The effect of earthworms (*Lumbricus rubellus*) and simulated tillage on soil organic carbon in a long-term microcosm experiment

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ABSTRACT

In a long-term (2.4 years) laboratory experiment, we studied carbon (C) loss from microcosms in which intact litter was placed on the soil surface with or without earthworms (*Lumbricus rubellus*) or was fragmented and mechanically mixed into the soil to simulate the tillage. Two soil and leaf litter combinations common in post-mining sites near Sokolov (Czech Republic) were used: clay with alder (*Alnus glutinosa*) and sand with willow (*Salix caprea*).

During the first 20 weeks, respiration was highest with surface litter with earthworms, lowest with mechanical mixing, and intermediate with surface litter minus earthworms. From week 20—80, respiration did not differ among the treatments. From week 80—126, respiration was highest when fragmented litter was mechanically mixed into soil. These results applied to both combinations of soil and litter types. At week 126, C stock was highest with surface-applied litter minus earthworms but most of the litter remained on the soil surface. In the mineral soil, the active C pool was significantly lower with surface-applied litter minus earthworms than in the other treatments. Based on microbial biomass and ergosterol content, microcosms with surface-applied litter minus earthworms were dominated by fungi while those with earthworms or litter that was mechanically mixed into soil were dominated by bacteria. Overall, the results indicate that C sequestration in soil is greater when litter is mixed into the soil by earthworms than by mechanical mixing.

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1. Introduction

Soil organic matter plays many important roles in terrestrial ecosystems including the maintenance of fertility, the enhancement of soil structure and porosity, and the storage of carbon (C) (Wolters, 2000). Soil organic C is a major pool in the global C cycle and because of its dynamic nature, soil organic C could serve as either a significant sink or source for atmospheric carbon dioxide (Post et al., 1982).

The principal source of soil organic C is plant biomass. Plant biomass decomposition is an important biological process driven by a range of complex and interacting factors, such as climate, biomass composition, soil physical and chemical properties, and soil organisms (Lavelle et al., 1997). Microorganisms are largely responsible for C and nitrogen (N) mineralization but are directly and indirectly affected by soil macrofauna and other soil invertebrates over a wide range of spatial and temporal scales (Anderson, 1988). The soil macrofauna contribute to plant biomass decomposition by digesting the biomass, increasing its surface area through fragmentation, and inoculating the biomass with microorganisms (Singh et al., 1999; Wolters, 2000).

C accumulation in soil is particularly important in the restoration of those ecosystems in which soil organic matter has been reduced by long-term tillage, deforestation, mining, or other disturbances (Lal et al., 2003). Tillage increases both the rate of organic matter decomposition and the leaching of nitrates and other nutrients from soil (Toyota et al., 2013) and changes the composition and abundance of soil microorganisms and invertebrates (Six et al., 1998). Non-tillage, in contrast, enhances soil aggregation and thereby reduces the decomposability of organic matter and increases C sequestration (Lal et al., 2003).

Relative to soil aggregation and other aspects of soil formation and function, earthworms are the most studied group of soil fauna (Pashanasi et al., 1992, Edwards and Bohlen, 1996, Pashanasi et al., 1996. Although the extent to which earthworms benefit soil formation and function may vary between soil types (Oades, 1993). Earthworms play a key role in removing plant litter, dung, and other

organic material from the soil surface and incorporating these materials into the soil (Martin, 1991). According to Raw (1962), earthworms *Lumbricus terrestris* in apple orchard in UK, consume up to 2 t of litter/ha/year, which can represent 100% of the annual litter fall. Edwards and Bohlen (1996) estimated that 7–90 t of top soil/ha/year passes through the guts of earthworms in temperate soils.

Earthworms enhance aggregation and are assumed to stimulate the formation of organo-mineral complexes (Shipitalo and Protz, 1988; Lee and Foster, 1991; Bossuyt et al., 2005). The adsorption of C on the surface of mineral particles is considered an important mechanism of C stabilization, a mechanism that may be enhanced by earthworm activity (Lavelle, 1988; Marinissen and Dexter, 1990). Aggregates physically protect soil organic matter and is crucial for the long-term storage and stabilization of soil C (Scullion and Malik, 2000; Six et al., 2004).

The overall objective of the current study was to clarify the mechanisms by which the epigeic earthworm *Lumbricus rubellus* affects different fractions of soil C. In a long-term laboratory experiment, we added leaf litter to the surface of soil microcosms with or without earthworms. In a third treatment, which did not include earthworms, we mechanically mixed fragmented litter into the soil to simulate tillage. We tested the hypothesis that carbon sequestration would be greater in microcosms with surface-applied litter and earthworms than in microcosms in which fragmented litter was mechanically mixed into the soil without earthworms. Despite fact that this is a laboratory experiment it is inspired real word situation in post mining sites near Sokolov where *L. rubellus* belongs to the most important early colonizers and two contrasting combination of litter and vegetation reflect common combination of substrate and vegetation found in field (Frouz et al., 2001; Mudrák and Frouz, 2012).

2. Materials and methods

2.1. Collection of materials

A laboratory experiment was performed with soil collected from two post-mining sites near Sokolov, Czech Republic. Both sites were ca. 10 years old (i.e., the soil had been deposited by mining operations 10 years earlier) and supported little vegetation. One site contained sand without any organic matter and the other contained tertiary clay. The soils were collected from a depth of 3–10 cm. The sand was passed through a 2-mm screen, and the clay was passed through a 5-mm screen. Neither the sand nor the clay contained earthworms. Carbon content in sand was 0.4% in clay 2.4% respectively. Dominant clay minerals in used clay were kaolinite and illite (Kříbek et al., 1998).

Two types of leaf litter were used: alder (*Alnus glutinosa*; C content 44.4) and willow (*Salix caprea*; C content 42.1%). Alder and willow are the dominant trees at post-mining sites near Sokolov. Alder has been widely planted as part of the restoration of clay soils (Frouz et al., 2001) and produces leaf litter with a C:N ratio of 23 (Frouz et al., 2013b). Willow naturally colonizes sandy or less weathered post-mining sites (Mudrák and Frouz, 2012) and produces leaf litter with a C:N ratio of 31(Frouz et al., 2013b) Alder litter was used with the clay soil, and willow litter was used with the sandy soil. Litter were collected at the time of natural litter fall, using litter traps consisting of nylon mesh sacks fixed on wooden frames (0.5 × 0.5 m) and located 0.5 m above the soil surface (Frouz et al., 2009). Collected materials were hand sorted to remove small branches and woody debris, and leaf litter was cut into pieces ca. 1 cm × 3 cm and homogenized. Leaf litter was then air dried and stored in paper sacks in the dark.

The epigeic earthworm *L. rubellus* inhabits the litter and upper layers of mineral soil and produces excrements (casts) with mixed organic and mineral particles (Frouz et al., 2007). *L. rubellus* is among the first colonisers of post-mining heaps (Frouz et al., 2013a,b). Earthworms were collected in the same locations as the litter (Frouz et al., 2009).

2.2. Experiment

Laboratory microcosms consisted of 250-ml glass bottles containing mineral soil and surface litter and from each of the two sites. The mineral layer (100 g per microcosm, dry weight equivalent) was moistened to field capacity with distilled water.

Before the leaf litter was added to the microcosms, its moisture content was increased to 70% (g of water/100 g dry litter). For remoistening with minimal leaching, the litter was placed in a plastic bag and repeatedly sprayed with distilled water. The moistened leaf litter were added to the microcosms in one of two ways: it was applied to the soil surface (or to the top of the existing litter layer when reapplied as described later), or it was crushed by hand while still dry, passed through a 1-mm sieve, moistened, and then mixed by spoon into the mineral soil in the microcosm. Each microcosm with surface litter contained two or zero specimens of *L. rubellus*; earthworms were not added to the microcosms with mechanically incorporated litter. The litter was added at the beginning of experiment and three times thereafter. The new litter was added within 3 weeks after the previously added litter had disappeared from the soil surface in microcosms with earthworms. This resulted in a total of four additions of litter and four periods after litter addition (Fig. 1). Each addition contained 4 g (dry weight equivalent) of litter, so that a total of 16 g was added to each microcosm. The microcosms were weekly checked throughout the experiment to confirm that the earthworms were alive and active, in several cases dead earthworm was observed, this was removed and replaced by another earthworm the same size. In two cases more then two worms were observed, when those reach size about
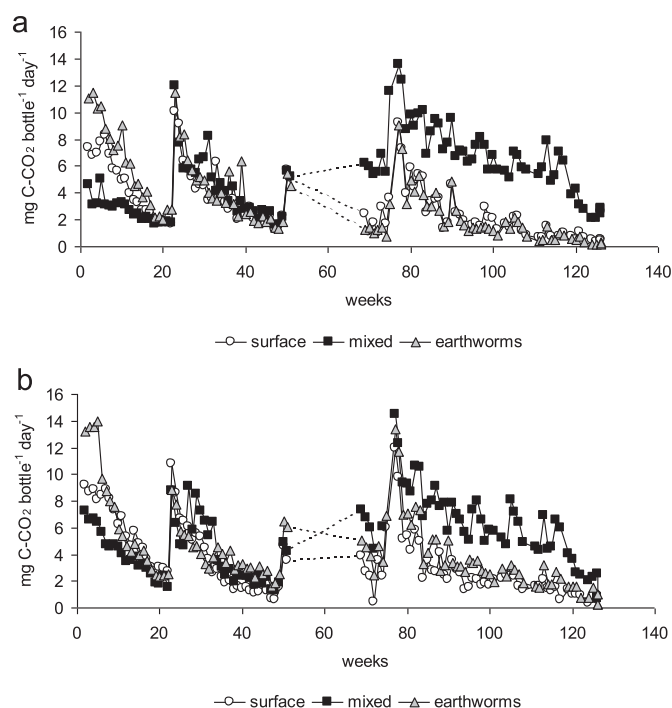

Fig. 1. Changes in the soil respiration in soil microcosms (bottles) over 126 weeks as affected by litter and earthworm treatments; treatments are described in Table 1. Microcosms contained (a) alder litter on a clay substrate or (b) willow litter on a sandy substrate. Litter was added four times, as indicated by arrows, resulting in four post-addition periods (I–IV).