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Biopore effects on phosphorus biogeochemistry in subsoils

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ABSTRACT

Biopores are characterised by high concentrations of plant available nutrients and provide preferential pathways for root growth into the subsoil, thereby potentially enabling plants to access phosphorus (P) resources located in the subsoil. Here, we sampled biopores from a replicated agricultural field trial in Klein-Altendorf, Germany, to analyse their nutrient composition and P speciation as determined by Hedley sequential extraction and X-ray absorption near edge structure (XANES) spectroscopy. In addition, we analysed the oxygen isotopic composition of HCl P ($\delta^{18}O_{HCl\ P}$) as an indicator of long-term effects of biological P turnover. We found that biopore effects were most pronounced in the subsoil, where the concentration of easily extractable (labile) P tended to be greater in biopores than in bulk soil, as evident in both Hedley sequential extraction and XANES spectroscopy. We assume that these findings result from inputs of organic matter from the topsoil as well as an input of Ca-particles into subsoil biopores by earthworm activity. Biologically cycled P was subsequently precipitated as Ca-P as evident by $\delta^{18}O_{HCl\ P}$ values close to equilibrium in biopores even at great depths. When incubating bulk soil samples with ^{18}O-labelled water, however, we observed a significant increase of $\delta^{18}O_{HCl\ P}$ values in the topsoil, but only small if any changes of $\delta^{18}O_{HCl\ P}$ values in the subsoil. Thus, biopores present hotspots of P cycling in the subsoil, but the effect of biopores on overall P turnover in the bulk subsoil is limited.

1. Introduction

In agricultural soils, phosphorus (P) is a limiting element for plant nutrition despite being present in large amounts: most P is bound in chemical forms not readily available to plants (Cross and Schlesinger, 1995; Kruse et al., 2015). Also, substantial amounts of P are stored in the subsoil (Kautz et al., 2013; Barej et al., 2014) and are therefore not immediately within the reach of roots of most cereal crops. Here, biopores, i.e. pores created by earthworms or by tap-rooted pre-crops, have often been suggested as a means to increase plant access to these subsoil resources (Passioura, 2002; McKenzie et al., 2009; Kautz et al., 2013).

Beneficial effects of biopores have been assigned, on the one hand, to their role in providing preferential paths for root growth, especially in compacted subsoils, due to lower penetration resistance and better aeration (Stirzaker et al., 1996; Valentine et al., 2012; Gaiser et al., 2013). Root growth in biopores might further be associated with nutrient mobilisation and cycling by microorganisms of the rhizosphere. On the other hand, earthworms transport organic matter from the soil surface into biopores and line the walls with casts, which exhibit high microbial activity (Don et al., 2008; Uksa et al., 2015). As a result of both, biopores may potentially present hotspots for nutrient acquisition due to enhanced biological cycling of nutrients as well as due to increased availability of nutrients for plant uptake. For P, it has been demonstrated that earthworm casts in biopores are characterised by significantly higher contents of plant available P relative to the bulk soil (Kuczak et al., 2006; Barej et al., 2014) although the activity of P-releasing enzymes such as acid phosphatase is not significantly elevated in these biopore linings (Jégou et al., 2001; Don et al., 2008). However, even as biopores in subsoils may be stable for several decades (Beven and Germann, 1982; Hagedorn and Bundt, 2002), the stimulation of microbial activity and related nutrient cycling might cease soon after roots have decayed or after earthworms have abandoned the pore (Don et al., 2008; Kuzyakov and Blagodatskaya, 2015). As a result, beneficial effects of earthworm activity do not necessarily translate into an increased P availability in the bulk soil (Vos et al., 2014). Also, roots growing along biopores may still be able to grow into the surrounding bulk

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soil in order to make use of P resources at greater depths (Perkons et al., 2014; Han et al., 2015). Beyond the scale of an individual biopore, it thus remains unclear whether and to which extent biopores affect subsoil P cycling in general and at time-scales exceeding the lifetime of a single root or earthworm.

Phosphorus availability is mainly controlled by the chemical bonding forms of P in soils. Sequential extraction procedures such as the one described by Hedley et al. (1982) have been introduced to assess P pools of different chemical speciation. Yet, as these procedures are operational, there are limitations in assigning a defined P fraction to a certain degree of bioavailability; moreover, different sample timing, handling and pre-treatment, as well as the accuracy of organic and inorganic P assessment may alter fraction yields (Cross and Schlesinger, 1995; Negassa and Leinweber, 2009). The above-mentioned limitations can partly be overcome by X-ray absorption near edge structure (XANES) spectroscopy, as it enables an element-specific and non-invasive direct elemental speciation in the solid phase. This approach has been applied, e.g., to determine effects of P fertilisation on P speciation in soils (Beauchemin et al., 2003; Eriksson et al., 2015).

Another powerful tool to trace biological processes involved in the P cycle is the analysis of the oxygen (O) isotopic composition of phosphate ($\delta^{18}O_P$) (Frossard et al., 2011). Under ambient environmental conditions the P–O bond in phosphate is stable when biological activity is absent, i.e. there will be no exchange of O atoms with water (Tudge, 1960; Kolodny et al., 1983; O'Neil et al., 2003). An exchange of O atoms will only occur in the presence of enzymes. In the soil environment, some enzymes, especially phosphohydrolases, are exuded by plants and microorganisms to promote the mineralisation of organic phosphate (P_o) (Zimmermann et al., 2003; Richardson et al., 2011). During this process, O atoms from the surrounding soil water will be incorporated into the newly formed phosphate molecule. This has been shown for phosphomonoesterases (Liang and Blake, 2006; von Sperber et al., 2014), phosphodiesterases (Liang and Blake, 2009) and phytases (von Sperber et al., 2015; Wu et al., 2015). Furthermore, the activity of the ubiquitous intracellular enzyme pyrophosphatase leads to a complete O exchange between phosphate and water (Chang and Blake, 2015; von Sperber et al., 2016), and thus to a complete equilibration of O isotope ratios. In light of these biochemical processes, the $\delta^{18}O_P$ values observed in soil samples have provided valuable information on the biological cycling of available P either in the natural environment (Angert et al., 2012; Tamburini et al., 2012; Gross et al., 2015) or in incubation studies using ^{18}O labelled phosphate (Melby et al., 2013) or ^{18}O labelled water (Gross and Angert, 2015). All of these studies demonstrated that labile soil P pools approach the expected oxygen isotopic equilibrium in soils. Yet, for more stable P pools, such as HCl P (a proxy of Ca-P), the changes in $\delta^{18}O_P$ values upon labelling were generally small (Zohar et al., 2010; Joshi et al., 2016). It has been suggested that HCl P does not participate in biological P cycling but can incorporate the signal of biologically cycled P due to the continuous precipitation of secondary Ca-P minerals (Joshi et al., 2016). Thus, the $\delta^{18}O_P$ signature of HCl P ($\delta^{18}O_{HCl\ P}$) integrates long periods of time, which will cause a shift in $\delta^{18}O_{HCl\ P}$ values but will not result in full equilibration. However, Amelung et al. (2015) reported that in an arable topsoil, $\delta^{18}O_{HCl\ P}$ values were close to equilibrium and only with increasing depth, $\delta^{18}O_{HCl\ P}$ values shifted progressively towards the isotopic signature of the parent material. Hence, in the long-term, even a putatively biologically inert P pool might in fact be part of the P cycle especially in the topsoil, possibly via the precipitation and dissolution of secondary minerals.

Similar effects might be observed for biopores, which over the course of decades integrate multiple generations of earthworms and roots and could thus enhance P cycling especially in the subsoil. Therefore, we resampled the bulk soil and also biopores at the site described in Amelung et al. (2015) and analysed them by a combination of Hedley sequential extraction, XANES spectroscopy and $\delta^{18}O_P$ of inorganic P extracted by 1 M HCl, in order to elucidate the effects of biopores on the biological turnover of the stable Ca-P pool. Further, we incubated bulk soil samples with ^{18}O-labelled water and analysed the change in their $\delta^{18}O_{HCl\ P}$ values to determine to which extent P cycling in the bulk subsoil affects the HCl P pool. We hypothesised that (1) due to P inputs by plant roots and especially earthworms, microbial P mobilisation and turnover in subsoil biopores is higher than in bulk soil indicated by greater concentrations of labile P in the Hedley sequential extraction and XANES spectroscopy and that (2) this higher cycling will shift $\delta^{18}O_{HCl\ P}$ values of Ca-P closer to equilibrium values. However, we assume that (3) outside of biopores the microbial P turnover and subsequent precipitation as Ca-P is low, which should be reflected by $\delta^{18}O_{HCl\ P}$ values further away from equilibrium and only slow changes in $\delta^{18}O_{HCl\ P}$ values in the subsoil during the incubation.

2. Materials and methods

2.1. Site and soil sampling

Samples were collected from a haplic Luvisol at an eight-year field trial at the Research Station Campus Klein-Altendorf of the University of Bonn. The field trial was established to observe the effect of pre-cropping on the abundance of biopores in the soil as well as their utilisation by following cereal crops. The pre-crops under study were alfalfa (*Medicago sativa* L.), chicory (*Cichorium intybus* L.) and tall fescue (*Festuca arundinacea* Schreb.). For a more detailed description of the field trial see, e.g., Kautz et al. (2014) and a general characterisation of the soil is given by Vetterlein et al. (2013). The tall fescue treatments were previously sampled and analysed for its bulk soil $\delta^{18}O_P$ signature in the study by Amelung et al. (2015). For the present study, we sampled three field replicates of the treatment with alfalfa (*Medicago sativa* L.) as a pre-crop for three years, followed by five years of a cereal-rapeseed rotation. The crop in the year of sampling was barley (*Hordeum vulgare* L.) and samples were collected in April, seven months after sowing. Biopores were identified as round voids in the soil (~5 mm in diameter) characterised by linings of a darker colour and in a few cases containing barley roots. Biopore material was collected by opening a biopore in 10 cm increments within specified depth intervals of 0–30, 30–45, 45–55, 55–75 and 75–105 cm depth. We sampled the linings of the biopores (~1 mm) until the bulk soil of a brighter colour appeared. All material from biopores was combined into a composite sample for each depth interval of each field replicate, respectively. As a reference, we also sampled bulk soil material excluding all areas around biopores. Samples were dried at 40 °C and sieved to 2 mm grain size.

2.2. General soil parameters

All samples were analysed for total elemental composition by microwave digestion in *aqua regia* and subsequent measurement using inductively coupled plasma-optical emission spectroscopy (ICP-OES; Thermo Fisher iCAP™* 7600). For the determination of the concentrations of total carbon (C), total nitrogen (N), and the C:N ratio we used dry combustion followed by the heat conductivity detection of the released trace gases (vario MICRO cube, Elementar, Hanau, Germany). For bulk soil samples we additionally checked for the presence of inorganic C by adding HCl to an aliquot of each sample and we determined pH ($CaCl_2$) at a 1:2.5 soil:solution ratio; for biopore samples there were not sufficient sample amounts for these measurements. For bulk and biopore samples we