Engineering the plant microbiota in the context of the theory of ecological communities

Asmaâ Agoussar and Etienne Yergeau*

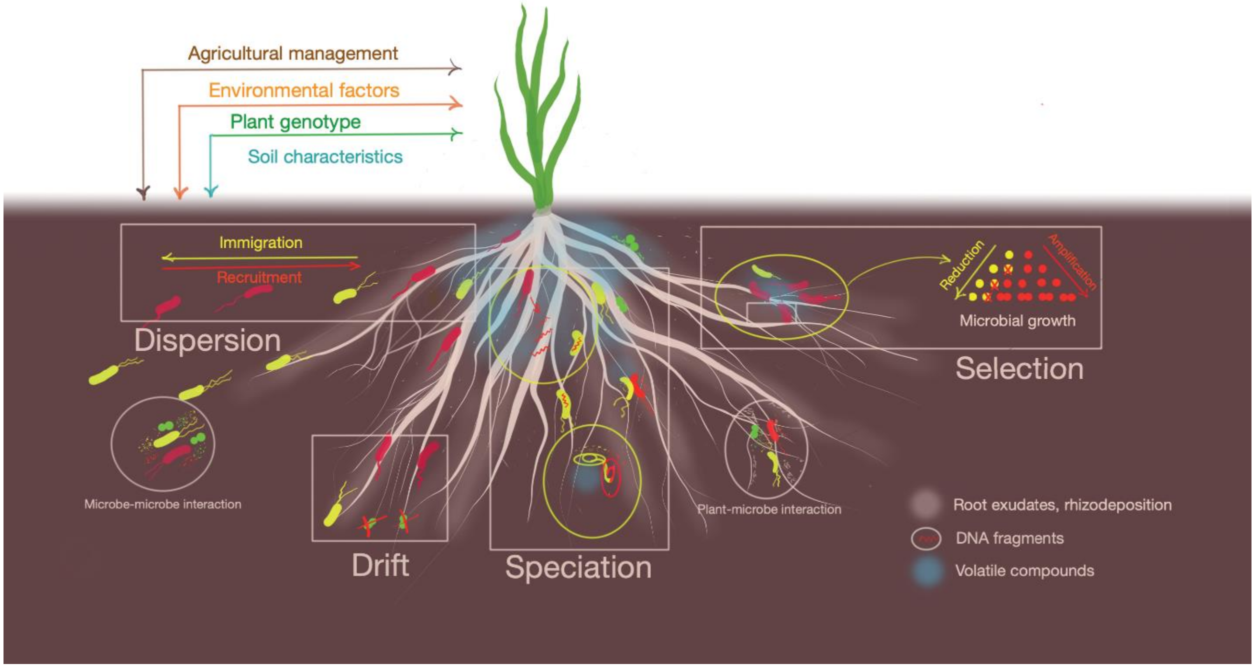
Institut national de la recherche scientifique, Centre Armand-Frappier Santé Biotechnologie, 531
boul. des Prairies, Laval (Québec), H7V 1B7, Canada

*Corresponding author: etienne.yergeau@inrs.ca; +1 450 687-5010, ext. 8881

Abstract

Crop-associated microorganisms are known to have a determining influence on crop growth and resistance to stresses. Indeed, microorganisms can deter pathogens, reduce stress levels, improve nutrition, and stimulate growth. However, the microbial communities associated with a plant are rarely optimal for agricultural needs. But how can we engineer crops-associated microbial communities? An interesting framework to address this question is the theory of ecological communities that stipulates four processes by which communities can change: 1) selection, 2) dispersal, 3) speciation and 4) ecological drift. Of these, speciation and dispersal can result in the addition of new species to the plant microbiota, whereas selection and drift can lead to the loss of species. We believe that if these mechanisms are sufficiently understood, they could be harnessed to purposefully engineer the crop microbiota. Here, we will discuss the recent efforts to modify the phenotype of plants that are aligned with these ecological processes.

Graphical abstract



Introduction

Due to global changes, biotic and abiotic pressure on agriculture will only increase in the future, resulting in lower yields and lower quality produce while the global population climbs toward the 9 billion mark. The unprecedented rate of these changes calls for novel, rapid approaches to improve the resistance and resilience of crops to stresses. One such approach is to engineer the crop-associated microbial communities, as microorganisms are known to provide vital service to plants such as increasing nutrition, deterring pathogens, protecting from stresses, and stimulating growth, amongst others. However, because of various co-evolutionary mechanisms, microbial communities interacting stably with the plant host are not necessarily providing the optimal services to the plant and might even be deleterious. The question then becomes: How can we revert this stable equilibrium and engineer the plant microbial communities for optimal services to the crops? An interesting theoretical framework to start addressing this question is the theory of ecological communities [1] that stipulates that there are four high-level processes by which ecological communities can be modified (Fig. 1): 1) selection, by reduction or amplification of community members already present within the host, 2) dispersal, by recruitment or immigration of new community members from the environment (soil, water or air), 3) speciation, by gradual mutation or, perhaps more importantly for bacteria, horizontal gene transfer (HGT) and 4) ecological drift. We believe that, if correctly understood in their proper theoretical framework, some of these ecological processes could be harnessed to purposefully modify the microbial communities of crops. Here, we will review plant microbiota engineering efforts made in the last few years through the lens of the theory of ecological communities. Since ecological drift is the random component of community dynamics, by definition it cannot be harnessed in the context of microbiota engineering, so it will not be included in our discussion.

Engineering the plant microbiota

1. Selection

When facing a stress or under varying environmental conditions, plant microbial communities undergo several modifications in term of their composition, diversity, and the relative and absolute abundances of its various members [2,3]. This in turn, results in shifts at the functional level [2], which can be either beneficial or deleterious for plant fitness and resilience to the changing conditions. In view of the remarkably high diversity of microorganisms present in the plant environment and the dynamic nature of microbial communities, there must be ways to orient these shifts toward increased beneficial services.

Microorganisms associated to crops could be selected directly, by acting on environmental variables, such as nutrient and water availability, soil pH, soil oxygen levels, crop identity, or other biotic or abiotic factors. Arguably, this type of selection has been carried out empirically by farmers around the world since the dawn of agriculture. However, with a more microbially- and ecologically-informed framework, selection-driven approaches using simple and already known interventions have a huge potential. For example, as even closely related plant genotypes were shown to harbor different microbial community compositions [3,4,5] and functions [2], a microbially-informed crop variety selection could increase resilience to stress likely to occur during the growing season. Interestingly, some engineering attempts using inoculation (covered in the next section about dispersal) failed to introduce the desired microorganisms, but resulted in clear shifts in the plant and soil microbial community composition [6]. It was also shown that roots of common beans growing in a soil inoculated with the fungi *Metarhizium* harbored several bacterial taxa that were not found in the roots of the plants growing in the uninoculated soils, even though no differences were observed in the abundance of the inoculated *Metarhizium* [7].

Alternatively, microorganisms could be selected indirectly, by acting on the plant selection mechanisms [8]. This would be most interesting and practical in the plant rhizosphere, where crops typically channel 3 to 5% of their net photosynthates [9], creating a strongly selective environment for microorganisms. Some studies have reported mechanisms by which plant exudation can be modified [10,11,12], which would provide an indirect method to alter the root-associated microbial communities. For example, Arabidopsis plants that were genetically modified to exude octopine had a rhizosphere that was significantly enriched in octopine degraders [13], providing a proof of concept that plants exudation patterns could be genetically engineered to steer "selection" processes in the rhizosphere. Another interesting study conditioned soils by seeding Arabidopsis for five generation in the same soil and exposing them to an aboveground pathogen [14]. When plants were grown in this conditioned soil, they exhibited altered root exudation patterns and rhizosphere microbiota and an improved disease resistance, suggesting that following exposure to an aboveground pathogen, plants associate with beneficial rhizosphere microorganisms through a shift in their root exudation patterns and that this can benefit the following generation of plants [14]. This also highlights that foliar application of certain microorganisms could be used to steer "selection" in the rhizosphere microbial communities, toward more beneficial services to the plant.

In addition to rhizodeposits, other factors are emerging as having a defining factor on the plant microbial communities. For instance, many gaseous compounds such as volatile organic compounds (VOCs) [15] or hydrogen [16] were shown to have a determining influence on plant- and soil-associated microorganisms, which led to the suggestion that the plant rhizosphere does in fact extend much farther than the millimetric zone of soil surrounding the roots [17]. However, it is still unclear how microorganisms within this extended rhizosphere would affect plant phenotype, and if they could be manipulated. In one study, hydrogen was infused in soil which led to a modification of the bacterial community [16] but it was not shown if this would have subsequently impacted crops. Some studies also reported that bacteria-emitted VOCs can inhibit the growth of fungal pathogens [18], and, conversely, fungal pathogens VOCs were shown to affect bacterial metabolism [19].

Another novel approach would be to use plant small RNAs to modify the activities of the plant-associated microbial communities. Indeed, small RNAs, and most particularly microRNAs (miRNAs), were shown to be involved in cross-kingdom communication [20,21], and their presence in the plant environment was suggested to have a key influence on the rhizosphere microbial communities [22]. The expression of plant miRNAs was shown to be regulated by micro-peptides (miPEPs) [23], which could be used as a tool to increase the transcription of specific miRNAs [24,25], suggesting an interesting avenue for modulating the gene expression and community composition of the plant microbiota.

Since the selection of microbes in the plant rhizosphere is thought to be based more on functions than taxonomy [26,27] we could extend our reflection on selection processes to gene expression. In that case, the shifts in the transcriptome and proteome of existing communities would be transient selection events, which would similarly lead to a differently functioning microbial community. In fact, shifts in microbial gene expression are not necessarily linked to shifts in microbial community composition, and can lead to different functioning within an otherwise identical microbial community [28]. However, without an explicit theoretical framework, it is difficult to interpret the results observed. For instance, there are three different forms of selection (constant, negative frequency-dependent and positive frequency-dependent) and the outcome in term of the abundance of the target microorganisms will depend on the form of selection that is at play.

2. Dispersal

The dispersal approach is the most frequently used in plant microbiota engineering efforts, mostly through the inoculation of single or multiple beneficial microorganisms, with the expectation that the inoculated organisms would establish themselves among the plant microbial communities and provide beneficial services to the plant. Many ecological mechanisms are counteracting this directed dispersal approach, such as priority effects, where microorganisms already occupying a niche are likely to have the upper hand on newcomers [29]. Other mechanisms, such as the one described in the “Kill the Winner” hypothesis [30], could also reduce the efficiency of microorganisms with rapid growth rates that are typically used in the inoculant industry, through preferred predation by soil animals or preferred lysis by phages. Additionally, diversity is known since a long time to be a buffer to invasion by plants and animals [31], and in the case of soil microbial communities, to determine the invasion by bacterial plant pathogens [32].

Recent work has pointed out that the seed microbiome was the preferred source of microorganisms during soybean development, preventing colonization from environmental (e.g., soil) microorganisms [33]. This could complicate any inoculation efforts that are not aiming specifically at the seed microbiome. Inoculating seeds during flower development was successful for introducing beneficial bacteria that could modulate the plant phenotype [34], which led to the patenting of the approach [35]. Similarly, the plant compartment origin of the inoculated isolates was shown to have a crucial effect on the growth and nutrient assimilation of the inoculated crops [36], which is further compounded by the variability of isolates coming from the same compartment [18].

Multi-isolates inoculants were often shown to outperform single isolates [18,33,37,38,39,40], potentially related to an enhanced robustness and adaptability conferred by the functional redundancy within the multi-isolate consortium. It was suggested that the creation of complex synthetic communities (SynComs) [41] could provide better inoculants, but it is still debatable if these would be able to outcompete the communities already interacting with the plant. Multiple inoculations of leaves and soil resulted in a shift in the resident microbial communities, which was not necessarily conducive to a better colonization in subsequent inoculations [42,43]. Finally, because of conflicting microbial traits requirements during the typical process of creating an inoculum, current approaches were suggested to be inadequate to generate ideal soil microbial inoculants [44].

An approach at the intersection of dispersal and speciation is the inoculation of genetically engineered strains in the plant environment. For instance, cloned strains capable of fixing nitrogen and of excreting large amounts of this fixed nitrogen in their environment were shown to colonize the root environment under different field conditions and could still be re-isolated from roots after 12 weeks [45]. Similarly, bacterial isolates engineered for phytate solubilization improved the growth of Arabidopsis plants supplemented with 0.8 mM sodium phytate [46], whereas endophytic isolates modified to express the ACC deaminase gene increased the resistance of banana to *Fusarium* wilt and promoted plant growth [47]. Similarly at the intersection of dispersal and speciation, it has been shown that in the longer-term (5 to 15 years), inoculated N-fixing strains evolved, resulting in an altered N-fixing efficiency, among other traits [48]. These results along with concerns related to non-target effects of the inoculants on soil microbial communities in the longer term [49], indicates that more research should be carried out to ascertain the innocuity of dispersal-based approaches on ecosystem functions.

Here again, without putting these results in the appropriate theoretical framework, it is difficult to explain why some “dispersal” efforts are successful and other are not. Recent work in that field showed that soybean-associated microbial communities were mostly assembled through niche-based processes [50], making it more difficult for exogenous microorganisms to invade these plant environments. However, neutral assembly processes also appeared to play a role in some plant compartments or at some plant growth stages, suggesting that these might be more amenable to microbial inoculations [50]. Modelling of the plant microbial communities based on the theory of ecological communities could also help predict the conditions necessary for successful inoculations without the need for large scale experimentations on many isolated microbial strains. In one such example, after categorizing a library of bacterial isolates for their effect on Arabidopsis phosphate content and testing the effect of a few consortia constructed from these strains, it was possible using neural network to accurately model and predict the effect of untested communities on Arabidopsis phosphate content [51]. More such predictive modelling efforts will be necessary to fully harness the potential of the “dispersal” approach.

3. Speciation

Since the definition of bacterial species is difficult and controversial, we will restrict our discussion on the apparition of new traits in a population already present in the plant environment. Within the framework of plant microbiota engineering, horizontal gene transfer mechanisms are particularly interesting, as it very rapidly changes the metabolic capacity of the plant microbiome [52], especially when compared to speciation through the accumulation of mutations. There are three main mechanisms of horizontal gene transfer, which could occur in the plant environment: 1) natural transformation, where bacteria pick up DNA fragments from the environment, 2) transduction, where bacteria are transformed by the action of phages, and 3) conjugation, where bacteria exchange a plasmid or a transposon. Interestingly, the rhizosphere is a known hotspot for HGT [52].

A classic example of conjugation in the plant environment is the study of Taghavi and coauthors [53], where poplars were inoculated with an endophytic *Burkholderia* strain harboring a plasmid expressing constitutively the toluene degradation gene. Poplar inoculated with the strain grew better in the presence of toluene, and toluene was more effectively removed from the soil. However, the inoculated strain could not be found in the plants, but the plasmid was readily detected, suggesting it had been transferred to resident microorganisms. This was shown to occur both in the presence and absence of toluene, highlighting that a selective pressure is not necessary to initiate HGT events. Two other HGT approaches based on conjugation were shown to be efficient to purposefully modify in place the gut [54] and the soil [55] microbial communities. For instance, Brophy and colleagues (2018) used inducible integrative and conjugative elements (ICE) to transfer DNA to either a single strain or a simple bacterial community directly in a soil. They reported that approximately 1 in 800 of the recipient strains were transformed, and that the ICE was detected in 4 out of the 6 members of the community [55]. The strength of this approach is that virtually any gene could be integrated in the ICE and transferred to, for instance, rhizosphere microbial communities.

Although these approaches are clearly extremely powerful, very few studies have attempted to harness HGT mechanisms for plant microbiome engineering. In the context of community ecology, speciation-based mechanisms need to work together with positive selection processes for the approach to be successful. A bacterium acquiring a new trait will not be able to establish and thrive in the plant microbial community without being positively selected. Nevertheless, these approaches, like the inoculation of genetically engineered microbial isolates, would have to be accepted by the public and the regulators before being used at a larger scale, and more studies would be needed to be able to control the persistence and host range of the inoculated DNA.

Conclusion

The plant microbiota engineering efforts discussed above were mostly conducted empirically without necessarily putting the approach in its theoretical context. We believe that by putting these efforts in the context of the theory of ecological communities, it will be possible to better choose and tailor an engineering approach, and to select which processes to combine. In addition, this theoretical framework allows for prediction and modelling, which will be invaluable tools to refine our plant microbiota engineering efforts without the necessity for substantial trial-and-error experimentation. With this framework in place, we can already predict that methods that aim at modifying microbial communities that are already in place (speciation and selection) are more promising and ecologically sounder than methods that aim at adding new microorganisms or replacing already established microbial communities (dispersal). In line with this, when inoculating wheat rhizosphere with a highly complex soil extract inoculum, it was calculated that, in the best case, 7% of the microbial communities of wheat plants under water stress was recruited from the inoculum, and that the vast majority of the changes observed were due to the selection of microorganisms already present in the plant environment [56]. Putting our plant microbiota engineering efforts back in the theory of ecological communities offer an exciting window toward increasing beneficial services to the plant.

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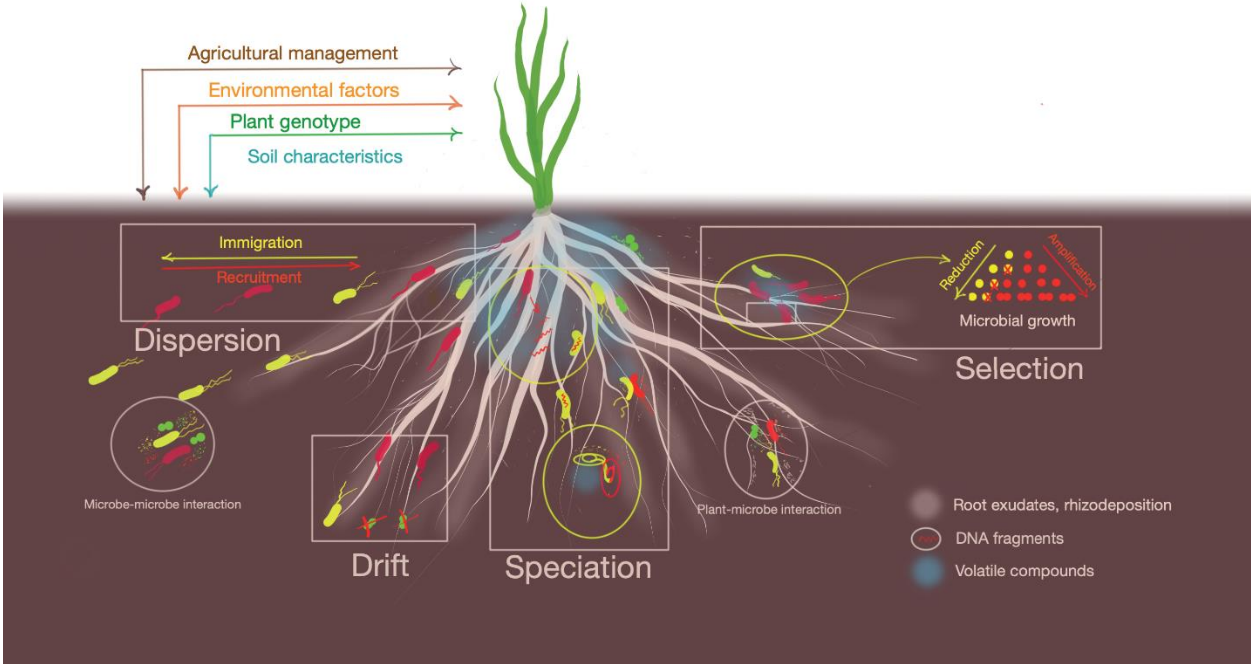
Figure 1.


Agricultural management
Environmental factors
Plant genotype
Soil characteristics
Immigration
Recruitment
Dispersion
Reduction
Amplification
Microbial growth
Selection
Microbe-microbe interaction
Drift
Speciation
Plant-microbe interaction
Root exudates, rhizodeposition
DNA fragments
Volatile compounds

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This study tried to harness the "dispersal" mechanism by inoculating soil microbial extract to wheat plants suffering from drought, hypothesizing that the plant would select adequate microorganisms from this "buffet". Unfortunately, the plants under stress appeared to mostly promote microorganisms from their already associated community.