



Response of C and N transformations in birch soil to coniferous resin volatiles

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ABSTRACT

The purpose of this study was to examine the effects of the resin volatile compounds of two coniferous tree species, Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) on C and N transformations in soil under silver birch (*Betula pendula* L.). Humus layers from two study sites were used, referred here as a N-poor soil (C:N ratio 30) and a N-rich soil (C:N ratio 19.5). In addition to these, N-poor soil added with arginine was used to ensure that the soil was not N-limited. Humus layers were subjected to resin treatments during a 28-day incubation period in the laboratory. The most abundant volatile compound in both resins was α -pinene; in spruce resin β -pinene was also abundant. Resins and pure α -pinene increased CO₂-C production, i.e. C mineralization, in both soils. In contrast, net N mineralization was clearly decreased in both soils, and net nitrification was completely stopped. There was no consistent effect on soil microbial biomass C or N. Based on these results, we conclude that volatile resin compounds affect C and N transformations in soil, but the mechanism behind these effects is still unclear.

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1. Introduction

Canal oleoresins are sticky compounds emitted from coniferous trees after injury or wounding. These resins consist predominantly of a mixture of volatile terpenes and solid terpenoids (Back, 2000, 2002). Terpenes are a complex group of secondary metabolites that occur in almost all plants. They are hydrocarbons built up of various numbers of isoprene (isopentane) C₅ units and include essential oils, resins, carotenoids and rubber. The composition of terpenes is dependent on the plant species (Obst, 1998). In conifers, terpenes are important components in the defence against mechanical wounds, attacks by insects and fungi, and perhaps even chemical threats from air pollutants (Back, 2000; Strömwall and Petersson, 2000). Soil microbes may also produce terpenes (Stahl and Parkin, 1996).

Monoterpenes (C₁₀H₁₆), which are formally composed of two molecules of isoprene, are widely distributed in plants, but occur in large amounts mainly in conifers. Monoterpenes evaporate easily into the air and thus are predominant among the volatile terpenes in conifers (Strömwall and Petersson, 2000). Monoterpenes have also been suggested to play a role in allelopathy, causing, e.g. inhibition of germination or regulatory growth (Rice, 1984). There is

evidence that monoterpenes may play an important role in controlling N cycling in forest soils. Certain volatile monoterpenes have been shown to inhibit net mineralization of N (Bremner and McCarty, 1988, 1996; White, 1986, 1991, 1994; Smolander et al., 2006) and net nitrification (White, 1986, 1991, 1994; Paavola et al., 1998) and to decrease the amount of C and N in the soil microbial biomass (Smolander et al., 2006). The impact of monoterpenes on soil microbes is complex since, while they may stimulate activity and growth of some microbial groups, they may inhibit others (Amaral and Knowles, 1998).

In the soil atmosphere under silver birch, volatile monoterpenes have been reported to be scarce, whereas in the soil atmosphere under Norway spruce and Scots pine they were very common (Smolander et al., 2006). Previous studies have shown that microbial biomass C and N and microbial activities in C and N transformations are often highest in birch soil (Priha and Smolander, 1999; Priha et al., 2001; Smolander and Kitunen, 2002; Smolander et al., 2005). Whether the different amounts of monoterpenes in birch and coniferous soils are partly responsible for this is not known.

The aim of this study was to study the chemical composition of volatile compounds from the canal resin of two major coniferous tree species in Finnish forest ecosystems, Scots pine and Norway spruce, and the effects of these volatile resin compounds on C and N transformations in birch soil. A laboratory experiment was conducted to determine: (1) the effective compounds in volatile resin

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and (2) the response of microbial C and N transformations in birch soil to these compounds. Birch soil was used because it would have been very difficult to separate the effect of added monoterpenes and monoterpenes already in the soil, which would be the case if conifer soils were used. Two different kinds of study sites were selected, a naturally N-poor (C:N ratio 30) and a naturally N-rich (C:N ratio 19.5) study site. To see whether additions of N could counteract the effects of volatile resin compounds and to ensure that there is enough N to study N transformations, there was also a treatment with arginine addition in N-poor soil.

Our hypothesis was that volatile compounds of resins affect microbial activity and C and N transformations after contact with soil and thus can partly explain the reasons behind different microbial processes in soils beneath different tree species.

2. Materials and methods

2.1. Study sites and sampling

Two different study sites were used, referred in this paper as a N-poor and a N-rich site. The first study site, N-poor-site, which had originally been a homogenous Norway spruce stand, was a tree-species experiment in Kivalo (66°20'N/26°40'E), northern Finland. This site included adjacent 76-year-old stands that were dominated by silver birch, Scots pine and Norway spruce growing on Hylocomnium–Myrtillus site type (Cajander, 1949). Each stand contained three study plots (25 m × 25 m), but for this study only the birch plots were used. The history and characteristics of the study site have previously been described in detail (Smolander and Kitunen, 2002).

The second study site, N-rich site, was located in Kerimäki (61°51'N/29°22'E), southeastern Finland. This site, which had originally been a Norway spruce stand growing on Oxalis–Myrtillus site type (Cajander, 1949), was clear-cut 14 years ago and planted with silver birch seedlings one year after clear-cutting. Samples for this study were taken from previously N-fertilized plots, N-fertilization totalling 860 kg N ha⁻¹ during 30 years, the last application having been given three years before clear-cutting. The history and characteristics of the study site have previously been described in detail (Smolander et al., 2000).

Samples of humus layer (Of+Oh) were taken from the sample sites, from Kivalo in August 2005 and from Kerimäki in September 2006. For each sample set, laboratory experiments were performed in autumn directly after soil collection. From each site about 50 soil cores (diameter 5.8 cm) were collected systematically and then combined to give one composite sample per site. Samples were sieved through a 4 mm mesh. Dry weight (105 °C, 16 h) was determined, and organic matter content measured as loss-on-ignition (550 °C, 4 h). Soil pH was measured from a soil–water suspension of 10 ml soil in 25 ml of ultrapure water. Soil properties of both study sites are presented in Table 1.

Spruce and pine resins were collected from Ruotsinkylä, Tuusula, southern Finland (60°21'N/24°59'E). The study site was

a 60- to 70-year-old mixed Norway spruce–Scots pine stand growing on Myrtillus site type (Cajander, 1949). Mature spruce and pine trees, several individuals of each species, were artificially wounded and containers were placed below these wounds to collect the resin. Resin was collected at various intervals in autumn 2005 and summer–autumn 2006. Samples of pine and spruce resins from different individual trees of each species were pooled. Resin was stored at –20 °C before use in laboratory experiments.

2.2. Laboratory experiments

The effects of volatile resin compounds on soil C and N transformations were studied in a laboratory experiment where the soil samples were incubated at constant moisture (60% water holding capacity) and temperature (14 °C). The treatments with N-poor soil were vapours from (–)- α -pinene (Aldrich Chemical Company, Inc., Germany) and spruce resin. Treatments with N-rich soil were the same, except that a pine resin treatment was added. Soil samples (amount equal to 8 g fresh weight) were added to 125 ml glass bottles and covered with gas-tight septa; to obtain a moisture content of 60% water holding capacity, ultrapure water was added. With N-poor soil samples, to ensure that the soil was not N-limited, to half of the bottles arginine was added with the water (1 mg arginine N per 1 g soil organic matter).

To absorb resins and α -pinene, steel nets (2.5 cm × 5 cm for α -pinene, 2.5 cm × 3 cm for resins, mesh size 0.2 mm) were cleaned with acetone. Resins were melted in a warm (ca. 60 °C) water bath, and liquid resins and α -pinene were absorbed in the nets. Then the nets, hanging from gas-tight septa caps, were placed in the bottles. Bottles with only soil were used as controls. The incubation bottles were thoroughly aerated by flushing them with air every second day. Half of the samples were incubated for 10 days (N-poor soil)/14 days (N-rich soil), the other half for 28 days (both soils).

All the following analyses were made using three replicate bottles in each treatment. Net N mineralization and net nitrification were determined after KCl extraction, as described by Smolander et al. (1995). Net N mineralization was estimated as the accumulation of NH₄-N and (NO₂ + NO₃)-N during incubation. CO₂-C production was monitored with GC (Smolander et al., 1994); CO₂ was measured 24 h after aeration. At the end of incubation, amounts of C and N in the microbial biomass were determined using the fumigation–extraction method (Smolander et al., 1994).

For the experiment with N-rich soil, the concentrations of volatile monoterpenes in bottles were measured at the beginning of the experiment, three times during the experiment at 7-day intervals and at the end of the experiment by injecting 50 μ l air into a gas chromatograph–mass spectrometer (HP6890-HP5973, Hewlett Packard, USA) and using an external-standard method. Electron ionization with 70 eV and mass range of *m/z* 40–300 were applied. The analytical column used was HP-5 (Agilent, Germany), and the temperature was programmed from 60 °C to 230 °C (45 °C/min). The external standard was α -pinene (Aldrich Chemical Company, Inc., Germany). Concentrations of other monoterpenes were measured as α -pinene equivalents.

2.3. Statistical analysis

To determine whether differences between the means of different treatments were significant, one-way analysis of variance (ANOVA) was performed. When needed, microbial biomass and net N mineralization data were transformed by log₁₀ or ln-transformations to achieve the same variation in all treatments. Significant differences of means by treatments were determined by Tukey's test using a significance level of *P* < 0.05.

Table 1
Characteristics of the soil humus layers studied

	Kivalo	Kerimäki
	N-poor	N-rich
o.m. (g kg ⁻¹ d.w.)	772	340
C _{tot} (g kg ⁻¹ o.m.)	600*	532
N _{tot} (g kg ⁻¹ o.m.)	20*	27
C-to-N ratio	30*	19.5
pH	4.2	4.5

Abbreviations: o.m. = organic matter, d.w. = dry weight. Values marked with * taken from Smolander and Kitunen, 2002.

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