Bridging microbial functional traits with localized process rates at soil interfaces

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Abstract: In this review, we introduce microbially mediated soil processes, the players, their functional traits, and their link to the processes at the biogeochemical interfaces (e.g., rhizosphere, detritusphere, (bio)-pores, and aggregate surfaces) considering biotic and abiotic dynamic drivers. Thereafter, the review mainly focuses on the strengths and weaknesses of current approaches for assessing process localization and corresponding estimation of process rates. We also discuss the challenges for modelling of microbially mediated processes in heterogeneous soil micro-habitats.

Key words: *rhizosphere*, *mycorrhizosphere, detritusphere, (bio)-pores, soil aggregates, soil priming, trophic interactions, statistical analysis of process locations*

Relevant microbially mediated soil processes

In terrestrial ecosystems, most critical biochemical processes belowground are performed by soil microorganisms (Fierer, 2017; Brussaard, 2012), and a broad range of microbial functions contribute to essential ecosystem services, such as soil fertility and resilience/resistance to abiotic and biotic stress (Mulder et al. 2011). One major category of microbial functions in terrestrial ecosystems is the decomposition and transformation of organic compounds entering the soil predominantly as plant materials.

An essential part of C assimilated via plant primary production enters the soil through roots, and root-derived organic matter inputs called rhizodeposits, which range in complexity from cells and lysates to small organic molecules. These are released as a consequence of tissue turnover to polysaccharide mucilage and proteins and biochemically diverse root exudates (Farrar et al., 2003; Nguyen, 2003). The production rate and the quality of rhizodeposits, including sloughed off border cells, mucilage, and root exudates, are governed by plant species and even genotype (Lesuffleur et al. 2007; Mönchgesang et al. 2016), the growth rate and age of an individual plant (Gransee and Wittenmayer 2000; Zhalnina et al. 2018), and by root morphology, e.g. root hairs (Datta et al., 2011; Poirier et al., 2018).

Generally, plants adapt their source-sink relationships dynamically under the varying abiotic-biotic environment, ensuring seed production to provide offspring and/or supporting growth (Smith et al., 2018). Consequently, at different time scales, carbon (C) allocation and distribution patterns in the plant body and the rhizosphere vary widely in magnitudes of allocation rate, compound variety, and quality (Brüggemann et al., 2011). For example, the net C allocation from shoot to root strongly depends on plant phenological stage, with a greater allocation to roots at young plant stages (40 to 60% of photosynthetically fixed C) than at reproductive and ripening stages (less than 15%), (Swinnen et al., 1994). According to temporal profiles, C allocation within the root system is also highly dynamic and differs between internal structures of root organs (Jahnke et al., 2009). The proportion of root carbon moving into the rhizosphere as rhizodeposition ranges from 5 to 10% of photosynthetically fixed C (Jones et al., 2009). Plant-derived resources fuel microbial activity and growth and are further transferred into the microbial food web (Kramer et al., 2016; Hünninghaus et al., 2019). Continuous transformation of primary and secondary C-input into soil requires a selection of mechanisms most suited to the environmental conditions. Such environmental selection results in co-occurrence of numerous decomposition-related processes, leading to the creation of specific interfaces such as rhizosphere, detritusphere, (bio)-pores, and aggregate surfaces (Fig. 1).

Figure. 1.

Soil microorganisms are resilient main decomposers of organic substrates in soils (Brussard, 2012) due to microbial functional redundancy and variability in organic matter degradation pathways (Fierer, 2017; Louca et al., 2018; Maron et al., 2018). The primary organic matter input is microbially transformed into cell constituents or excreted by the cell as labile metabolic products (Bradford, 2016). Moreover, microbial respiration during the transformation of organic matter results in carbon losses from the soil and in CO_2 emission to the atmosphere. An essential fraction of organic C, however, is assimilated within microbial biomass, which is further re-utilized after microbial death by multi-stage microbial succession (Morrien, 2016). Products and residues from dead microorganisms (necromass) serve as a secondary source of soil organic substrates, finally resulting in sequestration of up to 40% of primary C input (Miltner et al., 2014; Buckeridge et al. 2020).

Microorganisms decompose organic substrate to maintain their metabolic requirements and to enable growth. Microbial growth and anabolic reactions require not only C and energy but also a general stoichiometric composition of nutrients (e.g., N and P), which microorganisms have to immobilize from a multiphase (gaseous, liquid, and solid) soil environment (Zechmeister-Boltenstern et al., 2015). Soil microorganisms are able to mobilize and release nutrients, increasing their availability for plants in case microbial stoichiometric requirements are fulfilled (Hodge et al., 2000; Griffiths et al., 2012). Thus, the metabolic activity of soil microorganisms can cause both positive and negative consequences at the ecosystem level, such as i) C sequestration and losses during decomposition and transformation of soil organic matter or 2) nutrients mobilization and losses through leaching of mineral nitrogen and phosphates. These processes can also cause greenhouse gases (N_2O, CO_2, CH_4) emission. The direction and intensity of consequences of microbial metabolic activity are dependent on the functional traits of the organisms performing the ecologically relevant processes belowground and the abiotic and biotic conditions these organisms encounter in their habitats.

Relevant players, their functional traits, and link to the processes

Even though the *active* fraction of a predominantly *dormant* microbial community can be small in nutrient-poor or stressed environments (Jones and Lennon 2010; Barnard et al. 2013), soil microorganisms are among the most abundant **players** in the process of decomposition and transformation of soil organic matter (McGuire et al. 2010). Fresh input of labile organic substrates may enormously increase both fractions of active microorganisms (Blagodatsky et al. 2000) and decomposition of soil organic matter (SOM), thus, causing the “priming effect” (Arsjad and Giddens 1966). These decomposition processes mainly rely on the production of

extracellular enzymes facilitating oxidation or hydrolysis of diverse and complex SOM compounds (Nanipieri et al., 2012). The decomposition intensity of SOM after input of fresh substrate is *mediated by the environment* (Fig.1) and depends on microbial community traits, which can be sub-divided into the three groups (Fig.1). Microbial traits in the first group are very dynamic, e.g., the size of microbial fraction maintaining activity/alert state (*active* biomass) and the time required for the dormant microorganisms to switch to active growth (i.e., *lag-time,* T_{lag}). The second group represents *intrinsic functional traits* of the microbial population, such as maximal specific growth rate (μ_m), generation time (T_g), and an affinity of extracellular enzyme systems (K_m) to soil organic substrates used for microbial growth. The third group refers to phenotypic traits at the level of functional genes, e.g., related to internal microbial metabolism, extracellular resource acquisition, or to stress tolerance (Malik et al., 2018).

The environmentally mediated strategy of the microbial community is based, therefore, on the selection and activation of the populations with *intrinsic functional traits* mostly suited to the individual microhabitat within heterogeneous soil pore spaces. Thus, beyond the quality and regularity of substrate input, the biotic and abiotic environment (Huang et al. 2014), such as soil structure (Berg and Smalla, 2009), presence of organisms (Scheffknecht et al. 2006) and nutritional status (Jones et al. 2004; Hinsinger 2001) affect microbial functional traits in contrasting soil habitats. In soils covered by vegetation, microbial functional traits are in tight interactions with the physiological and morphological *traits of plant* roots. The growing root tip and its rhizodeposits turn the bulk soil into a **rhizosphere** soil with its specific physical, chemical, and biological characteristics, which convert it to a hotspot of biological activity compared to the surrounding bulk soil (Reinhold-Hurek et al., 2015; Goberna et al., 2007).

Rhizosphere interactions within and between interfaces

Plants modulate their surrounding environment either *actively*, i.e., producing exudates and exo-enzymes or *passively* through root and litter detritus (Kaiser et al., 2015), thus **interacting** with the corresponding microbiomes of other **soil interfaces** (e.g., detritusphere, (bio)-pores, and aggregate surfaces). Considering the active role of roots crossing, penetrating, and even forming aggregates, biopores, and detritus, we mainly focus here on the **rhizosphere** and its overlap with other relevant interfaces.

Rhizosphere

Soil - plant interactions are mainly localized in the rhizosphere and extend several millimeters from the root surface into the soil (Dazzo and Gantner 2012). The intensity of root-soil

interactions is demonstrated by pronounced distance gradients from the root surface (rhizoplane) through the rhizosphere to bulk soil), (Kuzyakov and Razavi 2019). Formation of ***chemical*** gradients in the rhizosphere is governed by the input of labile root exudation, changing their localization in accordance with root growth. Root exudation boosts the activity, and modulates a community structure of soil microorganisms, therewith explaining ***spatial biological*** gradients in the rhizosphere (Kuzyakov and Blagodatskaya, 2015). Rhizodeposition fluctuating in space and time due to root growth stimulates the "rhizosphere priming effect" (Cheng et al. 2014; Keiluweit et al. 2015; Nie et al. 2015), which is relatively short-term because the carbon from rhizodeposits is rapidly converted to microbial biomass, and is partly released by microbial respiration; consequently, decomposition rates are reduced in the absence of fresh C input (De Graaff et al. 2010). Furthermore, the broad spectrum of compounds in rhizodeposits (el Zahar Haichar et al. 2014) modulates biological activities in a compound mixture dependent manner. Especially the presence of certain phenolic compounds and sugars can exert profound negative and positive influences on community composition and functional potential of microorganisms (Badri et al. 2009; Chaparro et al. 2013), affect microbial growth, respiration, and decomposing activity (Chigineva et al. 2009; Zwetsloot et al. 2018).

Over time, a decrease in root exudations, e.g., due to a switch from vegetatative to regenerative growth (Aulakh et al., 2001; De-la-Peña et al., 2010), reduces the abundance of rhizosphere microorganisms (Chaparro et al., 2014; Schmidt and Eickhorst, 2014), ultimately leading to a downregulation of enzyme production. Therefore, the temporal-spatial shifts in the rhizosphere gradients of microbial activity impact soil functions such as decomposition and nutrient mobilization (Kuzyakov and Xu, 2013).

As the rhizosphere is one of the most dynamic interfaces actively developing in the local environment, the interactions of the rhizosphere with other interfaces e.g., aggregates, porosphere, and detritusphere can essentially affect functional traits of dominating microbial populations and the rates of microbially – mediated soil processes.

Mycorrhizosphere

Mycorrhizal fungi form a widespread symbiosis with the roots of most land plants, where the fungus delivers mineral nutrients to the mycorrhizal host plant and takes up plant sugars and lipids. The plant C flows to the soil through mycorrhizal roots, together with the external mycorrhizal mycelium, which are defined as "mycorrhizosphere" (van der Heijden et al. 2015), while the fungal hyphae as habitat for microorganisms are generally referred to a "hyphosphere". The external mycelium that may extend centimeters from root surface to nutrient patches provides plant C rapidly, in hours, to soil microorganisms (Drigo et al. 2010;

Kaiser et al. 2015; Gorka et al. 2017), and this resource is used not only for growth by bacteria and fungi but also as a “priming” resource for decomposition (Fontaine et al., 2003). Priming via mycorrhizosphere has been suggested for the widely distributed arbuscular mycorrhizal symbiosis (Cheng et al. 2012; Gui et al. 2017), but ectomycorrhizal fungi of forest trees possess enzymatic capabilities to degrade organic matter themselves to liberate soil organic N, and may either promote or slow down decomposition (Averill et al. 2014; Brzostek et al. 2015; Lindahl and Tunlid 2015). Consequently, arbuscular mycorrhizal symbiosis may interact with a functionally distinct microbiota compared to the microbiota that develops in response to direct microbe-root interactions. It may act at different trophic levels and allocate C into the food web for stimulating N mobilization from OM and subsequent transfer to the plant host (Koller et al., 2013b; Hünninghaus et al., 2019).

Rhizosphere – detritusphere interactions

The photosynthates are released into the soil not only in the form of soluble root exudates but also as a plant detritus (e.g. leaf litter) and rhizo-detritus: dead/decaying root cap, border cells, and, root tissues, thus, forming a rhizosphere – detritusphere interface (Marschner et al., 2012). Due to the spatial and chemical heterogeneity of rhizodeposits and rhizo-detritus in soil, the composition and diversity of the microbial communities are specific within a few mm from the root surface and along the growing root (Chen et al. 2017). Therefore, the specificity of the bacterial community at the overlap of rhizosphere – detritusphere interfaces is determined by a combination of root type (Swinnen et al., 1994a,b; Jahnke et al., 2009), root age, root turnover rate and rhizodeposition (Kawasaki et al., 2016; Steer & Harris, 2000). Root activity modulates decomposition processes in the detritusphere by altering the structure of the microbial community feeding on detritus, e.g., distinct microbial taxa decomposed ^{13}C-labeled rice straw in the rhizosphere and bulk soil (Maarastawi et al. 2018). As a result, essentially, less ^{13}C is assimilated by the microorganisms from the straw in the rhizosphere versus bulk soil due to the higher availability of (labile) carbon in the rhizosphere. The availability of detritus, in turn, reduces the consumption of root exudates by rhizosphere microbiota (Maarastawi et al. 2019), indicating that detritusphere processes are modulating rhizosphere processes and demonstrating interactions between the rhizosphere - detritusphere interfaces. Moreover, this overlap of interfaces has consequences for intra- and inter-specific interactions of the soil biota. For example, changes in litter quality in the root zone alter not only bacterial community structure and function but also cause strong feedback of bacterial grazers thus, affecting links in the soil *food web networking* (Koller et al., 2013a).

Differences in the quality of organic matter input induce contrasting competition situations within the rhizosphere and detritusphere. In the rhizosphere, the majority of easily available

organic C, such as sugars, amino acids, and carboxylic acids, are released through living roots (Jones et al., 2009). In the detritusphere, such easily-degraded monomers are rapidly used up, leaving behind higher molecular-weight compounds such as cellulose or lignin, therewith supporting different functional guilds (Pepe-Ranney et al. 2016; Pascault et al. 2013). Generally, root morphological properties (e.g., root hairs, fine roots, mycorrhiza) intensify a release of exudates and thus, increase the microbial activity, functionality, and consequently substrate utilization, therewith stimulating nutrient mobilization in the rhizosphere. However, at the same time, the plant takes up high levels of nutrients from the rhizosphere and can thus be a strong competitor for nutrient resources, therewith reducing microbial growth (Bonkowski et al., 2000; Blagodatskaya et al., 2014). Therefore, competition for nutrients can exist in the rhizosphere occurring mainly between plants and microorganisms (Kuzyakov and Xu, 2013), while competition in the detritusphere occurs primarily within or between microbial species (Esperschütz et al. 2011).

Rhizosphere – porosphere interactions.

The rhizosphere processes are strongly influenced by interactions with the porosphere, forming a specific microbiome, enriched in copiotrophic bacteria (Uksa et al., 2015). Interactions with the porosphere alter the spatial expansion of the rhizosphere in soil, as root growth and architecture are affected by biopores (Han et al., 2015). Soil pores of different origin (e.g., root or earthworm derived) serve as habitats for microorganisms, as well as conduits for chemical transport and water flow and thus play a key role in controlling rates of soil biochemical processes (Kravchenko et al., 2015; Negassa et al., 2015). The porosphere conditions influence microbial functioning due to the presence of roots, hyphae (Pagliai and Denobili, 1993; Quigley et al., 2018), O_2 levels (Keiluweit et al., 2016; 2017), or root exudate composition. Thus, it is not surprising that various groups of microorganisms are preferentially localized in pores of different sizes (Ruamps et al., 2013) or origin. C substrates localized in large pores are typically processed faster than in small pores (Killham et al., 1993), and dissolved organic matter in small pores is more complex and hence, is less decomposed than that in larger pores (Bailey et al., 2017; Toosi et al., 2017). Such a difference is not limited just to size but also to the origin of pores. For instance, *biopores* of decomposed roots or drilosphere made by earthworms can host different varieties of microbes with distinct rate and efficiency of growth (Hoang et al., 2020; Ma et al., 2017). The complexity of such systems increases even more, when these spheres penetrate each other. For instance, when earthworms reuse biopores of decomposed roots leaving behind the pore wall coatings or when roots grow within the drilosphere (Pagenkemper et al. 2013, 2015).

Rhizosphere – aggregate interactions

Roots and associated fungal hyphae enmesh particles and release agglutinating compounds, thus building up aggregates. Soil is defined as a group of primary soil particles (and/or smaller aggregates) that cohere to each other more strongly than surrounding non-cohesive particles and are considered as soil structural building units (Tisdall, 1996). A hierarchy of soil aggregates ranges from macroaggregates (> 250 µm) that are unstable and susceptible to soil management to the more stable microaggregates (<250 µm) (Six et al. 2004). The group of microaggregates is not homogeneous and is organized even at the smallest scale <2 µm, (Totsche et al., 2018). The primary structural units of microaggregates are composed of silicates, metal oxyhydroxides, organic matter, as well as microbial debris (Chenu and Plante, 2006). The role of roots is especially relevant in the formation of 53 - 250 µm micro-aggregates, while the formation of smaller aggregates (< 53 µm) is mainly governed by microorganisms, clay particles, and physicochemical forces (Tisdall and Oades, 1982; Dultz et al., 2018; Rillig and Mummey, 2006). Due to relatively fast root growth and its associated rhizodeposition, aggregate formation, and turnover in the rhizosphere and the root-mediated shaping/structuring of aggregate surfaces are highly dynamic processes (Wang et al. 2020). The rhizosphere is a remarkable interface, where the *aggregatosphere* interacts with the *detritusphere*, because root detritus (sloughed root cells, dead root fragments and residues) provides a substrate for microbial metabolism. Both microbial metabolic products and rhizodeposits form sticky polymeric substances (Redmile-Gordon et al., 2014), which are involved in the enmeshing and/or gluing of aggregates (Golchin et al., 1994) by binding mineral soil particles and organic fragments in the way of homoaggregation as well as in heteroaggregation (Dultz et al., 2019). The aggregate stability at the rhizosphere – detritusphere interface is directly related to biotic factors such as root biomass, macro-/micro-faunal and microbial activity, all of those being involved in the structuring of aggregate surfaces (Golchin et al., 1994) by modifying the soil biotic and abiotic environment. This results in structural and functional self-organization of the pore space, which improves microbial habitats (Young and Crawford, 2004). In turn, aggregates, as a habitat for organisms, not only organize the soil microbiome but also serve as 'concurrent incubators' that provide a refuge for microbes against predation (Hemkemeyer et al., 2014; Raynaud and Nunan, 2014).

Relevant dynamic drivers of microbially mediated soil processes

Bio-physical conditions:

The role of roots in aggregate formation has essential implications on rhizosphere physical properties like oxygen (O_2) diffusion, which affects both microbiome and physiological root

activities. Radial root growth and shrinkage create gaps along the root surface (Carminati et al., 2009) that may serve as conduits for preferential gas transport and enhance the replenishment of O_2 consumed by aerobic respiration in deeper soil layers (Uteau et al. 2013). Growing roots also create new pores in the rhizosphere because of water extraction, which results in intensified drying and wetting cycles (Materechera et al., 1992; Rasse et al., 2000) or in local stress concentration forming shear cracks, thus enhancing pore network connectivities in the root surrounding (Aravena et al., 2011). Air-filled porosity below a threshold of 12-15% is not sufficient to deliver enough O_2 for root respiration of *Zea mays*, thus reducing the rates of root elongation due to low O_2 levels (Grabler and Siemer (1968). This requirement of maize for air-filled porosity is higher than the 10% rule-of-thumb proposed in earlier studies (Wesseling and van Wijk, 1957; Grable, 1966; Robinson, 1964)); nevertheless the root's ability to modify its surrounding soil structure helps to circumvent this issue (Lucas et al. 2019). The action of the root modifying its environment to ensure fast O_2 transport (Hinsinger et al., 2009) facilitates organic matter turnover in the rhizosphere (Jones and Hinsinger, 2008) due to intensified microbial activity compared to the bulk soil (Nunan et al., 2003). Although most of the soil's respiratory activity (microbial and root respiration) takes place in the rhizosphere (Raynaud, 2010; Kuzyakov, 2002), only a few studies describe the spatial distribution of O_2 in structured aerobic rhizosphere soil also considering the water regime. Water content around 30–40% of soil field capacity generally ensures high respiration rates, pointing out the importance of assessing the moisture level when estimating the required O_2 supply (Balogh et al. (2011). At high moisture levels, microorganisms accelerate their metabolism, and at the same time, more pores are blocked by water bridges limiting oxygen diffusion. Redox potential gradients revealed reduced O_2 consumption with increasing distance from the rhizosphere (Fischer et al. (1989). The gradients of redox potential were most pronounced at the root tips extending up to 3 mm from the root surface. Oxygen limitation was detected at matric potentials exceeding a threshold value around -3 kPa up to field capacity, showing a clear gradient while approaching the root's surface that sharply decreased at a distance of 2-3 mm from the root surface (Uteau et al., 2015). Modelled O_2 consumption in the rhizosphere demonstrated dynamic microbiome responses to O_2 supply and the importance of the soil's structure around roots (Uteau et al., 2015).

Biochemical conditions:

Up to one-third of photosynthates allocated to the roots is released to the soil, i.e., is 'lost' by the plant (Pierret et al. 2007). Such losses through rhizodeposition (Lynch & Whipps 1991) and release of protons (Ayres et al. 2009; Andrianarisoa et al. 2010; Cesarz et al. 2013) serve as plant's investment to develop and modify the physical and biochemical properties of the rhizosphere environment to improve the uptake of nutrients (Augusto et al. 2002). Root

exudates in the form of low molecular weight solutes strongly affect nutrient solubility, microbial activities and the turnover of microbial biomass, but also interactions between plants (Bertin et al. 2003; Helal & Sauerbeck, 1986; Vives-Peris et al. 2020), and the production of extracellular enzymes (Asmar et al. 1994) hereby, indirectly influencing nutrient availability (Grayston et al. 1997; Hamilton & Frank, 2001; Herman et al. 2006; Landi et al. 2006). Roots can by-pass its surrounding soil volume by self-regulation via the production of root hairs and exudates, by which more photosynthetic resources are allocated belowground (Pages 2002). Furthermore, the release of signaling molecules such as abscisic acid present in root exudates (Hartung et al. 1994) promotes the selection of particular microbial taxa within the vicinity of the root system (Oger et al. 1997; Marschner et al. 2004), allowing efficient complementary functioning of roots with microorganisms for nutrient mobilization. The release of H^+ by roots into soils is one of the dominant mechanisms of plants for nutrient mobilization and maintenance of electrochemical potential in the rhizosphere (Marschner, 2012). Among various plants, legumes acidify rhizosphere soil strongly (Israel and Jackson, 1982; Haynes 1983), while some other plants (e.g., most of the cereals) release OH– ions by roots (Youssef and Chino 1989). Overall, the ability of plant species to influence the rhizosphere pH depends on the initial soil pH as well as N fertilization (Kuzyakov and Razavi 2019).

Trophic interactions:

An additional relevant driver of microbial processes are interactions with soil organisms of higher trophic levels (Scheu et al. 2005). For example, rhizobacteria are top-down regulated by grazers, particularly by protists (Bonkwoski et al. 2004). Grazing strongly affects the composition and functional evolution of microbial communities and fosters C- and N mineralization from detritus for plant uptake (Alphei et al. 1996; Geisen et al. 2018). Those mineralizsation processes are depending on the spatial arrangements, size as well as detritus quality (Bonkowski et al. 2000; Koller et al. 2013). During decomposition of labile and recalcitrant C fractions of detritus, protist communities themselves undergo a temporal succession on fine spatial and temporal scales (Hünninghaus et al. 2017). Microbial processes are also shaped by interactions with soil fauna (Bonkowski et al. 2000). For example, density-dependent and selective feeding of funigifore soil fauna affect the balance between mycorrhizal and saprotrophic fungi, nutrient mobilization, and thus plant performance (Klironomos & Ursic 1998; Scheu & Tiunov 2005). However, soil faunal activity affects soil physical structures such as pores and (micro)habitats (Maraun et al. 1999; Eisenhauer 2010). Thereby, faunal activity imposed spatial restrictions on soil organisms to sense and access food resources shaping trophic interactions (Erktan et al. 2020). The given examples already highlight the temporal and spatial complexity of multitrophic interactions as drivers for microbial processes. In turn, higher trophic level organisms, such as bacterial feeding

nematodes or protists, can strongly feed-back on bacterial respiration and nutrient mobilization, with the latter process being directly relevant for plant growth (Bonkowski, 2004; Brüggemann et al., 2011).

Relevant scales for processes localization

Ecological relevance of a soil process (gaseous emission, carbon sequestration, nutrients cycling, or leaching) is generally determined at the macro-scale, e.g., at the landscape or soil profile level. Such approaches are important for global budget estimations. Understanding the mechanisms and spatial distribution of these processes requires, however, more precise mesocosm studies, while a shift to the micro- and even to the nano-scales is necessary to find links between rates and distinctly local processes in soil microhabitats.

Recent progress in process visualization at the meso-scale (root-scale) was achieved by novel microsensors techniques and by soil zymography. These approaches enable to monitor the ***two***-dimensional distribution of soil properties (such as pH or oxygen concentration (Blossfeld and Gansert, 2007; Blossfeld et al., 2013) and intensity of SOM decomposition (e.g., by CO_2 and hydrolytic enzymes activity). Zymography coupling with X-ray CT is very promising for the **3D** reconstruction of enzymatic processes within soil pore space. A 4D visualization of dynamic development of the process within soil volume still remains a challenge.

Relevant approaches for processes localization

Destructive:

Techniques to identify microorganisms are nowadays mostly based on DNA sequencing approaches as DNA has high information content about taxonomy (Table 1). In case DNA sequencing is not limited to PCR-amplified phylogenetic markers but applied for metagenomic sequencing, information about the functional potential of the microbiome is obtained. To identify or quantify more specifically active decomposers, different methods have been developed, including meta-transcriptomics (Bai et al. 2019, Antunes et al. 2016), nanoSIMS (Stryhanyuk et al. 2018), stable isotope probing of DNA, RNA, or PLFAs (Hünninghaus et al. 2019, Hannula et al. 2012; Maarastawi et al. 2019) and other techniques that relate abundance to metabolically active microbial consortia (Emerson et al. 2017; Baldrian et al. 2012). To predict process rates, focus on the quantitative parameters of a process rather than the mere structure of the microbial community is needed, which can be provided by expression rates of genes being involved in specific processes, determined by RT-qPCR to quantify gene expression of specific functional genes, or by metatranscriptomics, in which transcript numbers can be taken as a proxy for gene expression rates. However, it has to be kept in mind that gene expression is not necessarily linked to enzyme activity (Nannipieri, 2003).

Recently, qSIP was proposed as an approach to quantify the metabolic activity of all specific groups of microorganisms that contribute to a substrate conversion process (Hungate et al., 2015; Papp et al., 2020). The goal to quantify the incorporation of a stable isotope label in specific groups of microorganisms was also achieved by combining microarray analysis with nanoscale secondary ion mass spectrometry (nanoSIMS), even though this approach is not widely used (Mayali et al. 2011). The use of isotopically labeled substrates, which are incorporated into microbial biomolecules, as a proxy for microbial activity contributing to a decomposition process of SOM or SOM components requires caution because incorporation of label to various types of biomolecules such as nucleic acids or membrane lipids is affected by the turnover times of these biomolecules (Malik et al. 2015).

Table 1.

Non-destructive:

The development of new approaches and concepts is not evenly distributed among the interfaces (hotspots); particularly, modern viewpoints are mostly presented for the rhizosphere (Table 1). For instance, most of the *in situ* techniques have been adapted for imaging of rhizosphere properties and processes (Oburger and Schmidt 2016). The approaches such as i) optodes for measurement of CO_2, pH, O_2 (Blossfeld et al., 2011; Rudolph et al., 2013), ii) sensitive gels (pH-indicators (Römheld, 1986), iii) zymography for enzyme activity (Spohn & Kuzyakov 2013; Razavi et al., 2019), iv) DGT gel (Diffusive Gradient in Thin-films) for elements (Fresno et al., 2017), v) imaging of radioactive isotopes: ^{14}C (Pausch and Kuzyakov, 2011), ^{33}P, ^{32}P; ^{40}Ca for nutrients and neutron imaging for water (Carminati, 2013), enabled visualization of spatio-temporal patterns of rhizosphere properties and rhizosphere processes (Kuzyakov and Razavi 2019). Such novel techniques revealed a multiscale (time and/or space) look at plant-microbiome interactions and their functionality (Baveye et al., 2018).

Despite visualization techniques enabling quantitative estimations based on calibration, many of them still remain qualitative or semi-quantitative and do not show consistent correspondence to the process rates/activity obtained by destructive sampling. For example, for the approaches based on the application of the sensor gel or membrane to the soil surface (i.e., optodes or zymography), essential methodological uncertainties occur related to the diffusion of targeted colored/fluorescent molecules (substrates or products of reaction) between soil and membrane as well as within the membrane (Guber et al., 2018, 2019).

Possible solutions for this problem could be i) a combination of activity hotspots localization by zymography with precise destructive micro-sampling after visualization (Tian et al., 2019) or visualization of the processes at the microscopic scale avoiding attachment of artificial sensors/membranes (Table 1). Certain disagreement also occurs between molecular approaches (identifying plant and microbial traits by functional genes) and estimation of the process rates, e.g., by enzymatic activity (Nilsson et al., 2019; Wei et al., 2019). Such a disagreement confirms that gene existence does not necessarily reflect the activity of the corresponding protein (Nannipieri, 2018). Hence, a quantitative estimation of process rates and the magnitude of changes in pools and fluxes is necessary at interfaces like the rhizosphere to clarify, for instance, how inoculants modulate the resident microbiome, how pathogenic attack affects the activity of the complex microbiota of hotspots, how grazing activities by protists, nematodes or bacteriophages control the extinction of species or how the rhizosphere microbiome responds to abiotic stresses (e.g., salinity, drought, heat). This remains currently a challenging task, considering the diversity of carbon compounds in the rhizosphere and the challenges regarding their analysis (Oburger and Jones 2018; van Dam and Bouwmeester 2016).

Prediction based on statistical analysis of process locations

A range of rhizosphere process-related parameters (e.g., pH, CO_2, P/Mn content, enzymes activity) are satisfactorily visualized in 2D by application of sensor membranes to the root – soil interface (Blossfeld and Gansert, 2007; Blossfeld et al., 2013), but localization of these parameters within soil volume requires undesirable destructive sampling (Table 1). From CT-based three-dimensional root localization within soil domain, the probability distribution of the distance of a randomly selected location to the nearest root (Schlüter et al., 2018) can be computed. Combining these two techniques potentially enables developing a probabilistic 3D model which predicts the pH-values in the three-dimensional soil surrounding a plant root, based on 2D measurements of pH-values but still remains a challenge. In this way, the spatially resolved arrays of rhizosphere relevant parameters and the 3D architecture of root systems can be co-localized, e.g., using Gaussian random fields (Histopulos, 2020), defined by the location-dependent mean value function and covariance function (Blossfeld and Gansert, 2007; Blossfeld et al., 2013). By incorporating further properties into the mean and covariance functions of the Gaussian random field, the model is easily extensible to account for local heterogeneity in the soil as well as topological and morphological properties of the root architecture like, e.g., branching, root tips or root age. Given the 3D architecture of the considered root system, the parameters of these functions are estimated from the 2D

information (Weber et al., 2020) and the fitted Gaussian random fields are used to generate realistic values in the 3D soil domain surrounding the root system, by means of Monte Carlo simulation (Lang and Potthoff, 2011). However, the direct link of root internal spatio-temproal C allocation and dynamics for rhizosphere processes is yet not fully understood. The co-registration of MRI–PET (Jahnke et al. 2009) opens the door for 3D non-invasive analysis of plant structures and recently fixed C- transport processes within a root structure that may change in response to genomic, developmental or environmental challenges.

A stereological technique gives a further promising perspective to overcome the need for expensive 3D imaging of plant roots, combining extensive model-based simulation of virtual root systems in 3D with methods of machine learning. This stereological technique employs root architecture models like, e.g., CPlantBox (Schnepf et al., 2018a,b) and corresponding spatially resolved values of parameter of interest with the consequent application of methods of deep learning, e.g., training convolutional networks (Goodfellow et al., 2016). This enables to predict the spatially resolved distribution of rhizosphere process in the three-dimensional soil surrounding a plant root, using 2D measurements only.

Emergent properties of microbial activity in soil

Traditionally, total microbial biomass, potential enzyme activities, substrate-induced respiration and organic matter content in a given volume of soil have been used to predict decomposition activity and to model the fate of organic matter. To assess how the microscale generates macroscopic behavior, the so-called emerging properties, microscale heterogeneity, the dynamics of substrate properties and microbial activities need to be taken into account (Baveye et al. 2018). This aim is multi-disciplinary and extremely challenging. It requires to link the spatial distribution of soil organic matter (Rawlins et al. 2016; Müller et al. 2016; Peth et al., 2014) with it's biophysical and biochemical properties combined as well as with decomposer microorganisms and the respective traits and activities in the contexts of space and time (Baveye et al. 2018). Promising techniques in taking the soil micro-heterogeneity in account are reproducible systems mimicking the soil that can be used for hypothesis testing (Tecon et al. 2017). Novel characterization techniques are increasingly used to systematically track the characteristics of organic C conversion at soil micro-interface (Table 1).

The transformation process of organic matter and its influencing factors are discussed at the scale of micro-ecological systems. Progress in near-edge X-ray spectromicroscopy (NEXAFS), scanning transmission X-ray microscopy (STXM), X-ray absorption spectroscopy, micro-fluorescence spectroscopy, and nanoSIMS, applied to soil thin sections, revealed

distinct spatial heterogeneity in the chemical composition of soils over minute distances (Lehmann et al., 2005). Pulse-labeling experiments in combination with NanoSIMS enable to trace the uptake, storage, and translocation of stable isotopes (Vidal et al. 2018). The development of novel detection technologies, such as NEXAFS and X-ray photoelectron spectroscopy (XPS) during the last decades, has greatly enriched our understanding of the microscopic distribution characteristics of SOM (Amelung et al. 2002). XPS has been successfully adapted to determine the chemical composition of SOM occluded in different aggregate size fractions. In addition, the spatial distribution of elements at a resolution of < 3 µm can be mapped in selected regions of coatings, mineral-organic associations and aggregates using electron probe microanalysis (EPMA). Significant advances related to molecular markers and detection sensitivity now also enable to better localize specific bacteria in soils and their spatial distribution at the micrometer scale to be determined in thin sections (Gutiérrez Castorena et al. 2016, Eickhorst and Tippkötter 2008). All this information can in principle, be combined and translated into 3D distributions using recently developed statistical algorithms.

The current challenge of modern ecology is an application of these state of the art and cutting edge methodologies for presise localization of biochemical processes considering interactions within and between soil interfaces as well as identifying and linking functional traits of plants and of microbial populations that contribute to the rates of soil processes relevant at ecosystem level.

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980

981 **Table and Figure:**

982

983 **Table 1.** Comparison of process monitored by various approaches.

Method Process/traits	***Predictive***	***Destructive***	***Non-destructive***	***Precise micro-sampling***
Root exudation	Exporter gene presence	Gene expression	Marker construct or knockout, autoradiography	Microdissection
Root/microbial traits & functions	Geochip, functional marker genes, specific marker genes, qPCR	RT-qPCR, qSIP, molecular biomarkers	scanning transmission X-ray microscopy	NanoSIMS, FISH, X-ray microscopy
pH	Correlative statistics	Suspension	Optodes	Microsensors
P solubilization	*pho* genes presence	Phosphatases & phytases	Zymography	Hotspots sampling
Respiration CO2, O2	Mechanistic Models based on local difussivities	Basal respiration	Optodes, Clark-type-based glas microelectrodes, dyes, soil cores	Ion beam slicing (down to micrometer thickness)
C and N transformation	Functional marker genes	Enzymatic approaches	Zymography	Hotspots sampling

Figure Description:

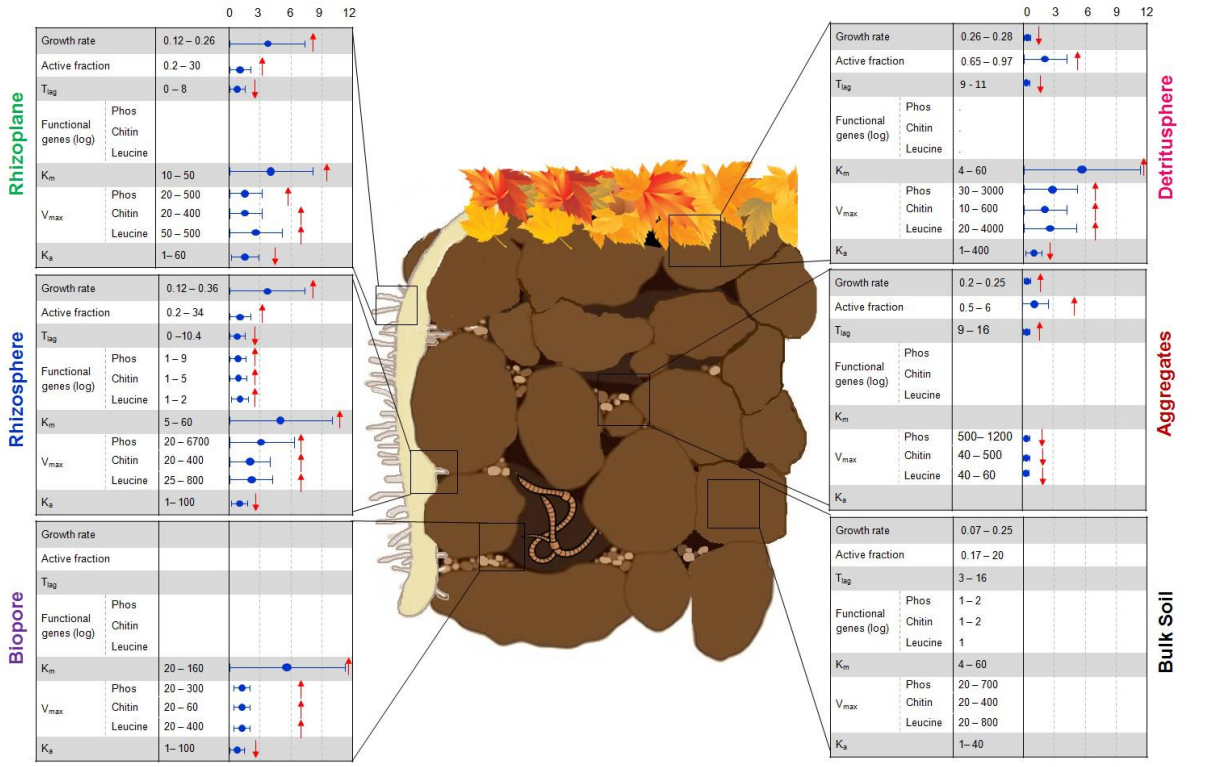

Fig 1. Linking microbial functional traits to process rates in the soil hotspheres. First column (left): Growth rate (h^{-1}), active fraction (% of total biomass), T_{lag} (h), Functional genes (log copies / g dry soil), K_m , enzyme affinity to the substrate (µmol g^{-1} soil), V_{max} , enzyme activity (Phos – phosphatase, Chitin – chitinase, Leucine – leucine-aminopeptidase; nmol g^{-1} h^{-1}), K_a (h^{-1}). Column (middle): ranges of original values based on literature, column (right): times of changes in comparison with bulk soil. The arrows show the increased ↑ or decreased ↓ trend compared to bulk soil. References used for this figure can be find in the supplementary material. After modification from ©Nature Education 2012.