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Rolling in the deep: Priming effects in earthworm biopores in topsoil and subsoil

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ABSTRACT

Priming effect is the change of soil organic matter (SOM) decomposition due to the addition of labile carbon (C) sources. Earthworms incorporate organic matter into their burrow-linings thereby creating preferred habitats for microorganisms, but the roles of such burrows in priming effect initiation is unknown. Here we study the mechanisms driving SOM decomposition in top- and subsoil biopores and additionally in the rhizosphere. Given the topsoil was newly formed after ploughing 10 months prior to sampling, we hypothesized that (1) SOM accessibility, enzyme activities and efficiency of enzymatic reaction (K_a) are main drivers of different priming effect in biopores vs. bulk soil and rhizosphere, subsoil vs. topsoil and (2) the production of microbial enzymes in biopores depends on microbial community composition. To test these hypotheses, biopores formed by *Lumbricus terrestris* L. and bulk soil were sampled from topsoil (0–30 cm) and two subsoil depths (45–75 and 75–105 cm). Additionally, rhizosphere samples were taken from the topsoil. Total organic C (C_{org}), total N (TN), total P (TP) and enzyme activities involved in C-, N-, and P-cycling (cellobiohydrolase, β-glucosidase, xylanase, chitinase, leucine aminopeptidase and phosphatase) were measured. Priming effects were calculated as the difference in SOM-derived CO_2 from soil with or without ^{14}C-labeled glucose addition.

Enzyme activities (V_{max}) and the catalytic efficiency (K_a) were higher in biopores compared to bulk soil and the rhizosphere, indicating that the most active microbial community occurred at this site. Negative correlations between some enzymes and C:N ratio in bulk soil are explained by higher content of fresh organic C in the topsoil, and the corresponding C and nutrient limitations in the subsoil. The positive correlation between enzyme activities and Corg or TN in biopores, however, was associated with the decrease of C and TN with pore age in the subsoil. In the subsoil, priming effect in biopores was 2.5 times higher than bulk soil, resulting from the favorable conditions for microorganisms in biopores and the stimulation of microbial activities by earthworm mucus. We conclude that earthworm burrows provide not only the linkage between top- and subsoil for C and nutrients, but strongly increase microbial activities and accelerate SOM turnover in subsoil, contributing to nutrient mobilization for roots.

1. Introduction

The earthworm *Lumbricus Terrestris* L. is an anecic species inhabiting one single vertical burrow (drilosphere) throughout its entire life (Don et al., 2008), transporting fresh plant detritus from the soil surface downwards while mixing it with mineral soil particles (Lee, 1985; Brown et al., 2000). Earthworms alter soil structure (Lavelle, 1997), distribute litter carbon (C) throughout the entire soil profile (Jégou et al., 2000) and accelerate C turnover over longer time scale (Yavitt et al., 2015). Along burrows, the improved air circulation, enrichment of soil organic matter (SOM) and nutrients, as well as the water retention may reduce or even override the biogeochemical differences between top- and subsoil.

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Earthworm burrowing activities affect soil C stocks in the topsoil and subsoil altering microbial activities, for which enzyme activity is a sensitive indicator.

Metabolic enzymes are proteins produced by microbiota, plant roots and soil fauna to hydrolyze SOM. Thus, by stimulating microbial communities (Don et al., 2008), distributing extracellular enzymes (exoenzymes; Hoang et al., 2016a) and enhancing enzyme activities (Dempsey et al., 2013), earthworms indirectly and directly affect enzyme activities. Moreover, earthworm mucus, which is a water-soluble mixture of low molecular weight carbohydrates, acts as a primer for plant residue mineralization (Bityutskii et al., 2012). Similar to earthworms, also plant roots not only produce enzymes (Asmar et al., 1994) but also alter enzyme activities through modification of root morphology, exudation and interaction with microorganisms (Asmar et al., 1994; Fontaine et al., 2007; Razavi et al., 2016a). In order to understand the specificity of these biological processes (Bar-Even et al., 2011) and the sensitivity of enzymes to biotic effect (earthworms and roots) (German et al., 2012), kinetic enzyme parameters are approximated by the Michaelis-Menten equation. Other than the maximal catalytic reaction rate (V_{max}) and substrate affinity (K_m), the catalytic efficiency (K_a – determined as V_{max}/K_m) should be considered to reflect the association between historic catalytic properties of enzymes and microbial competition for available substrates (Kovarova-Kovar and Egli, 1998; Moscatelli et al., 2012; Tischer et al., 2015). The catalytic efficiency (V_{max}/K_m) represents the formation or dispersion of an enzyme-substrate complex in soil. Higher value of V_{max}/K_m suggests that dispersion of enzyme-substrate complex occurs faster than its formation, i.e. more SOM is decomposed by microorganisms (Gianfreda et al., 1995; Ekberli et al., 2006; Kizilkaya and Ekberli, 2008; Razavi et al., 2017). Thus, the catalytic efficiency indicates altered SOM decomposition in soil microhabitats as compared to bulk soil.

Priming effect is defined as a short-term change in SOM turnover caused by organic C addition (Kuzyakov et al., 2000). Priming effect can be divided into two processes: apparent priming effect and real priming effect. Apparent priming effect is connected to microbial turnover, while the real priming effect links to SOM turnover. These processes are differently regulated between topsoil and subsoil by the availability of fresh C inputs (De Graaff et al., 2014), physical accessibility of decomposer to substrates (Salomé et al., 2010; De Graaff et al., 2014) and different response of microbial community to SOM inputs (Sanaullah et al., 2016). However, the processes controlling priming in biopores have not been investigated at all (Brown, 1995; Kuzyakov, 2010). Labile C incorporated into the subsoil biopores may accelerate old C decomposition, inducing C turnover in this layer (Don et al., 2008). Meanwhile, Salomé et al. (2010) suggested exoenzyme access to substrate as a foundation of C turnover in the subsoil contrarily to topsoil.

While plant residues are the primary source of microorganism-stimulating C inputs to biopores, living roots also impact soil microorganisms *via* rhizodeposition and exudation of organic compounds (sugars, amino acids, organic acids) (Pausch et al., 2013a). Winter barley (*Hordeum vulgare* L.), for example, is characterized by a fibrous root system that transfers 17% of total assimilated C to belowground pools (roots, microorganisms, soil organic matter) (Kuzyakov and Domanski, 2000). 70% of the whole root system, however, is found in the upper 30 cm of the soil profile, where nutrient contents are highest (Lucas et al., 2000; Steingrobe et al., 2001). Despite the short root exudate lifetimes of no more than a few days (Pausch and Kuzyakov, 2011; Kuzyakov and Blagodatskaya, 2015), the rhizosphere is critically important microbial hotspot in soil (Blagodatskaya and Kuzyakov, 2008; Kuzyakov and Blagodatskaya, 2015). In contrast, earthworm burrows can exist for even longer than the lifetime of *Lumbricus Terrestris* L. itself (Tiunov and Scheu, 1999; Stromberger et al., 2012) and can be re-occupied by succeeding generations. The longevity of biopores and rhizosphere affects the stability of their corresponding microbial activities through microhabitat persistence, C content, and nutrient availability and, therefore, regulates SOM decomposition by microorganisms.

The goal of this study was to investigate the effects of earthworm activity in biopores (burrows) and of root activity on altered SOM decomposition (priming effect), and whether this priming effect is depth dependent. Based on this investigation, we compared SOM mineralization induced by glucose supply in biopores and rhizosphere. Accordingly, we hypothesized that (i) priming effects are more pronounced in biopores than in bulk soil due to more accessible SOM presented by earthworm activity; (ii) the production of microbial enzymes in biopores depends on microbial community composition; (iii) enzyme activities and efficiency of enzymatic reaction (K_a) play the main roles driving different priming effect in subsoil vs. topsoil, biopores vs. bulk soil. To this end, we measured enzyme activities and SOM-derived CO_2 associated with earthworm burrows, rhizosphere and bulk soil at different soil depths (0–30 cm, 45–75 cm and 75–105 cm), and assessed the role of biopores in the priming effect, especially in the subsoil.

2. Material and methods

2.1. Soil sampling and sample preparation

The study site belongs to the research station at Campus Klein-Altendorf (50° 37′ N, 6° 59’ E) south-west of Bonn, Germany. The topsoil was ploughed 10 months prior sampling to grow winter barley (*Hordeum vulgare* L.). Winter barley was sown at a density of 320 grains m^{-2} on 2nd October, 2014. The soil is classified as a Haplic Luvisol (WRB). The topsoil and subsoil characteristics are given in Vetterlein et al. (2013). Sampling of drilosphere, rhizosphere and bulk soil was carried out in April 2015 from three independent plots (each 2 m × 2 m). The season affects earthworm abundance and according to Spurgeon and Hopkin (1999), highest caches are found in spring and winter, and lowest in summer and autumn in an unmanaged grassland in England. Moreover, temperature variation alters soil egestion rates (Curry et al., 1995). We sampled soil in April as this was their most abundant time and also the right time to excavate the worm burrows (Curry et al., 1995).

Drilosphere was collected within the innermost part of burrows (Tiunov and Scheu, 1999) at 3 depths (0–30 cm, 45–75 cm, 75–105 cm). In order to implement field sampling, a soil pit was dug to 150 cm to expose a soil profile. We did not remove the plants before excavating earthworm burrows so as to prevent top-burrow destruction in the topsoil. Burrow pores were carefully opened on one side with a sharp knife to reveal the burrow walls, according to Hoang et al. (2016b). A micro-spoon (5 × 100 mm) was acquired to scratch the dark surface of cast along burrow walls within each 10 cm increment of soil depth. This layer of drilosphere is supposed to be within a few millimeters (Parkin and Berry, 1999) or up to 1 cm in thickness (Jégou et al., 2000). We therefore tried to sample soil materials in more or less 2 mm thickness of burrow walls. Rhizosphere soil adhering to roots was collected only from the topsoil (0–30 cm) (Grayston et al., 1998). Bulk soil was considered as soil at a distance greater than 2 cm from root or earthworm pores and was collected from 0 to 30, 45–75, and 75–105 cm depths. However, due to very high density of roots in the topsoil, bulk soil was partly affected by rhizosphere. Samples were stored field-fresh at 5 °C (<one month) until use. Before the main experiments started, root litter and plant debris had been removed with tweezers. Soil samples were divided into 3 subsamples for analyzing (i) water content, total C (TC), total N (TN) and total P (TP), (ii) enzyme