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The fate of catechol in soil as affected by earthworms and clay

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ABSTRACT

The effect of endogeic earthworms (*Octolasion tyrtaeum*) and the availability of clay (Montmorillonite) on the mobilization and stabilization of uniformly ^{14}C-labelled catechol mixed into arable and forest soil was investigated in a short- and a long-term microcosm experiment. By using arable and forest soil the effect of earthworms and clay in soils differing in the saturation of the mineral matrix with organic matter was investigated. In the short-term experiment microcosms were destructively sampled when the soil had been transformed into casts. In the long-term experiment earthworm casts produced during 7 days and non-processed soil were incubated for three further months. Production of CO_2 and $^{14}CO_2$ were measured at regular intervals. Accumulation of ^{14}C in humic fractions (DOM, fulvic acids, humic acids and humin) of the casts and the non-processed soil and incorporation of ^{14}C into earthworm tissue were determined.

Incorporation of ^{14}C into earthworm tissue was low, with 0.1 and 0.44% recovered in the short- and long-term experiment, respectively, suggesting that endogeic earthworms preferentially assimilate non-phenolic soil carbon. Cumulative production of CO_2-C was significantly increased in casts produced from the arable soil, but lower in casts produced from the forest soil; generally, the production of CO_2-C was higher in forest than in arable soil. Both soils differed in the pattern of $^{14}CO_2$-C production; initially it was higher in the forest soil than in the arable soil, whereas later the opposite was true. *Octolasion tyrtaeum* did not affect $^{14}CO_2$-C production in the forest soil, but increased it in the arable soil early in the experiment; clay counteracted this effect. Clay and *O. tyrtaeum* did not affect integration of ^{14}C into humic fractions of the forest soil. In contrast, in the arable soil *O. tyrtaeum* increased the amount of ^{14}C in the labile fractions, whereas clay increased it in the humin fraction.

The results indicate that endogeic earthworms increase microbial activity and thus mineralization of phenolic compounds, whereas clay decreases it presumably by binding phenolic compounds to clay particles when passing through the earthworm gut. Endogeic earthworms and clay are only of minor importance for the fate of catechol in soils with high organic matter, clay and microbial biomass concentrations, but in contrast affect the fate of phenolic compounds in low clay soils.

1. Introduction

Phenolic compounds are the most widespread secondary plant metabolites with several thousand different compounds known (Siqueira et al., 1991; Hättenschwiler and Vitousek, 2000). They comprise up to 60% of plant dry mass (Northup et al., 1998) being responsible for UV protection, defence against pathogens and herbivores, and contribute to the colouring of plant tissues (Hättenschwiler and Vitousek, 2000). Chemically phenolic compounds are composed of an aromatic ring with one (phenol) or more (polyphenol) hydroxyl substituents and functional derivates such as esters, methyl esters or glycosides (Kuiters, 1990). They are ubiquitously distributed in the soil forming up to 10% of the total soluble organic carbon (Gallet and Keller, 1999) entering the soil on two different pathways: they are leached by rain from the tree canopy and are present in above- and below-ground litter, of which the latter is the dominant pathway (Hättenschwiler and Vitousek, 2000; Meier et al., 2008). In the soil phenolic compounds undergo various transformations primarily due to biological activity. They may rapidly degrade and mineralize, dissolve or be leached, adsorb to clay and humic particles, build chelates with metal ions like aluminium or iron ions, or be transformed into humic substances. These transformations are mediated by heterotrophic microorganisms altering phenolic compounds together with amino acids and proteins thereby forming stable humic substances (Martin and Haider, 1980; Vinken et al., 2005). Hence, phenolic compounds are

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stabilized in the soil matrix, mobilized as CO_2 or leached depending on biotic and abiotic soil conditions.

Phenolic compounds alter the composition and activity of the soil decomposer community. They restrict the activity and abundance of soil fauna and stimulate or inhibit the activity and biomass of soil microorganisms, thereby significantly affecting litter degradation and nutrient cycling (Kuiters, 1990; Northup et al., 1995; Hättenschwiler and Vitousek, 2000). The polyphenol concentration of litter is one of the most important factors determining litter palatability for decomposer soil fauna (Hendriksen, 1990; Tian et al., 2000).

Earthworms form a major part of the soil decomposer macrofauna and play an important role in organic matter processing and nutrient cycling in temperate ecosystems. Particularly endogeic earthworms which live in non-permanent burrows in the upper mineral soil ingesting soil and organic matter equivalent to two to five times their own body mass per day, forming organo-mineral complexes and thereby strongly affecting microbial activity and decomposition of organic matter (Scheu, 1995; Guggenberger et al., 1996; Marhan and Scheu, 2006; Mummey et al., 2006). Due to preferential feeding on microsites rich in organic matter and low assimilation efficiency earthworm casts are rich in nutrients and provide favourable conditions for microorganisms (Tiunov and Scheu, 1999). However, although casts are microbial hotspots, organic matter is generally protected from microbial degradation in inner compartments (Guggenberger et al., 1995), but degradation of organic matter enclosed in earthworm casts changes in time (Tiunov and Scheu, 1999). In fresh casts mineralization is enhanced since microorganisms utilise easily available nutrients from processed soil aggregates and organic matter primarily on the cast surface. In aging casts the availability of nutrients declines, nutrients become limited due to the reduced accessibility of microorganisms to nutrients enclosed inside cast aggregates, resulting in decreased carbon mineralization. When casts break up again enclosed nutrients become exposed and mineralization increases again. Recent studies have shown that the soil matrix may alter these processes by affecting the stability of casts. For example, Marhan and Scheu (2006) demonstrated that the availability of a humus unsaturated mineral soil matrix increases the stabilization of organic matter in earthworm casts. Further, clay increases the stability of cast aggregates and protects the organic matter therein against microbial degradation (Feller and Beare, 1997; Wolters, 2000).

Despite the high concentration of polyphenols and their persistence in the soil, little attention has been paid to the interaction of soil fauna and the soil mineral matrix in affecting the fate of phenolic compounds in soils. Considering the growing interest in the stabilization of carbon in soils, knowledge on the biotic and abiotic mechanisms affecting the fate of phenolic compounds in soils is required. We analysed the effect of the endogeic earthworm species *Octolasion tyrtaeum* (Savigny) and the availability of clay on the fate of catechol in arable and forest soil. By using arable and forest soil the effect of clay and earthworms on the stabilization of catechol in soils differing in the saturation of the mineral matrix with organic matter was investigated. Catechol was used as a model substance of monomeric phenols being commonly formed during microbial degradation of many naturally occurring and anthropogenic aromatic substances and regarded as a precursor of soil humic substances (Haider et al., 1975; Ji and Schäffer, 2002). Uniformly ^{14}C-labelled catechol was used enabling us to follow the fate of catechol in soils. Two experiments were set up to investigate short- and long-term processes.

2. Materials and methods

2.1. Experimental set-up

Arable soil was sampled from the long-term fertilization experiment in Bad Lauchstädt (Germany, Saxony-Anhalt). The long-term annual mean temperature in Bad Lauchstädt is 8.7 °C and the annual precipitation is 484 mm. The soil is a Mollisol, Haplic Chernozem loam (FAO classification) with 17.6% clay, 70.5% silt, 10.6% fine sand (63–250 μm) and 1.3% coarse sand (>250 μm). The soil was of neutral pH (6.9; 0.01 M $CaCl_2$, 1/2.5 w/v) with 1.8% C and 0.2% N. Forest soil was sampled in a 130-year old beech wood on limestone near Göttingen (Germany, Southern Lower Saxony). Long-term annual mean temperature in Göttingen is 7.9 °C and annual rainfall is 720 mm. The soil is shallow and of Redzina type with 29.6% clay, 61.4% silt, 6.3% fine sand (63–250 μm) and 2.7% coarse sand (>250 μm). It was of neutral pH (7.0; 0.01 M $CaCl_2$, 1/2.5 w/v) with 13.0% C and 1.0% N. Soil samples were taken from the upper 10 cm of the study sites, sieved (4 mm) to remove stones and large plant residues and defaunated by freezing at −28 °C for 5 days. Two weeks before the experiments were set up the soil samples were kept at 4 °C.

Adult specimens of *O. tyrtaeum* were collected by digging and hand-sorting in the beech forest described above. Earthworms were transferred to the laboratory, identified and kept in containers filled with soil from the beech forest for 4 weeks at 4 °C. Two weeks before the experiments were set up earthworms for the arable soil treatments were incubated in containers with soil of the arable field to avoid contamination with forest soil.

The short-term experiment was set up in 36 microcosms consisting of glass flasks (height 107 mm, diameter 100 mm), closed at the top by lids. The soils were packed into small plastic vessels (height 40 mm, diameter 30 mm) and placed into the microcosms. Small plastic vessels filled with 3 ml alkali (1 N NaOH) were placed into the microcosms to absorb CO_2 released from the soil. One half of the microcosms were filled with 5 g dry weight arable soil, the other with 5 g dry weight forest soil. Before adding the soil 0.5 g dry weight clay (Bentonite, Montmorillonite; Riedel-de Haën, Seelze, Germany) was mixed homogeneously into half of the microcosms to establish the clay treatments. ^{14}C -labelled catechol prepared as described in detail in Ji and Schäffer (2002) was added as a water solution into the soils resulting in a final specific radioactivity of 39 and 37 kBq g^{-1} dry weight soil in the arable and forest soil, respectively. The soils were adjusted to a final water content of 60% of the maximum water holding capacity and mixed homogeneously. One individual of *O. tyrtaeum* was added per microcosm to half of the soil and soil–clay treatments to establish the following treatments: arable (A) and forest soil (F), arable (AC) and forest soil with clay (FC), arable (AOt) and forest soil with *O. tyrtaeum* (FOt) and arable (ACOt) and forest soil with clay and *O. tyrtaeum* (FCOt). Four replicates per treatment were established; four empty microcosms served as control for quantification of the mineralization of ^{14}C catechol. Microcosms were incubated in darkness in a climate chamber at 20 °C until the soil in the earthworm treatments was transformed into casts.

The long-term experiment was set up in 40 microcosms consisting of Perspex tubes (height 135 mm, diameter 60 mm) with ceramic plates at the base fixed on a box. Microcosms were closed by lids at the top. Small vessels attached to the lids were filled with 3 ml alkali (1 N NaOH) to absorb CO_2. Microcosms were drained by lowering the atmospheric pressure (−0.2 to −0.4 bar) in a box below the ceramic plates. Leaching water of each microcosm was sampled in separate vessels placed in the box. Treatments were established as described above but with 10-fold amount of soil, clay and earthworm individuals per microcosm. The final specific radioactivity of ^{14}C catechol in the soil for this experiment was 0.93 kBq g^{-1} dry weight soil. Treatments with earthworms were replicated five times, those without four times; four empty microcosms served as control for quantification of mineralization of ^{14}C catechol. Microcosms were incubated in darkness in a climate chamber at 20 °C and watered once a week with 10 ml distilled water. After the soil had been transformed into casts, earthworms