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Microbial processing of plant residues in the subsoil – The role of biopores

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ABSTRACT

Most subsoil carbon (C) turnover occurs in biopore hotspots such as root channels and earthworm burrows. Biopores allocate large C amounts into the subsoil, where a vast capacity for long-term C sequestration is predicted. We hypothesise that organic matter (OM) cycling in biopores depends on their origin.

Earthworm and root biopores were induced under field conditions and were sampled from the subsoil (45–75 and 75–105 cm) after two years of biopore formation. The effects of biopore formation on OM decomposition were studied by biomarkers: neutral sugars, cutin and suberin-derived lipids, lignin-derived phenols and free lipids. The degradation stage of OM was biopore type-specific but was only governed by the soil depth in root biopores. Degradation of OM increased from earthworm biopores to root biopores and bulk soil. Hemicelluloses (GM/AX ratio) were more strongly degraded than lignin side-chains (relative change from initial values). Two years of microbial processing during biopore formation increased the GM/AX ratio in earthworm biopores from 0.65 to 1.05 and in root biopores from 0.15 to 1.35 (both relative to source biomasses). Root biopores and bulk soil had the highest GM/AX ratios (1.2–1.3), hinting to rapid processing of plant residues and accumulation of microbial residues. The regular, frequent OM inputs by earthworms stimulated microbial growth and processing of mostly bioavailable OM and, thus, relatively enriched more persistent OM (e.g. lignin). Syringyl subunits of lignin underwent low (ratio changed from 0.35 to 0.55 relative to initial input) and vanillyl subunits underwent almost no processing in earthworm biopores indicating the preferential microbial utilisation of the easily available compounds frequently replenished by earthworm activity. After two years of decomposition of the root detritus, mainly structural plant material was enriched in root biopores. Short periods (6 months) of earthworm activity effectively recharged the highly processed OM in root biopores with fresh OM.

In total, deep-rooting catch crops and short-term earthworm activities promote C accumulation in the subsoil followed by biopore-specific microbial processing predominantly governed by the C input frequency. As root biopores are up to 40 times more common than earthworm biopores, they dominate the OM input into subsoils. Such C inputs create several years lasting hotspots for preferential root growth and nutrient mobilisation in the subsoil. We conclude that root- and earthworm-derived biopores are vertical pathways for plant C from the soil surface into the subsoil and for intensive processing of litter C and sequestration of microbial necromass.

1. Introduction

Steadily increasing carbon (C) dioxide concentrations in the atmosphere are driving global climate change (IPCC, 2014). Its prominent projected consequences such as rising global mean temperatures and more variable precipitation (Li et al., 2009; Pal et al., 2004) are deemed unfavourable by society and therefore are to be mitigated (Tompkins et al., 2010; Urry, 2015). Sequestering carbon dioxide from the atmosphere in soils is being discussed as a viable option for reducing carbon dioxide concentrations (Lal et al., 2015; Poeplau and Don, 2015). Soils are the largest pool in the terrestrial C cycle (Scharlemann et al., 2014; Schimel, 1995) and are comparatively easy to manage in comparison to geological or marine pools. This holds particularly true for croplands as they are under management. Usually, mostly the topsoils are considered for plant nutrition in arable fields as the main C cycling and nutrient pools are found in the top part of the soil (Kautz et al., 2013). Recently, the subsoils, i.e. the soil below the ploughed horizon, have been pushed into the centre of attention of soil science. Subsoils have the capacity of storing large amounts of C when their mineral phases (clay, iron oxides) are not saturated (Kell, 2012). The large land cover of cropland

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(FAOSTAT, 2017), their manageability and the potential C storage capacity of subsoils make them attractive as long-term C sinks (Dignac et al., 2017; Torres-Sallan et al., 2017). However, for successful C sequestration, the dynamics of C in subsoils need to be better understood first.

Carbon reaches the subsoil predominantly through leaching as dissolved organic carbon (Kalbitz and Kaiser, 2008; Kindler et al., 2011). It involves potentially strong microbial modifications to the leached C compounds (e.g. respiration) and depends on the water solubility of the allocated compounds (Kaiser and Kalbitz, 2012). Large amounts of C may be also brought into the subsoils by roots or soil fauna such as earthworms, i.e. in biopores (Kautz, 2015; Stirzaker et al., 1996). Compared to leaching, organic matter (OM) ends up faster in the subsoil (Munyankusi et al., 1994), but in defined vertical macropores. They feature not just elevated C stocks, but also strong and intense microbial activity relative to the non-biopore bulk soil (Hoang et al., 2016). Hence, they are considered as hotspots, i.e. small soil volumes with much higher microbial processing rates than the average soil conditions (Kuzyakov and Blagodatskaya, 2015). The bulk subsoil is characterised by low C stocks, high radiocarbon age and slow microbial cycling (Rumpel et al., 2002; Rumpel and Kögel-Knabner, 2011). Biopores could contribute to higher C stocks in the subsoil because with higher C inputs, more C may remain stabilised as soil organic matter (SOM). Conversely, more easily available C may cause priming of older, potentially stabilised SOM, which would cause a loss of C (Fontaine et al., 2007; Kuzyakov, 2010). Studies on microbial communities and their residues in biopores have hinted to different functions of biopores, i.e. nutrient cycling *vs.* C sequestration – depending on their genesis, i.e. root vs earthworm-derived biopores (Banfield et al., 2017). C stabilisation may vary mechanistically among different hotspot types. Earthworms may change the texture of the soil in their burrows through selective feeding (Zhang and Schrader, 1993) and OM is strongly physically and chemically modified during the gut passage (Cheshire and Griffiths, 1989; Thakuria et al., 2010). Depending on the biopore-specific C inputs, the resulting microbial necromass may further contribute to long-term C sequestration (Miltner et al., 2012; Six et al., 2006). If C turnover depends on the biopore type, this will have consequences for the total subsoil C turnover, which mainly takes place in hotspots (Kuzyakov and Blagodatskaya, 2015).

The chemical composition of OM, its sources and degradation can be assessed by biomarkers (Amelung et al., 2008; Simoneit, 2005). Biomarkers are organic molecules whose detection indicates presence of an organism, tissue, secreted metabolites or their past presence. For instance, root influence is reflected by the contents of mid-chain ω-hydroxy alkanoic acids and lignin-derived phenols (Armas-Herrera et al., 2016; Spielvogel et al., 2008; Thevenot et al., 2010). The degradation stage of the OM is assessed by biomarker ratios, e.g. contents of oxidised/reduced lignin-derived phenols (Thevenot et al., 2010). Ideally, undecomposed source biomasses (e.g. crop shoots as earthworm food) are collected before the experiment and characterised for their biomarker composition together with the soil samples (Gunina and Kuzyakov, 2015). The change of the biomarker pattern of the undecomposed source biomass to the biomarker pattern in soil can be related to the experiment duration, herein termed 'microbial processing'.

To better understand C dynamics in hotspots and their relevance for subsoil C turnover, we conducted a biomarker study to link the biopore type and soil depth with C processing. Biomarker ratios known to characterise the degradation state of plant residues were used to reconstruct microbial processing by relating them to microbial biomarkers. In a field experiment over five years, three biopore types were induced by either (1) growing tap-rooted chicory (*Cichorium intybus* L.) for three years followed by two years of fallow (root biopores), (2) at least ≥ 3 years of earthworm activities (native earthworm biopores) or (3) 6 months of earthworm incubation into root biopores (earthworm-incubated biopores). We sampled the material on the inner walls of the biopores and analysed it for lignin-derived phenols, cutin and suberin-derived biomarkers, neutral sugars and free lipids. Proxies describing the processing of plant residues were calculated from these data and compared among the biopore types.

2. Material and methods

2.1. Sampling

The study was conducted on the Campus Klein-Altendorf experimental research station near Bonn, Germany. The location had a mean annual temperature of 9.6 °C, a mean annual precipitation of 625 mm and featured a Haplic Luvisol (Hypereutric, Siltic; IUSS Working Group WRB, 2008). A characterisation of the soil and its genetic horizons was given in Vetterlein et al. (2013). The experimental set-up was previously described in Banfield et al. (2017). In short, three biopore types were induced in the field and studied in two subsoil depths (45–75 cm; 75–105 cm), namely root biopores, native earthworm biopores and a combination of both: earthworm-incubated biopores (Fig. S1, Supplementary Material). Each treatment combination was replicated four times in different locations of the same agricultural field.

(I) *Root biopores:* from 2009 to 2012 chicory (*Cichorium intybus* L., var. Puna) was grown to induce taproots of at least 4 mm diameter in the subsoil. In 2012, the topsoil (0–45 cm) was temporarily removed and live roots were mapped in 45 cm depth. After filling the disturbed topsoil back, the plots were kept fallow and did not receive any clover-grass. Roots had two years to decay until sampling in autumn 2014.

(II) *Earthworm-incubated biopores*: after 1.5 years of root decomposition, earthworms (*Lumbricus terrestris* L.), a native earthworm species in Central Europe, were inserted into a subset of at least 25 root biopores per field replicate for six months. In spring 2012, the topsoil (0–45 cm) was temporarily removed, and earthworms were incubated into previously identified and mapped 1.5-year-old root pores. Prior to incubation, an elastomer tag was injected into each earthworm body to allow re-discovery of the earthworms (Butt and Lowe, 2007). Earthworms were fed with clover-grass (shoots of *Trifolium repens* L., *T. pratense* L. and *Lolium perenne* L.) put on the soil surface until four weeks prior to sampling.

(III) *Native earthworm biopores* (colonised with predominately *L. terrestris*) were treated similarly to the earthworm-incubated biopores: plots were kept fallow from 2012 on and grass-clover material was placed on the soil surface as food. Native earthworm biopores were identified at the end of the experiment in September 2014 as follows: after removing the topsoil, a new soil surface was prepared in −45 cm and covered with litter for three days. Biopores with visible earthworm middens were considered colonised with earthworms.

(IV) *Bulk soil* samples, i.e. soil not containing any macroscopic biopores, were taken from areas next to the biopore plots. These plots were kept fallow and did not receive any clover grass amendment.

In September 2014, after removing the topsoil down to −45 cm, soil around each identified biopore was manually removed first down to −75 cm, then to −105 cm. The biopores were opened vertically and the inner wall coating was sampled by shaving it off with micro spatulas once, i.e. approximately 1 mm (Andriuzzi et al., 2013). Thirty-two samples were taken: four field replicates were taken from each treatment (three biopore types + bulk soil) and from two subsoil depths (45–75 cm; 75–105 cm). Each sample was pooled from about 25 biopores.

2.2. Biomarker analyses

Contents of *neutral sugars* from hemicelluloses and microbial