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Plant secondary compounds and soil microbial processes in carbon and nitrogen cycling in relation to tree species

Sanna Kanerva

Department of Applied Chemistry and Microbiology
Faculty of Agriculture and Forestry
University of Helsinki

Academic dissertation

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Author: Sanna Kanerva

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Supervisor:
Docent Aino Smolander, Finnish Forest Research Institute, Vantaa, Finland

Pre-Examiners:
Professor Pertti Martikainen, University of Kuopio, Finland
Professor Tryggve Persson, Swedish University of Agricultural Sciences, Sweden

Opponent:
Docent Laura Höijer, Maj and Tor Nessling Foundation, Finland

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ABSTRACT

The aim of this study was to explore soil microbial activities related to C and N cycling and the occurrence and concentrations of two important groups of plant secondary compounds, terpenes and phenolic compounds, under silver birch (*Betula pendula* Roth), Norway spruce (*Picea abies* (L.) Karst) and Scots pine (*Pinus sylvestris* L.) as well as to study the effects of volatile monoterpenes and tannins on soil microbial activities. The study site, located in Kivalo, northern Finland, included ca. 70-year-old adjacent stands dominated by silver birch, Norway spruce and Scots pine. Originally the soil was very probably similar in all three stands. All forest floor layers (litter (L), fermentation layer (F) and humified layer (H)) under birch and spruce showed higher rates of CO_2 production, greater net mineralisation of nitrogen and higher amounts of carbon and nitrogen in microbial biomass than did the forest floor layers under pine. Concentrations of mono-, sesqui-, di- and triterpenes were higher under both conifers than under birch, while the concentration of total water-soluble phenolic compounds as well as the concentration of condensed tannins tended to be higher or at least as high under spruce as under birch or pine. In general, differences between tree species in soil microbial activities and in concentrations of secondary compounds were smaller in the H layer than in the upper layers. The rate of CO_2 production and the amount of carbon in the microbial biomass correlated highly positively with the concentration of total water-soluble phenolic compounds and positively with the concentration of condensed tannins.

Exposure of soil to volatile monoterpenes and tannins extracted and fractionated from spruce and pine needles affected carbon and nitrogen transformations in soil, but the effects were dependent on the compound and its molecular structure. Monoterpenes decreased net mineralisation of nitrogen and probably had a toxic effect on part of the microbial population in soil, while another part of the microbes seemed to be able to use monoterpenes as a carbon source. With tannins, low-molecular-weight compounds (also compounds other than tannins) increased soil CO_2 production and nitrogen immobilisation by soil microbes while the higher-molecular-weight condensed tannins had inhibitory effects. In conclusion, plant secondary compounds may have a great potential in regulation of C and N transformations in forest soils, but the real magnitude of their significance in soil processes is impossible to estimate.

Keywords: C and N cycling, Norway spruce, phenolic compounds, Scots pine, silver birch, soil microbial processes, terpenes

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THE AUTHORS' CONTRIBUTION

Sanna Kanerva performed most of the experimental work, calculated and interpreted the results and was the main author in papers I, II, IV and V. In paper III she participated in field measurements and manuscript preparation.

CONTENTS

1 INTRODUCTION

1.1 Tree species affect soil properties

1.1.1 Relations between tree species and soil properties

In several studies, soil chemical and microbiological characteristics have been shown to be affected by the dominant tree species (e.g. Miles and Young 1980, Mikola 1985, Bauhus et al. 1998, Priha and Smolander 1999, Côté et al. 2000, Priha et al. 2001, Grayston and Prescott 2005). There are studies in which the soil properties under certain tree species have been compared with the properties of treeless soil, for example, those studies in which agricultural soil is compared with afforested former agricultural soil (e.g. Ritter et al. 2003) or where the effects on heathland (dominated by *Calluna vulgaris*) following colonization by trees (birch) is examined (Miles and Young 1980), as well as studies where the effects of different tree species on soil properties have been compared (Mikola 1985, Priha and Smolander 1997, Bauhus et al. 1998, Smolander and Kitunen 2002).

Among the most widely studied tree species in Europe and North America are for example birch (*Betula* sp.), spruce (*Picea* sp.) pine (*Pinus* sp.) Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), oak (*Quercus* sp.) and aspen (*Populus* sp.) (e.g. Gardiner 1968, Miles and Young 1980, Mikola 1985, Priha and Smolander 1997, Bauhus et al. 1998, Côté et al. 2000, Thomas and Prescott 2000, Verchot et al. 2001, Smolander and Kitunen 2002, Ritter et al. 2003, Templer et al. 2003, Grayston and Prescott 2005, Lejon et al. 2005, Prescott and Vesterdal 2005, Templer 2005). Several other tree species, such as cedar (*Thuja* sp.), hemlock (*Tsuga* sp.), beech (*Fagus* sp.), maple (*Acer* sp.) and balsam fir (*Abies balsamea* (L.) Mill.) have also been studied in relation to soil properties (e.g. Bauhus et al. 1998, Côté et al. 2000, Verchot et al. 2001, Templer et al. 2003, Grayston and Prescott 2005, Prescott and Vesterdal 2005, Templer 2005). In these studies, the effects of tree species have been reported, for example, on soil pH, carbon (C) and nitrogen (N) transformations, C and N in the microbial biomass (C_{mic} and N_{mic}, respectively) and their proportion of the soil organic C (C_{org}) and total N (N_{tot}), respectively, dissolved organic carbon (DOC) in soil, characteristics of the soil organic matter, soil nutrient concentrations, base saturation, amount of exchangeable calcium, and structure of the soil microbial community.

Birch (*Betula* sp.) has a reputation in forestry history as a soil-improving species, especially compared to spruce. Norway spruce (*Picea abies* (L.) Karst) has been found to change soil fertility gradually in an unfavorable direction by lowering the soil pH, decomposition rates and concentration of exchangeable nutrients, by increasing soil C-to-N ratio and by enhancing podsolisation (Nihlgård 1971, Mikola 1985, Binkley and Valentine 1991, Ranger and Nys 1994, Priha and Smolander 2000, Menyailo et al. 2002a).

The improving effect of birch on soil fertility was mentioned already in the forestry literature of the nineteenth century, although in the twentieth century the references to this effect became more numerous (Gardiner 1968). Birch, especially silver birch (*B. pendula* Roth) and white birch (*B. pubescens* Ehrh.) but also paper birch (*B. papyrifera* Marsh.), has been reported to favor conversion of mor humus to mull, to increase pH values, concentration of nutrients, base saturation, C mineralisation, content of organic N and net N mineralisation, and to decrease the soil C-to-N ratio in soil. The proportions of microbial biomass C and N from soil organic C and total N, respectively, have been found to be higher in the forest floor under birch than under the coniferous species studied (Gardiner 1968, Miles and Young 1980, Miles 1981, Mikola 1985, Bauhus et al. 1998, Brandtberg et al. 2000, Côté et al. 2000).

Decomposition of cellulose has been found to be more active under silver and white birch than under Norway spruce (Mikola 1985) or heather (Miles and Young 1980, Miles 1981) at sites that originally were similar, and birch soil has been shown to favour the presence of earthworms (Miles and Young 1980, Miles 1981, Saetre 1998). When soil chemistry and microbial activities in soils under silver birch, Norway spruce and Scots pine (*Pinus sylvestris* L.) were compared, soil pH, C_{mic} and N_{mic}, mineralisation rate of C and rate of net N mineralisation, denitrification potential and denitrification enzyme activity tended to be higher, or at least in the same order of magnitude, in birch soil than in soils under Norway spruce and Scots pine (Priha 1999, Priha and Smolander 1999, Priha et al. 2001, Smolander and Kitunen 2002, Smolander et al. 2005).

Despite the abovementioned facts, the improving effect of birch on soil characteristics compared to conifers is not self-evident and depends on the coniferous species involved in the comparison (Menyailo et al. 2002b). Some soil properties, such as pH, are found to be increased by birch in most of the studies where birch is compared to conifers, while the effects of birch on certain other properties, such as mineralisation of C and N, seem to vary more. Study site, its fertility and the age of the stand seem to affect the differences observed between birch and conifers (Mikola 1985, Bauhus et al. 1998, Priha and Smolander 1999, Priha et al. 2001). For example, in the study of Priha and Smolander (1997) on an afforested former field and in the study of Menyailo et al. (2002a) on the site of an artificial afforestation experiment, ca. 20-30 years did not seem long enough to cause significant vertical differentiation between tree species, although Priha and Smolander (1997) observed clear differences between silver birch, Norway spruce and Scots pine in terms of ground vegetation and the microbiological characteristics of the litter samples. On the other hand, in 60 to 70-year old mature stands of silver birch, Norway spruce and Scots pine growing on forest soil that originally was similar, the transformations in soil C and N differed in many respects (Mikola 1985, Priha and Smolander 1999, Priha et al. 2001, Smolander and Kitunen 2002).

Miles (1981) suggested that the improving effects of birch are most rapid on nutrient-rich sites, but there are also studies in which the results indicated the opposite. Priha and Smolander (1999) and Priha et al. (2001) found that differences between tree species were seen both in the humus layer and in the mineral soil at the more fertile sites, while at the less fertile sites the differences were obvious only in the humus layer. Mikola (1985) reported that in peat soil the effect of birch on soil C-to-N ratio as well as on pH developed in only 10 years. On the other hand, according to Mikola (1985), the difference in soil pH between equally aged birch and spruce stands was more obvious in a former clearcut spruce stand growing on relatively poor sandy soil than in stands located on a finer textured, former agricultural field. In addition, Priha and Smolander (1997) found no differences in soil pH, C-to-N ratio, microbial biomass C and N or C and N mineralisation rates between birch, spruce and pine growing 23-24 years on a fertile former agricultural field.

The reputation of birch as a soil-improving species has also been criticised. Based on data from the literature, in order to determine whether birch has soil-improving effects, Miller (1984) developed models of the rates of above-ground nutrient cycling but found that nutrient cycling in birchwoods is comparable to that in forests of other species with similar rates and patterns of growth. Binkley (1995), on the other hand, questioned whether tree species affects soil properties in general and expected so-called common-garden experiments to lead to better understanding of the effects of different tree species on soil properties. In common-garden experiments, tree species are planted adjacent to each other in soil that originally is similar, and there are enough replications. Currently many conclusions about the effects of tree species on soil properties are derived from unreplicated experiments for which there

is a strong suspicion that the parent material under the different tree species is dissimilar, since properly replicated experiments that are old enough are not available (Binkley 1995). Moreover, according to Binkley (1995), trees should not be classified as 'degrading' or 'improving' species since there is no evidence that any species pushes all soil variables in unfavourable directions. In addition, in the few common-garden experiments no relationship was found between forest floor characteristics and tree growth or availability of nitrogen or phosphorus (Binkley 1995).

1.1.2 Why does birch differ from conifers?

As reviewed by Priha and Smolander (2000), explanations given for differences in the soils under birch and conifers have included microclimatic conditions, differences in ground vegetation cover, number of roots and amount and quality of root exudation as well as chemical composition of the litter.

Climatic factors are usually more favourable in birch than in coniferous stands. In birch stands, due to the smaller shading effect of the birch canopy, temperature is higher and there is more light than in spruce stands. Frost in wintertime is stronger under spruce than under birch because of a thinner snow cover under the coniferous species (Priha and Smolander 2000).

Differences in thermal and light conditions under birch and conifers result in different cover and species of ground vegetation, which, in turn, contributes to litter production and thus to changes in soil properties. Mosses are more abundant under spruce, while herbs and grasses may dominate under birch and contribute to the amount and quality of plant litter under birch (Mikola 1985). Moss litter has lower pH and decomposes more slowly than the dead parts of most herbs and grasses (Mikola 1954).

The composition and amount of birch and coniferous aboveground litters differ between different stands and different years (Mikola 1985, Johansson 1995). Viro (1955) found that the annual litter fall for birch is lower than that for pine and spruce, while Mikola (1985) reported somewhat higher values in birch stands than in spruce stands. This indicates the importance of study site, age of the stand, stem density in the stand and sampling year for the differences observed between birch and conifers. Birch leaf litter has higher concentrations of nutrients, especially N, K and Mg and in some cases also P, more water-soluble substances and simple carbohydrates than do the needle litters of spruce and pine (Viro 1955, Nykvist 1963, Berg and Wessén 1984, Mikola 1985, Johansson 1995). In addition, the pH of birch leaves is, on average, higher than that of spruce or pine needles (Mikola 1985). Pine needle litter, on the other hand, contains twice as much of ethanol-soluble compounds as spruce and birch (Berg and Wessén 1984, Johansson 1995). Spruce needle litter contain more lignin than birch and pine needle litters. In addition, the structure of lignin may also differ between tree species (Crawford and Crawford 1978, Berg 1986). Concentrations of cellulose in needle litters are similar but are much lower in birch leaf litter (Berg and Wessén 1984, Johansson 1995).

Miller (1984) proposed that the beneficial effect of birch on soil properties may occur underground since the chemical attributes of birch leaf litter were comparable to those of deciduous species that do not have the same reputation for soil improvement. In seedlings (Priha et al. 1998) and mature trees (Ostonen et al. 2007) the number of roots and root tips, as well as the specific root area and specific root length, have been found to be higher in silver birch than in Scots pine or in Norway spruce. However, not only the length or mass of roots determines the changes in microbial activities; differences in root activities per unit of root or differences in the quality of root exudates are also important (Priha. et al. 1998). Priha et al. (1998) reported that microbial biomass and activity in soil were stimulated by the roots

of silver birch and Scots pine seedlings, while the seedlings of Norway spruce had no effect. In the study of Bradley and Fyles (1995), soils in which paper birch seedlings had grown showed higher rates of CO_2 production, more available C in the soil and higher rates of net N mineralisation than did soils in which five other tree species had grown. This suggested that large amounts of labile C compounds from the roots in conjunction with rapid mineral-N uptake by birch roots can stimulate microbial communities to acquire nutrients from the soil. Roots and microbes can also compete with each other for nutrients and water (Parmelee et al. 1993). Priha and Smolander (2003) showed, using ^{15}N, that Scots pine and Norway spruce seedlings were more efficient competitors for added N than rhizosphere microbes were, while the opposite was true for silver birch seedlings. They concluded that this was due to more available C sources (root exudates) in the rhizosphere of birch, which increased competition between birch and microbes for N.

Rate of litter decomposition is determined by the quality of litter in terms of the abundance of its different components and its physical structure, as well as by environmental conditions such as water, temperature, oxygen and nutrient availability, soil texture and chemistry (Berg 1986, Fioretto et al. 2005, Mukhortova 2005). Concentrations of lignin and N are particularly important when decomposition rates of different litters are compared. There is evidence that some broadleaf litters such as birch (*B. pubescens* or *B. papyrifera* Marsh.), trembling aspen (*P. tremuloides* Michx.) or red alder (*Alnus rubra* Bong.) litters decompose faster than needle litter, but only during the first year of decomposition; after that, mass loss of broadleaf litter is slower than that of needle litter so that the differences between the two types of litter decline over time (Berg and Wessén 1984, Prescott et al. 2000, 2004). The high mass loss of birch leaves at the beginning of decomposition is due to release of water-soluble organic compounds (Nykvist 1963, Berg and Wessén 1984), while wax compounds on the surface of needle litter effectively prevent the release of water-soluble compounds. Decomposition of root litter may also have a marked effect on differences between soils under birch, spruce and pine since roots make up a significant portion of the forest biomass and often turn over more rapidly than foliage (Vogt 1986). However, not much is known about the decomposition of birch, spruce and pine root litter. In conclusion, more information is needed to better understand nutrient cycling and the dynamics of soil organic matter under these tree species.

1.2 Plant secondary compounds

1.2.1 Definition and occurrence

Plant secondary compounds, also called as plant secondary metabolites, are a very broad and diverse group of chemical compounds. The main classes of secondary compounds are terpenes, phenolic compounds and alkaloids. The definition of plant secondary compounds is not simple; plant primary metabolites are substances that are fundamental for plant cells, such as nucleic acids and proteins. Therefore, plant secondary compounds are ‘everything else’ that a plant produces – although some of the ‘secondary’ compounds are also vital to the very existence of the plant (Obst 1998). For example, part of the phenolic compounds are considered to be secondary compounds, but an important phenol, lignin, which is the most abundant organic structure on Earth after cellulose (Strack 1997), is a plant primary metabolite. Secondary compounds are usually involved in physiological plant mechanisms such as signaling and interaction with the surrounding environment and defense against biotic and abiotic factors.

Secondary compounds may make up from 1% to one-third of the dry weight of wood (Obst 1998). The concentration of these compounds in various parts of plants differs. In general, larger amounts occur in the bark, heartwood, roots, branch bases and wound tissues. The composition of plant secondary compounds is species-specific in spite of high variation within plant species, and the concentrations vary among species, but also from tree to tree and from season to season (Obst 1998).

Due to the enormous number of different compounds belonging to the plant secondary compounds, there are overlaps and confusions in their definitions and nomenclature. The huge number of different structures in this group of compounds makes their analysis demanding, and there is not always consensus among researchers as to which method is most appropriate for the compounds of interest. Only a few secondary compounds are available commercially, which makes studying them and their effects even more difficult. Two large and multifunctional groups of plant secondary compounds, terpenes and phenolic compounds, are introduced in the following sections.

1.2.2 Terpenes

Terpenes represent the largest group of plant secondary compounds: thousands of terpenes have been isolated, purified and their structure has been elucidated. They are found throughout nature and occur in almost all plants (Obst 1998). Terpenes are hydrocarbons derived from isoprene (isopentane) C_5 units (Fig. 1a); monoterpenes are compounds of two isoprene-derived units (C_{10}), sesquiterpenes contain three isoprene units (C_{15}), diterpenes four (C_{20}), sesterterpenes five (C_{25}), triterpenes six (C_{30}) and tetraterpenes eight isoprene units (C_{40}). Terpenes > C_{40} are called polyterpenes. Some terpenes, such as mono- and sesquiterpenes, can be volatile, whereas the others generally are not (Langenheim 1994). A few examples of terpenes are α- and β-pinene (mono-), different kinds of resin acids (di-), sterols (tri-), carotenoids (tetra-) and rubber (polymeric isoprene) (Obst 1998).

In plant cells terpenes are formed in the cytosol, in plastids and in mitochondria (Bramley 1997) but are then stored in specialized secretory structures, which protect the plant's metabolic processes from their toxic effects (Langenheim 1994). Isopentenyl pyrophosphate (IPP) (Fig. 1b), the essential precursor needed in terpene biosynthesis, can be formed through the mevalonate pathway, also known as the HMG-CoA (hydroxymethylglutaryl-CoA) reductase pathway (Bramley 1997), or through the MEP (2-Methyl-D-erythritol-4-phosphate) pathway, which is initiated from C5-sugars (Rohmer et al. 1996, Duvold et al. 1997, Rohmer 1999). The MEP pathway was found recently and is not as well-known as the mevalonate pathway. Different terpenes are formed from IPP and its isomer dimethylallyl pyrophosphate (DMAPP) (Fig. 1c) through the chain-lengthening reactions (Fig. 2). DMAPP and IPP produce geranyl pyrophosphate (GPP), which can be used for biosynthesis of monoterpenes or can produce farnesyl pyrophosphate (FPP) with the IPP molecule. FPP can be used for production of sesquiterpenes, production of geranylgeranyl pyrophosphate (GGPP) through a chain-lengthening reaction or by synthesis of triterpenes. GGPP is needed for production of diterpenes; it can be converted to geranylfarnesyl pyrophosphate (GFPP) through a chain-lengthening reaction or to tetraterpenes through dimerization, and it is also the precursor for polyterpenes. GFPP is needed for production of sesterterpenes (Bramley 1997).

Terpenes have many functions in plants; for example, they act as plant hormones, plant growth regulators, defence mechanisms against herbivores and pathogens, and as compounds that influence (directly or indirectly) the growth and development of neighbouring plants and

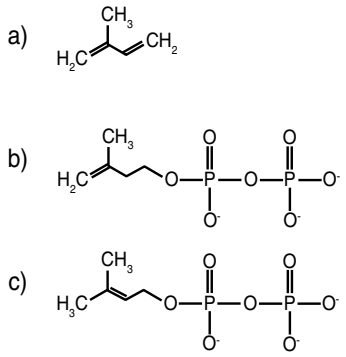

Fig. 1. Molecular structure of (a) isoprene, (b) isopentenyl pyrophosphate (IPP), and (c) dimethylallyl pyrophosphate (DMAPP). Terpenes are built up of various numbers of isoprene units through its activated forms, IPP and DMAPP.

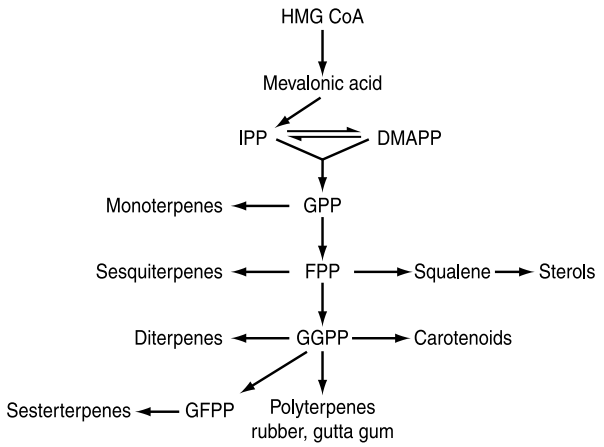

Fig. 2. General pathway for biosynthesis of terpenes (Modified from Bramley 1997). Abbreviations are given in the text.

micro-organisms, i.e. allelochemicals. Essential oils, latexes, and resinous exudates from plants are often composed mainly of terpenoids (Rice 1984, Dev 1989, Langenheim 1994, Bramley 1997, Obst 1998). Humans have used terpenes, for example, as perfumes, flavouring agents, waterproofing materials, insect repellents, fungicides, medicines and as raw materials for the synthesis of numerous products (Bramley 1997, Obst 1998).

According to the model calculations made by Lindfors and Laurila (2000), the average emissions of biogenic volatile organic compounds (VOCs) from the forests in Finland are 319 kilotonnes per annum and are dominated by monoterpenes, which contribute approximately 45% of the annual total. VOCs, especially monoterpenes, can form secondary organic aerosols that can scatter or absorb solar radiation, which modifies therefore the radiative balance of the atmosphere; it is currently taught that the net effect on climate is cooling, but quantitative estimates are highly uncertain (IPCC 2001, Kanakidou et al. 2004). In addition, volatile terpenes can be the source of some acid deposition, can interact with reactive gases to produce ozone, and can increase the atmospheric lifetime of methane (Langenheim 1994).

The concentration of terpenes in plant leaves is usually about 1–2% of the dry weight, although higher concentrations may occur in leaves and other plant organs (Langenheim

1994). The composition of terpenes is dependent on the plant species (Obst 1998); for example, mono-, sesqui- and diterpenes are typical for conifers, while birch contains higher terpenes (Dev 1989).

1.2.3 Phenolic compounds

Phenolic compounds are defined chemically by the presence of at least one aromatic ring bearing one (phenols) or more (polyphenols) hydroxyl substituents, including their functional derivatives. A few examples of phenolic secondary compounds in plants are flavonoids and tannins (Strack 1997); but as mentioned above, not all plant phenolic compounds, are considered to be secondary compounds (e.g. lignins). (Poly)phenols can be roughly divided into two groups: (1) low-molecular-weight compounds and (2) oligomers and polymers of relatively high molecular weight (Harborne 1997). Low-molecular-weight phenolic compounds occur universally in higher plants; some of them are common in various plant species while others are species specific. Lower molecular weight phenolic compounds, such as hydroquinones, are found, for example, in species of *Rosaceae* and *Ericaceae* (Strack 1994). Condensed tannins of higher molecular weight are the most abundant polyphenols in woody plants, but they are usually absent from herbaceous plants (reviewed by Hättenschwiler and Vitousek 2000). For example, *Acacia, Quercus, Betula, Salix* and *Pinus* are examples of tannin-containing trees (Obst 1998). Low nutrient content in soil, especially lack of nitrogen and phosphorus, has been found to increase the content of polyphenolic compounds in plants (Davies et al. 1964 and references therein). Other factors influencing the phenolic content of plants are intensity of light and ultraviolet (especially UV-B) radiation, temperature, pollutants, stress from soil dryness or high salinity, and chemical treatments (reviewed by Keskitalo 2001).

Phenolic compounds are formed in plant cells in the cytosol or in cellular membranes (Keskitalo 2001). The most important pathway in plants that produce phenolic compounds is the shikimate/arogenate pathway, which leads to formation of three aromatic amino acids. One of these, L-phenylalanine, is the precursor for the phenylalanine/hydroxycinnamate pathway, where most of the phenolic compounds are synthesized (Strack 1997) (Fig. 3). However, specific synthese routes for different compounds can be very complex and branched. In plants, phenolic compounds are located in cell vacuoles, cell walls, and epidermal cells on the plant surface; the location is dependent on the function of the compound (reviewed by Keskitalo 2001).

Phenolic compounds perform numerous functions in plants. For example, they play an important role in cell wall structures for mechanical support and barriers against microbial invasion; they are contributors to plant colors, may protect plants from damaging ultraviolet light, herbivores, insects and microbes, are signal molecules for nodulation in the legume-*Rhizobium* symbiosis, and may act as allelochemicals (Rice 1984, Haslam 1989, Harborne 1997, Strack 1997). Phenolic compounds are also of great importance to humans; plants rich in polyphenols have been used in leather tanning, phenolic compounds contribute to the taste of food and drink, and some of them are used as pharmaceuticals (Haslam 1989).

Tannins are commonly defined as water-soluble phenolic compounds ranging in molecular weight from 500 to 3000 Daltons that have the ability to precipitate proteins (Bate-Smith and Swain 1962). They are widely distributed, are common in both gymnosperms and angiosperms (Obst 1998) and are located in plant cell vacuoles (Strack 1997). Tannins can be separated into two classes based on their chemistry and origin. One class is hydrolysable tannins (HT), which is further divided into gallotannins and ellagitannins. They are made up of gallic acid or hexahydroxydiphenic acid esters, respectively, linked to a sugar moiety

(Figs. 4a and b). Another class is condensed tannins (CT), also called proanthocyanidins. Condensed tannins are polymers of three-ring flavanol monomer units joined by C-C bonds. The monomer units that make up CT can be further grouped according to the number of OH groups on the B-ring: the most common groups are procyanidins (PC) having a dihydroxy B ring, while prodelphinidins (PD) have a trihydroxy B-ring (Fig. 4c). Monomer units can also have different C-2 – C-3 stereochemistry (*cis* or *trans*). Linkages between monomers are typically C-4 -> C-8, although C-4 -> C-6 linkages can also be found (Fig. 4d). The stereochemistry and chain length of condensed tannins vary. The structural type of tannin is important when its reactivity is considered (Kraus et al. 2003a, Nierop et al. 2006a).

Hydrolysable tannins occur less frequently in plants than CTs do. Condensed tannins are found in the leaves of all ferns and gymnosperms and in about half the families (the woody members) of angiosperms, while hydrolysable tannins occur only in the dicotyledons, in some 15 of the 40 orders (Harborne 1997). While gymnosperms and monocots produce only CTs, dicots can produce either CTs or HTs or a mixture of the two (reviewed by Kraus et al. 2003b). In woody species, foliar concentrations of tannins commonly range from 15 to 25% dry weight (reviewed by Kraus et al. 2004a), but leaves and bark may contain up to 40% tannin by dry weight (Kraus et al. 2003a and references therein). Information about tannin concentrations in roots is more limited, but the tannin concentrations reported in roots range from 1 to 35% dry weight (reviewed by Kraus et al. 2003b). Concentration of condensed tannins can be lower in plant roots than in leaves (Kraus et al. 2004b), but there is also evidence that in fine roots (< 2mm) the concentration can be higher than in leaves of the same plant species (Gallet and Lebreton 1995), so roots can be an important contributor of tannins to the soil.

1.3 Plant secondary compounds in soil

Since production of secondary compounds varies not only by plant species, but also from season to season, inputs of secondary compounds in the ecosystem are expected to be variable and complex. Plant secondary compounds enter the soil mainly by two pathways: (1) as leachates from above- and belowground plant parts and (2) in above- and belowground plant litter. Concentrations in soils are not directly related to concentrations in trees and ground vegetation due to degradation and transformation of the compounds. Because of the huge number of different secondary compounds, their effects, biological as well as abiotic ones, may be very different in the soil environment, depending on the compound and its structure. Possibly some of the compounds can be valuable substrates for soil microbial population, but there does not exist much information on the availability or toxicity of specific compounds for soil microbes.

Analysis of secondary compounds from soil is more difficult than their analysis from plants due to the fact that some of secondary compounds may be tightly adsorbed on soil material, their concentrations in soil can be very low, and the soil matrix may cause problems in the analytical procedures. Some compounds that are not defined as secondary compounds in plants, such as lignin, may produce degradation products in soil that are categorized as secondary compounds. It should be noted that all secondary compounds found in soil are not derived from plants, since soil microbes may also produce some phenolic compounds (Swift et al. 1979) and terpenes (Stahl and Parkin 1996). In this study, the term ‘secondary compound’ is used for certain phenolic compounds and terpenes independently of their origin.

Traditionally it has been assumed that certain plant secondary compounds are produced for defense against plant pathogens as well as against insect and mammalian herbivores (e.g. Langenheim 1994, Harborne 1997), but nowadays there is increasing evidence to suggest

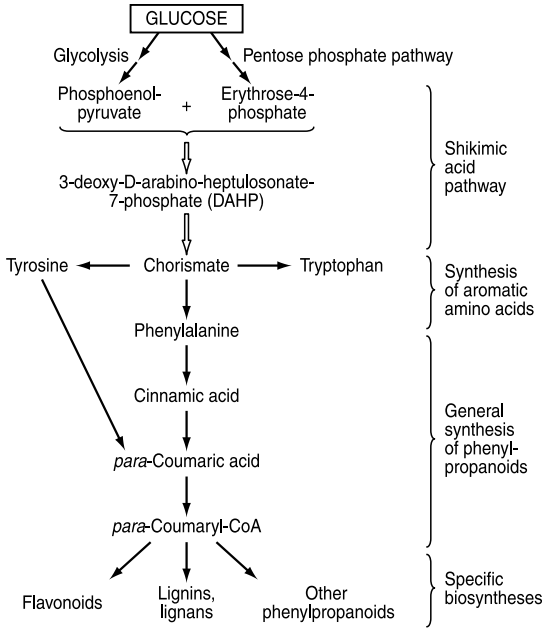

Fig. 3. Biosyntheses associated with the formation of phenolic compounds (Modified from Keskitalo 2001).

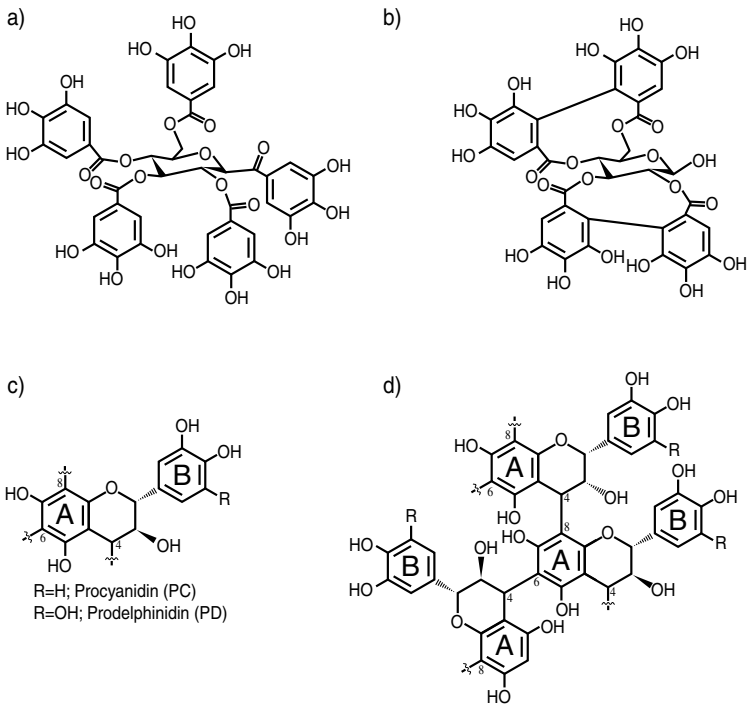

Fig. 4. Molecular structures of (a) a simple gallotannin, (b) a simple ellagitannin, (c) a basic unit of condensed tannin and (d) a condensed tannin trimer showing different intermolecular linkages (C-4 -> C-8 and C-4 -> C-6) (Modified from Kraus et al. 2003b).

that some secondary compounds in plants, like certain terpenes or phenolic compounds, play an important role also in plant-plant and plant-litter-soil interactions. These compounds may influence resource competition, nutrient dynamics, microbial ecology, mycorrhizae and even abiotic factors in soil (Wardle et al. 1998, Inderjit and Weiner 2001).

Certain monoterpenes have been suggested to inhibit germination or to regulate plant growth (Rice 1984). The impact of monoterpenes on soil microbes is complex since, while they may inhibit activity and growth of some microbial groups, they may stimulate others (Amaral and Knowles 1998). Monoterpenes have been found to inhibit net mineralisation of N (White 1986, 1991, 1994, Bremner and McCarty 1988) and net nitrification in soil (White 1986, 1991, 1994, Paavolainen et al. 1998). Inhibition of autotrophic nitrification is suggested to be a result of direct action of monoterpenes on the ammonia monooxygenase (AMO) enzyme (White 1988), the first enzyme in the ammonia oxidation pathway in autotrophic nitrifying bacteria. In a whole-cell pure culture experiment, Ward et al. (1997) reported that certain monoterpenes abundant in coastal redwood (*Sequoia sempervirens*) significantly inhibited growth of *Nitrosomonas europaea*, and that the degree of inhibition varied between monoterpenes. One of the monoterpenes studied, β-pinene, seemed to have stimulatory effects. These results indicate the importance of the molecular structure of a given monoterpene when its inhibitive effects are considered. Ward et al. (1997) expected that monoterpenes could exhibit two kinds of inhibitory effects: a specific inhibition of AMO by competitive or non-competitive inhibition at low concentrations, and general toxicity at high concentrations. Methane monooxygenase enzyme is in many ways similar to AMO. Amaral and Knowles (1998) showed that certain monoterpenes, especially (-)-α-pinene, effectively inhibited methane oxidation by the soil and, except for β-myrcene, inhibited methane oxidation by a metanotrophic (*Methylosinus trichosporium* OB3b) culture.

Certain phenolic compounds, especially tannins, have been shown to affect soil C and N transformations; complex proteins and possibly other N-containing compounds, metal ions and other macromolecules like polysaccharides, induce toxicity to microbes and inhibit enzyme activities in soil (Basaraba 1964, Baldwin et al. 1983, Schimel et al. 1996, Bradley et al. 2000, Fierer et al. 2001, reviewed by Schofield et al. 2001, Kraus et al. 2004a, Nierop et al. 2006b). Some phenolic acids have been found to inhibit nitrification in soil suspension (Rice and Pancholy 1973), although opposite conclusions have also been reached (McCarty and Bremner 1986, Bremner and McCarty 1996).

There is strong evidence that tannins play an important role in interspecies competition. In many studies the results have suggested that individual plants, due to the tannins they contain, may be important in nutrient cycling on the ecosystem level (Schimel et al. 1996, Bradley et al. 2000, Fierer et al. 2001, Kraus et al. 2004b, Nierop et al. 2006b). For example, Schimel et al. (1996) and Fierer et al. (2001) studied the effects of the foliage tannins of balsam poplar (*Populus balsamifera*) on soil under thin-leaf alder (*A. tenuifolia*) and Fierer et al. (2001) also studied their effects on soil under balsam poplar. Bradley et al. (2000) studied the effects of tannins purified from foliage of ericaceous shrub (*Kalmia angustifolia* L.) and balsam fir (*A. balsamea* (L.) Mill) on humus under black spruce (*P. mariana* (Mill.) B.S.P.); Kraus et al. (2004a) studied how purified tannins from foliage of Bishop pine (*P. muricata*), huckleberry (*Vaccinium ovatum*), manzanita (*Arctostaphylos nummularia*), rhododendron (*Rhododendron macrophyllum*) and salal (*Gaultheria shallon*) affected the A horizon of the soil under Bishop pine. Nierop et al. (2006b) studied the effects of condensed tannins purified from needles of Corsican pine (*P. nigra* var. *maritima*) on litter (F1 horizon) collected from a Corsican pine forest. In the abovementioned studies, net N mineralisation in soil decreased due to the addition of tannins, although the effects on C mineralisation were more variable.

It is widely thought that the most important effect of tannins on biogeochemical cycling is their ability to precipitate proteins. Northup et al. (1995, 1998) found a negative correlation between the phenolic content of *Pinus muricata* litter and release of mineral N. They suggested that tannins form strong complexes with proteins that are sparingly soluble and recalcitrant to decomposition and that high levels of polyphenols and tannins not only inhibit N mineralisation, but can also shift N cycling from mineral pathways to ones dominated by organic compounds. This efficiently monopolizes the N in litter into a form for which the plant's associated mycorrhizae have been shown to have a competitive acquisition advantage, and minimises nitrogen availability to competing organisms (Northup et al. 1995). On the other hand, there is evidence that ectomycorrhizal fungi are poor at breaking down phenolic-protein complexes compared to free-living saprophytic fungi or ericoid mycorrhizal fungi (Bending and Read 1996), and Wu et al. (2003) showed that pretreatment of protein-tannin complex by saprotrophs was necessary to make its N available to ectomycorrhizal fungi from red pine (*P. resinosa*). In addition, plants may compensate for the slow rates of nutrient cycling, associated with litter containing large amounts of tannins, by increasing the production of fine roots (Fischer et al. 2006).

Tannin reactivity in soil has been suggested to be dependent on structural characteristics such as condensed versus hydrolysable tannins and procyanidin versus prodelphinidin content of the condensed tannins (Kraus et al. 2004b, Nierop et al. 2006a, b). According to Hernes et al. (2001), PDs may be structurally less stable and thus more prone to chemical transformation by abiotic processes than PCs are. Nierop et al. (2006a) reported that PDs bind to or react more strongly with soil organic matter than PCs do. Different protein-binding capacity for the PC-type and the PD-type condensed tannins have been suggested to affect the total amount of extractable free condensed tannins in forest soils (Hernes et al. 2001, Maie et al. 2003). Molecular weight and degree of polymerisation of tannins or other phenolic compounds also seem to be important factors when their influence on soil nutrient cycling is considered. Schimel et al. (1996) and Fierer et al. (2001) demonstrated that high-molecular-weight phenolic compounds from balsam poplar acted as a general microbial inhibitor, while the effects of lower-molecular-weight phenolic compounds were less predictable and depended on prior exposure of the soil microbial community to related molecules; microbial communities previously exposed to smaller chain tannins were more likely to use them as a C substrate, while in the communities that had limited exposure to tannins they were more likely to prove toxic. However, it remained unclear whether the lower-molecular-weight compounds in particular also contained compounds other than phenolic ones, and therefore the effects observed may not have been caused solely by phenolic compounds.

Since the data concerning the effects of plant secondary compounds in soils have been obtained largely from artificial experiments, it remains uncertain whether the effects are similar in natural conditions. In addition, only a few compounds have been studied. For example, there is information on the effects of tannin addition on soil processes, but only tannins from a few plant species have been studied. With regard to terpenes, there are a few studies on the effects of monoterpenes but none on higher terpenes. It would be valuable to better understand the effects of secondary compounds from different plant species on soil N availability due to the fact that N usually is the nutrient restricting productivity of boreal forests. On the other hand, secondary compounds may also contribute to keeping nutrients in forest ecosystems via their effects on N mineralisation, immobilisation and nitrification processes. In the future, composition of tree species may change in forests for example due to the climate change; whether changes in the composition of plant secondary compounds in soil cause significant changes in soil N cycling is not known. To summarize, the role of plant

secondary compounds in soil processes is still unclear and needs further investigation due to the possibly great potential of the compounds to affect soil characteristics.

2 AIM OF THE STUDY

The aim of this study was to explore the effects of plant secondary compounds on soil microbial activities in C and N cycling under three different tree species; silver birch, Norway spruce and Scots pine. Since the effects of tree species are probably first seen in the litter layer - unless the effects of roots are more important than the effects of aboveground litter and leachates - plant secondary compounds and soil microbial activities were studied separately in litter (L), fermentation (F) and humified (H) layers of the forest floor, in a more detailed study than previous related studies. Studying the layers separately also provides information on how deep the effects of tree species can be seen. An additional aim was to explore the soil response to added tannins and volatile monoterpenes. The amounts and composition of phenolic compounds and terpenes, as well as microbial activities, were compared in the L, F and H layers in the forest floors under silver birch, Norway spruce and Scots pine (I, II); and occurrence and concentrations of volatile organic compounds, in particular, volatile monoterpenes, were compared in soil atmosphere under these tree species (III). The response of soil C and N transformations to certain volatile monoterpenes (III) and to tannins extracted from spruce and pine needles (IV, V) were studied in laboratory incubation experiments.

3 MATERIALS AND METHODS

The methods applied here are described in more detail in original papers I-V and in the references therein.

3.1 Study site

The stands used in this study were located in Kivalo, northern Finland (66°20'N/26°40'E), and were dominated by silver birch (*Betula pendula* Roth), Norway spruce (*Picea abies* (L.) Karst) and Scots pine (*Pinus sylvestris* L.) (Fig. 5) growing in soil that originally was very probably similar in all three stands. Parent material was till soil on Precambrian bedrock, and the soil type was podzolic and humus type mor. The site type was *Hylocomium-Myrtillus* (Cajander 1949). Originally, the study site had been a homogenous Norway spruce stand, which had been clear-cut and prescribed burned in 1926. The spruce stand was planted in 1930, the birch stand was naturally regenerated, and the pine stand was established after unsuccessful sowing of spruce, pine being favoured in cleaning of the seedling stand. The coniferous stands also contained species other than the dominant one (based on stem density, the spruce stand contained 75% spruce, 17% birch and 8% pine; and the pine stand contained 88% pine and 12% birch). Herbs and grasses were more abundant in the birch stand than in the coniferous stands, and the moss cover was most even in the pine stand (Nieminen and Smolander 2006). Three study plots (25 m x 25 m) were placed in each stand.

a)

b)

c)

Fig. 5. Stands dominated by (a) silver birch *(Betula pendula* Roth), (b) Norway spruce (*Picea abies* (L.) Karst) and (c) Scots pine (*Pinus sylvestris* L.) at the Kivalo study site.

3.2 Soil sampling and chemical determinations

In papers I and II, for determination of soil microbial characteristics and two plant secondary compound groups, phenolic compounds and terpenes, 20 cores (core diameter 19 cm) were taken systematically from the forest floors of all plots. The samples were divided into L, F, and H layers and combined to give one composite sample per plot and layer. The litter layer consisted of fresh or slightly decomposed litter from trees and understory; the F layer

consisted of partly decomposed litter, the origin of which was mainly identifiable; and the H layer consisted of decomposed organic matter, the origin of which could not be identified. F and H layer samples were sieved, the litter was cut into smaller pieces, and the samples were stored at 1°C until used. In papers III-V, in order to study soil response to added tannins or volatile monoterpenes, 20-30 cores (core diameter 58 mm) were taken systematically from the humus layer (F + H) of all plots (IV, V) or 20 cores were taken systematically from two birch plots (III). The samples were combined to give one composite sample per plot, and the composite samples from each plot were combined to give one sample that represented one stand (IV, V) or the two plots (III). After the green plant material was removed, the samples were sieved and stored at 4°C until used.

The dry matter content of the soil samples was determined by drying the samples for 24 h at 105°C and then measuring the soil organic matter (o.m.) content as loss-on-ignition at 550°C (II, IV). Soil pH was measured in soil suspended in H_2O (1/2.5, v/v) (I). Total C, H and N in soil were measured from air-dried samples using an automatic CHN analyser (I, II).

3.3 Plant sampling

For analysis of terpenes and phenolic compounds, undamaged bulk green birch leaves and spruce and pine needles were collected in September 2004; and samples from the four dominant species of ground vegetation (blueberry (*Vaccinium myrtillus* L.), lingonberry (*Vaccinium vitis-idaea* L.), feather moss (*Pleurozium schreberi* (Brid.) Mitt.) and wavy hair-grass (Deschampsia flexuosa (L.) Trin.)) were collected in August 2005 (II). All plant samples were dried at 40°C; leaves of blueberry and lingonberry were separated from stems, and the plants were finely ground. Ground plant material was stored at -20°C until used.

For extraction and fractionation of tannins for the soil incubation experiment, which was done in order to study the effects of added tannins in soil, undamaged bulk green needles were collected from the pine and spruce plots in spring 2001 (IV, V). After collection, the needles were freeze dried and finely ground.

3.4 Determination of plant secondary compounds in plant and soil samples

3.4.1 Terpenes

Sesqui-, di- and triterpenes were determined from samples taken from the L, F and H layers of the forest floors, from birch leaves and spruce and pine needles, and from samples taken from the ground vegetation (II). Samples were extracted with acetone, extracts were evaporated to dryness and re-dissolved in chloroform (determination of sesquiterpenes and diterpenes other than resin acids) or in pyridine + N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (determination resin acids and triterpenes). The concentration of terpenes was determined with a gas chromatograph – mass spectrometer (GC-MS). For identification of terpenes, literature (Pohjola 1993), mass spectrometric data and authentic reference compounds were all used.

3.4.2 Total phenolic compounds

Concentrations of total water-soluble phenolic compounds were determined from samples taken from the L, F and H layers of the forest floors, from birch leaves and coniferous needles

and from the samples taken from the ground vegetation with the Folin-Ciocalteu method (II). Low- and high-molecular-weight phenolic compounds were separated by casein precipitation. Concentration of phenolic compounds was measured with a spectrophotometer based on formation of a coloured complex between phenols and alkaline Folin-Ciocalteu reagent.

3.4.3 Condensed tannins

Soluble condensed tannins were determined from samples taken from the L, F and H layers of the forest floors and from birch leaves and spruce and pine needles (II) as well as from the fractions extracted from spruce and pine needles (IV, V) with modified acid-butanol assay (proanthocyanidin assay). The extractant used for soil samples and for leaves and needles was 70% aqueous acetone. Concentration of condensed tannins in the samples was measured with a spectrophotometer.

3.5 Measurement of VOCs in the field

Volatile organic compounds were measured from all stands using two methods: passive samplers and a chamber method (III). In the passive sampler method, organic vapour monitors were inserted into holes made in the soil. After 32 days the adsorbed VOCs were eluted from the disc with dichloromethane and analyzed with a GC-MS (III). In the chamber method, VOCs were collected five times from the soil atmosphere with a stainless steel cylindrical cap (diameter 19 cm, depth 12 cm, volume 3,4 l). The cap was hammered into the soil and a sorbent sampling tube containing activated carbon was connected to the chamber. Air was pumped out of the soil through the tube at a certain rate of flow for 6 min. For comparison, VOCs in ambient air were also collected. VOCs in the sorbent tubes were desorbed with carbon disulfide:methanol solution and analyzed by static headspace gas chromatography (HSGC) (III).

3.6 Determination of soil response to volatile monoterpenes

Birch soil was exposed to vapours from (-)-α-pinene, (-)-β-pinene, Δ-3-carene or myrcene, and incubated at constant moisture (60% of the water holding capacity, WHC) and temperature (14°C) for 6 weeks (III). The effects of monoterpenes on soil C and N transformations were monitored (see methods below). To ensure that the soil was not N-limited, arginine was added to half of the treatments.

3.7 Determination of soil response to added tannins

3.7.1 Extraction, fractionation and analysis of tannins

Tannins from spruce and pine needles were extracted and, based on the degree of polymerisation, were fractionated into four fractions (IV). Briefly, the ground needle material was soaked in hexane, extracted twice with acetone-water and filtered, then concentrated and extracted with ethyl acetate. The dried ethyl acetate fraction was labeled F1, while the water-acetone fraction was loaded into a Sephadex LH-20 column, and fractionated into fractions F2, F3 and F4 by eluting with 100% ethanol, with 100% methanol and with acetone-water (70:30), respectively.

Tannin fractions were needed for the incubation experiment in order to explore the soil response to added tannins (IV, V). A commercial tannic acid product containing hydrolysable tannins was also included. All needle fractions and the commercial tannic acid product were analyzed using thin layer chromatography (TLC) and reversed-phase and normal-phase high-performance liquid chromatography (RP- and NP-HPLC) coupled with an ultraviolet (UV) detector and electrospray-ionization mass spectrometer (ESI-MS). Concentration of the condensed tannins was measured from the needle fractions (see the methods above). The content of low-molecular-weight substances other than condensed tannins in the fractions were analysed with gas chromatography-mass spectrometry GC-MS (IV). To aid handling and application to soils, the fractions and the commercial tannic acid product were mixed with silica gel (IV, V).

3.7.2 Incubation experiment

Four needle fractions isolated from spruce or pine needles and the commercial tannic acid product were added to spruce, pine or birch soils (IV, V); the soils were incubated at constant moisture (60% WHC) and temperature (14°C) for 6 weeks, and C and N transformations were monitored (see methods below). To determine whether the effects of the fractions could be counteracted by adding N, all the same treatments were also done with addition of arginine.

3.7.3 Effects of fractions on soil bacteria and fungi

To assess the availability or inhibition of these amendments to soil bacteria and fungi, water extracts of the needle fractions and the commercial tannic acid product were used (IV). The rate of bacterial growth was measured using the ^{3}H-thymidine incorporation technique on bacteria extracted from soil, and the rate of fungal growth was measured using ^{14}C-acetate incorporation into ergosterol.

3.8 Measurement of soil C and N transformations

CO_2 production was measured several times during 8-week (I) or 6-week (III-V) soil incubations by sampling the headspace and analyzing the amount of CO_2 on a gas chromatograph.

In an aerobic incubation experiment in the laboratory, net ammonification and net nitrification were studied at constant temperature (14°C) and moisture (60% WHC) for 10 weeks (I) and in incubation experiments done in order to study the effects of volatile monoterpenes and added tannins in soil, described in sections 3.6 and 3.7.2, respectively (III-V). To calculate net ammonification and nitrification, initial concentrations of NH_4^+-N and (NO_2^- + NO_3^-)-N from non-incubated samples were subtracted from the final (post-incubation) concentrations of NH_4^+-N and (NO_2^- + NO_3^-)-N. DON was calculated as the difference between total N and inorganic N (IV, V).

Gross rates of NH_4^+ production and consumption were determined by the isotope dilution method (I, V). The ^{15}N content of the soil extracts was determined by the diffusion method and the ^{15}N/^{14}N ratio by mass spectrometry.

Microbial biomass C and N were measured by the fumigation-extraction method, in which C and N in microbial cells is released by fumigating soil with chloroform vapour that induces lysis of the cells (I, III-V).

3.9 Statistical analysis

Means of the measured characteristics between tree species (I, II), between the L, F and H layers (I, II) and between the monoterpene (III) or needle fraction (IV, V) treatments were compared by analysis of variance (ANOVA). When needed, transformations were made to fulfill the assumptions of the ANOVA. Significant differences between means were determined by Tukey's test. When the assumptions of ANOVA were not fulfilled (non-homogenous variances), non-parametric Kruskal-Wallis one-way analysis of variance was used (V). To describe the relationships between certain chemical and microbial characteristics of soil, Pearson correlation coefficients were calculated (I, II).

4 RESULTS AND DISCUSSION

The site used in this study included adjacent stands dominated by silver birch, Norway spruce and Scots pine growing in soil that originally was very probably similar in all three stands. In each of these stands, three replicate study plots were established; therefore, the experiment was not a true replicated experiment, suffering as it did from pseudoreplication, a problem in many large-scale ecological experiments. However, no appropriate, old-enough experiments on effects of tree species are available. In this study, the stands were established on the same geological formation and very possibly on soil that originally had been similar. Therefore, it is justified to assume that no other factors influence soil properties more than tree species does.

4.1 Soil chemical properties and microbial activities in the forest floor layers under birch, spruce and pine

Chemical properties and microbial activities in soil were affected by both tree species and the forest floor layer (I). In general, differences between tree species in the soil chemical properties and microbial activities were smaller in the H layer than in the L and F layers (I, Table 1). The more evident effect of tree species on upper than on lower soil layers is in accordance with the findings of other studies (Mikola 1985, Priha and Smolander 1999, Brandtberg et al. 2000). As mentioned in the introduction, both site fertility and age of the tree stand apparently affect the depth of the effects of tree species (Mikola 1985, Priha and Smolander 1999, Priha et al. 2001). In this study, it is possible that ca. 70 years is not long enough for tree species to considerably change the characteristics of the H layer on this particular study site, and that the effect of aboveground litter is stronger than that of root litter and root exudates.

Soil pH and C-to-N ratio varied according to tree species and the forest floor layer. The pH values varied from 3.6 in the spruce H layer to 5.2 in the birch L layer, and under all tree species were significantly higher in the L layer than in the F and H layers (I, Table 1). Birch always showed higher pH values than conifers did. In the L and F layers, the C-to-N ratio was highest under pine and lowest under birch whereas in the H layer there were no differences between tree species (I, Table 1). These results are in accordance with those from other studies in which the soil pH has been higher and the soil C-to-N ratio lower in the humus layer under birch than under conifers (Mikola 1985, Priha and Smolander 1999, Smolander et al. 2005).

Table 1. Soil pH, C-to-N ratio, mineralisation rate of C (C_{min}), rate of net N mineralisation (N_{min}) and C and N in microbial biomass (C_{mic} and N_{mic}, respectively) for litter (L), fermentation (F) and humified (H) layers under birch, spruce and pine. (o.m. = organic matter)

	pH	C-to-N ratio	C_{min}	N_{min}	C_{mic}	N_{mic}
			($g\ CO_2$-C kg^{-1} o.m. 8 $weeks^{-1}$)	(mg kg^{-1} o.m. 10 $weeks^{-1}$)	(g kg^{-1} o.m.)	(g kg^{-1} o.m.)
L						
Birch	5.2	34	60	120	21	2.2
Spruce	4.5	42	61	120	22	1.7
Pine	4.2	65	51	5	18	1.1
F						
Birch	4.3	24	28	528	14	2.2
Spruce	3.9	29	26	551	11	1.8
Pine	3.8	35	17	18	10	1.2
H						
Birch	3.8	34	10	27	9	1.3
Spruce	3.6	34	12	18	8	1.2
Pine	3.7	34	7	13	7	1.0

Differences between tree species in pH and in the C-to-N ratio were greater near the surface than deeper in the soil, which was also observed by Mikola (1985).

Birch and spruce forest floors seemed to be more active than the pine forest floor, with all layers in the former two species having higher rates of C and net N mineralisation and greater amounts of C and N in the microbial biomass (I, Table 1). The higher, or at least as high, microbial activities under birch compared to conifers have been reported previously at other sites (Priha and Smolander 1999, Priha et al. 2001, Smolander et al. 2005). The ranking of spruce and pine seems to be more dependent on the study site; in a few other studies (Priha and Smolander 1999, Priha et al. 2001), microbial activities were either similar to or higher in pine soil than in spruce soil; and spruce, in particular, has been mentioned previously as a soil-degrading species (Nihlgård 1971, Mikola 1985, Ranger and Nys 1994, Priha and Smolander 2000). On this particular study site, spruce soil already previously was shown to have higher rates of C mineralisation and net N mineralisation than the soil under pine (Smolander and Kitunen 2002). This discrepancy may be explained by differences in the study sites: the spruce stand in this study was not closed and it contained ground vegetation that resembled that in the pine and birch stands. The spruce stands of previous studies (Priha and Smolander 1999, Priha et al. 2001) were closed stands with only mosses and a thick needle layer on the ground. In addition, the fact that in the present study the coniferous stands also contained birch (the spruce stand more than the pine stand, 19 and 6% of the basal area of all tree species, respectively) may have affected the results. The spruce stand also contained some pines. Perhaps the birch mixture in the spruce stand was enough to increase microbial activities, since Brandtberg et al. (2000) observed that, compared with pure spruce plots, birch (*B. pendula* and *B. pubescens*) admixture (12% or more of the basal area) on Norway spruce plots increased pH, base saturation and exchangeable concentrations of Ca and Mg in the LF layer of the forest floor.

The higher rates of C mineralisation and the higher values for C and N in the microbial biomass under spruce, and especially under birch, than under pine (I, Table 1) could be partly due to the differences in the temperature or moisture in the soils under different tree species, to the different composition of the leaf and needle litter, or to the effect of roots. The concentration of water-soluble compounds is higher in birch leaf litter than in needle litter (Johansson 1995). The stimulating effect of birch roots on soil microbes has been reported by Bradley and Fyles (1995), Priha et al. (1998) and Priha and Smolander (2003). In addition, the rate of decomposition of broadleaf root litter is reported to be faster than that of coniferous species, due to the different chemical composition of the roots (Silver and Miya 2001). Differences between tree species may also be due to differences in ground vegetation, since herbs and grasses were more abundant in the birch stand than in the coniferous stands, and the moss cover was most even in the pine stand (Nieminen and Smolander 2006), which could also explain the differences between spruce and pine. Moss litter has a lower pH and decomposes more slowly than the dead parts of most herbs and grasses (Mikola 1954).

The amounts of C_{mic} and N_{mic} of the total organic C and N in soil were higher in the L layer than in the F layer and especially the H layer (I), which may indicate higher C immobilisation (Anderson and Domsch 1989) and especially greater N immobilisation in the microbial biomass of the L layer than in the lower layers. This is supported by Kiikkilä et al. (2006), who discussed that fresh litter contains a considerable amount of more easily degradable material, while in the deeper layers, fresh input is obtained only via leaching from the L layer or from degradation of dead roots or root exudates. In contrast, low percentages of C_{mic}/C_{tot} and N_{mic}/N_{tot} may indicate a low-quality substrate (Bauhus et al. 1998); therefore, the lowest C_{mic}/C_{tot} percentage in the F and H layers under pine may suggest lower availability and/or degradability of organic substrates provided by pine litter than by the litters of spruce and birch (Lejon et al. 2005). However, a high percentage of N_{mic} from N_{tot} may also indicate lack of N, which means that a higher proportion of soil N is immobilised in microbes.

The amount of inorganic N in soil reveals the amount of N available at a given moment but does not reveal anything about the rates of its formation and use. Gross N mineralisation describes the 'real' rate of formation of inorganic N in soil, while net N mineralisation is a good estimate of the amount of easily mineralisable N and describes the rate of formation of N available for plants, i.e. the amount of N formed in soil during a certain time period after the immobilisation of N is subtracted. Net nitrification is the net rate of formation of nitrite + nitrate in soil. In this study, net nitrification was always negligible, and therefore net N mineralisation in these soils is similar to net ammonification. Negligible net nitrification is often the case in Finnish forest soils, unless these soils are managed with nitrogen fertilization, liming or clear-cutting (reviewed by Smolander et al. 2000) or exposed to N deposition, for example, from fur farms (Martikainen et al. 1993).

In the L and F layers, birch and spruce had considerably higher values for net N mineralisation during the 10-week incubation than pine did, although, due to the high variation, the differences between tree species were not significant. In the H layer, all tree species showed low net mineralisation of N. In the pine forest floor, formation of inorganic N was very low in all layers (I, Table 1). The best predictor of the rate of net N mineralisation seemed to be the concentration of NH_4-N (I), which is consistent with the findings of Thomas and Prescott (2000). As reviewed by Berg (1986), the process of litter decomposition can be divided into two phases. In the first phase there is often net uptake of major nutrients by microbes, while in the second phase, net release of nutrients normally starts (Berg 1986). Therefore, the lower rates of net N mineralisation in the L layer under birch and spruce, compared to F, could be due to higher rates of immobilisation in L, a finding which is supported by high rates of CO_2

production and high C_{mic} values in the L layer. The relatively low C_{min}/N_{min} ratio of birch and spruce in the F layer (I) also suggests that a large proportion of N mineralised in the F layer is not immobilised (Thomas and Prescott 2000). On the other hand, the high C_{min}/N_{min} ratio in the F layer under pine may indicate lack of N; and according to Côté et al. (2000), high C_{min}/N_{min} ratio indicates low-quality organic matter in soil.

Rate of gross N mineralisation was determined using ^{15}N (I). Despite the fact that the recovery of ^{15}N was generally very low and may have affected the reliability of the results, the results for gross N mineralisation support the conclusions above; rates of gross N production were slightly higher in the F layer than in the L layer. In addition, production rates were slightly higher in the birch, and especially in the spruce, F layer than were the rates of consumption. In spruce and birch forest floors, gross mineralisation of N was lower in the H layer than in the L and F layers, while in pine forest floor there were no differences between layers. In the L layer and especially in the F layer, pine showed much lower rates of gross N mineralisation than birch or pine did, possibly indicating that pine soil was more deficient in N than birch or spruce soils were.

When the effects of silver birch, Norway spruce and Scots pine on soil microbial activities are compared in this and in other studies (Priha and Smolander 1999, Priha et al. 2001), there is no consensus about the ranking of the tree species. In particular, the ranking of spruce and pine varies; in this study, spruce soil showed unexpectedly high microbial activities compared to birch and pine. Therefore, the results of this study emphasize the importance of the characteristics of the study site: the age and density of the stand, the amount of trees other than the dominant species in the stand and the composition of ground vegetation.

4.2 Terpenes and phenolic compounds in the forest floor layers under birch, spruce and pine

As mentioned in the introduction, it may be difficult to determine amounts of secondary compounds in soil. For example, measuring the concentrations of monoterpenes is challenging since these compounds are very volatile. However, with both methods (passive samplers and chamber method) used in this study, the relative proportions of different monoterpenes in the soil atmosphere were similar, although the concentrations measured with passive samplers were considerably lower than those obtained with the chamber method since these methods measure different things (III). The highest sum concentrations of volatile monoterpenes in the soil atmosphere were in pine soil; these concentrations were intermediate in spruce soil and low or negligible in birch soil. The most abundant monoterpene was always α-pinene, which is the major monoterpene present in spruce and pine (Manninen et al. 2002). The second most abundant monoterpene under spruce was β-pinene and under pine Δ-3-carene and myrcene. Both spatial and temporal variations in monoterpene concentrations in the soil atmosphere were large, as has also been reported for monoterpene emissions from a *Pinus pinea* stand (Staudt et al. 1997). There exist few studies of volatile monoterpenes in the soil atmosphere. In these studies, the concentration of monoterpenes ranged from 2 mg m^{-3} of soil atmosphere in a mature Norway spruce stand measured with passive samplers (Paavolainen et al. 1998) to 3560 mg m^{-3} in the air of a carboy containing litter from *Pinus monophylla* and incubated at 38°C (Wilt et al. 1993a), indicating that the results depend on the method used. In the present study, concentrations with passive samplers ranged from 0 to 8.7 mg m^{-3} (mean) and with chamber method from 1.5 to 106 mg m^{-3} in the soil atmosphere.

In the L, F and H layers of the forest floor, concentrations of sesqui-, di- and triterpenes showed trends between tree species similar to those for monoterpenes in the soil atmosphere,

since, in particular, concentrations of sesqui- and diterpenes were considerably higher under conifers than under birch (II, Table 2). Pine always showed the highest concentrations. The difference between birch and conifers was to be expected, since the total concentration of sesqui-, di- and triterpenes was 2-3 times higher in pine and spruce needles than in birch leaves (II). Both coniferous species contained sesqui-, di- and triterpenes, while in birch leaves diterpenes were absent. In the coniferous soils there were several sesquiterpenes, but none of them clearly dominated. The most abundant diterpenes under all tree species were dehydroabietic acid and pinifolic acid. Concentrations of triterpenes did not differ as much as did concentrations of sesqui- and diterpenes between birch and conifer soils, although again birch had the lowest and pine the highest values (II, Table 2). The most abundant triterpene under all tree species was β-sitosterol. The higher relative proportion of triterpenes than sesqui- or diterpenes in the deeper layers compared to the L layer may indicate the lower degradability or the weaker ability to adsorb on soil particles of triterpenes compared to sesqui- and diterpenes, or be due to the input of fresh root litter in the lower layers (Dijkstra et al. 1998).

In all layers, the concentration of total water-soluble phenolic compounds as well as the concentration of condensed tannins was higher or at least as high under spruce as under birch or pine (II, Table 2). This is consistent with the results of Kuiters and Denneman (1987) in the humus layer of soils under silver birch, Norway spruce and pine (*P. nigra* Arnold). The concentration of total water-soluble phenolic compounds ranged from ca. 1.2 to 3.7 g tannic acid equivalents (TAE) kg^{-1} o.m. in different soils, being higher than those observed by Kuiters and Denneman (1987), Gallet and Lebreton (1995) and Smolander et al. (2005) for

Table 2. Relative concentrations of sesqui-, di- and triterpenes, total water-soluble phenolic compounds and condensed tannins for litter (L), fermentation (F) and humified (H) layers under silver birch, Norway spruce and Scots pine. The highest concentration of each compound is expressed as 100, and the other concentrations are related to that value. Relative values can only be compared within one compound. A value of 100 in the table represents concentrations 4.0, 14.7 and 1.7 g kg^{-1} organic matter (o.m.) for sesqui-, di- and triterpenes, respectively, and 3.7g tannic acid equivalents kg^{-1} o.m. and 3.6 g kg^{-1} o.m. for total water-soluble phenolic compounds and condensed tannins, respectively.

	Sesquiterpenes	Diterpenes	Triterpenes	Total phenolic compounds	Condensed tannins
L					
Birch	3	2	40	75	96
Spruce	49	70	53	100	87
Pine	100	100	100	89	53
F					
Birch	3	1	22	47	51
Spruce	6	8	30	54	100
Pine	12	22	43	38	45
H					
Birch	2	2	12	40	24
Spruce	3	4	15	44	58
Pine	4	8	19	31	15

unground Norway spruce or silver birch humus, while concentrations of condensed tannins were of the same order of magnitude as those present in the humus layer of Norway spruce or silver birch sites (Lorenz et al. 2000, Smolander et al. 2005). Due to the large number of analytical methods and problems in choosing the appropriate standards (Hagerman and Butler 1989), polyphenol concentrations in plants and soils reported in the literature differ greatly and might not be comparable with each other, as is widely known among researchers working with phenolic compounds. Folin-Ciocalteu's reagent reacts with the –OH groups in the phenols, and therefore its reactivity increases with substitution number; thus the results are very dependent on the standard used (Box 1983). In addition, it is important to note that different types of tannins react differently in the assays used to quantify them (Hagerman and Butler 1989, Kraus et al. 2003a). Therefore, the various methods with different extractions and different standards as well as different ways of expressing the results explain why the values for total phenolic compounds apparently are lower than those for condensed tannins. However, although colorimetric methods involve problems (Appel et al. 2001), more sophisticated methods used in structural studies of phenolic compounds (IV, Kraus et al. 2003a) are not quantitative enough.

Under all tree species the concentration of total water-soluble phenolic compounds was significantly higher in the L layer than in the lower layers, mainly due to the fact that high-molecular-weight phenolic compounds were significantly more abundant in the L layer than in the F or H layers (II, Table 2). In the F layer, and especially in the H layer, low-molecular-weight phenolic compounds comprised a greater amount of total water-soluble phenolic compounds than did the high-molecular-weight phenolic compounds, probably due to degradation of high-molecular-weight phenolic compounds, leaching of the low-molecular-weight phenolic compounds to deeper soil layers, and/or due to root degradation and exudates. Spruce needles contained considerably more total water-soluble phenolic compounds than pine needles and birch leaves did (II), due to the higher concentration of high-molecular-weight phenolic compounds. This was also reflected in the phenolic concentrations in the soil.

Distribution of condensed tannins between the layers differed between tree species. Under birch and pine, concentrations of condensed tannins decreased from the L layer to the lower layers, but under spruce, the highest concentration was detected in the F layer (II, Table 2). In addition, in the F and H layers, spruce had 2-4 times higher concentrations than birch or pine did. Spruce roots may contain 4 times more tannins than spruce needles do (Gallet and Lebreton 1995), which could contribute to the high values in the F and H layers. It is also possible that, due to their different structural characteristics, condensed tannins of pine, and especially those of birch, were more easily degraded in soil than were those of spruce (Nierop et al. 2006a). Analysis of the spruce and pine needle fractions revealed that the main anthocyanidin type of condensed tannins in pine needles was prodelphinidin, while in spruce needles procyanidins dominated (IV). These results are in accordance with other studies on pine (*P. sylvestris, P. muricata, P. contorta* ssp. *Bolanderi, P. ponderosa, P. maritima* var. *nigra* and *P. banksiana*) needles (Kraus et al. 2003b, Hernes and Hedges 2004, Nierop et al. 2005) and with spruce (*P. abies* and *P. mariana*) needles (Maie et al. 2003, Nierop et al. 2005). In birch (*B. pubescens* ssp. *czerepanovii* (Orlova) Hämet-Ahti) leaves the main type of anthocyanidin has been reported to be PD (Ossipova et al. 2001), while in the leaves of some other birch species (e.g. *B. resinifera* and *B. papyrifera*), PCs may dominate (reviewed by Ossipova et al. 2001). As discussed in the introduction, the concentration of PD in soil may decrease faster than the concentration of PC due to the less stable structure of PDs and because PDs are more prone to transformation processes. Other factors that could have affected the

concentrations of condensed tannins in soil are leaching and immobilisation (Hernes et al. 2001, Maie et al. 2003).

The fact that the concentrations of most of the secondary compounds studied were higher in the L layer than in the F or H layers is in accordance with the findings of other studies on diterpenoid acids and highly volatile monoterpenes (Wilt et al. 1988, 1993b, White 1991, 1994, Dijkstra et al. 1998). The decrease in concentrations of secondary compounds with depth may suggest that these compounds are not easily leached to the lower soil layers from the aboveground litter, or more probably, that they are partly degraded in the litter layer, which is supported by the results of other studies (Lorenz et al. 2000, Kainulainen and Holopainen 2002, Stark et al. 2007). Persistence of terpenes and phenolic compounds in the soil seemed to differ; the percentage decrease in the concentrations of terpenes with depth, in particular concentrations of sesqui- and diterpenes, in spruce and pine soils was markedly higher than the percentage decrease in the concentration of total water-soluble phenolic compounds.

The concentration and composition of soil secondary compounds are affected not only by the dominant tree species but also by species of ground vegetation. For example, lingonberry and blueberry contained considerably higher concentrations of total water-soluble phenolic compounds than birch leaves or coniferous needles did (II), which is in accordance with the results of Gallet and Lebreton (1995), who found that the concentrations of total phenols and the phenolic family compounds – tannins, flavonoids and phenolic acids – were higher in blueberry leaves than in spruce needles. In addition, the content of total phenolics has been reported to be lower in the litter of grass species than in the litter of dwarf shrubs (Barford and Lajtha 1992). However, not only the concentration of secondary compounds in the litter but also the amount of litter input affects the concentrations of secondary compounds in soil. At this study site, the amount of litter fall is not yet known. It is possible that, due to different site fertility and occurrence of ground vegetation in the study of Smolander et al. (2005), concentrations of sesqui- and triterpenes were higher in the humus layer under birch than under spruce, which is opposite to the results of the present study. However, tree species, including the different soil characteristics, did not seem to affect concentrations of terpenes or total water-soluble phenolic compounds in the ground vegetation (II).

4.3 Soil microbial activities in relation to plant secondary compounds

Some general observations were made concerning the relations between secondary compounds and soil microbial activities. In the microbiologically active birch soil, concentrations of mono-, sesqui- and diterpenes were considerably lower than those in the coniferous soils, while pine soil, showing the lowest activities, had the highest concentrations of terpenes (I-III). On the other hand, the greater amount of water-soluble phenolic compounds seemed to an indicator of the higher microbial activities in the soil (I, II).

Pearson correlation coefficients between the concentrations of total water-soluble phenolic compounds, condensed tannins and total terpenes as well as soil microbial activities were calculated in order to determine whether there were any linear relationships between them (II). Rate of C mineralisation and amount of C_{mic} correlated highly positively with concentration of total water-soluble phenolic compounds (r = 0.93, (Fig. 6a) and r = 0.91, respectively). In addition, the rate of C mineralisation and the amounts of C_{mic} and N_{mic} all correlated positively with the concentration of condensed tannins (r = 0.64, (Fig. 6b); r = 0.61 and r = 0.62, respectively). However, even a significant correlation between variables does not necessarily indicate a causal relationship, although it has been suggested that polyphenols

may accelerate rates of litter decay because they are leached quickly or used by microbes as a source of C (Valachovic et al. 2004). On the other hand, Loranger et al. (2002) found a negative correlation between initial concentration of phenolics (including tannins) in leaf litter and the rate of decomposition in semi-evergreen tropical forests. The amount of C mineralised in this study cannot be explained solely by the amount of water-soluble phenolic compounds in these soils. Instead, the correlations mentioned above may suggest that soils containing greater amounts of water-soluble phenolic compounds contain larger amounts of easily available C sources for microbes. Therefore, it is possible that the amount of phenolic compounds in soil may indicate the character and decomposition level of soil organic matter rather than being C sources themselves.

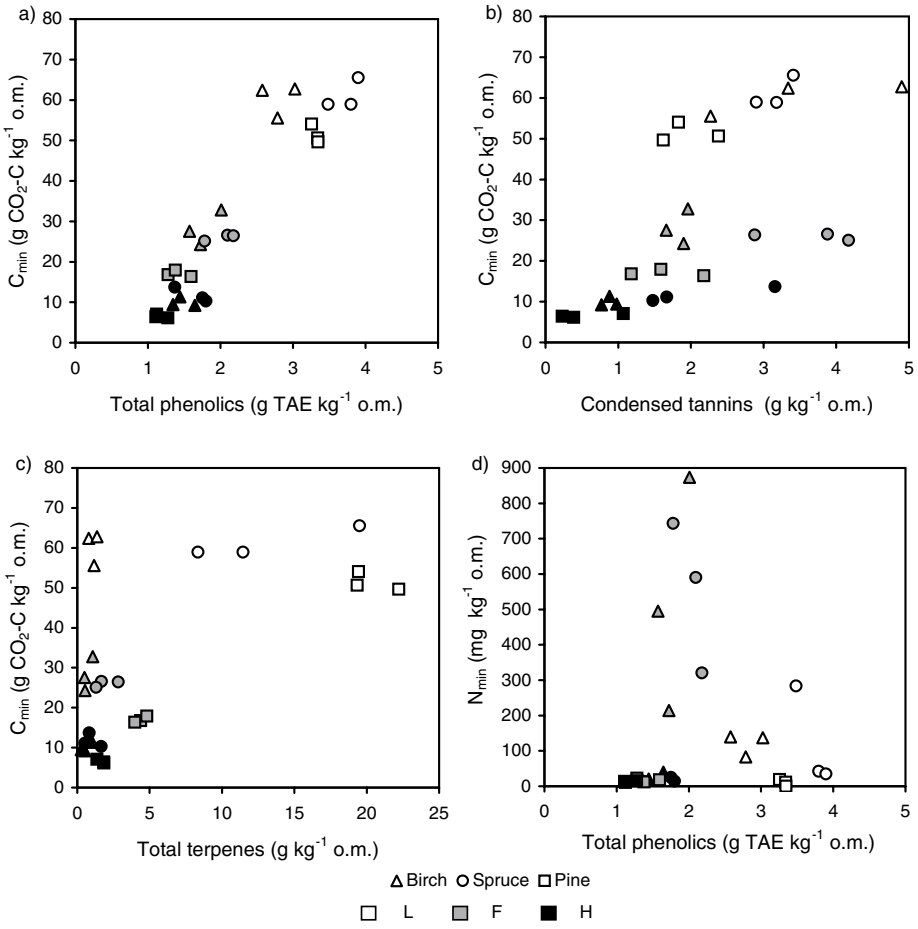

Fig. 6. Scatter plots for (a) total water-soluble phenolics and cumulative CO_2 production, (b) condensed tannins and cumulative CO_2 production, (c) total terpenes and cumulative CO_2 production and for (d) total water-soluble phenolics and net N mineralisation in different layers of soil organic horizon under silver birch, Norway spruce and Scots pine, n=27. (C_{min} = cumulative CO_2 production rate during 8-week incubation, N_{min} = rate of net N mineralisation during 10-week incubation, TAE = tannic acid equivalents).

Other significant correlations found in this study were between concentration of total water-soluble phenolic compounds and the C-to-N ratio (r = 0.61), between concentration of condensed tannins and rate of net N mineralisation (r = 0.40), and between concentration of total terpenes and C-to-N ratio (r=0.89), rate of C mineralisation (r = 0.59, Fig. 6c) and amount of C_{mic} (r = 0.57). Tree species and soil layer greatly affected the correlations. For example, without birch the correlations between concentration of total terpenes and rate of C mineralisation (r = 0.85, n = 18) and between concentration of total terpenes and amount of C_{mic} (r = 0.80, n = 18) were higher than when birch was included. When only birch and spruce L and F layers (i.e. the layers showing some net N mineralisation) were included, a negative correlation was found between concentration of total water-soluble phenolic compounds and rate of net N mineralisation (r = -0.76, n = 12), although with the whole dataset the correlation was not significant (Fig. 6d). This correlation suggests immobilisation of N with greater amount of phenolic compounds and supports the former conclusion that soils containing larger amounts of phenolic compounds contain larger amounts of easily available C sources for microbes. A negative correlation between phenolic compounds and net N mineralisation was also found by Northup et al. (1995, 1998) with *Pinus muricata* litter, which suggested that tannins form strong complexes with proteins that are sparingly soluble and recalcitrant to decomposition.

4.4 How does soil respond to volatile monoterpenes and tannins?

Effects of volatile monoterpenes and tannins extracted and fractionated from spruce and pine needles on soil microbial activities were studied in laboratory incubation experiments (III-V). Addition of volatile monoterpenes to birch soil was a more radical operation than addition of tannins to birch, spruce and pine soils, since birch soil contains only a very small amount of monoterpenes naturally, while tannins are more abundant in soils under birch, spruce and pine, although some structural differences can occur. Both groups of compounds affected soil C and N transformations, but the effects were dependent on the compound and its molecular structure.

4.4.1 Effects of volatile monoterpenes in soil

Samples of humus layer from the birch stand were exposed to vapours from the most abundant monoterpenes ((-)-α-pinene, (-)-β-pinene, Δ-3-carene or myrcene) in the coniferous soil atmosphere (III). All monoterpene treatments increased CO_2 production in the soil but simultaneously decreased net N mineralisation (III, Table 3). With addition of N (arginine) the trends were similar. After the first 12 days, the increasing effect of myrcene on CO_2 production in soil was lower than were the increasing effects of the other monoterpenes. An inhibitive effect of monoterpenes on net N mineralisation in soil has also been reported by other studies (White 1986, 1991, 1994, Bremner and McCarty 1988, 1996, Paavolainen et al. 1998). A simultaneous increase in CO_2 production, also reported for other soils after exposure to monoterpenes (Amaral and Knowles 1998, Paavolainen et al. 1998), suggests that the decreased net N mineralisation was due to immobilisation of N by soil microbes. However, the response of C and N in the soil microbial biomass to different monoterpenes varied (III, Table 3). Both α- and β-pinene decreased the amount of C_{mic} and N_{mic}, although not always significantly. In addition, Δ-3-carene decreased C_{mic} and N_{mic}, but only when arginine was added. Myrcene decreased N_{mic} when arginine was added, but had no significant effect without

arginine. Due to different responses of microbial biomass C and N to the terpene treatments, response of the C-to-N ratio of microbial biomass varied depending on the compound. The considerable decrease observed in microbial biomass simultaneously with increased CO_2 production indicates a smaller but more active microbial population in the soil. The different response of microbial biomass C and N to different monoterpenes indicates the importance of the specific molecular structure of a certain monoterpene when its effects on soil are considered. This is in accordance with White (1988, 1994), who suggested that the inhibitory activity of monoterpenes varies according to the molecular structure of the compound. However, it is also possible that the differences in the effects of different monoterpenes may be partly due to different concentrations. Altogether these results suggest that monoterpenes had a toxic effect on part of the microbial population in soil, while those microbes that were able to use monoterpenes as a carbon source flourished.

4.4.2 Effects of tannins in soil

Tannins extracted and fractionated from spruce and pine needles were added to spruce and pine soils (IV), respectively, and to birch soil (V). Birch soil was used to determine whether coniferous tannins could provide an explanation for the lower microbial activities often found in coniferous soils compared to birch soil. Use of one soil, birch, also allowed comparison between spruce and pine needle tannins.

Four needle fractions of spruce and pine were prepared for the experiment and analysed in order to determine their tannin contents, polymeric composition of tannins in different fractions, and the amount and character of compounds other than tannins (IV). Analyses revealed that both Norway spruce and Scots pine needle fractions F3 and F4 contained polymers of condensed tannins that were longer than those in the F1 and F2 fractions; therefore, fractions F1 and F2 are hereafter also called ‘light fractions’ and F3 and F4 ‘heavier fractions’. Fractions F3 and F4 consisted mainly of condensed tannins (55–87%) while F1 and F2 contained only 1.7–5.5% condensed tannins. Spruce needles contained more procyanidin than prodelphinidin units, while in pine needles prodelphinidin units were dominant. In addition, HPLC-ESI-MS analysis confirmed that the commercial tannic acid product, also added to soils, contained a mixture of galloylglucoses (hydrolysable tannins) of different molecular sizes. Acid-butanol assay with tannic acid gave no indication of condensed tannins. Minor amounts of several compounds other than condensed tannins (e.g. low-molecular-weight phenolic compounds and terpenes) were found in light fractions, especially in F1. The rest were probably other needle constituents such as chemically neutral and also higher-molecular-weight compounds like waxes, chlorophyll and terpenoids, which are soluble in organic solvents. F2 probably contained more polar compounds, such as phenols and oligomeric phenols. Therefore, it is not justified to specify all the effects of fractions F1 and F2 treatments as ‘tannin effects’; therefore they are called ‘effects of light fractions’.

Effects of light needle fractions F1 and F2 on soil microbial activities differed from the effects of heavier fractions F3 and F4 (IV, V, Table 4). The main trends caused by the fractions were similar regardless of the soil to which the fractions were added or the tree species origin of the fractions. Both spruce and pine fractions F1 and F2 and the commercial tannic acid product always sharply increased CO_2 production during the first days after addition. After that, C mineralisation settled to the same level as the control. In contrast, throughout the incubation experiment both spruce and pine F3 and F4 fractions decreased rates of C mineralisation relative to the control. With both spruce and pine fractions F1 and F2 and with tannic acid in all soils; there was net N immobilisation in the absence of arginine; and they

Table 3. Effects of volatile monoterpenes on rates of C and net N mineralisation (C_{min} and N_{min}, respectively) and on amounts of C and N in microbial biomass (C_{mic} and N_{mic}, respectively) in birch soil. Only treatments without arginine addition are given since when arginine was added, trends between the different treatments were mainly the same. Effects of monoterpene treatments are compared to the control. Explanations for the symbols: +++ > ++ > +, --- < -- < -, 0 = no effect compared to the control.

	C_{min}	N_{min}	C_{mic}	N_{mic}
α-pinene	+++	---	---	0/-
β-pinene	+++	---	---	-
Δ-3-carene	++	---	0/-	0/-
Myrcene	+	---	+	+

Table 4. Effects of needle fractions F1-F4 and commercial tannic acid product (TA) on rates of C and net N mineralisation (C_{min} and N_{min}, respectively), on amounts of C and N in microbial biomass (C_{mic} and N_{mic}, respectively) and on concentration of dissolved organic nitrogen (DON) in birch, spruce and pine soils. Only treatments without arginine addition are given since when arginine was added, the trends in the different treatments were mainly the same. Effects of tannin treatments are compared to the control. Explanations for the symbols: +++ > ++ > +, --- < -- < -, 0 = no effect compared to the control.

	C_{min}	N_{min}	C_{mic}	N_{mic}	DON
Spruce fr. in spruce soil					
F1	+++	---	+	0	-
F2	+	-	-	0	-
F3	-	++	-	0	--
F4	-	++	-	0	--
TA	++	---	0	+++	-
in birch soil					
F1	++	--	0/+	0	0/-
F2	+	0	0	0	-
F3	-	++	0/-	+	--
F4	-	++	0/-	0	--
Pine fr. in pine soil					
F1	+	-	0	+	0/-
F2	++	-	0/-	0/+	0/-
F3	-	0	--	0/+	--
F4	-	+	-	-	--
TA	+	-	-	0/+	-
in birch soil					
F1	++	--	+	++	+
F2	++	--	+	+	+
F3	-	++	0	0	--
F4	-	++	-	-	--
TA	++	---	0	0	+

showed significantly lower values than the control did, except for spruce F2 in the birch soil. With heavier fractions, net N immobilisation was similar or lower than in the control, and when arginine was added, heavier fractions showed similar or higher net N mineralisation than the control did.

The observation that the effects caused by the lighter fractions F1 and F2 were mainly opposite to those caused by the heavier fractions F3 and F4 is consistent with the results of Fierer et al. (2001) with *Populus balsamifera* leaf fractions. Lighter fractions seemed to act as a C source for microbes since they increased CO_2 production in soil, while the heavier fractions were inhibitors. Several other studies have also indicated that low-molecular-weight phenolic compounds can be readily metabolised by microbes, thus stimulating CO_2 production and microbial growth in soil (Sparling et al. 1981, Blum and Shafer 1988, Schimel et al. 1996). In this study, light fractions did not generally affect amounts of microbial biomass C and N much, especially in the absence of arginine. This is consistent with the results of Kraus et al. (2004b), who found no effect on the amounts of C and N in the microbial biomass due to additions of purified tannins from different plant species. Decreased net mineralisation of N by light fractions (IV, V, Table 4) is not necessarily a consequence of a reduction in gross mineralisation of N, although results for gross N mineralisation at the end of the incubation pointed to this: gross N mineralisation was decreased compared to the control when it was studied with a mixture of spruce F1 and F2 in birch soil (V). More probably, mineralised N was immobilised by soil microbes since CO_2 production in soil increased shortly after addition of the fractions. This indicates that the compounds in those fractions - whether low-molecular-weight phenolic compounds or not - were easily metabolised, as has also been seen in other studies with different plant species (Basaraba 1964, Schimel et al. 1996, Fierer et al. 2001, Castells et al. 2003, Kraus et al. 2004b).

Inhibitive effects of heavier fractions on soil microbial activities can be explained in different ways. Tannins may inhibit exoenzyme activity and complex proteinaceous substrates or possibly also other N-containing organic compounds (Kumar and Horigome 1986, Schimel et al. 1996, Bradley et al. 2000, Kraus et al. 2003b, Nierop et al. 2006a). There is also evidence that the protein-precipitating capacity of tannins increases with increasing degree of polymerisation of the tannins (Kumar and Horigome 1986) and that high-molecular-weight tannins precipitate more protein than low-molecular-weight tannins do (Kraus et al. 2003a). In this study, fractions F3 and F4 contained longer tannin polymers than fractions F1 and F2 did (IV). Therefore it is likely that protein precipitation could have played an important role in inhibiting C mineralisation in soils treated with heavier fractions. Most fractions seemed to reduce the concentration of DON slightly (IV, V, Table 4), but heavier fraction treatments slightly more than light fraction treatments. This points to protein precipitation by the heavier fractions, since most of protein-tannin complexes probably do not appear in DON, due to their weak extractability.

Inhibition of C mineralisation by heavier fractions may also have been due to toxic effects. Since heavier-fraction treatments with addition of N showed results for CO_2 production in soil that were similar to treatments without added N, it is possible that N was not a limiting factor in these soils. More probably, the N addition was not large enough to overcome the negative effects of the precipitation of organic N compounds or the inhibition mechanism of heavier fractions was other than substrate complexation, e.g. toxicity. This conclusion is also supported by C in the microbial biomass, since in some cases C_{mic} was slightly decreased by heavier fractions, which could indicate direct toxic effects of tannins on the microbial community or decreased enzyme activities (Kraus et al. 2004b). The experiment with spruce fractions F1 + F2 and F3 + F4 in birch soil done in order to study the rates of gross N mineralisation

(V) showed that, at the beginning of the incubation, with both fraction treatments the rates of production were slightly higher than the rates of consumption, while after 40 days incubation, F1 + F2 had similar rates of production and consumption and F3 + F4 had slightly higher rates of consumption than production. In addition, F3 + F4 showed significantly lower rates of consumption at the beginning of the incubation than the control did. But since the rates of gross N mineralisation were measured only at the beginning and at the end of the 40-day incubation period, it is not possible to know the relation between the rates of production and consumption of N during the 40-day incubation. However, the slight increases in the rates of net N mineralisation by heavier fractions compared to the control (IV, V, Table 4) were likely a consequence of reduced microbial activity and N uptake rather than the result of the gross mineralisation of N becoming more effective. The rate of N mineralisation may not have decreased as much as the rate of N immobilisation, which would result in accumulation of mineral N in the soil.

The effects of tannic acid resembled the effects of light fractions more than those of heavier fractions. Regardless of soil type, tannic acid seemed to be a relatively easily available source of C for microbes (IV, V, Table 4). This is consistent with the results of Nierop et al. (2006b), who found that tannic acid induced a rapid short-term effect resulting in high CO_2 production and net N and P immobilisation. Birch contains hydrolysable tannins naturally, while spruce and pine do not (Waterman and Mole 1994, Ossipova et al. 2001); but information on the hydrolysable tannin contents of ground vegetation is scarce.

When the effects of spruce and pine fractions were compared in birch soil, some differences were found (V). Since pine fraction F2 increased C mineralisation slightly more and decreased net N mineralisation more than spruce F2 did, pine fraction F2 seemed to be somewhat easier for the microbes to use than spruce fraction F2 was. Pine fractions F1 and F2 also increased C_{mic} and N_{mic}, while spruce lighter fractions had no effect. With N addition, differences in the response of N_{mic} to fractions from different tree species were even more obvious. These differences between the lighter fractions of spruce and pine may be due to differences in compounds other than tannins, but tannins may also have affected the results. Some differences between the heavier fractions of spruce and pine were also observed. With arginine addition, pine F3 increased N_{mic}, while spruce heavier fractions had no effect. Without, and especially with, arginine the heavier fractions of spruce decreased DON relatively more than the heavier fractions of pine did. This suggests that protein precipitation, discussed by Kumar and Horigome (1986) and Kraus et al. (2003b), may have been more effective with heavier fractions of spruce than with heavier fractions of pine, because low concentrations of DON might indicate formation of weakly soluble protein-tannin complexes, which may not appear in the DON. The predominance of prodelphinidins in pine needle tannins and of procyanidins in spruce needle tannins (IV) may have affected the differences observed in the effects of spruce and pine fractions. For example, different protein-binding capacities for the PD-type and the PC-type condensed tannins have been suggested to affect the total amount of extractable free condensed tannins in forest soils (Hernes et al. 2001, Maie et al. 2003). There are also suggestions that PDs may be less stable than PCs (Hernes et al. 2001) and that they react more strongly with soil organic matter and therefore reduce net N mineralisation more strongly than PCs do (Nierop et al. 2006a). In addition, PD monomers have been suggested to be more inhibitory than PC monomers (Kraus et al. 2003b, Nierop et al. 2006a), which is, however, opposite to the results of this study. One reason for the discrepancy may be that the exact ratios of PD:PC in the fractions of this study are unknown. In addition, spruce F3 and F4 contained somewhat longer tannin polymers than pine F3 and F4 did, which may have added to the inhibitory effects of the spruce fractions.

5 CONCLUSIONS

- The results of this study showed that different tree species could affect C and N transformations in soil. All forest floor layers (L, F and H) under birch and spruce showed higher rates of CO_2 production and net N mineralisation and greater amounts of microbial biomass C and N, than did the forest floor layers under pine.

- The concentration of monoterpenes in the soil atmosphere and sesqui-, di-, and triterpenes in the forest floor layers were higher under pine and spruce than under birch, while the concentrations of total water-soluble phenolic compounds and condensed tannins tended to be higher or at least as high under spruce as under birch or pine.

- In general, differences in the soil microbial activities and in the concentrations of secondary compounds between tree species were smaller in the H layer than in the upper layers.

- Rate of CO_2 production and amount of C in microbial biomass in soil were positively correlated with concentration of total water-soluble phenolic compounds and condensed tannins. This indicates that water-soluble phenolic compounds can be indicators of the character and decomposition level of soil organic matter.

- The role of terpenes as regulators of soil microbial activity may be important since pine soil, which showed the lowest activity, also had the highest concentrations of mono-, sesqui-, di- and triterpenes, while in the microbiologically active birch soil, the concentrations of terpenes were very low.

- Exposure of soil to volatile monoterpenes and to spruce and pine needle tannins affected C and N transformations in soil, but the effects were dependent on the compound and its molecular structure. Monoterpenes decreased net mineralisation of N and probably had a toxic effect on part of the microbial population in soil, while some other microbes were able to use them as a carbon source.

- The degree of polymerization of condensed tannins significantly influenced the soil processes. Low-molecular-weight compounds (also other than tannins) increased CO_2 production and decreased net N mineralisation, while the higher-molecular-weight compounds had inhibitory effects.

In conclusion, plant secondary compounds may have a great potential in regulation of C and N transformations in forest soils, but the real magnitude of their significance in soil processes is impossible to estimate. In the future research, more attention should be paid on development of methods to study soil organic chemistry; combining the knowledge of soil processes and organic matter characteristics will give a better understanding about the significance of secondary compounds in forest ecosystems.

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