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When biodiversity preservation meets biotechnology: The challenge of developing synthetic microbiota for resilient sustainable crop production

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Abstract

Agriculture needs to develop novel strategies and practices to meet the increasing global food demand, in an ecological and economical sustainable framework. The plant-associated microbiota is gaining increasing attention as part of these strategies since it strongly contributes to plant health, nutrition, and resilience to environmental perturbations. However, plant domestication has brought to the reduction of the plant abilities to recruit a beneficial microbiota. It is becoming clear that successful use of the plant microbiota requires a multifaceted approach where microbiologist, geneticists, plant scientists, agronomists, and computational biologists can develop ways and solutions to modify both the plant microbiota and plant's ability to recruit it, directed to increase crop performances. Here, while briefly reviewing the state-of-the-art in plant microbiota research, we focus the attention on the need to discover, understand and use the microbiota associated with wild relatives of crops and with neglected crops, which harbour the microbiota biodiversity needed for developing efficient bioinoculant solutions. In particular, we emphasize the convergence of in situ plant biodiversity preservation with microbiome preservation, which provides added value to nature and habitat conservation, as living collections of microbiome biodiversity. The heuristic value of bioinoculants (viz., synthetic communities) and the need of proper computational models to predict the outcome of their applications is also discussed toward a systems-biology-guided synthetic microbiota development.

KEYWORDS
ecosystems services, endophytes, microbiota modeling, plant microbiome, rhizosphere

1 | THE NEED OF FEEDING THE WORLD IN A MICROBIOLOGICAL PERSPECTIVE

1.1 | The challenge of sustainable food production

Under current trends, the world population is expected to reach 9.8 billion people by 2050. To meet the increasing demand for food and feed, agricultural production should increase by 50%–69% over the 2010–2050 period. To date, agricultural land area exploitable for food production covers 38% of the global land surface. Two-thirds of this is used as meadows and pastures, while just one-third consists of cropland. From 1961 to the present day, there has been a continuous reduction of global cropland area per capita, from about 0.45 hectares per capita to 0.21 hectares per capita. Increasing global food demand and difficult production conditions, worsened by climate change, is threatening food security situation (Funk & Brown, 2009). Indeed, climate change will impact many elements of the crop production environment; atmospheric carbon dioxide concentration and the average temperature will increase, droughts will be more frequent and severe, intense precipitation events will lead to flooding, and extreme environments will be more common.

The stability of climate experienced during Holocene contributed to the development of agriculture, that is a predictable stable production of food (Richerson et al., 2001). Climate instability, characterized by droughts, floods, cyclones, unseasonal rains, and heat and cold waves, is a major factor limiting crop production. For this reason, we are now facing the challenge to maintain agricultural productivity, trying to reduce climate change effects on human nutrition. Currently, research is aimed at developing new climate-resilient crop varieties and identifying stress-resistant cultivars. Traits characterizing crop adaptation to environmental stresses can be identified through genomic tools, then selected and potentially transferred into specific crops.

Moreover, with the worsening of the climatic conditions, the risk of relying on a few crops, such as for instance rice, wheat, soybean, and maize, could compromise food and nutritional security. One of the strategies should consider widening the portfolio of staple crops, including underutilized crops, with high nutritional content and often adapted to hostile abiotic conditions (Gregory et al., 2019). However, on a complementary side, agrobiotechnologies are forced to find solutions to let present-day crops also more resilient to environmental stress. The crop microbiome, that is the community of microbes inhabiting crop tissues and the plant–soil interface is among the recognized key elements contributing to plant resilience (Trivedi et al., 2020).

Here, while briefly summarizing the state-of-the-art of crop microbiome, we are focusing the attention on the plant microbiome, as a genetic resource, to be preserved and exploited for increasing crop resilience and productivity.

1.2 | The solution of microbial inoculations of plants

The exploitation of the plant and soil microbiome, that is the use of the wide genetic and functional diversity of beneficial microorganisms that colonize plants tissues and the rhizosphere soil, has emerged as an encouraging strategy to face agricultural challenges related to climate change (Qiu et al., 2019). Among these microorganisms, plant growth-promoting microorganisms (PGPM) can be used to formulate consortia able to promote overall plants health and yield (Figure 1). Indeed, plant growth-promoting Rhizobacteria (PGPR), plant growth-promoting bacteria (PGPB), and arbuscular mycorrhizal fungi (AMF) can provide several functions, including biological nitrogen fixation, solubilization of soil phosphorus and potassium, siderophores production, phytohormone (auxin) production and the stimulation of plant defence mechanisms, that mitigate both biotic and abiotic stress.

Traditional bioinoculants are based on a single strain characterized by growth-promoting (PGP) activities. However, while in vitro studies are often giving interesting results, in-field applications produce variable and sometimes unsatisfactory results, indicating the urgency to find solution to limit the lab-to-the field gap (Sessitsch et al., 2019) (Box 1). Suggestions for the need of a systematic approach in developing successful bioinoculant formulation have been indicated (Trivedi et al., 2020). In the last few years, thanks to advancements in multiomics and computational technologies, synthetic microbial communities (SynComs), small consortia of microorganisms designed to promote overall plant productivity as well as outcompete and/or suppress pathogens, have gained momentum (de Souza et al., 2020).

However, despite the progress made, the knowledge gap on detailed mechanistic intra- and inter-kingdom interactions needs to be filled through evaluation of trophic exchanges in the rhizosphere and endosphere (the zone including the internal part) of the plant.

2 | THE PHYTOSPHERE AS A MICROBIAL VAULT OF BIODIVERSITY

2.1 | Genes, taxa and the microbial diversity

Microbes are found everywhere. Moreover, prokaryotic microbes are different from *macrobes* (plants and animals), not only because of obvious biological differences in their cell structure/morphology and physiology, but also because they challenge some basic epistemological notions of natural sciences, as that of species. The biological species concept, *sensu* Mayr, cannot be applied to microbes, since they do not have sexual reproduction, and genomic studies have clarified that a prokaryotic taxon is composed of many different nonoverlapping genes sets (the pangenome concept) (Doolittle & Papke, 2006). This means that when considering an environment, we cannot simply observe a group of taxa, carrying a defined set of genes, but mainly a group of similar genes (i.e., functions) that can be carried by different taxa. Most of these genes are acquired by horizontal gene transfer (HGT) driven by mobile genetic elements (MGEs) when microbes colonize the same environment. We can thus consider the dynamic interplay among hosts, MGEs, and environments as network that shapes functions within communities of an environment (Skippington & Ragan, 2011). Each ecosystem undergoes variation when subjected to stressful factors (i.e., climatic changes) and, typically, the result is a wave of biodiversity loss. The ability to

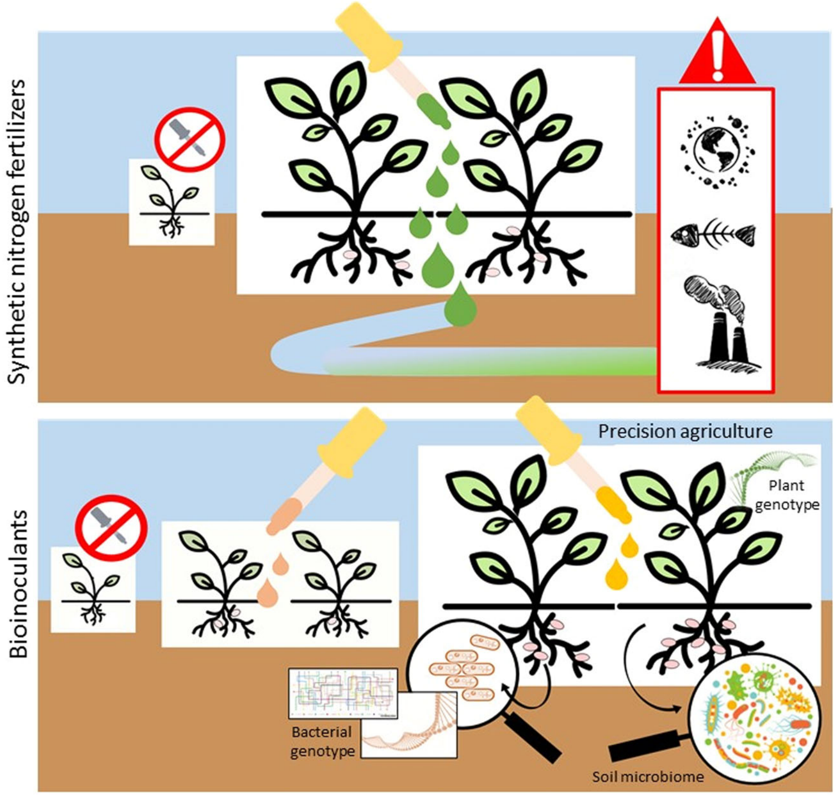

FIGURE 1 Bioinoculants represent a sustainable complement to conventional practices. For instance, bioinoculants with the ability to fix atmospheric nitrogen can complement synthetic nitrogen fertilization and can represent a smart solution in light of precision agriculture, being present and more active in contact with the plant and when the plant needs more nitrogen. Moreover, specificity for plant genotypes and soil types allows to reduce the lab-to-the-field gap, increasing plant colonization and survival in the environment.

maintain or recover its functions in response to external influences, also defined as ecosystem resilience, is connected to the skill of the species to counteract adverse events (Oliver et al., 2015). Functional diversity gives us a mechanistic link between ecosystem and species resilience.

A variegate functional composition of a microbiota should be preserved to make the community capable of facing environmental perturbations (Walker et al., 2022). Some measurables are needed to define the relationship between ecosystem function and diversity. Species richness is questioned, since it implies that all species are potentially equal with respect to function. This concept is clear when talking about predators and plants but should be considered valid also when talking about microorganisms, for the uncoupling between taxa and gene functions (Bengtsson, 1998). The *keystone species* concept, defined as those limited number of organisms and groups of organisms that seem to control the critical processes necessary for ecosystem functioning, as much as it is highly relevant for identification of ecological drivers (Banerjee et al., 2018), does not provide a quantitative measure of the involved processes nor does it describe the link between diversity and function (Banerjee et al., 2018). To relate a measure of diversity to ecosystem function, a possible solution is to analyze the diversity of functional groups (i.e., the number of functional groups) (Moore & de Ruiter, 1991). However, this suffers from some biases, first, the arbitrariness with which functional groups are defined: no identical species exist and species can be observed focusing on different functions. Functional groups can be defined in relation to feeding relationships (e.g., pathotroph, phototroph, osmotroph, and phagotroph), involvement in biogeochemical cycles (e.g., nitrogen-fixers, sulphur oxidizers) but also nontrophic effects, such as nutrient distribution and environmental structure and conditions (e.g., psychrophilic and piezophiles).

Striking examples of the importance of functions over taxa are coming from plant microbiology, for instance, when considering diazotrophs, microbes which carry out atmospheric nitrogen fixation. Symbiotic nitrogen fixers (called rhizobia) are part of the microbiota associated with the root of leguminous plants, where they provide the plant with fixed nitrogen. Rhizobia are a clear example of a functional group of microbes of polyphyletic origin. Rhizobia can be found in both alpha and beta proteobacteria. Additionally, even in the same genus (e.g., *Sinorhizobium*), the functional gene set required for symbiosis and nitrogen fixation (i.e., for being a rhizobium) can be scattered among distantly related strains and also be of different origin (Fagorzi et al., 2020).

As pointed out by M. O'Malley, this uncoupling between taxa and functions has profound consequences on the way we look at life evolution and biodiversity (O'Malley, 2014). In other words, when speaking about biodiversity in microbes, conserving genes can be more relevant than preserving taxa, as it is on the contrary the case for *macrobes* biodiversity, challenging some assumptions we have in biology and nature conservation (O'Malley & Dupré, 2007).

2.2 | Biodiversity in the phytosphere

The microbiota of the phytosphere is known to harbour a wide variety of microbes, including beneficial, neutral, and pathogenic ones. Beneficial microbes are considered a primary resource when

BOX 1. Most pressing questions in plant microbiota research

The high variability in the efficacy of bioinoculants, combined with the lack, in many cases, of agronomic indication on their use, risk producing a lack of confidence in the use of bioinoculants as a complementary or alternative solution to high-input agronomic practices. An effort is therefore needed, both in terms of basic research and technological applications in the field through the transfer of scientific knowledge, to shorten the lab-to-the-field gap (Sessitsch et al., 2019), and to develop successful bioinoculant synthetic microbial communities. This development must be accompanied by providing guidelines to farmers for their use, as well as novel agronomic practices.

In 2017, Busby et al. (2017a). listed five research priorities for plant microbiome research, including novel model systems for understanding the rules of microbiome assembly and the interactions between host plant genotype, microbiome, environment, and management. We think these questions are still valid after 5 years. Progresses have been made, especially on basic knowledge (for a recent review see Trivedi et al., 2020), but, still, the limiting factor is how to incorporate knowledge into management practices. Ideally, a scientific approach similar to drug validation, consisting of a discovery phase and laboratory proofs of efficacy, followed by trials in controlled conditions and in open field, should be applied. All these steps must require consensus on standards (e.g., number of conditions, replicates, yield reporting). We think we need to answer at least five main questions (Figure 2), spanning from basic understanding of the plant microbiomes (Questions 1 and 2), to the interplay among host, microbiome, and environment (Question 3), to the development and use of effective bioinoculants (Questions 4 and 5).

These questions require a complementary work on additional priorities, as (i) the understanding of microbiome assemblage and the prediction of their functionality, (ii) the promotion of culturing plant-associated microbiota, and (iii) the scouting of the microbiome diversity in landraces, nonconventional crops, nonmodel plants. A tight connection with farmers and agronomists is then pivotal to develop novel effective microbiome-based crop management on classical and new crops.

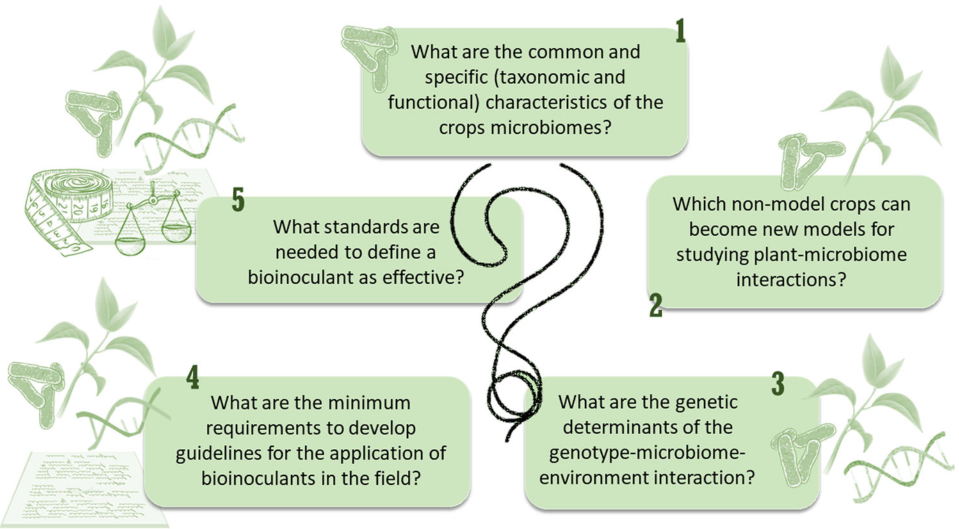

FIGURE 2 Main questions in the understanding and exploiting plant microbiota. Five most pressing questions (Questions 1–5) on research challenges in the plant microbiota and in the agronomic translation of the knowledge are reported.

talking about protection from disease, stress resistance, and growth in biomass. To carry out these functions, microbes act by increasing nutrient mobilization to plants, producing phytohormones, and manipulating plant functional traits, including the immune system (Friesen et al., 2011). Among these microorganisms, bacteria and fungi are predominant. The rhizosphere can contain up to 10^{11} microbial cells per gram of root and more than 30,000 prokaryotic species (Mendes et al., 2011). About a few thousand bacterial and fungal taxa have been reported from plant tissues (Berendsen et al., 2012). The biodiversity of microorganisms in the phytosphere is naturally variable, impacted by continuous environmental variations spanning from roots physiology to the aboveground part growth (e.g., the canopy), strongly affected by aerial dispersion of the microorganisms (Oukala et al., 2021).

Preserving this astounding biodiversity would be ideal but appears to be impossible in reality. What should be preserved? Taxa or the functions (genetic repertoire) they carry on? Are they all equally relevant? Which rational for the choice of what to preserve should we apply? And, finally, how to preserve since several bacterial taxa are still recalcitrant to cultivation or uncultivable?

Nikolay Vavilov was a central figure in the concept of biodiversity preservation. He created the largest collection of plant germplasm, scouting plants from all over the world. His research led to the identification of the centres of origins of cultivated plants and highlighted the importance of preserving biodiversity. Vavilov's collection is still active at the N.I. Vavilov All Russian Institute of Plant Genetic Resources (VIR) and initiative as the Svalbard Global Seed Vault (https://www.seedvault.no/) are in the frame of Vavilov's legacy.

However, after the introduction of the holobiont concept following the recognition of the large number and great importance of microorganisms associated with plants and animals, it is evident that preservation of seeds is only a part of our safeguard to a future food production. Host plants and their microbes need to be preserved together to guarantee adequate effectiveness of future crop improvement. Concerning the human microbiota initiative for its preservation are ongoing, as “The microbial vault” (https://www.microbiotavault.org/). This initiative is based on biobanking of human faecal samples, microbial isolation and culturing and long-term storage, under the recognition that gut microbiome is the one that mostly impacts human holobiont health.

Similar to human gut microbiota preservation, global efforts toward the analysis and future conservation of microbes associated to the main crops are active (https://www.globalsustainableagriculture.org/). Soil biodiversity hotspots have recently been identified, which should guide prioritization of conservation efforts (Guerra et al., 2022). However, here the challenge is much higher than for human microbiota: we need to preserve the microbiota of many different host species, all of those which can provide food and healthy ecosystems.

Conserving microbes is not an easy task. Many relevant microbes can be nonculturable under currently used conditions and efforts on plant microbiota culturomics initiatives are needed for increasing the culturability and have adequate collections of microorganisms (Mapelli et al., 2022). Though this kind of initiatives can be feasible for some staple crops, they cannot be applied to wild relatives, minor crops, and so on, as the nonmodel plants cited above. A solution is to preserve the plant in situ, on its local environment. Preserving the plant means preserving its microbiome, which is even containing more genetic information than the plant itself. Indeed, we should consider that a single prokaryotic taxon can contain a large genetic diversity, due to a possible open pangenome. Many prokaryotic species colonizing soil and having multiple niches (as soil and plant tissue) normally harbour a large dispensable genome, which determines variable phenotypes with respect to plant interaction (see e.g., Fagorzi et al., 2021). Consequently, though the genetic repertoire needed to interact with plants can be relatively small (Levy et al., 2018), an extensive set of modifier genes, relevant for plant growth promotion can be differentially present in the same taxon. On the other hand, the number of prokaryotic taxa which can be prioritized for such analyses and preservation could be relatively limited. A recent meta-analysis (Riva, 2019) showed that Gammaproteobacteria, Bacilli and Actinobacteria were the predominant groups which constituted core of the plant-associated microbiota, and within them, core genera were *Pseudomonas*, *Pantoea*, *Stenotrophomonas*, *Enterobacter*, *Bacillus*, *Staphylococcus*, *Paenibacillus*, *Microbacterium*, and *Curtobacterium*. However, we cannot exclude that this relatively limited number of taxa simply reflects a limited ability to culture other taxa, prompting to a renewed effort on novel microbial cultural methods (Mapelli et al., 2022).

2.3 | Promoting phytosphere-friendly breeding programs

The process of crop domestication arose around 10,000 years ago, when human civilization became largely dependent on agricultural products, providing a continuous food supply and promoting a sedentary lifestyle (Richerson et al., 2001). Consequently, plant species domestication has significantly contributed to human civilization, but, on the other hand, led to a strong loss of genetic diversity in modern crop cultivars due to a selection of a few species and germplasm, which better adapt and respond to agronomic practices (Evenson & Gollin, 2003). Domestication affected root architecture (i.e., root length and branching), root exudate production and/or composition, and plant defence mechanisms, reducing the ability of the plant to establish beneficial associations with rhizospheric microbes, which may have prominent effects on plant growth and health. Recent studies reported changes in the rhizospheric microbiota of plants such as common bean, lettuce, barley, rice, and *Arabidopsis* with the general trend of diminished Bacteroidetes and increased Proteobacteria and Actinobacteria in comparison to wild relatives (see for instances Bulgarelli et al., 2015; Kim et al., 2020). If we assume that plants partially depend on the microbial community present in the rhizospheric soil as an effect of natural selection, a loss or variation of the traits needed to recruit host-specific root microbiota in modern crop cultivars may have occurred compared to their wild relatives, which are genetically more diverse and adapted to preagricultural soils. For instance, wild plant relatives represent a “living library” of both diverse microbial and plant genetic traits that may have been lost during domestication and can be used as to improve the resistance of modern cultivars to biotic and abiotic stresses (Raaijmakers & Kiers, 2022). Moreover, assessing and accessing the microbiome of indigenous plants and their native habitats represents a new strategy to further exploit microbes and plant traits in modern agriculture. Consequently, molecular breeding and marker-assisted selection can be seen as biotechnological tools to improve beneficial plant–microbe interactions in crop systems and to face an increasing global food demand. This represents one of the major challenges due to the impossibility to expand croplands and the need to reduce the usage of synthetic fertilizers and pesticides.

Since the interaction between host plant and its microbiota is controlled, at least partially, by the plant genome, a prerequisite for the rational manipulation of the plant microbiota for agriculture and to exploit soil microbes for sustainable crop production is the identification of the plant genes shaping microbiota composition in the rhizosphere. This is particularly relevant for the microbiota associated with wild relatives of the crops, which, having evolved under marginal soil conditions, may represent an untapped resource for low-input agriculture.

Recently, by combining microbiota analysis and quantitative genetics, a locus harbouring a gene encoding an immune receptor with a major effect on the composition of rhizosphere microbiota was identified, confirming the strong link between immunity and beneficial microbiome recruitment (Hacquard et al., 2017) A similar approach was applied to tomato comparing the microbiome of diverse hybrid populations of wild and domesticated cultivars (Oyserman et al., 2022). Here both plant (e.g., iron regulator) and bacterial traits (polysaccharides, vitamins, sulphur, and iron metabolisms) associated to tomato quantitative trait loci (QTLs) were found. A link between the genetics plant and its microbiome have also been in the understanding of the heterosis in maize, a still not-well clear phenomenon called also hybrid vigor, where first-generation offspring outperform their parents (Wagner et al., 2021). These studies demonstrated the power of an integrated approach based on bacterial and plant genomics and directions for plant breeding programs and selection of microbial functions. QTLs can be targeted to obtain improved plant–microbe interactions, identifying "missing microbes" or functions lost during the domestication process, and provide model studies to dissect the molecular details driving plant–bacteria interactions.

2.4 | Wild relatives and nonmodel plants: Microbial biodiversity hotspots?

One of the greatest challenges to face when putting into practices the knowledge of the plant microbiomes is defining methods and principles to translate the results acquired on model plant species to real crops. Often real crops are nonmodel species or genotypes different from model systems (Busby et al., 2017b). Ideally, to allow a smoother translation of knowledge and methods, agricultural germplasms for which close relatives exist as model species, should be prioritized (Vaccaro et al., 2022). Crops, such as alfalfa, chickpea, lentil, landraces of rice, maize, and wheat can represent an excellent opportunity to take advantage of, since they hold their own specific features, but at the same time they can have relatives as model species (such as *Medicago truncatula* for alfalfa) (Vaccaro et al., 2022). Since wild relatives of crops evolved under marginal soil conditions, the microbiome associated with them is expected to harbour functions helping the plant to cope with low nutrients inputs and environmental heterogeneity, two conditions normally found in natural habitats. Because of that their microbiome can represent an untapped resource for the challenges presented by low-input agriculture (Escudero-Martinez & Bulgarelli, 2019). Moreover, microbiomes of wild relatives and nonmodel species, represent the benchwork to investigate the structure and the evolution of the plant-associated microbial communities. Then, they may provide mechanistic insights which could be ultimately useful and critical to rationally design synthetic microbial communities (Vaccaro et al., 2022).

Examples of insights gained from nonmodel plant species can be found in the identification of novel genetic determinants of host–bacteria interactions. A study on local crop varieties of alfalfa (*Medicago sativa*) revealed the existence of genotypic interactions influencing the phenotypic traits of the interaction with the rhizobial nitrogen-fixing symbiont *Sinorhizobium meliloti* (Fagorzi et al., 2021). Here, many genes (up to more than 1000 on a single strain) were influenced in their expression by the strain genotype (35%), by the genotype of the variety of host plant tested (16%), and by strain-by-host plant genotype interactions (29%). These data indicate from one side that alfalfa can largely benefit from breeding programs considering the selectivity of the symbiotic rhizobia recruitment, and, on the other side, that local varieties can host a large genetic diversity in their symbiotic microbiome. Another study on a Mexican indigenous maize landrace shed light on the vault of microbial biodiversity hosted in nonmodel plants. Here, it was shown that the microbiota associated to the mucilage secreted by aerial roots provides unexpected biological nitrogen fixation abilities which contribute to plant nutrition (Van Deynze et al., 2018). Since diazotrophic microbial associations with cereals is stirring a large interest in low-input agriculture, this example highlights the importance of research on nonmodel plants for discovering novel microbial functions and planning novel microbe-oriented plant.

In our opinion, research on landraces and consequently their preservation can be highly important not only to save important germplasm for breeding, *sensu* Vavilov, but also to save the plant-associated microbial diversity (which is yet to be investigated in such plants) and which can harbour functions for sustainable food production. For instance, concerning climate-resilient nonmodel crops of high nutritional importance, such as *Eragrostis tef* (teff), *Chenopodium quinoa* (quinoa), *Setaria italica* (foxtail millet), *Amaranthus* spp., no publication on their native microbiota is available which should prompt prioritization in future research programs (Gregory et al., 2019).

In conclusion, we should shift from plant preservation to holobiont preservation and here is when biodiversity and habitat preservation meets biotechnology (as exploitation of genetic resources). In our opinion, this concept of habitat conservation which includes the microbiome, must be considered when biodiversity reserves are planned and managed as well as when traditional, local or neglected crops are valorized in programs at the FAO Globally Important Agricultural Heritage Systems (GIAHS) (https://www.fao.org/giahs/). However, for a rational decision of (nonmodel) plant holobiont biodiversity hotspots studies on their microbiome must increase, as to date information is scarce.

3 | BIOTECHNOLOGY ON BIODIVERSITY: TOWARD THE UNDERSTANDING OF THE RULES OF ENGAGEMENT OF SYNTHETIC MICROBIOTA

As previously discussed, the relationship between microorganisms and plants is not a binary host-microbial interaction but a complex community assembly, summarized by the concept of holobiont, which considers the emerging interactions and outcomes of hosts and their microbiota (Theis et al., 2016). In response to such complexity, the process of bioinoculants design has been driven by new approaches. *Syn*thetic microbial *Com*munities (SynComs) relies on microbial ecology concepts, but their specific design balances between well-established theories of ecology and the need of predictive modelling to face the paucity of specific physiological data on microbes' functions and host-microbes cross talks. The general idea is to take advantage from the presence of a core plant microbiota and create a consortium of (several) strains which can mimic the taxonomy, functions, and effects of a healthy native plant microbiota. Several formulations have been tested to enhance yield and nutrient content of different crops, confirming the importance of the development of SynComs for agricultural use (Maitra et al., 2022). However, various strategies need to be applied to effectively engineer plant microbiomes, working either on plant side or on the microbial side (French et al., 2021). Moreover, abiotic factors (e.g., physicochemical soil characteristics) must be considered, since they can act directly on the microbial side by determining, for instance, the persistence in soil and microbial activity, and indirectly, by changing amount and type of plant-secreted chemical compounds. Recent computational methods, including mathematical models, Machine Learning and AI, are strengthening the development of efficient inoculants for plant's benefits (Leggieri et al., 2021; de Souza et al., 2020; Vorholt et al., 2017). These methods help to make use of the still limited knowledge about the interactions between microbes and plants and to take into consideration the many variables of the system (either biotic or abiotic). The most common methods which can be used to rationally design SynCom, span from statistical methods based on taxa occurrences to methods relying on metabolic exchanges (Figure 3).

Models of inter-species interaction from theoretical ecology, as Resource Ratio Theory (RRT), Maximum Power Principle (MPP), and Black Queen Theory (BQT), have been successfully employed to analyze interspecies microbial interactions (Dunham, 2007). The RRT (Figure 3a) is a model that could explain the conditions under which natural selection may favour cooperation over competition. Moreover, theories dealing with mutualism have spread out in recent years, including game theory (see next paragraph). MPP (Figure 3b) may provide predictions on communities clustering benefits looking for maximum power outcomes in ecological situations, while BQT can explain genome evolution in symbiotic bacteria (Morris et al., 2012).

Game theory (GT) is another general mathematical framework to model strategic interactions among a number of agents (players) where the payoff of each agent is not only a function of its own strategy (action) but also a function of other players' strategies. Cooperation and competition have a fitness face-off in game theory. Interactions among players are costly, the energy spent is a cost (loss) and the resources are benefits (gain): the net between gain and loss is the payoff. In GT the payoff for any player depends not only on its own strategy, but the strategies of the other players in the environment (Figure 3c). GT can help to understand the evolution of mutualism between microbes and hosts and, by establishing a cost for the mutualism, how much we can gain increasing the microbial counterpart (Leggieri et al., 2021; Zomorrodi & Segrè, 2017).

Still concerning mathematical frameworks, population dynamic models, as the generalized Lotka-Volterra (LV) models have been used to model bacteria-bacteriophage interactions, microbial gut interactions, and microbial communities' interactions, as models to predict outcomes in plant–microbe interactions (Vorholt et al., 2017).

However, we should consider that natural microbial communities are characterized by a high-level complex in their structure. The self-organization of the community itself depends on factors such as the heterogeneity of the habitat, natural gradients, interaction abundances, and many others. Spatial models, where the dynamics of the system across different locations is studied with partial differential equations, can be applied in such cases. The most widely used model is the reaction-diffusion equation, which determines the density of each species at different times and different locations in space caused by diffusion and population dynamics. This class of models has been used in combination to population-based models to study a variety of ecological phenomena related to spatial effects such as range expansion and diffusion-based spatial patterning. Recently, spatial models have been used to understand and predict the root-to-shoot circuit in *Arabidopsis* (Hou et al., 2021), where, under low light, leaves induce a modulation of the rhizosphere microbiota through physiological changes in roots. Altered microbiota, in turn, responds by alleviating plant stress due to low photosynthetic activity.

Going to prediction of taxa and genes (i.e., metabolic functions) relevance to the assembly and functionality of the microbiome, generalized linear models, as the Poisson regression, are well-established methods. The Poisson regression model is a technique used to describe count data as a function of a set of predictor variables. The Poisson distribution occupies a central position in both ecological theory and practice and allows to model time-series data in microbial ecology. Poisson regression has been used to predict plant status from rhizosphere microbiota (Bacci et al., 2018), providing the basis for the identification of relevant "predictor" taxa of the outcomes which can be prioritized in SynCom constitution (Hou et al., 2021).

Machine learning (ML) is another relevant technology for making sense of multi-omics data on plant-associated microbiome and to detect insightful data patterns (Figure 3d). ML can be used to understand how different organisms in a microbial community affect each other's growth to form complex networks of interdependencies. For example, Random Forest (RF) can be used to predict microbial interactions given trait descriptions (features) for each member of the community or extrapolate trait combinations that are causally relevant to species interactions (DiMucci et al., 2018). ML has been variously used in host–microbiota interaction, for instance, to link host plant (rice) functional traits to the core microbial community (Guo et al., 2021).

Finally, coming to the possibility to predict the metabolic outcomes from genome or a metagenome, metabolic *gen*ome-scale network *re*constructions (GENREs) are becoming popular. GENREs are intracellular network mathematical models consisting of a compilation of all biochemical reactions occurring in an organism (Fang et al., 2020). These models start from genome annotation and defining a stoichiometric matrix, are forced to solution by constraints (e.g., mass balance, steady-state conditions, etc.), and typically by applying a biomass-producing

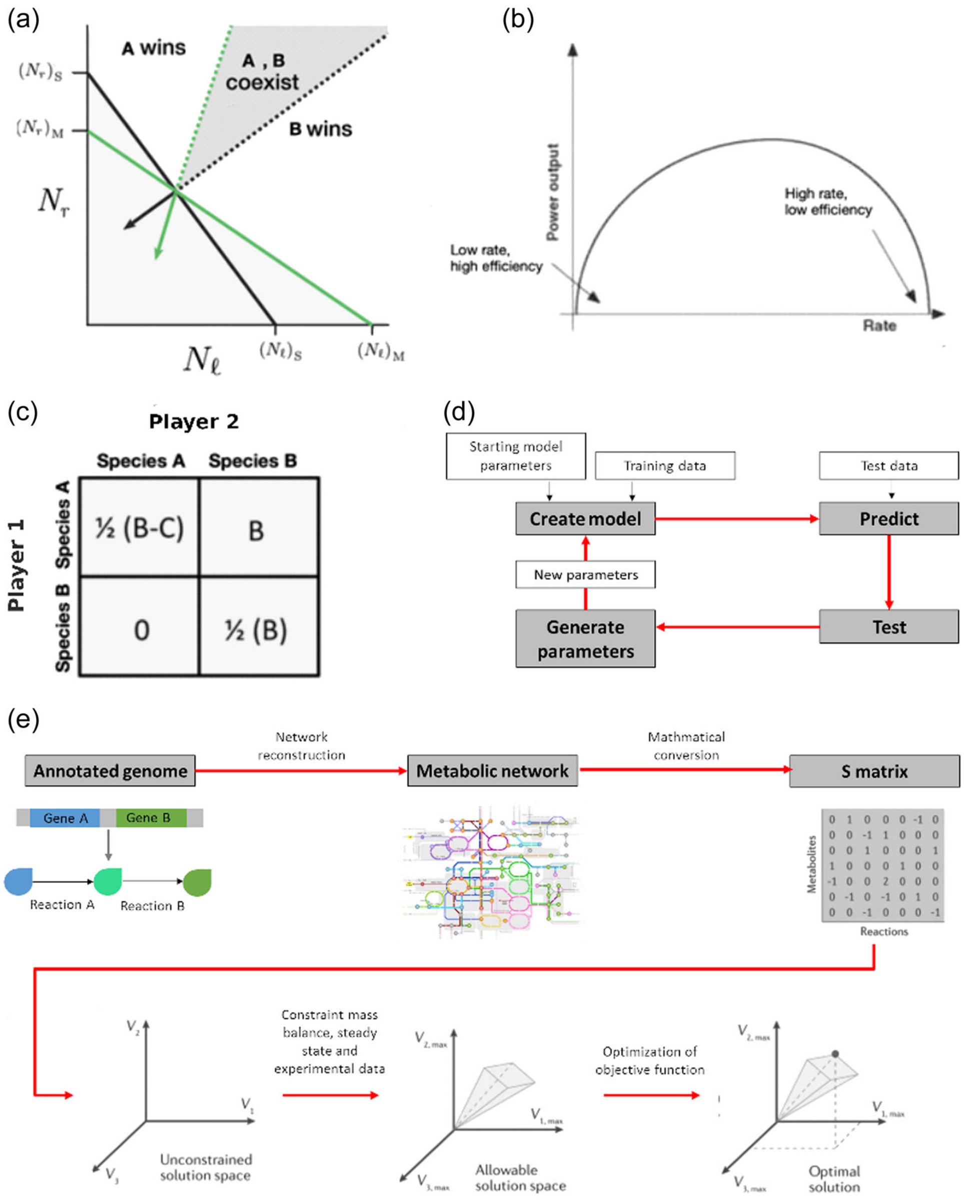

FIGURE 3 Developing model to investigate population dynamics and metabolic networks. (a) Scheme for the RRT modelling the competition between two or more species (A and B) for a limiting resource; prediction of outcomes including coexistence of species can be derived from single species abundance and rates of resource utilization; application of this modelling spans from conflicts in symbiosis (e.g., symbiotic nitrogen-fixing microbiota) to social interaction among members of the microbiota. (b) MPP is a thermodynamic-based model, where systems self-organize to increase energetic quantities (i.e., metabolic rate); optimum of metabolic fluxes between microbiota and the plant can be predicted in this way; (c) GT models strategic interactions among players where the payoff depends on the strategies of both players. Payoffs for two species: B "benefits," and C "costs," allowing to predict the cost of sustaining a microbiota with respect to the gain in plant biomass. (d) ML workflow starting from a training data set (e.g., in vitro plant-microbe interaction studies) used to create a first model, then tested on real data, which turns out in improved model parameters used to update the model in a recursive way; applications of ML includes studies of association between ecosystem functions and members of a microbiome. (e) Example of workflow in GENREs, where genome annotation provides the basis for the metabolic network reconstruction and the following stoichiometric (S) matrix, which is then solved applying constrains and an objective function to estimate the overall fluxes of reactions; modified from (Fang et al., 2020).

reaction (Figure 3e). The prediction of fluxes for each reaction in the model is an indication of relative importance of reactions and pathways to cell's growth capacity. Application of this modelling to communities is still challenging; however, predictions of metabolic exchanges in simplified systems, such as the rhizobium-legume symbiosis (diCenzo et al., 2020) have been done. For the rhizobium-legume symbiosis, the metabolic model reconstruction of the exchanges in the root nodule (the nitrogen-fixing symbiotic structure) allowed to predict the trade-off values of fixed nitrogen, plant biomass, and root nodule biomass to estimate the maximum nitrogen-fixing capacity of the holobiont.

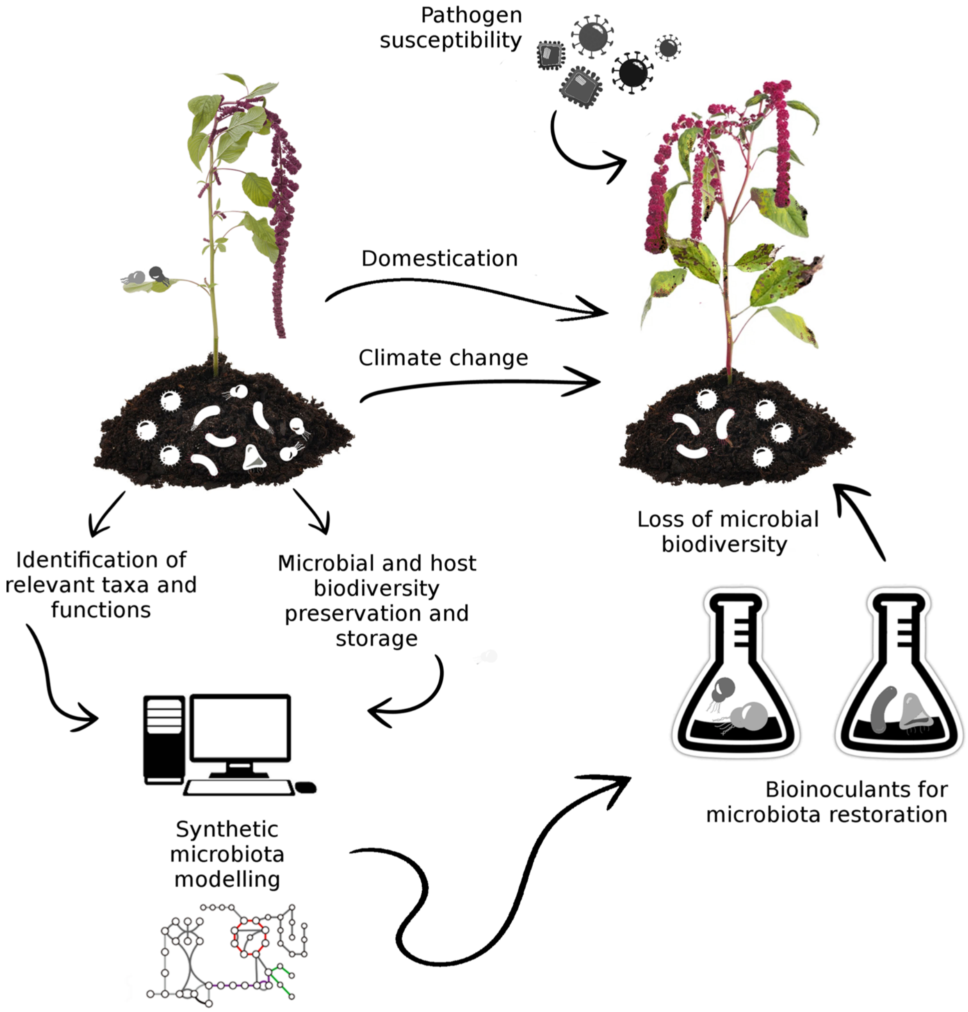

FIGURE 4 Bioinoculants development in a system biology approach. Natural environments represent a rich reservoir of genes and related functions that can be lost during domestication processes and as an effect of climate changes. The study, preservation, and storage of the microbial and host biodiversity lay the groundwork for the development of bioinoculants for microbiota restoration in case of augmented pathogen susceptibility or stress-related effect on the plant. Data obtained from microbiota and functions present in the natural soil can be integrated to create metabolic models that allow a synthetic biology approach to test hypotheses on plant-microbe interaction.

Although the number of approaches, briefly reviewed above, is allowing to dissect interactions among different (micro) organisms and the environment, to rationally design synthetic communities, these methods are not widely used. From one side, the expertize needed to master such models is not commonly found among microbiologists, re-proposing the classical division between lab scientists and computational scientists. Such division can be overcome by introducing more computational skills in educational programs in biological disciplines and by the recognition of the need of interdisciplinarity in research teams. On the other side, we should recognize that, despite a high number of reports and experiments performed on plant-associated microbiomes, relatively few are based on experiments designed to produce data which can be reused in clean computational models. The lack of shared standards, but also the enormous number of variables inherent to biological investigations (i.e., the many environmental variables) are serious limitations. The need to have a consensus on how data are reported is urgent and proposals have been presented (Pacheco et al., 2022), while we still need more data on real crops (often from nonmodel plant species/genotypes) in field and controlled conditions (Vaccaro et al., 2022).

4 | CONCLUSIONS AND FUTURE DIRECTIONS

The intense agricultural production, combined with land degradation, as a result also of global warming and climate alterations, is leading to a general depauperating of microbial genetic resources present in agroecosystems. This decrease in microbial diversity is having serious consequences, including reduction of soil fertility and increased sensitivity of plants to biotic and abiotic stresses. Soil health is a key factor for high crop productivity and requires conscious management of organic and inorganic elements. The integration of natural components such as bioinoculants improves efficiency of agronomic management (Maitra et al., 2022; de Souza et al., 2020). Indeed, bioinoculants are a core component among the Green Revolution Technologies because of the positive evidence shown by the plants teamed with microorganisms, which enhance the assimilation of nutrients such as nitrogen, resulting in an amelioration of plant's health (Maitra et al., 2022). However, the design of bioinoculants is still facing difficulties, due to our lack of knowledge of the microbes inhabiting the phytosphere, including their taxonomy, functions, and interactions. Programs for bioinoculant development should rely on various data and competences, from-omics data, to culturing and computational biology predictions. Such programs should also take into consideration the holobiont view of a plant, where plant genotypes and microbiome genotypes are interacting to provide increased plant functional traits and consequently that plant microbiomes are plant-genotype dependent (Figure 4). In the perspective of preserving microbes for future applications, we must strongly consider that wild relatives of crops, and wild plants, often harbour the most important part of the microbiomes needed for increased plant resilience to environmental perturbations. For sustainable agriculture, we need then to promote a novel approach to conservation biology, as the conservation of the holobiont. Conserving the holobiont means viewing the conservation of an organism (the plant in our case) as the conservation of its associated microorganisms also, which can be key for the agriculture of the future. In turn, conserving the holobiont means preserving the environment where the holobiont lives, including microbial endemism, since plant-associated microbes change under ex situ growth conditions.

AUTHOR CONTRIBUTIONS

Camilla Fagorzi and Alessio Mengoni conceived the idea. Camilla Fagorzi was responsible for organizing the whole manuscript, contributing to drafting Section 2 and finalizing figures. Lisa Cangioli contributed to drafting Section 2. Francesca Vaccaro contributed to drafting Section 1. Iacopo Passeri contributed to drafting Section 3. Camilla Fagorzi and Alessio Mengoni collected contributions from co-authors and finalized the writing of the manuscript. Alessio Mengoni secured funding and provided guidance to undertake the work. All authors provided feedback on the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

No data are reported.

ETHICS STATEMENT

The authors declare that they followed the ethics policies of the journal.

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