THE PROTIST MICROBIOTA OF MAIZE AND THEIR FUNCTIONAL ROLES FOR THE SELF-ORGANIZATION OF THE RHIZOSPHERE BACTERIAL MICROBIOME

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“Falling between order and chaos, the moment of complexity is the point at which self-organizing systems emerge to create new patterns of coherence and structures of behaviour.”

M.C. Taylor, 2001

Abstract

Plants engage in extensive exchanges with their belowground environment. The soil is a habitat rich in diverse organisms, many of which rely on plant-derived resources. While some of these organisms are beneficial to plants, others are detrimental, necessitating that plants employ a range of strategies to manage both beneficial and harmful interactions. In addition to biotic interactions, plants must adapt to the local abiotic conditions of the soil, including its type, texture, compaction, and the availability of water and mineral nutrients—factors that also significantly influence microbial communities. Moreover, plant roots are not uniform along their longitudinal axis and undergo continuous maturation as the root tip advances through the soil. This dynamic growth leads to spatiotemporal variation within the rhizosphere. Additionally, gradients of substrate concentration, highest near the root surface and decreasing toward more distant regions in the surrounding soil, define the spatial extent of the root's influence on its microenvironment. This ever-changing system is remarkably resilient to environmental perturbations, likely arising from cascades of feedback loops involving plant roots, the microbiome, and soil components. In other words, self-organization appears to play a crucial role in holistic rhizosphere functions.

As part of the priority program “Rhizosphere Spatiotemporal Organisation – a Key to Rhizosphere Functions” (SPP 2089), we aimed to identify spatiotemporal patterns within the rhizosphere and elucidate the underlying mechanisms. Knowledge in this field remains limited, as spatially resolved research on the rhizosphere is challenging due to the high diversity of influencing factors and the difficulty of obtaining high-resolution soil samples. Our specific focus was on the role of protists, key predators of bacteria in soil. Employing high-throughput amplicon sequencing as our primary analytical tool, we investigated protist and bacterial community dynamics in the rhizosphere of *Zea mays* L. across a series of studies.

We observed distinct protist and bacterial diversity as well as co-occurrence patterns in different root regions. In young root regions, community assembly was predominantly driven by random processes, likely due to high resource availability, whereas deterministic drivers, such as protistan predation, dominated in regions where lateral roots had already emerged and resources were more limited. To explore the influence of specific plant organs on the spatial microbiome assembly, we manipulated root caps and root hairs. The root cap emerged as a determinant of microbial community assembly, while root hairs had a more limited effect. Network analyses integrating microbial relative abundances and differential gene expression highlighted taxon-specific plant responses, suggesting active plant-mediated shaping of microbial communities. Microbial respiration measurements, when mucilage, root exudates, or specific carbon-containing compounds were added to soil, showed distinct growth

activation in bacteria. This suggests that the composition of rhizodeposits, which varies along the root axis or in response to environmental factors, and their concentration, which changes with distance from the root, may influence microbial metabolism. Additionally, we demonstrated the significance of an abiotic factor—soil texture—in microbial community assembly. Increasing soil particle size promoted lateral root growth while restricting primary root elongation and enhanced selectivity in microbiome recruitment. Changes in root morphology, along with altered microbial mobility within the soil matrix, likely influenced microbial community assembly. Finally, we found that feeding complementarity among protist species increased with protist diversity, altering bacterial community structure in ways that could affect plant performance. Surprisingly, this complementarity did not enhance nitrogen uptake by plants as we had anticipated.

Across studies, protist community assembly consistently aligned with bacterial assembly patterns, with network analyses suggesting significant protist-bacteria interactions. These findings underscore the important role of protists in shaping bacterial communities, positioning them as crucial components of the self-organizing rhizosphere network. This research represents one of the first spatially resolved assessments of rhizosphere microbial community structures. By incorporating protists, it provides a more comprehensive understanding of microbial assembly processes, addressing a critical gap in rhizosphere research.

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General Introduction

Carbon dynamics in the rhizosphere

Photosynthetically active terrestrial plants play a significant role in fixing atmospheric carbon by assimilating CO_2. This carbon (C) is not permanently stored in plant biomass. Instead, a high portion of it is translocated belowground, where it enters complex food webs of interacting organisms before ultimately being dissimilated (Fig. 1). Through plant roots between 10 and 40% of the photosynthetically fixed C is actively and passively released into the soil, fueling microbial growth (Nguyen, 2003). This C flux involves a diverse array of C compounds whose composition varies between plant species and further depends on the spatial and temporal conditions of the root (Farrar et al., 2003). Among plant species the proportions of C allocated above and belowground varies, while from there the further distribution is species independent (Pausch and Kuzyakov, 2018). Through the application of tracer techniques employing C isotopes, it was demonstrated that, for example, wheat transferred 26% of the total assimilated C below ground (Kuzyakov and Domanski, 2000). Of this portion, only 52% was sustainably incorporated into root biomass, while 12% ended up in microorganisms and soil organic matter, and 15% was promptly dissimilated during root and microbial respiration.

The availability of C to soil microorganisms significantly boosts their growth and activity in the rhizosphere, as they are extremely C-limited (Schnepf et al., 2022). With an increase of C in soil, their demand for other growth essential nutrients like phosphorus (P) and nitrogen (N) increases. These nutrients not only play a vital role for bacteria but also for plants. By releasing C to the rhizosphere, ironically, plants promote the growth of their competitors for growth-limiting nutrients (Jackson et al., 1989). Additionally, rhizodeposition represents a significant loss of energy for the plant. Considering the costs, it must be assumed that overall, plants benefit from the increased microbial activity in the rhizosphere. This may be attributed to enhanced nutrient turnover due to microbial activity or direct, specific plant-microbe interactions (Dakora and Phillips, 2002). It is not yet fully understood how plant-beneficial microbial communities in the rhizosphere are established and what exact role rhizodeposition plays in this context.

The plant holobiont concept

With the finding that plant associated microorganisms such as archaea, bacteria, fungi and protists positively affect plant health and productivity (Raaijmakers and Paulitz, 2008), research on plant-microbe interactions gained considerable attention. It is now well established that microbes can improve disease suppression in plants (Mendes et al., 2011), prime their immune system (Lanoue et al., 2009; Van der Ent et al., 2009), facilitate nutrient acquisition (Heijden et al., 2015), and enhance their tolerance to abiotic stresses (Rolli et al., 2015). The host plants in turn provide nutrition and diverse habitats to microorganisms, leading to rhizosphere colonization by specialized inhabitants (Thrall et al., 2006). Plants, along with their associated microorganisms, have been recently considered a 'plant-holobiont' comparable to the mammalian 'gut-holobiont', which is only well functioning in collaboration with the correct microbiome. The term describes a multicellular organism with its symbiotic microbiota, which have not been eliminated through natural selection and contribute additional traits to the host's genome. In this concept, the holobiont constitutes a superorganism upon which natural selection acts as if it were an integrated unit (Guerrero et al., 2013; Vandenkoornhuyse et al., 2015; Mesny et al., 2023). It is questionable if this concept is fully applicable to plants with their entire associated microorganisms, as selective pressure can also favor pathogens and negatively affect host fitness.

The recourse needs of plants and microorganisms overlap only in small parts – a situation which diminishes competition and promotes cooperation, providing a good starting point for the establishment of close symbioses. Examples of such symbioses include nitrogen-fixing diazotrophs (Franche et al., 2009) and mycorrhizal fungi, which supply their hosts with mineral nutrients such as P (Parniske, 2008).

Explaining the evolutionary preservation of loose associations, such as those between plant growth promoting pseudomonads and plants, is more challenging. Various *Pseudomonas* species release antifungal metabolites indirectly beneficial for plant growth (Cook et al., 1995). However, *Pseudomonas fluorescens* benefits even more from plants when they are infected by fungal pathogens, as infection can increase nutrient availability in the rhizosphere through leaking from damaged root tissue (Mazzola and Cook, 1991). Generally, increased root exudation levels caused by few microbial taxa benefit the entire microbial community, which would destabilize cooperation according to evolutionary theory (Velicer, 2003). This raises the question how microorganisms that invest into plant health and growth and finally increased exudation levels, are not outcompeted by commensals that benefit without having any costs. Denison *et al*. (2003) discussed why strains which have lost their plant beneficial traits but still benefit from the expense of their parental strain, do not gain dominance. This question remains to be answered as it is unclear whether plants can selectively favor strains which are most beneficial. However, to maintain the integrity and functionality of the plant-microbe system, there is a necessity of mechanisms to deal with non-cooperating microbes and 'cheaters'. Loo *et al.* (2024) were unable to reconstitute patterns of the spatial ectorhizosphere colonization observed in a natural community using a synthetic community composed of 60 members. The reduced diversity and the lack of predators in their experimental set-up may explain their results. Interactions among microbes, such as competition and predation, likely play a significant role in shaping microbial communities and might have a regulatory effect when it comes to 'cheating'.

Protists as shapers of bacterial communities and functions

It is long known that bacterivores, especially bacterial feeding protists, exert a strong grazing pressure on rhizosphere bacterial communities and liberate substantial amounts of N for plant uptake, i.e. the 'microbial loop' in soil (Clarholm, 1985; Kuikman and Van Veen, 1989; Kuikman et al., 1990, 1991) (Fig. 1). For instance, the release of N from bacteria by protists was demonstrated to serve as a primary source for plant N-uptake through arbuscular mycorrhizal fungi (Koller et al., 2013b, 2013a; Rozmoš et al., 2021). Grazing significantly enhances the turnover of rhizosphere bacterial communities (Alphei et al., 1996), but since protists feed selectively rather than randomly, predation can alter the composition of bacterial rhizosphere communities in a highly deterministic manner (Rosenberg et al., 2009; Trap et al., 2016). Protists and bacteria have coevolved for at least 500 Mio years before multicellular organisms evolved. Consequently, bacteria developed various highly adapted defense strategies against protists (reviewed in Jousset (2012)). These strategies include contact-dependent defenses, such as those involving the type VI secretion system (Flues et al., 2017), and long-distance defenses due to the release of toxic secondary compounds. For example, pseudomonads can produce numerous secondary metabolites to become resistant to grazing (Jousset et al., 2006; Mazzola et al., 2009; Amacker et al., 2020). The fact that the supernatant of an amoeba culture alone is sufficient to upregulate the production of defense compounds in bacteria (Jousset and Bonkowski, 2010) clearly demonstrates that the expression of bacterial (defense) traits is an adaptive strategy and that there is a trade-off between the bacterial investments in specific defense compounds, such as diacetyl-phloroglucinol (DAPG) (Jousset et al., 2010). Interestingly, DAPG is a highly protective metabolite against fungal and oomycete pathogens in the rhizosphere of plants (Raaijmakers and Weller, 1998; De Souza et al., 2003). However, the production of this secondary metabolite by a small bacterial cell is associated with significant trade-offs in growth, and therefore this highly plant protective trait is not stably expressed in these bacteria. Pseudomonads possess a naturally high mutation rate in the signaling pathways for secondary metabolite production, known as "phase change" (Broek et al., 2003). These pseudomonads have higher growth rates and do not invest in cooperation with their neighbors, potentially leading to the collapse of bacterial defense traits. Protist predators however, selecting for non-defended bacteria, shift the *Pseudomonas* populations in favor of those expressing defense traits (Jousset et al., 2009). Grazing resistance confers a double advantage for pseudomonads: they are not consumed, and more important, their non-defended competitors are preferably consumed by protists (Jousset et al., 2008). Accordingly, predation by protists can alter bacterial community composition in the rhizosphere in a highly deterministic manner, underscoring the importance of stabilizing the expression of specific defense traits in bacterial populations for the benefit of plant performance (Nguyen et al., 2020; Getzke et al., 2023).

Most studies on soil microbial communities primarily focus on fungi and prokaryotes, neglecting higher trophic level predators like protists. However, to comprehensively understand the dynamics of microbial community assembly, it is crucial to also consider protists due to their significant ecological roles. Protists are ubiquitous in soil (Finlay, 2002; Urich et al., 2008; Bates et al., 2013), with densities ranging from 10^4 - 10^7 individuals per gram of dry soil (Adl and Gupta 2006). The phyla Cercozoa and Endomyxa consistently constitute 20-30% of protist communities, alongside Amoebozoa, making them predominant taxa among terrestrial protists (Urich et al., 2008; Bates et al., 2013; Geisen et al., 2015; Grossmann et al., 2016; Singer et al., 2021). Cercozoa and Endomyxa are morphologically and functionally diverse (Dumack et al., 2019a), comprising flagellates, ameboflagellates, naked amoebae, and testate amoebae, some of which are free-living heterotrophs or autotrophic algae, while others are endophytes (Bass et al., 2009a, 2009b; Howe et al., 2009; Hess et al., 2012; Burki and Keeling, 2014). Notably, these taxonomic groups include both parasites that infect plants and animals (e.g. Ascetosporea and Phytomyxea (Neuhauser et al., 2014; Bass et al., 2019)), as well as organisms that benefit plants and establish symbiotic relationships (Cavalier-Smith and Chao, 2003; Dumack et al., 2021). The high occurrence of Cercozoa and Endomyxa and their functional diversity make them valuable indicators, providing insights into the general role of protists in soil ecosystems.

In contrast to the polyphyletic protists, the monophyletic grouping of the phyla Cercozoa and Endomyxa within Rhizaria (Cavalier-Smith and Chao, 2003) makes them amenable to primer-based high-throughput sequencing targeting the small subunit ribosomal rRNA gene (18S). When 'universal eukaryote primers' are used to encompass the entire range of protists (Stoeck et al., 2010; Bates et al., 2012), the results often exhibit bias towards specific taxa and include a high proportion of non-protist DNA in soil samples, mainly originating from multicellular organisms like plants and fungi. Consequently, this approach is unsuitable for revealing structural effects from environmental influences (Lentendu et al., 2014; Dupont et al., 2016; Fiore-donno et al., 2016).

Taken together, Cercozoa and Endomyxa are well-suited targets for 18S amplicon sequencing-based methods. Exploring these taxa facilitates a deeper comprehension of the ecological role of protists in terrestrial habitats. Consequently, we chose to focus on Cercozoa and Endomyxa as representatives of the soil protist community when utilizing high-throughput sequencing methods.

Mechanisms of plant microbiome recruitment

Plants release a high variety of rhizodeposits to the surrounding soil, which contain easily available C compounds, like sugars and organic acids (Santangeli et al., 2024). Rhizodeposits are exuded actively, like mucilage at the root tip or are lost passively, like root exudates in the subsequent elongation zone of the root (Jones et al., 2009). The easily available C molecules serve as energy source for soil microorganisms, whose growth is strongly C limited (Blagodatskaya and Kuzyakov, 2015; Niedeggen et al., 2024). Thus, only fast-growing (i.e. copiotrophic) microorganisms are able to colonize the advancing root (Zelenev et al., 2005; Benizri et al., 2007). Further, it is hypothesized that plants are able to attract specific microorganisms from their direct environment through the release of signal molecules or suppress others through defense mechanisms. In this scenario plants would actively select their specific rhizosphere microbiome (Hartmann and Schmid, 2009; Berg et al., 2014; Stéphane Hacquard et al., 2015). Both mechanisms lead to different microbial communities in the rhizosphere compared to bulk soil, the so-called "rhizosphere effect".

To facilitate the adjustment of exudation, different membrane transporters play a role (Sasse et al., 2018). Close to the root apex, where apoplastic flow is not inhibited by Casparian strips, or endodermal suberization, hydrophilic compounds can diffuse freely along a concentration gradient. Ion movement in or out of the root can be further facilitated by channel proteins or via active transport, utilizing ATP or proton gradient driven transporters. However, the role of microorganisms in the regulation of these transport mechanisms remains largely unknown. Research by for example Rudrappa et al. (2008) suggests that active transport is affected by plant-microbe interactions. They demonstrated that pathogen-treated plants respond by increasing the expression of ALMT1, a malate efflux transporter. This upregulation led to the attraction of the biocontrol agent *Bacillus subtilis.*

One mechanism by which the innate immune system of plants can detect and react to specific microorganisms is through the ‘zigzag model’ (Ngou et al., 2022). It involves two classes of immune receptors. First are the pattern recognition receptors (PRR) which recognize microbe-associated molecular patterns (MAMPs). They are localized on the plant cell surface, where they can bind to molecules such as chitin or flagellin, initiating intracellular signaling pathways that result in an antimicrobial response. This pathway is known as MAMP-triggered immunity (MTI). The second class of receptors are nucleotide-binding leucine-rich repeat (NLR) proteins, which detect pathogen-derived effectors that typically counteract MTI. Upon detection, NLRs activate effector triggered immunity (ETI), which effectively halts pathogenic growth. NLRs are primarily localized inside plant cells.

PRRs can exhibit high diversity among locally adapted genotypes, and it was shown that they enhance the fitness of plants in presence of the respective local bacteria with their specific MAMPs (Vetter et al., 2016). Interestingly, plants employ similar mechanisms to detect both pathogens and mutualists, resulting in comparable immune responses (Van Wees et al., 2008). However, the presence of pathogens and mutualists imposes different selective pressures on plants due to their contrasting effects on plant fitness. Furthermore, the plant immune response not only limits the growth of pathogens but also affects a wide range of phylogenetically unrelated microbes. Hacquard et al. (2017) proposed that the innate immune system of plants may primarily function to restrain microbial growth in general. For instance, the activation of the indole glucosinolate pathway upon perception of specific MAMPs serves as a crucial mechanism to defend against pathogens but also prevent the overgrowth of beneficial endophytes, which could potentially become pathogenic at high abundances. PRRs might have also evolved for crosstalk between symbionts and hosts.

Directionality of microbial assembly

Released rhizodeposits radially diffuse away from the root surface into the soil (Hinsinger et al., 2005). Additionally, rhizosphere organisms have to overcome different barriers first for colonizing the root surface and second for entering the plant host (Reinhold-Hurek et al., 2015). Accordingly, current models of microbial community assembly mainly consider a radial process of community assembly, proceeding from bulk soil over the rhizosphere towards the rhizoplane and finally the endosphere (Lundberg et al., 2012; Edwards et al., 2015; Reinhold-Hurek et al., 2015; Stephane Hacquard et al., 2015).

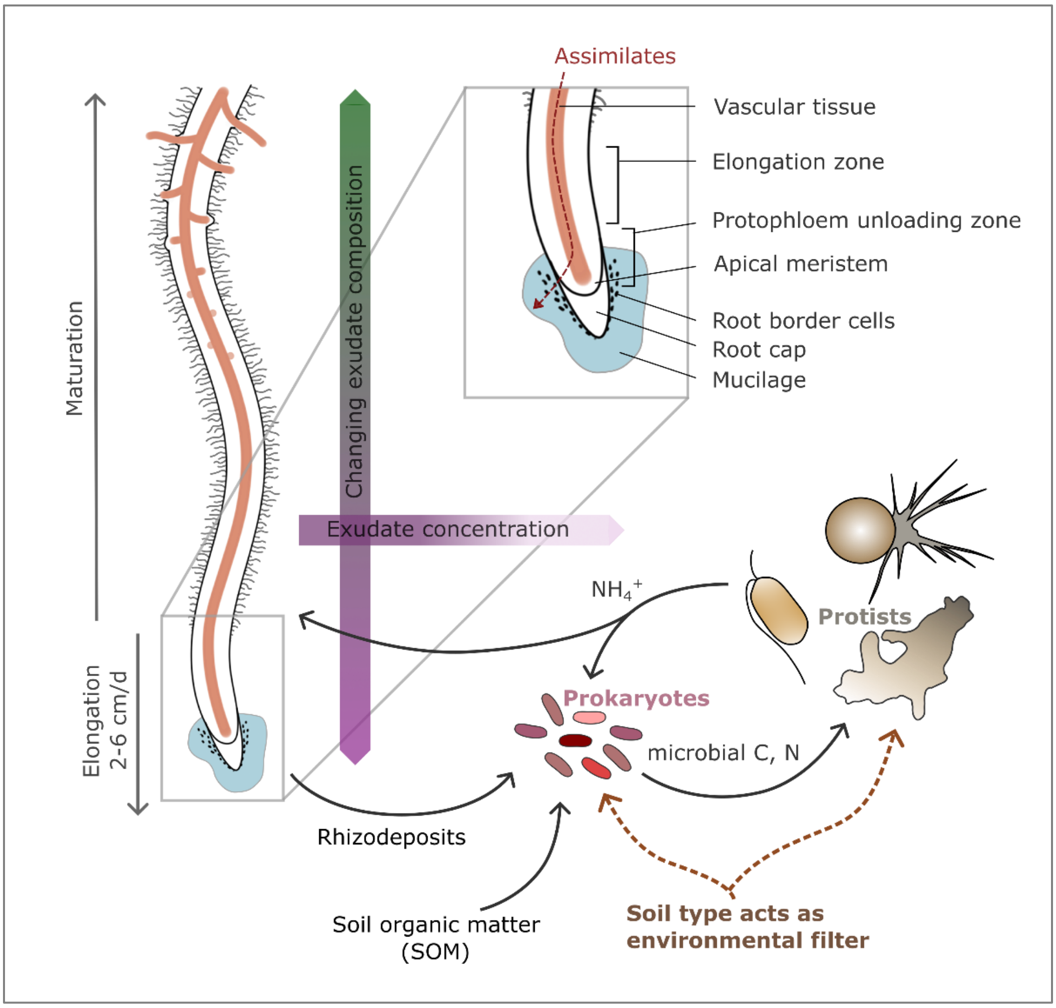

Figure 1 Simplified visualization of rhizosphere processes illustrating possible ways of rhizodeposition and microbial interactions. Roots grow and mature while releasing carbon-containing compounds spatially distinctly along the root axis. These compounds diffuse radially from the root. The major portion is exuded as mucilage at the root tip, while release of compounds through the unloading zone, active transport in mature root regions, or leakage from lateral root emergence sites is also possible. The released carbon enters the microbial food web upon consumption by prokaryotes, stimulating their growth and activity. Certain released molecules may exert specific effects on microbial community composition. Higher trophic level protists prey on these prokaryotes, thereby top-down influencing community structure and remobilizing a portion of the nitrogen fixed within bacterial biomass, rendering it available for plant uptake. Soil type acts as an additional environmental filter determining habitat conditions and influencing predator-prey dynamics.

When examining the community assembly in the rhizosphere, a comprehensive understanding cannot be achieved by solely considering radial processes. Such a unilateral approach fails to account for the multidimensional nature of the system. In particular, the morphological heterogeneity along the longitudinal root axis of young roots needs to be considered (Fig. 1). Different regions of roots fulfill different functions, and each region contributes uniquely to the overall rhizosphere microbiome assemblage. During maturing, root parts with specific functions undergo changes, and with this the conditions for inhabiting microbes change as well. While growth the root tip moves through the soil, consistently entering bulk soil and triggering dormant bacteria into activity (Niedeggen et al., 2024). In young maize roots this can happen with a speed of several centimeters a day. Meanwhile, the part above the root elongation zone does not change its position but matures. Microbes may either migrate along the root or remain in the same region, facing changing conditions, as rhizodeposition varies across different stages of root development. Fast growing taxa that might dominate in first stages of community assembly are replaced by specialists. Those might be more effective in facilitating rhizodeposits, are safe from predation or are resistant against antimicrobial compounds released by plants.

Root tip

The root tip is the first part of the root which comes into contact with bulk soil. At the apical root meristem, newly formed cells contribute to root elongation, but also to the formation of a root cap (Clowes and Wadekar, 1988; Hawes et al., 2003). Root cap cells are continuously generated and disposed as root border cells, a unique developmental pattern among plant organs, which leads to a finite size of the root cap (Barlow, 2003). The disposed border cells typically remain viable several days in most plant species (Vermeer and McCully, 1982; Hawes et al., 2003). The root cap, including detached border cells excrete large amounts of mucilage to the soil. In maize, mucilage is estimated to make up 20 to 25% of the total carbon released through roots (Chaboud, 1983). It is primarily composed of high-molecular weight carbohydrates. A meta study revealed that on average mucilage contains 78.4% polysaccharides, 7.3% proteins, 5.6% minerals, 3.1% lipids, and 5.7% other compounds (Nazari, 2021). The constant replacement of the root cap and the secretion of mucilage allow roots to push themselves through the soil. The cap protects stem cells from mechanical stress, while mucilage and border cells lubricate root growth by reducing the friction.

As shown in maize, mucilage additionally contains a variety of chemical compounds and enzymes, such as carboxylates and acid phosphatase, which can facilitate the mobilization of phosphorus, as well as others that serve as chelators for iron uptake (Ma et al., 2010).

Additionally, soil acidifying uronic acid leads to the mobilization of cations bound to the soil matrix (Morel et al., 1986; Edmond Ghanem et al., 2010; Nazari, 2021).

However, as noted by Hawes et al. (2003), mucilage and border cells serve another important purpose, specifically in the acquisition and interaction with microorganisms. Effects on microorganisms and nematodes include chemoattraction and repulsion (Hawes and Smith, 1989; Zhao et al., 2000), stimulation and inhibition of bacterial growth (Gochnauer et al., 1990), induction of nodulation genes (Zhu et al., 1997), species-specific inhibition of growth of pathogenic fungi (Jaroszuk-Ściseł et al., 2009) and their suppression through chitinases and peroxidases (Ordentlich et al., 1988; Wen et al., 2007), as well as release of phytoalexins and other antibiotics (Brigham et al., 1999). Moreover, mucilage contains nutrients that are not readily accessible to all microbes, as specific enzymes are required to break it down to utilize those nutrients. This may lead to the attraction of specific microbes capable of solubilizing mucilage (Pozzo et al., 2018; Amicucci et al., 2019). The polysaccharides of maize mucilage are primarily composed of monosaccharides, such as galactose, fucose, mannose, arabinose, glucose, and xylose (arranged in decreasing quantity). Due to its composition and high quantity in the rhizosphere, mucilage was considered a primary C source for rhizobacteria (Iijima et al., 2000). However, *in situ* analysis of microbial communities show that the root tip is not heavily colonized (Lagopodi et al., 2002; Gamalero et al., 2005; Humphris et al., 2005; Schmidt et al., 2018; Charura et al., 2024), likely due to the various defense mechanisms, that keep it safe from infections and the continuous movement through the soil. Dennis et al. (2010) hypothesized that pathogens, which are attracted by signal molecules released at the root apex, such as specific flavonoids (Hirsch et al., 2003) or strigolactones (Akiyama et al., 2005), require some time to react. Consequently, they only infect roots at the elongation zone, as the root has continued to grow. According to Hawes et al. (2012), carbon-based exudates may not always function as passive nutrient bases, but rather have the ability to trap, immobilize, and inhibit microbial growth in certain cases.

Root elongation zone

In the root elongation zone, assimilates are transported from the metaphloem to the protophloem. Subsequently, they are directed towards the root tip and the unloading zone, where lateral distribution takes place (Oparka et al., 1994; Ross-Elliott et al., 2017) (Fig. 1). Farrar et al. (2003) proposed that within the root elongation zone, a high portion of sucrose diffuses passively out of the root, following the concentration gradient between the interior and exterior of the root. This passive diffusion is unlike what occurs in mature parts of the root, where the movement of solutes is hindered by a layer of suberized endodermal cells.

Consequently, the root elongation zone could act as the primary site for the passive release of most primary metabolites, thereby stimulating microbial proliferation and potentially resulting in an increased microbial load in the adjacent root hair zone. Furthermore, the root elongation zone has been identified as both the mycorrhizal infection zone (Brunner and Scheidegger, 1992) and the primary site of infection by fungal pathogens. Notably, *Nectria haematococca* infections in pea (*Pisum sativum* L.) were predominantly confined to the region of root elongation, while older tissues and most root tips remained uninfected (Gunawardena and Hawes, 2002). The exact composition of root exudates released at the site of root elongation appears to be difficult to determine (van Dam and Bouwmeester, 2016).

Root hair zone

In the maturation zone, proximately to the elongation zone, differentiated epidermal cells, namely trichoblasts, form root hairs. They play a crucial role in various functions, primarily facilitating an increased surface area of the root for enhanced exchange between the root and its surrounding environment (Peterson and Farquhar, 1996; Gilroy and Jones, 2000). The role of root hairs in the uptake of water and drought sensing (Kwasniewski et al., 2016; Carminati et al., 2017; Hu et al., 2018) but also in the uptake of N, P, Fe and other mineral nutrients has been well recognized (Ma et al., 2001; Zhang et al., 2018; Bienert et al., 2021; Saengwilai et al., 2021). Plants can even adjust root hair length and density as needed.

To facilitate the uptake of poorly accessible nutrients through root hairs, plants exude protons (H^+), organic acids, chelating compounds and enzymes, including phosphatases, but the exact location of their release is mostly unknown (Yan et al., 2004; Marschner et al., 2011). The decreased phosphatase activity in a root-hairless mutant of barley (*Hordeum vulgare*) (Holz et al., 2020) points towards a role of root hairs in the synthesis or activation of phosphatase. Also microorganisms benefit from the mobilization of nutrients in the root hair region and might compete with the plant for these resources. Generally, exudation of carbon-rich compounds in the root hair zone is relatively lower when compared to younger root regions, which might be reflected in low microbial growth rates. Despite this, significant crosstalk occurs between plants and microorganisms in this region, as evidenced by numerous instances of plant interactions with both beneficial and pathogenic microorganisms (Buddrus-Schiemann et al., 2010; Libault et al., 2010). The clubroot disease-causing rhizarian protist *Plasmodiophora brassicae* for example penetrates the epidermis and gains access to the cortical tissue of its host through root hairs (Ingram and Tommerup, 1972; Hwang et al., 2012).

Root-microbe interactions in the root hair zone appear to differ from those in root regions lacking root hairs. However, a common method for sampling the "rhizosphere" involves removing roots from the soil and washing off the soil adhering to the entire root to investigate the microbial community. This approach may result in an overrepresentation of microbes associated with the root hair zone, as soil tends to adhere preferentially to those parts, thereby increasing the relative amount of root hair associated soil in the sample.

Lateral root breakage sites

As the root matures, lateral root primordia develop from a subset of pericycle cells, breaking through the overlaying cell layers and finally emerge (Péret et al., 2009). Resulting breakage sites can serve as potential entry points for microorganisms and pathogens (Gopalaswamy et al., 2000; Lagopodi et al., 2002; Sprague et al., 2007).

By using a tryptophan biosensor strain, Jaeger III et al. (1999) observed elevated levels of tryptophan released in the region of lateral root emergence in the annual grass *Avena barbata*. They assumed that their findings, combined with the higher activity of glutamate dehydrogenase (Wallace, 1973) (associated with NH_4^+ assimilation into amino acids) in the mature section of roots (in maize) indicate a general increase in amino acid synthesis. This could result in the leakage of amino acids through the breakage sites during lateral root emergence and may lead to a different composition of rhizodeposits compared to younger parts of the root and consequently to an altered microbiome composition.

Park et al. (2004) demonstrated an increased release of benzoxazinoids around breakage sites of the epidermis during lateral root emergence in *Zea mays*. Benzoxazinoids have been found to influence the root microbiome either directly or by regulating root metabolism, as suggested by correlations between benzoxazinoid-controlled root secondary metabolites, particularly flavonoids, and specific bacterial taxa (Cotton et al., 2019).

Several specific molecules, such as flavonoids, coumarins, phenolics, indoles, amino acids, and proteins, have been identified to participate in plant-microbe communication and influence the composition of the rhizosphere microbial community. Although the precise sites of their release are still not fully understood, rhizodeposition along the longitudinal root axis is heterogenous. This raises the question of whether plants are able to locally react to microbial colonization, which would be reflected in changes in gene expression along the root.

Soil texture

Soil type was identified as a key determinant of rhizosphere community structure, often exerting a stronger influence than plant species (Marschner et al., 2001; Araújo Da Silva et al., 2003; Sapp et al., 2017; Dumack et al., 2020; Bourceret et al., 2022). Soil type classification is complex, considering factors such as composition, structure, particle size, mineral and organic matter content, pH level, drainage properties, and nutrient availability. These factors not only affect soil microorganisms but also influence plants, which in turn shape microbial communities, complicating the isolation of effects individual factors have, particularly in natural settings. For instance, Marschner *et al.* (2001) observed root zone dependent variation in bacterial species diversity in sandy soil and clay, but not in loamy sand. The soil type further influenced the strength of the plant species effect, indicating an interaction between soil type, plant species, and location along the root in influencing rhizosphere community composition.

Soil types are commonly categorized into texture classes like sand (0.05 - 2.0 mm), silt (0.002 - 0.05 mm) and clay (smaller than 0.002 mm) based on particle size. Soil texture influences pore structure, aggregate formation (Kim et al., 2008), and soil water content (Fierer, 2017), thereby determining the habitable space for soil microbiota (Gupta and Germida, 1988; Mummey et al., 2006; Vos et al., 2013). Pores with small neck sizes, for example, restrict the entry of bacterivorous predators such as protists while allowing the movement of smaller bacteria (Rutherford and Juma, 1992), thereby protecting bacteria from predation (Wright et al., 1995). Soil water content and water films are vital for microorganisms as the aqueous phase in soil provides their living space, and its connectivity governs nutrient and gaseous fluxes (Erktan et al., 2020). Unconnected water films can impede their access to nutrients and movement, affecting competition and predator-prey dynamics (Or et al., 2007)

Soil constitution further changes with depth, typically resulting in increased soil density and reduced pore size and aeration in deeper layers of arable soil (Hamza and Anderson, 2005; Berisso et al., 2012). Microorganisms are most abundant in upper soil layers, with their abundance and diversity decreasing with depth (Fierer et al., 2003; Eilers et al., 2012; Pausch et al., 2018; Sosa-Hernández et al., 2018; Degrune et al., 2019; Yim et al., 2022). Degrune *et al.* (2019) found increased relative abundance of certain Cercozoan (protist) families in subsoil compared to topsoil, suggesting adaptations to smaller pore sizes typical of compacted subsoils. Additionally, Pausch *et al.* (2018) identified distinct depth-dependent patterns while investigating carbon stocks in the soil food web. They concluded that specific communities are shaped by depth-dependent abiotic and biotic habitat properties. Such properties might include low oxygen and nutrient concentrations as well as small pore size. These factors were also considered relevant by Yim *et al.* (2022), who conducted a laboratory experiment to assess the impact of varying depths in both loam and sand on fungal, bacterial and Cercozoan communities within pots planted with *Zea Mays*.

Distinguishing the effects of plants on microorganisms from those of soil texture or depth is challenging due to their combined influence on root growth and, consequently, on the interactions between roots and microbes.

Aims and Hypotheses

The research in this thesis was conducted as part of the priority program 2089 - "Rhizosphere Spatiotemporal Organization: A Key to Rhizosphere Functions" funded by the German Research Foundation (DFG). The central hypothesis of the priority program posited that resilience in the rhizosphere emerges from self-organized spatiotemporal pattern formation. The theory of self-organization suggests that patterns emerge at a global level solely from numerous interactions among lower-level components (Camazine et al., 2003). To understand the properties of such systems, individual components cannot be studied in isolation; instead, the interactions among the system's components must be considered. Therefore, within this project, the rhizosphere was approached as a self-organized system, with self-organization driven by feedback loops between the root, microbiome, and soil.

The aim of this PhD thesis was to uncover major underlying mechanisms for the assembly of root microbiota by dissecting the influence of soil structure, root traits, and bacterial and protist co-occurrences and interactions, with a particular focus on the protistan phyla Cercozoa and Endomyxa. Assisted by meta-genome analyses, our goal was to unravel changes in bacterial and protist identity and function, as well as to identify significant protist-bacteria interactions.

Accordingly, the hypotheses of this thesis were:

H1 Root microbiome assembly occurs from tips to older root sections.

H2 Protists play an important role in microbial assembly.

H3 The root cap and the root hair zone are critical drivers for microbiome assembly.

H4 Rhizosphere control points: If plant roots actively recruit their microbiomes, root gene expression should correlate with the occurrence of specific microbial taxa.

H5 Exudate composition and concentration influence bacterial metabolism and growth.

H6 Soil texture affects the morphology of the root system and thereby feeds back on microbial assembly.

H7 Protist species differ in their grazing selectivity and intensity (trait diversity) and increasing diversity of protists leads to improved exploitation of bacterial prey.

H8 Protists enhance plant growth by driving the microbial loop, increasing nitrogen uptake.

Chapter Summary

Chapter I: Microbial assembly along the root

Assembly patterns of the rhizosphere microbiome along the longitudinal root axis of maize (*Zea mays* L.)

The rhizosphere of plants hosts a diverse array of microorganisms spanning multiple trophic levels. These rhizosphere communities exhibit distinct compositions, diversities and microbial quantities compared to those found in bulk soil. It is hypothesized that plants selectively recruit a specific subset of microorganisms from the surrounding soil for their benefit. However, the reality appears to be quite intricate due to two main factors: firstly, plant roots are spatially heterogenous and continuously grow and mature, and secondly, interactions among microorganisms and their community dynamics likely influence the assembly process. To find patterns that elucidate the process of microbial community assembly along roots, we conducted high throughput amplicon sequencing of rhizosphere microorganisms at specific root regions and subsequent network analysis. By considering not only prokaryotes but also their predators (i.e. protists), we aimed to provide insight into the role of multitrophic relationships.

Chapter II: Plant drivers of rhizosphere microbiome dynamics

Root cap is an important determinant of rhizosphere microbiome assembly

Plants develop root zone specific organs, including root caps and root hairs, each serving different functions that remain incompletely understood. Root caps and root hairs likely impact microorganisms in structure specific ways through the divergent release of rhizodeposits. In this chapter we aimed to determine whether root caps and root hairs exert specific influences on microbiome assembly, and if such effects mutually influence each other.

To actively shape their microbial community, a comprehensive machinery of genomic pathways in the plant must react to the presence of specific microbes. Through correlation analysis of the relative abundances of microbial taxa and root zone specific expression levels of plant genes, we aimed to identify potential molecular rhizosphere control points.

Microbial utilisation of maize rhizodeposits applied to agricultural soil at a range of concentrations

Composition and quantity of rhizodeposits likely play a role in bacterial growth patterns and mineralization. By assessing microbial respiration patterns in response to varying concentrations of different components of rhizodeposits, we aimed to understand how these parameters influence bacterial growth dynamics. This understanding allows for more accurate predictions of how rhizodeposition drives microbial carbon and nutrient dynamics in the soil.

Chapter III: The role of soil texture

Responses of root architecture and the rhizosphere microbiome assembly of maize (*Zea mays L.*) to a soil texture gradient

Soil texture, i.e. the fractions of different sized particles, determines soil porosity, aggregate formation and water retention. These factors not only affect root growth but also shape the physical habitat for microorganisms and their ability to move freely through the soil matrix. The reciprocal relationship between soil texture and its effects on root growth and microbial assembly remains unclear. To address this, we investigated how a gradient in soil texture impacts root growth and architecture. Concurrently, we monitored the corresponding shifts in microbial diversity to understand how microbial assembly is influenced. Additionally, we conducted co-occurrence network analysis to explore whether networks will reflect differences in microbial motility, particularly of larger predators, due to soil texture.

Chapter IV: Complementary feeding by protists

Effects of protist species richness on the composition of the rhizosphere bacterial microbiome and plant performance

Protists are important bacterivorous predators in soil, but they do not graze on prokaryotes uniformly. Complementary feeding modes between different taxa likely allow a more thorough exploitation of bacterial prey. In this chapter we aimed to find out how increased species richness and different species composition of protists affects prokaryote communities, their function, and how this feeds back on plant growth and N uptake.

Chapter I: Microbial assembly along the root

Assembly patterns of the rhizosphere microbiome along the longitudinal root axis of maize (*Zea mays L.)*

Reference:

Rüger, L., Feng, K., Dumack, K., Freudenthal, J., Chen, Y., Sun, R., Wilson, M., Yu, P., Sun, B., Deng, Y., Hochholdinger, F., Vetterlein, D., Bonkowski M., 2021. Assembly patterns of the rhizosphere microbiome along the longitudinal root axis of maize (Zea mays L.). Frontiers in microbiology, 12, 614501. doi: 10.3389/fmicb.2021.614501

Author contributions:

This study was designed and planned together with Michael Bonkowski, based on the experimental framework provided by Doris Vetterlein. Experiments, laboratory work, bioinformatics, and statistical analyses were primarily conducted by the author, with support from co-authors as acknowledged in the publication. The manuscript was written by the author under the guidance of Michael Bonkowski.

Chapter II: Plant drivers of rhizosphere microbiome dynamics — Part 1

Root cap is an important determinant of rhizosphere microbiome assembly

Reference:

Rüger, L., Ganther, M., Freudenthal, J., Jansa, J., Heintz-Buschart, A., Tarkka, M.T., Bonkowski, M., 2023. Root cap is an important determinant of rhizosphere microbiome assembly. New Phytologist, 239, 1434-1448. doi: 10.1111/nph.19002

Author contributions:

This study was designed and planned in collaboration with Michael Bonkowski, Minh Ganther, and Mika Tarkka. Experiments, laboratory work, bioinformatics, and statistical analyses were conducted equally by the author and Minh Ganther. Network analysis was performed by Jule Freudenthal with the support of Anna Heintz-Buschart, while the analysis of prokaryota was carried out by Jan Jansa. All authors contributed to the writing of the manuscript.

Chapter II: Plant drivers of rhizosphere microbiome dynamics – Part 2

Microbial utilisation of maize rhizodeposits applied to agricultural soil at a range of concentrations

Reference:

Niedeggen, D., Rüger, L., Oburger, E., Santangeli, M., Ahmed, M., Vetterlein, D., Blagodatsky, S., Bonkowski, M., 2024. Microbial utilisation of maize rhizodeposits applied to agricultural soil at a range of concentrations. European Journal of Soil Science, 75:4, e13530. doi: 10.1111/ejss.13530

Author contributions:

Daniela Niedeggen: Conceptualization; formal analysis; methodology; validation; visualization; writing – review and editing; writing – original draft; data curation. Lioba Rüger: Conceptualization; methodology. Eva Oburger: Validation; writing – review and editing; supervision. Michael Santangeli: Writing – review and editing; methodology. Mutez Ahmed: Validation; methodology; conceptualization. Doris Vetterlein: Writing – review and editing; conceptualization. Sergey Blagodatsky: Validation; writing –review and editing; formal analysis; supervision; methodology. Michael Bonkowski: Conceptualization; validation; writing – review and editing; writing – original draft; project administration; supervision; funding acquisition.

Chapter III: The role of soil texture

Responses of root architecture and the rhizosphere microbiome assembly of maize (*Zea mays L.*) to a soil texture gradient

Reference:

Rüger, L., Feng, K., Chen, Y., Sun, R., Sun, B., Deng, Y., Vetterlein, D., Bonkowsk, M., 2023. Responses of root architecture and the rhizosphere microbiome assembly of maize (*Zea mays L.)* to a soil texture gradient. Soil biology and Biochemistry, 181, 109026. doi: 10.1016/j.soilbio.2023.109026

Author contributions:

This study was designed and planned in collaboration with Michael Bonkowski, based on the experimental framework provided by Doris Vetterlein. Experiments, laboratory work, bioinformatics, and statistical analyses were primarily conducted by the author. Network analysis was carried out by Kai Feng and Ye Deng. Bo Sun, Yan Chen, and Ruibo Sun provided processed sequencing data for bacteria and archaea. The manuscript was written by the author and Kai Feng, under the guidance of Michael Bonkowski.

Chapter IV: Complementary feeding by protists

Effects of protist species richness on the composition of the rhizosphere bacterial microbiome and plant performance

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Abstract

Protists, key bacterivorous predators in soil, play a critical role in regulating bacterial communities and nutrient cycling. Their grazing behavior is non-uniform, with complementary feeding strategies among different taxa potentially enabling more efficient exploitation of bacterial prey. Protist predation has been linked to enhanced nitrogen (N) availability to plants through the microbial loop, where protists release significant N from bacterial biomass. We conducted an inoculation experiment to examine how increasing protist species richness influences prokaryote communities, their functions, and the nitrogen uptake and performance of maize. Our results reveal protist species-specific shifts in bacterial community composition, suggesting that complementary feeding strategies, possibly linked to protist functional traits such as feeding mode (e.g., flagellates versus amoebae), drive these changes. A net biodiversity effect was observed on bacterial operational taxonomic unit (OTU) richness, likely due to reduced dominance of certain bacterial taxa, allowing less competitive taxa to proliferate. Additionally, protist diversity had a slight effect on the metabolic potential of bacterial communities, as reflected in their ability to utilize diverse carbon sources. Despite these effects on microbial community structure and function, we did not observe an increase in plant nitrogen uptake, suggesting that enhanced prey exploitation did not translate into measurable benefits for plant N nutrition under our experimental conditions.

Introduction

High throughput sequencing studies have revealed an unsuspected diversity of bacterivorous protists in the rhizosphere of plants (Fiore-Donno et al., 2020; Fiore-Donno et al., 2019; Rüger et al., 2023; Rüger et al., 2021; Taerum et al., 2021), emphasizing the importance of protistan grazers in regulating the composition and functions of bacterial microbiomes. Bacterivory among protists, however, is not a uniform trait, and different taxa evolved highly divergent strategies for preying on bacteria (reviewed in Bonkowski et al., 2019). Accordingly, an extensive literature exists on the specific selective pressures of diverse bacterivorous protistan taxa and their impact on the expressed traits and composition of bacterial communities from aquatic (Šimek et al., 1997; Posch et al., 1999, 2001; Jürgens and Matz, 2002; Pfandl et al., 2004), and soil ecosystems (Rosenberg et al., 2009; Hünninghaus et al., 2017; Amacker et al., 2020, 2022; Bahroun et al., 2021; Liu et al., 2024). Complementary feeding modes likely allow a more thorough exploitation of bacterial prey, posing the question of how increased species richness of protistan bacterivores affects microbiome composition and feeds back on plant performance.

Protists are known to enhance plant N nutrition and growth by releasing significant amounts of N from consumed bacterial biomass, a process known as microbial loop in soil (Bonkowski and Clarholm, 2012; Clarholm, 1985; Kuikman, and Van Veen, 1989). Enhanced top-down control through complementary grazing pressure due to different feeding impacts of individual protist taxa (Glücksman et al., 2010; Flues et al., 2017) could then increase the amount of N available to plants (Bonkowski et al., 2000; Bonkowski et al., 2021). As most predation pressure is directed towards undefended, fast-growing bacteria (Sherr et al., 1992; Posch et al., 1999), more intense top-down control at higher protist species richness might also enhance microbiome diversity when subdominant, rare bacterial taxa are increasingly released from their competitors (Saleem et al., 2012). This increased biodiversity might positively impact plant growth and health (Saleem et al., 2019).

In particular bacterial taxa with grazing-resistance traits are favored under protist predation pressure (Amacker et al., 2020; Jousset and Bonkowski, 2010; Jousset et al., 2010; Song et al., 2015), leading to predator-specific shifts of bacterial rhizosphere microbiomes. Feeding modes of protists range from highly selective consumption of individual bacteria to engulfment of whole colonies (Bonkowski et al., 2019). An important question is then if the effect of multiple bacterivores in a community may overyield the average effects of the single predators. Despite multiple potential benefits to plants (Jousset, 2017; Gao et al., 2018; Xiong et al., 2020), only few studies ever investigated complementary effects of increased protist species richness on ecosystem functions (Glücksman et al., 2010; Saleem et al., 2012, 2013; Hünninghaus et al., 2017).

To better understand the role of protist diversity in the plant rhizosphere we set up a microcosm experiment to investigate how increased species richness and the presence of distinct functional groups of protists (i.e. flagellates vs amoebae) affect plant performance through grazing-induced shifts of the rhizobacterial microbiome. We hypothesized that increasing protist species richness, through complementarity in selectivity and consumption of bacteria leads to i) additive shifts in alpha and beta diversity of bacterial prey, and consequently ii) a more thorough consumption of bacterial biomass and thus improved coupling of N release and plant uptake via the microbial loop and iii) overyield in plant performance.

Materials and Methods

Experimental Set-Up

Microcosm chambers consisted of glass tubes (150 mm height, 60 mm diameter) with a lysimeter plate at the bottom (Hoskin Scientific, Canada). 20 microcosm chambers each were mounted on an air-tight PVC box (50 x 60 cm, 20 cm height) (Bonkowski, 2019). Three boxes were set up with 60 microcosms in total. To mimic natural soil matric potential, the microcosm chambers were continuously drained by applying vacuum to the box. The microcosm chambers were closed with PVC lids, with a central opening for the plant shoot, two openings for aeration and one for watering as in Bonkowski et al. (2000). The latter openings were protected by sterile filters (0.2 µm) to prevent contaminations from the air or during watering. The microcosms were wrapped in aluminum foil to protect soil and roots from light. Glass and metal materials were sterilized by autoclaving (120°C, 30 min), PVC-boxes with 70% ethanol and the set up was performed under sterile conditions.

A protist free bacterial inoculum was obtained by shaking, centrifugating (4500 x g for 15 min) and filtering (3.0 and 1.2 µm pore size filters, Satorius, Germany) a soil slurry derived from 1 kg fresh loamy soil suspended in 1000 ml of wheat grass (WG) medium (0.15 g wheat grass powder (Raab Vitalfood GmbH, Rorhbach, Germany) per 1000 ml sterile H_2O_{dest}) as described in Bonkowski (2019). To confirm the absence of protist and fungal contaminants, the filtrate was cultured in WG medium for 10 days. Microcosms were filled with 560 g (soil dry weight) autoclaved (30 min at 120°C) loamy sand, consisting of 17% of an agricultural loamy soil and 83% of a quartz sand (WF 33, Quarzwerke Weferlingen, Germany) (Vetterlein et al., 2021). The soil was subsequently inoculated with 1 ml of bacterial suspension, followed by the addition of 90 ml of sterile H_2O_{dest}. The microcosms were then incubated in the dark at 20°C for one week.

Clonal cultures of four flagellate species (*Allapsa* sp., *Paracercomonas* sp., *Neocercomonas* sp. (Cercozoa, Rhizaria); *Spumella* sp. (Chrysophyta, Stramenopiles)) and of three amoeba species (*Rhogostoma radagasteri* (Cercozoa, Rhizaria); *Flamella* sp. (Amoebozoa); *Allovahlkampfia* sp. (Heterolobosea, Excavata)) were kept in WG medium. In addition, an axenic culture of *Acanthamoeba castellanii* ATCC 30010 (Amoebozoa) (Koller et al. 2013) was used as amoeba inoculum. All cultures were washed three times by centrifugation with sterile Waris-H medium (McFadden and Melkonian, 1986) for 30 min at 1000 x g to remove excess bacteria. Subsequently, protistan culture densities were measured microscopically using a counting chamber. One day before planting, each microcosm was inoculated with ~20.000 protist cells in total (approx. 500 µl) of either monocultures, or mixed combinations of 2, 4 or 8 different protist species according to Table 1 (Schmid et al., 2017). All microcosms received 200 µl of a mixed bacterial filtrate from all protist cultures in addition to the bacterial inoculum. Microcosms of control treatments were inoculated only with bacteria and no protists. Accordingly, the experiment features a substitutive, partially factorial design in which all protist treatments had the same initial protistan cell density (i.e. maximum evenness) (Schmid et al., 2017).

Zea mays seeds (inbred line B73) were surface sterilized with 10% H_2O_2 under vacuum for 10 min, rinsed with water and sown individually in cylindrical plastic tubes (30 mm height, 17 mm diameter) filled with 5 g of sterile, moist soil (see Bonkowski, 2019). Both ends of the tubes were sealed with sterilized PARAFILM® and placed for germination at 20 °C in the dark. After germination, 60 tubes with seedlings of similar size were selected. One tube with a seedling was placed into the central hole of the lid of each microcosm. The opening of the tubes around the stem of each seedling was sealed with kneading mass (Teroson GmbH, Heidelberg, Germany). The microcosms were placed in a climate chamber with a day–night regime of 12/12 h (350 µmol m^{-2} s^{-1} photosynthetically active radiation) at 24 °C/18 °C and 65% humidity. Plants were watered through a sterile filter (0.2 µm) every second day with 60 ml of autoclaved deionized water. Leachate was continuously removed by applying underpressure to lysimeter plates below the microcosm chambers (Bonkowski, 2019).

Table 1 Experimental design with 21 different combinations of four flagellate (F1, F2, F3, F4) and four amoeba species (A1, A2, A3, A4) as well as a control treatment to test for the effects of protist species richness (1-8 species) on plant performance, microbial biomass and bacterial community structure.

Protist species																							
Allapsa sp.	F1		x								x				x				x		x		x
Paracercomonas sp.	F2			x							x					x				x	x		x
Neocercomonas sp.	F3				x							x					x		x			x	x
Spumella sp.	F4					x						x						x		x		x	x
Flamella sp.	A1						x						x		x				x		x		x
Rhogostoma radagasteri	A2							x					x			x				x	x		x
Allovahlkampfia sp.	A3								x					x			x		x			x	x
Acanthamoeba castellanii	A4									x				x				x		x		x	x
No. of species		0	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	4	4	4	4	8
No. of replicates		8	4	4	4	4	4	4	4	4	1	1	1	1	1	1	1	1	1	1	1	1	8

Sampling

After 17 days of growth, microcosms were destructively sampled. Shoots were cut above the soil and roots were rinsed from adherent soil. Subsequently, the plant material was dried at 60 °C for 48 h, weighed, milled and analyzed for C and N content with a C/N element analyzer (Flash 2000 Thermo Fisher Scientific GmbH, Germany). The soil was mixed and 1 g fresh wt was taken for DNA extraction; the remaining soil was stored at 4 °C until further analysis.

As a measure of microbial functional changes in response to protistan grazing, we compared the ability of culturable bacteria to metabolize 31 different C-substrates of increasing complexity (BIOLOG™ EcoPlates, Biolog Inc., Hayward, CA, USA) as in Griffiths et al. (2001). Briefly, 5 g soil per sample were suspended in 45 ml 0.85 % NaCl, vortexed for 3 min and centrifuged at 150 x g for 30 min. After the samples had settled for a further 10 min, EcoPlates were inoculated with 125 µl of the supernatant, giving an absorbance of 0.4 at a wavelength of 590 nm. Optical density was measured at 590 nm wavelength after 48 h of incubation. Absorbances 1.4 times higher than the control well without C source were considered as positive substrate utilization. The number of metabolized substrates was determined for each sample and Pielou's evenness was calculated from OD values for comparison of metabolic profiles.

Quantification and amplicon-sequencing of bacteria

For DNA-extraction and purification the FastDNA® SPIN Kit for soil and the GENECLEAN® SPIN Kit (MP Biomedicals, Santa Ana, CA, USA) were used, following the manufacturer's instructions. Extracted DNA was used for quantification and sequencing of bacteria.

Bacterial community abundance was determined by quantitative polymerase chain reaction (qPCR) as described in (Bukovská et al., 2021). Briefly, for calibration amplicons were generated from soil DNA extracts with the primers Eub338F (5'-ACTCCTACGGGAGGCAGCAG-3') and Eub518R (5'-ATTACCGCGGCTGCTGG-3'), using the TP HS DNA-free 2x Master Mix (TopBio, Vestec, Czech Republic). After purification with the QIAquick PCR purification kit (Qiagen, Holden, Germany) and quantification by Picogreen fluorescence, amplicons were serially diluted to reach a broad range of fragment copy numbers. The qPCR was performed in a total volume of 20 µl containing 10 µl Luna Universal qPCR 2x Master Mix (New England Biolabs, M3003), 2 µl template DNA, 0.5 µl of 10 µM forward primer Eub 338 and 0.5 µl of 10 µM reverse primer Eub518. A LightCycler 480 II Instrument (Roche Molecular Systems, Rotkreuz, CHE) was used to repeat the following cycling steps 55 times, after initial denaturation at 95°C for 5 min: denaturation at 95 °C for 10 s (ramp 4.4 °C s-1), annealing at 55 °C for 20 s (ramp 2.0 °C s-1), and amplification at 72 °C for 25 s (ramp 4.4°C s-1). The qPCR results were recorded as the second derivation maximum of the amplification curves. Unusually low melting temperatures (Tm) in melting curve profiles were used to indicated false positives (e.g., due to non-specific primer dimer formation).

For sequencing, amplicons of an approximately 350 bp long fragment of the prokaryotic V4 region of the 16S rRNA gene were generated. The forward primer 515F (5'-GTGCCAGCMGCCGCGGTAA-3') (Caporaso et al., 2011) and the reverse primer 806R (5'-GGACTACNVGGGTWTCTAAT-3') (Apprill et al., 2015) were used. Double indexing of amplicons with Nextera XT indexes provided unique index combinations per sample. After amplicon concentrations were measured using Picogreen fluorescence, amplicons were brought to equimolar concentrations, mixed and sequenced on a 2X300 MiSeq Illumina platform (Illumina Inc., San Diego, CA, United States) at the Joint Microbiome Facility, Vienna University, Austria. Sequences were demultiplexed to individual samples and sequencing adapters were removed using cutadapt (Martin, 2011). All further steps were carried out in SEED2 (Větrovský et al., 2018). Forward and reverse sequence reads were merged with a minimum overlap of 20 bp and maximum 15% mismatches. An average quality score below 30, a per-base quality score less than 7, or a sequence length of less than 200 bp were exclusion criteria. Potentially chimeric sequences were identified and removed. Remaining sequences were clustered into operational taxonomic units (OTUs) at 97 % similarity level (using usearch v. 8.1.1861 embedded in SEED2 software) and identified by comparing (using Blastn) with the SILVA SSU database. Sequences belonging to Eukaryota (mitochondria, chloroplasts) were removed and the remaining data were rarefied to 26,000 sequences per sample. Sequences were clustered again at 97% similarity level. The most abundant sequences from each OTU were used for identification via RDP classifier with the SILVA database as reference. Finally, OTUs represented by less than 100 reads were removed. For further statistical analysis, relative abundances of the OTUs per sample were calculated.

Statistical analyses

Statistical analysis was performed in R version 4.0.3 (R Core Team, 2020). The packages dplyr (Wickham et al., 2018) and tidyr (Wickham et al., 2024) were used for data manipulation, vegan (Oksanen et al., 2023), emmeans (Lenth, 2024), nlme (Pinheiro et al., 2020) and MuMIn (Barton, 2020) for model fitting and calculations and ggplot2 (Wickham, 2016) and gridExtra (Auguie, 2017) for data visualization.

Differences in bacterial community composition when microcosms were inoculated with various protist species combinations (Table 1), were analysed by Non-Metric Multidimensional Scaling (NMDS) based on Bray-Curtis dissimilarities (relative abundance OUT matrix). Differences in bacterial communities between treatments were tested by a permutational multivariate analysis of variance (PERMANOVA, vegan::adonis2), pairwise adonis (vegan::pairwise.adonis2) and comparison of group dispersion (vegan::betadisper). Additionally, bacterial OTU-richness and Pielou evenness was calculated from the rarefied data (Oksanen et al., 2023). The increase of protist species richness was hypothesized to cause complementary effects on bacterial diversity and function. These can be either additive, equivalent to the average effect of each species in monocultures, or overyielding, i.e. greater than the average effect of each species in monocultures. To determine if bacterial diversity, abundance and function share a linear relationship with protist diversity, linear mixed-effects models were employed on additive effects and net biodiversity effects (NBE; i.e. overyielding). To account for a block effect caused by the position of microcosms in the climate chamber and to determine the protist species richness effect on each response variable, first the difference between treatment (i.e., microcosms incubated with protists) and control samples (without protists) within the same microcosm box was calculated for all data.

Subsequently, the net biodiversity effect (NBE) was calculated as follows:

$$NBE = \mathrm{Y}_o - \mathrm{Y}_e = \mathrm{Y}_o - \frac{1}{n}\sum_{i=1}^{n} \overline{M}_i$$

where Y_o represents the observed yield from a mixed culture for a variable of interest, $\overline{M}_i$represents the mean yield of a constituent species in monoculture, and n is the total number of species in the mixed culture. The NBE was tested using a linear mixed-effects (LME) model fitted by restricted maximum likelihood (REML) method and subsequent ANOVA. Further R^2 values based on fixed terms alone (marginal R^2) or both fixed and random terms (conditional R^2) were computed. Random term selection was based on the Akaike information criterion (AIC). Since the experimental design features species composition as a unit of replication for species richness, the species composition variable was assigned to the random term, as well as the variation explained by the block effect.

In order to analyze if certain protist taxa led to increased plant performance, shoot and root biomass and plant C- and N-contents of the protist-free control were compared to protist treatments containing only one protist species using type I SS analysis of variance (ANOVA, emmeans::contrast). Plant performance variables were subsequently analyzed for linear relationships with protist diversity using LME as above.

Results

The total bacterial richness in the experiment consisted of 468 OTUs. Among these, 262 bacterial OTUs (~56%) were shared across all inoculation treatments and the control group, which received only the bacterial inoculum (Figure 1). An additional 72 OTUs (~15%) were exclusively found in the inoculation treatments but were absent in the control. Forty-nine OTUs (10%) were absent in the four-protist species treatment.

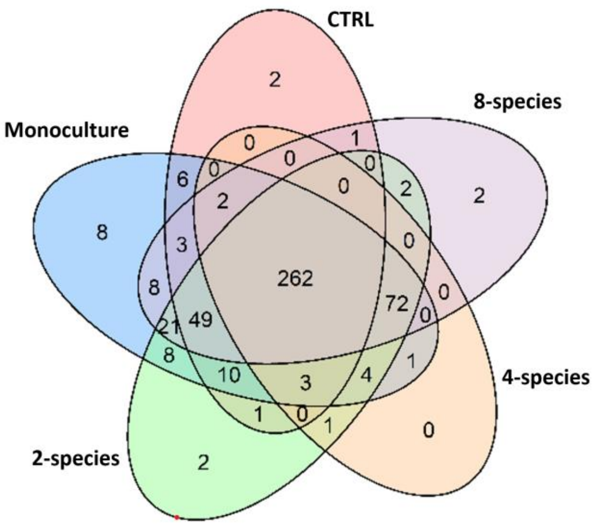

Figure 1 Venn diagram illustrating the overlap of bacterial OTUs across treatments, including the control with no protist species added (CTRL), monoculture treatment, and treatments with 2, 4, and 8 protist species.

The individual protist species caused distinct shifts of bacterial community composition (Figure 2a, Table S1). Accordingly, protist identity explained 60% of the variation in bacterial community composition (PERMANOVA, $F_{7,\,22}$ = 5.5, R^2= 0.602, p = 0.001). Effects of flagellate and amoeba functional groups were clearly separated (PERMANOVA, $F_{1,\,29}$ = 3.2, R^2= 0.093, p = 0.001). Protist species richness, without taking protist identity into account, exerted a clear directional shift of bacteria community composition (Figure 2b; PERMANOVA, $F_{4,\,49}$ = 4.2, R^2= 0.243, p = 0.001). Beta diversity dispersal, did not differ among control, monoculture, and 8-species treatments.

We further investigated whether an increase in protist species richness caused no, additive or overyielding effects on bacterial OTU richness, evenness, bacterial abundance or functional diversity. Upon normalization to the control, no significant additive linear relationships of protist species richness on bacterial richness, abundance or functions were found (Figure 2c). However, a significant positive relationship was found between number of protist species and the NBE on bacterial OUT richness (mR^2=0.157, p=0.027) while overyielding effects on bacterial evenness were only marginally significant (mR^2=0.09, p=0.084). Although the cultivable bacterial communities on BIOLOG™ Ecoplates did not utilize a broader range of carbon sources, higher protist species richness resulted in a marginally significant increase in NBE for the evenness of carbon-source exploitation by bacteria (mR^2=0.093, p=0.06).

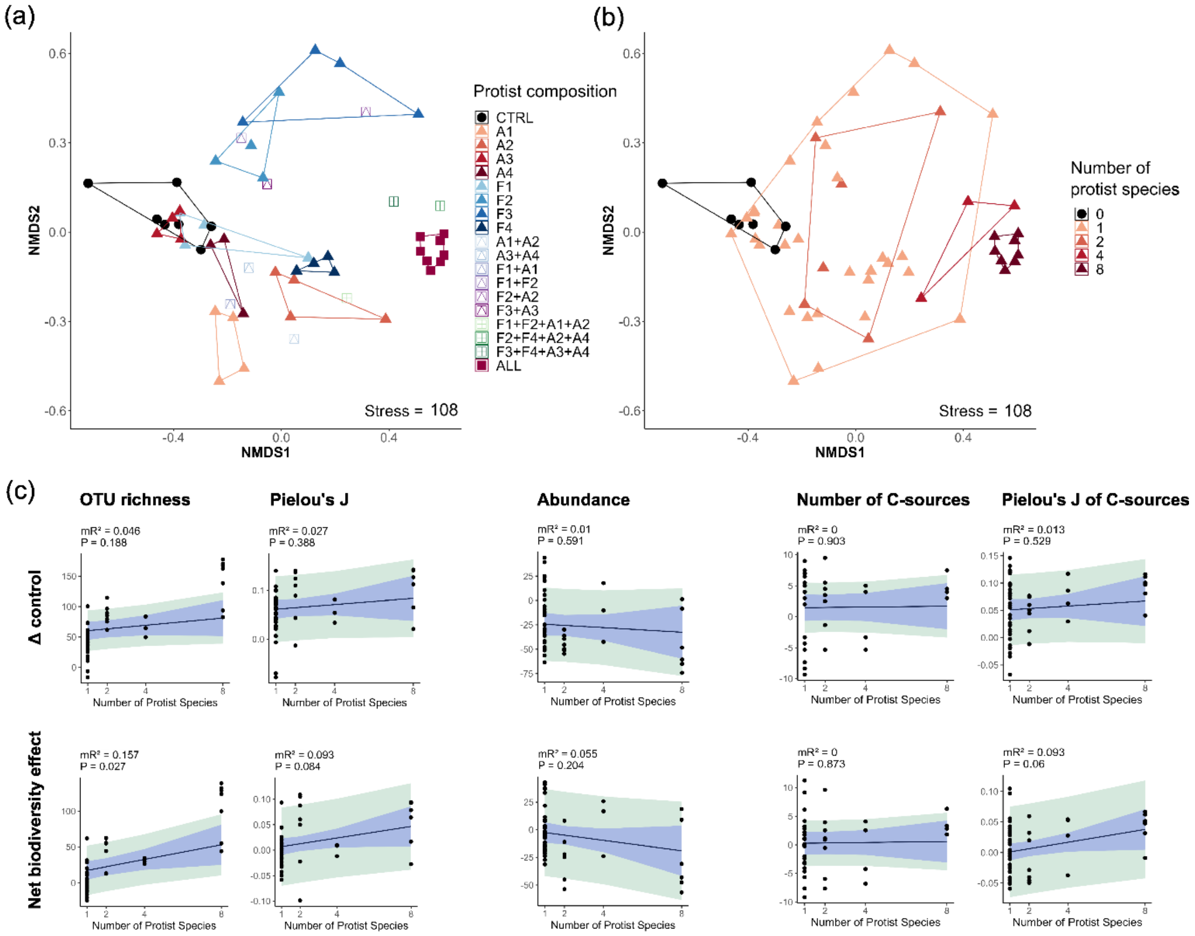

Figure 2 Bacterial community structure, abundance and function. (a, b) Nonmetric multidimensional scaling (NMDS) of Bray-Curtis dissimilarities of bacterial communities in soil inoculated with (a) combinations of four flagellate (F1, F2, F3, F4) and four amoeba species (A1, A2, A3, A4) and (b) at increasing richness of protist species. The control treatment (CTRL) contained no protists, while the "ALL" treatment included all eight protist species. (c) Linear regression plots with the standard error (blue shaded area) and the standard error accounting for residual variance (green shaded area). The plots illustrate whether increased species richness of protists (x-axis) had additive effects relative to the control (Δ control, upper panel), or caused overyielding (Net Biodiversity Effect, lower panel) on bacterial OTU richness, evenness, abundance (16S rRNA gene copy numbers g^{-1}), numbers and evenness of C sources utilized on BIOLOG™ EcoPlates. The marginal R^2 (mR^2) and p-values (P) of the linear mixed-effects (LME) analysis are displayed above each plot.

Among protist species, only the monoculture of *Rhogostoma* (A2) had a significant positive effect on maize shoot biomass (Figure 3a; Table S2; contrast analysis, *p* = 0.023). An increase in the number of protist species did not lead to a significantly enhanced shoot or root biomass and no positive NBE was observed (Figure 3b). Only the proportion of C (%) in maize shoots increased with the number of protist species (mR^2=0.24, p=0.002) and also the NBE increased (mR^2=0.36, p<0.001). No effects were observed on root C (%) and shoot and root N (%).

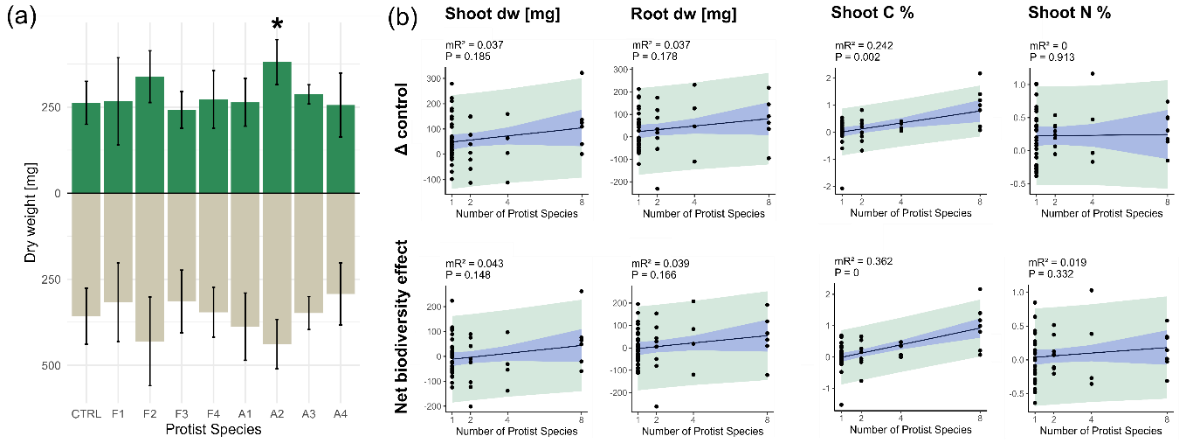

Figure 3 Plant performance. Bar plots of shoot (green) and root (tan) (a) biomass (mg dry wt). Asterisks above bars indicate significant differences (P<0.05) between treatment. (b) Linear regression plots with the standard error (blue shaded area) and the standard error for the residual variance (green shaded area). The plots illustrate whether increased species richness of protists (x-axis) had additive effects relative to the control (Δ control, upper panel), or caused overyielding (Net Biodiversity Effect, lower panel) on shoot and root dry weight (mg), C content (%) and N content (%). The marginal R² (mR²) and p-values (P) of the linear mixed-effects (LME) analysis are displayed above each plot.

Discussion

Studies in aquatic (Šimek et al., 1997; Batani et al., 2016) and terrestrial ecosystems have convincingly demonstrated strong grazing impacts of bacterivore protists on bacterial community composition (Flues et al., 2017; Oliverio et al., 2020), associated with significant changes in bacterial defense traits (Jousset et al., 2009; Jousset and Bonkowski, 2010; Amacker et al., 2020). This is in line with the species-specific differences in bacterial community composition observed in protistan monocultures in the present study. Notably, bacterial communities consumed by flagellates or amoebae showed clear distinctions. Flagellates exhibit diverse feeding modes, specializing in suspended or surface-bound bacteria, while slight variations in prey-handling times drive trade-offs, influencing predator selectivity and bacterial predator-avoidance strategies. (Boenigk and Arndt, 2002; Matz et al., 2004). Even closely related flagellate species may differ significantly in respect to selectivity and grazing impact (Boenigk, 2008; Glücksman et al., 2010; Flues et al., 2017). Amoebae in contrast are strict biofilm grazers (Huws et al. 2005). Despite being still selective, amoeba are able to engulf whole bacterial colonies and thus exert a completely different selection pressure on their prey than flagellates (Matz, 2007; Jousset et al., 2009). The clear distinction between bacterial communities under flagellate vs. amoebae grazing likely reflects general outcomes of grazing impacts between these divergent functional groups on bacterial community composition.

In a mixed protist community, complementary feeding might prevent the suppression of subdominant bacterial taxa as efficient feeding directed to a broad range of fast-growing taxa relieves slow-growing taxa from competition (Abrams, 1992; Saleem et al., 2012). It allows coexistence of subdominant bacteria, thus enhancing species richness and evenness as observed. At the same time, protist diversity was not significantly related with a reduction of bacterial abundance (qPCR). Due to the fast reproduction times of bacteria, especially in the rhizosphere where C is not limiting bacterial growth (Rüger et al., 2023), bacterivory is often associated with increased microbial turnover instead of a clear decrease in microbial abundance (Alphei et al., 1996). Saleem et al. (2013) suggested that an increasing protist species richness leads to a decrease in prey abundance, due to the more effective exploitation of the food source by predator complementarity. Others postulated a positive effect of predators on prey populations (Brown et al. 2004) and productivity (Griffiths, 1986; Bonkowski et al., 2000). With regard to prey abundance these opposite effects may cancel each other out and further depend on predator identity and composition and the availability of C for bacterial production (Hünninghaus et al., 2017). Metabolic profiling suggested that protist diversity and the resulting bacterial OTU richness led to an increasingly even breakdown of the various carbon sources provided in BIOLOG™ EcoPlates. The higher number of bacterial taxa increases the possibility, that the community contains one or more specialized species for the metabolization each of the specific carbon sources. These specialists might still not dominate the community, due to complementary grazing by protists.

Niche complementarity assumes that certain functional traits of an organism such as morphology, grazing preference or motility will lead to the specialized exploitation of a 'niche' which leads to increased yield performance in mixtures relative to monocultures (Loreau, 2004). On the other hand, functional redundancy describes distinct species who occupy the same ecological niches and are thus expected to yield more in monocultures under less interspecific competition. Niche complementarity arises from efficient resource partitioning that can be accounted for by temporal, spatial or resource use differentiation (Saleem et al., 2019). As the root grows, hotspots of carbon exudation and guilds of stimulated bacterial biomass shift along with it. Furthermore, the heterogeneity of the soil contributes to stratification of microbial communities. The temporal and spatial flux of carbon and microbial communities may positively influence niche differentiation thereby reducing the occupation of the same niche (i.e. site of stimulated bacterial abundance) by multiple protist predators.

This has already been shown for protists in rice cultivars (Asiloglu and Murase, 2017) in which flagellates, amoeba and ciliates, differed in abundance and spatial colonization of rice roots, presumably as a result of increased interspecific competition.

In this study, among the treatments with individual protist species, only inoculation with Rhogostoma radagasteri resulted in increased shoot biomass. However, no overall linear relationship was observed between protist species richness and plant biomass or the proportion of incorporated nitrogen. Effective protistan predation in soil can boost the nutrient – especially nitrogen - turnover (Clarholm, 1985; Bonkowski et al., 2000; Bonkowski and Clarholm, 2012), and consequently affect plant growth (Kreuzer et al., 2006; Herdler et al., 2008; Asiloglu et al., 2020). In this study, we found that protist diversity did not enhance proportional nitrogen incorporation but it is particularly noteworthy that, despite the lack of significant effects in single species treatments, a linear relationship between protist species richness and proportional carbon content in shoots was observed.

This study is one of the few biodiversity-ecosystem functioning experiments to examine the relationship between protists and their impact on plant performance across a species richness gradient. We observed distinct bacterial communities in the presence of different protist species, and found changes in bacterial function and plant performance, such as increased carbon content in shoots, with increasing protist species richness. These findings highlight the importance of protist trait diversity and suggest complementary feeding strategies among protists. The functional and nutritional changes to microbiome functioning prompted by protist bactivory have been supported by various studies. However, to better understand their impact on plant health, the definition of functional diversity must be beyond basic traits like morphology and locomotion. Although many questions concerning the protist grazing efficiencies and intraspecific competition remain yet unanswered elements of microbiome functioning, future projects must carefully balance the use of well-studied, model taxa for comparative studies with a diverse representation of topological traits, metabolic function and sensitivity to abiotic factors.

Author contributions

This study was designed and planned in collaboration with Michael Bonkowski. The experiments and laboratory work were conducted by the author and Monica Wilson. Jan Jansa provided the processed bacterial sequencing data. Statistical analyses were performed by the author with assistance from Juanjuan Huang and Nico Eisenhauer. The manuscript was written by the author under the guidance of Michael Bonkowski.

Data availability statement

The original data are available upon request and are archived in the data repository of the research group in which the work was carried out.

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References

Abrams PA (1992) Why don't predators have positive effects on prey populations? Evol Ecol 6:449–457. doi: 10.1007/bf02270691

Alphei J, Bonkowski M, Scheu S (1996) Protozoa, Nematoda and Lumbricidae in the rhizosphere of Hordelymus europaeus (Poaceae): faunal interactions, response of microorganisms and effects on plant growth. Oecologia 106:111–126. doi: 10.1007/BF00334413

Amacker N, Gao Z, Agaras BC, et al (2020) Biocontrol traits correlate with resistance to predation by protists in soil Pseudomonads. Front Microbiol 11:1–13. doi: 10.3389/fmicb.2020.614194

Amacker N, Gao Z, Hu J, et al (2022) Protist feeding patterns and growth rate are related to their predatory impacts on soil bacterial communities. FEMS Microbiol Ecol 98:. doi: 10.1093/femsec/fiac057

Apprill A, Mcnally S, Parsons R, Weber L (2015) Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquatic Microbial Ecology 75:129–137. doi: 10.3354/ame01753

Asiloglu R, Murase J (2017) Microhabitat segregation of heterotrophic protists in the rice (Oryza sativa L.) rhizosphere. Rhizosphere 4:82–88. doi: 10.1016/j.rhisph.2017.08.001

Asiloglu R, Shiroishi K, Suzuki K, et al (2020) Protist-enhanced survival of a plant growth promoting rhizobacteria, Azospirillum sp. B510, and the growth of rice (Oryza sativa L.) plants. Applied Soil Ecology 154:103599. doi: 10.1016/j.apsoil.2020.103599

Auguie B, (2017) gridExtra: Miscellaneous functions for "Grid" graphics (R package version 2.3). https://CRAN.R-project.org/package=gridExtra

Bahroun A, Jousset A, Mrabet M, et al (2021) Protists modulate Fusarium root rot suppression by beneficial bacteria. Applied Soil Ecology 168:104158. doi: 10.1016/j.apsoil.2021.104158

Batani G, Pérez G, Martínez de la Escalera G, et al (2016) Competition and protist predation are important regulators of riverine bacterial community composition and size distribution. J Freshw Ecol 31:609–623. doi: 10.1080/02705060.2016.1209443

Barton K (2020) *MuMIn*: Multi-Model Inference. R package Version 1.43.17. https://CRAN.R-project.org/package=MuMIn

Boenigk J (2008) Nanoflagellates: functional groups and intraspecific variation. Denisia 23:331–335

Boenigk J, Arndt H (2002) Bacterivory by heterotrophic flagellates: community structure and feeding strategies. Antonie Van Leeuwenhoek 81:465–480. doi: 10.1023/A:1020509305868

Bonkowski M (2019) Microcosm Approaches to Investigate Multitrophic Interactions between Microbial Communities in the Rhizosphere of Plants. In: Reinhardt D, Sharma AK (eds) Methods in Rhizosphere Biology Research. Springer Singapore, Singapore, pp 255-270

Bonkowski M, Clarholm M (2012) Stimulation of plant growth through interactions of bacteria and protozoa: testing the auxiliary microbial loop hypothesis. Acta Protozool 51:237–247. doi: 10.4467/16890027AP.12.019.0765

Bonkowski M, Dumack K, Fiore-Donno AM (2019) The Protists in soil - A token of untold eukaryotic diversity. In: van Elsas JD, Trevors JT, Soares Rosado A, Nannipieri P (eds) Modern Soil Microbiology (3rd edition). Boca Raton, USA, CRC Press, pp 125-140

Bonkowski M, Griffiths B, Scrimgeour C (2000) Substrate heterogeneity and microfauna in soil organic ‘ hotspots ’ as determinants of nitrogen capture and growth of ryegrass. Applied Soil Ecology 14:37–53

Brown DH, Ferris H, Fu S, Plant R (2004) Modeling direct positive feedback between predators and prey. Theor Popul Biol 65:143–152. doi: 10.1016/j.tpb.2003.09.004

Bukovská P, Rozmoš M, Kotianová M, et al (2021) Arbuscular mycorrhiza mediates efficient recycling from soil to plants of nitrogen bound in chitin. Front Microbiol 12:574060. doi: 10.3389/fmicb.2021.574060

Caporaso JG, Lauber CL, Walters WA, et al (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. PNAS 108:4516–4522. doi: 10.1073/pnas.1000080107

Clarholm M (1985) Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. Soil Biol Biochem 17:181–187. doi: 10.1016/0038-0717(85)90113-0

Fiore-donno AM, Richter-Heitmann T, Bonkowski M (2020) Contrasting responses of protistan plant parasites and phagotrophs to ecosystems, land management and soil properties. Front Microbiol 11:1–33. doi: 10.3389/fmicb.2020.01823

Fiore-Donno AM, Richter-Heitmann T, Degrune F, et al (2019) Functional traits and spatio-temporal structure of a major group of soil protists (rhizaria: Cercozoa) in a temperate grassland. Front Microbiol 10:. doi: 10.3389/fmicb.2019.01332

Flues S, Bass D, Bonkowski M (2017) Grazing of leaf-associated Cercomonads (Protists: Rhizaria: Cercozoa) structures bacterial community composition and function. Environ Microbiol 19:3297–3309. doi: 10.1111/1462-2920.13824

Gao Z, Karlsson I, Geisen S, et al (2018) Protists: puppet masters of the rhizosphere microbiome. Trends Plant Sci 24:165–176. doi: 10.1016/j.tplants.2018.10.011

Glücksman E, Bell T, Griffiths RI, Bass D (2010) Closely related protist strains have different grazing impacts on natural bacterial communities. Environ Microbiol 12:3105–3113. doi: 10.1111/j.1462-2920.2010.02283.x

Griffiths BS (1986) Mineralization of nitrogen and phosphorus by mixed cultures of the ciliate protozoan Colpoda steinii, the nematode Rhabditis SP. and the bacterium *Pseudomonas fluorescens*. Soil Biol Biochem 18:637–641. doi: 10.1016/0038-0717(86)90087-8

Griffiths BS, Bonkowski M, Roy J, Ritz K (2001) Functional stability , substrate utilisation and biological indicators of soils following environmental impacts. 16:49–61

Herdler S, Kreuzer K, Scheu S, Bonkowski M (2008) Interactions between arbuscular mycorrhizal fungi (Glomus intraradices, Glomeromycota) and amoebae (Acanthamoeba castellanii, Protozoa) in the rhizosphere of rice (Oryza sativa). Soil Biol Biochem 40:660–668. doi: 10.1016/j.soilbio.2007.09.026

Hünninghaus M, Koller R, Kramer S, et al (2017) Changes in bacterial community composition and soil respiration indicate rapid successions of protist grazers during mineralization of maize crop residues. Pedobiologia - International Journal of Soil Biology 62:1–8. doi: 10.1016/j.pedobi.2017.03.002

Huws SA, McBain AJ, Gilbert P (2005) Protozoan grazing and its impact upon population dynamics in biofilm communities. J Appl Microbiol 98:238–244. doi: 10.1111/j.1365-2672.2004.02449.x

Jousset A (2017) Application of protists to improve plant growth in sustainable agriculture. In: Rhizotrophs: plant growth promotion to bioremediation. Springer, Singapore, pp 263–273

Jousset A, Bonkowski M (2010) The model predator *Acanthamoeba castellanii* induces the production of 2, 4, DAPG by the biocontrol strain *Pseudomonas fluorescens* Q2-87. Soil Biol Biochem 42:1647–1649. doi: 10.1016/j.soilbio.2010.05.018

Jousset A, Rochat L, Péchy-Tarr M, et al (2009) Predators promote defence of rhizosphere bacterial populations by selective feeding on non-toxic cheaters. ISME J 3:666–674. doi: 10.1038/ismej.2009.26

Jousset A, Rochat L, Scheu S, et al (2010) Predator-prey chemical warfare determines the expression of biocontrol genes by rhizosphere-associated *Pseudomonas fluorescens*. Appl Environ Microbiol 76:5263–5268. doi: 10.1128/aem.02941-09

Jürgens K, Matz C (2002) Predation as a shaping force for the phenotypic and genotypic composition of planktonic bacteria

Koller R, Rodriguez A, Robin C, et al (2013) Protozoa enhance foraging efficiency of arbuscular mycorrhizal fungi for mineral nitrogen from organic matter in soil to the benefit of host plants. New Phytologist 199:203–211. doi: 10.1111/nph.12249

Kreuzer K, Adamczyk J, Iijima M, et al (2006) Grazing of a common species of soil protozoa (*Acanthamoeba castellanii*) affects rhizosphere bacterial community composition and root architecture of rice (Oryza sativa L.). Soil Biol Biochem 38:1665–1672. doi: 10.1016/j.soilbio.2005.11.027

Kuikman PJ, Van Veen JA (1989) The impact of protozoa on the availability of bacterial nitrogen to plants*. Biol Fertil Soils 8:13–18. doi: 10.1007/BF00260510

Liu C, Wang Y, Zhou Z, et al (2024) Protist predation promotes antimicrobial resistance spread through antagonistic microbiome interactions. ISME J 18:. doi: 10.1093/ismejo/wrae169

Loreau M (2004) Does functional redundancy exist? OIKOS 104:606–611. doi: 10.1111/j.0030-1299.2004.12685.x

Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J 17:10–12. doi: 10.14806/ej.17.1.200

Matz C, Bergfeld T, Rice SA, Kjelleberg S (2004) Microcolonies, quorum sensing and cytotoxicity determine the survival of *Pseudomonas aeruginosa* biofilms exposed to protozoan grazing. Environ Microbiol 6:218–226. doi: 10.1111/j.1462-2920.2004.00556.x

Oksanen J, Blanchet F G, Friendly M, et al (2023) *vegan: Community Ecology Package* (R package version 2.6-6). https://CRAN.R-project.org/package=vegan

Oliverio AM, Geisen S, Delgado-baquerizo M, et al (2020) The global-scale distributions of soil protists and their contributions to belowground systems. Science Advanced 6:eaax8787. doi: 10.1126/sciadv.aax8787

Pfandl K, Posch T, Boenigk J (2004) Unexpected effects of prey dimensions and morphologies on the size selective feeding by two bacterivorous flagellates (Ochromonas sp. and Spumella sp.). Journal of Eukaryotic Microbiology 51:626–633. doi: 10.1111/j.1550-7408.2004.tb00596.x

Pinheiro J, Bates D, DebRoy S, Sarkar D and R Core Team (2020) *nlme*: Linear and nonlinear mixed effects models. R package Version 3.1-149. https://CRAN.R-project.org/package=nlme

Posch T, Jezbera J, Vrba J, et al (2001) Size selective feeding in Cyclidium glaucoma (Ciliophora, Scuticociliatida) and its effects on bacterial community structure: A study from a continuous cultivation system. Microb Ecol 42:217–227. doi: 10.1007/s002480000114

Posch T, Šimek K, Vrba J, et al (1999) Predator-induced changes of bacterial size-structure and productivity studied on an experimental microbial community. Aquatic Microbial Ecology 18:235–246. doi: 10.3354/ame018235

Rosenberg K, Bertaux J, Krome K, et al (2009) Soil amoebae rapidly change bacterial community composition in the rhizosphere of Arabidopsis thaliana. ISME J 3:675–684. doi: 10.1038/ismej.2009.11

Rüger L, Feng K, Chen Y, et al (2023) Responses of root architecture and the rhizosphere microbiome assembly of maize (*Zea mays* L.) to a soil texture gradient. Soil Biol Biochem 181:. doi: 10.1016/j.soilbio.2023.109026

Rüger L, Feng K, Dumack K, et al (2021) Assembly patterns of the rhizosphere microbiome along the longitudinal root axis of maize (*Zea mays* L.). Front Microbiol 12:1–14. doi: 10.3389/fmicb.2021.614501

Saleem M, Fetzer I, Dormann CF, et al (2012) Predator richness increases the effect of prey diversity on prey yield. Nat Commun 3:. doi: 10.1038/ncomms2287

Saleem M, Fetzer I, Harms H, Chatzinotas A (2013) Diversity of protists and bacteria determines predation performance and stability. ISME J 7:1912–1921. doi: 10.1038/ismej.2013.95

Saleem M, Hu J, Jousset A (2019) More than the sum of its parts: microbiome biodiversity as a driver of plant growth and soil health. The Annual Review of Ecology, Evoltion, and Systematics 50:6.1-6.24. doi: 10.1146/annurev-ecolsys-110617-062605

Schmid B, Baruffol M, Wang Z, Niklaus PA (2017) A guide to analyzing biodiversity experiments. Journal of Plant Ecology 10:91–110. doi: 10.1093/jpe/rtw107

Sherr BF, Sherr EB, McDaniel J (1992) Effect of protistan grazing on the frequency of dividing cells in bacterioplankton assemblages. Appl Environ Microbiol 2381–2385. doi: 10.1128/aem.58.8.2381-2385.1992

Šimek K, Vrba J, Pernthaler J, et al (1997) Morphological and Compositional Shifts in an Experimental Bacterial Community Influenced by Protists with Contrasting Feeding Modes. Appl Environ Microbiol 63:587–595. doi: 10.1128/aem.63.2.587-595.1997

Song C, Mazzola M, Cheng X, et al (2015) Molecular and chemical dialogues in bacteria-protozoa interactions. Nature Publishing Group 1–13. doi: 10.1038/srep12837

Taerum SJ, Micciulla J, Corso G, et al (2022) 18S rRNA gene amplicon sequencing combined with culture-based surveys of maize rhizosphere protists reveal dominant, plant-enriched and culturable community members. Environ Microbiol Rep 14:110–118. doi: 10.1111/1758-2229.13038

Větrovský T, Baldrian P, Morais D (2018) SEED 2: a user-friendly platform for amplicon high-throughput sequencing data analyses. Bioinformatics 34:2292–2294. doi: 10.1093/bioinformatics/bty071

Vetterlein D, Lippold E, Schreiter S, et al (2021) Experimental platforms for the investigation of spatiotemporal patterns in the rhizosphere—Laboratory and field scale. Journal of Plant Nutrition and Soil Science 184:35–50. doi: 10.1002/jpln.202000079

Wickham H (2016) ggplot2: elegant graphic for data analysis. New York, USA: Springer-Verlag.

Wickham H, François R, Henry L, Müller K (2018) dplyr: *A grammar of data manipulation.* R Package Version 1.0.6. https://CRAN.R-project.org/package=dplyr.

Wickham H, Vaughan D, Girlich M (2024). *tidyr: Tidy Messy Data*. R package version 1.3.1, https://github.com/tidyverse/tidyr, https://tidyr.tidyverse.org

Xiong W, Song Y, Yang K, et al (2020) Rhizosphere protists are key determinants of plant health. Microbiome 827:1–9. doi: 10.1186/s40168-020-00799-9

Supplementary material

Table S1 Results of pairwise PERMANOVA (adonis) analyses showing differences in bacterial beta diversity between control and protist monoculture treatments, as well as among individual protist monoculture treatments. The table includes the specific comparisons, F-values, degrees of freedom (df), R-squared values, and p-values. Comparisons with p-values below 0.05 were considered statistically significant.

Comparison	**F-value**	**df**	**R^2**	**p-value**
CTRL_vs_F1	3.693765	1 _ 9	0.29099	**0.001**
CTRL_vs_F2	5.089512	1 _ 9	0.361227	**0.006**

CTRL_vs_F3	6.355991	1 _ 9	0.41391	**0.003**
CTRL_vs_A2	6.259815	1 _ 9	0.410216	**0.002**
CTRL_vs_A3	2.781261	1 _ 9	0.236075	**0.004**
CTRL_vs_F4	7.243054	1 _ 9	0.445917	**0.006**
CTRL_vs_ALL	20.32739	1 _ 13	0.60993	**0.001**
CTRL_vs_A4	2.406447	1 _ 8	0.231246	**0.021**
CTRL_vs_A1	5.516169	1 _ 9	0.380002	**0.007**
F1_vs_F2	4.637498	1 _ 6	0.435958	**0.03**
F1_vs_F3	6.072864	1 _ 6	0.503018	**0.03**
F1_vs_A2	4.605936	1 _ 6	0.434279	**0.032**
F1_vs_A3	4.025206	1 _ 6	0.401509	**0.035**
F1_vs_F4	7.618665	1 _ 6	0.559428	**0.029**
F1_vs_ALL	12.343	1 _ 10	0.552432	**0.001**
F1_vs_A4	3.734153	1 _ 5	0.427535	**0.02**
F1_vs_A1	5.365036	1 _ 6	0.472065	**0.025**
F2_vs_F3	3.859459	1 _ 6	0.391447	**0.029**
F2_vs_A2	5.108793	1 _ 6	0.459887	**0.02**
F2_vs_A3	4.64425	1 _ 6	0.436315	**0.025**
F2_vs_F4	9.735185	1 _ 6	0.618689	**0.037**
F2_vs_ALL	12.97047	1 _ 10	0.564659	**0.005**
F2_vs_A4	4.073292	1 _ 5	0.448932	**0.028**
F2_vs_A1	6.246755	1 _ 6	0.510074	**0.024**
F3_vs_A2	4.842374	1 _ 6	0.446616	**0.031**
F3_vs_A3	6.465701	1 _ 6	0.518679	**0.043**
F3_vs_F4	8.00501	1 _ 6	0.571582	**0.032**
F3_vs_ALL	8.706046	1 _ 10	0.465413	**0.004**
F3_vs_A4	4.530091	1 _ 5	0.475346	**0.025**
F3_vs_A1	5.918478	1 _ 6	0.49658	**0.027**
A2_vs_A3	4.965575	1 _ 6	0.452833	**0.036**
A2_vs_F4	6.253147	1 _ 6	0.51033	**0.033**
A2_vs_ALL	9.304348	1 _ 10	0.481982	**0.004**
A2_vs_A4	3.203367	1 _ 5	0.390494	**0.033**
A2_vs_A1	5.073484	1 _ 6	0.458165	**0.032**
A3_vs_F4	10.61941	1 _ 6	0.638976	**0.03**
A3_vs_ALL	16.62232	1 _ 10	0.624375	**0.007**
A3_vs_A4	3.750239	1 _ 5	0.428587	**0.02**
A3_vs_A1	5.86246	1 _ 6	0.494203	**0.023**
F4_vs_ALL	10.1465	1 _ 10	0.503636	**0.001**
F4_vs_A4	6.567223	1 _ 5	0.567744	**0.03**
F4_vs_A1	8.554775	1 _ 6	0.587764	**0.022**
ALL_vs_A4	10.78527	1 _ 9	0.545116	**0.004**
ALL_vs_A1	13.42186	1 _ 10	0.573048	**0.002**
A4_vs_A1	4.146061	1 _ 5	0.453317	**0.028**

Table S2 Results of contrast analysis comparing shoot and root dry weight, as well as shoot and root C:N ratios, of maize plants treated with protist monocultures versus control plants without protists. The table specifies the comparisons made, along with the corresponding estimate, standard error (SE), degrees of freedom (df), t-ratio, p-value, response variable, and the unit of the response variable.

Contrast	Estimate	SE	df	t-ratio	p-value	Response variable	Unit
CTRL.vs.A1	1.750	49.330	29	0.035	0.972	Shoot dw	mg
CTRL.vs.A2	118.500	49.330	29	2.402	**0.023**	Shoot dw	mg
CTRL.vs.A3	24.750	49.330	29	0.502	0.620	Shoot dw	mg
CTRL.vs.A4	-6.500	49.330	29	-0.132	0.896	Shoot dw	mg
CTRL.vs.F1	4.750	49.330	29	0.096	0.924	Shoot dw	mg
CTRL.vs.F2	76.250	49.330	29	1.546	0.133	Shoot dw	mg
CTRL.vs.F3	-20.250	49.330	29	-0.411	0.684	Shoot dw	mg
CTRL.vs.F4	9.750	49.330	29	0.198	0.845	Shoot dw	mg
CTRL.vs.A1	30.250	58.465	29	0.517	0.609	Root dw	mg
CTRL.vs.A2	80.750	58.465	29	1.381	0.178	Root dw	mg
CTRL.vs.A3	-9.500	58.465	29	-0.162	0.872	Root dw	mg
CTRL.vs.A4	-64.750	58.465	29	-1.108	0.277	Root dw	mg
CTRL.vs.F1	-41.000	58.465	29	-0.701	0.489	Root dw	mg
CTRL.vs.F2	72.750	58.465	29	1.244	0.223	Root dw	mg
CTRL.vs.F3	-43.000	58.465	29	-0.735	0.468	Root dw	mg
CTRL.vs.F4	-11.750	58.465	29	-0.201	0.842	Root dw	mg
CTRL.vs.A1	0.172	0.153	29	1.122	0.271	Shoot C:N	ratio
CTRL.vs.A2	-0.058	0.153	29	-0.380	0.707	Shoot C:N	ratio
CTRL.vs.A3	0.084	0.153	29	0.547	0.589	Shoot C:N	ratio
CTRL.vs.A4	0.243	0.153	29	1.586	0.124	Shoot C:N	ratio
CTRL.vs.F1	0.231	0.153	29	1.508	0.142	Shoot C:N	ratio
CTRL.vs.F2	0.002	0.153	29	0.013	0.989	Shoot C:N	ratio
CTRL.vs.F3	0.243	0.153	29	1.587	0.123	Shoot C:N	ratio
CTRL.vs.F4	0.140	0.153	29	0.911	0.370	Shoot C:N	ratio
CTRL.vs.A1	-0.260	0.143	29	-1.822	0.079	Root C:N	ratio
CTRL.vs.A2	-0.222	0.143	29	-1.558	0.130	Root C:N	ratio
CTRL.vs.A3	-0.184	0.143	29	-1.293	0.206	Root C:N	ratio
CTRL.vs.A4	-0.278	0.143	29	-1.951	0.061	Root C:N	ratio
CTRL.vs.F1	-0.122	0.143	29	-0.855	0.400	Root C:N	ratio
CTRL.vs.F2	-0.242	0.143	29	-1.698	0.100	Root C:N	ratio
CTRL.vs.F3	-0.259	0.143	29	-1.819	0.079	Root C:N	ratio
CTRL.vs.F4	-0.238	0.143	29	-1.671	0.106	Root C:N	ratio

General Discussion

In this thesis, the role of protists in microbial community assembly and their functional contributions to the rhizosphere processes of *Zea mays* were investigated. As outlined in the introduction, the rhizosphere is a dynamic and complex habitat where plants and microbes interact, microbes compete and prey on each other, and all processes are shaped by local soil conditions. Additionally, the root matures along the longitudinal axis, resulting in distinct regions with varying compositions of rhizodeposits. To date, research investigating microbial community assembly along the longitudinal root axis, particularly in natural soil, remains limited. One reason for this knowledge gap is the challenge of obtaining location-specific samples from plants grown in natural soil. We developed a method to collect root region-specific samples for microbiome analysis, enabling us to characterize spatially distinct microbial communities within the rhizosphere. Incorporating bacterivorous protists into our analyses added an often-overlooked layer of complexity, providing a more comprehensive understanding of microbial community assembly processes. Through the combination of high spatial resolution sampling and the incorporation of protists, this work contributes to elucidating the mechanisms underlying rhizosphere self-organization.

Predation as driver of the rhizosphere microbial community assembly

To understand the fundamental characteristics of rhizosphere microbial assembly, two hypotheses were initially tested: (H1) that microbial assembly occurs not only radially, as previously demonstrated (Lundberg et al., 2012; Edwards et al., 2015; Reinhold-Hurek et al., 2015; Stephane Hacquard et al., 2015), but also along the longitudinal root axis and (H2) that protists play a significant role in this process. We compared bacterial and protist communities across different root regions and bulk soil, revealing a shift in beta diversity from bulk soil to the root tip, and from the root tip along the root axis towards older root regions. Co-occurrence network analysis suggested that mechanisms driving microbial assembly vary along the root axis. In young root regions, we observed high variation in microbial diversity and few correlations between bacterial and protist taxa (Chapter I, Rüger et al., 2021). This suggests that selection was primarily random, possibly driven by priority effects, where early-colonizing species establish dominance through niche preemption and niche modification (Fukami, 2015). Due to the high amount of carbon-rich rhizodeposition in the young root region, fast-growing copiotrophs that first encountered the growing root may have been favored in this area. In contrast, in older root regions microbial communities exhibited less variation, decreased diversity, and a higher number of bacteria-protist associations, suggesting a more significant role of deterministic selection mechanisms. Competition for less abundant resources in these regions likely favored better competitors, i.e., more specialized taxa (Nemergut et al., 2013). In the older root, the growth limitation of prokaryotes likely led to an increasingly important role of selective predation by protists (Matz and Kjelleberg, 2005; Jousset, 2012). Our data suggest that plants do not shape the prokaryote microbiome alone. Instead, interactions among microorganisms from different trophic levels also play a significant role, particularly in root regions with limited resource availability. Selectively feeding bacterivorous protists can exert top-down control on bacteria, specifically reducing certain taxa (Rosenberg et al., 2009; Glücksman et al., 2010; Flues et al., 2017), a factor often overlooked in rhizosphere research.

We consistently found that protist community assembly patterns were comparable to those of prokaryotes, and network analyses found strong co-correlations between protists and prokaryotes, indicating a dominant role of protists in shaping bacterial communities (Rüger et al., 2021, 2023b, 2023a). In the context of a food web, the release of rhizodeposits may favor specific bacterial taxa. In turn, their increased abundance could influence the community composition of selectively feeding protists at the next trophic level, leading to positive co-occurrences. Conversely, protists can exert top-down control by selectively feeding on bacteria, potentially resulting in negative co-occurrences and altering the net effect of plant metabolites on bacterial communities. Protists may thus play a critical role in stabilizing bacterial rhizosphere microbiomes (Dumack et al., 2020).

Recently, there is strong interest in trait-based ecology, as species traits are under direct selection pressure and influence the fitness of species. Particularly in taxonomically diverse communities, species traits may better reflect changes in community responses than taxonomy (Bui et al., 2020). Recent efforts have been made to define a set of functional traits common to all protists (Giachello et al., 2023). However, it is questionable whether traits as broad as “consumer”, “parasite” or “autotroph” provide meaningful insights for a better functional understanding of soil processes (Oliverio et al., 2020; Nguyen et al., 2021; Singer et al., 2021; Mazel et al., 2022). Even seemingly straightforward effect traits like fungivory or bacterivory can vary significantly between protist taxa, leading to profoundly different functional outcomes. Fungivory may be restricted to feeding on fungal spores, single-celled yeasts or fungal filaments (Chakraborty et al., 1983; Petz et al., 1985; Dumack et al., 2016, 2019b). Similarly, bacterivory encompasses diverse feeding modes including grasping or direct interception of single bacterial cells, suspension and filter feeding, or surface grazing of bacterial colonies in biofilms (Boenigk and Arndt, 2000, 2002, Heaton et al., 2001; Parry, 2004). Even closely related taxa can exhibit distinct selectivity, resulting in varied functional consequences (Rosenberg et al., 2009; Glücksman et al., 2010; Flues et al., 2017). Therefore, we conducted an experiment combining bacterivores from very different phylogenetic origins, which could be categorized into two morphological classes: flagellates and amoebae. The rationale was that if feeding modes differ, they should complement each other, and greater protist species richness would consequently lead to more efficient exploitation of bacterial prey (H7). As a result, grazing by a more diverse protist community could influence plant growth through enhanced nitrogen remobilization from the consumed bacterial biomass in the soil (Clarholm, 1985; Kuikman and Van Veen, 1989; Kuikman et al., 1990, 1991) (H8). To assess this, we grew *Zea mays* plants in sterilized soil, inoculated with a natural bacterial community and varying numbers of protist species. We found evidence that increasing protist diversity enhanced the exploitation of a broader range of prey. This had implications for bacteria community composition, reflected by a more balanced capacity of bacterial communities to metabolize diverse carbon sources. Surprisingly, however, while the shoot carbon content increased, we found no evidence of enhanced nitrogen remobilization, which was anticipated to result in increased nitrogen uptake by plants (Chapter IV). Complementary feeding by diverse protist communities may result in a balanced reduction of various bacterial species, preventing r-strategists from dominating the community and allowing subdominant rare taxa to grow (Sherr et al., 1992). This could promote greater bacterial diversity and the expansion of taxa with functionally redundant traits, ultimately enhancing the rhizosphere functionality and ensuring its stability. The resulting reduction in costly competition among bacteria may lead to investing more into plant-beneficial functions (Rahman et al., 2023)

The plant’s role in shaping microbial communities along roots

Current research suggests that plants selectively recruit a subset or core microbiome from the bulk soil microbiome, with the composition influenced by plant species or genotype (Bulgarelli et al., 2012; Lundberg et al., 2012; Simonin et al., 2019). The establishment of these rhizosphere microbiomes is largely attributed to factors such as carbon-rich rhizodeposition, signaling, and defense mechanisms (Oger et al., 2004; Hartmann and Schmid, 2009; Hawes et al., 2012), all of which can exhibit spatial variation along the root axis. Additionally, as root cells continuously mature and the root tip progresses into the soil, it is likely that roots influence microbial communities in a dynamic and spatially divergent manner. However, only a limited number of studies have investigated the specific root regions where microbial recruitment occurs, and the plant-derived mechanisms underlying root region-specific microbial selection remain largely unclear. Nevertheless, variations in bacterial abundance along the root in both artificial settings (Massalha et al., 2017) and natural soil (Semenov et al., 1999) support the concept of root region-specific microbial community establishment.

By manipulating root organs likely involved in plant–microbe interactions, specifically root caps and root hairs, we aimed to identify drivers of microbial assembly and recruitment (H3, H4). We compared community assembly patterns of maize plants that either had their root caps removed, or lacked root hairs, using root hairless *rth3* mutant plants (Chapter II, Rüger et al., 2023b). Manipulation of the root cap influenced microbial communities even in older root regions and affected the next higher trophic level: protists. This demonstrates that plants significantly impact microbiome assembly locally with implications for its progression during root maturation. Root border cells at the root tip may play a key role. They secrete large amounts of mucilage and antimicrobial proteins and peptides, protecting the delicate apical root meristem from microbial colonization (Driouich et al., 2013, 2021). In a recent study, Charura et al. (2024) demonstrated that delayed programmed root cap cell death in the *Arabidopsis smb-3* mutant led to abnormal colonization patterns of the beneficial fungus *Serendipita indica*, including hypercolonization along the root. Even the meristematic zone was colonized, whereas wild-type plants exhibited minimal to no microbial presence at the root tip. The enhanced colonization at the root tip of *smb-3* mutant plants likely influenced fungal colonization along the root, with hypercolonization delaying the beneficial effects of *Serendipita indica*. Their findings align with our observation that manipulation of the root cap impact colonization patterns with ripple effects in older root regions. However, their artificial setting, focusing on a single fungal species, may not reflect natural environments with complex microbial networks. Additionally, accumulated dead cells in older *smb-3* root regions could locally impact fungal abundance. Another study identified the root tip as a key determinant in shaping the rhizosphere microbiome and the ability to colonize the root tip a crucial trait of "rhizosphere competent" bacteria (Lugtenberg et al., 2001). In accordance, Dupuy and Silk (2016) concluded from their models that the successful attachment to the root tip gives bacteria an advantage in proliferating along the root as they initially get access to exudate carbon.

In our study, manipulation of root caps, resulted in the upregulation of genes involved in defense, especially within the phenylpropanoid-flavonoid biosynthesis pathway. This upregulation suggests that the plant may have compensated for the protective function of the missing cap. At the time of sampling (six days after decapping), the root had likely already replaced the missing root cap. Replacement usually takes 3-4 days in *Zea mays* (Barlow, 1974).

However, any potential initial change in colonization at the root tip might have progressed during root maturation, requiring ongoing defense efforts from the plant.

In conclusion, the root cap along with its associated defense mechanisms and programmed cell death, plays a critical role in protecting sensitive young root tissues and the meristematic zone. Consequently, these mechanisms impact the ongoing microbial assembly, ultimately playing a role in shaping the microbial community composition along the longitudinal root axis. The defense responses at the root tip might be relatively nonspecific, primarily serving to prevent overgrowth that could lead to detrimental effects, rather than significantly contributing to the recruitment of specific microbial taxa.

We did not observe an overall effect of the absence of root hairs on microbial communities. Despite antimicrobial compounds like the coumarin scopoletin have been reported to be increasingly secreted in the root hair zone (Robe et al., 2021). Scopoletin inhibits the fungal pathogens *Fusarium oxysporum* and *Verticillium dahliae*, while plant-beneficial rhizobacteria *Pseudomonas simiae* WCS417 and *Pseudomonas capeferrum* WCS35 are resistant to its antimicrobial properties (Stringlis et al., 2018; Robe et al., 2021). Scopoletin may primarily target fungi rather than bacteria or protists. Additionally, *rth3* mutant plants may have compensated for the absence of root hairs by releasing higher levels of secondary metabolites from epidermal cells other than trichoblasts.

Generally, little is known about the mechanisms underlying the active selection of core microbiota by plants. If plants actively recruit microbial taxa, then gene expression in roots should correlate to taxa in the rhizosphere microbiome. Network analysis integrating root transcriptomic data and microbial community data indeed revealed relationships between plant gene expression and the presence of specific bacterial and protist taxa. Notably, a member of the Sandonidae family (Rhizaria: Cercozoa) showed a negative association with *ethylene-responsive transcription factor 55* (*ERF55*) involved in stress response regulation, and *Cercomonas* (Rhizaria: Cercozoa) was negatively associated with *pathogenesis-related protein 10* (*PR10*), a gene implicated in effector-triggered immunity (ETI). However, the study did not reveal whether these effects are indirect, mediated by the regulatory impact of protist feeding on bacteria, or stem from direct protist-plant interactions. Overall, our findings strongly suggest cross-communication between plants and microbes across different trophic levels in the rhizosphere.

However, metabolite export transporters, which have been sparsely described, likely play a central role in selection of microbiota (Sasse et al., 2018). Investigating transporters is challenging, as it requires root-region-specific plant transcriptomic data. To evaluate the effects specific transporters may have on the microbial community and its assembly along the root, additionally, plant genetic engineering techniques can be used. Interestingly, a recent study by Loo et al. (2024) found that the sugar uniporters SWEET2, SWEET4 and SWEET12 exhibited spatially distinct accumulation patterns, as demonstrated using SWEET-GUS translational fusion plants. These patterns were dependent on the presence of microorganisms (heat-killed bacteria were added to an agar-based "artificial soil"). Loss of function mutants *sweet2*, *sweet4* and *sweet11;12* showed altered spatial abundance of metabolites, and the colonization pattern of a 60-member synthetic microbiota community in the endosphere was impaired. The study suggests that sugar efflux, regulated spatially by SWEET transporters, may enable plants to actively influence microbiomes at specific locations along the root. Although so far only been demonstrated for endophytic bacteria, it is possible that a greater number of such mechanisms exist than are currently recognized.

Placing studies like those of Charura et al. (2024), Loo et al. (2024) and our findings (Rüger et al., 2021, 2023b) in context can enhance our understanding of the interplay between various mechanisms along the longitudinal root axis. Examining not only the effects of plants on a global level but also differentiating between root sections provides insight into the distinct mechanisms at play. This differentiation highlights that roots respond with high spatial specificity in defense against or interaction with microbes, with implications for the entire root system. In the case of SWEET transporters, the findings suggest that plants can even actively respond in a location-specific manner, influencing bacterial growth. This strategy is likely to benefit the plant, as it would not persist evolutionarily without providing adaptive advantages. However, further research is needed to investigate the active release of specific compounds by plants, including their spatial distribution along the root, as this could offer deeper insights into how plants selectively recruit soil microbes and influence their colonization patterns in a targeted manner.

We further demonstrated that a natural soil microbial community exhibited different C-thresholds for growth activation, depending on the substrate identity (Chapter II, Niedeggen et al., 2024). We tested the mineralization of several C compounds typically found in root exudates (glucose, sucrose, arabinose, citric acid, oxalic acid, and aspartate), as well as exudates and mucilage from *Zea mays*. Interestingly, at high concentrations, mucilage induced an initial lag phase, followed by two temporally shifted bacterial growth peaks. Microbial taxa may utilize different carbon sources at different times, with some groups consuming certain substrates later. This delayed utilization could be further influenced by the presence of compounds that inhibit the microbes' ability to process these substrates. Plants may produce antibiotics and microbial inhibitors to prevent the rapid degradation of substances crucial for their function (Hawes et al., 2000; Bais et al., 2006). We also observed distinct bacterial growth patterns in response to exudates from early- and late-season maize plants, with late-season exudates showing a similar C-threshold as sugars. The first aligns with findings by Santangeli et al. (2024), who reported that late-season exudates contain over 50% soluble carbohydrates. Differences in microbial utilization of the tested compounds further support the idea that the composition of rhizodeposits effects microbial growth patterns. As the distance from the root surface increases, the concentration of C-compounds released by plants decreases rapidly. When this concentration falls below a certain threshold, bacterial growth ceases. The exact value of this threshold concentration appears to depend on the specific composition of the rhizodeposits.

Can plants alone maintain a beneficial rhizosphere microbiome?

Rhizosphere microorganisms influence each other through a complex network of competition and predation, resulting in microbial assembly along the root which continuously progresses into the soil. Additionally, the plant shapes the spatial patterning of the rhizosphere microbiome through the localized passive release of metabolites, and presumably through active release in response to prevailing conditions. However, plant defense mechanisms are often not targeted exclusively at a single pathogen but instead affect a range of microbes, leading to their suppression. The plant’s immune system may respond more specifically to endospheric microbes i.e., through Pattern-Triggered Immunity (PTI) and Effector-Triggered Immunity (ETI) (Stephane Hacquard et al., 2017; Ngou et al., 2022). Interestingly, beneficial organisms can also activate these same immune pathways, eventually priming the plant's immune system (Pieterse et al., 2014). Others suggested that plants release a range of specific antimicrobial or stimulatory compounds to select for specific microbes (Hartmann and Schmid, 2009).

However, when introducing a bacterial synthetic community (SynCom) in a rhizotron experiment, Loo et al. (2024) found that spatial patterning along the root, similar to that observed in natural communities, was established in the endosphere but not in the rhizosphere. This, together with the observed interrelation between bacterial communities and their predators (Chapter IV and Rüger et al., 2021), suggests that the plant alone may not be sufficient to shape the bacterial community in the ectorhizosphere. It raises the question of whether the plant holobiont can be sustained in the absence of selective top-down control exerted by predation, particularly considering that microbial predation evolved prior to the establishment of microbe-plant interactions.

In natural soil environments, bacteria are constantly exposed to predation by nematodes and protists, which are the primary predators of bacteria in soil (Yeates et al., 1993; Trap et al., 2016). Selective feeding behavior of predators can shape bacterial communities, influencing their function by favoring specific traits, as demonstrated in a recent study Liu et al. (2024). Using metagenomics in combination with controlled experiments, they showed that protist predation induces the enrichment of bacterial antibiotic resistance genes. Antibiotic-producing bacteria maintained a high relative abundance under predation pressure. Bacteria produce antibiotics as a defense mechanism, which has been shown to inhibit the growth of protists (Matz et al., 2004; Mazzola et al., 2009). However, to survive, bacteria must also protect themselves from these antibiotics, likely driving the observed increase in antibiotic resistance in the presence of protists (Nguyen et al., 2023). Additionally, bacterial predators and bacteriophages play a role in the top-down control of soil microbiota, potentially increasing selective pressure on microbial communities (Lueders et al., 2006; Kuzyakov and Mason-Jones, 2018). For example, various members of myxobacteria are known to be micropredators with broad prey spectra (Morgan et al., 2010). By using metatranscriptomics, Petters et al. (2021) investigated the position of myxobacteria in the soil microbial food web. They found a consistently high abundance of the potentially bacterivorous myxobacteria across all 11 tested soils; in 10 of these, myxobacteria were more abundant than protists with less variability. Myxobacteria likely contribute to controlling bacterial abundances and by doing so, they occupy different ecological niches than protists. Their smaller cell size might enable them to penetrate soil pores that are inaccessible to protists, granting them access to additional prey. However, compared to protists, which feed phagotrophically, myxobacteria secrete promiscuously active lytic enzymes— a predation strategy that appears less selective overall but exerts selective pressure favoring distinct bacterial adaptations. Using ^{13}C-labelled biomass of *Pseudomonas putida* and *Arthrobacter globiformis*, (Zhang and Lueders, 2017) demonstrated that myxobacteria preferentially consumed C from *Pseudomonas putida*, while protists, particularly amoebae, consumed both but exhibited niche differentiation between the rhizosphere and bulk soil. This finding underscores niche partitioning between bacterial and protistan predators, further driven by soil compartments.

Viruses, particularly bacteriophages, play a crucial role in the top-down control of bacterial communities in aquatic environments. In seawater, it is estimated that approximately 20% of bacterial cells are lysed by viruses (Suttle, 2007). Similarly, in soil, viruses have a high potential to impact bacterial communities. This was demonstrated by Liu et al. (2023), showing that viral activity can significantly influence microbial community composition. The host specificity of viruses makes them particularly effective in targeting specific bacterial populations. For instance, a study by Wang et al. (2019) successfully tested bacteriophages as biocontrol agents against bacterial wilt disease caused by *Ralstonia solanacearum*, while leaving the native rhizosphere microbiota unaffected. The host specific infection, the ubiquitous occurrence and the high abundances of up to 10^{10} g^{-1} soil of viruses (Williamson et al., 2017), suggests that they significantly contribute to shaping soil microbial communities.

Predation exerts selective pressure on bacteria, shaping bacterial communities and driving evolutionary processes. However, microbes also evolve as they adapt to plants. (Li et al., 2021a) showed that in the rhizosphere of *Arabidopsis*, *Pseudomonas protegens* rapidly evolved into mutualists within six plant growth cycles. This adaptation was associated with enhanced competitiveness for root exudates, and improved tolerance to scopoletin. In turn, the plant exhibited reduced phytotoxicity, increased transcription of the MYB72 transcription factor which regulates scopoletin production in roots, and enhanced growth. In another study, (Li et al., 2021b) further demonstrated the dynamic nature of trait correlation networks of *Pseudomonas protegens*. Initially, two distinct modules were identified: one related to stress resistance and the other to resource use. The network rapidly restructured, forming new modules and losing the stress resistance module during adaptation to the plant.

In summary, plants can indeed respond locally to the presence of specific bacteria, and these responses might be particularly critical for endospheric plant-microbe interactions. They further drive evolutionary adaptation of rhizosphere microorganisms. Outside the roots, the constant selective pressure exerted by higher trophic-level organisms likely plays an additional significant role in maintaining rhizosphere microbiomes. Bacteria consequently face the challenge of adapting to a multitude of selective pressures exerted by plants, viruses, and predators. Friman and Buckling (2014) demonstrated that PNM phages limited the attenuating effect of the protist *Tetrahymena thermophila* on the virulence of *Pseudomonas aeruginosa*. This was likely due to the phages' impact on population dynamics and their role in introducing conflicting selection pressures. In another study Friman and Buckling (2013) demonstrated that *Pseudomonas fluorescens* diversifies into defense specialists when coevolved simultaneously with the BW25Φ2 phage and the protist *Tetrahymena thermophila*. Their findings revealed a fitness trade-off between defenses against these two enemies. Notably, their results indicated that strong pairwise coevolution can persist even within complex communities.

In the rhizosphere, numerous equilibria must be maintained to sustain a healthy plant and a functioning microbial community. Despite—or perhaps because of—its complexity and the multitude of interactions, the rhizosphere remains remarkably stable, typically supporting robust plant growth. This stability likely stems from the functional redundancy of the involved players and their capacity for rapid adaptation. It is a self-organized system, where countless interactions converge to maintain balance and resilience amidst ever-changing environmental and biological pressures.

Synthetic communities for assessing plant microbe interactions

Presumably, predation and competition play important roles in maintaining bacterial communities and their beneficial effects on plants. Hence, the question arises as to how meaningful it is to work with synthetic communities (SynComs) in this research field. SynComs, commonly used in the study of plant-microbe interactions, typically exclude higher trophic-level organisms, such as protists. These communities consist of a defined set of bacterial strains isolated from soil, each cultured separately before being combined and introduced into experimental settings. This approach has become a widely used method in the field (Marín et al., 2021). Compared to holistic approaches using culture-independent techniques, the use of SynComs provides greater certainty regarding the traits of individual members, enabling the analysis of causal relationships. However, the results of SynCom experiments may not fully capture the complex rhizosphere dynamics observed in natural environments. Bacterial SynComs are typically composed of 3 to 200 bacterial taxa, intended to simulate microbial diversity, which is estimated to include 2,000 to 50,000 bacterial taxa in one gram of soils (Schloss and Handelsman, 2006; Roesch et al., 2007), and consequently an even greater number of interactions among these species. Additionally, the cultivability of the used bacterial strains biases the selection of taxa by researchers strongly. Fifty-three bacterial phyla are widely acknowledged, but representatives from only 27 of these phyla have been successfully cultivated and described, with most of these phyla represented by only a few isolates (Coleman and Whitman, 2005). This raises the question of how realistic such approaches are. While the use of SynComs can be an efficient method for identifying mechanisms of plant-microbe interactions, it falls short in providing insights into microbial community dynamics. For instance, taxa that act as strong competitors in low-diversity environments may become weak competitors when required to share a specific niche.

The absence of higher trophic-level organisms, such as protists, overlooks the essential contributions of these organisms to plant health and performance. Protists increase nitrogen availability for plants through the microbial loop (Clarholm, 1985; Kuikman and Van Veen, 1989; Kuikman et al., 1990, 1991). Additionally selective feeding by protists can shift the composition of bacterial communities (Rosenberg et al., 2009; Trap et al., 2016; Flues et al., 2017). By doing so, protists alter the competitive dynamics among and within bacterial taxa in the rhizosphere, favoring those with defensive capabilities linked to pathogen suppression (Jousset et al., 2009). Since several protists have been shown to feed on plant pathogens, such as the fungus *Fusarium culmorum* (Geisen et al., 2016), they also hold potential for controlling the abundance of plant pathogens in soil.

The use of SynComs to study community dynamics primarily highlights the complexity of microbial interaction networks as small changes in the composition easily change the dominance of individual strains. While demonstrating how certain taxa may have advantages over others in artificial settings, this approach cannot fully reveal how these taxa behave in natural ecosystems.

Environmental influences – soil texture

Predator-prey and plant-microbe interactions are significantly influenced by habitat characteristics, such as soil texture, density, and the associated pore size distribution. Research indicates that bacteria residing in smaller pores are less susceptible to predation, as larger microbial predators are unable to access these confined spaces (Elliott et al., 1980; Rutherford and Juma, 1992b). Furthermore, soil texture plays a critical role in microbial mobility, affecting their ability to move towards or along plant roots (Wang et al., 2005). Our findings demonstrate that soil texture influences root architecture, with coarser soil (i.e., increased sand content) leading to a shorter primary root, which is compensated for by longer and more numerous lateral roots (Chapter III, Rüger et al., 2023a). This alteration in root architecture, combined with changes in soil texture, directly impacted the assembly of microbial communities within the rhizosphere. Additionally, shifts in the network topology of these microbial communities showed higher connectivity in sandy soils, likely due to larger pores facilitating interactions among a broader range of microbial taxa.

Additionally, soil texture and compaction can reduce gaseous diffusion by decreasing the volume and number of air-filled pores within the soil matrix. This restriction in diffusion can limit the movement of volatile organic compounds (VOCs), such as ethylene, and can thereby impair plant-microbe interactions. Ethylene, a key plant hormone, plays an essential role in regulating plant growth, development, and stress responses, contributing to stress tolerance (Sasidharan and Voesenek, 2015; Thao et al., 2015). Plant associated soil microorganisms often have the capacity to sense ethylene and to detect environmental stressors relevant to plant health (Carlos et al., 2016). In response, many of these microorganisms can modulate ethylene levels: some reduce ethylene by deaminating its precursor, 1-aminocyclopropane-1-carboxylate (ACC), promoting root elongation even under soil compaction (Glick et al., 1998; Glick, 2005), while others may increase ethylene levels by producing it themselves (Weingart and Völksch, 1997). These interactions suggest a co-evolution of ethylene signaling pathways between plants and their associated microbial communities (Ravanbakhsh et al., 2018). Additionally, ethylene has been shown to act as a fungistatic agent (Smith, 1973). Consequently, reduced ethylene concentrations in the soil around roots, due to compaction, could promote fungal growth.

Conclusively, reduced diffusion of VOCs due to soil texture and compaction likely affects signaling between plants and microbiota, with potential consequences for plant health and rhizosphere colonization. In our study (Rüger et al., 2023a), changes in soil texture may have led to impaired VOC diffusion, ultimately altering root selection for rhizosphere microbiota.

Engineering microbial communities

A key objective in understanding the complex processes underlying rhizosphere microbiome assembly is to foster beneficial interactions in the rhizosphere, which could ultimately enhance plant health and productivity. There is a need for studies that integratively examine the rhizosphere microbiome and the mechanisms essential for plant-microbe interactions, enabling prediction and management of microbiota (Philippot et al., 2013). This may also encompass top-down regulation through predation as well as the influence of environmental conditions.

Two primary approaches to manipulate the plant-associated microbiome have been discussed in the literature: first, through inoculation with a specific set of microorganisms, and second, through plant breeding (Wille et al., 2019). Bio-inoculants containing single or combined species applied via seed coating or inoculation of soil have already demonstrated high efficacy, significantly increasing yields by up to 50% (Tarafdar and Rao, 1997; Valverde et al., 2006; Chandra and Kumar, 2008; Hu et al., 2017). Constraints of such inoculants include their limited viability, as well as their effectiveness being influenced by abiotic conditions and potential incompatibility with the native soil microbiome. We demonstrated that different soil textures, for example, influence rhizosphere microbiome assembly (Rüger et al., 2023a). Additionally, previous studies, including our own (Chapter IV), indicate that microbial predators alone significantly impact bacterial community composition and structure (Rosenberg et al., 2009;

Glücksman et al., 2010; Flues et al., 2017), highlighting the challenges associated with intervening in complex plant-microbe interaction networks.

Jacquiod et al. (2022) emphasized that especially the stability of beneficial microbial communities is essential for their efficiency. The authors selectively extracted soil microbiomes from plants with either high or low leaf greenness as a proxy for plant fitness. After several plant generations, they could establish stable microbiomes with consistent effects on leaf greenness, either positive or negative, respectively. Functional redundancy may play a crucial role in maintaining community stability, as soil and the rhizosphere are dynamic systems influenced by a variety of abiotic and biotic factors (Bissett et al., 2013). The concept of functional redundancy refers to a situation where a group of organisms performs the same function at similar rates across a range of conditions. If high functional redundancy leads to stochastic filling of ecological niches, functional composition becomes more relevant than species composition. Consequently, functional traits should be considered in addition to species diversity when studying the rhizosphere microbiome.

The second approach, to engineer the rhizosphere microbiome through plant breeding, is based on the hypothesis that plants and their associated microorganisms, the plant holobiont, function as a single unit of selection and adaptation (Guerrero et al., 2013; Mesny et al., 2023). However, selection may not always occur at the holobiont level. Nonetheless, breeding strategies should also consider the capacity of the plant to influence microbiome composition with a gain in plant fitness. Previous breeding may have inadvertently led to the loss of traits essential for plant-microbe communication or defense by focusing only on yield or on two-way pathogen host interactions under artificial settings. Such losses can be mitigated by selecting for beneficial plant traits in living soil, ideally reflecting the target environment and representing reservoirs of genetic and ecological potential (Oyserman et al., 2018; Wille et al., 2019).

Closing remarks

Through the research presented in this thesis, we advance the understanding of microbial assembly in the rhizosphere at high spatial resolution, identifying and discussing various factors that influence assembly processes. These findings strongly support the concept of the rhizosphere as a self-organized system. A particular focus is placed on protists, which are critical predators of bacteria in soil, emphasizing their role in shaping microbial dynamics. This work brings us closer to predicting processes within soil ecosystems, which could ultimately pave the way for the development of sustainable and still high-yielding agricultural practices.

References

Akiyama, K., Matsuzaki, K., Hayashi, H., 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435, 824–827. doi:10.1038/nature03608

Alphei, J., Bonkowski, M., Scheu, S., 1996. Protozoa, Nematoda and Lumbricidae in the rhizosphere of Hordelymus europaeus (Poaceae): faunal interactions, response of microorganisms and effects on plant growth. Oecologia 106, 111–126. doi:10.1007/BF00334413

Amacker, N., Gao, Z., Agaras, B.C., Latz, E., Kowalchuk, G.A., Valverde, C.F., Jousset, A., Weidner, S., 2020. Biocontrol traits correlate with resistance to predation by protists in soil Pseudomonads. Frontiers in Microbiology 11, 1–13. doi:10.3389/fmicb.2020.614194

Amicucci, M.J., Galermo, A.G., Guerrero, A., Treves, G., Nandita, E., Kailemia, M.J., Higdon, S.M., Pozzo, T., Labavitch, J.M., Bennett, A.B., Lebrilla, C.B., 2019. Strategy for structural elucidation of polysaccharides: elucidation of a maize mucilage that harbors diazotrophic bacteria. Analytical Chemistry 91, 7254–7265. doi:10.1021/acs.analchem.9b00789

Araújo Da Silva, K.R., Salles, J.F., Seldin, L., Van Elsas, J.D., 2003. Application of a novel Paenibacillus-specific PCR-DGGE method and sequence analysis to assess the diversity of Paenibacillus spp. in the maize rhizosphere. Journal of Microbiological Methods 54, 213–231. doi:10.1016/S0167-7012(03)00039-3

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., Vivanco, J.M., 2006. The Role of Root Exudates in Rhizosphere Interactions with Plants and Other Organisms. Annual Review of Plant Biology 57, 233–266. doi:10.1146/annurev.arplant.57.032905.105159

Barlow, P., 1974. Regeneration of the cap of primary roots of Zea mays. New Phytologist 73, 937–954. doi:10.1111/j.1469-8137.1974.tb01323.x

Barlow, P.W., 2003. The root cap: cell dynamics, cell differentiation and cap function. Journal of Plant Growth Regulation 21, 261–286. doi:10.1007/s00344-002-0034-z

Bass, D., Chao, E.E.Y., Nikolaev, S., Yabuki, A., Ishida, K. ichiro, Berney, C., Pakzad, U., Wylezich, C., Cavalier-Smith, T., 2009a. Phylogeny of novel naked filose and reticulose Cercozoa: Granofilosea cl. n. and Proteomyxidea revised. Protist 160, 75–109. doi:10.1016/j.protis.2008.07.002

Bass, D., Howe, A.T., Mylnikov, A.P., Vickerman, K., Chao, E.E., Smallbone, J.E., Snell, J., Cabral, C., Cavalier-smith, T., 2009b. Phylogeny and classification of Cercomonadida (Protozoa, Cercozoa): Cercomonas, Eocercomonas, Paracercomonas, and Cavernomonas gen. nov. Protist 160, 483–521. doi:10.1016/j.protis.2009.01.004

Bass, D., Ward, G.M., Burki, F., 2019. Ascetosporea. Current Biology. doi:10.1016/j.cub.2018.11.025

Bates, S.T., Berg-Lyons, D., Lauber, C.L., Walters, W.A., Knight, R., Fierer, N., 2012. A preliminary survey of lichen associated eukaryotes using pyrosequencing. Lichenologist 44, 137–146. doi:10.1017/S0024282911000648

Bates, S.T., Clemente, J.C., Flores, G.E., Walters, W.A., Parfrey, L.W., Knight, R., Fierer, N., 2013. Global biogeography of highly diverse protistan communities in soil. ISME Journal 7, 652–659. doi:10.1038/ismej.2012.147

Benizri, E., Nguyen, C., Piutti, S., Slezack-Deschaumes, S., Philippot, L., 2007. Additions of maize root mucilage to soil changed the structure of the bacterial community. Soil Biology and Biochemistry 39, 1230–1233. doi:10.1016/j.soilbio.2006.12.026

Berg, G., Grube, M., Schloter, M., Smalla, K., 2014. The plant microbiome and its importance for plant and human health. Frontiers in Microbiology 5. doi:10.3389/fmicb.2014.00491

Berisso, F.E., Schjønning, P., Keller, T., Lamandé, M., Etana, A., De Jonge, L.W., Iversen, B. V., Arvidsson, J., Forkman, J., 2012. Persistent effects of subsoil compaction on pore size distribution and gas transport in a loamy soil. Soil and Tillage Research 122, 42–51. doi:10.1016/j.still.2012.02.005

Bienert, M.D., Werner, L.M., Wimmer, M.A., Bienert, G.P., 2021. Root hairs: The villi of plants. Biochemical Society Transactions. doi:10.1042/BST20200716

Bissett, A., Brown, M. V., Siciliano, S.D., Thrall, P.H., 2013. Microbial community responses to anthropogenically induced environmental change: Towards a systems approach. Ecology Letters 16, 128–139. doi:10.1111/ele.12109

Blagodatskaya, E., Kuzyakov, Y., 2015. Microbial hotspots and hot moments in soil: concept & review. Soil Biology and Biochemistry 83, 184–199. doi:10.1016/j.soilbio.2015.01.025

Boenigk, J., Arndt, H., 2002. Bacterivory by heterotrophic flagellates: community structure and feeding strategies. Antonie van Leeuwenhoek 81, 465–480. doi:10.1023/A:1020509305868

Boenigk, J., Arndt, H., 2000. Particle handling during interception feeding by four species of heterotrophic nanoflagellates. Journal of Eukaryotic Microbiology 47, 350–358. doi:10.1111/j.1550-7408.2000.tb00060.x

Bourceret, A., Guan, R., Dorau, K., Mansfeldt, T., Omidbakhshfard, A., Medeiros, D.B., Fernie, A.R., Hofmann, J., Sonnewald, U., Mayer, J., Gerlach, N., Bucher, M., Garrido-Oter, R., Spaepen, S., Schulze-Lefert, P., 2022. Maize field study reveals covaried microbiota and metabolic changes in roots over plant growth. MBio 13. doi:10.1128/mbio.02584-21

Brigham, L.A., Michaels, P.J., Flores, H.E., 1999. Cell-specific production and antimicrobial activity of naphthoquinones in roots of Lithospermum erythrorhizon. Plant Physiology 119, 417–428. doi:10.1104/pp.119.2.417

Broek, D. Van Den, Chin-a-woeng, T.F.C., Eijkemans, K., Mulders, I.H.M., Guido, V., Lugtenberg, B.J.J., 2003. Biocontrol traits of Pseudomonas spp. are regulated by phase variation. The American Phytopathological Society 16, 1003–1012. doi:10.1094/MPMI.2003.16.11.1003

Brunner, I., Scheidegger, C., 1992. Ontogeny of synthesized Picea abies (L.) Karst.–Hebeloma crustuliniforme (Bull. ex St Amans) Quél. ectomycorrhizas. New Phytologist 120, 359–369. doi:10.1111/j.1469-8137.1992.tb01076.x

Buddrus-Schiemann, K., Schmid, M., Schreiner, K., Welzl, G., Hartmann, A., 2010. Root colonization by Pseudomonas sp. DSMZ 13134 and impact on the indigenous rhizosphere bacterial community of barley. Microbial Ecology 60, 381–393. doi:10.1007/s00248-010-9720-8

Bui, V.B., Ziegler, T., Bonkowski, M., 2020. Morphological traits reflect dung beetle response to land use changes in tropical karst ecosystems of Vietnam. Ecological Indicators 108. doi:10.1016/j.ecolind.2019.105697

Bulgarelli, D., Rott, M., Schlaeppi, K., Ver, E., Themaat, L. Van, Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E., Peplies, J., Gloeckner, F.O., Amann, R., Eickhorst, T., Schulze-lefert, P., 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature 488, 91–95. doi:10.1038/nature11336

Burki, F., Keeling, P.J., 2014. Rhizaria. Current Biology. doi:10.1016/j.cub.2013.12.025

Carlos, M.H.J., Stefani, P.V.Y., Janette, A.M., Melani, M.S.S., Gabriela, P.O., 2016. Assessing the effects of heavy metals in ACC deaminase and IAA production on plant growth-promoting bacteria. Microbiological Research 188–189, 53–61. doi:10.1016/j.micres.2016.05.001

Carminati, A., Passioura, J.B., Zarebanadkouki, M., Ahmed, M.A., Ryan, P.R., Watt, M., Delhaize, E., 2017. Root hairs enable high transpiration rates in drying soils. Source: The New Phytologist 216, 771–781. doi:10.2307/90015071

Chaboud, A., 1983. Isolation, purification and chemical composition of maize root cap slime. Plant and Soil 73, 395–402. doi:10.1007/BF02184316

Chakraborty, S., Old, K.M., Warcup, J.H., 1983. Amoebae from a take-all suppressive soil which feed on Gaeumannomyces graminis tritici and other soil fungi, Soil i?io/. Eiochem.

Chandra, R., Kumar, R., 2008. Influence of PGPR and PSB on Rhizobium leguminosarum Bv. viciae Strain Competition and Symbiotic Performance in Lentil. World Journal of Agricultural Sciences 4, 297–301.

Charura, N.M., Llamas, E., De Quattro, C., Vilchez, D., Nowack, M.K., Zuccaro, A., 2024. Root cap cell corpse clearance limits microbial colonization in Arabidopsis thaliana. ELife. doi:10.7554/eLife.96266.1

Clarholm, M., 1985. Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. Soil Biology and Biochemistry 17, 181–187. doi:10.1016/0038-0717(85)90113-0

Clowes, F.A.L., R. Wadekar, 1988. Modelling of the root cap of Zea mays L, in relation to temperature 108, 259–262. doi:10.1111/j.1469-8137.1988.tb04160.x

Coleman, D.C., Whitman, W.B., 2005. Linking species richness, biodiversity and ecosystem function in soil systems. Pedobiologia 49, 479–497. doi:10.1016/j.pedobi.2005.05.006

Cook, R.J., Thomashow, L.S., Weller, D.M., Fujimoto, D., Mazzola, M., Bangera, G., Kim, D.-S., 1995. Molecular mechanisms of defense by rhizobacteria against root disease. doi:10.1073/pnas.92.10.4197

Cotton, T.E.A., Pétriacq, P., Cameron, D.D., Meselmani, M. Al, Schwarzenbacher, R., Rolfe, S.A., Ton, J., 2019. Metabolic regulation of the maize rhizobiome by benzoxazinoids. The ISME Journal 13, 1647–1658. doi:10.1038/s41396-019-0375-2

Dakora, F.D., Phillips, D.A., 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments, Plant and Soil. doi:10.1023/A:1020809400075

De Souza, J.T., Arnould, C., Deulvot, C., Lemanceau, P., Gianinazzi-Pearson, V., Raaijmakers, J.M., 2003. Effect of 2,4-diacetylphloroglucinol on pythium: Cellular responses and variation in sensitivity among propagules and species. doi:10.1094/PHYTO.2003.93.8.966

Degrune, F., Dumack, K., Fiore-donno, A.M., Bonkowski, M., 2019. Distinct communities of Cercozoa at different soil depths in a temperate agricultural field. FEMS Microbiology Ecology 95. doi:10.1093/femsec/fiz041

Denison, R.F., Bledsoe, C., Kahn, M., O'Gara, F., Simms, E.L., Thomashow, L.S., 2003. Cooperation in therhizosphere and the "free rider" problem. Ecology 84, 838–845.

Dennis, P.G., Miller, A.J., Hirsch, P.R., 2010. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiology Ecology 72, 313–327. doi:10.1111/j.1574-6941.2010.00860.x

Driouich, A., Follet-Gueye, M.-L., Vicré-Gibouin, M., Hawes, M., 2013. Root border cells and secretions as critical elements in plant host defense. Plant Biology 16, 489–495. doi:10.1016/j.pbi.2013.06.010

Driouich, A., Gaudry, A., Pawlak, B., Moore, J.P., 2021. Root cap-derived cells and mucilage: a protective network at the root tip. Protoplasma 258, 1179–1185. doi:10.1007/s00709-021-01660-y

Dumack, K., Feng, K., Flues, S., Sapp, M., Schreiter, S., Grosch, R., Rose, L., Deng, Y., Smalla, K., Bonkowski, M., 2020. What drives the assembly of plant-associated protist microbiomes? 120, 1–23.

Dumack, K., Fiore-Donno, A.M., Bass, D., Bonkowski, M., 2019a. Making sense of environmental sequencing data: Ecologically important functional traits of the protistan groups Cercozoa and Endomyxa (Rhizaria). Molecular Ecology Resources 1–6. doi:10.1111/1755-0998.13112

Dumack, K., Müller, M.E.H., Bonkowski, M., 2016. Description of Lecythium terrestris sp. nov. (Chlamydophryidae, Cercozoa), a Soil Dwelling Protist Feeding on Fungi and Algae. Protist 167, 93–105. doi:10.1016/j.protis.2016.01.001

Dumack, K., Pundt, J., Bonkowski, M., 2019b. Food Choice Experiments Indicate Selective Fungivorous Predation in Fisculla terrestris (Thecofilosea, Cercozoa). Journal of Eukaryotic Microbiology 66, 525–527. doi:10.1111/jeu.12680

Dumack, K., Sapp, M., Steimker, T. Von, M, A.T., Rose, L.E., Bonkowski, M., 2021. A call for research: a resource of core microbial symbionts of the Arabidopsis thaliana microbiome ready and awaiting experimental exploration. Phytobiomes Journal 5, 362–366. doi:10.1094/pbiomes-11-20-0080-a

Dupont, A.Ö.C., Griffiths, R.I., Bell, T., Bass, D., 2016. Differences in soil micro-eukaryotic communities over soil pH gradients are strongly driven by parasites and saprotrophs. Environmental Microbiology 18, 2010–2024. doi:10.1111/1462-2920.13220

Dupuy, L.X., Silk, W.K., 2016. Mechanisms of early microbial establishment on growing root surfaces. Vadose Zone Journal 15. doi:10.2136/vzj2015.06.0094

Edmond Ghanem, M., Han, R.-M., Classen, B., Quetin-Leclerq, J., Mahy, G., Lutts, S., Ruan, C.-J., Qin, P., Pérez-Alfocea, F., Lutts, S., 2010. Mucilage and polysaccharides in the halophyte plant species Kosteletzkya virginica: Localization and composition in relation to salt stress. Journal of Plant Physiology 167, 382–392. doi:10.1016/j.jplph.2009.10.012

Edwards, J., Johnson, C., Santos-medellín, C., Lurie, E., Podishettyb, N.K., Bhatnagarc, S., Eisenc, J.A., Sundaresana, V., 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. PNAS PLUS 20, E911–E920. doi:10.1073/pnas.1414592112

Eilers, K.G., Debenport, S., Anderson, S., Fierer, N., 2012. Digging deeper to find unique microbial communities: The strong effect of depth on the structure of bacterial and archaeal communities in soil. Soil Biology and Biochemistry 50, 58–65. doi:10.1016/j.soilbio.2012.03.011

Elliott, E.T., Anderson, R. V, Coleman, D.C., Cole, C. V, 1980. Habitable pore space and microbial trophic interactions. Oikos 35, 327–335. doi:https://doi.org/10.2307/3544648

Erktan, A., Or, D., Scheu, S., 2020. The physical structure of soil: determinant and consequence of trophic interactions. Soil Biology and Biochemistry 148, 107876. doi:10.1016/j.soilbio.2020.107876

Farrar, J., Hawes, M., Jones, D., Lindow, S., 2003a. How roots control the flux of carbon to the rhizosphereRHIZOSPHERE, Special Feature Ecology. doi:10.1890/0012-9658(2003)084[0827:HRCTFO]2.0.CO;2

Farrar, J., Hawes, M., Jones, D., Lindow, S., 2003b. How roots control the flux of carbon to the rhizosphere. Ecological Society of America 84, 827–837.

Fierer, N., 2017. Embracing the unknown: Disentangling the complexities of the soil microbiome. Nature Reviews Microbiology 15, 579–590. doi:10.1038/nrmicro.2017.87

Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. Soil Biology & Biochemitry 35, 167–176. doi:10.1016/S0038-0717(02)00251-1

Finlay, B.J., 2002. Global dispersal of free-living microbial eukaryote species. Science 296, 1061–1063. doi:10.1126/science.1070710

Fiore-donno, A.M., Weinert, J., Wubet, T., Bonkowski, M., 2016. Metacommunity analysis of amoeboid protists in grassland soils. Nature Publishing Group 1–13. doi:10.1038/srep19068

Flues, S., Bass, D., Bonkowski, M., 2017. Grazing of leaf-associated Cercomonads (Protists: Rhizaria: Cercozoa) structures bacterial community composition and function. Environmental Microbiology 19, 3297–3309. doi:10.1111/1462-2920.13824

Franche, C., Lindström, K., Elmerich, C., 2009. Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant and Soil. doi:10.1007/s11104-008-9833-8

Friman, V.P., Buckling, A., 2014. Phages can constrain protist predation-driven attenuation of Pseudomonas aeruginosa virulence in multienemy communities. ISME Journal 8, 1820–1830. doi:10.1038/ismej.2014.40

Friman, V.P., Buckling, A., 2013. Effects of predation on real-time host-parasite coevolutionary dynamics. Ecology Letters 16, 39–46. doi:10.1111/ele.12010

Fukami, T., 2015. Historical contingency in community assembly: integrating niches, species pools, and priority effects. Annu. Rev. Ecol. Evol. Syst. 46, 1–23. doi:10.1146/annurev-ecolsys-110411-160340

Gamalero, E., Lingua, G., Tombolini, R., Avidano, L., Pivato, B., Berta, G., 2005. Colonization of tomato root seedling by Pseudomonas fluorescens 92rkG5: spatio–temporal dynamics, localization, organization, viability, and culturability. Microbial Ecology 50, 289–297. doi:10.1007/s00248-004-0149-9

Geisen, S., Tveit, A.T., Clark, I.M., Richter, A., Svenning, M.M., Bonkowski, M., Urich, T., 2015. Metatranscriptomic census of active protists in soils. ISME Journal 9, 2178–2190. doi:10.1038/ismej.2015.30

Getzke, F., Amine Hassani, M., Crüsemann, M., Malisic, M., Zhang, P., Ishigaki, Y., Böhringer, N., Fernández, A.J., Wang, L., Ordon, J., Ma, K.W., Thiergart, T., Harbort, C.J., Wesseler, H., Miyauchi, S., Garrido-Oter, R., Shirasu, K., Schäberle, T.F., Hacquard, S., Schulze-Lefert, P., 2023. Cofunctioning of bacterial exometabolites drives root microbiota establishment. Proceedings of the National Academy of Sciences of the United States of America 120. doi:10.1073/pnas.2221508120

Giachello, S., Cantera, I., Carteron, A., Marta, S., Cipriano, C., Guerrieri, A., Bonin, A., Thuiller, W., Ficetola, G.F., 2023. Toward a common set of functional traits for soil protists. Soil Biology and Biochemistry 187. doi:10.1016/j.soilbio.2023.109207

Gilroy, S., Jones, D.L., 2000. Through form to function: root hair development and nutrient uptake. Trends in Plant Science 5, 1360–1385. doi:10.1016/S1360-1385(99)01551-4

Glick, B.R., 2005. Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiology Letters. doi:10.1016/j.femsle.2005.07.030

Glick, B.R., Penrose, D.M., Li, J., 1998. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria, J. theor. Biol. doi:10.1006/jtbi.1997.0532

Glücksman, E., Bell, T., Griffiths, R.I., Bass, D., 2010. Closely related protist strains have different grazing impacts on natural bacterial communities. Environmental Microbiology 12, 3105–3113. doi:10.1111/j.1462-2920.2010.02283.x

Gochnauer, M.B., Sealey, L.J., M. E. McCully, 1990. Do detached root-cap cells influence bacteria associated with maize roots? Plant, Cell and Environment 13, 793–801. doi:10.1111/j.1365-3040.1990.tb01095.x

Gopalaswamy, G., Kannaiyan, S., O'Callaghan, K.J., Davey, M.R., Cocking, E.C., 2000. The xylem of rice (Oryza sativa) is colonized by Azorhizobium caulinodans. The Royal Society 267, 103–107. doi:10.1098/rspb.2000.0973

Grossmann, L., Jensen, M., Heider, D., Jost, S., Glücksman, E., Hartikainen, H., Mahamdallie, S.S., Gardner, M., Hoffmann, D., Bass, D., Boenigk, J., 2016. Protistan community analysis: Key findings of a large-scale molecular sampling. ISME Journal 10, 2269–2279. doi:10.1038/ismej.2016.10

Guerrero, R., Margulis, L., Berlanga, M., 2013. Symbiogenesis: The holobiont as a unit of evolution. International Microbiology 16, 133–143. doi:10.2436/20.1501.01.188

Gunawardena, U., Hawes, M.C., 2002. Tissue specific localization of root infection by fungal pathogens: Role of root border cells. MPMI 15, 1128–1136. doi:10.1094/MPMI.2002.15.11.1128

Gupta, V.V.S.R., Germida, J.J., 1988. Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. Soil Biology and Biochemistry 20, 777–786. doi:10.1016/0038-0717(88)90082-X

Hacquard, Stephane, Garrido-Oter, R., Gonza, A., Spaepen, S., Ackermann, G., Lebeis, S., McHardy, A.C., Dangl, J.L., Knight, R., Ley, R., Schulze-Lefert, P., 2015. Review microbiota and host nutrition across plant and animal kingdoms. Cell Host & Microbe 17, 603–616. doi:10.1016/j.chom.2015.04.009

Hacquard, Stéphane, Garrido-Oter, R., González, A., Spaepen, S., Ackermann, G., Lebeis, S., McHardy, A.C., Dangl, J.L., Knight, R., Ley, R., Schulze-Lefert, P., 2015. Microbiota and host nutrition across plant and animal kingdoms. Cell Host and Microbe 17, 603–616. doi:10.1016/j.chom.2015.04.009

Hacquard, Stéphane, Spaepen, S., Garrido-Oter, R., Schulze-Lefert, P., 2017. Annual Review of Phytopathology Interplay Between Innate Immunity and the Plant Microbiota. doi:10.1146/annurev-phyto-080516

Hacquard, Stephane, Spaepen, S., Garrido-Oter, R., Schulze-Lefert, P., 2017. Interplay between innate immunity and the plant microbiota. Annual Review of Phytopathology 55, 565–589. doi:10.1146/annurev-phyto-080516- 035623

Hamza, M.A., Anderson, W.K., 2005. Soil compaction in cropping systems A review of the nature, causes and possible solutions. Soil and Tillage Research 82, 121–145. doi:10.1016/j.still.2004.08.009

Hartmann, A., Schmid, M., 2009. Plant-driven selection of microbes. Plant Soil 321, 235–257. doi:10.1007/s11104-008-9814-y

Hawes, M.C., Bengough, G., Cassab, G., Ponce, G., 2003. Root caps and rhizosphere. Journal of Plant Growth Regulation 21, 352–367. doi:10.1007/s00344-002-0035-y

Hawes, M.C., Curlango-Rivera, G., Xiong, Z., Kessler, J.O., 2012a. Roles of root border cells in plant defense and regulation of rhizosphere microbial populations by extracellular DNA ‘ trapping .’ Plant Soil 355, 1–16. doi:10.1007/s11104-012-1218-3

Hawes, M.C., Curlango-Rivera, G., Xiong, Z., Kessler, J.O., 2012b. Roles of root border cells in plant defense and regulation of rhizosphere microbial populations by extracellular DNA ‘ trapping .’ Plant Soil 355, 1–16. doi:10.1007/s11104-012-1218-3

Hawes, M.C., Gunawardena, U., Miyasaka, S., Zhao, X., 2000. The role of root border cells in plant defense. Trends in Plant Science 5, 1360–1385. doi:10.1016/s1360-1385(00)01556-9

Hawes, M.C., Smith, L.Y., 1989. Requirement for chemotaxis in pathogenicity of Agrobacterium tumefaciens on roots of soil-grown pea plantst. Journal of Bacteriology 171, 5668–5671. doi:10.1128/jb.171.10.5668-5671.1989

Heijden, M.G.A. Van Der, Bruin, S. De, Luckerhoff, L., Logtestijn, R.S.P. Van, Schlaeppi, K., 2015. A widespread plant-fungal-bacterial symbiosis promotes plant biodiversity , plant nutrition and seedling recruitment. The ISME Journal 10, 389–399. doi:10.1038/ismej.2015.120

Hess, S., Sausen, N., Melkonian, M., 2012. Shedding Light on vampires: The phylogeny of vampyrellid amoebae revisited. PLoS ONE 7. doi:10.1371/journal.pone.0031165

Hinsinger, P., Gobran, G.R., Gregory, P.J., Wenzel, W.W., 2005. Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes. New Phytologist 168, 293–303. doi:10.1111/j.1469-8137.2005.01512.x

Hirsch, A.M., Bauer, W.D., Bird, D.M., Cullimore, J., Tyler, B., Yoder, J.I., 2003. Molecular Signals and Receptors: Controlling Rhizosphere Interactions between Plants and other Organisms. Ecology 84, 858–868. doi:10.1890/0012-9658(2003)084[0858:MSARCR]2.0.CO;2

Holz, M., Zarebanadkouki, M., Carminati, A., Becker, J.N., Spohn, M., 2020. The effect of root hairs on rhizosphere phosphatase activity. Journal of Plant Nutrition and Soil Science 183, 382–388. doi:10.1002/jpln.201900426

Howe, A.T., Bass, D., Vickerman, K., Chao, E.E., Cavalier-smith, T., 2009. Phylogeny, taxonomy, and astounding Genetic diversity of Glissomonadida ord . nov ., the dominant gliding zooflagellates in soil (Protozoa: Cercozoa). Protist 160. doi:10.1016/j.protis.2008.11.007

Hu, J., Wei, Z., Weidner, S., Friman, V.P., Xu, Y.C., Shen, Q.R., Jousset, A., 2017. Probiotic Pseudomonas communities enhance plant growth and nutrient assimilation via diversity-mediated ecosystem functioning. Soil Biology and Biochemistry 113, 122–129. doi:10.1016/j.soilbio.2017.05.029

Hu, L., Robert, C.A.M., Cadot, S., Zhang, X., Ye, M., Li, B., Manzo, D., Chervet, N., Steinger, T., Heijden, M.G.A. Van Der, Schlaeppi, K., Erb, M., 2018. Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. Nature Communications 9, 2738. doi:10.1038/s41467-018-05122-7

Humphris, S.N., Bengough, A.G., Griffiths, B.S., Kilham, K., Rodger, S., Stubbs, V., Valentine, T.A., Young, I.M., 2005. Root cap influences root colonisation by Pseudomonas fluorescens SBW25 on maize. FEMS Microbiology Ecology 54, 123–130. doi:10.1016/j.femsec.2005.03.005

Hwang, S.-F., Strelkov, S.E., Feng, J., Gossen, B.D., Howard, R.J., 2012. Plasmodiophora brassicae: A review of an emerging pathogen of the Canadian canola (Brassica napus) crop. Molecular Plant Pathology. doi:10.1111/j.1364-3703.2011.00729.x

Iijima, M., Griffiths, B., Bengough, A.G., 2000. Sloughing of cap cells and carbon exudation from maize seedling roots in compacted sand. New Phytologist 145, 477–482. doi:10.1046/j.1469-8137.2000.00595.x

Ingram, D.S., Tommerup, I.C., 1972. The life history of Plasmodiophera brassicae Woron. Proceedings of the Royal Society B 180, 103–112. doi:10.1098/rspb.1972.0008

Jackson, L.E., Schimel, J.P., Firestone, M.K., 1989. Short-term partitioning of ammonium and nitrate between plants and microbes in an annual grassland. Soil Bid. Biochem 5, 409–415. doi:10.1016/0038-0717(89)90152-1

Jaeger III, C.H., Lindow, S.E., Miller, W., Clark, E., 1999. Mapping of sugar and amino acid availability in soil around roots with bacterial sensors of sucrose and tryptophan. Applied and Environmental Microbiology 65, 2685–2690. doi:10.1128/AEM.65.6.2685-2690.1999

Jaroszuk-Ściseł, J., Kurek, E., Rodzik, B., Winiarczyk, K., 2009. Interactions between rye (Secale cereale) root border cells (RBCs) and pathogenic and nonpathogenic rhizosphere strains of Fusarium culmorum. Mycological Research 113, 1053–1061. doi:10.1016/j.mycres.2009.07.001

Jones, D.L., Nguyen, C., Finlay, R.D., 2009. Carbon flow in the rhizosphere : carbon trading at the soil – root interface 5–33. doi:10.1007/s11104-009-9925-0

Jousset, A., 2012a. Ecological and evolutive implications of bacterial defences against predators. Environmental Microbiology 14, 1830–1843. doi:10.1111/j.1462-2920.2011.02627.x

Jousset, A., 2012b. Ecological and evolutive implications of bacterial defences against predators. Environmental Microbiology 14, 1830–1843. doi:10.1111/j.1462-2920.2011.02627.x

Jousset, A., Bonkowski, M., 2010. The model predator Acanthamoeba castellanii induces the production of 2, 4, DAPG by the biocontrol strain Pseudomonas fluorescens Q2-87. Soil Biology and Biochemistry 42, 1647–1649. doi:10.1016/j.soilbio.2010.05.018

Jousset, A., Lara, E., Wall, L.G., Valverde, C., 2006. Secondary metabolites help biocontrol strain Pseudomonas fluorescens CHA0 to escape protozoan grazing. Applied and Environmental Microbiology 72, 7083–7090. doi:10.1128/AEM.00557-06

Jousset, A., Rochat, L., Péchy-Tarr, M., Keel, C., Scheu, S., Bonkowski, M., 2009. Predators promote defence of rhizosphere bacterial populations by selective feeding on non-toxic cheaters. The ISME Journal 3, 666–674. doi:10.1038/ismej.2009.26

Jousset, A., Rochat, L., Scheu, S., Bonkowski, M., Keel, C., 2010. Predator-prey chemical warfare determines the expression of biocontrol genes by rhizosphere-associated Pseudomonas fluorescens. Applied and Environmental Microbiology 76, 5263–5268. doi:10.1128/aem.02941-09

Jousset, A., Scheu, S., Bonkowski, M., 2008. Secondary metabolite production facilitates establishment of rhizobacteria by reducing both protozoan predation and the competitive effects of indigenous bacteria. Functional Ecology 22, 714–719. doi:10.1111/j.1365-2435.2008.01411.x

Kim, J.-S., Dungan, R.S., Crowley, D., 2008. Microarray analysis of bacterial diversity and distribution in aggregates from a desert agricultural soil. Biology and Fertility of Soils 44, 1003–1011. doi:10.1007/s00374-008-0291-5

Koller, R., Rodriguez, A., Robin, C., Scheu, S., Bonkowski, M., 2013a. Protozoa enhance foraging efficiency of arbuscular mycorrhizal fungi for mineral nitrogen from organic matter in soil to the benefit of host plants. New Phytologist 199, 203–211. doi:10.1111/nph.12249

Koller, R., Scheu, S., Bonkowski, M., Robin, C., 2013b. Protozoa stimulate N uptake and growth of arbuscular mycorrhizal plants. Soil Biology and Biochemistry 65, 204–210. doi:10.1016/j.soilbio.2013.05.020

Kuikman, P.J., Jansen, A.G., Van Veen, J.A., 1991. 15N-nitrogen mineralization from bacteria by protozoan grazing at different soil moisture regimes. Soil Biology and Biochemistry 23, 193–200.

Kuikman, P.J., Jansen, A.G., Van Veen, J.A., Zehnder, A.J.B., 1990. Protozoan predation and the turnover of soil organic carbon and nitrogen in the presence of plants. Biol Fertil Soils 10, 22–28. doi:10.1007/BF00336120

Kuikman, P.J., Van Veen, J.A., 1989. The impact of protozoa on the availability of bacterial nitrogen to plants*. Biology and Fertility of Soils 8, 13–18. doi:10.1007/BF00260510

Kuzyakov, Y., Domanski, G., 2000. Carbon input by plants into the soil. J. Plant Nutr. Soil Sci. 163, 421–431.

Kuzyakov, Y., Mason-Jones, K., 2018. Viruses in soil: Nano-scale undead drivers of microbial life, biogeochemical turnover and ecosystem functions. Soil Biology and Biochemistry. doi:10.1016/j.soilbio.2018.09.032

Kwasniewski, M., Daszkowska-Golec, A., Janiak, A., Chwialkowska, K., Nowakowska, U., Sablok, G., Szarejko, I., 2016. Transcriptome analysis reveals the role of the root hairs as environmental sensors to maintain plant functions under water-deficiency conditions. Journal of Experimental Botany 67, 1079–1094. doi:10.1093/jxb/erv498

Lagopodi, A.L., Ram, A.F.J., Lamers, G.E.M., Punt, P.J., Van den Hondel, C.A.M.J.J., Lugtenberg, B.J.J., Bloemberg, G. V, 2002. Novel aspects of tomato root colonization and infection by Fusarium oxysporum f. sp. radicis-lycopersici revealed by confocal laser scanning microscopic analysis using the green fluorescent protein as a marker. MPMI 15, 172–179. doi:10.1094/MPMI.2002.15.2.172

Lanoue, A., Burlat, V., Henkes, G.J., Koch, I., Schurr, U., Röse, U.S.R., 2009. De novo biosynthesis of defense root exudates in response to Fusarium attack in barley. New Phytologist 185, 577–588.

Lentendu, G., Wubet, T., Chatzinotas, A., Wilhelm, C., Buscot, F., Schlegel, M., 2014. Effects of long-term differential fertilization on eukaryotic microbial communities in an arable soil: A multiple barcoding approach. Molecular Ecology 23, 3341–3355. doi:10.1111/mec.12819

Li, E., de Jonge, R., Liu, C., Jiang, H., Friman, V.P., Pieterse, C.M.J., Bakker, P.A.H.M., Jousset, A., 2021a. Rapid evolution of bacterial mutualism in the plant rhizosphere. Nature Communications 12. doi:10.1038/s41467-021-24005-y

Li, E., Ryo, M., Kowalchuk, G.A., Bakker, P.A.H.M., Jousset, A., 2021b. Rapid evolution of trait correlation networks during bacterial adaptation to the rhizosphere. Evolution 75, 1218–1229. doi:10.1111/evo.14202

Libault, M., Brechenmacher, L., Cheng, J., Xu, D., Stacey, G., 2010. Root hair systems biology. Trends in Plant Science 15, 641–650. doi:10.1016/j.tplants.2010.08.010

Liu, C., Ni, B., Wang, X., Deng, Y., Tao, L., Zhou, X., Deng, J., 2023. Effect of forest soil viruses on bacterial community succession and the implication for soil carbon sequestration. Science of the Total Environment 892. doi:10.1016/j.scitotenv.2023.164800

Liu, C., Wang, Y., Zhou, Z., Wang, S., Wei, Z., Ravanbakhsh, M., Shen, Q., Xiong, W., Kowalchuk, G.A., Jousset, A., 2024. Protist predation promotes antimicrobial resistance spread through antagonistic microbiome interactions. The ISME Journal 18. doi:10.1093/ismejo/wrae169

Loo, E.P.-I., Durán, P., Pang, T.Y., Westhoff, P., Deng, C., Durán, C., Lercher, M., Garrido-Oter, R., Frommer, W.B., 2024. Sugar transporters spatially organize microbiota colonization along the longitudinal root axis of Arabidopsis. Cell Host & Microbe. doi:10.1016/j.chom.2024.02.014

Lueders, T., Kindler, R., Miltner, A., Friedrich, M.W., Kaestner, M., 2006. Identification of bacterial micropredators distinctively active in a soil microbial food web. Applied and Environmental Microbiology 72, 5342–5348. doi:10.1128/AEM.00400-06

Lugtenberg, B.J.J., Dekkers, L., Bloemberg, G. V, 2001. Molecular determinants of rhizosphere colonization by Pseudomonas. Annual Review of Phytopathology 39, 461–490. doi:10.1146/annurev.phyto.39.1.461

Lundberg, D.S., Lebeis, S.L., Paredes, S.H., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektson, A., Kunin, V., Rio, T.G. del, Edgar, R.C., Eickhorst, T., Ley, R.E., Hugenholtz, P., Tringe, S.G., Dangl, J.L., 2012a. Defining the core Arabidopsis thaliana root microbiome. Nature 488, 86–90. doi:10.1038/nature11237

Lundberg, D.S., Lebeis, S.L., Paredes, S.H., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektson, A., Kunin, V., Rio, T.G. del, Edgar, R.C., Eickhorst, T., Ley, R.E., Hugenholtz, P., Tringe, S.G., Dangl, J.L., 2012b. Defining the core Arabidopsis thaliana root microbiome. Nature 488, 86–90. doi:10.1038/nature11237

Ma, W., Muthreich, N., Liao, C., Franz-Wachtel, M., Schütz, W., Zhang, F., Hochholdinger, F., Li, C., 2010. The mucilage proteome of maize (Zea mays L.) primary roots. Journal of Proteome Research 9, 2968–2976. doi:10.1021/pr901168v

Ma, Z., Bielenberg, D.G., Brown, K.M., Lynch, J.P., 2001. Regulation of root hair density by phosphorus availability in Arabidopsis thaliana. Plant, Cell and Environment 24, 459–467. doi:10.1046/j.1365-3040.2001.00695.x

Marín, O., González, B., Poupin, M.J., 2021. From Microbial Dynamics to Functionality in the Rhizosphere: A Systematic Review of the Opportunities With Synthetic Microbial Communities. Frontiers in Plant Science. doi:10.3389/fpls.2021.650609

Marschner, P., Crowley, D., Rengel, Z., 2011. Rhizosphere interactions between microorganisms and plants govern iron and phosphorus acquisition along the root axis - model and research methods. Soil Biology and Biochemistry 43, 883–894. doi:10.1016/j.soilbio.2011.01.005

Marschner, P., Yang, C., Lieberei, R., Crowley, D.E., 2001. Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biology and Biochemistry 33, 1437–1445.

Massalha, H., Korenblum, E., Malitsky, S., Shapiro, O.H., Aharoni, A., 2017. Live imaging of root-bacteria interactions in a microfluidics setup. Proceedings of the National Academy of Sciences of the United States of America 114, 4549–4554. doi:10.1073/pnas.1618584114

Matz, C., Deines, P., Boenigk, J., Arndt, H., Eberl, L., Kjelleberg, S., Jürgens, K., 2004. Impact of Violacein-Producing Bacteria on Survival and Feeding of Bacterivorous Nanoflagellates. Applied and Environmental Microbiology 70, 1593–1599. doi:10.1128/AEM.70.3.1593-1599.2004

Matz, C., Kjelleberg, S., 2005. Off the hook – how bacteria survive protozoan grazing. TRENDS in Microbiology 13. doi:10.1016/j.tim.2005.05.009

Mazel, F., Malard, L., Niculita-Hirzel, H., Yashiro, E., Mod, H.K., Mitchell, E.A.D., Singer, D., Buri, A., Pinto, E., Guex, N., Lara, E., Guisan, A., 2022. Soil protist function varies with elevation in the Swiss Alps. Environmental Microbiology 24, 1689–1702. doi:10.1111/1462-2920.15686

Mazzola, M., Cook, R.J., 1991. Effects of fungal root pathogens on the population dynamics of biocontrol strains of fluorescent pseudomonads in the wheat rhizosphere, APPLIED AND ENVIRONMENTAL MICROBIOLOGY. doi:10.1128/aem.57.8.2171-2178.1991

Mazzola, M., De Bruijn, I., Cohen, M.F., Raaijmakers, J.M., 2009. Protozoan-induced regulation of cyclic lipopeptide biosynthesis is an effective predation defense mechanism for Pseudomonas fluorescens. Applied and Environmental Microbiology 75, 6804–6811. doi:10.1128/AEM.01272-09

Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J.H.M., Piceno, Y.M., DeSantis, T.Z., Andersen, G.L., Bakker, P.A.H.M., Raaijmakers, J.M., 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332, 1093–1097. doi:10.1126/science.1202007

Mesny, F., Hacquard, S., Thomma, B.P., 2023. Co-evolution within the plant holobiont drives host performance. EMBO Reports 24. doi:10.15252/embr.202357455

Morel, J.L., Mench, M., Guckert, A., 1986. Measurement of Pb2+, Cu2+ and Cd2+ binding with mucilage exudates from maize (Zea mays L.) roots. Biology and Fertility of Soils 29–34. doi:10.1007/BF00638958

Morgan, A.D., MacLean, R.C., Hillesland, K.L., Velicer, G.J., 2010. Comparative analysis of myxococcus predation on soil bacteria. Applied and Environmental Microbiology 76, 6920–6927. doi:10.1128/AEM.00414-10

Mummey, D., Holben, W., Six, J., Stahl, P., 2006. Spatial stratification of soil bacterial populations in aggregates of diverse soils. Microbial Ecology 51, 404–411. doi:10.1007/s00248-006-9020-5

Nazari, M., 2021. Rhizosphere plant mucilage components and their functions in the rhizosphere. Rhizosphere 18, 100344. doi:10.1016/j.rhisph.2021.100344

Nemergut, D.R., Schmidt, S.K., Fukami, T., Neill, S.P.O., Bilinski, T.M., Stanish, L.F., Knelman, J.E., Darcy, J.L., Lynch, R.C., Wickey, P., Ferrenberg, S., 2013. Patterns and Processes of Microbial Community Assembly. Microbiology and Molecular Biology Reviews 77, 342–356. doi:10.1128/MMBR.00051-12

Neuhauser, S., Kirchmair, M., Bulman, S., Bass, D., 2014. Cross-kingdom host shifts of phytomyxid parasites. doi:10.1186/1471-2148-14-33

Ngou, B.P.M., Ding, P., Jones, J.D.G., 2022. Thirty years of resistance: Zig-zag through the plant immune system. Plant Cell. doi:10.1093/plcell/koac041

Nguyen, B.-A.T., Chen, Q.-L., He, J.-Z., Hu, H.-W., 2020. Microbial regulation of natural antibiotic resistance: Understanding the protist-bacteria interactions for evolution of soil resistome. Science of the Total Environment 705. doi:10.1016/j.scitotenv.2019.135882

Nguyen, B.A.T., Chen, Q.L., Yan, Z.Z., Li, C., He, J.Z., Hu, H.W., 2021. Distinct factors drive the diversity and composition of protistan consumers and phototrophs in natural soil ecosystems. Soil Biology and Biochemistry 160. doi:10.1016/j.soilbio.2021.108317

Nguyen, C., 2003. Rhizodeposition of organic C by plants: mechanisms and controls. Agronomie 23, 375–396. doi:10.1051/agro

Nguyen, T.B.A., Bonkowski, M., Dumack, K., Chen, Q.L., He, J.Z., Hu, H.W., 2023. Protistan predation selects for antibiotic resistance in soil bacterial communities. ISME Journal 17, 2182–2189. doi:10.1038/s41396-023-01524-8

Niedeggen, D., Rüger, L., Oburger, E., Santangeli, M., Mutez, A., Vetterlein, D., Blagodatsky, S., Bonkowski, M., 2024. Microbial utilisation of maize rhizodeposits applied to agricultural soil at a range of concentrations. European Journal of Soil Science 75. doi:10.1111/ejss.13530

Oger, P.M., Mansouri, H., Nesme, X., Dessaux, Y., 2004. Engineering root exudation of Lotus toward the production of two novel carbon compounds leads to the selection of distinct microbial populations in the rhizosphere. Microbial Ecology 47, 96–103. doi:10.1007/s00248-003-2012-9

Oliverio, A.M., Geisen, S., Delgado-baquerizo, M., Maestre, F.T., Turner, B.L., Fierer, N., 2020. The global-scale distributions of soil protists and their contributions to belowground systems. Science Advanced 6, eaax8787. doi:10.1126/sciadv.aax8787

Oparka, K.J., Duckett, C.M., Prior, O.A.M.I., Fisher, D.B., 1994. Real-time imaging of phloem unloading in the root tip of Arabidopsis. The Plant Journal 6, 759–766. doi:10.1046/j.1365-313X.1994.6050759.x

Or, D., Smets, B.F., Wraith, J.M., Dechesne, A., Friedman, S.P., 2007. Physical constraints affecting bacterial habitats and activity in unsaturated porous media – a review. Advances in Water Resources 30, 1505–1527. doi:10.1016/j.advwatres.2006.05.025

Ordentlich, A., Elad, Y., Chet, I., 1988. The role of chitinase of Serratia marcescens in biocontrol of Sclerotium rolfsii. Ecology and Epidemiology 78, 84–88. doi:10.1094/phyto-78-84

Oyserman, B.O., Medema, M.H., Raaijmakers, J.M., 2018. Road MAPs to engineer host microbiomes. Current Opinion in Microbiology. doi:10.1016/j.mib.2017.11.023

Park, W.J., Hochholdinger, F., Gierl, A., 2004. Release of the benzoxazinoids defense molecules during lateral- and crown root emergence in Zea mays. Journal of Plant Physiology 161, 981–985. doi:10.1016/j.jplph.2004.01.005

Parniske, M., 2008. Arbuscular mycorrhiza: The mother of plant root endosymbioses. Nature Reviews Microbiology. doi:10.1038/nrmicro1987

Pausch, J., Hünninghaus, M., Kramer, S., Scharroba, A., Scheunemann, N., Butenschoen, O., Marhan, S., Bonkowski, M., Kandeler, E., Scheu, S., Kuzyakov, Y., Ruess, L., 2018. Carbon budgets of top- and subsoil food webs in an arable system. Pedobiologia 69, 29–33. doi:10.1016/j.pedobi.2018.06.002

Pausch, J., Kuzyakov, Y., 2018. Carbon input by roots into the soil : Quantification of rhizodeposition from root to ecosystem scale. Global Change Biology 24, 1–12. doi:10.1111/gcb.13850

Péret, B., Larrieu, A., Bennett, M.J., 2009. Lateral root emergence: a difficult birth. Journal of Experimental Botany 60, 3637–3643. doi:10.1093/jxb/erp232

Peterson, R.L., Farquhar, M.L., 1996. Root hairs: specialized tubular cells. The Botanical Review 62, 1–40. doi:10.1007/BF02868919

Petters, S., Groß, V., Söllinger, A., Pichler, M., Reinhard, A., Maria, M., Tim, B., 2021. The soil microbial food web revisited : Predatory myxobacteria as keystone taxa ? The ISME Journal. doi:10.1038/s41396-021-00958-2

Petz, W., Foissner, W., Adam, H., 1985. Culture, food selection and growth rate in mycophagous ciliate Grossglocknearia acuta Foissner, 1980: First evidence of autochthonous soil ciliates. Soil Biol. Biochem 17, 871–875.

Philippot, L., Raaijmakers, J.M., Lemanceau, P., Putten, W.H. Van Der, 2013. Going back to the roots : the microbial ecology of the rhizosphere. Nature Reviews Microbiology 789–799. doi:10.1038/nrmicro3109

Pieterse, M.J., Zamioudis, C., Berendsen, R.L., Weller, D.M., Wees, S.C.M. Van, Bakker, P.A.H.M., 2014. Induced systemic resistance by beneficial microbes. Annual Review of Phytopathology 52, 347–375. doi:10.1146/annurev-phyto-082712-102340

Pozzo, T., Higdon, S.M., Pattathil, S., Hahn, M.G., Bennett, A.B., 2018. Characterization of novel glycosyl hydrolases discovered by cell wall glycan directed monoclonal antibody screening and metagenome analysis of maize aerial root mucilage. PLoS ONE 13, e0204525. doi:10.1371/journal.pone.0204525

Raaijmakers, J.M., Paulitz, T.C., 2008. The rhizosphere : a playground and battlefield for soilborne pathogens and beneficial microorganisms 341–361. doi:10.1007/s11104-008-9568-6

Raaijmakers, J.M., Weller, D.M., 1998. Natural plant protection by 2,4-diacetylphloroglucinol-producing Pseudomonas spp. in take-All decline soils. Molecular Plant-Microbe Interactions MPMI 11, 144–152. doi:10.1094/MPMI.1998.11.2.144

Rahman, A., Manci, M., Nadon, C., Perez, I.A., Farsamin, W.F., Lampe, M.T., Le, T.H., Torres Martínez, L., Weisberg, A.J., Chang, J.H., Sachs, J.L., 2023. Competitive interference among rhizobia reduces benefits to hosts. Current Biology 33, 2988-3001.e4. doi:10.1016/j.cub.2023.06.081

Ravanbakhsh, M., Sasidharan, R., Voesenek, L.A.C.J., Kowalchuk, G.A., Jousset, A., 2018. Microbial modulation of plant ethylene signaling: ecological and evolutionary consequences. Microbiome 6, 1–10. doi:10.1186/s40168-018-0436-1

Reinhold-Hurek, B., Bünger, W., Burbano, C.S., Sabale, M., Hurek, T., 2015. Roots Shaping Their Microbiome: Global Hotspots for Microbial Activity. Annual Review of Phytopathology 53, 403–424. doi:10.1146/annurev-phyto-082712-102342

Robe, K., Conejero, G., Gao, F., Lefebvre-Legendre, L., Sylvestre-Gonon, E., Rofidal, V., Hem, S., Rouhier, N., Barberon, M., Hecker, A., Gaymard, F., Izquierdo, E., Dubos, C., 2021a.

Coumarin accumulation and trafficking in Arabidopsis thaliana: a complex and dynamic process. New Phytologist 229, 2062–2079. doi:10.1111/nph.17090

Robe, K., Conejero, G., Gao, F., Lefebvre-Legendre, L., Sylvestre-Gonon, E., Rofidal, V., Hem, S., Rouhier, N., Barberon, M., Hecker, A., Gaymard, F., Izquierdo, E., Dubos, C., 2021b. Coumarin accumulation and trafficking in Arabidopsis thaliana: a complex and dynamic process. New Phytologist 229, 2062–2079. doi:10.1111/nph.17090

Roesch, L.F.W., Fulthorpe, R.R., Riva, A., Casella, G., Hadwin, A.K.M., Kent, A.D., Daroub, S.H., Camargo, F.A.O., Farmerie, W.G., Triplett, E.W., 2007. Pyrosequencing enumerates and contrasts soil microbial diversity. ISME Journal 1, 283–290. doi:10.1038/ismej.2007.53

Rolli, E., Marasco, R., Vigani, G., Ettoumi, B., Mapelli, F., Deangelis, M.L., Gandolfi, C., Casati, E., Previtali, F., Gerbino, R., Pierotti Cei, F., Borin, S., Sorlini, C., Zocchi, G., Daffonchio, D., 2015. Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. Environmental Microbiology 17, 316–331. doi:10.1111/1462-2920.12439

Rosenberg, K., Bertaux, J., Krome, K., Hartmann, A., Scheu, S., Bonkowski, M., 2009. Soil amoebae rapidly change bacterial community composition in the rhizosphere of Arabidopsis thaliana. The ISME Journal 3, 675–684. doi:10.1038/ismej.2009.11

Ross-elliott, T.J., Jensen, K.H., Haaning, K.S., Wager, B.M., Knoblauch, J., Howell, A.H., Mullendore, D.L., Monteith, A.G., Paultre, D., Yan, D., Otero, S., Bourdon, M., Sager, R., Lee, J., Knoblauch, M., Oparka, K.J., 2017. Phloem unloading in Arabidopsis roots is convective and regulated by the phloem- pole pericycle. Elife 6, e24125. doi:10.7554/eLife.24125

Rozmoš, M., Bukovská, P., Hršelová, H., Kotianov, M., Dudáš, M., Gančarčíková, K., Jansa, J., 2021. Organic nitrogen utilisation by an arbuscular mycorrhizal fungus is mediated by specific soil bacteria and a protist. The ISME Journal 16, 1–10. doi:10.1038/s41396-021-01112-8

Rudrappa, T., Czymmek, K.J., Paré, P.W., Bais, H.P., 2008. Root-secreted malic acid recruits beneficial soil bacteria. Plant Physiology 148, 1547–1556. doi:10.1104/pp.108.127613

Rüger, L., Feng, K., Chen, Y., Sun, R., Sun, B., Deng, Y., Vetterlein, D., Bonkowski, M., 2023a. Responses of root architecture and the rhizosphere microbiome assembly of maize (Zea mays L.) to a soil texture gradient. Soil Biology and Biochemistry 181. doi:10.1016/j.soilbio.2023.109026

Rüger, L., Feng, K., Dumack, K., Freudenthal, J., Chen, Y., Sun, R., Wilson, M., Yu, P., Sun, B., Deng, Y., Hochholdinger, F., 2021. Assembly patterns of the rhizosphere microbiome along the longitudinal root axis of maize (Zea mays L.). Frontiers in Microbiology 12, 1–14. doi:10.3389/fmicb.2021.614501

Rüger, L., Ganther, M., Freudenthal, J., Jansa, J., Heintz-Buschart, A., Tarkka, M.T., 2023b. Root cap is an important determinant of rhizosphere microbiome assembly. New Phytologist 239, 1434–1448. doi:10.1111/nph.19002

Rutherford, P.M., Juma, N.G., 1992a. Influence of texture on habitable pore space and bacterial-protozoan populations in soil. Biology and Fertility of Soils 12, 221–227. doi:10.1007/BF00336036

Rutherford, P.M., Juma, N.G., 1992b. Influence of texture on habitable pore space and bacterial-protozoan populations in soil. Biology and Fertility of Soils 12, 221–227. doi:10.1007/BF00336036

Saengwilai, P., Strock, C., Rangarajan, H., Chimungu, J., Salungyu, J., Lynch, J.P., 2021. Root hair phenotypes influence nitrogen acquisition in maize. Annals of Bontany 128, 849–858. doi:10.1093/aob/mcab104

Santangeli, M., Steininger-Mairinger, T., Vetterlein, D., Hann, S., Oburger, E., 2024. Maize (Zea mays L.) root exudation profiles change in quality and quantity during plant development – A field study. Plant Science 338. doi:10.1016/j.plantsci.2023.111896

Sapp, M., Ploch, S., Fiore-Donno, A.M., Bonkowski, M., Rose, L.E., 2017. Protists are an integral part of the Arabidopsis thaliana microbiome. Environmental Microbiology 20, 30–43. doi:10.1111/1462-2920.13941

Sasidharan, R., Voesenek, L.A.C.J., 2015. Ethylene-mediated acclimations to flooding stress. Plant Physiology 169, 3–12. doi:10.1104/pp.15.00387

Sasse, J., Martinoia, E., Northen, T., 2018. Feed Your Friends : Do Plant Exudates Shape the Root Microbiome ? Trends in Plant Science 23, 25–41. doi:10.1016/j.tplants.2017.09.003

Schloss, P.D., Handelsman, J., 2006. Toward a census of bacteria in soil. PLoS Computational Biology 2, 0786–0793. doi:10.1371/journal.pcbi.0020092

Schmidt, H., Nunan, N., Höck, A., Eickhorst, T., Kaiser, C., Woebken, D., Raynaud, X., 2018. Recognizing patterns: Spatial analysis of observed microbial colonization on root surfaces. Frontiers in Environmental Science 6. doi:10.3389/fenvs.2018.00061

Schnepf, A., Carminati, A., Ahmed, M.A., Ani, M., Benard, P., Bentz, J., Bonkowski, M., Knott, M., Diehl, D., Duddek, P., Kröner, E., Javaux, M., Landl, M., Lehndorff, E., Lippold, E., Lieu, A., Mueller, C.W., Oburger, E., Otten, W., Portell, X., Phalempin, M., Prechtel, A., Schulz, R., Vanderborght, J., Vetterlein, D., 2022. Linking rhizosphere processes across scales: Opinion. Plant and Soil 478, 5–42. doi:10.1007/s11104-022-05306-7

Semenov, A.M., Van Bruggen, A.H.C., Zelenev, V. V, 1999. Moving Waves of Bacterial Populations and Total Organic Carbon along Roots of Wheat.

Sherr, B.F., Sherr, E.B., McDaniel, J., 1992. Effect of protistan grazing on the frequency of dividing cells in bacterioplankton assemblages. APPLIED AND ENVIRONMENTAL MICROBIOLOGY 2381–2385. doi:10.1128/aem.58.8.2381-2385.1992

Simonin, M., Terzi, V., Ngonkeu, E.L., Diouf, D., 2019. Influence of plant genotype and soil on the wheat rhizosphere microbiome : identification of a core microbiome across eight African and European soils. doi:10.1101/777383

Singer, D., Seppey, C.V.W., Lentendu, G., Dunthorn, M., Bass, D., Belbahri, L., Blandenier, Q., Debroas, D., de Groot, G.A., de Vargas, C., Domaizon, I., Duckert, C., Izaguirre, I., Koenig, I., Mataloni, G., Schiaffino, M.R., Mitchell, E.A.D., Geisen, S., Lara, E., 2021a. Protist taxonomic and functional diversity in soil, freshwater and marine ecosystems. Environment International 146. doi:10.1016/j.envint.2020.106262

Singer, D., Seppey, C.V.W., Lentendu, G., Dunthorn, M., Bass, D., Belbahri, L., Blandenier, Q., Debroas, D., de Groot, G.A., de Vargas, C., Domaizon, I., Duckert, C., Izaguirre, I., Koenig, I., Mataloni, G., Schiaffino, M.R., Mitchell, E.A.D., Geisen, S., Lara, E., 2021b. Protist taxonomic and functional diversity in soil, freshwater and marine ecosystems. Environment International 146. doi:10.1016/j.envint.2020.106262

Smith, A.M., 1973. Ethylene as a cause of soil fungistasis. Nature 311–313. doi:10.1038/246311a0

Sosa-Hernández, M.A., Roy, J., Hempel, S., Kautz, T., Köpke, U., Uksa, M., Schloter, M., Caruso, T., Rillig, M.C., 2018. Subsoil arbuscular mycorrhizal fungal communities in arable soil differ from those in topsoil. Soil Biology and Biochemistry 117, 83–86. doi:10.1016/j.soilbio.2017.11.009

Sprague, S.J., Watt, M., Kirkegaard, J.A., Howlett, B.J., 2007. Pathways of infection of Brassica napus roots by Leptosphaeria maculans. New Phytologist 176, 211–222. doi:10.1111/j.1469-8137.2007.02156.x

Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M.D.M., Breiner, H.W., Richards, T.A., 2010. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Molecular Ecology 19, 21–31. doi:10.1111/j.1365-294X.2009.04480.x

Stringlis, I.A., Yu, K., Feussner, K., Jonge, R. De, Bentum, S. Van, Verk, M.C. Van, Berendsena, R.L., Bakkera, P.A.H.M., Feussnerb, I., Pietersea, C.M.J., 2018. MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. PNAS 115, E5213–E5222. doi:10.1073/pnas.1722335115

Suttle, C.A., 2007. Marine viruses - Major players in the global ecosystem. Nature Reviews Microbiology 5, 801–812. doi:10.1038/nrmicro1750

Tarafdar, J.C., Rao, A. V., 1997. Response of arid legumes to VAM fungal inoculation. Symbiosis 22, 265–274.

Thao, N.P., Khan, M.I.R., Anh Thu, N.B., Thi Hoang, X.L., Asgher, M., Khan, N.A., Tran, L.S.P., 2015. Role of ethylene and its cross talk with other signaling molecules in plant responses to heavy metal stress. Plant Physiology 169, 73–84. doi:10.1104/pp.15.00663

Thomas Cavalier-Smith, Ema E.-Y. Chao, 2003. Phylogeny and classification of phylum Cercozoa (protozoa). Protist 154, 341–358. doi:10.1078/143446103322454112

Thrall, P.H., Hochberg, M.E., Burdon, J.J., Bever, J.D., 2006. Coevolution of symbiotic mutualists and parasites in a community context. Trends in Ecology and Evolution 22, 120–126. doi:10.1016/j.tree.2006.11.007

Trap, J., Bonkowski, M., Plassard, C., Blanchart, E., 2016. Ecological importance of soil bacterivores for ecosystem functions. Plant Soil 398, 1–24. doi:10.1007/s11104-015-2671-6

Urich, T., Lanzén, A., Qi, J., Huson, D.H., Schleper, C., Schuster, S.C., 2008. Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. PLoS ONE 3. doi:10.1371/journal.pone.0002527

Valverde, A., Burgos, A., Fiscella, T., Rivas, R., Velázquez, E., Rodríguez-Barrueco, C., Cervantes, E., Chamber, M., Igual, J.M., 2006. Differential effects of coinoculations with Pseudomonas jessenii PS06 (a phosphate-solubilizing bacterium) and Mesorhizobium ciceri C-2/2 strains on the growth and seed yield of chickpea under greenhouse and field conditions. Plant and Soil 287, 43–50. doi:10.1007/s11104-006-9057-8

van Dam, N.M., Bouwmeester, H.J., 2016. Metabolomics in the rhizosphere: Tapping into belowground chemical communication. Trends in Plant Science. doi:10.1016/j.tplants.2016.01.008

Van der Ent, S., Van Hulten, M., Pozo, M.J., Czechowski, T., Udvardi, M.K., Pieterse, M.J., Ton, J., 2009. Priming of plant innate immunity by rhizobacteria and β-aminobutyric acid: differences and similarities in regulation. New Phytologist 183, 419–431. doi:10.1111/j

Van Wees, S.C.M., Van Der Ent, S., Pieterse, C.M.J., 2008. Plant immune responses triggered by beneficial microbes. Current Opinion in Plant Biology 11, 443–448. doi:10.1016/j.pbi.2008.05.005

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., Dufresne, A., 2015. The importance of the microbiome of the plant holobiont. New Phytologist. doi:10.1111/nph.13312

Velicer, G.J., 2003. Social strife in the microbial world. Trends in Microbiology. doi:10.1016/S0966-842X(03)00152-5

Vermeer, J., McCully, M.E., 1982. The rhizosphere in Zea: new insight into its structure and development. Planta 156, 45–61. doi:10.1007/BF00393442

Vetter, M., Karasov, T.L., Bergelson, J., 2016. Differentiation between MAMP Triggered Defenses in Arabidopsis thaliana. PLoS Genetics 12. doi:10.1371/journal.pgen.1006068

Vos, M., Wolf, A.B., Jennings, S.J., Kowalchuk, G.A., 2013. Micro-scale determinants of bacterial diversity in soil. FEMS Microbiology Reviews 37, 936–954. doi:10.1111/1574-6976.12023

Wallace, W., 1973. The distribution and characteristics of nitrate reduetase and glutamate dehydrogenase in the maize seedling. Plant Physiol 52, 191–196. doi:10.1104/pp.52.3.191

Wang, W., Shor, L.M., Leboeuf, E.J., Wikswo, J.P., Kosson, D.S., 2005. Mobility of protozoa through narrow channels. Applied and Environmental Microbiology 71, 4628–4637. doi:10.1128/AEM.71.8.4628

Wang, X., Wei, Z., Yang, K., Wang, J., Jousset, A., Xu, Y., Shen, Q., Friman, V.P., 2019. Phage combination therapies for bacterial wilt disease in tomato. Nature Biotechnology 37, 1513–1520. doi:10.1038/s41587-019-0328-3

Weingart, H., Völksch, B., 1997. Ethylene production by Pseudomonas syringae pathovars in vitro and in planta. Applied and Environmental Microbiology 63, 156–161. doi:10.1128/aem.63.1.156-161.1997

Wen, F., VanEtten, H.D., Tsaprailis, G., Hawes, M.C., 2007. Extracellular proteins in pea root tip and border. Plant Physiology 143, 773–783. doi:10.1104/pp.106.091637

Wille, L., Messmer, M.M., Studer, B., Hohmann, P., 2019. Insights to plant–microbe interactions provide opportunities to improve resistance breeding against root diseases in grain legumes. Plant Cell and Environment. doi:10.1111/pce.13214

Williamson, K.E., Fuhrmann, J.J., Wommack, K.E., Radosevich, M., 2017. The Annual Review of Virology is online at virology.annualreviews.org Downloaded from www. Annu. Rev. Virol 4, 15. doi:10.1146/annurev-virology

Wright, D.A., Killham, K., Glover, L.A., Prosser, J.I.M.I., 1995. Role of pore size location in determining bacterial activity during predation by protozoa in soil 61, 3537–3543. doi:10.1128/aem.61.10.3537-3543.1995

Yan, X., Liao, H., Beebe, S.E., Blair, M.W., Lynch, J.P., 2004. QTL mapping of root hair and acid exudation traits and their relationship to phosphorus uptake in common bean. Plant and Soil 265, 17–29. doi:10.1007/s11104-005-0693-1

Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W., Georgieva, S.S., 1993. Feeding habits in soil nematode families and genera - An outline for soil ecologists. JOURNAL OF NEMATOLOGY SEPTEMBER 25, 315–331.

Yim, B., Ibrahim, Z., Rüger, L., Ganther, M., Maccario, L., Sørensen, S.J., Heintz-Buschart, A., Tarkka, M.T., Vetterlein, D., Bonkowski, M., Blagodatskaya, E., Smalla, K., 2022. Soil texture is a stronger driver of the maize rhizosphere microbiome and extracellular enzyme activities than soil depth or the presence of root hairs. Plant and Soil 478, 229–251. doi:10.1007/s11104-022-05618-8

Zelenev, V. V, Bruggen, A.H.C. Van, Semenov, A.M., 2005. Modeling wave-like dynamics of oligotrophic and copiotrophic bacteria along wheat roots in response to nutrient input from a growing root tip. Ecological Modelling 188, 404–417. doi:10.1016/j.ecolmodel.2005.01.046

Zhang, C., Simpson, R.J., Kim, C.M., Warthmann, N., Delhaize, E., Dolan, L., Byrne, M.E., Wu, Y., Ryan, P.R., 2018. Do longer root hairs improve phosphorus uptake? Testing the hypothesis with transgenic Brachypodium distachyon lines overexpressing endogenous RSL genes. New Phytologist 217, 1654–1666. doi:10.1111/nph.14980

Zhang, L., Lueders, T., 2017. Micropredator niche differentiation between bulk soil and rhizosphere of an agricultural soil depends on bacterial prey. FEMS Microbiology Ecology 93. doi:10.1093/femsec/fix103

Zhao, X., Schmitt, M., Hawes, M.C., 2000. Species-dependent effects of border cell and root tip exudates on nematode behavior. The American Phytopathological Society 90, 1239–1245. doi:10.1094/PHYTO.2000.90.11.1239

Zhu, Y., Pierson, L.S., Hawes, M.C., 1997. Induction of microbial genes for pathogenesis and symbiosis by chemicals from root border cells. Plant Physiology 115, 1691–1698. doi:10.1104/pp.115.4.1691

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Rüger, L., Feng, K., Dumack, K., Freudenthal, J., Chen, Y., Sun, R., Wilson, M., Yu, P., Sun, B., Deng, Y., Hochholdinger, F., Vetterlein, D., Bonkowski M., 2021. Assembly patterns of the rhizosphere microbiome along the longitudinal root axis of maize (*Zea mays* L.). Frontiers in microbiology, 12, 614501. doi: 10.3389/fmicb.2021.614501

Rüger, L., Ganther, M., Freudenthal, J., Jansa, J., Heintz-Buschart, A., Tarkka, M.T., Bonkowski, M., 2023. Root cap is an important determinant of rhizosphere microbiome assembly. New Phytologist, 239, 1434-1448. doi: 10.1111/nph.19002

Niedeggen, D., Rüger, L., Oburger, E., Santangeli, M., Ahmed, M., Vetterlein, D., Blagodatsky, S., Bonkowski, M., 2024. Microbial utilisation of maize rhizodeposits applied to agricultural soil at a range of concentrations. European Journal of Soil Science, 75:4, e13530. doi: 10.1111/ejss.13530

Rüger, L., Feng, K., Chen, Y., Sun, R., Sun, B., Deng, Y., Vetterlein, D., Bonkowsk, M., 2023. Responses of root architecture and the rhizosphere microbiome assembly of maize (*Zea mays L.)* to a soil texture gradient. Soil biology and Biochemistry, 181, 109026. doi: 10.1016/j.soilbio.2023.109026

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