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

3

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23 **Running title:**

24 **root microbiota composition dynamics**

25

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30 **Abstract**

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

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

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

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

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

55 **Introduction**

56 Plant development and survival are largely dependent on a remarkable diversity of
57 microorganisms (Compant et al., 2019; Lemanceau et al., 2017; Vandenkoornhuysen et
58 al., 2015). These microorganisms, either epiphytes or endophytes, can live in all plant
59 organs and tissues. All plants worldwide are colonized by many different types of
60 microorganisms, the most frequently studied being bacteria and fungi (Müller et al.,
61 2016). These plant-associated microorganisms that form plant microbiota are deeply
62 involved in supplying the plant with water and nutrients (Trivedi et al., 2020;
63 Vandenkoornhuysen et al., 2015). They help their host face biotic and abiotic stresses
64 such as drought and solar radiation (Trivedi et al., 2020; Vannier et al., 2015; Yang et
65 al., 2013). It is worth noting that microbial communities display dynamic fluctuations.
66 In continental ecosystems, dynamic fluctuations through seasons have been evidenced
67 and were found correlated with environmental constraints including temperature and
68 precipitation (Liu & Howell, 2021). Deterministic processes of microbiota assembly
69 driving changes through time can thus be hypothesized. In grafted plants, as far as we
70 know, determinism in changes through time in the microbiota composition within their
71 host-plant (i.e. the microbiota endosphere) has never been formally analyzed.

72 Endospheric microorganisms associated with plants originate from the environment,
73 mostly from the soil, which thus serves as a microbial reservoir (Vandenkoornhuysen et
74 al., 2015; Xiong et al., 2021; Zarraonaindia et al., 2015). The processes behind plant
75 colonization shaping microbial assemblage composition are thought to be mainly
76 deterministic, i.e. shaped by host selection (Morrison-Whittle et al., 2015; Xiong et al.,
77 2021; and in grafted plants, Biget et al., 2023; Dries et al., 2021; Marasco et al., 2022;
78 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; Mougél 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

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

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

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

130 **Materials and Methods**

131 **Experimental design and study site**

132 We selected individual vine plants in a single 66-ha vineyard located in Margaux,
133 France, (45°2'12.73" N; 0°40'9.84" W). Organic and biodynamic practices have been
134 used in the vineyard for 12 years. We sampled 25 fields (8 plant individuals per field),
135 the number of fields containing Cabernet Sauvignon CS (12 fields) and Merlot M (13
136 fields) clones (Fig. 1) all of similar age ranges. These two cultivars representing the
137 upper part (scion) were grafted on rootstock 101-14 mainly. However, it can be
138 highlighted that overall, three rootstocks types were used according to different
139 combinations (Table S1) and M and CS rootstocks are not strictly identical. Within one
140 plot, the plants were of the same genotype (i.e. same scion and rootstock clones). We
141 analyzed the composition of the microbial community associated with roots of the two
142 grapevine cultivars in September 2018 (S18), June 2019 (J19), and September 2019
143 (S19) (June: flowering stage and September: mature grapes before harvest). The same
144 individual grapevines were sampled in the three sampling campaigns (Fig. 1). The
145 region is characterized by a temperate climate with average temperatures of 14.2 °C
146 (2018) and 14 °C (2019); and cumulative annual precipitation of 934mm (2018) and
147 1,112mm (2019). Fig. S1 shows the average daily temperature and cumulative
148 precipitation per day recorded in the three sampling campaigns, two in 2018 and one in
149 2019 by the DEMETER weather station located in Margaux, [http://www.meteo-](http://www.meteo-agriculture.eu)
150 [agriculture.eu](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

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

190 **Raw data processing and clustering**

191 Base calling was performed on the Miseq instrument with CASAVA v1.8 software
192 (Illumina). Sequence data trimming consisted of removing primers and deleting reads
193 containing unidentified bases using Cutadapt software. The sequences were then
194 processed using the FROGS pipeline following the recommendations (Escudié et al.,
195 2018). The FROGS pipeline uses SWARM as a sequence agglomeration strategy to
196 produce “sequence clusters”.

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

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

206 The resulting contingency tables contained 14 765 700 reads for bacteria and 12 483
207 626 reads for fungi. The sequenced negative control did not contain any detectable
208 contaminating DNA. Rarefaction curves were produced using R (“vegan” package).
209 Sequencing depths of both bacterial and fungal microbiota were sufficient to limit
210 sequence cluster subsampling (Fig. S2). To perform statistical analysis, because the
211 slopes of the curves tended toward a plateau (Fig. S2), we normalized the number of
212 reads at 8 500 and 3 700 reads for the bacterial- and fungal- dataset respectively. For
213 the bacteria, we discarded only 13 samples (13/960 samples representing 1.4%) to
214 rarefy at 8500 reads per sample. For the fungi, we discarded 37 samples (37/960
215 samples representing 3.85%) to rarefy at 3700 reads per sample. None of the fields were
216 composed of less than four out of the eight samples.

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

223 **Statistical analyses**

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

234 *Effect of the sampling date on microbial assemblage structure*

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

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

249 We used Wald tests applied to linear mixed models to test whether the sampling date
250 impacted microbial richness. We constructed all the models using the richness index as
251 the explanatory variable, the “individual grapevine” as random effect, and the
252 “sampling date” as fixed effect. The linear mixed models and subsequent tests were
253 performed using the R package ‘lme4’ (Bates et al., 2014), ‘car’ (Fox & Weisberg,
254 2018) and ‘emmeans’ (Searle et al., 1980).

255 *Sampling dates differences on stochastic and deterministic assemblage processes*

256 The Sloan neutral community model (NCM) was used to determine the influence of
257 neutral processes on the bacterial and fungal community assembly inhabiting the root
258 (Sloan et al., 2006). This neutral model predicts the relationship between sequence
259 cluster detection frequency and their relative abundance (Burns et al., 2016;
260 Venkataraman et al., 2015). The model predicts that species that are more abundant in
261 the metacommunity (total source pool) are more expected to disperse and be randomly
262 present in different sampling conditions, whereas species with low abundance are more
263 likely to get lost due to ecological drift (Burns et al., 2016). In this study, we conducted
264 neutral model analyses assuming the three-date metacommunity (i.e., the total dataset
265 of a given cultivar) was the source of microbial communities. One neutral model was
266 generated per sampling date and per taxonomic group (fungi and bacteria). The
267 adequacy of model fitting was assessed through R^2 values ($0 < R^2 \leq 1$) (Sloan et al., 2006).
268 The sequence clusters from each matrix were then separated into three classes
269 depending on their occurrence frequency based on a 95% confidence interval of the
270 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.

291 **Results**

292 **Description of the microbial composition of root endosphere**

293 The Cabernet Sauvignon (CS) matrices contained 945 (12 fields, 288 samples) and 523
294 (12 fields, 278 samples) bacterial and fungal sequence clusters respectively while the
295 Merlot (M) matrices contained 943 (13 fields, 307 samples) bacterial sequence clusters
296 and 519 (13 fields, 519 samples) fungal sequence clusters.

297 **Effect of the sampling date on microbial community composition and richness**

298 The effect of sampling date on microbial community composition was analyzed
299 through PCoA. PCoA analyses demonstrated clear differences in both bacterial and
300 fungal communities among sampling dates, whatever the cultivar ($p < 0.001$; Fig. 2).

301 The effect of the sampling date explained 44.2% and 41.6% (R^2 in Fig. 2A, B) of the
302 variance of the bacterial community and 25.2% and 26.9% of the variance of the fungal
303 community (R^2 in Fig. 2C, D) in Cabernet Sauvignon and Merlot respectively.

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

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

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

318 To decipher the stochastic and deterministic processes in shaping microbial
319 communities depending on the sampling date, we conducted neutral community model
320 analyses (NCM) (Fig. 4A, B). Overall, the occurrence frequency of bacteria analyses
321 was partly defined by the neutral model ($R^2=35.5\sim43.9$ for Cabernet Sauvignon and
322 $R^2=29.5\sim44.1$ for Merlot; Fig. 4A, B). However, for the fungal taxa, we did not record
323 a proper fit to the model (negative R^2 values Fig. S6A, B) for three out of the six models.
324 Proper fits were found only for the J19 and S19 analyses ($R^2=14.8$ and $R^2=6.14$; Fig.
325 S6A) and the J19 community analyses ($R^2=15.5$; Fig. S6B) for Cabernet Sauvignon
326 and Merlot, respectively.

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

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

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

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

353 **Discussion**

354 **Composition of the grapevine root endosphere**

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

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

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

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

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

449 **Stochastic versus deterministic process of assembly of the grapevine root** 450 **endosphere over time**

451 Recent advances in statistical analyses have allowed to partition deterministic and
452 ecologically neutral processes of assembly using a null model framework (Burns et al.,
453 2016) (see Materials and Methods for the rationale). For fungi, the absence of a fit of
454 the neutral community model (NCM) analysis did not allow partitioning with statistical
455 support for stochastic and deterministic processes of assembly. However, considering
456 bacteria, we were able to estimate that the deterministic part accounted for 43 to 84%
457 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

483 oxygen and pH conditions thus stimulating rhizospheric microbial growth in these
484 specific conditions (McRose et al., 2023). It is thus not only the control of carbon
485 released by plants but also the physicochemical status which modulates the rhizosphere
486 composition. Modifications in rhizosphere microbiota and in plant microbiota
487 evidenced herein over time may thus be a consequence, at least partially, of both
488 changes in the root secondary metabolites excreted (Dang et al., 2021; Kaur & Suseela,
489 2020; Voges et al., 2019) and physicochemical modifications related to water
490 availability. The rhizosphere being the antechamber of the endosphere, this process
491 should act together with the plant immune system and the mechanism of preferential C
492 allocation to the best cooperators (Kiers et al., 2011). This process would explain the
493 determinism observed in microbiota assembly in root-endosphere samples.

494 **Convergence of temporal shifts in the microorganisms root-endosphere** 495 **composition between the two cultivars**

496 A key result of this work is the convergence of the temporal dynamics of
497 microorganisms between the two cultivars, Cabernet Sauvignon and Merlot. The
498 rootstock genotypes used in Cabernet Sauvignon (CS) are not strictly comparable to
499 Merlot (M) (Table S1). At least two plots contained a specific rootstock genotype
500 associated with Merlot scion. From this and because we have recently demonstrated
501 that rootstock is the key driver of endospheric microbiota composition (Biget et al.,
502 2023) we expected to observe differences among grafted Cabernet Sauvignon and
503 Merlot. Conversely to the expectation over drivers than plant material can be the
504 observed convergence in the microbiota composition. It can be argued that the
505 environmental conditions (lower water availability) in September might cause a
506 convergent change in the endospheric microbiota composition. It is possible that in

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

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

532 **Conclusion**

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

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808 **Statements and Declarations**

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815 **Competing interests**

816 The authors declared no conflicts of interest.

817 **Author contribution**

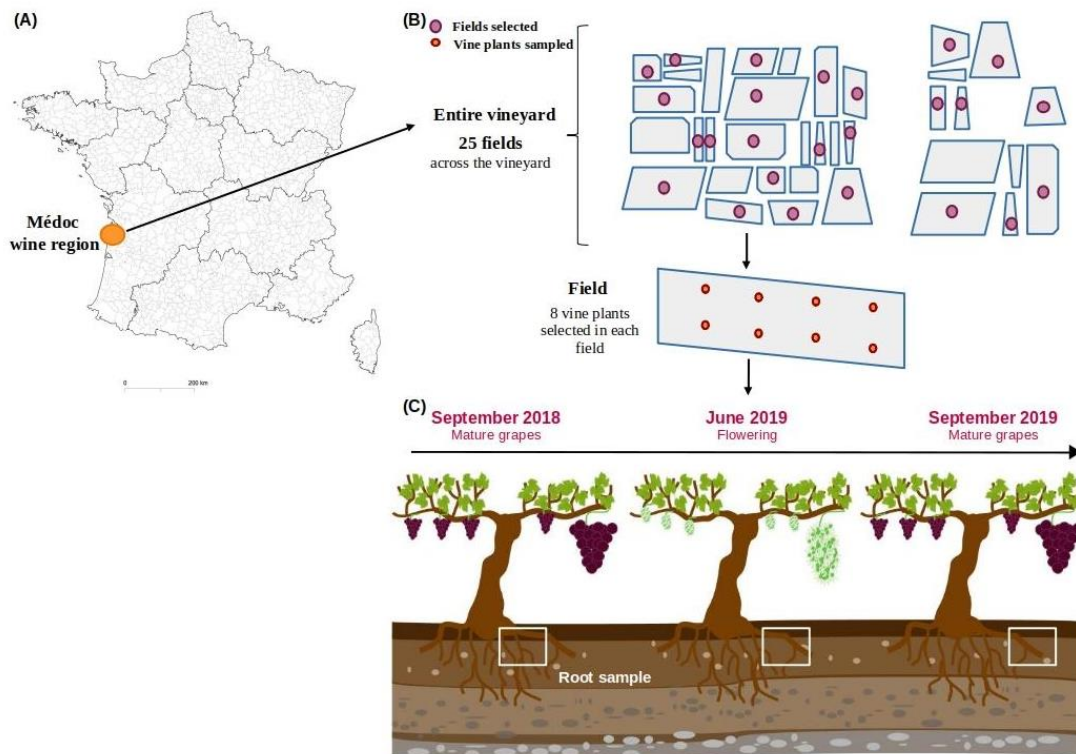
818 MB, CM, SP, VC and PV conceived the project. PV, CM and MB set up the
819 methodology of the study. MB, CM, OJ, NL and PV conducted the sampling. MB
820 conducted lab experiments with the help of AM. RCV and SMC performed the
821 sequencing and MB and TW analyzed the sequence data. MB and TW performed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

824 **Data Accessibility**

825 Data are available on Figshare <https://figshare.com/s/6c6ca1fee254f88e0598>.

826 Sequence data are available at European Nucleotide Archive (ENA) under accession
827 number PRJEB47615.

828 **Figures**



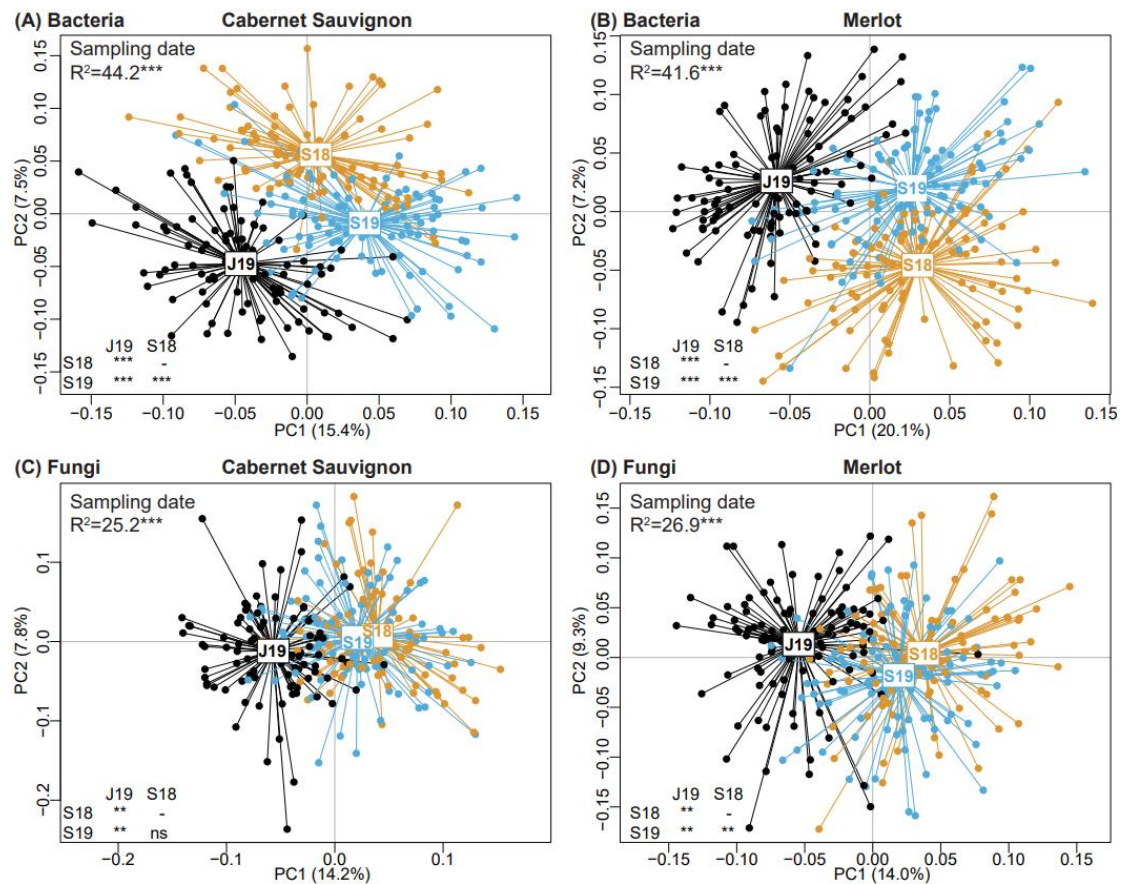
829

830 **Fig.1 Experimental design and sampling scheme.**

831 (A) Map of the study site located near Bordeaux (France) in the Medoc wine region.

832 (B) Scheme representing the nearby location of the 25 fields across the vineyard and
833 the 8 equidistant grapevines sampled in each field.

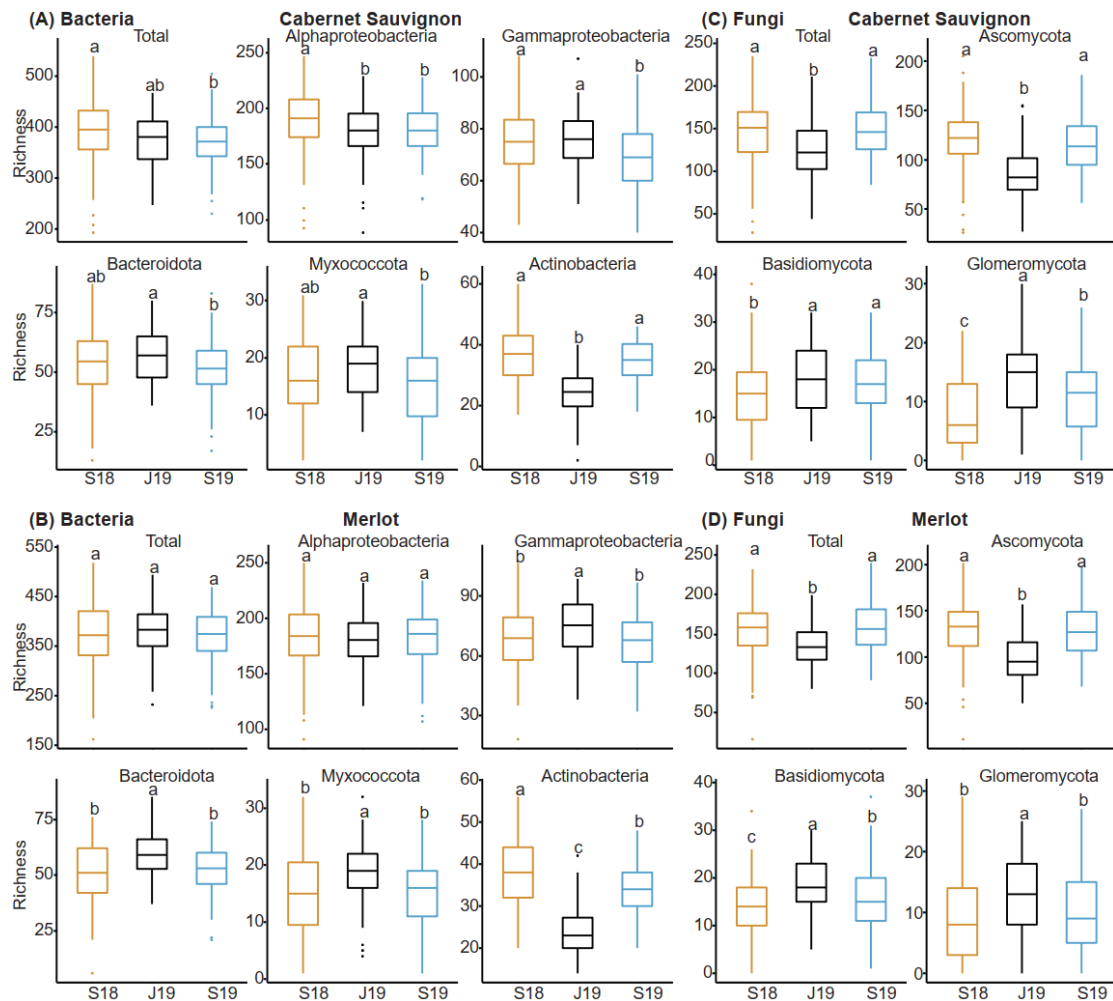
834 (C) Sampling dates of root collection associated with plant developmental stage:
835 September 2018 and 2019 (S18/S19 - mature grapes) and June 2019 (J19 - flowering
836 period).



837

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

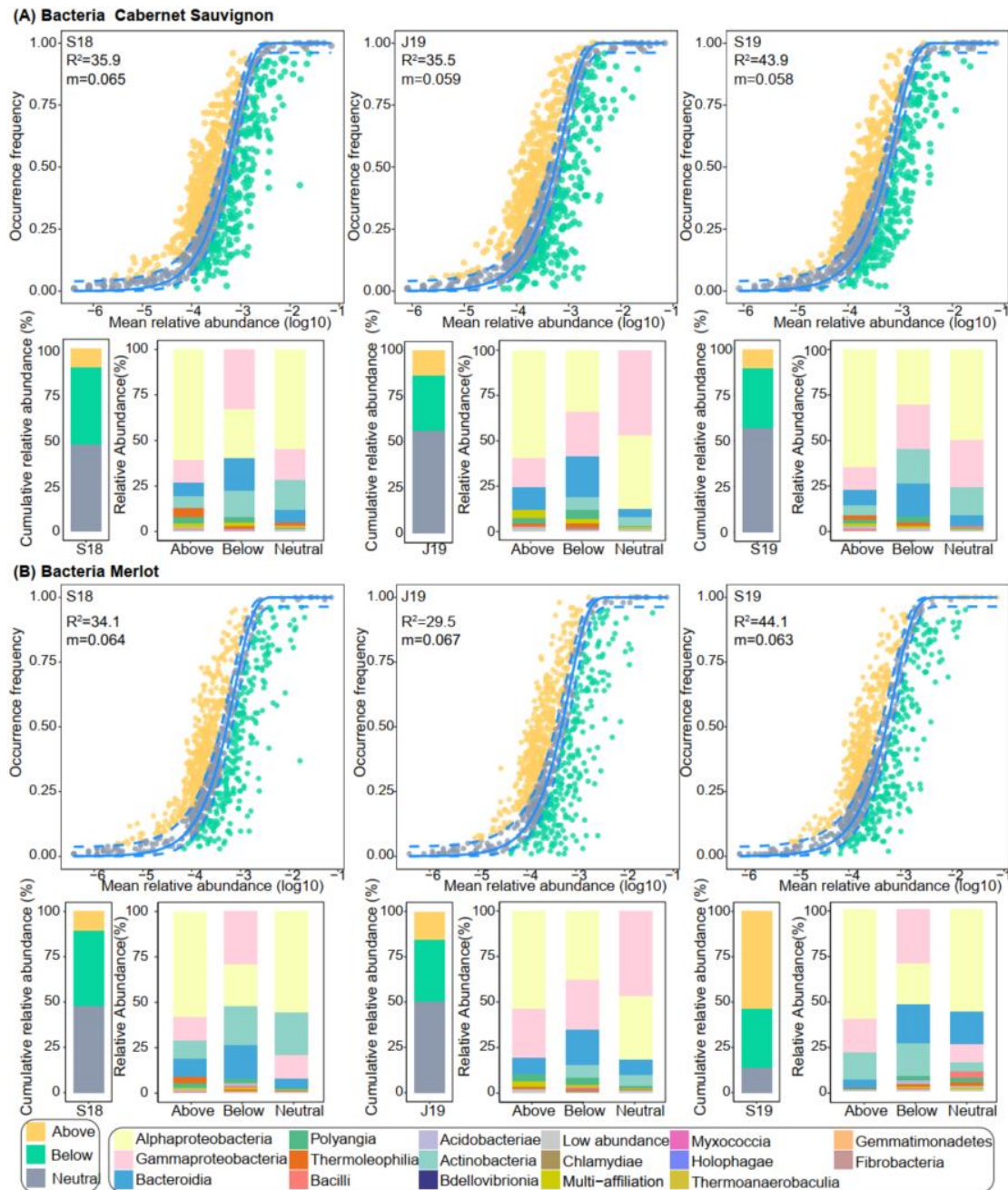
840 Analyses were done on the sequence-cluster matrix of the bacterial (A, B) and fungal
 841 (C, D) communities and for the two cultivars considered: the Cabernet-Sauvignon (A,
 842 C) and the Merlot (B, D). Results from the permutation tests testing the effect of the
 843 sampling dates (***: $p < 0.001$) 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.**

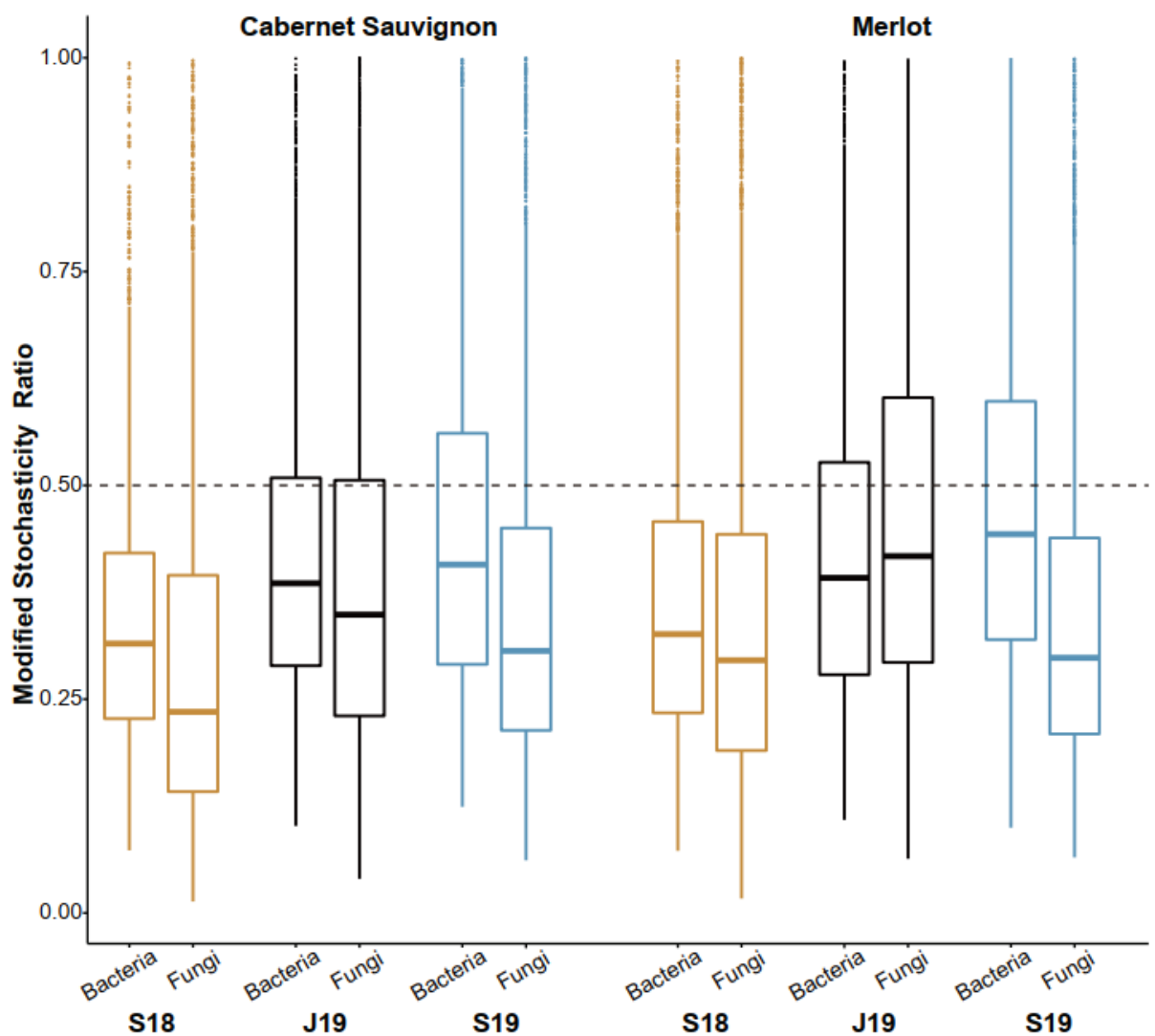
850 Linear mixed models for each sampling campaign were used to test whether the
 851 sampling date influenced microbial richness (grapevine individual considered as
 852 random effect). (A), (B) Results of bacterial communities for Cabernet-Sauvignon and
 853 Merlot cultivars respectively. (C), (D) 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.



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

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

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



870

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

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