Rosén, K., 1996. Carbon and nitrogen in coniferous forest soils after clear felling and harvest of different intensity. Forest Ecology and Management 82:19–32.
http://dx.doi.org/10.1016/0378-1127(95)03697-0

Ossipova, S., Ossipov, V., Haukioja, E., Loponen, J., Pihlaja K., 2001. Proanthocyanidins of mountain birch leaves: quantitation and properties. Phytochemical Analysis 12: 128-133. http://dx.doi.org/10.1002/pca.568

Paavolainen, L., Smolander, A., 1998. Nitrification and denitrification in soil from a clear-cut Norway spruce (*Picea abies*) stand. Soil Biology and Biochemistry 30: 775-781. http://dx.doi.org/10.1016/S0038-0717(97)00165-X

Peñuelas, J., Llusia, J., 2003. BVOCs: plant defense against climate warming? Trends in Plant Science 8: 105-109.
http://dx.doi.org/10.1016/S1360-1385(03)00008-6

Persson, T., Wiren, A., 1995. Nitrogen mineralization and potential nitrification at different depths in acid forest soils. Plant and Soil 168-169: 55-65. http://dx.doi.org/10.1007/BF00029313

Pietikäinen, J., Fritze, H., 1995. Clear-cutting and prescribed burning in coniferous forest: comparison of effects on soil fungal and total microbial biomass, respiration activity and nitrification. Soil Biology and Biochemistry 27: 101–109. http://dx.doi.org/10.1016/0038-0717(94)00125-K

Piirainen, S., Finér, L., Starr, M., 2015. Changes in forest floor and mineral soil carbon and nitrogen stocks in a boreal forest after clear-cutting and mechanical site preparation. European Journal of Soil Science 66: 735-743. http://dx.doi.org/10.1111/ejss.12264

Pohjola, J., 1993. A Headspace Gas Chromatographic Study on the Variation of Needle Volatile Terpenes in Scots Pine (*Pinus sylvestris* L.) (Academic dissertation). Department of Pharmacy, University of Helsinki, Finland, ISBN 952-90- 4754-1, p. 172.

Poikolainen, J., Piispanen J., Karhu J., Kubin E., 2009. Long-term changes in nitrogen deposition in Finland (1990–2006) monitored using the moss Hylocomium splendens. Environmental Pollution 157: 3091-3097. http://dx.doi.org/10.1016/j.envpol.2009.05.018

Popova, M., Trusheva, B., Gyosheva, M., Tsvetkova, I., Bankova, V., 2009. Antibacterial triterpenes from the threatened wood-decay fungus *Fomitopsis rosea*. Fitoterapia 80: 263-266. http://dx.doi.org/10.1016/j.fitote.2009.02.008

Prescott, C., Maynard, D.G., Laiho, R., 2000. Humus in northern forests: friend or foe? Forest Ecology and Management 133: 23-36. http://dx.doi.org/10.1016/S0378-1127(99)00295-9

Preston, C., Bhatti, J., Flanagan, L., Norris, C., 2006. Stocks, chemistry and sensitivity to climate change of dead organic matter along the Canadian Boreal Forest Transect Case Study. Climate Change 74: 223–251. http://dx.doi.org/10.1007/s10584-006-0466-8

Qualls, R.G., Haines, B.L., Swank , W.T., Tyler, S.W., 2000. Soluble organic and inorganic nutrient fluxes in clear-cut and mature deciduous forests. Soil Science Society of America Journal 64: 1068-1077. http://dx.doi.org/10.2136/sssaj2000.6431068x

Rawat; M.S.M., Pant, G., Prasad, D., Joshi, R.K., Pande, C.B., 1998. Plant growth inhibitors (Proanthocyanidins) from *Prunus armeniaca*. Biochemical Systematics and Ecology 26: 13-23. http://dx.doi.org/10.1016/S0305-1978(97)00080-X

Robertson, S.M.C., Hornung, M., Kennedy, V.H., 2000. Water chemistry of throughfall and soil water under four tree species at Gisburn, northwest England, before and after felling. Forest Ecology and Management 129: 101-117. http://dx.doi.org/10.1016/S0378-1127(99)00156-5

Rosen, K., Lundmark-Thelin, A., 1987. Increased nitrogen leaching under piles of slash – a consequence of modern harvest techniques. Scandinavian Journal of Forest Research 2: 21-29. http://dx.doi.org/10.1080/02827588709382443

Schimel, J.P., Bennet, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85: 591-602. http://dx.doi.org/10.1890/03-8002

Schimel, J.P., Cates, R.G., Ruess, R., 1998. The role of balsam poplar secondary chemicals in controlling soil nutrient dynamics through succession in the Alaskan taiga. Biogeochemistry 42: 221-234. http://dx.doi.org/10.1023/A:1005911118982

Schimel, J.P., Van Cleve, K.V., Cates, R.G., Thomas, T.P., Reichardt, P.B., 1996. Effects of balsam poplar (*Populus balsamifera*) tannins and low molecular weight phenolics on microbial activity in taiga floodplain soil: implications for changes in N cycling during succession. Canadian Journal of Botany 74: 84-90. http://dx.doi.org/10.1139/b96-012

Schmidt, M.W.I, Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kogel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478: 49-56. http://dx.doi.org/10.1038/nature10386

Smania, E.F.A., Monache, F.D., Smania, A., Yunes, R.A., Cuneo, R.S., 2003. Antifungal activity of sterols and triterpenes isolated from *Ganoderma annulare*. Fitoterapia 74: 375-377. http://dx.doi.org/10.1016/S0367-326X(03)00064-9

Smolander, A., Kanerva, S., Adamczyk, B., Kitunen, V., 2012. Nitrogen transformations in boreal forest soils – does composition of plant secondary compounds give any explanations? Plant and Soil 350: 1-26. http://dx.doi.org/10.1007/s11104-011-0895-7

Smolander, A., Ketola, R., Kotiaho, T., Kanerva, S., Suominen, K., Kitunen, V., 2006. Volatile monoterpenes in soil micro air under birch and conifers: effects on soil N transformations. Soil Biology and Biochemistry 38: 3436-3442. http://dx.doi.org/10.1016/j.soilbio.2006.05.019

Smolander, A., Kitunen, V., 2002. Soil microbial activities and characteristics of dissolved organic C and N in relation to tree species. Soil Biology and Biochemistry 34: 651-660. http://dx.doi.org/10.1016/S0038-0717(01)00227-9

Smolander, A., Kitunen, V., Kukkola, M., Tamminen, P., 2013. Response of soil organic layer characteristics to logging residues in three Scots pine thinning stands. Soil Biology and Biochemistry 66: 51-59. http://dx.doi.org/10.1016/j.soilbio.2013.06.017

Smolander, A., Kitunen, V., Tamminen, P., Kukkola, M., 2010. Harvest of logging residue in Norway spruce thinning stands: Long-term changes in organic layer properties. Soil Biology and Biochemistry 42: 1222-1228. http://dx.doi.org/10.1016/j.soilbio.2010.04.015

Smolander, A., Levula, T., Kitunen, V., 2008. Response of litter decomposition and soil C and N transformations in a Norway spruce thinning stand to harvest of logging residue. Forest Ecology and Management 256: 1080-1086. http://dx.doi.org/10.1016/j.foreco.2008.06.008

Smolander, A., Loponen, J., Suominen, K., Kitunen, V., 2005. Organic matter characteristics and C and N transformations in the humus layer under two tree species, *Betula pendula* and *Picea abies*. Soil Biology and Biochemistry 37: 1309-1318. http://dx.doi.org/10.1016/j.soilbio.2004.12.002

Smolander, A., Paavolainen, L., Malkonen, E., 2000. C and N transformations in forest soil after mounding for regeneration. Forest Ecology and Management 134: 17-28. http://dx.doi.org/10.1016/S0378-1127(99)00242-X

Smolander, A., Saarsalmi, A., Tamminen, P., 2015. Response of soil nutrient content, organic matter characteristics and growth of pine and spruce seedlings to logging residues. Forest Ecology and Management 357: 117-125. http://dx.doi.org/10.1016/j.foreco.2015.07.019

Stahl, P.D., Parkin, T.B., 1996. Microbial production of volatile organic compounds in soil microcosms. Soil Science Society of America Journal 60: 821–828. http://dx.doi.org/10.2136/sssaj1996.03615995006000030020x

Stark, S., Hilli, S. Willför, S., Smeds A. I., Reunanen, M., Penttinen M., Hautajärvi R., 2012. Composition of lipophilic compounds and carbohydrates in the accumulated plant litter and soil organic matter in boreal forests. European Journal of Soil Science 63: 65-74. http://dx.doi.org/10.1111/j.1365-2389.2011.01411.x

Steinbrecher, R., Ziegler, H., 1997. Monoterpene production by plants. In: Rennenberg H., Eschrich W., Ziegler H., (eds) Trees- contribution to modern tree physiology. Backhuys Publishers, Leiden.

Strömvall, A-N, Petersson G. 1991. Conifer monoterpenes emitted to air by logging operations. Scandinavian Journal of Forest Research 6: 253-258. http://dx.doi.org/10.1080/02827589109382666

Stursova, M., Sinsabaugh, R.L., 2008. Stabilization of oxidative enzymes in desert soil may limit organic matter accumulation. Soil Biology and Biochemistry 40: 550–553. http://dx.doi.org/10.1016/j.soilbio.2007.09.002

Tamminen, P., Saarsalmi, A., Smolander, A., Kukkola, M., Helmisaari, H.-S., 2012. Effects of logging residue harvest in thinnings on amounts of soil carbon and nutrients in Scots pine and Norway spruce stands. Forest Ecology and Management 263: 31-38. http://dx.doi.org/10.1016/j.foreco.2011.09.015

Tarvainen, V., Hakola, H., Hellén, H., Bäck, J., Hari, P., Kulmala, M., 2005. Temperature and light dependence of the VOC emissions of Scots pine. Atmospheric Chemistry and Physics 5: 989-998.
http://dx.doi.org/10.5194/acpd-4-6691-2004

Terrill, T.H., Rowan, A.M., Douglas, G.B., Barry, T.N., 1992. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. Journal of the Science of Food and Agriculture 58: 321–329. http://dx.doi.org/10.1002/jsfa.2740580306

Tharayil, N., Suseela, V., Triebwasser, D.J., Preston, C.M., Gerard, P.D., Jeffrey S Dukes, J.S., 2011. Changes in the structural composition and reactivity of *Acer rubrum* leaf litter tannins exposed to warming and altered precipitation: climatic stress-induced tannins are more reactive. New Phytologist 191: 132-145.
http://dx.doi.org/10.1111/j.1469-8137.2011.03667.x

Thiffault, E., Hannam, K.D., Paré, D., Titus, B.D., Hazlett, P.W., Maynard, D.G., Brais, S., 2011. Effects of forest biomass harvesting on soil productivity in boreal and temperate forests – A review. Environmental Reviews 19: 278-309.
http://dx.doi.org/10.1139/a11-009

Thimmappa, R., Geisler, K., Louveau, T., O'Maille, P., Osbourn, A., 2014. Triterpene Biosynthesis in Plants. The Annual Review of Plant Biology 5: 225-57. http://dx.doi.org/10.1146/annurev-arplant-050312-120229

Triebwasser, D., Tharayil, N., Preston, C., Gerard, P.D., 2012. The susceptibility of soil enzymes to inhibition by leaf litter tannins is dependent on the tannin chemistry, enzyme class and vegetation. New Phytologist 196: 1122-1132.
http://dx.doi.org/10.1111/j.1469-8137.2012.04346.x

Uribe, S., Ramirez, J., Pena, A., 1985. Effects of β-pinene on yeast membrane function. Journal of Bacteriology 161: 1195–1200.

Uusitalo, M., Kitunen, V., Smolander, A., 2008. Response of C and N transformations in birch soil to coniferous resin volatiles. Soil Biology and Biochemistry 40: 2643-2649. http://dx.doi.org/10.1016/j.soilbio.2008.07.009

Väisänen, M., Martz, F., Kaarlejärvi, E., Julkunen-Tiitto, R., Stark, S, 2013. Phenolic responses of mountain crowberry (*Empetrum nigrum* ssp. *hermaphroditum*) to global

climate change are compound specific and depend on grazing by reindeer (*Rangifer tarandus*). Journal of Chemical Ecology 39: 1390-9.
http://dx.doi.org/10.1007/s10886-013-0367-z

Wall, A., 2008. Effect of removal of logging residue on nutrient leaching and nutrient pools in the soil after clear-cutting in a Norway spruce stand. Forest Ecology and Management 256: 1372-1383.
http://dx.doi.org/10.1016/j.foreco.2008.06.044

Ward, B.B., Courtney, K.J., Langenheim, J.H., 1997. Inhibition of Nitromonas europaea by monoterpenes from coastal redwood (*Sequoia sempervirens*) in whole-cell studies. Journal of Chemical Ecology 23: 2583-2598.
http://dx.doi.org/10.1023/B:JOEC.0000006668.48855.b7

Waterman, P.G., Mole, S., 1994. Analysis of phenolic plant metabolites. In: Lawton, J.H., Likens, G.E. (Eds.), Methods in Ecology. Blackwell Scientific Publications, Oxford, pp. 92-95.

White, C.S., 1986. Volatile and water-soluble inhibitors of nitrogen mineralisation and nitrification in a ponderosa pine ecosystem. Biology and Fertility of Soils 2: 97-104.
http://dx.doi.org/10.1007/BF00257586

White, C.S., 1991. The role of monoterpenes in soil nitrogen cycling processes in ponderosa pine. Results from laboratory bioassays and field studies. Biogeochemistry 12: 43-68.
http://dx.doi.org/10.1007/BF00002625

White, C.S., 1994. Monoterpenes: their effects on ecosystem nutrient cycling. Journal of Chemical Ecology 20: 1381-1406.
http://dx.doi.org/10.1007/BF02059813