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The fate of ^{14}C-naphthalene in soil microcosms containing Scots pine seedlings and enchytraeids

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Abstract

The fate of freshly spiked and aged ^{14}C-naphthalene associated residues as investigated in the presence and absence of ectomycorrhizal Scots pine seedlings and enchytraeid worms, in a factorial experimental design. Microcosms were used which enabled the ^{14}C-labelled naphthalene associated residues to be quantified, including plant lipids which acted as an additional naphthalene sink within the microcosms. The presence of plant roots altered the availability of the ^{14}C-naphthalene and associated residues to degrading microbes. Mineralisation and volatilisation of ^{14}C naphthalene in freshly spiked soil were lower in the presence of Scots pine. Conversely, in soil aged for 180 d, Scots pine increased mineralisation, and bioavailability of naphthalene. Root-mediated processes, microbial activity and enchytraeids interact with desorption, bioavailability and mineralisation of naphthalene.

Keywords: Naphthalene; Scots pine; Enchytraeids; Ageing; Desorption

1. Introduction

Naphthalene is a polycyclic aromatic hydrocarbon (PAH), composed of two fused carbon rings, listed by the US EPA as a priority pollutant (Niu et al., 2003). It is produced by a number of industries, mainly from the incomplete combustion of fossil fuels (Wild and Jones, 1995), and is found at elevated concentrations in nearly all terrestrial ecosystems, largely through historical deposition (Liu et al., 2000). Once in the soil, naphthalene mobility and degradation may be affected by mineralisation, volatilisation, sequestration, bioaccumulation, leaching and photodegradation.

The fate and distribution of this type of non-polar contaminants are strongly influenced by sorptive interactions with soil; increasing contact time with soil results in a reduction in the bioavailability of the compound, known as the ageing process (Semple et al., 2003).

In the UK, coniferous forests cover 1.6 million ha, 6% of the land area (UK Agriculture, 2006). Naphthalene can be transported atmospherically (van Brummelen et al., 1996), remaining in the atmosphere for several days (Bodnár et al., 2005), travelling long distances to coniferous forests where it can be adsorbed to the lipophilic needle cuticles through gaseous and particulate dry and wet deposition (Horstmann and McLachlan, 1998). Naphthalene is incorporated into the organic horizon of the soil after the needles are shed, and concentrations of 154 $\mu g\,kg^{-1}$ naphthalene, have been reported in coniferous forest soil (Wild and Jones, 1995), which may remain constant with increasing soil depth (Krauss et al., 2000). Little is known regarding the complex interplay of biota in the coniferous forest system. Enchytraeids are highly abundant in UK coniferous forests, with up to 100,000 m^{-2} of soil (Killham, 1994). *Cognettia* are non-specific feeders, they consume large quantities of material and strip of the organic matter, by doing this they are essential in bioturbation of the soil, and subsequently increase soil microbial numbers. Thus,

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positively affecting humification and decomposition of organic matter, releasing nutrients (especially carbon and nitrogen), which can subsequently be taken up by plants (Briones and Ineson, 2002; Setälä, 1995).

The aim of the present study was to investigate the effects of Scots pine seedlings, with and without enchytraeids, on the fate of naphthalene that was both freshly spiked and aged in soil. Progressive quantification of PAH volatilisation and mineralisation was recorded through the use of specially designed glass microcosms and ^{14}C-labelled naphthalene. Desorption rates of naphthalene were also investigated to identify the availability and temporal mobility of naphthalene in soil.

2. Materials and methods

2.1. Field sampling

A single soil sample was collected from Deepsyke, Edinburgh (British National Grid Reference NT 184525); a 1.5 ha, 15 yr^{-2} old unfertilised plantation of Sitka spruce (*Picea sitchensis* Bong. Carr) with soil characteristics pH 2.8 ± 0.1, moisture content, 81% ± 0.01, carbon content, 53% ± 0.4, and nitrogen content, 2% ± 0.04. Samples were taken approximately 50 m within the forest, from the O-horizon (top 10 cm of soil) where enchytraeids are most abundant (Didden and de Fluiter, 1998). All soil samples were stored at 12 °C.

Mycorrhizal Scots pine seedlings (1.5–2 yr old, mean total length 15 cm) were collected from a Scots pine plantation in Aberdeenshire (British National Grid Reference NJ 437028). As seedlings were collected from the field mycorrhiza were present on roots. Seedlings were watered every 2 d and allowed a 1-month acclimatisation period in a greenhouse. The majority of soil was carefully removed from the root system prior to being transplanted into microcosms.

2.2. Enchytraeid extraction

Enchytraeids were extracted from the Deepsyke soil using O'Connors' (1955) wet funnel method. The funnel was filled with tap water until the soil was waterlogged and a 60 W light bulb was placed over the funnel. After 24 h the water was collected from the base of the funnel from which the enchytraeids *Cognettia sphagnetorum* and *C. glandulosa* were collected, as identified under a LEICA Mz8 microscope (× 16).

2.3. Microcosm design

Microcosms were constructed out of Quickfit glass in such a way that air was drawn in through a volatilisation trap. The volatile trap consisted of 0.8 g glass wool per microcosm, from 150 g glass wool coated with 20 ml mineral oil added in 1 l hexane. Air flowed over the soil and then through another volatile trap (7.2 g glass wool per microcosm) followed by the 1 M $NaOH_{(aq)}$ (10 ml) $^{14}CO_2$ trap. Air was drawn continuously from the top of each microcosm at a rate of 17.5 ml min^{-1} by a multi-channel Watson-Marlow^{TM} peristaltic pump. Microcosms were left in a growth cabinet (Sanyo) at 10 °C, one period of 16 h daylight per day, for 91 d. Traps were replaced on 2, 5 and 7 d, and weekly thereafter.

2.4. Fate of ^{14}C-naphthalene residues in soil

The invertebrate community in the Deepsyke soil was killed by two 24 h freeze-thaw cycles at −80 °C, and sieved to 1 cm. Approximately 15 g of sieved soil was then spiked with 20 μg g^{-1} of naphthalene, containing 90 Bq g^{-1} of [uniformly labelled-^{14}C] naphthalene (Sigma-Aldrich, UK), added to a gram of soil, dissolved in 300 μl hexane (156.3 pM). This amount of naphthalene was chosen, as it is a realistic concentration that can be found in coniferous forests (Wild and Jones, 1993). This soil was placed in a 250 ml round bottom flask and mixed manually. Soil was gradually added until there was 100 g per microcosm (Reid et al., 1998). Three microcosms were inoculated with 50 enchytraeids (equivalent to a density of 33,000 m^{-2}). Three microcosms received a Scots pine seedling and 50 enchytraeids, and a further 3, received only a Scots pine seedling. Three unspiked microcosms without enchytraeids and seedlings were prepared as controls. Soil received 4 ml of water every 14 d.

For naphthalene aged in soil approximately 15 g of sieved soil was then spiked with 20 μg of naphthalene, containing 250 Bq of [uniformly labelled-^{14}C] naphthalene, added per gram of soil, dissolved in 5 ml of hexane, and mixed manually in a 500 ml Duran bottle. Progressively more soil was gradually added until there was 200 g of soil per bottle. Duran bottles were left upside down in a growth cabinet (Sanyo) at 10 °C, for one period of 16 h daylight per day. After 180 d of ageing, 3 × 67 g of soil from the same Duran bottle was re-moistened and put into three 250 ml round bottom flasks.

Three microcosms were not inoculated with organisms; another 3 were inoculated with 50 enchytraeids (equivalent to a density of 33,000 m^{-2}). A further 6 microcosms had a field grown Scots pine seedling transplanted into them, with 3 of these also being inoculated with 50 enchytraeids.

2.4.1. Mineralisation of ^{14}C-naphthalene residues in soil

The rate of mineralisation was measured by adding 1 ml of Hionic Fluor scintillation fluid (Perkin-Elmer, UK) to 100 μl of 1 M $NaOH_{(aq)}$. The ^{14}C activity of a further 100 μl 1 M $NaOH_{(aq)}$ was determined after washing with hexane (5 ml) to remove any dissolved volatile organic ^{14}C-activity. ^{14}C-activity was then added to the volatilisation results.

2.4.2. Volatilisation of ^{14}C-naphthalene residues

The volatile traps consisted of 7.2 g glass wool per microcosm, from 150 g glass wool coated with 20 ml mineral