

Hochschule Geisenheim University
and
Justus Liebig University Giessen
Faculty 09 - Agricultural Sciences, Nutritional Sciences,
and Environmental Management

Microbial structure and diversity in the rhizosphere of grape vines (*Vitis vinifera* L.)

Submitted in fulfilment of the requirements for the degree
Doktorin der Agrarwissenschaften (Dr. agr.)

Submitted by

Leonie Dries, M. Sc.

Born on February 4, 1994

in Rüdesheim am Rhein, Germany

Geisenheim, December 2022

This thesis was accepted on 21st December 2023.

as a doctoral dissertation in fulfilment of the requirements for the degree

“Doktorin der Agrarwissenschaften”

by Hochschule Geisenheim University and Justus Liebig University Giessen

Examination Committee:

Supervisor and Reviewer: Prof. Dr. Otmar Löhnertz

2nd Reviewer: Prof. Dr. Sylvia Schnell

3rd Reviewer: Prof. Dr. Tillmann Lüders

1st Examiner: Prof. Dr. Christoph Müller

2nd Examiner: Prof. Dr. Kai Voss-Fels

Head of the Committee: Prof. Dr. Ilona Leyer

Statutory declaration

“I declare that this thesis has been composed solely by myself, without the unpermitted aid of others, except where explicitly stated in the text. All text passages taken in letter or in spirit from published writings and all information based on oral statements are clearly referenced as such.

I have adhered to the principles of good scientific practice as laid down in the Statutes of Hochschule Geisenheim University and Justus-Liebig-Universität Giessen to ensure good scientific practice in the studies I have carried out and which are referred to as such in the doctoral thesis.”

§ 16 (2) Doctoral regulations Hochschule Geisenheim University (2018)

Geisenheim, 20.12.2022

Leonie Dries

“When the wind's against you
Remember this insight
That's the optimal condition
For birds to take flight”

Enter Shikari

Table of contents

List of Abbreviations	VI
1. Summary.....	1
1.1 Zusammenfassung	1
1.2 Summary	3
1.3 Author Contribution.....	5
1.3.1 List of publications.....	7
2. Introduction	9
2.1 Soil – the fundamental of our life	11
2.1.1 The importance of soil health.....	12
2.1.2 The soil microbial community	13
2.2 Digging deeper – the rhizosphere	15
2.3 Grapevine and its microbiome	20
2.4 Aim of this study.....	23
2.5 References.....	25
Chapter 1 – Rhizosphere engineering: leading towards a sustainable viticulture?	36
Chapter 2 – Rootstocks Shape Their Microbiome - Bacterial Communities in the Rhizosphere of Different Grapevine Rootstocks.....	48
Chapter 3 – The metabolic active bacterial communities of grapevine rhizosphere in dependence on rootstock and scion variety	61
Chapter 4 - The metabolic active bacterial communities in the rhizosphere of grapevine (<i>Vitis vinifera</i> L. cv. Pinot Noir) are affected by different nitrogen fertilization levels and sampling depths.....	87
3. Discussion.....	115
References.....	125
Acknowledgements	133

List of Abbreviations

%	percentage
°C	degree Celsius
~	approximately
*	significant
&	and
ACC	Acetyl-CoA-Carboxylase
ALDEx2	ANOVA-Like Differential Expression
ASV	amplicon sequence variant
bp	base pair
C	carbon
cDNA	complementary DNA
Cl ⁻	chlorine
clr	center log ratio
cm	centimetre
CO ₂	carbon dioxide
cv.	cultivar
DNA	deoxyribonucleic acid
DWD	Deutscher Wetterdienst
et al.	et alia
FDW	Forschungsring des Deutschen Weinbaus
Fe	iron
Fox	<i>Fusarium oxysporum</i>
GmbH	Gesellschaft mit beschränkter Haftung
ha	hectares
i.e.	lat. <i>id est</i>
IR	induced resistance
ISR	induced systemic resistance

K	potassium
kg	kilograms
kg/ha	kilograms per hectares
LDA	linear discriminant analysis
LEfSe	Linear discriminant analysis Effect Size
mg	milligramm
min	minute
mm	millimetre
mM	millimolar
mRNA	messenger ribonucleic acid
N	nitrogen
NGS	next generation sequencing
NH ₄	ammonium
NO ₃	nitrate
ns	not significant
O ₂	oxygen
OIV	International Organization of Vine and Wine
P	phosphorus
PCoA	Principal Coordinate Analysis
PCR	polymerase chain reaction
PERMANOVA	Permutational Multivariate Analysis of Variance
PERMDISP	Permutational Multivariate Analysis of Dispersion
PGPB	plant growth promoting bacteria
PGB(R)B	plant growth promoting (rhizo)bacteria
pH	lat. <i>pondus hydrogenii</i> or <i>potentia hydrogenii</i>
PSB	P-solubilizing bacteria
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
S	sulphur

spec.	species
sp.	species
spp.	species
tRNA	transfer ribonucleic acid
VDLUFA	Verband deutscher landwirtschaftlicher Untersuchungs- und Forschungsanstalten e. V.
vs	versus
Zn	zinc

1. Summary

1.1 Zusammenfassung

Mikroorganismen sind ein wesentlicher Bestandteil der Rhizosphäre und die Aktivität und Zusammensetzung der mikrobiellen Populationen in der Rhizosphäre beeinflussen die Wechselwirkungen zwischen Pflanzen und dem Boden erheblich. Darüber hinaus können die Mikroorganismen in der Rhizosphäre die Entwicklung, das Wachstum und die Vitalität der Pflanzen positiv beeinflussen. In Weinbergen beeinflussen Bewirtschaftungspraktiken sowohl das Wurzelwachstum der Reben als auch die Mikroorganismen der Rhizosphäre direkt, aber die genaue Wirkungsweise ist weitgehend unbekannt. In jüngster Zeit rücken jedoch zwei neue Forschungsansätze zur Verbesserung des Wachstums und der Gesundheit der Weinrebe immer mehr in den Mittelpunkt: das „Plant Engineering“ und das „Rhizosphere Engineering“ (Kapitel 1). Beim „Plant Engineering“ wird das Wissen über die Wechselwirkungen zwischen Pflanze und Mikrobiom für Pflanzenzüchtungsstrategien genutzt. Beim „Rhizosphären-Engineering“ werden mikrobielle Gemeinschaften durch die Zugabe spezifischer Düngemittel, Nährstoffe oder durch Bioinokulation mit bestimmten Bakterien und/oder Pilzen verändert. Zusammengenommen bieten diese neuen Methoden ein Potenzial für eine nachhaltigere Entwicklung eines pestizid- und düngerreduzierten Weinbaus in der Zukunft.

Ziel dieser Studie war es daher, das bakterielle Rhizobiom von Weinreben (*Vitis vinifera* L.) in verschiedenen Versuchsanordnungen zu untersuchen. Durch die Charakterisierung des bakteriellen 16S rRNA-Gens kann eine Aussage über die vorhandenen bakteriellen Gemeinschaften und deren Diversität getroffen und unter verschiedenen Umweltbedingungen verglichen werden. Dazu wurde zunächst eine Methode etabliert, die es erlaubt, Rhizosphären- und Wurzelmaterial von Weinrebenpflanzen zu gewinnen und für die Sequenzierung und weitere Analysen aufzubereiten. Dies gilt sowohl für Weinreben, die in Gewächshäusern unter kontrollierten Bedingungen angebaut werden, als auch für Varianten unter Freilandbedingungen. Die Versuchspläne zielten darauf ab, Erkenntnisse über die bakterielle Diversität der Rhizosphäre von Weinreben unter Einbeziehung verschiedener Rebsorten, verschiedener Unterlagen sowie verschiedener Düngungsmaßnahmen zu gewinnen.

Das erste Experiment verfolgte das Ziel, die Variabilität des Rhizosphärenmikrobioms zu untersuchen, die sich durch den Genotyp des Wurzelstocks ergibt und gleichzeitig den Einfluss

aller anderen Umweltfaktoren zu minimieren. Dies wurde durch die Untersuchung von vier ungepfropften Rebenunterlagen gleichen Alters ermöglicht, die auf demselben Boden unter denselben klimatischen Bedingungen angebaut und identisch bewirtschaftet wurden. Die Bakteriengemeinschaften, die mit der Rhizosphäre der Unterlagen 1103 Paulsen, 140 Ruggeri, 161-49 Couderc und Kober 5BB assoziiert sind, wurden mit der ampliconbasierten Sequenzierungstechnik charakterisiert, die auf die Regionen V4-V5 des 16S rDNA-Gens abzielt. Die vier Unterlagsreben wiesen Ähnlichkeiten in Bezug auf die Struktur der bakteriellen Assemblage (richness und evenness) auf. Dennoch wurden Unterschiede in der Zusammensetzung der bakteriellen Gemeinschaften festgestellt. Tatsächlich rekrutierten alle untersuchten Unterlagen Gemeinschaften mit unterscheidbaren Merkmalen, was die Rolle des Genotyps der Unterlage als treibende Kraft für die Bakterienzusammensetzung bestätigt (Kapitel 2).

Um die Auswirkungen von Edelreis und Unterlage zu entflechten, wurde die rRNA (V4-V5-Region der 16S rRNA) aus der Rhizosphäre der Rebsorten Riesling und Müller-Thurgau, ungepfropft bzw. auf verschiedene Unterlagsreben gepfropft, in drei Versuchsanordnungen in zwei verschiedenen Jahren sequenziert. Die bioinformatische Analyse mit Werkzeugen, die auf die Robustheit von Kompositionsdaten ausgelegt sind, zeigte, dass die untersuchten Unterlagen oder Edelreiser bzw. Kombinationen bakterielle Gemeinschaften mit unterscheidbaren Merkmalen rekrutierten. Statistische Unterschiede wurden zwischen unveredeltem Riesling und Müller-Thurgau, zwischen veredeltem Riesling und unveredeltem Riesling sowie zwischen unveredeltem Müller-Thurgau und veredeltem Müller-Thurgau festgestellt. Damit wurde die Rolle des Genotyps von Edelreiser und Unterlagen als Einflussfaktor für die Struktur und Zusammensetzung der Bakteriengemeinschaften in der Rhizosphäre von Reben bestätigt (Kapitel 3).

Um die Auswirkungen unterschiedlicher Stickstoffdüngungen auf das Mikrobiom der Rhizosphäre zu untersuchen, wurde 2020 und 2021 ein experimenteller Weinberg mit Reben (*Vitis vinifera* L. cv. Pinot Noir) in Großcontainern zum Zeitpunkt des Austriebs untersucht. Die Reben wurden mit zwei unterschiedlichen Stickstoffgaben pro Jahr (N0 25 kg/ha und N1 75 kg/ha) individuell gedüngt. Zur Untersuchung der jeweiligen Bakteriengemeinschaften wurde Rhizosphärenmaterial entnommen und anschließend eine RNA-Extraktion mit anschließender Sequenzierung zur Charakterisierung des aktiven Bakterioms durchgeführt. Die

Studie ergab signifikante Unterschiede in den bakteriellen Gemeinschaften in der Rhizosphäre in Abhängigkeit von der Düngemenge und der Beprobungstiefe. Die Ergebnisse könnten dazu beitragen, die Wirkung der Stickstoffdüngung auf das Mikrobiom zu verstehen und langfristig die Düngung im Weinbau zu verringern, was zu einer nachhaltigeren Bewirtschaftung der Weinreben führen würde (Kapitel 4).

Die Ergebnisse dieser Studien können übergeordnet dabei helfen, mit den neuen Erkenntnissen eine gezieltere Pflanzenernährung in Form von genaueren Düngeempfehlungen zu ermöglichen, um die damit verbundenen Kosten auf wirtschaftliche und umweltfreundliche Weise zu senken. Eine genauere Kenntnis der Spezifität und Dynamik des Mikrobioms in der Rhizosphäre der Weinrebe unter verschiedenen Bewirtschaftungspraktiken, Rebsorten, Unterlagen und Düngungsniveaus sollte gezielte Anpassungsstrategien für immer häufiger auftretende Extremjahre ermöglichen und zu einem nachhaltigeren Weinbau führen.

1.2 Summary

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. 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 the grapevine growth and health: plant engineering and rhizosphere engineering (Chapter 1). In plant engineering, knowledge about plant-microbiome interactions is used for plant breeding strategies. In rhizosphere engineering, microbial communities are modified by adding specific fertilizers, 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 a pesticide- and fertilizer-reduced viticulture in the future.

Consequently, the aim of this study was to investigate the bacterial rhizobiome of grapevines (*Vitis vinifera* L.) under different experimental designs. By characterizing the bacterial 16S rRNA gene, a statement on the existing bacterial communities and their diversity can be made and compared under different environmental conditions. For this, a method has to be established

that allows to obtain rhizosphere and root material of grapevine plants, and to prepare it for sequencing and further analyses. This applies both to grapevines cultivated in greenhouses under controlled conditions as well as to variants under field conditions. The experimental designs aimed to gain knowledge regarding the bacterial diversity of the grapevine rhizosphere including different grape varieties, different grapevine rootstocks as well as different fertilization measures.

The first experiment aimed to investigate the variability of the rhizosphere microbiome resulting from the genotype of the rootstock while minimizing the effect of all other environmental factors. This was made possible by investigating four ungrafted grapevine rootstock varieties of the same age, grown on the same soil under the same climatic conditions and managed identically. The bacterial communities associated with the rhizosphere of the rootstocks 1103 Paulsen, 140 Ruggeri, 161-49 Couderc, and Kober 5BB were characterized with the amplicon based sequencing technique, targeting regions V4-V5 of 16S rDNA gene. The four rootstocks showed similarities concerning the structure of the bacteria assemblage (richness and evenness). Nonetheless, differences were detected in the composition of the bacterial communities. Indeed, all investigated rootstocks recruited communities with distinguishable traits, thus confirming the role of rootstock genotype as driver of the bacteria composition (Chapter 2).

In order to disentangle the effect of scion and rootstock, the rRNA (V4–V5 region of 16S rRNA) extracted from the rhizosphere of the grape varieties Riesling and Mueller-Thurgau ungrafted vs. grafted on different rootstocks were sequenced in three experimental set-ups in two different years. The bioinformatic analysis with tools designed to be robust for compositional data showed, that the investigated rootstocks or scions or combinations, respectively, recruited bacterial communities with distinguishable traits. Statistical differences were revealed between ungrafted Riesling and Mueller-Thurgau, and between grafted Riesling and ungrafted Riesling, and ungrafted Mueller-Thurgau and grafted Mueller-Thurgau. Thus, confirming the role of scion and rootstock genotype as a driver of the structure and composition of bacterial communities in the rhizosphere of grapevines (Chapter 3).

In order to investigate the impact of different levels of nitrogen (N) fertilization on the rhizosphere microbiome an experimental vineyard with vines (*Vitis vinifera* L. cv. Pinot Noir) in large containers was examined in 2020 and 2021 at bud break. The vines were fertilized with

two different levels of nitrogen added per year (N0 25 kg/ha and N1 75 kg/ha) individually. In order to examine the respective bacterial communities, rhizosphere material was sampled and a subsequent RNA-extraction followed by next generation sequencing was performed. The study revealed significant differences in the bacterial communities in the rhizosphere regarding fertilization levels and sampling depths. The results may help to understand the effect of nitrogen fertilization on the microbiome and, in the long term, reduce fertilization in viticulture, leading to more sustainable vine management (Chapter 4).

Hence, superordinate, the future aim is to use new insights to enable more precise plant nutrition in the form of more accurate fertilization recommendations in order to reduce the associated costs in an economical and environmentally friendly way. A more precise knowledge of the specificity and dynamics of the grapevine rhizosphere microbiome under different management practices, grape varieties, rootstocks, and fertilization levels, should enable targeted adaptation strategies for increasingly frequent extreme years leading to a more sustainable viticulture.

1.3 Author Contribution

Chapter 1 is based on the following peer-reviewed publication:

Dries, Leonie; Hendgen, Maximilian; Schnell, Sylvia; Löhnertz, Otmar; Vortkamp, Anne (2021): Rhizosphere engineering: leading towards a sustainable viticulture? In *OENO One* 55 (2), pp. 353–363. DOI: 10.20870/oeno-one.2021.55.2.4534.

Leonie Dries carried out literature research and writing. Maximilian Hendgen, Sylvia Schnell, Otmar Löhnertz and Anne Vortkamp contributed to writing. Otmar Löhnertz and Anne Vortkamp coordinated the project. All authors gave final approval for publication.

Chapter 2 is based on the following peer-reviewed publication:

Dries, Leonie, Bussotti, Simone, Pozzi, Carlo, Kunz, Robert, Schnell, Sylvia, Löhnertz, Otmar, Vortkamp, Anne (2021). Rootstocks Shape Their Microbiome-Bacterial Communities in the

Rhizosphere of Different Grapevine Rootstocks. *Microorganisms*, 9(4).
<https://doi.org/10.3390/microorganisms9040822>

Conceptualization of the study was done by Leonie Dries, Anne Vortkamp and Simone Bussotti, methodology was carried out by Leonie Dries, Simone Bussotti, and Robert Kunz. Leonie Dries led the writing and statistical analyses, and supervised the laboratory work. Otmar Löhnertz and Anne Vortkamp and Sylvia Schnell coordinated the project, review, and editing. All authors gave final approval for publication. Leonie Dries and Simone Bussotti share the first authorship.

Chapter 3 is based on the following manuscript, submitted to a peer-reviewed journal:

Dries, Leonie, Ratering, Stefan, Bussotti, Simone, Löhnertz, Otmar, Vortkamp, Anne, Schnell, Sylvia. The metabolic active bacterial communities of grapevine rhizosphere in dependence on rootstock and scion variety. Submitted to *OENO One* (Chapter 3)

Leonie Dries carried out sample taking, laboratory work, statistical analyses, and writing. Stefan Ratering contributed to statistical analyses. Simone Bussotti assisted in sample taking. Otmar Löhnertz, Anne Vortkamp and Sylvia Schnell coordinated the design of the study, and editing. All authors gave final approval for publication.

Chapter 4 is based on the following manuscript:

Dries, Leonie et al. The metabolic active bacterial communities in the rhizosphere of grapevine (*Vitis vinifera* L. cv. Pinot Noir) are affected by different nitrogen fertilization levels and sampling depths. (Chapter 4)

Leonie Dries carried out sample taking, laboratory work, statistical analyses, and writing. Stefan Ratering contributed to statistical analyses. Otmar Löhnertz, Anne Vortkamp and Sylvia Schnell coordinated the design of the study.

1.3.1 List of publications

International peer reviewed scientific publications

Dries, L., Hendgen, M., Schnell, S., Löhnertz, O., & Vortkamp, A. (2021). Rhizosphere engineering: leading towards a sustainable viticulture? *OENO One*, 55(2), 353–363. (Chapter 1)

Dries, L., Bussotti, S., Pozzi, C., Kunz, R., Schnell, S., Löhnertz, O., & Vortkamp, A. (2021). Rootstocks Shape Their Microbiome - Bacterial Communities in the Rhizosphere of Different Grapevine Rootstocks. *Microorganisms*, 9(4). (Chapter 2)

Submitted publication

Dries, L., Ratering, S., Bussotti, S., Löhnertz, O., Vortkamp, A., & Schnell, S. The metabolic active bacterial communities of grapevine rhizosphere in dependence on rootstock and scion variety. *Submitted to OENO One* (Chapter 3)

Publication in preparation

Dries, L. et al. The metabolic active bacterial communities in the rhizosphere of grapevine (*Vitis vinifera* L. cv. Pinot Noir) are affected by different nitrogen fertilization levels and sampling depths. (Chapter 4)

Other publications

Dries, L., Soethe, N., Nehring, E., Wu, W., Shahzad, W., Xue, C., & Geilfus, C-M. MgSO₄-leaf application stabilizes yield of sweet pepper (*Capsicum* L.) under aphid (*Myzus persicae*) attack: a strategy to reduce use of pesticides. *Submitted to Plants People Planet*

Dries, L., Franzisky, B. L., Becker, C., Mueller, R. C., Goettmann, J., & Geilfus, C-M. Facing upcoming challenges in viticulture: The potential key role of root exudates. *Submitted to Plant and Soil*

Dries, L. (2022): Rund um die Wurzel. Der deutsche Weinbau (6) S. 20 - 22. *Non peer-reviewed publication.*

Chapters 1-4 have all been submitted to peer-reviewed, international, scientific journals and have either been published or accepted for publication, or are currently under review, or in preparation for submitting. I am the first author or shared first author of all publications. I designed and planned the experimental designs with my supervisors, took the samples and conducted sample preparation for sequencing. I performed the bioinformatics analysis for all chapters. Finally, I wrote the manuscripts. My co-authors supported with sample taking or sample preparation, funding, and reviewing or editing the manuscripts. The publications and manuscripts represent self-contained units that can be read independently. This may result in some redundancy of content between the chapters, and/or between the chapters and parts of the dissertation. The supplemental material of Chapter 2 is available online at <https://www.mdpi.com/article/10.3390/microorganisms9040822/s1>, Table S1: Amplicon Sequenced Variants Table. The supplemental material of Chapter 3 and 4 is not available in the printed version of the dissertation, since the high amount of excel tables. The supplemental material of these two chapters can be found in the digital submission of the dissertation or upon request to Leonie Dries.

2. Introduction

Rising temperatures, drought and extreme weather phenomena are just some of the effects of climate change in progress. During the last years, the annual mean temperatures in Germany, and also in Geisenheim (Figure 1), have increased rapidly, and an end of this increase is currently hardly assessable. Geisenheim is located in the middle of the wine-growing region Rheingau with approximately 3200 ha vineyards, where primarily the grape variety Riesling is cultivated (Deutsches Weininstitut GmbH 2019). Grapevines (*Vitis vinifera* L.) are highly sensitive to climatic changes, especially with regard to temperatures. This makes them particularly interesting for forecasting the consequences of climate change and the corresponding development of adaptation strategies. Furthermore, grapevines are one of the economically most important fruit crops worldwide (Álvarez-Pérez et al. 2017; Andreolli et al. 2016) mainly used for wine production. 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 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 such as *Plasmopara viticola* and *Botrytis cinerea* (Lakkis et al. 2019; Yamamoto et al. 2000).

In addition, in vineyards, grapevines are cultivated for very long periods of time. The grapevines remain in the vineyard for at least 25 to 30 years and are usually replanted when the plant becomes over-aged, with no change of area. The vineyard ecosystem is often characterized by dry, sunny slopes with soils rich in skeletal nutrients and poor in water, with potentially high mineralization rates and a specially adapted animal and plant community. In this context, cultivation takes place on soils with an extreme range of variation in terms of biological, physical and chemical properties of the soil. Thus, viticulture is confronted with several challenges: Rising temperatures, higher risk of sunburn, drought stress, extreme weather

conditions and pathogenic pressure during increasing bans on pesticides or fungicides, respectively. Moreover, climate change has an impact on the ripening phase, the yeast-assimilable nitrogen (YAN, Bell and Henschke 2005) and thus a change in the aroma profile (Pons et al. 2017). Thus, the need is high for alternative methods to be able to carry out winegrowing and viticulture in the future. Also, the challenges of maintaining and promoting soil health is a concern for viticulture.

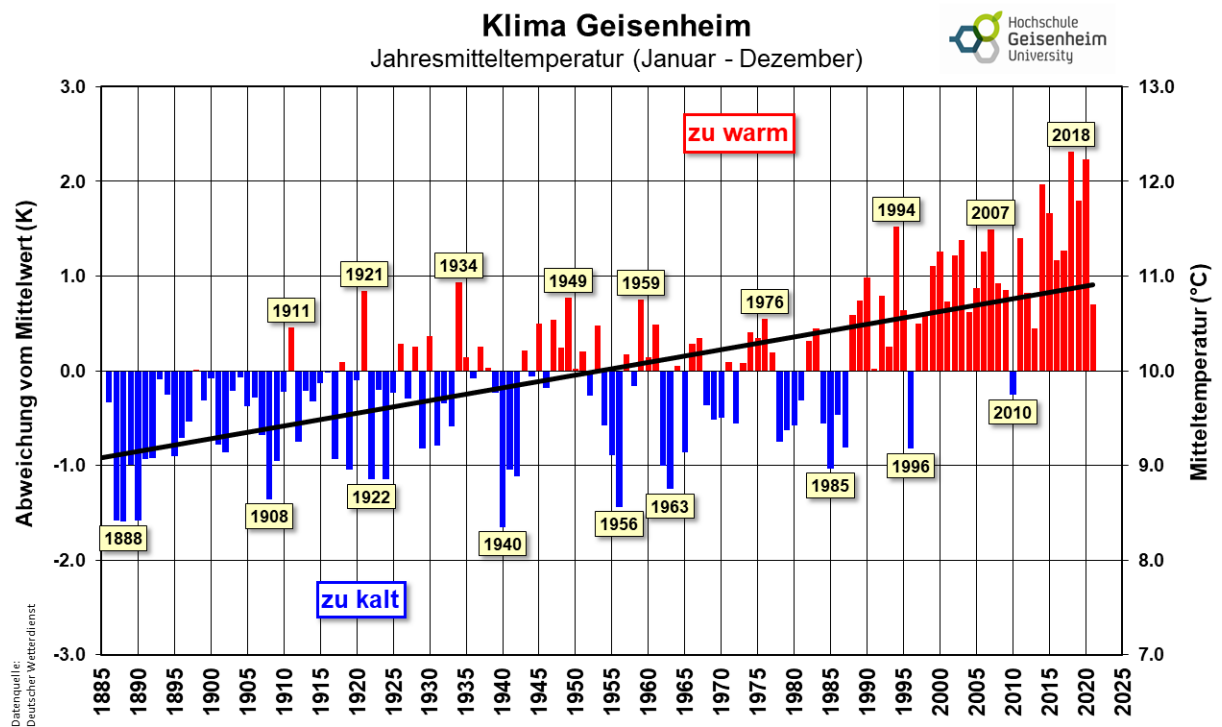


Figure 1. Annual mean temperatures in °C in Geisenheim from 1885 to 2021. Blue bars indicate lower temperatures; red bars indicate higher temperatures compared to the yearly average temperature (Source: Deutscher Wetterdienst (DWD)).

2.1 Soil – the fundamental of our life

“Land, then, is not merely soil;

it is a fountain of energy flowing through a circuit of soils, plants, and animals.”

Aldo Leopold, A Sand County Almanac, 1949

Soil is defined as a transformation product of mineral and organic substances of the outer earth crust interspersed with water, air and living organisms with its own morphological organization. The totality of soils forms the pedosphere and soil formation (pedogenesis) refers to the process by which soils are formed which is influenced by six factors: climate, parent material, time, relief, organics, and humans.

However, soil as the fundament of terrestrial life provides various essential functions. This includes, for example, control functions such as storage, filtering, buffering, and transformer functions, the basis of life for microorganisms, plants, animals and humans, the basis for agricultural use, raw materials, and areas for settlement and traffic as well as historical functions as a component of landscapes and as an archive of landscape history. For this reason, soil is considered to be particularly worthy of protection, which has been specified in the ‘Bundesbodenschutzgesetz’ since 1998. This states, that the sustainable safeguarding of soil fertility and the performance of the soil as a natural resource must be ensured through agricultural use. Consequently, the importance of soil for global food and nutrition security, the agroecosystem, the environment, and human life has exponentially shifted research questions toward soil health.

2.1.1 The importance of soil health

A healthy, diverse and active soil life is of great importance. According to the European Commission (European Commission 2021) soils are considered healthy when they are in good chemical, biological and physical condition and are thus able to permanently provide as many of the following ecosystem services on a permanent basis:

- provide food and biomass production, including agriculture and forestry;
- absorb, store and filter water and transform nutrients and substances, thus protecting groundwater bodies;
- provide the basis for life and biodiversity, including habitats, species and genes;
- act as a carbon reservoir;
- provide a physical platform and cultural services for humans and their activities;
- act as a source of raw materials;
- constitute an archive of geological, geomorphological and archaeological heritage.

Hence, basing on the above, soil health is the ability of soil to provide a range of ecosystem and agricultural functions and services. This implies, the maintenance and development of soil health and fertility is the key factor for our agricultural productivity. The productivity of agricultural systems is greatly dependent on the functional processes of the soil microbiome (as reviewed by Pankhurst et al. 1996).

All ecosystem services mentioned before as well as maintaining soil health are also relevant for vineyard soils. In vineyard soils, one of the main issues regarding soil health is the problem of nitrate leaching, and they are often considered as an important driver for nitrate contamination of groundwater. The reason for this is the special type of viticultural management. A high nitrogen pollution potential result, for instance, from the short vegetation period of about 150-180 days (May to October), a late start to vegetation (late April/early May), the low nutrient efficiency of grapevines, drought stress problems, or high mineralization rates in case of repeated tillage (Nendel and Kersebaum, 2004). The mineralization of nitrogen and the nitrogen cycle is regulated completely by soil microorganisms (as reviewed by Jetten 2008). Therefore, for maintaining soil health and agricultural services, it is not only important to consider the physical and chemical properties of the soil, but also the soil biology, and, in specifically, the soil microorganisms.

2.1.2 The soil microbial community

Numerous biotic and abiotic processes take place in the soil, which are linked to key ecosystem functions such as the cycling of nitrogen and other nutrients (Köberl et al. 2020). To provide such soil functions, diverse and active soil microorganisms are required (Di Giacinto et al. 2020). For analysis of the soil bacterial community molecular methods are used which include DNA or RNA extractions from the soil samples and for taxonomic structure analysis the 16S RNA gene is amplified and sequenced in a metabarcode approach. Bioinformatic tools can be used to determine the taxonomic affiliation and frequency of the bacteria as well as diversity parameters for the sequence data. The results can be summarized as microbiome data which can be completed by sequencing of the total DNA (functional metagenomics) or the expressed RNA (rRNA, mRNA and tRNA for transcriptomics). The results of soil microbiome studies are often linked to soil health and agricultural productivity. The closer examination of the microbial soil life is increasingly becoming the focus of international research, especially in view of climate change and the changing environmental conditions in this context (Figure 2A). The microbiome, which comprises all microorganisms, including fungi and bacteria, is central to a healthy soil life and plays a major role in the vitality of the plants being cultivated. The positive effects of soil microorganisms on plant health and the associated mechanisms of action have already been presented in many reviews (for example in Babalola 2010; Lugtenberg and Kamilova 2009; Trivedi et al. 2020; Nazli et al. 2020).

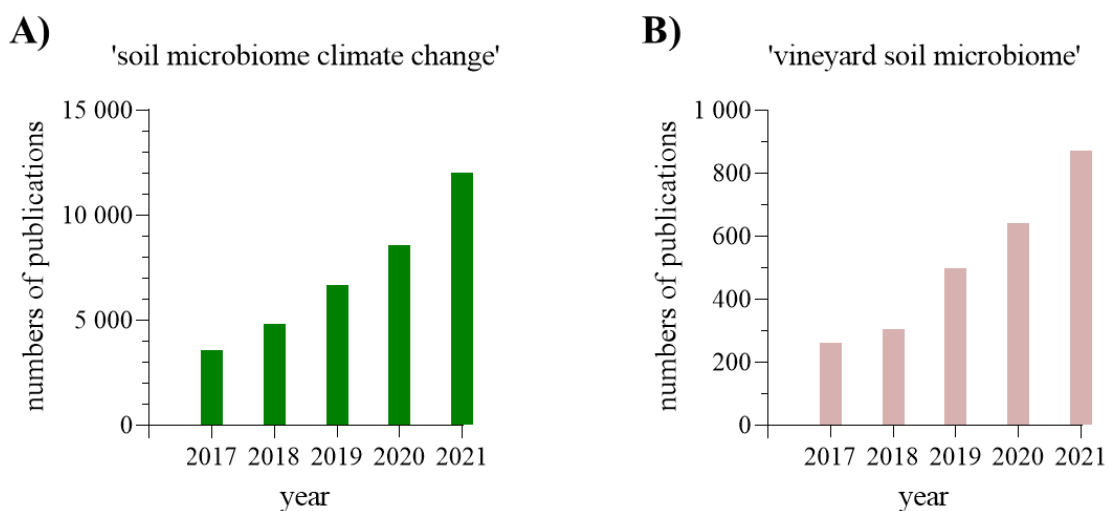


Figure 2. Number of published articles per year in google scholar for A) key word ‘soil microbiome climate change’ and B) key word ‘vineyard soil microbiome’.

The composition of bacterial communities in soils are shaped by different environmental factors such as soil pH, temperatures, or soil texture (Figure 3, as reviewed by Fierer et al. 2007). In addition, Wang et al. (2019) investigated soil pH affecting the bacterial communities in agricultural soil. Tan et al. (2020) discovered mean annual precipitation and soil pH as the major environmental factors shaping soil bacterial communities in maize soils.

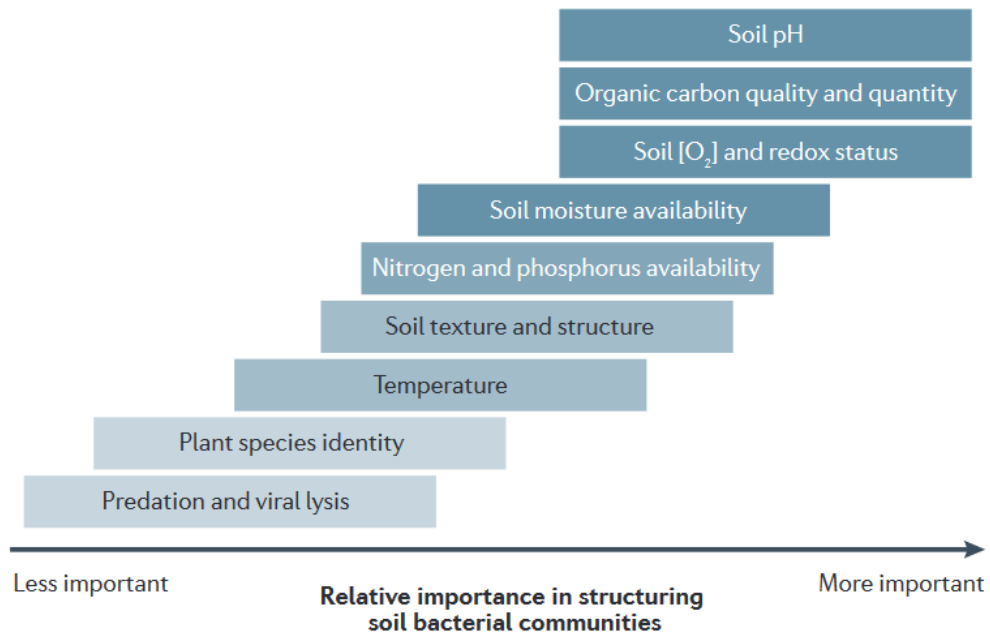


Figure 3. Biotic and abiotic factors that can influence the composition of soil bacterial communities (as reviewed by Fierer 2017). A hierarchy of biotic and abiotic factors that can influence soil bacterial communities, and their relative importance in influencing the structure of soil bacterial communities across space or time. ‘Importance’ is defined here as the ease of detecting the effects of these factors on the overall composition of soil bacterial communities. These factors are not necessarily independent and can correlate with one another (for example, soil texture can influence soil moisture availability). Furthermore, the importance of these factors will depend on the soils under investigation and the bacterial lineage in question. The shading of each box qualitatively indicates how well we understand the specific effects of each factor on bacterial communities; darker shades highlight factors that have been reasonably well-studied.

2.2 Digging deeper – the rhizosphere

Agricultural productivity is based on a microbial activity, much of which takes place in soil (Bakker et al. 2012), dependent on the functional processes of the soil microorganisms (Vega-Avila et al. 2015). However, the most important microorganisms in terms of plant nutrition, plant health and crop quality, inhabits the soil that directly surrounds the plant's root space – the rhizosphere (Berg and Smalla 2009). The term 'rhizosphere' was first defined by Lorenz Hiltner in 1904 (Hartmann et al. 2008). The definition of the rhizosphere is now characterized as the area of the soil that is physically, chemically and biologically influenced by the living root of the plant (Bertin et al. 2003; Ryan et al. 2009; Ottow 2011; Figure 4). Furthermore, the rhizosphere is characterized by high concentrations of plant-derived organic exudates, the release of root cap mucilage and of root border cells (Vieira et al. 2020). Thus, in terms of root growth and community development of macro- and microbiota, the rhizosphere is of great importance (Bertin et al. 2003). The intimacy of this interface between plants and their environment is essential for the acquisition of water and nutrients and for beneficial interactions with soil-borne microorganisms (Ryan et al. 2009).

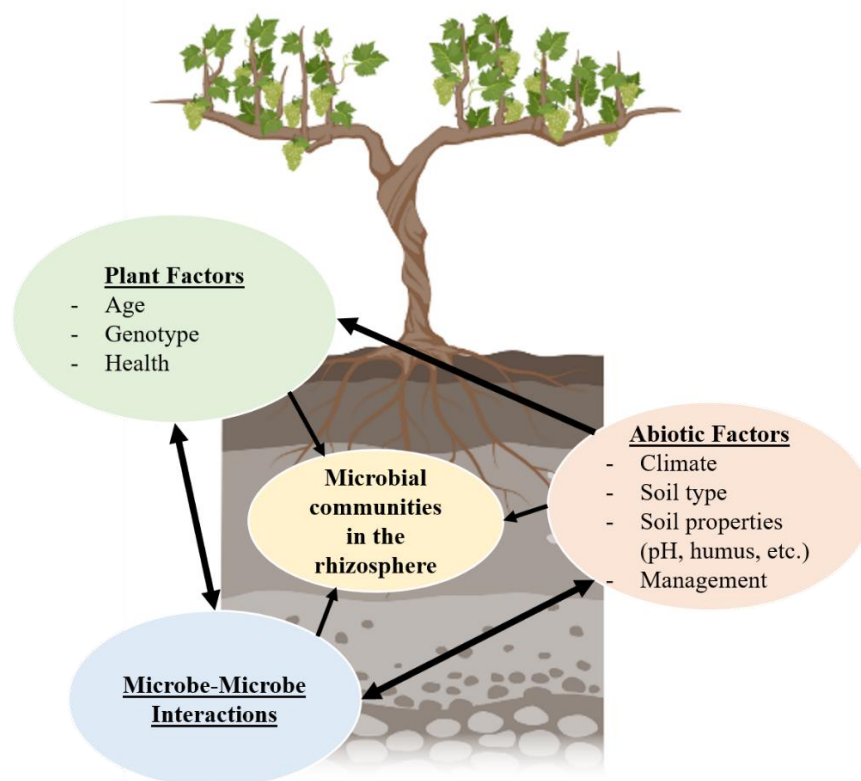


Figure 4. Various factors shape the microbial communities in the rhizosphere. (Created with Biorender.com)

In particular, the rhizosphere microorganisms can positively influence plant development, general vitality and plant growth by providing and acquiring nutrients. These microorganisms influence plant fitness in various ways, for example, via pathogen suppression, nitrogen conversion, phosphorus availability, mineralization and preservation of soil organic matter, stimulation of plant growth and fruit development (Berendsen et al. 2012; Schlaeppli and Bulgarelli 2015; Durán et al. 2018; Hu et al. 2018; Zhang et al. 2019a; Li et al. 2020). Consequently, the involved bacteria are also referred to as plant-growth promoting (rhizo)bacteria (PGP(R)B) (Trotel-Aziz et al. 2008; Compant et al. 2010). PGP(R)B can have a positive effect on plant growth via direct or indirect mechanisms. Direct mechanisms include, for instance, the mobilization of nutrients. Many of the PGP(R)B make poorly available nutrients, such as Phosphorus (P), Zinc (Zn), and Iron (Fe), available to the plant. Phosphorus is one of the most important macronutrients for the plant as it is involved in many physiological and biochemical processes such as cell division, photosynthesis, respiration, root system development, and biosynthesis of macromolecules (Sharma et al. 2013). However, only a small amount of phosphorus is present in the soil in plant-available form (Sharma et al. 2013). Various microorganisms are an essential component of the natural phosphorus cycle and thus involved in the transformation of phosphates in soil. The bacteria that have the ability to make phosphorus soluble in soil are called P-solubilizing bacteria (PSB). These PSB can convert insoluble inorganic phosphates into a plant-available form (Hakim et al. 2021). These PSB include microorganisms such as *Arthrobacter*, *Bacillus*, *Brevundimonas*, *Delftia*, or *Enterobacter* (Sharma et al. 2013).

Zinc is also an important plant micronutrient that plays numerous important roles in the plant life cycle, such as in DNA transcription (Hakim et al. 2021). In most soils, zinc is in inaccessible or unavailable form to the plant (Saraf 2017). PGP(R)B such as *Acinetobacter*, *Bacillus thuringiensis*, *Burkholderia cenocepacia*, or *Gluconacetobacter diazotrophicus* can increase the availability of zinc by making complexes of zinc soluble and alleviating zinc deficiency in plants (Saravanan et al. 2011).

Iron is also among the micronutrients that play an important role in cellular processes essential for plant development and growth since it serves as an important co-factor in enzymes of, photosynthesis and respiration, and synthesis and protection of DNA. This nutrient is also difficult for the plant to obtain, especially in calcareous soils. Deficiency of iron leads to decreased photosynthesis, lower yield and quality of fruits in the plant (Zhang et al. 2019b).

PGPR such as *Agrobacterium tumefaciens*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, or *Mycobacterium* make iron in the soil available to the plant, thereby promoting growth and productivity (Hakim et al. 2021).

Nitrogen (N) is among the most important nutrients for plants, as the macronutrient is essential for the synthesis of nucleotides and proteins including enzymes (Tisdale et al. 1993). Our atmosphere is composed of 80 % molecular nitrogen, but it is not available to plants in this particular form. Indeed, plants can only uptake nitrogen in the form of ammonium and nitrate through their roots. Specific PGP(R)B processes make it possible to convert molecular nitrogen from the air into ammonium via biological nitrogen fixation, thus making it available to the plant. Nitrogen fixation can be catalyzed by soil free-living bacteria and archaea aiming to cover majority their own N-need. Nevertheless, the nitrogen metabolisms of these free-living microorganisms also contribute to the N-supply of plants. Besides a specified symbiosis between rhizobacteria such as *Rhizobium*, *Bradyrhizobium*, *Ensifer*, *Azorhizobium* and *Mesorhizobium*, and legumes exists which more specifically manages the C and N exchange between the symbiotic partners (Sindhu et al. 2019).

Moreover, PGP(R)B can produce phytohormones which act as chemical messengers and can influence the physiological functions of the plant even at very low concentrations. Phytohormones can be divided into five different classes: Auxin, Cytokinin, Gibberellin, Abscicic Acid, and Ethylene (Oleńska et al. 2020). Many different taxa of PGP(R)B can produce these phytohormones, thereby promoting plant growth. Auxin controls almost all processes related to plant development. PGP(R)B that produce auxin improve root growth as well as growth and yield of a wide variety of crops (Ali et al. 2014; Imran et al. 2014). Mitigation of abiotic stresses such as drought, salinity, and soil contamination are also achieved by auxin-producing PGP(R)B (Kudoyarova et al. 2019). These PGP(R)B include for example *Bacillus thuringiensis*, *B. amyloliquefaciens*, *B. simplex*, *Enterobacter aerogenes*, or *Moraxella pluranimalium* (Hakim et al. 2021).

However, rhizobacteria also provide indirect mechanisms such as biological control of pathogens through the production of ACC deaminases, antibiotics, lytic enzymes, and induced systemic resistance (ISR). The diversity of PGP(R)B is not fully discovered and varies with plant species or cultivar, soil composition, crop rotation, and other environmental conditions. Among the indirect mechanisms of PGP(R)B is the biological control of pathogens. The application of PGP(R)B to the soil is an environmentally friendly and effective way to promote

sustainable plant growth and soil fertility, as it has the potential to reduce the need for chemical pesticides or fungicides. For instance, some PGP(R)B, such as *Bacillus*, *Pseudomonas*, *Azospirillum lipoferum*, and *Rhizobium* are able to produce ACC deaminases, helping the plant to better survive stress conditions (Ali et al. 2020). ACC deaminase reduces the level of pathogen-induced ethylene in plants by degrading the precursor of ethylene (Saraf et al. 2010). Thus, PGP(R)B producing ACC deaminase act as effective biological regulators against phytopathogens such as *Botrytis cinerea*, *Rhizoctonia solani*, *Fusarium oxysporum*, or *Xanthomonas oryzae*. Similarly, also abiotic stress is mitigated by ACC deaminase producing PGB(R)B as demonstrated for *Hartmannibacter diazotrophicus* for salt stressed barley plants (Suarez et al. 2015). Several other mechanisms have been reported for rhizobacteria-mediated tolerance of the plant to abiotic stressors (Asghari et al. 2020). In this context, the tolerance level strongly depends on the ability of PGP(R)B to stimulate the plant system to express transcription factors, flush out reactive oxygen species, or stabilize biomass (Habib et al. 2016; Chatterjee et al. 2020). Inoculation of the soil with these PGP(R)B can reduce abiotic stresses such as drought stress, salt stress, or heat stress.

All these characteristics are known, but the interactions of microbial communities and the roles of individual candidates remain to be elucidated. Recent technological developments in this regard have paved the way for more in-depth testing and associated elucidation. A different investigation approach than, for example, the isolation and further propagation of microorganisms from the root zone alone by classical cultivation methods, is a molecular biological investigation followed by a bioinformatic evaluation of microbial communities and their respective abundance in the rhizosphere. To examine the microbiome more closely, molecular biological methods such as 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). Bulgarelli et al. (2012) described a method in which rhizosphere and endophytic bacterial communities can be compared and characterized at the DNA level. This was investigated for *Arabidopsis thaliana* cultured under laboratory conditions using two different natural soils (Bulgarelli et al. 2012). In 2015, Bulgarelli et al. conducted a similar study with barley (*Hordeum vulgare*) (Bulgarelli et al. 2015). Wild and domesticated barley were also grown in natural soil under laboratory conditions and the bacterial communities of the rhizosphere and the communities of endophytes

were studied, compared and taxonomically evaluated at the 16S rRNA level (Bulgarelli et al. 2015). As also postulated by Pèrez-Jaramillo et al. (2016), the genetic diversity and abundance of different bacterial taxa was clearly seen in the direct comparison between wild and domesticated barley. However, the referred studies again deal with annual plants and model organisms such as *Arabidopsis thaliana*, which facilitates sampling.

Systematic rhizosphere investigations with molecular biological evaluation have so far mostly been carried out with the model organism *Arabidopsis thaliana*, barley (*Hordeum vulgare*) or similar plants. Since these plants are annual plants, it is easy to use the complete plant and to secure rhizosphere sample material. In the case of the permanent crop wine, such investigations are more difficult, since grapevines can root very far to a depth of about 6 meters, but also up to 32 meters in some cases (as reviewed by Ollat et al. 2015).

Furthermore, previous research usually deals with the consideration of the taxonomic diversity of the microorganisms located in the rhizosphere. In order to gain a better understanding of all the complex processes of the rhizosphere microbiome, an investigation at the 16S rRNA level is recommended. Thus, an insight into the respective "status quo" of the active microbiome can be gained, as well as possible statements about the recruitment of the microorganisms on the part of the grapevine can take place. A more precise knowledge of the processes may in future allow better conclusions to be drawn on the actual recycling of nutrients. The knowledge on expressed enzymes ideally is completed with chemical measurements in the close proxy of the root. These measurements typically require higher sample amounts compared to molecular analysis also because different nutrients are analyzed using various methods. Beside the important plant nutrients N, P and K also trace elements are required in viticulture. The sum of these results could lead to a more targeted fertilization recommendation, for example regarding the timing and optimization of nitrogen fertilization, as well as a targeted use of foliar fertilizers and fertigation measures or even management recommendations (especially in response to extreme years). Fertilizer and/or pesticide use could be reduced while maintaining healthy plants, adequate yield and increasing sustainable management.

2.3 Grapevine and its microbiome

As already indicated, the microbiome, especially that of the rhizosphere, is of great relevance for the grapevine health. In the last five years, more than 2500 papers about the vineyard soil microbiome have been published, revealing the increasing interest of the scientific community in this research area (Figure 2B). However, there is not only a rising interest in the role of the soil microbiome for grapevine health, but also for wine quality since it has been demonstrated that the (endophytic) microbiome of grapevines has a major impact on the regional *terroir* of wines (Pacifico et al. 2019; Zarraonaindia et al. 2015) and may correlate with fruit and wine quality (Zhou et al. 2020). The definition of the *terroir* concept is given by the International Organization of Vine and Wine (OIV 2010) as: “a concept which refers to an area in which collective knowledge of the interactions between the identifiable physical and biological environment and applied viticultural practices develops, providing distinctive characteristics for the products originating from this area. “Terroir” includes specific soil, topography, climate, landscape characteristics and biodiversity features” (OIV 2010). Soil and landscape characteristics have already been studied, however, the impact of the microbiome of grapevines on the *terroir* is still unraveled (Belda et al. 2017; Vaudour et al. 2015).

The vineyard microbiome has been studied many times (such as by Hendgen et al. 2018), but only general soil samples have been studied, or soil samples taken "near the roots" (at different depths) and these defined as rhizosphere in the following. Of course, this does not include fine root hairiness of the vine, a detailed consideration of chemotaxis and especially a good reproducibility of the results can consequently not be guaranteed. It is therefore of great importance to first develop a method that allows actual rhizosphere (i.e. soil immediately surrounding the root) as well as fine roots to be sampled and examined. Furthermore, some studies deal only with fungal (such as Schreiner 2003; Pancher et al. 2012; Holland et al. 2014), and others only with microorganisms of bacterial origin (Marasco et al. 2018; Burns et al. 2016; Burns et al. 2015). Schreiner (2003) was able to show that there are minor differences in the ability to form mycorrhizae-colonization between different grapevine rootstocks, but that other factors, have a major influence on the colonization of roots by arbuscular mycorrhiza fungi. In addition, Holland et al. (2014) observed that arbuscular mycorrhiza fungal communities differed significantly from host identity (grapevine or interrow plant roots). A study conducted by Pancher et al. (2012) indicates that fungal communities in vines from organically managed

vineyards differ from those from vineyards using integrated pest management. In contrast, Burns et al. (2015; 2016) investigated the soil bacterial diversity and composition by geographic features (Burns et al. 2015) and vineyard management. In another study conducted by Marasco et al. (2018), it was shown, that richness, diversity and bacterial community networking in the root compartments were significantly influenced by the rootstocks.

It has also been shown that, interestingly, microbial communities in vineyards are present regardless of season and time of year and are subject to little variation (Corneo et al. 2013). In their study, Corneo et al. (2013) found that temperature has no effect on microbial community structure. They conclude that the microorganisms are probably able to adapt quickly to seasonal temperature variations, but in return are more sensitive to permanent, stable differences in physico-chemical parameters (Corneo et al. 2013).

In vineyards, management practices influence soil characteristics, consequently affect the grapevine root system, and further impact the overall assemblies of the microbial communities (Vega-Avila et al. 2015). During the vegetation period, vineyard soils are subjected to a series of management practices to promote the growth and health of the grapevines, such as tillage, weed control, fertilization, and the use of pesticides and fungicides (Cordero et al. 2020; Hendgen et al. 2018).

Agricultural, or viticultural production respectively, is confronted to continuous challenges for improving crop yield while optimizing the use of fertilizers, water, and pesticides (Cordero et al. 2020). Moreover, adaption to climate change, including rising temperatures, drought, incoming radiation, and disruptive weather patterns due to increasing levels of atmospheric CO₂, is a key to the future of viticulture. The vegetative and reproductive cycles of the grapevine are depending heavily on weather and climatic conditions (van Leeuwen and Destrac-Irvine 2017; Naulleau et al. 2020). Therefore, a new approach for facing future challenges in viticulture must be developed, also with regard to the reduction of fertilizer and pesticides (as reviewed by Dries et al. 2021).

The microbiome of the rhizosphere can be described as very heterogeneous, which also depends to a large extent on the type of vineyard management. It has already been shown that microbial diversity in organically managed vineyards is demonstrably different from the diversity of conventionally managed areas. Hendgen et al. (2018) cite higher cumulative soil respiration, greater microbial mass, higher microbial activity, and better microbial-to-organic carbon ratios

as characteristic effects of bioorganic or biodynamic management. Vega-Avila et al. (2015) also detected higher bacterial diversity in the ecological variants studying different vineyards in Argentina. DNA extraction and next generation sequencing were performed in both studies to obtain a comprehensive overview of taxonomic microbial diversity and abundances (Hendgen et al. 2018; Vega-Avila et al. 2015).

In contrast to Vega-Avila et al. (2015) and Hendgen et al. (2018), which describe the status quo in a vineyard soil with respect to microbial communities, other studies address specific questions and individual bacterial "candidates" after DNA isolation from the vineyard rhizosphere has occurred. Salomon et al. (2014) described a molecular study of rhizosphere soil from an Argentine vineyard and subsequent selection of two bacterial strains for further study. Moreover, Salomon et al. conducted additional *in vitro* studies using potted grapevines with *Vitis vinifera* to test the two bacterial strains (*Bacillus licheniformis* Rt4M10 and *Pseudomonas fluorescens* Rt6M10) to gain further insight regarding the PGP(R)B selected (Salomon et al. 2014). Despite the progress made in describing the grapevine microbiome and its effects on growth, yield, and product quality, little is known about how different factors such as grape varieties and rootstocks influence the grapevine microbiome (Awad et al. 2020).

The grapevine rootstock affects the whole plant physiology and is responsible for different parameters such as phenology, biomass accumulation, grafting compatibility, rooting and propagation ability, resistance to nematodes and pathogens, tolerance to lime, salinity, drought, and nutrient uptake (for instance shown by Gong et al. 2011; Sivritepe et al. 2010; Granett et al. 2001; Ollat et al. 2015; May 1994; Smart et al. 2006). For example, it was shown by Gong et al. (2011), among others, that the rootstock 140 Ruggeri (*Vitis berlandieri* x *Vitis rupestris*) has the ability to exclude Cl⁻, while vines grafted on K 51-40 (*Vitis champinii* x *Vitis riparia* 'Gloire') can accumulate high concentrations of Cl⁻ when grown under saline conditions. In another study conducted by Sivritepe et al. (2010), that not only the rootstock has a supporting role in the susceptibility to salt stress, but also the genotype of the scion plays a dominant role in determining the biomass and the accumulation of inorganic ions in the grafted vines grown under salt stress. They were able to show that the scions of the grapevine cultivars 'Müsküle' and 'Sultana' reacted differently to salinity (Sivritepe et al. 2010). In a chapter written by Ollat et al. (2015), the authors show a comprehensive table of different grapevine rootstocks and their water stress adaptation, ranging from very low to very high. For example, the rootstock Börner (*Vitis riparia* 183 Geisenheim × *Vitis cinerea* Arnold) shows high water stress adaptation,

whereas the rootstock 125 AA (*Vitis riparia* × *Vitis berlandieri*) shows medium water stress adaptation (as reviewed by Ollat et al. 2015).

However, not only resistance to abiotic factors, but also to biotic factors can be affected by the rootstocks of the vine. Granett et al. (2001) summarize that the use of resistant rootstocks derived from native American *Vitis* has become the most important means of controlling grapevine phylloxera (*Daktulosphaira vitifoliae*).

In conclusion, a better understanding of gradual differences in microbiological activities of the rhizosphere microorganisms of grapevines under the different conditions such as grape variety, rootstock, grapevine scion and rootstock combinations, fertilization and management needs to be investigated.

2.4 Aim of this study

The variety of vineyard sites, climatic conditions, soil types, and grape varieties across the world's wine-growing regions results in the unavailability of a general type of adaptation strategy for vines facing climate change challenges (Schultz and Jones 2010). Therefore, an approach for characterizing and understanding the structure and function of the rhizobiome is considered as a first step towards its manipulation (Del Orozco-Mosqueda et al. 2022). Hence, superordinate, the future aim is to use new insights to enable more precise plant nutrition in the form of more accurate fertilization recommendations in order to reduce N-leaching, to increase aroma formation and maintain yeast-utilizable N content, and to reduce the associated costs in an economical and environmentally friendly way. A more precise knowledge of the specificity and dynamics of the grapevine rhizosphere microbiome under different management practices, grape varieties, rootstocks, and fertilization levels, should enable targeted adaptation strategies for increasingly frequent extreme years.

Consequently, the aim of this study was to investigate the bacterial rhizobiome of grapevines (*Vitis vinifera* L.) under different experimental designs. By characterizing the bacterial 16S RNA gene, a statement on the existing bacterial communities and their diversity can be made and compared under different environmental conditions. For this, a method has to be established that allows to obtain rhizosphere and root material of grapevine plants, and to prepare it for sequencing and further experiments. This applies both to grapevines cultivated in greenhouses under controlled conditions as well as to variants under field conditions. The experimental designs aimed to gain knowledge regarding the bacterial diversity of the grapevine rhizosphere

including different grape varieties, different grapevine rootstocks as well as different fertilization measures.

The investigations of this study were founded on the following questions:

1. Is it possible to “engineer” the grapevine rhizosphere for a more sustainable viticulture? (Chapter 1)
2. Are there differences between the bacterial communities of the grapevine rhizosphere regarding the grape variety, the rootstock, or different combinations, respectively? (Chapter 2 and 3)
3. Are there differences between the bacterial communities of the grapevine rhizosphere regarding different nitrogen-fertilization levels? (Chapter 4)

The first question was answered in the form of an extensive literature review. For this purpose, publications of other authors were consulted, read, critically evaluated and summarized. To answer the second experimental question, different approaches were taken, sampling potted grapevines (the grape varieties Riesling and Mueller-Thurgau and their rootstocks) on the one hand and grapevine in tubes (four different rootstocks without scion) on the other. For the third question, large containers of grapevines grown under field conditions with two different nitrogen-fertilization levels were investigated.

2.5 References

- Ali, Saira; Hameed, Sohail; Imran, Asma; Iqbal, Mazhar; Lazarovits, George (2014): Genetic, physiological and biochemical characterization of *Bacillus sp.* strain RMB7 exhibiting plant growth promoting and broad spectrum antifungal activities. In *Microbial cell factories* 13, p. 144. DOI: 10.1186/s12934-014-0144-x.
- Ali, Saira; Hameed, Sohail; Shahid, Muhammad; Iqbal, Mazhar; Lazarovits, George; Imran, Asma (2020): Functional characterization of potential PGPR exhibiting broad-spectrum antifungal activity. In *Microbiological research* 232, p. 126389. DOI: 10.1016/j.micres.2019.126389.
- Álvarez-Pérez, José Manuel; González-García, Sandra; Cobos, Rebeca; Olego, Miguel Ángel; Ibañez, Ana; Díez-Galán, Alba et al. (2017): Use of Endophytic and Rhizosphere Actinobacteria from Grapevine Plants To Reduce Nursery Fungal Graft Infections That Lead to Young Grapevine Decline. In *Applied and environmental microbiology* 83 (24). DOI: 10.1128/AEM.01564-17.
- Andreolli, Marco; Lampis, Silvia; Zapparoli, Giacomo; Angelini, Elisa; Vallini, Giovanni (2016): Diversity of bacterial endophytes in 3 and 15 year-old grapevines of *Vitis vinifera* cv. Corvina and their potential for plant growth promotion and phytopathogen control. In *Microbiological research* 183, pp. 42–52. DOI: 10.1016/j.micres.2015.11.009.
- Asghari, Susan; Harighi, Behrouz; Ashengroph, Morahem; Clement, Christophe; Aziz, Aziz; Esmaeel, Qassim; Ait Barka, Essaid (2020): Induction of systemic resistance to *Agrobacterium tumefaciens* by endophytic bacteria in grapevine. In *Plant Pathol* 69 (5), pp. 827–837. DOI: 10.1111/ppa.13175.
- Awad, Murad; Giannopoulos, Georgios; Mylona, Photini V.; Polidoros, Alexios N. (2020): Genotype May Influence Bacterial Diversity in Bark and Bud of *Vitis vinifera* Cultivars Grown under the Same Environment. In *Applied Sciences* 10 (23), p. 8405. DOI: 10.3390/app10238405.
- Babalola, Olubukola Oluranti (2010): Beneficial bacteria of agricultural importance. In *Biotechnol Lett* 32 (11), pp. 1559–1570. DOI: 10.1007/s10529-010-0347-0.
- Bakker, Matthew G.; Manter, Daniel K.; Sheflin, Amy M.; Weir, Tiffany L.; Vivanco, Jorge M. (2012): Harnessing the rhizosphere microbiome through plant breeding and agricultural management. In *Plant Soil* 360 (1-2), pp. 1–13. DOI: 10.1007/s11104-012-1361-x.

- Belda, Ignacio; Zarraonaindia, Iratxe; Perisin, Matthew; Palacios, Antonio; Acedo, Alberto (2017): From Vineyard Soil to Wine Fermentation: Microbiome Approximations to Explain the “terroir” Concept. In *Frontiers in microbiology* 8, p. 821. DOI: 10.3389/fmicb.2017.00821.
- Bell, S.-J. and Henschke, P.A. (2005) Implications of nitrogen nutrition for grapes, fermentation and wine. *Australian Journal of Grape and Wine Research* 11, 242–295. DOI: 10.1111/j.1755-0238.2005.tb00028.x
- Berendsen, Roeland L.; Pieterse, Corné M. J.; Bakker, Peter A. H. M. (2012): The rhizosphere microbiome and plant health. In *Trends in plant science* 17 (8), pp. 478–486. DOI: 10.1016/j.tplants.2012.04.001.
- Berg, Gabriele; Smalla, Kornelia (2009): Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. In *FEMS microbiology ecology* 68 (1), pp. 1–13. DOI: 10.1111/j.1574-6941.2009.00654.x.
- Berlanas, Carmen; Berbegal, Mónica; Elena, Georgina; Laidani, Meriem; Cibriain, José Félix; Sagües, Ana; Gramaje, David (2019): The Fungal and Bacterial Rhizosphere Microbiome Associated With Grapevine Rootstock Genotypes in Mature and Young Vineyards. In *Frontiers in microbiology* 10, p. 1142. DOI: 10.3389/fmicb.2019.01142.
- Bertin, Cecile; Yang, Xiaohan; Weston, Leslie A. (2003): The role of root exudates and allelochemicals in the rhizosphere. In *Plant and Soil* (256), pp. 67–83.
- Bulgarelli, Davide; Garrido-Oter, Ruben; Münch, Philipp C.; Weiman, Aaron; Dröge, Johannes; Pan, Yao et al. (2015): Structure and function of the bacterial root microbiota in wild and domesticated barley. In *Cell host & microbe* 17 (3), pp. 392–403. DOI: 10.1016/j.chom.2015.01.011.
- Bulgarelli, Davide; Rott, Matthias; Schlaeppi, Klaus; van Loren Themaat, Emiel ver; Ahmadinejad, Nahal; Assenza, Federica et al. (2012): Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. In *Nature* 488 (7409), pp. 91–95. DOI: 10.1038/nature11336.
- Burns, Kayla N.; Bokulich, Nicholas A.; Cantu, Dario; Greenhut, Rachel F.; Kluepfel, Daniel A.; O’Geen, A. Toby et al. (2016): Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: Differentiation by vineyard management. In *Soil Biology and Biochemistry* 103, pp. 337–348. DOI: 10.1016/j.soilbio.2016.09.007.

- Burns, Kayla N.; Kluepfel, Daniel A.; Strauss, Sarah L.; Bokulich, Nicholas A.; Cantu, Dario; Steenwerth, Kerri L. (2015): Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: Differentiation by geographic features. In *Soil Biology and Biochemistry* 91, pp. 232–247. DOI: 10.1016/j.soilbio.2015.09.002.
- Carro-Huerga, Guzmán; Compant, Stéphane; Gorfer, Markus; Cardoza, Rosa E.; Schmoll, Monika; Gutiérrez, Santiago; Casquero, Pedro A. (2020): Colonization of *Vitis vinifera* L. by the Endophyte *Trichoderma* sp. Strain T154: Biocontrol Activity Against *Phaeoacremonium minimum*. In *Frontiers in plant science* 11, p. 1170. DOI: 10.3389/fpls.2020.01170.
- Chatterjee, Poulami; Kanagendran, Arooran; Samaddar, Sandipan; Pazouki, Leila; Sa, Tong-Min; Niinemets, Ülo (2020): Influence of *Brevibacterium linens* RS16 on foliage photosynthetic and volatile emission characteristics upon heat stress in *Eucalyptus grandis*. In *The Science of the total environment* 700, p. 134453. DOI: 10.1016/j.scitotenv.2019.134453.
- Compant, Stéphane; Brader, Günter; Muzammil, Saima; Sessitsch, Angela; Lebrihi, Ahmed; Mathieu, Florence (2013): Use of beneficial bacteria and their secondary metabolites to control grapevine pathogen diseases. In *BioControl* 58 (4), pp. 435–455. DOI: 10.1007/s10526-012-9479-6.
- Compant, Stéphane; Clément, Christophe; Sessitsch, Angela (2010): Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. In *Soil Biology and Biochemistry* 42 (5), pp. 669–678. DOI: 10.1016/j.soilbio.2009.11.024.
- Cordero, Jorge; Freitas, J. Renato de; Germida, James J. (2020): Bacterial microbiome associated with the rhizosphere and root interior of crops in Saskatchewan, Canada. In *Canadian journal of microbiology* 66 (1), pp. 71–85. DOI: 10.1139/cjm-2019-0330.
- Corneo, Paola E.; Pellegrini, Alberto; Cappellin, Luca; Roncador, Marco; Chierici, Marco; Gessler, Cesare; Pertot, Ilaria (2013): Microbial community structure in vineyard soils across altitudinal gradients and in different seasons. In *FEMS Microbiol Ecol* 84 (3), pp. 588–602. DOI: 10.1111/1574-6941.12087.
- Del Orozco-Mosqueda, Ma Carmen; Fadiji, Ayomide Emmanuel; Babalola, Olubukola Oluranti; Glick, Bernard R.; Santoyo, Gustavo (2022): Rhizobiome engineering: Unveiling

- complex rhizosphere interactions to enhance plant growth and health. In *Microbiological research* 263, p. 127137. DOI: 10.1016/j.micres.2022.127137.
- Deutsches Weininstitut GmbH (2019): German Wine Manual. Available online at https://www.deutscheweine.de/fileadmin/user_upload/9732_Seminarhandbuch2019Web_engl.pdf, checked on 10/11/2022.
- Di Giacinto, Sofia; Friedel, Matthias; Poll, Christian; Döring, Johanna; Kunz, Robert; Kauer, Randolph (2020): Vineyard management system affects soil microbiological properties. In *OENO One* 54 (1). DOI: 10.20870/oeno-one.2020.54.1.2578.
- Dries, Leonie; Hendgen, Maximilian; Schnell, Sylvia; Löhnertz, Otmar; Vortkamp, Anne (2021): Rhizosphere engineering: leading towards a sustainable viticulture? In *OENO One* 55 (2), pp. 353–363. DOI: 10.20870/oeno-one.2021.55.2.4534.
- Durán, Paloma; Thiergart, Thorsten; Garrido-Oter, Ruben; Agler, Matthew; Kemen, Eric; Schulze-Lefert, Paul; Hacquard, Stéphane (2018): Microbial Interkingdom Interactions in Roots Promote *Arabidopsis* Survival. In *Cell* 175 (4), 973-983.e14. DOI: 10.1016/j.cell.2018.10.020.
- European Commission (2021): COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT, THE COUNCIL, THE EUROPEAN ECONOMIC AND SOCIAL COMMITTEE AND THE COMMITTEE OF THE REGIONS EU Soil Strategy for 2030 Reaping the benefits of healthy soils for people, food, nature and climate. Document 52021DC0699 (COM/2021/699 final).
- Fierer, Noah (2017): Embracing the unknown: disentangling the complexities of the soil microbiome. In *Nature reviews. Microbiology* 15 (10), pp. 579–590. DOI: 10.1038/nrmicro.2017.87.
- Fierer, Noah; Bradford, Mark A.; Jackson, Robert B. (2007): Toward an ecological classification of soil bacteria. In *Ecology* (88(6)), pp. 1354–1364.
- Giri, Bhoopander; Prasad, Ram; Wu, Qiang-Sheng; Varma, Ajit (2019): Biofertilizers for Sustainable Agriculture and Environment 55. DOI: 10.1007/978-3-030-18933-4.
- Gong, Haijun; Blackmore, Deidre; Clingeleffer, Peter; Sykes, Steve; Jha, Deepa; Tester, Mark; Walker, Rob (2011): Contrast in chloride exclusion between two grapevine genotypes and its variation in their hybrid progeny. In *Journal of experimental botany* 62 (3), pp. 989–999. DOI: 10.1093/jxb/erq326.

- Granett, J.; Walker, M. A.; Kocsis, L.; Omer, A. D. (2001): Biology and management of grape phylloxera. In *Annual review of entomology* 46, pp. 387–412. DOI: 10.1146/annurev.ento.46.1.387.
- Habib, Sheikh Hasna; Kausar, Hossain; Saud, Halimi Mohd (2016): Plant Growth-Promoting Rhizobacteria Enhance Salinity Stress Tolerance in Okra through ROS-Scavenging Enzymes. In *BioMed research international* 2016, p. 6284547. DOI: 10.1155/2016/6284547.
- Hakim, Sughra; Naqqash, Tahir; Nawaz, Muhammad Shoib; Laraib, Iqra; Siddique, Muhammad Jawad; Zia, Rabisa et al. (2021): Rhizosphere Engineering With Plant Growth-Promoting Microorganisms for Agriculture and Ecological Sustainability. In *Front. Sustain. Food Syst.* 5, Article 617157. DOI: 10.3389/fsufs.2021.617157.
- Hartmann, Anton; Rothballer, Michael; Schmid, Michael (2008): Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. In *Plant Soil* 312 (1-2), pp. 7–14. DOI: 10.1007/s11104-007-9514-z.
- Hendgen, Maximilian; Hoppe, Björn; Döring, Johanna; Friedel, Matthias; Kauer, Randolph; Frisch, Matthias et al. (2018): Effects of different management regimes on microbial biodiversity in vineyard soils. In *Scientific reports* 8 (1), p. 9393. DOI: 10.1038/s41598-018-27743-0.
- Holland, Taylor C.; Bowen, Pat; Bogdanoff, Carl; Hart, Miranda M. (2014): How distinct are arbuscular mycorrhizal fungal communities associating with grapevines? In *Biol Fertil Soils* 50 (4), pp. 667–674. DOI: 10.1007/s00374-013-0887-2.
- Hu, Lingfei; Robert, Christelle A. M.; Cadot, Selma; Zhang, Xi; Ye, Meng; Li, Beibei et al. (2018): Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. In *Nature communications* 9 (1), p. 2738. DOI: 10.1038/s41467-018-05122-7.
- Imran, Asma; Saadalla, Marryam Jumma Abdulla; Khan, Sami-Ullah; Mirza, Muhammad Sajjad; Malik, Kauser Abdulla; Hafeez, Fauzia Yusuf (2014): *Ochrobactrum* sp. Pv2Z2 exhibits multiple traits of plant growth promotion, biodegradation and N-acyl-homoserine-lactone quorum sensing. In *Ann Microbiol* 64 (4), pp. 1797–1806. DOI: 10.1007/s13213-014-0824-0.
- Jetten, Mike S. M. (2008): The microbial nitrogen cycle. In *Environmental microbiology* 10 (11), pp. 2903–2909. DOI: 10.1111/j.1462-2920.2008.01786.x.

- Karimi, Battle; Cahurel, Jean-Yves; Gontier, Laure; Charlier, Laurent; Chovelon, Marc; Mahé, Héloïse; Ranjard, Lionel (2020): A meta-analysis of the ecotoxicological impact of viticultural practices on soil biodiversity. In *Environ Chem Lett* 18 (6), pp. 1947–1966. DOI: 10.1007/s10311-020-01050-5.
- Klein Goldewijk, Kees; Beusen, Arthur; Doelman, Jonathan; Stehfest, Elke (2017): Anthropogenic land use estimates for the Holocene – HYDE 3.2. In *Earth Syst. Sci. Data* 9 (2), pp. 927–953. DOI: 10.5194/essd-9-927-2017.
- Köberl, Martina; Wagner, Philipp; Müller, Henry; Matzer, Robert; Unterfrauner, Hans; Cernava, Tomislav; Berg, Gabriele (2020): Unraveling the Complexity of Soil Microbiomes in a Large-Scale Study Subjected to Different Agricultural Management in Styria. In *Frontiers in microbiology* 11, p. 1052. DOI: 10.3389/fmicb.2020.01052.
- Kudoyarova, Guzel; Arkhipova, Tatiana; Korshunova, Tatiana; Bakaeva, Margarita; Loginov, Oleg; Dodd, Ian C. (2019): Phytohormone Mediation of Interactions Between Plants and Non-Symbiotic Growth Promoting Bacteria Under Edaphic Stresses. In *Frontiers in plant science* 10, p. 1368. DOI: 10.3389/fpls.2019.01368.
- Lakkis, Sara; Trotel-Aziz, Patricia; Rabenoelina, Fanja; Schwarzenberg, Adrian; Nguema-Ona, Eric; Clément, Christophe; Aziz, Aziz (2019): Strengthening Grapevine Resistance by *Pseudomonas fluorescens* PTA-CT2 Relies on Distinct Defense Pathways in Susceptible and Partially Resistant Genotypes to Downy Mildew and Gray Mold Diseases. In *Frontiers in plant science* 10, p. 1112. DOI: 10.3389/fpls.2019.01112.
- Li, Jiangang; Mavrodi, Olga V.; Hou, Jinfeng; Blackmon, Chazden; Babiker, Ebrahiem M.; Mavrodi, Dmitri V. (2020): Comparative Analysis of Rhizosphere Microbiomes of Southern Highbush Blueberry (*Vaccinium corymbosum* L.), Darrow’s Blueberry (*V. darrowii* Camp), and Rabbiteye Blueberry (*V. virgatum* Aiton). In *Frontiers in microbiology* 11, p. 370. DOI: 10.3389/fmicb.2020.00370.
- Lugtenberg, Ben; Kamilova, Faina (2009): Plant-growth-promoting rhizobacteria. In *Annu. Rev. Microbiol.* 63 (1), pp. 541–556. DOI: 10.1146/annurev.micro.62.081307.162918.
- Marasco, Ramona; Rolli, Eleonora; Fusi, Marco; Michoud, Grégoire; Daffonchio, Daniele (2018): Grapevine rootstocks shape underground bacterial microbiome and networking but not potential functionality. In *Microbiome* 6 (1), p. 3. DOI: 10.1186/s40168-017-0391-2.
- May, P. (1994): Using grapevine rootstocks: the Australian perspective: Winetitles.

- Naulleau, Audrey; Gary, Christian; Prévot, Laurent; Hossard, Laure (2020): Evaluating Strategies for Adaptation to Climate Change in Grapevine Production-A Systematic Review. In *Frontiers in plant science* 11, p. 607859. DOI: 10.3389/fpls.2020.607859.
- Nazli, Farheen; Najm-ul-Seher; Khan, Muhammad Yahya; Jamil, Moazzam; Nadeem, Sajid Mahmood; Ahmad, Maqshoof (2020): Soil Microbes and Plant Health. In Imran Ul Haq, Siddra Ijaz (Eds.): *Plant Disease Management Strategies for Sustainable Agriculture through Traditional and Modern Approaches*, vol. 13. 1st ed. 2020. Cham: Springer International Publishing; Imprint Springer (Springer eBook Collection, 13), pp. 111–135.
- Nendel, C., Kersebaum, K., 2004. A simple model approach to simulate nitrogen dynamics in vineyard soils. *Ecological Modelling* 177 (1-2), 1–15. DOI: 10.1016/j.ecolmodel.2004.01.014.
- OIV (2010): General assembly of the international organisation of vine and wine. Resolution OIV/VITI 333/2010.
- OIV (2016): General assembly of the international organisation of vine and wine. Available online at <http://www.oiv.int/en/statistiques/recherche>, checked on 10/11/2022.
- Oleńska, Ewa; Małek, Wanda; Wójcik, Małgorzata; Swiecicka, Izabela; Thijs, Sofie; Vangronsveld, Jaco (2020): Beneficial features of plant growth-promoting rhizobacteria for improving plant growth and health in challenging conditions: A methodical review. In *The Science of the total environment* 743, p. 140682. DOI: 10.1016/j.scitotenv.2020.140682.
- Ollat, N.; Peccoux, A.; Papura, D.; Esmenjaud, D.; Marguerit, E.; Tandonnet, J.-P. et al. (2015): Rootstocks as a component of adaptation to environment, pp. 68–108. DOI: 10.1002/9781118735985.ch4.
- Ottow, Johannes C.G. (2011): *Mikrobiologie von Böden. Biodiversität, Ökophysiologie und Metagenomik*. Berlin, Heidelberg: Springer Berlin Heidelberg (Springer-Lehrbuch). Available online at <http://nbn-resolving.org/urn:nbn:de:bsz:31-epflicht-1622396>.
- Pacifico, Davide; Squartini, Andrea; Crucitti, Dalila; Barizza, Elisabetta; Lo Schiavo, Fiorella; Muresu, Rosella et al. (2019): The Role of the Endophytic Microbiome in the Grapevine Response to Environmental Triggers. In *Frontiers in plant science* 10, p. 1256. DOI: 10.3389/fpls.2019.01256.
- Pancher, Michael; Ceol, Marco; Corneo, Paola Elisa; Longa, Claudia Maria Oliveira; Yousaf, Sohail; Pertot, Ilaria; Campisano, Andrea (2012): Fungal endophytic communities in

- grapevines (*Vitis vinifera* L.) respond to crop management. In *Applied and environmental microbiology* 78 (12), pp. 4308–4317. DOI: 10.1128/AEM.07655-11.
- Pankhurst, C. E.; Ophel-Keller, K.; Doube, B. M.; Gupta, V. V. S. R. (1996): Biodiversity of soil microbial communities in agricultural systems. In *Biodivers Conserv* 5 (2), pp. 197–209. DOI: 10.1007/BF00055830.
- Pérez-Jaramillo, Juan E.; Mendes, Rodrigo; Raaijmakers, Jos M. (2016): Impact of plant domestication on rhizosphere microbiome assembly and functions. In *Plant molecular biology* 90 (6), pp. 635–644. DOI: 10.1007/s11103-015-0337-7.
- Pons, Alexandre, Allamy, Lucile, Schüttler, Armin, Rauhut, Doris, Thibon, Cecile, Darriet, Philippe (2017). What is the expected impact of climate change on wine aroma compounds and their precursors in grape?. *OENO one*, 51(2-3), 141-146. DOI: 10.20870/oeno-one.2016.0.0.1868
- Ryan, Peter R.; Dessaux, Yves; Thomashow, Linda S.; Weller, David M. (2009): Rhizosphere engineering and management for sustainable agriculture. In *Plant Soil* 321 (1-2), pp. 363–383. DOI: 10.1007/s11104-009-0001-6.
- Salmon, Jean-Michel; Samson, Alain; Beaujouan, Maxime (2020): How to adapt winegrowing and winemaking practices to improve organic wine production sustainability? In *Org. Agr.* 10 (S1), pp. 131–138. DOI: 10.1007/s13165-020-00317-z.
- Salomon, María Victoria; Bottini, Rubén; Souza Filho, Gonçalo Apolinário de; Cohen, Ana Carmen; Moreno, Daniela; Gil, Mariana; Piccoli, Patricia (2014): Bacteria isolated from roots and rhizosphere of *Vitis vinifera* retard water losses, induce abscisic acid accumulation and synthesis of defense-related terpenes in in vitro cultured grapevine. In *Physiologia plantarum* 151 (4), pp. 359–374. DOI: 10.1111/ppl.12117.
- Saraf, Meenu (2017): Zinc Biofortification: Strategy to Conquer Zinc Malnutrition through Zinc Solubilizing PGPR's. In *BJSTR* 1 (1). DOI: 10.26717/BJSTR.2017.01.000158.
- Saraf, Meenu; Jha, Chaitanya Kumar; Patel, Dhara (2010): The Role of ACC Deaminase Producing PGPR in Sustainable Agriculture 18, pp. 365–385. DOI: 10.1007/978-3-642-13612-2_16.
- Saravanan, V. S.; Kumar, M. Rohini; Sa, T. M. (2011): Microbial Zinc Solubilization and Their Role on Plants, pp. 47–63. DOI: 10.1007/978-3-642-21061-7_3.

- Schlaeppli, Klaus; Bulgarelli, Davide (2015): The plant microbiome at work. In *Molecular plant-microbe interactions : MPMI* 28 (3), pp. 212–217. DOI: 10.1094/MPMI-10-14-0334-FI.
- Schreiner, R. P. (2003): Mycorrhizal Colonization of Grapevine Rootstocks under Field Conditions. In *Am J Enol Vitic.* 54 (3), pp. 143–149. Available online at <https://www.ajevonline.org/content/54/3/143.short>.
- Schultz, Hans R.; Jones, Gregory V. (2010): Climate Induced Historic and Future Changes in Viticulture. In *Journal of Wine Research* 21 (2-3), pp. 137–145. DOI: 10.1080/09571264.2010.530098.
- Sharma, Seema B.; Sayyed, Riyaz Z.; Trivedi, Mrugesh H.; Gobi, Thivakaran A. (2013): Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. In *SpringerPlus* 2, p. 587. DOI: 10.1186/2193-1801-2-587.
- Sindhu, Satyavir S.; Sharma, Ruchi; Sindhu, Swati; Sehrawat, Anju (2019): Soil Fertility Improvement by Symbiotic Rhizobia for Sustainable Agriculture. In *Soil Fertility Management for Sustainable Development: Springer, Singapore*, pp. 101–166. Available online at https://link.springer.com/chapter/10.1007/978-981-13-5904-0_7.
- Sivritepe, N.; Sivritepe, H. Ö.; Celik, H.; Katkat, A. V. (2010): Salinity Responses of Grafted Grapevines: Effects of Scion and Rootstock Genotypes. In *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* (38(3)), pp. 193–201. DOI: 10.15835/nbha3834677.
- Smart, David R.; Schwass, Erin; Lakso, Alan; Morano, Lisa (2006): Grapevine Rooting Patterns: A Comprehensive Analysis and a Review. In *American Journal of Enology and Viticulture* 57 (1), pp. 89–104. Available online at <https://www.ajevonline.org/content/57/1/89>.
- Suarez, Christian; Cardinale, Massimiliano; Ratering, Stefan; Steffens, Diedrich; Jung, Stephan; Montoya, Ana M. Zapata et al. (2015): Plant growth-promoting effects of *Hartmannibacter diazotrophicus* on summer barley (*Hordeum vulgare* L.) under salt stress. In *Applied Soil Ecology* 95, pp. 23–30. DOI: 10.1016/j.apsoil.2015.04.017.
- Tan, Wenjun; Wang, Junman; Bai, Wenqing; Qi, Jiejun; Chen, Weimin (2020): Soil bacterial diversity correlates with precipitation and soil pH in long-term maize cropping systems. In *Scientific reports* 10 (1), p. 6012. DOI: 10.1038/s41598-020-62919-7.
- Tisdale, S. L.; Nelson, W. L.; Beaton, J. D.; Havlin, J. L. (1993): Elements required in plant nutrition (Soil fertility and fertilizers. MacMillan Publ. Co., New York).

- Trivedi, Pankaj; Leach, Jan E.; Tringe, Susannah G.; Sa, Tongmin; Singh, Brajesh K. (2020): Plant-microbiome interactions: from community assembly to plant health. In *Nat Rev Microbiol* 18 (11), pp. 607–621. DOI: 10.1038/s41579-020-0412-1.
- Trotel-Aziz, Patricia; Couderchet, Michel; Biagiatti, Sylvie; Aziz, Aziz (2008): Characterization of new bacterial biocontrol agents *Acinetobacter*, *Bacillus*, *Pantoea* and *Pseudomonas spp.* mediating grapevine resistance against *Botrytis cinerea*. In *Environmental and Experimental Botany* 64 (1), pp. 21–32. DOI: 10.1016/j.envexpbot.2007.12.009.
- van Leeuwen, Cornelis; Destrac-Irvine, Agnès (2017): Modified grape composition under climate change conditions requires adaptations in the vineyard. In *OENO One* 51 (2), p. 147. DOI: 10.20870/oeno-one.2016.0.0.1647.
- Vaudour, E.; Costantini, E.; Jones, G. V.; Mocali, S. (2015): An overview of the recent approaches to terroir functional modelling, footprinting and zoning. In *SOIL* 1 (1), pp. 287–312. DOI: 10.5194/soil-1-287-2015.
- Vega-Avila, A. D.; Gumiere, T.; Andrade, P. A. M.; Lima-Perim, J. E.; Durrer, A.; Baigori, M. et al. (2015): Bacterial communities in the rhizosphere of *Vitis vinifera* L. cultivated under distinct agricultural practices in Argentina. In *Antonie van Leeuwenhoek* 107 (2), pp. 575–588. DOI: 10.1007/s10482-014-0353-7.
- Vieira, Selma; Sikorski, Johannes; Dietz, Sophie; Herz, Katharina; Schrupf, Marion; Bruelheide, Helge et al. (2020): Drivers of the composition of active rhizosphere bacterial communities in temperate grasslands. In *The ISME journal* 14 (2), pp. 463–475. DOI: 10.1038/s41396-019-0543-4.
- Wang, Cheng-yu; Zhou, Xue; Guo, Dan; Zhao, Jiang-hua; Yan, Li; Feng, Guo-zhong et al. (2019): Soil pH is the primary factor driving the distribution and function of microorganisms in farmland soils in northeastern China. In *Ann Microbiol* 69 (13), pp. 1461–1473. DOI: 10.1007/s13213-019-01529-9.
- Yamamoto, T.; Iketani, H.; Ieki, H.; Nishizawa, Y.; Notsuka, K.; Hibi, T. et al. (2000): Transgenic grapevine plants expressing a rice chitinase with enhanced resistance to fungal pathogens. In *Plant cell reports* 19 (7), pp. 639–646. DOI: 10.1007/s002999900174.
- Zarraonaindia, Iratxe; Owens, Sarah M.; Weisenhorn, Pamela; West, Kristin; Hampton-Marcell, Jarrad; Lax, Simon et al. (2015): The soil microbiome influences grapevine-associated microbiota. In *mBio* 6 (2). DOI: 10.1128/mBio.02527-14.

- Zhang, Jingying; Liu, Yong-Xin; Zhang, Na; Hu, Bin; Jin, Tao; Xu, Haoran et al. (2019a): NRT1.1B is associated with root microbiota composition and nitrogen use in field-grown rice. In *Nature biotechnology* 37 (6), pp. 676–684. DOI: 10.1038/s41587-019-0104-4.
- Zhang, Xinxin; Di Zhang; Sun, Wei; Wang, Tianzuo (2019b): The Adaptive Mechanism of Plants to Iron Deficiency via Iron Uptake, Transport, and Homeostasis. In *International journal of molecular sciences* 20 (10). DOI: 10.3390/ijms20102424.
- Zhou, Jia; Cavagnaro, Timothy R.; Bei, Roberta de; Nelson, Tiffanie M.; Stephen, John R.; Metcalfe, Andrew et al. (2020): Wine Terroir and the Soil Bacteria: An Amplicon Sequencing-Based Assessment of the Barossa Valley and Its Sub-Regions. In *Frontiers in microbiology* 11, p. 597944. DOI: 10.3389/fmicb.2020.597944.

Chapter 1 – Rhizosphere engineering: leading towards a sustainable viticulture?

Rhizosphere engineering: leading towards a sustainable viticulture?

✉ Leonie Dries^{1*}, Maximilian Hendgen¹, Sylvia Schnell², Otmar Löhnertz¹ and Anne Vortkamp^{1,3}

¹Department of Soil Science and Plant Nutrition, Hochschule Geisenheim University, Von-Lade-Str. 1, 65366 Geisenheim, Germany

²Institute of Applied Microbiology, Research Center for BioSystems, Land Use, and Nutrition (IFZ), Justus-Liebig University Giessen, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany

³REACH EUREGIO Start-up Center, University of Muenster, Geiststr. 24-26, 48151 Muenster, Germany. Former affiliation: Department of Soil Science and Plant Nutrition, Hochschule Geisenheim University, Von-Lade-Str. 1, 65366 Geisenheim, Germany

✉ *corresponding author: leonie.dries@hs-gm.de

✉ Associate editor: Astrid Forneck

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; Zarraonaindia *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 (Pacífico *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 apply 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).

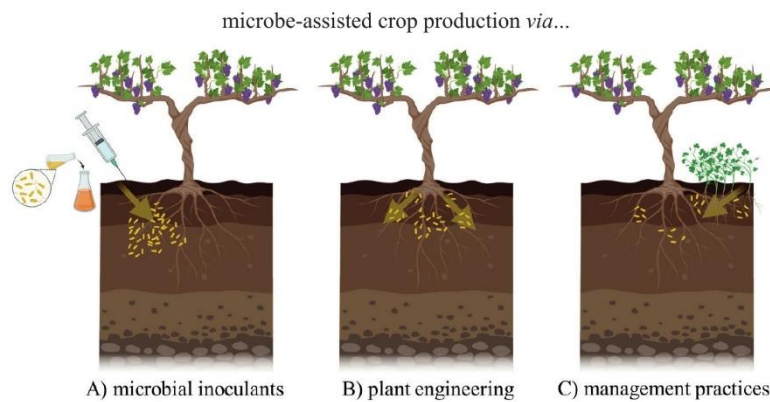


FIGURE 1. Microbe-assisted crop production via different methods through the rhizosphere.
 A) Increasing beneficial microorganisms via microbial inoculants. B) Enhancing beneficial microorganisms through plant breeding or plant engineering. C) Stimulating beneficial microorganisms through management 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).

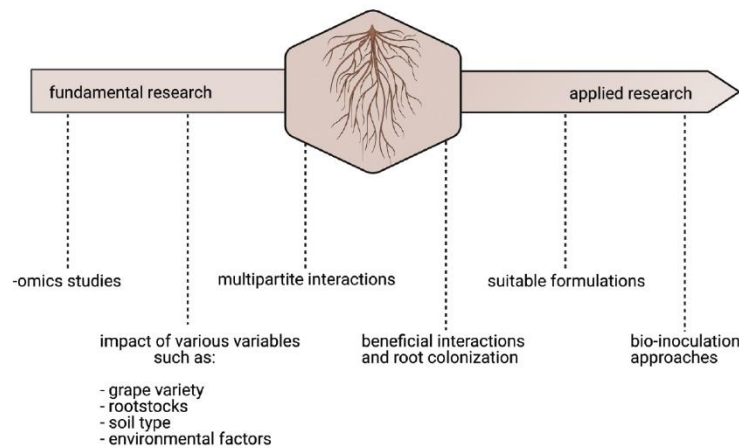


FIGURE 2. Steps to be considered in further studies.
 Several steps need to be considered that lead to more sustainable viticulture using rhizosphere engineering. Created with BioRender.com.

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.

Acknowledgement: We thank Forschungsring des Deutschen Weinbaus (FDW) for funding this project.

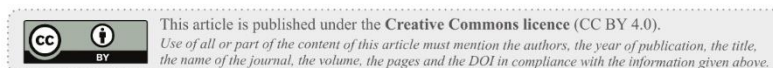
REFERENCES

- Aguín, O., Mansilla, J. P., Vilariño, A., & Sainz, M. J. (2004). Effects of Mycorrhizal Inoculation on Root Morphology and Nursery Production of Three Grapevine Rootstocks. *American Journal of Enology and Viticulture* (55:1), 108–111.
- Ahkami, A. H., Allen White, R., Handakumbura, P. P., & Jansson, C. (2017). Rhizosphere engineering: Enhancing sustainable plant ecosystem productivity. *Rhizosphere*, 3, 233–243. <https://doi.org/10.1016/j.rhisph.2017.04.012>
- Álvarez-Pérez, J. M., González-García, S., Cobos, R., Olego, M. Á., Ibañez, A., Díez-Galán, A., Garzón-Jimeno, E., & Coque, J. J. R. (2017). Use of Endophytic and Rhizosphere Actinobacteria from Grapevine Plants to Reduce Nursery Fungal Graft Infections That Lead to Young Grapevine Decline. *Applied and Environmental Microbiology*, 83(24). <https://doi.org/10.1128/AEM.01564-17>
- Andreolli, M., Lampis, S., Zapparoli, G., Angelini, E., & Vallini, G. (2016). Diversity of bacterial endophytes in 3 and 15 year-old grapevines of *Vitis vinifera* cv. Corvina and their potential for plant growth promotion and phytopathogen control. *Microbiological Research*, 183, 42–52. <https://doi.org/10.1016/j.micres.2015.11.009>
- Andreolli, M., Zapparoli, G., Lampis, S., Santi, C., Angelini, E., & Bertazzon, N. (2021). In Vivo Endophytic, Rhizospheric and Epiphytic Colonization of *Vitis vinifera* by the Plant-Growth Promoting and Antifungal Strain *Pseudomonas protegens* MP12. *Microorganisms*, 9(2). <https://doi.org/10.3390/microorganisms9020234>
- Bach, E., Seger, G. D. d. S., Fernandes, G. d. C., Lisboa, B. B., & Passaglia, L. M. P. (2016). Evaluation of biological control and rhizosphere competence of plant growth promoting bacteria. *Applied Soil Ecology*, 99, 141–149. <https://doi.org/10.1016/j.apsoil.2015.11.002>
- Bakker, M. G., Manter, D. K., Sheffin, A. M., Weir, T. L., & Vivanco, J. M. (2012). Harnessing the rhizosphere microbiome through plant breeding and agricultural management. *Plant and Soil*, 360(1-2), 1–13. <https://doi.org/10.1007/s11104-012-1361-x>
- Berg, G., & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology*, 68(1), 1–13. <https://doi.org/10.1111/j.1574-6941.2009.00654.x>
- Berlanas, C., Berbegal, M., Elena, G., Laidani, M., Cibriain, J. F., Sagües, A., & Gramaje, D. (2019). The Fungal and Bacterial Rhizosphere Microbiome Associated With Grapevine Rootstock Genotypes in Mature and Young Vineyards. *Frontiers in Microbiology*, 10, 1142. <https://doi.org/10.3389/fmicb.2019.01142>

- Bokulich, N. A., Thorngate, J. H., Richardson, P. M., & Mills, D. A. (2014). Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(1), E139-48. <https://doi.org/10.1073/pnas.1317377110>
- Brožová, J. (2002). Exploitation of the Mycoparasitic Fungus *Pythium oligandrum* in Plant Protection. *Plant Protection Science* (Vol. 38, No. 1), 29–35.
- Brunori, E., Farina, R., & Biasi, R. (2016). Sustainable viticulture: The carbon-sink function of the vineyard agro-ecosystem. *Agriculture, Ecosystems & Environment*, *223*, 10–21. <https://doi.org/10.1016/j.agee.2016.02.012>
- Burns, K. N., Bokulich, N. A., Cantu, D., Greenhut, R. F., Kluepfel, D. A., O'Geen, A. T., Strauss, S. L., & Steenwerth, K. L. (2016). Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: Differentiation by vineyard management. *Soil Biology and Biochemistry*, *103*, 337–348. <https://doi.org/10.1016/j.soilbio.2016.09.007>
- Carro-Huerga, G., Compant, S., Gorfer, M., Cardoza, R. E., Schmoll, M., Gutiérrez, S., & Casquero, P. A. (2020). Colonization of *Vitis vinifera* L. By the Endophyte *Trichoderma* sp. Strain T154: Biocontrol Activity Against *Phaeoacremonium minimum*. *Frontiers in Plant Science*, *11*, 1170. <https://doi.org/10.3389/fpls.2020.01170>
- Compant, S., Brader, G., Muzammil, S., Sessitsch, A., Lebrihi, A., & Mathieu, F. (2013). Use of beneficial bacteria and their secondary metabolites to control grapevine pathogen diseases. *BioControl*, *58*(4), 435–455. <https://doi.org/10.1007/s10526-012-9479-6>
- Compant, S., Clément, C., & Sessitsch, A. (2010). Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry*, *42*(5), 669–678. <https://doi.org/10.1016/j.soilbio.2009.11.024>
- Compant, S., Mitter, B., Colli-Mull, J. G., Gangl, H., & Sessitsch, A. (2011). Endophytes of grapevine flowers, berries, and seeds: Identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization. *Microbial Ecology*, *62*(1), 188–197. <https://doi.org/10.1007/s00248-011-9883-y>
- Compant, S., Samad, A., Faist, H., & Sessitsch, A. (2019). A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research*, *19*, 29–37. <https://doi.org/10.1016/j.jare.2019.03.004>
- Contreras, F., Díaz, J., Rombolá, A. D., & La Luz Mora, M. de (2019). Prospecting intercropping between subterranean clover and grapevine as potential strategy for improving grapevine performance. *Current Plant Biology*, *19*, 100110. <https://doi.org/10.1016/j.cpb.2019.100110>
- Cordero, J., Freitas, J. R. de, & Germida, J. J. (2020). Bacterial microbiome associated with the rhizosphere and root interior of crops in Saskatchewan, Canada. *Canadian Journal of Microbiology*, *66*(1), 71–85. <https://doi.org/10.1139/cjm-2019-0330>
- Dessaux, Y., Grandclément, C., & Faure, D. (2016). Engineering the Rhizosphere. *Trends in Plant Science*, *21*(3), 266–278. <https://doi.org/10.1016/j.tplants.2016.01.002>
- Deyett, E., & Rolshausen, P. E. (2019). Temporal Dynamics of the Sap Microbiome of Grapevine Under High Pierce's Disease Pressure. *Frontiers in Plant Science*, *10*, 1246. <https://doi.org/10.3389/fpls.2019.01246>
- Di Gaspero, G., & Cattonaro, F. (2010). Application of genomics to grapevine improvement. *Australian Journal of Grape and Wine Research*, *16*, 122–130. <https://doi.org/10.1111/j.1755-0238.2009.00072.x>
- Di Giacinto, S., Friedel, M., Poll, C., Döring, J., Kunz, R., & Kauer, R. (2020). Vineyard management system affects soil microbiological properties. *OENO One*, *54*(1). <https://doi.org/10.20870/oeno-one.2020.54.1.2578>
- Figueroa-López, A. M., Cordero-Ramírez, J. D., Martínez-Álvarez, J. C., López-Meyer, M., Lizárraga-Sánchez, G. J., Félix-Gastélum, R., Castro-Martínez, C., & Maldonado-Mendoza, I. E. (2016). Rhizospheric bacteria of maize with potential for biocontrol of *Fusarium verticillioides*. *SpringerPlus*, *5*, 330. <https://doi.org/10.1186/s40064-016-1780-x>
- Gattullo, C. E., Mezzapesa, G. N., Stellacci, A. M., Ferrara, G., Occhiogrosso, G., Petrelli, G., Castellini, M., & Spagnuolo, M. (2020). Cover Crop for a Sustainable Viticulture: Effects on Soil Properties and Table Grape Production. *Agronomy*, *10*(9), 1334. <https://doi.org/10.3390/agronomy10091334>
- Giri, B., Prasad, R., Wu, Q.-S., & Varma, A. (Eds.). (2019). *Biofertilizers for Sustainable Agriculture and Environment* (Vol. 55). Springer International Publishing. <https://doi.org/10.1007/978-3-030-18933-4>
- González-García, S., Álvarez-Pérez, J. M., Sáenz de Miera, L. E., Cobos, R., Ibañez, A., Díez-Galán, A., Garzón-Jimeno, E., & Coque, J. J. R. (2019). Developing tools for evaluating inoculation methods of biocontrol *Streptomyces* sp. Strains into grapevine plants. *PLoS One*, *14*(1), e0211225. <https://doi.org/10.1371/journal.pone.0211225>
- Gu, Y., Dong, K., Geisen, S., Yang, W., Yan, Y., Gu, D., Liu, N., Borisjuk, N., Luo, Y., & Friman, V.-P. (2020). The effect of microbial inoculant origin on the rhizosphere bacterial community composition and plant growth-promotion. *Plant and Soil*, *452*(1-2), 105–117. <https://doi.org/10.1007/s11104-020-04545-w>
- Hendgen, M., Hoppe, B., Döring, J., Friedel, M., Kauer, R., Frisch, M., Dahl, A., & Kellner, H. (2018). Effects of different management regimes on microbial biodiversity in vineyard soils. *Scientific Reports*, *8*(1), 9393. <https://doi.org/10.1038/s41598-018-27743-0>

- Hohmann, P., Schlaeppi, K., & Sessitsch, A. (2020). Micrope 2019 - emerging research priorities towards microbe-assisted crop production. *FEMS Microbiology Ecology*, *96*(10). <https://doi.org/10.1093/femsec/fiaa177>
- Karimi, B., Cahurel, J.-Y., Gontier, L., Charlier, L., Chovelon, M., Mahé, H., & Ranjard, L. (2020). A meta-analysis of the ecotoxicological impact of viticultural practices on soil biodiversity. *Environmental Chemistry Letters*. Advance online publication. <https://doi.org/10.1007/s10311-020-01050-5>
- Klein Goldewijk, K., Beusen, A., Doelman, J., & Stehfest, E. (2017). Anthropogenic land use estimates for the Holocene – HYDE 3.2. *Earth System Science Data*, *9*(2), 927–953. <https://doi.org/10.5194/essd-9-927-2017>
- Köberl, M., Wagner, P., Müller, H., Matzer, R., Unterfrauner, H., Cernava, T., & Berg, G. (2020). Unraveling the Complexity of Soil Microbiomes in a Large-Scale Study Subjected to Different Agricultural Management in Styria. *Frontiers in Microbiology*, *11*, 1052. <https://doi.org/10.3389/fmicb.2020.01052>
- Lakkis, S., Trotel-Aziz, P., Rabenoelina, F., Schwarzenberg, A., Nguema-Ona, E., Clément, C., & Aziz, A. (2019). Strengthening Grapevine Resistance by *Pseudomonas fluorescens* PTA-CT2 Relies on Distinct Defense Pathways in Susceptible and Partially Resistant Genotypes to Downy Mildew and Gray Mold Diseases. *Frontiers in Plant Science*, *10*, 1112. <https://doi.org/10.3389/fpls.2019.01112>
- Lewandowski, I., Haerdlein, M., & Kaltschmitt, M. (1999). Sustainable Crop Production: Definition and Methodological Approach for Assessing and Implementing Sustainability. *Crop Sci.*(39), 184–193.
- Li, J., Mavrodi, O. V., Hou, J., Blackmon, C., Babiker, E. M., & Mavrodi, D. V. (2020). Comparative Analysis of Rhizosphere Microbiomes of Southern Highbush Blueberry (*Vaccinium corymbosum* L.), Darrow's Blueberry (*V. darrowii* Camp), and Rabbiteye Blueberry (*V. virgatum* Aiton). *Frontiers in Microbiology*, *11*, 370. <https://doi.org/10.3389/fmicb.2020.00370>
- Liang, H., Wang, X., Yan, J., & Luo, L. (2019). Characterizing the Intra-Vineyard Variation of Soil Bacterial and Fungal Communities. *Frontiers in Microbiology*, *10*, 1239. <https://doi.org/10.3389/fmicb.2019.01239>
- Marasco, R., Rolli, E., Fusi, M., Michoud, G., & Daffonchio, D. (2018). Grapevine rootstocks shape underground bacterial microbiome and networking but not potential functionality. *Microbiome*, *6*(1), 3. <https://doi.org/10.1186/s40168-017-0391-2>
- Mendes, L. W., Raaijmakers, J. M., Hollander, M. de, Mendes, R., & Tsai, S. M. (2018). Influence of resistance breeding in common bean on rhizosphere microbiome composition and function. *The ISME Journal*, *12*(1), 212–224. <https://doi.org/10.1038/ismej.2017.158>
- Novello, G., Gamalero, E., Bona, E., Boatti, L., Mignone, F., Massa, N., Cesaro, P., Lingua, G., & Berta, G. (2017). The Rhizosphere Bacterial Microbiota of *Vitis vinifera* cv. Pinot Noir in an Integrated Pest Management Vineyard. *Frontiers in Microbiology*, *8*, 1528. <https://doi.org/10.3389/fmicb.2017.01528>
- OIV (2016). The International Organisation of Vine and Wine. <http://www.oiv.int/en/statistiques/recherche>
- Pacifico, D., Squartini, A., Crucitti, D., Barizza, E., Lo Schiavo, F., Muresu, R., Carimi, F., & Zottini, M. (2019). The Role of the Endophytic Microbiome in the Grapevine Response to Environmental Triggers. *Frontiers in Plant Science*, *10*, 1256. <https://doi.org/10.3389/fpls.2019.01256>
- Ryan, P. R., Dessaux, Y., Thomashow, L. S., & Weller, D. M. (2009). Rhizosphere engineering and management for sustainable agriculture. *Plant and Soil*, *321*(1-2), 363–383. <https://doi.org/10.1007/s11104-009-0001-6>
- Salmon, J.-M., Samson, A., & Beaujouan, M. (2020). How to adapt winegrowing and winemaking practices to improve organic wine production sustainability? *Organic Agriculture*. Advance online publication. <https://doi.org/10.1007/s13165-020-00317-z>
- Schreiner, R. P. (2003). Mycorrhizal Colonization of Grapevine Rootstocks under Field Conditions. *American Journal of Enology and Viticulture* (54:3), 143–149.
- Silva-Valderrama, I., Toapanta, D., Miccono, M. d. L. A., Lolas, M., Díaz, G. A., Cantu, D., & Castro, A. (2020). Biocontrol Potential of Grapevine Endophytic and Rhizospheric Fungi Against Trunk Pathogens. *Frontiers in Microbiology*, *11*, 614620. <https://doi.org/10.3389/fmicb.2020.614620>
- Stempien, E., Pierron, R. J. G., Adendorff, I., van Jaarsveld, W., Halleen, F., & Mostert, L. (2020). Host defence activation and root colonization of grapevine rootstocks by the biological control fungus *Trichoderma atroviride*. *Phytopathologia Mediterranea*(59 (3)), 615–626. <https://doi.org/10.14601/Phyto-11137>
- Tartaglia, M., Bastida, F., Sciarrillo, R., & Guarino, C. (2020). Soil Metaproteomics for the Study of the Relationships Between Microorganisms and Plants: A Review of Extraction Protocols and Ecological Insights. *International Journal of Molecular Sciences*, *21*(22). <https://doi.org/10.3390/ijms21228455>
- Taye, Z. M., Helgason, B. L., Bell, J. K., Norris, C. E., Vail, S., Robinson, S. J., Parkin, I. A. P., Arcand, M., Mamet, S., Links, M. G., Dowhy, T., Siciliano, S., & Lamb, E. G. (2019). Core and Differentially Abundant Bacterial Taxa in the Rhizosphere of Field Grown *Brassica napus* Genotypes: Implications for Canola Breeding. *Frontiers in Microbiology*, *10*, 3007. <https://doi.org/10.3389/fmicb.2019.03007>

- Trotel-Aziz, P., Couderchet, M., Biagiatti, S., & Aziz, A. (2008). Characterization of new bacterial biocontrol agents *Acinetobacter*, *Bacillus*, *Pantoea* and *Pseudomonas* spp. mediating grapevine resistance against *Botrytis cinerea*. *Environmental and Experimental Botany*, *64*(1), 21–32. <https://doi.org/10.1016/j.envexpbot.2007.12.009>
- Úrbez-Torres, J. R., Tomaselli, E., Pollard-Flamand, J., Boulé, J., Gerin, D., & Pollastro, S. (2020). Characterization of *Trichoderma* isolates from southern Italy, and their potential biocontrol activity against grapevine trunk disease fungi. *Phytopathologia Mediterranea*(59 (3)), 425–439. <https://doi.org/10.14601/Phyto-11273>
- Velásquez, A., Vega-Celedón, P., Fiaschi, G., Agnolucci, M., Avio, L., Giovannetti, M., D'Onofrio, C., & Seeger, M. (2020). Responses of *Vitis vinifera* cv. Cabernet Sauvignon roots to the arbuscular mycorrhizal fungus *Funnelformis mosseae* and the plant growth-promoting rhizobacterium *Ensifer meliloti* include changes in volatile organic compounds. *Mycorrhiza*, *30*(1), 161–170. <https://doi.org/10.1007/s00572-020-00933-3>
- Vieira, S., Sikorski, J., Dietz, S., Herz, K., Schrupf, M., Bruelheide, H., Scheel, D., Friedrich, M. W., & Overmann, J. (2020). Drivers of the composition of active rhizosphere bacterial communities in temperate grasslands. *The ISME Journal*, *14*(2), 463–475. <https://doi.org/10.1038/s41396-019-0543-4>
- Wille, L., Messmer, M. M., Studer, B., & Hohmann, P. (2019). Insights to plant-microbe interactions provide opportunities to improve resistance breeding against root diseases in grain legumes. *Plant, Cell & Environment*, *42*(1), 20–40. <https://doi.org/10.1111/pce.13214>
- Yacoub, A., Gerbore, J., Magnin, N., Chambon, P., Dufour, M.-C., Corio-Costet, M.-F., Guyoneaud, R., & Rey, P. (2016). Ability of *Pythium oligandrum* strains to protect *Vitis vinifera* L., by inducing plant resistance against *Phaeoaniella chlamydospora*, a pathogen involved in Esca, a grapevine trunk disease. *Biological Control*, *92*, 7–16. <https://doi.org/10.1016/j.biocontrol.2015.08.005>
- Yacoub, A., Gerbore, J., Magnin, N., Haidar, R., Compant, S., & Rey, P. (2018). Transcriptional analysis of the interaction between the oomycete biocontrol agent, *Pythium oligandrum* and the roots of *Vitis vinifera* L. *Biological Control*, *120*, 26–35. <https://doi.org/10.1016/j.biocontrol.2017.02.007>
- Yacoub, A., Haidar, R., Gerbore, J., Masson, C., Dufour, M.-C., Guyoneaud, R., & Rey, P. (2020). *Pythium oligandrum* induces grapevine defence mechanisms against the trunk pathogen *Neofusicoccum parvum*. *Phytopathologia Mediterranea*(59 (3)), 565–580. <https://doi.org/10.14601/Phyto-11270>
- Yamamoto, T., Iketani, H., Ieki, H., Nishizawa, Y., Notsuka, K., Hibi, T., Hayashi, T., & Matsuta, N. (2000). Transgenic grapevine plants expressing a rice chitinase with enhanced resistance to fungal pathogens. *Plant Cell Reports*, *19*(7), 639–646. <https://doi.org/10.1007/s002999900174>
- Zarraonaindia, I., Owens, S. M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., Bokulich, N. A., Mills, D. A., Martin, G., Taghavi, S., van der Lelie, D., & Gilbert, J. A. (2015). The soil microbiome influences grapevine-associated microbiota. *MBio*, *6*(2). <https://doi.org/10.1128/mBio.02527-14>
- Zucca, G., Smith, D. E., & Mitry, D. J. (2009). Sustainable viticulture and winery practices in California: What is it, and do customers care? *International Journal of Wine Research* (1), 189–194. <https://doi.org/10.2147/IJWR.S5788>



**Chapter 2 – Rootstocks Shape Their Microbiome - Bacterial
Communities in the Rhizosphere of Different Grapevine
Rootstocks**

Article

Rootstocks Shape Their Microbiome—Bacterial Communities in the Rhizosphere of Different Grapevine Rootstocks

Leonie Dries ^{1,*}, Simone Bussotti ^{1,†}, Carlo Pozzi ², Robert Kunz ¹, Sylvia Schnell ³, Otmar Löhnertz ¹ and Anne Vortkamp ⁴

¹ Department of Soil Science and Plant Nutrition, Hochschule Geisenheim University, 65366 Geisenheim, Germany; Simone.bussotti.enol@gmail.com (S.B.); Robert.kunz@hs-gm.de (R.K.); Otmar.loehnertz@hs-gm.de (O.L.)

² Department of Agricultural and Environmental Sciences, University of Milano, 20133 Milano, Italy; carlo.pozzi@unimi.it

³ Research Center for BioSystems, Land Use, and Nutrition (IFZ), Institute of Applied Microbiology, Justus-Liebig University Giessen, 35392 Giessen, Germany; sylvia.schnell@umwelt.uni-giessen.de

⁴ REACH EUREGIO Start-up Center, University of Muenster, 48151 Muenster, Germany; Anne.Vortkamp@wiwi.uni-muenster.de

* Correspondence: Leonie.dries@hs-gm.de; Tel.: +49-(0)6722-502-433

† These authors contributed equally to this work.



Citation: Dries, L.; Bussotti, S.; Pozzi, C.; Kunz, R.; Schnell, S.; Löhnertz, O.; Vortkamp, A. Rootstocks Shape Their Microbiome—Bacterial Communities in the Rhizosphere of Different Grapevine Rootstocks. *Microorganisms* **2021**, *9*, 822. <https://doi.org/10.3390/microorganisms9040822>

Academic Editor: Denis Faure

Received: 24 March 2021

Accepted: 12 April 2021

Published: 13 April 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: The microbiota associated with the rhizosphere is responsible for crucial processes. Understanding how the plant and its bacterial community interact is of great importance to face the upcoming agricultural and viticultural challenges. The composition of the bacterial communities associated with the rhizosphere of grapevines is the result of the interaction between many drivers: biogeography, edaphic factors, soil management and plant genotype. The experimental design of this study aimed to reduce the variability resulting from all factors except the genotype of the rootstock. This was made possible by investigating four ungrafted grapevine rootstock varieties of the same age, grown on the same soil under the same climatic conditions and managed identically. The bacterial communities associated with the rhizosphere of the rootstocks 1103 Paulsen, 140 Ruggeri, 161-49 Couderc and Kober 5BB were characterized with the amplicon based sequencing technique, targeting regions V4–V5 of 16S rRNA gene. Linear discriminant analysis effect Size (LEfSe) analysis was performed to determine differential abundant taxa. The four rootstocks showed similarities concerning the structure of the bacteria assemblage (richness and evenness). Nonetheless, differences were detected in the composition of the bacterial communities. Indeed, all investigated rootstocks recruited communities with distinguishable traits, thus confirming the role of rootstock genotype as driver of the bacteria composition.

Keywords: viticulture; metabarcoding; microbiota; soil; vineyard soil

1. Introduction

The continuous improvement of cultivation-independent techniques is driving a paradigm shift in the field of biology; the expression of a specific phenotype is no longer determined solely by the interaction between genotype and environment but has to take into account the host-associated microorganisms (microbiota) [1]. The microbiome (the genome of the microbiota) provides adaptability and metabolic diversity, broadening the plant capacity to overcome environmental changes and to cope with challenging conditions [2,3]. The microbes can be divided in epiphytes when they colonize the exterior surfaces or in endophytes when they colonize the interior surfaces [4]. Moreover, the distribution of the microbiota is not even throughout the plant, which can be interpreted as an assembly of several niches. The main distinction is between the above ground and the belowground part. However, agricultural productivity is based on microbial activity, much of which occurs in the soil [5]. The productivity of agricultural systems

is highly dependent on the functional processes of microbial communities in the soil, more specifically in the rhizosphere [6]. In particular, the rhizosphere microbiome can positively influence plant development, overall vigor and plant growth by providing nutrients, and has generally been targeted to identify positive relationships between plants and microorganisms [7,8]. However, even if there are no physical barriers, the composition of the soil microbiota is not the same as the rhizosphere microbiota. The soil serves as the initial inoculum, but then the plant actively recruits the bacteria from the soil [9]. Thus, the rhizospheric communities are a subset of the more complex soil microbiota [10]. This selection occurs at the level of the root compartment, which consists of the rhizosphere, rhizoplane and root endosphere [11,12]. Due to this selection, the choice of the right rootstock for recruitment of the soil microbiota is crucial, which is also relevant for grapevine. Even though rootstocks were adopted for their resistance to *Phylloxera*, it is necessary to take into account several other factors, such as grafting compatibility, rooting and propagation ability, resistance to nematodes and pathogens, tolerance to lime, salinity, drought and nutrient uptake [13]. However, the impact of genetic variation on the composition of the microbiome of grapevine species, particularly in the root compartment, is poorly studied [14]. And it can be expected that different rootstocks differentially select their microbial communities from the surrounding soil [4]. Consequently, rootstock genotype affects the selection and recruitment of bacteria that colonize aboveground plant organs such as flowers and fruits and can alter fruit quality [4,15]. Thus, rootstocks could play a role in determining the plant-associated bacterial community by affecting the microbial terroir [16]. This study considered rootstocks derived from the breeding of the most commonly used *Vitis riparia*, *Vitis berlandieri* and *Vitis rupestris* [17]. The experimental design of this study aimed to reduce the variability resulting from environmental factors to unravel the effect of the genotype of the grapevine rootstock on the bacterial communities in the rhizosphere. This was made possible by using ungrafted grapevines of the same age, grown in the same soil under the same climatic conditions and managed identically.

2. Materials and Methods

2.1. Vineyard Location and Vines

The experiment site was located at Geisenheim University, Germany (49°59' N, 7°57' E; 96 m above sea level) in the wine-growing region of Rheingau. The climate of the region is temperate oceanic (Köppen-Geiger classification: Cfb) [18]. The mean annual temperature of the 1986–2010 period is 10.5 °C and the total annual precipitation averages 543.0 mm [19]. The grapevines were planted in a soil mixture (two parts loess soil with one part sand) in cylindrical tubes with a diameter of 15 cm and a height of 120 cm in the year 2015 (Figure 1). The tubes are perforated at the bottom side allowing the water to drain. The following rootstocks were selected for the investigations: 1103 Paulsen (*V. berlandieri* x *V. rupestris*, clone 1 Gm, “1103 P” when abbreviated), 140 Ruggeri (*V. berlandieri* x *V. rupestris* clone 4 Gm, “140 Ru” when abbreviated), 161-49 Couderc (*V. berlandieri* x *V. riparia* clone 3 Gm, “161-49 C” when abbreviated) and Kober 5 BB (*V. berlandieri* x *V. riparia* clone 13-3 Gm).

2.2. Rhizosphere and Soil Sampling

Rhizosphere and soil sampling was performed in July 2020. The tubes with the grapevines were extracted from the ground. Subsequently, each tube was opened using an angle grinder, which completely exposed the root system. This procedure ensured accurate selection and sampling of the rhizosphere soil, which was taken in the very close proximity to the roots. Three replicates per rootstock genotype were investigated. For each replicate, two rhizosphere soil samples (>2 g each) for microbiome analyses were collected and stored at −80 °C. Additionally, for each replicate one soil sample (>100 g) was collected for soil analyses and stored at 4 °C.



Figure 1. Experimental vineyard. The grapevine rootstocks are planted in cylindrical tubes with a diameter of 15 cm and a height of 120 cm.

2.3. Soil Analyses

All soil analyses were performed at the Department of Soil Science and Plant Nutrition, Hochschule Geisenheim University. The soil samples were dried for three days at 30 °C and sieved to a size of 2 mm. For all soil samples (12 samples, $n = 3$ per rootstock) the pH level as well as the concentration of phosphorus (P), potassium (K) and magnesium (Mg) were measured as described previously by Hendgen et al. [20], according to the VDLUFA database of methods [21]. Statistical analyses were conducted with Microsoft® Excel®.

2.4. DNA Extraction, Sample Preparation and Ion Torrent Sequencing

DNA from 500 mg rhizosphere soil per sample was extracted according to Lueders et al. [22] and Mettel et al. [23] with the following modification: instead of the described sodium dodecyl sulfate (SDS) solution, TPM (50 mM TrisHCl, 1.7% Polyvinylpyrrolidone, 20 mM $MgCl_2$) was used. Extracted DNA was solved in water and stored at -80 °C. The Ion Torrent Polymerase-Chain-Reactions (PCRs) were conducted as described in Kaplan et al. [24]. The first Ion Torrent PCR was performed with KAPA HiFi Polymerase kit (Roche Sequencing Store, Basel, Switzerland) according to the manufacturer's protocol. During amplification of the partial sequence of the hypervariable regions (V4-V5), the primer 926R (5'-CCGYCAATTYMTTTRAGTTT-3' [25]) and 520F (5'-AYTGGGYDTAAAGNG-3' [26]) were used. Amplification parameters were 3 min at 95 °C followed by 35 cycles at 98 °C for 20 s, 55 °C for 30 s, 72 °C for 30 s and finally 72 °C for 5 min. The amplified PCR product was confirmed with agarose gel electrophoresis and used as the template of the second Ion Torrent PCR reaction and barcoding according to Kaplan et al. [24]. The PCR was performed with the following amplification parameters: 3 min at 95 °C followed by 10 cycles at 98 °C for 20 s, 55 °C for 30 s, 72 °C for 30 s and finally 72 °C for 7 min. The PCR products were loaded onto an agarose gel and subsequently purified using NucleoSpin® Gel and PCR Clean-up (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). Further, this product was purified with DNA purification beads NucleoMagVR NGS clean-up kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). The Ion Torrent Sequencing was done according to Kaplan et al. [24].

2.5. Bioinformatic Analysis of the Sequencing Data

Bioinformatic analysis was performed with QIIME 2 2020.8 [27]. The obtained raw sequences were demultiplexed using cutadapt [28] with no errors allowed in the barcode sequences. Quality control, sequence denoising, clustering to amplicon sequence variants

(ASVs) (i.e., 100% operational taxonomic units) [29], dereplication and removal of chimera sequences were done with DADA2 [30] (via q2-dada2). Thereby the first 15 bp were deleted and all sequences were cut to a length of 330 bp. Taxonomy was assigned to the ASVs using the q2-feature classifier [31], which uses naïve Bayes machine-learning classifiers [32] to assign taxonomies based on sequence k-mer frequencies. As a reference database, a trained version of the Silva ribosomal database [33] version 137 was used. All ASVs belonging to chloroplasts and mitochondria were removed. All ASVs were aligned with mafft [34] (via q2-alignment) and used to construct a phylogeny with fasttree2 [35] (via q2-phylogeny). Alpha-diversity metrics (Faith's Phylogenetic Diversity [36]—measures of microbiome richness, and Pielou's Evenness) were calculated in R version 4.0.2 [37] using the packages phyloseq version 1.34.0 [38] and picante version 1.8.2 [39]. Beta-diversity metrics (weighted UniFrac [40], unweighted UniFrac [41] and Bray-Curtis dissimilarity [42]—measures of microbiome composition dissimilarity), and Principal Coordinate Analysis (PCoA) were estimated using q2-diversity after samples were rarefied to 2220 sequences per sample. The distance matrices were also analyzed by the PERMANOVA test [43], followed by the PERMDISP test [44] to ensure the homogeneity of dispersion among the four rootstocks. Both the PERMANOVA and PERMDISP tests were performed using 999 permutations. Linear discriminant analysis effect Size (LEfSe) was run to assess differences in bacteria distribution among the rootstocks [45]. The online Galaxy platform [46] was used. The threshold for the logarithmic linear discriminant analysis (LDA) score was set at 2.0 and the non-parametric factorial Kruskal Wallis sum-rank test ($\alpha = 0.05$) was performed with an all-against-all strategy. Significant taxa were visualized with a bar graph and in a taxonomic cladogram.

3. Results

3.1. Soil Analyses

The main chemical characteristics of the sampled soils were measured and a Kruskal-Wallis H test was conducted. The pH levels (Table 1) ranged between 7.53 ± 0.09 and 7.60 ± 0.00 without a statistical difference ($p = 0.89$). The P, K and Mg contents (Table 1) were also statistically similar (respectively, $p = 0.34$, $p = 0.63$, $p = 0.15$).

Table 1. Main chemical characteristics of the soil of the four different rootstocks. Phosphorus (P), potassium (K) and magnesium (Mg) values are expressed in mg kg^{-1} .

Soil Characteristics	1103 P	140 Ru	161-49C	Kober 5BB
pH	7.53 ± 0.12	7.53 ± 0.09	7.60 ± 0.00	7.57 ± 0.12
P	117.72 ± 9.42	194.75 ± 76.05	152.60 ± 19.82	140.97 ± 52.48
K	165.98 ± 20.33	237.90 ± 137.09	193.64 ± 19.56	157.68 ± 58.68
Mg	116.61 ± 12.39	86.45 ± 52.66	152.79 ± 15.04	138.72 ± 22.57

3.2. High-Throughput Amplicon Sequencing

The Ion Torrent sequencing generated 910,225 16S rRNA gene V4-V5 amplicon sequences, 72% of which (642,114) passed the Ion Torrent Sequencing quality selection. After barcoding errors cleaning, the remaining 578,539 reads were demultiplexed to assign each read to the respective sample. Four samples were removed from the subsequent analysis due to the low number of reads (<2827 features; one sample per rootstock). The denoised and filtered reads were clustered into 2034 amplicon sequence variants (ASVs).

3.3. Diversity and Richness of Bacterial Communities

Pielou's evenness and Faith's phylogenetic diversity were used to calculate alpha-diversity (i.e., the diversity within the group). All measures indicated that no significant differences between the structure of the rhizosphere communities were observable (Figure 2). Pielou's evenness reported values approaching 1, meaning that bacteria were evenly distributed among species. To calculate beta-diversity (i.e., the diversity between

groups) the estimators Bray-Curtis, unweighted UniFrac and weighted UniFrac were used. The distance matrices were ordered on a PCoA (Figure 3). The permutation test with pseudo-F ratios revealed a significant effect of the factor “rootstock genotype” on beta-diversity for Bray-Curtis ($p = 0.001$), unweighted UniFrac ($p = 0.001$) and weighted UniFrac ($p = 0.002$) indices. PERMDISP was run to assess the homogeneity of the dispersion. The test led to a p-value of 0.131 for Bray-Curtis, 0.572 for unweighted UniFrac and 0.258 for weighted UniFrac.

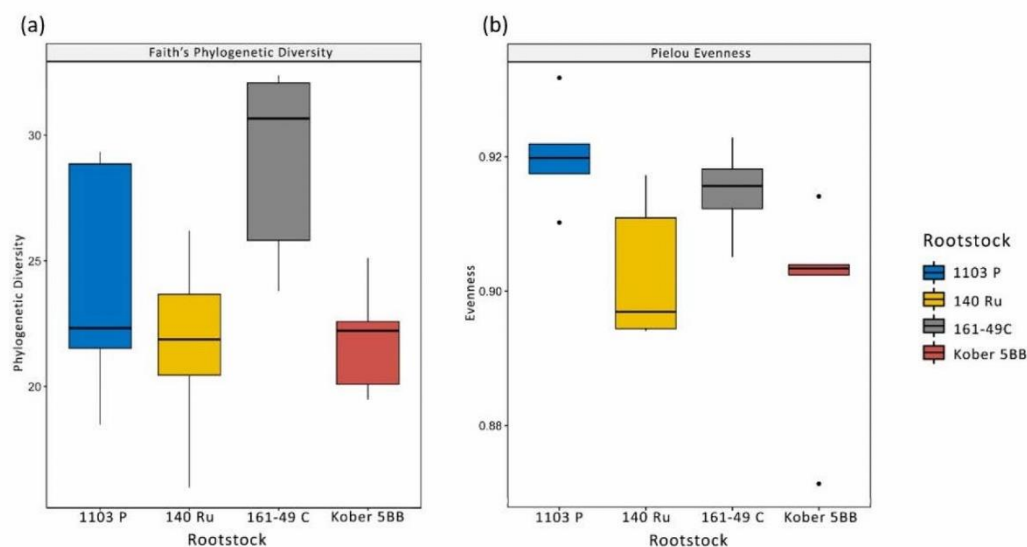


Figure 2. Boxplots reporting the results of alpha diversity analysis. (a) Faith’s phylogenetic plot showing similar values for the four rootstocks. (b) Pielou’s evenness plot showing values higher than 0.895 for all the rootstocks.

3.4. Taxonomy and Distribution among Rootstocks

The taxonomy was assigned using a trained classifier against the Silva database. The 2034 different ASVs, all belonging to the bacteria domain, clustered into 36 different phyla, 97 classes, 199 orders, 257 families and 365 genera (Supplementary Materials Table S1). The rhizosphere was dominated by *Acidobacteriota* (35%), *Proteobacteria* (22%), *Latescibacteriota* (15%), *Methylomirabilota* (6%) and *Gemmatimonadota* (4%). The most represented classes were *Vicinamibacteria* (17%), *Gammaproteobacteria* (15%), *Latescibacterota* (15%), *Acidobacteriae* (9%), *Alphaproteobacteria* (7%), *Methylomirabilia* (6%) and *Gemmatimonadetes* (4%). The linear discriminant analysis effect size (LEfSe) detected 68 bacterial clades differently distributed among the rootstocks (Figures 4 and 5). Significant differences (LDA score > 2.0) were found for each taxonomic level: phylum (2), class (6), order (15), family (20) and genus (25). *Proteobacteria* was the most heterogeneously distributed phylum (21 clades, 12 belonging to *Alphaproteobacteria* and 9 belonging to *Gammaproteobacteria*), followed by the phyla *Chloroflexi* (11 clades), *Methylomirabilota* (7 clades) and *Myxococcota* (7 clades). The rootstock 1103 Paulsen reported the highest number of differentially abundant taxa (32).

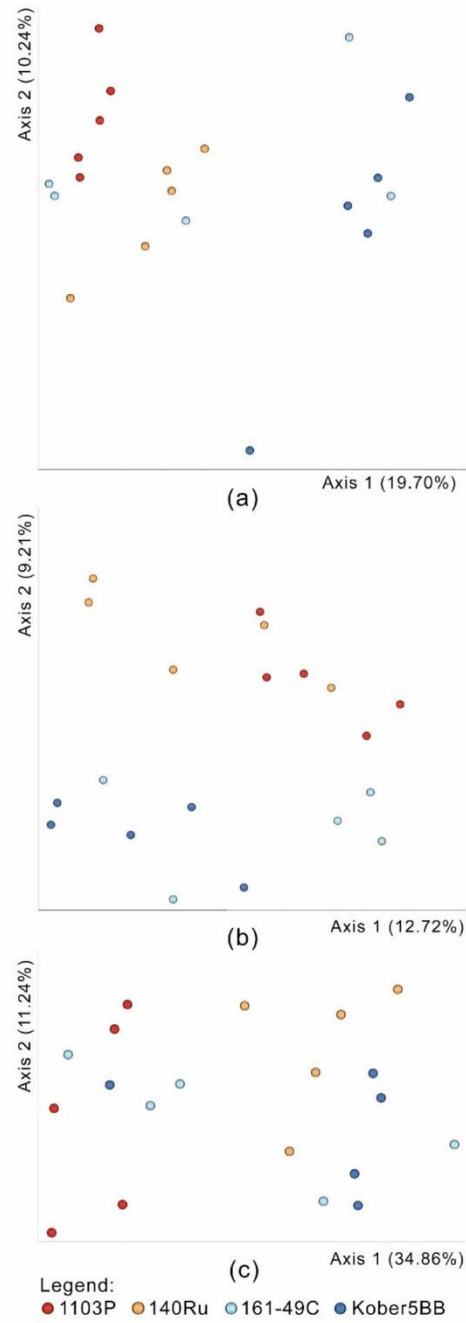


Figure 3. Principal Coordinate Analyses (PCoAs) reporting the distance matrices of the beta diversity indexes: (a) Bray-Curtis, (b) unweighted UniFrac and (c) weighted UniFrac.

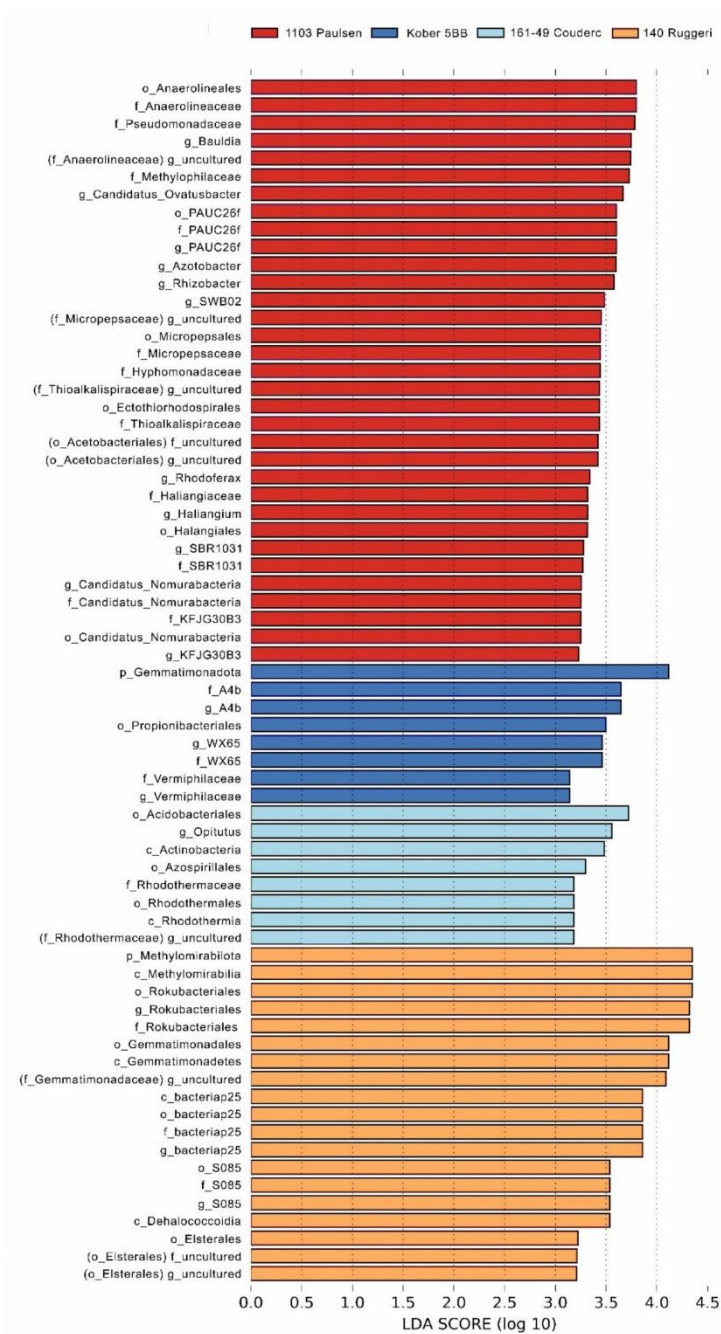


Figure 4. Histogram of the linear discriminant analysis (LDA) scores reveals the most differentially abundant taxa among the four rootstocks 1103 Paulsen, 140 Ruggeri, 161-49 Couderc and Kober 5BB.

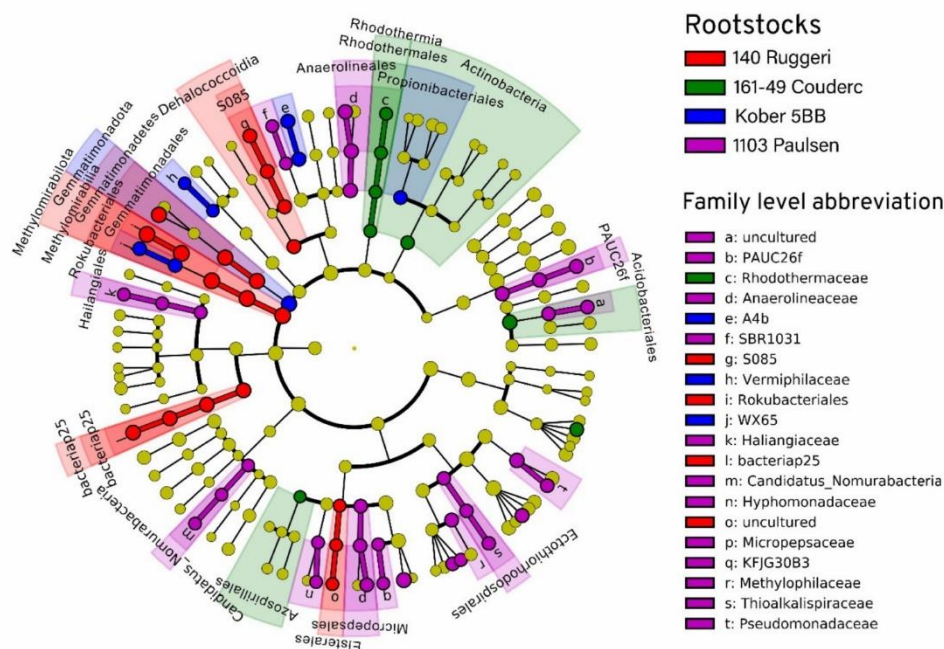


Figure 5. Linear discriminant analysis (LDA) effect size taxonomic cladogram comparing the bacterial diversity of the four rootstocks. Each ring represents a taxonomic level, with kingdom, phylum, class, order, family and genus appearing from the center to the periphery. Each node represents a taxonomic unit found in the dataset. For each significantly discriminating taxon detected, the corresponding node and branch region in the taxonomic cladogram is colored according to the highest ranked group for that taxon. If the taxon is not significantly different between rootstocks, the corresponding node is colored yellow.

4. Discussion

The experimental design of this study aimed at minimizing the variability from all factors but the rootstock genotype. This was made possible by using grapevines of the same age, located in the same soil sharing also the same climatic conditions, and managed identically. The amplicon sequencing analysis revealed that rootstock rhizospheres recruited complex bacterial communities mainly composed of *Acidobacteriota*, *Proteobacteria*, *Latescibacterota*, *Methylomirabilota* and *Gemmatimonadota*. These results are in partial agreement with the data of other recent studies [4,14,16]. According to Berlanas et al. [14], the dominant phyla found in two vineyards located in Northeastern Spain were *Proteobacteria*, *Actinobacteria*, *Acidobacteria* and *Bacteroidetes*. Similarly, Marasco et al. [16] reported phylum *Proteobacteria* as prevalent, followed by *Actinobacteria*, *Bacteroidetes*, *Chloroflexi* and *Acidobacteria* in an Oltrepò Pavese (Italy) vineyard. These results highlight that there is a core of bacteria always recruited by grapevine despite the different conditions (e.g., biogeography, and climatic or edaphic factors). Among them, *Proteobacteria* is commonly the dominant phylum followed by *Actinobacteria*. Both phyla play an important role in carbon cycling and production of secondary metabolites [47]. *Proteobacteria* comprise organisms with a broad variety of metabolic capabilities; alpha-, beta-, gamma- and delta- *Proteobacteria* are the classes mostly described in soil studies. In this study, only *Gamma**proteobacteria* and *Alphaproteobacteria* were found. Both classes are considered copiotrophs (r-strategist) and can easily colonize environments characterized by abundance of nutrients, such as the rhizosphere [48]. Instead, *Actinobacteria* are described as oligotrophic k-strategists

growing slowly and with low nutritional requirements [49]. In this project, the percentage of ASVs assigned to this phylum was rather low (0.56%) in comparison to other studies. Conversely, the percentage shown by *Acidobacteriota* phylum was unusually high (~35%), and greater than *Proteobacteria* phylum (~22%). *Acidobacteriota* phylum is one of the most widespread and abundant on the planet [50], but its abundance is reported to decrease moving from bulk soil to rhizosphere [2]. Information about *Acidobacteriota* functionality is scarce since there are issues in cultivating the majority of the members of this phylum [51]. A negative correlation between the abundance of *Acidobacteriota* and concentration of the organic carbon in soil was reported [50]. This, together with the low growth rate, may indicate this phylum as oligotrophic [50]. Additionally, Zarraonaindia et al. [4] reported high abundance of *Acidobacteriota* together with unusually low abundance of *Actinobacteria*. It may be suggested that *Acidobacteriota* can replace *Actinobacteria* when some biotic or abiotic factors hamper the normal thriving of the latter. Indeed, both phyla are described as oligotrophic k-strategist [50] and occupy the same niche. Remarkably, this study is the first to report the occurrence of the phylum *Latescibacterota* (previously known as WS3) among the most abundant phyla in vineyard soils. *Gemmatimonadota* phylum was first associated with vineyard soils by Novello et al. [52], and this study confirmed their presence in the grapevine rhizosphere. *Gemmatimonadota* is reported as one of the top nine phyla found in soils [53]. Nonetheless, little is known about its metabolic capabilities since most of its members are uncultivated. It was observed that the microbial communities associated with the four rootstocks did not significantly differ in evenness and richness. Indeed, all the four rootstocks showed a bacterial community with no clearly dominating species and with similar phylogenetic richness. This is consistent with the theory of a well conserved core of bacteria associated with the grapevine rhizosphere [14]. This study, however, showed that rootstocks 1103P, 140 Ru, 161-49C and Kober 5BB were able to assemble distinct bacterial microbiota at the rhizosphere level. The percentage explained by weighted (46%) and unweighted UniFrac (22%) suggests that the differences are mainly driven by a different recruitment of the same bacteria. Moreover, the LEfSe analysis detected 68 clades significantly differentially recruited by the four investigated rootstocks. These differences confirm the rootstock genotype as a substantial driver of the bacterial communities associated with the rhizosphere. The rootstock genotype may exert its influence on the rhizosphere associated communities through specific root exudates [54–56] and by means of its immune system. Indeed, it has been proven that the immune system can affect not only the microbial composition of the root interior surfaces but also the communities thriving near the root [57,58]. Marasco et al. [16] have investigated whether different rootstocks affect the recruitment of the bacteria from the surrounding soil comparing ungrafted and grafted grapevines of the *Vitis vinifera* cultivar Barbera, all cultivated in the same soil. They concluded that the rootstock can influence the diversity and richness of the bacterial communities associated with the root and that the rootstock is a factor determining the specificity of the microbiota independently of the scion cultivar. D'Amico et al. [59] have highlighted the importance of the microbiota recruited by the rootstock with a cultivation-independent technique: the same scion (*V. vinifera* cv Lambrusco) was grafted onto two different rootstocks (1103 Paulsen and Kober 5BB) in the same vineyard, where only the grapevines grafted on 1103 Paulsen had potassium absorption problems. The analysis of the microbiota with an amplicon-based approach has revealed that the rootstock 1103 Paulsen is not able to successfully recruit several potassium solubilizing microorganisms [59]. Thus, the knowledge of the microbiota associated with diverse rootstocks is valuable to make the right choice at the moment of the implant, to intervene when the choice is already taken, and also for breeders to select for rootstocks with a specific microbiota.

5. Conclusions

These results show that the rootstock genotype affects the composition but not the structure of the bacterial community in the rhizosphere. However, recognizing the associations between bacteria and rootstock is crucial but still not sufficient to exploit this

information from a practical perspective. In conclusion, it is essential to understand the recruitment mechanisms of grapevine to microbial communities and to identify the beneficial taxa in a more detailed context. Following studies should use a whole genome sequencing approach and combine it with other meta-omics methods. For example, the use of metatranscriptomics and metaproteomics together allows the characterization of the microbial communities thriving in the rhizosphere and simultaneously the comprehension of their role. However, applying several -omics methods will inevitably lead to a better comprehension of the rhizosphere microbiome in relation to soil type, grapevine variety, rootstock and environmental conditions.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/microorganisms9040822/s1>, Table S1: Amplicon Sequenced Variants Table.

Author Contributions: Conceptualization, A.V., L.D. and S.B.; methodology, L.D., S.B. and R.K.; formal analysis, S.B.; investigation, L.D. and S.B.; resources S.S. and O.L.; data curation, L.D. and S.B.; writing—original draft preparation, L.D. and S.B.; writing—review and editing, L.D., S.B., C.P., S.S., R.K., O.L. and A.V.; visualization, S.B.; supervision, C.P., S.S., O.L. and A.V.; project administration, L.D., A.V. and O.L.; funding acquisition, A.V. and O.L. All authors have read and agreed to the published version of the manuscript.

Funding: We thank Forschungsring des Deutschen Weinbaus (FDW) for funding this project.

Data Availability Statement: The sequencing reads from this study are openly available in the National Center for Biotechnology Information (ncbi) under accession no. PRJNA716534. This data can be found at <https://www.ncbi.nlm.nih.gov/bioproject/716534>.

Acknowledgments: We appreciate and acknowledge the members of the Department of Soil Science and Plant Nutrition, Hochschule Geisenheim University, for their assistance in sample taking and soil analyses. We thank Joachim Schmid, Department of Grapevine Breeding, Hochschule Geisenheim University, for the opportunity to sample the experimental vineyard. We thank Annette Reineke and Olivia Herczynski, Department of Crop Protection, Hochschule Geisenheim University, for the opportunity to use the molecular biology laboratory and for their helpful advices. We are very grateful to Bellinda Schneider, Justus-Liebig University Gießen, for technical support with Ion Torrent Sequencing. We further thank Maximilian Hendgen for carefully proofreading the manuscript. We acknowledge support by the German Research Foundation (Deutsche Forschungsgemeinschaft DFG)—project number 432888308—and the Open Access Publishing Fund of Geisenheim University.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Bordenstein, S.R.; Theis, K.R. Host Biology in Light of the Microbiome: Ten Principles of Holobionts and Hologenomes. *PLoS Biol.* **2015**, *13*, e1002226. [[CrossRef](#)]
- Rosenberg, E.; Zilber-Rosenberg, I. Microbes Drive Evolution of Animals and Plants: The Hologenome Concept. *mBio* **2016**, *7*, e01395. [[CrossRef](#)] [[PubMed](#)]
- Bulgarelli, D.; Schlaeppi, K.; Spaepen, S.; Ver Loren van Themaat, E.; Schulze-Lefert, P. Structure and functions of the bacterial microbiota of plants. *Annu. Rev. Plant Biol.* **2013**, *64*, 807–838. [[CrossRef](#)] [[PubMed](#)]
- Zarraonaindia, I.; Owens, S.M.; Weisenhorn, P.; West, K.; Hampton-Marcell, J.; Lax, S.; Bokulich, N.A.; Mills, D.A.; Martin, G.; Taghavi, S.; et al. The soil microbiome influences grapevine-associated microbiota. *mBio* **2015**, *6*. [[CrossRef](#)] [[PubMed](#)]
- Bakker, M.G.; Manter, D.K.; Sheflin, A.M.; Weir, T.L.; Vivanco, J.M. Harnessing the rhizosphere microbiome through plant breeding and agricultural management. *Plant Soil* **2012**, *360*, 1–13. [[CrossRef](#)]
- Vega-Avila, A.D.; Gumiere, T.; Andrade, P.A.M.; Lima-Perim, J.E.; Durrer, A.; Baigori, M.; Vazquez, F.; Andreote, F.D. Bacterial communities in the rhizosphere of *Vitis vinifera* L. cultivated under distinct agricultural practices in Argentina. *Antonie Van Leeuwenhoek* **2015**, *107*, 575–588. [[CrossRef](#)] [[PubMed](#)]
- Ryan, P.R.; Dessaux, Y.; Thomashow, L.S.; Weller, D.M. Rhizosphere engineering and management for sustainable agriculture. *Plant Soil* **2009**, *321*, 363–383. [[CrossRef](#)]
- Taye, Z.M.; Helgason, B.L.; Bell, J.K.; Norris, C.E.; Vail, S.; Robinson, S.J.; Parkin, I.A.P.; Arcand, M.; Mamet, S.; Links, M.G.; et al. Core and Differentially Abundant Bacterial Taxa in the Rhizosphere of Field Grown *Brassica napus* Genotypes: Implications for Canola Breeding. *Front. Microbiol.* **2019**, *10*, 3007. [[CrossRef](#)]
- Gilbert, J.A.; van der Lelie, D.; Zarraonaindia, I. Microbial terroir for wine grapes. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 5–6. [[CrossRef](#)]

10. Berendsen, R.L.; Pieterse, C.M.J.; Bakker, P.A.H.M. The rhizosphere microbiome and plant health. *Trends Plant Sci.* **2012**, *17*, 478–486. [[CrossRef](#)]
11. Edwards, J.; Johnson, C.; Santos-Medellín, C.; Lurie, E.; Podishetty, N.K.; Bhatnagar, S.; Eisen, J.A.; Sundaresan, V. Structure, variation, and assembly of the root-associated microbiomes of rice. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E911–E920. [[CrossRef](#)] [[PubMed](#)]
12. Bonito, G.; Reynolds, H.; Robeson, M.S.; Nelson, J.; Hodkinson, B.P.; Tuskan, G.; Schadt, C.W.; Vilgalys, R. Plant host and soil origin influence fungal and bacterial assemblages in the roots of woody plants. *Mol. Ecol.* **2014**, *23*, 3356–3370. [[CrossRef](#)]
13. Granett, J.; Walker, A.M.; Kocsis, L.; Omer, A.D. Biology and management of grape phylloxera. *Annu. Rev. Entomol.* **2001**, 387–412. [[CrossRef](#)] [[PubMed](#)]
14. Berlanas, C.; Berbegal, M.; Elena, G.; Laidani, M.; Cibriain, J.F.; Sagües, A.; Gramaje, D. The Fungal and Bacterial Rhizosphere Microbiome Associated With Grapevine Rootstock Genotypes in Mature and Young Vineyards. *Front. Microbiol.* **2019**, *10*, 1142. [[CrossRef](#)] [[PubMed](#)]
15. Bokulich, N.A.; Thorngate, J.H.; Richardson, P.M.; Mills, D.A. Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, E139–E148. [[CrossRef](#)] [[PubMed](#)]
16. Marasco, R.; Rolli, E.; Fusi, M.; Michoud, G.; Daffonchio, D. Grapevine rootstocks shape underground bacterial microbiome and networking but not potential functionality. *Microbiome* **2018**, *6*, 3. [[CrossRef](#)] [[PubMed](#)]
17. Dry, P.R.; Coombe, B.G.; Anderson, C.J. (Eds.) *Viticulture*, 2nd ed.; Winetitles Pty.: Adelaide, Australia, 2005; ISBN 0975685007.
18. Reineke, A.; Selim, M. Elevated atmospheric CO₂ concentrations alter grapevine (*Vitis vinifera*) systemic transcriptional response to European grapevine moth (*Lobesia botrana*) herbivory. *Sci. Rep.* **2019**, *9*, 2995. [[CrossRef](#)] [[PubMed](#)]
19. DWD Climate Data Center. *Historical Daily Station Observations (Temperature, Pressure, Precipitation, Sunshine Duration, etc.) for Germany*; Version v006; DWD: Köln, Germany, 2018.
20. Hendgen, M.; Hoppe, B.; Döring, J.; Friedel, M.; Kauer, R.; Frisch, M.; Dahl, A.; Kellner, H. Effects of different management regimes on microbial biodiversity in vineyard soils. *Sci. Rep.* **2018**, *8*, 9393. [[CrossRef](#)] [[PubMed](#)]
21. Bassler, R. (Ed.) Band I: Die Untersuchung von Böden. In *Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik*; VDLUFA-Verlag: Darmstadt, Germany, 2011.
22. Lueders, T.; Manefield, M.; Friedrich, M.W. Enhanced sensitivity of DNA- and rRNA-based stable isotope probing by fractionation and quantitative analysis of isopycnic centrifugation gradients. *Environ. Microbiol.* **2004**, *6*, 73–78. [[CrossRef](#)]
23. Mettel, C.; Kim, Y.; Shrestha, P.M.; Liesack, W. Extraction of mRNA from soil. *Appl. Environ. Microbiol.* **2010**, *76*, 5995–6000. [[CrossRef](#)]
24. Kaplan, H.; Ratering, S.; Felix-Henningsen, P.; Schnell, S. Stability of in situ immobilization of trace metals with different amendments revealed by microbial ¹³C-labelled wheat root decomposition and efflux-mediated metal resistance of soil bacteria. *Sci. Total Environ.* **2019**, *659*, 1082–1089. [[CrossRef](#)]
25. Quince, C.; Lanzen, A.; Davenport, R.J.; Turnbaugh, P.J. Removing noise from pyrosequenced amplicons. *BMC Bioinf.* **2011**, *12*, 38. [[CrossRef](#)]
26. Claesson, M.J.; O’Sullivan, O.; Wang, Q.; Nikkilä, J.; Marchesi, J.R.; Smidt, H.; de Vos, W.M.; Ross, R.P.; O’Toole, P.W. Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. *PLoS ONE* **2009**, *4*, e6669. [[CrossRef](#)]
27. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [[CrossRef](#)]
28. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J.* **2011**, *17*, 10. [[CrossRef](#)]
29. Callahan, B.J.; McMurdie, P.J.; Holmes, S.P. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME J.* **2017**, 2639–2643. [[CrossRef](#)] [[PubMed](#)]
30. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13*, 581–583. [[CrossRef](#)]
31. Bokulich, N.A.; Kaehler, B.D.; Rideout, J.R.; Dillon, M.; Bolyen, E.; Knight, R.; Huttley, G.A.; Gregory Caporaso, J. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2’s q2-feature-classifier plugin. *Microbiome* **2018**, *6*, 90. [[CrossRef](#)] [[PubMed](#)]
32. Pedregosa, F.; Varoquaux, G.; Gramfort, A.; Michel, V.; Thirion, B.; Grisel, O.; Blondel, M.; Prettenhofer, P.; Weiss, R.; Dubourg, V.; et al. Scikit-learn: Machine learning in python. *J. Mach. Learn. Res.* **2011**, *12*, 2825–2830.
33. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F.O. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* **2013**, *41*, D590–D596. [[CrossRef](#)]
34. Katoh, K.; Misawa, K.; Kuma, K.-i.; Miyata, T. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **2002**, 3059–3066. [[CrossRef](#)] [[PubMed](#)]
35. Price, M.N.; Dehal, P.S.; Arkin, A.P. FastTree 2—Approximately Maximum-Likelihood Trees for Large Alignments. *PLoS ONE* **2010**, *5*, e9490. [[CrossRef](#)] [[PubMed](#)]
36. Faith, D.P. Conservation evaluation and phylogenetic diversity. *Biol. Conserv.* **1992**, *61*, 1–10. [[CrossRef](#)]
37. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2020.

38. McMurdie, P.J.; Holmes, S. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* **2013**, *8*, e61217. [[CrossRef](#)]
39. Kembel, S.W.; Cowan, P.D.; Helmus, M.R.; Cornwell, W.K.; Morlon, H.; Ackerly, D.D.; Blomberg, S.P.; Webb, C.O. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* **2010**, *26*, 1463–1464. [[CrossRef](#)]
40. Lozupone, C.A.; Hamady, M.; Kelley, S.T.; Knight, R. Quantitative and qualitative beta diversity measures lead to different insights into factors that structure microbial communities. *Appl. Environ. Microbiol.* **2007**, *73*, 1576–1585. [[CrossRef](#)]
41. Lozupone, C.; Knight, R. UniFrac: A new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* **2005**, *71*, 8228–8235. [[CrossRef](#)]
42. Bray, J.R.; Curtis, J.T. An Ordination of the Upland Forest Communities of Southern Wisconsin. *Ecol. Monogr.* **1957**, *27*, 325–349. [[CrossRef](#)]
43. Anderson, M.J. *Permutational Multivariate Analysis of Variance (PERMANOVA)*; Wiley & Sons: Hoboken, NJ, USA, 2017; pp. 1–15. [[CrossRef](#)]
44. Anderson, M.J. Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* **2006**, *62*, 245–253. [[CrossRef](#)]
45. Segata, N.; Izard, J.; Waldron, L.; Gevers, D.; Miropolsky, L.; Garrett, W.S.; Huttenhower, C. Metagenomic biomarker discovery and explanation. *Genome Biol.* **2011**, *12*, R60. [[CrossRef](#)]
46. Afgan, E.; Baker, D.; Batut, B.; van den Beek, M.; Bouvier, D.; Cech, M.; Chilton, J.; Clements, D.; Coraor, N.; Grünig, B.A.; et al. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. *Nucleic Acids Res.* **2018**, *46*, W537–W544. [[CrossRef](#)] [[PubMed](#)]
47. Jenkins, S.N.; Waite, I.S.; Blackburn, A.; Husband, R.; Rushton, S.P.; Manning, D.C.; O'Donnell, A.G. Actinobacterial community dynamics in long term managed grasslands. *Antonie Van Leeuwenhoek* **2009**, *95*, 319–334. [[CrossRef](#)]
48. Fierer, N.; Bradford, M.A.; Jackson, R.B. Toward an ecological classification of soil bacteria. *Ecology* **2007**, 1354–1364. [[CrossRef](#)] [[PubMed](#)]
49. Fierer, N.; Schimel, J.P.; Holden, P.A. Variations in microbial community composition through two soil depth profiles. *Soil Biol. Biochem.* **2003**, *35*, 167–176. [[CrossRef](#)]
50. Kielak, A.M.; Barreto, C.C.; Kowalchuk, G.A.; van Veen, J.A.; Kuramae, E.E. The Ecology of Acidobacteria: Moving beyond Genes and Genomes. *Front. Microbiol.* **2016**, *7*, 744. [[CrossRef](#)] [[PubMed](#)]
51. Navarrete, A.A.; Kuramae, E.E.; de Hollander, M.; Pijl, A.S.; van Veen, J.A.; Tsai, S.M. Acidobacterial community responses to agricultural management of soybean in Amazon forest soils. *FEMS Microbiol. Ecol.* **2013**, *83*, 607–621. [[CrossRef](#)]
52. Novello, G.; Gamalero, E.; Bona, E.; Boatti, L.; Mignone, F.; Massa, N.; Cesaro, P.; Lingua, G.; Berta, G. The Rhizosphere Bacterial Microbiota of *Vitis vinifera* cv. Pinot Noir in an Integrated Pest Management Vineyard. *Front. Microbiol.* **2017**, *8*, 1528. [[CrossRef](#)]
53. Janssen, P.H. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Appl. Environ. Microbiol.* **2006**, *72*, 1719–1728. [[CrossRef](#)]
54. Matthews, A.; Pierce, S.; Hipperson, H.; Raymond, B. Rhizobacterial Community Assembly Patterns Vary Between Crop Species. *Front. Microbiol.* **2019**, *10*, 581. [[CrossRef](#)]
55. Badri, D.V.; Chaparro, J.M.; Zhang, R.; Shen, Q.; Vivanco, J.M. Application of natural blends of phytochemicals derived from the root exudates of *Arabidopsis* to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. *J. Biol. Chem.* **2013**, *288*, 4502–4512. [[CrossRef](#)]
56. Bulgarelli, D.; Rott, M.; Schlaeppi, K.; Ver Loren van Themaat, E.; Ahmadinejad, N.; Assenza, F.; Rauf, P.; Huettel, B.; Reinhardt, R.; Schmelzer, E.; et al. Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature* **2012**, *488*, 91–95. [[CrossRef](#)] [[PubMed](#)]
57. Liu, Z.; Beskrovnyaya, P.; Melnyk, R.A.; Hossain, S.S.; Khorasani, S.; O'Sullivan, L.R.; Wiesmann, C.L.; Bush, J.; Richard, J.D.; Haney, C.H. A Genome-Wide Screen Identifies Genes in Rhizosphere-Associated *Pseudomonas* Required to Evade Plant Defenses. *mBio* **2018**, *9*. [[CrossRef](#)]
58. Lebeis, S.L.; Paredes, S.H.; Lundberg, D.S.; Breakfield, N.; Gehring, J.; McDonald, M.; Malfatti, S.; Glavina del Rio, T.; Jones, C.D.; Tringe, S.G.; et al. PLANT MICROBIOME. Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science* **2015**, *349*, 860–864. [[CrossRef](#)] [[PubMed](#)]
59. D'Amico, F.; Candela, M.; Turrone, S.; Biagi, E.; Brigidi, P.; Bega, A.; Vancini, D.; Rampelli, S. The Rootstock Regulates Microbiome Diversity in Root and Rhizosphere Compartments of *Vitis vinifera* Cultivar Lambrusco. *Front. Microbiol.* **2018**, *9*, 2240. [[CrossRef](#)] [[PubMed](#)]

Chapter 3 – The metabolic active bacterial communities of grapevine rhizosphere in dependence on rootstock and scion variety

The metabolic active bacterial communities of grapevine rhizosphere in dependence on rootstock and scion variety

Leonie Dries^{1,*}, Stefan Ratering², Simone Bussotti³, Otmar Löhnertz¹, Anne Vorkamp⁴, and Sylvia Schnell²

¹Department of Soil Science and Plant Nutrition, Hochschule Geisenheim University, 65366 Geisenheim, Germany; leonie.dries@hs-gm.de; otmar.loehnertz@hs-gm.de

²Research Center for BioSystems, Land Use, and Nutrition (IFZ), Institute of Applied Microbiology, Justus-Liebig University Giessen, 35392 Giessen, Germany; stefan.ratering@umwelt.uni-giessen.de; sylvia.schnell@umwelt.uni-giessen.de

³AGRION, Fondazione per la Ricerca l'Innovazione e lo Sviluppo Tecnologico dell'Agricoltura Piemontese, Manta (CN), 12030, Italy

⁴REACH EUREGIO Start-up Center, University of Muenster, 48151 Muenster, Germany; anne.vorkamp@wiwi.uni-muenster.de

*corresponding author: leonie.dries@hs-gm.de

Abstract

The rhizosphere is the space where crucial processes for the productivity of viticultural systems take place. The composition of the bacterial communities associated with the rhizosphere of grapevines is known to depend on plant genotype. However, the genotype of grafted grapevines differs between scion and rootstock; the role of each genotype is unclear. In order to disentangle the effect of scion and rootstock, the rRNA (V4–V5 region of 16S rRNA) extracted from the rhizosphere of the grape varieties Riesling and Mueller-Thurgau ungrafted vs. grafted on different rootstocks were sequenced in three experimental set-ups in two different years. The bioinformatic analysis with tools designed to be robust for compositional data showed, that the investigated rootstocks or scions or combinations, respectively, recruited bacterial communities with distinguishable traits. Statistical differences were revealed between ungrafted Riesling and Mueller-Thurgau, and between grafted Riesling and ungrafted Riesling, and ungrafted Mueller-

Thurgau and grafted Mueller-Thurgau. Thus, confirming the role of scion and rootstock genotype as a driver of the structure and composition of bacterial communities in the rhizosphere of grapevines.

Keywords: vineyard soil, viticulture, grape variety, microbiome, compositional data analysis, metatranscriptome

Introduction

Plants are colonized both below and above ground by a variety of microbes that serve for their mutualistic benefits. In particular, the microbiota associated with the plant rhizosphere (rhizosphere microbiota) are involved in important processes such as growth modulation, defense responses and nutrients uptake (Berendsen et al., 2012; Durán et al., 2018; Hu et al., 2018; Li et al., 2020; Schlaeppli & Bulgarelli, 2015; Zhang et al., 2019). In general, the rhizosphere microbiome is often described as a positive interaction between plants and microorganisms (Ryan et al., 2009; Taye et al., 2019), involving numerous previously unknown taxa (Vieira et al., 2020). To date, the majority of rhizobiome studies has focused on model plants such as *Arabidopsis thaliana* (Alegria Terrazas et al., 2016) or annual crop plants such as barley (Bulgarelli et al., 2013), canola, wheat, pea, and lentil (Cordero et al., 2020), oilseed rape (Etesami & Alikhani, 2016) and maize (Peiffer et al., 2013). However, for supporting crop growth, especially under difficult conditions (Timmusk et al., 2014), it is also remarkably important to consider the rhizosphere microorganisms of perennial plants such as grapevines (Marasco et al., 2018).

Microbial communities associated with grapevines and wine, respectively, have already been extensively studied (Burns et al., 2016; Contreras et al., 2019; Gattullo et al., 2020; Hendgen et al., 2018; Holland et al., 2014). In vineyard soil, the microbial communities have been described for example as a function of spatial distribution or management practices (Bokulich et al., 2014; Hendgen et al., 2018; Holland et al., 2014; Vega-Avila et al., 2015). Nonetheless, the bulk of these studies were based on soil samples taken only near the grapevines, not on veritable rhizosphere samples. The rhizosphere, however, is the location where crucial processes for the productivity of the agricultural systems take place mediated by microorganisms. Therefore, the

processes and microorganisms in the rhizosphere need to be considered in order to study the direct interaction between the grapevine root and the surrounding soil.

The choice of grapevine variety impacts the microorganisms in the grapevine rhizosphere (Berlanas et al., 2019; Marasco et al., 2018). However, it should be noted that since the nineteenth century, *Vitis vinifera* cultivars have been cultivated as scions, grafted onto *Phylloxera*-resistant *Vitis* sp. rootstocks. Despite this, it is still in the beginning to uncover the mechanisms underlying the mechanisms of grafted grapevines and the interactions between the grafted scion and the rootstock (as reviewed by Gautier et al., 2019). In future research, rootstock selection is therefore also inevitable with regard to their microbial community (Zarraonaindia et al., 2015). Vink et al. (2021) investigated differences in the microbial communities in the rhizosphere of 11-year-old grapevine of four scion cultivars and four rootstock types regarding alpha- and beta-diversity indices. Thus, they concluded that bacterial diversity is affected by both scion and rootstock variety. However, this effect depends on the diversity measures and the specific rootstock-scion combinations considered (Vink et al., 2021). Nevertheless, the knowledge about the influence of the rootstock, the grape variety and their interaction is still limited (Berlanas et al., 2019). Therefore, with the aim to investigate all these factors independently, a study was carried out under controlled conditions to reduce the variability resulting from all factors except the genotype of the rootstock and scion with potted grapevines. In three different experimental designs, the rhizobiome of two ungrafted *Vitis vinifera* varieties (Riesling and Mueller-Thurgau) were compared with the ones of Riesling and Mueller-Thurgau grapevines grafted on different rootstocks. In order to get a better insight into the active microbiome of the rhizosphere of the grapevines, a metabarcoding analysis was performed on the extracted RNA (Carvalhais et al., 2012; Garoutte et al., 2016; Turner et al., 2013) that indicates microbial activity (Singh et al., 2018). Additionally, to the best of our knowledge, no compositional data analyses of 16S rRNA data of the grapevine rhizosphere microbiome with different scions and rootstock cultivars has been carried out before. Data derived from high-throughput sequencing of biological samples must be considered as compositions rather than counts, as ratio-based analyses can lead to qualitatively incorrect conclusions (Fernandes et al., 2014; McLaren et al., 2019; Quinn et al., 2019). Due to the controlled experimental conditions, the effect of the grape variety and rootstock respectively on the metatranscriptome of the bacterial communities in the rhizosphere could be investigated.

Materials and methods

2.1 Plant material

The independent experiments were located at Hochschule Geisenheim, University, Germany. The grapevines were planted in pots (15 cm x 15 cm x 18.5 cm) with soil (Einheitserde Typ ED 73, H. Nitsch & Sohn GmbH & Co. KG, Kreuztal, Germany) in 2019, and 2020 respectively (Table 1). The grapevines were grown in an open greenhouse under field conditions for six months. They were additionally watered whenever it was required. In May 2019 ungrafted Mueller-Thurgau und Riesling grapevines, as well as soil-filled pots without grapevines used as controls, were examined. In the same year, additional Riesling grapevines grafted on four different rootstocks (*Vitis berlandieri* Planch. x *Vitis riparia* Michx. cv. SO4; *Vitis riparia* x *Vitis cinerea* Engelm. cv. Boerner; *Vitis berlandieri* Planch. x *Vitis riparia* Michx. cv. 125AA; and *Vitis berlandieri* Planch. x *Vitis riparia* Michx. cv. Teleki 8 B) were investigated. In 2020 ungrafted Mueller-Thurgau and Riesling grapevines, soil-filled pots without grapevines as control, Riesling grapevines with four different rootstocks (*Vitis berlandieri* Planch. x *Vitis riparia* Michx. cv. SO4; *Vitis riparia* x *Vitis cinerea* Engelm. cv. Boerner; *Vitis berlandieri* Planch. x *Vitis riparia* Michx. cv. 125AA; and *Vitis berlandieri* Planch. x *Vitis riparia* Michx. cv. Teleki 8 B) and Mueller-Thurgau with three different rootstocks (*Vitis berlandieri* Planch. x *Vitis riparia* Michx. cv. SO4; *Vitis berlandieri* Planch. x *Vitis riparia* Michx. cv. 125AA; and *Vitis berlandieri* Planch. x *Vitis riparia* Michx. cv. Kober 5 BB) were examined.

Table 1. Grape varieties and rootstocks used for rhizosphere sampling at three different experimental time points. Number of plants sampled (n).

time point	grape variety (n)	rootstock
May 2019	Mueller-Thurgau (4)	ungrafted
	Riesling (4)	ungrafted
	No plant (4)	Control
October 2019	Riesling (5)	SO4
	Riesling (5)	125AA
	Riesling (5)	Boerner
	Riesling (5)	8B
October 2020	Riesling (9)	ungrafted
	Riesling (3)	SO4

Riesling (3)	125AA
Riesling (3)	Boerner
Riesling (3)	8B
Mueller-Thurgau (9)	ungrafted
Mueller-Thurgau (3)	SO4
Mueller-Thurgau (3)	125AA
Mueller-Thurgau (3)	Kober 5BB
No plant (9)	Control

2.2 Rhizosphere sampling

Rhizosphere sampling was performed at three different time points for the three experimental set-ups: May 2019, October 2019, and October 2020. A total of four Riesling, four Mueller-Thurgau and four controls were sampled in May 2019. Five samples per grapevine rootstock were collected in October 2019. Additionally, nine controls, nine ungrafted Riesling and Mueller-Thurgau, and three samples per grapevine rootstock were collected in October 2020. Only soil attaching to the roots was considered as rhizosphere soil for sampling and microbiome analyses.

2.3 RNA extraction, Reverse-Transcriptase-PCR, and Ion Torrent Sequencing

RNA extraction from the rhizosphere soil and cDNA amplification were done according to Rosado-Porto et al. (Rosado-Porto et al., 2021). First Ion Torrent PCR was performed with a KAPA HiFi Polymerase kit (VWR International GmbH, Darmstadt, Germany) amplifying the partial sequence of the hypervariable regions (V4 and V5) of the 16S rRNA gene with the primer 520F (5'-AYTGGGYDTAAAGNG-3', (Claesson et al., 2009)) and 926R (5'-CCGYCAATTYMTTTRAGTTT-3', (Engelbrektson et al., 2010)). Amplification parameters were 3 min at 95 °C followed by 35 cycles at 98 °C for 20 s, 55 °C for 30 s, 72 °C for 30 s and finally 72 °C for 5 min. Second Ion Torrent PCR with primers including barcodes and Ion Torrent sequencing adapters was conducted with the PCR product from the first Ion Torrent PCR as suggested by Berry et al. (2011). The PCR was performed with the following amplification parameters: 3 min at 95 °C followed by 10 cycles at 98 °C for 20 s, 55 °C for 30 s, 72 °C for 30 s and finally 72 °C for 7 min. The PCR products were applied to an 1 % agarose

gel and subsequently purified using NucleoSpin® Gel and PCR Clean-up (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). Further, this product was purified with DNA purification beads NucleoMagVR NGS clean-up kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). The Ion Torrent Sequencing was done according to Kaplan et al. (2019).

2.4 Bioinformatic analysis of the sequencing data

Bioinformatic analysis was performed with QIIME 2 2020.11 (Bolyen et al., 2019). The obtained raw sequences were demultiplexed using cutadapt (Martin, 2011) with no errors allowed in the barcode sequences. Quality control, sequence denoising, clustering to amplicon sequence variants (ASVs), dereplication and removal of chimera sequences were conducted with DADA2 (Callahan et al., 2016) (via q2-dada2). Thereby the first 15 base pairs (bp) were removed and the sequences were cut to a length of 312 bp (May 2019), 317 bp (October 2019), and 312 bp (October 2020), respectively. Afterwards, the taxonomy was assigned as described previously by Dries et al. (Dries, Bussotti, et al., 2021) and all ASVs belonging to chloroplasts and mitochondria were removed. All ASVs were aligned with MAFFT (Katoh & Standley, 2013) (via q2-alignment) and used for constructing a phylogeny with fasttree2 (Price et al., 2010) (via q2-phylogeny). The feature table was filtered for the 20 dominant bacterial families and taxabarplots were created with GraphPad Prism version 9.3.1 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). Alpha-diversity metrics (Shannon, Simpson, and observed features) and significances were calculated with Kruskal-Wallis or PERMANOVA (Anderson, 2017), respectively. Beta-diversity was measured using DEICODE (Martino et al., 2019) with a filtered beta-diversity ordination file. For this purpose, all feature counts below 10 and all sample counts below 500 were removed. Beta-diversity for all time points was visualized within a compositional biplot, displaying the eight most important features. From the ASVs shown as arrows in the DEICODE graphs, a more accurate taxonomic affiliation was done with a pairwise alignment at the online database EzBioCloud (Yoon et al., 2017). The distance matrices were also analyzed by the PERMANOVA test, using 999 permutations. Significance of differential abundance was determined using ALDEx2 (Fernandes et al., 2013). Therefore, all feature frequency counts below 10 and all sample counts below 2 were removed.

Results

3.1. Bacterial alpha-diversity across the different experimental set-ups

The 20 dominating bacterial families for the three time points are shown in Figure 1 and 2. Control soil showed clear differences of the 20 dominating bacterial families compared to the ungrafted Mueller-Thurgau and Riesling (Figure 1A). In the rhizosphere of Riesling grapevine grafted onto four different rootstocks (SO4, 125AA, Boerner, 8B) the dominating bacterial families in the rhizosphere showed no clear differences of the 20 dominating families comparing the four rootstocks (Figure 1B). For time point October 2020 (Figure 2), some differences were found between Riesling and Mueller-Thurgau ungrafted compared to the grafted varieties and the control of the 20 dominating bacterial families.

For ungrafted grape varieties Mueller-Thurgau and Riesling, significant effects of the grape variety were found in alpha-diversity indices Shannon and observed features (Table 2). No statistical differences were found regarding alpha-diversity Simpson index. However, while the Shannon–Wiener index is strongly influenced by species richness and by rare species, the Simpson index gives more weight to evenness and common species. Nonetheless, shows Shannon index both richness and abundance. For grape variety Riesling with four different rootstocks, examined in October 2019, and for time point October 2020, no statistical differences were found in the alpha-diversity (Shannon, Simpson, and observed features).

Table 2. Results of Kruskal-Wallis pairwise tests of alpha-diversity indices per ungrafted grape variety or control, respectively for time point May 2019. Significant differences (corrected p-value < 0.05) are indicated with *, no differences are indicated with ns = not significant. For time point October 2019 and October 2020, no statistical differences were measured, thus they are not listed in this table.

time point	grape variety	shannon	simpson	observed features
May 2019	Mueller-Thurgau vs. Riesling	0.03 *	1.00 ns	0.03 *
	Mueller-Thurgau vs. Control	0.25 ns	1.00 ns	0.56 ns
	Riesling vs. Control	0.03 *	1.00 ns	0.03 *

3.2. Bacterial beta-diversity across the different experimental set-ups

Compositional beta-diversity metric was calculated using a robust Aitchison PCA via DEICODE. Aitchison distance is a Euclidean distance between samples after center log ratio (clr) transformation. For all experimental set-ups and time points significant differences in the beta-diversity of the bacterial communities were detected (Figure 3). The determinant of the bacterial communities in the rhizosphere is the grape variety and rootstock, or grape variety and rootstock combination, respectively. PERMANOVA pairwise results reveal statistical differences (Table 2) for time point May 2019 between Mueller-Thurgau and Riesling ($p = 0.03$), Mueller-Thurgau and control ($p = 0.03$), and Riesling and control ($p = 0.03$). No statistical significances for time point October 2019 were detected between the rootstocks ($p > 0.05$), so no arrows illustrating ASVs strongly influencing the principal component axis are visible. Moreover, statistical differences were detected for time point October 2020 between Mueller-Thurgau ungrafted vs. Mueller-Thurgau grafted onto different rootstocks, Mueller-Thurgau ungrafted vs. Riesling ungrafted, Mueller-Thurgau ungrafted vs. Riesling grafted onto different rootstocks, and Mueller-Thurgau ungrafted vs. the control ($p < 0.05$, Table 3). Statistical differences were also detected between Mueller-Thurgau grafted vs. Riesling ungrafted, Mueller-Thurgau grafted vs. the control, Riesling ungrafted vs. Riesling grafted onto different rootstocks, Riesling ungrafted vs. the control, and Riesling grafted vs. the control ($p < 0.5$, Table 3). However, no statistical difference in the beta-diversity were detected between Mueller-Thurgau grafted and Riesling grafted ($p = 0.21$, Table 3).

DEICODE allows the display of a biplot by showing not only the Aitchison distances but also the taxa (in the form of an arrow) that most strongly influence principal component axes. The eight most important taxa influencing the principal component axes shown in each figure are members of the phyla *Verrucomicrobia*, *Proteobacteria*, *Acidobacteria*, and *Chloroflexi*. According to the taxonomic classification, *Verrucomicrobia*, *Proteobacteria*, *Acidobacteria* and *Chloroflexi* also form the main composition of the predominant phyla in the rhizosphere of the different rootstock or shoot genotypes or combinations. A bacterium related to *Nevshia terrae* influenced the rhizosphere microbiome of Mueller-Thurgau, whereas *Nevshia soli* and *Longimicrobium terrae* influenced the rhizosphere microbiome of Riesling (Figure 3). A sequence next relative to the bacterium *Racemicystis persica* (KX443485, 91.06 %) belonging

to the *Proteobacteria* phylum and *Polyangiaceae* family is one of the eight most important features in two of the three experimental set-ups (Figure 3).

Table 3. Bacterial beta-diversity results of PERMANOVA pairwise tests. Significant differences (corrected p-value < 0.05) are indicated with *, pairs with no statistical differences are not shown in this table. In October 2019, no significant differences were measured.

time point	grape variety	PERMANOVA
May 2019	Mueller-Thurgau vs. Riesling	0.03 *
	Mueller-Thurgau vs. Control	0.03 *
	Riesling vs. Control	0.03 *
October 2020	Mueller-Thurgau ungrafted vs. Mueller-Thurgau grafted	0.001 *
	Mueller-Thurgau ungrafted vs. Riesling ungrafted	0.002 *
	Mueller-Thurgau ungrafted vs. Riesling grafted	0.001 *
	Mueller-Thurgau ungrafted vs. Control	0.001 *
	Mueller-Thurgau grafted vs. Riesling ungrafted	0.001 *
	Mueller-Thurgau grafted vs. Riesling grafted	0.21 ns
	Mueller-Thurgau grafted vs. Control	0.001 *
	Riesling ungrafted vs. Riesling grafted	0.001 *
	Riesling ungrafted vs. Control	0.001 *
	Riesling grafted vs. Control	0.001 *

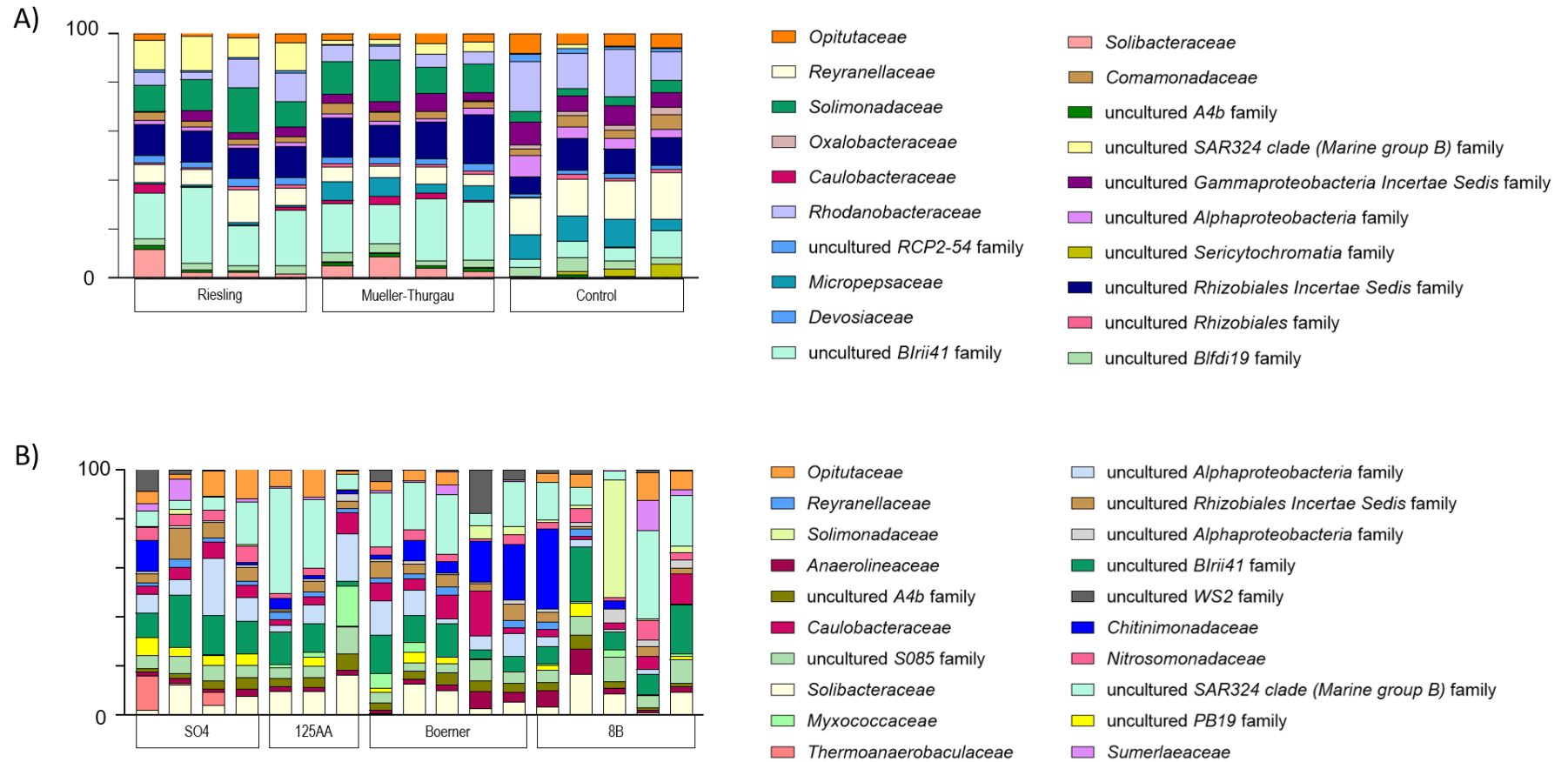


Figure 1. Relative abundance of different bacterial families (in %) in the rhizosphere of grapevine. A) The 20 most dominating bacterial families in the rhizosphere of ungrafted Riesling, ungrafted Mueller-Thurgau, and a control (time point May 2019). B) The 20 most dominating bacterial families in the rhizosphere of Riesling grapevine grafted onto four different rootstocks (SO4, 125AA, Boerner, 8B, time point October 2019).

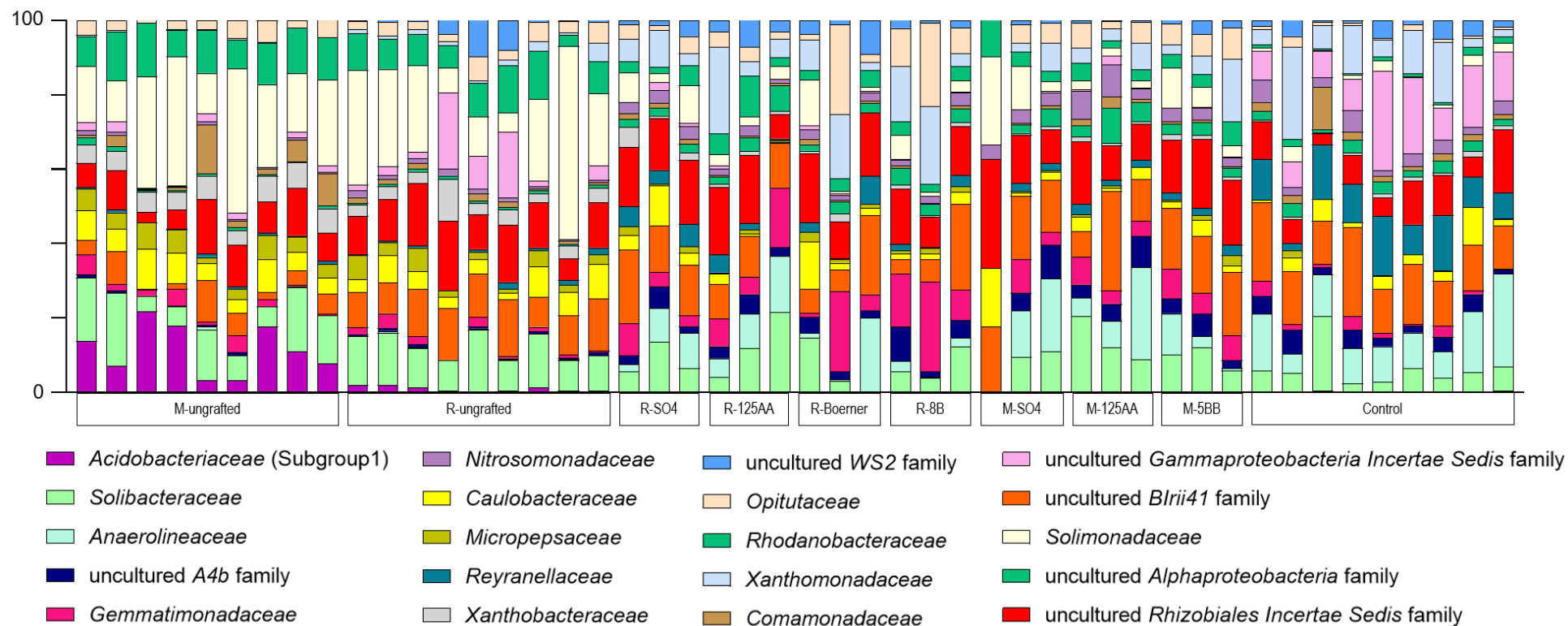


Figure 2. Relative abundance of different bacterial families (in %) in the rhizosphere of grapevine. The 20 most dominating bacterial families in the rhizosphere of ungrafted Mueller-Thurgau, ungrafted Riesling, Riesling grafted onto different rootstocks (SO4, 125AA, Boerner, and 8B), Mueller-Thurgau grafted onto different rootstocks (SO4, 125AA, 5BB), and a control.

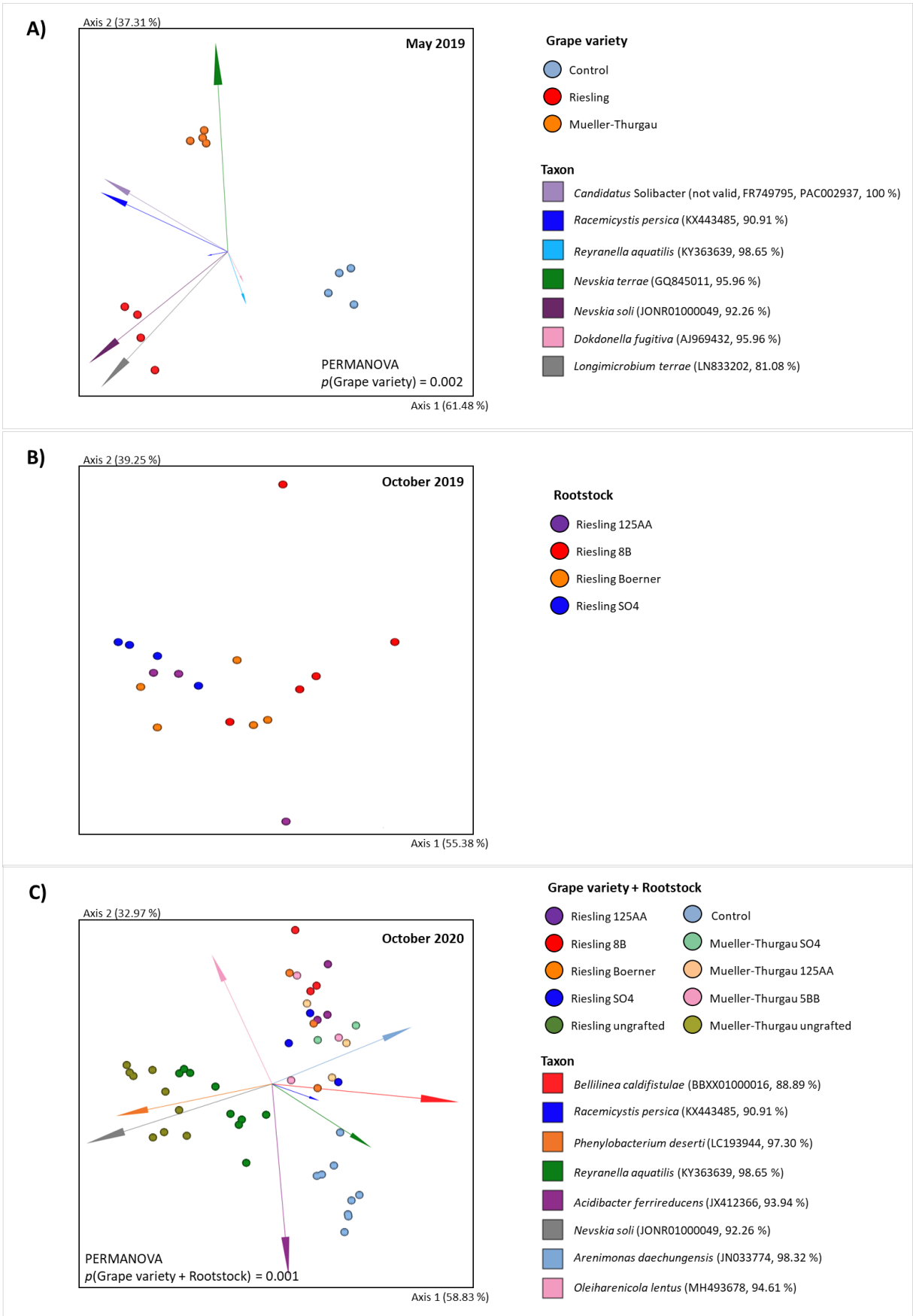


Figure 3. PCoA biplots calculated based on robust Aitchison community dissimilarity distance matrix with arrows that illustrate the ASVs that strongly influence the principal component axis. Taxonomic affiliations of the arrows showed the next related sequences with accession numbers found by pairwise alignment at EzBioCloud Database. Numbers in the brackets show the percentage of identity of the ASVs sequence with the next related sequence. A) Beta-diversity of bacterial rhizosphere communities for ungrafted Riesling and ungrafted Mueller-Thurgau grape variety, and a control (time point May 2019). Statistical differences were detected between all groups (PERMANOVA, $p = 0.002$). B) Beta-diversity of bacterial rhizosphere communities for Riesling grape variety with four different rootstocks (time point October 2019). No statistical differences were detected (PERMANOVA, $p > 0.05$). C) Beta-diversity of bacterial rhizosphere communities for Riesling grape variety ungrafted and with four different rootstocks, Mueller-Thurgau grape variety ungrafted and with three different rootstocks, and a control (October 2020). Statistical differences were detected (PERMANOVA, $p = 0.001$).

3.3 Changes on the rhizosphere microbial community

Compositional differential abundance analyses indicated that several bacterial genera in the rhizosphere were affected. ALDEx2 demonstrated that for time point May 2019, in total 26 bacterial genera differed according to the grape variety. Bacterial genera with highest fold changes belonged to *Rhodospirillaceae*, *Opitutaceae*, *Burkholderiaceae*, *Polyangiaceae*, and “*Solibacteraceae*” (Tables 1 - 3, Supplementary Material). An uncultured bacterium relative to the *Candidatus Solibacter* showed the highest fold changes (fold change 1640.65 Control vs. Mueller-Thurgau and fold change 862.6 Control vs. Riesling). This bacterial genus is also displayed in Figure 3A) as an ASV strongly influencing the principal component axis. For time point October 2019, no genera differ according to the different rootstocks and Riesling grape variety. For time point October 2020, ALDEx2 demonstrated in total 560 bacterial genera differing according to the grape variety or rootstock or combinations, respectively. Highest log fold changes showed *Nevskiaceae* (also displayed in Figure 3C), *Acidobacteriaceae*, “*Solibacteraceae*”, *Comamonadaceae*, *Caulobacteraceae* (*Phenylobacterium deserti*, also shown in Figure 3C), *Opitutaceae*, and *Steroidobacteraceae*. Statistical different genera were detected for time point October 2020 between Mueller-Thurgau ungrafted vs. Mueller-Thurgau grafted onto different rootstocks, Mueller-Thurgau ungrafted vs. Riesling ungrafted, Mueller-Thurgau ungrafted vs. Riesling grafted onto different rootstocks, Mueller-Thurgau ungrafted

vs. the control, Riesling ungrafted vs. Riesling grafted onto different rootstocks, and Riesling grafted vs. the control (Tables 4 – 9, Supplementary Material). Between Riesling grafted vs. Mueller-Thurgau grafted, no statistical differences regarding the bacterial genera were detected.

Discussion

The experimental design of this study aimed at minimizing the variability coming from all factors except grape variety and rootstock genotype using grapevines in pots under the same environmental conditions. The differences in the bacterial communities between the two grape varieties Riesling and Mueller-Thurgau, ungrafted and grafted onto different rootstocks, were investigated using compositional data analyses. The observed ASVs revealed that the rootstock and scion rhizosphere, respectively, recruited complex bacterial communities mainly composed of *Proteobacteria*, *Verrucomicrobiota*, *Chloroflexi*, *Myxococcota*, *Acidobacteria*, and *Gemmatimonadota*. Also, a similar study by Dries, Bussotti et al. (2021) with ungrafted grapevine rootstocks showed the phyla *Proteobacteria*, *Acidobacteria*, and *Gemmatimonadota* as some of the predominant bacterial communities in the rhizosphere. Other studies have come to comparable conclusions, independent of factors such as grape variety and rootstock (Berlanas et al., 2019; Coller et al., 2019; Marasco et al., 2018; Novello et al., 2017; Torres et al., 2021; Vink et al., 2021; Zarraonaindia et al., 2015).

The dominating families give a higher resolution on taxonomic level which is more suitable for comparison than phyla level. During all experimental time points *Opitutaceae*, *Reyranellaceae*, “*Solibacteraceae*”, and *Solimonadaceae* were found as dominating families among others (Figure 1 and 2). This is also consistent with a study conducted by Marasco et al. (2018). They revealed “*Solibacteraceae*” as one of the families shaping the topology of the bacterial network in the grafted root system (Marasco et al., 2018). *Comamonadaceae* (Figure 1A and 2) were also found in a study by D’Amico et al (2018) in the rhizosphere of grapevine rootstocks 5BB and Paulsen 1103. As a wider variety of bacteria are found in soil, it can be assumed that soil serves as a primary reservoir for potential plant-associated bacteria (Zarraonaindia et al., 2015). However, soil as well as rhizosphere can also form a path of infection for soil-borne pathogens (Berlanas et al., 2019). For grapevines, this includes pathogens such as *Fusarium oxysporum*

(Freire Cruz & Carvalho Pires, 2014) or *Sorosphaera viticola* (Neuhauser S., Huber L., Kirchmair M., 2009).

The alpha-diversity indices in this study revealed higher observed features for ungrafted Riesling grape variety in May 2019 (Table 2). This may indicate that ungrafted Riesling grapevines were able to recruit a higher number of bacteria in their rhizosphere than Mueller-Thurgau grapevines. The underlying reason for this varying colonization could originate from the root exudates. Indeed, root exudates are strongly influenced by the cultivar and they are considered among the most important factors in the recruitment of the microbiome (Kusstatscher et al., 2021; Marasco et al., 2018; Wei et al., 2019). As a key role in the rhizosphere ecosystem, it is essential to understand the root exudation patterns to unravel the subsequent effects on the surrounding soil and microbial communities (Yee et al., 2021). These results are also consistent with previous studies, concluding the grapevine variety as the major factor shaping the vineyard microbiome (Berlanas et al., 2019), but rootstock genotype takes a subordinate position (Vink et al., 2021). The alpha-diversity indices for Riesling and Mueller-Thurgau grafted onto different grapevine rootstocks revealed no statistical differences (Table 2). However, three out of four rootstocks used for this experiment have emerged from the same breedings: *Vitis berlandieri* Planch. x *Vitis riparia* Michx., which may be an explanation of the statistical same bacterial communities in the rhizosphere. The fact, that most cultivated grapevines are genetic chimeras with two different genotypes (Marín et al., 2021), complicates the separation of the genotypes from each other regarding their related microbial communities. Hence, all the studies indicate that certain taxa are always apparently present at all times in the rhizosphere of the grapevine.

The beta-diversity revealed statistical differences in the three experimental set-ups. Pairwise PERMANOVA results showed statistical differences between ungrafted Mueller-Thurgau and ungrafted Riesling grape varieties, but no statistical differences between different rootstocks. The results indicate, that the grape variety may be a driving factor of the bacterial communities in the rhizosphere, but the grafted grapevine scion and rootstock combination are a less driving factor. This is also in accordance to a former study, showing the ungrafted grapevine rootstocks as a driver of the bacterial communities (Dries, Bussotti, et al., 2021). Thus, it might be concluded that grafting grapevines onto rootstocks implies a change in bacterial communities. Moreover, Vink et al. (2021) revealed in their study that the main determinant of the bacterial

communities was scion variety for the alpha-diversity and a significant interaction between scion and rootstocks regarding the beta-diversity. However, the authors did not refer to ungrafted grape varieties. In another study conducted by Berlanas et al. (2019), they described the rootstock genotype as the most important factor in shaping the microbiome. Wright et al. (2022) revealed that the rootstock was a significant factor driving the root microbiome with grape variety New York Muscat ungrafted and grafted onto two different rootstocks.

Comparing the ASVs strongly influencing the principal component axis from the PCoA biplot (Figure 3) with the results of the compositional differential abundance analyses (ALDEx2, Tables 1 – 3, Supplemental Material) for time point May 2019 revealed ASVs next relative to *Nevskia terrae*, uncultured *Candidatus Solibacter*, and uncultured *Deltaproteobacteria* as those taxa appearing in both analyses. *Nevskia terrae* was already described as a bacterium isolated from soil in Korea (Kim et al., 2011), belonging to the *Xanthomonadaceae*. Some members of this family are already described as plant-growth promoting (Cutiño-Jiménez et al., 2020). *Candidatus Solibacter* was described previously as a bacteria inhabiting the rhizosphere of walnut trees (Bai et al., 2020), and *Deltaproteobacteria* in rice (Zhang et al., 2018). For time point October 2020, all eight displayed ASVs strongly influencing the principal component axis from the DEICODE beta-diversity also appeared in the compositional abundance analyses (ALDEx2, Tables 4 – 9, Supplemental Material). The next relative *Phenylobacterium deserti* was first isolated from desert soil (Khan et al., 2017), other *Phenylobacterium* were also isolated from different soil samples (Khan et al., 2018; Li et al., 2019). The next relative of uncultured “*Acidibacter*” belongs to *Gammaproteobacteria*, members of this class are known for plant growth-promoting traits, inhabiting the rhizosphere (Madhaiyan et al., 2017), root nodules (Ibáñez et al., 2009) or plant tissue (Madhaiyan et al., 2020). However, further research on this topic is needed to reveal all effects of rootstock and grapevine scion combinations on the bacterial communities in the rhizosphere. In addition, the effects on the growth and health of the vine must be revealed from a practical perspective. A thorough understanding of microorganisms in vineyard soil, and the complex relationships between microbial communities, soil properties and plant are crucial for enhancing plant productivity, grape production, biogeochemical processes and vineyard management practices (Di Liu et al., 2019; Dries, Hendgen, et al., 2021; Holland et al., 2014; Liang et al., 2019; Yee et al., 2021). Inferring, the colonization of the microorganisms in the rhizosphere and the root exudation patterns of grapevine must be investigated. Future research in this field is inevitably required to provide a

better understanding of the rhizospheric grapevine microbiome in the context of root exudates, grape variety, rootstock as well as different environmental conditions.

Conclusion

The results from these experimental designs reveal differences in the bacterial communities in the rhizosphere of grafted or ungrafted grapevine varieties, respectively. The bacteria in the rhizosphere of grapevine are affected by both grapevine variety and scion-rootstock combination. While differences were observed between ungrafted vs. grafted grape varieties, especially in terms of beta-diversity, no differences were observed between the different rootstocks. Thus, the grapevine cultivar appears to have a predominant role compared with the rootstock in shaping the rhizosphere microbiota. Future research is needed, to provide a better understanding of the differing microorganisms in the grapevine rhizosphere regarding the scion-rootstock combinations. Moreover, the effects on the grapevine growth and health, and also on the wine quality, have to be revealed.

Acknowledgements

We thank Forschungsring des Deutschen Weinbaus (FDW) for funding this project. We appreciate and acknowledge the members of the Department of Soil Science and Plant Nutrition, Hochschule Geisenheim University, for their assistance in sample taking. We are very grateful to Bellinda Schneider, Justus-Liebig University Gießen, for technical support with Ion Torrent Sequencing. We thank the Department of Crop Protection, Hochschule Geisenheim University, for the opportunity to use the molecular biology laboratory and for their helpful advice. We further thank Maximilian Hendgen for carefully proofreading the manuscript.

References

- Alegria Terrazas, R., Giles, C., Paterson, E., Robertson-Albertyn, S., Cesco, S., Mimmo, T., Pii, Y., & Bulgarelli, D [D.] (2016). Plant-Microbiota Interactions as a Driver of the Mineral Turnover in the Rhizosphere. *Advances in Applied Microbiology*, 95(95), 1–67. <https://doi.org/10.1016/bs.aambs.2016.03.001>
- Anderson, M. J. (2017). *Permutational Multivariate Analysis of Variance (PERMANOVA)*. Wiley Statistics Reference Online, 1–15. <https://doi.org/10.1002/9781118445112.stat07841>
- Bai, Y.-C., Chang, Y.-Y., Hussain, M., Lu, B., Zhang, J.-P., Song, X.-B., Lei, X.-S., & Pei, D. (2020). Soil Chemical and Microbiological Properties Are Changed by Long-Term Chemical Fertilizers That Limit Ecosystem Functioning. *Microorganisms*, 8(5). <https://doi.org/10.3390/microorganisms8050694>
- Berlanas, C., Berbegal, M., Elena, G., Laidani, M., Cibriain, J. F., Sagües, A., & Gramaje, D [David] (2019). The Fungal and Bacterial Rhizosphere Microbiome Associated With Grapevine Rootstock Genotypes in Mature and Young Vineyards. *Frontiers in Microbiology*, 10, 1142. <https://doi.org/10.3389/fmicb.2019.01142>
- Berry, D., Ben Mahfoudh, K., Wagner, M., & Loy, A. (2011). Barcoded primers used in multiplex amplicon pyrosequencing bias amplification. *Applied and Environmental Microbiology*, 77(21), 7846–7849. <https://doi.org/10.1128/AEM.05220-11>
- Bokulich, N. A., Thorngate, J. H., Richardson, P. M., & Mills, D. A. (2014). Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *Proceedings of the National Academy of Sciences of the United States of America*, 111(1), E139-48. <https://doi.org/10.1073/pnas.1317377110>
- Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., . . . Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, 37(8), 852–857. <https://doi.org/10.1038/s41587-019-0209-9>
- Bulgarelli, D [Davide], Schlaeppi, K., Spaepen, S., van Loren Themaat, E. ver, & Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology*, 64, 807–838. <https://doi.org/10.1146/annurev-arplant-050312-120106>

- Burns, K. N., Bokulich, N. A., Cantu, D., Greenhut, R. F., Kluepfel, D. A., O'Geen, A. T., Strauss, S. L., & Steenwerth, K. L. (2016). Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: Differentiation by vineyard management. *Soil Biology and Biochemistry*, 103, 337–348. <https://doi.org/10.1016/j.soilbio.2016.09.007>
- Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). Dada2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13(7), 581–583. <https://doi.org/10.1038/nmeth.3869>
- Carvalhais, L. C., Dennis, P. G., Tyson, G. W., & Schenk, P. M. (2012). Application of metatranscriptomics to soil environments. *Journal of Microbiological Methods*, 91(2), 246–251. <https://doi.org/10.1016/j.mimet.2012.08.011>
- Claesson, M. J., O'Sullivan, O., Wang, Q., Nikkilä, J., Marchesi, J. R., Smidt, H., Vos, W. M. de, Ross, R. P., & O'Toole, P. W. (2009). Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. *PLoS ONE*, 4(8), e6669. <https://doi.org/10.1371/journal.pone.0006669>
- Coller, E., Cestaro, A., Zanzotti, R., Bertoldi, D., Pindo, M., Larger, S., Albanese, D., Mescalchin, E., & Donati, C. (2019). Microbiome of vineyard soils is shaped by geography and management. *Microbiome*, 7(1), 140. <https://doi.org/10.1186/s40168-019-0758-7>
- Contreras, F., Díaz, J., Rombolà, A. D., & La Luz Mora, M. de (2019). Prospecting intercropping between subterranean clover and grapevine as potential strategy for improving grapevine performance. *Current Plant Biology*, 19, 100110. <https://doi.org/10.1016/j.cpb.2019.100110>
- Cordero, J., Freitas, J. R. de, & Germida, J. J. (2020). Bacterial microbiome associated with the rhizosphere and root interior of crops in Saskatchewan, Canada. *Canadian Journal of Microbiology*, 66(1), 71–85. <https://doi.org/10.1139/cjm-2019-0330>
- Cutiño-Jiménez, A. M., Menck, C. F. M., Cambas, Y. T., & Díaz-Pérez, J. C. (2020). Protein signatures to identify the different genera within the *Xanthomonadaceae* family. *Brazilian Journal of Microbiology: [Publication of the Brazilian Society for Microbiology]*, 51(4), 1515–1526. <https://doi.org/10.1007/s42770-020-00304-2>
- D'Amico, F., Candela, M., Turrone, S., Biagi, E., Brigidi, P., Bega, A., Vancini, D., & Rampelli, S. (2018). The Rootstock Regulates Microbiome Diversity in Root and Rhizosphere Compartments of *Vitis vinifera* Cultivar Lambrusco. *Frontiers in Microbiology*, 9, 2240. <https://doi.org/10.3389/fmicb.2018.02240>

- Di Liu, Zhang, P [Pangzhen], Chen, D., & Howell, K. (2019). From the Vineyard to the Winery: How Microbial Ecology Drives Regional Distinctiveness of Wine. *Frontiers in Microbiology*, 10, 2679. <https://doi.org/10.3389/fmicb.2019.02679>
- Dries, L., Bussotti, S., Pozzi, C., Kunz, R., Schnell, S., Löhnertz, O., & Vortkamp, A. (2021). Rootstocks Shape Their Microbiome-Bacterial Communities in the Rhizosphere of Different Grapevine Rootstocks. *Microorganisms*, 9(4). <https://doi.org/10.3390/microorganisms9040822>
- Dries, L., Hendgen, M., Schnell, S., Löhnertz, O., & Vortkamp, A. (2021). Rhizosphere engineering: leading towards a sustainable viticulture? *OENO One*, 55(2), 353–363. <https://doi.org/10.20870/oeno-one.2021.55.2.4534>
- Engelbrektson, A., Kunin, V., Wrighton, K. C., Zvenigorodsky, N., Chen, F., Ochman, H., & Hugenholtz, P. (2010). Experimental factors affecting PCR-based estimates of microbial species richness and evenness. *The ISME Journal*, 4(5), 642–647. <https://doi.org/10.1038/ismej.2009.153>
- Etesami, H., & Alikhani, H. A. (2016). Rhizosphere and endorhiza of oilseed rape (*Brassica napus* L.) plant harbor bacteria with multifaceted beneficial effects. *Biological Control*, 94, 11–24. <https://doi.org/10.1016/j.biocontrol.2015.12.003>
- Fernandes, A. D., Macklaim, J. M., Linn, T. G., Reid, G., & Gloor, G. B. (2013). Anova-like differential expression (ALDEx) analysis for mixed population RNA-Seq. *PLoS ONE*, 8(7), e67019. <https://doi.org/10.1371/journal.pone.0067019>
- Fernandes, A. D., Reid, J. N., Macklaim, J. M., McMurrough, T. A., Edgell, D. R., & Gloor, G. B. (2014). Unifying the analysis of high-throughput sequencing datasets: Characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. *Microbiome*, 2, 15. <https://doi.org/10.1186/2049-2618-2-15>
- Freire Cruz, A., & Carvalho Pires, M. de (2014). Soil-Borne Plant Pathogens Associated to Decline of Grapevine Grown in Greenhouse. *Journal of Plant Physiology & Pathology*, 02(01). <https://doi.org/10.4172/2329-955X.1000115>
- Garoutte, A., Cardenas, E., Tiedje, J., & Howe, A. (2016). Methodologies for probing the metatranscriptome of grassland soil. *Journal of Microbiological Methods*, 131, 122–129. <https://doi.org/10.1016/j.mimet.2016.10.018>
- Gattullo, C. E., Mezzapesa, G. N., Stellacci, A. M., Ferrara, G., Occhiogrosso, G., Petrelli, G., Castellini, M., & Spagnuolo, M. (2020). Cover Crop for a Sustainable Viticulture: Effects

- on Soil Properties and Table Grape Production. *Agronomy*, 10(9), 1334. <https://doi.org/10.3390/agronomy10091334>
- Gautier, A. T., Chambaud, C., Brocard, L., Ollat, N., Gambetta, G. A., Delrot, S., & Cookson, S. J. (2019). Merging genotypes: Graft union formation and scion-rootstock interactions. *Journal of Experimental Botany*, 70(3), 747–755. <https://doi.org/10.1093/jxb/ery422>
- Hendgen, M., Hoppe, B., Döring, J., Friedel, M., Kauer, R., Frisch, M., Dahl, A., & Kellner, H. (2018). Effects of different management regimes on microbial biodiversity in vineyard soils. *Scientific Reports*, 8(1), 9393. <https://doi.org/10.1038/s41598-018-27743-0>
- Holland, T. C., Bowen, P., Bogdanoff, C., & Hart, M. M. (2014). How distinct are arbuscular mycorrhizal fungal communities associating with grapevines? *Biology and Fertility of Soils*, 50(4), 667–674. <https://doi.org/10.1007/s00374-013-0887-2>
- Ibáñez, F., Angelini, J., Taurian, T., Tonelli, M. L., & Fabra, A. (2009). Endophytic occupation of peanut root nodules by opportunistic *Gammaproteobacteria*. *Systematic and Applied Microbiology*, 32(1), 49–55. <https://doi.org/10.1016/j.syapm.2008.10.001>
- Kaplan, H., Ratering, S., Felix-Henningsen, P., & Schnell, S. (2019). Stability of in situ immobilization of trace metals with different amendments revealed by microbial ¹³C-labelled wheat root decomposition and efflux-mediated metal resistance of soil bacteria. *The Science of the Total Environment*, 659, 1082–1089. <https://doi.org/10.1016/j.scitotenv.2018.12.441>
- Katoh, K., & Standley, D. M. (2013). Mafft multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, 30(4), 772–780. <https://doi.org/10.1093/molbev/mst010>
- Khan, I. U., Habib, N., Xiao, M., Huang, X., Khan, N. U., Im, W.-T., Ahmed, I., Zhi, X.-Y., & Li, W.-J. (2018). *Phenylobacterium terrae* sp. Nov., isolated from a soil sample of Khyber-Pakhtun-Khwa, Pakistan. *Antonie Van Leeuwenhoek*, 111(10), 1767–1775. <https://doi.org/10.1007/s10482-018-1064-2>
- Khan, I. U., Hussain, F., Habib, N., Wadaan, M. A. M., Ahmed, I., Im, W.-T., Hozzein, W. N., Zhi, X.-Y., & Li, W.-J. (2017). *Phenylobacterium deserti* sp. Nov., isolated from desert soil. *International Journal of Systematic and Evolutionary Microbiology*, 67(11), 4722–4727. <https://doi.org/10.1099/ijsem.0.002366>

- Kim, S.-J., Weon, H.-Y., Kim, Y.-S., Park, I.-C., Son, J.-A., & Kwon, S.-W. (2011). *Nevskia terrae* sp. Nov., isolated from soil. *International Journal of Systematic and Evolutionary Microbiology*, 61(Pt 5), 1226–1229. <https://doi.org/10.1099/ijms.0.021238-0>
- Kusstatscher, P., Adam, E., Wicaksono, W. A., Bernhart, M., Olimi, E., Müller, H., & Berg, G. (2021). Microbiome-Assisted Breeding to Understand Cultivar-Dependent Assembly in *Cucurbita pepo*. *Frontiers in Plant Science*, 12, 642027. <https://doi.org/10.3389/fpls.2021.642027>
- Li, X., Yu, Y., Choi, L., Song, Y., Wu, M., Wang, G., & Li, M. (2019). *Phenylobacterium soli* sp. Nov., isolated from arsenic and cadmium contaminated farmland soil. *International Journal of Systematic and Evolutionary Microbiology*, 69(5), 1398–1403. <https://doi.org/10.1099/ijsem.0.003325>
- Liang, H., Wang, X., Yan, J., & Luo, L. (2019). Characterizing the Intra-Vineyard Variation of Soil Bacterial and Fungal Communities. *Frontiers in Microbiology*, 10, 1239. <https://doi.org/10.3389/fmicb.2019.01239>
- Madhaiyan, M., Poonguzhali, S., Saravanan, V. S., Selvapravin, K., Duraipandiyam, V., & Al-Dhabi, N. A. (2017). *Pseudomonas sesami* sp. Nov., a plant growth-promoting *Gammaproteobacteria* isolated from the rhizosphere of *Sesamum indicum* L. *Antonie Van Leeuwenhoek*, 110(7), 843–852. <https://doi.org/10.1007/s10482-017-0859-x>
- Madhaiyan, M., Saravanan, V. S., Blom, J., Smits, T. H. M., Rezzonico, F., Kim, S.-J., Weon, H.-Y., Kwon, S.-W., Whitman, W. B., & Ji, L. (2020). *Phytobacter palmae* sp. Nov., a novel endophytic, N₂ fixing, plant growth promoting *Gammaproteobacterium* isolated from oil palm (*Elaeis guineensis* Jacq.). *International Journal of Systematic and Evolutionary Microbiology*, 70(2), 841–848. <https://doi.org/10.1099/ijsem.0.003834>
- Marasco, R., Rolli, E., Fusi, M., Michoud, G., & Daffonchio, D. (2018). Grapevine rootstocks shape underground bacterial microbiome and networking but not potential functionality. *Microbiome*, 6(1), 3. <https://doi.org/10.1186/s40168-017-0391-2>
- Marín, D., Armengol, J., Carbonell-Bejerano, P., Escalona, J. M., Gramaje, D [D.], Hernández-Montes, E., Intrigliolo, D. S., Martínez-Zapater, J. M., Medrano, H., Mirás-Avalos, J. M., Palomares-Rius, J. E., Romero-Azorín, P., Savé, R., Santesteban, L. G., & Herralde, F. de (2021). Challenges of viticulture adaptation to global change: tackling the issue from the roots. *Australian Journal of Grape and Wine Research*, 27(1), 8–25. <https://doi.org/10.1111/ajgw.12463>

- Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, 17(1), 10. <https://doi.org/10.14806/ej.17.1.200>
- Martino, C., Morton, J. T [James T.], Marotz, C. A., Thompson, L. R., Tripathi, A., Knight, R., & Zengler, K. (2019). A Novel Sparse Compositional Technique Reveals Microbial Perturbations. *MSystems*, 4(1). <https://doi.org/10.1128/mSystems.00016-19>
- McLaren, M. R., Willis, A. D., & Callahan, B. J. (2019). Consistent and correctable bias in metagenomic sequencing experiments. *ELife*. Advance online publication. <https://doi.org/10.7554/eLife.46923.001>
- Neuhauser S., Huber L., Kirchmair M. (2009). *Sorosphaera viticola*, a plasmodiophorid parasite of grapevine. *Phytopathol Mediterr.*(48(1)), 136–139.
- Novello, G., Gamalero, E., Bona, E., Boatti, L., Mignone, F., Massa, N., Cesaro, P., Lingua, G., & Berta, G. (2017). The Rhizosphere Bacterial Microbiota of *Vitis vinifera* cv. Pinot Noir in an Integrated Pest Management Vineyard. *Frontiers in Microbiology*, 8, 1528. <https://doi.org/10.3389/fmicb.2017.01528>
- Peiffer, J. A., Spor, A., Koren, O., Jin, Z., Tringe, S. G., Dangl, J. L., Buckler, E. S., & Ley, R. E. (2013). Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proceedings of the National Academy of Sciences of the United States of America*, 110(16), 6548–6553. <https://doi.org/10.1073/pnas.1302837110>
- Price, M. N., Dehal, P. S., & Arkin, A. P. (2010). FastTree 2 - Approximately Maximum-Likelihood Trees for Large Alignments. *PLoS ONE*(5(3)), 1–10. <https://doi.org/10.1371/journal.pone.0009490.t001>
- Quinn, T. P., Erb, I., Gloor, G., Notredame, C., Richardson, M. F., & Crowley, T. M. (2019). A field guide for the compositional analysis of any-omics data. *GigaScience*, 8(9). <https://doi.org/10.1093/gigascience/giz107>
- Rosado-Porto, D., Ratering, S., Cardinale, M., Maisinger, C., Moser, G., Deppe, M., Müller, C., & Schnell, S. (2021). Elevated Atmospheric CO₂ Modifies Mostly the Metabolic Active Rhizosphere Soil Microbiome in the Giessen FACE Experiment. *Microbial Ecology*. Advance online publication. <https://doi.org/10.1007/s00248-021-01791-y>
- Singh, P., Gobbi, A., Santoni, S., Hansen, L. H., This, P., & Péros, J.-P. (2018). Assessing the impact of plant genetic diversity in shaping the microbial community structure of *Vitis vinifera* phyllosphere in the Mediterranean. *Frontiers in Life Science*, 11(1), 35–46. <https://doi.org/10.1080/21553769.2018.1552628>

- Timmusk, S., Abd El-Daim, I. A., Copolovici, L., Tanilas, T., Kännaste, A., Behers, L., Nevo, E., Seisenbaeva, G., Stenström, E., & Niinemets, Ü. (2014). Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: Enhanced biomass production and reduced emissions of stress volatiles. *PLoS ONE*, 9(5), e96086. <https://doi.org/10.1371/journal.pone.0096086>
- Torres, N., Yu, R., & Kurtural, S. K. (2021). Inoculation with Mycorrhizal Fungi and Irrigation Management Shape the Bacterial and Fungal Communities and Networks in Vineyard Soils. *Microorganisms*, 9(6). <https://doi.org/10.3390/microorganisms9061273>
- Turner, T. R., Ramakrishnan, K., Walshaw, J., Heavens, D., Alston, M., Swarbreck, D., Osbourn, A., Grant, A., & Poole, P. S. (2013). Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants. *The ISME Journal*, 7(12), 2248–2258. <https://doi.org/10.1038/ismej.2013.119>
- Vega-Avila, A. D., Gumiere, T., Andrade, P. A. M., Lima-Perim, J. E., Durrer, A., Baigori, M., Vazquez, F., & Andreote, F. D. (2015). Bacterial communities in the rhizosphere of *Vitis vinifera* L. Cultivated under distinct agricultural practices in Argentina. *Antonie Van Leeuwenhoek*, 107(2), 575–588. <https://doi.org/10.1007/s10482-014-0353-7>
- Vieira, S., Sikorski, J., Dietz, S., Herz, K., Schrumpf, M., Bruelheide, H., Scheel, D., Friedrich, M. W., & Overmann, J. (2020). Drivers of the composition of active rhizosphere bacterial communities in temperate grasslands. *The ISME Journal*, 14(2), 463–475. <https://doi.org/10.1038/s41396-019-0543-4>
- Vink, S. N., Dini-Andreote, F., Höfle, R., Kicherer, A., & Salles, J. F. (2021). Interactive Effects of Scion and Rootstock Genotypes on the Root Microbiome of Grapevines (*Vitis spp.* L.). *Applied Sciences*, 11(4), 1615. <https://doi.org/10.3390/app11041615>
- Wei, F., Zhao, L., Xu, X., Feng, H., Shi, Y., Deakin, G., Feng, Z., & Zhu, H. (2019). Cultivar-Dependent Variation of the Cotton Rhizosphere and Endosphere Microbiome Under Field Conditions. *Frontiers in Plant Science*, 10, 1659. <https://doi.org/10.3389/fpls.2019.01659>
- Yee, M. O., Kim, P., Li, Y., Singh, A. K., Northen, T. R., & Chakraborty, R. (2021). Specialized Plant Growth Chamber Designs to Study Complex Rhizosphere Interactions. *Frontiers in Microbiology*, 12, 625752. <https://doi.org/10.3389/fmicb.2021.625752>
- Yoon, S.-H., Ha, S.-M., Kwon, S., Lim, J., Kim, Y., Seo, H., & Chun, J. (2017). Introducing EzBioCloud: A taxonomically united database of 16S rRNA gene sequences and whole-

genome assemblies. *International Journal of Systematic and Evolutionary Microbiology*, 67(5), 1613–1617. <https://doi.org/10.1099/ijsem.0.001755>

Zarraonaindia, I., Owens, S. M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., Bokulich, N. A., Mills, D. A., Martin, G., Taghavi, S., van der Lelie, D., & Gilbert, J. A. (2015). The soil microbiome influences grapevine-associated microbiota. *MBio*, 6(2). <https://doi.org/10.1128/mBio.02527-14>

Zhang, J., Zhang, N., Liu, Y.-X., Zhang, X., Hu, B., Qin, Y., Xu, H., Wang, H., Guo, X., Qian, J., Wang, W., Zhang, P [Pengfan], Jin, T., Chu, C., & Bai, Y. (2018). Root microbiota shift in rice correlates with resident time in the field and developmental stage. *Science China. Life Sciences*, 61(6), 613–621. <https://doi.org/10.1007/s11427-018-9284-4>

Chapter 4 - The metabolic active bacterial communities in the rhizosphere of grapevine (*Vitis vinifera* L. cv. Pinot Noir) are affected by different nitrogen fertilization levels and sampling depths

The metabolic active bacterial communities in the rhizosphere of grapevine (*Vitis vinifera* L. cv. Pinot Noir) change with different nitrogen fertilization levels and sampling depths

Abstract

The nitrogen cycle, which is regulated by soil microorganisms, as well as the related issue of nitrate leaching is furthermore of great importance, particularly in viticulture. In vineyards, many management practices impact soil characteristics like root growth or microbial communities, respectively. In order to investigate the impact of different levels of nitrogen (N) fertilization on the rhizosphere microbiome an experimental vineyard with vines (*Vitis vinifera* L. cv. Pinot Noir) in large containers was examined in 2020 and 2021 at bud break. The vines were fertilized with two different levels of nitrogen added per year (N0 25 kg/ha and N1 75 kg/ha) individually. In order to examine the respective bacterial communities, rhizosphere material was sampled and a subsequent RNA-extraction followed by next generation sequencing was performed. The study revealed significant differences in the bacterial communities in the rhizosphere regarding fertilization levels and sampling depths. The results may help to decrease nitrogen fertilization in viticulture, leading to a more sustainable management of grapevines.

1. Introduction

Vineyard soils are often considered as the main driver for nitrate contamination of groundwater, and therefore, one of the main issues regarding vineyard soil health is the problem of nitrate leaching. This is due to the special type of viticultural management: a high nitrogen pollution potential result, for instance, from the short vegetation period of about 150-180 days (May to October), a late start to vegetation (late April/early May), the low nutrient efficiency of grapevines, drought stress problems, or high mineralization rates in case of repeated tillage (Nendel and Kersebaum, 2004). Viticulture is facing many challenges such as climate change and the necessary reduction of pesticide and fertilizer inputs. Given that the productive life of a commercial vineyard may reach more than 40 years, considering the long-term impacts of

viticultural practices is very important (Longbottom and Petrie, 2015). Furthermore, this also offers the opportunity to improve the environmental footprint of vineyard operations (Longbottom and Petrie, 2015). Since this perennial crop has strong, decade-long tie to its site, there is ample potential to address current and future challenges by manipulating its rhizobiome to maximize the provision of soil ecosystem services. Unravelling the grapevine rhizobiome under different conditions such as different fertilization levels, will be key for sustainable viticulture. However, there is still a considerable gap of knowledge about the underlying mechanisms because the sampling of grapevine rhizosphere in the field is challenging. However, in regarding to soil health and the agricultural services, it is not only important to consider the physical and chemical properties of the soil, but also the soil microbiome, more specifically, the microbiome in the rhizosphere. The rhizosphere microbiome is often referred to as a positive interaction between plants and microorganisms (Ryan et al., 2009; Taye et al., 2019) and is involved in important processes such as growth modulation, defense responses, and nutrient uptake (Berendsen et al., 2012; Schlaeppli and Bulgarelli, 2015; Durán et al., 2018; Hu et al., 2018; Li et al., 2020). Moreover, the mineralization of nitrogen and the nitrogen cycle is regulated completely and across all stages by soil microorganisms (as reviewed by Jetten, 2008).

Microbial communities associated with grapevines and wine, respectively, have already been extensively studied (Holland et al., 2014; Burns et al., 2016; Hendgen et al., 2018; Contreras et al., 2019; Gattullo et al., 2020). In vineyard soil, the microbial communities have been described for example as a function of spatial distribution or management practices (Bokulich et al., 2014; Holland et al., 2014; Vega-Avila et al., 2015; Hendgen et al., 2018). Nonetheless, the bulk of these studies were based on soil samples taken only near the grapevines, not on veritable rhizosphere samples. The rhizosphere, however, is the location where crucial processes for the productivity of the agricultural systems take place mediated by microorganisms. Therefore, the processes and microorganisms in the rhizosphere need to be considered in order to study the direct interaction between the grapevine root and the surrounding soil. Therefore, with the aim to investigate all these factors independently, a study was carried out under controlled conditions to reduce the variability resulting from all factors except the fertilization levels with grapevines in large containers under field condition. In two sampling years (2020 and 2021), the rhizobiome of two differently fertilized grapevines were compared. In order to get a better insight into the active microbiome of the rhizosphere of the grapevines, a metabarcoding analysis

was performed on the extracted RNA (Carvalhais et al., 2012; Turner et al., 2013; Garoutte et al., 2016) that indicates microbial activity (Singh et al., 2018). Additionally, to the best of our knowledge, no compositional data analyses of 16S rRNA data of the grapevine rhizosphere microbiome with different fertilization levels under controlled conditions has been carried out before. Data derived from high-throughput sequencing of biological samples must be considered as compositions rather than counts, as ratio-based analyses can lead to qualitatively incorrect conclusions (Fernandes et al., 2014; McLaren et al., 2019; Quinn et al., 2019). Due to the controlled experimental conditions, the effect of the fertilization level on the metatranscriptome of the bacterial communities in the rhizosphere could be investigated.

2. Material and Methods

2.1 Grapevines

The experiments were located at Hochschule Geisenheim, University, Germany. The grapevines (*Vitis vinifera* L. cv. Pinot Noir, rootstock SO4 Kl.31 Op) were planted into large containers (240 l) in 2010. Due to the closed system of the large containers, it was possible to irrigate and fertilize each grapevine individually under field conditions. In this experimental design, a high-fertilized variant N 1 (75 kg of nitrogen added per ha per year) was compared with a low-fertilized variant N 0 (25 kg of nitrogen added per ha per year).

2.2 Rhizosphere and soil sampling

Rhizosphere and soil sampling were performed in April/May (bud break) in 2020 and 2021. For each time point, two containers of N 1 and two containers of N 0 were taken out of the ground. The large containers were divided into three sections: 0 – 30 cm, 30 – 60 cm and 60 – 90 cm. 64 samples for microbiome studies and 16 samples for soil analyses were taken. Only soil attaching to the roots was considered as rhizosphere soil for sampling and microbiome analyses. Samples for microbiome analyses were stored at -80 °C, and samples for soil analyses were stored at 4 °C.

2.3 Soil analyses

All soil analyses were performed at the Department of Soil Science and Plant Nutrition, Hochschule Geisenheim University according to the VDLUFA database of methods (Bassler, 2011). For a detailed description of the conducted analyses, see Hendgen et al. (2018). The results are displayed as mean values in tables 1 and 2.

2.4 RNA extraction, Reverse-Transcriptase-PCR, and Ion Torrent Sequencing

RNA extraction from the rhizosphere soil and cDNA amplification were done according to Rosado-Porto et al. (2021). First Ion Torrent PCR was performed with a KAPA HiFi Polymerase kit (VWR International GmbH, Darmstadt, Germany) amplifying the partial sequence of the hypervariable regions (V4 and V5) of the 16S rRNA gene with the primer 520F (5'-AYTGGGYDTAAAGNG-3', (Claesson et al., 2009)) and 926R (5'-CCGYCAATTYMTTTRAGTTT-3', (Engelbrektson et al., 2010)). Amplification parameters were 3 min at 95 °C followed by 35 cycles at 98 °C for 20 s, 55 °C for 30 s, 72 °C for 30 s and finally 72 °C for 5 min. Second Ion Torrent PCR with primers including barcodes and Ion Torrent sequencing adapters was conducted with the PCR product from the first Ion Torrent PCR as suggested by Berry et al. (2011). The PCR was performed with the following amplification parameters: 3 min at 95 °C followed by 10 cycles at 98 °C for 20 s, 55 °C for 30 s, 72 °C for 30 s and finally 72 °C for 7 min. The PCR products were applied to an 1 % agarose gel and subsequently purified using NucleoSpin® Gel and PCR Clean-up (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). Further, this product was purified with DNA purification beads NucleoMagVR NGS clean-up kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). The Ion Torrent Sequencing was done according to Kaplan et al. (2019).

2.5 Bioinformatic analysis of the sequencing data

Bioinformatic analysis was performed with QIIME 2 2020.11 (Bolyen et al., 2019). The obtained raw sequences were demultiplexed using cutadapt (Martin, 2011) with no errors allowed in the barcode sequences. Quality control, sequence denoising, clustering to amplicon sequence variants (ASVs), dereplication and removal of chimera sequences were conducted

with DADA2 (Callahan et al., 2016) (via q2-dada2). Thereby the first 15 base pairs (bp) were removed and the sequences were cut to a length of 312 bp (May 2019), 317 bp (October 2019), and 312 bp (October 2020), respectively. Afterwards, the taxonomy was assigned as described previously by Dries et al. (2021a) and all ASVs belonging to chloroplasts and mitochondria were removed. All ASVs were aligned with MAFFT (Katoh and Standley, 2013) (via q2-alignment) and used for constructing a phylogeny with fasttree2 (Price et al., 2010) (via q2-phylogeny). The feature table was filtered for the 20 dominant bacterial families and taxabarplots were created with GraphPad Prism version 9.3.1 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). Alpha-diversity metrics (Shannon, Simpson, and observed features) and significances were calculated with Kruskal-Wallis or PERMANOVA (Anderson, 2017), respectively. Beta-diversity was measured using DEICODE (Martino et al., 2019) with a filtered beta-diversity ordination file. For this purpose, all feature counts below 10 and all sample counts below 500 were removed. Beta-diversity for all time points was visualized within a compositional biplot, displaying the eight most important features. From the ASVs shown as arrows in the DEICODE graphs, a more accurate taxonomic affiliation was done with a pairwise alignment at the online database EzBioCloud (Yoon et al., 2017). The distance matrices were also analyzed by the PERMANOVA test, using 999 permutations. Significance of differential abundance was determined using ALDEx2 (Fernandes et al., 2013). Therefore, all feature frequency counts below 10 and all sample counts below 2 were removed.

3. Results

Soil parameters

The main chemical characteristics of the sampled soils were measured. The pH levels (Table 1) ranged between 7.2 and 7.7.

Table 1. Main chemical characteristics of the soil of the large containers in 2020 and 2021. Phosphorus (P₂O₅), potassium (K) and magnesium (Mg) values are expressed in mg kg⁻¹.

year	fertilization	sample type	depth	Mg	P ₂ O ₅	K	pH-value
2020	N 0	bulk soil	-	18	44	9.85	7.5
	N 0	rhizosphere	0 - 30 cm	15.5	46	10.5	7.5
	N 0	rhizosphere	30 - 60 cm	14	39	11	7.65
	N 0	rhizosphere	60 - 90 cm	14.5	42	14	7.65
	N 0	interrow	0 - 30 cm	9	16	25	7.25
	N 0	interrow	30 - 60 cm	11	14.5	16.5	7.45
	N 0	interrow	60 - 90 cm	10.5	6	11.5	7.35
	N 1	bulk soil	-	15	26	7.15	7.55
	N 1	rhizosphere	0 - 30 cm	16	30	7.45	7.5
	N 1	rhizosphere	30 - 60 cm	15	24	7.35	7.55
	N 1	rhizosphere	60 - 90 cm	13.5	41.5	10.2	7.5
	N 1	interrow	0 - 30 cm	9	15	25	7.2
	N 1	interrow	30 - 60 cm	11	17	16.5	7.35
	N 1	interrow	60 - 90 cm	12.5	12.5	15.5	7.4
2021	N 0	bulk soil	-	11.5	43.5	11	7.65
	N 0	rhizosphere	0 - 30 cm	10.5	38.5	7.5	7.7
	N 0	rhizosphere	30 - 60 cm	9	42	10	7.7
	N 0	rhizosphere	60 - 90 cm	11	49	14.5	7.7
	N 0	interrow	0 - 30 cm	7	45	24.5	7.4
	N 0	interrow	30 - 60 cm	7.5	43.5	17.5	7.55
	N 0	interrow	60 - 90 cm	9	24	12.5	7.55
	N 1	bulk soil	-	14	34	7	7.6
	N 1	rhizosphere	0 - 30 cm	13.5	25	6	7.5
	N 1	rhizosphere	30 - 60 cm	13	42	7.5	7.65
	N 1	rhizosphere	60 - 90 cm	13.5	29	6	7.45
	N 1	interrow	0 - 30 cm	7.5	44.5	23.5	7.3
	N 1	interrow	30 - 60 cm	9	49.5	16.5	7.3
	N 1	interrow	60 - 90 cm	9.5	40.5	11	7.4

Moreover, the Nitrogen amount (NO₃-N) were measured. There was no NH₄-N detected in the soil samples, therefore, these values are not displayed in Table 2.

Table 2. Nitrogen amount (NO₃-N) of the large containers in 2020 and 2021.

year	fertilization	sample type	depth	NO₃-N [kg/ha]
2020	N 0	bulk soil	-	4.0
	N 0	rhizosphere	0 - 30 cm	4.0
	N 0	rhizosphere	30 - 60 cm	5.1
	N 0	rhizosphere	60 - 90 cm	3.4
	N 0	interrow	0 - 30 cm	3.5
	N 0	interrow	30 - 60 cm	4.3
	N 0	interrow	60 - 90 cm	2.9
	N 1	bulk soil	-	4.3
	N 1	rhizosphere	0 - 30 cm	2.4
	N 1	rhizosphere	30 - 60 cm	2.3
	N 1	rhizosphere	60 - 90 cm	1.6
	N 1	interrow	0 - 30 cm	1.9
	N 1	interrow	30 - 60 cm	1.7
	N 1	interrow	60 - 90 cm	1.2
2021	N 0	bulk soil	-	8.9
	N 0	rhizosphere	0 - 30 cm	7.5
	N 0	rhizosphere	30 - 60 cm	5.5
	N 0	rhizosphere	60 - 90 cm	4.9
	N 0	interrow	0 - 30 cm	2.7
	N 0	interrow	30 - 60 cm	1.9
	N 0	interrow	60 - 90 cm	1.4
	N 1	bulk soil	-	8.9
	N 1	rhizosphere	0 - 30 cm	6.8
	N 1	rhizosphere	30 - 60 cm	3.4
	N 1	rhizosphere	60 - 90 cm	4.1
	N 1	interrow	0 - 30 cm	3.9
	N 1	interrow	30 - 60 cm	1.9
	N 1	interrow	60 - 90 cm	4.3

Bacterial alpha-diversity across the different experimental set-ups

The 20 dominating bacterial families for the experimental setup are shown in Figures 1 and 2. In the rhizosphere of Pinot Noir grapevine under different fertilization levels, the bacterial families in the rhizosphere showed only slight differences.

For fertilization levels, significant effects were found in alpha-diversity indices Simpson, Shannon and observed features for bud break 2020, but not for 2021 (Table 3). However, while

the Shannon–Wiener index is strongly influenced by species richness and by rare species, the Simpson index gives more weight to evenness and common species. Nonetheless, shows Shannon index both richness and abundance. Regarding sampling depths (Table 4), for 2020 statistical differences were found in alpha-diversity indices Simpson, Shannon and observed features between 0 – 30 cm and 30 – 60 cm, 0 – 30 cm and 60 – 90 cm, and 30 – 60 cm and 60 – 90 cm. Moreover, for 2021, statistical differences were found between 0 – 30 cm and 60 – 90 cm, and 30 – 60 cm and 60 – 90 cm for alpha-diversity indices Simpson, Shannon and observed features. Between sampling depth 60 – 90 cm and bulk soil, statistical differences were found regarding the observed features ($p = 0.03$).

Table 3. Results of Kruskal-Wallis pairwise tests of alpha-diversity indices per fertilization for time point bud break 2020 and 2021. Significant differences (corrected p-value < 0.05) are indicated with *, no differences are indicated with ns = not significant.

fertilization (N0 vs. N1)	year	simpson	shannon	observed features
	2020	$p = 0.01$ *	$p = 0.02$ *	$p = 0.03$ *
	2021	$p = 0.1$ ns	$p = 0.4$ ns	$p = 0.7$ ns

Table 4. Results of Kruskal-Wallis pairwise tests of alpha-diversity indices per sampling depth for time point bud break 2020 and 2021. Significant differences (corrected p-value < 0.05) are indicated with *, no differences are indicated with ns = not significant.

sampling depth	year	group 1	group 2	simpson	shannon	observed features
	2020	0-30 cm	30-60 cm	0,01 *	< 0,01 *	< 0,01 *
		0-30 cm	60-90 cm	< 0,01 *	< 0,01 *	< 0,01 *
		0-30 cm	bulk soil	0,35 ns	0,33 ns	0,52 ns
		30-60 cm	60-90 cm	0,01 *	< 0,01 *	0,01 *
		30-60 cm	bulk soil	0,63 ns	0,33 ns	0,17 ns
		60-90 cm	bulk soil	0,29 ns	0,16 ns	0,16 ns
	2021	0-30 cm	30-60 cm	0,26 ns	0,21 ns	0,08 ns
		0-30 cm	60-90 cm	0,03 *	< 0,01 *	< 0,01 *
		0-30 cm	bulk soil	1,00 ns	0,82 ns	0,88 ns
		30-60 cm	60-90 cm	0,04 *	0,03 *	0,02 *
		30-60 cm	bulk soil	0,58 ns	0,42 ns	0,26 ns
		60-90 cm	bulk soil	0,11 ns	0,06 ns	0,03 *

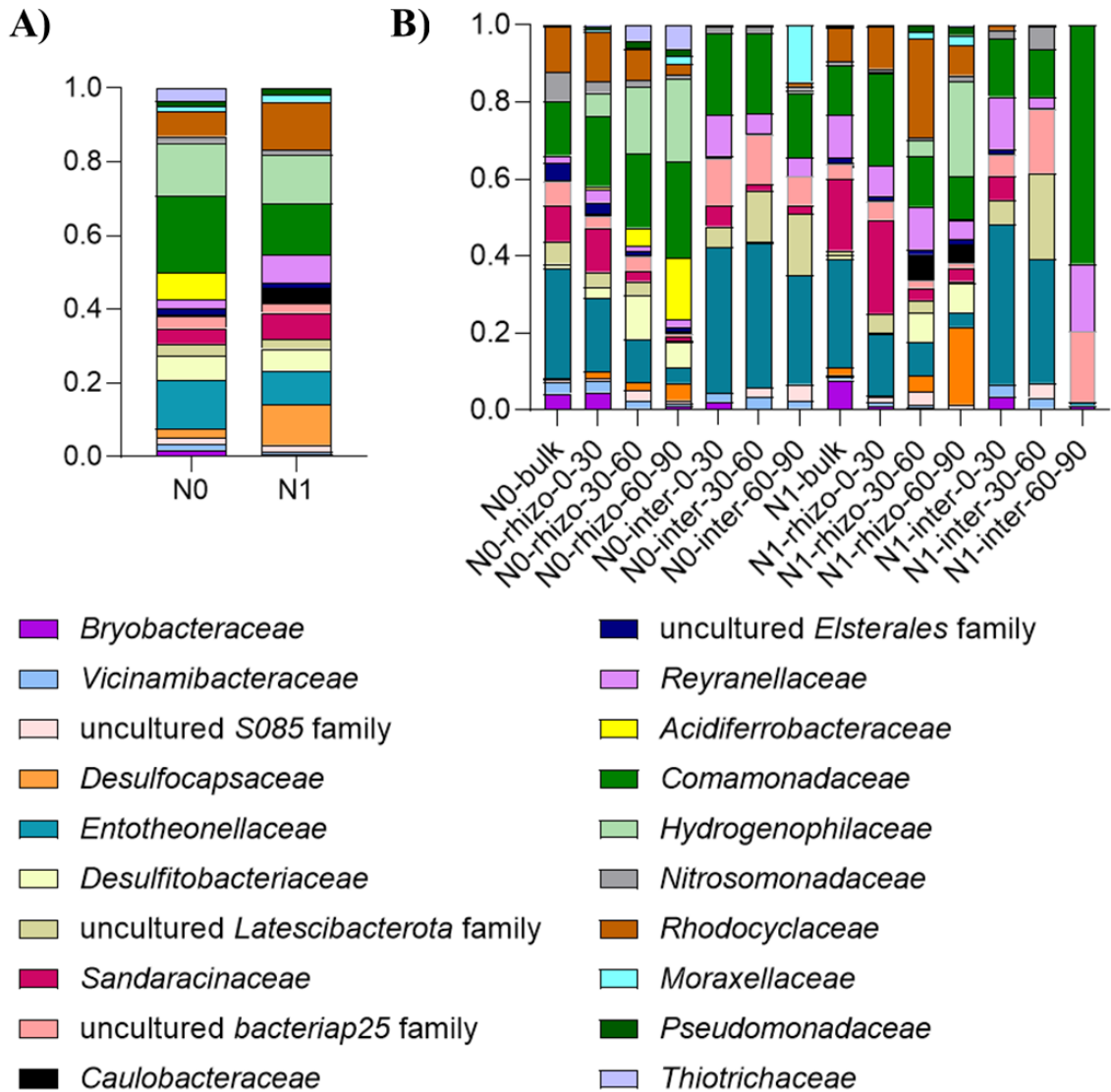


Figure 1. Relative abundance of different bacterial families (in %) in the rhizosphere of grapevine. A) The graph shows the 20 most dominating bacterial families in the rhizosphere of Pinot Noir under the two different fertilization levels N0 and N1 (time point bud break 2020). B) The graph shows the bacterial families regarding the different sampling depths (time point bud break 2020).

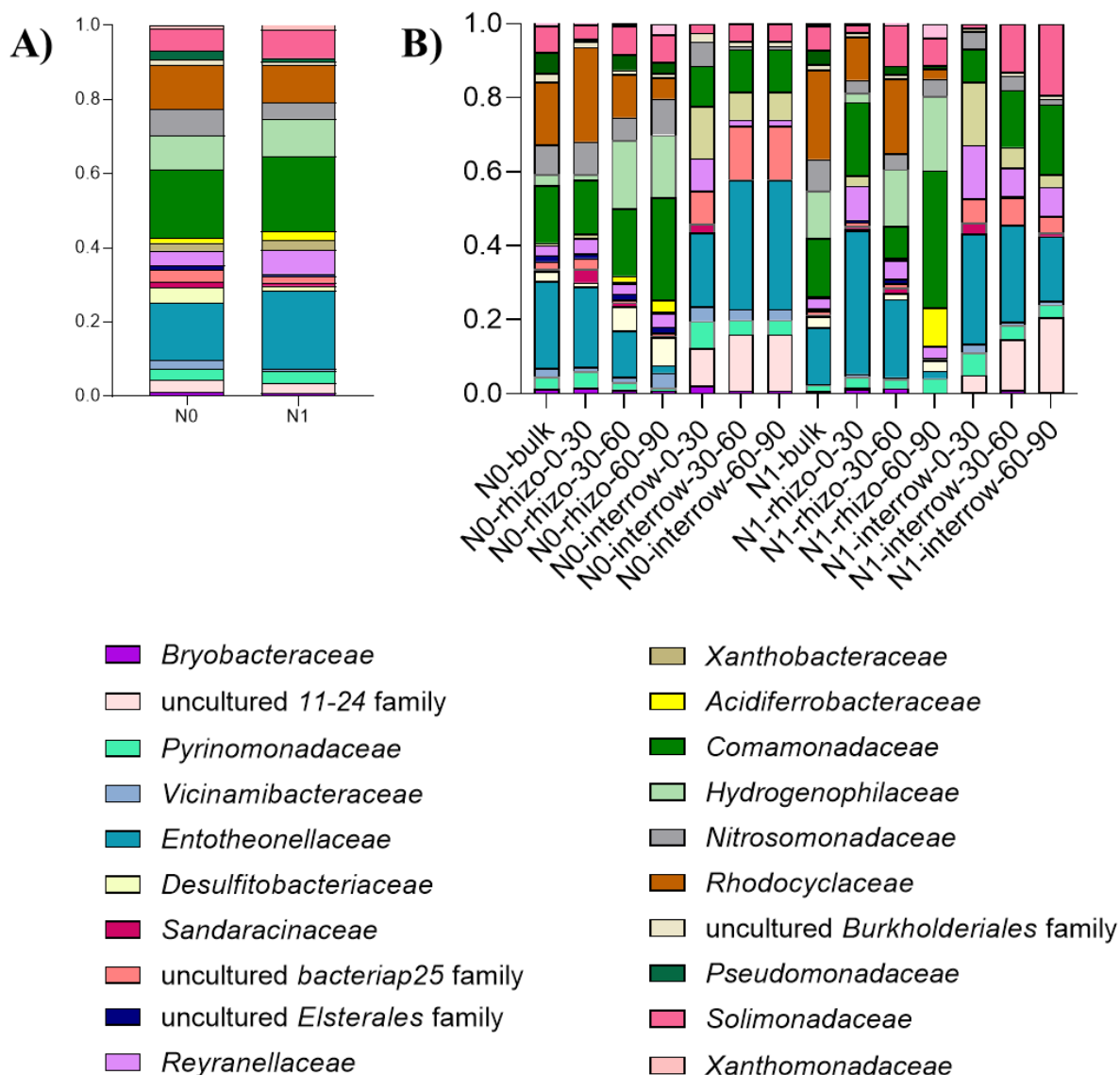


Figure 2. Relative abundance of different bacterial families (in %) in the rhizosphere of grapevine. A) The graph shows the 20 most dominating bacterial families in the rhizosphere of Pinot Noir under the two different fertilization levels N0 and N1 (time point bud break 2021). B) The graph shows the bacterial families regarding the different sampling depths (time point bud break 2021).

Bacterial beta-diversity across the different experimental set-ups

Compositional beta-diversity metric was calculated using a robust Aitchison PCA via DEICODE. Aitchison distance is a Euclidean distance between samples after center log ratio (clr) transformation. For all experimental set-ups and time points significant differences in the beta-diversity of the bacterial communities were detected (Figures 3 and 4). The determinant of the bacterial communities in the rhizosphere is the fertilization level and sampling depth, respectively. PERMANOVA pairwise results reveal statistical differences for the two time points bud break 2020 and bud break 2021 between different fertilization levels ($p = 0.001$ and $p = 0.001$) and different sampling depths ($p = 0.001$ and $p = 0.004$).

DEICODE allows the display of a biplot by showing not only the Aitchison distances but also the taxa (in the form of an arrow) that most strongly influence principal component axes. The eight most important taxa influencing the principal component axes shown in each figure are members of the phyla *Bacillota*, *Pseudomonadota*, *Nitrospinae/Tectomicrobia* group, and *Proteobacteria*. According to the taxonomic classification, *Proteobacteria*, *Nitrospinae/Tectomicrobia* group, and *Acidobacteria* form the main composition of the predominant phyla in the rhizosphere of Pinot Noir under different fertilization levels. The sequences next relative to the bacteria *Reyranella soli* (JX260424, 99.36 %), *Desulfosporosinus fructosivorans* (KX822015, 98.73 %), *Nitrospinales* spec., and *Thiobacillus* spec. are belonging to the most important features in both sampled years (Figures 3 and 4).

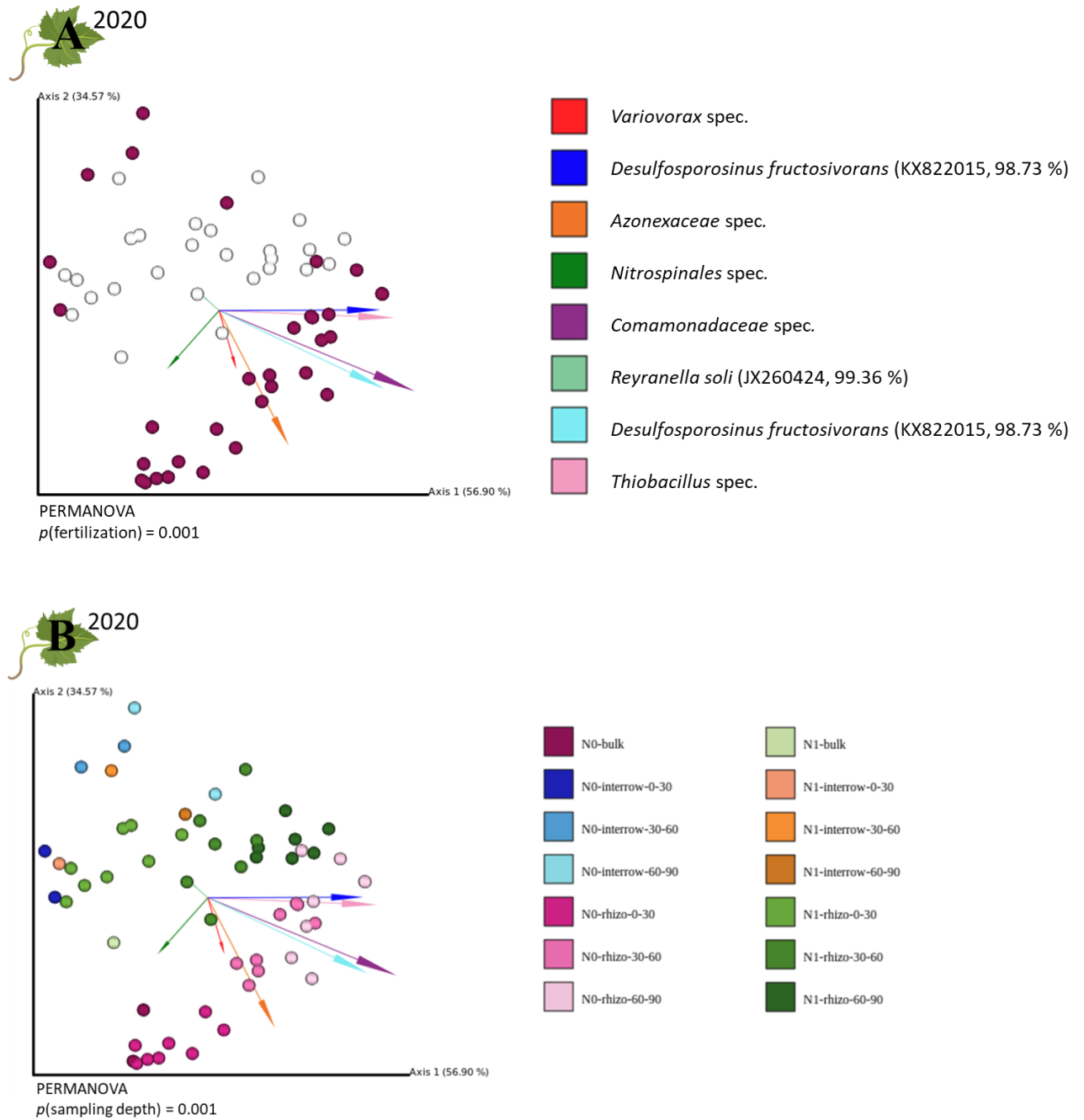


Figure 3. PCoA biplots calculated based on robust Aitchison community dissimilarity distance matrix with arrows that illustrate the ASVs that strongly influence the principal component axis. Taxonomic affiliations of the arrows showed the next related sequences with accession numbers found by pairwise alignment at EzBioCloud Database. Numbers in the brackets show the percentage of identity of the ASVs sequence with the next related sequence. A) Beta-diversity of bacterial rhizosphere communities for Pinot Noir under different fertilization levels (time point bud break 2020). Statistical differences were detected between all groups

(PERMANOVA, $p = 0.001$). B) Beta-diversity of bacterial rhizosphere communities for Pinot Noir under different sampling depths (time point bud break 2020). Statistical differences were detected between all groups (PERMANOVA, $p = 0.001$).

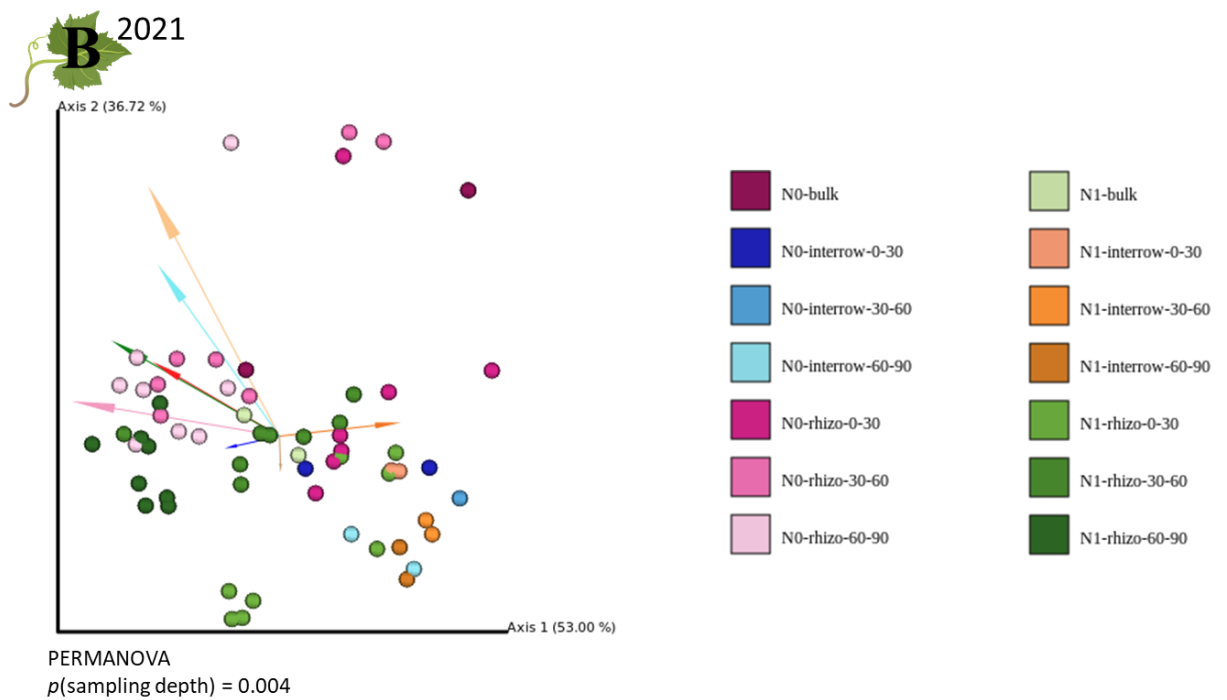
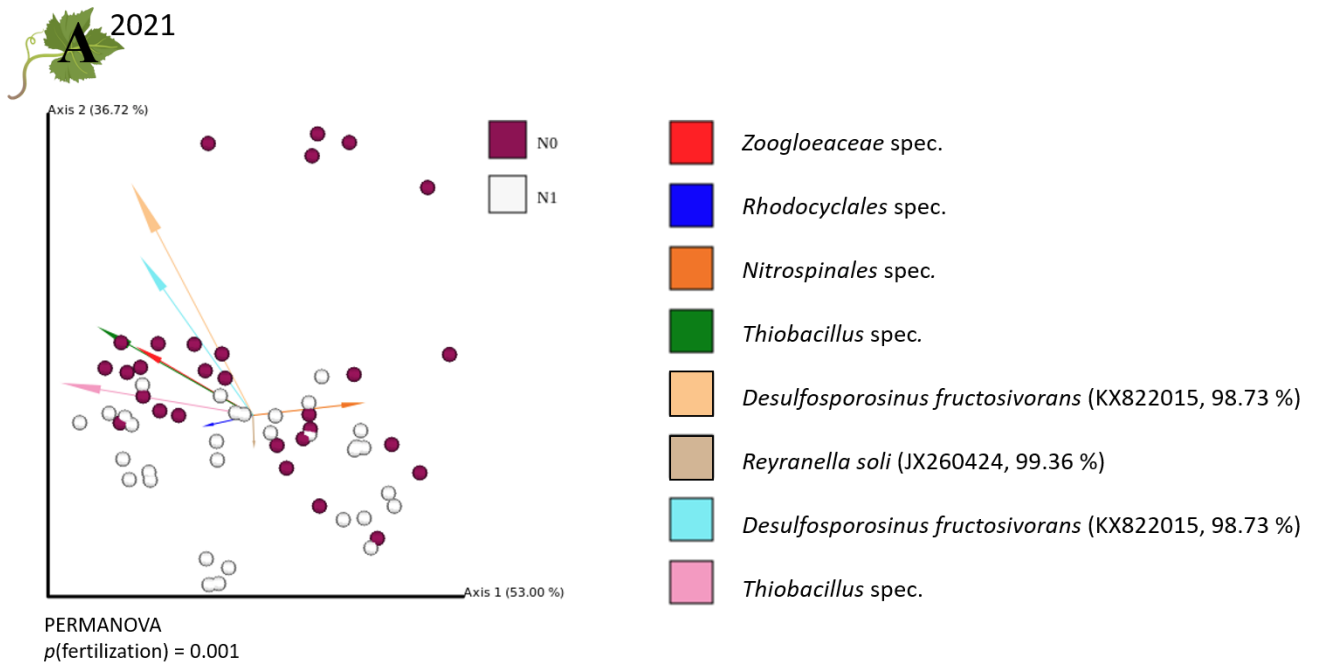


Figure 4. PCoA biplots calculated based on robust Aitchison community dissimilarity distance matrix with arrows that illustrate the ASVs that strongly influence the principal component axis. Taxonomic affiliations of the arrows showed the next related sequences with accession numbers found by pairwise alignment at EzBioCloud Database. Numbers in the brackets show the percentage of identity of the ASVs sequence with the next related sequence. A) Beta-diversity of bacterial rhizosphere communities for Pinot Noir under different fertilization levels (time point bud break 2021). Statistical differences were detected between all groups (PERMANOVA, $p = 0.001$). B) Beta-diversity of bacterial rhizosphere communities for Pinot Noir under different sampling depths (time point bud break 2021). Statistical differences were detected between all groups (PERMANOVA, $p = 0.004$).

Changes on the rhizosphere microbial community

Compositional differential abundance analyses indicated that several bacterial genera in the rhizosphere were affected. ALDEx2 demonstrated that for time point bud break 2020, in total 44 bacterial genera differed according to the fertilization level. Bacterial genera with highest fold changes belonged to *Nitrosomonadaceae*, *Rhodocyclaceae*, and *Entotheonellaceae*. For time point bud break 2021, ALDEx2 demonstrated in total nine bacterial genera differing according to the fertilization level, mainly composed of *Rhodocyclaceae*, *Solimonadaceae*, and *Nitrosomonadaceae*.

4. Discussion

The experimental design of this study aimed at minimizing the variability coming from all factors except nitrogen fertilization levels and sampling depths using grapevines in large containers under field conditions.

The differences in the bacterial communities between the two experimental set ups, were investigated using compositional data analyses. The observed ASVs revealed that the grapevine rhizosphere under the two different nitrogen levels recruited complex bacterial communities mainly composed of *Proteobacteria*, *Nitrospinae/Tectomicrobia* group, and *Acidobacteria*. A former study by (Dries et al., 2021a) with ungrafted grapevine rootstocks showed the phyla

Proteobacteria and *Acidobacteria* as some of the predominant bacterial communities in the rhizosphere. Other studies have come to comparable conclusions (Zarraonaindia et al., 2015; Novello et al., 2017; Marasco et al., 2018; Berlanas et al., 2019; Coller et al., 2019; Torres et al., 2021; Vink et al., 2021).

The dominating families give a higher resolution on taxonomic level which is more suitable for comparison than phyla level. During all experimental time points *Entotheonellaceae*, *Comamonadaceae*, *Hydrogenophilaceae*, and *Rhodocyclaceae* were found as dominating families among others (Figure 1 and 2). Comparing them with the results of the compositional differential abundance analyses (ALDEx2) for time point bud break 2020 and 2021 revealed also ASVs next relative to *Rhodocyclaceae*, *Entotheonellaceae*, *Hydrogenophilaceae*, and *Comamonadaceae*. *Comamonadaceae* were also found in a study by D'Amico et al. (2018) in the rhizosphere of grapevine. To the best of our knowledge, *Entotheonellaceae*, *Hydrogenophilaceae* and *Rhodocyclaceae* were not described for the rhizosphere of grapevine before. As a wider variety of bacteria are found in soil, it can be assumed that soil serves as a primary reservoir for potential plant-associated bacteria (Zarraonaindia et al., 2015). However, soil as well as rhizosphere can also form a path of infection for soil-borne pathogens (Berlanas et al., 2019). For grapevines, this includes pathogens such as *Fusarium oxysporum* (Freire Cruz and Carvalho Pires, 2014) or *Sorosphaera viticola* (Neuhauser S., Huber L., Kirchmair M., 2009). Changes in the 20 most dominating bacterial families between 2020 and 2021 were detected. These changes could be due to the different sampled containers and different weather conditions in the two sampled years. However, 14 of 20 families remained the same, among them *Reyraneliaceae*, *Acidiferrobacteraceae*, and *Pseudomonadaceae*. Members of *Pseudomonadaceae* family are already known as plant-growth promoting bacteria, beneficial for plant health (as reviewed by Roquigny et al., 2017).

Löhnertz (1988) estimated the average N requirement of the vine to be 50 kg/ha per year. This ensures optimal vegetative growth if only grapes are exported from the vineyard. However, in this experimental design, leaves and cut wood were not returned to the soil. In this study, the NO_3^- levels in the soil samples are not far apart between the two experimental conditions. This could be due to the fact that grapevines require about 92 kg/ha of N per year (Löhnertz, 1988), so both experimental conditions took up the same amount of nitrogen, even at different fertilization levels. However, bacterial conditions were different because of the different

fertilizer rates and sampling depths. In most cases, NH_4^+ was not found in the soil samples because it is rapidly nitrified in soils (as described in Verdenal et al., 2021). This was also true for this study, as no NH_4^+ was found in the samples.

The alpha-diversity indices in this study revealed statistical differences regarding the fertilization level in 2020, but not in 2021. This may indicate that the bacteria changed during the year, which could be due to weather conditions, or other abiotic or biotic factors.

The underlying reason for this varying colonization could originate from the root exudates. Indeed, root exudates are strongly influenced by the cultivar and they are considered among the most important factors in the recruitment of the microbiome (Marasco et al., 2018; Wei et al., 2019; Kusstatscher et al., 2021). As a key role in the rhizosphere ecosystem, it is essential to understand the root exudation patterns to unravel the subsequent effects on the surrounding soil and microbial communities (Yee et al., 2021).

The beta-diversity revealed statistical differences in the two experimental set-ups. Pairwise PERMANOVA results showed statistical differences between Pinot Noir under different nitrogen fertilization levels and in different sampling depths. The results indicate that both the level of nitrogen fertilizer and the different depths at which the samples were taken have an effect on metabolically active bacteria in the rhizosphere of grapevine. This is also in accordance to former studies, showing that the rhizosphere microbiome was strongly affected by the level of nitrogen fertilizer in soybean (Ikeda et al., 2010) or Chinese cabbage (Qi et al., 2022). Moreover, Kang et al. (2022) demonstrated that moderate nitrogen fertilization modulated enhanced root colonization by plant growth promoting bacteria, significantly promoting plant growth and nitrogen use efficiency. They also suggest that rational nitrogen fertilization is critical to promote beneficial rhizosphere interactions for sustainable agricultural production (Kang et al., 2022). Regarding sampling depths, it was already shown by different studies (for instance by Ekelund et al., 2001; Fierer et al., 2003), that sampling depth has an impact on the bacterial communities. In a study conducted by Steenwerth et al. (2008), to investigate morphology, depth and grapevine root frequency influencing microbial communities in a Pinot noir vineyard, it was shown that the distribution of soil microbial communities could have been explained with depth. Thus, they conclude that compared to other systems, the distinct patterns in soil microbial communities as influenced by depth and root distribution in this Pinot noir vineyard suggest that vineyard management practices and deep

grapevine root distribution combine to cultivate a unique microbial community in these soil profiles (Steenwerth et al., 2008). Furthermore, they hypothesize that nutrient turnover and decomposition may be unique in these soils (Steenwerth et al., 2008). However, the authors did not refer to a DNA or RNA sequencing method to describe the bacterial communities more deeply. Comparing the ASVs strongly influencing the principal component axis from the PCoA biplot (Figures 3 and 4) between time point bud break 2020 and time point bud break 2021 revealed ASVs next relative to *Nitrospinales* spec., *Thiobacillus* spec., *Reyranella soli*, and *Desulfosporosinus fructosivorans* as those taxa appearing in both analyses. *Reyranella soli* was already described as a bacterium isolated from forest soil in Korea (Kim et al., 2013), belonging to the genus *Reyranella*. Some members of this genus are already described as denitrifying bacteria (Chen et al., 2020; Pessi et al., 2022). *Desulfosporosinus fructosivorans* was described previously as a bacterium inhabiting the subsurface sediments of the Baltic Sea (Vandieken et al., 2017). Members of this genus are known for sulfate-reducing (Hausmann et al., 2019; Sato et al., 2019). In contrast, the studied *Thiobacillus* spp. in the samples of the two years is related to sulfur-oxidizing bacteria and believed to play a major role in the sulfur cycle (Kumar et al., 2020). Kumar et al. (2020) further review, that *Thiobacillus* and its related genera are believed to play an important role in crop production through S-oxidation, P-solubilization, and solubilization of other nutrients. However, further research on this topic is needed to reveal all effects of fertilization on the bacterial communities in the rhizosphere. In addition, the effects on the growth and health of the vine must be revealed from a practical perspective. A thorough understanding of microorganisms in vineyard soil, and the complex relationships between microbial communities, soil properties and plant are crucial for enhancing plant productivity, grape production, biogeochemical processes and vineyard management practices (Holland et al., 2014; Di Liu et al., 2019; Liang et al., 2019; Dries et al., 2021b; Yee et al., 2021). Inferring, the colonization of the microorganisms in the rhizosphere and the root exudation patterns of grapevine must be investigated. Future research in this field is inevitably required to provide a better understanding of the rhizospheric grapevine microbiome in the context of fertilization, rooting depth, root exudates, grape variety, rootstock as well as different environmental conditions.

5. Conclusion

The results from these experimental designs reveal differences in the bacterial communities in the rhizosphere of Pinot Noir under different nitrogen fertilization levels and sampling depths, respectively. The bacteria in the rhizosphere of grapevine are affected by both nitrogen fertilization level and sampling depth. Future research is needed, to provide a better understanding of the differing microorganisms in the grapevine rhizosphere regarding the tested treatments. Moreover, the effects on the grapevine growth and health, and also on the wine quality, have to be revealed. These results may help to reduce nitrogen fertilization in viticulture, leading to a more sustainable management of grapevines.

Acknowledgements

We thank Forschungsring des Deutschen Weinbaus (FDW) for funding this project. We appreciate and acknowledge the members of the Department of Soil Science and Plant Nutrition, Hochschule Geisenheim University, for their assistance in sample taking and soil analyses. We thank the Department of Pomology, Hochschule Geisenheim University, for assistance in sample taking. We are very grateful to Bellinda Schneider, Justus-Liebig University Gießen, for technical support with Ion Torrent Sequencing. We thank the Department of Crop Protection, Hochschule Geisenheim University, for the opportunity to use the molecular biology laboratory and for their helpful advice.

References

- Anderson, M.J., 2017. Permutational Multivariate Analysis of Variance (PERMANOVA). Wiley Statistics Reference Online, 1–15. 10.1002/9781118445112.stat07841.
- Bassler, R. (Ed.), 2011. Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik. Band I: Die Untersuchung von Böden.
- Berendsen, R.L., Pieterse, C.M.J., Bakker, P.A.H.M., 2012. The rhizosphere microbiome and plant health. *Trends in plant science* 17 (8), 478–486. 10.1016/j.tplants.2012.04.001.
- Berlanas, C., Berbegal, M., Elena, G., Laidani, M., Cibriain, J.F., Sagües, A., Gramaje, D., 2019. The Fungal and Bacterial Rhizosphere Microbiome Associated With Grapevine Rootstock Genotypes in Mature and Young Vineyards. *Frontiers in microbiology* 10, 1142. 10.3389/fmicb.2019.01142.
- Berry, D., Ben Mahfoudh, K., Wagner, M., Loy, A., 2011. Barcoded primers used in multiplex amplicon pyrosequencing bias amplification. *Applied and environmental microbiology* 77 (21), 7846–7849. 10.1128/AEM.05220-11.
- Bokulich, N.A., Thorngate, J.H., Richardson, P.M., Mills, D.A., 2014. Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *Proceedings of the National Academy of Sciences of the United States of America* 111 (1), E139-48. 10.1073/pnas.1317377110.
- Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K.B., Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciulek, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh,

- P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., Hippel, M. von, Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K.C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature biotechnology* 37 (8), 852–857. 10.1038/s41587-019-0209-9.
- Burns, K.N., Bokulich, N.A., Cantu, D., Greenhut, R.F., Kluepfel, D.A., O’Geen, A.T., Strauss, S.L., Steenwerth, K.L., 2016. Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: Differentiation by vineyard management. *Soil Biology and Biochemistry* 103, 337–348. 10.1016/j.soilbio.2016.09.007.
- Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature methods* 13 (7), 581–583. 10.1038/nmeth.3869.
- Carvalhais, L.C., Dennis, P.G., Tyson, G.W., Schenk, P.M., 2012. Application of metatranscriptomics to soil environments. *Journal of microbiological methods* 91 (2), 246–251. 10.1016/j.mimet.2012.08.011.
- Chen, S., Qi, G., Ma, G., Zhao, X., 2020. Biochar amendment controlled bacterial wilt through changing soil chemical properties and microbial community. *Microbiological research* 231, 126373. 10.1016/j.micres.2019.126373.
- Claesson, M.J., O’Sullivan, O., Wang, Q., Nikkilä, J., Marchesi, J.R., Smidt, H., Vos, W.M. de, Ross, R.P., O’Toole, P.W., 2009. Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. *PLoS ONE* 4 (8), e6669. 10.1371/journal.pone.0006669.
- Coller, E., Cestaro, A., Zanzotti, R., Bertoldi, D., Pindo, M., Larger, S., Albanese, D., Mescalchin, E., Donati, C., 2019. Microbiome of vineyard soils is shaped by geography and management. *Microbiome* 7 (1), 140. 10.1186/s40168-019-0758-7.
- Contreras, F., Díaz, J., Rombolà, A.D., La Luz Mora, M. de, 2019. Prospecting intercropping between subterranean clover and grapevine as potential strategy for improving grapevine performance. *Current Plant Biology* 19, 100110. 10.1016/j.cpb.2019.100110.
- D’Amico, F., Candela, M., Turrioni, S., Biagi, E., Brigidi, P., Bega, A., Vancini, D., Rampelli, S., 2018. The Rootstock Regulates Microbiome Diversity in Root and Rhizosphere

- Compartments of *Vitis vinifera* Cultivar Lambrusco. *Frontiers in microbiology* 9, 2240. 10.3389/fmicb.2018.02240.
- Di Liu, Zhang, P., Chen, D., Howell, K., 2019. From the Vineyard to the Winery: How Microbial Ecology Drives Regional Distinctiveness of Wine. *Frontiers in microbiology* (10:2679). 10.3389/fmicb.2019.02679.
- Dries, L., Bussotti, S., Pozzi, C., Kunz, R., Schnell, S., Löhnertz, O., Vorkamp, A., 2021a. Rootstocks Shape Their Microbiome-Bacterial Communities in the Rhizosphere of Different Grapevine Rootstocks. *Microorganisms* 9 (4). 10.3390/microorganisms9040822.
- Dries, L., Hendgen, M., Schnell, S., Löhnertz, O., Vorkamp, A., 2021b. Rhizosphere engineering: leading towards a sustainable viticulture? *OENO One* 55 (2), 353–363. 10.20870/oenone.2021.55.2.4534.
- Durán, P., Thiergart, T., Garrido-Oter, R., Agler, M., Kemen, E., Schulze-Lefert, P., Hacquard, S., 2018. Microbial Interkingdom Interactions in Roots Promote *Arabidopsis* Survival. *Cell* 175 (4), 973-983.e14. 10.1016/j.cell.2018.10.020.
- Ekelund, F., Rønn, R., Christensen, S., 2001. Distribution with depth of protozoa, bacteria and fungi in soil profiles from three Danish forest sites. *Soil Biology and Biochemistry* 33 (4-5), 475–481. 10.1016/S0038-0717(00)00188-7.
- Engelbrektsen, A., Kunin, V., Wrighton, K.C., Zvenigorodsky, N., Chen, F., Ochman, H., Hugenholtz, P., 2010. Experimental factors affecting PCR-based estimates of microbial species richness and evenness. *The ISME journal* 4 (5), 642–647. 10.1038/ismej.2009.153.
- Fernandes, A.D., Macklaim, J.M., Linn, T.G., Reid, G., Gloor, G.B., 2013. ANOVA-like differential expression (ALDEx) analysis for mixed population RNA-Seq. *PLoS ONE* 8 (7), e67019. 10.1371/journal.pone.0067019.
- Fernandes, A.D., Reid, J.N., Macklaim, J.M., McMurrough, T.A., Edgell, D.R., Gloor, G.B., 2014. Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. *Microbiome* 2, 15. 10.1186/2049-2618-2-15.
- Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. *Soil Biology and Biochemistry* 35 (1), 167–176. 10.1016/S0038-0717(02)00251-1.

- Freire Cruz, A., Carvalho Pires, M. de, 2014. Soil-Borne Plant Pathogens Associated to Decline of Grapevine Grown in Greenhouse. *J Plant Physiol Pathol* 02 (01). 10.4172/2329-955X.1000115.
- Garoutte, A., Cardenas, E., Tiedje, J., Howe, A., 2016. Methodologies for probing the metatranscriptome of grassland soil. *Journal of microbiological methods* 131, 122–129. 10.1016/j.mimet.2016.10.018.
- Gattullo, C.E., Mezzapesa, G.N., Stellacci, A.M., Ferrara, G., Occhiogrosso, G., Petrelli, G., Castellini, M., Spagnuolo, M., 2020. Cover Crop for a Sustainable Viticulture: Effects on Soil Properties and Table Grape Production. *Agronomy* 10 (9), 1334. 10.3390/agronomy10091334.
- Hausmann, B., Vandieken, V., Pjevac, P., Schreck, K., Herbold, C.W., Loy, A., 2019. Draft Genome Sequence of *Desulfosporosinus fructosivorans* Strain 63.6FT, Isolated from Marine Sediment in the Baltic Sea. *Microbiology resource announcements* 8 (31). 10.1128/MRA.00427-19.
- Hendgen, M., Hoppe, B., Döring, J., Friedel, M., Kauer, R., Frisch, M., Dahl, A., Kellner, H., 2018. Effects of different management regimes on microbial biodiversity in vineyard soils. *Scientific reports* 8 (1), 9393. 10.1038/s41598-018-27743-0.
- Holland, T.C., Bowen, P., Bogdanoff, C., Hart, M.M., 2014. How distinct are arbuscular mycorrhizal fungal communities associating with grapevines? *Biol Fertil Soils* 50 (4), 667–674. 10.1007/s00374-013-0887-2.
- Hu, L., Robert, C.A.M., Cadot, S., Zhang, X., Ye, M., Li, B., Manzo, D., Chervet, N., Steinger, T., van der Heijden, M.G.A., Schlaeppli, K., Erb, M., 2018. Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature communications* 9 (1), 2738. 10.1038/s41467-018-05122-7.
- Ikeda, S., Okubo, T., Kaneko, T., Inaba, S., Maekawa, T., Eda, S., Sato, S., Tabata, S., Mitsui, H., Minamisawa, K., 2010. Community shifts of soybean stem-associated bacteria responding to different nodulation phenotypes and N levels. *The ISME journal* 4 (3), 315–326. 10.1038/ismej.2009.119.
- Jetten, M.S.M., 2008. The microbial nitrogen cycle. *Environmental microbiology* 10 (11), 2903–2909. 10.1111/j.1462-2920.2008.01786.x.
- an Kang, Zhang, N., Xun, W., Dong, X., Xiao, M., Liu, Z., Xu, Z., Feng, H., Zou, J., Shen, Q., Zhang, R., 2022. Nitrogen fertilization modulates beneficial rhizosphere interactions

- through signaling effect of nitric oxide. *Plant Physiology* 188 (2), 1129–1140. 10.1093/plphys/kiab555.
- Kaplan, H., Ratering, S., Felix-Henningsen, P., Schnell, S., 2019. Stability of in situ immobilization of trace metals with different amendments revealed by microbial ¹³C-labelled wheat root decomposition and efflux-mediated metal resistance of soil bacteria. *The Science of the total environment* 659, 1082–1089. 10.1016/j.scitotenv.2018.12.441.
- Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular biology and evolution* 30 (4), 772–780. 10.1093/molbev/mst010.
- Kim, S.-J., Ahn, J.-H., Lee, T.-H., Weon, H.-Y., Hong, S.-B., Seok, S.-J., Whang, K.-S., Kwon, S.-W., 2013. *Reyranella soli* sp. nov., isolated from forest soil, and emended description of the genus *Reyranella* Pagnier et al. 2011. *International journal of systematic and evolutionary microbiology* 63 (Pt 9), 3164–3167. 10.1099/ijs.0.045922-0.
- Kumar, M., Zeyad, M.T., Choudhary, P., Paul, S., Chakdar, H., Singh Rajawat, M.V., 2020. *Thiobacillus*. *Beneficial Microbes in Agro-Ecology*, 545–557. 10.1016/B978-0-12-823414-3.00026-5.
- Kusstatscher, P., Adam, E., Wicaksono, W.A., Bernhart, M., Olimi, E., Müller, H., Berg, G., 2021. Microbiome-Assisted Breeding to Understand Cultivar-Dependent Assembly in *Cucurbita pepo*. *Frontiers in plant science* 12, 642027. 10.3389/fpls.2021.642027.
- Li, J., Mavrodi, O.V., Hou, J., Blackmon, C., Babiker, E.M., Mavrodi, D.V., 2020. Comparative Analysis of Rhizosphere Microbiomes of Southern Highbush Blueberry (*Vaccinium corymbosum* L.), Darrow's Blueberry (*V. darrowii* Camp), and Rabbiteye Blueberry (*V. virgatum* Aiton). *Frontiers in microbiology* 11, 370. 10.3389/fmicb.2020.00370.
- Liang, H., Wang, X., Yan, J., Luo, L., 2019. Characterizing the Intra-Vineyard Variation of Soil Bacterial and Fungal Communities. *Frontiers in microbiology* 10, 1239. 10.3389/fmicb.2019.01239.
- Löhnertz, O., 1988. Untersuchungen zum zeitlichen Verlauf der Nährstoffaufnahme bei *Vitis vinifera* (cv. Riesling): Geisenheim, Universität Giessen.
- Longbottom, M.L., Petrie, P.R., 2015. Role of vineyard practices in generating and mitigating greenhouse gas emissions. *Australian Journal of Grape and Wine Research* 21, 522–536. 10.1111/ajgw.12197.

- Marasco, R., Rolli, E., Fusi, M., Michoud, G., Daffonchio, D., 2018. Grapevine rootstocks shape underground bacterial microbiome and networking but not potential functionality. *Microbiome* 6 (1), 3. 10.1186/s40168-017-0391-2.
- Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet j.* 17 (1), 10. 10.14806/ej.17.1.200.
- Martino, C., Morton, J.T., Marotz, C.A., Thompson, L.R., Tripathi, A., Knight, R., Zengler, K., 2019. A Novel Sparse Compositional Technique Reveals Microbial Perturbations. *mSystems* 4 (1). 10.1128/mSystems.00016-19.
- McLaren, M.R., Willis, A.D., Callahan, B.J., 2019. Consistent and correctable bias in metagenomic sequencing experiments. *eLife* (8:e46923). 10.7554/eLife.46923.001.
- Nendel, C., Kersebaum, K., 2004. A simple model approach to simulate nitrogen dynamics in vineyard soils. *Ecological Modelling* 177 (1-2), 1–15. 10.1016/j.ecolmodel.2004.01.014.
- Neuhauser S., Huber L., Kirchmair M., 2009. *Sorosphaera viticola*, a plasmodiophorid parasite of grapevine. *Phytopathol Mediterr.* (48(1)), 136–139.
- Novello, G., Gamalero, E., Bona, E., Boatti, L., Mignone, F., Massa, N., Cesaro, P., Lingua, G., Berta, G., 2017. The Rhizosphere Bacterial Microbiota of *Vitis vinifera* cv. Pinot Noir in an Integrated Pest Management Vineyard. *Frontiers in microbiology* 8, 1528. 10.3389/fmicb.2017.01528.
- Pessi, I.S., Viitamäki, S., Virkkala, A.-M., Eronen-Rasimus, E., Delmont, T.O., Marushchak, M.E., Luoto, M., Hultman, J., 2022. In-depth characterization of denitrifier communities across different soil ecosystems in the tundra. *Environmental microbiome* 17 (1), 30. 10.1186/s40793-022-00424-2.
- Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2 - Approximately Maximum-Likelihood Trees for Large Alignments. *PLoS ONE* (5(3)), 1–10. 10.1371/journal.pone.0009490.t001.
- Qi, Y., Wu, Z., Zhou, R., Hou, X., Yu, L., Cao, Y., Jiang, F., 2022. Nitrogen Reduction with Bio-Organic Fertilizer Altered Soil Microorganisms, Improved Yield and Quality of Non-Heading Chinese Cabbage (*Brassica campestris ssp. chinensis* Makino). *Agronomy* 12 (6), 1437. 10.3390/agronomy12061437.
- Quinn, T.P., Erb, I., Gloor, G., Notredame, C., Richardson, M.F., Crowley, T.M., 2019. A field guide for the compositional analysis of any-omics data. *GigaScience* 8 (9). 10.1093/gigascience/giz107.

- Roquigny, R., Novinscak, A., Biessy, A., Filion, M., 2017. *Pseudomonadaceae*: From Biocontrol to Plant Growth Promotion, 39–68. 10.1007/978-981-10-4862-3_3.
- Rosado-Porto, D., Ratering, S., Cardinale, M., Maisinger, C., Moser, G., Deppe, M., Müller, C., Schnell, S., 2021. Elevated Atmospheric CO₂ Modifies Mostly the Metabolic Active Rhizosphere Soil Microbiome in the Giessen FACE Experiment. *Microbial ecology*. 10.1007/s00248-021-01791-y.
- Ryan, P.R., Dessaux, Y., Thomashow, L.S., Weller, D.M., 2009. Rhizosphere engineering and management for sustainable agriculture. *Plant Soil* 321 (1-2), 363–383. 10.1007/s11104-009-0001-6.
- Sato, Y., Hamai, T., Hori, T., Aoyagi, T., Inaba, T., Kobayashi, M., Habe, H., Sakata, T., 2019. *Desulfosporosinus spp.* were the most predominant sulfate-reducing bacteria in pilot- and laboratory-scale passive bioreactors for acid mine drainage treatment. *Applied microbiology and biotechnology* 103 (18), 7783–7793. 10.1007/s00253-019-10063-2.
- Schlaeppli, K., Bulgarelli, D., 2015. The plant microbiome at work. *Molecular plant-microbe interactions : MPMI* 28 (3), 212–217. 10.1094/MPMI-10-14-0334-FI.
- Singh, P., Gobbi, A., Santoni, S., Hansen, L.H., This, P., Péros, J.-P., 2018. Assessing the impact of plant genetic diversity in shaping the microbial community structure of *Vitis vinifera* phyllosphere in the Mediterranean. *Frontiers in Life Science* 11 (1), 35–46. 10.1080/21553769.2018.1552628.
- Steenwerth, K.L., Drenovsky, R.E., Lambert, J.-J., Kluepfel, D.A., Scow, K.M., Smart, D.R., 2008. Soil morphology, depth and grapevine root frequency influence microbial communities in a Pinot noir vineyard. *Soil Biology and Biochemistry* 40 (6), 1330–1340. 10.1016/j.soilbio.2007.04.031.
- Taye, Z.M., Helgason, B.L., Bell, J.K., Norris, C.E., Vail, S., Robinson, S.J., Parkin, I.A.P., Arcand, M., Mamet, S., Links, M.G., Dowhy, T., Siciliano, S., Lamb, E.G., 2019. Core and Differentially Abundant Bacterial Taxa in the Rhizosphere of Field Grown *Brassica napus* Genotypes: Implications for Canola Breeding. *Frontiers in microbiology* 10, 3007. 10.3389/fmicb.2019.03007.
- Torres, N., Yu, R., Kurtural, S.K., 2021. Inoculation with Mycorrhizal Fungi and Irrigation Management Shape the Bacterial and Fungal Communities and Networks in Vineyard Soils. *Microorganisms* 9 (6). 10.3390/microorganisms9061273.

- Turner, T.R., Ramakrishnan, K., Walshaw, J., Heavens, D., Alston, M., Swarbreck, D., Osbourn, A., Grant, A., Poole, P.S., 2013. Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants. *The ISME journal* 7 (12), 2248–2258. 10.1038/ismej.2013.119.
- Vandieken, V., Niemann, H., Engelen, B., Cypionka, H., 2017. *Marinisorobacter balticus* gen. nov., sp. nov., *Desulfosporosinus nitroreducens* sp. nov. and *Desulfosporosinus fructosivorans* sp. nov., new spore-forming bacteria isolated from subsurface sediments of the Baltic Sea. *International journal of systematic and evolutionary microbiology* 67 (6), 1887–1893. 10.1099/ijsem.0.001883.
- Vega-Avila, A.D., Gumiere, T., Andrade, P.A.M., Lima-Perim, J.E., Durrer, A., Baigori, M., Vazquez, F., Andreote, F.D., 2015. Bacterial communities in the rhizosphere of *Vitis vinifera* L. cultivated under distinct agricultural practices in Argentina. *Antonie van Leeuwenhoek* 107 (2), 575–588. 10.1007/s10482-014-0353-7.
- Verdenal, T., Dienes-Nagy, Á., Spangenberg, J.E., Zufferey, V., Spring, J.-L., Viret, O., Marin-Carbonne, J., van Leeuwen, C., 2021. Understanding and managing nitrogen nutrition in grapevine: a review. *OENO One* 55 (1), 1–43. 10.20870/oenone.2021.55.1.3866.
- Vink, S.N., Dini-Andreote, F., Höfle, R., Kicherer, A., Salles, J.F., 2021. Interactive Effects of Scion and Rootstock Genotypes on the Root Microbiome of Grapevines (*Vitis spp.* L.). *Applied Sciences* 11 (4), 1615. 10.3390/app11041615.
- Wei, F., Zhao, L., Xu, X., Feng, H., Shi, Y., Deakin, G., Feng, Z., Zhu, H., 2019. Cultivar-Dependent Variation of the Cotton Rhizosphere and Endosphere Microbiome Under Field Conditions. *Frontiers in plant science* 10, 1659. 10.3389/fpls.2019.01659.
- Yee, M.O., Kim, P., Li, Y., Singh, A.K., Northen, T.R., Chakraborty, R., 2021. Specialized Plant Growth Chamber Designs to Study Complex Rhizosphere Interactions. *Frontiers in microbiology* 12, 625752. 10.3389/fmicb.2021.625752.
- Yoon, S.-H., Ha, S.-M., Kwon, S., Lim, J., Kim, Y., Seo, H., Chun, J., 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *International journal of systematic and evolutionary microbiology* 67 (5), 1613–1617. 10.1099/ijsem.0.001755.
- Zarraonaindia, I., Owens, S.M., Weisenborn, P., West, K., Hampton-Marcell, J., Lax, S., Bokulich, N.A., Mills, D.A., Martin, G., Taghavi, S., van der Lelie, D., Gilbert, J.A., 2015.

The soil microbiome influences grapevine-associated microbiota. *mBio* 6 (2).
10.1128/mBio.02527-14.

3. Discussion

Superordinate, the future aim for sustainable viticulture is to use new insights to enable more precise plant nutrition in the form of more accurate fertilization recommendations in order to reduce the associated costs in an economical and environmentally friendly way. A more precise knowledge of the dynamics of the grapevine rhizosphere microbiome under different management practices, grape varieties, rootstocks, and fertilization levels, should enable targeted adaptation to increasingly frequent extreme years. The variety of vineyard sites, climatic conditions, soil types, and grape varieties across the world's wine-growing regions results in the unavailability of a general type of adaptation strategy towards climate change scenarios (Schultz and Jones 2010). Therefore, a new approach regarding characterizing the structure and function of the rhizobiome as a first step towards its manipulation is inescapable. Consequently, the aim of this study was to investigate the bacterial rhizobiome of grapevines (*Vitis vinifera* L.) under different experimental designs. By characterizing the bacteria, a statement on the existing bacterial communities can consequently be made. To characterize the diversity of the existing bacteria of the rhizosphere in the vineyard ecosystem, which adapts to changing environmental conditions, first, a method was established that allows to obtain rhizosphere material of grapevine plants, and to prepare it for sequencing and further experiments. This applies both to grapevines cultivated in greenhouses under controlled conditions as well as to variants under field conditions. The experimental designs aimed to gain knowledge regarding the bacterial diversity of the grapevine rhizosphere including different grape varieties, different grapevine rootstocks as well as different fertilization measures.

In conclusion, the studies revealed statistical differences in the bacterial communities in the rhizosphere under different conditions. To be more precise, differences between different rootstocks and scion varieties (Chapter 2 and 3), and different nitrogen fertilization levels and sampling depths (Chapter 4) were unravelled. The experimental design of the studies aimed at minimizing the variability coming from all factors except grape variety and rootstock genotype, or nitrogen levels and sampling depths, respectively, using grapevines in pots or large containers under the same environmental conditions.

In Chapter 3, the differences in the bacterial communities between the two grape varieties Riesling and Mueller-Thurgau, ungrafted and grafted onto different rootstocks, were investigated using compositional data analyses. The observed ASVs revealed that the rootstock

and scion rhizosphere, respectively, recruited complex bacterial communities mainly composed of *Proteobacteria*, *Verrucomicrobiota*, *Chloroflexi*, *Myxococcota*, *Acidobacteria*, and *Gemmatimonadota*. The similar study (Chapter 2) with ungrafted grapevine rootstocks showed the phyla *Proteobacteria*, *Acidobacteria*, and *Gemmatimonadota* as some of the predominant bacterial communities in the rhizosphere. The observed ASVs in Chapter 4 revealed also that the grapevine rhizosphere under the two different nitrogen levels recruited complex bacterial communities mainly composed of *Proteobacteria*, *Nitrospinae/Tectomicrobia* group, and *Acidobacteria*. Other studies have come to comparable conclusions, independent of factors such as grape variety and rootstock (Berlanas et al. 2019; Collier et al. 2019; Marasco et al. 2018; Novello et al. 2017; Torres et al. 2021; Vink et al. 2021; Zorraonaindia et al. 2015). According to Berlanas et al. (2019), the dominant phyla found in two vineyards located in Northeastern Spain were *Proteobacteria*, *Actinobacteria*, *Acidobacteria* and *Bacteroidetes*. Similarly, Marasco et al. (2018) reported phylum *Proteobacteria* as prevalent, followed by *Actinobacteria*, *Bacteroidetes*, *Chloroflexi* and *Acidobacteria* in an Italian vineyard. These results highlight that there is a core of bacteria always recruited by grapevine despite the different conditions (e.g., biogeography, and climatic or edaphic factors). Among them, *Proteobacteria* is commonly the dominant phylum followed by *Actinobacteria*. Both phyla play an important role in carbon cycling and production of secondary metabolites (Jenkins et al. 2009). *Proteobacteria* comprise organisms with a broad variety of metabolic capabilities; alpha-, beta-, gamma- and delta-*Proteobacteria* are the classes mostly described in soil studies. The dominating families give a higher resolution on taxonomic level which is more suitable for comparison than phyla level. During all experimental time points of Chapter 3, *Opitutaceae*, *Reyraneliaceae*, “*Solibacteraceae*”, and *Solimnadaceae* were found as dominating families among others (Figure 1 and 2). This is also consistent with a study conducted by Marasco et al. (2018). They revealed “*Solibacteraceae*” as one of the families shaping the topology of the bacterial network in the grafted root system (Marasco et al. 2018). *Comamonadaceae* (Figure 1A and 2) were also found in a study by D'Amico et al. (2018) in the rhizosphere of grapevine rootstocks 5BB and Paulsen 1103. As a wider variety of bacteria are found in soil, it can be assumed that soil serves as a primary reservoir for potential plant-associated bacteria (Zorraonaindia et al. 2015). However, soil as well as rhizosphere can also form a path of infection for soil-borne pathogens (Berlanas et al. 2019). For grapevines, this includes pathogens such as *Fusarium oxysporum* (Freire Cruz and Carvalho Pires 2014) or *Sorosphaera*

viticola (Neuhauser S., Huber L., Kirchmair M. 2009). Knowledge about plant–microbial interactions could be added to plant breeding programs to create and maintain healthy and beneficial microbial communities in the rhizosphere. 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. 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). Moreover, little is known about the metabolic capabilities of the bacteria since most of them are uncultivated. However, this doctoral thesis contributed a part to unravelling the mystery.

The alpha-diversity indices in Chapter 3 revealed higher observed features for ungrafted Riesling grape variety in May 2019 (Table 2). This may indicate that ungrafted Riesling grapevines were able to recruit a higher number of bacteria in their rhizosphere than Mueller-Thurgau grapevines. The underlying reason for this varying colonization could originate from the root exudates. Indeed, root exudates are strongly influenced by the cultivar and they are considered among the most important factors in the recruitment of the microbiome (Kusstascher et al. 2021; Marasco et al. 2018; Wei et al. 2019). As a key role in the rhizosphere ecosystem, it is essential to understand the root exudation patterns to unravel the subsequent effects on the surrounding soil and microbial communities (Yee et al. 2021). The rhizobiome is strongly influenced by plant root exudates (for more details see Sasse et al. 2018) which are characterized by high concentrations of volatile and non-volatile plant-derived organic compounds, released root cap mucilage and root border cells (Calvo et al. 2017). Root exudates represent the main input of carbon into the soil (Kuzyakov et al. 2007). The rhizobiome and grapevine root exudates are considered as a plant shield against pathogens and an active tissue for nutritional supplementation (Dias et al. 2013). Plant-specific microbiomes play a crucial role in supporting plant health and adaptation to changing environmental conditions (Marasco et al. 2018). Furthermore, the composition of microbial communities and potential interactions may help predicting plant health (Xiong et al. 2019).

Despite its potential key role in plant nutrition and plant protection, this root-soil interface in the field is largely understudied due to the physico-chemical and (micro)biological complexity (Brunel et al. 2020). It remains unclear, for instance, whether exudate profiles are solely shaped by plant-driven processes or if the microbial community also influences these (Jones et al. 2019).

However, the results of Chapter 3 are also consistent with previous studies, concluding the grapevine variety as the major factor shaping the vineyard microbiome (Berlanas et al. 2019), but rootstock genotype takes a subordinate position (Vink et al. 2021). The alpha-diversity indices for Riesling and Mueller-Thurgau grafted onto different grapevine rootstocks revealed no statistical differences (Table 2). However, three out of four rootstocks used for this experiment have emerged from the same breedings: *Vitis berlandieri* Planch. x *Vitis riparia* Michx., which may be an explanation of the statistical same bacterial communities in the rhizosphere. The fact, that most cultivated grapevines are genetic chimeras with two different genotypes (Marín et al. 2021), complicates the separation of the genotypes from each other regarding their related microbial communities. Hence, all the studies indicate that certain taxa are always apparently present at all times in the rhizosphere of the grapevine.

The beta-diversity revealed statistical differences in the three experimental set-ups. Pairwise PERMANOVA results showed statistical differences between ungrafted Mueller-Thurgau and ungrafted Riesling grape varieties, but no statistical differences between different rootstocks. The results indicate, that the grape variety may be a driving factor of the bacterial communities in the rhizosphere, but the grafted grapevine scion and rootstock combination are a less driving factor. This is also in accordance to the study of Chapter 2, showing the ungrafted grapevine rootstocks as a driver of the bacterial communities. Thus, it might be concluded that grafting grapevines onto rootstocks implies a change in bacterial communities.

In Chapter 2, it was observed that the microbial communities associated with the four rootstocks did not significantly differ in evenness and richness. Indeed, all the four rootstocks showed a bacterial community with no clearly dominating species and with similar phylogenetic richness. This is consistent with the theory of a well conserved core of bacteria associated with the grapevine rhizosphere (Berlanas et al. 2019). Chapter 2, however, showed that rootstocks 1103P, 140 Ru, 161-49C and Kober 5BB were able to assemble distinct bacterial microbiota at the rhizosphere level. The percentage explained by weighted (46%) and unweighted UniFrac (22%) suggests that the differences are mainly driven by a different recruitment of the same bacteria. Moreover, the LEfSe analysis detected 68 clades significantly differentially recruited by the four investigated rootstocks. These differences confirm the rootstock genotype as a substantial driver of the bacterial communities associated with the rhizosphere. The rootstock genotype may exert its influence on the rhizosphere associated communities through specific

root exudates (Badri et al. 2013; Bulgarelli et al. 2012; Matthews et al. 2019) and by means of its immune system. Indeed, it has been proven that the immune system can affect not only the microbial composition of the root interior surfaces but also the communities thriving near the root (Lebeis et al. 2015; Liu et al. 2018). Marasco et al. (2018) have investigated whether different rootstocks affect the recruitment of the bacteria from the surrounding soil comparing ungrafted and grafted grapevines of the *Vitis vinifera* cultivar Barbera, all cultivated in the same soil. They concluded that the rootstock can influence the diversity and richness of the bacterial communities associated with the root and that the rootstock is a factor determining the specificity of the microbiota independently of the scion cultivar. D'Amico et al. (2018) have highlighted the importance of the microbiota recruited by the rootstock with a cultivation-independent technique: the same scion (*V. vinifera* cv. Lambrusco) was grafted onto two different rootstocks (1103 Paulsen and Kober 5BB) in the same vineyard, where only the grapevines grafted on 1103 Paulsen had potassium absorption problems. The analysis of the microbiota with an amplicon-based approach has revealed that the rootstock 1103 Paulsen is not able to successfully recruit several potassium solubilizing microorganisms (D'Amico et al. 2018). Thus, the knowledge of the microbiota associated with diverse rootstocks is valuable to make the right choice at the moment of the implant, to intervene when the choice is already taken, and also for breeders to select for rootstocks with a specific microbiota.

Moreover, Vink et al. (2021) revealed in their study that the main determinant of the bacterial communities was scion variety for the alpha-diversity and a significant interaction between scion and rootstocks regarding the beta-diversity. However, the authors did not refer to ungrafted grape varieties. In another study conducted by Berlanas et al. (2019), they described the rootstock genotype as the most important factor in shaping the microbiome. Wright et al. (2022) revealed that the rootstock was a significant factor driving the root microbiome with grape variety New York Muscat ungrafted and grafted onto two different rootstocks.

Comparing the ASVs in Chapter 3 strongly influencing the principal component axis from the PCoA biplot (Figure 3) with the results of the compositional differential abundance analyses (ALDEx2, Tables 1 – 3, Supplemental Material) for time point May 2019 revealed ASVs next relative to *Nevskia terrae*, uncultured *Candidatus Solibacter*, and uncultured *Deltaproteobacteria* as those taxa appearing in both analyses. *Nevskia terrae* was already described as a bacterium isolated from soil in Korea (Kim et al. 2011), belonging to the

Xanthomonadaceae. Some members of this family are already described as plant-growth promoting (Cutíño-Jiménez et al. 2020). *Candidatus Solibacter* was described previously as a bacterium inhabiting the rhizosphere of walnut trees (Bai et al. 2020), and *Deltaproteobacteria* in rice (Zhang et al. 2018). For time point October 2020, all eight displayed ASVs strongly influencing the principal component axis from the DEICODE beta-diversity also appeared in the compositional abundance analyses (ALDEx2, Tables 4 – 9, Supplemental Material). The next relative *Phenylobacterium deserti* was first isolated from desert soil (Khan et al. 2017), other *Phenylobacterium* were also isolated from different soil samples (Khan et al. 2018; Li et al. 2019). The next relative of uncultured “*Acidibacter*” belongs to *Gammaproteobacteria*, members of this class are known for plant growth-promoting traits, inhabiting the rhizosphere (Madhaiyan et al. 2017), root nodules (Ibáñez et al. 2009) or plant tissue (Madhaiyan et al. 2020).

Comparing the ASVs in Chapter 3 strongly influencing the principal component axis from the PCoA biplot, *Entotheonellaceae*, *Comamonadaceae*, *Hydrogenophilaceae*, and *Rhodocyclaceae* were found as dominating families among others (Figure 1 and 2) during both time points. Comparing them with the results of the compositional differential abundance analyses (ALDEx2) for time point bud break 2020 and 2021 revealed also ASVs next relative to *Rhodocyclaceae*, *Entotheonellaceae*, *Hydrogenophilaceae*, and *Comamonadaceae*. *Comamonadaceae* were also found in a study by D'Amico et al. (2018) in the rhizosphere of grapevine. To the best of our knowledge, *Entotheonellaceae*, *Hydrogenophilaceae* and *Rhodocyclaceae* were not described for the rhizosphere of grapevine before.

Changes in the 20 most dominating bacterial families between 2020 and 2021 were detected. These changes could be due to the different sampled containers and different weather conditions in the two sampled years. However, 14 of 20 families remained the same, among them *Reyranellaceae*, *Acidiferrobacteraceae*, and *Pseudomonadaceae*. Members of *Pseudomonadaceae* family are already known as plant-growth promoting bacteria, beneficial for plant health (as reviewed by Roquigny et al., 2017).

The alpha-diversity indices in this study revealed statistical differences regarding the fertilization level in 2020, but not in 2021. This may indicate that the bacteria changed during the year, which could be due to weather conditions, or other abiotic or biotic factors.

The beta-diversity revealed statistical differences in the two experimental set-ups. Pairwise PERMANOVA results showed statistical differences between Pinot Noir under different nitrogen fertilization levels and in different sampling depths. The results indicate that both the level of nitrogen fertilizer and the different depths at which the samples were taken have an effect on metabolically active bacteria in the rhizosphere of grapevine. This is also in accordance to former studies, showing that the rhizosphere microbiome was strongly affected by the level of nitrogen fertilizer in soybean (Ikeda et al., 2010) or Chinese cabbage (Qi et al., 2022). Moreover, Kang et al. (2022) demonstrated that moderate nitrogen fertilization modulated enhanced root colonization by plant growth promoting bacteria, significantly promoting plant growth and nitrogen use efficiency. They also suggest that rational nitrogen fertilization is critical to promote beneficial rhizosphere interactions for sustainable agricultural production (Kang et al., 2022). Löhnertz (1988) estimates the average N requirement of the vine to be 50 kg/ha per year. This ensures optimal vegetative growth if only grapes are exported from the vineyard. However, in this experimental design, leaves and cut wood were not returned to the soil. In this study, the NO_3^- levels in the soil samples are not far apart between the two experimental conditions. This could be due to the fact that grapevines require about 92 kg/ha of N per year (Löhnertz, 1988), so both experimental conditions took up the same amount of nitrogen, even at different fertilization levels. However, bacterial conditions were different because of the different fertilizer rates and sampling depths. In most cases, NH_4^+ was not found in the soil samples because it is rapidly nitrified in soils (as described in Verdenal et al., 2021). This was also true for this study, as no NH_4^+ was found in the samples.

Regarding sampling depths, it was already shown by different studies (for instance by Ekelund et al., 2001; Fierer et al., 2003), that sampling depth has an impact on the bacterial communities. In a study conducted by Steenwerth et al. (2008), to investigate morphology, depth and grapevine root frequency influencing microbial communities in a Pinot noir vineyard, it was shown that the distribution of soil microbial communities could have been explained with depth. Thus, they conclude that compared to other systems, the distinct patterns in soil microbial communities as influenced by depth and root distribution in this Pinot noir vineyard suggest that vineyard management practices and deep grapevine root distribution combine to cultivate a unique microbial community in these soil profiles (Steenwerth et al., 2008). Furthermore, they hypothesize that nutrient turnover and decomposition may be unique in these soils (Steenwerth et al., 2008). However, the authors did not refer to a DNA or RNA sequencing method to

describe the bacterial communities more deeply. Comparing the ASVs strongly influencing the principal component axis from the PCoA biplot (Figures 3 and 4) between time point bud break 2020 and time point bud break 2021 revealed ASVs next relative to *Nitrospinales* spec., *Thiobacillus* spec., *Reyranella soli*, and *Desulfosporosinus fructosivorans* as those taxa appearing in both analyses. *Reyranella soli* was already described as a bacterium isolated from forest soil in Korea (Kim et al., 2013), belonging to the genus *Reyranella*. Some members of this genus are already described as denitrifying bacteria (Chen et al., 2020; Pessi et al., 2022). *Desulfosporosinus fructosivorans* was described previously as a bacterium inhabiting the subsurface sediments of the Baltic Sea (Vandieken et al., 2017). Members of this genus are known for sulfate-reducing (Hausmann et al., 2019; Sato et al., 2019). In contrast, the studied *Thiobacillus spp.* in the samples of the two years is related to sulfur-oxidizing bacteria and believed to play a major role in the sulfur cycle (Kumar et al., 2020). Kumar et al. (2020) further review, that *Thiobacillus* and its related genera are believed to play an important role in crop production through S-oxidation, P-solubilization, and solubilization of other nutrients.

However, further research on this topic is needed to reveal all effects of rootstock and grapevine scion combinations on the bacterial communities in the rhizosphere. In addition, the effects on the growth and health of the vine must be revealed from a practical perspective. A thorough understanding of microorganisms in vineyard soil, and the complex relationships between microbial communities, soil properties and plant are crucial for enhancing plant productivity, grape production, biogeochemical processes and vineyard management practices (Di Liu et al. 2019; Holland et al. 2014; Liang et al. 2019; Yee et al. 2021). Inferring, the colonization of the microorganisms in the rhizosphere and the root exudation patterns of grapevine must be investigated. Future research in this field is inevitably required to provide a better understanding of the rhizospheric grapevine microbiome in the context of root exudates, grape variety, rootstock as well as different environmental conditions.

It has been shown, that the (endophytic) microbiome of grapevines has a major impact on the regional terroir of wines (Zarraonaindia et al., 2015). Furthermore, Bokulich et al. (2014) note that regional factors could influence the “microbial terroir” of grape surfaces within global viticulture. 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 investigation of the interactions between the grapevines and their microbiome could 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). 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).

In conclusion, this dissertation with the topic "Microbial structure and diversity in the rhizosphere of grapevine (*Vitis vinifera* L.)" has contributed to shed more light on the world of bacterial communities in the rhizosphere of grapevine under different conditions. It was found that the bacterial communities are influenced by various factors such as genotype, rootstock and nitrogen fertilization. Future research must now aim at assessing these results against practical applications. Only once it has been understood why different microorganisms are found and what effect they have on the grapevine, the rhizobiome can be manipulated to the advantage of the plant.

References

- Ahkami, Amir H., Richard Allen White, Pubudu P. Handakumbura, and Christer Jansson. 2017. Rhizosphere engineering: Enhancing sustainable plant ecosystem productivity. *Rhizosphere* 3: 233–243.
- Badri, Dayakar V., Jacqueline M. Chaparro, Ruifu Zhang, Qirong Shen, and Jorge M. Vivanco. 2013. Application of natural blends of phytochemicals derived from the root exudates of *Arabidopsis* to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. *The Journal of biological chemistry* 288 (7): 4502–4512.
- Bai, Yong-Chao, Ying-Ying Chang, Muzammil Hussain, Bin Lu, Jun-Pei Zhang, Xiao-Bo Song, Xia-Shuo Lei, and Dong Pei. 2020. Soil Chemical and Microbiological Properties Are Changed by Long-Term Chemical Fertilizers That Limit Ecosystem Functioning. *Microorganisms* 8 (5).
- Bakker, Matthew G., Daniel K. Manter, Amy M. Sheflin, Tiffany L. Weir, and Jorge M. Vivanco. 2012. Harnessing the rhizosphere microbiome through plant breeding and agricultural management. *Plant and Soil* 360 (1-2): 1–13.
- Berg, Gabriele, and Kornelia Smalla. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS microbiology ecology* 68 (1): 1–13.
- Berlanas, Carmen, Mónica Berbegal, Georgina Elena, Meriem Laidani, José Félix Cibriain, Ana Sagües, and David Gramaje. 2019. The Fungal and Bacterial Rhizosphere Microbiome Associated With Grapevine Rootstock Genotypes in Mature and Young Vineyards. *Frontiers in microbiology* 10: 1142.
- Bokulich, N.A., Thorngate, J.H., Richardson, P.M., Mills, D.A., 2014. Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *Proceedings of the National Academy of Sciences of the United States of America* 111 (1), E139-48. 10.1073/pnas.1317377110.
- Brunel, Caroline, Robin Pouteau, Wayne Dawson, Michael Pester, Kelly S. Ramirez, and Mark van Kleunen. 2020. Towards Unraveling Macroecological Patterns in Rhizosphere Microbiomes. *Trends in plant science* 25 (10): 1017–1029.
- Bulgarelli, Davide, Matthias Rott, Klaus Schlaeppi, Emiel ver van Loren Themaat, Nahal Ahmadinejad, Federica Assenza, Philipp Rauf, Bruno Huettel, Richard Reinhardt, Elmon Schmelzer, Joerg Peplies, Frank Oliver Gloeckner, Rudolf Amann, Thilo Eickhorst, and

- Paul Schulze-Lefert. 2012. Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature* 488 (7409): 91–95.
- Burns, K.N., Bokulich, N.A., Cantu, D., Greenhut, R.F., Kluepfel, D.A., O'Geen, A.T., Strauss, S.L., Steenwerth, K.L., 2016. Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: Differentiation by vineyard management. *Soil Biology and Biochemistry* 103, 337–348. 10.1016/j.soilbio.2016.09.007.
- Calvo, Olga C., Jürgen Franzaring, Iris Schmid, Matthias Müller, Nolwenn Brohon, and Andreas Fangmeier. 2017. Atmospheric CO₂ enrichment and drought stress modify root exudation of barley. *Global change biology* 23 (3): 1292–1304.
- Chen, S., Qi, G., Ma, G., Zhao, X., 2020. Biochar amendment controlled bacterial wilt through changing soil chemical properties and microbial community. *Microbiological research* 231, 126373. 10.1016/j.micres.2019.126373.
- Coller, Emanuela, Alessandro Cestaro, Roberto Zanzotti, Daniela Bertoldi, Massimo Pindo, Simone Larger, Davide Albanese, Enzo Mescalchin, and Claudio Donati. 2019. Microbiome of vineyard soils is shaped by geography and management. *Microbiome* 7 (1): 140.
- Cutiño-Jiménez, Ania M., Carlos Frederico Martins Menck, Yusdiel Torres Cambas, and Juan Carlos Díaz-Pérez. 2020. Protein signatures to identify the different genera within the *Xanthomonadaceae* family. *Brazilian journal of microbiology: [publication of the Brazilian Society for Microbiology]* 51 (4): 1515–1526.
- D'Amico, Federica, Marco Candela, Silvia Turrone, Elena Biagi, Patrizia Brigidi, Alessia Bega, Davide Vancini, and Simone Rampelli. 2018. The Rootstock Regulates Microbiome Diversity in Root and Rhizosphere Compartments of *Vitis vinifera* Cultivar Lambrusco. *Frontiers in microbiology* 9: 2240.
- Di Liu, Pangzhen Zhang, Deli Chen, and Kate Howell. 2019. From the Vineyard to the Winery: How Microbial Ecology Drives Regional Distinctiveness of Wine. *Frontiers in microbiology* 10: 2679.
- Dias, Armando C. F., Francisco Dini-Andreote, Silja Emilia Hannula, Fernando Dini Andreote, Michele de Cássia Pereira E Silva, Joana Falcão Salles, Wietse de Boer, Johannes van Veen, and Jan Dirk van Elsas. 2013. Different selective effects on rhizosphere bacteria exerted by genetically modified versus conventional potato lines. *PLoS ONE* 8 (7): e67948.

- Ekelund, F., Rønn, R., Christensen, S., 2001. Distribution with depth of protozoa, bacteria and fungi in soil profiles from three Danish forest sites. *Soil Biology and Biochemistry* 33 (4-5), 475–481. 10.1016/S0038-0717(00)00188-7.
- Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. *Soil Biology and Biochemistry* 35 (1), 167–176. 10.1016/S0038-0717(02)00251-1.
- Freire Cruz, Andre, and Marcio de Carvalho Pires. 2014. Soil-Borne Plant Pathogens Associated to Decline of Grapevine Grown in Greenhouse. *Journal of Plant Physiology & Pathology* 02 (01).
- Hausmann, B., Vandieken, V., Pjevac, P., Schreck, K., Herbold, C.W., Loy, A., 2019. Draft Genome Sequence of *Desulfosporosinus fructosivorans* Strain 63.6FT, Isolated from Marine Sediment in the Baltic Sea. *Microbiology resource announcements* 8 (31). 10.1128/MRA.00427-19.
- Holland, Taylor C., Pat Bowen, Carl Bogdanoff, and Miranda M. Hart. 2014. How distinct are arbuscular mycorrhizal fungal communities associating with grapevines? *Biology and Fertility of Soils* 50 (4): 667–674.
- Ibáñez, Fernando, Jorge Angelini, Tania Taurian, María Laura Tonelli, and Adriana Fabra. 2009. Endophytic occupation of peanut root nodules by opportunistic *Gammaproteobacteria*. *Systematic and applied microbiology* 32 (1): 49–55.
- Ikeda, S., Okubo, T., Kaneko, T., Inaba, S., Maekawa, T., Eda, S., Sato, S., Tabata, S., Mitsui, H., Minamisawa, K., 2010. Community shifts of soybean stem-associated bacteria responding to different nodulation phenotypes and N levels. *The ISME journal* 4 (3), 315–326. 10.1038/ismej.2009.119.
- Jenkins, Sasha N., Ian S. Waite, Adrian Blackburn, Rebecca Husband, Steven P. Rushton, David C. Manning, and Anthony G. O'Donnell. 2009. Actinobacterial community dynamics in long term managed grasslands. *Antonie van Leeuwenhoek* 95 (4): 319–334.
- Jones, Piet, Benjamin J. Garcia, Anna Furches, Gerald A. Tuskan, and Daniel Jacobson. 2019. Plant Host-Associated Mechanisms for Microbial Selection. *Frontiers in plant science* 10: 862.
- an Kang, Zhang, N., Xun, W., Dong, X., Xiao, M., Liu, Z., Xu, Z., Feng, H., Zou, J., Shen, Q., Zhang, R., 2022. Nitrogen fertilization modulates beneficial rhizosphere interactions

- through signaling effect of nitric oxide. *Plant Physiology* 188 (2), 1129–1140. 10.1093/plphys/kiab555.
- Khan, Inam U., Neeli Habib, Min Xiao, Xing Huang, Nasr Ullah Khan, Wan-Taek Im, Iftikhar Ahmed, Xiao-Yang Zhi, and Wen-Jun Li. 2018. *Phenylobacterium terrae* sp. nov., isolated from a soil sample of Khyber-Pakhtun-Khwa, Pakistan. *Antonie van Leeuwenhoek* 111 (10): 1767–1775.
- Khan, Inam U., Firasat Hussain, Neeli Habib, Mohammed A. M. Wadaan, Iftikhar Ahmed, Wan-Taek Im, Wael N. Hozzein, Xiao-Yang Zhi, and Wen-Jun Li. 2017. *Phenylobacterium deserti* sp. nov., isolated from desert soil. *International journal of systematic and evolutionary microbiology* 67 (11): 4722–4727.
- Kim, Soo-Jin, Hang-Yeon Weon, Yi-Seul Kim, In-Cheol Park, Jung-A Son, and Soon-Wo Kwon. 2011. *Nevskia terrae* sp. nov., isolated from soil. *International journal of systematic and evolutionary microbiology* 61 (Pt 5): 1226–1229.
- Kim, S.-J., Ahn, J.-H., Lee, T.-H., Weon, H.-Y., Hong, S.-B., Seok, S.-J., Whang, K.-S., Kwon, S.-W., 2013. *Reyranella soli* sp. nov., isolated from forest soil, and emended description of the genus *Reyranella* Pagnier et al. 2011. *International journal of systematic and evolutionary microbiology* 63 (Pt 9), 3164–3167. 10.1099/ij.s.0.045922-0.
- Kumar, M., Zeyad, M.T., Choudhary, P., Paul, S., Chakdar, H., Singh Rajawat, M.V., 2020. *Thiobacillus*. *Beneficial Microbes in Agro-Ecology*, 545–557. 10.1016/B978-0-12-823414-3.00026-5.
- Kusstascher, Peter, Eveline Adam, Wisnu Adi Wicaksono, Maria Bernhart, Expedito Olimi, Henry Müller, and Gabriele Berg. 2021. Microbiome-Assisted Breeding to Understand Cultivar-Dependent Assembly in *Cucurbita pepo*. *Frontiers in plant science* 12: 642027.
- Kuzyakov, Yakov, Paul W. Hill, and David L. Jones. 2007. Root exudate components change litter decomposition in a simulated rhizosphere depending on temperature. *Plant and Soil* 290 (1-2): 293–305.
- Lebeis, Sarah L., Sur Herrera Paredes, Derek S. Lundberg, Natalie Breakfield, Jase Gehring, Meredith McDonald, Stephanie Malfatti, Tijana Glavina del Rio, Corbin D. Jones, Susannah G. Tringe, and Jeffery L. Dangl. 2015. PLANT MICROBIOME. Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science (New York, N.Y.)* 349 (6250): 860–864.

- Li, Xuexue, Ying Yu, Lina Choi, Yali Song, Minghan Wu, Gejiao Wang, and Mingshun Li. 2019. *Phenylobacterium soli* sp. nov., isolated from arsenic and cadmium contaminated farmland soil. *International journal of systematic and evolutionary microbiology* 69 (5): 1398–1403.
- Liang, Hebin, Xiaowen Wang, Junwei Yan, and Lixin Luo. 2019. Characterizing the Intra-Vineyard Variation of Soil Bacterial and Fungal Communities. *Frontiers in microbiology* 10: 1239.
- Liu, Zhexian, Polina Beskrovnaya, Ryan A. Melnyk, Sarzana S. Hossain, Sophie Khorasani, Lucy R. O'Sullivan, Christina L. Wiesmann, Jen Bush, Joël D. Richard, and Cara H. Haney. 2018. A Genome-Wide Screen Identifies Genes in Rhizosphere-Associated *Pseudomonas* Required to Evade Plant Defenses. *mBio* 9 (6).
- Löhnertz, O., 1988. Untersuchungen zum zeitlichen Verlauf der Nährstoffaufnahme bei *Vitis vinifera* (cv. Riesling): Geisenheim, Universität Giessen.
- Madhaiyan, Munusamy, Selvaraj Poonguzhali, Venkatakrisnan Sivaraj Saravanan, Kumaran Selvapravin, Veeramuthu Duraipandiyan, and Naif Abdullah Al-Dhabi. 2017. *Pseudomonas sesami* sp. nov., a plant growth-promoting *Gammaproteobacteria* isolated from the rhizosphere of *Sesamum indicum* L. *Antonie van Leeuwenhoek* 110 (7): 843–852.
- Madhaiyan, Munusamy, Venkatakrisnan Sivaraj Saravanan, Jochen Blom, Theo H. M. Smits, Fabio Rezzonico, Soo-Jin Kim, Hang-Yeon Weon, Soon-Wo Kwon, William B. Whitman, and Lianghai Ji. 2020. *Phytobacter palmae* sp. nov., a novel endophytic, N₂ fixing, plant growth promoting *Gammaproteobacterium* isolated from oil palm (*Elaeis guineensis* Jacq.). *International journal of systematic and evolutionary microbiology* 70 (2): 841–848.
- Marasco, Ramona, Eleonora Rolli, Marco Fusi, Grégoire Michoud, and Daniele Daffonchio. 2018. Grapevine rootstocks shape underground bacterial microbiome and networking but not potential functionality. *Microbiome* 6 (1): 3.
- Marín, D., J. Armengol, P. Carbonell-Bejerano, J. M. Escalona, D. Gramaje, E. Hernández-Montes, D. S. Intrigliolo, J. M. Martínez-Zapater, H. Medrano, J. M. Mirás-Avalos, J. E. Palomares-Rius, P. Romero-Azorín, R. Savé, L. G. Santesteban, and F. de Herralde. 2021. Challenges of viticulture adaptation to global change: tackling the issue from the roots. *Australian Journal of Grape and Wine Research* 27 (1): 8–25.

- Matthews, Andrew, Sarah Pierce, Helen Hipperson, and Ben Raymond. 2019. Rhizobacterial Community Assembly Patterns Vary Between Crop Species. *Frontiers in microbiology* 10: 581.
- Mendes, Lucas W., Jos M. Raaijmakers, Mattias de Hollander, Rodrigo Mendes, and Siu Mui Tsai. 2018. Influence of resistance breeding in common bean on rhizosphere microbiome composition and function. *The ISME journal* 12 (1): 212–224.
- Neuhauser S., Huber L., Kirchmair M. 2009. *Sorosphaera viticola*, a plasmodiophorid parasite of grapevine. *Phytopathol Mediterr.* (48(1)): 136–139.
- Novello, Giorgia, Elisa Gamalero, Elisa Bona, Lara Boatti, Flavio Mignone, Nadia Massa, Patrizia Cesaro, Guido Lingua, and Graziella Berta. 2017. The Rhizosphere Bacterial Microbiota of *Vitis vinifera* cv. Pinot Noir in an Integrated Pest Management Vineyard. *Frontiers in microbiology* 8: 1528.
- Pessi, I.S., Viitamäki, S., Virkkala, A.-M., Eronen-Rasimus, E., Delmont, T.O., Marushchak, M.E., Luoto, M., Hultman, J., 2022. In-depth characterization of denitrifier communities across different soil ecosystems in the tundra. *Environmental microbiome* 17 (1), 30. 10.1186/s40793-022-00424-2.
- Qi, Y., Wu, Z., Zhou, R., Hou, X., Yu, L., Cao, Y., Jiang, F., 2022. Nitrogen Reduction with Bio-Organic Fertilizer Altered Soil Microorganisms, Improved Yield and Quality of Non-Heading Chinese Cabbage (*Brassica campestris ssp. chinensis* Makino). *Agronomy* 12 (6), 1437. 10.3390/agronomy12061437.
- Ryan, Peter R., Yves Dessaux, Linda S. Thomashow, and David M. Weller. 2009. Rhizosphere engineering and management for sustainable agriculture. *Plant and Soil* 321 (1-2): 363–383.
- Roquigny, R., Novinscak, A., Biessy, A., Fillion, M., 2017. *Pseudomonadaceae*: From Biocontrol to Plant Growth Promotion, 39–68. 10.1007/978-981-10-4862-3_3.
- Sasse, Joelle, Enrico Martinoia, and Trent Northen. 2018. Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? *Trends in plant science* 23 (1): 25–41.
- Sato, Y., Hamai, T., Hori, T., Aoyagi, T., Inaba, T., Kobayashi, M., Habe, H., Sakata, T., 2019. *Desulfosporosinus spp.* were the most predominant sulfate-reducing bacteria in pilot- and laboratory-scale passive bioreactors for acid mine drainage treatment. *Applied microbiology and biotechnology* 103 (18), 7783–7793. 10.1007/s00253-019-10063-2.
- Schultz, Hans R., and Gregory V. Jones. 2010. Climate Induced Historic and Future Changes in Viticulture. *Journal of Wine Research* 21 (2-3): 137–145.

- Steenwerth, K.L., Drenovsky, R.E., Lambert, J.-J., Kluepfel, D.A., Scow, K.M., Smart, D.R., 2008. Soil morphology, depth and grapevine root frequency influence microbial communities in a Pinot noir vineyard. *Soil Biology and Biochemistry* 40 (6), 1330–1340. 10.1016/j.soilbio.2007.04.031.
- Taye, Zelalem M., Bobbi L. Helgason, Jennifer K. Bell, Charlotte E. Norris, Sally Vail, Stephen J. Robinson, Isobel A. P. Parkin, Melissa Arcand, Steven Mamet, Matthew G. Links, Tanner Dowhy, Steven Siciliano, and Eric G. Lamb. 2019. Core and Differentially Abundant Bacterial Taxa in the Rhizosphere of Field Grown *Brassica napus* Genotypes: Implications for Canola Breeding. *Frontiers in microbiology* 10: 3007.
- Torres, Nazareth, Runze Yu, and S. Kaan Kurtural. 2021. Inoculation with Mycorrhizal Fungi and Irrigation Management Shape the Bacterial and Fungal Communities and Networks in Vineyard Soils. *Microorganisms* 9 (6).
- Vandieken, V., Niemann, H., Engelen, B., Cypionka, H., 2017. *Marinisporobacter balticus* gen. nov., sp. nov., *Desulfosporosinus nitroreducens* sp. nov. and *Desulfosporosinus fructosivorans* sp. nov., new spore-forming bacteria isolated from subsurface sediments of the Baltic Sea. *International journal of systematic and evolutionary microbiology* 67 (6), 1887–1893. 10.1099/ijsem.0.001883.
- Verdenal, T., Dienes-Nagy, Á., Spangenberg, J.E., Zufferey, V., Spring, J.-L., Viret, O., Marin-Carbonne, J., van Leeuwen, C., 2021. Understanding and managing nitrogen nutrition in grapevine: a review. *OENO One* 55 (1), 1–43. 10.20870/oeno-one.2021.55.1.3866.
- Vink, Stefanie N., Francisco Dini-Andreote, Rebecca Höfle, Anna Kicherer, and Joana Falcão Salles. 2021. Interactive Effects of Scion and Rootstock Genotypes on the Root Microbiome of Grapevines (*Vitis spp.* L.). *Applied Sciences* 11 (4): 1615.
- Wei, Feng, Lihong Zhao, Xiangming Xu, Hongjie Feng, Yongqiang Shi, Greg Deakin, Zili Feng, and Heqin Zhu. 2019. Cultivar-Dependent Variation of the Cotton Rhizosphere and Endosphere Microbiome Under Field Conditions. *Frontiers in plant science* 10: 1659.
- Wille, Lukas, Monika M. Messmer, Bruno Studer, and Pierre Hohmann. 2019. Insights to plant-microbe interactions provide opportunities to improve resistance breeding against root diseases in grain legumes. *Plant, cell & environment* 42 (1): 20–40.
- Wright, A. H., Shawkat Ali, Zoë Migicovsky, Gavin M. Douglas, Svetlana Yurgel, Adèle Bunbury-Blanchette, Jeff Franklin, Sarah J. Adams, and Allison K. Walker. 2022. A

Characterization of a Cool-Climate Organic Vineyard's Microbiome. *Phytobiomes Journal* 6 (1): 69–82.

Xiong, Li, Xiaoyu Liu, Giovanni Vinci, Riccardo Spaccini, Marios Drosos, Lianqing Li, Alessandro Piccolo, and Genxing Pan. 2019. Molecular changes of soil organic matter induced by root exudates in a rice paddy under CO₂ enrichment and warming of canopy air. *Soil Biology and Biochemistry* 137: 107544.

Yee, Mon O., Peter Kim, Yifan Li, Anup K. Singh, Trent R. Northen, and Romy Chakraborty. 2021. Specialized Plant Growth Chamber Designs to Study Complex Rhizosphere Interactions. *Frontiers in microbiology* 12: 625752.

Zarraonaindia, I., Owens, S. M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., Bokulich, N. A., Mills, D. A., Martin, G., Taghavi, S., van der Lelie, D., & Gilbert, J. A. (2015). The soil microbiome influences grapevine-associated microbiota. *MBio*, 6(2). <https://doi.org/10.1128/mBio.02527-14>

Zhang, Jingying, Na Zhang, Yong-Xin Liu, Xiaoning Zhang, Bin Hu, Yuan Qin, Haoran Xu, Hui Wang, Xiaoxuan Guo, Jingmei Qian, Wei Wang, Pengfan Zhang, Tao Jin, Chengcai Chu, and Yang Bai. 2018. Root microbiota shift in rice correlates with resident time in the field and developmental stage. *Science China. Life sciences* 61 (6): 613–621.

Acknowledgements

After now three years and four months my PhD journey is finally over. The time was characterized by many hours in the lab, a lot of time in front of the computer, a lot of time at home (no thanks to Corona Virus!), a lot of coffee, many doubts, fears and feeling left alone. But also characterized by good times with my colleagues, meeting and working with great people, a research topic that I hope to continue working on throughout my life, and an incredible personal growth. In the following I would like to thank everyone who was involved in the process of writing this thesis.

First of all, I would like to thank the whole Department of Soil Science and Plant Nutrition of the Hochschule Geisenheim University. I have felt very well taken care of since day one and I am very grateful to have become a member of this family and team. Thank you for taking care of me, helping me through this time and watching me grow.

I would like to express my sincere gratitude to my supervisor Otmar Löhnertz for giving me the opportunity to work in this project and for supporting me even out of your retirement. I would also like to thank Anne Vortkamp for giving me the opportunity to work in this project, for the wine on your balcony and kind words during struggling times. I would like to express my deepest thanks to Robert Kunz and Maximilian Hendgen who supported me with all of their knowledge. Thank you for teaching me how to be a scientist and for keeping me on track.

I would like to thank Sylvia Schnell for supervising me and the collaboration in this project. Thank you for your advice and listening to all my questions. Thank you, Stefan Ratering, for answering even the hundredth bioinformatics question in a helpful and kind way. Thanks to Bellinda Schneider for your support whenever I ran into a problem or had question about laboratory work and for the Ion Torrent Sequencing. Moreover, I would like to thank the whole Institute of Applied Microbiology for helping me and discussing all questions that I had.

I would like to thank Annette Reineke and Olivia Hercynski for using your molecular lab and your help during my work. Further I would like to thank the Department of Molecular Plant Sciences for using the laboratory equipment.

Finally, I must express my thanks to my family and friends for supporting me in many ways along the way. I am truly thankful for your love, friendship and patience, that were a key motivation throughout my research journey.