RHIZOSPHERE: A COMPLEX DETERMINANT OF SOIL MICROBIAL COMMUNITY

Atilade Adedayo ADEDEJI[*,**], Olubukola Oluranti BABALOLA[*]

[*]*Food Security and Safety Niche Area, Faculty of Natural and Agricultural Sciences, North-West University, Private Bag X2046, Mmabatho 2735, South Africa*
[**]*Department of Biochemistry and Microbiology, School of Environmental and Biological Sciences, Rutgers University, New Brunswick, New Jersey, USA*
Corresponding Author: olubukola.babalola@nwu.ac.za

Abstract. Several attempts have been made by researchers to evaluate the abundance and distribution of microorganisms in the soil following the first discovery and publication of the estimated number of prokaryotes that could be occupying the soil. Many described this information based on the relatedness of the community structure to the function of ecosystem. It was revealed that the amount and heterogeneity of microbial species inhabiting the soil are significant for the continued sustenance of plant growth and development, as a broad assortment of microbes are involved in vital soil functions. Current studies further explain the roles of the rhizosphere in defining the arrangement and composition of the soil microbes, the ability of plants to specifically shape their microbial community, the interplay between plants and soil in shaping their community. Furthermore, the bulk of soil microbes are yet to be cultured and their functions still largely unknown. With the advent of molecular biology, there is a growing concern about the possible effects of difficult-to-culture microbial species in soil environments and the contributing factors to their dynamics. This review consequently deploys old and recent molecular tools in describing these variables and introduces metagenomics as a modern tool to unravel the dynamics and community functional potential focusing on up-to-date data in describing them.

Keywords: exudates; metagenomics; rhizosphere; root signaling; microbiome.

INTRODUCTION

Soil microbial community describes one of the most beneficial and largest reservoirs of biodiversity on earth [68]. Members of this community hold meaningful interactions with plants present in the soil as the individual microbial populations are necessary for biological processes that contribute to plant performance and productivity. Microbes mediate processes that sustain soil functions. They exercise varying effects on crop growth and development, mobilization and transformation of nutrients in biochemical cycles and soil productivity [55]. Microorganisms in the soil also contribute substantially to plant health and development by preventing attachment or adherence of pathogenic species to plant parts, inhibiting pathogen spread and proliferation, inducing systemic resistance thereby improving plant growth [2]. They also provide plants with nutrients [63], increase the plants' tolerance to drought [17] and even protect plants against herbivore [51].

Bacterial population deduced to be present in one gram of soil may approach 10^{10}–10^{11} cells [52] and fungal hyphae can be estimated to be 200 m/cm^3 [36]. The abundance, richness and composition of these microbes are subjective. They are sensitive to modifications which may be influenced by various biotic and abiotic factors [73] such that, in changing environments, minute shifts in soil microbial composition may drive notable changes in health, growth and how nutrients are transformed in plant-soil system [7], plant developments via either beneficial or deleterious interactions that influence root and shoot development, nutrient demand, growth and resistance to biotic and abiotic stresses [17].

The implied diversity and dynamic composition of the soil microbiota also bears direct relation to soil function, structure and aggregation. Considering the dynamics of these microbes, it was argued that the effects of the physiological activities of plants should be taken into account as a more important factor than any other non-living factor that influences soil microbiome. This is due to the resultant consequences on the activities of the wide varieties of organisms present in the ecological system of the soil. It was deduced that the existence of plants does not only undividedly have direct effects on the inhabitation of soil microbes but also got some clear influences on the abiotic determinants that shape their growth and distribution indirectly. A different study reported that the properties of the soil and the physiographic determinants are the paramount components when defining the composition, structure and abundance of the soil microbial communities; and in return, these soil microorganisms can have vital consequences on the developments of soil aggregates [10]. Thus, as the importance of soil microbiota cannot be underestimated for the long-term sustainability of agricultural systems, a quantitative description of soil microbial structure as influenced by the region in which they both coexist is of great significance.

Over the years, many approaches employed in studying microbial diversity have shown several limitations. Soil microbial consortia have been challenging to be fully described largely due to the extensive diversity of their phenotypes, genotypes and crypticity [41]. Currently, less than 1% of this diversity could still be cultivable by traditional methods [68]. Nevertheless, the discovery and use of new microbial identification methods is increasingly gaining more scientific reputation and correcting the perspective of microbial ecology. The composition, structure and function of microbial consortia can now be estimated through metagenomics. Metagenomic methods offer the plausibility to evaluate the overall heterogeneity directly by circumventing the constraints posed by cultivation-based techniques. Several researchers have applied metagenomics in the study of different range of

soil environments [31]. The use has waxed greatly and indispensably advancing studies in microbial ecology; however, we have to be cautious of the biases involved.

Soil: a unique environment

The soil environment is very intricate. The soil is fundamental and irreplaceable; it represents a diverse, highly heterogeneous environment and provides several key functions to the ecosystems [20]. The soil is formed by an aggregation of geological parent matter, glacial and geomorphological antiquity, the presence and actions of biological species, specific cultural or anthropogenic history and disturbance regimes. The different elements of the solid fractions that make up the soil (sand, clay, silt and organic matter) represent an innumerable assortment of microhabitats. The soil as a habitat for several organisms is consequently open to differing conditions which may be ramified into abiotic, biotic and nutritional requirements over the micrometre scale. The exact characteristics of a habitat housing a community of organisms is determined by a complex interplay of geology, climate and vegetation (see Fig 1). Therefore, it is possible for one to hypothesize that in a "stable" system specific microhabitat is filled with organisms that have the best capacity to find a role and become stabilized. These organisms together form the key catalysts of the biochemical processes in soil ecosystems. Therefore, microhabitat and organismal biospheres determine the microbial processes in soil, diversities and species richness [53, 54]. The totality of the fresh weight of organisms below temperate grasslands can be more than 45 tonnes per hectare, matching or exceeding the above-ground biomass. Of these, bacterial species are the most abundant as they precede archaeal species which show abundance10-fold less. Fungi, nonetheless, also occupy significant niche and they oftentimes contribute the most to the total microbial biomass in soil ecosystems [1]. However, the soil structure, heterogeneity and discontinuous system, disparity in nutrient abundance and differences in energy sources cause microbial populations to occupy very distinct microhabitats. Soil as microhabitats are seemingly dynamic and changes over time as the measures of the environments rely solely on the size of the organisms present. Even in cases where the usable space is unrestricted in the soil, these microorganisms still occupy spaces that are favorable to their existence and may represent a minute proportion (usually not up to 5% of the entire space).

Another unique distinguishing feature of the soil as a microhabitat is the ability possessed by the solid phase to accumulate essential organic molecular compounds and growth factors which include proteins and nucleic acids. The amount and activities of these biological molecules generally influence the actions and occurrence of extracellular enzymes accumulated in clay minerals or trapped within humic molecules as they sustain enzyme activity, protect them against proteolysis as well as thermic and pH denaturation [47]. Deoxyribonucleic acid (DNA) molecules bound to particles of sand and clay and humic molecules are commonly safeguarded from degradation by nucleases, but can still be picked up by competent bacterial cells in a bioprocess known as transformation. The buildup of organic syntheses by soil colloids slow down microbial activity and could affect the community structure.

In the soil, the breakdown of soil organic matter is impacted by resident microbes via enzymes that catalyze reactions needed for life processes, the formation of organic matter and soil structure. Enzymes usually produced, accumulated and inactivated have great effects on nutrient cycling processes and consequent microbial diversity, such that soil enzyme activities can be an indicator of biochemical processes in the soil and possible alterations in the soil management. Soil enzyme activity can be used to indicate the intensity of certain biochemical processes. Soil enzyme activity can be used as a unique integrative biological indicator of the intensity of certain biochemical processes, underlying soil evaluations due to the close relationship of soil enzymes with soil biology and the rapid response to changes in soil management. Thus, a good understanding of the microhabitat is essential for improved crop productivity and soil health.

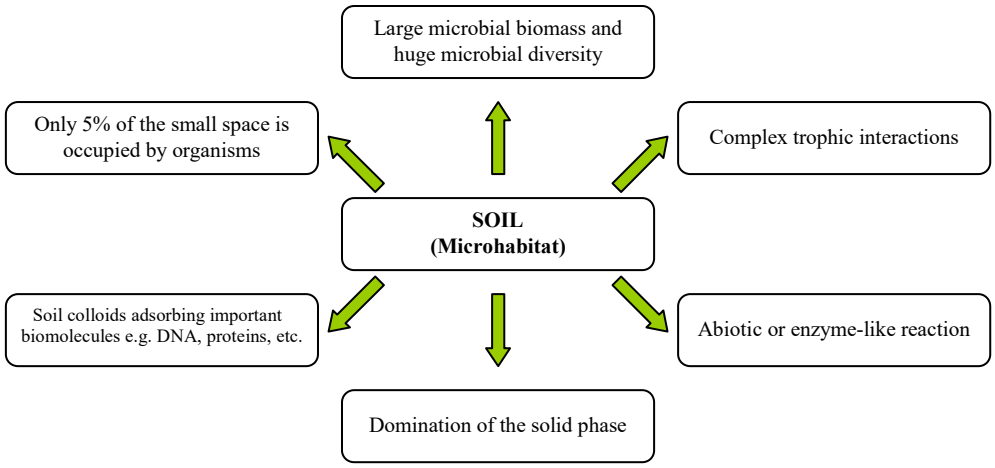

Figure 1. Selected characteristics of the soil as a microhabitat

Microbes in the soil

Soil microbes generally measure less than 100 µm and are one of the most plenteous and distinct assortments of soil organisms. Investigating a gram of soil, estimates of tens of thousands of species are present. Bacteria and fungi are the most studied of all soil microbes. Bacterial species, are typically single-celled prokaryotes; they need soil water films to thrive and also to survive in the soil matrix. Filamentous fungi, however, are less constrained biological entities as they can cross air-filled pore spaces. More than 25 distinct bacterial phyla have been identified within the soil ecosystem. Hence, most soils appear to be strongly dominated by bacteria from the following genus - *Acidobacteria, Proteobacteria, Actinobacteria, Bacteriodes*, and *Firmicutes* [35]. Fungal species in soil include the following phyla*, Basidiomycota, Ascomycota,* and *Glomeromycota* existing as single populations or aggregated with plants in associations like mycorrhizal communities. Our previous study on beneficial soil microorganisms also indicated the abundance of beneficial microbes belonging to the genus *Bacillus, Pseudomonas, Bradyrhizobium, Rhodococcus, Agrobacterium* and *Enterobacter* [2]. Archaea and viruses are also present within soil environments [18]. Archaea are single-celled prokaryotes but are unique in their evolutionary history and their tolerance to environmental stress. Viruses are typically extremely small [once referred to as filterable particles] and consist of nucleic acids surrounded by a protein coat resulting in the term 'nucleocapsid'. Microorganisms in the soil contribute many essential ecosystem functions. These include decomposition and degradation of organic matter, carbon and nutrient cycling, modification of soil structure, disease suppression, plant's growth regulation, development and primary productivity. Bacteria and fungi play notable roles in the soil as the primary decomposers of organic matter as well as agents that mobilize mineral nutrients and elements. These microorganisms are therefore determinants of both the rate at which nutrients become available to plants and the amount of carbon stored in soils.

The rhizosphere effects: the root of the matter

The region of soil unearthed in the range of about 2 mm in length from the root surface is referred to as the rhizosphere. Rhizosphere is a chemically complex zone having a changing microbiome [22, 67]. Usually, the rhizosphere comprises the plant roots and the neighboring soil, more often this region are seen to include rhizoplanes which are attachment of microbial biofilms . This is a widespread definition coined more than one hundred years ago by Hiltner [24], and later modified by Pinton to be the precincts of the soil that are under the control of the root and the tissues of the roots colonized by microbes [49]. In this region, a strong relationship exists between soil biota and aboveground vegetation. This significantly changes both the physical and chemical characteristics of the soil and further goes on to modify the community of microbes in the near root region.

The characteristics of the soil in near proximity of plant roots can be transformed by series of processes that take place during the phase of growth. These processes in turn modify the near root microbiota. During the growth period, exudes with low molecular mass [e.g, sugars, amino acids and organic acids], polymerized sugar [that is, mucilage], root border cells and dead root cap cells are released. These rhizodeposits are utilized as source of carbon and energy by soil microbes and they account for an estimated 25% of the carbon allotted to the roots of cereals and grasses [32]. These deposits in the near root regions also comprise secondary metabolites, which may include anti-microbial substances, nematicides and flavonoids [48], usually associated with the establishment of symbiosis or pest and pathogen resistance. The Soil pH, another vital determinant of the soil microbial structure, could rise or drop by up to two units in the root region due to the ion that will be released and uptaken. Uptake of water and respiration in the root affect the soil oxygen pressure, thereby impacting microbial respiration. Also, as chelators such as phytosiderophores, sequester metallic micronutrients are released, they have significant effects on the nutrients availability around the root region. However, untangling the influence of these drivers is complex, as the ways of influence are interconnected. For instance, the measure and manner of influence of roots on the features of soil could vary depending on the type of soil, the species of plants and the feedback response of the root region microbial occupants. In addition, the characterization of the near root community could be questioned by several changes of properties of the soil along the region of the root as it relates to the age and physiological state of the plant.

Key players in soil microbial distribution

Root exudates

Generally, plant root exudates are metabolic response of the plants and they mediate interactions in both the roots of plants and the microbes in the near regions [12]. Comparing one plant to the other, the type, chemical constituents and amount of these exudates differ and could directly or indirectly impact the corresponding composition and abundance of near root microbes. Consequently, this shapes the rhizosphere to be a 'hotspot' microhabitat where there is an increasing microbial interaction, abundance and exchange of genetic materials.

Plant root releases close to 10 to 250 mg C/g which is an estimated 5%–21% of the photosynthetically fixed carbon by plants is exuded most commonly as amino acids, soluble sugars or secondary metabolites [3]. The rates of exudation of these substances differ widely among species and environmental conditions, this influence changes in soil parameters and feedback to affect the growth of plants and microbial consortia [57]. Mostly these carbon sources supplied by plants to

the microbes after breakdown return in form of minerals [34]. Consequently, the released materials [minerals] create unique environments for the microorganisms and alter the input of nutrients in the soil. The resource-altered environment then creates substantial effects on the configuration of soil bacterial communities [50], in this root region, microorganisms usually give a unique response to the minerals released. Considering the aforementioned that plants release different root exudates, it could be easily inferred that the difference in the compositions of root exudates will most likely select distinct rhizosphere communities [41]. Also, the specific metabolites secreted within the root region can arouse increased responses in many soil microbes. As an example, flavonoids from plants can be an attractant not just for symbionts like *Bradyrhizobium ejaponicum*, but may also be for disease-causing organisms like *Phytophthora sojae*. Similarly, flavonoids also enhance plant-fungal relationship in germination of spore and branching of hyphae. In addition, they influence quorum sensing. Likewise, constitutive secondary defensive metabolic substances, which include pyrrolizidine alkaloids, can modify the near root microbial environment by promoting tolerant or resistant microbes or in some other circumstances, microbes that breakdown these substances.

Antimicrobial substances

In the rhizosphere, a nutrimental rich environment, plants and microbes interact and exchange nutrients that may not even be directly available. The microbes in the near root are involved in key functions such as promoting the growth and development of the plant, nutrient acquisition, yields, disease and insect resistance mechanisms while the photosynthetic produce from plants is used as both a substrate and energy source for rhizosphere microbial support [42]. In this regard, the plants do not only offer these nutrients for these microbes, some species of plants also hold some distinct antimicrobial metabolites present in their plant root secretions which could ward off some susceptible species of microorganism. Some of such plants are employed in herbal medicine. For instance, chamomile, thyme and eucalyptus, and other related secondary metabolic products of such plants [11] affect underground diversity. Interestingly, some of these antimicrobial inducing plants can also hold significant consequences on the communities of the soil microbes. Furthermore, in the near root, microbial community interactions can also be impacted by substances produced by other microbes. For instance, a study by Jones et al highlighted that *Streptomyces* growth around the root was favored by interplays with the yeast *Saccharomyces cerevisiae* via the emission of trimethylamine (TMA), a volatile substance which increases the pH around the root. It was identified that the TMA synthesized considerably modified the root region and distinctively decreases the availability of iron, this consequently impacted the viability and structure of resident organisms[33]

Signaling and interconnections of the plant microbiome

The connection existing among plants and millions of microbes entails great communication [66]. Quite a number of signatures encoding communications through quorum sensing and different signaling molecules have been identified in metagenomes of microbe in close association with plants [8]. Nevertheless, the mechanism involved in the interaction of this community to bring about a structured microbiome still lacks proper understanding. Volatile organic substances are liable for 'microbial small talk' but can also act as long-distance messengers for communication with the plant host [59].

Soil and plant types interplay to shape microbial community

Composition of microorganisms in the soil mediates vital processes in the soil that could affect plants growth and development [29]. For instance, microbes in the soil stimulate nutrient cycling and enhance the availability of nutrients to plants [4]. Some specific groups of microorganisms have the ability to fix nitrogen [6] and make nutrients available to plants [60], which consequently extend to affect the global nutrient cycles. Furthermore, microbes in close association with the root can also modify specific plant characteristics such as its ability to protect against diseases [58], root architecture [74], and the ability of the plant to withstand water scarce conditions. Commonly, the mechanism utilized involves translocation, mineralization and mobilization of soil P, K and Fe through the production of phytochormone (cytokinins, gibberellins and auxins). Together with antimicrobial substances to protect the crops against diseases.

Recent advances in molecular biology which allows the study of the genetic material directly obtained from the soil has further afforded scholars opportunities to examine a much wider spectrum of microbes resident in the near root region. In an experiment using PCR-denaturing gradient gel electrophoresis [DGGE] to investigate the 16S rRNA gene fragments, it was first reported that the composition of bacteria species in the near root region is usually influenced by multiple interplays which involve the type of soil, species of plants and the region occupied by the root when they investigated three plant species (Grape, chickpea and Sudan grass) planted in three Californian soils (sandy, loamy and clay) [39]. Other similar studies indicated that either the species of plants or type of soil are usually the most considerable determinants when examining the community construction of the near root microbial community. It was further identified in another investigation on the extent to which the rhizosphere will be plant-dependent and if the resultant effect is promoted when the same crop is grown for

two continuous years. It was recorded that the planted potato, strawberry, and oilseed rape was observed in the second year that the plant-dependence changed in the relative bacterial compositions [63]. This was not limited to species of plants alone, as cultivar can also alter the structure of the near root microbe [27]. However, the interplay between plant and soil types and the structure of the rhizosphere microbiota is a more elaborate subject and exceeds the scope of this review.

Changes in microbial diversity during plant developmental stages

Plant species possess different kinds of root architectural pattern, metabolism and growth strategies that influence the microbial quality and diversity of soil [70]. Current data also show that the numerous actions of microorganisms and their corresponding abundance can depend on the plant species [14; 72]. Furthermore, the balance of the microbial community varies according to certain period owing to the differing and dynamic root exudates which could vary during the life processes and how the plant responds as season changes [37]. Similarities among species of plants revealed that there are clear observable differences among plant rhizosphere communities when evaluating the community structure and function at specific period of time along their growth phase, with the biggest changes observed in young plants [28, 62]. Furthermore a work conducted on the influences of cultivars and growth of plants on the rhizosphere community composition revealed that cultivars had a near root effect on bacteria community and the stages of growth modified the betaproteobacterial communities greatly [27]. It is indicative that the community of microorganisms inhabiting the rhizosphere of a plant is not constant but changes over time with the same plant type.

Specific plants, specific microbial community

Commonly, rhizosphere microbial communities have lesser diversity than those of the bulk soil [23]. Out of the prevailing population of microorganisms inhabiting the bulk soil, the root of the plant creates an environment suitable for the survival and thriving of specific microorganisms in the rhizosphere. The plant roots usually do not do this alone but rather collectively with some other significant drivers which include the genotype of the plants and the soil type [19]. From earlier reports, a mere relationship between the different compositions of the bacterial community and plants were initially documented [21]. However, subsequent discoveries showed there was more to the relationship. Viebahn et al observed that the microbial consortia in the rhizospheres of individual plant species occupying a particular soil were also usually different [68]. It was inferred that considering the significance of mutualistic/ parasitic interplay existing between plants and microorganisms in microbial food webs, a robust influence of a particular species of plant on soil fungal and bacterial community composition can possibly be expected. Also, an extensively studied association between rhizobia–legume interactions further pinpoint the singular effects of plants on microbial diversity and it precision [64].

When exudates are released in the roots, they encourage relationships connecting specific microbes and plant species [16, 61]. This interplay could alter the composition of microbial consortia in the root in a manner that favors specific plants [9]. Badri et al. observed that a mutant Arabidopsis ABC transporter that synthesizes chemical compounds (phenolics) better than sugars in relation to the wild type gave notable modifications in the native community of microbes in the soil [3]. The resulting modifications in root exudate synthesis were observed to favor beneficial bacterial communities which included plant growth promoting rhizobacteria, microorganisms that fix Nitrogen and metal remediator. In a similar experiment, Micallef et al. also noted that Arabidopsis ecotypes did not only exude specific sets of substances but that the changes in the root exudates allowed a distinguished near root bacterial communities which may be favorable for the existence of the plant [43]. Furthermore, benzoxazinoids released in moderate quantities from the root of some cereal plants was identified to influence the survival of rhizosphere microorganisms. In maize (*Zea mays*), there is a natural antimicrobial substance [benzoxazinoid] called 2,4-dihydroxy7-methoxy-2H-1,4-benzoxazine-3(4H)-one (DIMBOA). Nacke et al. observed that *Pseudomonas putida* KT2440 does not just tolerate DIMBOA but the compound also chemotactically attracts them [47]. However, in the roots of a mutant species of the maize, KT2440 were notably not present as much as the wild-type plants, pointing that DIMBOA particularly allows this plant beneficial bacterium. This suggests that these microorganisms were specifically enriched in the soil to further protect them from diseases causing organisms. Badri et al. further highlighted that by adding specific mix of native chemicals obtained from Arabidopsis, root exudates created a different near root community of microorganisms which appear to possess the trait to break down atrazine or included more mutualistic microbes [3]. Also, in a purely isolated exudate of seeds, young plants and rootlets of tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativus*) and sweet pepper (*Capsicum annuum*), the most common constituent was organic acids. The strength possessed by strains of rhizobacterial to survive in vitro on citric acid as the only source of carbon seemed to correspond to their potentiality to colonize the root. See Table 1 for specific plants identified with specific microbial community.

Plant species composition alter soil microbial community

A molecular-based experiment by Wardle et al. documented that in a field, when specific plants were removed from an assortment, the removed plant had a

Table 1. Specific Bacterial phyla dominating rhizosphere and assemblages

Host Species	Dominating Phyla
Cultivated rice [*Oryza sativa*] [c]	*Actinobacteria, Proteobacteria,*
Cultivated potato [*Solanum tuberosum*][a]	*Actinobacteria, Proteobacteria*
Oak [*Quercus* spp.]	*Acidobacteria, Actinobacteria Proteobacteria*
Poplar [*Populus deltoides*]	*Acidobacteria Proteobacteria*
Cultivated potato [*Solanum tuberosum*]a	*Actinobacteria, Bacteroidetes Firmicutes, Proteobacteria*
Thale cress [*Arabidopsis thaliana*]	*Acidobacteria Actinobacteria Bacteroidetes Proteobacteria*
Wild oat [*Avena fatua*][a]	*Actinobacteria, Firmicutes Proteobacteria*
Cultivated maize [*Zea mays]*[b]	*Proteobacteria*
Sugar beet [*Beta vulgaris*][a]	*Actinobacteria,Firmicutes Proteobacteria*

[a]data obtained with phylochip. [b]data obtained with a specific system-designed 16S rRNA gene microarray. [c]data obtained from whole-metagenome shotgun and 16S rRNA gene clone library
Table culled from Mendes et al [41]

noticeable impact on the community of microorganisms; nevertheless, there was no observable difference in the total biomass of bacterial and fungal species [70]. To their disappointment, when they tried to aim at a more pronounced shift in the soil microbial diversity, they only identified mere temporary root-induced influences. Furthermore, throughput shotgun sequencing employed in a study of soil microbial consortia in close relationship to antarctic vascular plants carried out by Molina-Montenegro et al. in a view to studying how microorganisms influence changes in plants under unfavorable conditions resulted in that bacterial species had a soaring relative richness in the sites (98%) which was far more than Archaea (0.22%) and Eukaryota (1.77%), among the bacterial Phyla, *Proteobacteria, Actinobacteria, Bacteroidetes, Acidobacteria* and *Firmicutes* were the largely abundant, estimating almost 85% of the sequences in the near root soil samples [44]. These identified Phyla have often been reported to abound in other soil samples with specific plants [26]. They also make up an essential root microbiome where they play pivotal role in promoting the growth of plants due to their ability to acquire nutrient and tolerate unfavorable conditions [13]. A conceivable explanation for the role and observed relative abundance of these Phyla is that specific plant composition in a specific habitat could shape the root region by selectively favoring specific species across these sites [5, 38]. Bakker et al. mentioned that the species richness of nearby plants caused a major alteration in the structure of the *Streptomyces* spp. community of neighboring vegetal species [4]. As plant richness increases, the community of *Streptomyces* decreases and there were observable increases and relatedness in the new community. The more distinct the community of plant is, the more diverse the composition of roots exudates found in such environment, and this consequently influences the diversity of microorganisms inhabiting such region.

Notable methodological approaches in the study of soil microbial community

Taxonomic and methodological limitations have to an extent hindered the study of species and genetic diversity in microbial communities. Over the years, the methodologies employed in the investigation of the rhizosphere have been rooted deeply in the use of several culture-based procedures and molecular technique. As quite a number of culture media were composed in a bid to heighten the recovery and isolation of several groups of organisms within soil microbial communities. Scientific developments further birthed the introduction of a biolog-based method for the direct examination and study of the potential activities of soil microbial communities, commonly referred to as community-level physiological profiling (CLPP). However, a fundamental challenge associated with many conventional physiological and biochemical approaches was their dependence on the study of phenotypic expressions (e.g., enzymes, respiration, and catabolic potential), and despite the demonstration of metabolic activities, many microbial populations are yet unculturable under laboratory conditions. Furthermore, the resulting metabolic fingerprints seem to be a less-accurate, weak or false representation of the in-situ functional diversity in a typical consortium of microbes [62]. In addition, as a result of weak gene expression following the test conditions, using biochemical test methods resulted in fairly common negative results. Several procedures have been identified to surmount this challenge. These approaches include the use of signature lipid biomarkers (SLB) which include phospholipid fatty acids (PLFA), nucleic acid technologies (molecular biology) such as amplified rDNA restriction analysis (ARDRA), Denaturing Gradient Gel Electrophoresis/ Temperature Gradient Gel Electrophoresis (DGGE/TGGE), terminal restriction fragment length polymorphism, ribosomal intergenic spacer length polymorphism. However, these PCR-based techniques are in source reproducible and robust, they are predisposed to possible bents. Benefits and drawbacks of different techniques are summed in Table 2.

Metagenomics: the new way of seeing the soil

The use and advancement of metageomic tools in the study of soil microbial consortia offer a new way of thinking and system-level perspective of microbial diversity. In lieu of analyzing just one organism or single function, this approach explores the whole consortium of genes in a community, allowing the building of a framework of genes and functions on which to establish systems about community structure

Table 2: Common techniques adopted in the investigation of soil microbial communities before metagenomics

Method	Advantages	Weaknesses
DGGE/ TGGE	Renders full sequences that can be subjected to additional analysis	Gel-to-gel variation PCR primer design (GC clamp) only short sequences < 400 base pair (bp) can be analyzed using TGGE
SSCP	Presents full sequences that can be subject to further analysis Technically simple gel preparation Variant folding of single strand molecules	Complicated DNA preparation (two purification steps) Only short sequences < 200 bp can be analyzed
T-RFLP	Technically simple High discrimination power	Loss of some variability (sequences not cleaved or cleaved near to primer)
LH-PCR/ ARISA	Technically simple	Low discrimination power
Microarrays	No bias due to PCR	Detects only sequences corresponding to probes Detection limit lower than in PCR-based methods
PLFA analysis	Can cover whole communities across kingdoms Quantitative description of the community	Low taxonomic separation limited to community composition analysis

Source: Garbeva et al. [19]
Abbreviations: DGGE/ TGGE- Denaturing Gradient Gel Electrophoresis/ Temperature Gradient Gel Electrophoresis; SSCP- Single-strand conformation polymorphism analysis, T-RFLP- Terminal Restriction Length Polymorphism, LH-PCR/ ARISA- Length Heterogeneity-Polymerase Chain Reaction/ Automated Ribosomal Intergenic Spacer Analysis, PLFA-phospholipid fatty acids

and function. Metagenomics involves the genomic investigation of microbial communities [68]. This approach entails the direct isolation of DNA from an environmental sample [water, soil, gut], and then analyses the DNA sample afterwards, such that it further unveils the diversity concealed within environmental samples. Metagenomics has a high power of genomic analysis, such that when the 16S and 18S rRNA are sequenced, the regions of microbe resident in the natural samples otherwise permits a straightforward classification of genera, circumventing the stress to isolate and culture individual microbial species. Nonetheless, the complexity linked with metagenomic DNA results from its build-up as it is a composition of genomes from several distinct organisms. This could consequently result in a challenging analysis and relatively intricate approach.

However, with this improvement and popularization of metagenomics, a tremendous amount of research on the heterogeneity of microbial consortia have been conducted and also in progress [40; 45]. Metagenomic approach has also been efficiently utilized by several researchers in recent times to advance comprehensively their description of taxonomic and functional diversity of soil microorganisms [30; 71]. One of such reported developments was Nacke et al. who showed that about 10% of environmental microbial sequences could possibly be lost from classical PCR-based Small Subunit ribosomal RNA gene surveys, which often include members of the Candidate Phyla Radiation (CPR) and also uncharacterized Archaea [45]. This report underscores previous approaches and further provides fruitful avenues for describing additional phylogenetic lineages. Furthermore, the arrival of next-generation sequencing (NGS) now allows scholars to investigate large sequences of a specific genome. The arrival of NGS is increasingly changing sequencing technology and the landscape of metagenomics. Still, these unexplored microbial niches are in great need in premises where metagenomics tools are being utilized to unveil the hidden potential of such valuable environment. For instance, a recent study in Portugal by Romao et al. used NGS in combination with cultivation-based approaches to study the community of fungi and prokaryotes for the occurrence of potentially diseases causing organism in beach sands in Portugal [56]. The study highlighted that cultivation-based fungal enumeration showed low and variable concentrations of the species targeted (yeasts and dermatophytes) [57]. This otherwise showed that the population was inadequately represented in the community when analyzed by NGS targeting the ITS1 region. Conversely, NGS showed that uncultivable *Purpureocillium liliacinum* were present among the complete fungal community. It was also reported that cultivable fecal indicator bacterial concentrations were moderate during the investigation and were not similar to the communities marked by NGS. This further buttresses the importance of metagenomics in the understanding of the biochemical functions of uncultivable microorganisms and their interplay within their environments. Nevertheless, it is believed that metagenomics is still underutilized as this breakthrough in microbial ecology holds a great promise for tapping the rich genetic resources, phylogenetic and functional diversity of microorganisms that appears difficult to culture.

Approaches to metagenomics

Metagenomics is divided into two main approaches, which are geared at numerous parts of the local microbial community associated with the soil habitat. The first technique, which is also identified as ‘sequence-driven metagenomics’, the DNA obtained from the soil is sequenced and analyzed with bioinformatics and computational tools The metagenomic sequences will then be subjected to comparison with sequences present in an open and accessible database such as Genebank. The genes are then assembled in groups of much related function, and the natures of proteins that conduct those functions. The construction of metagenome library involves successive steps which include: [1] Recovery of whole

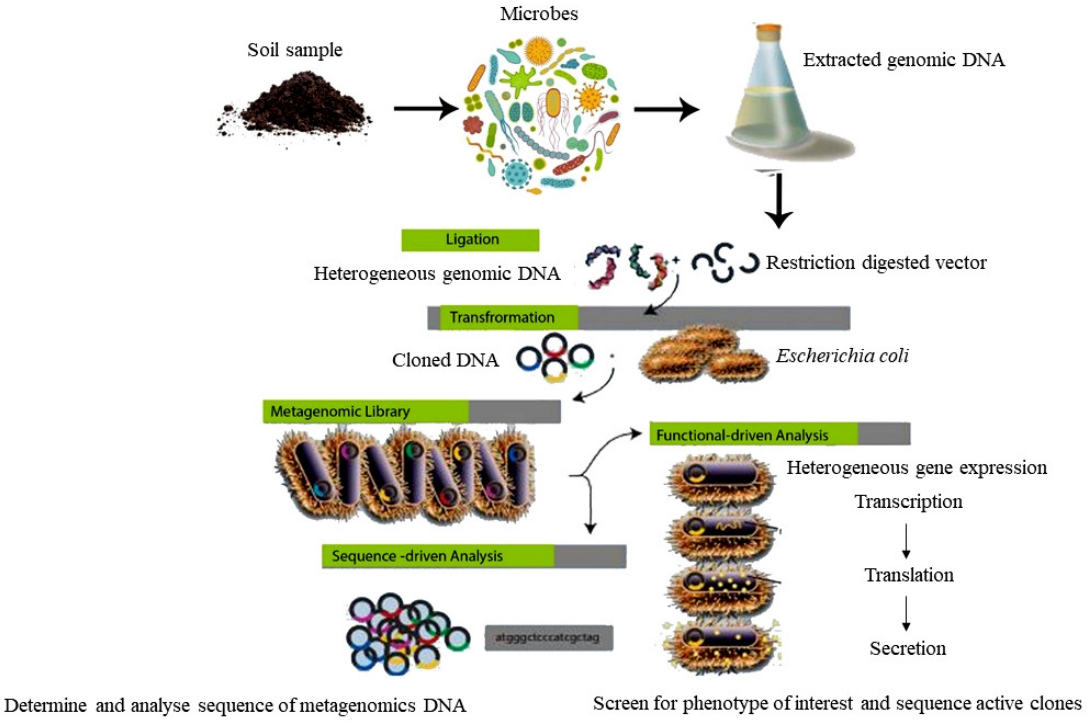

Figure 2. Construction and analysis of metagenomic libraries.

DNA from an environmental sample; [2] shotgun cloning of random DNA fragments in a proper vector; and [3] reconstructing the clones into a host bacterium as well as screening for positive clones. Metagenome libraries built of small DNA fragments in the range of 2–3 kb render high-grade coverage of the metagenome of an environment than those with larger fragments. Reports show that to recover the genomes from limited groups of microbial communities, not less than 1011 genomic clones will be required [30]. Small-insert DNA libraries are also important to select for phenotypes that are encoded by singular genes and for reconstructing the metagenomes for genotypic analysis. Large-fragment metagenomic libraries (100– 200 kb) are advantageous while reviewing multigene biochemical pathways. See Fig. 2 for construction and analysis of metagenomic libraries.

In the other method termed 'function-driven metagenomics', the isolated DNA from the soil is also obtained and filled into an alternate host as a storage technique, but instead of proceeding to a sequencing step, the captured fragments of DNA will be screened, or 'cloned', for a specific function. It is required that the surrogate host is devoid of this function so that acquisition of the function by the host following the metagenomic DNA expression can solely be said to be a function of the presence of the metagenomic DNA. In function-driven metagenomic investigations, libraries are screened on the basis of a preferred and distinctive phenotypic expression on a specific medium. This approach was used in a study by Tringe et al. who performed compositional and functional comparisons of microbial communities from two nutrient-poor and two nutrient-enriched environments [65]. The major concern of the approach was centered on gene function rather than genome composition, thus overcoming limitations experienced when assembling genome from complex environments. Researchers however, demonstrated that gene function and structure differed in nutrient-limited as compared to nutrient-abundant environments. Functional metagenomics can consequently be viewed as a reliable explorative tool for the identification and characterization of new genes [46], metabolic traits, bioactive compounds [15] or pathways [25] from yet to be cultured soil microorganism.

Limitations and way Forward

Studies have shown that the two approaches have been very effective in appraising the diversity of function of the microbial world. Nonetheless, both methods still possess their benefits and weaknesses. The sequence driven approach, on the one hand, is still confined by existing information. For instance, if metagenomic gene information is not an identified function collected in the databases, then, limited information can be extracted about the gene sequences. However, one way to solving such challenges confronted by soil microbial ecologists is to drive the generation of a wide catalog of all microbial consortia members and functions for at least a reference soil. This comprehensive reference dataset would cast more light and be a pool of the yet unknown structure of a soil microbial species frequency distribution. This could also, possibly be a prospective reference for evaluating community composition shifts across soil landscapes. The function driven analysis, on the other hand, can define genes that have not been identified to anything earlier examined as genes are distinguished by their displayed function instead of sequence.

However, the shortcoming is that the common genes from organisms in wild communities are not shown simply by the selected surrogate host. Furthermore, a very weak level or no expression of the preponderance environmental genes could also be an issue. In another instance, enhanced gene expression can be obtained by inputting metagenomic DNA into several supplementary alternate hosts such as *Streptomyces, Bacillus, Pseudomonas, and Agrobacterium*. Thus, regarding the inadequate ability of *E. coli* to express genes from different taxonomic groups of organisms, additional shuttle vectors with extended host range are required.

CONCLUSION AND FUTURE PERSPECTIVE

From this review, we have been able to demonstrate the inherent ability of plants and soil types in shaping their own microbial community. Furthermore, it was suggestive that the composition microorganisms can be altered solely or synergistically by the types of plant or/and soil. In some cases, microbiomes are suitably formed by specific plants based on their metabolic and physiological responses or shaped to complement the beneficial effects they confer. Microbial diversity and balance is a key for healthy plants. Traditional knowledge and current perceptions form a clearer picture on how composition could go a long way to determine the ability of the plants to resist other disease-causing organisms. New insights further showed how notable microbial diversity can play key roles as antagonistic phytopathogens. Despite the fact that plant microbial diversity depends on these factors, the secondary metabolites which originate from plants often trigger the arrangements of species compositions and should be considered in future screening strategies. Usually, microbes associated with vegetal create a network which can be influenced by soil and plant types. This network models the soil and plant microbiomes. However, it is still left to reason that those plants that modify their microbiota in a manner that is profitable to their reproductive success and survival will be favored during evolutionary selection. Meanwhile, it is important to highlight that the factors affecting microbial diversity in the soil are not just limited to the points discussed [69] (see Fig 3) and microbial structures are not solely influenced by these factors but also by their functions. Also, by the close relationship with microorganisms from the same soil environment, plants at times can easily reach a better fitness advantage than if they are in relationship with microorganisms from other soil environments. New developments in investigating and understanding the diversity of microorganisms are wrought with taxonomic and methodological deficiencies.

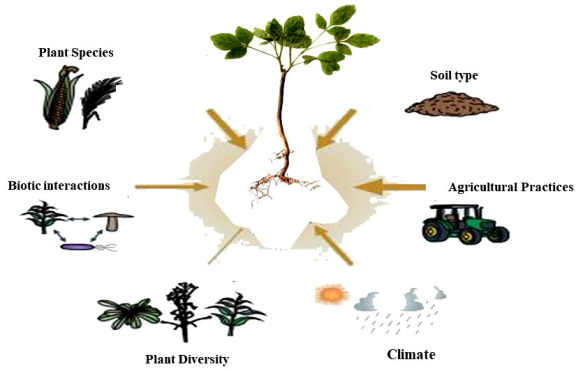

Figure 3. Major determinants of soil microbial community structure

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REFERENCES

[1] Aislabie, J., Deslippe, J.R., (2013): Soil microbes and their contribution to soil services. pp. 143-161. In: Dymond, J.R. (ed.): Ecosystem services in New Zealand – conditions and trends. Manaaki Whenua Press, Lincoln, New Zealand.
[2] Babalola, O.O., (2010): Beneficial bacteria of agricultural importance. Biotechnology letters, 32(11): 1559-1570.
[3] Badri, D.V., Chaparro, J.M., Zhang, R., Shen, Q., Vivanco, J.M., (2013): Application of natural blends of phytochemicals derived from the root exudates of Arabidopsis to the soil reveal that phenolic related compounds predominantly modulate the soil microbiome. Journal of Biological Chemistry, 288(7): 4502-4512.
[4] Baker, N.R., Khalili, B., Martiny, J.B.H., Allison, S.D., (2018): Microbial decomposers not constrained by climate history along a Mediterranean climate gradient in southern California. Ecology, 99(6): 1441-1452.
[5] Bakker, M.G., Bradeen, J.M., Kinkel, L.L., (2013): Effects of plant host species and plant community richness on streptomycete community structure. FEMS Microbiology Ecology, 83(3): 596-606.
[6] Boyd, E. and Peters, J.W., (2013): New insights into the evolutionary history of biological nitrogen fixation. Frontiers in Microbiology, 201(4): 1-12.
[7] Bragazza, L., Bardgett, R.D., Mitchell, E.A., Buttler, A., (2015): Linking soil microbial communities to vascular plant abundance along a climate gradient. New Phytologist, 205: 1175-1182.
[8] Bragina, A., Berg, C., Cardinale, M., Shcherbakov, A., Chebotar, V., Berg, G., (2012): Sphagnum mosses harbour highly specific bacterial diversity during their whole lifecycle. The ISME journal, 6: 802-813.
[9] Broeckling, C.D., Broz, A.K., Bergelson, J., Manter, D.K., Vivanco, J.M., (2008): Root exudates regulate soil fungal community composition and diversity. Applied and Environmental Microbiology, 74(3): 738-744
[10] Bronick, C.J. and Lal, R., (2005): Soil structure and management: a review. Geoderma, 124(2): 3-22.
[11] Buchanan, B.B., Gruissem, W., Jones, R.L., (2000): Biochemistry , molecular biology of plants, 2nd Edition. American Society of Plant Physiologists Rockville, Maryland, pp. 300-308.
[12] Chaparro, J.M., Badri, D.V., Bakker, M.G., Sugiyama, A., Manter, D.K., Vivanco, J.M., (2013): Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions. PLoS One, 8(2): e55731.
[13] Chen, Z., Zheng, Y., Ding, C., Ren, X., Yuan, J., Sun, F., Li, Y., (2017): Integrated metagenomics and molecular ecological network analysis of bacterial community composition during the phytoremediation of cadmium-contaminated soils by bioenergy crops. Ecotoxicology and Environmental Safety, 145: 111-118.

[14] Clairmont, L.K., Stevens, K.J., Slawson, R.M., (2019): Site-specific differences in microbial community structure and function within the rhizosphere and rhizoplane of wetland plants is plant species dependent. Rhizosphere, 9: 56-68.

[15] Craig, J.W., Chang, F.-Y., Kim, J.H., Obiajulu, S.C., Brady, S.F., (2010): Expanding small-molecule functional metagenomics through parallel screening of broad-host-range cosmid environmental DNA libraries in diverse proteobacteria. Applied and Environmental Microbiology, 76: 1633-1641.

[16] El Zahar Haichar, F., Marol, C., Berge, O., Rangel-Castro, J.I., Prosser, J.I., Balesdent, J.M., Heulin, T., Achouak, W., (2008): Plant host habitat and root exudates shape soil bacterial community structure. The ISME journal, 2: 1221-1230.

[17] Enebe, M.C., Babalola, O.O., (2018): The impact of microbes in the orchestration of plants' resistance to biotic stress: a disease management approach. Applied Microbioly and Biotechnology, 103: 9-25.

[18] Fierer, N., Bradford, M.A., Jackson, R.B., (2007): Toward an ecological classification of soil bacteria. Ecology, 88(6): 1354-1364.

[19] Garbeva, P., Van Elsas, J., Van Veen, J., (2008): Rhizosphere microbial community and its response to plant species and soil history. Plant and Soil, 301(1-2): 19-32.

[20] García-Orenes, F., Morugán-Coronado, A., Zornoza, R., Cerdà, A., Scow, K., (2016): Correction: Changes in Soil Microbial Community Structure Influenced by Agricultural Management Practices in a Mediterranean Agro-Ecosystem. PLoS One, 8(11): e80522.

[21] Germida, J.J., Siciliano, S.D., Renato De Freitas, J., Seib, A.M., (1998): Diversity of root-associated bacteria associated with field-grown canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.). FEMS Microbiology ecology, 26(1): 43-50.

[22] Haldar, S., Sengupta, S., (2015): Plant-microbe cross-talk in the rhizosphere: insight and biotechnological potential. The open microbiology journal, 9: 1-7.

[23] Hein, J.W., Wolfe, G.V., Blee, K.A., (2008): Comparison of rhizosphere bacterial communities in *Arabidopsis thaliana* mutants for systemic acquired resistance. Microbial ecology, 55(2): 333-343.

[24] Hiltner, L.T., (1904): Uber nevere erfahrungen und probleme auf dem gebiet der boden bakteriologie und unter besonderer Beurchsichtigung der Grundungung und Broche. *Arbeiten.* Deutsche Landwirtschaft Gesellschaft Berlin, 98: 59-78.

[25] Illeghems, K., Weckx, S., De Vuyst, L., (2015): Applying meta-pathway analyses through metagenomics to identify the functional properties of the major bacterial communities of a single spontaneous cocoa bean fermentation process sample. Food microbiology, 50: 54-63.

[26] Imchen, M., Kumavath, R., Barh, D., Avezedo, V., Ghosh, P., Viana, M., Wattam, A.R., (2017): Searching for signatures across microbial communities: Metagenomic analysis of soil samples from mangrove and other ecosystems. Scientific Reports, 7(1): 1-13.

[27] İnceoğlu, Ö., Salles, J.F., Van Elsas, J.D., (2012): Soil and cultivar type shape the bacterial community in the potato rhizosphere. Microbial ecology, 63(2): 460-470.

[28] İnceoğlu, Ö., Salles, J.F., Van Overbeek, L., Van Elsas, J.D., (2010): Effects of plant genotype and growth stage on the betaproteobacterial communities associated with different potato cultivars in two fields. Applied and Environmental Microbiology, 76(11): 3675-3684.

[29] Jacoby, R., Peukert, M., Succurro, A., Koprivova, A., Kopriva, S., (2017): The role of soil microorganisms in plant mineral nutrition - current knowledge and future directions. Frontiers in plant science, 8: 1617.

[30] Jiang, H., Huang, L., Yang, J., Wu, G., (2018): Chapter 23 - A Microbial Analysis Primer for Biogeochemists. In: Environmental Geochemistry (Second Edition), pp. 599-609.

[31] Jiménez, D.J., Chaves-Moreno, D.,Van Elsas, J.D., (2015): Unveiling the metabolic potential of two soil-derived microbial consortia selected on wheat straw. Scientific Reports, 5: 13845.

[32] Jones, D.L., Nguyen, C., Finlay, R.D., (2009): Carbon flow in the rhizosphere: carbon trading at the soil–root interface. Plant and Soil, 321(1-2): 5-33.

[33] Jones, S.E., Pham, C.A., Zambri, M.P., McKillip, J., Carlson, E.E. and Elliot, M.A., (2019): Streptomyces volatile compounds influence exploration and microbial community dynamics by altering iron availability. Molecular Biology and Physiology, 10(2) :171-190.

[34] Larsen, J., Jaramillo-López, P., Nájera-Rincon, M. , González-Esquivel, C., (2015): Biotic interactions in the rhizosphere in relation to plant and soil nutrient dynamics. Journal of soil science and plant nutrition, 15(2): 449-463.

[35] Lauber, C.L., Hamady, M., Knight, R., Fierer, N., (2009): Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology, 75(15): 5111-5120.

[36] Leake, J.R., (2004): Myco-heterotroph/epiparasitic plant interactions with ectomycorrhizal and arbuscular mycorrhizal fungi. Current Opinion in Plant Biology, 7(4): 422-428.

[37] Li, H., Su, J.-Q., Yang, X.-R., Zhu, Y.-G., (2019): Distinct rhizosphere effect on active and total bacterial communities in paddy soils. Science of The Total Environment, 649: 422-430.

[38] Mahoney, A.K., Yin, C., Hulbert, S.H., (2017): Community structure, species variation, and potential functions of rhizosphere-associated bacteria of different winter wheat (*Triticum aestivum)* cultivars. Frontiers in plant science, 8: 132-146.

[39] Marschner, P., Yang, C.-H., Lieberei, R., Crowley, D.E., (2001): Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biology and Biochemistry, 33(11): 1437-1445.

[40] Mašínová, T., Bahnmann, B.D., Větrovský, T., Tomšovský, M., Merunková, K., Baldrian, P., (2017): Drivers of yeast community composition in the litter and soil of a temperate forest. FEMS Microbiology ecology, 93(2): 1-10.

[41] Mcpherson, M.R., Wang, P., Marsh, E.L., Mitchell, R.B., Schachtman, D.P., (2018): Isolation and analysis of microbial communities in soil, rhizosphere, and roots in perennial grass experiments. Journal of visualized experiments, 137: 57932.

[42] Mendes, R., Kruijt, M., De Bruijn, I., Dekkers, E., Van Der Voort, M., Schneider, J.H., Piceno, Y.M., Desantis, T.Z., Andersen, G.L., Bakker, P.A., (2011): Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science, 332(6033):1097-1100.

[43] Micallef, S.A., Channer, S., Shiaris, M.P., Colón-Carmona, A., (2009): Plant age and genotype impact the progression of bacterial community succession in the Arabidopsis rhizosphere. Plant signaling, behavior, 4(8): 777-780.

[44] Molina-Montenegro, M.A., Ballesteros, G.I., Castro-Nallar, E., Meneses, C., Torres-Díaz, C., Gallardo-Cerda, J., (2018): Metagenomic exploration of soils microbial communities associated to Antarctic vascular plants. PeerJ Preprints, 6: 26501-26508.

[45] Nacke, H., Goldmann, K., Schöning, I., Pfeiffer, B., Kaiser, K., Castillo-Villamizar, G.A., Schrumpf, M., Buscot, F., Daniel, R., Wubet, T., (2016): Fine spatial scale variation of soil microbial communities under European beech and Norway spruce. Fronters in Microbiology, 7: 2067-2081.

[46] Nacke, H., Will, C., Herzog, S., Nowka, B., Engelhaupt, M., Daniel, R., (2011): Identification of novel lipolytic genes and

gene families by screening of metagenomic libraries derived from soil samples of the German Biodiversity Exploratories. FEMS Microbiology ecology, 78(1): 188-201.
[47] Nannipieri, P., Kandeler, E., Ruggiero, P., (2002): Enzyme activities and microbiological and biochemical processes in soil. pp. 1-33. In: Burns, R.G., Dick, R.P. (eds.): Enzymes in the environment. Marcel Dekker, New York.
[48] Oldroyd, G.E., (2013): Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. Nature Reviews Microbiology, 11(4): 252-263.
[49] Pinton, R., Varanini, Z., Nannipieri, P., (2007): The rhizosphere: biochemistry and organic substances at the soil-plant interface, CRC press, pp. 4-6.
[50] Ramirez, K.S., Lauber, C.L., Knight, R., Bradford, M.A., Fierer, N., (2010): Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. Ecology, 91(12): 3463-3470.
[51] Rasmann, S., Bennett, A., Biere, A., Karley, A., Guerrieri, E., (2017): Root symbionts: Powerful drivers of plant above-and belowground indirect defenses. Insect science, 24(6): 947-960.
[52] Raynaud, X., Nunan, N., (2014): Spatial ecology of bacteria at the microscale in soil. PLoS One, 9(1): 87217-87226.
[53] Ritz, K., (2005): Underview: origins and consequences of belowground biodiversity. Biological diversity and function in soils. Cambridge University Press, Cambridge, pp. 381-401.
[54] Roesch, L.F., Fulthorpe, R.R., Riva, A., Casella, G., Hadwin, A.K., Kent, A.D., Daroub, S.H., Camargo, F.A., Farmerie, W.G., Triplett, E.W., (2007): Pyrosequencing enumerates and contrasts soil microbial diversity. The ISME Journal, 4(1): 283-290.
[55] Roger-Estrade, J., Anger, C., Bertrand, M., Richard, G., (2010): Tillage and soil ecology: partners for sustainable agriculture. Soil and Tillage Research, 111(1): 33-40.
[56] Romão, D., Staley, C., Ferreira, F., Rodrigues, R., Sabino, R., Veríssimo, C., Wang, P., Sadowsky, M., Brandão, J., (2017): Next-generation sequencing and culture-based techniques offer complementary insights into fungi and prokaryotes in beach sands. Marine Pollution Bulletin, 119(1): 351-358.
[57] Rúa, M.A., Antoninka, A., Antunes, P.M., Chaudhary, V.B., Gehring, C., Lamit, L.J., Piculell, B.J., Bever, J.D., Zabinski, C., Meadow, J.F., (2016): Home-field advantage? evidence of local adaptation among plants, soil, and arbuscular mycorrhizal fungi through meta-analysis. BMC Evolutionary Biology, 16(1): 122-137.
[58] Santhanam, R., Weinhold, A., Goldberg, J., Oh, Y., Baldwin, I.T., (2015): Native root-associated bacteria rescue a plant from a sudden-wilt disease that emerged during continuous cropping. Proceedings of the National Academy of Sciences, 112(36): 5013-5020.
[59] Schmidt, R., Köberl, M., Mostafa, A., Ramadan, E.M., Monschein, M., Jensen, K.B., Bauer, R., Berg, G., (2014): Effects of bacterial inoculants on the indigenous microbiome and secondary metabolites of chamomile plants. Frontiers in Microbiology, 5: 64-75.
[60] Shakeel, M., Rais, A., Hassan, M.N., Hafeez, F.Y., (2015): Root associated Bacillus sp. improves growth, yield and zinc translocation for basmati rice (*Oryza sativa*) varieties. Frontiers in Microbiology, 6: 1286-1298.
[61] Singh, A., Moody, G., Wu, S.F., Wu, Y.W., Ghimire, N.J., Yan, J.Q., Mandrus, D.G., Xu, X.D., Li, X.Q., (2014): Coherent electronic coupling in atomically thin MoSe2. Physical Review Letters, 112(21): 216804-216809.
[62] Smalla, K., Wieland, G., Buchner, A., Zock, A., Parzy, J., Kaiser, S., Roskot, N., Heuer, H., Berg, G., (2001): Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Applied and Environmental Microbiology, 67(10): 4742-4751.
[63] Smith, S.E., Smith, F.A., (2011): Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. Annual review of plant biology, 62: 227-250.
[64] Thrall, P.H., Laine, A.-L., Broadhurst, L.M., Bagnall, D.J., Brockwell, J., (2011): Symbiotic effectiveness of rhizobial mutualists varies in interactions with native Australian legume genera. PLoS One, 6(8): 23545-23556.
[65] Tringe, S.G., Von Mering, C., Kobayashi, A., Salamov, A.A., Chen, K., Chang, H.W., Podar, M., Short, J.M., Mathur, E.J., Detter, J.C., (2005): Comparative metagenomics of microbial communities. Science, 308(5721): 554-557.
[66] Venturi, V., Keel, C., (2016): Signaling in the rhizosphere. Trends in Plant Science, 21(3): 187-198.
[67] Viebahn, M., Veenman, C., Wernars, K., Van Loon, L.C., Smit, E., Bakker, P.A., (2005): Assessment of differences in ascomycete communities in the rhizosphere of field-grown wheat and potato. FEMS Microbiology ecology, 53(2): 245-253.
[68] Vitorino, L., Bessa, L., (2018): Microbial diversity: the gap between the estimated and the known. Diversity, 10(2): 46-65.
[69] Wardle, D., Nicholson, K., Bonner, K., Yeates, G., (1999): Effects of agricultural intensification on soil-associated arthropod population dynamics, community structure, diversity and temporal variability over a seven-year period. Soil Biology and Biochemistry, 31(12): 1691-1706.
[70] Weir, T.L., Perry, L., Gilroy, S., Vivanco, J., (2010): The role of root exudates in rhizosphere interactions with plants and other organisms. Annual review of plant biology, 57: 233-266.
[71] Wolińska, A., (2019): Metagenomic Achievements in microbial diversity determination in croplands: a review. in: microbial diversity in the genomic era. pp 15-35. In. Das, S., Dash, H.R. (eds): Academic press.
[72] Yu, H., Ling, N., Wang, T., Zhu, C., Wang, Y., Wang, S., Gao, Q., (2019): Responses of soil biological traits and bacterial communities to nitrogen fertilization mediate maize yields across three soil types. Soil and Tillage Research, 185: 61-69.
[73] Zhang, X., Ning, T., Han, H., Sun, T., Li, G., Li, Z., Lal, R., (2018): Effects of waxy maize relay intercropping and residue retention on rhizosphere microbial communities and vegetable yield in a continuous cropping system. Pedosphere, 28(1): 84-93.
[74] Zhou, C., Guo, J., Zhu, L., Xiao, X., Xie, Y., Zhu, J., Ma, Z., Wang, J., (2016): *Paenibacillus polymyxa* BFKC01 enhances plant iron absorption via improved root systems and activated iron acquisition mechanisms. Plant Physiology and Biochemistry, 105: 162-173.

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