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Response of soil C and N transformations to tannin fractions originating from Scots pine and Norway spruce needles

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Abstract

Tannins are polyphenolic compounds that may influence litter decomposition, humus formation, nutrient (especially N) cycling and ultimately, plant nutrition and growth. The aim of this study was to determine the response of C and N transformations in soil to tannins of different molecular weight from Norway spruce (*Picea abies* (L.) Karst) and Scots pine (*Pinus sylvestris* L.) needles, tannic acid and cellulose. Arginine was added to test whether the soil microbial community was limited by the amount of N, and arginine + tannin treatments were used to test whether the effects of tannins could be counteracted by adding N. Soil and needle samples were taken from adjacent 70-year-old Scots pine and Norway spruce stands located in Kivalo, northern Finland. Tannins were extracted from needles and fractioned based on molecular weight; the fractions were then characterized by LC–MS and GC–MS. Light fractions contained tannin monomers and dimers as well as many other compounds, whereas heavy fractions consisted predominantly of polymerized condensed tannins. Spruce needles contained more procyanidin than prodelphinidin units, while in pine needles prodelphinidin units seemed to be dominant. The fractions were added to soil samples, pine fractions to pine soil and spruce fractions to spruce soil, and incubated at 14 °C for 6 weeks. CO_2 evolution was followed throughout the experiment, and the rates of net mineralization of N and net nitrification, concentration of dissolved organic N (DON) and amounts of microbial biomass C and N were measured at the end of the experiment. The main effects of the fractions were similar in both soils. Light fractions strongly enhanced respiration and decreased net N mineralization, indicating higher immobilization of N in the microbial biomass. On the contrary, heavy fractions reduced respiration and slightly increased net N mineralization, suggesting toxic or protein-precipitating effects. The effects of tannic acid and cellulose resembled those of light fractions. DON concentrations generally decreased during incubation and were lower with heavy fractions than with light fractions. No clear differences were detected between the effects of light and heavy fractions on microbial biomass C and N. Treatments that included addition of arginine generally showed trends similar to treatments without it, although some differences between light and heavy fractions became more obvious with arginine than without it. Overall, light fractions seemed to act as a labile source of C for microbes, while heavy fractions were inhibitors.

Keywords: Forest soil; Microbial activities; Mineralization; Nitrogen cycling; Norway spruce; Polyphenols; Scots pine; Tannins

1. Introduction

Tannins are polyphenolic compounds with the ability to form stable complexes with proteins and other compounds. They can be divided in to two main classes: condensed tannins, which are also called proanthocyanidins, and hydrolyzable tannins. Condensed tannins can be further divided into subclasses such as procyanidins and prodelphinidins. While gymnosperms and monocots produce only condensed tannins, dicots can produce either condensed or hydrolyzable tannins or a mixture of the two (reviewed by Kraus et al., 2003). In woody species, foliar concentrations of tannins commonly range from 15 to 25% dry weight (reviewed by Kraus et al., 2004). Tannins may influence litter decomposition rates, humus formation, nutrient (especially N) cycling and ultimately, plant nutrition and growth (e.g. Schimel et al., 1996; Bradley et al., 2000; Fierer et al., 2001). Tannins from various plant species have been shown to affect N mineralization, induce toxicity in microbes and affect enzyme activities in soil (Schimel et al., 1996; Bradley et al., 2000; Fierer et al., 2001; Kraus et al., 2003 and references therein). Hence, there is very strong evidence that tannins play an important role in interspecific competition, and many studies have suggested that individual plants may be important in nutrient cycling on the ecosystem level (Schimel

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et al., 1996; Chen and Stark, 2000; Fierer et al., 2001; Castells et al., 2003; Kraus et al., 2004).

Concentration and composition of phenolic compounds in the soil seem to vary depending on the plant species growing in it (e.g. Kuiters and Denneman, 1987; Smolander et al., 2005). Tannin reactivity in soil has been suggested to be based on such characteristics as condensed versus hydrolyzable tannins and procyanidin versus prodelphinidin content of the tannins (Kraus et al., 2004). In addition, molecular weight or degree of polymerization of tannins or phenolic compounds seems to be an important factor when their influence on soil nutrient cycling is considered. Schimel et al. (1996) and Fierer et al. (2001) demonstrated that high molecular weight phenolics from balsam poplar acted as a general microbial inhibitor, while the effects of lower molecular weight phenolics 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.

Norway spruce (*Picea abies* (L.) Karst) and Scots pine (*Pinus sylvestris* L.) are the dominant tree species in Finland. Both similarities and differences have been reported in the C and N transformations of soil under these species (Priha and Smolander, 1997, 1999; Smolander and Kitunen, 2002). The aim of this study was to find out the response of soil C and N transformations to tannins of different molecular weight from Norway spruce and Scots pine needles. Fractions prepared from spruce and pine needles were added to spruce and pine soils, respectively, to examine their effects on microbial activities by measuring CO_2 evolution, net mineralization of N and net nitrification rates, concentrations of dissolved organic nitrogen (DON) and amounts of C and N in the microbial biomass. The availability or inhibition of these fractions to bacteria and fungi was also assessed.

2. Materials and methods

2.1. Study site, soil and needle sampling and chemical analysis

The stands used in this study were adjacent 70-year-old stands in Kivalo, northern Finland (66°20′N/26°40′E), which were dominated by Scots pine, Norway spruce or silver birch (*Betula pendula* Roth) growing in soil that originally was similar in all three stands. The soil type was podzolic and humus type mor. Three study plots (25×25 m) were placed in each stand. The coniferous stands also contained species other than the dominant one (the spruce stand contained 76% spruce and 24% other species, and the pine stand contained 88% pine and 12% tree species other than pine). For a more detailed description of the study site and the tree stands, see Smolander and Kitunen (2002).

In August 2001 soil samples (20–30 cores, core diameter 58 mm) were taken systematically from the humus layer of spruce and pine plots. 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. After the green plant material was removed, the samples were sieved through a 4.0 mm mesh and stored in plastic bags at 4 °C until used. Content of soil organic matter (o.m.) was measured as loss-on-ignition at 550 °C. The soil characteristics have been described earlier (Smolander and Kitunen, 2002); the pH (H_2O) of both conifer soils was 4.0, and the C-to-N ratio in spruce soil was 37 and in pine soil 32.

Undamaged bulk green needles were collected from the pine and spruce plots in spring 2001. After collection, the needles were freeze dried and finely ground.

2.2. Extraction, fractionation and analysis of tannin fractions

Tannins were extracted and fractioned as described in Fierer et al. (2001) but with some modifications. The ground plant material (1300 g spruce/1070 g pine needles) was placed in a steel container and 5 l of hexane was added. The material was soaked in hexane and stirred occasionally with a power drill; at the end the solvent was decanted from the plant material. The plant material was extracted again with 2.5 l hexane, stirred and decanted. This procedure was repeated two more times. After that, the remaining plant material was extracted with acetone–water (70:30) overnight. The next day the suspension was stirred and filtered through a filter paper (S & S 589[3]). The filtrate was collected in a glass bottle. Needles were extracted again with acetone–water (70:30), stirred 30 min and filtered. The procedure was repeated once overnight. The three acetone–water extracts were combined and concentrated by roto-evaporation. This concentrated extract was then extracted with 100% ethyl acetate for 30 min at 200 rev min^{-1}. Ethyl acetate and water were separated with a siphon, and the procedure was repeated three more times. The ethyl acetate fractions were combined and roto-evaporated to dryness. This fraction was labelled F1.

The acetone–water fraction was loaded onto a Sephadex LH-20 column that had previously been equilibrated with methanol–water (50:50). The column was eluted with methanol–water (50:50) followed by acetone–water (70:30) until the eluate was colourless. The acetone–water fraction was concentrated by roto-evaporation.

The concentrated acetone–water fraction was loaded into a clean Sephadex LH-20 column that had previously been equilibrated with methanol–water (50:50). The acetone–water fraction was eluted with 100% ethanol, and the eluate was concentrated by roto-evaporation and labelled F2.

The extract in the LH-20 column was then eluted with 100% methanol. The eluate was concentrated and labelled F3. Finally, the extract in the column was extracted with acetone–water (70:30), and the eluate was concentrated and labelled F4.

All four fractions were analyzed using thin layer chromatography (TLC) to determine their composition. The solvent system for TLC analyses was toluene/acetone/formic acid (3/6/1). The components in each fraction were detected by UV-light. In addition, the compositions of the fractions, in terms of number and types of monomer units, molecular weights of