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Effects of addition of maize litter and earthworms on C mineralization and aggregate formation in single and mixed soils differing in soil organic carbon and clay content

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ABSTRACT

C mineralization and aggregate stability directly depend upon organic matter and clay content, and both processes are influenced by the activity of microorganisms and soil fauna. However, quantitative data are scarce. To achieve a gradient in C and clay content, a topsoil was mixed with a subsoil. Single soils and the soil mixture were amended with 1.0 mg maize litter C g soil^{-1} with and without endogeic earthworms (*Aporrectodea caliginosa*). The differently treated soils were incubated for 49 days at 15 °C and 40% water holding capacity. Cumulative C mineralization, microbial biomass, ergosterol content and aggregate fractions were investigated and litter derived C in bulk soil and aggregates were determined using isotope analyses. Results from the soil mixture were compared with the calculated mean values of the two single soils. Mixing of soil horizons differing in carbon and clay content stimulated C mineralization of added maize residues as well as of soil organic matter. Mixing also increased contents of macro-aggregate C and decreased contents of micro-aggregate C. Although *A. caliginosa* had a stimulating effect on C mineralization in all soils, decomposition of added litter by *A. caliginosa* was higher in the subsoil, whereas *A. caliginosa* decreased litter decomposition in the soil mixture and the topsoil. Litter derived C in macro-aggregates was higher with *A. caliginosa* than with litter only. In the C poor subsoil amended with litter, *A. caliginosa* stimulated the microbial community as indicated by the increase in microbial biomass. Furthermore, the decrease of ergosterol in the earthworm treated soils showed the influence of *A. caliginosa* on the microbial community, by reducing saprotrophic fungi. Overall, our data suggest both a decrease of saprotrophic fungi by selective grazing, burrowing and casting activity as well as a stimulation of the microbial community by *A. caliginosa*.

Introduction

Decomposition of soil organic carbon (SOC) as well as soil aggregation are important processes that regulate soil functions. These processes are influenced by the quality and quantity of SOC and clay (Swift et al., 1979; Denef and Six, 2005). The activity of the microbial and faunal decomposer community also affects these processes (Ketterings et al., 1997; van Breemen and Finzi, 1998). Furthermore, the processes are highly interlinked, since aggregation decreases the accessibility of organic matter and promotes the occlusion of plant residues (Denef et al., 2001) resulting in a decrease of C-mineralization. Correspondingly it has been widely shown for topsoils that intensive disturbance of aggregates increases CO_2 production (Gupta and Germida, 1988; Franzluebbers, 1999).

Generally, one may expect decreased C mineralization in subsoils compared with topsoils, since subsoils are often characterized by a low content of organic matter and reduced microbial activity, resulting in a higher mean C residence time than for topsoils (Trumbore, 2000). On the other hand, subsoils are mostly less aggregated (Taylor et al., 2002). Therefore, comparison of C mineralization between topsoils and subsoils does not give consistent results. For instance, mineralization of topsoil SOC over a wide temperature and moisture range was twice that in subsoils (Lomander et al., 1998). In contrast, no consistent difference in C mineralization between topsoil and subsoil was observed over a wide range of water potentials in a short-term incubation experiment (Fierer

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Table 1
pH values, C and N content, soil texture, and water holding capacity of the soils (mean ± standard deviation, $n = 5$; soil texture $n = 1$).

Soil	pH $CaCl_2$	C (mg g^{-1})	N (mg g^{-1})	C/N	Clay (%)	Silt (%)	Sand (%)	WHC (%)
Topsoil A	5.68 ± 0.02	16.9 ± 0.04	1.8 ± 0.01	9.2	24	68	7	69.1
Subsoil B	5.84 ± 0.02	1.4 ± 0.14	0.1 ± 0.01	11.3	11	57	33	37.8
Mixed soil AB	5.72 ± 0.02	8.9 ± 0.07	0.9 ± 0.01	9.6	18[a]	62[a]	20[a]	53.2

[a] Calculated mean of A and B.

et al., 2003). Salomé et al. (2010) found higher or equal C mineralization in topsoil and subsoil. The reasons for these different results are not sufficiently understood.

Mixing of different soil horizons is a naturally occurring process and is accomplished in temperate ecosystems predominantly by anecic earthworms (Lavelle and Spain, 2001). The mixing activities of earthworms may increase C mineralization. For instance, increasing bioturbation by *Aporrectodea caliginosa* resulted in high C mineralization in an agricultural and a forest soil (Wolters and Schaefer, 1993). Such an increase in C mineralization after the mixing of different soil substrates may be caused by the increased contact between microorganisms and substrate (Salomé et al., 2010). Overall, the effect on C mineralization and aggregation by earthworms has mostly been attributed to comminution of litter and processing of soil mixtures and soil litter mixtures in the gut, or selective feeding of the organisms, leaving hot spots enriched in mineral nutrient and labile carbon (Edwards, 2004).

In addition to a stimulating effect on C mineralization, the activity of earthworms may also stabilize C, as has been observed in earthworm casts (Scheu and Wolters, 1991). This stabilization has been attributed to the enclosure of labile C in cast or stable aggregates (Shipitalo et al., 1988; Marinissen and Dexter, 1990; McInerney et al., 2001; Bossuyt et al., 2005). Marhan and Scheu (2005) reported that while for a forest soil C mineralization was less for earthworm casts than for soil that had not been consumed by earthworms, this was not the case for an arable soil of lower clay content. Thus, the effect of earthworms on C mineralization may mainly depend on the contents of clay and organic matter, which regulate the processes of organic residues comminution and aggregate enclosure.

The effect of earthworms on C mineralization and sequestration processes may be mediated by their influence on the microbial biomass. However, studies on effects of earthworms on microbial biomass have yielded contrasting results. In one study, gut passage did not change the microbial biomass (Daniel and Anderson, 1992), but in another active microbial biomass increased (Scheu, 1992). In contrast, Zhang et al. (2000) concluded from a short term incubation experiment with the anecic earthworm *Metaphire guillelmi* that microorganisms were used by earthworms as a secondary food resource, and that passage through the earthworm gut decreased the total soil microbial biomass. Similarly, microbial biomass as well as respiration rate decreased in soils to which *Lumbricus terrestris* had been added (Devliegher and Verstraete, 1995). However, in another study, microbial biomass and respiration have been shown to be greater in fresh earthworm casts than in the parent soil (Tiunov and Scheu, 2000; Aira et al., 2003).

In general, the processes that govern C mineralization and aggregation in topsoils and subsoils are not completely understood. This is especially true for the effects of earthworm activity on these processes. To elucidate the interaction of aggregation and C mineralization in the presence of earthworms, incubation experiments were conducted with a topsoil, a subsoil and a mixture of the two. Treatments included the addition of maize litter and *A. caliginosa*. Stable isotope analysis in bulk soil as well as in aggregates enabled the differentiation between both origins of organic C, C derived from soil organic matter and maize litter. It is hypothesized that (1) mixing of the two horizons results in a linear relationship between organic C and clay content as well as C-mineralization and aggregation and (2) earthworms increase litter decomposition but that at the same time an increase of aggregation and occlusion of maize derived C in aggregates may reduce the expected increased C-mineralization mediated by earthworms.

Materials and methods

Soils, earthworms and maize litter

An incubation experiment was conducted with three different soil materials. Topsoil A was obtained from the upper soil layer (0–10 cm) of a rendzic Leptosol (IUSS Working Group WRB, 2006) derived from shell limestone covered with loess. The soil was under permanent grassland, located at Drakenberg near Göttingen (Southern Lower Saxony, Germany). Subsoil B was taken from a lower soil layer (170–180 cm) of a haplic Luvisol derived from loess under beech forest, located near Burg Ludwigstein (Northern Hessia). Soil AB was prepared by mixing equal amounts of soil from topsoil A and subsoil B. Characteristics of the soils are shown in Table 1. Measured values of AB differed from the mean of A and B by less than 7%. Endogeic earthworms of the species *Aporrectodea caliginosa* were sampled with an electrical octet-method (Thielemann, 1986; Schmidt, 2001). For the incubation experiment, only adult earthworms were used and kept on moist paper for 48 h to void their guts. During the 49 days of incubation (described below) the weight of the earthworms did not change. Maize was grown in a greenhouse and green leaves were harvested after 66 days. Chemical properties of maize leaves (mean ± standard deviation, $n = 3$) were 431 (±1) mg C g^{-1}, 32 mg N g^{-1} (±0.4) dry weight, and $\delta^{13}C$ was −12.4‰ (±0.1) V-PDB.

Incubation experiment

Soils were sieved (2 mm), homogenized, remaining litter particles were removed and soil equivalent to 400 g DW was incubated in 3-litre glass jars. Soils were pre-incubated for 3 days at 45% water holding capacity and 15 °C in permanent darkness. The incubation experiment was conducted with five replicates per treatment as follows: (1) control without any addition, (2) addition of 1.0 mg C g $soil^{-1}$ green maize leaves (chopped in 10 mm pieces and mixed into the soil), and (3) addition of four adults of *A. caliginosa* with 1.0 mg C g $soil^{-1}$ green maize leaves (chopped in 10 mm pieces and mixed into the soil). Soils were incubated for 49 days under the same conditions as during the pre-incubation. Evolved CO_2 was absorbed in 10 ml (control) and 50 ml (litter treatments) NaOH solution (1.0 M) placed in jars, which were changed after 4–7 days. A 10 ml aliquot was back-titrated by adding 1 M HCl after addition of 5 ml saturated $BaCl_2$ solution (Zibilske, 1992). Incubation vessels were flushed with air after changing of the NaOH solution. Desiccation of samples was checked by weighing, but no addition of water was necessary.

Aggregate fractionation

Aggregate fractionation was conducted by sieving in distilled water using the method described by Haynes (1993). Briefly,