Root-rhizosphere-soil interactions in biopores

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Abstract

Background Even with extensive root growth, plants may fail to access subsoil water and nutrients when root-restricting soil layers are present. Biopores, created from decaying roots or soil fauna, reduce penetration resistance and channel root growth into the deeper soil. Further positive effects on plants result from biopore traits, as the pore walls are enriched in nutrients, microbial abundance, and activity relative to bulk soil. However, negative effects on plant growth have also been observed due to root clumping in biopores, less root-soil contact than in the surrounding bulk soil and leaching of nutrients.

Scope We discuss methods for biopore research, properties of biopores and their impact plant performance based on a literature review and own data. We elucidate potential implications of altered root-soil contact for plant growth and the consequences of root growth in pores for the rhizosphere microbiome.

Conclusions Biopores play an important but ambiguous role in soils. The effects of biopores on plant growth depend on soil properties such as compaction and moisture in an as-yet-unresolved manner. However, pore properties and root-soil contact are key parameters affecting plant yield. Knowledge gaps exist on signaling pathways controlling root growth in pores and on mechanisms modifying rhizosphere properties inside biopores. The degree to which negative effects of biopores on plant growth are compensated in the bulk soil is also unclear. Answering these questions requires interdisciplinary research efforts and novel imaging methods to improve our dynamic understanding of root growth and rhizosphere processes within biopores and at the rhizosphere-biopore interface.

Keywords Biopore · Root growth · Soil microbiome · Pore system · Soil hotspot

Introduction

Plant roots explore the soil for resources such as water and nutrients. However, many soils have root-restricting layers that prevent efficient plant growth (Schneider and Don 2019; Schneider et al. 2021). As a result, huge amounts of resources remain unused,
particularly from the subsoil (below 0.3 m soil depth), which usually contains more than half of the soil water and nutrient stocks (Schneider and Don 2019; Gocke et al. 2021). However, root growth may benefit from soil biopores, which can act as preferential growth paths into the subsoil, because the penetration resistance for roots growing in such pores is lower than in the bulk soil (Valentine et al. 2012). Especially in scenarios where roots re-enter into the bulk soil below root restricting layers (Athmann et al. 2013; Kautz et al. 2013; Han et al. 2015), soil biopores may increase access to subsoil resources.

There are different pore systems in soil, which can be classified according to size and thus water availability, but also according to continuity, shape, abundance, and genesis (e.g., Johnson et al. 1960). Pores form according to the spatial arrangement of soil particles, and therefore as a result of aggregation processes, as well as from biological processes such as root growth or faunal activity. The pores inside microaggregates (<250 μm) are generally smaller than root hairs or even fungal hyphae, so they are not considered here (for a review of structures inside microaggregates, see Totsche et al. 2018). Pores may be formed without the presence of biota: these abiogenic pores are mainly cracks formed by drying and shrinking processes in clay-rich soils (Dexter 1991; McMahon and Christy 2000) or, less frequently, from freezing of water within the soil profile (Kaab 2008; Lu and Liu 2017). Gaseous exchange may contribute to their formation (Johnson et al. 1960). However, such strictly abiogenic pores are frequently transient in their occurrence, as cracks may close during swelling upon rewetting, or as soil material falls into these cracks. Abiogenic pores can persist when soil conditions do not change or when they are subsequently used by soil fauna or roots, although this changes pore properties towards those of biogenic pores. Thus, biogenic pores occur and persist more frequently in most soils than abiogenic pores, so this review will mostly focus on effects observed in pores derived from biological processes. However, even if biogenic pores derive from various types of soil fauna (such as ground nesting bees, ants, spiders) (Colloff et al. 2010), most of the literature focuses on earthworm biopores.

In the second half of the 19th century, Hugo Thiel was one of the first researchers to investigate roots growing in pre-existing channels. He concluded that these channels were made by roots of previously growing plants or the soil fauna, such as earthworms, insects, or nematodes (Thiel 1865). Later, the term ‘biopore’ was introduced, which describes pores derived from biological activity (e.g., Dexter 1986; Blackwell et al. 1990; Stirzaker et al. 1996). Studies focusing on biopores created and inhabited by earthworms have also coined the term ‘drilosphere’ to describe the specific conditions induced by earthworms. As opposed to the general biopore concept, the drilosphere includes not only the wall of the pore modified by earthworms, but also the gut and skin of the earthworms, their burrows, cast, and middens (Brown et al. 2000). Here, cast is material excreted by earthworms, while middens refer to the mixture of litter, cast, and mucus (Bottinelli and Capowiez 2021). For more information on the history of biopore research, the review of Kautz (2015) is recommended.

Biopores are mostly tubular, round-shaped channels that are vertically orientated in the soil, but horizontal biopores exist (Kautz 2015). While the total pore volume in soils can be up to 50% of the total soil volume (Amelung et al. 2018), biopores only account for 1–10% of the total soil volume (Banfield et al. 2017a) and are part of the soil macropore system. Some studies have defined a threshold of >50 μm for the minimum diameter of a biopore (Rouquerol et al. 1994), while others have applied >1000 μm as the lower size limit (Kim et al. 2010). However, these limits are usually defined by technical requirements and do not represent a systematic consensus in the literature. Hence, research about biopores involves pores of different sizes ranging from those less than 1 mm (Volkmar 1996) to greater than 5 mm (e.g., Athmann et al. 2013).

As a result of root decay or soil faunal activity in the pore, biopore walls may be enriched with nutrients (Stirzaker et al. 1996; Athmann et al. 2013) and characterized by high levels of microbial activity (Athmann et al. 2017; Hoang et al. 2017; Banfield et al. 2018). Based on these properties, soil pores have been suggested as a major hotspot of nutrient uptake for plant roots in the soil (Hoang et al. 2016a, b). However, this suggestion disregards the fact that, especially in pores with a diameter wider than the root diameter, root-soil contact may be reduced (Landl et al. 2019), thus potentially limiting plant nutrient uptake from the pore. Moreover, when growing in contact with soil, roots are typically associated
with specific microbial communities, the rhizosphere microbiome, which may control nutrient mobilization and mineralization (Berendsen et al. 2012).

Biopore formation and maintenance in soil depends heavily on agricultural management as well as on soil properties, climatic conditions, and other abiotic factors. Under natural soil conditions, such as under permanent vegetation, the life cycle of aggregates, and thus the turnover of the pore system, is likely to be slow. In managed ecosystems undergoing tillage, in turn, aggregates and associated pore systems are interrupted and soil structures may decay rapidly, particularly in the plough horizon (Lobe et al. 2011; Lucas et al. 2019; Or et al. 2021). Hence, in annual cropping systems, the contribution of biopores to plant nutrition is usually restricted to the subsoil when the surface soil is ploughed; only in no-till and perennial ecosystems can biopore continuity extend into the surface soil. Subsequent biopore utilization by crops then depends on factors such as soil and climate. In Australia, for instance, White and Kirkegaard (2010) reported root clumping in biopores and no re-entry of the roots into the surrounding bulk soil. In Europe, by contrast, Athmann et al. (2013) detected a re-entry of roots into the soil matrix and less root clumping. These two regions face different soil and climatic conditions, with Australia having old, hard soils (Kandosols according to Australian soil classification; likely Ultisols according to US Soil Taxonomy; a translation of Kandosols into WRB classification is not easily feasible; Morand 2013) and dry climates, but Germany being characterized by postglacial loess soils with lower penetration resistance (Alfisols according to US soil taxonomy) and more precipitation. Several factors, including different degrees of subsoil compaction, different clay mineralogies, and different climates, lead to different shrink-swell and freeze-thaw dynamics, and therefore to a different development and performance of the pores. Accordingly, when discussing data from different soils and climates, the specific site conditions must be considered.

Here, we aim to highlight the connection of biopore properties to root-soil contact and the interaction with the rhizosphere microbiome of roots growing in the pores, as well as potential consequences for plant nutrient acquisition. The first section of this review provides a brief overview of methods currently employed for biopore research to provide a critical evaluation of advantages and disadvantages that may affect the observations reported in later sections on biopore properties, root growth, and microbial activity in pores. We also provide examples from our own experiments to highlight various aspects of biopore research.

Analysis of biopores

The selection of methods to analyze biopore features, which often includes a simultaneous assessment of root growth in biopores, is a critical aspect in biopore research. Different approaches for the evaluation of root growth and root architecture have been described; Table 1 provides a brief summary of methods and obtained parameters characterizing pores. Overall, the methods can be differentiated into invasive and non-invasive methods, although the latter are often applied only to small undisturbed soil cores or pot experiments and hence mainly consider early stages of plant development. Additionally, for all methods observing root growth, the differentiation of root growth in the pore, along the pore wall or in the bulk soil is especially critical, as differentiation and cut-off criteria are within the researcher’s estimation and have not been systematically defined yet. A consistent classification is also needed in order to reliably identify and quantify root-soil contact zones within pores.

Among non-invasive methods, the main goal of endoscopy, magnetic resonance imaging (MRI), X-ray computed tomography (CT), and neutron CT applications is the visualization of physical pore characteristics, such as pore diameter, circularity, area and connectivity. For endoscopy, this can be done quite easily by visual inspection of a pore via the endoscope camera, as shown in an exemplary picture of roots growing in pores (Fig. 1). Endoscopy has no direct effect on plant growth or on the soil bacterial community, but careless introduction of the endoscope into the pore can damage the root or the pore wall. Furthermore, endoscopy is limited to larger pores because of technical constraints.

The MRI technique is not restricted to the visualization of a single large root and can capture whole root systems. Thus, MRI scans provide information on root architecture and root distribution in soil, among other things, and can therefore be used to visualize root growth inside and outside pores based
| Method                        | Principle                                                                 | Potential parameters                                                                 | Reference                                      |
|-------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------------------|------------------------------------------------|
| **non-invasive**               |                                                                           |                                                                                       |                                                |
| Endoscopy                     | Probe attached to a tube with light-conducting optical fibers; a small camera transmits the image to a monitor | Pore structure; pore characteristics (e.g., diameter, circularity, area, connectivity); root growth inside pores | Athmann et al. 2013, 2019                      |
| Magnetic resonance imaging (MRI) | Manipulation of the orientation of the magnetic moment of atomic nuclei (mainly the protons of hydrogen atoms in water); imaging occurs by detecting the realignment to another external magnetic field | Pore structure; pore characteristics (e.g., diameter, circularity, area, connectivity), root growth inside pores | Metzner et al. 2015; van Dusschoten et al. 2016 |
| X-ray tomography (X-ray CT)   | 3D visualization of attenuation coefficients for X-rays directed at a sample; these coefficients are correlated with the density of the material (e.g., soil or root) | Root water uptake in situ; pore structure; pore characteristics (e.g., diameter, circularity, area, connectivity); root growth inside pores | Pierret et al. 2002; Taina et al. 2008; De Gryze et al. 2006; Ganther et al. 2020; Leue et al. 2019 |
| Neutron tomography            | 3D images are produced by detecting the absorption of neutrons produced by a neutron source | Soil moisture distribution; pore structure; pore characteristics (e.g., diameter, circularity, area, connectivity); root growth inside pores | Mawodza et al. 2020                           |
| **invasive**                  |                                                                           |                                                                                       |                                                |
| Profile wall method           | Soil is removed down to a few meters of depth to create a vertical wall onto which a rectangular frame consisting of identical grids is applied to count roots extending from the profile wall | Root length density; biopore frequency; pore wall characteristics; root growth inside pores | Han et al. 2015; Barej et al. 2014; Hoang et al. 2016a, b; Banfield et al. 2018; Haas and Horn 2018; Rodionov et al. 2019 |
| Soil core collection          | Collection of soil cores and careful dissection                          | Root growth inside pores; pore wall characteristics; water flow in the core           | White and Kirkegaard 2010; Stewart et al. 1999; Shougakpam et al. 2010                   |
| Isotope tracing               | Stable isotope ratios of nutrients change during biological transformation processes Root labelling, such as with radioactive isotopes, will transfer the radionuclide signal into the biopores of the decaying roots | Nutrient turnover and biological activity in pore walls Biopore history, utilization of pores by subsequent plant roots | Bauke et al. 2017a Banfield et al. 2017b |

Table 1 Principles of methods useful for biopore research, when studied along with plant root growth in pores, and parameters that can be obtained when applying these methods.
on the clustering of roots (Fig. 2). However, relating root distribution quantitatively to pore size distribution remains challenging. The assessment of root growth in pores can be improved by additional scans of a pre-processed control pot without plants, where pore walls are coated with silicone rubber materials to become visible in MRI scans (personal communication Robert Koller, Forschungszentrum Jülich, Germany). Via comparative post-processing of scan images, root growth within pores can be more precisely inferred. However, this is currently only feasible in pot experiments with predefined pore arrangements that are standardized across pots. Besides, the detection of fine roots such as laterals can be challenging with MRI, and paramagnetic ions strongly affect the resolution of the MRI scans. The abundance of paramagnetic iron frequently increases with increasing clay content; indeed, the loamy soil used in our exemplary scans did not show the best possible resolution (Fig. 2) as formerly reported for pure sand mixtures (Jahnke et al. 2009). However, a major advantage of MRI is that it is considered to have no effects on plant development (van Dusschoten et al. 2016), which is especially important when the scanning occurs over multiple days (Pflugfelder et al. 2021).

In contrast to MRI, X-ray or neutron tomography make it possible to visualize root growth in pores due to different densities, and thus different attenuation or absorption coefficients of soil, roots, and water- or air-filled pores (Helliwell et al. 2013). The X-ray attenuation is related to the density of the material, so with X-ray the soil is visible, but not water. However, neutrons are attenuated by hydrogen and boron, so water is detectable with neutron tomography. With additional contrast agents or modelling approaches, CT can also visualize water flow in the pore system. Unlike MRI, paramagnetic ions do not interfere with the analyses. Recent findings have shown X-Ray CT to have only a marginal impact on plant gene expression, with any effect being gone after 24 h, and no effect on the bacterial community structure (Ganther et al. 2020); however, these findings are likely to be dose-dependent and require more research (Zappala et al. 2013). The best resolution of root growth and of associated water distribution and water flow is achieved with neutron tomography, which makes it possible to analyze moisture gradients within the soil or pore (Mawodza et al. 2020).

An advantage of invasive methods, such as the profile wall method or collection of soil cores, is that...
they make it possible to simultaneously collect samples for further downstream analyses. Additionally, compared to MRI or CT scans, for example, roots growing in bulk soil can be assessed more reliably in a profile wall, thus allowing for a quantitative analysis of root growth within the pores. As the profile wall method is usually applied in the field, the analysis is not performed in reference to individual plants, but to a 2D soil section. The digitalization of root and pore distribution in a profile wall and subsequent image processing with automated algorithms (e.g., AI rootpainter; Smith et al. 2020) has opened up new research opportunities with faster and less subjective assessments of root growth. Nevertheless, an analysis of the pore structure in profile walls is limited because pores need to be opened and will therefore be partially destroyed. Similarly, the connectivity of the overall pore system, as well as root-soil contact zones across the full root system, cannot be studied effectively and conclusively.

Finally, if the aim is to study responses in root growth rather than pore characteristics, biogenic or abiogenic pores can also be artificially created. Abiogenic pores, for example, are made with steel rods (Pfeifer et al. 2014; Bauke et al. 2017a), resulting in individual uniform macropores with compacted pore walls, but without the biological features typical of biopores. In some cases, abiogenic pores are incubated with earthworms, resulting in biopores (Miller et al. 2009; Athmann et al. 2019). Besides, biological pores can be formed by direct pre-incubation of the soil with earthworms or pre-cultivation of plants. Biopores derived from plant roots are obtained by pre-cultivating plants in the pot and harvesting the above-ground biomass, while the roots remain in the pots and decay for a few months before the plant of interest is grown in the same pot (Stirzaker et al. 1996; Banfield et al. 2017b).

Across all experimental settings, both invasive and non-invasive methods will not provide information on physiological or biogeochemical characteristics. For these purposes, a subsequent destructive sampling is needed. Follow-up analyses may include high resolution imaging of roots or soil material; for example, by scanning electron microscopy (SEM) in combination with energy dispersive X-ray (EDX) spectroscopy for mapping of element distribution, or microscopy to visualize microbial root colonization. Furthermore, standard analyses of soil, root, and microbial materials for characteristics such as soil organic matter or nutrient contents, enzyme activities, or microbial community structure are valuable tools. If these analyses are intended, an additional combination with enzymatic mapping (zymography; Hoang et al. 2016a, b), stable isotope (e.g., Grabmaier et al. 2014; Bauke et al. 2017b), or radioisotope analyses (e.g., Banfield et al. 2017b; Bauke et al. 2017a) may prove advantageous for identifying processes occurring within pores. This can be further substantiated by root transcriptomic analyses or microbial metatranscriptomics. For a more detailed discussion of methods that provide information on soil processes, see Blagodatskaya et al. (2021).

In summary, the selection of methods depends on the specific information needed on pore structure, pore connectivity, root growth, and root distribution, or on microbial or plant-driven processes, which usually demands the collection of material for specific downstream analyses. Many imaging studies have been conducted in pots or have used soil columns, which mostly cover the early stages of plant development. Most invasive studies have relied on sampling of biopore wall material from a few predefined depth intervals in field studies, but have hardly considered the whole soil profile. For a better understanding of nutrient availability and uptake by plant roots in biopores, information at higher spatio-temporal resolution would be advantageous. Besides pore properties changing with soil depth, likewise, root, and rhizosphere processes also show spatially explicit patterns along the root axis (Bonkowski et al. 2021). Hence, a combination of different image-based approaches, in combination with analytical methods based on guided sample collection, are required. Such combined high-resolution approaches make it possible to identify regions of different root-soil-contact and thus to study its impact on nutrient uptake, but also on microbial nutrient cycling at the pore wall and its interface with the rhizosphere.

Biopore origin and history

Biopores derive from decaying roots or soil faunal activity (Kautz 2015), with one preceding the other; or with physically generated macropores such as fine cracks providing first biopore directions. Figure 3 provides a conceptual overview of biopore creation and reuse over time. Knowing the origin of a biopore
can improve our understanding of the different characteristics of biopores, such as the increased nutrient concentrations or activity of specific microbial communities. This can provide directions for the promotion of different biopore types for specific agricultural benefits. However, it is often difficult to distinguish between a pore created by roots or by earthworms.

In some studies, the pore type is largely pre-defined by the experimental design; that is, when pores are artificially created or when a particular field management is expected to favor the development of specific types of biopores. In a long-term field experiment, deep-reaching biopores were created by lucerne roots (*Medicago sativa* L.) or by growing taprooted chicory (*Cichorium intybus* L.) for three consecutive years, followed by a decay period of two years (Kautz and Köpke 2010; Athmann et al. 2013). As a result, biopores were considered to be mainly root-derived. In contrast, Banfield et al. (2017a) enforced the formation of earthworm pores by keeping a plot fallow for approximately two years. Afterwards, the topsoil was removed and the subsoil surface was covered with litter for three days. Pores with an accumulation of earthworm middens at the pore entrance were identified as earthworm biopores. However, it remained unclear whether pores had been colonized by roots in the pre-fallow phase. Hence, such experimental settings can provide indications on the current predominant pore use, but they cannot completely exclude cross-over effects resulting from pore reuse.

In studies where the origin of biopores is not defined by the experimental design, the reconstruction of biopore origin is more challenging. Options for characterizing pore wall chemistry include...
destructive sampling, such as by gene or biomarker analyses for reconstructing past root growth (Banfield et al. 2018), by identifying earthworm linings in biopore walls (Athmann et al. 2013), or by laser-ablation isotope ratio mass spectrometry after C3/C4 vegetation changes (Rodionov et al. 2019; Sosa et al. 2021). A distinction of biopores based on free extractable fatty acids in pore wall linings was achieved by Hafner et al. (2015). Decaying plant roots consisted of saturated and unsaturated C16 and C18 free extractable fatty acids and chains with >19 C atoms. Earthworm-derived organic matter consisted of <10% dicarboxylic acids and >10% unsaturated fatty acids, whereas the rhizosphere included 10–20% dicarboxylic acids and >10% unsaturated fatty acids. By contrast, >15% dicarboxylic acids and <10% unsaturated fatty acids were found in the bulk soil. These differences in fatty acid distribution were consistent throughout the soil profile to a depth of 105 cm (Hafner et al. 2015). However, these findings only considered one Alfisol (US Soil Taxonomy), and hence need to be corroborated for other soils and environmental settings. Besides using fatty acids as indicators for pore types, the microbial community structure in the pore walls may contain markers – that is specific microbial taxa – that allow for conclusions on pore history, as the rhizosphere, like earthworm pore walls and casts, support the development of specific microbiomes (see below).

Zhang et al. (2018) suggested that a morphological distinction of biogenic and abiogenic pores may be feasible as biopores are mostly wide, cylindrical pores that sustain their shape over the full pore length. In contrast, abiogenic pores are frequently smaller, not connected and rather irregular in shape, although these characteristics may change over time due to biotic and abiotic processes. Within biopores, distinct morphological characteristics may point to root or earthworm origin (Haas and Horn 2018). As root hairs and lateral roots can penetrate the pore wall, numerous microscale pores, and thus a very porous wall, partly with root residues inside, are found when the biopore is predominantly formed by plant roots (Koebernick et al. 2017, 2019). In contrast, pores created by earthworms contained rather wavy pore walls of lower porosity (Haas and Horn 2018). Thus, the visual inspection of biopores, along with chemical and microbiological analyses of pore linings, appears to be a promising strategy for drawing conclusions on pore origin.

Moreover, land use and site properties can help to reconstruct spatial patterns in pore occurrence. Smaller biopores are preferentially found in the upper part of a soil profile and are associated with decaying roots. As root mass decreases with depth, biopores created from decaying roots are less abundant in deeper soil layers (Stolze et al. 2018), and pores created by earthworms and other soil fauna become more dominant. Therefore, the ratio of root-derived biopores versus soil faunal biopores is likely to change along the soil profile towards relatively more biopores being created by soil fauna with increasing soil depth. However, ecosystem-specific deviations from this pattern can be expected as, for example, tree roots can penetrate deeper into the soil than the soil fauna usually does, resulting in deep root-derived biopores. In agricultural soils, the majority of biopores persists in the subsoil, as tillage disturbs the three-dimensional structure of the topsoil (Ehlers et al. 1983; Or et al. 2021). Hence, the topsoil mostly features recently developed small-scale pores and the ratio of large biopores (>6 mm) to small biopores (<4 mm) increases with soil depth (Stolze et al. 2018). Any factor promoting the growth of deep roots and earthworm activity also promotes biopore formation. For example, organic fertilizer application can increase earthworm abundance, as the organic material serves as a direct food source for earthworms (Tiwari 1993; Iordache and Borza 2010). Moreover, climatic conditions and soil mechanical states are crucial for the abundance of earthworms (Ruiz et al. 2021). However, fertilization will also promote root growth into deeper soil layers (Zhou et al. 2013; Zhang et al. 2018). Hence, a simple depth-related differentiation of root- or earthworm-derived pores is not generally possible.

The formation and decay of biopores is also subject to temporal dynamics. The initial formation process can be studied at revegetation sites, where significantly more pores were observed after 11–20 years than at sites aged 3–5 and 6–10 years (Colloff et al. 2010). Similarly, in a chronosequence of reclamation sites in a mining area in Germany, biopore formation occurred primarily during the first 12 years, but stagnated thereafter, indicating that a steady state had been reached (Lucas et al. 2019). Such a steady state requires that either the formation of new biopores is
compensated by the decay of others within this time interval or that earthworms mainly re-utilize existing biopores. The latter assumption was supported by Han et al. (2015), who observed an increased abundance of earthworms, but no increase in biopore formation over three years of fodder cropping. Thus, it is likely that earthworms re-used existing pores including root-derived pores, resulting in a mix of root and earthworm effects within these pores.

Taken together, the differentiation of pores remains a challenging task in natural soils when considering that pores undergo temporal changes; that is, that existing biogenic or abiogenic pores are reused by plant roots as well as by earthworms, which modify pore characteristics and leave their own footprints at the pore walls. Little is known about the degree to which microorganisms or specific substances exuded by the roots or earthworms, such as mucilage or gut residues, alter pore wall morphologies, (micro-)biology and related functions and can be used as signatures for plant and earthworm derived pore traits to reconstruct pore origin or even history.

Effects of biopores on plant growth

When are roots growing in pores?

The way roots use biopores, regarding the portion of the root growing in the pore (e.g., Atkinson et al. 2020) and regarding the usage of the pore by one or several roots, is highly variable (e.g., Han et al. 2017). This has implications for root-soil contact and, accordingly, for nutrient and water uptake. Also, the growth of individual roots within pores can differ. Roots can grow vertically within the pore with or without pore wall contact, cross a pore and re-enter the bulk soil, or follow the pore for a while and then re-enter the soil. For barley roots, even a spiral growth along the pore wall has sometimes been observed (Athmann et al. 2013; Pfeifer et al. 2014). However, this growing pattern is considered to occur less frequently.

Root growth towards and inside pores has been termed trematropism (Dexter 1986). Later work has suggested that root growth in relation to pores is not only guided by trematropism, but likely affected by further environmental stimuli (Pfeifer et al. 2014). Besides gravitropism, which is widely known to induce directional root growth, further root tropisms have been described to guide root growth in soil in general, such as thigmotropism (directional root growth as a response to a stimulus of touch; Lee et al. 2020), oxytropism (roots follow a gradient in oxygen concentration; Muthert et al. 2020; Porterfield and Musgrave 1998), hydrotropism (roots follow a gradient in water content; Eapen et al. 2005; Barej et al. 2014) and nutritropism (roots follow a gradient in nutrient concentration; Yamazaki et al. 2020). The relevance of these tropisms for root growth in relation to soil pore structure remains largely unknown, despite evidence that different tropisms will act interactively such as trematropism and oxytropism (Pfeifer et al. 2014; Moulton et al. 2020). The relevance and interplay of different tropisms will become clearer once a deeper understanding of these mechanisms controlling root growth is available, including the identification of the specific environmental stimuli, the plant sensing mechanisms, and the signaling cascades that lead to directed growth responses. These aspects remain particularly unknown for trematropism, which may be multi-faceted. Clarifying the mechanisms underlying trematropism will lead to a better understanding of the apparently contrasting findings about root growth in pores. For example, Athmann et al. (2019) observed only a marginal use of biopores by cereals or faba bean plants. Similarly, a rhizotron study by Bauke et al. (2017a), observed no impact of artificial macropores in the subsoil on root growth and nutrient uptake. In contrast, Stewart et al. (1999) found that around 80% of blue grass and tall oat grass roots grew in biopores.

As an intermediary between root growth in bulk soil and in pores, roots can also grow in close proximity to pores; that is, within 1–3 mm of a pore wall (Stewart et al. 1999). These roots may benefit from the enrichment of organic matter and therefore nutrients in the biopore sheet (Fig. 3), while the short distance to the empty pore space ensures sufficient supply with air and water towards the root. Moreover, roots maintain intensive soil contact in this way, which is necessary to, for example, anchor the plant in the soil and for nutrient uptake (Stewart et al. 1999). However, it is also possible that the root is not able to penetrate further into the bulk soil due to soil compaction around the pore. This discussion of possibly underlying reasons for observed root growth patterns illustrates that root growth in relation to pores has so far mostly been explained by specific soil traits, while
the underlying plant mechanisms leading to observed root growth patterns remain to be unraveled.

One factor that evidently controls root growth in pores is bulk soil density, which has accordingly been addressed in a number of studies. De Freitas et al. (1999) compared root growth of maize in soils with bulk densities of 1.4, 1.6, and 1.8 Mg m\(^{-3}\) and observed preferential root growth in pores when soil bulk density was at or above 1.6 Mg m\(^{-3}\). Likewise, wheat roots were preferentially growing in bulk soils at a lower bulk density of 1.2 Mg m\(^{-3}\), with only 12.5\% of roots growing in biopores, while this increased to 68.8\% of roots in pores in the compacted soil with a density of 1.6 Mg m\(^{-3}\) (Atkinson et al. 2020). Thus, higher soil bulk density stimulates root growth towards and within biopores. This may even result in root clumping inside biopores, as has been observed for some hardened soils in Australia (Stewart et al. 1999; Pankhurst et al. 2002). White and Kirkegaard (2010) found 30–40\% of the wheat roots clumped together inside pores of the topsoil, and this percentage increased to 85 to 100\% in the more compacted subsoil. In an exemplary pot experiment, we demonstrated enhanced clumping of maize roots in pores for soil compacted to a bulk density of 1.8 Mg m\(^{-3}\) compared to a soil with 1.4 Mg m\(^{-3}\) bulk density (Fig. 4).

Soil bulk density is closely related to penetration resistance for roots growing in bulk soil. A penetration resistance of >2 MPa has been reported as a threshold value limiting root elongation (Bengough et al. 2011), while a penetration resistance of 2.8 MPa resistance prevented roots from re-entering the bulk soil from a pore (Atkinson et al. 2020). Hence, root length is promoted, especially in wide biopores, where root-soil contact is strongly reduced and penetration resistance decreases towards 0 MPa. Increased root growth rates inside the pores of otherwise compacted soil have been observed repeatedly (Valentine et al. 2012; Atkinson et al. 2020). However, the effect of penetration resistance may be modulated by soil moisture, as soil resistance increases with decreasing water content. Volkmar (1996) investigated root growth in soils with water potentials of -0.01, -0.1, and -1.5 MPa; that is, with increasing penetration resistance. Biopore-associated root growth was only observed at the two most negative water potentials; that is, in dry conditions. This again underlines the relevance of climatic conditions for biopore research, as discussed above for the diverging observations in Australian and European studies, and highlights the need for comparative biopore research in different climatic settings.

Besides soil bulk density and moisture content, the morphological properties of biopores determine the extent of root growth therein. In a field experiment with barley, an increase of fine roots was observed in a soil with mainly small pores, which had been created by a fine rooted pre-crop. By contrast, the growth of medium (2–5 mm) and large roots (>5 mm) was increased in a soil with wider biopores created from taprooted pre-crops (Han et al. 2017). Hence, the axial and radial pressure derived from the pore structure influences root elongation, as well as radial expansion of the roots within a pore (Han et al. 2017). In addition to pore diameter, the orientation of pores within the soil affects both the extent of root growth within the pore and the tendency for roots to grow

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**Fig. 4** Maize roots growing inside artificial pores (6 mm inner diameter). (A) Soil compacted to 1.8 Mg m\(^{-3}\) bulk density. All roots grow inside pores and clump together. (B) Soil compacted to 1.4 Mg m\(^{-3}\) bulk density. Only some roots grow in pores, growing either attached to the pore wall or establishing soil contact via their lateral roots. For information on the experimental design, see Supp. 3
out of the pore into the bulk soil. Hirth et al. (2005) reported that 78% of ryegrass (*Lolium perenne*) roots grew for a few millimeters in biopores with an angle of 40°-from-horizontal before re-entering the soil; by comparison, in a setting with vertical pores, only 13% of the roots grew within the pores and re-entered the bulk soil. Similar results were produced in a computer model by Landl et al. (2017), who found that 18% and 60% (reliant on the parametrization) of the simulated average root length fraction was growing in 40° inclined pores, whereas with 90° inclination (that is, in vertical pores), 83–98% of the roots remained inside the pores. Further, lower roughness and microporosity of the pore wall may keep the roots inside the pores, while higher roughness and microporosity may facilitate re-entry into the bulk soil (Hirth et al. 2005). A microporous structure of the pore wall facilitates root hairs to anchor the root at the pore wall, which may be crucial for successful outgrowth into the bulk soil (Bengough et al. 2016).

Apart from such physical pore properties, incremental evidence suggests that increased nutrient availability may stimulate root growth in biopores, as six times more roots were observed in artificial pores with a high nutrient content than in artificial sand-filled or empty pores (McKee 2001). N-species such as nitrate and ammonia, for instance, are known to modulate root elongation and architecture (e.g., Lima et al. 2010; Ötvös et al. 2021), and these mineral species as well as other soil nutrients may be enriched inside pore walls after fertilization or due to enhanced microbial activities (e.g., Bundt et al. 2001; Fuhrmann et al. 2019). However, these latter effects are investigated less frequently and are therefore difficult to isolate systematically.

Finally, plant characteristics are also relevant for the extent of root growth within pores. Pores are used differently by the specific root systems developed by different plant species. Accordingly, the root growth of barley, wheat (fibrous root system) and faba bean (not fibrous roots and not a distinct taproot) was only marginally increased in biopores compared to bulk soil, whereas observations for the growth of oilseed rape roots (taproot) in biopores were more variable (Athmann et al. 2019; Huang et al. 2020). Likewise, exploration strategies can be plant-species-specific and affect root growth in pores: while the taproots of oilseed rape were mainly growing in the center of a pore with only laterals establishing pore wall contact and extending into the pore wall, the fibrous roots of wheat were mostly growing attached to pore walls, establishing a high root-soil contact area (Athmann et al. 2013). Besides, several wheat roots often grew within the same pore. It can be assumed that different exploration strategies of roots growing in biopores are genetically driven, as genes responsible for various aspects of root growth can vary among species and genotypes. Islam et al. (2021) compared a deep rooting rice variant with a shallow rooting genotype and found greater root length density, increased root abundance, and a higher branching of roots for the deep rooting genotype compared to the shallow rooting one. By contrast, Zhou et al. (2021) provided evidence that the development of the wheat root system in the field is more strongly affected by the soil macropore system than by genotype. Hence, genotype-specific differences in root growth within pores are currently not consistently understood.

Taken together, different soil properties, pore characteristics and the plant itself determine the extent and appearance of plant root growth in pores. Further studies and hypothesis-driven research are now needed to better understand the underlying mechanisms that determine and control the extent to which plant roots grow within pores. It is likely that phytohormones and other signaling pathways play important roles in directing root growth towards and within biopores. However, these aspects demand further investigation, not only in general (Izzo and Aronne 2021), but specifically in the context of pore colonization. For example, there is no conclusive evidence from the literature about whether root growth into or out of a pore is indeed directional and, if so, which parameters trigger these growth patterns.

**Are biopores beneficial for plant growth and agriculture?**

Biopores and their specific properties can provide favorable conditions for root growth, but at the same time may also limit plant performance (Table 2). Depending on the pore and root diameter, only part of the root surface will be in contact with the soil when growing along a pore wall, but the modulating beneficial or detrimental effects of biopores for root growth are yet to be resolved (Carminati et al. 2013). Currently, only minimal information is available about root-soil contact zones inside biopores, mainly
because of the absence of techniques for reliable quantification (Athmann et al. 2013; see also previous chapter on methods for studying biopores for a more detailed discussion).

It has been suggested that roots grow preferentially in pores with a larger diameter than their own (White and Kirkegaard 2010). This will result in lower root-soil contact as part of the root surface is facing towards the empty pore space. However, additional lateral roots and root hairs growing along the pore wall may again increase the root surface area and hence root-soil contact (White and Kirkegaard 2010; Han et al. 2017). By contrast, a reduction in root-soil contact is reinforced by root clumping inside of pores, as observed, for example, in the hard soil of southern Australia (White and Kirkegaard 2010). Consequently, root-soil contact of individual roots is limited when growing in pores, and multiple roots may even compete for contact space along the pore wall.

However, the roots that are in contact with the soil may benefit from enhanced nutrient availability in the pore wall. The biopore linings created by earthworms are usually enriched with nutrients, such as N, P, and C and likely also basic cations, when compared with the bulk soil (Jégou et al. 2001; Milleret et al. 2009; Barej et al. 2014; Bauke et al. 2017b). Further, biopores are known to represent hotspots of microbial abundance and activity, as will be discussed in more detail below. Both nutrient cycling (Bauke et al. 2017b) and plant nutrient uptake from earthworm-derived linings along the pore walls are enhanced (Grabmaier et al. 2014). Likewise, enriched nutrient concentrations have been reported for pore walls created by decaying roots. Higher concentrations of organic C, total N, bicarbonate-extractable P, Ca, Cu, Fe, and Mn were observed in the soil within 1–10 mm of the pore wall surface (Pankhurst et al. 2002), although more recent studies indicate a gradient in nutrient concentrations from the pore wall towards the bulk soil (Petzoldt and Kautz 2021). Also in abiogenic pores, such as in cracks of rice paddy fields, an enrichment of nutrients and elevated microbial activity has been observed (Fuhrmann et al. 2019). This enrichment is probably due to a leakage of nutrients from the nutrient-rich topsoil into the pores or from fertilizer translocation. It is known that soluble organic compounds from decaying plant residues and organic matter can enter the biopores with percolation of water (Kaiser and Kalbitz 2012). These compounds, being rich in nutrients (Erinle et al. 2020), are transported along the pores, thus contributing to the formation of nutrient hotspots, especially in the subsoils, as well as to respective nutrient losses from the topsoil.

Soil pores also contribute to increased diffusion of oxygen and transport of water into deeper soil layers. As biopores increase the air permeability in soil (Dziejkowski et al. 1997), roots in pores will gain a higher supply of oxygen, which is needed to sustain metabolic processes (Laan et al. 1989). Sufficient aeration of the soil is essential for plant growth and productivity (Grable 1966; Stepniewski and Stepniewska 2009; Ben-Noah and Friedman 2018). This is especially relevant in deeper layers, where oxygen concentration is lower than at the soil surface. Moreover, pores enable fast drainage
of water after a heavy rainfall, which results in faster and better aeration of the roots, both in the pore and in bulk soil (McMahon and Christy 2000). Larger biopores especially enhance water percolation (Pitkänen and Nuutinen 1998) and help reduce soil erosion in sloped terrain (Pitkänen and Nuutinen 1998). However, as water draining through the pores percolates too quickly to be taken up by the roots, these processes will not be addressed in further detail here. For information on water flow in macropores, we refer to the review of Jarvis (2007).

Pores do not only provide preferential flow paths for water, but also for root growth due to the low penetration resistance, thus forming ‘highways for the roots’ (Passioura 2002) towards water and nutrient resources in deeper soil horizons. Accordingly, a significantly increased rooting depth was observed for plants in field experiments with a high number of large biopores (Huang et al. 2020). It has been suggested that root growth, especially in deep soil layers, is only possible via biopores (White and Kirkegaard 2010; Gao et al. 2016). Deep rooting via biopores provides better access to water resources in the subsoil (MacKenzie et al. 2009; Gaiser et al. 2012; Landl et al. 2019) or even to weathered bedrock (Sternberg et al. 1996). This additional water supply is particularly important during dry spells (Gaiser et al. 2012; Landl et al. 2019; Seidel et al. 2019). Similarly, root growth via biopores should provide better access to nutrient resources in the subsoil (Han et al. 2017, Seidel et al. 2019). For more detailed information about deep rooting strategies, see also the opinion article of Gao et al. (2016).

Despite these advantages of deep roots, it must be kept in mind that enhanced root growth towards water and nutrient resources into deeper soil horizons represents a substantial investment in below-ground carbon allocation. The photosynthetic assimilates directed to the roots may be at the expense of yield. This is especially critical under conditions where roots become trapped in the pores and cannot re-enter the bulk soil (Atkinson et al. 2020), which will then even decrease water and nutrient uptake from deeper soil layers. Given the lack of quantitative proof, more research should be directed towards a specific calculation of C and nutrient balances within plants growing with or without biopores, with the aim of estimating thresholds for root biomass production in deeper soil layers in return for additional nutrient and water resources accessed.

In the broader agricultural and environmental context, the positive effects reported for water percolation via pores into the subsoil can have trade-offs, particularly when nutrient leaching contaminates groundwater reservoirs (Refsgaard et al. 1999; Lehmann and Schrotth 2009). Similarly, pesticides can leach quickly through pores into the groundwater (Roulier and Jarvis 2003). The amount of chemicals or nutrients leached through biopores depends heavily on rainfall intensity. With heavy rainfall soon after the application of fertilizer or pesticides, substantial leaching can be observed, whereas it may be almost lacking upon drizzles (Jarvis 2007).

Thus, biopores provide advantages and risks for root growth, plant yield, and agriculture. We suggest that the final balance between benefits and risks for plants depends on both climate and soil conditions and is modulated by root traits. Assessing the benefits and drawbacks of root growth in pores under different plant growth conditions and identifying root-traits that provide advantages in this context represents a promising way to increase resilience under changing environmental conditions. With extreme climate events, the benefits provided by pores in terms of increased plant resilience to environmental stress such as dry spells with limited water, and thus also limited nutrient supply in the surface soil, are likely to outweigh the risks in a positive manner. The same applies to soils with root restricting layers and soils prone to subsoil compaction or erosion. However, in terms of improved soil pore management, it should be kept in mind that, with more heavy rainfall events in future climates, the leaching of nutrients and chemicals would likely be increased.

Quantifying the impact of biopores on plant nutrient uptake and yield

Due to the above-mentioned restrictions (Table 2), some studies reported no responses, or even negative responses, of plant growth to pores in soil (Passioura and Stirzaker 1993; Bauke et al. 2017a). The ideal root system benefiting from the presence of biopores is likely one that requires little C for root growth and exudation, but grows quickly through biopores into the subsoil. This might not require maximum root soil contact but an optimized one, guaranteeing maximum
deep root survival in extreme seasons. Under such circumstances, the plant may acquire subsoil water and nutrient resources in case they are needed, and disregard them when not required. Given these pre-requisites, biopores may improve plant nutrition, irrespective of their small contribution to the overall soil volume. The first evidence to support this assumption appeared in 1895, when Schultz-Lupitz showed an increased yield of potato when cultivated after lupine as pre-crop in the field (Schultz-Lupitz 1891). However, it is likely that this effect resulted from the additional N supply to the potatoes from symbiotic N fixation during lupine cultivation, rather than or in addition to positive effects of lupine-derived biopores. The role of biopores, which are introduced into soils by specific crop rotation regimes, for yield increase has since then been addressed in further studies. Stirzaker et al. (1996) observed an increase in total leaf area of 10–25% for barley when cultivated after lucerne, ryegrass, canola and clover, and also when grown in hard soil under low soil moisture with artificial pores filled with peat compared to plants grown in hard bulk soil (Stirzaker et al. 1996). A more recent study by Dresemann et al. (2018) confirmed the positive effects on yield by reporting a higher shoot dry matter and N uptake of winter wheat when grown in the presence of earthworm biopores. When plants were grown in soil containing a high phosphorus concentration but low moisture content, the shoot dry matter increased by 66% in the presence of pores. By contrast, the shoot dry matter increased by only 39% under high soil moisture content compared to plant growth in soil without biopores. Similar patterns were seen for N uptake (101% vs. 62%) and P uptake (83% vs. 33%). The authors explained these effects mainly with better access to deep water when roots grew in biopores (Dresemann et al. 2018). However, an increased mobility of the nutrients due to biopores may also be a possible explanation. Dry soil limits N uptake due to low N mobility (Gauer et al. 1992), whereas solute transport in biopores may increase the mobility of N. It also seems reasonable to speculate that deep roots in biopores are able to intercept nutrients, especially N, that are otherwise leached through the pores into the even deeper ground. The interception of nutrients could be especially important during rainy seasons (for example, a wet winter with heavy rainfall in Europe). Modelling studies have supported estimates of increased biomass production and grain yield in soils with biopores (Mboh et al. 2018), as well as higher P and higher N uptake from deep soil layers for plants being cultivated after lucerne as pre-crop (Seidel et al. 2019). Again, this was especially relevant during dry spells and in relatively dense soils (Mboh et al. 2018; Seidel et al. 2019). These studies confirm the beneficial effect of biopores on plant nutrient uptake and plant yield, although it should be stressed that many of the reported findings are currently co-observations, such as examining the presence of pores in soil along with higher yield. The specific mechanisms underlying such correlations and their respective role in plant performance frequently remain to be elucidated. Moreover, the observed correlations are likely to be modulated by different conditions, such as internal factors like root-soil contact in pores, pore properties, root traits, and rhizosphere processes, as well as by external factors like the prevailing climatic conditions that affect nutrient and water uptake. Such modulating effects also need to be mechanistically elucidated in future studies.

**Biopores as microbial hotspots in soil**

Microorganisms play a major role in soil. They contribute to microaggregation and related pore-size distribution (Totsche et al. 2018), as well as to all relevant biogeochemical processes like soil C sequestration, decomposition of organic material, and nutrient cycling (Geisseler et al. 2010; Fierer 2017; Jacoby et al. 2017). Hence, they directly or indirectly contribute to plant nutrition (Jacoby et al. 2017; Jansson and Hofmockel 2018). However, only a small amount of the soil volume is occupied by microorganisms and microbial activity is known to be rather unevenly distributed in soil, primarily linked to so-called hotspots with higher process rates (Kuzyakov and Blagodatskaya 2015; Hoang et al. 2016a, b). Biopores are considered to represent such microbial hotspots in soil due to the higher concentrations and better availability of organic carbon compounds (Kuzyakov and Blagodatskaya 2015; see also discussion of biopore properties in previous chapters). Hence, biopores should represent a major interface of root growth and microbial activity in soils. We observed a dense microbial colonization of roots growing in pores and especially of root hairs that established contact with the pore wall (Fig. 5). However, for this exemplary microscopic analysis, plants were grown in a pot.
experiment with artificial pores, created mechanically without any impact of previous plant growth or faunal activity. Biological processes within pores are highly likely to imprint additional differences in the microbiome and its activities, leading to even greater microbiological differences between pores and surrounding bulk soil.

Microbial community structure in biopores

Only a limited number of studies have analyzed differences in microbial community composition between pore and bulk soil. Uksa et al. (2015) studied the bacterial community structure using DNA based community profiling in agricultural soil profiles. They showed that differences exist in bacterial community composition in the bulk, biopore, and rhizosphere soil, confirming that each of these compartments in a soil hosts specific microbiota. It may seem obvious that the observed microbiological differences between matrix soil and pore wall soil are linked to the higher availability of carbon substrates in pores (Fig. 3), which is in agreement with the observation that copiotrophic bacteria such as Proteobacteria and Bacteroidetes were more predominantly detected in the rhizosphere and in the pore wall than in bulk soil. Proteobacteria and Bacteroidetes are known to be abundant in carbon-rich environments and are involved in the metabolization of labile carbon inputs (Fierer et al. 2007). By contrast, oligotrophic bacteria such as Acidobacteria, Actinobacteria, Gemmatimonadetes, Planctomycetes, and Verrucomicrobia were observed at higher relative abundance in the bulk soil (Uksa et al. 2015). Especially, Acidobacteria are known to be associated with low C mineralization rates (Fierer et al. 2007). That higher C and nutrient availability in biopore soil is supporting microbial life is substantiated by a 26–35 times higher recovery of phospholipid fatty acids (PLFAs) in biopore soil than in bulk soil samples (Banfield et al. 2017a). Thus, the limited number of available studies suggests that the biopore microbiota is different from that in bulk soil in terms of community composition and abundance, likely caused by C availability. Fewer differences may be present in the case of abiogenic pores, according to results obtained from cracks in a rice paddy field (Fuhrmann et al. 2019). Changes in bacterial community composition between pore wall and bulk soil were not evident in Fuhrmann et al.’s work and differences in bacterial population size remained weak.

Whether and to what extent different pore types support specific microbiomes remains currently largely unclear. A comparative analysis of PLFA profiles by Banfield et al. (2017a) indicated that
Gram-positive bacteria were more common in root pores compared to earthworm pores, whereas Gram-negative bacteria were more abundant in earthworm pores. Similarly, fungal PLFAs were more common in the earthworm pores. Banfield et al. again attribute these differences to C availability, which is lower in root biopores than in earthworm pores. The higher abundance of fungi in earthworm pores may appear unexpected, considering that earthworms are digesting fungal biomass and interrupt the hyphal network (Medina-Sauza et al. 2019). However, substrate availability may counteract the negative effects of earthworms here. Clearly, more studies are needed to obtain broader insight.

Taken together, the available studies provide initial evidence that pore microbiomes are not only different from those in bulk soil, but may even be pore-type specific; an aspect that clearly deserves more attention to deepen our understanding of microbial players and processes occurring in different soil compartments and pore types. Knowledge about specific differences in the microbiome between pore types may have the potential to improve our abilities to differentiate pore types in field-based studies if we can, for example, define indicator taxa. However, the extent to which similar or identical taxa are consistently enriched in the linings of different pore types, or the extent to which other biotic or abiotic factors may modulate pore-specific selection mechanisms, remains unknown.

Dissimilarities in community structure are often accompanied by differences in metabolic processes. Uksa et al. (2014) evaluated the abundance of two selected functional groups of bacteria, nitrifiers and denitrifiers, by real-time PCR targeting functional marker genes (\textit{nirS, nirK, nosZ} and \textit{amoA}). This revealed a higher abundance of both functional groups in the pore walls than in bulk soil. Similarly, a higher abundance of cellulose utilizers in pores was reported earlier (Karsten and Drake 1995). However, as mentioned above, the aeration in biopores is increased, which could lead to decreased denitrification rates (Rohe et al. 2021).

No depth-dependent decline of the nitrogen-cycling populations was observed in the biopores, which is in contrast to the bulk soil (Uksa et al. 2014). This suggests that the higher N cycling rates in pores compared to bulk soil are independent of soil depth (Uksa et al. 2014). Likewise, the total microbial community composition seems not to display depth-dependent patterns within biopores (Uksa et al. 2014; Banfield et al. 2017a). At the same time, community compositional differences between biopore and bulk soil were more pronounced in the subsoil than in the topsoil (Uksa et al. 2015), which is explained by greater heterogeneity and spatial separation of hot-spots in the subsoil compared to the frequently tilled topsoil. Furthermore, the topsoil is more nutrient-rich and may therefore show weaker gradients between the bulk soil and the biopore coating. Based on these findings, it can be concluded that the usual vertical stratification of the soil microbiome is apparently overlain by pore-specific traits that control microbial community composition and population size, largely independent of soil depth.

Besides nutrient-cycling microorganisms, plant-beneficial or pathogenic microorganisms affect plant performance, and their occurrence should be carefully evaluated in pores to draw conclusions about possible implications on plant performance upon root growth in pores. We are not aware of any study evaluating plant beneficial microorganisms such as mycorrhizal fungi in pores or at roots growing inside biopores, but some data are available for pathogens. Pankhurst et al. (2002) and Pierret et al. (1999) found higher populations of the root pathogen \textit{Pythium} in pores and the surrounding 1–3 mm of soil in comparison to bulk soil. Otten et al. (2004) reported that large pores represent a preferential growth pathway of the pathogenic fungus \textit{Rhizoctonia solani}. Lastly, Watt et al. (2006) observed increased root colonization by filamentous bacteria when they were in contact with remnant roots. Bearing in mind that remnant roots may have been infected with pathogens, root-root contact may support the transmission of pathogens. Thus, the above-mentioned studies suggest that root growth in pores may increase the risk of pathogen infection (thus listed in Table 2 as a further potential disadvantage). However, with the elevated total microbial activity in this organic matter-rich environment, antagonistic potentials might also be elevated, which has not been evaluated yet either. Another aspect to consider when discussing the presence of pathogens in soil pores is the increased risk of pathogen translocation. Like other microorganisms, pathogens, as well as viruses, can be transported through pores into lower soil layers and even to the groundwater, contaminating it with pathogens, potentially...
even with human pathogens (Guzman et al. 2009). In summary, the microbiome in biopores has not gained much attention yet in biopore research, even though it may hold important benefits or harm for plant yield and health. Analyzing microbial community composition and metabolic traits of the microbiome will provide valuable information about microbial processes occurring in biopores, which control C- and nutrient cycling and, thus, nutrient availability for plants in this particular soil compartment.

**Microbial activity in biopores**

As the enrichment of organic matter in biopores occurs in conjunction with a higher abundance of specific microbial groups, it has been hypothesized that the mineralization of organic matter and mobilization of nutrients occurs at higher rates in biopores than in bulk soil (Athmann et al. 2013; Blagodatskaya et al. 2014). This hypothesis has been reported in different studies based on an analysis of microbial enzyme activities or microbial organic matter decomposition, studied by degradation analyses of biomarkers such as neutral sugars, cutin and suberin-derived lipids, lignin-derived phenols, and free lipids. The ratios of these biomarkers hint at the degradation state of plant residues. The differences in microbial community structure and abundance between pores and bulk soil, as well as between different pore types, are reflected by metabolic activity. Banfield et al. (2018) reported that hemicellulose and lignin are degraded to a higher degree in root biopores than earthworm pores and bulk soil, as these were the main carbon sources available from the plant residues after easily-degrade plant biomolecules had been metabolized. In earthworm pores, by contrast, lignin was only marginally degraded, suggesting that microorganisms may have access to other, more easily available C sources derived from, for example, earthworm casts, in this habitat.

Athmann et al. (2017), who analyzed enzymes involved in the C-cycle (xylanase, cellobiohydrolase, β-glucosidase) and N-cycle (chitinase, chitotriosidase, leucine aminopeptidase), observed increased enzyme activities within root biopores and root biopores following earthworm incubation in comparison to bulk soil. Hoang et al. (2017) confirmed this, and also studied the priming effect (organic matter decomposition stimulated by the addition of labile C sources) in biopores by measuring the CO₂ derived from organic matter decomposition, finding that it was 2.5 times greater in pores compared to the bulk soil. They concluded that biopores increase microbial activities and organic matter turnover and thus contribute to nutrient mobilization for plants. That the walls of abiogenic pores may also support increased microbial activities compared to bulk soil is suggested by the findings of an isotope labelling experiment in abiogenic cracks of a rice paddy field (Fuhrmann et al. 2019). A higher ¹⁵N uptake into fungal residues was measured by compound-specific isotope tracing of amino sugars 24 h after ¹⁵N-urea application. However, changes in microbial community composition between pore wall and bulk soil were not evident in that study, and differences in bacterial population size remained weak, indicating that abiogenic pores may be less distinct from bulk soil than biogenic pores.

There is evidence that bacterial abundance inside biopores is not depth-dependent in a field study (Uksa et al. 2014; Banfield et al. 2017a). However, a recent mesocosms study by Hoang et al. (2020) revealed depth-dependent differences in substrate turnover along biopores. The authors conducted a labelling experiment with ¹⁴C-wheat straw fed to earthworms and found higher substrate turnover rates in topsoil biopores than in those allocated in the subsoil. The authors attributed this effect to more efficient enzymes in the topsoil than in the subsoil (Hoang et al. 2020). Also, Athmann et al. (2017) found a higher enzyme activity in the 45–75 cm soil layer than in the 75–105 cm soil layer. However, the topsoil is known to be favored by *Lumbricus terrestris* (Hoang et al. 2020), leading to higher enzyme activity in topsoil biopores. In summary, there is conclusive evidence for higher microbial activity in pores and thus higher substrate turnover rates. The consequences of this higher microbial activity and higher nutrient turnover rates in selected pores for overall plant growth remain to be studied.

**Linking biopore and rhizosphere processes and traits**

Intensive research on the rhizosphere microbiome and on rhizosphere processes (Mendes et al. 2013; Philippot et al. 2013; Gao et al. 2016; Venturi and Keel 2016; Busby et al. 2017; Sasse et al. 2018; Oburger and Schmidt 2016; Santoyo et al. 2017;
Garcia and Kao-Kniffin (2018) has been done in the past. Moreover, first insights in pore microbiology and processes have been established. However, despite the fact that these two hotspots of microbial activity and nutrient cycling meet in nature, we are not aware of any studies that have addressed rhizosphere processes within biopores. Considering that roots in biopores should rather suffer from the disadvantage of reduced root-soil contact, the interactive and potentially additive effects of nutrient cycling by biopore and rhizosphere microbial communities may hold the key to explaining the observed beneficial effects of biopores on overall crop performance. However, these interactions are likely to be depth-dependent.

Regarding the microbial community structure, some analogies can be established between biopores and the rhizosphere. As both hotspots are nutrient-rich, copiotrophic bacteria have an ecological advantage. In particular, Proteobacteria and Bacteroidetes have been found to be abundant in both biopores (Uksa et al. 2015) and rhizosphere (Bulgarelli et al. 2013). Knowing that the microbial community of the rhizosphere is strongly influenced by plant traits, it can be expected that the microbiome in the biopore is also altered due to plant root growth and in dependence on plant traits, which can vary depending on the plant species and its developmental and physiological state (Sasse et al. 2018).

Moreover, it can be assumed that microorganisms in the rhizosphere benefit from rhizodeposition (Sasse et al. 2018), which depends, among other factors, on root growth in the pore, the extent of root-soil-contact zones, and the spatial dimension of the rhizosphere created along a pore wall. If the root has full soil contact in small pores, the microbiome of the whole biopore might be affected. However, in larger pores, these interferences are expected to be largely limited to pore regions with direct root contact. Kuzyakov and Razavi (2019) found a distribution of root exudates up to 10 mm away from the root; that is, at a magnitude that extends well into the biopore sheath. It is important to note that nutrient concentrations in the rhizosphere are usually considered to decline from the soil towards the root, due to ongoing nutrient uptake, whereas nutrient gradients in the biopores point to elevated nutrient contents at the biopore lining; that is, in direct vicinity to the root (Petzoldt and Kautz 2021). When both spheres overlap, the rhizosphere community inside biopores may be able to overcome nutrient limitations resulting from plant nutrient uptake more easily than their counterparts in a rhizosphere soil that developed from bulk soil. However, it remains to be studied how nutrient concentrations and gradients in the pore wall change in space and over time when influenced by root-related processes. This applies in a similar way to the microbiota that develops in dependence of these processes.

It is possible that the above-mentioned phyla are even more strongly promoted in the interface of biopore and rhizosphere hotspots. However, it is also conceivable that other taxa are promoted instead or in addition. Evidence for the stimulation of additional taxa in soil regions where two hotspots meet comes from the first studies that addressed this aspect. Maarastawi et al. (2018) found that degradation of plant residue in the detritusphere is carried out by a partially different microbiota when the detritusphere overlaps with the rhizosphere. The detritusphere is a thin layer of soil surrounding plant residues and predominantly occurring in the topsoil. It is influenced by plant residue decomposition and is therefore known to be rich in nutrients (Erinle et al. 2020). The same study also showed that the presence of detritus reduces the uptake of rhizodeposits in microorganisms to some extent (Maarastawi et al. 2019). Likewise, interferences were recently reported by Nuccio et al. (2020), who revealed that higher taxonomic and functional diversity occurs in the overlapping rhizosphere–detritusphere than in the individual hotspots. Evidence for a potential impact of biopores on the root microbiome was provided by Zhou et al. (2020). On investigating wheat and chickpea roots in soil cores with decaying roots and soil cores without decaying roots, they found that the detritusphere microbiome controls the microbiome of the rhizosphere in a stronger way than the plant species (Zhou et al. 2020). While these studies demonstrate that alterations can be expected when biopores and rhizosphere overlap, the specific consequences for the microbiome, the processes they control, and the plant performance remain to be understood.

Focusing on the processes, the mobilization of nutrients is known to occur at increased rates in both biopores and rhizosphere in comparison with the bulk soil. The microorganisms and involved exoenzymes are more abundant in both types of hotspots due to the
higher availability of easy-to-degrade organic C compounds (Dijkstra et al. 2013; Hoang et al. 2017), but it remains unknown how such effects add up or cancel each other out when rhizosphere and biopores overlap. In principle, rhizodeposition from roots growing inside biopores may induce priming effects, leading to an increased mobilization of nutrients from the soil organic matter (SOM) in the biopores, accompanied by higher growth and activity of microorganisms than that seen in biopores or the rhizosphere alone. To substantiate this assumption, more data are required on root exudation within biopores, on exudate composition, as well as on its competitive consumption by microorganisms that inhabit either one or both hotspots in soil. Taken together, interferences are expected from rhizodeposition into biopore soil, leading to alterations in the microbiome and the processes in this particular soil compartment. The effects are likely to be spatially restricted to those parts of the pore that are in direct contact with the root, and they are likely to depend on pore age as well as characteristics of the rhizodeposition process, although these cannot currently be substantiated due to the lack of adequate data.

Conclusions

Biopores play an important but ambiguous role in soils. They are hotspots of microbial activity and nutrient mineralization and represent preferential flow paths for root growth, gas exchange, and water percolation. As a result, they replenish the subsoil with water and nutrient resources and provide access to these when needed, but also facilitate leaching losses after heavy rain. For plants, they may facilitate root growth and subsoil access due to improved tropism, gravitropism, oxytropism, nutritropism, and hydrotropism. However, the effects on plants change with soil bulk density: the more the bulk soil is compacted, the more biopores promote root growth and resource acquisition; however, root competition for biopores also increases and an increasing number of roots may be trapped within these pores, eventually even resulting in negative effects on plant performance. Hence, there is no static number of biopore density, size, and abundance for optimum plant growth support; it depends on soil and likely also climatic conditions. The final role of biopores for crop growth is triggered by root-soil contact, which changes along biopores as well as for crop species, and possibly over time. Little is yet known about the soil, climate, and management effects on root-soil contact, nor on specific rhizosphere traits of roots in pores that potentially differ from those of roots growing in bulk soil. To better understand variations in root-soil interactions in biopores, spatially resolved analyses of rhizosphere processes are mandatory, which requires the application of small-scale analyses, such as in-situ endoscopy for visualizing root growth and targeted destructive sample collection, at best guided by imaging approaches such as CT or MRI. Moreover, small-scale resolved and temporal analysis of the microbiota is needed in order to better understand biogeochemical processes in biopores.

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Data availability Not applicable.

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Declarations

Conflicts of interest/Competing interests The authors declare that they have no conflicts of interest.

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