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REVIEW

The endophytome (plant-associated microbiome): methodological approaches, biological aspects, and biotech applications

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Abstract

Similar to other organisms, plants establish interactions with a variety of microorganisms in their natural environment. The plant microbiome occupies the host plant's tissues, either internally or on its surfaces, showing interactions that can assist in its growth, development, and adaptation to face environmental stresses. The advance of metagenomics and metatranscriptomics approaches has strongly driven the study and recognition of plant microbiome impacts. Research in this regard provides comprehensive information about the taxonomic and functional aspects of microbial plant communities, contributing to a better understanding of their dynamics. Evidence of the plant microbiome's functional potential has boosted its exploitation to develop more ecological and sustainable agricultural practices that impact human health. Although microbial inoculants' development and use are promising to revolutionize crop production, interdisciplinary studies are needed to identify new candidates and promote effective practical applications. On the other hand, there are challenges in understanding and analyzing complex data generated within a plant microbiome project's scope. This review presents aspects about the complex structuring and assembly of the microbiome in the host plant's tissues, metagenomics, and metatranscriptomics approaches for its understanding, covering descriptions of recent studies concerning metagenomics to characterize the microbiome of non-model plants under different aspects. Studies involving bio-inoculants, isolated from plant microbial communities, capable of assisting in crops' productivity, are also reviewed.

Keywords Endophytes · Bioinformatics · Environmental stress · Metagenomics · Metatranscriptomics · Microbial communities

Introduction

Microorganisms are the most abundant and diverse forms of life, estimated to constitute up to 60% of the Earth's biomass (Singh et al. 2009). Widely distributed in nature, they can withstand extreme environmental conditions, presenting mechanisms that allow their adaptation to the most diverse niches (Jayadev and Navami 2014). Microbial interactions play a fundamental role in balancing various natural processes, including those established between plants and microorganisms. Due to the ability to inhabit multiple environments with high microbial richness, plants interact with microorganisms essential for ecosystem balance (Kumar et al. 2016).

Such "plant-microorganism" interactions can occur in different ways and levels. Almost all the plant organs interact with microbes at some stage of their development, from germination to senescence. In general, in addition to plants providing a protected habitat for microorganisms, several organic and inorganic compounds they produce represent a rich source of nutrients, thus providing an environment favorable to the colonization of microbial communities. In contrast, microbial communities also can interfere (directly or indirectly) in plant physiology through pathogenic, commensal, mutualistic, or amensalistic interactions (Kumar et al. 2016; Schirawski and Perlin 2018).

Plant colonization by various microorganisms can influence plant health (beneficial, neutral, or harmful) and development (Müller et al. 2016). The processes of colonization

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and establishment of beneficial or pathogenic interactions between plant-microorganisms require the modulation of the plant immune system by microorganisms, thus leading to a complex and intricate relationship (Chadha 2019). The ability of plants to simultaneously benefit from the microbiota's favorable roles and tolerate or resist pathogenic activities performed by different microorganisms has aroused several researchers' attention over the years.

The microbiome—a set of microorganisms associated with a host plant species is composed of bacterial communities and other organisms, including viruses, fungi, archaea, and nematodes, which contribute to relevant functions in the biology of the host (Orozco-Mosqueda et al. 2018). It plays an essential role in plant physiology through involvement in biotic and abiotic factors (Kaul et al. 2017). Besides, it exerts a positive influence on plant development by contributing microorganisms associated with the plant, including nutrition, protection against pathogens, and stress tolerance (Mitter et al. 2019). Consequently, elucidating and understanding aspects of the plant microbiome is essential for identifying microorganisms that can be better explored to promote plants' growth and health under different conditions.

In turn, endophytes regard plant endosymbiotic microorganisms—often bacteria or fungi. They colonize plants' inter- and/or intracellular locations during all or part of the plant's life cycle, with no apparent disease symptoms. Endophytes are known to enhance plant growth and nutrient gain, sometimes improving the plant's ability to tolerate abiotic and biotic stresses. Some endophytes produce phytohormones and other bioactive compounds of biotechnological interest, including pharmaceutical drugs (Gouda et al. 2016).

To distinguish the whole endophyte community (here called "*endophytome*") from other microorganisms that inhabit plants (including pathogenic ones) is challenging. In this context, studies on plant microbiome have opened new possibilities to infer who are "*the good and the bad guys*" or even who is there without significant consequences. Thus, there are still fundamental questions that need to be clarified, including the interaction (at the molecular level) between host-microorganisms and the potential of applying this knowledge in various areas of expertise (van der Heijden and Schlaeppi 2015). Although different microorganisms colonize plants, most microbiome research studies are related to isolated taxonomic groups (mainly prokaryotes). Thus, the joint analysis of other microbial groups and their interactions within the microbiota and with the plant host is a concept that is on the rise and can contribute to the elucidation of the critical microbiome's functional characteristics (Berg 2015).

Given the significance of the microbiome for host plants, further investigations are required to fill the gaps concerning the complex communication between microorganisms and plants and exploit these resources to promote plant species' tolerance to adverse conditions. Therefore, new studies integrating data from interdisciplinary areas have been carried out, ranging from microbiology and molecular biology to agronomy. In this context, the present review presents an overview of the plant microbiome, the plant niches where microbial communities' interactions are established, and the main tools (including bioinformatics) that help study those communities at the omics level. Emerging studies on non-model plant microbiomes are addressed and, finally, the prospects for its application are discussed, aiming to promote more sustainable agricultural production.

Characterization of the microbiome and its communities in plant tissues

Microorganisms associated with plants can inhabit from the host's surface (epiphytes) to the internal tissues (endophytes) (Kumar et al. 2017b), whereas endophytic seem to have a most important functional role. In plants, interactions with the microbiota can occur in three distinct compartments: (i) the rhizosphere (in the soil, under the influence of the roots) (Fig. 1a); (ii) the endosphere (in the internal tissues) (Fig. 1b), and (iii) the phyllosphere (in the aerial part) (Fig. 1c) (Sekar and Kandavel 2010). These microenvironments provide biotic and abiotic conditions necessary to maintain plant-associated microbial life.

The plant microbiome's structuring comprises a dynamic process reflecting changes in the microbial community, given the environmental conditions and plant developmental stage (Müller et al. 2016). The complex interaction between the processes of selection, dispersion, drift, and speciation influences a given community's structuring and dynamics (Vellend 2016). The balance between these processes in the plant-microbiome relationship depends on the plant's genotype, soil composition, stress type (biotic/abiotic), and agricultural practices.

It has been proposed that the host species strongly influence the composition of the plant microbiome (Thijs et al. 2016). A comparative study was carried out between the rhizosphere's microbial communities and the roots endosphere of *Setaria italic* Roem. & Schult. (Poaceae) and its wild relative *S. viridis* (L.) Thell. Such studies reveal distinct communities between both species, especially regarding Betaproteobacteria and Firmicutes and, among archaea, the Methanobacteria and Methanomicrobia. Such differences reflect the critical role of the genotype in selecting members of the prokaryotic community involved in the plant-soil interaction (Chaluvadi and Bennetzen 2018).

In contrast, the composition of the root bacterial community of selected lycopods, ferns, angiosperms, and gymnosperms indicated the soil as the primary determining factor. However, a significant correlation with the phylogeny of host plants was identified (Yeoh et al. 2017). Similar results were

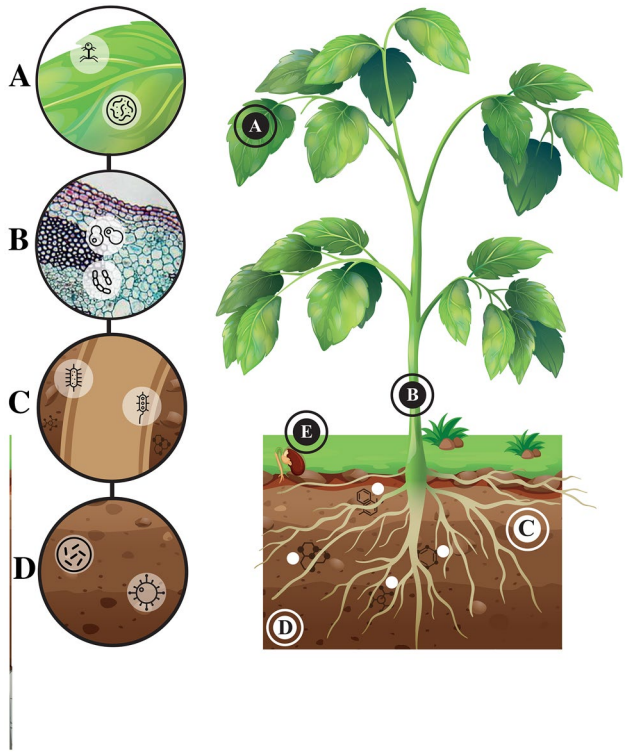

Fig. 1 Schematic representation of a plant in its environment, indicating its main compartments where microbial communities can inhabit: phyllosphere (**a**); endosphere (**b**); rhizosphere (**c**); and those present in the soil (**d**). Microorganisms horizontal transmission can be established with the host plant from the air or soil microbiome. In the latter case, root exudates (white circles) play an important role in recruiting microorganisms from the soil to the rhizosphere. In contrast, vertical transmission can occur through advancing colonization in different plant tissues by microorganisms or between generations of plants through seeds (**e**)

found in studying the rhizosphere and endosphere of different rice cultivars (*Oryza sativa* L., Poaceae), where the soil played a more substantial role than the host genotype in the formation of the microbial community (Xu et al. 2020). Considering the phyllosphere, the host species also assumes an essential role in the composition of bacterial communities, as observed in seven different tree species in Brazil's Atlantic forest (Lambais et al. 2014).

One of the primary determining factors for the acquisition and establishment of the microbiome in different compartments of the host plant is the "transmission pattern" (Simon et al. 2019). The so-called "horizontal transmission" can occur through the air-plant (phyllosphere) interface or through the soil, which acts as an essential source of microbial diversity associated with the host plant. In this case, soil microorganisms are attracted to the rhizosphere through root exudates (represented by white circles in Fig. 1). Part of the microorganisms penetrate the plant's roots and colonize the internal tissues. In turn, "vertical transmission" assumes its role when colonization proceeds to the host plant (seeds) above the soil surface (Zheng and Gong 2019). Wind, water and animals can be involved in the vertical transmission of endophytic microorganisms, but this may occur between generations of plants through seeds. Such endophytes are transmitted from generation to generation since seed-borne transmission assures their presence in seedlings of the next generation (Fig. 1e) (Shade et al. 2017). An interplay between both horizontal and vertical transmission is assumed. In this sense, plant-soil biotic interactions lead to the gradual colonization of a subgroup of microorganisms in the established *continuum* of plant compartments from the soil to the host tissues (Vandenkoornhuyse et al. 2015).

Rhizosphere—microbial diversity recruited from the soil

The rhizosphere (Fig. 1c) comprises the soil's narrow region under the influence of the plant's roots, characterized by a richness in microorganisms, considered one of the most dynamic environments on the planet (Philippot et al. 2013). Several microorganisms inhabit this environment, including bacteria, fungi, protozoa, and multicellular organisms, such as nematodes and some invertebrates. The processes of root exudation, in the form of primary and secondary metabolites, besides signaling in the rhizosphere, influence the selection and colonization of microbial communities in this region (Venturi and Keel 2016).

The rhizosphere's recruitment of microorganisms is expected, mainly due to the exudation of root compounds (white circles, Fig. 1), which mediate the interaction between plant and soil microorganisms (Fig. 1d). Consequently, plants can alter their exudates' composition, thus modifying the rhizospheric microbiome, attracting beneficial microorganisms (such as mycorrhizal fungi and growth-promoting rhizobacteria) from the soil, or inhibiting the spread of pathogenic microorganisms (Vives-Peris et al. 2020).

Together with its physical–chemical properties and biogeographic processes, the soil influences the rhizospheric microbiome formation, given its role in defining the root exudation pattern (Philippot et al. 2013). According to Turner et al. (2013b) the rhizospheric microbiome varies between plant species, considering that different hosts can control, harbor, and select various prokaryotic and eukaryotic microorganisms from the soil. For example, in a study on the rhizospheric plant community in temperate forests, based on 16S rRNA (ribosomal RNA) meta-barcode, significant differences were identified between the OTUs (Operational Taxonomic Units) of *Milium effusum* Lour (Poaceae) and *Stachys sylvatica* Torr (Lamiaceae), demonstrating the influence of the plant species in determining the composition of the bacterial community of the rhizospheric soil (Ma et al. 2019). Using the same approach (16S rRNA) to analyze the

rhizosphere of 19 herbaceous plant species, Dawson et al. (2017) identified OTUs that responded to specific plant taxa. Some plant taxa were related to critical microbial members that influenced plant growth during beneficial interactions, despite representing a small number.

Independent cultivation approaches reveal that the microbial diversity of the rhizosphere is underestimated. Among the various microorganisms characterized as beneficial for plant development and health, nitrogen-fixing bacteria, PGPR (Plant Growth Promoting Rhizobacteria), and mycorrhizal fungi have drawn particular attention. PGPR as an alternative to replacing agrochemicals is a promising approach to developing more sustainable agriculture (Shailendra Singh 2015). PGPR promotes plant growth directly through the production of plant hormones or the availability of nutrients, or indirectly, through antimicrobial metabolites for biocontrol (Arora et al. 2013). As an example of the benefits, Kumari et al. (2018) demonstrated that bacteria isolated from the rhizosphere of mung beans (*Vigna radiata* R. Wilczek, Leguminosae) had significant PGPR attributes, such as phosphate solubilization and phytohormone production, in addition to the ability to inhibit (in vitro) the growth of the phytopathogen *Rhizoctonia solani*, the causative agent of root rot in this species. In a metagenomic study of *Paspalum scrobiculatum* L. (Poaceae), Prabha et al. (2019) identified high taxonomic diversity in the rhizosphere associated with the metabolic capacities of microbial communities to promote plant growth and development, including carbon fixation, nitrogen, phosphorus, response to stress, and phytohormone synthesis. Thus, different microbial members of the rhizosphere may be potential candidates for improving plant development and health during plant–soil interactions.

Endosphere—a selective niche

For a long time, microorganisms inside the plants were erroneously associated with pathogens, which promoted damage in the development and loss of crop yield (Andreote et al. 2014). It has been considered that microorganisms that occupy the endosphere (or internal tissues such as root, stem, or leaves), called endophytes (Turner et al. 2013a) without causing deleterious symptoms, were non-pathogenic. Still, depending on the environmental conditions or host defense response, some can be considered latent pathogens (Schulz and Boyle 2005).

According to their lifestyle, endophytic microorganisms can be classified into two categories: (i) mandatory endophytes, which require the metabolism of the host plant for its growth and survival, whose transmission occurs vertically or through vectors; and (ii) facultative endophytes, which may or may not live within the host plant (and in other habitats) at some stage in their life cycle (Hardoim et al. 2008). Thus, endophytic microorganisms can gain access and colonize the internal tissues of the plant by horizontal (soil) and/or vertical (seed) transmission (Omomowo and Babalola 2019).

Seeds are essential vehicles in establishing the endophytome between successive plant generations through vertical transmission, acting as an initial inoculum in the formation of the microbiome of descendant plants (Shade et al. 2017). Microorganisms that inhabit the seed embryo or endosperm are more likely to be transferred vertically than those present in the outer seed coating (Barret et al. 2016). In addition, the seed endophytome also represents a reservoir of microorganisms able to colonize plant tissues, playing a crucial role in later stages of development, promoting nutrient absorption and resistance to pathogens (Rybakova et al. 2017; Bergna et al. 2018), among other features.

As demonstrated by Rodríguez et al. (2020), the association of healthy seeds with environmental sources of microorganisms ensures the establishment and vigor in the initial stages of seedling development during the assembly of the endophytome. Endophytes from external sources (soil, rhizosphere, phyllosphere, and environment) are also important for assembling the seed microbiome, favoring early plant establishment and vigor (Rodríguez et al. 2020).

The structure of the root endophytic community is guided by three main factors: (i) the soil, including geographical, geological, and edaphoclimatic aspects, which influence the microorganisms that can occupy the soil, affecting the community of endophytes; (ii) the host plant, whose species, stage of development and health affects microbial colonization; (iii) the endophytic microorganism, its ability to penetrate and to colonize the root tissue (Gaiero et al. 2013); and an additional aspect (iv) should be considered regarding the genomic and epigenomic features of each individual of a given species.

Among the factors that influence the bacterial spectrum before endophytic root colonization, the roots' architecture and the composition of the root exudates deserve mention, besides mycorrhization or existing wounds in the penetration region. Once colonization has occurred, other factors are responsible for selecting the bacterial spectrum within the root, such as the dimension of the intercellular space, the disposition of nutrients in apoplastic fluids, besides plant response to endophytic colonization (Hallmann and Berg 2006).

Campisano et al. (2017) proposed that seasonal changes throughout the year and temperature affect the succession of endophytic communities in the stem of grape plants (*Vitis vinifera* L., Vitaceae) more intensely than in the roots. This study comprised a controlled experiment with different air temperatures (15, 25, or 35 °C), inducing a significant increase in the microbial diversity of stems, which was reversed along the evaluated time. Furthermore, evaluation under field conditions during different seasons (every three

months) affected the stem microbial composition more than the root microbiome. The results suggested that grapevine roots offer a more stable compartment for the endophytome, being less susceptible to environmental disturbances.

In general, the microbial diversity of the endosphere tends to be lower than that of the rhizosphere, considering that only a subset of the microorganisms colonizes the rhizoplane. An even smaller fraction is released and proliferates in the endosphere (Edwards et al. 2015). The selectivity of the endosphere is also a determinant of lower microbial diversity. For example, Lundberg et al. (2012) demonstrated that under controlled conditions, the microbiome of *Arabidopsis thaliana* L. (Brassicaceae) suffers a drastic loss in diversity from the rhizosphere compartment in the root endosphere. In this research, several bacterial taxa typically present in the soil and rhizosphere were absent in the endosphere. Such an absence was due to three possible factors: (i) the activity of the host's immune system; (ii) to the overcoming by successful colonizers of the endosphere; or (iii) to the metabolic inability to colonize this niche.

Some bacterial and fungal endophytes have demonstrated essential roles in plant growth and development of economically relevant crops when subjected to normal environmental conditions (Díaz-González et al. 2020) or even when exposed to salt stress (Khan et al. 2020). Endophytes can promote the development of plants under different conditions of environmental stresses, such as drought and nitrogen deficiency (Rho et al. 2018). Some endophytic microorganisms can also induce phytohormones' expression, essential for plant growth, thus essential in arid environmental conditions, besides acting in biological control of phytopathogens (Asaf et al. 2017; Liotti et al. 2018).

Phyllosphere—dynamics and microbial colonization on plant surfaces

The phyllosphere comprises the entire surface of the plant above the ground, predominantly represented by the leaves (Morris and Kinkel 2002). The phyllosphere microbiome comprises different genera of bacteria, filamentous fungi, yeasts, algae, and, occasionally, protozoa and nematodes, with bacteria representing the most abundant group in the leaves (Lindow and Brandl 2003).

The microbial community must undergo constant adaptations since the phyllosphere is directly influenced by abiotic factors, as environmental conditions, climate, ultraviolet radiation, water, and nutrient availability, besides biotic factors as host antimicrobial compounds. Consequently, microbial proliferation tends to be uneven in composition and distribution along the leaf surface (Vorholt 2012; Copeland et al. 2015).

At the species or cultivar level, some studies have shown that plant genotype plays a relevant role in establishing bacterial and fungal communities in the phyllosphere, besides geographic location or season of the year. This was the case for cereal crops (Sapkota et al. 2015) and arboreal species in a forest environment (Laforest-Lapointe et al. 2016). On the other hand, geographic location was identified as a dominant factor in the phyllosphere microbiome of three *Tamarix* (Tamaricaceae) species in different Israel and the USA regions. In these plants, bacterial and fungal communities of the phyllosphere growing under different climatic conditions were distinct (Finkel et al. 2011). Another study highlighted that (i) seasonal changes, (ii) genotypic factors, and (iii) geographic area (respectively, in this order of relevance) were determinants of the phyllosphere microbiome composition and diversity of arboreal gymnosperms in China (Bao et al. 2020).

The most critical transport sources for forming microbial communities in the phyllosphere include air, water, and soil (Bulgarelli et al. 2013). However, insects, seeds, tree shoots, sedimentation, rain splashes, harvesting, and cultivation practices have also been fundamental (Kumar et al. 2017b). Once in the phyllosphere (by any of these sources), microbial colonization occurs; it tends to be restricted to specific locations, such as stomata, at the base of trichomes, and under the cuticle. Also, most are found in aggregates or microbial biofilms (Leveau and Lindow 2001; Whipps et al. 2008). Microbial communities in the phyllosphere are often associated with plant development, including their participation in the nitrogen fixation process (Abadi et al. 2020), in promoting growth (Mehanathan et al. 2016), and protection against phytopathogens (Wiraswati et al. 2019). Thus, the interactions between the phyllosphere microbiome, the plant, and the multiple environmental factors influence this microbial community's modulation and favor heterogeneity in species abundance.

Modern molecular technologies have elucidated the importance of the phyllosphere microbiome, offering new opportunities to study microbial communities in structural, functional, and ecological terms. In this sense, recent advances in high-performance omics approaches have the potential to deepen the understanding of microbial communities in the phyllosphere and explore their impact on the ecosystem (Bringel and Couée 2015; Rossmann et al. 2017). In the case of humans, apparently, the irregular distribution of the microbiota along the skin's surface is associated with moisture and the availability of nutrients. They are also critical components in the protection against undesirable pathogens (Byrd et al. 2018). In the case of plants, can it be said that this distribution is analogous?

Metagenomics as a tool for microbiome analysis

The cultivation and classification of new microorganisms figure among the main objectives of microbiology

(Handelsman 2004). For years, members of the plant microbiome have been characterized by traditional techniques involving isolation and cultivation (Yoo et al. 2017). However, it is estimated that only a small portion (about 1:1000) of microorganisms from environmental samples can be cultivated by conventional methods (Pace 2018). From this perception, there is an emerging demand to develop new approaches beyond cultivation to access the diversity and ecological functionality of the "microbial world" (Riesenfeld et al. 2004).

The independent culture methods mainly analyze nucleic acids extracted directly from the target sample, enabling access to the microbial community (Correia et al. 2012). Among the currently available approaches for studying the microbiome (Fig. 2), metagenomics stands out (Zeyaullah et al. 2009). In metagenomics, the genome evaluated is a microbial community in a given environment, not the genome of a single microorganism, as assumed in conventional genomics (Guazzaroni et al. 2009).

Metagenomics studies are based on the combination of genomics, bioinformatics, and systems biology for the joint investigation of microbial genomes, collected directly from an environmental sample. Advances, including lower cost of next-generation sequencing (NGS) technologies, new

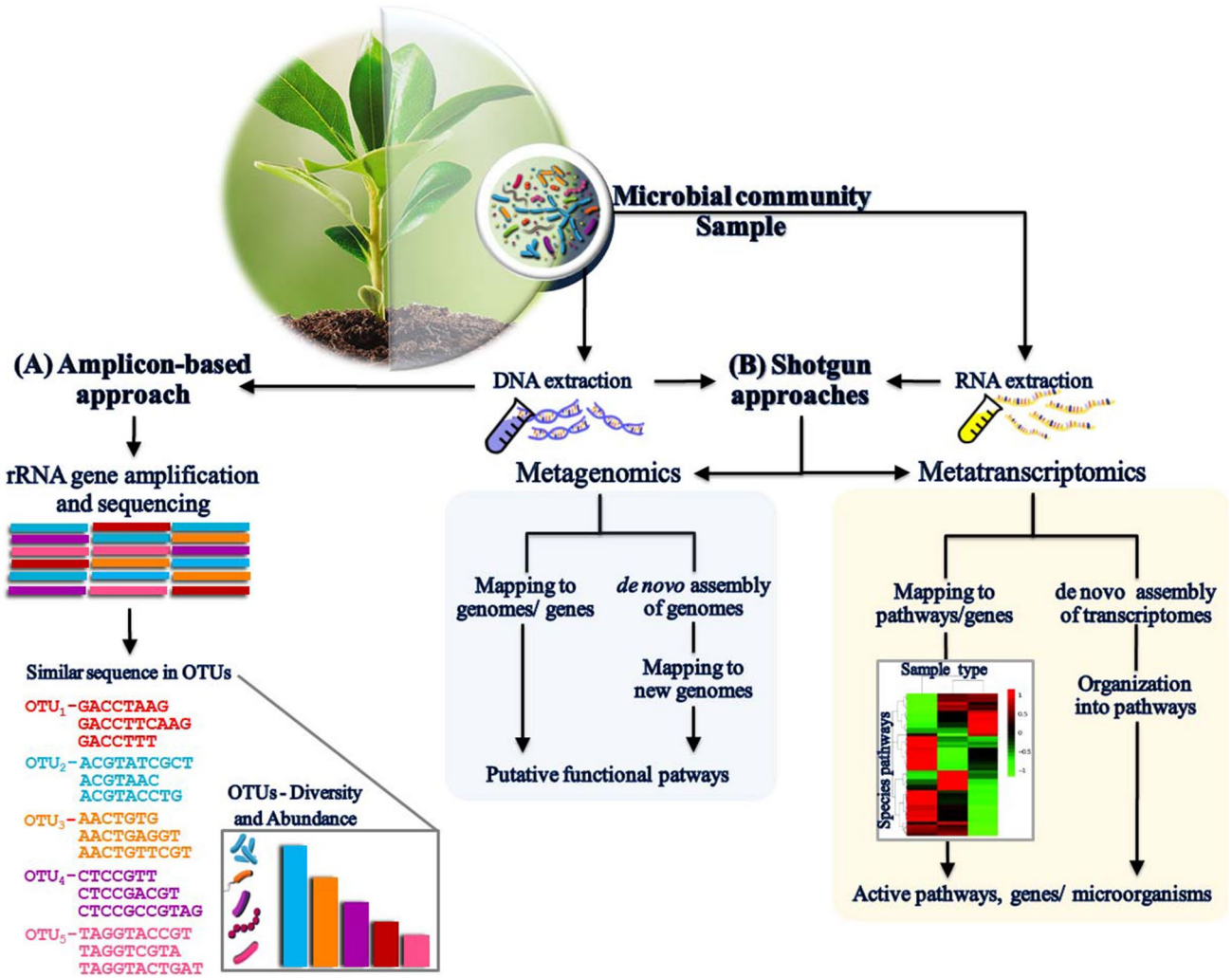

Fig. 2 Different sequencing and bioinformatics strategies based on cultivation-independent methods to study the plant microbiome. **a** The amplicon-based approach. Includes the amplification and sequencing of variable regions within rRNA genes (16S, 18S, and ITS). The filtered sequences are grouped into OTUs, which are taxonomically classified based on sequence homology. **b** Shotgun approaches include metagenomics and metatranscriptomics. Metagenomics comprises the whole metagenome sequencing (WMS), followed by mapping against reference genomes/genes or de novo assembly. Subsequently, the abundance of genes, genomes, and the functional potential of the retrieved sequences can be estimated. In contrast, metatranscriptomics encompasses the sequencing of the complete metatranscriptome, followed by mapping RNA sequences and reference genes to identify active pathways, genes, and microorganisms. The combined application of different methods contributes to a more holistic understanding of the composition and functional characteristics of the plant microbiome

bioinformatics tools, in addition to high-throughput screening methods (HTS), have promoted substantial progress in metagenomic studies (Kumar et al. 2015). In the last decade, different plant compartments, including the rhizosphere (Goss-Souza et al. 2019), phyllosphere (Khoiri et al. 2021), and endosphere (Zhang et al. 2020a), have been the subject to such studies.

A metagenomic analysis begins with collecting a specific environmental sample containing a microbial community of interest, DNA extraction, followed by the sequencing and bioinformatic analysis steps, which involve steps from quality control to comparative analysis (Fig. 3) (Stanchev et al. 2016). Sampling is a primordial and essential step in metagenomic projects, as it is decisive in the quality of the data and, consequently, for the interpretation of the results obtained (Thomas et al. 2012). The main objectives in sampling are to ensure a sufficient amount of microbial biomass for sequencing purposes and minimize contamination of environmental samples. Therefore, care is required in optimizing the collection and storage methods according to the sample of interest (Quince et al. 2017).

The efficient extraction of plant DNA is necessary to guarantee that the microbial community associated with each analyzed plant tissue is properly accessed (Fadiji and Babalola 2020). The isolated DNA must represent the totality of microbial cells present in the sample, have a sufficient amount and high quality to ensure the success of the subsequent stages (Thomas et al. 2012). However, the extraction and purification of high-quality DNA, associated with the lack of a standard extraction method for all environmental samples, constitute some of the main obstacles faced by metagenomic studies (Kunin et al. 2008). Several commercial kits were developed for DNA extraction from different types of samples. However, a careful literature review and validation of the protocol or kit of choice is highly recommended to select the most appropriate extraction method (Izard 2015).

Metagenomic analyzes can be divided into two distinct approaches: amplicon and shotgun metagenomics (Gilbert and Dupont 2011), the first also called meta-barcode. Both can be applied to metagenomic projects in an isolated or joint way to promote a better understanding of the structural diversity and/or functional potential of microbial communities, as well as their changes in response to external factors (National Research Council 2007). Further details are presented in the following sections.

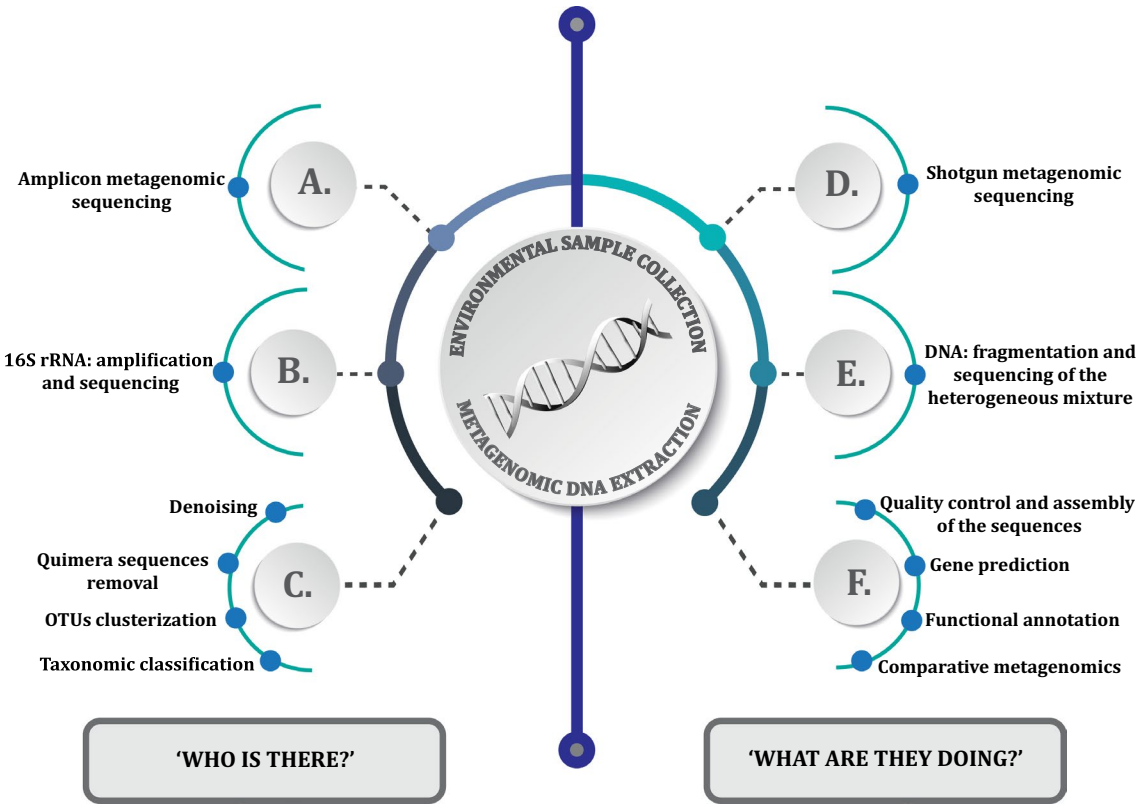

Fig. 3 Flowchart of the main approaches used in metagenomic projects and studies. DNA from metagenomic samples can be used for analyzes based on amplification and sequencing of marker genes (**a**–**c**) or in analyzes based on total sequencing (**d**–**f**)

Amplicon metagenomics

The study of microbial diversity by amplicon metagenomics is based on analyzing universal and conserved target genes, such as rRNA genes (Li et al. 2014). The 18S rRNA gene is the most commonly used for studying the phylogenetic and taxonomic diversity of eukaryotes (Tanabe et al. 2016) since it has highly conserved regions. However, it also anchors eight hypervariable areas that enable identification at the genus and species level, using sequence data available in public databases (Ki 2012). Likewise, the 16S rRNA gene sequence is used to identify prokaryotes (bacteria and archaea) (Petrosino et al. 2009). In studies of the diversity of fungi in a microbial community, the most frequently used gene is the ITS (Internal Transcribed Spacers). In most cases, it contains a sufficient degree of variation to allow differentiation at the species level (Seifert 2009).

The amplicon metagenomics approach for the identification of microbial diversity involves obtaining DNA directly from an environmental sample (e.g., rhizosphere, phyllosphere, or endosphere), the PCR amplification of 16S, 18S, and/or ITS genes, sequencing the amplicons and analyzing amplicon similarity against databases (ribosomal genes or ITSs) available (Fig. 3) (Silva et al. 2015). The necessary steps for the execution and data processing involve (i) removal of "noise" sequences (denoising); (ii) removal of chimeras; (iii) clustering of OTUs; and (iv) taxonomic classification. The denoising stage is essential because it filters "noise" sequences generated from PCR or sequencing (Kim et al. 2013). Among the tools used for this purpose are Denoiser (Reeder and Knight 2010) and DADA2 (Rosen et al. 2012). Then the removal of chimeric sequences must be performed, as they make it difficult to identify original sequences during the analysis (Edgar et al. 2011). UCHIME (Edgar et al. 2011) is an accurate tool for this type of analysis.

The OTU clustering step consists of aligning and grouping the reads based on their similarity. OTUs are determined from the sequence grouping, using a defined percentage of the difference between them, with a similarity limit of 97%, 95%, and 80% for species, genus, and phylum, respectively (Schloss and Handelsman 2005). Initially, reads are pre-grouped (reducing computational time), and OTUs are selected from 2 approaches: (i) de novo, whose sequences are compared to each other and grouped by similarity, without the use of a reference (Di Bella et al. 2013), through tools such as Cd-hit (Li and Godzik 2006), and UCLUST (Edgar 2010); and (ii) taxonomy-based, where sequences are compared and grouped into OTUs based on their similarity with sequences deposited in databases (Di Bella et al. 2013), using the BLAST tool (Altschul et al. 1997), for example. The taxonomic classification stage allows inferring a taxonomic lineage to an OTU by searching specifically marker genes in reference databases. Some of the databases frequently applied in this type of analysis include Greengenes (DeSantis et al. 2006) and Ribosomal Database Project (RDP) (Cole et al. 2009) for analysis of 16S metagenomic data; Silva (Pruesse et al. 2007), for 16S and 18S; and Unite (Kõljalg et al. 2013) for ITS (Oulas et al. 2015). Thus, analyses based on marker genes are favored because databases cover genes from millions of species and accessions (Breitwieser et al. 2017). A collection of software used for amplicon-based taxonomic annotation of metagenomics data sets is presented in Table 1.

Despite its importance as a successful method for characterizing microbial communities, the amplicon metagenomic approach has some major limitations for more comprehensive studies. Since it is based on PCR amplification, there may be an underestimation of species since not all rRNA genes amplify equally (Aguilar and Grasso 2010).

Due to the sequence homology between the bacterial 16S rRNA gene and the organellar DNA (mitochondria and chloroplast) of the host, the application of universal prokaryotic PCR primers results in the co-amplification of the plant host's DNA and consequent substantial presence of plant sequences in the generated dataset (Lefèvre et al. 2020). This co-amplification is generally more problematic for studying endophytic communities (de Souza et al. 2016). Although their removal in silico is feasible, such an abundance impacts coverage of actual microbial diversity (Lefèvre et al. 2020). To overcome this limitation, a promising approach is the application of a Peptide Nucleic Acid (PNA) PCR clamp, which selectively binds to a target region of the plant genome and inhibits its amplification during PCR (Kawasaki and Ryan 2021). This technique has been efficiently applied and significantly reduces host DNA co-amplification during library preparation in different plant species (de Souza et al. 2016; Fitzpatrick et al. 2018; Lefèvre et al. 2020).

Furthermore, although the amplicon metagenomics approach allows obtaining data about the taxonomic composition of a microbial community, information about functional aspects remains unavailable (Aguilar and Grasso 2010). Therefore, the amplicon metagenomic strategy can answer the question: "*who is there*?" by accessing the taxonomic diversity of the microbial diversity. On the other hand, information on the functional diversity of the studied microbiome, which response to the question: "*what are they doing*?" can be obtained only through the shotgun metagenomic approach (DeCastro et al. 2016).

Shotgun metagenomics

Shotgun metagenomics is based on sequencing the total DNA of a sample. Considering the advances in the NGS technologies and the development of increasingly efficient bioinformatics tools, shotgun metagenomic sequencing has

Table 1 Examples of software used for functional and taxonomic annotation of metagenomic data

Software	Application	References	Website
Parallel-META3	Data mining on taxonomy and metabolic function in a large number of metagenome data sets. Shotgun and amplicon 16S/18S and ITS rRNA metagenomic sequences are accepted	Jing et al. (2017)	http://bioinfo.single-cell.cn/parallel-meta.html
Metaxa2	Taxonomic classification based on rRNA sequences	Bengtsson-Palme et al. (2015)	http://microbiology.se/software/metaxa2/
QIIME	Read quality demultiplexing and filtering, OTU selection, taxonomic annotation and phylogenetic reconstruction, and diversity analyzes	Caporaso et al. (2010)	http://qiime.org/
SPINGO	Taxonomic classification of 16S rRNA gene sequences down to the species level	Allard et al. (2015)	https://github.com/GuyAllard/SPINGO
CLARK	Classification of short metagenomic reads at the level of genus/species	Ounit et al. (2015)	http://clark.cs.ucr.edu/
MetaCluster-TA	Taxonomic annotation based on binning of reads and contigs	Wang et al. (2014)	https://i.cs.hku.hk/~alse/MetaCluster/index.html
MetaErg	Automated taxonomic and functional annotation pipeline of metagenomic data	Dong and Strous (2019)	https://github.com/xiaoli-dong/metaerg
MetaPhlAn2	Definition of the taxonomic composition of microbial communities from shotgun metagenomic sequencing data	Truong et al. (2015)	https://huttenhower.sph.harvard.edu/metaphlan2/
COGNIZER	Functional annotation of metagenomic datasets	Bose et al. (2015)	http://metagenomics.atc.tcs.com/function/cognizer
MetaStorm	Functional and taxonomic annotation of metagenomic data, enabling a customized analysis	Arango-Argoty et al. (2016)	http://bench.cs.vt.edu/MetaStorm/
LMAT	Taxonomic annotation of metagenomic data sets	Ames et al. (2013)	https://sourceforge.net/projects/lmat/
Genometa	Taxonomic annotation of metagenomic short-reads	Davenport et al. (2012)	https://webext.mh-hannover.de/genomics/genometa/
MyTaxa	Taxonomic classification of metagenomic data	Luo et al. (2014)	http://enve-omics.ce.gatech.edu/mytaxa/
MOCAT2	Pipeline covering assembly of metagenomic sequence, gene prediction, and functional annotation of metagenomic data sets	Kultima et al. (2016)	https://mocat.embl.de

been used to characterize microbial communities in terms of structure, function, and diversity in different organisms and environments (Abraham et al. 2020; Hagagy et al. 2021; Fadiji et al. 2021).

In this methodology, the DNA of the microbiome is isolated, fragmented, and sequenced (Mande et al. 2012). The result of sequencing is the generation of millions of reads, which undergo a series of bioinformatics analyzes, including quality control (QC), assembly, gene prediction, gene annotation, taxonomic, and comparative analysis (Fig. 3) (Ladoukakis et al. 2014).

In the QC step, low-quality reads and contaminants are filtered (Zhou et al. 2014) using tools such as FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and PRINSEQ5 (Schmieder and Edwards 2011). The assembly maps the sequence data to reconstruct the target, grouping the reads into contigs and contigs into scaffolds (Miller et al. 2010). Two strategies used for assembly are 'reference' and/or 'de novo', the latter using the OLC approach (Overlap-Layout-Consensus) or an algorithm based on Bruijn's graph (DBG) (Ji et al. 2011). Specifically, because it involves several genomes from a sample, metagenomic assembly requires algorithms to separate data from different species (Di Bella et al. 2013). Among the assemblers developed for metagenomic data, we can mention MetaSPAdes (Nurk et al. 2017) and MetaVelvet (Namiki et al. 2012).

The mapping of genes within a genome is based on the detection of ORFs (Open Read Frames) followed by determining their viability for translation into functional proteins, thus aiming to determine candidate nucleotide sequences for coding genes (Ladoukakis et al. 2014). Some tools were designed for the detection of genes from the properties of

metagenomic data (Di Bella et al. 2013) as FragGeneScan (Kim et al. 2015) and Metagenomics Gene Caller (MGC) (El Allali and Rose 2013), for instance.

Once the coding gene sequences are determined, it is necessary to predict their functions through functional annotation. The annotation is performed using the non-redundant (nr) database of sequences with known functions (Thomas et al. 2012). Examples of such databanks are UniProt (UniProt Consortium 2012), KEGG (Kanehisa et al. 2004), and PFAM (Bateman et al. 2004). Also, large-scale databases such as MG-RAST (Glass et al. 2010) aggregated information from several reference databases, gathered in a single structure, optimizing the automation of processing and functional annotation (Thomas et al. 2012). In the analysis, the taxonomic classification of the various microbial groups present in the sample is considered (Mande et al. 2012). Contigs or reads are compared to available sequences in databanks, assigning them the classification in species or genera. Tools for this purpose include PhyloPythiaS+ (Gregor et al. 2016) and TACOA (Diaz et al. 2009); or even considering the sequence similarity analysis tools available in databases (Oulas et al. 2015), such as MG-RAST v.4 (Meyer et al. 2019) and MEGAN6 (Huson et al. 2016). Additional examples of software for taxonomic and functional annotation of metagenomic data are compiled in Table 1.

In the last step, metagenomic data sets can be compared with MEGAN (Huson and Weber 2013), IMG/M v.5.0 (Chen et al. 2019), and parallel-meta (Su et al. 2014). These programs are based on algorithms that make it possible to compare both functional and taxonomic content with statistical support (Ladoukakis et al. 2014).

The main advantages of the shotgun metagenomic approach comprise the possibility of investigating the total diversity of organisms in a community, as this is not limited by the use of primer pairs in the amplification of target genes (Petrosino et al. 2009), allowing to compare, concomitantly, the relative abundance of all the different organisms present in a given sample. This approach also makes it possible to obtain the entire repertoire of microbial genes that encode a series of essential metabolic functions (Fadiji and Babalola 2020). Among the challenges, the difficulty in computerizing complex metagenomic data, the predominance of the host's DNA, and the higher cost of sequencing the complete metagenome. However, advances in this research area have led to the circumvention of these obstacles, making it feasible to execute this strategy in a large number of projects and laboratories (Sharpton 2014).

Metagenomics and non-model plant microbiomes

NGS technologies and bioinformatics have become more efficient and affordable, allowing a broader characterization of plant microbiomes. This benefited especially non-model plants, whose cultivation (of the plant itself) is sometimes difficult, and even more, the cultivation and identification of its microbiome with traditional methods. Thus, metagenomic approaches have been successfully applied to evaluate microbiome diversity and functional characteristics of the model and non-model plants (Simon et al. 2019) and compare them to each other.

Since the description of the microbiome associated with the root of healthy *A. thaliana* plants through metagenomics (Lundberg et al. 2012), several other non-model plants (including crops) had their microbiomes characterized. Investigations of plant microbiomes aim to promote the descriptive or structural analysis of microbial communities associated with plants under different conditions (Sánchez-Cañizares et al. 2017), as illustrated in Table 2, including host plant species (Marasco et al. 2018), developmental stage (Xiong et al. 2021) and temporal differences (Ou et al. 2019).

Studies have explored the structure and function of the microbiome of non-model species and cultivation under both natural and controlled conditions (Table 2) (Busby et al. 2017; Nadarajah 2019). Some of them investigated the microbiome of invasive plants under both conditions, using metagenomic approach 16S rRNA, providing a description of microbial communities' structural diversity in separate compartments. Studies in this context have identified bacterial members associated with Plant Growth Promoters (PGP), indicating that the microbiome is potentially involved in the colonization capacity of invasive plants in new environments (Cheng et al. 2019; Zhang et al. 2020c), often with challenging environmental conditions (Table 2).

Research with emphasis on the microbiome exploitation of important cultivated species includes, for example, crops such as corn (*Zea mays* L., Poaceae), soybean (*Glycine max* (L.) Merr), and rice (*O. sativa*) (Table 2). Using metagenomic shotgun associated with metabarcoding (16S rRNA and ITS), Xiong et al. (2021) proposed that bacterial communities in leaves and roots of maize played a critical role in early developmental stages, while fungal communities showed a similar pattern in the late developmental stages. This result indicates that the developmental stage of maize strongly influences microbiome assembly, with bacterial and fungal communities possibly having distinct and beneficial ecological roles on the microbiome and host performance throughout plant growth. Using 18S rDNA sequencing, Andreo-Jimenez et al. (2019) analyzed the endophytic fungal diversity of rice. They identified that the richness of fungal OTUs in the root endosphere increased when the plants were subjected to water deficit. The authors proposed that the recruitment of fungi by the roots aimed to mitigate the effects of drought on the host plant.

The use of metagenomics in non-model plants has enabled the investigation of the effects of abiotic stresses in

Table 2 Synthetic description of exemplary studies on different aspects of non-model plant microbiomes reported in the literature, using metagenomics approaches (SA: sequencing of amplicon; SS: DNA shotgun sequencing)

Host plant(s)	Source	Microbiome understudy	Approach	Main conclusions	References
Ageratina adenophora (Spreng.) R.M. King & H. Rob. (Asteraceae)	Experimental conditions	Roots, stems, and leaves endosphere	SA (16S rRNA)	The endophytic bacterial community showed significantly higher diversity in the roots and stems of control plants than plants treated with tetracycline. The reduced abundance of endophytic bacterial members in the treated plants was accompanied by adverse effects on plant development, suggesting its relevance to host plant performance and health	Zhang et al. (2020c)
Oryza sativa	International Rice Research Institute (IRRI, Los Baños, Philippines)	Root endosphere	SA (18S rRNA)	The fungal community of the root endosphere changed when plants were subjected to water stress. Enrichment of endophytic fungi in the roots possibly helped to mitigate the effects of drought on the host plant	Andreo-Jimenez et al. (2019)
Morus alba L., *M. laevigata* Wall., *M. atropurpurea* Roxb. e *M. atropurpurea* Roxb	Fields of China	Branch endosphere	SA (16S rRNA)	The composition of the endophytic bacterial community showed differences between samples obtained from the plants in autumn and spring. Taxonomic analysis suggested that mulberry trees can selectively recruit species of bacterial genera to adapt to the adverse autumn environment	Ou et al. (2019)
Stipagrostis sabulicola (Pilg.) De Winter, *S. seelyae* De Winter, and *Cladoraphis spinosa* (L.f.) S.M.Phillips (Poaceae)	Dunes of the Namib Desert, Africa	Rhizosphere and roots	SA (16S rRNA e ITS)	In severe desertic biomes, microbial recruitment of the rhizosphere-root niche is more affected by the sand conditions concerning the genotypes of different plant species. The predominance of bacteria over fungi in the rhizosphere of the studied taxa	Marasco et al. (2018)

Table 2 (continued)

Host plant(s)	Source	Microbiome understudy	Approach	Main conclusions	References
Alhagi sparsifolia Shap. (Fabaceae)	Desert region, China	Rhizosphere and root endosphere	SA (16S rRNA) and SS	Rhizosphere and root endosphere harbor different microbial taxa and abundances. Different rates of bacterial endophytes improve the plant tolerance to drought	Zhang et al. (2020b)
Senecio vulgaris L. (Asteraceae)	Shennongjia Forest District, Hubei Province, China	Rhizosphere and leaf and root endosphere	SA (16S rRNA)	Higher microbial diversity was detected in the rhizosphere compared to the endosphere (roots and leaves were sampled). *Brevundimonas diminuta* and *Rhizobium leguminosarum* were abundant in roots and leaves, possibly involved in the adaptive capacity of the invasive plant species to challenging environments	Cheng et al. (2019)
Zea mays L. (Poaceae)	Fields of China	Rhizoplane, root endosphere, phylloplane, leaf endosphere, and grain	SA (16S rRNA and ITS) and SS	The plant development stage plays a deterministic role in the assembly of the microbiome, with bacterial communities enriched in the early stages and fungal communities in the late stages. Such microbial succession can play important ecological roles throughout plant development	Xiong et al. (2021)
Oryza sativa	Central Agricultural University (CAU), India	Stems and roots endosphere	SA (16S rRNA) and SS	Plant growth stages impacted the composition of the endophytic bacterial community in the root and shoot. The enrichment of antioxidant-producing genes in the endophytic microbiome indicates the possibility of their contribution to the antioxidant activity of host plants	Singha et al. (2021)

Table 2 (continued)

Host plant(s)	Source	Microbiome understudy	Approach	Main conclusions	References
Glycine max	Fields of China	Seeds and roots endosphere	SA (ITS)	Soybean roots and seeds have different endophytic fungal communities. The most outstanding amount in specific fungal genera and members was identified in the roots, indicating that soil conditions impact the diversity of the fungal community after seed germination	Yang et al. (2018)
Elymus nutans Griseb. (Poaceae)	Qinghai Tibet Plateau, China	Seeds endosphere	SA (16S rRNA e ITS)	The *E. nutans* seed endosphere microbiome presents a higher number of positive associations between bacterial and fungal members. Such microbial networks possibly impact the establishment of fungal and bacterial associations fundamental in the next generation of plants	Guo et al. (2021)
Agave tequilana F.A.C.Weber, *Agave deserti* Engelm, *Agave salmiana* Otto ex Salm-Dyck (Agavaceae), *Myrtillocactus geometrizans* Console, and *Opuntia robusta* H.L.Wendl. ex Pfeiff (Cactaceae)	Fields of Mexico	Rhizosphere, phyllosphere and root and leaf endosphere	SA (16S rRNA e ITS)	The plant niche and biogeography mainly influence the composition of the microbiome. Different plant niches have specific bacterial taxa and functions, possibly necessary for survival in arid environments	Citlali et al. (2018)
Ephedra przewalskii, Nitraria sphaerocarpa, Reaumuria soongorica, Salsola passerine, and *Sympegma regelii*	Anxi Extreme Arid Desert, China	Leaves, stems, and roots endosphere	SA (ITS)	The diversity of the endosphere fungal microbiome of xerophytic species in an extreme desert environment is strongly driven by host plant identity. Characteristics of these plants (morphological, physiological, and genetic) possibly shape the endophytic fungal microbiome	Zuo et al. (2021)

Table 2 (continued)

Host plant(s)	Source	Microbiome understudy	Approach	Main conclusions	References
Echinocactus platyacanthus Link & Otto, *Ferocactus latispinus* Haw. Britton & Rose, *Ferocactus pilosus* Galeotti Werderm *and Stenocereus queretaroensis* F.A.C. Weber Buxb. (Cactaceae)	Fields of Mexico	Seeds endoseminal and episeminal	SA (16S rRNA e ITS)	Different compositions between the episeminal and endoseminal bacterial and fungal communities were identified in the seeds among the cactus species. Bacterial and fungal diversity showed the high dominance of specific genera in seeds. Such dominance may result from selection by the host due to the beneficial functions performed by the microorganisms during germination and plantlet development	Mascot-Gómez et al. (2021)

structuring the microbiome of plant species capable of inhabiting places with challenging environmental conditions. This approach is promising since the microbiome may also benefit the plant's survival or adaptation to environmental conditions (Ahlawat et al. 2018). In this context, studies involving the composition and influence of the plant microbiome on adaptation or tolerance to different environmental stresses, such as drought and salinity, have received particular attention (Table 2). Analyzing the microbiome of *Alhagi sparsifolia* Shap. (a Leguminosae of desert environments) using metagenomic approaches, Zhang et al. (2020b) found that different plant compartments (rhizosphere and root endosphere) presented other microbial taxa and variable abundances, proposing that the endosphere houses endophytic taxa that contribute to plant drought resistance. Using a similar approach, Zuo et al. (2021) demonstrated that the fungal microbiome of five xerophytic shrubs inhabiting an extremely acidic ecosystem varied among different host plant species, indicating that even under identical environmental conditions, host identity strongly influences the structuring and selection of the fungal community in the inner tissues of leaves, stems, and roots. Furthermore, the dominance of specific fungal members in the endosphere characterized with beneficial effects may be related to the adaptive and ecological success of the studied species in arid environments.

Metatranscriptomics as a tool for studying the microbiome

Metagenomics comprises an initial stage for studies in the microbiome, providing data on the taxonomic profile and whole metagenome sequencing (WMS), allowing potential functional profiling of a microbial community (Aguiar-Pulido et al. 2016). On the other hand, metatranscriptomics covers the sequencing of an environmental sample's transcriptome, making it possible to analyze the set of expressed RNAs of the associated microbiota (Marchesi and Ravel 2015). In this way, metatranscriptomics complements metagenomic data, allowing the accurate obtaining of the transcriptional profile of the genomes that make up a microbial community submitted to a particular environmental condition (Chandra et al. 2014).

In general, studies with metatranscriptomics involve the following steps: (i) collecting the samples; (ii) isolation of total RNA (for each sample); (iii) removal of rRNA, aiming at enriching the protein-coding mRNA (when the target of the study is gene expression); (iv) cDNA synthesis; (v) sequencing, using NGS technologies; and (vi) data processing and analysis (bioinformatics), including QC, assembly, and differential gene expression analysis (Pérez-Pantoja and Tamames 2015). Metatranscriptomics analyses provide a better understanding of the microbiome

functions and enable the discovery of new genes for biotechnological applications (Mukherjee and Reddy 2020).

Since its introduction in the early 2000s, the number of studies based on metatranscriptomics has increased significantly, with investigations on the most varied ecosystems. It has not been different considering plant microbiomes since metatranscriptomics allows us to identify specific functions of microbial members and transcripts involved in the microbiome-plant interaction (Shakya et al. 2019).

Metatranscriptomic analysis of leaves of *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) revealed genes differentially expressed in fungal communities (endophytic and epiphytic) in plants subjected to simulated global warming conditions (compared to natural conditions in maritime Antarctica), demonstrating the influence of climatic factors on the modulation of the microbial metatranscriptome. Genes induced in the simulated condition and related to the metabolic and molecular pathways of the involved microorganisms may assist in the adaptation of the species to adverse environmental conditions (Ballesteros et al. 2020). Through independent analysis of the rRNA and mRNA data sets, it was demonstrated that the combined condition of prolonged heat waves and elevated atmospheric CO_2 (eCO_2) has significant effects on the composition and activity of the rhizosphere microbiome in European pastures (Bei et al. 2019). A considerable decline in fungal abundance was observed in the rhizosphere, with reduced nitrogen metabolism. It increased the production of secondary metabolites in the rhizosphere and roots as a strategy to defend the plant against the extreme heat conditions associated with eCO_2 (Bei et al. 2019).

Metatranscriptomics approach allowed the identification of active microbial communities and the expression of a gene associated with the microbiome in the plant carbohydrate metabolism during the fruit ripening process in different watermelon (*Citrullus lanatus* (Thunb.) Mansf., Cucurbitaceae) cultivars (Saminathan et al. 2018). A similar approach was used to study the xylem sap of vines during the bleeding period to identify their microbiome composition and metabolic activity. *Proteobacteria*, *Basidiomycota*, and *Ascomycota* were the most abundant phyla. The functional analysis pointed out the microbiota activity associated with the growth of microorganisms and resistance to diseases in the xylem sap during the bleeding period (Zheng et al. 2019).

Like any other approach, metatranscriptomics also has limitations. For example, in some cases, RNA extraction (from complex samples) is not a very easy procedure (Bashiardes et al. 2016). Also, the lack of high-quality reference genomes for assigning transcripts to specific microorganisms is a limiting factor (Levy et al. 2018). Despite these difficulties, new tools have been continuously developed in this area and help study active members, gene expression, and pathways involved in microbiomes (Shakya et al. 2019).

Microbiome–plant interactions as an alternative for sustainable agricultural production

Given the world population's constant growth, the great demand for food production makes intensive agricultural output necessary. However, the application of this practice can trigger a series of environmental imbalances, including drought, soils that are poorer in nutrients and more saline, contaminated, increased incidence of pests, pollution of rivers, among others (Browne et al. 2013). In this scenario, plant and soil microbiome exploration as a promising approach to agricultural sustainability has been increasingly relevant (Barea 2015), especially the here called "endophytome".

The identification of endophytic members of the microbiome with beneficial activities emerges as a powerful strategy to be considered in promoting more sustainable agriculture, reducing the application of fertilizers, and combating phytopathogens to boost better crop yields. Therefore, a greater understanding of the aspects involved in plant-endophytome interaction and the influence of this set of microbes on better plant productivity is necessary. Singh et al. (2020) consider that only then can new methods be explored in the manipulation of the microbiome for the development of sustainable agriculture.

There are different strategies to promote the engineering of the plant microbiome. Among them is the possibility of selection mediated by hosts. Another alternative is to inoculate strains directly into the soil or plant tissues, including rhizosphere, seeds, and seedlings. There is also tissue atomization or direct injection of strains into tissues or wounds of the plant (Orozco-Mosqueda et al. 2018).

The host-mediated microbiome selection approach comprises a technique that applies artificial selection to improve host performance. In this case, microbial communities are selected indirectly through host fitness characteristics that have evolved between generations due to the influence of the microbiome (Mueller and Sachs 2015).

The best strategy for applying endophytic microorganisms in agricultural systems is still not well established, although the most used method consists of direct inoculation in soil or seeds (Cocq et al. 2017). This option was efficiently applied in sugarcane (*Saccharum officinarum* L., Poaceae) research (da Silva et al. 2012), for example. The spraying of flowers with an inoculum of the endophytic microorganism of interest has also been suggested, aiming at vertical transmission (via seeds) to plants' next generation. Following this strategy, Mitter et al. (2017) successfully managed to introduce *Paraburkholderia phytofirmans* PsJN (a powerful grown-promoting bacterium) in the flowers of

various crops of agronomic interest, with consequent inclusion in the microbiome on the seeds of the progenies. Even tested in the field, this strategy comprises a new tool to supply beneficial bacteria to plants, helping their development and agricultural production (Mitter et al. 2017).

The effects of inoculants on the native plant-associated (indigenous) microbiome was recently reviewed by Berg et al. (2021). According to the authors, the modulation of the indigenous microbiome is influenced by the action of inoculants, depending on the sampling time (after application), resulting in six types of modulation: (i) transient changes; (ii) stabilization or increase in microbial diversity; (iii) stabilization or enhancement of microbiome uniformity; (iv) restoration of dysbiosis/compensation of a phytopathogen-induced change; (v) targeted changes to members of the plant's beneficial native microbiome; and (vi) phytopathogen suppression. Considering microbial composition, the effects of microbial inoculants lead to changes that simultaneously enrich potentially beneficial members of the indigenous microbiome while suppressing potential pathogens (Berg et al. 2021).

Although different microbiome approaches to improving agricultural productivity have proliferated, there are still some practical challenges to the effectiveness of these strategies in the field. The successful introduction of microbial inoculants depends on their ability to compete with the native plant-associated microbiome (Batista and Singh 2021). To overcome this limitation, selecting indigenous plant microorganisms as inoculants emerges as a promising procedure to explore their potential in promoting plant growth and agricultural productivity (Kouadio et al. 2017; May et al. 2021). In this sense, the selection of endophyte strains adapted to the plant can improve the survival chances and inoculum performance in promoting beneficial effects on plant development (Qiu et al. 2019). Nevertheless, further studies are needed in order to broaden the understanding of how the indigenous microbiome interacts with microbial inoculants to improve microbiome management methods (French et al. 2021).

Another constraint is because a large part of environmental microorganisms cannot be cultivated. Also, the activity of several microbes may be influenced by specific biotic and abiotic factors. Therefore, the integration of emerging approaches in agriculture, synthetic biology, big data, and metagenomics is mandatory to provide a factual basis for exploring the maximum potential of biotechnological applications of the plant microbiome in the development of agricultural and environmental sustainability (Singh et al. 2018).

Among the main objectives of agricultural biotechnology is developing microbial inoculants capable of increasing plant growth and repressing plant diseases, aiming to reduce the dependence on chemical fertilizers and pesticides (Adesemoye et al. 2009). For the development of these inoculants, however, interdisciplinary research studies are necessary, capable of optimizing parameters including, selection of beneficial microorganisms, analysis and selection of those with positive effects on the plant, as well as detecting and evaluating the performance of most promising inoculants in the soil (Pereg and McMillan 2015).

Microbial biotechnology plays an essential role in promoting sustainable agriculture in different ways, including managing biotic and abiotic stresses, increasing productivity, acquiring nutrients, and promoting bioremediation (Singh 2019). Some relevant studies involving potential applications of plant microbes to increase agricultural production sustainably will be reviewed below.

Management of biotic and abiotic stresses

Given the current climate change scenario, the development of crops tolerant to various environmental stresses is essential. Although the genome of the plant itself can confer this type of tolerance, the innumerable interactions with microbial communities can assist in the greater efficiency of this process (Farrar et al. 2014). Rhizobacterial species (*Bacillus* spp. and *Pantoea* spp.) isolated from cactus species in a semiarid environment were inoculated in corn seedlings (*Z. mays*) and conferred a capacity to promote plant growth under water stress conditions (Kavamura et al. 2013). Likewise, rhizobacteria isolated from *S. italic* have been identified as potential bioinoculants to assist agricultural production in arid regions due to their proven stimulation activity in the germination and growth of seedlings of this species under water deficit (Niu et al. 2018).

Soil salinity comprises another abiotic stress that negatively impacts agricultural production, sometimes leading to soil infertility. Microbiomes isolated from cultures adapted to saline ecosystems can promote plant growth (Yadav and Saxena 2018). For instance, arbuscular mycorrhizal fungi (AMF) isolated from areas and plants affected by salinity conferred tolerance to salt stress in inoculated corn plants (Estrada et al. 2013). A consortium of three strains isolated from PGPR tolerant to salinity from wheat plants increased the growth and yield of this culture under experimental and field saline conditions through PGPR biofertilization (inoculation) technology. Such a strategy has emerged as an alternative to wheat biofertilizer in saline environments, reducing chemical fertilizers (Rajput et al. 2018).

Several phytopathogens lead to a series of diseases responsible for significant losses in crop and harvest productivity worldwide. In this context, using beneficial microorganisms as a control alternative to limit chemical pesticides has encouraged studies over the past decades (Syed Ab Rahman et al. 2018).

Microbial plant communities as a source of biocontrol agents (BCAs) have generated great enthusiasm as a method

of sustainable control of plant pathogens, deserving more comprehensive research, especially regarding practical applications in cultivated plants (Massart et al. 2015). For instance, fungi isolated from the endosphere of strawberry leaves (*Fragaria vesca* L., Rosaceae), especially *Paecilomyces*, induced a high mortality rate of the pathogen *Duponchelia fovealis*, emerging as a promising alternative for biocontrol (Amatuzzi et al. 2018).

Biofertilizers for increased productivity

A biofertilizer comprises a substance containing potentially beneficial living microorganisms that colonize their interior or rhizosphere when applied to plant seeds or added to the soil, increasing crop productivity and nutrients supply to the host plant. Microbial biofertilizers represent one of the main alternatives for sustainable agricultural production, minimizing the need to use chemical fertilizers (Reddy et al. 2020; Kour et al. 2020). They improve soil fertility by fixing atmospheric nitrogen, solubilizing insoluble phosphates, and producing plant growth substances in the soil (Mazid and Khan 2015). Biofertilizers include different genera and species of microorganisms. They are categorized into four groups according to their beneficial mechanisms of action: (i) nitrogen fixation (e.g., *Azotobacter*, *Rhizobium*, *Azospirillum*, and *Cyanobacteria*); (ii) phosphate solubilization (e.g., *Pseudomonas striata*, *Bacillus polymyxa*, *Penicillium*, and *Aspergillus*); (iii) composting microorganisms (e.g., *Bacillus* spp.); and (iv) PGPRs (e.g., *Pseudomonas* and *Bacillus* species) (Pathak and Kumar 2016; Khan et al. 2018).

Studies in plant microbiomes revealed unexplored microbial consortia that can be used as effective biofertilizers (Meena and Busi 2019). In this perspective, under field conditions, Khaitov et al. (2020) analyzed the effect of the inoculation of rhizobia strains (*Rhizobium phaseoli*—R9 and *Mesorhizobium cicero*—R6) isolated from the rhizospheres of 2 legumes—soybean, *G. max*. and chickpea (*Cicer arietinum* L.)—in common bean seeds (*Phaseolus vulgaris* L.). Co-inoculation increased the growth, root nodulation, and yield of *P. vulgaris*, probably due to higher nitrogen absorption. This indicates that this alternative is better than the inoculation of a single rhizobium strain for biofertilization, improving productivity under salinity. Another example regards endophytic fungi isolated from rice and inoculated as consortia in cucumber seeds (*Cucumis sativus* L., Cucurbitaceae) were able to efficiently increase plant growth, highlighting the potential use of endophytic fungal isolates as biofertilizers (Syamsia et al. 2021).

Inoculants and their influence on the acquisition of nutrients

Plant growth and productivity require the presence of nutrients in the soil–root interaction zone. The availability of nutrients at this interface is influenced by many factors, including the activity of roots and microorganisms in the rhizosphere. The use of microbial inoculants as an alternative to the application of chemical fertilizers for crop nutrition comprises a promising approach to improve plant development based on more sustainable practices. Evidence indicates that the inoculation of plants with PGPR increases soil nutrients' bioavailability for plants (Pii et al. 2015). In this context, 4 PGPR (*Pseudomonas* sp., *Pseudomonas* sp., *Bacillus pumilus*, and *Paenibacillus polymyxa*) were analyzed to investigate their effects on growth, nutrient uptake, and photosynthetic activity in Habanero pepper (*Capsicum chinense* Jacq., Solanaceae). As a result of the inoculation during sowing, there was a significant increase in growth parameters, photosynthetic activity, and the absorption of nutrients. Seedlings inoculated with *P. polymyxa* showed higher acquisition of P and K compared to controls. Later, inoculation with this rhizobacterium was pointed out as an alternative for reducing fertilizers in agricultural production (Castillo-Aguilar et al. 2017). Using similar approaches, Kumar et al. (2017a) analyzed the effect of indigenous PGPR isolated from different crops on growth and obtaining nutrients in wheat cultivation. The inoculation of a consortium of 3 PGPR, besides having positive effects, helped in plants growth and yield, also leading to a significant increase in the levels of macro and micronutrients (N, P, Cu, Zn, Mn, and Fe) in wheat grains, also in-field conditions.

Arbuscular mycorrhiza (AM) is the most frequent type of mycorrhizal association. They play a vital role in acquiring macro- and micronutrients for the host plant (Hodge and Storer 2015). These fungi promote an increase in the acquisition of nitrogen, phosphorus, and other soil nutrients by exchanging photosynthetically fixed carbon by the host plant (Kaul et al. 2017). Ortas et al. (2019) observed under field conditions that in different horticultural plants, the effects of inoculation of mycorrhizal fungi on plant yield were higher without phosphorus addition, which led to better plant growth and nutrient absorption (P and Zn). These results showed that mycorrhizal inoculants could reduce the dependence on phosphorus fertilization crop plants.

Microorganisms in the promotion of bioremediation

Bioremediation involves the use of living organisms, plants, and/or microorganisms to mitigate or eliminate environmental pollutants (Chibuike and Obiora 2014). As forms of bioremediation, phytoremediation in plant tissues and rhizoremediation in the rhizosphere are likely to happen

naturally in plants. Such a process can be improved by the association with microorganisms capable of helping plants survive in contaminated environments or even in the degradation of contaminants (Eevers et al. 2017). So, phytoremediation is a sustainable, cheaper, and ecological alternative to conventional physicochemical pollutant remediation methods, also applicable in economically emerging countries (Shmaefsky 2020).

Iram et al. (2019) evaluated the effects of inoculation of fungi of the Trichocomaceae family on corn and sunflower (*Helianthus annuus* L., Asteraceae) for the uptake of heavy metals in contaminated soils of agricultural importance. The study demonstrated that corn and sunflower plants, inoculated with fungi using three different methods, significantly increased the phytoremediation potential to absorb heavy metals in contaminated soils in the Gujranwala region (Pakistan). In another study, Liu et al. (2021) analyzed the effect of using endophytic bacteria-isolated from *Phytolacca acinosa* Roxb. (Phytolaccaceae) growing in cadmium (Cd) contaminated soil—as inoculants. As a result, inoculated plants of *P. acinosa* presented increased growth and capacity of accumulating Cd, demonstrating the potential use of endophytes in soil remediation, including those contaminated by heavy metals.

PGPR inoculation may also assist the phytoremediation process; as observed by Khan et al. (2017) in a greenhouse experiment, the rhizobacteria *Bacillus subtilis* 189 and *Pseudomonas fluorescens* RB4 were inoculated into seedlings of *Catharanthus roseus* (L.) G. Don (Apocynaceae) to identify their effects on phytoremediation in soils contaminated with copper (Cu) and lead (Pb) in Pakistan. As a result, the co-inoculation of the two rhizobacteria significantly increased the growth of shoots in contaminated soils, plant biomass and resulted in a more significant accumulation of heavy metals (Cu and Pb) in shoots of *C. roseus* grown in the contaminated soils as compared with non-inoculated control plants (Khan et al. 2017).

Conclusions and perspectives

The plant microbiome interacts with both the internal and the superficial tissues of the host plant. Its structure and performance are influenced by a series of intrinsic (genome, plant immune system, developmental stage, etc.) and extrinsic factors (such as soil properties and environmental factors). Consequently, there are noticeable differences in the microbiome's composition between internal and superficial niches on the same plant and between different plant individuals or taxa.

The affordable cost of sequencing and new bioinformatics tools contribute to a more comprehensive description of the structural and microbial metabolic composition of the model and non-model plants. The development of metagenomic and metatranscriptomic approaches allows trespassing limitations of culture-dependent microbiological techniques to study and understand microbiomes. Despite that, the isolation and lab cultivation of specific components of the plant microbiome remains challenging.

Also, sequences from microbiotic origin have been detected in sequencing plant genomes and transcriptomes, mainly being ignored or reported as 'no hits". Revisiting raw data of such projects should bring many interesting evidences concerning plant associated-microorganisms.

There is no doubt that essential roles are provided through microbiome-plant interactions in promoting plant growth and defense against biotic and abiotic stresses. Indeed, recent advances in understanding plant microbial communities through metagenomics demonstrate the enormous untapped potential of the plant microbiome. Evidence points out that the inoculation of microorganisms isolated from specific plant microbial communities increases plant growth, yield, and productivity, through different processes.

However, there is a long way to go. Detailed information about the plant-microbiome-environment interaction is available to a limited number of plant species, mainly cultivated, herbaceous, and temperate. Information on tropical plants, specially adapted to challenging environments (so-called extremophiles), is necessary and should shed new light on the complexity of survival in such environments. In this context, the microbiome of pioneer plants, capable of establishing in degraded environments or soils contaminated by heavy metals, should receive investments and special attention.

There are no reports of adverse effects related to the application of endophytes in improving crop production. Therefore, the implementation of these practices in a sustainable way worldwide is promising and can revolutionize agriculture. However, it demands standardized processes that depend on interdisciplinary efforts, especially regarding developing marketed products and appropriate strategies for manipulating plant microbiomes associated with a given culture and environment.

The main question remains: Is the plant microbiome defined by the soil it inhabits, or is the soil microbiome defined/influenced by the plants (and organisms) that colonize a given environment? Examples of both situations can be observed.

Also, few studies discuss the balance between endophytes and known pathogenic microorganisms within the same microbiome. The general idea is that latent pathogenic species may be harmful to the plant under a sudden stressful condition. On the other hand, such "unwanted hosts" may be responsible for a state of alertness, important for constitutive plant defense.

Research efforts on wild plants in their natural environment (also relatives of crop species) may be more elucidative since many factors can affect studies under greenhouse conditions or experimental fields.

The future application of systems biology approaches is mandatory to bring an integrated view of both plant and microbiome molecular processes in a holistic approach.

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Declarations

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References

Abadi VJM, Sepehri M, Rahmani HA et al (2020) Diversity and abundance of culturable nitrogen-fixing bacteria in the phyllosphere of maize. J Appl Microbiol. https://doi.org/10.1111/jam.14975

Abraham BS, Caglayan D, Carrillo NV et al (2020) Shotgun metagenomic analysis of microbial communities from the Loxahatchee nature preserve in the Florida Everglades. Environ Microbiome 15:2. https://doi.org/10.1186/s40793-019-0352-4

Adesemoye AO, Torbert HA, Kloepper JW (2009) Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58:921–929. https://doi.org/10.1007/s00248-009-9531-y

Aguiar-Pulido V, Huang W, Suarez-Ulloa V et al (2016) Metagenomics, metatranscriptomics, and metabolomics approaches for microbiome analysis. Evol Bioinforma 12:5–16. https://doi.org/10.4137/ebo.s36436

Aguilar M, Grasso DH (2010) Metagenómica. In: Levitus G, Echenique V, Rubinstein C, et al (eds) Biotecnología y mejoramiento vegetal II. Instituto Nacional de Tecnología Agropecuaria, Argentina

Ahlawat OP, Tiwari R, Pratap Singh G (2018) Metagenomics of wheat rhizosphere for abiotic stress management. Wheat Barley Res 10:64–77. https://doi.org/10.25174/2249-4065/2018/79291

Allard G, Ryan FJ, Jeffery IB, Claesson MJ (2015) SPINGO: a rapid species-classifier for microbial amplicon sequences. BMC Bioinform 16:324. https://doi.org/10.1186/s12859-015-0747-1

Altschul SF, Madden TL, Schäffer AA et al (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402. https://doi.org/10.1093/nar/25.17.3389

Amatuzzi RF, Cardoso N, Poltronieri AS et al (2018) Potential of endophytic fungi as biocontrol agents of *Duponchelia fovealis* (Zeller) (Lepidoptera:Crambidae). Braz J Biol 78:429–435. https://doi.org/10.1590/1519-6984.166681

Ames SK, Hysom DA, Gardner SN et al (2013) Scalable metagenomic taxonomy classification using a reference genome database. Bioinformatics 29:2253–2260. https://doi.org/10.1093/bioinformatics/btt389

Andreo-Jimenez B, Vandenkoornhuyse P, Van AL et al (2019) Plant host and drought shape the root associated fungal microbiota in rice. PeerJ 7:e7463. https://doi.org/10.7717/peerj.7463

Andreote FD, Gumiere T, Durrer A (2014) Exploring interactions of plant microbiomes. Sci Agric 71:528–539. https://doi.org/10.1590/0103-9016-2014-0195

Arango-Argoty G, Singh G, Heath LS et al (2016) MetaStorm: a public resource for customizable metagenomics annotation. PLoS ONE 11:e0162442. https://doi.org/10.1371/journal.pone.0162442

Arora NK, Tewari S, Singh R (2013) Multifaceted plant-associated microbes and their mechanisms diminish the concept of direct and indirect PGPRs. Plant microbe symbiosis: fundamentals and advances. Springer, Berlin, pp 411–449

Asaf S, Khan MA, Khan AL et al (2017) Bacterial endophytes from arid land plants regulate endogenous hormone content and promote growth in crop plants: an example of *Sphingomonas* sp. and *Serratia marcescens*. J Plant Interact 12:31–38. https://doi.org/10.1080/17429145.2016.1274060

Ballesteros GI, Torres-Díaz C, Bravo LA et al (2020) In silico analysis of metatranscriptomic data from the Antarctic vascular plant *Colobanthus quitensis*: responses to a global warming scenario through changes in fungal gene expression levels. Fungal Ecol 43:100873. https://doi.org/10.1016/j.funeco.2019.100873

Bao L, Gu L, Sun B et al (2020) Seasonal variation of epiphytic bacteria in the phyllosphere of *Gingko biloba*, *Pinus bungeana* and *Sabina chinensis*. FEMS Microbiol Ecol 96:17. https://doi.org/10.1093/femsec/fiaa017

Barea JM (2015) Future challenges and perspectives for applying microbial biotechnology in sustainable agriculture based on a better understanding of plant-microbiome interactions. J Soil Sci Plant Nutr. https://doi.org/10.4067/S0718-95162015005000021

Barret M, Guimbaud J, Darrasse A, Jacques M (2016) Plant microbiota affects seed transmission of phytopathogenic microorganisms. Mol Plant Pathol 17:791–795. https://doi.org/10.1111/mpp.12382

Bashiardes S, Zilberman-Schapira G, Elinav E (2016) Use of metatranscriptomics in microbiome research. Bioinform Biol Insights 10:19–25. https://doi.org/10.4137/BBI.S34610

Bateman A, Coin L, Durbin R et al (2004) The Pfam protein families database. Nucleic Acids Res 32:D138-141. https://doi.org/10.1093/nar/gkh121

Batista BD, Singh BK (2021) Realities and hopes in the application of microbial tools in agriculture. Microb Biotechnol 14:1258–1268. https://doi.org/10.1111/1751-7915.13866

Bei Q, Moser G, Wu X et al (2019) Metatranscriptomics reveals climate change effects on the rhizosphere microbiomes in European grassland. Soil Biol Biochem 138:107604. https://doi.org/10.1016/j.soilbio.2019.107604

Bengtsson-Palme J, Hartmann M, Eriksson KM et al (2015) metaxa2: improved identification and taxonomic classification of small and large subunit rRNA in metagenomic data. Mol Ecol Resour 15:1403–1414. https://doi.org/10.1111/1755-0998.12399

Berg G (2015) Beyond borders: investigating microbiome interactivity and diversity for advanced biocontrol technologies. Microb Biotechnol 8:5–7. https://doi.org/10.1111/1751-7915.12235

Berg G, Kusstatscher P, Abdelfattah A et al (2021) Microbiome modulation—toward a better understanding of plant microbiome response to microbial inoculants. Front Microbiol 12:803. https://doi.org/10.3389/fmicb.2021.650610

Bergna A, Cernava T, Rändler M et al (2018) Tomato seeds preferably transmit plant beneficial endophytes. Phytobiomes J 2:183–193. https://doi.org/10.1094/PBIOMES-06-18-0029-R

Bose T, Haque MM, Reddy C, Mande SS (2015) COGNIZER: a framework for functional annotation of metagenomic datasets. PLoS ONE 10:e0142102. https://doi.org/10.1371/journal.pone.0142102

Breitwieser FP, Lu J, Salzberg SL (2017) A review of methods and databases for metagenomic classification and assembly. Brief Bioinform 20:1125–1136. https://doi.org/10.1093/bib/bbx120

Bringel F, Couée I (2015) Pivotal roles of phyllosphere microorganisms at the interface between plant functioning and atmospheric trace gas dynamics. Front Microbiol. https://doi.org/10.3389/fmicb.2015.00486

Browne P, Barret M, Morrissey JP, O'Gara F (2013) Molecular-based strategies to exploit the inorganic phosphate-solubilization ability of *Pseudomonas* in sustainable agriculture. In: de Bruijn FJ (ed) Molecular microbial ecology of the rhizosphere. Wiley, Hoboken, pp 615–628

Bulgarelli D, Schlaeppi K, Spaepen S et al (2013) Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807–838. https://doi.org/10.1146/annurev-arplant-050312-120106

Busby PE, Soman C, Wagner MR et al (2017) Research priorities for harnessing plant microbiomes in sustainable agriculture. PLoS Biol 15:e2001793

Byrd AL, Belkaid Y, Segre JA (2018) The human skin microbiome. Nat Rev Microbiol 16:143–155. https://doi.org/10.1038/nrmicro.2017.157

Campisano A, Albanese D, Yousaf S et al (2017) Temperature drives the assembly of endophytic communities' seasonal succession. Environ Microbiol 19:3353–3364. https://doi.org/10.1111/1462-2920.13843

Caporaso JG, Kuczynski J, Stombaugh J et al (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336. https://doi.org/10.1038/nmeth.f.303

Chadha S (2019) Plant-microbe interaction: gene-to-metabolite network. In: Abdelrahman M (ed) Bioactive molecules in plant defense. Springer, pp 75–100

Chaluvadi S, Bennetzen JL (2018) Species-associated differences in the below-ground microbiomes of wild and domesticated Setaria. Front Plant Sci. https://doi.org/10.3389/fpls.2018.01183

Chandra D, Meena SC, Chattopadhyay A, Saharan V (2014) Metagenomics and complementary approaches for the study of microbial communities. Microbial Biodivers Sustain Agric 21

Chen I-MA, Chu K, Palaniappan K et al (2019) IMG/M vol 5.0: an integrated data management and comparative analysis system for microbial genomes and microbiomes. Nucleic Acids Res 47:D666–D677. https://doi.org/10.1093/nar/gky901

Cheng D, Tian Z, Feng L et al (2019) Diversity analysis of the rhizospheric and endophytic bacterial communities of *Senecio vulgaris* L. (Asteraceae) in an invasive range. PeerJ 6:e6162

Chibuike GU, Obiora SC (2014) Heavy metal polluted soils: effect on plants and bioremediation methods. In: Applied and environmental soil science. https://doi.org/10.1155/2014/752708

Citlali F-G, Desgarennes D, Flores-Núñez VM, Partida-Martínez LP (2018) The microbiome of desert CAM Plants: lessons from amplicon sequencing and metagenomics. In: Metagenomics. Elsevier, pp 231–254

Cocq KL, Gurr SJ, Hirsch PR, Mauchline TH (2017) Exploitation of endophytes for sustainable agricultural intensification. Mol Plant Pathol 18:469–473. https://doi.org/10.1111/mpp.12483

Cole JR, Wang Q, Cardenas E et al (2009) The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res 37:D141–D145

Copeland JK, Yuan L, Layeghifard M et al (2015) Seasonal community succession of the phyllosphere microbiome. Mol Plant Microbe Interact 28:274–285. https://doi.org/10.1094/MPMI-10-14-0331-FI

Correia A, Alves A, Henriques I (2012) Métodos de estudo de comunidades bacterianas: diversidade filogenética e molecular, resistoma e elementos genéticos móveis. Magazine Da SPM 1:1–7

da Silva MF, de Souza AC, de Oliveira PJ et al (2012) Survival of endophytic bacteria in polymer-based inoculants and efficiency of their application to sugarcane. Plant Soil 356:231–243. https://doi.org/10.1007/s11104-012-1242-3

Davenport CF, Neugebauer J, Beckmann N et al (2012) Genometa - a fast and accurate classifier for short metagenomic shotgun reads. PLoS ONE 7:e41224. https://doi.org/10.1371/journal.pone.0041224

Dawson W, Hör J, Egert M et al (2017) A small number of low-abundance bacteria dominate plant species-specific responses during rhizosphere colonization. Front Microbiol. https://doi.org/10.3389/fmicb.2017.00975

de la Castillo-Aguilar C, C, Garruña R, Zúñiga-Aguilar JJ, Guzmán-Antonio AA, (2017) PGPR inoculation improves growth, nutrient uptake and physiological parameters of *Capsicum chinense* plants. Phyton 86:199–204

de Souza RSC, Okura VK, Armanhi JSL et al (2016) Unlocking the bacterial and fungal communities assemblages of sugarcane microbiome. Sci Rep 6:1–15. https://doi.org/10.1038/srep28774

DeCastro M-E, Rodríguez-Belmonte M, González-Siso M-I (2016) Metagenomics of Thermophiles with a focus on discovery of novel thermozymes. Front Microbiol 7:1521. https://doi.org/10.3389/fmicb.2016.01521

del Orozco-Mosqueda M, C, Rocha-Granados M del C, Glick BR, Santoyo G, (2018) Microbiome engineering to improve biocontrol and plant growth-promoting mechanisms. Microbiol Res 208:25–31. https://doi.org/10.1016/j.micres.2018.01.005

DeSantis TZ, Hugenholtz P, Larsen N et al (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72:5069–5072. https://doi.org/10.1128/AEM.03006-05

Di Bella JM, Bao Y, Gloor GB et al (2013) High throughput sequencing methods and analysis for microbiome research. J Microbiol Methods 95:401–414. https://doi.org/10.1016/j.mimet.2013.08.011

Diaz NN, Krause L, Goesmann A et al (2009) TACOA: taxonomic classification of environmental genomic fragments using a kernelized nearest neighbor approach. BMC Bioinform 10:56. https://doi.org/10.1186/1471-2105-10-56

Díaz-González S, Marín P, Sánchez R et al (2020) Mutualistic fungal endophyte *Colletotrichum tofieldiae* Ct0861 colonizes and increases growth and yield of maize and tomato plants. Agronomy 10:1493. https://doi.org/10.3390/agronomy10101493

Dong X, Strous M (2019) An integrated pipeline for annotation and visualization of metagenomic contigs. Front Genet. https://doi.org/10.3389/fgene.2019.00999

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Edgar RC, Haas BJ, Clemente JC et al (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200. https://doi.org/10.1093/bioinformatics/btr381

Edwards J, Johnson C, Santos-Medellín C et al (2015) Structure, variation, and assembly of the root-associated microbiomes of rice. Proc Natl Acad Sci 112:E911–E920. https://doi.org/10.1073/pnas.1414592112

Eevers N, White JC, Vangronsveld J, Weyens N (2017) Bio- and phytoremediation of pesticide-contaminated environments. In: Advances in botanical research. Elsevier, pp 277–318

El Allali A, Rose JR (2013) MGC: a metagenomic gene caller. BMC Bioinform 14:S6. https://doi.org/10.1186/1471-2105-14-S9-S6

Estrada B, Aroca R, Maathuis FJM et al (2013) Arbuscular mycorrhizal fungi native from a Mediterranean saline area enhance maize tolerance to salinity through improved ion homeostasis. Plant Cell Environ 36:1771–1782. https://doi.org/10.1111/pce.12082

Fadiji A, Babalola O (2020) Metagenomics methods for the study of plant-associated microbial communities: a review. J Microbiol Methods 170:105860. https://doi.org/10.1016/j.mimet.2020.105860

Fadiji AE, Ayangbenro AS, Babalola OO (2021) Unveiling the putative functional genes present in root-associated endophytic microbiome from maize plant using the shotgun approach. J Appl Genet 62:339–351. https://doi.org/10.1007/s13353-021-00611-w

Farrar K, Bryant D, Cope-Selby N (2014) Understanding and engineering beneficial plant–microbe interactions: plant growth promotion in energy crops. Plant Biotechnol J 12:1193–1206. https://doi.org/10.1111/pbi.12279

Finkel OM, Burch AY, Lindow SE et al (2011) Geographical location determines the population structure in phyllosphere microbial communities of a salt-excreting desert tree. Appl Environ Microbiol 77:7647–7655. https://doi.org/10.1128/AEM.05565-11

Fitzpatrick CR, Lu-Irving P, Copeland J et al (2018) Chloroplast sequence variation and the efficacy of peptide nucleic acids for blocking host amplification in plant microbiome studies. Microbiome 6:144. https://doi.org/10.1186/s40168-018-0534-0

French E, Kaplan I, Iyer-Pascuzzi A et al (2021) Emerging strategies for precision microbiome management in diverse agroecosystems. Nat Plants 7:256–267. https://doi.org/10.1038/s41477-020-00830-9

Gaiero JR, McCall CA, Thompson KA et al (2013) Inside the root microbiome: bacterial root endophytes and plant growth promotion. Am J Bot 100:1738–1750. https://doi.org/10.3732/ajb.1200572

Gilbert JA, Dupont CL (2011) Microbial metagenomics: beyond the genome. Ann Rev Mar Sci 3:347–371. https://doi.org/10.1146/annurev-marine-120709-142811

Glass EM, Wilkening J, Wilke A et al (2010) Using the metagenomics RAST server (MG-RAST) for analyzing shotgun metagenomes. Cold Spring Harb Protoc. https://doi.org/10.1101/pdb.prot5368

Goss-Souza D, Mendes LW, Borges CD et al (2019) Amazon forest-to-agriculture conversion alters rhizosphere microbiome composition while functions are kept. FEMS Microbiol Ecol. https://doi.org/10.1093/femsec/fiz009

Gouda S, Das G, Sen SK et al (2016) Endophytes: a treasure house of bioactive compounds of medicinal importance. Front Microbiol. https://doi.org/10.3389/fmicb.2016.01538

Gregor I, Dröge J, Schirmer M et al (2016) PhyloPythiaS+: a self-training method for the rapid reconstruction of low-ranking taxonomic bins from metagenomes. PeerJ 4:e1603. https://doi.org/10.7717/peerj.1603

Guazzaroni M-E, Beloqui A, Golyshin PN, Ferrer M (2009) Metagenomics as a new technological tool to gain scientific knowledge. World J Microbiol Biotechnol 25:945–954. https://doi.org/10.1007/s11274-009-9971-z

Guo J, Bowatte S, Hou F (2021) Diversity of endophytic bacteria and fungi in seeds of *Elymus nutans* growing in four locations of Qinghai Tibet Plateau, China. Plant Soil 459:49–63. https://doi.org/10.1007/s11104-020-04608-y

Hagagy N, Hamedo H, Elshafi N, Selim S (2021) Shotgun metagenomic sequencing of extremophilic community from Soda Lake, Ga'ar Lake, in Wadi Al-Natrun. Egypt Egyptian J Exp Biol. https://doi.org/10.5455/egyjebb.20210309063625

Hallmann J, Berg G (2006) Spectrum and population dynamics of bacterial root endophytes. In: Schulz BJE, Boyle CJC, Sieber TN (eds) Microbial root endophytes. Springer, Berlin, pp 15–31

Handelsman J (2004) Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev 68:669–685. https://doi.org/10.1128/MMBR.68.4.669-685.2004

Hardoim PR, van Overbeek LS, van Elsas JD (2008) Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol 16:463–471. https://doi.org/10.1016/j.tim.2008.07.008

Hodge A, Storer K (2015) Arbuscular mycorrhiza and nitrogen: implications for individual plants through to ecosystems. Plant Soil 386:1–19. https://doi.org/10.1007/s11104-014-2162-1

Huson DH, Weber N (2013) Microbial community analysis using MEGAN. Methods Enzymol 531:465–485. https://doi.org/10.1016/B978-0-12-407863-5.00021-6

Huson DH, Beier S, Flade I et al (2016) MEGAN Community edtion - interactive exploration and analysis of large-scale microbiome sequencing data. PLOS Comput Biol 12:1004. https://doi.org/10.1371/journal.pcbi.1004957

Iram S, Basri R, Ahmad KS, Jaffri SB (2019) Mycological assisted phytoremediation enhancement of bioenergy crops *Zea mays* and *Helianthus annuus* in heavy metal contaminated lithospheric zone. Soil Sediment Contam 28:411–430

Izard J (2015) Steps in metagenomics: let's avoid garbage in and garbage out. In: Metagenomics for microbiology. Elsevier, pp 1–23

Jayadev A, Navami SS (2014) Bioprospecting microorganisms of marine environment—a review. Asian J Microbiol Biotechnol Environ Exp Sci 16:137–143

Ji Y, Shi Y, Ding G, Li Y (2011) A new strategy for better genome assembly from very short reads. BMC Bioinform 12:493. https://doi.org/10.1186/1471-2105-12-493

Jing G, Sun Z, Wang H et al (2017) Parallel-META 3: comprehensive taxonomical and functional analysis platform for efficient comparison of microbial communities. Sci Rep. https://doi.org/10.1038/srep40371

Kanehisa M, Goto S, Kawashima S et al (2004) The KEGG resource for deciphering the genome. Nucleic Acids Res 32:D277-280. https://doi.org/10.1093/nar/gkh063

Kaul S, Choudhary M, Sharma T, Dhar MK (2017) Harnessing the plant microbiome: a key towards sustainable agriculture. In: Singh DP, Singh HB, Prabha R (eds) Plant-microbe interactions in agro-ecological perspectives. Springer, Singapore, pp 307–322

Kavamura VN, Santos SN, da Silva JL et al (2013) Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought. Microbiol Res 168:183–191. https://doi.org/10.1016/j.micres.2012.12.002

Kawasaki A, Ryan PR (2021) Peptide Nucleic Acid (PNA) clamps to reduce co-amplification of plant DNA during PCR amplification of 16S rRNA genes from endophytic bacteria. Methods Mol Biol 2232:123–134. https://doi.org/10.1007/978-1-0716-1040-4_11

Khaitov B, Vollmann J, Pyon JY, Park KW (2020) Improvement of salt tolerance and growth in common bean (*Phaseolus vulgaris* L.) by co-inoculation with native rhizobial strains. J Agric Sci Technol 22:209–220

Khan WU, Ahmad SR, Yasin NA et al (2017) Effect of *Pseudomonas fluorescens* RB4 and *Bacillus subtilis* 189 on the phytoremediation potential of *Catharanthus roseus* (L.) in Cu and Pb-contaminated soils. Int J Phytorem 19:514–521. https://doi.org/10.1080/15226514.2016.1254154

Khan NT, Jameel N, Khan MJ (2018) Microbes as biofertilizers. J Biomed Eng Biosci 2:10. https://doi.org/10.32474/OAJBEB.2018.02.000150

Khan MA, Asaf S, Khan AL et al (2020) Plant growth-promoting endophytic bacteria augment growth and salinity tolerance in rice plants. Plant Biol 22:850–862. https://doi.org/10.1111/plb.13124

Khoiri AN, Cheevadhanarak S, Jirakkakul J et al (2021) Comparative metagenomics reveals microbial signatures of sugarcane phyllosphere in organic management. Front Microbiol. https://doi.org/10.3389/fmicb.2021.623799

Ki J-S (2012) Hypervariable regions (V1–V9) of the dinoflagellate 18S rRNA using a large dataset for marker considerations. J Appl Phycol 24:1035–1043. https://doi.org/10.1007/s10811-011-9730-z

Kim M, Lee K-H, Yoon S-W et al (2013) Analytical tools and databases for metagenomics in the next-generation sequencing era. Genomics Inf 11:102–113. https://doi.org/10.5808/GI.2013.11.3.102

Kim D, Hahn AS, Wu S-J, et al (2015) FragGeneScan-plus for scalable high-throughput short-read open reading frame prediction. In: 2015 IEEE conference on computational intelligence in bioinformatics and computational biology (CIBCB). IEEE, Niagara Falls, pp 1–8

Kõljalg U, Nilsson RH, Abarenkov K et al (2013) Towards a unified paradigm for sequence-based identification of fungi. Mol Ecol 22:5271–5277. https://doi.org/10.1111/mec.12481

Kouadio ANM-S, Nandjui J, Krou SM et al (2017) A native arbuscular mycorrhizal fungus inoculant outcompetes an exotic commercial species under two contrasting yam field conditions. Rhizosphere 4:112–118. https://doi.org/10.1016/j.rhisph.2017.10.001

Kour D, Rana KL, Yadav AN et al (2020) Microbial biofertilizers: bioresources and eco-friendly technologies for agricultural and environmental sustainability. Biocatal Agric Biotechnol 23:101487. https://doi.org/10.1016/j.bcab.2019.101487

Kultima JR, Coelho LP, Forslund K et al (2016) MOCAT2: a metagenomic assembly, annotation and profiling framework. Bioinformatics 32:2520–2523. https://doi.org/10.1093/bioinformatics/btw183

Kumar S, Krishnani KK, Bhushan B, Brahmane MP (2015) Metagenomics: retrospect and prospects in high throughput age. Biotechnology Research International. https://www.hindawi.com/journals/btri/2015/121735/. Accessed 28 Mar 2020

Kumar V, Baweja M, Singh PK, Shukla P (2016) Recent developments in systems biology and metabolic engineering of plant–microbe interactions. Front Plant Sci. https://doi.org/10.3389/fpls.2016.01421

Kumar A, Maurya BR, Raghuwanshi R et al (2017a) Co-inoculation with enterobacter and rhizobacteria on yield and nutrient uptake by wheat (*Triticum aestivum* L.) in the alluvial soil under indo-gangetic plain of India. J Plant Growth Regul 36:608–617. https://doi.org/10.1007/s00344-016-9663-5

Kumar J, Singh D, Ghosh P, Kumar A (2017B) Endophytic and epiphytic modes of microbial interactions and benefits. In: Singh DP, Singh HB, Prabha R (eds) Plant–microbe interactions in agro-ecological perspectives. Springer, Singapore, pp 227–253

Kumari P, Meena M, Upadhyay RS (2018) Characterization of plant growth promoting rhizobacteria (PGPR) isolated from the rhizosphere of *Vigna radiata* (mung bean). Biocatal Agric Biotechnol 16:155–162. https://doi.org/10.1016/j.bcab.2018.07.029

Kunin V, Copeland A, Lapidus A et al (2008) A bioinformatician's guide to metagenomics. Microbiol Mol Biol Rev 72:557–578

Ladoukakis E, Kolisis FN, Chatziioannou AA (2014) Integrative workflows for metagenomic analysis. Front Cell Dev Biol. https://doi.org/10.3389/fcell.2014.00070

Laforest-Lapointe I, Messier C, Kembel SW (2016) Host species identity, site and time drive temperate tree phyllosphere bacterial community structure. Microbiome 4:27. https://doi.org/10.1186/s40168-016-0174-1

Lambais MR, Lucheta AR, Crowley DE (2014) Bacterial community assemblages associated with the phyllosphere, dermosphere, and rhizosphere of tree species of the Atlantic Forest are host taxon dependent. Microb Ecol 68:567–574. https://doi.org/10.1007/s00248-014-0433-2

Lefèvre E, Gardner CM, Gunsch CK (2020) A novel PCR-clamping assay reducing plant host DNA amplification significantly improves prokaryotic endo-microbiome community characterization. FEMS Microbiol Ecol 96:110. https://doi.org/10.1093/femsec/fiaa110

Leveau JHJ, Lindow SE (2001) Appetite of an epiphyte: quantitative monitoring of bacterial sugar consumption in the phyllosphere. Proc Natl Acad Sci 98:3446–3453. https://doi.org/10.1073/pnas.061629598

Levy A, Conway JM, Dangl JL, Woyke T (2018) Elucidating bacterial gene functions in the plant microbiome. Cell Host Microbe 24:475–485. https://doi.org/10.1016/j.chom.2018.09.005

Li W, Godzik A (2006) Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22:1658–1659. https://doi.org/10.1093/bioinformatics/btl158

Li X-R, Lv Y, Meng H et al (2014) Analysis of microbial diversity by pyrosequencing the small-subunit ribosomal RNA without PCR amplification. Appl Microbiol Biotechnol 98:3777–3789. https://doi.org/10.1007/s00253-014-5583-0

Lindow SE, Brandl MT (2003) Microbiology of the phyllosphere. Appl Environ Microbiol 69:1875–1883. https://doi.org/10.1128/AEM.69.4.1875-1883.2003

Liotti RG, da Silva Figueiredo MI, da Silva GF et al (2018) Diversity of cultivable bacterial endophytes in *Paullinia cupana* and their potential for plant growth promotion and phytopathogen control. Microbiol Res 207:8–18. https://doi.org/10.1016/j.micres.2017.10.011

Liu C, Lin H, Li B et al (2021) Endophyte inoculation redistributed bioavailable Cd and nutrient in soil aggregates and enhanced Cd accumulation in *Phytolacca acinosa*. J Hazard Mater 416:125952. https://doi.org/10.1016/j.jhazmat.2021.125952

Lundberg DS, Lebeis SL, Paredes SH et al (2012) Defining the core *Arabidopsis thaliana* root microbiome. Nature 488:86–90. https://doi.org/10.1038/nature11237

Luo C, Rodriguez-R LM, Konstantinidis KT (2014) MyTaxa: an advanced taxonomic classifier for genomic and metagenomic sequences. Nucleic Acids Res 42:e73–e73. https://doi.org/10.1093/nar/gku169

Ma S, De Frenne P, Boon N et al (2019) Plant species identity and soil characteristics determine rhizosphere soil bacteria community composition in European temperate forests. FEMS Microbiol Ecol. https://doi.org/10.1093/femsec/fiz063

Mande SS, Mohammed MH, Ghosh TS (2012) Classification of metagenomic sequences: methods and challenges. Brief Bioinform 13:669–681

Marasco R, Mosqueira MJ, Fusi M et al (2018) Rhizosheath microbial community assembly of sympatric desert speargrasses is independent of the plant host. Microbiome 6:215. https://doi.org/10.1186/s40168-018-0597-y

Marchesi JR, Ravel J (2015) The vocabulary of microbiome research: a proposal. Microbiome 3:31. https://doi.org/10.1186/s40168-015-0094-5

Mascot-Gómez E, Flores J, López-Lozano NE (2021) The seed-associated microbiome of four cactus species from Southern Chihuahuan Desert. J Arid Environ 190:104531. https://doi.org/10.1016/j.jaridenv.2021.104531

Massart S, Martinez-Medina M, Jijakli MH (2015) Biological control in the microbiome era: challenges and opportunities. Biol Control 89:98–108. https://doi.org/10.1016/j.biocontrol.2015.06.003

May A, Coelho LF, Pedrinho A et al (2021) The use of indigenous bacterial community as inoculant for plant growth promotion in soybean cultivation. Arch Agron Soil Sci. https://doi.org/10.1080/03650340.2021.1964017

Mazid M, Khan TA (2015) Future of bio-fertilizers in indian agriculture: an overview. Int J Agric Food Res. https://doi.org/10.24102/ijafr.v3i3.132

Meena H, Busi S (2019) Use of microbial biofertilizers technology in agro-environmental sustainability. In: New and future developments in microbial biotechnology and bioengineering. Elsevier, pp 199–211

Mehanathan T, Deivamani M, Senthilkumar P, Ramanathan N (2016) Efficacy of phyllosphere microorganisms on growth of bhendi. J Innov Agric 3:7–10

Meyer F, Bagchi S, Chaterji S et al (2019) MG-RAST version 4-lessons learned from a decade of low-budget ultra-high-throughput metagenome analysis. Brief Bioinform 20:1151–1159. https://doi.org/10.1093/bib/bbx105

Miller JR, Koren S, Sutton G (2010) Assembly algorithms for next-generation sequencing data. Genomics 95:315–327. https://doi.org/10.1016/j.ygeno.2010.03.001

Mitter B, Pfaffenbichler N, Flavell R et al (2017) A new approach to modify plant microbiomes and traits by introducing beneficial bacteria at flowering into progeny seeds. Front Microbiol. https://doi.org/10.3389/fmicb.2017.00011

Mitter B, Brader G, Pfaffenbichler N, Sessitsch A (2019) Next generation microbiome applications for crop production—limitations and the need of knowledge-based solutions. Curr Opin Microbiol 49:59–65. https://doi.org/10.1016/j.mib.2019.10.006

Morris CE, Kinkel LL (2002) Fifty years of phyllosphere microbiology: significant contributions to research in related fields. Phyllosphere microbiology, Lindow, S., E. Hecht-Poinar and V. Elliott. APS Press, St. Paul

Mueller UG, Sachs JL (2015) Engineering microbiomes to improve plant and animal health. Trends Microbiol 23:606–617. https://doi.org/10.1016/j.tim.2015.07.009

Mukherjee A, Reddy MS (2020) Metatranscriptomics: an approach for retrieving novel eukaryotic genes from polluted and related environments. 3 Biotech 10:71. https://doi.org/10.1007/s13205-020-2057-1

Müller DB, Vogel C, Bai Y, Vorholt JA (2016) The plant microbiota: systems-level insights and perspectives. Annu Rev Genet 50:211–234. https://doi.org/10.1146/annurev-genet-120215-034952

Nadarajah KK (2019) Microbes: an important resource for sustainable agriculture. In: Kumar V, Prasad R, Kumar M, Choudhary DK (eds) Microbiome in plant health and disease. Springer, Singapore, pp 53–77

Namiki T, Hachiya T, Tanaka H, Sakakibara Y (2012) MetaVelvet: an extension of Velvet assembler to de novo metagenome assembly from short sequence reads. Nucleic Acids Res 40:e155. https://doi.org/10.1093/nar/gks678

National Research Council (2007) The new science of metagenomics: revealing the secrets of our microbial planet. In: The new science of metagenomics: revealing the secrets of our microbial planet. National Academies Press

Niu X, Song L, Xiao Y, Ge W (2018) Drought-tolerant plant growth-promoting rhizobacteria associated with foxtail millet in a semi-arid agroecosystem and their potential in alleviating drought stress. Front Microbiol. https://doi.org/10.3389/fmicb.2017.02580

Nurk S, Meleshko D, Korobeynikov A, Pevzner PA (2017) metaSPAdes: a new versatile metagenomic assembler. Genome Res 27:824–834. https://doi.org/10.1101/gr.213959.116

Omomowo OI, Babalola OO (2019) Bacterial and fungal endophytes: tiny giants with immense beneficial potential for plant growth and sustainable agricultural productivity. Microorganisms 7:481. https://doi.org/10.3390/microorganisms7110481

Ortas I, Iqbal T, Yücel YC (2019) Mycorrhizae enhances horticultural plant yield and nutrient uptake under phosphorus deficient field soil condition. J Plant Nutr 42:1152–1164. https://doi.org/10.1080/01904167.2019.1609500

Ou T, Xu W, Wang F et al (2019) A microbiome study reveals seasonal variation in endophytic bacteria among different mulberry cultivars. Comput Struct Biotechnol J 17:1091–1100. https://doi.org/10.1016/j.csbj.2019.07.018

Oulas A, Pavloudi C, Polymenakou P et al (2015) Metagenomics: tools and insights for analyzing next-generation sequencing data derived from biodiversity studies. Bioinform Biol Insights 9:75–88. https://doi.org/10.4137/BBI.S12462

Ounit R, Wanamaker S, Close TJ, Lonardi S (2015) CLARK: fast and accurate classification of metagenomic and genomic sequences using discriminative k-mers. BMC Genomics 16:236. https://doi.org/10.1186/s12864-015-1419-2

Pace NR (2018) The small things can matter. PLoS Biol. https://doi.org/10.1371/journal.pbio.3000009

Pathak DV, Kumar M (2016) Microbial inoculants as biofertilizers and biopesticides. In: Singh DP, Singh HB, Prabha R (eds) Microbial inoculants in sustainable agricultural productivity. Springer, New Delhi, pp 197–209

Pereg L, McMillan M (2015) Scoping the potential uses of beneficial microorganisms for increasing productivity in cotton cropping systems. Soil Biol Biochem 80:349–358. https://doi.org/10.1016/j.soilbio.2014.10.020

Pérez-Pantoja D, Tamames J (2015) Prokaryotic metatranscriptomics. In: McGenity TJ, Timmis KN, Nogales B (eds) Hydrocarbon and lipid microbiology protocols. Springer, Berlin, pp 69–98

Petrosino JF, Highlander S, Luna RA et al (2009) Metagenomic pyrosequencing and microbial identification. Clin Chem 55:856–866. https://doi.org/10.1373/clinchem.2008.107565

Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11:789–799. https://doi.org/10.1038/nrmicro3109

Pii Y, Mimmo T, Tomasi N et al (2015) Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. Biol Fertil Soils 51:403–415. https://doi.org/10.1007/s00374-015-0996-1

Prabha R, Singh DP, Gupta S et al (2019) Rhizosphere metagenomics of *Paspalum scrobiculatum* L. (Kodo Millet) reveals rhizobiome multifunctionalities. Microorganisms 7:608. https://doi.org/10.3390/microorganisms7120608

Pruesse E, Quast C, Knittel K et al (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res 35:7188–7196. https://doi.org/10.1093/nar/gkm864

Qiu Z, Egidi E, Liu H et al (2019) New frontiers in agriculture productivity: optimised microbial inoculants and in situ microbiome engineering. Biotechnol Adv 37:107371. https://doi.org/10.1016/j.biotechadv.2019.03.010

Quince C, Walker AW, Simpson JT et al (2017) Shotgun metagenomics, from sampling to analysis. Nat Biotechnol 35:833–844. https://doi.org/10.1038/nbt.3935

Rajput L, Imran A, Mubeen F, Hafeez FY (2018) Wheat (*Triticum aestivum* L.) growth promotion by halo-tolerant PGPR-consortium. Soil Environ 37:178–189

Rascovan N, Carbonetto B, Perrig D et al (2016) Integrated analysis of root microbiomes of soybean and wheat from agricultural fields. Sci Rep 6:28084. https://doi.org/10.1038/srep28084

Reddy GC, Goyal RK, Puranik S et al (2020) Biofertilizers toward sustainable agricultural development. In: Varma A, Tripathi S, Prasad R (eds) Plant microbe symbiosis. Springer, Cham, pp 115–128

Reeder J, Knight R (2010) Rapidly denoising pyrosequencing amplicon reads by exploiting rank-abundance distributions. Nat Methods 7:668–669. https://doi.org/10.1038/nmeth0910-668b

Rho H, Hsieh M, Kandel SL et al (2018) Do endophytes promote growth of host plants under stress? A meta-analysis on plant stress mitigation by endophytes. Microb Ecol 75:407–418. https://doi.org/10.1007/s00248-017-1054-3

Riesenfeld CS, Schloss PD, Handelsman J (2004) Metagenomics: genomic analysis of microbial communities. Annu Rev Genet 38:525–552. https://doi.org/10.1146/annurev.genet.38.072902.091216

Rodríguez CE, Antonielli L, Mitter B et al (2020) Heritability and functional importance of the *Setaria viridis* bacterial seed microbiome. Phytobiomes J 4:40–52. https://doi.org/10.1094/PBIOMES-04-19-0023-R

Rosen MJ, Callahan BJ, Fisher DS, Holmes SP (2012) Denoising PCR-amplified metagenome data. BMC Bioinform 13:283. https://doi.org/10.1186/1471-2105-13-283

Rossmann M, Sarango-Flores SW, Chiaramonte JB et al (2017). In: Pylro V, Roesch L (eds) The Brazilian microbiome. Springer, Cham, pp 7–20

Rybakova D, Mancinelli R, Wikström M et al (2017) The structure of the *Brassica napus* seed microbiome is cultivar-dependent and affects the interactions of symbionts and pathogens. Microbiome 5:104. https://doi.org/10.1186/s40168-017-0310-6

Saminathan T, García M, Ghimire B et al (2018) Metagenomic and metatranscriptomic analyses of diverse watermelon cultivars reveal the role of fruit associated microbiome in carbohydrate metabolism and ripening of mature fruits. Front Plant Sci. https://doi.org/10.3389/fpls.2018.00004

Sánchez-Cañizares C, Jorrín B, Poole PS, Tkacz A (2017) Understanding the holobiont: the interdependence of plants and their microbiome. Curr Opin Microbiol 38:188–196. https://doi.org/10.1016/j.mib.2017.07.001

Sapkota R, Knorr K, Jørgensen LN et al (2015) Host genotype is an important determinant of the cereal phyllosphere mycobiome. New Phytol 207:1134–1144. https://doi.org/10.1111/nph.13418

Schirawski J, Perlin M (2018) Plant–microbe interaction 2017—the good, the bad and the diverse. Int J Mol Sci 19:1374. https://doi.org/10.3390/ijms19051374

Schloss PD, Handelsman J (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Appl Environ Microbiol 71:1501–1506. https://doi.org/10.1128/AEM.71.3.1501-1506.2005

Schmieder R, Edwards R (2011) Quality control and preprocessing of metagenomic datasets. Bioinformatics 27:863–864. https://doi.org/10.1093/bioinformatics/btr026

Schulz B, Boyle C (2005) The endophytic continuum. Mycol Res 109:661–686. https://doi.org/10.1017/S095375620500273X

Seifert KA (2009) Progress towards DNA barcoding of fungi. Mol Ecol Resour 9:83–89. https://doi.org/10.1111/j.1755-0998.2009.02635.x

Sekar S, Kandavel D (2010) Interaction of plant growth promoting rhizobacteria (PGPR) and endophytes with medicinal plants—new avenues for phytochemicals. J Phytol 2:91–100

Shade A, Jacques M-A, Barret M (2017) Ecological patterns of seed microbiome diversity, transmission, and assembly. Curr Opin Microbiol 37:15–22. https://doi.org/10.1016/j.mib.2017.03.010

Shailendra Singh GG (2015) Plant growth promoting rhizobacteria (pgpr): current and future prospects for development of sustainable agriculture. J Microbial Biochem Technol. https://doi.org/10.4172/1948-5948.1000188

Shakya M, Lo C-C, Chain PSG (2019) Advances and challenges in metatranscriptomic analysis. Front Genet. https://doi.org/10.3389/fgene.2019.00904

Sharpton TJ (2014) An introduction to the analysis of shotgun metagenomic data. Front Plant Sci. https://doi.org/10.3389/fpls.2014.00209

Shmaefsky BR (2020) Principles of phytoremediation. In: Shmaefsky BR (ed) Phytoremediation: in-situ applications. Springer, Cham, pp 1–26

Silva LP, Caruso CS, Maia M (2015) A metagenômica na busca de enzimas hidrolíticas bacterianas. Revista Eletrônica Estácio Saúde 4:193–210

Simon J-C, Marchesi JR, Mougel C, Selosse M-A (2019) Host-microbiota interactions: from holobiont theory to analysis. Microbiome 7:5. https://doi.org/10.1186/s40168-019-0619-4

Singh R (2019) Microbial biotechnology: a promising implement for sustainable agriculture. In: New and future developments in microbial biotechnology and bioengineering. Elsevier, pp 107–114

Singh BK, Campbell CD, Sorenson SJ, Zhou J (2009) Soil genomics. Nat Rev Microbiol 7:756–756. https://doi.org/10.1038/nrmicro2119-c1

Singh BK, Trivedi P, Singh S et al (2018) Emerging microbiome technologies for sustainable increase in farm productivity and environmental security. Microbiol Aust 39:17–23. https://doi.org/10.1071/MA18006

Singh S, Kumar V, Singh S et al (2020) Global scenario of plant–microbiome for sustainable agriculture: current advancements and future challenges. In: Yadav A, Singh J, Rastegari A, Yadav N (eds) Plant microbiomes for sustainable agriculture. Springer, Cham, pp 425–443

Singha KM, Singh B, Pandey P (2021) Host specific endophytic microbiome diversity and associated functions in three varieties of scented black rice are dependent on growth stage. Sci Rep 11:12259. https://doi.org/10.1038/s41598-021-91452-4

Stanchev K, Sicheritz-Ponten T, Nordahl Petersen T (2016) Big data in metagenomics: a pipeline for automated metagenomic analysis (OMEGA)

Su X, Pan W, Song B et al (2014) Parallel-META 2.0: enhanced metagenomic data analysis with functional annotation, high performance computing and advanced visualization. PLoS ONE 9:e89323. https://doi.org/10.1371/journal.pone.0089323

Syamsia S, Idhan A, Firmansyah AP et al (2021) Combination on endophytic fungal as the plant growth-promoting fungi (PGPF) on cucumber (*Cucumis sativus*). Biodivers J Biol Divers. https://doi.org/10.13057/biodiv/d220315

Syed Ab Rahman SF, Singh E, Pieterse CMJ, Schenk PM (2018) Emerging microbial biocontrol strategies for plant pathogens. Plant Sci 267:102–111. https://doi.org/10.1016/j.plantsci.2017.11.012

Tanabe AS, Nagai S, Hida K et al (2016) Comparative study of the validity of three regions of the 18S-rRNA gene for massively parallel sequencing-based monitoring of the planktonic eukaryote community. Mol Ecol Resour 16:402–414. https://doi.org/10.1111/1755-0998.12459

Thijs S, Sillen W, Rineau F et al (2016) Towards an enhanced understanding of plant–microbiome interactions to improve phytoremediation: engineering the metaorganism. Front Microbiol. https://doi.org/10.3389/fmicb.2016.00341

Thomas T, Gilbert J, Meyer F (2012) Metagenomics: a guide from sampling to data analysis. Microb Inf Exp 2:3. https://doi.org/10.1186/2042-5783-2-3

Truong DT, Franzosa EA, Tickle TL et al (2015) MetaPhlAn2 for enhanced metagenomic taxonomic profiling. Nat Methods 12:902–903. https://doi.org/10.1038/nmeth.3589

Turner TR, James EK, Poole PS (2013a) The plant microbiome. Genome Biol. https://doi.org/10.1186/gb-2013-14-6-209

Turner TR, Ramakrishnan K, Walshaw J et al (2013b) Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants. ISME J 7:2248–2258. https://doi.org/10.1038/ismej.2013.119

UniProt Consortium (2012) Reorganizing the protein space at the Universal Protein Resource (UniProt). Nucleic Acids Res 40:D71-75. https://doi.org/10.1093/nar/gkr981

van der Heijden MG, Schlaeppi K (2015) Root surface as a frontier for plant microbiome research. Proc Natl Acad Sci 112:2299–2300

Vandenkoornhuyse P, Quaiser A, Duhamel M et al (2015) The importance of the microbiome of the plant holobiont. New Phytol 206:1196–1206. https://doi.org/10.1111/nph.13312

Vellend M (2016) The theory of ecological communities (MPB-57). Princeton University Press

Venturi V, Keel C (2016) Signaling in the rhizosphere. Trends Plant Sci 21:187–198. https://doi.org/10.1016/j.tplants.2016.01.005

Vives-Peris V, de Ollas C, Gómez-Cadenas A, Pérez-Clemente RM (2020) Root exudates: from plant to rhizosphere and beyond. Plant Cell Rep 39:3–17. https://doi.org/10.1007/s00299-019-02447-5

Vorholt JA (2012) Microbial life in the phyllosphere. Nat Rev Microbiol 10:828–840. https://doi.org/10.1038/nrmicro2910

Wang Y, Leung HCM, Yiu SM, Chin FYL (2014) MetaCluster-TA: taxonomic annotation for metagenomic data based on assembly-assisted binning. BMC Genomics 15:S12. https://doi.org/10.1186/1471-2164-15-S1-S12

Whipps JM, Hand P, Pink D, Bending GD (2008) Phyllosphere microbiology with special reference to diversity and plant genotype. J Appl Microbiol 105:1744–1755. https://doi.org/10.1111/j.1365-2672.2008.03906.x

Wiraswati MN, Rusmana I, Nawangsih AA, Wahyudi AT (2019) Antifungal activities of bacteria producing bioactive compounds isolated from rice phyllosphere against *Pyricularia oryzae*. J Plant Protect Res 59:10. https://doi.org/10.24425/jppr.2019.126047

Xiong C, Singh BK, He J-Z et al (2021) Plant developmental stage drives the differentiation in ecological role of the maize microbiome. Microbiome 9:171. https://doi.org/10.1186/s40168-021-01118-6

Xu Y, Ge Y, Song J, Rensing C (2020) Assembly of root-associated microbial community of typical rice cultivars in different soil types. Biol Fertil Soils 56:249–260. https://doi.org/10.1007/s00374-019-01406-2

Yadav AN, Saxena AK (2018) Biodiversity and biotechnological applications of halophilic microbes for sustainable agriculture. J Appl Biol Biotechnol 6:48–55

Yang H, Ye W, Ma J et al (2018) Endophytic fungal communities associated with field-grown soybean roots and seeds in the Huang-Huai region of China. PeerJ 6:e4713. https://doi.org/10.7717/peerj.4713

Yeoh YK, Dennis PG, Paungfoo-Lonhienne C et al (2017) Evolutionary conservation of a core root microbiome across plant phyla along a tropical soil chronosequence. Nat Commun 8:1–9. https://doi.org/10.1038/s41467-017-00262-8

Yoo K, Lee TK, Choi EJ et al (2017) Molecular approaches for the detection and monitoring of microbial communities in bioaerosols: a review. J Environ Sci 51:234–247. https://doi.org/10.1016/j.jes.2016.07.002

Zeyaullah M, Kamli MR, Islam B et al (2009) Metagenomics: an advanced approach for non-cultivable micro-organisms. Biotechnol Mol Biol Rev 4:49–54

Zhang H, Zhang Q, Chen S et al (2020a) Enterobacteriaceae predominate in the endophytic microbiome and contribute to the resistome of strawberry. Sci Total Environ 727:138708. https://doi.org/10.1016/j.scitotenv.2020.138708

Zhang L, Zhang W, Li Q et al (2020b) Deciphering the root endosphere microbiome of the desert plant *Alhagi sparsifolia* for drought resistance-promoting bacteria. Appl Environ Microbiol 86:e02863-e2919. https://doi.org/10.1128/AEM.02863-19

Zhang M, Wang H, Hussain M et al (2020c) Identification and functional assessment of endophytic bacterial diversity in *Ageratina adenophora* (Sprengel) and their interactions with the host plant. S Afr J Bot 134:99–108. https://doi.org/10.1016/j.sajb.2020.07.038

Zheng Y, Gong X (2019) Niche differentiation rather than biogeography shapes the diversity and composition of microbiome of *Cycas panzhihuaensis*. Microbiome 7:152. https://doi.org/10.1186/s40168-019-0770-y

Zheng T, Zhang K, Zhu X et al (2019) Integrated metatranscriptome and transcriptome reveals the microbial community composition and physiological function of xylem sap on grapevine during bleeding period. Genes Genom 41:1095–1111. https://doi.org/10.1007/s13258-019-00841-7

Zhou Q, Su X, Ning K (2014) Assessment of quality control approaches for metagenomic data analysis. Sci Rep 4:6957. https://doi.org/10.1038/srep06957

Zuo Y, Li X, Yang J et al (2021) Fungal endophytic community and diversity associated with desert shrubs driven by plant identity and organ differentiation in extremely arid desert ecosystem. J Fungi 7:578. https://doi.org/10.3390/jof7070578