water status. Other means of maintaining leaf turgor when the air or the soil becomes drier are, for example, to reduce leaf area or to increase leaf-level osmolyte supply. Thus, leaf turgor is the result of a suite of plant hydraulic traits and processes. This makes Ψ_L at turgor loss (Ψ_{tlp}), the point a plant must avoid to maintain control over water loss, a promising trait to quantify the relative isohydry of a plant [8]. Although we expect Ψ_{tlp} to be a good approximation of full stomatal closure, in rare cases stomata may remain partly open at leaf water potentials below Ψ_{tlp}. Those exceptions, however, do not make Ψ_{tlp} a less-promising predictor of plant hydraulic behavior and thus of relative iso/anisohydry. The Ψ_{tlp} value of a given plant can vary in response to changing Ψ_S and VPD within the physiological (e.g., osmotic adjustment) and structural (e.g., hydraulic architecture) boundaries of the plant [9]. This, in turn, will result in intraspecific variation of apparent iso/anisohydry with environmental conditions (Figure 2), and may provide valuable insights concerning the physiological and structural ability of a species to acclimate to different moisture regimes.

In general, the environment plays a major role in determining the degree of iso/anisohydry of a plant, and direct comparison of the stringency of water-status regulation across individuals and species is only meaningful under comparable environmental conditions. Thus, applying the iso/anisohydry concept should be constrained to either comparing the responses of different species under similar environmental conditions or the response of a single species across environments (Figure 2). In the former case, interspecific differences in relative isohydry as measured by Ψ_{tlp} may well be apparent [8], underlining the strength of the iso/anisohydry concept for typifying species in a defined framework.

Concluding Remarks

The way plants regulate their water status as measured by water potential is highly complex. Any concept holistically describing this regulation should thus account for the interplay of plant hydraulic traits/processes and their dependence on environmental conditions. The iso/anisohydry concept has long been applied to describe plant water-status regulation, but with varying success, mainly because of persistent misconceptions. It is now clear that a continuum of coordination and trade-offs among coevolved traits leads to a continuum of stringency of plant water-status regulation [10]. This stringency, in turn, can be characterized as spanning the continuum of relative isohydry to anisohydry [11], and not as a dichotomy, which any approach using this nomenclature should account for. A promising approach is based on the turgor loss point Ψ_{tlp} as a proxy for iso/anisohydry, because Ψ_{tlp} is coordinated with a suite of plant hydraulic traits, whereas differences in Ψ_{tlp}, and thus in relative iso/anisohydry, for a given species reflect the ability of that species to adjust to different environments [11]. We therefore believe that, provided the concept being used is clearly defined and the relevant environmental conditions reported, assessments of iso/anisohydry may contain considerable information and should not be abandoned.

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Forum

Microbiome Applications from Lab to Field: Facing Complexity

Angela Sessitsch,[1,*] Nikolaus Pfaffenbichler,[1] and Birgit Mitter[1]

Plant microbiota are the subject of new product developments, primarily aimed at improving plant health, nutrition, and stress resilience. However, current application of microbials in the field faces multiple

challenges and we propose that multiple aspects need to be considered, for example, understanding the complexity and ecological behaviour of natural microbiota.

The Complexity and Potential of Plant-Associated Microbiota

In the last decade microbiome research has received tremendous attention and it has become evident that microbiota associated with higher organisms have highly important functions supporting health, growth, and well-being of their hosts [1,2]. The term **holobiont** (see Glossary) has been coined [3], recognising that the host and all associated microbiota interact and perform in a concerted manner. For plants, the associated microbiota have been termed the accessory plant genome, taking into account that the plant actively recruits highly diverse microorganisms, which in turn perform important functions for their host [4]. Multiple functions have been described for plant microbiota, such as their role for the protection of plants against pests and pathogens as well as their importance for plant growth and stress resilience (Box 1). These functions can be tapped into to address the challenges agriculture is currently facing, such as sustainable food production. The world population is growing substantially and food production has to increase accordingly. At the same time, a changing climate and soil degradation present more challenging growing conditions for crops. According to a recent report [5], 52% of all fertile, food-producing soils globally are now classified as degraded, and it has been projected that this will lead to a 12% decline in global food production over the next 25 years. Furthermore, our society demands sustainable and nonhazardous agricultural practices. The application of microorganisms to improve plant production has huge potential, particularly under adverse conditions, and to reduce the use of chemical fertilisers and pesticides. However, current application of **microbials** in the field faces multiple challenges, and practices adapted to the application of chemicals may not be transferable to microbials. We propose that field application of microbials requires the consideration of multiple aspects, ranging from appropriate **formulation** design to new concepts based on understanding the complexity and ecological behaviour of natural microbiota.

Challenges of Microbial Field Applications

Usually, strains are screened for plant growth-promoting characteristics in the laboratory under highly artificial conditions, also partly using model host plants. After the selection of microbial strains for a specific application, strains are usually tested in greenhouse experiments, often showing significant effects, even when **non-sterile soil** is used for experimentation. However, when applied under field conditions, effects are highly variable and often lack consistency, which restricts microbiota applicability (Figure 1).

Challenge 1: Delivery of Microbial Inoculants

Successful long-term establishment of microbial inoculants, colonisation of the target niches in the plant environment, as well as expressing the relevant plant growth and health-promoting effects, are key issues to be considered. Taking into account the huge numbers and diversity of soil microorganisms in the soil/plant environment, in which the inoculant has to establish, appropriate numbers of active cells have to be applied. This requires the delivery of microorganisms together with suitable formulations, which should protect microbial cells from desiccation and other adverse conditions. For some microorganisms, such as for

Glossary

Community evenness: refers to how close in numbers each community member (e.g., microbial species) in an environment is.
Endophyte: a microorganism living at least part of its lifecycle in the plant interior.
Formulation: compounds and procedures to ensure microbial viability and to protect them from deleterious environmental parameters.
Holobiont: the association between a host and other organisms, such as, for example, microorganisms/microbiota.
Metagenome: all the genetic material present in an environmental sample, consisting of the genomes of many individual organisms.
Microbials: microorganisms applied within a microbial product.
Non-sterile soil: (natural) soil containing high numbers of diverse microorganisms [versus soil that has been sterilised (e.g., via gamma-irradiation or heat treatment) and depleted of microorganisms].
Quorum sensing: the regulation of gene expression in response to fluctuations in cell-population density.
Rhizosphere: the soil surrounding roots and influenced by root exudates.

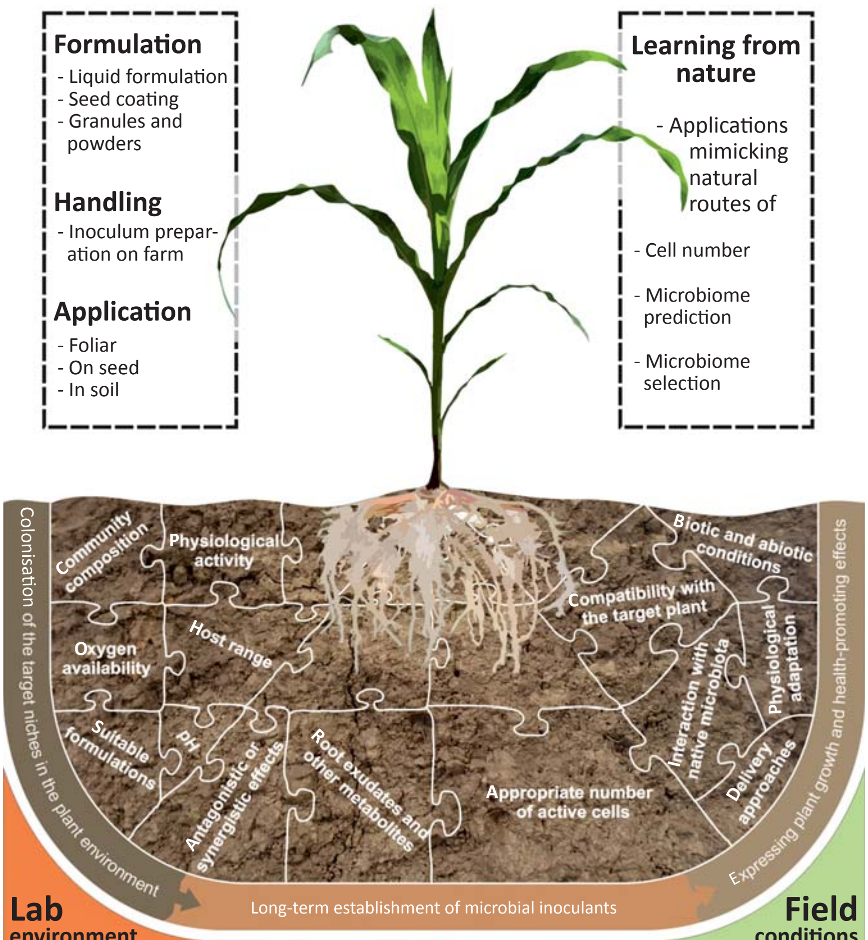
Box 1. Microbial Traits with Beneficial Effects on Plant Growth and Health

Plant Growth-Promoting Activities

Plant growth-promoting effects of microorganisms are often based on microbial plant hormone production, the modulation of hormone levels in plants or plant gene expression, increased stress tolerance, and immobilisation of heavy metals or degradation of toxic compounds. Microbes can also provide nutrients and essential vitamins to plants, improve nutrient uptake, and influence plant secondary metabolism.

Biocontrol Activities

Plant-associated microbes often have the potential to control plant pathogens. Antagonism is mediated either directly by niche occupation, production of antimicrobial compounds, suppression of the development or virulence of the pathogen, or indirectly by inducing plant resistance or stimulating additional organisms capable of inhibiting pest or pathogens.



Artificial/highly controlled conditions used for screening and testing of microbial strains	Highly complex environment with high microbial diversity, versatile environmental/climatic conditions, variable stress conditions; agro-management practices influencing microbial diversity and activity
Availability and high throughput potential of screening methods determine strain selection poorly predicting field efficacy and particularly yield traits	Complex (plant and microbial) mechanisms in place determining plant traits, not easily predictable from simplified lab assays
Colonisation and competitive ability of inoculants strains rarely determined	Poor competitions and colonisation in natural (agro)environments of many inoculant strains
Use of model plants because of high throughput potential in screenings, but low prediction power for interaction of microbes with crops/other plants.	Use of crops not bred/optimised for beneficial plant–microbe interactions

Trends in Plant Science

Figure 1. Challenges of Microbial Inoculation.

endospore-producing *Bacillus* strains, such formulations are available and these strains are also highly resistant due to their ability to produce endospores. However, many microorganisms, particularly gram-negative bacteria, are highly sensitive and require special formulation and/or delivery approaches.

Challenge 2: Strain Establishment and Plant Colonisation

Strain establishment is likely to depend on numerous factors in addition to initial cell dosage, such as physiological activity, compatibility with the target plant, as well as abiotic and biotic conditions of the receiving environment. Usually, microorganisms are produced in rather rich media not considering prevailing conditions of the soil/plant environment. Physiological adaptation might reduce the competitive ability of an introduced strain and limit its establishment.

Plant colonisation by microorganisms is not a random process. Plants produce root exudates and other metabolites, which act as signals and nutrient sources attracting microorganisms. For establishment, the colonising microorganisms do not only have to be able to recognise and metabolise these substances, they also have to be able to cope with other prevailing conditions, such as pH or oxygen availability. Among the microorganisms there are strains, which can be considered as generalists colonising a wide range of host plants, whereas others are more restricted. Microorganisms may also colonise only specific niches in the plant environment.

Inoculant microorganisms have to compete with a highly diverse microflora. Depending on the size, diversity, and community interactions of the resident populations, an introduced inoculant strain may establish well or only poorly. De Roy *et al*. [6] have shown that under unstressed conditions, **community evenness** greatly determines community stability or invasion. Additionally, community composition, as well as ongoing interactions, might play an important role. For example, some community members may exhibit antagonistic or synergistic effects towards the introduced cells (e.g., by the production of certain antimicrobial compounds, co-metabolism,

quorum sensing, and other mechanisms). Microbial interactions may also influence the activity of the introduced microorganism affecting also beneficial, plant growth-promoting traits and introduced strains may also interact with the resident microflora.

Challenge 3: Potential Concerns and Hurdles in Regulatory Approval

Due to the fact that microorganisms are well known for their pathogenicity, there is concern that the application of microbials poses a risk for either plant health and/or food safety. As a high number of cells is introduced to the environment, it is of high importance to warrant that the applied microbials do not harm humans, animals, or the environment (e.g., other plants) in any way. Usually microorganisms undergo rigorous safety assessment before approval, unless microorganisms are applied, which are 'generally considered as safe' (GRAS concept) due to a long history of safe application (e.g., rhizobia). However, due to the rigorous requirements in many countries to prove safety and partly also efficacy, regulatory approval may take a few years, especially for microbial biocontrol products. Nevertheless, regulatory approval and time to market of microbials is generally faster and cheaper than approval of chemical products or genetically modified plants.

Consideration for Successful Field Application

Consideration 1: Formulations for Microbials and Alternative Delivery Approaches

The perception of microbial products in agriculture is strongly driven by the experiences with synthetic chemical fertilisers and plant protection agents. Therefore, microbials are developed mostly as liquid formulations for foliar applications, as seed coatings and pellets, or granules and powders. To overcome the limitations in shelf-life some microbial products require preparation or handling by the farmer, and successful application depends often on handling skills and storage capacity of the farmer. However, microbial products are living organisms and therefore *per se* more variable and less predictable than chemical compounds. Keeping this in mind, it is no surprise that the rationale used for development and application of agrochemicals has only been moderately successful for microorganisms.

In the past decades we have learnt much about the routes and modes of plant colonisation by microorganisms [7] and molecular mechanisms of plant–microbe interactions [2,8]. This knowledge could guide new developments of microbial crop protection and strengthening agents by mimicking the processes by which microbiota interact with plants in nature. One such novel approach is to introduce plant beneficial microorganisms into the seed microbiome, thereby making use of the plant seed as a protective carrier for the microbe [9]. In this approach, bacterial formulations are sprayed on flowers of crop plants and upon colonising the flower, thereby the bacteria become incorporated into the progeny seed. Similar to the natural seed microbiota, the introduced microbial strain is protected from the strong competitive pressure in soil and **rhizosphere** and can colonise the next-generation plant at an early stage of plant development. This is only one example of how nature could provide guidance in the development of new and innovative concepts for the implementation of beneficial plant–microbe interactions in agriculture. Generally, the application of plant beneficial **endophytes** [1], which have the capacity to colonise different plant tissues internally, has advantages as endophytes escape fierce competition in the rhizosphere and may establish a long-lasting interaction with their host.

Consideration 2: Microbiome-Based Concepts

Knowledge of plant microbiome build-up and dynamics, and its association with plant phenotypic traits under field conditions, could lead to the development of strategies to optimise the microbiota for plant benefit. Evidence-based prediction of disorders or other complex phenotypes from microbiome and **metagenome** data has been a focus in human microbiome research for many years and bioinformatics tools for prediction of microbiome-phenotype associations have been developed [10]. Only recently, similar concepts have been applied to model optimal plant–microbiome combinations (e.g., metagenome-wide association study and machine learning were used to predict crop productivity from bulk soil metagenome data [11]).

A consequence of modelling microbiomes for the plant's benefit is the development of means and strategies to modulate or select microbiomes. It is well understood that the soil is a major determinant of plant microbiota [12]. Furthermore, the plant genotype, as well as agricultural management and cropping practices, shape plant microbiota [13,14]. With this in mind, intelligent cropping or management practices could be used to foster desired microbial consortia in soil [13]. Furthermore, the plant-protective activity of plant-associated microbiota was found to depend on nutrient availability [15]. So the fertilisation regime might also be a means to manage microbiomes for the plant's benefit.

Concluding Remarks

In conclusion, field application of microbials requires the consideration of multiple aspects, ranging from appropriate formulation design suited to enable survival and shelf-life of microorganisms, to new concepts based on understanding the complexity and ecological behaviour of natural microbial communities (Figure 1).

Practices adapted to the application of chemical treatments might be only to a limited extent transferable to exploit the whole potential of microbiome understanding in regard to their role in crop production. Ultimately, a constant transfer of knowledge from plant microbiome research taking field conditions into account could drive innovation in microbiome applications for agriculture [16] and help to bridge the gap between laboratory results and performance on the field.

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