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 biogeochemical interfaces (e.g., rhizosphere, detritusphere, (bio)-pores, and aggregate surfaces). A conceptual view emphasizes the central role of the rhizosphere in interactions with other biogeochemical interfaces considering biotic and abiotic dynamic drivers. We discuss an applicability of three groups of traits based on microbial physiology, activity state and genomic functional traits to reflect microbial growth in soil. The sensitivity and credibility of modern molecular approaches to estimate microbial specific growth rates demands further development. A link between functional traits determined by physiological (e.g., respiration, biomarkers) and genomic (e.g., genome size, number of ribosomal gene copies per genome, expression of catabolic versus biosynthetic genes) approaches is strongly affected by environmental conditions such as C and nutrients availability and ecosystem type. We address, therefore, the role of soil physico-chemical conditions and trophic interactions as drivers of microbially mediated soil processes at relevant

scales for process localization. The strengths and weaknesses of current approaches (destructive, non-destructive and predictive) for assessing process localization and corresponding estimation of process rates are bridged to the challenges for modelling of microbially mediated processes in heterogeneous soil micro-habitats. Finally, we introduce a conceptual self-regulatory mechanism based on flexible structure of active microbial community. Microbial taxa best suited to each successional stage of substrate decomposition, become dominating and alter the community structure. The rates of decomposition of organic compounds, therefore, are dependent on functional traits of dominating taxa and microbial strategy, which are selected and driven by the local environment.

Key words: *rhizosphere*, *mycorrhizosphere, detritusphere, (bio)-pores, soil aggregates, soil priming, trophic interactions, 3D process localization*

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, resilience and 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 material. Majority of microorganisms are capable of breaking down labile compounds derived from fresh plant litter or rhizodeposits, thus ensuring functional redundancy. Other processes rely on more specialized microorganisms in the breakdown of recalcitrant compounds, which usually occur at later stages of organic matter decomposition (Pepe-Ranney et al 2016, Bastian et al 2009). Functional redundancy in the soil microbiome or more generally biodiversity thereby provides ecosystem resilience (Fierer, 2017; Louca et al., 2018; Maraun et al., 2018) and is crucial for ecosystem multifunctionality (Wagg et al 2014; 2019).

The *primary* input organic substances in soil 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 material results in carbon losses from the soil and in CO_2 emission to the atmosphere. An essential fraction of organic C that is assimilated within microbial biomass is further re-utilized after microbial death by multi-stage microbial succession (Morrien, 2016). Plant-derived resources are therewith further transferred into the microbial food web (Kramer et al., 2016; Hünninghaus et al., 2019). Products from living

microorganisms and in particular residues of dead microorganisms (necromass) serve also as a *secondary* source of soil organic substrates, finally resulting in sequestration of up to 40% of primary C input (Miltner et al., 2012; Kallenbach et al 2016; Buckeridge et al., 2020).

Microorganisms decompose organic substrates 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 mobilize from a multiphase (gaseous, liquid, and solid) soil environment (Zechmeister-Boltenstern et al., 2015). In case their own stoichiometric requirements are fulfilled, they can moreover release nutrients, therewith increasing the availability for plants (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) nutrient mobilization, possibly followed by losses through leaching of mineral nitrogen and phosphates. These processes can also cause greenhouse gas (N_2O, CO_2, CH_4) emissions. 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, e.g. in the rhizosphere by rhizodeposition, may enormously increase the fraction of active microorganisms (Blagodatsky et al. 2000) and therewith the decomposition of soil organic matter (SOM), thus, causing the well-known “rhizosphere priming effect” (Arsjad and Giddens 1966; Cheng et al., 2003). Moreover, both SOM stabilization and destabilization in the rhizosphere are driven by different other processes, not only rhizodeposition, but also root turnover, as well as nutrients and water uptake by the plant (Dijkstra et al., 2020).

SOM transformation processes mainly rely on the production of extracellular enzymes facilitating oxidation or hydrolysis of diverse and complex SOM compounds (Nannipieri et al., 2012). The decomposition rate of SOM is mediated by the molecular nature of the SOM as well as by the degree of biotic interactions (Sokol et al., 2018). Moreover, it depends on microbial community traits, which can be sub-divided into three groups (Fig.1). Microbial traits in the first group are very dynamic, e.g., the size of microbial fraction maintaining activity or 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. Bimodal classical concepts based on functional traits (Panikov, 1995; Morris and Blackwood, 2007) exploit the general ecological principles classifying microorganisms e.g., by resource requirements (copiotrophs versus oligotrophs), by spatial mobility (zymogenous vs autochtonous), or by growth and efficiency (r- versus K-strategists). These concepts are often not capable to cover the great diversity of microbial functions and life strategies. They have thus been complemented by other concepts such as the competitor-ruderal-stress-tolerator life strategy concept (Ho et al. 2013, Krause et al. 2014), which has very recently been further developed to a high yield, resource acquisition and stress tolerance concept, (Malik et al., 2020). However, it remains challenging to identify proxies for specific traits, which can serve as quantitative measures of a category. For example, r- and K- strategists can be differentiated by the values of maximum specific growth rates (μ_m), and by enzyme affinities to a substrate (K_m), experimentally determined under *in situ* soil conditions (Blagodatskaya et al., 2014; Tian et al., 2020). In contrast, quantitative estimation of traits specifically responsible, e.g., for stress tolerance or for resource acquisition remained challenging. Recent developments of molecular approaches, however, provide potential for microbial trait differentiation based on information about genome size, number of ribosomal gene copies per genome, or quantification of functional marker genes or their transcripts by omics approaches (Malik et al., 2020). The idea that multiple versus single *rrn* operons in a genome correspond to faster versus slower growing taxa raises a question: how to relate gene copy numbers (single vs multi-copy genes) encoding certain functions with microbial ability to grow under *in situ* soil conditions. At the physiological level, the growth rate is dependent on a balance between two fractions of the total proteome (Scott et al., 2014), which are growth-independent (i.e., related to maintenance function) and growth-dependent (i.e., ribosomal and metabolic proteins). In accordance with this concept, a physiological approach based on substrate-induced growth respiration (SIGR) estimates microbial specific growth rates considering a partitioning of microbial respiration into growth-related and growth-independent fractions (Panikov, 1995). Microbial specific growth rates determined by SIGR in contrasting soil microhabitats (Fig.1) are comparable, but are reasonably slower as compared with exponential growth of *E.coli* in pure culture (You et al., 2013). As the SIGR approach estimates non-limited microbial growth in soil amended with an access of glucose and nutrients, a complementary approach has been developed to determine bacterial and fungal growth by incorporation of trace amounts of

139 labeled ^{3}H-leucine, ^{3}H-thymidine or ^{14}C-acetate, respectively (Bååth, 2001; Bååth et al., 2001).
140 The specific bacterial growth rates obtained by trace labeling (e.g., 0.33 h^{-1}) are in a good
141 correspondence with SIGR (Meisner et al., 2013). However, several orders of magnitude
142 slower rates obtained for growth on glucose by quantitative stable isotope probing (qSIP) with
143 an ^{18}O-labeled water approach (Li et al., 2019) reflect rather the flaws in experimental design
144 (assumption of steady-state after glucose addition; 7 days incubation time, which is too long
145 compared to exponential growth occurring within 20-48 h after soil activation with substrate,
146 see e.g., Meisner et al., 2013; Loeppmann et al., 2020) than the realistic microbial specific
147 growth rates (see also the section 'Relevant approaches for processes localization' below).

148 A larger genome size corresponded to slower growth induced by glucose; this relationship,
149 however was weakened in both non-amended and nutrient rich soil, thus indicating a
150 regulatory role of the environment under natural soil conditions (Li et al., 2019). Reduced
151 growth rates under either C or nutrient limitation might cause a contrasting response of genes
152 involved in bacterial metabolism. Thus, expression of C catabolic genes increased with
153 decreasing growth rates under C limitation but it decreased under N limitation (You et al.,
154 2013). In contrast, the expression of biosynthetic genes followed the opposite growth-rate
155 dependence as the catabolic genes did. Under environmental control, growth rates of
156 individual taxa can vary by the factor of two in non-amended soils of contrasting ecosystems
157 (Morrissey et al., 2019).

158 The environmental selection results, therefore, in the activation of populations with *intrinsic*
159 *functional traits* mostly suited to the individual microhabitat within heterogeneous soil pore
160 spaces. Thus, beyond the quality and regularity of substrate input, the biotic and abiotic
161 environment (Huang et al. 2014), such as soil structure (Berg and Smalla, 2009), presence of
162 organisms (Scheffknecht et al. 2006) and nutritional status (Jones et al. 2004; Hinsinger 2001)
163 affect microbial functional traits in contrasting soil habitats. In soils covered by vegetation,
164 microbial functional traits are also affected by the physiological and morphological *traits of*
165 *plant*s.

166 **Rhizosphere processes and interactions within and between interfaces**

167 Plants are the major primary producers in terrestrial ecosystems and are thus predominantly
168 responsible for organic C input into the soil. They modulate their surrounding soil environment
169 either *actively*, i.e., producing exudates and exo-enzymes or *passively* through root and litter
170 detritus (Kaiser et al., 2015), thus **interacting** with the corresponding microorganisms near
171 the roots or of other **soil interfaces** (e.g., detritusphere, (bio)-pores, and aggregate surfaces).
172 Considering the active role of roots crossing, penetrating, and even forming aggregates,

biopores, and detritus, we mainly focus in the following on the **rhizosphere** and its overlap with other relevant interfaces.

Rhizosphere

An essential part of C assimilated via plant primary production enters the soil through roots by the process called rhizodeposition. 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). Thus, specific microbial communities develop in the endosphere, the rhizoplane, and the rhizosphere, i.e., within a few mm from the root surface (Edwards et al., 2015; Vidal et al., 2018) and along the growing root (Chen et al. 2017; Schmidt et al., 2018). Continuous root growth and turnover drives the spatial distribution and transformation of primary and secondary C-input into soil. This C-input is determined by a combination of factors related to root type (Swinnen et al., 1994a,b; Jahnke et al., 2009), root age, root turnover rate and their specific rhizodeposition processes (Kawasaki et al., 2016; Steer & Harris, 2000). Root growth also leads to the creation of specific interfaces between the rhizosphere and other interfaces such as the detritusphere, (bio)-pores or aggregate surfaces (Fig. 1).

Rhizodeposits range in complexity from cells and lysates to small organic molecules. Rhizodeposits are released as a consequence of tissue turnover, sloughed off border cells, by mucilage release, or the secretion of biochemically diverse root exudates (Farrar et al., 2003; Nguyen, 2003; Jones et al 2009). The production rate and the quality of rhizodeposits 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 type or root hairs (Datta et al., 2011; Poirier et al., 2018; Cotton et al., 2019).

Generally, plants adapt their source-sink relationships dynamically under the varying abiotic-biotic environment, ensuring seed production to provide offspring and 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 the plant functional type with a mean value of 21% for crops and 33% for grasses (Pausch and Kuzyakov, 2018), and 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 C moving into the rhizosphere as rhizodeposition ranges from 1.3 - 20% of photosynthetically fixed C for crops, trees and perennial grasses (Jones et al., 2009; Kaiser et al., 2015; Pausch and Kuzyakov, 2018) to 20 – 30% for cereals and mycorrhizal plants (Jakobsen and Rosendahl, 1990; Kuzyakov and Domanski, 2000) and even up to 50 – 60% (Lynch and Whipps, 1990; van Dam and Bouwmeester, 2016) with substantial uncertainty that's still apparent with regard to ecosystem type, climate conditions, etc.

Soil - plant interactions in the rhizosphere 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 the 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 into 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, Zhalnina et al., 2018; Pett-Ridge et al., 2020) modulates biological activities in a compound mixture dependent manner. Especially the presence of certain sugars or secondary metabolites such as phenolic compounds can exert profound negative and positive influences on community composition and the functional potential of microorganisms (Badri et al. 2009; Chaparro et al. 2013, Cotton et al. 2019), affecting 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 vegetative 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; Nuccio et al., 2020). As the rhizosphere is one of the most dynamic interfaces actively developing in the local environment, the interaction 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 in these interfaces.

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 the 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 the mycorrhizosphere has been suggested for the widely distributed arbuscular mycorrhizal symbiosis (Cheng et al. 2012), and priming by arbuscular mycorrhizal fungi is associated with modifications in soil microbial community composition (Nuccio et al. 2013; Gui et al. 2017; Herman et al 2012). Consequently, arbuscular mycorrhizal symbiosis may influence different trophic levels by enhancing C allocation into the food web, and stimulating N mobilization from OM and subsequent transfer to the plant host (Koller et al., 2013b; Hünninghaus et al., 2019). By contrast, ectomycorrhizal fungi themselves produce extracellular enzymes and free radicals that release N from organic compounds (Nicolás et al., 2019; reviewed by Lindahl and Tunlid 2015). By two distinct mechanisms relating to litter decomposition stage and soil depth, they either suppress or stimulate decomposition (Brzostek et al. 2015; Sterkenburg et al. 2018). In topsoil with fresh litter, ectomycorrhizal competition for N decreases decomposition rate (Averill et al. 2014), whereas in deeper soil layers with litter at later stages of decomposition, the ectomycorrhizal fungi contribute to decomposition (Sterkenburg et al. 2018).

Rhizosphere – detritusphere interactions

Plant photosynthates are released into the soil not only in the form of soluble root exudates, but also as plant detritus (e.g. leaf litter and rhizo-detritus). Thus, rhizodeposition processes overlap with leaf litter and dead root tissue degradation processes, forming a rhizosphere – detritusphere interface. Due to the spatial and chemical heterogeneity of rhizodeposits and rhizo-detritus in soil, the microbial communities and activities in these two spheres as well as in the overlapping sphere are specific (Marschner et al., 2012, Nuccio et al., 2020). It has been shown that root activity modulates decomposition processes in the detritusphere by altering

the structure of the microbial community feeding on detritus. Distinct microbial taxa were involved in ^{13}C-labeled rice straw degradation in the rhizosphere compared to bulk soil (Maarastawi et al. 2018). As a result, essentially, less ^{13}C was assimilated by the microorganisms from the straw in the rhizosphere versus bulk soil, likely due to the higher availability of (labile) carbon in the rhizosphere. The availability of detritus, in turn, reduces the consumption of root exudates by the rhizosphere microbiota (Maarastawi et al. 2019), indicating that detritusphere processes are modulating rhizosphere processes and demonstrating interactions between the rhizosphere - detritusphere interfaces. A recent study by Nuccio et al. (2020) observed higher taxonomic and functional diversity in the combined rhizosphere-detritusphere, suggesting that coexistence of rhizosphere guilds is facilitated by niche differentiation. This observation was based on a metatranscriptomics study where the expression of genes responsible for decomposition of different organic substrates was studied comparatively in soils that originated either from growing roots, from decaying root material, or the overlapping spheres. Spatial and temporal niche differentiation by functional genes responsible for similar functions clearly confirmed a strong redundancy of the functions in the rhizosphere and detritusphere. For example, for various functions (genome classes), the number of active genomes shared between rhizosphere and detritusphere amounted 28% (housekeeping gene gyrase A, B), 32%, 67% and 50% (oligosaccharide hydrolases, cellulases and xylanases encoding genes, respectively) of total number of functionally active taxa (data extracted from Fig. 3 in Nuccio et al., 2020). Moreover, rhizosphere organisms expressed genes involved in the consumption of primary carbon compounds as well as breakdown products, indicating that they profit from synergistic consumption processes. Thus, such an 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 webs* (Koller et al., 2013a). Therefore, successional changes of bacterial and fungal populations in the course of plant development may lead to a corresponding succession of protist communities in the rhizosphere which translates into less complex and dense protist networks during plant senescence (Ceja-Navarro et al., 2021).

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; Nuccio et al., 2020). Generally, root morphological properties (e.g., root hairs, fine roots, mycorrhiza)

intensify a release of exudates and thus, increase 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 other soil organisms (Kuzyakov and Xu, 2013), while competition in the detritusphere occurs primarily within or between microbial species (Esperschütz et al. 2011). Due to contrasting quality of C sources (root-derived low molecular weight C versus more macromolecular organic compounds in detritus), the abundance and successional changes of a microbial community may differ essentially in intensity and dynamics between rhizosphere and detritusphere. These differences and dynamics in turn may be a major determinant of predatory bacteria, RNA viral and RNA phage dynamics. Functional traits of predatory versus non-predatory bacteria revealed themselves as faster growth and much faster C assimilation rates, which were comparable to the C flow through viruses and were substantially higher than that in predatory eukaryotes (Starr et al., 2020; Hungate et al. 2021). There is an evidence that changes in structure and dynamics of multi-level trophic interactions corresponded to the differences in energy flow between rhizosphere and detritusphere. Thus, not only microbial community but also the community of RNA eukaryotic viruses as well as the community of phages inhabiting the detritusphere was more distinct in structure from bulk soil than the rhizosphere community (Starr et al., 2019). As a result, successional changes in community structure are driven to a large extent by mycoviruses and phages, bacterial predators and protozoan grazers, demonstrating clear temporal population dynamics and patchy spatial distribution.

Rhizosphere – porosphere interactions

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 the drilosphere made by earthworms can host different varieties of microbes with distinct rates and efficiencies 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 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 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) provide 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 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, microbial, macro- and micro-faunal activity, all of those being involved in the structuring of aggregate surfaces (Golchin et al., 1994) by modifying the soil biotic and abiotic environment. Furthermore, positive correlation between soil water-stable aggregates and content of

extracellular polysaccharides (EPS) indicated that soil abiotic conditions such as pH and water potential are the primary controllers of both, aggregate stability and microbial EPS production (Sher et al., 2020). 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. Reduced redox potential at root surfaces due to high O_2 consumption rates (Fischer et al. 1989) forms a notorious gradient of oxygen concentrations, which goes from very low at the root surface to

average soil concentration at around 15 mm distance (Keiluweit et al., 2015). 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 a proper 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 (Clarholm, 1985; Bonkowski et al. 2004). Grazing strongly affects the composition and functional evolution of microbial communities and fosters C- and

458 N mineralization from detritus for plant uptake (Alphei et al. 1996; Geisen et al. 2018). Those
459 mineralization processes are depending on the spatial distribution, size as well as detritus
460 quality (Bonkowski et al. 2000; Koller et al. 2013). During decomposition of labile and
461 recalcitrant C fractions of detritus, protist communities themselves undergo a temporal
462 succession on fine spatial and temporal scales (Hünninghaus et al. 2017). Microbial processes
463 are also shaped by interactions with the soil fauna (Bonkowski et al. 2000). For example,
464 density-dependent and selective feeding of funigifore soil fauna affect the balance between
465 mycorrhizal and saprotrophic fungi, nutrient mobilization, and thus plant performance
466 (Klironomos & Ursic 1998; Scheu & Tiunov 2005). However, soil faunal activity affects soil
467 physical structures such as pores and (micro)habitats (Maraun et al. 1999; Eisenhauer 2010).
468 Thereby, faunal activity imposed spatial restrictions on soil organisms to sense and access
469 food resources shaping trophic interactions (Erktan et al. 2020). The given examples already
470 highlight the temporal and spatial complexity of multitrophic interactions as drivers for
471 microbial processes. In turn, higher trophic level organisms, such as bacterial feeding
472 nematodes or protists, can strongly feed-back on bacterial respiration and nutrient
473 mobilization, with the latter process being directly relevant for plant growth (Bonkowski, 2004;
474 Brüggemann et al., 2011).
475
476 **Relevant scales for processes localization**

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

483 Recent progress in process visualization at the meso-scale (root-scale) was achieved by novel
484 microsensor techniques and by soil zymography. These approaches enable to monitor the
485 ***two***-dimensional distribution of soil properties (such as pH or oxygen concentration (Blossfeld
486 and Gansert, 2007; Blossfeld et al., 2013) and intensity of SOM decomposition (e.g., by CO_2
487 and hydrolytic enzyme activities). Zymography coupling with X-ray CT is very promising for
488 the **3D** reconstruction of enzymatic processes within soil pore space, in particular, if the
489 integration of 2D chemical imaging (in this case zymography) and 3D (µ)X-ray CT is further
490 coupled with modeling of pore scale processes (Roose et al. 2016). For example, there are
491 spatially explicit models for nutrient uptake by roots and root hairs based on X-ray CT (Daly et
492 al. 2016) that could be coupled with the **3D** reconstruction of enzymatic processes for a
493 comprehensive insight into enzyme driven nutrient mobilization in the rhizosphere. A 4D

visualization of dynamic developments of a process within the soil volume as well as a shift the zymography from meso- to the microscopic scale still remains a challenge. Such a challenge can be realized through coupled visualization of soil processes and estimation of localized process rates. This requires a methodology considering biotic and abiotic drivers, functionally and phylogenetically diverse players, and multiple resolution scales of soil biochemical processes.

Relevant approaches for processes localization

Already existing and newly-developing methods for the determination of localized process rates in soil can be differentiated by three groups based on i) *destructive* sampling disturbing soil microcosms; ii) non-destructive imaging *in situ* techniques, and iii) prediction by modelling.

Destructive approaches:

Techniques to identify microorganisms are nowadays mostly based on DNA sequencing approaches as DNA has a high information content about taxonomy (Table 1). When DNA sequencing is not limited to specific PCR-amplified phylogenetic or functional markers but is applied to metagenomic sequencing, information on the broad functional potential of the microbiome is obtained. To identify or quantify more specifically the active decomposers, different methods have been developed, including meta-transcriptomics (Antunes et al. 2016; Bei et al., 2019; Yergeau et al 2018, Nuccio et al 2020), nanoSIMS (Pett-Ridge and Firestone, 2017; Vidal 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 (Malik et al., 2020), and this 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 level does not necessarily correlate with protein abundance or 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). While comparisons of the obtained values within specific studies can provide valuable information about the performance of individual taxa under specific conditions and over a given period of time, a comparison of quantitative data between studies remains

difficult. Growth rates estimated by ^{18}O qSIP in soils without substrate input (0.0002 – 0.001 h^{-1}, data extracted from Fig. 4 in Morrissey et al., 2019) are 2 - 3 orders of magnitude lower as compared with exponential microbial growth on glucose (Fig.1). Likewise, an up to 2 times underestimation of growth rates can be obtained by ^{18}O qSIP as compared with ^{13}C qSIP depending on the ecosystem (according to Fig. 3 in Li et al., 2019). Strong disagreements in growth rates, e.g. on glucose between qSIP applied at a weekly basis (0.07 – 0.3 $week^{-1}$, Morrissley et al., 2019) and SIGR applied at an hour basis (Fig.1) are due to differences in the experimental design, which need to be taken into account: application of qSIP in one week after substrate input mirrors substrate-induced successional changes and substrate re-utilization rather than bacterial growth rates. Fungal and bacterial growth rates are very dynamic and can rise or drop up to 7 – 10 times within one week after substrate addition; thus, a remarkable shift can occur between the peak values for growth of bacteria and fungi (Nannipieri et al., 1978; Silva-Sánchez et al., 2019). Therefore, the task to relate gene phenotypic traits to the *in situ* growth rates still remains a challenge.

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), (Mayali et al. 2011). Incorporation of isotopically labeled substrates into microbial biomolecules serve as quantitative proxy for microbial activity contributing to a decomposition process of SOM or SOM components. However, also this approach requires some caution when comparing quantitative data of studies using different time intervals and various types of biomolecules, because incorporation of isotope labels is affected by the turnover times of these biomolecules, e.g., nucleic acids are more quickly labeled relative to PLFAs and membrane lipids (Malik et al. 2015).

Table 1.

Non-destructive approaches:

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 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 and 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 or 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 or 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 the soil volume requires undesirable destructive sampling (Table 1). From CT-based three-dimensional root localization within the soil domain, the probability distribution

of the distance of a randomly selected location to the nearest root (Schlüter et al., 2018) can be computed, which potentially enables developing a probabilistic 3D model to co-localize the spatially resolved arrays of rhizosphere relevant parameters and the 3D architecture of root systems, e.g., using Gaussian random fields (Histopulos, 2020, Blossfeld and Gansert, 2007; Blossfeld et al., 2013). 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. Moreover, spatial resolution of predicted parameters can be defined by the underlying 2D measurements, thus enabling the investigation of various soil interfaces as outlined above. By co-registration of MRI–PET (Jahnke et al. 2009), a 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 may be established.

A stereological technique based on root architecture models like, e.g., CPlantBox (Schnepf et al., 2018 a, b) 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. Thus, the spatially resolved distribution of processes in the rhizosphere and other soil interfaces can be simulated in the three-dimensional soil surrounding a plant root, using 2D measurements only.

Conclusion and outlook: 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 its 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 into 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, as well as combined STXM-NanoSIMS (Keiluweit et al., 2012, Remusat et al., 2012), applied to soil thin sections, revealed distinct spatial heterogeneity in the chemical composition of soils over minute distances (Lehmann et al., 2005; Mueller et al., 2013). 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.

To conclude, this review suggested a conceptual view emphasizing the central role of the rhizosphere in interactions with other biogeochemical interfaces. The main and basic **drivers** of plant – microbial interactions, such as input of substrate through exudation and rhizodeposition, physico-chemical conditions (e.g., proton release and oxygen diffusion and transport) are already under intensive research. In contrast, the driving role of trophic interactions within and between interfaces including competition for nutrients and successional dynamics requires more specific studies at both higher and lower trophic levels, (e.g., protists, predatory bacteria and mycoviruses - phages). According to our concept, microorganisms are not the drivers but they are the most abundant and powerful **players** in the soil interfaces due to great diversity and specificity of genes encoding similar functions. Combination of phylogenic specificity and functional redundancy ensures sustainability of a soil microbial community by the use of functional traits (e.g., an ability to produce specific extracellular enzymes, fast or slow growth, an efficiency of metabolic pathways) as a **tool** to develop a microbial life strategy, which in turn, affects the rates of transformation of organic compounds in soil. Thus, the taxa with life strategy best adapted to the environment become dominating and alter the structure of active microbial community. This self-regulatory mechanism

maintains metabolic activity of microbial community in the course of successional decomposition of organic substrates entering the soil. However, the rates of substrate decomposition are dependent on functional traits of dominating taxa and microbial life strategy, which in turn are selected according to substrate quality and local environmental constrains, e.g., water and nutrients availability. Fast development of instrumental and molecular techniques fueled attempts to reconsider concepts of microbial life strategies with the goal to specify functional groups according to their ecological relevance. This requires identification and estimation of intrinsic traits by microbial physiology or phenotypic traits at the level of functional genes. Quantitative definition of functional traits based on genetic and isotopic approaches is very promising but demands further development with caution to the relevant resolution time and type of biomarker. Additional technique development is needed to ground-truth measurements of microbial growth in soil linking physiological and molecular approaches. Therefore, the current challenge of modern ecology is the further development of cutting edge methodologies for precise 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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1277 **Table and Figures:**

1278

1279 **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 CO_2, O_2	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 microbial hotspots. 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, the column (right) shows standardized times of changes in comparison with bulk soil "0, 3, 6, 9, 12". The arrows show the increased ↑ or decreased ↓ trend compared to bulk soil. References used for this figure can be find in the supplementary material. Please note that only experiments and analyses performed in soil as matrix were included. After modification from ©Nature Education 2012.

Fig. 2. Conceptual illustration of central role of the rhizosphere in interactions with other biogeochemical interfaces. The main **driver** of plant – microbial interactions is an input of substrate through exudation, which is controlled by biotic and abiotic factors. Microorganisms are the most powerful **players** in the soil interfaces, using functional traits (e.g., the ability to produce specific extracellular enzymes) as a **tool** to develop a microbial life strategy, which in turn influences the rates of transformation of organic compounds in the soil.

Figure 1.JPEG

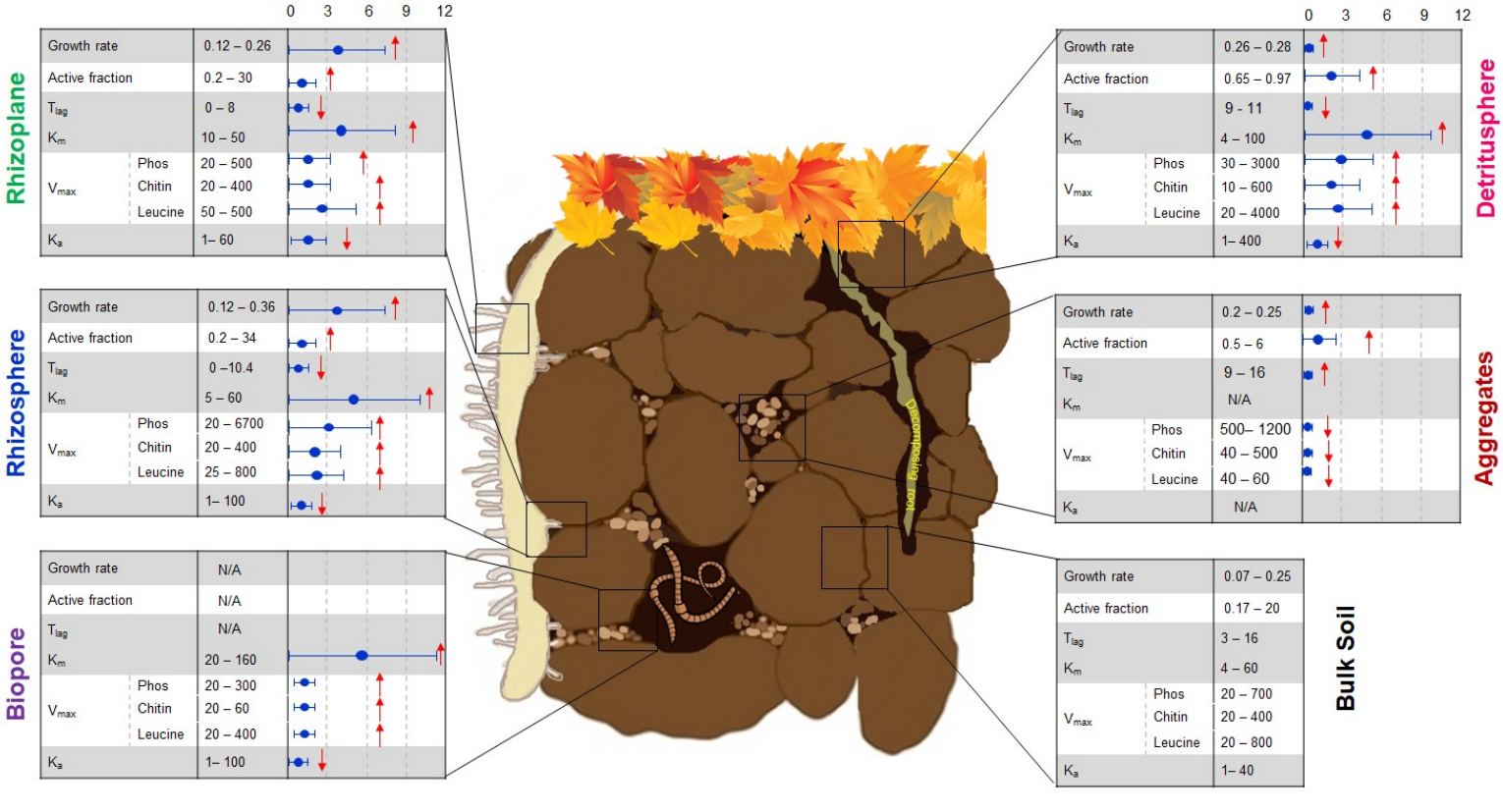

Figure 2.JPEG