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Corrigendum

Stabilization of soil organic matter by earthworms is connected with physical protection rather than with chemical changes of organic matter

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ABSTRACT

Earthworms are important drivers for the formation of soil structure and play a key role in soil organic matter (SOM) dynamics. Our previous long-term (126 weeks) laboratory experiment showed that carbon (C) loss declined through time in soil when litter was mixed and consumed by earthworms (*Lumbricus rubellus*). Eventually, the C loss was lower than in treatments where litter was mechanically mixed into soil with exclusion of earthworms. However, it is not clear if the solely physical manipulation of soil or biological activity of earthworms lead to different SOM quality, which would result in a distinction in C loss and consequently C sequestration. Thus, we differentiated between physical (mechanical mixing) and earthworm effects on SOM composition. Two types of soil were used in the experiment: clay and sand, and these were incubated with alder (*Alnus glutinosa*) and willow (*Salix caprea*) litter, respectively. The combination of soils and litter types corresponds to the natural combinations at the sampling sites.

To explain underlying mechanisms of a lower C loss in the earthworm vs. mechanically mixed treatment, we separated SOM fractions in order to gain pools defined in the Rothamsted model. Chemical differences between initial litter and the active and slow pool of SOM obtained by fractionation were studied. No significant differences between the earthworm and mechanically mixed treatment were found in C, nitrogen (N), and phenol contents, composition of major chemical groups of litter studied by solid-state ^{13}C NMR spectroscopy, and composition of aromatic components of SOM studied by analytical pyrolysis (Py GC/MS). This lack of differences in chemical composition suggests that greater SOM sequestration in the earthworm treatment is likely to be connected with physical protection of SOM inside cast aggregates rather than with chemical changes in SOM mediated by earthworms.

1. Introduction

Litter decomposition represents a crucial process leading to soil organic matter (SOM) formation and plays therefore an essential role in soil carbon (C) sequestration. Litter decomposition is driven by a complex interaction of various factors. Besides initial litter quality, climate and soil physical and chemical properties, the presence of soil fauna and various management practices play an important role in decomposition processes.

Earthworms as soil ecosystem engineers profoundly affect soil environment and thus litter decomposition (García-Palacios et al., 2014). They influence the storage of soil C and nitrogen (N) (Frouz et al., 2014; Ketterings et al., 1997; Lavelle and Martin, 1992; Zhang et al., 2003) through their high leaf litter consumption rates connected with the burrowing of plant material. Earthworms also positively influence soil physical properties such as aeration or water retention and infiltration (Bossuyt et al., 2005; Kladivko et al., 1997). An increase in earthworm density may therefore support an improvement of soil quality and consequently lead to a higher amount of sequestered C in soil.

The passage of soil and organic matter (OM) through the earthworm gut creates casts formed from differently sized aggregates. During aging, the casts become stable and SOM is highly protected inside these structures (Lavelle and Martin, 1992). Although earthworms belong to the most studied group of soil fauna (e.g. Edwards and Bohlen, 1995), the mechanism of SOM protection inside their casts is still not completely understood (Vidal et al., 2016).

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Many studies link the stabilization of SOM to physical protection within cast aggregates (e.g. Bossuyt et al., 2005; Lavelle and Martin, 1992; Six et al., 2004; Zhang et al., 2003). The pore diameters of cast aggregates are smaller (Görres et al., 2001) than in bulk soil and thus prevent microbial access to SOM within them (Bossuyt et al., 2005). Also, chemical changes of SOM composition inside casts were described (Guggenberger et al., 1996; Parle, 1963; Zhang et al., 2003). Earthworm casts are reported to be enriched in polysaccharides and lignin. Carbohydrates serve as cementing agents (Scullion and Malik, 2000), supporting enhanced stabilization of OM inside casts. Compared to initial litter, the relative content of carbohydrates may decrease and the relative contents of aliphatic and resistant lignin components may increase during the passage of OM through the guts of macrofauna as it was observed in fresh macrofauna casts (Frouz et al., 2015). Thus, it seems that the physico-chemical stabilization processes of SOM also play a role within casts.

In the present study, we investigate if earthworms influence the soil system mainly through mechanical activity or the earthworm impact comprises also chemical changes in OM that would alter C stabilization in soil. Our study benefited from a long-term (126 weeks) laboratory experiment (Frouz et al., 2014) comparing soil systems with and without presence of earthworms (*Lumbricus rubellus*). The same amount of litter was either consumed and mixed with soil by earthworms or was simply mixed and fragmented mechanically. The study by Frouz et al. (2014) showed that earthworms increased C mineralization shortly after they had been introduced into the system as compared to no-earthworm treatments. However, over a longer period of time, C mineralization decreased and consequently, C loss in soil was lower in earthworm treatments. In the present study, we especially focused on differences in chemical composition of SOM in the described treatments possibly being responsible for a lower C loss and a greater C sequestration in microcosms where OM was mixed into the soil by earthworm activity.

Two soil and leaf litter combinations were used in the experiment: clay with alder (*Alnus glutinosa*) and sand with willow (*Salix caprea*). These two combinations were used as they represent the most common combination of plant species and soil substrate in post-mining sites near Sokolov, where sampling was done. The post-mining sites provided the advantage of no earthworm occurrence and the soil substrates contained negligibly small contents of recent organic C. Thus, the impact of the earthworms could directly be detected. Incubation of two types of soil allowed us to observe whether soil particle size played a role in C loss and sequestration. Soil was fractionated according to Zimmermann et al. (2007) in order to obtain soil functional pools corresponding to those defined in the Rothamsted model. An active and a slow pool of SOM were used for chemical analysis. Investigating the mechanisms of how the C is sequestered in these treatments may substantially help to improve our understanding of C sequestration in post-mining sites and the role of earthworms in C sequestration in general.

2. Materials and methods

2.1. Collection of materials

The material used for the laboratory experiment was collected at post-mining sites near Sokolov, Czech Republic. The soil originated from two approximately 10 years old sites (i.e., the soil had been deposited by mining operations 10 years earlier as dumped material on the heaps) with little vegetation cover. One site consisted of sand and the other site contained tertiary clay with a predominance of kaolinite and illite (Frouz et al., 2013). Although small patches of toxic material exist in the mining area, the Cypris clays, which have been used in this experiment and which form the majority of overburden, do not have any toxicity issues; the same applies for the sand used (Frouz et al., 2005). Soil was collected from the top 10 cm following removal of the vegetation cover (i.e. of plants and litter). Sand material was passed through a 2-mm screen, and clay material was passed through a 5-mm screen. The C content was 4 g C kg^{-1} in sand and 24 g C kg^{-1} in clay material. Further soil substrate characterizations are given in Table 1.

Leaf litter used for the laboratory experiment corresponded to the dominant trees at Sokolov post-mining sites: alder (*Alnus glutinosa*) and willow (*Salix caprea*). Leaf litter was collected using litter traps (0.5 × 0.5 m frame consisting of a nylon mesh located 0.5 m above the ground) at the time of litter fall (October 2009). The collected litter was subsequently sorted to remove woody debris and small branches. Leaves were cut into pieces ca. 1 cm × 3 cm and air dried. The C content was 444 g C kg^{-1} in alder and 421 g C kg^{-1} in willow litter. Alder is a typical tree used for the controlled restoration of clay soils at Sokolov post-mining sites (Frouz et al., 2001), and willow is a characteristic colonizer of sandy soils at post-mining sites (Mudrák and Frouz, 2012). Based on these aspects, alder litter was incubated with the clayey soil and the willow litter with the sandy soil. The earthworms (*Lumbricus rubellus*) were collected at the same locations as the litter. The differentiation of *L. rubellus* from other earthworm species present at the sites was made based on general morphology on living specimen.

2.2. Experimental design and analysis

The experiment was designed as four different treatments. Two combinations of soil and litter (clay + alder; sand + willow) were treated either by mechanical mixing of litter into the soil, or by earthworm activity where the litter was left on the surface of incubated soil. Every combination had six replicates. In the experimental design, we also considered a treatment with neither earthworm activity nor mechanical mixing. However, because the litter biomass in this ‘control’ treatment was decomposed only in a relatively small area on the soil surface and likely some leaching of DOM, this treatment was inappropriate for the observation of differences in SOM.

Laboratory microcosms were 250-ml glass bottles filled with soil (sand or clay) and leaf litter (willow or alder). The mineral layer consisted of 100 g dry weight equivalent soil, which was moistened to field capacity determined on bulk soil (Kuráž et al., 2012). The leaf litters for the mechanical mixing with the two soils were homogenized using a 1-mm screen. The size of OM fragments was chosen according to previous findings (Frouz et al., 2011) where the size of OM fragments inside aggregates of earthworm origin were similar to that used in the present study. The leaf litters introduced into the earthworm treatments were pieces ca. 1 cm × 3 cm in size. Before adding the plant material to the microcosms, the leaf litter was moistened in plastic bags to 70% water content (g of water/100 g dry litter). This procedure minimized the leaching of soluble OM before addition to the microcosms.

Leaf litter was either added to the soil surface or mixed into the soil as homogenized pieces. In the earthworm treatments with the litter on top of the soil surface, two earthworms were added to each microcosm. In the mechanically mixed treatments, OM fragments were divided into four equal parts. The first part was spread uniformly on the surface and mixed into the soil using a spate for 5 min. Then, the next portion was added and the whole procedure was repeated until all litter was mixed into the soil. The litter was added to all treatments four times in total: once at the beginning and three times thereafter, always

Table 1
Chemical properties of the soil substrates used in the experiment. Chemical properties are from studies of Kuráž et al. (2012) and Frouz et al. (2016). The particle size distribution in the soil was determined using the FAO method.

Soil type	C (%)	N (%)	pH	Conductivity (μS cm^{-1})	Clay (%)
Clay	2.4	0.14	8.75	780	63
Sand	0.4	0.02	6.65	104.5	3