

Rhizosphere engineering: leading towards a sustainable viticulture?

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ABSTRACT

Microorganisms are a substantial component of the rhizosphere, and the activity and composition of rhizosphere microbial populations markedly affect interactions between plants and the soil environment. In addition, the microbiota of the rhizosphere can positively influence plant development, growth and vitality. In vineyards, management practices influence both grapevine root growth directly and the rhizosphere microbiota, but the exact mode of action is largely unknown. Recently, however, two new research approaches are increasingly coming into focus to enhance grapevine growth and health: plant engineering and rhizosphere engineering. In plant engineering, knowledge about plant-microbiome interactions is used for plant breeding strategies. In rhizosphere engineering, microbial communities are modified by adding specific fertilisers, nutrients or by bio-inoculation with certain bacteria and/or fungi. Taken together, these new methods suggest a potential for reaching a more sustainable development of pesticide-reduced viticulture in the future.

KEYWORDS

Plant microbiome, plant engineering, plant growth-promoting bacteria, plant-microbe interaction, vineyard soil, bio-inoculation, biocontrol

INTRODUCTION

The plant microbiome represents an area of active ongoing research efforts and represents a promising approach to increase crop productivity and optimise agricultural management strategies (Li *et al.*, 2020; Taye *et al.*, 2019). The productivity of agricultural systems is greatly dependent on the functional processes of soil microbial communities, especially within the rhizosphere (Bakker *et al.*, 2012). The quest for higher yields leads to an intensification in crop production, which results in a loss of soil fertility and quality (Gattullo *et al.*, 2020). However, soil quality and soil health are important factors for sustainable farming (Karimi *et al.*, 2020). Sustainability is a frequently used term (Lewandowski *et al.*, 1999), especially nowadays of increasing awareness of ongoing climate change, loss of biodiversity, and environmental pollution. Due to this increased awareness, and also in response to rising restrictions on synthetic pesticide usage, there has been a movement toward the adoption of more sustainable agricultural and viticultural practices (Andreolli *et al.*, 2021; Úrbez-Torres *et al.*, 2020; Zucca *et al.*, 2009).

Sustainability in general, or sustainable agriculture, respectively, is characterised by a systems perspective of natural and human resource management; it encompasses three objectives: environmental health, economic viability, and social and economic equity (Zucca *et al.*, 2009).

There are different approaches to achieve sustainable farming. On the one hand, it is possible to genetically modify plants in such a way that they become resistant to the pathogen. The study of Yamamoto *et al.* (2000) was the first report of grapevine being genetically modified for resistance against fungal diseases. They used transgenic grapevine plants harbouring a rice chitinase gene, which enhanced resistance to powdery mildew and anthracnose (Yamamoto *et al.*, 2000). As it takes many years to breed disease-resistant grapevines, the identification of resistance genes is a good starting point for improving grapevine cultivars (Yamamoto *et al.*, 2000). However, since genetically modified organisms (GMOs) lack social acceptance, two new research approaches are increasingly coming into focus for viticulture: plant engineering and rhizosphere engineering. Plant engineering looks at the extent to which the plant genotype can be optimised through breeding programs. In this way, the newly used fungus-resistant grape varieties were developed.

In rhizosphere engineering, the rhizosphere of the plant is studied and optimised. For instance, specific fertilisers or microbial inoculants can alter the composition of the microbial community in the rhizosphere. With these changes, better nutrient uptake or the induction of plant defence responses are aimed for. Both approaches are commonly used in agriculture (Taye *et al.*, 2019; Mendes *et al.*, 2018; Figueroa-López *et al.*, 2016), but with regard to viticulture, there is little experience. Therefore, this review aims to discuss whether these two options, especially rhizosphere engineering, offer an advantage for sustainable viticulture as well.

Grapevines (*Vitis vinifera* L.) are one of the economically most important fruit crops worldwide (Álvarez-Pérez *et al.*, 2017; Andreolli *et al.*, 2016) mainly used for wine production (Andreolli *et al.*, 2016; Compant *et al.*, 2013). During the growing season, vineyard soils are subject to a number of management practices intended to support the grapevines growth and health such as tillage, weed management, fertilisation and application of pesticides or fungicides (Cordero *et al.*, 2020; Hendgen *et al.*, 2018). Since grapevines are susceptible to various pathogens (Lakkis *et al.*, 2019; Trotel-Aziz *et al.*, 2008) and biotic and abiotic stresses, it is essential to protect the vines and grapes by applying pesticides and fungicides (Carro-Huerga *et al.*, 2020). Climate change further increases the need for protection (Karimi *et al.*, 2020) due to pathogen pressure and water limitation. In total, around 35 % of pesticides are used in viticulture (Compant *et al.*, 2013), which accounts for only 0.005 % of the world's arable land (Klein Goldewijk *et al.*, 2017; OIV, 2016). However, increased environmental awareness of consumers and producers promotes on the other hand the desire for sustainable viticulture (Giri *et al.*, 2019; Compant *et al.*, 2013) and a drastic reduction of all types of chemical inputs (Salmon *et al.*, 2020). To make viticulture more sustainable, alternative strategies are required to control serious grapevine diseases like *Plasmopara viticola* and *Botrytis cinerea* (Lakkis *et al.*, 2019; Yamamoto *et al.*, 2000). Thus, alternative methods for protecting the grapevines such as biocontrol (Compant *et al.*, 2013) or microbe-assisted crop production are inevitable. The use of such methods is particularly promising on the plant rhizosphere (Figure 1).

THE RHIZOSPHERE

Soil inhabits multiple biotic and abiotic processes, which are linked to key ecosystem functions like the cycling of carbon and other nutrients (Köberl *et al.*, 2020). In terms of plant nutrition, plant health and crop quality, the most important is the soil that directly surrounds the plant's root space – the rhizosphere (Berg and Smalla, 2009). The rhizosphere differs from bulk soil and is defined as the area of the soil that is physically, chemically and biologically influenced by the living root of the plant (Ryan *et al.*, 2009). It is characterised by high concentrations of plant-derived organic exudates, released root cap mucilage and root border cells (Vieira *et al.*, 2020). Due to these released substances, the rhizosphere contains the highest concentration and diversity of bacteria in the soil (Giri *et al.*, 2019).

In particular, the microbiome of the rhizosphere can positively influence plant development, vitality and growth by providing and acquiring nutrients and has frequently been targeted to identify positive plant–microbial relationships (Taye *et al.*, 2019).

It is known that certain beneficial bacteria influence the production of different growth regulators in plants (Giri *et al.*, 2019). They are therefore called “plant growth-promoting bacteria (PGPB)” (Compant *et al.*, 2010) or “plant growth-promoting rhizobacteria (PGPR)” (Trotel-Aziz *et al.*, 2008). PGPBs can promote plant growth by direct or indirect mechanisms (Compant *et al.*, 2019), which are linked to microbial metabolisms such as nitrogen fixation, phytohormone production, phosphate solubilisation, and pathogen suppression (Giri *et al.*, 2019; Pacifico *et al.*, 2019). In addition, by the stimulation of the host plant, these beneficial organisms also lead to increased enzymatic catalysis, enhanced water and nutrient uptake or defence responses (Pacifico *et al.*, 2019). Some of them can produce *e.g.*, auxin, cytokinin, or gibberellin, which affect plant growth as phytohormones (Compant *et al.*, 2019). In addition, PGPR plays an important role in the industry of sustainable agriculture (Giri *et al.*, 2019) as they can act as efficient biocontrol agents. Bacterial biocontrol agents protect their host plants against pathogens either by direct interaction with the pathogen or by induction of the host's resistance (Trotel-Aziz *et al.*, 2008). This so-called induced resistance (IR) occurs when plants develop their defensive capacity when they are triggered by pathogens (Trotel-Aziz *et al.*, 2008).

By contrast, the rhizosphere can also form the path of infection for soil-borne pathogens (Berlanas *et al.*, 2019).

Hence, the microorganisms living in and on the roots can influence plant fitness and provide effective protection against abiotic stress by induction of defence reactions (Li *et al.*, 2020). To provide such soil functions, a diverse and active soil microbiome is required (Di Giacinto *et al.*, 2020).

It has been shown, that the (endophytic) microbiome of grapevines has a major impact on the regional *terroir* of wines (Pacifico *et al.*, 2019; Zarronaindia *et al.*, 2015). Furthermore, Bokulich *et al.* (2014) note that regional factors could influence the “microbial *terroir*” of grape surfaces within global viticulture. However, over the past ten years, the microbiome of vineyard soils has gained raising attention as well (Novello *et al.*, 2017). Although, much progress has already been made in describing the grapevine microbiome and its effects on growth, yield and product quality, little is known about how different factors such as cultivars influence the grapevine microbiome (Awad *et al.*, 2020). The investigation of the soil microbial community could be a new strategy to develop a biological indicator for vineyard soil quality and health (Burns *et al.*, 2016). The soil microbial community of the rhizosphere can be investigated by cultivation-dependent and cultivation-independent approaches. Silva-Valderrama *et al.* (2020) isolated 378 different fungi and yeasts from multiple grapevine tissues using a cultivation-dependent approach. Additionally, Compant *et al.* (2011) cultivated and isolated bacteria and fungi from different grapevine tissues. Especially in the rhizosphere, they examined different species such as *Pseudomonas* spp. and *Bacillus* spp. (Compant *et al.*, 2011), which are also known to act as biocontrol agents (Figueroa-López *et al.*, 2016; Trotel-Aziz *et al.*, 2008). To examine the microbiome more closely, molecular biological methods like metagenomics and next-generation sequencing (NGS) have become essential (Berlanas *et al.*, 2019). Such techniques may also help to identify beneficial organisms to develop bio-inoculants that can be used not only to improve plant health but also to eliminate unwanted microbes (Giri *et al.*, 2019). Deyett and Rolshausen (2019) used a culture-independent approach and examined 2875 bacterial amplicon sequencing variants (ASVs) and 2694 fungal ASVs in 68 samples of the xylem sap of grapevine.

As the main bacterial phyla, they identified *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* (Deyett and Rolshausen, 2019). In a study conducted by Liang *et al.* (2019) *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, and *Firmicutes* were found in vineyard soil.

Furthermore, in the past few years, molecular markers for several agronomically relevant traits such as berry colour and weight or disease resistance against mildews have been revealed in grapevine (Di Gaspero and Cattonaro, 2010). This could help in the development of a biomarker strategy for manipulating plant microbiome ecosystems (Giri *et al.*, 2019).

PLANT ENGINEERING

Continuing advances in biotechnology and bioinformatics promote researchers to evaluate the microbiome to a greater depth, to include more replications and to account for variables such as genotype, time, and space (Taye *et al.*, 2019). In agricultural systems, the plant genotype is carefully controlled through breeding and cultivar selection (Bakker *et al.*, 2012). However, traditional approaches of plant breeding do not take the plant microbiome into account, although the importance of the rhizosphere microbiome for the plant ecosystem functioning has been widely recognised (Mendes *et al.*, 2018). Knowledge about plant–microbial interactions could be added to plant breeding programs to create and maintain healthy and beneficial microbial communities in the rhizosphere (Figure 1B). Integrating the knowledge on multifunctional interactions between crop plants and microbes in future agricultural systems and plant breeding will eventually lead to sustainable solutions to reduce the threat imposed by soil-borne pathogens (Wille *et al.*, 2019). By an analysis of the rhizosphere microbiomes of common bean cultivars with different degrees of *Fusarium oxysporum* (Fox) resistance, Mendes *et al.* (2018) showed that Fox resistance is based on plant genetic traits. Their data support the hypothesis that breeding for resistance may have unintentionally altered the rhizosphere microbiome composition, altering the frequency of beneficial microorganisms and traits that may contribute to plant growth or assist in protection against the pathogen. Considering that Fox resistance is based on genetic and chemical alterations in the plant, their findings suggest that the observed changes in the rhizosphere microbiome may enforce the first line of defence, limiting pathogen invasion through a higher abundance of specific microbial

groups and functions, high microbial diversity, abundance and a more complex network structure (Mendes *et al.*, 2018).

Based on their results, Taye *et al.* (2019) suggested that different *Brassica napus* genotypes have an extensive and selective control on associated rhizosphere bacterial genera. Given these controls are genetically based, they may represent potential breeding targets if the associated bacteria show to be positively correlated with yield or other positive traits in subsequent work (Taye *et al.*, 2019).

Traditional plant breeding approaches and advanced plant genome editing-based methods are promising ways to accumulate favourable alleles associated with stress tolerance in a plant genome (Ryan *et al.*, 2009). Given the wide range of genotypes that can be collected and/or generated per a specific plant species, genetic diversity is a potentially important asset in maintaining or increasing plant ecosystem values, *e.g.*, in controlling stability and stress resilience in native and cultivated ecosystems, productivity in cultivated ecosystems, and ecosystem functioning (Ahkami *et al.*, 2017).

It is of considerable interest to characterise the structure and composition of rhizosphere microbial communities as a first step towards its manipulation to improve crop performance (Shi *et al.*, 2019). Farmers influence the environment around the roots of their crops and pasture species every time they irrigate their fields or apply fertilisers (Ryan *et al.*, 2009). Progress toward sustainability is offered through the development of crop varieties that selectively enhance beneficial functions within the soil microbiome (Bakker *et al.*, 2012). Identifying genetically controlled positive plant-microbial interactions by comparing lines within breeding programs and across diversity panels is the first step in enabling plant breeders to develop varieties by selecting for genetic factors controlling beneficial plant–microbial interrelationships (Taye *et al.*, 2019).

Although each plant species is thought to select its specific microorganisms (Berg and Smalla, 2009), the understanding of the impact of the genetic variation between the microorganisms and the host plant is still incomplete, especially in grapevine species (Berlanas *et al.*, 2019). However, Berlanas *et al.* (2019) determined the grapevine genotype as the most important factor for shaping the microbiome in the grapevine rhizosphere. The investigation of the interactions between the grapevines and their microbiome

could therefore provide a new tool to improve plant breeding programs for grapevines, leading to an improvement in grape production and vineyard management practices (Bokulich *et al.*, 2014).

However, as *Vitis vinifera* grape varieties are usually grafted on *Phylloxera*-resistant *Vitis* sp. rootstocks, future research in rootstock selection is inevitable with regard to their microbial community as well (Zarraonaindia *et al.*, 2015). Thus, for example, rootstocks displaying a better performance regarding mycorrhiza development may be better suited for viticulture under water stress conditions (Schreiner, 2003). Nonetheless, the study of rootstock resistance remains difficult, since the plant genomes of rootstock cultivars could differ depending on the rootstock (Stempien *et al.*, 2020).

Besides the increasing intensity of agricultural land use, climate change also creates a need for improving rhizosphere ecosystems. Rhizosphere ecology and ecosystem function will be concerned by global climate change, including rising temperatures and disruptive weather patterns due to increasing levels of atmospheric CO₂ (Ahkami *et al.*, 2017). For instance, this will lead to more abiotic stresses like drought stress or flooding. This notion suggests that selection of both genotypes and species should be considered in ecosystem design and breeding programs (Ahkami *et al.*, 2017). Plant-specific microbiomes play an indisputable role in supporting plant health and adaptation to changing environmental conditions (Marasco *et al.*, 2018). Since viticulture already takes place in diverse environments, a variety of grapevine genotypes exists that can help in breeding to adapt vines to the changing climatic conditions (Brunori *et al.*, 2016).

RHIZOSPHERE ENGINEERING

It is important to note that impacting the rhizosphere via plant engineering can be a very complex process due to degradation or inactivation of the engineered compound in the soil, insufficient rate of exudation to influence the rhizosphere, limited knowledge about root exudates composition, and changes in exudate releasing time and level with plant development and external stimuli (Ryan *et al.*, 2009). Bioengineering of synthetic microbial communities for plant growth promotion, disease resistance, and stress tolerance or regulation presents a unique opportunity (Ahkami *et al.*, 2017). While hundreds of bacterial strains have been identified to have beneficial effects; engineering a sustainable synthetic

microbial community represents a significant challenge (Ahkami *et al.*, 2017). Microorganisms are a vital component of the rhizosphere, and the total biomass and composition of rhizosphere microbial populations markedly affect interactions between plants and the soil environment. There is considerable interest in developing methods for encouraging the proliferation of beneficial introduced or indigenous microbial populations that facilitate nutrient uptake (*e.g.*, rhizobia and mycorrhiza), promote plant growth directly, or suppress plant pathogens. Once the complexity of the rhizosphere is unravelled, one can attempt to create conditions most beneficial to plant growth by amending the soil, breeding or engineering better plants, and manipulating plant/microorganism interactions (Ryan *et al.*, 2009).

It has been shown that the grapevine microbiome can be shaped by various factors, like seasonality, plant genotype, age, pedo-climatic features, surrounding wild plants or presence of pathogens (Pacifico *et al.*, 2019). Furthermore, soil organisms can be impacted by management systems like organic, integrated or biodynamic farming (Hendgen *et al.*, 2018). The microbial community of vineyard soil is affected by various factors, like cover crop use, tillage, compost application, and management practices (Burns *et al.*, 2016, Figure 1C). It was shown that the bacterial community had a higher diversity in vineyard soils that were tilled less, biodynamically farmed, and had received compost application (Di Giacinto *et al.*, 2020; Burns *et al.*, 2016).

However, organic farming, in general, is considered to be a more sustainable form of agriculture (Brunori *et al.*, 2016). A study by Gattullo *et al.* (2020) showed that fescue (*Festuca arundinacea* Schreb.) cover crop strongly improves the soil microbiological and soil chemical properties of a table grape vineyard. Another study showed that an intercropping with subterranean clover (*Trifolium subterraneum* L.) improved grapevine plant performance (Contreras *et al.*, 2019).

However, there are more ways to engineer the rhizosphere. It is also possible to applicate specific fertiliser, nutrients and bioinoculants with bacteria and/or fungi (Figure 1A). In a study by Bach *et al.* (2016), the authors examined three bacteria (*Bacillus mycoides* B38V, *Burkholderia cepacia* 89, and *Paenibacillus riograndensis* SBR5) regarding their competitive characteristics *in vitro*. They showed that all three bacteria showed good rhizosphere competence through

hydrolytic enzyme production or antagonistic activities, so they might be tested as biocontrol agents against different plant diseases in future studies (Bach *et al.*, 2016). Figueroa-López *et al.* (2016) investigated three *Bacillus* strains from the rhizosphere of maize that showed antagonistic activity against *Fusarium verticillioides*. Furthermore, they suggest possible control mechanisms against these fungi by inoculating the *Bacillus* strains into the rhizosphere (Figueroa-López *et al.*, 2016).

Regarding grapevine, it has been discovered by Yacoub *et al.* (2016) that young vines can be protected by various inoculants of the oomycete *Pythium oligandrum* against a pathogen that is involved in the Esca disease complex. *P. oligandrum* is known as a biocontrol agent (reviewed by Brožová, 2002) and is available in different products for crop plants. It is also known that *Azotobacter* species including *A. vinelandii*, *A. beijerinckii*, *A. nigricans*, *A. salinestri* and *A. chroococcum* are used in various inoculants against plant pathogens and for nitrogen fixation (Giri *et al.*, 2019). Even in viticulture, a study by Trotel-Aziz *et al.* (2008) could show that some grapevine associated bacteria like *Acinetobacter lwoffii*, *Pseudomonas fluorescens*, *Pantoea agglomerans* and *Bacillus subtilis* can act as new biocontrol agents against *Botrytis cinerea*.

In a review by Compant *et al.* (2013) some beneficial bacteria and their secondary metabolites for controlling grapevine pathogen diseases are summed up. However, they concluded that there were not enough examples of biocontrol agents used for grapevine yet (Compant *et al.*, 2013). Yacoub *et al.* (2018) inoculated roots of Cabernet Sauvignon with *Pythium oligandrum*, a biocontrol agent. They concluded by transcriptomic analysis that several transcripts of vine induced defence systems against *P. oligandrum*. This study shows that inoculation with biocontrol agents could lead to changes in the whole plant root transcriptome due to a complex plant response (Yacoub *et al.*, 2018). In a further study, these authors showed that grapevine root treatment with *P. oligandrum* reduced wood necrosis (about 60 %) resulting from inoculation with *Neofusicoccum parvum* (Yacoub *et al.*, 2020). However, it is important to investigate the pathways within the plant to use it beneficially. In another study, Carro-Huerga *et al.* (2020) showed that inoculation with a *Trichoderma* strain biocontrol agent can protect the vine from *Phaeoacremonium minimum*, known as a pioneer fungus involved in

Grapevine Trunk Diseases (GTD) like Esca. *Trichoderma* spp. is known to have direct effects on GTD pathogens as a biocontrol agent, by activation of host defence genes, therefore, many formulations based on *Trichoderma* spp. strains have been already assessed for protection (Stempien *et al.*, 2020; Yacoub *et al.*, 2020). Stempien *et al.* (2020) showed the colonisation of *Trichoderma* spp. to three different tested rootstock cultivars, but activation of host defence was cultivar dependent. Thus, the use of *Trichoderma* spp. for grapevine root application needs to be studied for more rootstocks under field conditions (Stempien *et al.*, 2020). González-García *et al.* (2019) showed that *Streptomyces* sp. strains isolated from grapevine roots can be readily introduced into young grapevine plants by both immersion and injection into the rootstock as potential biocontrol agents to control fungal pathogens causing young grapevine decline (YGD). Additionally, Andreolli *et al.* (2021) examined inhibitory effects from the rhizospheric and plant growth-promoting strain *Pseudomonas protegens* MP12 toward different grapevine phytopathogens via soil inoculation when the vines were planted in the nursery. However, further studies are required to evaluate these results under field conditions (Andreolli *et al.*, 2021).

Furthermore, it was already shown by different studies that arbuscular mycorrhizas (AM) fungal inoculation enhanced the growth of grapevine rootstocks (Aguín *et al.*, 2004; Schreiner, 2003). In a study carried out by Velásquez *et al.* (2020), it was shown that the arbuscular mycorrhizal fungus *Funneliformis mosseae* IN101 and the plant growth-promoting rhizobacterium *Ensifer meliloti* TSA41 promoted the growth of vine plants, as both single inoculants and co-inoculants, increasing plant height and total dry weight. However, it would be more effective to produce mycorrhizal plants at the nursery than inoculate them in the field (Aguín *et al.*, 2004), as root tissues already colonised with AM could directly promote vine growth in the vineyard after planting out (Giri *et al.*, 2019; Aguín *et al.*, 2004). This could be a promising new technology to reduce the incidence of grapevine fungal infections through the root system (Álvarez-Pérez *et al.*, 2017).

However, it is not only important to have knowledge about the interaction between the plant and the beneficial microorganism but also to understand the colonisation and modulation of the resident microbiome (Compant *et al.*, 2019; Compant *et al.*, 2010).

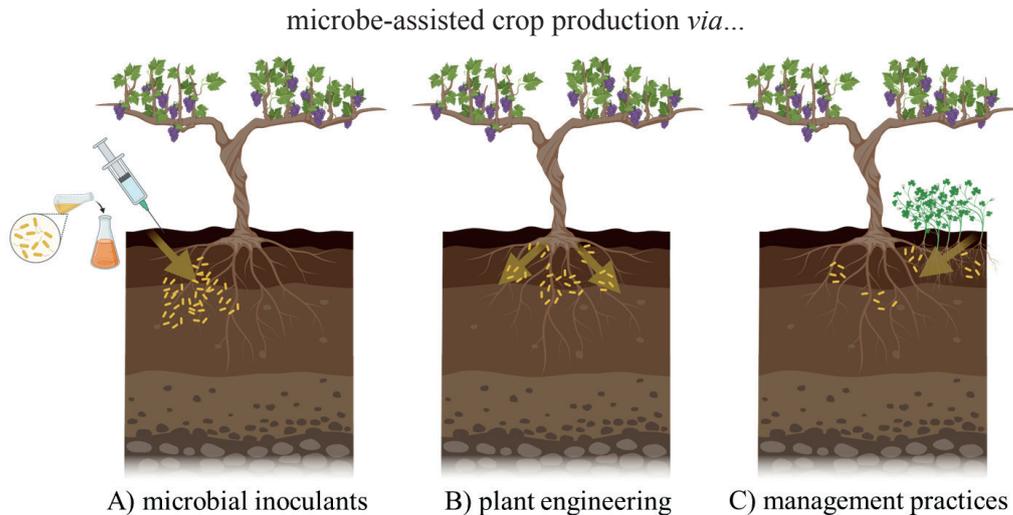


FIGURE 1. Microbe-assisted crop production via different methods through the rhizosphere. A) Increasing beneficial microorganisms via microbial inoculants. B) Enhancing beneficial microorganisms through plant breeding or plant engineering. C) Stimulating beneficial microorganisms through management practices like cover cropping or tillage. Created with BioRender.com. Adapted from Hohmann *et al.* (2020).

Only after successful colonisation of added inoculants, the plant health or growth will be improved (Compant *et al.*, 2010). However, applications of bioinoculants or biocontrol agents in the field often failed. In addition, the success of such organisms depends on the target crop, product availability, costs, application options and environmental conditions (Giri *et al.*, 2019). Therefore it is important to investigate in future studies how the sole usage of bacterial inoculants can improve soil quality and enhance plant health (Giri *et al.*, 2019).

CONCLUSION—WHAT’S NEXT?

To conclude, the emerging field of rhizosphere and ecosystem engineering is more than a promising way leading towards more sustainable viticulture. In future, it may be possible to conserve and restore soil microbial diversity and to support beneficial organisms exposed to intensive agricultural practices (Gu *et al.*, 2020). It should be noted, however, that finding beneficial microorganisms does not lead instantaneously to the holy grail of bio-inoculations. Many different factors need to be considered in future research (Figure 2).

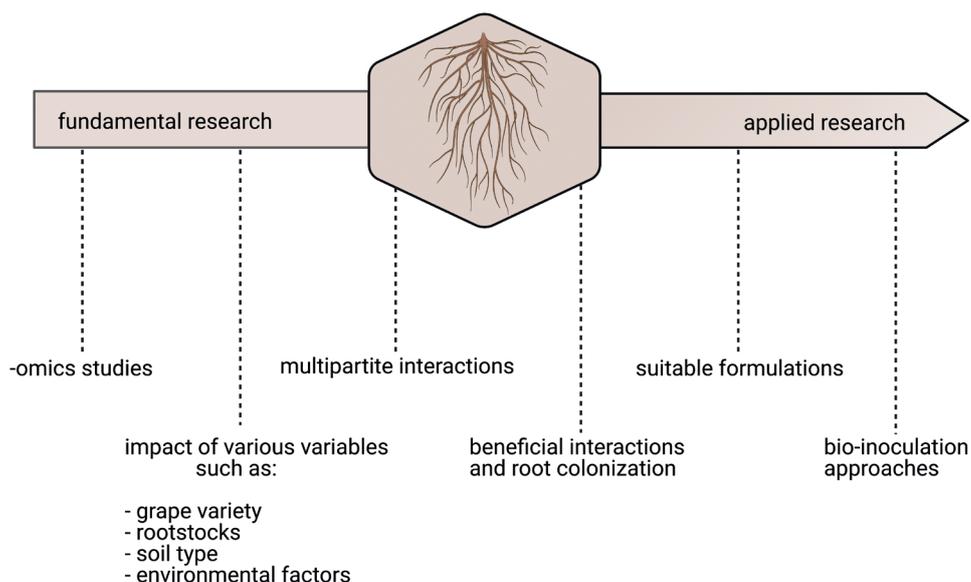


FIGURE 2. Steps to be considered in further studies. Several steps need to be considered that lead to more sustainable viticulture using rhizosphere engineering. Created with BioRender.com.

To achieve sustainable viticulture, even stronger and deeper identification and characterisation of (beneficial) microorganisms on grapevine roots is needed. Using a metabarcoding or a metagenomics approach, the microbial composition can be identified without time-consuming cultivation, leading to a better understanding of the microbiome of vineyard soil and rhizosphere. It is particularly important to consider the influence of different variables such as rootstock variations, plant genotype, and grape varieties. In addition, the impact of factors such as soil type, environmental conditions, climate change, management practices, and fertilisation levels should also be considered. To achieve this research objective, other multi-omics approaches should be taken into account. Metaproteomics is of great interest, especially to engineer the rhizosphere by altering the microbial community and/or the resident plants in response to environmental stress, and climate change to determine how the rhizosphere may respond to targeted engineering interventions to enhance its capabilities (Tartaglia *et al.*, 2020). Moreover, these results can be used for further studies of multipartite interactions between rhizosphere microorganisms and vines.

In the next step, the investigation of the beneficial mode of plant-microbe interaction needs to be elucidated. In addition, the colonisation patterns on the root surface are important to study. Only through understanding these two mechanisms, it is possible to modulate or optimise the cross-talk of plant roots and rhizosphere microbiota.

Final, suitable formulations of beneficial microorganisms for bio-inoculation need to be developed for viticulture. Some approaches for using biocontrol agents in viticulture have already been summarised in this review. However, further trials in bio-inoculation might be more effective when the interactions between the vine and its microbiome are studied more intensively.

Achieving these steps is an important task to reduce the application of pesticides or fertilisers and to improve soil quality and crop productivity (Dessaux *et al.*, 2016). All new findings of beneficial organisms or methods for rhizosphere engineering and the use of microbial inoculants as biocontrol agents offer the potential for reaching more sustainable viticulture.

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