Intra- and inter-annual changes in root endospheric microbial communities of grapevine are mainly deterministic

Marine Biget, Cendrine Mony, Tingting Wang, Ning Ling, Adèle Miteul, Olivier Jambon, Romain Causse-Védrines, Sophie Michon-Coudouel, Maxime Hervé, Véronique Chable, et al.

Intra- and inter-annual changes in root endospheric microbial communities of grapevine are mainly deterministic

Marine Biget[1,2], Cendrine Mony[1], Tingting Wang[1,3], Ning Ling[3], Adèle Miteul[1,4], Olivier Jambon[1], Romain Causse-Védrines [1,5], Sophie Michon-Coudouel [1,5], Maxime Hervé[2], Véronique Chable[6], Sabrina Pernet[7], Philippe Vandenkoornhuyse[1]#

[1] Université de Rennes 1, CNRS, UMR6553 ECOBIO (Ecosystèmes, biodiversité, évolution), F-35000 Rennes, France

[2] Université de Rennes 1, INRAE, Institut Agro, UMR IGEPP, F-35000, Rennes, France

[3] Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, National Engineering Research Center for Organic-based Fertilizers, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, Nanjing Agricultural University, Nanjing 210095, China

[4] Present address: Université Laval - Pavillon Paul Comtois - Sciences de l'Agriculture et de l'alimentation- 2425 Rue de l'Agriculture, Québec, QC G1V 0A6, Canada

[5] Université de Rennes 1, CNRS, UAR 3343 OSUR, Plateforme EcogenO, F-35000 Rennes, France

[6] Institut Agro, INRAE, UMR BAGAP, 35000, Rennes, France

[7] Château Palmer, lieu dit Issan, F-33460 Margaux, France

Running title:

root microbiota composition dynamics

#Address correspondence to Philippe Vandenkoornhuyse
philippe.vandenkoornhuyse@univ-rennes1.fr
UMR 6553 Ecobio, CNRS - University of Rennes 1, Avenue du General Leclerc, 35042 Rennes Cedex, France – Phone: +33 (0)2.23.23.50.07

Abstract

Background and aims Considering the plant microbiota, temporal changes are expected depending on plant development stages and environmental pressures because of modifications in plant requirements and available soil microbial reservoir.

Methods Herein, we analyzed the composition of root endosphere microbiota of grafted vine plants using two grapevine cultivars (Merlot and Cabernet-Sauvignon as scion grafted on rootstocks of different clones) as models both sampled in a single vineyard at three dates over a period of two growing years.

Results Highly conserved temporal patterns were found in the two cultivars. Intra-annual changes in microbial community composition were recorded whereas convergent microbial communities were observed on the two September dates. In particular, the increase in Actinobacteria and decrease in Glomeromycota in September were interpreted as shifts in the microbiota community patterns related to plant physiological requirements (e.g. water supply). A high proportion of non-random assembly of the root endospheric bacterial community confirmed the deterministic influence of the plant or/and the environment in microbial recruitment over time. The modified normalized stochasticity ratio (MST) showed that deterministic processes of assembly (MST<50%) were commonplace despite the changes in the root microbiota composition observed among sampling dates.

Conclusion Our study suggests an intra-annual rhythm of microbiota shifts, marginally random, with a succession within the root-microbiota endosphere likely governed by active plant filtering. A better knowledge of microbial-recruitment at work, seems important for both fundamental and applied perspectives.

53 **Keywords:** root microbiota, endosphere, temporal dynamics, deterministic assembly,
54 microbiota shifts, grapevine

Introduction

Plant development and survival are largely dependent on a remarkable diversity of microorganisms (Compant et al., 2019; Lemanceau et al., 2017; Vandenkoornhuyse et al., 2015). These microorganisms, either epiphytes or endophytes, can live in all plant organs and tissues. All plants worldwide are colonized by many different types of microorganisms, the most frequently studied being bacteria and fungi (Müller et al., 2016). These plant-associated microorganisms that form plant microbiota are deeply involved in supplying the plant with water and nutrients (Trivedi et al., 2020; Vandenkoornhuyse et al., 2015). They help their host face biotic and abiotic stresses such as drought and solar radiation (Trivedi et al., 2020; Vannier et al., 2015; Yang et al., 2013). It is worth noting that microbial communities display dynamic fluctuations. In continental ecosystems, dynamic fluctuations through seasons have been evidenced and were found correlated with environmental constraints including temperature and precipitation (Liu & Howell, 2021). Deterministic processes of microbiota assembly driving changes through time can thus be hypothesized. In grafted plants, as far as we know, determinism in changes through time in the microbiota composition within their host-plant (i.e. the microbiota endosphere) has never been formally analyzed.

Endospheric microorganisms associated with plants originate from the environment, mostly from the soil, which thus serves as a microbial reservoir (Vandenkoornhuyse et al., 2015; Xiong et al., 2021; Zarraonaindia et al., 2015). The processes behind plant colonization shaping microbial assemblage composition are thought to be mainly deterministic, i.e. shaped by host selection (Morrison-Whittle et al., 2015; Xiong et al., 2021; and in grafted plants, Biget et al., 2023; Dries et al., 2021; Marasco et al., 2022; Vink et al., 2021). Deterministic processes are assumed to be primarily linked to plant-

79 microorganism interactions. However, randomness and neutral processes can occur
80 (Werner & Kiers, 2015).

81 Two main mechanisms may be related to temporal fluctuations in the plant microbiota
82 community composition. Firstly, the development stage of the plant influences plant
83 microbiota over time (Chaparro et al., 2014; Copeland et al., 2015; Jumpponen & Jones,
84 2010; Mougel et al., 2006). This can be explained by changes in plant needs, which
85 thus modify the microbial assemblages recruited by the plants at the rhizosphere stage
86 and inside the roots (Berg & Smalla, 2009; Chaparro et al., 2014). Gradual changes
87 may also occur ranging from random colonization by species living close to the roots
88 to preferential selection of particular species by plants (i.e. host plant preference)
89 (Vandenkoornhuyse et al., 2002) and filtration by preferential carbon allocation to the
90 best cooperators (i.e. deterministic changes) (Kiers et al., 2011). In addition, microbiota
91 modifications can be linked to the development of new roots to colonize and to temporal
92 modifications in root characteristics, shape, and functions that may modify the space
93 available for microbial colonization (Iannucci et al., 2021; Pérez-Jaramillo et al., 2017;
94 Saleem et al., 2018) coming together with grape phenology with, for instance, drastic
95 changes in grape metabolism during grape ripening stage, leading to pectin and
96 cellulose degradation, acidity decreasing, and sugars accumulation in berries (Liu &
97 Howell, 2021; Renouf et al., 2005).

98 Secondly, environmental pressures influence plant microbiota over time. It has been
99 empirically noticed that microbial communities vary over time, both between seasons
100 and years (Liu & Howell, 2021; Zarraonaindia et al., 2015). In a year, climate
101 conditions may vary strongly depending on seasons, resulting in changes in soil
102 parameters (e.g. water availability, C/N ratio). These environmental changes over the

year can lead to dynamic changes in the reservoir of recruitable microorganisms, and hence in the composition of microorganisms recruited by the plant. In addition, plants respond to changes in environmental conditions and buffer abiotic stresses by recruiting particular microorganisms from the soil reservoir (Vannier et al., 2015; Vives-Peris et al., 2020). However, so far, very few studies have investigated temporal variations in plant microbiota endosphere, the most intimate fraction of the plant microbiota. We used herein grapevine as a model to assess the endospheric microbiota dynamics because grapevine has arguably a number of advantages, notably they are perennial, genetically homogeneous (i.e. herein, when made of particular scion clone grafted on a particular rootstock clone) and are usually managed very similarly at a given location and in a given vineyard domain.

We formulated different hypotheses. (i) The composition of both the bacteria and fungi on the grapevine varies over time. Assuming the existence of a major impact of the plant development stage as well as of modifications in the soil parameters in the different seasons, (ii) microbial differences between growing periods would be more pronounced than annual variations (same sampling date in two different years). (iii) If these temporal changes occur as a response to environmental constraints (changes in plant selection and abiotic conditions), these changes in the plant microbiota result from a combination of deterministic and stochastic processes, with the main factor driving recruitment being the soil reservoir, followed by plant-related factors (genotype/stage). To test these hypotheses, we sampled roots in a single vineyard domain located in Margaux (France). At this location, the bedrock is considered homogeneous and vineyard management is similar among fields. The root endosphere of grafted grapevine plant only have been analysed. Amplified fragment (amplicons) of fungal and bacterial small sub-unit rRNA gene obtained from root samples collected from the

same individual plants through time (three sampling campaigns) were sequenced to address the hypotheses.

Materials and Methods

Experimental design and study site

We selected individual vine plants in a single 66-ha vineyard located in Margaux, France, (45°2’12.73" N; 0°40’9.84" W). Organic and biodynamic practices have been used in the vineyard for 12 years. We sampled 25 fields (8 plant individuals per field), the number of fields containing Cabernet Sauvignon CS (12 fields) and Merlot M (13 fields) clones (Fig. 1) all of similar age ranges. These two cultivars representing the upper part (scion) were grafted on rootstock 101-14 mainly. However, it can be highlighted that overall, three rootstocks types were used according to different combinations (Table S1) and M and CS rootstocks are not strictly identical. Within one plot, the plants were of the same genotype (i.e. same scion and rootstock clones). We analyzed the composition of the microbial community associated with roots of the two grapevine cultivars in September 2018 (S18), June 2019 (J19), and September 2019 (S19) (June: flowering stage and September: mature grapes before harvest). The same individual grapevines were sampled in the three sampling campaigns (Fig. 1). The region is characterized by a temperate climate with average temperatures of 14.2 °C (2018) and 14 °C (2019); and cumulative annual precipitation of 934mm (2018) and 1,112mm (2019). Fig. S1 shows the average daily temperature and cumulative precipitation per day recorded in the three sampling campaigns, two in 2018 and one in 2019 by the DEMETER weather station located in Margaux, http://www.meteo-agriculture.eu.

151 **Sampling and DNA extraction**

152 On each date, 200 similar non-lignified vine root fragments (i.e. roots produced during
153 the current year) were collected to analyze variations in the grapevine root microbiota
154 over time (25 fields x 8 plant individuals). For each individual plant, ~ 1g of healthy
155 and non-lignified roots, of similar shape and diameter were picked close to the stem at
156 a depth of 5-20 cm and placed in individual hermetic bags and stored in cooled packs.
157 At the laboratory, the roots were thoroughly washed in tap water, then immersed in a
158 5‰ Triton X100 solution for 10 minutes and finally thoroughly rinsed several times
159 using sterile ultra-pure water (Lê Van et al., 2017). The washed roots were placed in
160 sterile micro-tubes and stored at -80 °C until DNA processing. A representative healthy
161 rootlet sub-sample (100 mg) of the cleaned roots was used for the DNA extraction.
162 Total root DNA was extracted and quantified at the GENTYANE platform (Clermont-
163 Ferrand, France) using magnetic bead technology (Sbeadex) on an Oktopure LGC
164 Genomics automat with Hoechst 33258 reagent. The DNA samples were then stored at
165 -20 °C until amplicon production.

166 **Molecular biology and sequencing**

167 All 600 extracted DNA were normalized at 10ng/µL to ensure PCR amplifications were
168 the same for all the samples. Endospheric fungi were targeted by amplifying an 18S
169 rRNA gene fragment with fungal primers NS22b (5′-
170 AATTAAGCAGACAAATCACT-3′) and SSU817 (5′-
171 TTAGCATGGAATAATRRAATAGGA-3′). Endophytic bacteria were analyzed by
172 PCR amplification of a 16S rRNA gene fragment (V5-V7 region of the 16S gene) using
173 the bacterial primers 799F (5′-AACMGGATTAGATACCCKG-3′) and 1223R (5′-
174 CCATTGTAGTACGTGTGTA-3′). The sets of primers were customized to

incorporate sample tags and Illumina® adaptors. To provide normalized PCR mix and avoid contamination, Illustra™PuReTaq Ready-to-go beads (GE Healhcare®) were used for both bacterial and fungal amplifications. The PCR conditions associated with the specific targets have been detailed previously (Vannier et al., 2018). The following molecular steps were performed on the EcogenO platform (Rennes, France): briefly, PCR products were purified with AMpureXP magnetic beads (Agencourt®) and quantified with a Quant-iT PicoGreen™ dsDNA Assay Kit to insure normalization of the PCR products at the same concentration. A second PCR using the Smartchip-Real Time PCR machine (Takara) performed multiplex tagging, the resulting tagged-amplicon pool was purified (AMpureXP, Agencourt®) and quantified using the Kapa Library Quantification Kit-Illumina®. The bacterial and fungal libraries were finally sequenced on an Illumina MiSeq (PE-2x250 and PE-2x300 cycles for bacterial- and fungal-amplifications respectively, with 3 sequencing runs each). As routine in amplicon sequencing at EcogenO platform, a PCR negative control passing all the steps of the library construction was included in the sequencing procedure.

Raw data processing and clustering

Base calling was performed on the Miseq instrument with CASAVA v1.8 software (Illumina). Sequence data trimming consisted of removing primers and deleting reads containing unidentified bases using Cutadapt software. The sequences were then processed using the FROGS pipeline following the recommendations (Escudié et al., 2018). The FROGS pipeline uses SWARM as a sequence agglomeration strategy to produce “sequence clusters”.

Briefly, the filtering steps recommended (Escudié et al., 2018) comprised a specialized algorithm for de-noising and a chimera removal step to exclude artificial sequence

clusters. Further filtering enabled removal of all sequence clusters identified in fewer than three samples and with a threshold of 0.005% of reads. After taxonomic affiliations using Silva138 16S rRNA for bacteria (Quast et al., 2012) and Phymyco-DB databases for fungi (Mahé et al., 2012), a final filtering step was applied to affiliated sequence clusters (95% coverage and 95% identity (BLAST)). The amplicon sequences did not contain any plastidial or plant mitochondrial DNA sequence. Bacterial or fungal DNA only was amplified and sequenced.

The resulting contingency tables contained 14 765 700 reads for bacteria and 12 483 626 reads for fungi. The sequenced negative control did not contain any detectable contaminating DNA. Rarefaction curves were produced using R ("vegan" package). Sequencing depths of both bacterial and fungal microbiota were sufficient to limit sequence cluster subsampling (Fig. S2). To perform statistical analysis, because the slopes of the curves tended toward a plateau (Fig. S2), we normalized the number of reads at 8 500 and 3 700 reads for the bacterial- and fungal- dataset respectively. For the bacteria, we discarded only 13 samples (13/960 samples representing 1.4%) to rarefy at 8500 reads per sample. For the fungi, we discarded 37 samples (37/960 samples representing 3.85%) to rarefy at 3700 reads per sample. None of the fields were composed of less than four out of the eight samples.

The final matrices containing 947 bacterial and 529 fungal sequence clusters were then used for statistical analyses. We calculated sequence cluster richness using the R "vegan" package. Index calculations and statistical analyses were performed for the dominant phyla (i.e., phyla representing the most sequence clusters with a threshold set at 5% of the total sequence clusters per date at the vineyard scale for the two microbial communities).

Statistical analyses

Statistical analyses were performed using R software version 4.0.4. We analyzed the effect of the sampling date (S18, J19 and S19) on root endospheric microbiota structure with a combination of multivariate analysis, univariate models, and neutral models. We checked if the microbial communities inhabiting the two cultivars differed using RDA (Redundancy analyses; Fig. S3). RDA were performed on Hellinger distances computed from sequence cluster matrices. The cultivar effect was assessed by a permutation test (999 repetitions) using the 'RVAideMemoire' package (Hervé, 2020). Because there was a significant effect of the cultivar in the two taxonomic groups (Fig. S3), all the subsequent statistical analyses were conducted separately on each cultivar to test the effect of the sampling date.

Effect of the sampling date on microbial assemblage structure

Partial principal coordinate analyses (PCoAs) were performed to assess the distribution pattern of root endospheric microbiota. PCoAs were based on Hellinger distances computed from sequence cluster matrices (Legendre & Gallagher, 2001). The individual grapevine was considered as a conditional effect to account for the biological dependency of the samples. The effect of the sampling date was assessed by a permutation test applied on the first PCoA factorial axes (function envfit () in the R 'vegan' package, 999 permutations). Pairwise comparisons were performed in the same way, with p-values adjusted using false discovery rate correction (Benjamini & Hochberg, 1995). PCoAs were also used to assess the effect of rootstocks on the microbial composition within each cultivar. Variation partitioning analysis (VPA) was used to test the effect of rootstock and sampling time on the community composition within each cultivar. We compared the difference in the Bray-Curtis distance to

compare the microbial variation between intra- and inter-annual time point under different cultivars and under the combination of scion and rootstock.

We used Wald tests applied to linear mixed models to test whether the sampling date impacted microbial richness. We constructed all the models using the richness index as the explanatory variable, the "individual grapevine" as random effect, and the "sampling date" as fixed effect. The linear mixed models and subsequent tests were performed using the R package 'lme4' (Bates et al., 2014), 'car' (Fox & Weisberg, 2018) and 'emmeans' (Searle et al., 1980).

Sampling dates differences on stochastic and deterministic assemblage processes

The Sloan neutral community model (NCM) was used to determine the influence of neutral processes on the bacterial and fungal community assembly inhabiting the root (Sloan et al., 2006). This neutral model predicts the relationship between sequence cluster detection frequency and their relative abundance (Burns et al., 2016; Venkataraman et al., 2015). The model predicts that species that are more abundant in the metacommunity (total source pool) are more expected to disperse and be randomly present in different sampling conditions, whereas species with low abundance are more likely to get lost due to ecological drift (Burns et al., 2016). In this study, we conducted neutral model analyses assuming the three-date metacommunity (i.e., the total dataset of a given cultivar) was the source of microbial communities. One neutral model was generated per sampling date and per taxonomic group (fungi and bacteria). The adequacy of model fitting was assessed through R^2 values ($0<R^2\leq1$) (Sloan et al., 2006). The sequence clusters from each matrix were then separated into three classes depending on their occurrence frequency based on a 95% confidence interval of the neutral community model predictions. The 95% confidence intervals around all fitting

271 statistics were obtained by bootstrapping with 1 000 replicates. The three categories
272 corresponded to the sequence clusters that were more frequent than expected (above
273 class), less frequent than expected (below class), and within the confidence interval
274 (neutral class). The model also calculated the estimate rate "m" (read as a measure of
275 dispersal limitation) using a non-linear least squares fitting (NLS- 'minpack.lm'
276 package) (Elzhov et al., 2010). The m value gives the probability of dispersal
277 substitution from the metacommunity due to random loss of species in the local
278 community (Burns et al., 2016). Therefore, higher m values likely show less dispersal
279 limitation. The R code used for this analysis originated from (Burns et al., 2016). We
280 further analyzed the sequence clusters specific to each class (above, below, and neutral
281 classes) and extracted their taxonomic affiliations to identify the main specific
282 taxonomic patterns that define each class associated with each sampling date.

283 The modified normalized stochasticity ratio (MST) was further used to quantify the
284 relative importance of deterministic and stochastic process in community assembly
285 with 50% as the boundary point between more deterministic (MST<50%) and more
286 stochastic (MST>50%) assembly (Ning et al., 2019). The MST value was calculated
287 based on the Jaccard distance estimated by using "tNST" functions in 'NST' package
288 (Ning et al., 2019). We divided all the samples into three time points to test the influence
289 of time series on the balance between determinism and stochasticity, and also divided
290 them into combinations of scion and rootstock.

Results

Description of the microbial composition of root endosphere

The Cabernet Sauvignon (CS) matrices contained 945 (12 fields, 288 samples) and 523 (12 fields, 278 samples) bacterial and fungal sequence clusters respectively while the Merlot (M) matrices contained 943 (13 fields, 307 samples) bacterial sequence clusters and 519 (13 fields, 519 samples) fungal sequence clusters.

Effect of the sampling date on microbial community composition and richness

The effect of sampling date on microbial community composition was analyzed through PCoA. PCoA analyses demonstrated clear differences in both bacterial and fungal communities among sampling dates, whatever the cultivar ($p < 0.001$; Fig. 2). The effect of the sampling date explained 44.2% and 41.6% (R^2 in Fig. 2A, B) of the variance of the bacterial community and 25.2% and 26.9% of the variance of the fungal community (R^2 in Fig. 2C, D) in Cabernet Sauvignon and Merlot respectively.

Regarding communities composition, J19 and S19 displayed the highest dissimilarity (Bray-Curtis distance) difference, while S18 and S19 presented the lowest difference, with similar ranges for all bacterial and fungal communities of both cultivars ($p<0.05$, Fig. S4; Fig. S5). The richness of the fungal microbiota ("all fungi") was mainly explained by Ascomycota (the most numerous in terms of richness), and richness was lowest in J19 for both cultivars compared to S18 and S19. Reciprocally, Glomeromycota richness was significantly higher in J19 for both cultivars (Fig. 3C, D) while Basidiomycota richness had the same pattern for Merlot cultivar only. In contrast to fungi, bacteria showed no clear pattern of variation among sampling dates, except for Actinobacteria (lowest richness in J19 whatever the cultivar; Fig. 3A, B).

Gammaproteobacteria, Bacteroidota and Myxococcota were richer in J19 than in S18 and S19 but only in the Merlot cultivar (Fig. 3B).

Effect of sampling date on the stochastic and deterministic assemblages of the microbial communities

To decipher the stochastic and deterministic processes in shaping microbial communities depending on the sampling date, we conducted neutral community model analyses (NCM) (Fig. 4A, B). Overall, the occurrence frequency of bacteria analyses was partly defined by the neutral model (R^2*=35.5~43.9* for Cabernet Sauvignon and R^2*=29.5~44.1* for Merlot; Fig. 4A, B). However, for the fungal taxa, we did not record a proper fit to the model (negative R^2 values Fig. S6A, B) for three out of the six models. Proper fits were found only for the J19 and S19 analyses (R^2*=14.8* and R^2*=6.14*; Fig. S6A) and the J19 community analyses (R^2*=15.5*; Fig. S6B) for Cabernet Sauvignon and Merlot, respectively.

In the two grapevine cultivars and on all three sampling dates, some sequence clusters of bacterial taxa occurred more frequently (10.7 to 53.9%, yellow dots) or less frequently (30.1 to 42%, green dots) than predicted by the neutral models (Fig. 4A, B, single bar charts). The yellow sequence clusters suggest active selection and maintenance by the host plant while the green taxa suggest active selection against the host (Fig. 4A, B). The stochastic fraction accounted for from 13.6 to 56.9% of cumulative microbial occurrence, thereby rejecting the null hypothesis of random assembly of the plant microbiota (hypothesis 4) (Fig. 4A, B, single bar charts).

Community composition defining the three classes (above, below and neutral partitions) was not evenly distributed among phyla (Fig. 4A, B, combined bar charts). Clear differences were observed for Alphaproteobacteria, Gammaproteobacteria, Bacteroidia

as well as Actinobacteria between the two deterministic partitions (Fig. 4A, B, “above” and “below” in the combined bar charts) indicating that within a taxonomic group some sequence clusters were deterministically selected and maintained by the host plant while others were negatively influenced by the host plant or by the local environment (Fig. 4A, B, “above” and “below” in bar charts).

To further quantify the relative importance of deterministic processes in shaping plant associated microbiota, and to complement the NCM analyses, the modified normalized stochastic ratio showed that MST value of bacterial and fungal communities was below the 50% boundary point, i.e. deterministic processes of assembly played a preponderant role in both bacterial and fungal communities composition in the vine root endosphere (Fig. 5; Fig. S7). The estimated minimum proportion of determinism in the community composition of endospheric microbial communities was 53% in S19 bacterial community of Merlot. MST values depended on sampling date with higher values for J19 compared to S18 and S19 for fungi; and increasing values across sampling dates for bacteria.

Discussion

Composition of the grapevine root endosphere

In this study, we analyzed the bacterial and fungal communities inhabiting the root endosphere and deciphered the temporal dynamics that structure microbial communities at the level of a vineyard. Proteobacteria (Alphaproteobacteria and Gammaproteobacteria) and Ascomycota were the main phyla forming the root endospheric microbiota in the grapevines in line with the results of most studies that analyzed grapevine microbial communities in different compartments, areas and climate (Bettenfeld et al., 2022). Although the microbial composition of the main phyla

remained stable and was similar to that described in most microbial studies of grapevine (Deyett et al., 2020; Swift et al., 2021), we observed specific changes in phyla abundances depending on the sampling date.

Intra- versus inter-annual variations affect both microbial composition and richness

In this present study, as expected, we observed consistent changes in the microbial communities of the grapevine roots depending on the sampling date, with changes in the composition, richness and selection of specific taxa (hypothesis 1). In both cultivars, we showed that intra-annual variations in the composition of microbial communities in the same year (June 2019 and September 2019) were stronger than those observed in a given growing period in two different years (September 2018 and September 2019) (hypothesis 2). Similarly, but in a different compartment, the rhizosphere, a recent study showed changes in the bacterial and fungal richness of grapevine related to the sampling date during the year (Berlanas et al., 2019). Elsewhere, in grafted grapevine, it has been shown that the combination of both scion and rootstock determine, at least in part, the root microbiota composition (Marasco et al., 2022) with a more pronounced effect of rootstock as driver of the deterministic process of assembly of the grapevine root microbiota (Biget et al., 2023). Considering the observed changes in the grapevine root microbiota composition through time, we noticed that (i) almost all scion and rootstock combinations showed less variation within than between years (Fig. S5) (ii) the assembly process considering the different time points under almost all combinations of rootstock and scion is deterministic (Fig S7) which is not coherent with the observation (i.e. less variation between years) despite a confirmed effect of the rootstock type on the microbial composition (Fig S8 and S9). Taken altogether, and

386 despite the limits of these analyses of the rootstock effect (i.e. redundancy between
387 factors field and rootstock-scion combination), the results (Figs S5-S9) suggest that the
388 changes observed among sampling dates in the grapevine root endosphere microbiota
389 composition are not a consequence of divergences related to the rootstock type but
390 rather seemingly convergent response patterns related to external effector.

391 Regarding the composition of the bacterial community, we particularly observed that
392 Actinobacteria were significantly richer in September than in June, whereas
393 Bacteroidota and Myxococcota were richer in June. Actinobacteria have been described
394 to improve plant mineral and hydric nutrition (Govindasamy et al., 2014) and may thus
395 have been recruited by the plant in greater numbers in September to buffer abiotic
396 stresses at the end of the summer. Monthly rainfall and mean temperature were 36.8
397 mm and 20.6°C, 134.0 mm and 20.9°C, 71.0 mm and 20.0°C for Sept 18, June 19 and
398 Sept 19 respectively. It can thus be suggested that the microbiota composition changes
399 are related to water availability. In support to this, analyses of synchronic effect of
400 drought on the microbiota composition have previously demonstrated changes in the
401 plant endosphere microbiota composition (Andreo-Jimenez et al., 2019).

402 Some genera among Actinobacteria, particularly *Streptomyces*, are known to control
403 pathogens by producing antibiotic molecules (Doumbou et al., 2001; Schrey & Tarkka,
404 2008). Thus, these *Streptomyces* can help to suppress phytopathogens and plant
405 diseases. We detected specific *Streptomyces* sequence clusters at the two September
406 sampling dates (the same sequence-clusters were identified in September 2018 and
407 September 2019, Table S3). Because the critical development stage of grapevines is at
408 the end of grape ripening, it can be suggested that the plants may secrete specific
409 exudate compounds to recruit Actinobacteria to face both biotic and abiotic stresses.

410 The increase in Bacteroidota in June can be explained by their role in plant nutrition.
411 Indeed, Bacteroidota have been reported to be involved in organic phosphorus
412 mineralisation (Lidbury et al., 2021). Recruiting Bacteroidota in June, when the plants
413 reach their highest vegetative growth phase, may help them to face increasing demand
414 for mineral nutrition. Changes in bacterial communities between the flowering and the
415 harvest periods (mature grapes) appeared to be in line with developmental grapevine
416 stages, both involving differential plant needs.

417 The composition of the fungal community was also strongly influenced by the sampling
418 date, with higher Basidiomycota (only for Merlot) and Glomeromycota richness in June
419 and higher Ascomycota richness in September. A recent study investigated intra-annual
420 changes in fungal communities of different bio-compartments, which supports our
421 results on the root endosphere (Liu & Howell, 2021). They correlated weather
422 conditions with changes in fungal composition and observed dramatic changes in
423 microbiota composition and diversity especially at the veraison stage, just after the fruit
424 set period (Liu & Howell, 2021). Glomeromycota forming Arbuscular Mycorrhiza (AM)
425 are known to be involved in supplying minerals and water to plants and in improving
426 resistance to plant diseases (Smith & Read, 2010). Thus, changes in the patterns of AM
427 fungal community composition over time that have already been evidenced (Bennett et
428 al., 2013; Vandenkoornhuyse et al., 2002) may be correlated with the plant
429 development stage and plant requirements over the seasons. Beyond this aspect, the
430 shifts in AM symbiosis composition may be partly attributable to a demonstrated
431 priority effect (i.e. the effect of order of arrival on subsequent community development)
432 (Werner & Kiers, 2015).

433 Many endospheric Basidiomycota and Ascomycota that form positive associations with

plants (i.e. endophytic fungi) are known as plant “protective agents" (Porras-Alfaro & Bayman, 2011) that promote plant resistance to environmental stresses including drought, salinity, heat, insects, and pathogens (Hardoim et al., 2015; Porras-Alfaro & Bayman, 2011). For instance, under drought conditions, rice has an enriched fungal endosphere that has been interpreted as positive recruitment to buffer drought conditions (Andreo-Jimenez et al., 2019). Fungal endophytes are known to induce a plant systemic resistance (ISR) while these fungal symbionts escape this immune response (Hardoim et al., 2015). In addition, it has been demonstrated *in vitro* that some of these recruited fungi are able to produce antifungal metabolites (Hardoim et al., 2015) which might modify the diversity of the root-fungal endosphere. Further analysis including an absolute quantification of each fungus composing the grapevine mycobiota might provide complementary information to reinforce conclusions related to the changes observed. More widely, the temporal changes in root-endosphere community composition over time raise the question of the extent of the determinism in the process of assembly.

Stochastic versus deterministic process of assembly of the grapevine root endosphere over time

Recent advances in statistical analyses have allowed to partition deterministic and ecologically neutral processes of assembly using a null model framework (Burns et al., 2016) (see Materials and Methods for the rationale). For fungi, the absence of a fit of the neutral community model (NCM) analysis did not allow partitioning with statistical support for stochastic and deterministic processes of assembly. However, considering bacteria, we were able to estimate that the deterministic part accounted for 43 to 84% for the two grapevine cultivars (Fig. 4) (hypothesis 3). This appeared consistent with

458 non-random assembly of bacterial communities inhabiting the root endosphere of
459 grafted grapevine plants, although the grafted plants were compared to the ungrafted
460 plants under the holobiont concept (Biget et al., 2023). In our study, we defined a new
461 non-random assemblage process of the grafted plant along a seasonal variation. This
462 new process seems independent from the rootstock genotype, a factor supposedly
463 inducing variance (i.e. a random-like part of the partitioning). The endosphere
464 composition dominated by deterministic assembly processes can be explained by active
465 rejection of bacteria by the host (bar charts showing the cumulative occurrence
466 frequencies of sequence clusters in Fig. 4), and to a lesser extent to sequence clusters
467 actively maintained and selected by the host plant. Previous studies have showed that
468 the endophytic communities in *Pinus* were structured by host age and environmental
469 factors (Carper et al., 2018). It has been shown in plant microhabitats (roots, shoots)
470 that deterministic processes of assembly were primarily responsible for community
471 assembly likely related to plant filtration processes (Guo et al., 2021). The determinism
472 governing the community assembly also suggested thinly tuned filtering processes in
473 grapevine root-endosphere composition over time in this study. The specific selection
474 of key taxa by the host plant can be interpreted as a process to face particular
475 environmental conditions or to meet specific needs. Increasing evidence has explained
476 selection and regulation of rhizospheric microbial communities through root exudates
477 (Broeckling et al., 2008; Chaparro et al., 2013; Chaparro et al., 2014; Voges et al. 2019;
478 Zhalnina et al., 2018). Depending on their development stage, plants can secrete
479 different types of compounds, including specific sugars and amino acids as well as more
480 complex secondary metabolites (Cotton et al., 2019; Jacoby et al., 2020; Voges et al.,
481 2019), that act as signals for microbial assemblages of the rhizosphere habitat (Chaparro
482 et al., 2014). It has been recently shown that coumarins solubilized iron under particular

oxygen and pH conditions thus stimulating rhizospheric microbial growth in these specific conditions (McRose et al., 2023). It is thus not only the control of carbon released by plants but also the physicochemical status which modulates the rhizosphere composition. Modifications in rhizosphere microbiota and in plant microbiota evidenced herein over time may thus be a consequence, at least partially, of both changes in the root secondary metabolites excreted (Dang et al., 2021; Kaur & Suseela, 2020; Voges et al., 2019) and physicochemical modifications related to water availability. The rhizosphere being the antechamber of the endosphere, this process should act together with the plant immune system and the mechanism of preferential C allocation to the best cooperators (Kiers et al., 2011). This process would explain the determinism observed in microbiota assembly in root-endosphere samples.

Convergence of temporal shifts in the microorganisms root-endosphere composition between the two cultivars

A key result of this work is the convergence of the temporal dynamics of microorganisms between the two cultivars, Cabernet Sauvignon and Merlot. The rootstock genotypes used in Cabernet Sauvignon (CS) are not strictly comparable to Merlot (M) (Table S1). At least two plots contained a specific rootstock genotype associated with Merlot scion. From this and because we have recently demonstrated that rootstock is the key driver of endospheric microbiota composition (Biget et al., 2023) we expected to observe differences among grafted Carbernet Sauvignon and Merlot. Conversely to the expectation over drivers than plant material can be the observed convergence in the microbiota composition. It can be argued that the environmental conditions (lower water availability) in September might cause a convergent change in the endospheric microbiota composition. It is possible that in

addition to the environmental stress, the plant growth developmental stage is also important to consider. If this is true, the high convergence observed in the endospheric microbiota composition can be interpreted as empirical evidence of deterministic selection during plant growth and development. This interpretation is in agreement with recent findings in the maize microbiome, i.e. the plant developmental stage as driver of differentiation in ecological functions of the plant-microbiota and consequently of the recruited microorganisms forming this microbiota (Xiong et al., 2021).

The root microbiota has been characterized as a combination of dynamic processes (primary colonization of microbes from the surrounding soil followed by host recruitment) (Bulgarelli et al., 2013). Our results suggest that these mechanisms – priority effect and host preference – are likely to be similar regarding the type of the cultivar analysed and greatly expand on the development of the host plant. Although variations in microbial composition depending on cultivar type and on the genotype have been proposed, most of the work done so far acknowledged that the geographical location and local environmental conditions (climate/soil parameters) had a bigger impact on microbial assembly (Bokulich et al., 2014; Marasco et al., 2018; Zarraonaindia et al., 2015; Zhang et al., 2019). We have worked herein at the level of a vineyard domain to limit the effect of bedrock and related soils characteristics, viticulture practices and environmental conditions and confirmed differences in the microorganism endosphere community composition in between Cabernet Sauvignon and Merlot (Fig. S3) and more importantly demonstrated that the two cultivars analyzed responded temporally in the same way, i.e. same key intra- and inter-cultivar variations in the microbial-endosphere structure through time. To confirm our results, the temporal dynamics of the microbiota should now be tested on a larger number of cultivars at a given location under similar viticultural practices.

Conclusion

The study of microbial temporal dynamics advanced our understanding of the mechanisms governing their assemblages and demonstrated tight relationships between microbial assemblages and plant phenology and adaptation to environmental conditions. Here, we showed similar patterns between the two cultivars, both cultivars displayed a deterministic recruitment of specific microorganisms and harbour similar trend in microbial communities variation. Beyond this framework, our understanding of the mechanisms behind the deterministic changes, including the relative effect of the host plant versus abiotic conditions, remains incomplete. Our results stress the complex interactions that take place between a host and its microbiota over time. Understanding microbial assembly rules would help preserve grapevine health, and possibly will allow to understand the particularity of vintages and intensify the spirit of the wine (*microbial terroir*). However, fundamental research is still needed to understand the mechanisms of microorganisms recruitment and dynamics in the endosphere. One promising prospect would be to analyse in gnotobiotic conditions (i.e. with the use of a culture collection) how plants attract specific microorganisms (e.g. dynamic analyses of exudates composition emitted) and also to better decipher the importance of source-sink transitions for carbon in relation to the developmental stage of the plants.

Acknowledgements

This work was supported by a grant from the CNRS-EC2CO program (HOLOBIONT project), CNRS-IRP program (M-AGRI project) and by the "*Agence Nationale de la Recherche et de la Technologie*" (ANRT) through an industrial CIFRE agreement between Château Palmer, Château Latour, Sovivins, Pépinière de Salettes and the University of Rennes 1 (Agreement N° 2017/1579). We are grateful to the GENTYANE and, within the ANAEE-France research infrastructure, to the EcogenO platforms for help with DNA extraction and amplicon sequencing, respectively. We are also grateful to the Genotoul platform Toulouse Midi-Pyrenees and GenOuest for access to their bioinformatics pipeline and storage facilities and the Ecolex platform for help during sampling. We deeply acknowledge all the trainees and people at Château Palmer for their help during the sampling campaigns, especially Sylvain Fries, Oriane Heulliet, Hélène Sarkis for their help performing this work. We are also very grateful to July Hémon (EcogenO, molecular analysis) and Achim Quaiser (sampling). We finally acknowledge Daphne Goodfellow for English editing.

References

Andreo-Jimenez, B., Vandenkoornhuyse, P., Lê Van, A., Heutinck, A., Duhamel, M., Kadam, N., Jagadish, K., Ruyter-Spira, C., & Bouwmeester, H (2019) Plant host and drought shape the root associated fungal microbiota in rice. Peer J 7: e7463. https://doi.org/10.7717/peerj.7463

Bates, D., Mächler, M., Bolker, B., & Walker, S (2014) Fitting linear mixed-effects models using lme4. J Stat Softw 67:201-210. https://doi.org/10.18637/jss.v067.i01

Benjamini, Y., & Hochberg, Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc 57:289-300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bennett, A. E., Daniell, T. J., Öpik, M., Davison, J., Moora, M., Zobel, M., Selosse, M.-A., Evans, D (2013) Arbuscular mycorrhizal fungal networks vary throughout the growing season and between successional stages. PloS One 8:e83241. https://doi.org/10.1371/journal.pone.0083241

Berg, G., & Smalla, K (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 68:1–13. https://doi.org/10.1111/j.1574-6941.2009.00654.x

Berlanas, C., Berbegal, M., Elena, G., Laidani, M., Cibriain, JF., Sagües, A., Gramaje, D (2019) The fungal and bacterial rhizosphere microbiome associated with grapevine rootstock genotypes in mature and young vineyards. Front Microbiol 10:1142. https://doi.org/10.3389/fmicb.2019.01142

Bettenfeld, P., Cadena i Canals, J., Jacquens, L., Fernandez, O., Fontaine, F., van Schaik, E., Courty, P.-E., & Trouvelot, S (2022) The microbiota of the grapevine holobiont: A key component of plant health. J Adv Res 40:1-15. https://doi.org/10.1016/j.jare.2021.12.008

Biget, M., Wang, T., Mony, C, Xu, Q., Lecoq, L., Chable, V., Theis, K.R., Ling, N., Vandenkoornhuyse, P (2023) Evaluating the hologenome concept by analyzing the root-endosphere microbiota of chimeric plants. iScience 26:106031. https://doi.org/10.1016/j.isci.2023.106031

Bokulich, N. A., Thorngate, J. H., Richardson, P. M., & Mills, D. A (2014) Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. Proc Natl Acad Sci USA 111: E139-E148. https://doi.org/10.1073/pnas.1317377110

Broeckling, C. D., Broz, A. K., Bergelson, J., Manter, D. K., & Vivanco, J. M (2008) Root exudates regulate soil fungal community composition and diversity. Appl Environ Microbiol 74:738-744. https://doi.org/10.1128/AEM.02188-07

Bulgarelli, D., Schlaeppi, K., Spaepen, S., Van Themaat, E. V. L., & Schulze-Lefert, P (2013) Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807-838. https://doi.org/10.1146/annurev-arplant-050312-120106

Burns, A. R., Stephens, W. Z., Stagaman, K., Wong, S., Rawls, J. F., Guillemin, K., & Bohannan, B. J. M (2016) Contribution of neutral processes to the assembly of gut microbial communities in the zebrafish over host development. ISME J 10:655-664. https://doi.org/10.1038/ismej.2015.142

Carper, D. L., Carrell, A. A., Kueppers, L. M., & Frank, A. C (2018) Bacterial endophyte communities in *Pinus flexilis* are structured by host age, tissue type, and environmental factors. Plant Soil 428:335-352. https://doi.org/10.1007/s11104-018-3682-x

Chaparro, J. M., Badri, D. V., Bakker, M. G., Sugiyama, A., Manter, D. K., & Vivanco, J. M (2013) Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions. PloS One 8:e55731. https://doi.org/10.1371/journal.pone.0055731

Chaparro, J. M., Badri, D. V., & Vivanco, J. M (2014) Rhizosphere microbiome assemblage is affected by plant development. ISME J 8:790-803. https://doi.org/10.1038/ismej.2013.196

Compant, S., Samad, A., Faist, H., & Sessitsch, A (2019) A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. J Adv Res 19:29-37. https://doi.org/10.1016/j.jare.2019.03.004

Copeland, J. K., Yuan, L., Layeghifard, M., Wang, P. W., & Guttman, D. S (2015) Seasonal community succession of the phyllosphere microbiome. Mol Plant Microbe Interact 28:274-285. https://doi.org/10.1094/MPMI-10-14-0331-FI

Cotton, TEA., Pétriacq, P., Cameron, DD., Meselmani, MA., Schwarzenbacher, R., Rolfe, SA., Ton, J (2019) Metabolic regulation of the maize rhizobiome by benzoxazinoids. ISME J 13: 1647–1658. https://doi.org/10.1038/s41396-019-0375-2

Dang, H., Zhang, T., Wang, Z., Li, G., Zhao, W., Lv, X., Zhuang, L (2021) Succession of endophytic fungi and arbuscular mycorrhizal fungi associated with the growth of plant and their correlation with secondary metabolites in the roots of plants. BMC Plant Biol 21:165. https://doi.org/10.1186/s12870-021-02942-6

Deyett, E., and Rolshausen, P.E (2020) Endophytic microbial assemblage in grapevine. FEMS Microbiol Ecol 96: fiaa053. https://doi.org/10.1093/femsec/fiaa053

Doumbou, C. L., Hamby Salove, M. K., Crawford, D. L., & Beaulieu, C (2001) Actinomycetes, promising tools to control plant diseases and to promote plant growth. Phytoprotection 82: 85-102. https://doi.org/10.7202/706219ar

Dries, L., Bussotti, S., Pozzi, C., Kunz, R., Schnell, S., Lohnertz, O., and Vortkamp, A (2021) Rootstocks shape their microbiome-bacterial communities in the rhizosphere of different grapevine rootstocks. Microorganisms 9: 822. https://doi.org/10.3390/microorganisms9040822

Elzhov, T. V., Mullen, K. M., Spiess, A., & Bolker, B (2010) Minpack. lm: R interface to the Levenberg-Marquardt nonlinear least-squares algorithm found in MINPACK. Plus support for bounds 1-2. http://CRAN.R-project.org/package=minpack.lm.

Escudié, F., Auer, L., Bernard, M., Mariadassou, M., Cauquil, L., Vidal, K., Maman, S., Hernandez-Raquet, G., Combes, S., & Pascal, G (2018) FROGS: find, rapidly, OTUs with galaxy solution. Bioinformatics 34:1287-1294. https://doi.org/10.1093/bioinformatics/btx791

Fox, J., & Weisberg, S (2018) An R companion to applied regression. Sage publications.

Govindasamy, V., Franco, C. M. M., & Gupta, V. V. S. R (2014) Endophytic actinobacteria: diversity and ecology. Adv Endophy Res, Springer, New Delhi 27-59. https://doi.org/10.1007/978-81-322-1575-2_2

Guo, J., Ling, N., Li, Y., Li, K., Ning, H., Shen, Q., Guo, S., & Vandenkoornhuyse, P (2021) Seed-borne, endospheric and rhizospheric core microbiota as predictors of plant functional traits across rice cultivars are dominated by deterministic processes. New Phytol 230:2047-2060. https://doi.org/10.1111/nph.17297

Hardoim, P. R., Van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., Döring, M., & Sessitsch, A (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 79: 293-320. https://doi.org/10.1128/MMBR.00050-14

Hervé, M., & Hervé, M. M (2020) RVAideMemoire: testing and plotting procedures for biostatistics. The R Foundation. See https://CRANR-projectorg/package= RVAideMemoire.

Iannucci, A., Canfora, L., Nigro, F., De Vita, P., & Beleggia, R (2021). Relationships between root morphology, root exudate compounds and rhizosphere microbial community in durum wheat. Appl Soil Ecol 158:103781. https://doi.org/10.1016/j.apsoil.2020.103781

Jacoby, RP., Chen, L., Schwier, M., Koprivova, A., Kopriva, S (2020) Recent advances in the role of plant metabolites in shaping the root microbiome. F1000Res 9:151. https://doi.org/10.12688/f1000research.21796.1

Jumpponen, A., Jones, KL (2010) Seasonally dynamic fungal communities in the Quercus macrocarpa phyllosphere differ between urban and nonurban environments. New Phytol 186:496–513. https://doi.org/10.1111/j.1469-8137.2010.03197.x

Kaur, S., Suseela, V (2020) Unraveling arbuscular mycorrhiza-induced changes in plant primary and secondary metabolome. Metabolites 10:335. https://doi.org/10.3390/metabo10080335

Kiers, E. T., Duhamel, M., Beesetty, Y., Mensah, J. A., Franken, O., Verbruggen, E., Fellbaum, C. R., Kowalchuk, G. A., Hart, M. M., & Bago, A (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science 333:880-882. https://doi.org/10.1126/science.1208473

Legendre, P., & Gallagher, E. D (2001) Ecologically meaningful transformations for ordination of species data. Oecologia 129:271-280. https://doi.org/10.1007/s004420100716

Lemanceau, P., Blouin, M., Muller, D., & Moënne-Loccoz, Y (2017) Let the core microbiota be functional. Trends Plant Sci 22:583-595. https://doi.org/10.1016/j.tplants.2017.04.008

Lê Van, A., Quaiser, A., Duhamel, M., Michon-Coudouel, S., Dufresne, A., Vandenkoornhuyse, P (2017) Ecophylogeny of the endospheric root fungal microbiome of co-occurring Agrostis stolonifera. PeerJ 5: e3454 https://doi.org/10.7717/peerj.3454

Lidbury, I. D. E. A., Borsetto, C., Murphy, A. R. J., Bottrill, A., Jones, A. M. E., Bending, G. D., Hammond, J. P., Chen, Y., Wellington, E. M. H., & Scanlan, D. J (2021) Niche-adaptation in plant-associated Bacteroidetes favours specialisation in organic phosphorus mineralisation. ISME J 15:1040-1055. https://doi.org/10.1038/s41396-020-00829-2

693 Liu, D., & Howell, K (2021) Community succession of the grapevine fungal
694 microbiome in the annual growth cycle. Environ Microbiol 23:1842-1857.
695 https://doi.org/10.1111/1462-2920.15172

696 Mahé, S., Duhamel, M., Le Calvez, T., Guillot, L., Sarbu, L., Bretaudeau, A., Collin,
697 O., Dufresne, A., Kiers, E. T., & Vandenkoornhuyse, P (2012) PHYMYCO-DB: a
698 curated database for analyses of fungal diversity and evolution. PloS One 7:e43117.
699 https://doi.org/10.1371/journal.pone.0043117

700 Marasco, R., Rolli, E., Fusi, M., Michoud, G., & Daffonchio, D (2018) Grapevine
701 rootstocks shape underground bacterial microbiome and networking but not potential
702 functionality. Microbiome 6:3. https://doi.org/10.1186/s40168-017-0391-2

703 Marasco, R., Alturkey, H., Fusi, M., Brandi, M., Ghiglieno, I., Valenti, L., and
704 Daffonchio, D (2022) Rootstock-scion combination contributes to shape diversity and
705 composition of microbial communities associated with grapevine root system. Environ
706 Microbiol 24: 3791-3808. https://doi.org/10.1111/1462-2920.16042

707 McRose, Darcy L., Li, J., and Newman, Dianne K (2023) The chemical ecology of
708 coumarins and phenazines affects iron acquisition by pseudomonads. Proc Natl Acad
709 Sci 120: e2217951120. https://doi.org/10.1073/pnas.2217951120

710 Morrison-Whittle, P., & Goddard, M. R (2015) Quantifying the relative roles of
711 selective and neutral processes in defining eukaryotic microbial communities. ISME J
712 9:2003-2011. https://doi.org/10.1038/ismej.2015.18

713 Mougel, C., Offre, P., Ranjard, L., Corberand, T., Gamalero, E., Robin, C., Lemanceau,
714 P (2006) Dynamic of the genetic structure of bacterial and fungal communities at
715 different developmental stages of *Medicago truncatula* Gaertn. cv. Jemalong line J5.
716 New Phytol 170:165–175. https://doi.org/10.1111/j.1469-8137.2006.01650.x

717 Müller, DB., Vogel, C., Bai, Y., Vorholt, JA (2016) The plant microbiota: systems-
718 level insights and perspectives. Annu Rev Genet 50:211–234.
719 https://doi.org/10.1146/annurev-genet-120215-034952

720 Ning, D., Deng, Y., Tiedje James, M., & Zhou, J (2019) A general framework for
721 quantitatively assessing ecological stochasticity. Proc Natl Acad Sci USA 116:16892-
722 16898. https://doi.org/10.1073/pnas.1904623116

723 Pérez-Jaramillo, J. E., Carrión, V. J., Bosse, M., Ferrão, L. F. V., De Hollander, M.,
724 Garcia, A. A. F., Ramírez, C. A., Mendes, R., & Raaijmakers, J. M (2017) Linking
725 rhizosphere microbiome composition of wild and domesticated *Phaseolus vulgaris* to
726 genotypic and root phenotypic traits. ISME J 11:2244-2257.
727 https://doi.org/10.1038/ismej.2017.85

728 Porras-Alfaro, A., & Bayman, P (2011) Hidden fungi, emergent properties: endophytes
729 and microbiomes. Annu Rev Phytopathol 49:291-315. https://doi.org/10.1146/annurev-
730 phyto-080508-081831

731 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., &
732 Glöckner, F. O (2012) The SILVA ribosomal RNA gene database project: improved
733 data processing and web-based tools. Nucleic Acids Res 41: D590-D596.
734 https://doi.org/10.1093/nar/gks1219

735 Renouf, V., Claisse, O., and Lonvaud-Funel, A (2005) Understanding the microbial
736 ecosystem on the grape berry surface through numeration and identification of yeast

and bacteria. Austral J Grape Wine Res 11: 316-327. https://doi.org/10.1111/j.1755-0238.2005.tb00031.x

Saleem, M., Law, A. D., Sahib, M. R., Pervaiz, Z. H., & Zhang, Q (2018) Impact of root system architecture on rhizosphere and root microbiome. Rhizosphere 6:47-51. https://doi.org/10.1016/j.rhisph.2018.02.003

Schrey, S. D., & Tarkka, M. T (2008) Friends and foes: streptomycetes as modulators of plant disease and symbiosis. Antonie Van Leeuwenhoek 94:11-19. https://doi.org/10.1007/s10482-008-9241-3

Searle, S. R., Speed, F. M., & Milliken, G. A (1980) Population marginal means in the linear model: an alternative to least squares means. Am Stat 34:216-221. https://doi.org/10.1080/00031305.1980.10483031

Sloan, W. T., Lunn, M., Woodcock, S., Head, I. M., Nee, S., & Curtis, T. P (2006) Quantifying the roles of immigration and chance in shaping prokaryote community structure. Environ Microbiol 8:732-740. https://doi.org/10.1111/j.1462-2920.2005.00956.x

Smith, S. E., Read, D. J (2010) Mycorrhizal symbiosis. Academic Press (Third edition). https://doi.org/10.1097/00010694-198403000-00011

Swift, J.F., Hall, M.E., Harris, Z.N., Kwasniewski, M.T., and Miller, A.J (2021) Grapevine microbiota reflect diversity among compartments and complex interactions within and among root and shoot systems. Microorganisms 9: 92. https://doi.org/10.3390/microorganisms9010092

Trivedi, P., Leach, J. E., Tringe, S. G., Sa, T., Singh, B. K (2020) Plant-microbiome interactions: from community assembly to plant health. Nat Rev Microbiol 18:607-621. https:// doi.org/10.1038/s41579-020-0412-1)

Vandenkoornhuyse, P., Husband, R., Daniell, T. J., Watson, I. J., Duck, J. M., Fitter, A. H., Young, J. P. W (2002) Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. Mol Ecol 11:1555-1564. https:// doi.org/10.1046/j.1365-294x.2002.01538.x)

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., Dufresne, A (2015) The importance of the microbiome of the plant holobiont. New Phytol 206:1196-1206. https://doi.org/10.1111/nph.13312

Vannier, N., Mony, C., Bittebière, A.-K., & Vandenkoornhuyse, P (2015) Epigenetic mechanisms and microbiota as a toolbox for plant phenotypic adjustment to environment. Front Plant Sci 6:1159. https://doi.org/10.3389/fpls.2015.01159

Vannier, N., Mony, C., Bittebiere, A.-K., Michon-Coudouel, S., Biget, M., & Vandenkoornhuyse, P (2018) A microorganisms' journey between plant generations. Microbiome 6:79. https://doi.org/10.1186/s40168-018-0459-7

Venkataraman, A., Bassis, CM., Beck, JM., Young, VB., Curtis, JL., Huffnagle., GB, Schmidt, TM (2015) Application of a neutral community model to assess structuring of the human lung microbiome. mBio 6:e02284-14. https://doi.org/10.1128/mBio.02284-14

Vink, S.N., Dini-Andreote, F., Höfle, R., Kicherer, A., and Salles, J.F (2021) Interactive effects of scion and rootstock genotypes on the root microbiome of grapevines (Vitis spp. L.). Appl Sci 11:1615. https://doi.org/10.3390/app11041615

781 Vives-Peris, V., de Ollas, C., Gómez-Cadenas, A., Pérez-Clemente, RM (2020) Root
782 exudates: from plant to rhizosphere and beyond. Plant Cell Rep 39:3–17.
783 https://doi.org/10.1007/s00299-019-02447-5

784 Voges, MJEEE., Bai., Y, Schulze-Lefert, P., Sattely, ES (2019) Plant-derived
785 coumarins shape the composition of an Arabidopsis synthetic root microbiome. Proc
786 Natl Acad Sci USA 116:12558–12565. https://doi.org/10.1073/pnas.1820691116

787 Werner, G. D. A., & Kiers, E. T (2015) Order of arrival structures arbuscular
788 mycorrhizal colonization of plants. New Phytol 205:1515-1524.
789 https://doi.org/10.1111/nph.13092

790 Xiong, C., Zhu, Y. G., Wang, J. T., Singh, B., Han, L. L., Shen, J. P., Li, P. P., Wang,
791 G. B., Wu, C. F., Ge, A. H (2021) Plant developmental stage drives the differentiation
792 in ecological role of the maize microbiome. Microbiome 9:171.
793 https://doi.org/10.1186/s40168-021-01118-6

794 Yang, T., Chen, Y., Wang, X.-X., & Dai, C.-C (2013) Plant symbionts: keys to the
795 phytosphere. Symbiosis 59:1-14. https://doi.org/10.1007/s13199-012-0190-2

796 Zarraonaindia, I., Owens, S. M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax,
797 S., Bokulich, N. A., Mills, D. A., Martin, G., & Taghavi, S (2015) The soil microbiome
798 influences grapevine-associated microbiota. mBio 6:e02527-02514.
799 https://doi.org/10.1128/mBio.02527-14

800 Zhalnina, K., Louie, K. B., Hao, Z., Mansoori, N., da Rocha, U. N., Shi, S., Cho, H.,
801 Karaoz, U., Loqué, D., & Bowen, B. P (2018) Dynamic root exudate chemistry and
802 microbial substrate preferences drive patterns in rhizosphere microbial community
803 assembly. Nat Microbiol 3:470-480. https://doi.org/10.1038/s41564-018-0129-3

804 Zhang, J., Wang, E. T., Singh, R. P., Guo, C., Shang, Y., Chen, J., & Liu, C (2019)
805 Grape berry surface bacterial microbiome: impact from the varieties and clones in the
806 same vineyard from central China. J Appl Microbiol 126:204-214.
807 https://doi.org/10.1111/jam.14124

Statements and Declarations

Funding

This work was supported by a grant from the CNRS-EC2CO program (HOLOBIONT project), CNRS-IRP program (M-AGRI project) and by the "Agence Nationale de la Recherche et de la Technologie" (ANRT) through an industrial CIFRE agreement between Château Palmer, Château Latour, Sovivins, Pépinière de Salettes and the University of Rennes 1 (Agreement N° 2017/1579).

Competing interests

The authors declared no conflicts of interest.

Author contribution

MB, CM, SP, VC and PV conceived the project. PV, CM and MB set up the methodology of the study. MB, CM, OJ, NL and PV conducted the sampling. MB conducted lab experiments with the help of AM. RCV and SMC performed the sequencing and MB and TW analyzed the sequence data. MB and TW performed the statistical analyses with advice from CM, MH and NL. MB wrote the first draft of the paper. All the authors gave their final approval for publication.

Data Accessibility

Data are available on Figshare https://figshare.com/s/6c6ca1fee254f88e0598.
Sequence data are available at European Nucleotide Archive (ENA) under accession number PRJEB47615.

Figures

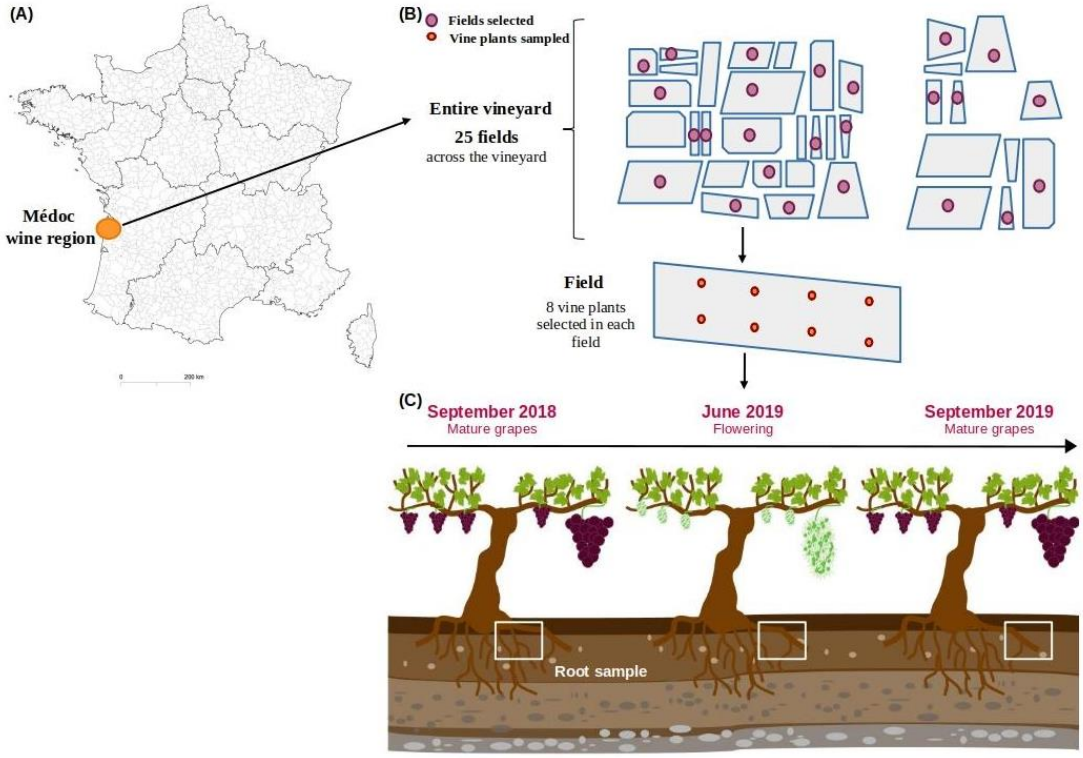
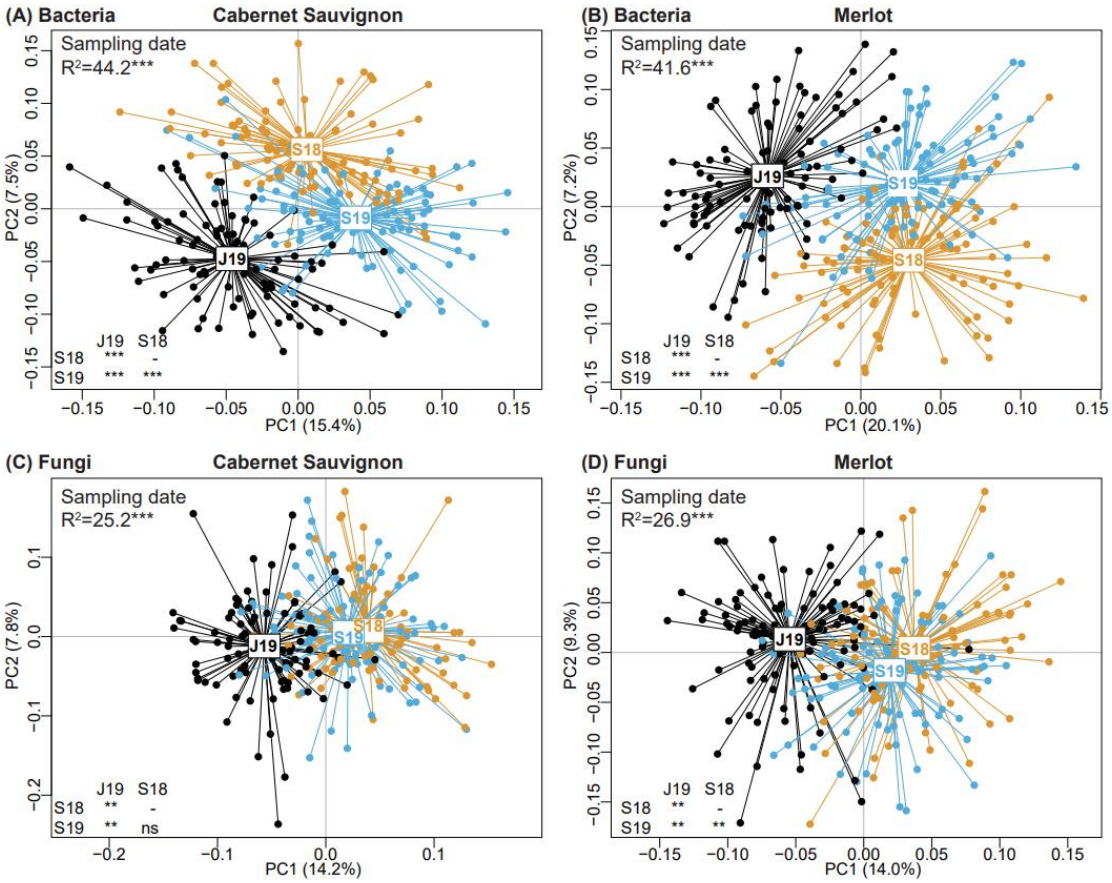


Fig.1 Experimental design and sampling scheme.

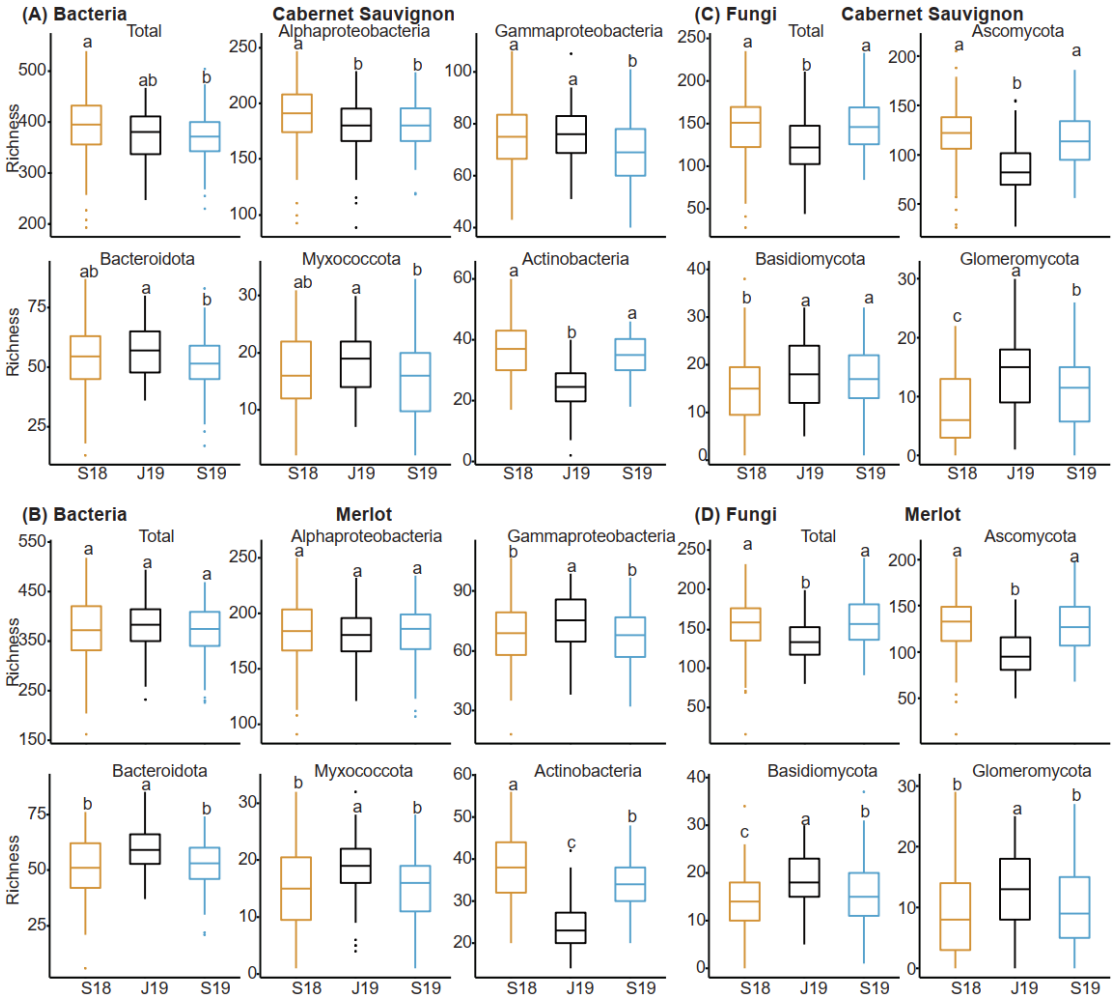
(A) Map of the study site located near Bordeaux (France) in the Medoc wine region. (B) Scheme representing the nearby location of the 25 fields across the vineyard and the 8 equidistant grapevines sampled in each field. (C) Sampling dates of root collection associated with plant developmental stage: September 2018 and 2019 (S18/S19 - mature grapes) and June 2019 (J19 - flowering period).



837

838 **Fig. 2 Principal coordinate analysis (PCoA) plots depicted the differences of the**
839 **microbial communities.**

840 Analyses were done on the sequence-cluster matrix of the bacterial (A, B) and fungal
841 (C, D) communities and for the two cultivars considered: the Cabernet-Sauvignon (A,
842 C) and the Merlot (B, D). Results from the permutation tests testing the effect of the
843 sampling dates (***: $p<0.001$) and R^2, percentage of variance explained, are included
844 on the plots. Statistical results from the pairwise comparisons are also described on the
845 plots (**: $p<0.01$; ***: $p<0.001$; ns: $p>0.05$). S18-yellow: September 2018; J19-black:
846 June 2019 and S19-blue: September 2019.



847

848 **Fig. 3 Effect of the sampling date on bacterial and fungal sequence-cluster richness**

849 **at the α-diversity scale.**

850 Linear mixed models for each sampling campaign were used to test whether the
851 sampling date influenced microbial richness (grapevine individual considered as
852 random effect). (A), (B) Results of bacterial communities for Cabernet-Sauvignon and
853 Merlot cultivars respectively. (C), (D) Results of fungal communities for the Cabernet-
854 Sauvignon and the Merlot cultivars respectively. S18-yellow: September 2018; J19-
855 black: June 2019 and S19-blue: September 2019.

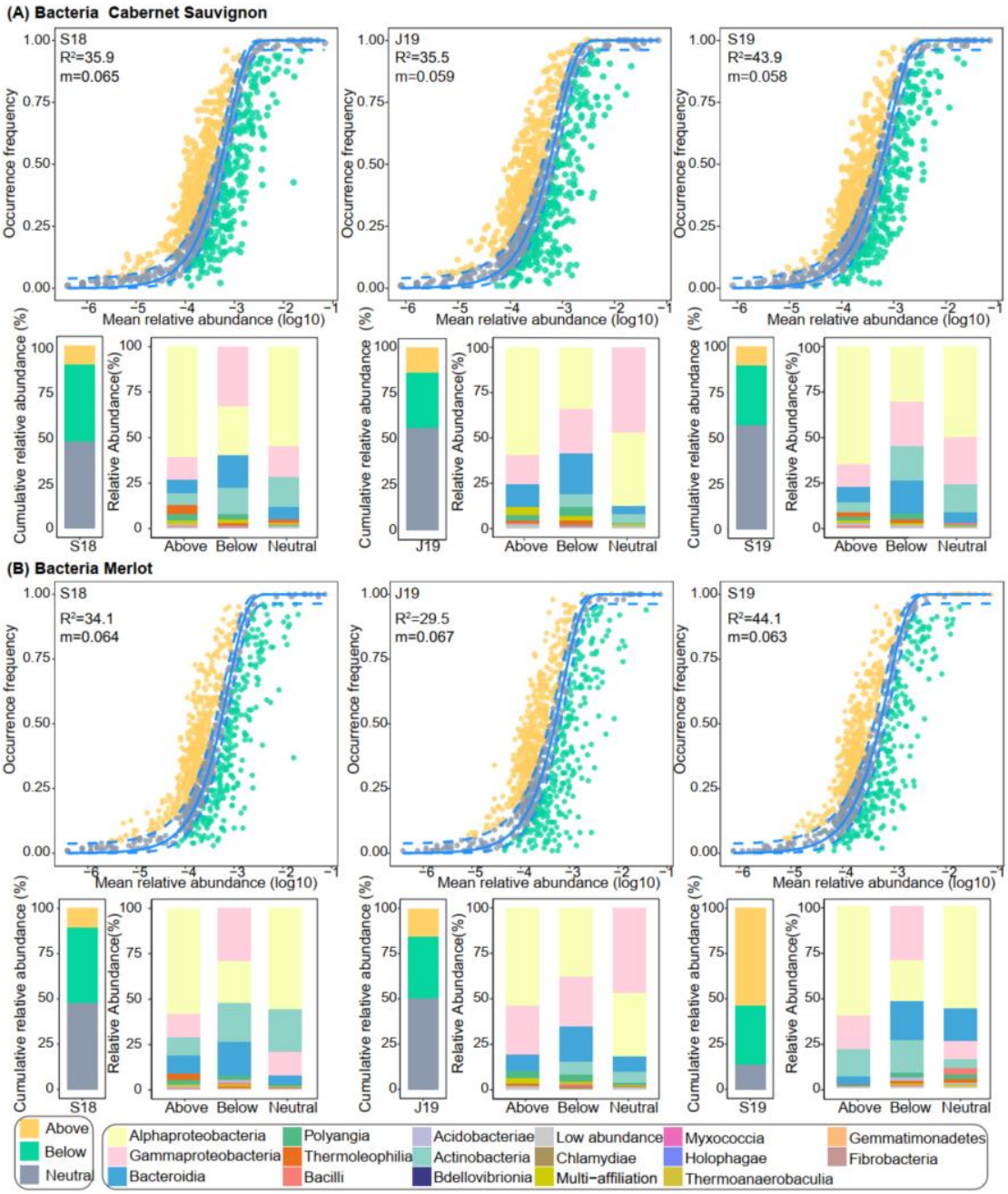


Fig. 4 Partition of deterministic and ecologically neutral processes of bacterial community assembly using neutral community model (NCM).

(A) S18/J19/S19-Cabernet-Sauvignon and (B) S18/J19/S19-Merlot, served as the community source to create neutral models. The first graphs showed the occurrence frequencies predicted through the NCM. The sequence-clusters occurring more frequently than expected were dotted in yellow while the ones occurring less frequently

were colored in green. The solid blue line indicates the best fit of the model and the dashed ones indicate 95% confidence intervals. The dashed lines indicate the envelope of neutrally. "m" (estimate rate) and R^2 ($0< R^2 \leq 1$, adequacy of the model fitting) are indicated on the graphs. The single bar charts indicated cumulative sequence-clusters occurrence frequencies of Above, Neutral and Below partitions; the combined bar charts represent ranked cumulative relative abundance of taxonomic groups of the three sequence-cluster partitions

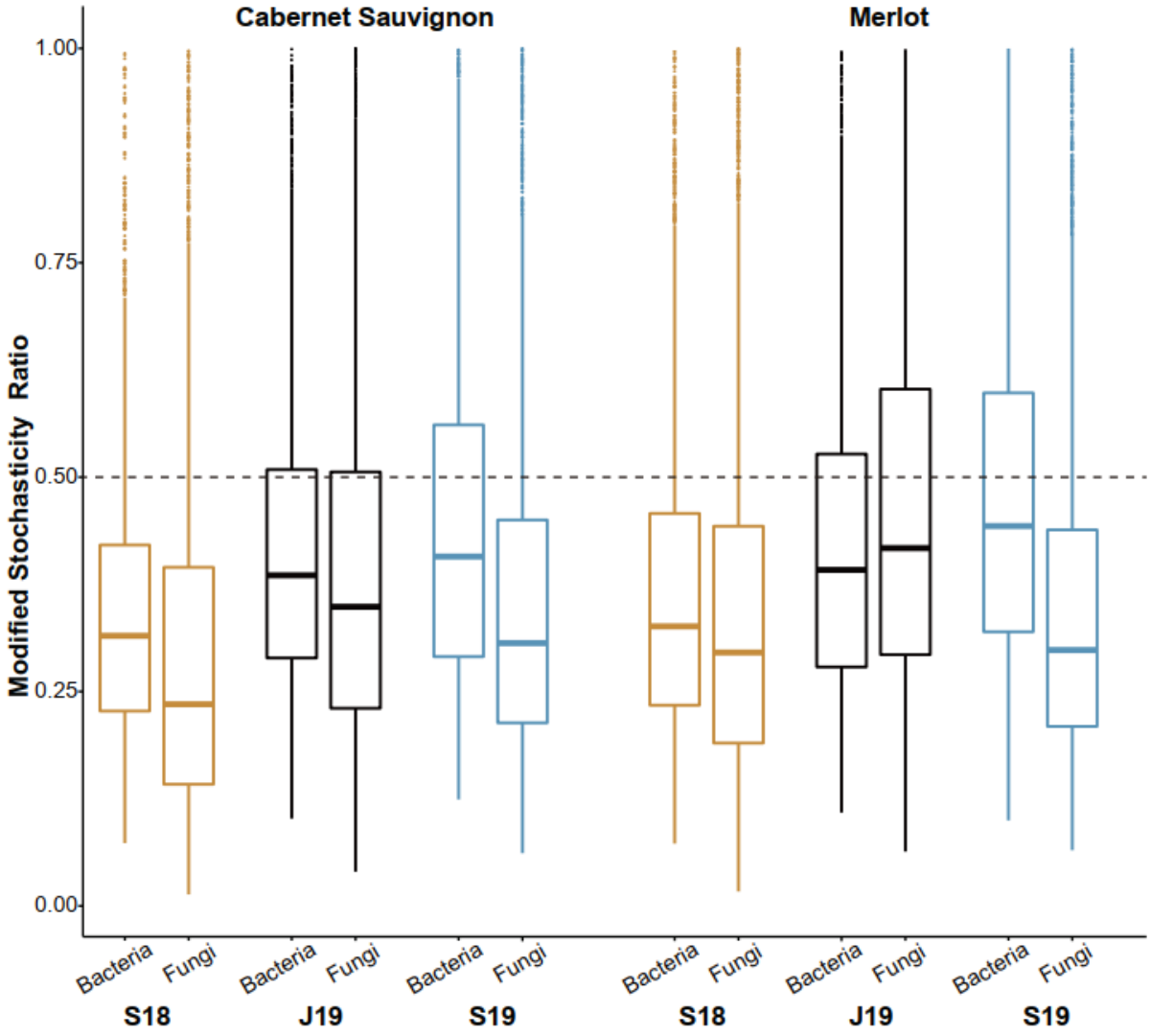


Fig. 5 Modified normalized stochasticity ratios (MST) estimated to measure the relative importance of deterministic processes of assembly.

Bar plots showed the bacterial and fungal MST values under different treatments. S18-yellow: September 2018; J19-black: June 2019 and S19-blue: September 2019.