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New insights into the biology, ecology and control of black-foot disease in grapevine
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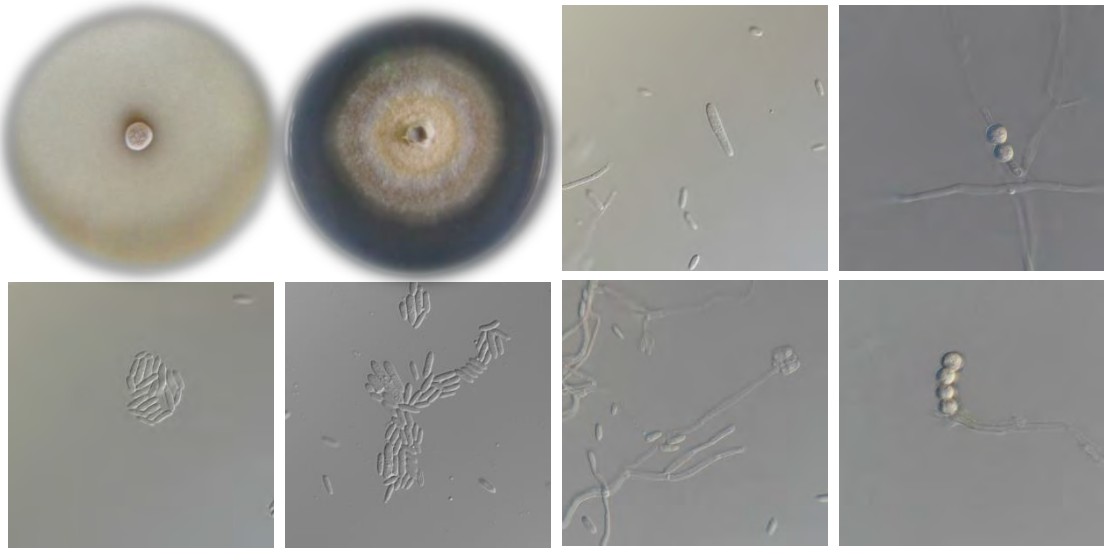

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Los abajo firmantes, el Dr. David Gramaje Pérez y el Dr. Enrique García-Escudero Domínguez, investigadores del Instituto de Ciencias de la Vid y del Vino (Gobierno de la Rioja, Universidad de La Rioja, CSIC).

CERTIFICAN:

Que el presente trabajo titulado "New insights into the biology, ecology anc control of black-foot disease in grapevine" ha sido realizado en el Departamento de Agricultura y Alimentación de la Universidad de La Rioja bajo nuestra dirección, por Carmen Berlanas Vicente, y reúne las condiciones exigidas para optar al grado de Doctor con Mención Internacional.

Lo que hacen constar en Logroño, a de enero de 2020

Dr. David Gramaje Pérez

Dr. Enrique García-Escudero Domínguez

List of publications obtained from the research carried out during the thesis

This Ph.D. thesis is submitted in *compendium form* and the works that are part of it are listed below:

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Table of contents

List of Figures

List of Tables

Summary

Grapevine trunk diseases (GTDs) are a growing concern in the wine sector worldwide. Among them, black-foot disease (BFD) has increased its incidence in grapevine nurseries and newly established vineyards. BFD is caused by numerous *Cylindrocarpon*-like asexual morphs species. This disease has been widely studied in Spain in recent years. However, the constant reclassification and taxonomic expansion of the species associated with BFD, the restriction in the application of fungicides, and the emergence in the use of high-throughput sequencing techniques has made necessary a review and an update of the knowledge obtained so far. In this Ph.D. thesis, the biology and ecology of the disease was studied in detail. The evaluation of different control strategies to improve disease management was also investigated.

Firstly, a wide collection of isolates associated to BFD, which were obtained from asymptomatic vines, were characterized. These isolates were studied with morphological and cultural characteristics as well as phylogenetic analyses of combined DNA sequences of the *his3*, *tef1* and *tub2* genes, and the ITS region. Two new species associated with the disease were described: *Dactylonectria riojana* and *Ilyonectria vivaria. Ilyonectria pseudodestructans* and *Neonectria quercicola* were isolated for the first time from grapevine in Spain, raising the total number of fungal species associated to BFD in our country to 17. The development of a semi-selective medium based on previous research allowed the identification and quantification of viable propagules of fungi associated with BFD from soil samples. The presence of BFD inoculum in rotating nursery fields and in mature vineyards was confirmed. Moreover, a positive correlation was established between Colony Forming Units (CFU) of BFD pathogens and the $CaCO_3$ concentration in soil. On the other hand, the fungal and bacterial microbiome of the rhizosphere of 5 rootstocks in two vineyards located in La Rioja and Navarra has been characterized by high-throughput amplicon sequencing (HTAS). The results showed that grapevine rootstock genotype was the most important factor in shaping the microbiome in a mature vineyard (25-year-old), but not in a young vineyard (7-year-old). However, several bacterial and fungal species were found in both vineyards, demonstrating the existence of a “core” microbiome conserved in the vineyard, regardless of the geographic region. In addition, a positive correlation has been observed between the

relative abundance of BFD pathogens obtained by HTAS and by qPCR. Moreover, the rhizosphere compartment of the "140 R" and "161-49 C" rootstocks harboured lower number of these pathogens than the "1103 P", "110 R" and "41 B" rootstocks. Finally, regarding control measures, the efficacy of white mustard biofumigation was compared with the use of propamocarb + fosetyl-Al, as well as the effect of Tusal® (*Trichoderma atroviride* T11 + *Trichoderma asperellum* T25) application. Biofumigation with *Brassica* sp. is a promising alternative to the use of chemical fungicides for BFD control, while the application of commercial products based on *Trichoderma* sp. on the roots before planting resulted ineffective for disease management.

Resumen

Actualmente, las enfermedades fúngicas de la madera de la vid son muy graves y han sido señaladas en muchos foros como una de las principales preocupaciones actuales del sector vitivinícola, si no la mayor. Entre estas enfermedades destaca la enfermedad del pie negro, cuya incidencia es creciente en viveros de vid y en nuevas plantaciones. Esta enfermedad está causada por numerosas especies con formas asexuales del tipo "*Cylindrocarpon*". El pie negro ha sido extensamente estudiado en España durante los últimos años. Sin embargo, la constante reclasificación y ampliación taxonómica de las especies asociadas a la enfermedad, la restricción en la aplicación de fungicidas y la emergencia en el uso de técnicas de secuenciación de nueva generación ha hecho necesario una revisión y actualización de los conocimientos obtenidos hasta ahora. En esta tesis se ha estudiado en detalle la biología y ecología de la enfermedad, y se han evaluado diversas estrategias de control.

En primer lugar, se han caracterizado una amplia colección de aislados asociados al pie negro obtenidos de vides asintomáticas. Estos aislados fueron analizados mediante el estudio de sus caracteres fenotípicos y la secuenciación de los genes *his3*, *tef1* y *tub2* y la región ITS. Como resultado, se describieron dos nuevas especies asociadas al pie negro de la vid: *Dactylonectria riojana* e *Ilyonectria vivaria*, y por primera vez se han aislado de vid *Ilyonectria pseudodestructans* y *Neonectria quercicola*, elevando a 17 el twtotal de especies fúngicas asociadas con la enfermedad en España. Además, se ha desarrollado un medio semi-selectivo, basado en un trabajo ya publicado, para identificar y cuantificar propágulos viables de hongos asociados al pie negro en muestras de suelo. El uso de este medio ha permitido confirmar la presencia de inóculo en campos de vivero en rotación y en viñedos adultos. Además, se ha establecido una relación positiva entre Unidades Formadoras de Colonias (UFC) de los patógenos de la enfermedad del pie negro y la concentración de $CaCO_3$ en el suelo. A continuación, se ha caracterizado el microbioma fúngico y bacteriano de la rizosfera de 5 portainjertos en dos viñedos localizados en La Rioja y Navarra mediante secuenciación de amplicones. Los resultados mostraron que el genotipo es determinante en la selección del microbioma residente en la rizosfera en el viñedo adulto (25 años), mientras que este factor no influía en la selección del microbioma en el viñedo joven (7 años). Sin embargo,

diversas especies bacterianas y fúngicas se encontraron en ambos viñedos, lo que demuestra la existencia de un microbioma conservado en el viñedo, independientemente de la región geográfica. Además, se ha observado una correlación positiva entre la abundancia relativa de patógenos de la enfermedad del pie negro obtenida mediante secuenciación masiva de amplicones y la obtenida mediante qPCR. También se ha comprobado que la rizosfera de los portainjertos “140 R” y “161-49 C” contiene menor cantidad de estos patógenos que los portainjertos“1103 P”, “110 R” y “41 B”. Por último, en cuanto a medidas de control, se ha comparado la eficacia de la biofumigación con mostaza blanca con el empleo de propamocarb + fosetyl-Al, así como el efecto de la aplicación de Tusal®, un producto comercial basado en las especies *Trichoderma atroviride* T11 y *Trichoderma asperellum* T25. Se ha confirmado que la biofumigación con *Brassica* sp. es una alternativa prometedora al uso de fungicidas químicos para el control de la enfermedad del pie negro, mientras que la aplicación de *Trichoderma* spp. sobre las raíces antes de la plantación resultó ser inefectiva para el manejo de la enfermedad.

CHAPTER 1.

GENERAL INTRODUCTION

CHAPTER 2.

OBJECTIVES AND OUTLINE OF THE THESIS

As highlighted in the General Introduction, black-foot disease (BFD) caused by a broad range *Cylindrocarpon*-like asexual morphs is particularly important in grapevine nurseries and new plantations in wine-producing countries around the world, including Spain.

Black-foot disease pathogens have a serious effect on the health and long-term productivity of vines and young vineyards, and there are no effective control measures once they are established in the plant. Although vines can eventually acquire this disease during the propagation process in grapevine nurseries, infected soil in nurseries and young vineyards are a major source of BFD infection and can result in the failure of new vineyards soon after planting.

The constant identification and description of new *Cylindrocarpon*-like asexual morphs associated with BFD, the emergence of novel high-throughput sequencing technologies and the restriction on the use of fungicides all over the word, required the implementation of new research approaches to study black-foot disease. Therefore, the aim of this thesis has been to gain a better understanding about the biology, ecology and control of BFD of grapevine.

Chapter 3 describes the characterization of *Cylindrocarpon*-like asexual morphs fungi isolated from visually symptomless vines and asymptomatic internal wood tissue of grafted plants. A semi-selective culture medium adapted from the literature was also developed to estimate BFD pathogens populations in soils and to examine how shifts in the abundance and composition of black-foot pathogens corresponded to changes in specific soil properties. Finally, the rizhosphere bacterial and fungal microbiota across grapevine rootstock genotypes was characterized, with special emphasis on the comparison between the relative abundances of sequences reads and DNA amount of BFD pathogens.

Chapter 4 evaluates the effect of combined soil and root treatments to control BFD of grapevine under field conditions. In particular, chemical and biological control, and biofumigation, were evaluated in this thesis.

Chapter 5 contains a general and summarizing discussion of the results obtained in this thesis. These data are reviewed in light of what was known prior to this study, leading to several suggestions for future research initiatives.

Finally, **chapter 6** presents as concluding remarks, the most important achievements of this thesis.

CHAPTER 3.

BIOLOGY AND ECOLOGY

Plant Disease 104:94-104. 2019.

Occurrence and diversity of black-foot disease fungi in symptomless grapevine nursery stock in Spain

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Abstract

In this study, 3,426 grafted grapevines ready to be planted from 15 grapevine nursery fields in Northern Spain were inspected from 2016 to 2018 for black-foot causing pathogens. In all, 1,427 isolates of black-foot pathogens were collected from the asymptomatic inner tissues of surface sterilized secondary roots and characterized based on morphological features and DNA sequence data of the nuclear ribosomal DNA-internal transcribed spacer region, histone H3, translation elongation factor 1-alpha and β-tubulin genes. Eleven species belonging to the genera *Dactylonectria*, *Ilyonectria*, *Neonectria* and *Thelonectria* were identified, including *Dactylonectria alcacerensis*, *D. macrodidyma*, *D. novozelandica*, *D. pauciseptata*, *D. torresensis*, *Ilyonectria liriodendri*, *I. pseudodestructans*, *I. robusta*, *Neonectria quercicola, Neonectria sp.* 1 and *Thelonectria olida*. In addition, two species are newly described, namely *D. riojana* and *I. vivaria*. Twenty-four isolates representing 13 black-foot species were inoculated onto grapevine seedlings cultivar 'Tempranillo'. The pathogenicity tests detected diversity in virulence among fungal species and between isolates within each species. The most virulent species was *D. novozelandica* isolate BV-0760, followed by *D. alcacerensis* isolate BV-1240 and *I. vivaria* sp. nov. isolate BV-2305. This study improves our knowledge on the etiology and virulence of black-foot disease pathogens, and opens up new perspectives in the study of the endophytic phase of these pathogens in grapevines.

Keywords: *Cylindrocarpon*-like fungi, endophyte, Nectriaceae, systematics, *Vitis vinifera*, tree fruits, etiology, pathogen diversity.

Plant and Soil 417:467-479. 2017.

Estimation of viable propagules of black-foot disease pathogens in grapevine cultivated soils and their relation to production systems and soil properties

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Abstract

Aims The study aimed to assess comparatively the accuracy and efficiency of three culture media protocols for estimating black-foot disease pathogens populations in soils and to examine how shifts in the abundance and composition of black-foot pathogens correspond to changes in specific soil properties.

Methods Firstly, culture media were compared by evaluating the mycelial growth of selected black-foot pathogens and by estimating the population of *Dactylonectria torresensis* from artificially infested soils. Secondly, the most efficient culture medium was selected for estimating the viable propagules of black-foot disease pathogens in eight naturally infested soils. An analysis of the soil physicochemical properties was conducted. Data were statistically analyzed in order to explore possible relationships between the studied variables.

Results Glucose-Faba Bean Rose Bengal Agar (GFBRBA) was selected as the most efficient culture medium. All naturally infested soils tested positive for the presence of black-foot pathogens. *D. torresensis* was the most frequently isolated species, followed by *Dactylonectria alcacerensis* and *Ilyonectria liriodendri.* A positive relationship between calcium carbonate and the Colony-Forming Units (CFUs) level of black-foot pathogens in soil was obtained.

Conclusions In this study, we provide an early, specific, and accurate detection of viable propagules of black-foot pathogens in soil, which is critical to understand the ecology of these fungi and to design effective management strategies.

Keywords: *Dactylonectria torresensis*, fungal ecology, grapevine trunk disease, soilborne inoculum.

Abbreviations

CEC	Cation Exchange Capacity
CFU	Colony-Forming Unit
EC	Electric conductivity
GFBA	Glucose-Faba Bean Agar
GFBRGA	Glucose-Faba Bean Rose Bengal Agar
ITS	Internal Transcribed Spacer
MRBA	Modified Rose Bengal Agar
NMDS	Non-Metric Multidimensional Scaling Analysis
LSD	Least Significant Difference
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PDAC	Potato Dextrose Agar supplemented with 250 mg l^{-1} of chloramphenicol
SNA	Spezieller Nährstoffarmer Agar
SOM	Soil Organic Matter

Frontiers in Microbiology 10:1142. 2019.

The fungal and bacterial rhizosphere microbiome associated with grapevine rootstock genotypes in mature and young vineyards

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Abstract

The microbiota colonizing the rhizosphere and the endorhizosphere contribute to plant growth, productivity, carbon sequestration and phytoremediation. Several studies suggested that different plants types and even genotypes of the same plant species harbor partially different microbiomes. Here, we characterize the rhizosphere bacterial and fungal microbiota across five grapevine rootstock genotypes cultivated in the same soil at two vineyards and sampling dates over two years by 16S rRNA gene and ITS high-throughput amplicon sequencing. In addition, we use quantitative PCR (qPCR) approach to measure the relative abundance and dynamic changes of fungal pathogens associated with black-foot disease. The objectives were to (1) unravel the effects of rootstock genotype on microbial communities in the rhizosphere of grapevine and (2) to compare the relative abundances of sequence reads and DNA amount of black-foot disease pathogens. Host genetic control of the microbiome was evident in the rhizosphere of the mature vineyard. Microbiome composition also shifted as year of sampling, and fungal diversity varied with sampling moments. Linear discriminant analysis identified specific bacterial (i.e., *Bacillus*) and fungal (i.e., *Glomus*) taxa associated with grapevine rootstocks. Host genotype did not predict any summary metrics of rhizosphere α- and β- diversity in the young vineyard. Regarding black-foot associated pathogens, a significant correlation between sequencing reads and qPCR was observed. In conclusion, grapevine rootstock genotypes in the mature vineyard were associated with different rhizosphere microbiomes. The latter could also have

been affected by age of the vineyard, soil properties or field management practices. A more comprehensive study is needed to decipher the cause of the rootstock microbiome selection and the mechanisms by which grapevines are able to shape their associated microbial community. Understanding the vast diversity of bacteria and fungi in the rhizosphere and the interactions between microbiota and grapevine will facilitate the development of future strategies for grapevine protection.

Keywords: bacterial and fungal recruitment, black-foot disease, microbial ecology, microbiome, rhizosphere, rootstock selection.

Abbreviations

OTU	Operational taxonomic unit
ITS	Internal transcribed spacer
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PERMANOVA	Permutational multivariate analysis of variance
QIIME	Quantitative insights into microbial ecology
qPCR	Quantitative polymerase chain reaction
rRNA	Ribosomal RNA
SIMPER	Similarity percentages

Introduction

Plants have evolved to cope with biotic and abiotic stresses in association with soil microorganisms (Lemanceau et al. 2017). These microorganisms are known as plant microbiota and, together with the plant, they form an holobiont (Liu et al. 2018). Plant-soil microbiome interactions are complex and, until recent times, the study of these relationships has been mainly focused in the pathogenicity of some microbial agents and how they use and compete for the resources (Philippot et al. 2013; Zancarini et al. 2013; Gilbert et al. 2014; Sapkota et al. 2015). Recent investigations have shown that soil microbiota can directly and indirectly interact with the plants improving their fitness and health (Sapkota et al. 2015). For example, these interactions help plants to deal with abiotic stress and diseases, improving the exchange of substances such as nitrogen or phosphate, or by acting as biocontrol agents through competition with pathogens (Reinhold-Hurek et al. 2015; Vega-Avila et al. 2015; Gallart et al. 2018).

Roots are surrounded by a narrow zone of soil known as rhizosphere. This area, which is influenced by the roots, has a high microbial diversity and its community structure is expected to be different than the one found in the bulk soil (Reinhold-Hurek et al. 2015). The rhizosphere microbiome community composition is affected by different factors, such as ambient conditions, soil properties and background microbial composition (Qiao et al. 2017). In addition, plants are able to shape their rhizosphere microbiome, as evidenced by the fact that different plant species host specific microbial communities when grown on the same soil (Aira et al. 2010; Berendsen et al. 2012; Bazghaleh et al. 2015).

As reviewed by Philippot et al. (2013), plant roots release a huge variety of carbon-containing compounds known as rhizodeposits (nutrients, exudates, border cells and mucilage) which make the rhizosphere more nutritive than the bulk soil, which is mostly mesotrophic/oligotrophic, inducing therefore changes on soil microbial communities. It has been reported that the biodiversity in the rhizosphere is lower than in the corresponding bulk soil (Reinhold-Hurek et al. 2015; Lemanceau et al. 2017) since carbon availability often limits microbial growth (Dennis et al. 2010). Rhizodeposits released by the plants considerably vary according to the age and development of plants, among species and even among different genotypes of the same species (Inceoğlu et al. 2010; Philippot et al. 2013; Gilbert et al. 2014; Bazghaleh

et al. 2015; Hacquard 2016; Wagner et al. 2016; Lemanceau et al. 2017; Qiao et al. 2017).

The rhizosphere is also the infection court where soil-borne pathogens establish a parasitic relationship with the plant. To infect root tissue, pathogens have to compete with members of the rhizosphere microbiome for available nutrients and microsites (Chapelle et al. 2016). Exploiting genetic variation in host plant species and understanding interactions between microbiota and their hosts plants will allow the rhizosphere microbiota to be incorporated into plant breeding programs to promote beneficial associations between plants and microorganisms.

Common grapevine (*Vitis vinifera* L.) is one of the most extensively grown and economically important woody perennial fruit crop worldwide with an annual production in 2014 exceeding 74 million tons of grapes and 30 million tons of wine (FAO 2018). Since the late 19th century, *V. vinifera* cultivars have been grafted onto resistant rootstock of other *Vitis* species and hybrids to combat the devastating root phylloxera pest. Several major criteria have been outlined for choosing rootstocks: resistance to phylloxera and nematodes, and adaptability to drought, salinity, limestone content and poor mineral nutrition (Reynolds and Wardle 2001). In addition, the rootstock influence may affect scion vigour, yields, and fruit and wine qualities (Warschefsky et al. 2016).

Plant genetic control over microbial communities in the rhizosphere has been reported for different genotypes of the same species (Aira et al. 2010; Bouffaud et al. 2012; Peiffer et al. 2013; Marques et al. 2014; Jiang et al. 2017; Gallart et al. 2018). However, within grapevine species, the impact of genetic variation on the composition of the bacterial and fungal microbiota is poorly understood. In a recent study, Marasco et al. (2018) observed that five grapevine genotypes influenced the bacterial microbiome from both the root tissues and the rhizosphere fractions at a single vineyard, sampling date and year.

To better understand the players and processes that operate in the rhizosphere, a variety of molecular techniques, such as metagenomics have been applied over the past decade. Here, we characterize the rhizosphere bacterial and fungal microbiota across five grapevine rootstock genotypes cultivated in the same soil at two vineyards and sampling dates over two years by 16S rRNA gene and ITS high-throughput

amplicon sequencing (HTAS). This design allowed us to evaluate the effect of the growing region, year, sampling date, grapevine genotype, and their interactions on the bacterial and fungal community diversity. In addition, we used quantitative Polymerase Chain Reaction (qPCR) approach to measure the relative abundance and dynamic changes of fungal pathogens associated with black-foot disease, one of the main soil-borne fungal diseases affecting grapevine production worldwide.

Materials and methods

Sample collection

Grapevine rhizosphere samples of five rootstocks ('110 R', '140 Ru', '1103 P', '41 B' and '161-49 C') were collected at two vineyards located in Aldeanueva de Ebro (abbreviated as 'Aldea') (La Rioja, Spain) and Olite (Navarra, Spain). Features of the selected rootstocks are reported in Supplementary Table 3.3.1. All the selected rootstocks were cultivated in the same vineyard and had been grafted onto 'Tempranillo' cultivar. Soil physicochemical properties showed significant differences between soil types. Climate and soil management practices for fertilization, irrigation and disease control also varied between vineyards (Supplementary Table 3.3.2). Aldea vineyard was 25-year-old vines at the moment of sampling and contained four randomized blocks of 48 vines per rootstock and block. Olite vineyard was 7-year-old vines at the moment of sampling and contained three randomized blocks of 15 vines per rootstock and block. In each vineyard, three rhizosphere samples were randomly collected per rootstock at two sampling dates (June and November) over two years (2016 and 2017). Sampled vines did not show any symptom of disease or nutrient deficiency. A total of 60 samples were collected per vineyard.

Rhizosphere soil samples were collected with a sterile spade close to the stem at depths of 40 to 50 cm, where the root system was denser. All samples were stored in sterile bags on dry ice at the time of sampling, and brought to the laboratory for further processing within 24 h from the time of sampling. The sampled roots with rhizosphere soil particles attached were placed in sterile tubes containing 9 mL of physiological solution (9 g/L NaCl). The tubes were vortexed for 5 min to detach the soil particles and then centrifuged at 4000 rpm for 5 min. The supernatant was discarded and the remaining soil fraction was used for DNA extraction.

DNA extraction and sequencing

The rhizosphere DNA was extracted from 0.5 g sample using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) and DNA samples were randomized across plates. The bacterial V4 region of the 16S rRNA gene was amplified using the protocol described by Lundberg et al. (2013). The universal primer pair 515F and 806R was used to generate bacterial-derived 16S rRNA amplicons. PNA PCR clamps were used to reduce host organelle contamination. The fungal ITS2 region was amplified using the universal primers ITS3/KYO2 and ITS4 (Toju et al. 2012). All primers were modified to include Illumina adaptors (www.illumina.com). Each 25 µL reaction contained 12.5 µL of HiFi HotStart Ready Mix (KAPA Biosystems, Woburn, MA, USA), 1.0 µL of each primer (10 µM), 2.5 µL of DNA template (5 ng/µL), and 8.0 µL PCR-grade water. PCR amplifications (performed in triplicate for each sample) consisted of a 3 min denaturation at 95°C; 25 cycles of 30 s at 95°C, 30 s at 55°C and 30 s at 72°C; and 5 min at 72°C. Samples were cleaned using the AMPure beads XP purification system (Beckman Coulter, UK) and sequenced on the Illumina MiSeq platform at the Fundación FISABIO (Valencia, Spain) facility using a 2 x 300 nucleotide paired reads protocol.

Data analysis

Raw forward and reverse reads for each sample were assembled into paired-end reads considering the minimum overlapping of 50 nucleotides and a maximum of one mismatch within the region using the fastq-join tool from the ea-tools suite (Aronesty 2011). The paired reads were then quality trimmed with a minimum of Q20. Sequences without either primer were discarded. Chimeric sequences were identified and filtered using the Usearch tool (Edgar 2010, 2018). The UClust algorithm (Edgar 2013) in QIIME (Caporaso et al. 2010) was used to cluster sequences at a 97% sequence similarity aginast UNITE dynamic database (Abarenkov et al. 2010) for ITS reads and Greengenes database (DeSantis et al. 2006) using the QIIME implementation of the RDP classifier for 16S rRNA reads (Caporaso et al. 2010). A tree was constructed from a gap-filtered alignment using FastTree (Price et al. 2009). A final OTU table was created excluding unaligned sequences and singletons. OTUs with no kingdom-level classification or matching chloroplast, mitochondrial or Viridiplantae sequences were then removed from the data set. Good's coverage values were calculated using the

Mothur computer software (Schloss et al. 2009). The rarefied OTU table and the phylogenetic tree were used as inputs for the subsequent analyses of α- and β-diversity. The OTU table was log transformed for statistical analysis (McMurdie and Holmes 2014). As a final filter, taxa whose total abundances were less than 1% of the mean abundance were excluded, and only the OTUs present in at least two-thirds of the replicates of each sample were selected.

Bacterial and fungal diversity, taxonomy distribution and statistical analysis

Biodiversity indexes and principle statistics analyses on taxonomic profiles were analyzed in R version 3.5 using the vegan (Oksanen et al. 2018) and Phyloseq packages (McMurdie and Holmes 2014). Data in each vineyard was analyzed separately due to the differences in soil chemistry and climate (Supplementary Table 3.3.2). Technical noise (variation attributable to sequencing depth or batch effects) was controlled by including MiSeq run as a random effect.

Within sample type, α-diversity estimates were calculated by analyzing the Chao1 richness and Shannon diversity in Phyloseq package, as implemented in the tool MicrobiomeAnalyst (Dhariwal et al. 2017). The normalized OTU table was analyzed using Bray Curtis metrics (Bray and Curtis 1957) and utilized to evaluate the β- diversity and to construct PCoA plots (Vázquez-Baeza et al. 2013) using MicrobiomeAnalyst. In order to compare bacterial and fungal communities composition and to partition of variance in different categories, Bray-Curtis distance matrices were subjected to PERMANOVA (Anderson 2001) using the adonis function with a permutation number of 999 available in the vegan package of R. PERMANOVA was performed to investigate which OTUs significantly differed in abundance among experimental factors.

The variance-partitioning model tests for effects of year, sampling date and genotype on microbiome communities, while year-by-genotype and date-by-genotype interaction terms describe how the distinct fungal and bacterial communities at different common rootstocks respond differently to each of these factors. The linear mixed models were fit using the lme4 package (Bates et al. 2015). Statistical significance of fixed predictors (Year + Sampling Date + Genotype + Year x Genotype + Date x Genotype) was assessed using Type III ANOVA with Satterthwaite's approximation of denominator degrees of freedom in the package InnerTest

(Kuznetsova et al. 2016), and of random effects (MiSeq run) using likelihood ratio tests. This model was used to predict community descriptors that were continuous and approximately normally distributed in α-diversity metrics (Shannon entropy and Chao1 estimated richness) as described above.

The Linear Discriminant Analysis Effect Size (LEfSe) algorithm was used to identify taxa (genus level or higher) that differed in relative abundance between the rootstocks (Segata et al. 2011). The online Galaxy Version 1.0 interface (The Huttenhower Lab) was used, the threshold for the logarithmic LDA score was set at 1.0 and the Wilcoxon *p*-value at 0.05. The results are displayed in a cladogram and a bar graph. A Similarity Percentages (SIMPER) analysis was performed with PRIMER 6 software to explore the dissimilarities between the rootstock factor. Summarized taxa tables at the phylum and genera levels were used to investigate the phylogenetic groups that contribute to the dissimilarity. Unclassified OTUs amounting to less than 3% of the relative abundance in the rhizosphere were discarded from the analysis, according to Marasco et al. (2018). The bacterial and fungal OTUs shared among vineyards and rootstocks were defined by a Venn-diagram analysis using the software available at (van de Peer et al. 2018).

Quantitative PCR amplification and quantification of black-foot disease pathogens

Quantitative PCR analyses were performed with the DNA extracted from the soil samples, as Agustí-Brisach et al. (2014) developed in previous research, using the primers YT2F and Cyl-R (Dubrovsky and Fabritius 2007; Tewoldemedhin et al. 2011). These primers amplify the main *Cylindrocarpon*-like asexual morphs associated with black-foot disease, in particular those belonging to the genera *Dactylonectria*, *Ilyonectria*, *Neonectria*, and *Thelonectria.* Rotor-Gene 6000 real-time rotary analyser (Qiagen, Hilden, Germany) was used to perform the qPCR amplifications. Each reaction contained 2 µL of DNA, 1x of SYBR Premix Ex Taq II (Tli RNase H Plus) (Takara Bio Inc., Shiga, Japan) and 0.4 µM of each primer. The reaction mix was adjusted to a final volume of 20 µL with sterile distilled water. The thermocycling profile consisted of 30 s at 95°C and 50 cycles of 10 s at 95°C, 10 s at 60°C, and 30 s at 72°C. To evaluate amplification specificity, melting curve analysis was performed at the end of the qPCR runs according to the manufacturer's recommendations. Each analysis included three

replicates of each sample, a non-template control reaction (water) and a positive control containing DNA extracted from a pure culture of the *D. torresensis* isolate GTMF DT097, obtained from the collection of the Instituto Agroforestal Mediterráneo, Universitat Politècnica de Valencia, Spain. *D. torresensis* is the most common fungal species associated with black-foot diseased vines in Italy (Carlucci et al. 2017), Portugal (Reis et al. 2013) and Spain (Berlanas et al. 2017). For DNA extraction, fungal mycelium of this isolate grown on potato dextrose agar (PDA, Biokar-Diagnostics, Zac de Ther, France) for 2 weeks at 25°C in darkness, was scraped from the surface of the plate with a sterile scalpel. Total DNA was extracted using the E.Z.N.A. Plant Miniprep kit (Omega Bio-Tek, Doraville, USA) following the manufacturer's instructions and mycelia was previously homogenized with 4 steel beads of 2.38 mm and 2 of 3 mm diameter (Qiagen, Hilden, Germany) using a FastPrep-24TM5G (MP Biomedicals, California, USA) at 5m/s for 20 s twice. DNA extracted was quantified with Invitrogen Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, USA).

DNA of the *Cylindrocarpon*-like asexual morphs species was quantified using a standard curve constructed with the isolate GTMF DT097, consisting of a dilution series from 275 µg/µL to 0.275 fg/µL. Quantitative PCR analysis were perform as previously explained and the standard curve was generated following the MIQE guidelines (Bustin et al. 2009), by plotting quantification cycle (C_q) values obtained for each specific DNA concentration, versus the logarithm of the initial concentration of isolate DNA. The mean DNA concentration and the standard deviation were determined from three replicates per dilution. Sensitivity of the qPCR assay was assessed using the standard curve to determine the minimum DNA concentration that can be detected. The amplification efficiency (E) and the coefficient of determination (R^2) of the standard curve were obtained using the Rotor-Gene 6000 Series software v. 1.7 (Qiagen, Hilden, Germany). Signal threshold levels were set automatically by the instrument software and the limit of detection (LOD) was identified by the last dilution when successful qPCR amplification of DNA occurred, accompanied by a melting curve peak temperature specific to *D. torresensis*.

Values from the *Cylindrocarpon*-like asexual morphs number of OTUs and DNA concentration were transformed by log ($n/N * 1000 + 1$). Where n was the number of OTUs or the DNA concentration detected on each sample and N was the total number

of OTUs and the total DNA concentration detected. An analysis of correlation between both transformed datasets was performed in R version 3.5 using the corrr package.

Results

High-throughput amplicon sequencing

After paired-end alignments, quality filtering and deletion of chimeric, singletons, and mitochondrial and chloroplast sequences, a total of 4,337,395 bacterial 16S rRNA sequences and 6,216,366 fungal internal transcribed spacer (ITS) sequences were generated from 117 (three samples were removed from the analysis due to the low number of sequence reads) and 120 samples, respectively, and assigned to 975 bacterial and 567 fungal operational taxonomic units (OTUs) (Supplementary Table 3.3.3). Good's coverage values indicated that on average 94.5% and 90.1% of the total species richness were accounted for in bacteria and fungal communities, respectively (Supplementary Table 3.3.4). Chao1 diversity estimator ranged from 143.6 to 549.5 in the bacterial microbiome, and from 90.5 to 254.9 in the fungal microbiome. Shannon diversity estimator ranged from 1.80 to 4.68 in the bacterial microbiome, and from 1.80 to 3.84 in the fungal microbiome (Supplementary Table 3.3.4).

Core grapevine phylogeny between vineyards

The two habitats used as vineyard sites (Aldeanueva del Ebro, abbreviated 'Aldea' in the figures and tables; and Olite) were separated by 45 km, and varied in most of soil physico-chemical properties (Supplementary Table 3.3.2). Bacterial communities of rhizosphere soil samples did not differ significantly between vineyards (Supplementary Table 3.3.5). However, α-diversity differed among sites when studying the fungal microbiota, and principal coordinates analysis (PCoA) of Bray Curtis data demonstrated that vineyard was the primary source of β-diversity (Supplementary Figure 3.3.1). Comparing the fungal and bacterial microbiota of the two vineyards, 82.9 and 58.7% of bacterial and fungal OTUs, respectively, were shared between vineyards, demonstrating the existence of a "core" grape phylogeny that is independent of the growing region (Figure 3.3.1).

The relative abundance of bacterial and fungal phyla detected across all samples is shown in Figure 3.3.1. In both vineyards, the bacterial phyla Proteobacteria (26.1% and

28.1% in Aldea and Olite, respectively) and Actinobacteria (24.1% and 18.5%) represented almost 50% of the total bacteria detected. These phyla were followed by Acidobacteria (13.7 and 16.4%), unidentified bacteria (11.4 and 11.7%) and Bacteroidetes (5.2 and 6.1%) (Figure 3.3.1).

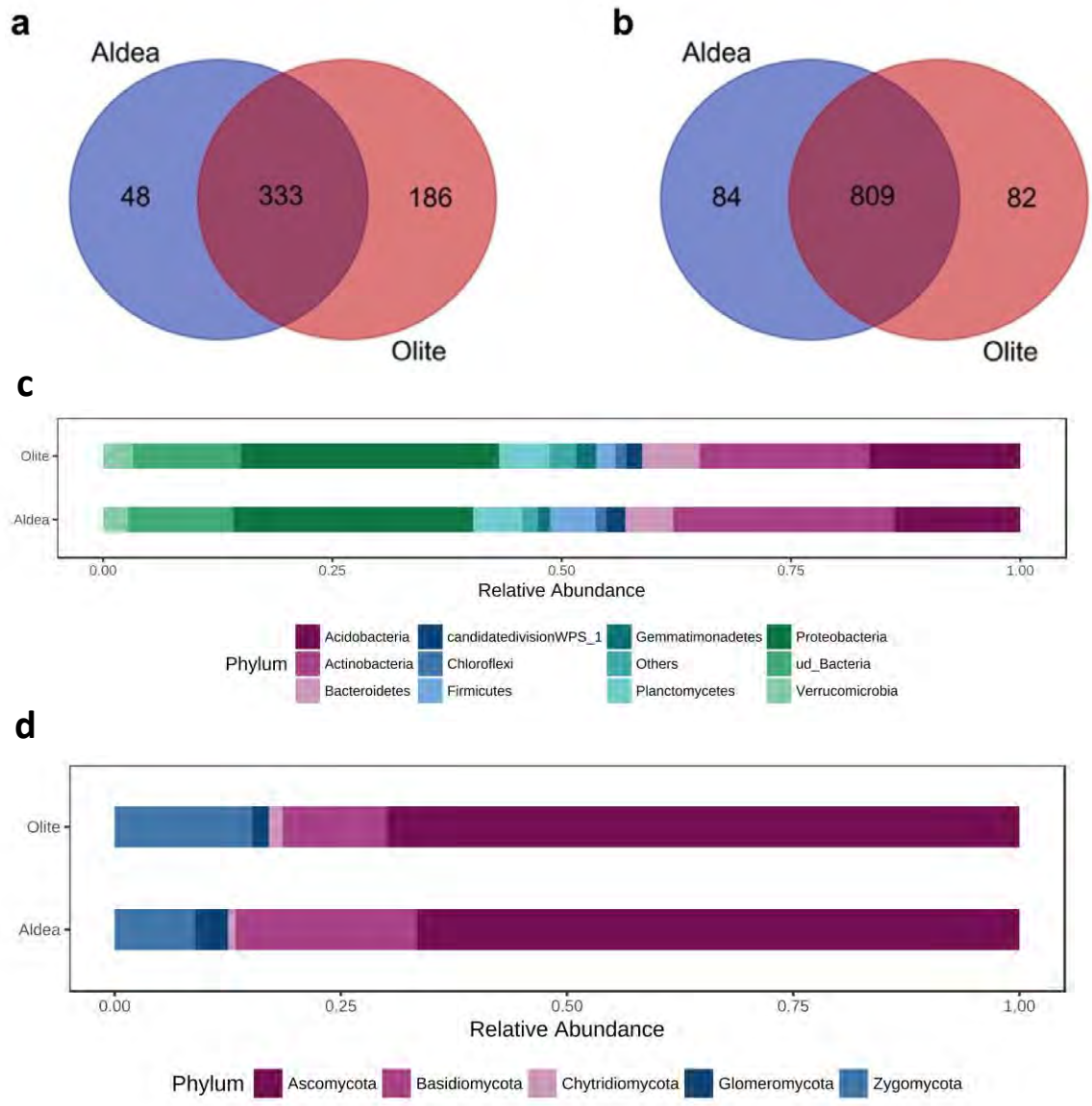

Figure 3.3.1. *Venn diagram illustrating the overlap of the OTUs identified in the fungal* ***(a)*** *and bacterial* ***(b)*** *microbiota between vineyards. Relative abundance of different bacterial* ***(c)*** *and fungal* ***(d)*** *phyla in the rootstock rhizospheres in both vineyards representing OTUs showing more than 1% relative abundance of all reads and present in at least 2/3 of replicates. Phyla representing less than 1% of the total reads are grouped in 'Others'.*

The most abundant families within the Proteobacteria phylum were unidentified families from the order Rhizobiales (13.0% and 10.4% in Aldea and Olite, respectively), unidentified families from the class Betaproteobacteria (9.8% and 13.0%) and Sphingomonadaceae (7.6% and 10.7%). The most abundant families within the

Actinobacteria phylum were unidentified Actinobacteria (29.1% and 22.5% in Aldea and Olite, respectively), Gaiellaceae (16.0% and 15.2%) and Streptomycetaceae (6.2% and 6.7%) (Supplementary Figure 3.3.2). Regarding the fungal taxa, the most abundant fungal phylum was Ascomycota (66.6% and 69.9% in Aldea and Olite, respectively), followed by Basidiomycota (20.1% and 11.5%) and Zygomycota (8.9 and 15.2%) (Figure 3.3.1). The most abundant families within the Ascomycota phylum were Nectriaceae (15.4%), unidentified Ascomycota (8.8%) and Bionectriaceae (9.1%) in Aldea vineyard, and Nectriaceae (17.7%), unidentified Ascomycota (11.1%), Pyronemataceae (9.6%) and Trichocomaceae (8.4%) in Olite vineyard (Supplementary Figure 3.3.2).

Host genetic influence on the rhizosphere microbiota

Bacterial and fungal diversity in rhizosphere soil samples differed significantly among rootstocks in Aldea vineyard. However, plant genotype did not predict Chao1 diversity (Table 3.3.1). Host genotype was the most important factor in structuring bacterial (R^2 = 0.65, P < 0.001) and fungal (R^2 = 0.86, P < 0.001) communities in the entire dataset, and also when the data were split by year and date (Table 3.3.1). A PCoA further demonstrated the variation in the total dataset could be attributed to host genotype in Aldea vineyard (Figure 3.3.2). In Olite vineyard, plant genotype had a much weaker influence on rhizosphere-associated bacterial and fungal communities. Host genotype did not predict any summary metrics of rhizosphere α and β-diversities (Tables 3.3.1 and 3.3.2).

The linear discriminant analysis effect size (LEfSe) detected 27 bacterial and 36 fungal clades in the rhizospheres, which discriminated the microbial communities between the different rootstock genotypes in Aldea vineyard (Figures 3.3.3 and 3.3.4). Both rootstocks ‘1103 P’ and ‘41 B’ showed higher number of differentially abundant bacterial clades (8 each) than the other rootstocks (5, 4 and 2 in ‘161-49 C’, ‘110 R’ and ‘140 Ru’, respectively). The dominant bacterial phyla were Firmicutes (37%) in rootstock ’41 B’, Actinobacteria and Planctomycetes (50% each) in rootstock ‘140 Ru’, and Actinobacteria in rootstocks ‘161-49 C’, ‘110 R’ and ‘1103 P’ (60%, 75% and 75%, respectively) (Figure 3.3.3). The dominant fungal phyla were Basidiomycota (73%) in rootstock ‘140 Ru’, and Ascomycota in rootstocks ‘41 B’, ‘161-49 C’, ‘110 R’ and ‘1103 P’ (75%, 100%, 36% and 71%, respectively) (Figure 3.3.4).

Table 3.3.1. Experimental factors predicting α-diversity of rhizosphere associated fungal and bacterial communities in Aldea and Olite vineyards.

Bacteria	Aldea		Olite	
	Shannon	Chao1	Shannon	Chao1
Genotype	$F_{4,54}$ = 3.47	$F_{4,54}$ =0.34	$F_{4,54}$ = 0.90	$F_{4,54}$ = 0.32
	***P* = 0.0134**	*P* = 0.8480	*P* = 0.4693	*P* = 0.8648
Year	$F_{1,57}$ = 6.83	$F_{1,57}$ = 17.39	$F_{1,57}$ = 4.66	$F_{1,57}$ = 7.55
	***P* = 7.3^{-09}**	***P* = 1.5^{-20}**	***P* = 1.6^{-04}**	***P* = 4.7^{-10}**
Year x Genotype	$F_{4,49}$ = 0.73	$F_{4,49}$ = 1.48	$F_{4,49}$ = 2.33	$F_{4,49}$ = 6.08
	***P* = 0.0122**	*P* = 0.3661	*P* = 0.0623	*P* = 0.2143
Date	$F_{1,57}$ = 0.05	$F_{1,57}$ = 0.18	$F_{1,57}$ = 0.68	$F_{1,57}$ = 0.13
	P = 0.9555	*P* = 0.8502	*P* = 0.4989	*P* = 0.8941
Date x Genotype	$F_{4,49}$ = 1.55	$F_{4,49}$ = 0.74	$F_{4,49}$ = 0.19	$F_{4,49}$ = 1.67
	P = 0.1812	*P* = 0.7702	*P* = 0.1802	*P* = 0.2561
MiSeq r	χ^2_1 = 0.55	χ^2_1 = 0.74	χ^2_1 = 0.28	χ^2_1 = 1.59
	P = 0.3623	*P* = 0.4565	*P* = 0.7712	*P* = 0.3421
Fungi				
Genotype	$F_{4,55}$ = 2.80	$F_{4,55}$ = 1.12	$F_{4,55}$ = 0.82	$F_{4,55}$ = 2.27
	***P* = 0.0232**	*P* = 0.3529	*P* = 0.5130	*P* = 0.0929
Year	$F_{1,58}$ = 0.95	$F_{1,58}$ = 10.62	$F_{1,58}$ = 0.37	$F_{1,58}$ = 5.25
	P = 0.3415	***P* = 3.2^{-15}**	*P* = 0.7112	***P* = 3.5^{-06}**
Year x Genotype	$F_{4,50}$ = 2.85	$F_{4,50}$ = 1.15	$F_{4,50}$ = 0.35	$F_{4,50}$ = 3.85
	P = 0.1126	*P* = 0.3601	*P* = 0.1831	*P* = 0.3126
Date	$F_{1,58}$ = 8.52	$F_{1,58}$ = 2.17	$F_{1,58}$ = 0.44	$F_{1,58}$ = 1.31
	***P* = 1.08^{-11}**	*P* = 0.0640	*P* = 0.6597	*P* = 0.1937
Date x Genotype	$F_{4,50}$ = 0.71	$F_{4,50}$ = 0.91	$F_{4,50}$ = 1.91	$F_{4,50}$ = 6.81
	***P* = 0.0112**	*P* = 0.2903	*P* = 0.6351	*P* = 0.7443
MiSeq run	χ^2_1 = 0.74	χ^2_1 = 2.92	χ^2_1 = 1.77	χ^2_1 = 0.12
	P = 0.4912	*P* = 0.2551	*P* = 0.8135	*P* = 0.7331

ANOVA, analysis of variance

Statistics describe linear random-intercept models of Shannon diversity and Chao1 richness in the rhizosphere. All *P* values were corrected for multiple comparisons using the sequential Bonferroni correction. Significance was assessed using Type III ANOVA with F tests for fixed effects and likelihood ratio tests for the random effect. Bold values indicate statistically significant results after correction for multiple comparisons $P < 0.05$.

The rootstock-pairs dissimilarity, due to phyla and genera contribution in the rhizosphere was calculated by SIMPER (similarity percentages) analysis (Supplementary Table 3.3.6). Higher microbiome dissimilarity among rootstocks was revealed in Aldea vineyard compared to Olite vineyard, considering bacterial (Supplementary Table 3.3.6A) and fungal phyla (Supplementary Table 3.3.6C), and bacterial (Supplementary Table 3.3.6B) and fungal genera (Supplementary Table 3.3.6D) distribution. Firmicutes and Acidobacteria were the major phyla that contribute to differentiate the bacterial communities associated with the different rootstock types in Aldea and Olite vineyards, respectively (Supplementary Table 3.3.6A). Several genera were predominant and determined the dissimilarities among rootstocks such as *Bacillus* in Aldea vineyard or *Aridibacer* in Olite vineyard. The genus *Bacillus* appeared to be rhizosphere genotype biomarker of '140 Ru' and '161-49 C' rootstocks (Supplementary Table 3.3.6B).

The fungal phyla Ascomycota and Basidiomycota contributed to the dissimilarity among rootstocks in Aldea vineyard, while only the phylum Basidiomycota contributed to differentiate fungal communities among rootstocks (Supplementary Table 3.3.6C). The fungal genera *Geopyxis*, *Clonostachys* and *Lecanicillium* determined the dissimilarities among rootstocks in Aldea vineyard, being *Geopyxis* a rhizosphere genotype biomarker of '110 R' rootstock and *Clonostachys* of '1103 P' and '140 Ru' rootstocks (Supplementary Table 3.3.6D). In Aldea vineyard, '161-49 C' rootstock showed the highest dissimilarity with the other rootstocks in bacterial and fungal microbiome distribution.

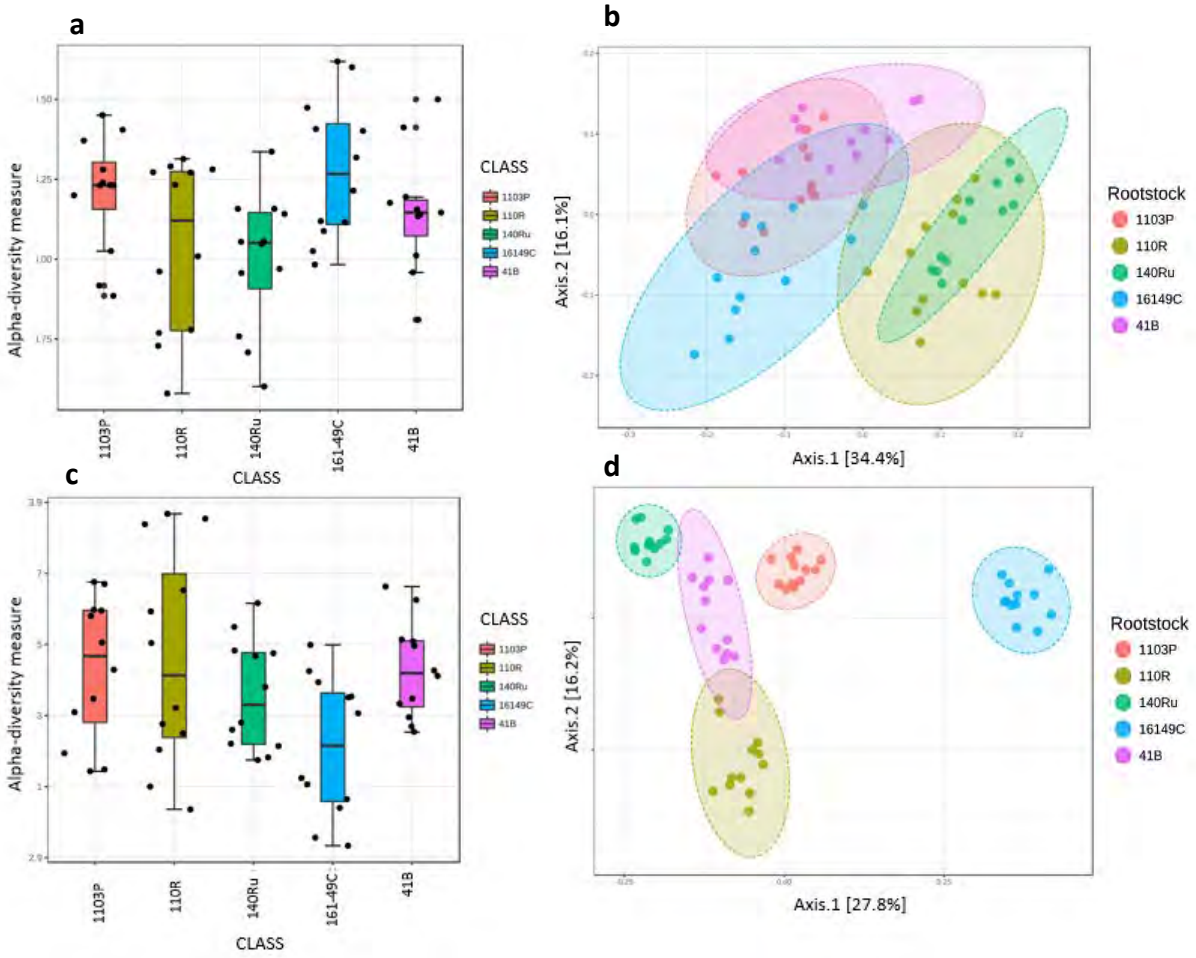

Figure 3.3.2. *Boxplot illustrating the differences in Shannon diversity measures of the bacterial* ***(a)*** *and fungal* ***(c)*** *communities in the grapevine rootstocks in Aldea vineyard. Principal Coordinate Analysis (PCoA) based on Bray Curtis dissimilarity metrics, showing the distance in the bacterial* ***(b)*** *and fungal* ***(d)*** *communities among grapevine rootstocks.*

Year strongly influenced microbiomes

Our results demonstrate that bacterial microbiome varied profoundly between years. This pattern was consistent to community-level measure of α- diversity in both Aldea and Olite vineyards (Table 3.3.1) Richness increased between 2016 and 2017 in

both vineyards (Supplementary Figure 3.3.3). However, year of sampling affected the Bray Curtis metric of β-diversity in only Olite vineyard (R^2 = 0.494) (Supplementary Figure 3.3.3). Regarding the fungal microbiome, richness also varied between vineyards and increased between 2016 and 2017 in both vineyards (Table 3.3.1; Supplementary Figure 3.3.4). However, year of sampling did not predict Shannon diversity and affected the Bray Curtis metric of β-diversity in only Olite vineyard (Table 3.9; Supplementary Figure 3.3.4). Sampling date also contributed to α-diversity variation indicating temporal changes in relative abundance of fungal OTUs in Aldea vineyard. Fungal composition decreased between June and November (Table 3.3.1; Supplementary Figure 3.3.5). Fungal community structure varied individually in each rootstock with date (R^2 ranging from 0.42 to 0.61), but not in the total dataset (R^2 < 0.1) (Table 3.3.2).

Rootstock-specific and shared bacterial and fungal assemblages

The rhizosphere compartments of grapevine rootstocks showed specific fungal and bacterial OTUs for each rootstocks and a cluster of shared OTUs. In Aldea, specific OTUs associated with most of the rootstocks ranged from 4.3 to 5.8% of their bacterial communities (Figure 3.3.5). Specific OTUs associated with the rootstocks '140 Ru', '1103 P', '41 B' and '110 R' represented less than 9% of their fungal communities, where the '161-49 C'-specific OTUs enriched only 4.5% of the relative abundance (Figure 3.3.5). In Olite, specific OTUs associated with most of the rootstocks represented less than 9% of their bacterial and fungal communities, with the exception of bacterial communities associated with 140 Ru rootstock that represented 21.3% of its total (Figure 3.3.6). The OTUs that were unique in each of the grapevine rootstock are shown in Supplementary Tables 3.3.7 and 3.3.8.

Quantification of black-foot disease pathogens using quantitative PCR

The standard curve, constructed with serial dilutions of the DNA of *Dactylonectria torresensis* isolate GTMF DT097, revealed high correlations between C_q and DNA, with R^2 value of 0.99 and reaction efficiency of 0.90. The minimum DNA concentration detectable of *D. torresensis* was at C_q value of the dilution D7 thus, the limit of detection (LOD) was established at 2.75 fg/µL.

DNA of *Cylindrocarpon*-like asexual morphs was detected in all rootstock rhizosphere samples, in both vineyards and years, with concentrations ranging from 0.39 pg/µL to 4.06 pg/µL in Aldea 2016, from 3.52 pg/µL to 14.14 pg/µL in Aldea 2017, from 0.88 pg/µL to 8.45 pg/µL in Olite 2016 and from 2.65 pg/µL to 59 pg/µL in Olite 2017. The year and vineyard factors had a significant effect on *Cylindrocarpon*-like asexual morphs DNA concentration detected ($P < 0.01$).

Table 3.3.2. Adonis test of category effect on bacterial and fungal Bray-Curtis distance matrix.

Bacteria		Aldea			Olite	
Dataset	Factor	R^2	*P* value	Factor	R^2	*P*-value
Total	Genotype	0.658	0.001	Genotype	0.058	0.015
	Year	0.163	0.001	Year	0.494	0.001
	Date	0.109	0.002	Date	0.059	0.004
'110 R'	Year	0.564	0.002	Year	0.438	0.005
	Date	0.028	0.116	Date	0.204	0.066
'140 Ru'	Year	0.235	0.006	Year	0.458	0.005
	Date	0.355	0.002	Date	0.092	0.333
'1103 P'	Year	0.220	0.011	Year	0.379	0.005
	Date	0.461	0.002	Date	0.174	0.036
'41 B'	Year	0.087	0.071	Year	0.453	0.005
	Date	0.670	0.002	Date	0.129	0.092
'161 49 C'	Year	0.228	0.003	Year	0.471	0.005
	Date	0.228	0.005	Date	0.221	0.040
2016	Genotype	0.868	0.001	Genotype	0.206	0.031
	Date	0.067	0.035	Date	0.165	0.001
2017	Genotype	0.768	0.001	Genotype	0.240	0.001
	Date	0.135	0.004	Date	0.138	0.002
June	Genotype	0.634	0.001	Genotype	0.145	0.365
	Year	0.110	0.005	Year	0.331	0.001
November	Genotype	0.831	0.001	Genotype	0.240	0.020
	Year	0.123	0.004	Year	0.354	0.001
Fungi						
Total	Genotype	0.864	0.001	Genotype	0.096	0.027
	Year	0.052	0.004	Year	0.564	0.001
	Date	0.084	0.001	Date	0.042	0.005
'110 R'	Year	0.183	0.122	Year	0.438	0.005
	Date	0.501	0.002	Date	0.204	0.066
'140 Ru'	Year	0.142	0.137	Year	0.458	0.005
	Date	0.615	0.002	Date	0.092	0.333
'1103 P'	Year	0.266	0.031	Year	0.379	0.005
	Date	0.496	0.002	Date	0.174	0.036
'41 B'	Year	0.241	0.033	Year	0.453	0.005
	Date	0.425	0.002	Date	0.129	0.092
'161 49 C'	Year	0.191	0.066	Year	0.471	0.005
	Date	0.472	0.002	Date	0.221	0.040
2016	Genotype	0.841	0.001	Genotype	0.144	0.305
	Date	0.110	0.002	Date	0.070	0.002
2017	Genotype	0.928	0.001	Genotype	0.274	0.001
	Date	0.130	0.002	Date	0.127	0.002
June	Genotype	0.808	0.001	Genotype	0.220	0.012
	Year	0.066	0.080	Year	0.289	0.001
November	Genotype	0.753	0.001	Genotype	0.200	0.003
	Year	0.105	0.004	Year	0.208	0.001

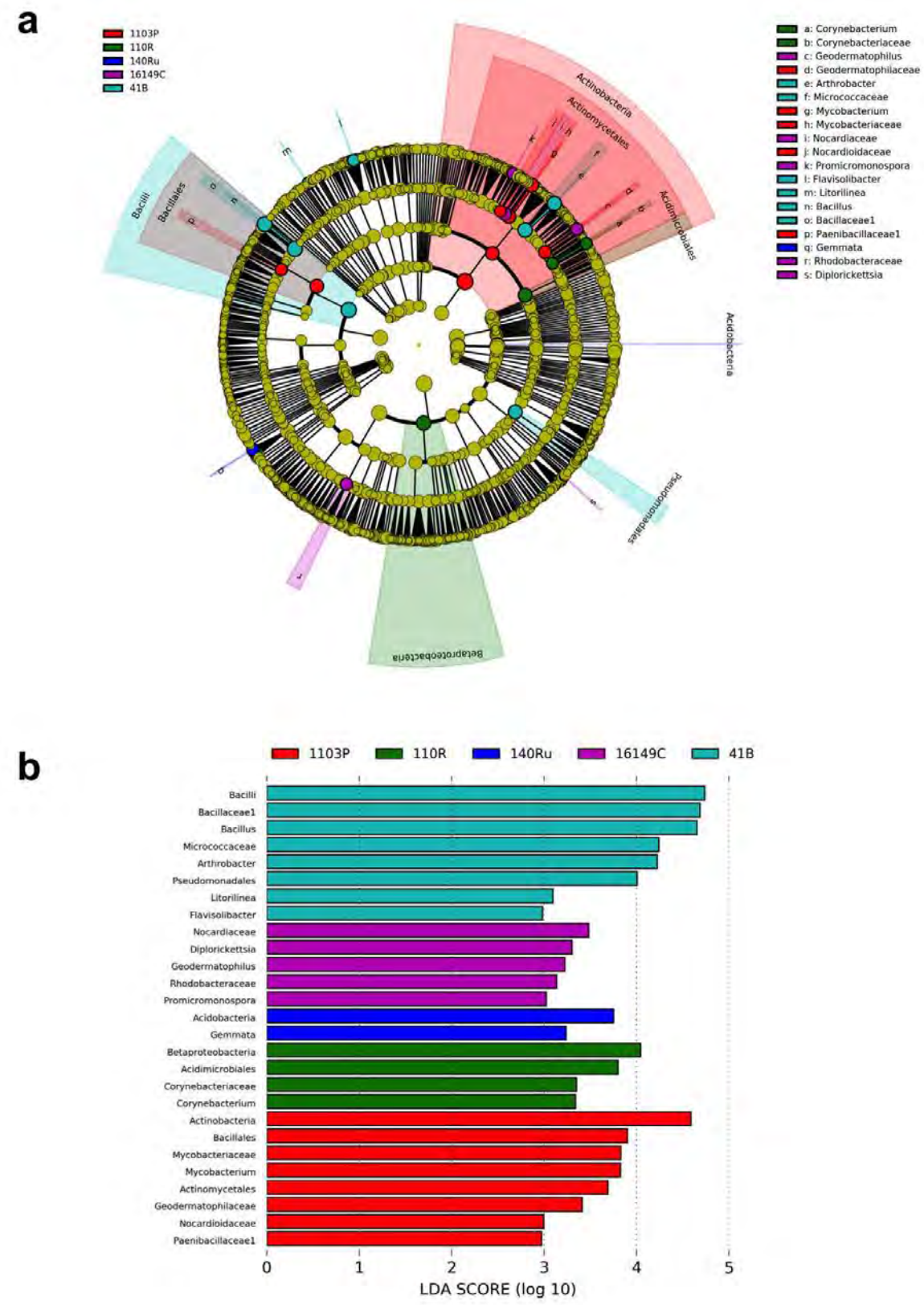

Figure 3.3.3. *LEfSe was used to identify the most differentially abundant taxa among grapevine rootstocks in Aldea vineyard. Cladogram generated by LEfSe indicating differences of bacteria* ***(a)*** *at phylum, class, family and genus levels between the five groups (relative abundance ≤ 0.5%). Each successive circle represents a phylogenetic level. Color regions indicate taxa enriched in the different rootstocks. Differing taxa are listed on the right side of the cladogram. Bar graph showing LDA scores for bacteria* ***(b)****. Only taxa meeting an LDA significant threshold < 2 are shown.*

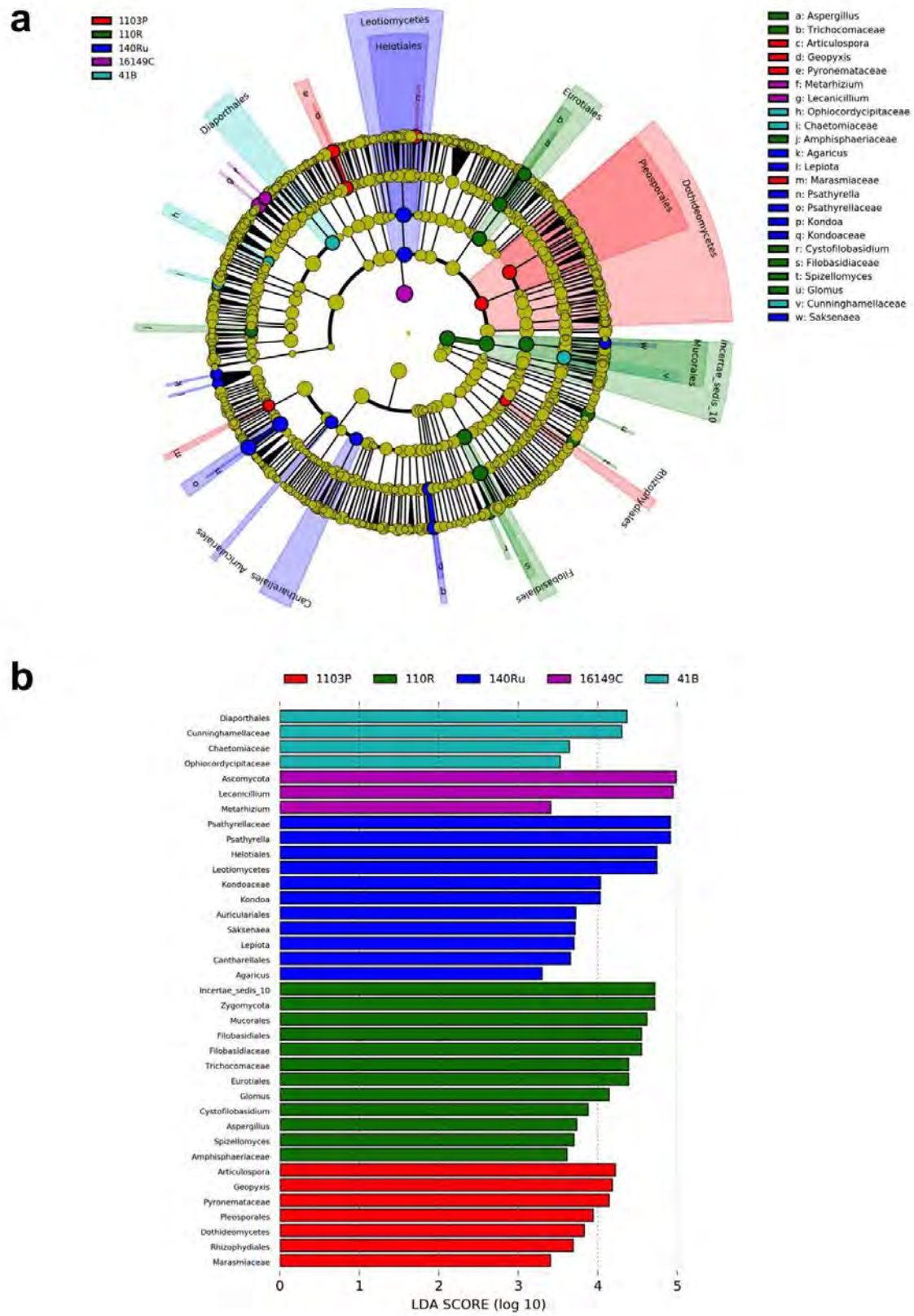

Figure 3.3.4. *LEfSe was used to identify the most differentially abundant taxa among grapevine rootstocks in Aldea vineyard. Cladogram generated by LEfSe indicating differences of fungi* ***(a)*** *at phylum, class, family and genus levels between the five groups (relative abundance ≤ 0.5%). Each successive circle represents a phylogenetic level. Color regions indicate taxa enriched in the different rootstocks. Differing taxa are listed on the right side of the cladogram. Bar graph showing LDA scores for fungi* ***(b).*** *Only taxa meeting an LDA significant threshold > 2 are shown.*

The concentration of DNA detected was significantly higher in Olite vineyard compared with Aldea vineyard, especially in year 2017. The rootstock factor had a significant effect on the DNA concentration detected in Aldea vineyard for 2017 samples (P = 0.0156). Rootstocks '161-49 C', '140 Ru', '1103 P' and '110 R' showed similar DNA concentrations values that were significantly lower when compared with '41 B' rootstocks (Figure 3.3.7). The analysis showed a positive significant correlation between the number of OTUs and the *Cylindrocarpon*-like asexual morphs DNA quantified using the real-time approach (P < 0.01, Spearman correlation coefficient = 0.72) (Figure 3.3.8).

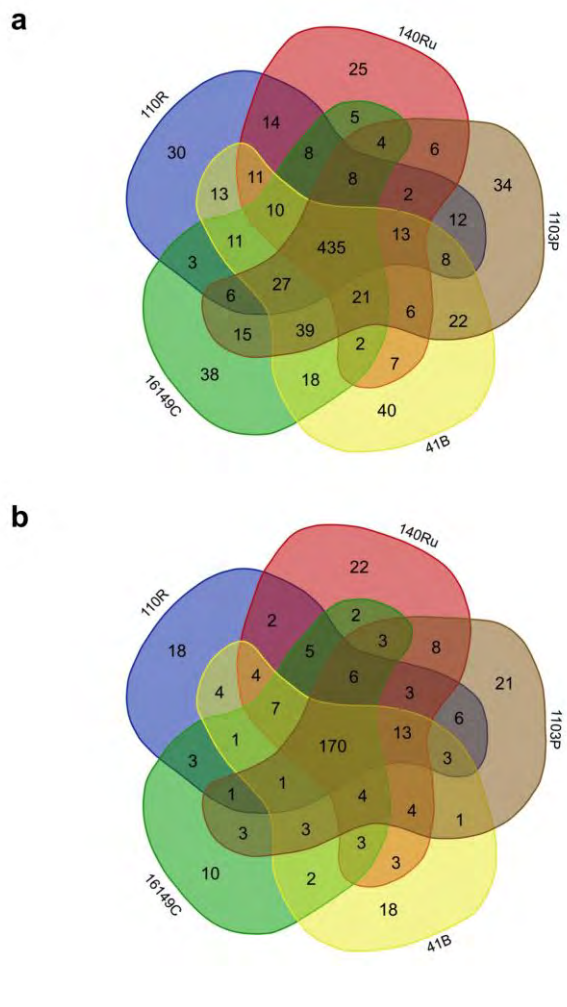

Figure 3.3.5. *Venn diagrams showing the common and exclusive bacterial* ***(a)*** *and fungal* ***(b)*** *OTUs of the rhizosphere of the grapevine rootstocks in Aldea vineyard.*

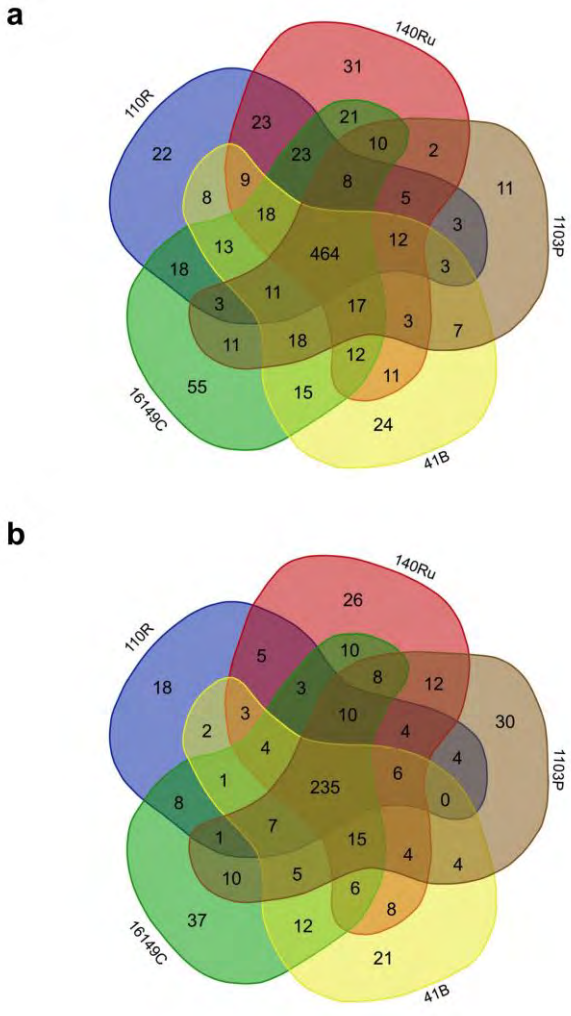

Figure 3.3.6. *Venn diagrams showing the common and exclusive bacterial* ***(a)*** *and fungal* ***(b)*** *OTUs of the rhizosphere of the grapevine rootstocks in Olite vineyard.*

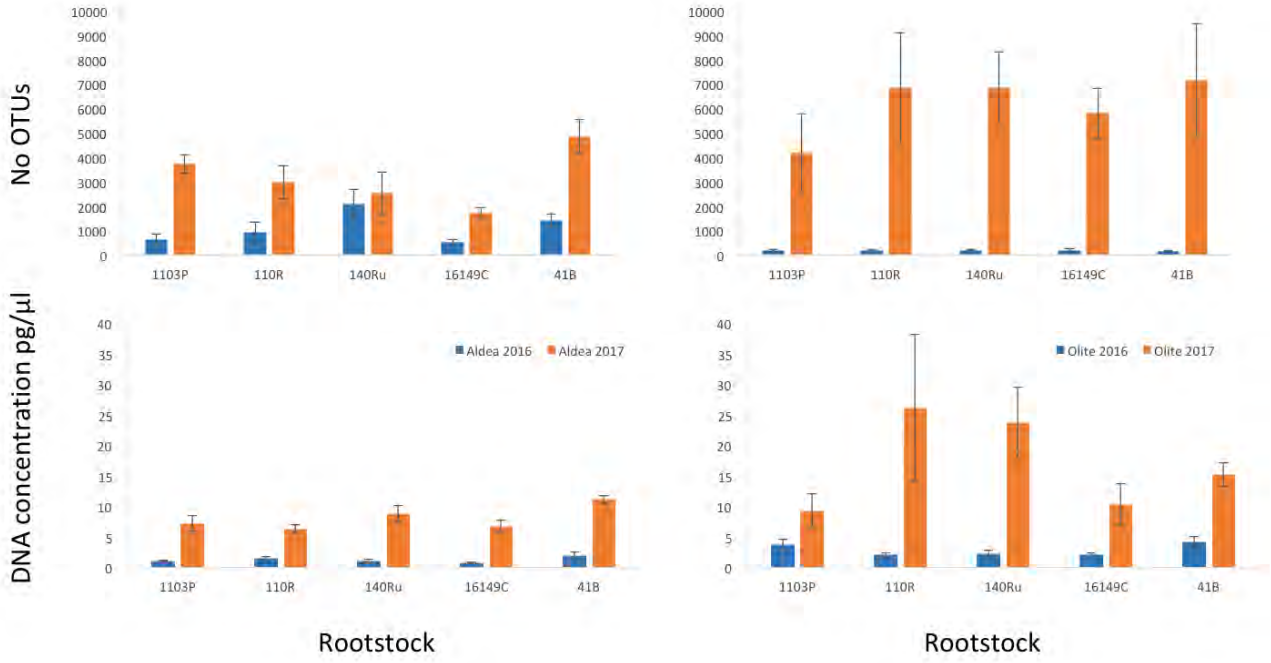

Figure 3.3.7. *Number of OTUs identified and DNA concentration of Cylindrocarpon-like asexual morphs for the five rootstocks analyzed in Aldea and Olite geographic regions in both years studies. Values are the mean of six replicates (3 samples per sampling time) and twelve replicates (3 samples per sampling time and 2 runs for each one) for qPCR and high-throughput amplicon sequencing analysis, respectively. Vertical bars represent the standard errors.*

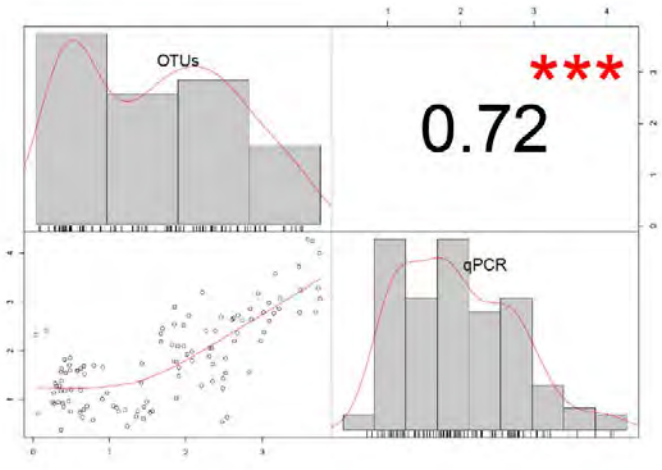

Figure 3.3.8. *The distribution of the number of OTUs and DNA concentration of Cylindrocarpon-like asexual morphs values are shown on the diagonal. The bivariate scatter plot with a fitted line is displayed on the bottom of the diagonal and the Spearman correlation value ($P < 0.05$) in indicated on the top of the diagonal.*

Discussion

In this study, we characterized the rhizosphere microbial community composition across five commercial grapevine rootstock genotypes cultivated in the same soil at two vineyards and sampling dates over two years. The analysis of bacterial and fungal populations in the grapevine rhizosphere targeting 16S rRNA and ITS region, respectively, have been proved effective in previous studies (Corneo et al. 2014; Holland et al. 2016; Longa et al. 2017; Manici et al. 2017; Stefanini and Cavalieri 2018). Especially for bacterial barcoding, the choice of partial sequence regions is pivotal and can significantly affect the results because the 16S rRNA gene regions have different divergence (Youssef et al. 2009). In our study, we used the V4 region because according to recent *in silico* studies (Youssef et al. 2009), V4 along with V5-V6, and V6-V7 regions were considered as the most suitable regions for metagenomic purposes because they provided estimates comparable to those obtained with the complete 16S rRNA gene sequence (Youssef et al. 2009).

Our study represents the first approach to investigate the rhizosphere fungal microbiome of grapevine by HTAS. In grapevine, the ecology of fungal communities is so far largely derived from the studies using pyrosequencing approach in bulk soil (Holland et al. 2016; Castañeda and Barbosa 2017; Longa et al. 2017) or ARISA fingerprinting (Likar et al. 2017) and PCR-DGGE (Manici et al. 2017) approaches in rhizosphere soil. Even though the ITS region was ratified by The Fungal Barcoding Consortium (Schoch et al. 2012) as the universal DNA barcode for the fungal kingdom using the same gene section proposed by White et al. (1990), some recent reports point out its limitations for specific taxa. This region does not work well with taxa having narrow or no barcode gaps in their ITS regions, such as *Fusarium* or *Trichoderma* (Schoch et al. 2012). In addition, the correct identification of morphologically similar cryptic species using the ITS regions is still problematic due to the lack of consensus in the lineage-specific cut-off value for species determination (Nilsson et al. 2008).

The bacterial microbiomes of the different rootstocks were largely composed of Proteobacteria and Actinobateria that accounted for almost 50% of the relative abundance in both vineyards. The predominant bacterial phyla found in this work is consistent with the results obtained in other studies in vineyard soil (Opsi et al. 2014;

Vega-Avila et al. 2015; Castañeda and Barbosa 2017; Longa et al. 2017; Marasco et al. 2018). Proteobacteria and Actinobacteria are known for their role in the carbon biochemical cycle and their production of second metabolites (Jenkins et al. 2009). The major fungal phyla detected in our study were largely composed of Ascomycota and Basiodiomycota that accounted for almost 75% of the relative abundance in both vineyards. Previous studies also agree on the most common fungal phyla detected in grapevines fields (Castañeda and Barbosa 2017; Longa et al. 2017; Manici et al. 2017). These results suggest that vineyard microbiome in Navarra and La Rioja regions is partially conserved.

The results obtained in the Aldea vineyard showed a significant fraction of variation in fungal and bacterial diversity (both the α- and β-diversity) that could be attributed to host genetics. Recent research indicated that rootstock genotypes could have a notable influence in shaping the bacteria taxa distribution in the root and rhizosphere systems of grapevine (Marasco et al. 2018). This effect of the host genotype in the rhizosphere microbiome has been reported in other woody crops, such as apple (Liu et al. 2018) and pines (Gallart et al. 2018), as well as in several annual crops, such as maize (Peiffer et al. 2013), potato (Inceoğlu et al. 2010) and chickpea (Bazghaleh et al. 2015). This could be due to the influence of the genotype in the root metabolism, including immune response and exudate composition, which impact in the rhizosphere microbiome (Wagner et al. 2016). Rootstocks show different level of tolerance to distinct diseases; and this could be decisive in their effect in the microbiome (Sapkota et al. 2015). Moreover, as reviewed by Liu et al. (2018), several studies hint to a possible co-evolution of the holobiont. However, further research is needed to validate this hypothesis. On the other hand, the Olite vineyard showed a lower microbiome dissimilarity among rootstocks, suggesting that the effect of genotype in shaping the microbiome might be influenced by other factors.

The differences between Olite and Aldea vineyards could lie in the soil physicochemical properties, in the soil and cultivar management practices, or in the age of the plants, being vines cultivated in Olite vineyard younger than in Aldea vineyard. Environmental heterogeneity, such as the soil physicochemical properties and moisture content have been identified as major factors shaping the spatial scaling of the rhizosphere microbiome in many previous studies (Costa et al. 2006; Tan et al.

2013; Schreiter et al. 2014;), including grapevine (Fernández-Calviño et al. 2010; Corneo et al. 2014; Burns et al. 2015; Zarraonaindia et al. 2015; Holland et al. 2016). Soil physicochemical properties can also influence the population structure of specific soil-borne pathogens. For instance, Berlanas et al. (2017) observed that excessive calcium carbonate in soil may increase black-foot disease inoculum density.

Field management practices have been also reported as an important driver of the microbiome diversity (Hacquard 2016; Santhanam et al. 2015; Sapkota et al. 2015; Gallart et al. 2018), including the grapevine soil microbiome (Vega-Avila et al. 2015; Likar et al. 2017; Longa et al. 2017;). Nevertheless, other studies showed a long-term effect of cultivation rather than field management on soil microbial diversity (Buckley and Schmidt 2001; Peiffer et al. 2013). Microbiome studies should consider the high degree of temporal variability in the sample design, because sampling the same point in different times can give different results due the variability of the own microbial community through time (Redford and Fierer 2009). The year to year variation found in our study could be explained by the different root response to distinct environmental factors, such as temperature or precipitation (Wagner et al. 2016). Further research is needed to determine if environment plays a much greater role than host genetics in determining the composition of the rhizosphere microbiome of grapevine.

Several studies have remarked the effect of the growth stage of the plant in its associated rhizosphere microbiome (Baudoin et al. 2002; Inceoğlu et al. 2010; Li et al. 2014; Okubo et al. 2014; Yuan et al. 2015; Wagner et al. 2016; Qiao et al. 2017). Changes in the quantity and quality of root exudates as plants develop have been proposed as the main source of variation of the rhizosphere microbiome composition present during different developmental stages of maize cultivars (Baudoin et al. 2002). However, most of the published studies are focused in annual plant systems. In grapevine, Manici et al. (2017) recently investigated shifts in bacterial and fungal communities between mature and young replaced vines in Italy. At a single sampling moment, these researchers concluded that long-term growth legacy overcame plant age in shaping rhizosphere microbiome (Manici et al. 2017). Further research is therefore needed to determine the long-term effect of the grapevine age on the associated microbiome as plants develop. This could be accomplished by comparing

the rhizosphere microbiome (i) in a single vineyard over time, or (ii) in two vineyards in close proximity with identical environmental conditions and soils, but with vines on different aging process.

Our results showed that the root system type is able to select specific bacterial and fungal OTUs as biomarkers for the different genotypes. Members of the bacterial genus *Bacillus*, which was only found in '140 Ru' and '161-49 C' rootstocks in Aldea vineyard, has wide diversity of physiological ability with respect to heat, pH and salinity. Therefore, *Bacillus* species can be found in a wide range of habitats, being a few of them pathogenic to vertebrates or invertebrates (Holt et al. 1994). *Bacillus subtilis* and *B. amyloliquefaciens* have been described as potential biocontrol agents against *Aspergillus parasiticus* and stem rot disease (Le et al. 2018; Siahmoshteh et al. 2018). *In vitro* assays of the heat stable metabolites of *B. subtilis* showed promising results in reducing the growth of the fungal trunk pathogens *Lasiodiplodia theobromae*, *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum* (Alfonzo et al. 2009). Rezgui et al. (2016) recently identified several *B. subtilis* strains inhabiting the wood tissues of mature grapevines in Tunisia with antagonistic traits against fungal trunk pathogens. On the other hand, some species of the arbuscular mycorrhizal (AM) fungal genus *Glomus*, one of the most differentially abundant taxa for '110 R' rootstock in Aldea vineyard, are catalogued as biocontrol agents (Tahat et al. 2010). For instance, inoculation of grapevine roots with *Rhizophagus irregularis* (syn. *Glomus intraradices*) reduced both the disease severity and the number of root lesions caused by black-foot disease pathogens (Petit and Gubler2006). AM fungi form one of the most interesting beneficial plant-micro-organism associations (Smith and Read 2008) and are known to colonize the roots of the majority of land plants, including grapevines (Schreiner and Mihara 2009; Trouvelot et al. 2015). Several genera within the Glomeromycota phylum have been identified from the rhizosphere samples obtainted in this study, namely *Claroideoglomus*, *Diversispora*, *Entrophosphora* and *Rhizophagus*. Trouvelot et al. (2015) reported that soil management can greatly impact the diversity of AM fungi. In fact, AM fungal communities are highly influenced by the soil characteristics but also to a smaller extent by the host plant development stage (Schreiner and Mihara 2009; Balestrini et al. 2010).

High-throughput amplicon sequencing is a powerful method for the analysis of microbial populations. It is accomplished by sequencing specific marker genes amplified directly from environmental DNA without prior enrichment or cultivation of the target population (Franzosa et al. 2015). The advantages of this approach is the detection of rare taxa at the genus level given the availability of large and comprehensive reference databases as well as several pipelines for bioinformatics analysis (Stefanini and Cavalieri 2018). Drawbacks of HTAS include the biased relative quantification of bacterial communities since bacterial species bear various number of copies of 16S rRNA genes, the sequencing of matrix (e.g., grape ITS, chloroplast 16S) and the low confidence for taxonomic assignment at the species level (Stefanini and Cavalieri 2018). A step forward consists of the understanding of how changes in the composition of microbial communities impact the population's biological functions (Ravin et al. 2015). Unfortunately, HTAS only allows inference of functional annotation while in whole-genome sequencing, functional annotation can be carried out by gene enrichment (Stefanini and Cavalieri 2018). A further drawback of using DNA-based metagenomic data to infer the biological functions potentially exploited by microbial populations is that the detected DNA may belong to dead organisms. However, an approach based on RNA sequencing would give a direct report of the functions achievable by the viable microbial populations. In grapevine, the study of the active fungal communities of internal grapevine wood by HTAS in extracted total RNA has been recently accomplished by Eichmeier et al. (2018).

The quantitative significance of next-generation sequencing data for microorganisms is often debated (Amend et al. 2010). Fortunately, we were able to compare the relative abundance of reads with the relative abundance of DNA of black-foot disease pathogens, and we observed significant positive correlation. From the fungal soilborne pathogens affecting grapevine, *Cylindrocarpon*-like asexual morphs associated with black-foot disease are among the most important limiting factor of the production worldwide (Halleen et al. 2006; Agustí-Brisach and Armengol 2013). Therefore, *Cylindrocarpon*-like asexual morphs can be considered model pathogens to monitor the healthy status of the grapevine planting material when analyzing the fungal microbial composition of soil/rhizosphere samples.

Grapevine rootstocks have different susceptibilities towards pathogens, including trunk disease pathogens (Alaniz et al. 2010; Eskalen et al. 2001; Gramaje et al. 2010; Brown et al. 2013; Billones-Baaijens et al. 2014), which may be an important factor in shaping not only pathogens abundance but also entire communities. Nevertheless, we did not observe a clear correlation between known disease resistances in individual genotypes and the fungal communities, although *Cylindrocarpon*-like asexual morphs were found in lower abundance in '161-49 C' rootstock by both high-throughput amplicon sequencing and qPCR approaches. The use of '161-49 C' rootstock was previously recommended within an integrated management program for other grapevine trunk diseases, such as Petri disease and esca (Gramaje et al. 2010).

Conclusion

We have studied the effects of genotype, year, sampling date and location on bacterial and fungal communities in the grapevine rhizosphere. We found that grapevine genotype was the most important factor in shaping the microbiome in the mature vineyard. Many bacterial and fungal species were found in all rootstocks and in both locations in our study, demonstrating the existence of a "core" grape phylogeny that is independent of the growing region. Interestingly, the rhizosphere compartments of '140 Ru' and '161-49 C' rootstocks, the latter showing high tolerance to esca and Petri disease pathogens in previous research (Gramaje et al. 2010), harboured lower number of black-foot pathogens than the other grapevine rootstocks. Also of interest was the presence of high relative abundance of the genus *Bacillus* in both grapevine rootstocks, a bacterial genus recognized as biocontrol agents. A more comprehensive study is needed to decipher the cause of the rootstock microbiome selection and the mechanisms by which grapevines are able to shape their associated microbial community. Understanding the vast diversity of bacteria and fungi in the rhizosphere and the interactions between microbiota and grapevine will facilitate the development of future strategies for grapevine protection.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

The study was conceived by CB, MB and DG. All authors contributed to the data collection. Data interpretation and manuscript preparation were performed by CB, MB, GE and DG. CB, MB, DG, GE and ML performed the experiments. CB, MB, GE and DG contributed to bioinformatics data analysis. All authors critically reviewed and edited the manuscript, and approved its publication.

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Data Availability Statement

The unrarefied OTU tables, and corresponding taxonomic classifications, sequence files and metadata for all samples used in this study have been deposited in Figshare (https://figshare.com/projects/Grapevine_rhizosphere_microbiome/36155).

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Supplementary material

Supplementary Tables

Supplementary Table 3.3.1 Information about rootstock selected in this study. The information is based in published studies (Martínez-Cutillas et al. 1990, Hidalgo 2002; Keller 2010).

Rootstock Type	Rootstock germplasm	Phylloxera resistance*	Nematode resistance	Grafted Scion Vigour	Tolerance			Ease of rooting
					Drought	Wet soil	Salt	
'110 R'	*V. berlandieri* x *V. rupestris*	High	Poor	Medium	High	Low	Poor	Low
'140 Ru'	*V. berlandieri* x *V. rupestris*	High	Low	High	High	Low	High	Poor
'1103 P'	*V. berlandieri* x *V. rupestris*	High	Medium	Medium	Medium	Low	High	Medium
'41 B'	*V. vinifera* x *V. berlandieri*	High	Poor	n.d.	High	Poor	Low	Medium
'161-49 C'	*V. berlandieri* x *V. riparia*	Excellent	Poor	Medium	Low	Low	Low	Low

* Excellent > High > Medium > Poor > Low

Supplementary Table 3.3.2. Physicochemical properties, soil management practices and climate of the two vineyard soils examined in this study. Values represent the mean±SE.

	Aldea vineyard	Olite vineyard
Coordinates	42,234961º, -1,899365º	42,252659º, 1,394441º
Altitude (m)	347	396
Physicochemical properties		
pH	8.4[a]±0.02	8.1
P mg/100g*	3.47±0.27	1.8±0.11
K mg/100g	15.52±0.59	17.3±0.44
S mg/100g*	4.37±0.38	0.9±0.27
Mg mg/100g*	25.57±0.29	15.0±0.26
Mn mg/100g	9.31±0.87	9.23±0.11
Fe mg/100g*	7.5±0.44	3.27±0.07
Ca mg/100g*	1570.92±220.81	1862.15±12.65
Na mg/100g*	6.08±0.19	1.46±0.05
SOM%*	0.74±0.02	1.75±0.01
Clay%	21.6±0.25	29.42±0.28
Sand%*	37.25±0.29	21.62±0.66
Silt%	41.12±0.05	49.0±0.41
CO_3Ca	15.05±0.03	18.67±0.23
CEC mekv/100g*	9.7±0.23	21.25±0.10
EC mS/cm	0.15	0.16
Assim. Ca mekv/100g*	10.2±0.19	20.4±0.15
Assim. Mg mekv/100g*	1.47±0.03	0.8±0.04
Soil temperature (ºC) (June)	25.7	22
Soil temperature (ºC) (November)	10.5	10.6
Soil management practices		
Irrigation system	Drip irrigation	Drip irrigation
Fertilization	1 application per year	6 applications per year
Pest management practices	5 spray treatments against powdery and downy mildew per year	6 spray treatments against powdery and downy mildew per year
Herbicide treatment	Yes	No
Climate		
Precipitation (mm)	529	462
Mean temperature (ºC)	13.5	12.8

[a]Average of 4 replicates. Asterisk indicates statistically significant results ($P < 0.05$)

Supplementary Table 3.3.3. Number of reads, total OTUs, richness (Chao1 estimates of species richness) or diversity (Shannon's index of diversity) indices expressed as average and standard deviation in the rootstock studied, for both bacteria and fungi analysis.

Aldea vineyard

Index	Bacteria				
	'110 R'	'140 Ru'	'1103 P'	'41 B'*	'161-49 C'
Reads	29769.8±13217.1	36838.8±20139.9	30159.1±10864.9	32881.7±11279.7	28512.2±10966.7
OTUs	611	577	658	683	650
Chao1	370.6±163.5	332.7±185.2	385.2±171.4	373.7±158.8	339.5±188.3
Shannon	4.0±0.7	4.1±0.5	4.2±0.2	4.2±0.3	4.3±0.2
Index	Fungi				
	'110 R'	'140 Ru'	'1103 P'	'41 B'	'161-49 C'
Reads	51139.7±29265.5	45952.4±28535.3	51436.2±28284.0	49659.7±16547.8	45420.4±29794.6
OTUs	246	259	250	241	224
Chao1	168.9±31.2	183.4±43.1	176.5±31.9	180.3±52.0	176.2±47.0
Shannon	3.4±0.1	3.3±0.1	3.4±0.2	3.3±0.3	3.1±0.4

Richness and diversity indices calculated at an even sequencing depth of 1,000 sequences/sample for both bacteria and fungi
*Sample DG126 was removed from the analysis due to the low number of sequence reads.

Olite vineyard

Index	Bacteria				
	'110 R'*	'140 Ru'	'1103 P'	'41 B'	'161-49 C'
Reads	37315.3±7632.7	4117.25±15790.9	39529±16251.5	34539.9±14862.6	58237.9±16430.6
OTUs	643	669	588	645	717
Chao1	400.5±66.0	413.5±103.8	427.6±102.9	373.6±123.9	400.5±99.8
Shannon	4.2±0.4	4.3±0.2	4.3±0.07	4.1±0.3	4.2±0.2
Index	Fungi				
	'110 R'	'140 Ru'	'1103 P'	'41 B'	'161-49 C'
Reads	53623.8±23695.8	57160.5±27945.5	49059.2±21623.0	40862.6±23264.0	61027.7±33281.9
OTUs	311	359	355	333	372
Chao1	189.2±64.2	223.7±33.6	213.7±51.3	202.1±33.1	224.4±43.9
Shannon	2.6±0.9	2.9±0.4	2.9±0.9	2.5±0.5	2.9±0.4

Richness and diversity indices calculated at an even sequencing depth of 1,000 sequences/sample for both bacteria and fungi
*Samples DG47 and DG48 were removed from the analysis due to the low number of sequence reads

Supplementary Table 3.3.4. Estimates of sample coverage and diversity indices at the genus level for bacteria and fungal profiles.

BACTERIA			
Sample ID	Good's coverage	Chao1 richness	Shannon diversity
DG01	1.000	275.8	3.85
DG02	0.995	265.9	3.73
DG03	0.991	315.4	3.75
DG04	0.999	220.3	3.70
DG05	0.994	175.0	4.33
DG06	0.988	155.1	4.21
DG07	1.000	180.2	4.03
DG08	0.981	210.1	3.85
DG09	0.933	161.1	4.04
DG10	0.921	250.4	4.10
DG11	0.995	321.7	4.25
DG12	0.973	335.0	4.11
DG13	0.933	245.0	3.99
DG14	0.933	245.1	3.88
DG15	0.999	219.9	3.95
DG16	0.987	240.9	3.80
DG17	0.931	276.8	4.68
DG18	0.910	299.9	3.75
DG19	0.923	310.0	3.85
DG20	0.962	325.4	4.15
DG21	0.987	260.5	4.30
DG22	0.987	305.4	4.25
DG23	0.892	216.6	4.23
DG24	0.900	351.8	4.11
DG25	0.903	391.8	4.04
DG26	0.987	255.0	3.95
DG27	0.911	200.8	3.89
DG28	0.987	428.9	3.99
DG29	0.927	335.6	4.01
DG30	0.987	350.4	3.97
DG31	0.994	402.5	3.97
DG32	0.991	265.7	3.99
DG33	0.991	270.2	4.23
DG34	1.000	221.4	4.26
DG35	1.000	170.6	4.29
DG36	0.995	170.7	3.95
DG37	0.981	145.7	4.33
DG38	0.988	143.6	3.98
DG39	0.981	158.8	3.91
DG40	0.991	230.4	3.80
DG41	0.909	399.1	4.08
DG42	1.000	225.7	4.09

DG43	1.000	403.6	4.09
DG44	0.909	175.9	4.12
DG45	0.999	224.5	3.58
DG46	0.987	214.4	3.42
DG49	0.994	221.1	3.65
DG50	0.895	215.9	3.66
DG51	0.958	380.9	3.77
DG52	0.910	235.9	4.10
DG53	0.895	270.6	3.41
DG54	0.899	390.7	3.64
DG55	0.895	399.8	3.76
DG56	0.920	448.1	3.80
DG57	0.962	461.6	2.49
DG58	0.958	365.7	3.67
DG59	0.900	375.7	4.35
DG60	0.910	378.6	4.15
DG61	0.999	410.1	4.33
DG62	0.855	419.3	4.21
DG63	0.995	448.4	4.15
DG66	0.981	465.6	4.21
DG67	0.988	465.2	4.09
DG68	0.981	410.4	4.13
DG71	0.995	503.0	3.96
DG72	0.995	499.8	3.95
DG73	0.960	470.4	4.01
DG76	0.995	518.5	4.10
DG77	0.855	475.6	3.94
DG78	1.000	460.7	4.12
DG81	0.960	475.6	4.21
DG82	0.994	500.1	4.15
DG83	1.000	501.2	4.17
DG86	0.884	426.6	4.68
DG87	0.910	446.7	3.76
DG88	0.899	455.8	3.85
DG91	0.910	478.0	3.86
DG92	0.899	480.9	4.02
DG93	0.884	449.9	4.05
DG96	0.905	515.6	4.25
DG97	0.967	535.9	4.30
DG98	0.899	462.1	4.29
DG101	0.905	501.0	4.14
DG102	0.884	508.0	4.21
DG103	0.905	445.5	4.20
DG106	0.904	456.8	4.19
DG107	0.884	425.9	4.15
DG108	0.899	485.9	4.23

DG111	0.994	470.2	3.54
DG112	0.981	465.7	4.24
DG113	0.889	480.8	4.51
DG116	0.995	501.8	4.52
DG117	0.949	525.0	4.35
DG118	1.000	485.1	4.35
DG121	0.949	519.4	4.12
DG122	0.973	490.5	4.27
DG123	0.973	485.7	4.28
DG127	0.995	405.2	4.35
DG128	0.991	406.3	3.98
DG131	0.889	410.0	4.11
DG132	0.889	445.0	4.13
DG133	0.995	443.7	4.01
DG136	0.911	448.9	4.11
DG137	0.857	540.1	4.24
DG138	0.908	525.3	4.31
DG141	0.912	250.2	4.15
DG142	0.900	251.2	4.13
DG143	0.900	445.6	4.11
DG146	0.851	510.5	4.10
DG147	0.896	509.8	4.21
DG148	0.884	505.0	3.76
DG151	0.911	475.0	4.21
DG152	0.904	549.5	4.20
DG153	0.893	234.4	4.11
DG156	0.884	421.3	4.19
DG157	0.896	446.7	4.19
DG158	0.911	447.5	4.13
Average	0.945	370.0	4.00

	FUNGI		
Sample ID	Good's coverage	Chao1 richness	Shannon diversity
DG01	0.966	103.0	3.41
DG02	0.991	130.5	3.43
DG03	0.991	130.6	3.50
DG04	0.872	155.7	3.52
DG05	0.952	156.0	3.30
DG06	0.930	175.1	3.54
DG07	0.891	140.1	3.57
DG08	0.988	138.3	3.55
DG09	0.875	139.0	3.55
DG10	0.991	170.3	3.83
DG11	0.873	140.5	3.33
DG12	0.872	110.4	3.34
DG13	0.829	119.4	3.51
DG14	0.973	172.0	3.50
DG15	0.853	175.1	3.54
DG16	0.875	165.2	2.55
DG17	0.855	180.3	1.80
DG18	0.971	196.2	2.68
DG19	0.891	163.7	2.76
DG20	0.992	90.8	1.80
DG21	0.829	241.0	3.60
DG22	0.856	185.6	3.25
DG23	0.905	190.7	3.33
DG24	0.875	200.3	3.34
DG25	0.971	204.1	3.50
DG26	0.849	170.1	3.41
DG27	0.849	175.8	3.34
DG28	0.853	175.6	3.32
DG29	0.875	147.5	3.30
DG30	0.905	223.6	3.29
DG31	0.904	215.8	3.04
DG32	0.890	216.0	3.10
DG33	0.930	165.3	3.12
DG34	0.991	112.4	3.43
DG35	0.973	128.5	3.11
DG36	0.944	141.1	3.13
DG37	0.930	225.5	3.18
DG38	0.952	165.5	3.15
DG39	0.872	225.8	3.04
DG40	0.855	148.0	3.02
DG41	0.930	173.0	3.14
DG42	0.973	164.3	3.21
DG43	0.905	160.2	3.22
DG44	0.966	135.1	3.24

DG45	0.872	138.5	3.14
DG46	0.837	90.5	2.75
DG47	0.973	155.8	2.87
DG48	0.857	161.0	3.10
DG49	0.952	162.5	3.05
DG50	0.857	165.6	3.03
DG51	0.835	225.5	3.09
DG52	0.849	205.4	2.87
DG53	0.880	185.9	2.91
DG54	0.991	224.0	3.36
DG55	0.890	203.6	3.34
DG56	0.830	140.7	2.99
DG57	0.829	148.6	3.13
DG58	0.900	181.5	3.12
DG59	0.831	182.6	3.50
DG60	0.904	159.1	3.84
DG61	0.836	208.3	3.34
DG62	0.844	206.4	3.59
DG63	0.849	223.5	3.56
DG66	0.904	244.9	3.54
DG67	0.896	189.0	3.51
DG68	0.966	190.7	3.44
DG71	0.896	250.7	3.44
DG72	0.857	211.8	3.82
DG73	0.880	188.7	3.58
DG76	0.844	177.6	3.59
DG77	0.893	207.3	3.33
DG78	0.971	185.4	3.44
DG81	0.833	250.5	3.58
DG82	0.930	220.0	2.65
DG83	0.845	205.0	2.51
DG86	0.952	225.7	2.65
DG87	0.911	210.1	2.23
DG88	0.893	208.4	2.51
DG91	0.860	203.4	3.65
DG92	0.860	192.1	3.62
DG93	0.930	191.9	3.10
DG96	0.829	235.0	3.12
DG97	0.893	240.4	3.70
DG98	0.911	202.5	2.90
DG101	0.899	191.5	3.55
DG102	0.896	226.0	3.43
DG103	0.880	212.2	3.13
DG106	0.902	203.4	3.15
DG107	0.956	221.3	3.12
DG108	0.938	175.1	3.26

DG111	0.944	183.6	3.31
DG112	0.934	240.9	2.92
DG113	0.845	215.3	3.34
DG116	0.893	186.6	3.33
DG117	0.881	210.0	2.93
DG118	0.872	245.5	3.42
DG121	0.833	220.4	3.32
DG122	0.911	231.5	3.24
DG123	0.905	227.6	3.25
DG126	0.846	214.2	3.21
DG127	0.890	223.3	3.24
DG128	0.841	201.1	3.26
DG131	0.841	223.1	2.24
DG132	0.842	220.6	3.35
DG133	0.833	218.7	3.41
DG136	0.896	180.6	3.45
DG137	0.966	220.5	3.23
DG138	0.967	221.9	3.24
DG141	0.923	254.9	3.25
DG142	0.994	230.7	3.25
DG143	0.900	215.0	3.03
DG146	0.847	240.1	2.99
DG147	0.848	222.0	3.11
DG148	0.988	191.2	3.23
DG151	0.841	223.3	3.14
DG152	0.993	248.9	3.15
DG153	0.960	190.3	3.25
DG156	0.950	222.8	3.23
DG157	0.860	234.8	3.11
DG158	0.983	241.9	3.24
Average	0.901	190.6	3.21

Supplementary Table 3.3.5. Experimental factors predicting α- and β-diversity of rhizosphere associated bacterial and fungal communities between vineyards.

	α-diversity		β-diversity
Bacteria	Shannon	Chao1	Bray Curtis
	$F_{1,115}$ = 0.27	$F_{1,115}$ = 1.42	R^2 = 0.19
	P = 0.7840	*P* = 0.1567	*P* = 0.1134
Fungi	$F_{1,117}$ = 2.15	$F_{1,117}$ = 1.37	R^2 = 0.69
	***P* = 0.033**	*P* = 0.1724	***P* < 0.001**

ANOVA, analysis of variance

All *P* values were corrected for multiple comparisons using the sequential Bonferroni correction. Significance was assessed using Type III ANOVA. Bold values indicate statistically significant results after correction for multiple comparisons, $P < 0.05$.

Supplementary Table 3.3.6. Similarity percentages (SIMPER) analysis determines the bacterial phyla **(A)** and genera **(B)**, and fungal phyla **(C)** and genera **(D)** contributions to the dissimilarity among rootstocks in the rhizosphere. In the upper part of the table the rootstock pairwise comparison of average dissimilarity percentage has been reported. In the lower part, the overall top one, two or three phyla/genera contributing to the pairwise dissimilarity were listed, reporting in parenthesis their relative contribution to the observed dissimilarity expressed as percentage.

(A) SIMPER analysis determined the bacterial phyla contributions in Aldea and Olite vineyards.

ALDEA	**'110 R'**	**'140 Ru'**	**'1103 P'**	**'41 B'**	**'161-49 C'**
'110 R'		9.2	16.1[#]	7.2	5.5[†]
'140 Ru'	Latescibacteria (21.3) Firmicutes (19.3) Planctomycetes (11.2)		8.2	10.1[#]	10.7[#]
'1103 P'	Acidobacteria (24.5) Firmicutes (15.6) Gemmatimonadetes (11.3)	Acidobacteria (31.5) Firmicutes (19.5) Planctomycetes (9.3)		11.0[#]	18.1[#]
'41 B'	Firmicutes (23.1) Nitrospirae (21.6) Actinobacteria (8.5)	Firmicutes (24.4) Acidobacteria (15.6) Latescibacteria (11.1)	Actinobacteria (19.4) Bacteroidetes (13.4) Planctomycetes (12.2)		8.4
'161-49 C'	Firmicutes (29.5) Acidobacteria (21.5) Nitrospirae (13.8)	Acidobacteria (32.4) Firmicutes (18.6) candidatedivisionWPS_1 (13.4)	Firmicutes (30.6) candidatedivisionWPS_1 (25.6) Verrucomicrobia (16.7)	Firmicutes (29.5) Actinobacteria (17.8) Acidobacteria (8.9)	
OLITE	**'110 R'**	**'140 Ru'**	**'1103 P'**	**'41 B'**	**'161-49 C'**
'110 R'		8.1	9.5	7.6	10.3[#]
'140 Ru'	No significant phyla		3.7	2.5	5.0
'1103 P'	Nitrospirae (14.4)	Nitrospirae (22.3) Acidobacteria (17.8)		5.1	1.8[†]
'41 B'	Parcubacteria (16.5) Acidobacteria (13.2)	Acidobacteria (25.0)	Acidobacteria (27.6)		4.8
'161-49 C'	Acidobacteria (21.6)	Acidobacteria (24.3) Nitrospirae (14.4) Ud_Bacteria (11.0)[¥]	Acidobacteria (25.4) Chlamydiae (10.1)	No significant phyla	

[#] Rootstock-pairs showing dissimilarity in phyla distribution higher than 10%.

[†] Rootstock-pair showing the lowest dissimilarity observed in phyla distribution.

¥ Ud: unidentified.

(B) SIMPER analysis determined the bacterial genera contributions in Aldea and Olite vineyards.

ALDEA	**'110 R'**	**'140 Ru'**	**'1103 P'**	**'41 B'**	**'161-49 C'**
'110 R'		5.5	14.8[#]	9.7	12.8[#]
'140 Ru'	*Corynebacterium* (24.3) Ud_Microbacteriaceae (23.5)[¥] Nocardioides (11.7)		9.9	6.3	6.1
'1103 P'	*Gp6* (26.0) Ud_Betaproteobacteria (25.6) *Gemmatimonas* (21.1)	*Bacillus* (26.6) *Gp4* (23.4) *Gp6* (14.2)		4.9	4.7[†]
'41 B'	*Ilumatobacter* (23.6) *Propionibacterium* (22.5) *Rubrobacter* (21.3)	*Bacillus* (25.7) *Nocardioides* (14.5) Ud_Bacillales (11.0)	*Mycobacterium* (25.1) Ud_Betaproteobacteria (22.0) *Ilumatobacter* (9.8)		5.1
'161-49 C'	Ud_Bacillales (25.6) *Bacillus* (12.3) Ud_Rhodocyclaceae (12.1)	*Bacillus* (24.5) *Gaiella* (15.5) *GP4* (8.8)	*Bacillus* (27.8) *Serratia* (18.4) *Pesudomonas* (14.0)	*Bacillus* (29.8) *Ilumatobacter* (22.6) *Propionibacterium* (20.5)	
OLITE	**'110 R'**	**'140 Ru'**	**'1103 P'**	**'41 B'**	**'161-49 C'**
'110 R'		3.6	3.8	2.5	2.1[†]
'140 Ru'	*Aquicella* (15.4) *Flavobacterium* (9.8) Ud_Bradyrhizobiaceae (9.7)[¥]		3.0	4.1	3.1
'1103 P'	*Aridibacter* (21.0) *Aquicella* (17.6) *Chitinophaga* (15.0)	*Aridibacter* (17.7) Ud_Cytophagales (12.3) *Vasilyevaea* (6.7)		6.8	3.5
'41 B'	No significant genera	*Gp6* (14.4) *Povalibacter* (14.1) Ud_Proteobacteria (10.9)	*Gp10* (17.0) *Gemmata* (11.4) *Flavobacterium* (11.2)		6.7
'161-49 C'	*Aridibacter* (16.7) Ud_Acidobacteria (16.1) *Gp5* (11.4)	*Gp6* (15.4) *Aridibacter* (15.2) *Blastocatella* (13.4)	*Gp10* (19.0) *Sphingomonas* (15.9) *Mycobacterium* (15.6)	Ud_Sphingomonadales (23.4) *Aeromicrobium* (21.0) *Povalibacter* (14.5)	

[#] Rootstock-pairs showing dissimilarity in phyla distribution higher than 10%.

[†] Rootstock-pair showing the lowest dissimilarity observed in phyla distribution.

¥ Ud: unidentified.

(C) SIMPER analysis determined the fungal phyla contributions in Aldea and Olite vineyards.

ALDEA	**'110 R'**	**'140 Ru'**	**'1103 P'**	**'41 B'**	**'161-49 C'**
'110 R'		8.2	16.2[#]	6.6[†]	25.3[#]
'140 Ru'	Ascomycota (29.3) Basidiomycota (24.1) Zygomycota (17.8)		9.8	22.7[#]	21.0[#]
'1103 P'	Ascomycota (6.3)	Basidiomycota (30.8) Zygomycota (9.2)		25.9[#]	12.8[#]
'41 B'	Basiodiomycota (22.9) Ascomycota (12.8) Glomeromycota (7.0)	Basidiomycota (22.4) Zygomycota (16.8) Ascomycota (8.4)	Glomeromycota (19.4) Basiodiomycota (12.4) Ascomycota (6.1)		29.7[#]
'161-49 C'	Ascomycota (27.5) Zygomycota (13.5)	Basidiomycota (35.5)	Glomeromycota (22.1)	Glomeromycota (19.5) Zygomycota (13.5) Ascomycota (13.5)	
OLITE	**'110 R'**	**'140 Ru'**	**'1103 P'**	**'41 B'**	**'161-49 C'**
'110 R'		2.2	1.9	3.2	1.5[†]
'140 Ru'	Basidiomycota (8.1)		1.7	2.9	3.3
'1103 P'	No significant phyla	No significant phyla		5.6	8.0
'41 B'	No significant phyla	No significant phyla	No significant phyla		9.2
'161-49 C'	Basidiomycota (8.8)	No significant phyla	No significant phyla	Basidiomycota (5.5)	

[#] Rootstock-pairs showing dissimilarity in phyla distribution higher than 10%.

[†] Rootstock-pair showing the lowest dissimilarity observed in phyla distribution.

(D) SIMPER analysis determined the fungal genera contributions in Aldea and Olite vineyards.

ALDEA	**'110 R'**	**'140 Ru'**	**'1103 P'**	**'41 B'**	**'161-49 C'**
'110 R'		7.2	7.1	7.8	12.7[#]
'140 Ru'	Ud_Auriculariales (19.0) [¥] *Geopyxis* (15.5) *Psathyrella* (15.1)		6.5	6.6	16.1[#]
'1103 P'	Ud_ Pleosporales (17.5) *Sporormiella* (15.9) *Articulospora* (15.3)	*Clonostachys* (15.5) *Lecanicillium* (14.6) *Scutellinia* (10.3)		6.3[†]	12.9[#]
'41 B'	Ud_Giomeraceae (13.1) *Gongronella* (13.0) *Geopyxis* (13.0)	*Clonostachys* (14.4) *Lecanicillium* (12.5) *Psathyrella* (9.1)	*Clonostachys* (8.1) Ud_Glomeraceae (8.1) Ud_Nectriaceae (8.0)		17.5[#]
'161-49 C'	*Geopyxis* (16.5) Ud_Glomeraceae (16.1) *Psathyrella* (15.6)	*Clonostachys* (9.4) *Cryptococcus* (9.1) *Davidiella* (9.0)	*Clonostachys* (9.2) *Cryptococcus* (9.2) *Lecanicillium* (9.2)	*Cryptococcus* (8.8) *Davidiella* (8.7) *Lecanicillium* (8.7)	
OLITE	**'110 R'**	**'140 Ru'**	**'1103 P'**	**'41 B'**	**'161-49 C'**
'110 R'		4.1	8.2	8.5	8.7
'140 Ru'	*Gymnopus* (5.6) *Spizellomyces* (4.6) *Pseudogymnoascus* (4.3)		4.2	2.7[†]	7.1
'1103 P'	*Calcarisporiella* (8.7)	*Geopyxis* (14.3) *Calcarisporiella* (11.7) *Scytalidium* (10.5)		12.8[#]	8.8
'41 B'	*Clavaria* (11.2) *Scytalidium* (6.7) *Penicillium* (4.5)	*Geopyxis* (17.5) *Scytalidium* (14.0) *Gymnopus* (13.8)	Ud_Basiodiomycota (6.1) Ud_Ceratobasidiaceae (5.5) *Calcarisporiella* (4.5)		4.1
'161-49 C'	Ud_Auriculariales (11.2) *Spizellomyeces* (10.3) *Geopyxis* (6.6)	*Scutellinia* (13.4) Ud_Auriculariales (13.2) *Gymnopus* (5.7)	*Scutellinia* (10.1) Ud_Basidiomycota (7.6) *Gymnoascus* (6.7)	Ud_Auriculariales (12.5) *Gymnoascus* (7.6) *Scytalidium* (5.9)	

[#] Rootstock-pairs showing dissimilarity in phyla distribution higher than 10%.

[†] Rootstock-pair showing the lowest dissimilarity observed in phyla distribution.

¥ Ud: unidentified.

Supplementary Table 3.3.7. Bacterial OTUs that were unique in each of the sample type.

	Rootstock				
Vineyard	'110 R'	'140 Ru'	'1103 P'	'41 B'	'161-49 C'
Aldea					
	Gp21	*Mobiluncus*	*Terriglobus*	*Pilimelia*	*Turicella*
	Pyrinomonas	*Planosporangium*	ud-Dermacoccaceae	*Gordonia*	ud-Demequinaceae
	Phytomonospora	*Plantactinospora*	*Pseudoclavibacter*	*Xylanibacterium*	*Barrientosiimonas*
	Crossiella	*Actinokineospora*	*Enteractinococcus*	*Micropruina*	*Dietzia*
	Atopobium	*Prevotella*	*Zhihengliuella*	ud-Prolixibacteraceae	*Amnibacterium*
	Vibrionimonas	*Rubrivirga*	*Rugosimonospora*	*Capnocytophaga*	*Krasilnikovia*
	Thermosporothrix	ud-Alicyclobacillaceae	*Tessaracoccus*	*Empedobacter*	*Brooklawnia*
	Elusimicrobium	*Thermicanus*	*Thermocatellispora*	*Epilithonimonas*	*Thermobispora*
	Halobacillus	*Abiotrophia*	*Bacteroides*	*Kyrpidia*	*Imperialibacter*
	Marininema	*Acetatifactor*	*Paludibacter*	*Geomicrobium*	*Filimonas*
	Hespellia	*ClostridiumXlVb*	*Tannerella*	*Piscibacillus*	*Falsibacillus*
	Peptoniphilus	ud-Lentisphaerae	*Bhargavaea*	*Gemella*	*Guggenheimella*
	Faecalibacterium	*Starkeya*	*Oxobacter*	*Jeotgalicoccus*	*Anaerovorax*
	Leptotrichia	Pandoraea	*Eisenbergiella*	*Desemzia*	*Lachnoanaerobaculum*
	Albidovulum	*Tepidiphilus*	*Pelotomaculum*	*Dolosigranulum*	*Stomatobaculum*
	Sandarakinorhabdus	*Simonsiella*	*ClostridiumIV*	*Streptococcus*	*Halobacteroides*
	Burkholderia	ud-Desulfobacteraceae	*Oscillibacter*	*Anaerobacter*	ud-Halobacteroidaceae
	Kingella	*Alishewanella*	*Anoxybacter*	*Natronincola*	*Ignavibacterium*
	Rivicola	ud-Chromatiaceae	*Megasphaera*	*Tepidanaerobacter*	*Pelagibacterium*
	Desulfohalobium	*Vulcaniibacterium*	*Camelimonas*	*Dehalobacter*	*Hansschlegelia*
	ud-Desulfohalobiaceae	*Leptonema*	*Prosthecomicrobium*	ud-Peptococcaceae2	*Aquamicrobium*
	Corallococcus	*Leptospira*	*Rhodomicrobium*	*Pseudobacteroides*	*Phreatobacter*
	Leclercia	*Spirochaeta*	*Methyloligella*	*Dialister*	*Defluviimonas*
	Marinomonas	ud-Spirochaetaceae	*Stella*	*Fusobacterium*	*Falsirhodobacter*

ud-Oceanospirillales	*Fervidobacterium*	*Nitrospirillum*	*Cereibacter*	*Rhodobacter*
Alkanindiges		*Anaplasma*	*Elioraea*	*Acidisoma*
Moraxella		*Advenella*	*Oceanibaculum*	*Defluviicoccus*
Aspromonas		*Malikia*	*Tistlia*	*Pigmentiphaga*
Acholeplasma		*Undibacterium*	*Limnobacter*	*Sulfurisoma*
Subdivision5_genera_incertae_sedis	ud-Desulfovibrionaceae	*Thiobacter*	*Halobacteriovorax*	
		ud-Desulfovibrionales	*Chitinibacter*	*Desulfocapsa*
		Ignatzschineria	*Snodgrassella*	*Desulfuromonas*
		Cloacibacillus	ud-Nitrosomonadaceae	ud-Syntrophaceae
		ud-Synergistaceae	*Azonexus*	*Campylobacter*
			Georgfuchsia	*Raoultella*
			Sulfuricurvum	ud-Methylococcaceae
			Sulfurimonas	*Luteibacter*
			Aggregatibacter	*Limisphaera*
			ud-Pasteurellaceae	
			ud-Pseudomonadales	

Olite

Actinospica	*Flaviflexus*	ud-Cryptosporangiaceae	*Citricoccus*	*Pyrinomonas*
Stackebrandtia	*Frigoribacterium*	*Rubricoccus*	*Zhihengliuella*	*Pseudoclavibacter*
ud-Glycomycetaceae	*Enteractinococcus*	ud-Paenibacillaceae2	*Capnocytophaga*	*Polymorphospora*
Amnibacterium	*Micropruina*	ud-Aerococcaceae	*Asinibacterium*	*Rugosimonospora*
Plantibacter	*Microbispora*	*Flavonifractor*	*Solitalea*	*Brooklawnia*
ud-Bifidobacteriaceae	*Atopobium*	*Pseudoflavonifractor*	*Thermicanus*	*Odoribacter*
ud-Deinococcales	*Alicyclobacillus*	*Vallitalea*	*Saccharibacillus*	*Algoriphagus*
ud-Enterococcaceae	*Aeribacillus*	*Undibacterium*	*Chungangia*	*Nibrella*
Lactococcus	*Geomicrobium*	*Psychrobacter*	*Alloiococcus*	*Imperialibacter*
Anaerosolibacter	ud-Bacillales_incertae_sedis	*Spirochaeta*	*Anaerobacter*	ud-Flammeovirgaceae

Sporanaerobacter	*Aerococcus*	Subdivision5_genera_incertae_sedis	ud-Clostridiaceae2	*Filimonas*
Garciella	*Fonticella*		*Stomatobaculum*	*Heliimonas*
Desulfitispora	*Tepidanaerobacter*		*Peptoniphilus*	*Arcticibacter*
ud-Peptococcaceae1	*Guggenheimella*		*Butyricicoccus*	ud-Chloroflexaceae
Ralstonia	*Acetatifactor*		ud-Erysipelotrichaceae	ud-Deinococcaceae
Hydrogenophilus	*Blautia*		*Ignavibacterium*	*Elusimicrobium*
Arcobacter	*Sporotomaculum*		*Oligosphaera*	*Candidatus Endomicrobium*
Marinobacter	ud-Peptococcaceae2	ud-Hyphomonadaceae	*Kyrpidia*	
Shewanella	*Gemmiger*		*Pandoraea*	*Halobacillus*
Kosakonia	*Hydrogenoanaerobacterium*	*Massilia*	*Marininema*	
Aggregatibacter	*Oscillibacter*		*Hafnia*	*Abiotrophia*
Leptonema	*Syntrophomonas*		*Klebsiella*	ud-Carnobacteriaceae
	Gluconobacter		ud-Oceanospirillales	*Oxobacter*
	Castellaniella		*Xiphinematobacter*	ud-Clostridiaceae3
	Sphaerotilus			*Anaerovorax*
	Azoarcus			*Anaerobacterium*
	Dechloromonas			*Anoxybacter*
	Celerinatantimonas			*Selenomonas*
	Methylobacter			*Camelimonas*
	Aspromonas			*Rhodomicrobium*
	SR1_genera_incertae_sedis		*Aquamicrobium*	
				Alsobacter
				Phreatobacter
				Methyloligella
				Falsirhodobacter
				Rhodobacter
				Acidisoma

		Magnetospirillum
		Orientia
		Sandarakinorhabdus
		Advenella
		Inhella
		Malikia
		Roseateles
		Paraherbaspirillum
		Thiobacillus
		ud-Neisseriaceae
		Halobacteriovorax
		Corallococcus
		ud-Syntrophaceae
		Campylobacter
		ud-Methylococcaceae
		Leptospira
		Anaeroplasma
		Limisphaera

Supplementary Table 3.3.8. Fungal OTUs that were unique in each of the simple type.

	Rootstock				
Vineyard	'110 R'	'140 Ru'	'1103 P'	'41 B'	'161-49 C'
Aldea					
	Astraeus	*Bipolaris*	*Amaurodon*	*Camarosporium*	*Ascosphaera*
	Debaryomyces	*Canalisporium*	*Amphinema*	Chaetomiaceae_ud	Capnodiales_ud
	Glomerales_ud	*Colletotrichum*	*Cadophora*	*Cyathus*	*Cladophialophora*
	Hymenoscyphus	*Crepidotus*	*Geotrichum*	*Dothiorella*	*Devriesia*
	Incertae_sedis_12_ud	*Cytospora*	Incertae_sedis_26_ud	*Haematonectria*	Morchellaceae_ud
	Lecythophora	*Guehomyces*	*Inocybe*	*Hebeloma*	*Mycenastrum*
	Leotiomycetes_ud	*Gymnopus*	*Phanerochaete*	*Lyophyllum*	Teratosphaeriaceae_ud
	Lycoperdaceae_ud	*Hypocrea*	*Pholiota*	*Oidiodendron*	Hyaloscyphaceae_ud
	Neophaeosphaeria	*Lacrymaria*	*Pilaira*	*Ophiosphaerella*	Incertae_sedis_25
	Pyrenochaeta	*Neofusicoccum*	*Pilidium*	Phaeosphaeriaceae_ud	*Filobasidiales*
	Rinodina	*Pisolithus*	*Podospora*	*Scleroderma*	
	Sarcinomyces	Polyporales_ud	Pyronemataceae_ud	*Tomentella*	
	Sphaeropsis	*Pringsheimia*	*Sarocladium*	Tricholomataceae_ud	
	Teloschistaceae_ud	*Thecaphora*	*Stagonospora*	*Typhula*	
	Tulostoma	Helotiaceae_ud	*Ascobolaceae*	Mycosphaerellaceae_ud	
	Teloschistaceae_ud	*Strophariaceae*	*Diatrypaceae*	Verrucariaceae_ud	
	Ascomycota_ud	*Filobasidiaceae*	*Pleurotaceae*	*Pezizaceae*	
	Volvariella	Incertae_sedis_12	*Ambisporaceae*	*Verrucaria*	
		Pezizales	*Cantharellales*		
		Verticillium	*Wallemia*		
		Xenasmatella	*Zygosaccharomyces*		
		Xylariaceae_ud			
Olite					
	Auricularia	*Alnicola*	*Arthroascus*	Ambisporaceae_ud	*Ascochyta*

Corticiales_ud
Dissoconium
Eutypa
Geopora
Glarea
Leotiomycetes_ud
Leveillula
Lycoperdaceae_ud
Paurocotylis
Phaeocytostroma
Phialocephala
Septoglomus
Stagonospora
Thanatephorus
Thelonectria
Tulostoma
Orbiliomycetes

Ampelomyces
Annulohypoxylon
Athelia
Calocybe
Cystolepiota
Eupenicillium
Fibroporia
Gymnoascaceae_ud
Heterobasidion
Neostagonospora
Plectania
Pluteus
Polyscytalum
Pringsheimia
Pseudovalsaria
Pyrenochaetopsis
Sphaerulina
Tapinella
Diatrypaceae
Marasmiaceae
Incertae_sedis_28
Archaeorhizomycetes
Cephalothecaceae
Diversisporaceae
Valsaria

Atheliaceae_ud
Battarrea
Bulleromyces
Ceratobasidium
Clavicipitaceae_ud
Crocicreas
Didymosphaeria
Entyloma
Gloeophyllum
Hemimycena
Lachnum
Lalaria
Millerozyma
Monacrosporium
Pilaira
Psathyrellaceae_ud
Saccharomycetaceae_ud
Sebacinales_ud
Sphaeropsis
Stropharia
Blumeria
Helotiaceae_ud
Incertae_sedis_2_ud
Incertae_sedis_26
Hymenochaetales
Onygenaceae
Sordariaceae
Volutella

Amphinema
Backusella
Caloplaca
Crepidotus
Cristinia
Eucasphaeria
Gliomastix
Lachnella
Mycena
Neoerysiphe
Neofusicoccum
Parasola
Parasola
Rosellinia
Simplicillium
Teloschistaceae_ud
Tylospora
Microbotryomycetes
Ustilaginales
Verticillium

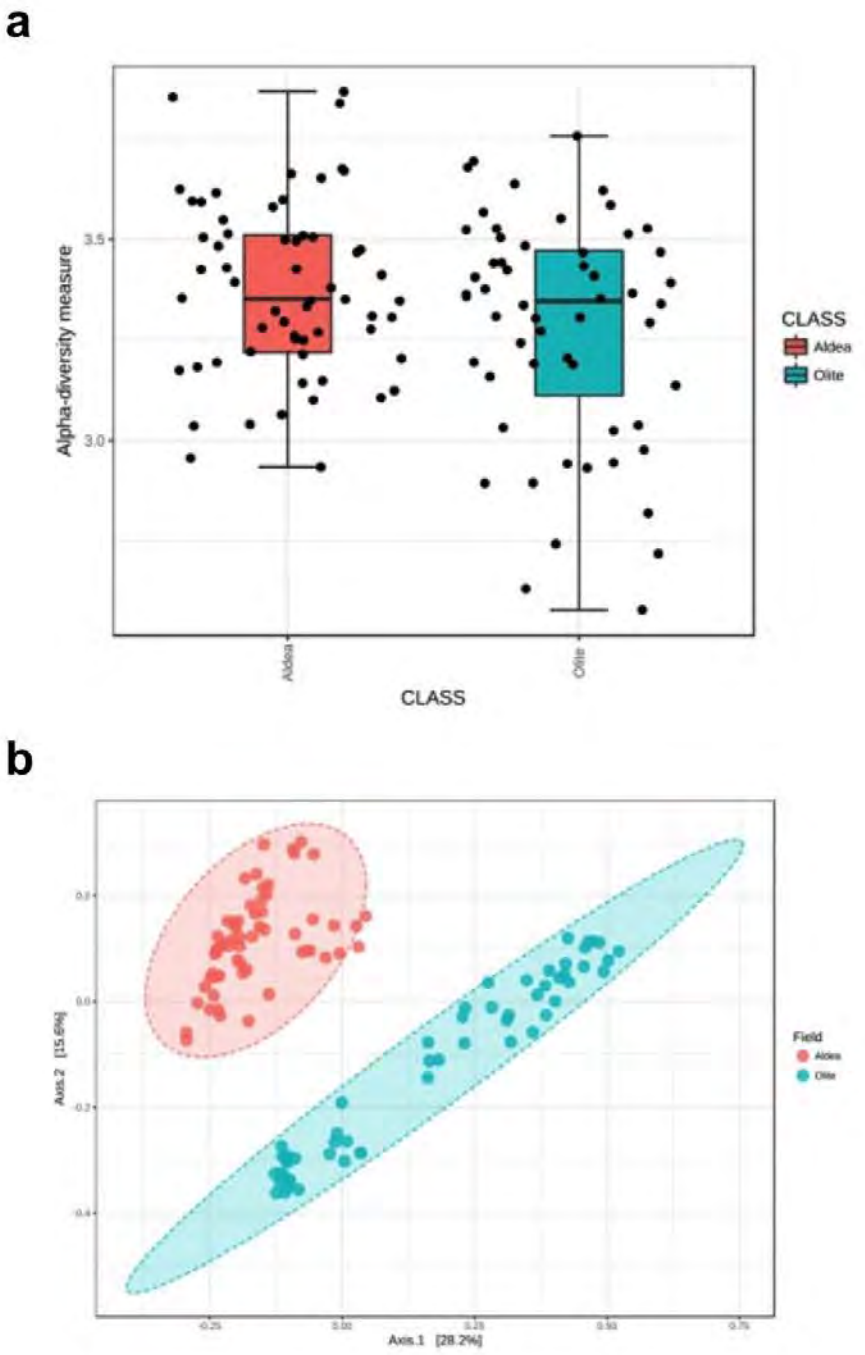
Polyporaceae_ud

Byssomerulius
Cephalotheca
Cistella
Claroideoglomus
Cochliobolus
Exobasidiomycetes_ud
Gnomonia
Hypoxylon
Incertae_sedis_12_ud
Incertae_sedis_2_ud
Leucopaxillus
Lichtheimia
Lophodermium
Monographella
Montagnulaceae_ud
Mycenastrum
Mycosphaerellaceae_ud
Myriodontium
Nemania
Oudemansiella
Phlebia
Plagiostoma

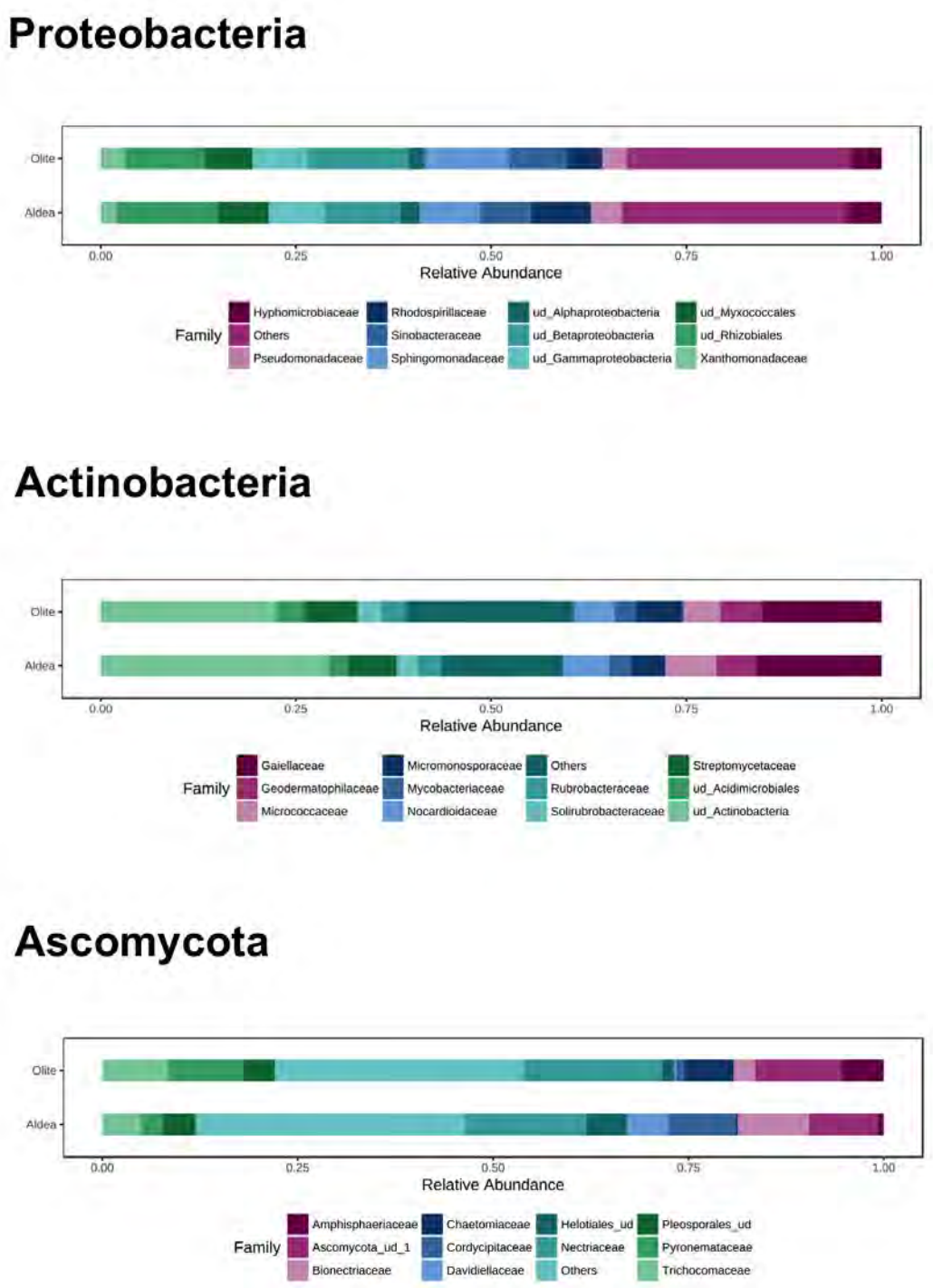
Pseudeurotiaceae_ud
Rhizopogon
Sporisorium
Trametes
Truncatella

	Williopsis	Tubeufiaceae_ud
		Valsaceae
		Incertae_sedis_25
		Ustilaginaceae
		Entylomatales
		Vuilleminia
		Wallemia
		Xylodon

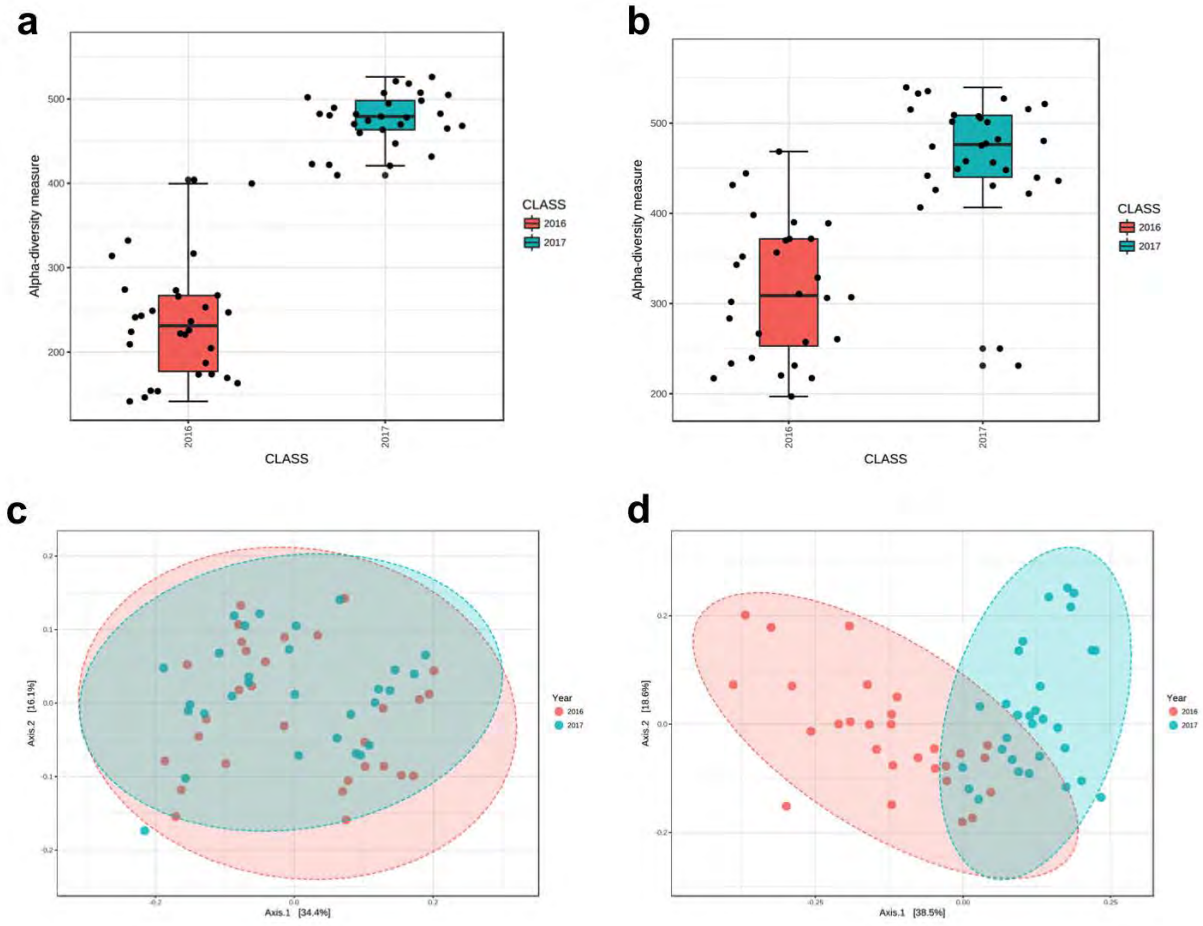
Supplementary Figures



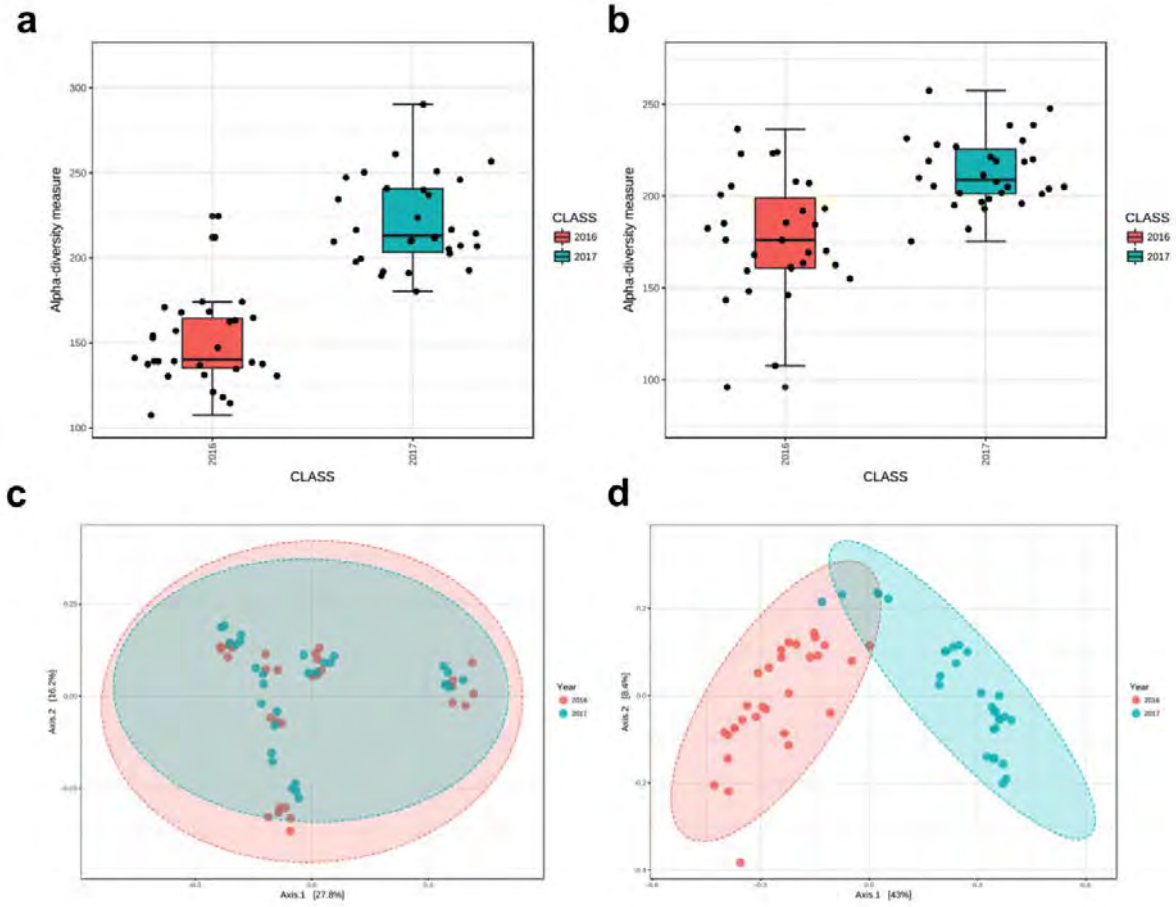
Supplementary Figure 3.3.1. *Boxplot illustrating the differences in Shannon diversity measures of the fungal communities between vineyards* ***(a)****. Principal Coordinate Analysis (PCoA) based on Bray Curtis dissimilarity metrics, showing the distance in the fungal communities between vineyards* ***(b)****.*



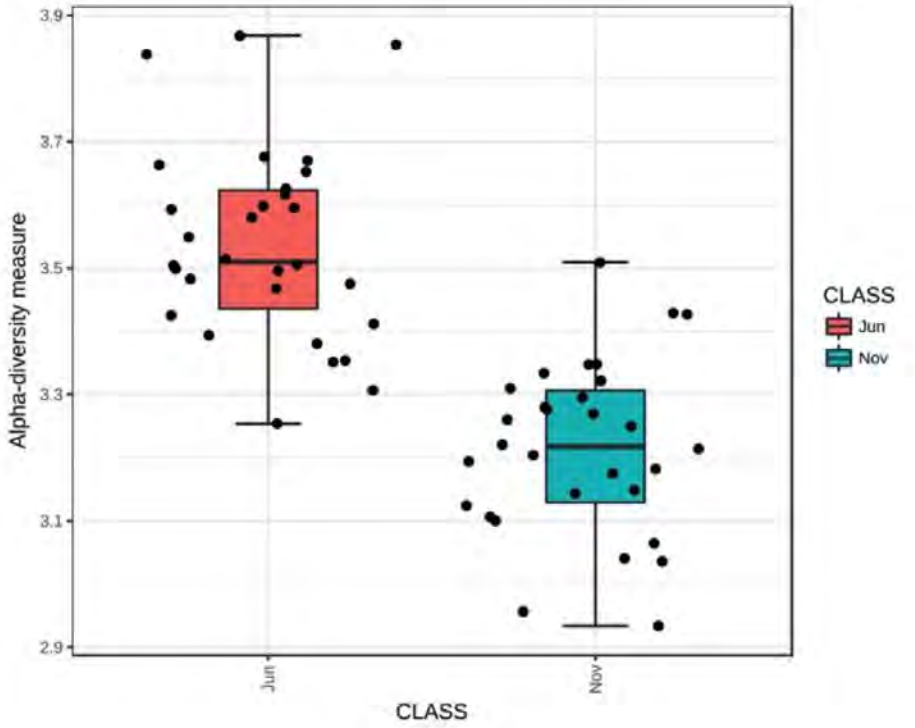
Supplementary Figure 3.3.2. *Relative abundance of the most abundant families within the phyla Actinobacteria, Proteobacteria and Ascomycota in both vineyards representing OTUs showing more than 1% relative abundance of all reads and present in at least 2/3 of replicates. Families representing less than 1% of the total reads are grouped in 'Others'.*



Supplementary Figure 3.3.3. *Boxplot illustrating the differences in Chao1 richness measures of the bacterial communities between years of sampling in the grapevine rootstocks in Aldea* ***(a)*** *and Olite* ***(b)*** *vineyards. Principal Coordinate Analysis (PCoA) based on Bray Curtis dissimilarity metrics, showing the distance in the bacterial communities among grapevine rootstocks in Aldea* ***(c)*** *and Olite* ***(d)*** *vineyards.*



Supplementary Figure 3.3.4. *Boxplot illustrating the differences in Chao1 richness measures of the fungal communities between years of sampling in the grapevine rootstocks in Aldea* ***(a)*** *and Olite* ***(b)*** *vineyards. Principal Coordinate Analysis (PCaA) based on Bray Curtis dissimilarity metrics, showing the distance in the fungal communities among grapevine rootstocks in Aldea* ***(c)*** *and Olite* ***(d)*** *vineyards.*



Supplementary Figure 3.3.5. *Boxplot illustrating the differences in Shannon diversity measures of the fungal communities between sampling dates in the grapevine rootstocks in Aldea vineyard.*

CHAPTER 4.

CONTROL

Pest Management Science 74:2864-2873. 2018.

Effect of white mustard cover crop residue, soil chemical fumigation and *Trichoderma* spp. root treatment on black-foot disease control in grapevine

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Abstract

Background Black-foot disease is one of the main soilborne fungal diseases affecting grapevine production worldwide. Two field experiments were established to evaluate the effect of white mustard cover crop residue amendment and chemical fumigation with propamocarb + fosetyl-Al combined with *Trichoderma* spp. root treatment on the viability of black-foot inoculum in soil and fungal infection in grafted plants and grapevine seedlings used as bait plants.

Results A total of 876 black-foot pathogens isolates were collected from grafted plants and grapevine seedlings used as bait plants in both fields. White mustard biofumigation reduced inoculum of *Dactylonectria torresensis* and the incidence and severity of black-foot of grapevine, but no added benefit was obtained when biofumigation was used with *Trichoderma* spp. root treatments. The effect of white mustard residues and chemical fumigation on populations of *D. torresensis* propagules in soil was inconsistent, possibly due to varying pretreatment inoculum levels.

Conclusion Biofumigation with white mustard plants had potential for improving control of black-foot disease in grapevines. This control strategy can reduce soil inoculum levels and protect young plants from infection, providing grape growers and nursery propagators with more tools for developing integrated and sustainable control systems.

Keywords*:* biocontrol*,* biofumigation, *Brassica* residues, fosetyl-Al, propamocarb, *Vitis vinifera* L.

CHAPTER 5.

GENERAL DISCUSSION

During the last few years, extensive research into black-foot disease (BFD) of grapevine has been carried out in Spain with significant advances on its etiology, epidemiology and control (Aroca et al. 2006; Alaniz et al. 2007, 2009, 2010, 2011a, b; Gramaje et al. 2010a; Agustí-Brisach et al. 2011a, 2012, 2013a, b, 2014, 2019; Tolosa-Almendros 2016; Martínez-Diz et al. 2018; Pintos et al. 2018). BFD affects particularly plant nursery stock and young vineyards (Halleen et al. 2006; Agustí-Brisach and Armengol 2012), and has been associated, together with Petri disease, to the young vine decline syndrome in almost all grapevine growing regions worldwide (Gramaje and Armengol 2011).

There has been a continuous changing of the taxonomic reclassification within the *Cylindrocarpon*-like asexual morphs fungi, with the identification and description of many new species from different genera associated with BFD. This fact, together with the still lack of complete information about the ecology and epidemiology of the disease, and the unavailable curative methods for its control, has increased the complexity of this pathosystem.

Different aspects of BFD have been studied in this thesis, but the overall objective was to obtain information about the biology and ecology of the disease, as well as to evaluate different control strategies, in order to improve the disease management. This concluding chapter discusses the results obtained in the previous chapters of the thesis and addresses areas of potential future research that have arisen based on the data generated in this study.

5.1 Biology and ecology

Up to 27 species in the genera *Campylocarpon*, *Cylindrocladiella*, *Dactylonectria*, *Ilyonectria*, *Neonectria*, *Pleiocarpon* and *Thelonectria* have been reported to cause BFD (Gramaje et al. 2018; Lawrence et al. 2019; Aigoun-Mouhous et al. 2019). In this thesis, 11 known species belonging to the genera *Dactylonectria*, *Ilyonectria*, *Neonectria* and *Thelonectria* have been identified from asymptomatic nursery stock, with *I. pseudodestructans* and *N. quercicola* reported for the first time in Spain. In addition, two novel species have been characterized, *Dactylonectria riojana* and *Ilyonectria vivaria*, bringing the total number of BFD pathogens isolated from grapevines in Spain to 17. Micromorphological characters, such as conidiophores morphology, macroconidia and

microconidia size and shape, and cultural characters were used to describe new fungal species. However, several studies indicated that such characters alone are not sufficient to differenciate among BFD fungi (Cabral et al. 2012a, c; Lombard et a. 2014, Lawrence et al. 2019), therefore being necessary the use of DNA sequences to get a confident species diagnosis when working with *Cylindrocarpon*-like asexual morphs. Traditionally, while working with ascomycetes, the ITS region, or partial sequences of the *tef1* and *tub2* genes have been used in molecular phylogenetic analysis, either as a single-gene or as a concatenation. Lawrence et al. (2019) studied the accurate species identification of the traditional gene set compared to the use of the *his3* locus, as suggested firstly by Cabral et al. (2012a, c), and confirmed that the use of multigene analysis including the *his3*, *tef1* and *tub2* genes increased the accuracy of *Cylindrocarpon*-like fungal species identification. In Chapter 3.1, *his3*, ITS, *tef1*, and *tub2* were used to confirm the identity of *D. riojana* and *I. vivaria*.

Dactylonectria torresensis was the most common species isolated from grapevines, which agrees with previous studies conducted in Algeria (Aigoun-Mouhous et al. 2019), Italy (Carlucci et al. 2017), Portugal (Reis et al. 2013) and Spain (Tolosa-Almendros et al. 2016). Recent findings also indicated that agricultural crops such as *Actinidia chinensis* (Erper et al. 2013), *Eriobotrya japonica* (Agustí-Brisach et al. 2016), *Malus domestica* (Manici et al. 2018), *Olea europea* (Nigro et al. 2019), or forest trees (Mora-Sala et al. 2018) represent other common niches for *D. torresensis*. However, even if *D. torresensis* has a high prevalence in some countries and hosts, it is not the most common fungal species associated with BFD everywhere. For example, *D. macrodidyma* is one of the most prevalent species in South Africa (Langenhoven et al. 2018), New Zealand (Mundy 2015) and Canada (Úrbez-Torres et al. 2014). *I. liriodendri* is also very frequently isolated in New Zealand (Mundy 2015) and Canada (Úrbez-Torres et al. 2014).

Dispersal of BFD pathogens through asymptomatic planting material might have great impact in other regions where the disease is not present or the fungal diversity associated with BFD is still low. This finding also highlights the urgent need to implement early, accurate and specific *in planta* detection and quantification of these fungi to prevent the spread of BFD in grapevine propagation material. The endophyte definition has changed many times over the last years. Recently, Hardoim et al. (2015) defined endophyte based on the colonization niche but not on the function. These authors

therefore considered the existence of both pathogenic and non-pathogenic endophytes (Hardoim et al. 2015). In this study, 13 fungal species associated with BFD colonized the root vascular tissue endophytically without causing any type of external or internal symptom in plants. However, only 5 out of the 13 species showed high degree of virulence. The most virulent species were *D. novozelandica*, *D. alcacerensis*, *D. macrodidyma* and *I. vivaria*, which were isolated, respectively, in only 3.22%, 2.94%, 3.85% and 0.21% of the plants. These results agree with those obtained by Cabral et al. (2012b), who found that minor species such as *I. lusitanica*, *D. estremocensis* and *I. europaea* were more virulent to grapevine than *D. macrodidyma* and *I. liriodendri*, species previously accepted as the main causal agents of BFD. Given these findings, the future prospects on BFD needs to investigate (i) how *Cylindrocarpon*-like asexual morphs colonize the grapevine endorhizosphere and establish themselves inside, and (ii) what triggers latents BFD fungi to transition from a non-pathogenic to pathogenic endophyte, and cause disease symptoms in grapevine.

The knowledge about the epidemiology of *Cylindrocarpon*-like asexual morphs in grapevine is inferred from studies conducted in other hosts (Booth 1966; Brayford 1993). These fungi are able to develop chlamydospores, resistant structures that allow them to survive for a long time in the soil. In recent years, the development of new molecular tools has been crucial for the correct detection and identification of *Cylindrocarpon*-like asexual morphs from soil samples (Cardoso et al. 2013; Agustí-Brisach et al. 2014). However, DNA-based methods are unable to distinguish between viable or dead organisms with intact genetic material (England et al. 1997; Demanèche et al. 2001). In this thesis, a semi-selective medium has therefore been developed to identify the active and viable *Cylindrocarpon*-like species directly from soil (Chapter 3.2). Glucose-Faba Bean Rose Bengal Agar (GFBRBA) medium was adapted from Hunter et al. (1980) and selected as the best option among three different media after several tests, including evaluation of mycelial growth and efficacy of inoculum recovery. BFD pathogens, mainly *D. torresensis*, were isolated from soils in mature and young vineyards, nursery fields with grapevine and in rotation. In a previous study, Cardoso et al. (2013) detected *D. macrodidyma* and *D. torresensis* from 5 out of 12 soils in Portugal by planting soil samples on PDA supplemented with chloramphenicol. The fact that viable inoculum of BFD was found in nursery fields during the standard crop rotation

procedure suggests that this approach is unsuccessful for the management of BFD, as others studies had previously reported in Portugal (Rego et al. 2009; Cardoso et al. 2013) and South Africa (Halleen et al. 2003; Langenhoven et al. 2018).

The development of the GFBRA medium has improved the knowledge on the ecology of BFD fungi in soil, and has allowed researchers (i) to characterize the genetic structure of *D. torresensis* populations in soil, and compare them with those collected from grapevine roots and asymptomatic secondary hosts such as weeds (Berlanas et al. 2019), and (ii) to compare the genomes of *D. torresensis* isolated from soil, asymptomatic grapevine roots and weeds (Gramaje et al. 2019).

The effect of the physicochemical properties of the studied soils on BFD pathogen populations was also evaluated. High amount of calcium carbonate in soil favoured the presence of BFD fungi. Carbon availability as well as others nutrients are affected by plants growth and microbial communities, which at the same time are influenced by that nutrient availability (Kaiser et al. 2010). Moreover, several studies suggest that soil physicochemical properties and moisture content can affect the grapevine rhizosphere microbiome (Fernández-Calviño et al. 2010; Corneo et al. 2014; Burns et al. 2015; Zarraonaindia et al. 2015; Holland et al. 2016). Therefore, population structure of specific soil-borne pathogens can also be altered by soil physicochemical properties. Further studies are needed to understand how soil properties affect both grapevine and BFD pathogen health in order to make effective management decisions.

Soil microbiome is not only affected by the soil physicochemical properties, but also by the plants. In fact, the plants, and their genotype, have a key role in the selection of the microbiome that inhabits their rhizosphere and root compartments (Aira et al. 2010; Berendsen et al. 2012; Bazghaleh et al. 2015). Therefore, knowledge on how the microbiome is affected by the plants can help in the management of soilborne pathogens, such as BFD fungi. In grapevine, microbes associated with the plant meanwhile it is growing and producing may influence the organoleptic properties of the wine (Zarraonaindia et al. 2015). On the other hand, grapevine microbiome also affects plant health, stress protection, productivity and plant development (Zarraonaindia and Gilbert 2015). Moreover, grapevine wine production is linked to the *Terroir*, and the knowledge of the biogeography patterns and spatio-temporal dynamics of the grapevine associated microorganisms is fundamental to recreate that characteristic

(Zarraonaindia and Gilbert 2015; Marasco et al. 2018). Several studies also suggests that microbiome components can have an important role as inhibitors of phytopathogenic bacteria and fungi (Haas and Keel 2003; Vacheron et al. 2013; Yu and Hochholdinger 2018). An effect of rhizosphere microbiome reducing pathogen growth has been found in grapevines (Zarraonaindia et al. 2015).

Molecular approaches based on high-throughput sequencing technology (NGS) have progressively replaced molecular markers to characterize microbial communities in nature, including soil samples. They allow the detection and identification of more microorganisms, including species that cannot be obtained in culture (Amann et al. 1995). The new advance in NGS have increased both the resolution and scope of fungal community analyses and have revealed a high diverse and complex microbiota of grapevine soils (Zarraonaindia et al. 2015; Holland et al. 2016; Marasco et al. 2018; Martínez-Diz et al. 2019b). NGS has allowed recovering data not only about the genera linked to pathogenic species, but also about genera associated to biocontrol activities, such as *Trichoderma* spp. or *Bacillus* spp. (dos Santos et al. 2016).

The fungal and bacterial microbiome was deeply studied in the rhizosphere of five rootstocks of young and mature grapevines in Chapter 3.3. A comparison of the relative abundances of sequence reads by NGS and DNA amount of BFD pathogens by qPCR was performed for the first time. The results showed that in the case of the bacterial communities the most common phylum were Proteobacteria and Actinobacteria. Regarding fungal microbiome, Ascomycota was the most abundant phyla. Previous studies conducted on grapevine soil bacterial and fungal communities share this taxonomic pattern, indicating that the selective forces shaping fungal root microbiome composition at a high taxonomic rank are consistent against several environmental conditions (Castañeda and Barbosa 2017; Longa et al. 2017; Manici et al. 2017). Moreover, the root system was able to select specific bacterial and fungal OTUs depending on the genotype. For instance, *Bacillus* spp. were only found in rootstocks '140 Ru' and '161-49 C', and some species of the arbuscular mycorrhizal fungal genus *Glomus* were one of the most differentially abundant taxa for '110 R' rootstock. Some species of both genera have been described as potential biocontrol agents (Tahat et al. 2010; Siahmoshteh et al. 2018). *Cylindrocarpon*-like asexual morphs DNA concentration detected was affected by the year and vineyard, and were found in lower abundance in

‘161-49 C’ rootstock by both high-throughput amplicon sequencing and real-time PCR (qPCR) approaches. The use of ‘161-49 C’ rootstock was previously recommended within an integrated management program for esca and Petri disease pathogens (Gramaje et al. 2010b). However, the use of this rootstock has decreased over the last years due to physiological problems detected in most vineyards in France, and in some regions in Italy and Germany (Spilmont et al. 2016).

Recently, more innovative molecular techniques such as the droplet digital PCR (ddPCR) or NGS based on RNA have been adapted to study the grapevine microbiome, particularly the fungal pathogens associated with grapevine trunk diseases (Úrbez-Torres et al. 2017; Eichmeier et al., 2018; Martínez-Diz et al. 2019a). On one hand, ddPCR detects and provides absolute quantification of lower target concentrations than qPCR (Úrbez-Torres et al. 2017). The protocol to quantify black-foot pathogens from soil and plants have been already established, confirming the usefulness of the technique for a better understanding of soil microbiome (Martínez-Diz et al. 2019a). On the other hand, sequencing of the community mRNA presents an even greater improvement for microbial ecology studies because, unlike other methods targeting DNA, this approach can differentiate between viable and dead microorganisms since it targets the metabolically active fraction of the microbiome (Keer and Birch 2003).

5.2 Control

To date, the control of BFD has been based on the use of chemical products and hot-water treatment (HWT) (Halleen et al. 2007; Rego et al. 2006; Alaniz et al. 2011a). However, due to the difficulties associated with the implantation of HWT as a standard process in nurseries (Gramaje and di Marco 2015), and the reduction of chemical control products due to environmental and public health concerns (Decoin 2001), an alternative to control BFD is needed. In order to fill this gap, the final aim of this thesis was to evaluate other management strategies such as the use of biocontrol agents and biofumigants which can be applied to nursery soils or to graftlings as a pre-planting strategy.

The efficacy of *Trichoderma atroviride* SC1 against Petri disease (Pertot et al. 2016; Berbegal et al. 2019) and BFD (Berbegal et al. 2019) have been proven in nurseries and in newly established vineyards, whereas biofumigation with *Brassica* sp. has shown

promising results against BFD (Bleach 2013). In Chapter 4.1, the effect of white mustard biofumigation and propamocarb+fosetyl-Al applied into the soil, and Tusal® (*Trichoderma atroviride* T11 + *Trichoderma asperellum* T25) applied as a pre-planting method into grapevine grafted roots was evaluated. Soil treatments were also evaluated in grapevine seedlings. In grafted plants, biofumigation with white mustard plants reduced disease incidence by 55.3% in Field 1 and 42.2% in Field 2 when compared to the no soil treatment control. Disease severity was also reduced with white mustard residues, suggesting that biofumigation is a valid alternative to chemical fungicides to reduce soil BFD inoculum levels. Reduction of disease incidence and severity in grafted plants was independent on the application of *Trichoderma* spp. as dips before planting. This finding agrees with previous research showing the limitations of the application of these biocontrol agents into the roots (Halleen et al. 2007; dos Santos et al. 2016). Halleen et al. (2007) dipped the plants for 1 min in the treatment, whereas dos Santos et al. (2016) drenched the commercial substrate with the biocontrol agents 14 days prior planting. Recent research suggested that the dipping of basal ends in dry formulation gives higher colonization than soaking the base of vines during 1 hour or field drenching (van Jaarsveld et al. 2019). Regarding grapevine seedlings, biofumigation reduced BFD severity in both fields of study by 13.6% and 25.1%, respectively. The future direction of research needs to evaluated a wide spectrum of brassicaceous plant species with different glucosinolate profiles in order to select potential biofumigants for BFD of grapevine in different regions.

All the knowledge generated by this thesis is now available to researchers, diagnostic laboratories, grapevine nurseries and growers. The results obtained in this study points to a need for alternative strategies to minimize the impact of BFD pathogens on the long-term sustainability of viticultural production worldwide. Although the emphasis of the thesis was put on a specific pathosystem on viticulture, other agricultural systems could equally benefit from our results.

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CHAPTER 6.

CONCLUSIONS

1. A wide diversity of black-foot disease pathogens were identified from visually symptomless vines and asymptomatic internal wood tissue of grafted vines, bringing the total number of *Cylindrocarpon*-like asexual morphs fungi isolated from grapevine in Spain to 17.
2. Two novel species, namely *Dactylonectria riojana* and *Ilyonectria vivaria*, found on asymptomatic grapevines were characterized.
3. High degree of virulence variability was noticed among the *Cylindrocarpon*-like asexual morphs fungi in Spain, with the prevalent species *Dactylonectria torresensis* showing low virulence on grapevine seedlings.
4. An early, specific, and accurate detection method of viable propagules of black-foot disease pathogens in soil based on the Glucose-Faba Bean Rose Bengal Agar (GFBRBA) medium was provided.
5. Viable inoculum of *Dactylonectria torresensis* was still present during the rotation cycle in grapevine nurseries as conidia or chlamydospores.
6. Colony Forming Units (CFU) of black-foot pathogens per gram of soil correlated positively with $CaCO_3$ concentration in soils.
7. Grapevine rootstock genotype was the most important factor in shaping the rhizosphere microbiome in a mature vineyard (25-year-old), but not in a young vineyard (7-year-old).
8. Many bacterial and fungal species were found in all rootstocks and in both locations/vineyards, demonstrating the existence of a “core” grape phylogeny that is independent of the growing region.
9. A significant positive correlation was observed between the relative abundance of high-throughput amplicon sequencing reads and the relative abundance of DNA of black-foot disease pathogens in soil.
10. The rhizosphere compartments of “140 Ru” and “161-49 C” rootstocks harboured lower number of black-foot pathogens than the other grapevine rootstocks evaluated (“1103 P”, “110 R” and “41 B”).
11. Biofumigation with white mustard plants showed potential for improving control of black-foot disease in grapevines.
12. The application of a *Trichoderma*-based commercial product as dips before planting was ineffective to control black-foot pathogens.

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