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Microscale analysis of soil characteristics and microbiomes reveals potential impacts on plants and fruit: vineyard as a model case study

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1	Microscale analysis	of soil characterist	ics and microbiomes reveals potential											
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25 Abstract

26 Aims

Soil microbiome roles in agriculture is becoming more and more important. This importance is also reflected on the way plants are seen: complex organisms formed by the plant itself plus the microbes inhabiting its tissues, including the ones on the surface of every organ and the ones adhered or in proximity to the roots. In addition, as already demonstrated, the microbial community associated with a specific soil is able to predetermine the health status of crops. For all the above mentioned reasons, defining the microbial composition of agricultural soils and the factors driving the assemblage is pivotal to achieve more sustainable agriculture and viticulture.

34 Methods

We aimed to investigate how the soil geological characteristics influence the microbiome composition associated with close geographically related vineyards. Moreover, we studied both the top (15 cm in depth) and deep (120 cm in depth) soil layers as anthropically influenced and almost-undisturbed soil, respectively.

39 **Results**

We observed slightly different microbial communities despite the close geographical proximity of the two vineyards, which is considered one of the main determinants of the soil microbiome composition. In addition, we found that the geological characteristics of the two soils influence both the root distribution and the accumulation of pathogen- and symbiont-related genera. Sensory profiles of the Grillo wines from the two different soils confirmed the tight link between soil origin and wine traits.

45 Conclusions

In the present study, we highlight that the geological characteristics of soil can influence soil microbial
composition and assemblage in close geographically related vineyards, with a potential effect on wine
features.

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49 Introduction

50 Soil microbial biomass has crucial roles in Earth's biogeochemical cycles in both natural and 51 human-managed ecosystems (Fierer 2017). Despite the challenges that living organisms face to survive 52 in such environments, if we consider viruses, bacteria and fungi, we can estimate that each gram of soil 53 can contain up to millions of individual microorganisms (Fierer et al. 2009). These organisms play key 54 roles in nutrient cycling, soil fertility and soil carbon sequestration, and they show both direct and 55 indirect effects on plant and animal health (Fierer et al. 2009; Serna-Chavez et al. 2013; Fierer 2017). 56 In this respect, some microorganisms have evolved the ability to associate with plants, forming 57 mutualistic symbioses (e.g. arbuscular mycorrhizal fungi [AMF] and rhizobacteria). Furthermore, the 58 importance of interactions between plants and microorganisms has been described as an additive 59 ecological function that can be a major trait in extending a plant's ability to adapt to many stressful 60 environmental conditions (Bulgarelli et al. 2012). Given this wide importance of microbes on plant 61 health, they are seen as a reservoir of additional genes and functions for their host; plants with their 62 interacting microbes are defined as the so-called holobiont (Zilber-Rosenberg and Rosenberg 2008).

63 Grapevine, as do many other crops that are represented by the same or very similar genotypes, 64 display differential geographical phenotypes in terms of morphological and sensorial signatures; these 65 differences are generally described as the terroir (Van Leeuwen and Seguin 2006). Viticulturists have 66 been selectively growing vine cultivars from local wild *Vitis vinifera* subsp. sylvestris varieties, which 67 present differences among grape size and shape, berry colour, flavour, yield and many other phenotypic 68 aspects (Arroyo-García et al. 2006). The most interesting individuals have been multiplied by vegetative 69 propagation for years, during which time genetic and somatic modifications have spontaneously 70 occurred. Those events have given rise to an intra-varietal variability associated with phenotypic and 71 biochemical variation, which has led to the description of grapevine clones (Pelsy 2010). Despite this 72 very detailed characterisation, the same grape clones (which are still vegetatively propagated) can show 73 differences among phenotypic characteristics and biochemical traits when grown in different 74 environments, confirming that the plant genome is not the only player able to shape the phenotype. If 75 we look at the final product (i.e. wine), it is well known that indigenous yeast and bacteria inhabiting 76 the berries' surface can have a wide impact on the flavour and aroma of typical wines (Pretorius 2000; 77 Capece et al. 2010; Tristezza et al. 2014; Knight et al. 2015). From this view, on their journey from the 78 vineyard to the winery, grapes are transformed into wine through microbial biochemical processes, with 79 unquestionable consequences for wine quality parameters.

80 Due to the above-mentioned importance of microbes associated with grape plants and their impacts 81 on wine characteristics, researchers have tried to address what are the main factors driving the microbial 82 terroir composition of the holobiont. Among all the parameters, one of the most important is the 83 geographical location. It has been demonstrated that there are clear delineations among local natural 84 populations of yeast inhabiting berries (Gayevskiy and Goddard 2012; Bokulich et al. 2014). Similarly, 85 a study analysed the impact of geographical distance on both fungal and bacterial communities (Miura 86 et al. 2017). The latter work clearly showed that spatial processes play an important role in structuring 87 the biogeographical pattern of grape-associated fungal communities but do not influence bacterial 88 communities. Interestingly, another main factor influencing the microbial *terroir* is the soil. Researchers 89 have demonstrated that the majority of organ-associated microbes reflect the ones found in the 90 surrounding soil, and their distribution is in turn influenced by the highly localised biogeographical 91 factors and vineyard management practices (Zarraonaindia et al. 2015). The strict relationship among 92 geographical location and microbiome structure was also recently reported among the famous Italian 93 wine region of Trentino (Coller et al. 2019). The authors suggested an inverse correlation between the 94 geographical location and the bacterial and fungal community structure.

As an additional level of complexity, we need to mention that *V. vinifera* cultivars are commonly grown using rootstocks. Rootstocks are used because of their ability to cope with certain biotic and abiotic factors, such as phylloxera (Granett et al. 1987), nematodes (Stirling and Cirami 1984), salinity (Upreti and Murti 2010) and water limitations (Berdeja et al. 2015), and rootstocks also play a role in the growth-defence trade-off balance (Chitarra et al. 2017). It has been demonstrated that the microbiome of the soil surrounding a plant is strongly influenced by the rootstock genotype (Marasco et al. 2018). Those authors reported that the genotype of the grape root system can select and recruit
 microbes that will then colonise the aboveground organs, ultimately influencing both fruit and wine
 qualities.

For all the above-mentioned reasons, we decided to study the microbial composition of bulk soil associated with the root of the grapevine cultivar Grillo in a limited geographical area to determine what are the most important players able to shape the wine typicity. We selected two different soil types that lead to the production of the same Grillo wine but with different organoleptic features (Scienza and Giorgianni 2015), and we analysed the soil chemical and microbial compositions, including the wine organoleptic profile. Furthermore, to understand the anthropic-mediated impact on the microbial communities, we analysed the soil at depths of 15 and 120 cm.

111 Methods

112 Vineyard location and sampling

Our study was conducted in two different vineyards located in the municipality of Menfi (Ag), Sicily, Italy. The two vineyards are characterized by the cultivation of the same Grillo RS297 clone, grafted onto 1103 Paulsen (1103P). Grillo is one of the most popular Sicilian varieties and it is the offspring of a natural cross between Catarratto bianco and Muscat of Alessandria. Nowadays Grillo is mainly cultivated in the Trapani province and in Sicily it accounts for more than 6.500 ha.

The first field that we took in consideration is located in Contrada Finocchio $(37^{\circ}37'06.0"N 12^{\circ}54'54.9"E)$ on a marly-limestone substratum (ML) at 115 meters above the see level. The second field is located eastward of Menfi, in Contrada Bertolino $(37^{\circ}35'02.7"N 13^{\circ}00'38.3"E)$ on a calcarenitic substratum (C) at 140 m a.s.l. The two vineyards are about 8 km far from each other, both of about 1 hectare $(10.000 m^2)$ and the sampling was performed in both vineyards at 15 cm in depth (designated as superficial = S) and 120 cm of depth (designated as deep = D) in correspondence of root profiling trenches.

125 In Menfi area the summers are warm, muggy and dry and the winters are long, cold, windy, and 126 partly cloudy defining a harsh Mediterranean environment. Over the course of the year the rainfall are

127 about 490 mm and the temperature typically vary from a minimum of 8°C during the winter to the maximum of 35°C in the summer season. In both vineyards we have chosen, vines were planted in 2002 128 129 and were subjected to standard cultural practices (soil, nutrition, irrigation, canopy and pest 130 management) routinely used in the Menfi area. The training system was Guyot consisted of one fruit 131 cane of 8/10 buds and its total length was about 0.6-0.8 m per vine. Vine spacing was 2.5 m \times 0.9 m 132 (intra row and inter vines) equal to 4.444 vines per hectare and the fruit cane was trained 0.7 m above 133 ground with one pairs of surmounting catch wires for a canopy wall extending about 1.5 m above the 134 fruit cane.

135

136 Grapevine root development

137 As the root systems of grapevines are capable to reach a large volume of soil exploration, 138 influenced mostly by soil conditions, the classical profile wall method was chosen as the most 139 appropriate one to determine root distribution and density (Böhm and Köpke, 1977; Böhm, 1979). In 140 February 2017, during the dormant period, six vines per vineyards with similar scion circumferences 141 (considering the first 50 cm above the scion-rootstock junction) were selected randomly along the field. 142 For each two vines, a trench of approximately 1.20 m deep was dug parallel to the vine row, first at 1.00 143 m and then at 0.40 m distances from the vine trunk. At each distance, roots were counted by using a 1.2 144 m high and 2.0 m wide grid system placed against the profile wall, the grid was divided in sub-grid 145 block with a size of 0.2 m x 0.2 m, two vines for each trench have been considered. Roots were plotted 146 in five depths (0-20 cm, 20-40 cm, 40-60 cm, 60-80 cm, 80-100 cm) and were classified into three root 147 diameter classes according to size: $\emptyset < 1.0 \text{ mm} = \text{fine roots}; \emptyset 1.0-3.0 \text{ mm} = \text{medium roots and } \emptyset > 3.0$ 148 mm = permanent roots. Each thickness class had its own symbol to distinguish between the roots drawn on the plan. Processed data are expressed as root number $/m^2$. A rooting index has been calculated 149 150 according to (Van Zyl, 1984). A high index reflects more thin roots relative to medium and thick roots 151 as a result of more favourable soil conditions.

Two soil moisture sensors (WaterScout SM100 Spectrum Technologies - Aurora , IL, USA) has been placed in both soils at two depths (40 and 110 cm), providing the Volumetric Water Content (VWC) of soil (Resolution: 0.1% VWC; Accuracy: 3% VWC @ EC < 8 mS/cm). The two sensors were connect to one station FL SENS USB (GMR Strumenti , Scandicci, IT) data logger.

156

157 Wine profiles

Grape composition at harvest was measured on a sample of 0.5 kg of berries collected randomly from each one of the two Grillo vineyards. Soluble solids were measured by refractometer (Atago PR32) at 20 °C, pH and titratable acidity (expressed as g/L of tartaric acid) were measured using an automatic titrator (Crison Micro TT 2022, Riera Principal, 34-36 08328, Alella (Barcelona), Spain) by titration with 0.1N NaOH. Winter pruning wood from 3 replications of 15 vines of each vineyard (total 45 vines) was weighed electronically by means of a hanging scale (CH, Kern, Germany) on 26th February 2018 as an indicator of vine canopy growth in 2017.

165 Using a standard protocol (Alabi et al., 2016), Settesoli winery provided a separate vinification 166 of the grapes coming from the two soils (about 7,000 kg of grapes per vinification). The tasting analysis 167 was carried out in Settesoli using the internal trained panel test made up of 13 oenologists (8 males, 5 168 females). Demographic aspects were recorded at the beginning of the first session and no information 169 about the nature of the study was provided in order to reduce bias. Twenty-five mL aliquots of each 170 wine at $20 \pm 1^{\circ}$ C were poured into wineglasses coded with a random three-digit number and covered to 171 avoid dispersion of volatiles. Wines were then presented during four evaluation sessions using a 172 randomized design with three replicates for each wine. For the quantitative evaluation of the intensity 173 of attributes (visual, olfactory, gustatory and retro-olfactory) a questionnaire providing discrete scale 174 responses with intervals from 1 to 9 has been used.

175

176 Soil DNA isolation and sequencing

Total nucleic acids were obtained as previously reported from 1 g of soil (Angel, 2012). DNA was then cleaned using the commercial ZymoBIOMICS DNA Kit (Zymo Research, CA, USA) according to manufacturer's protocols yielding 3 to 5 µg of DNA per extraction quantified using a NanoDrop One spectrophotometer (Thermo Fisher Scientific). DNA integrity was evaluated by electrophoresis on a 1% agarose gel in 1x TAE buffer (40 mM Tris-HCl, 20 mM acetic acid, 1 mM EDTA, pH 7.5) stained with Red Safe Nucleic Acid Staining Solution (Labotaq, Sevilla, Spain) and then visualized under UV light. Three biological replicates for each condition were obtained and used as independent samples.

Illumina tag screening of the V3-V4 hypervariable regions of the 16S rRNA gene was performed on the DNA by Macrogen Inc. (South Korea), using primers 341f and 785r to build the bacterial amplicon libraries (Kuczynski et al., 2012). The primer ITS3-ITS4 were used to amplify the highly variable spacer ITS2 of the rDNA fungal operon (Lindahl et al., 2013) by Macrogen, Inc. (South Korea). Sequencing of both bacterial and fungal libraries were done with the MiSeq Illumina apparatus.

189

190 Metaphylogenomic analyses, taxonomic distributions

191 PrinSeq v0.20.4 (Schmieder and Edwards, 2011) was used for a first strict quality control on raw 192 data that were then processed in Qiime 2 (Caporaso et al., 2010). A specific pipeline was used for fungal 193 analysis: retained reads were then used to identify the start and stop sites for the ITS region using the 194 hidden Markov models (HMMs) (Rivers et al., 2018), created for fungi and 17 other groups of 195 eukaryotes. Briefly, the software allows to distinguish true sequences from sequencing errors. In order 196 to distinguish true sequences from those containing errors, sequences have been sorted by abundance 197 and then clustered in a greedy fashion at a threshold percentage of identity (97%). Trimmed sequences 198 are then analysed with DADA2 (Callahan et al., 2016), which models and corrects Illumina-sequenced 199 amplicon errors. Sequence variants are then taxonomically classified through the UNITE database (we 200 selected the reference database built on a dynamic use of clustering thresholds) (Abarenkov et al., 2010). 201 For graphic representation, only genera with an average relative abundance higher than the settled 202 threshold (0.1%) were retained.

A different pipeline was used for bacteria: quality filtering was performed with DADA2 which is able to perform chimera removal, error-correction, sequence variant calling with reads truncated at 260 bp and displaying a quality score above 20. Obtained feature sequences were summarized and annotated using the RDP classifier (Cole et al., 2013) trained to the full length 16S database retrieved from the curated NCBI database.

208

209 Statistical analyses

210 Statistical analysis for microbiome data was performed with R (Version 3.4.4) using phyloseq 211 (version 1.24.0) to import, store and analyse data (McMurdie and Holmes, 2014). To convert data from 212 phyloseq, the official extension phyloseq to deseq2 was used (Love et al., 2014). Fungal and bacterial 213 communities were used to evaluate beta-diversity deriving from the Bray-Curtis distance matrix 214 (complete dataset was considered at the OTU level clustered with a cut-off threshold of 97% identity). 215 The matrix was further used as input to run a non-parametric multivariate analysis (PERMANOVA) (p 216 values were corrected with sequential Bonferroni significance) and non-metric multidimensional 217 scaling (NMDS) was calculated among both the fungal and bacterial diversity in the samples using 218 PAST (Hammer et al., 2001). Statistical analysis for the determination of significant differences 219 between roots number was carried out using Student-Newman-Keuls test ($p \le 0.05$) Statistica version 8 220 (StatSoft, Inc.).

For the wine attributes, the relative differences between wines were analysed and confirmed submitting the judgements to statistical analysis using the ANOVA method according to Alabi et al., 223 2016.

224

225 Results

226 Soil geological description

In the area of Contrada Finocchio, where the first vineyard is located, a middle-upper Oligocene lithological succession outcrops; it consists of open shelf carbonates, mostly represented by thick-

bedded whitish marly limestones, alternating with white or greyish marls. The observed thickness of 229 each level varies from a few centimetres up to 80-100 cm. Intercalations of nummulitic and 230 231 resedimented biocalcarenites are sometimes present. The above-described lithological complex is 232 ascribed to the lower portion of the so-called Ragusa Formation, particularly to the Leonardo Member. 233 The general characteristics of the succession are strongly influenced by the predominant presence of 234 compact limestones or of the more or less marly levels containing 20%–60% clay, in addition to other constituents. The permeability degree of the lithological complex is generally scarce due to the presence 235 236 of marly levels with very low permeability; however, the local presence of limestone with high 237 secondary permeability, due to fissuration induced by tectonic processes, allows a moderate drainage 238 of the groundwater and the formation of small aquifers with local importance.

239 East the town of Menfi, where Contrada Bertolino and the second vineyard are located, outcrops 240 a middle-upper Pliocene terrigenous succession locally known as the Marnoso-arenacea del Belice 241 formation. From the bottom, it comprises several tens of metres of fine to medium sandstones; the sandy 242 particles are mainly rounded quartz grains, but there are also levels of resedimented biocalcarenites. 243 They extent upwards to sandstones and coastal calcarenites and conglomerates. The substratum in the 244 area is formed of up to 50 m of a thick package of hemipelagic shales and marls as well as brownish 245 siltstones with interbedded siltstones and calcarenite mudstones. The Marnoso-arenacea del Belice 246 formation underlies the quaternary marine deposits, which are mostly represented by lower Pleistocene 247 vellowish, partially cemented calcarenites and biocalcarenites alternating with thick beds of 248 biocalcirudites, with thin intercalations of marl levels, conglomerate lenses and calcareous sand and 249 gravel. The total thickness of the quaternary complex varies from a few metres, as in the area of interest, 250 up to tens of metres. The natural porosity characteristics of the above-mentioned lithological complexes, 251 especially of the calcarenite levels and of the small cemented sands, allows good drainage and

circulation of the groundwater, which is partially confined by marly and loam/clay grain thin-beddedintercalations with low permeability.

254

255 Soil chemical analyses

The average values of physical and chemical characteristics of the two soils are reported in Supplementary Table 1. The pH is alkaline, but it is in an optimal pH range for plant nutrient uptake (Proffitt and Campbell-Clause 2012).

259

260 Root system distribution and development

261 Table 1 reports the root density at two distances from the vine trunk (0.40 and 1 m), dividing the roots into three diameter classes, and the average number of roots/m² found in the two soils along the 262 263 dug trench. In those soil conditions, we found that the total root number of C soil was higher compared 264 with ML soil 0.40 m from the vine trunk, while the mean root density did not vary significantly at 1 m 265 from the vine trunk. Considering the root size, we found thinner roots ($\emptyset < 1 \text{ mm}$) in ML soil 1 m from 266 the vine trunk and more abundant, thicker roots (Ø 1–3 mm and Ø > 3 mm) for both distances (0.4 and 267 1 m) in C soil, where the sandy texture allowed more root diffusion (Table 1). We found some significant 268 differences: an average ratio of 7.1 versus 3.8 at 0.4 m from the vine trunk (p < 0.05) and 10.9 versus 269 4.3 at 1 m from the vine trunk (p < 0.05) for ML and C soil, respectively (Supplementary Table 2).

270 Regarding the vertical distribution, in C soil, most roots ($62 \operatorname{roots}/0.04 \operatorname{m}^2$) were located between 271 0.40 and 0.60 m of soil depth and 0.40 m from the vine trunk. Indeed, for ML soil, we observed most 272 roots at the same depth (0.40–0.60 m) but at 1 m from the vine trunk (48 roots/0.2 m²).

273 Soil humidity was monitored during the season at two depths, 40 and 110 cm. The higher 274 humidity in silt, owing to marly limestone, permits a better water holding capacity. In C soil, due to its 275 high sandy texture and high drainage, the lower humidity indicates a reduced ability to water retain 276 (Supplementary Fig. 1).

278 Bacterial community diversity and composition

The bacterial community was analysed at both the family and the genus level: the number of retained sequences after chimera removal and taxonomical assignment ranged from 29,924 to 51,428. The diversity indices (Shannon and Simpson) indicate a significant difference between superficial and deep microbial composition in both soil types (Table 2). Interestingly both indices suggest that calcarenite superficial (CS) displays the same diversity as deep marly limestone (MLD).

284 The complete bacterial community composition for each sample type at the family level is 285 reported in Supplementary Table 3, with statistical results of pairwise comparisons reported in 286 Supplementary Table 4. To simplify, we describe results for the families that represent at least 2% of 287 the bacterial community (Fig. 1a). Regarding C soil, we found that the composition of superficial (CS) 288 and deep (CD) samples is quite different, as also indicated by the statistical analysis in Supplementary 289 Table 4 and in the PCoA (Supplementary Fig. 2). It is worth noting that CD displays more taxa and the 290 most variable composition compared with all the other samples. In detail, the superficial sample is richer 291 in Nitrososphaeraceae and Bacillaceae, whereas Sphingobacteriaceae and Comamonadaceae are more 292 abundant in the 120-cm depth sample (Supplementary Table 4). Based on pairwise comparison of 293 superficial calcarenite and marly limestone samples, only three families showed significant differences 294 in abundance. Among the three families, Bacillaceae and Nitrososphaeraceae are more abundant in CS 295 than MLS. Regarding ML soil, we observed 21 families that are more abundant in the superficial (MLS) 296 sample and 28 families that are more represented at the 120-cm depth (MLD) sample. Among the 297 differentially abundant families, Acidobacteriaceae, Sphingomonadaceae, Gemmatimonadaceae, 298 Nitrospiraceae, Acidimicrobiaceae and Verrucomicrobia subdivision 3 are overrepresented in MLS; in 299 comparison, Bacillaceae, Nitrososphaeraceae, Streptomycetaceae and Sphingobacteriaceae are 300 overrepresented in MLD. We then also compared the two deep soil types and observed a larger number 301 of differentially abundant families. There are 36 overrepresented families in MLD, including 302 Nitrososphaeraceae, Bacillaceae, Hyphomicrobiaceae, Rhodospirillaceae, Chitinophagaceae and 303 Sinobacteraceae, which belong to the top selected families representing about the 60% of microbial

304 composition. In parallel, 32 families are more abundant in CD, including *Sphingobacteriaceae*,
 305 *Comamonadaceae*, *Pseudomonadaceae*, *Nitrospiraceae*, *Acidobacteriaceae*, *Sphingomonadaceae* and
 306 *Cytophagaceae*.

307 Regarding the bacterial composition at the genus level (Supplementary Table 5), we decided to 308 retain only taxa representing at least 2% of the overall community (Fig. 1b). Pairwise comparisons were 309 done to determine the significant differences among sample types (Supplementary Table 6). First, we 310 started by comparing CS and CD. There are eight genera that are more abundant in CS, whereas 21 311 genera are more abundant in CD. Some of the genera more abundant in CD soil are Pedobacter, 312 Flavobacterium, Variovorax and Pseudomonas. When comparing CS and MLS, we observed only a 313 few differences: four genera are more abundant in CS, among them Flavobacterium, and only one is 314 more abundant in MLS. Then, we compared MLS to MLD, observing a large number of differentially abundant genera: 37 are more abundant in MLS whereas 50 are more abundant in MLD soil. Some of 315 316 the more abundant genera more in MLS are Acidobacterium, Sphingomonas, Nordella, 317 Stenotrophobacter, Nitrospira and Vicinamibacter. In parallel, some of the more abundant genera in 318 MLD are Flavobacterium, Pedobacter and Steroidobacter. Finally, we also compared the genera of 319 MLD and CD. Similarly to what we found for the families, this comparison produced the largest number 320 of differentially abundant genera: 61 are more abundant in MLD and 62 are more abundant in CD. 321 Among them, Steroidobacter and Thiobacter are more abundant in MLD and Pedobacter, 322 Flavobacterium, Variovorax, Acidobacterium, Nitrospira, Nordella, Vicinamibacter, Sphingomonas 323 and Stenotrophobacter are more abundant in CD.

324

325 Fungal community diversity and composition

We also analysed the fungal communities at both the family and the genus level: the number of retained sequences after chimera removal and taxonomical assignment ranged from 40,294 to 73,785. The diversity indices (Shannon and Simpson) indicate that in both soil types there is a significant difference between superficial and deep microbial composition (Table 3). The difference is moremarked in calcarenite than in marly limestone soil.

331 The complete fungal community composition for each sample type at the family level is reported 332 in Supplementary Table 5, with statistical results of pairwise comparisons reported in Supplementary 333 Table 6. For simplicity, we describe results for the families that represent at least the 2% of the fungal 334 community (Fig. 2a). As a first, general observation, we can note that at least the family level, the 335 composition of both CD and MLD is almost the same. There is only one family (Phaffomycetaceae, 336 which does not represent at least 2% of the fungal community) that shows a differential abundance 337 (Supplementary Table 6). When comparing CS and CD, four families are more abundant in CS and six 338 are more abundant in CD. Among the more abundant families in CD samples are *Botryosphaeriaceae*, 339 Togniniaceae and Chaetomiaceae, each of which represents at least 2% of the fungal community. We 340 then compared CS and MLS: eight families are more abundant in CS (among them the *Clodosporiaceae*) 341 and 13 are more abundant in MLS. Among the latter are Dermataceae and Togniniaceae. Finally, we 342 compared MLS and MLD: 13 families are more abundant in MLS (among them Dermataceae) and 10 343 families are more abundant in MLD. Among the latter group, Botryosphaeriaceae and Chaetomiaceae 344 are above the 2% threshold.

345 The complete fungal community composition for each sample type at the genus level is reported 346 in Supplementary Table 9, with statistical results of pairwise comparisons reported in Supplementary 347 Table 10. For simplicity, we describe results for the families that represent at least the 2% of the fungal 348 community (Fig. 2b). Similarly to what we observed for the family level, there are minimal genus-level 349 differences between CD and MLD. We observed only one genus that is more abundant in CD 350 (Stemphylium) and one more abundant in MLD (Cyberlindnera); both genera account for less than the 351 selected 2% threshold of the fungal community. When comparing CS and CD, 18 genera are more 352 abundant in CS (among them, only *Dactylonectria* is above the 2% threshold) and six genera are more 353 abundant in CD. Among the latter are Neofusicoccum, Camarosporium, Phaeoacremonium and 354 Humicola. When comparing the composition of the two superficial soils (CS vs MLS), we found more

differentially abundant genera (54 in total): 25 more abundant in CS and 29 more abundant in MLS. Regarding genera that represent at least 2% of the fungal community, *Chaetomium* is more abundant in CS whereas *Laetinaevia* and *Phaeoacremonium* are more abundant in MLS. For the MLS and MLD comparison, there are 28 more abundant genera in MLS (only *Laetinaevia* and *Dactylonectria* are above the 2% threshold), whereas there 19 are more abundant genera in MLD (only *Camarosporium* and *Neofusicoccum* are above the 2% threshold).

361 Specific fungal genera can have an important impact on plant development; hence, we also 362 focussed on pathogenic (Fig. 3) and mycorrhizal (Fig. 4) fungi. Regarding pathogenic fungi - we 363 selected the genera that are usually reported as grape pathogens and which are already reported in soil 364 - in both soils, *Neofusicoccum* species are more abundant in the 120-cm depth samples than in 365 superficial ones. On the contrary, *Ilyonectria* is more abundant in both soil types in the superficial layer. 366 In addition, in calcarenite soil, we observed a significant accumulation of *Phaeoacremonium* in CD. 367 Moreover, the genus Cadophora is more abundant in MLS than MLD. Regarding mycorrhiza, in both 368 soils *Glomus* is more abundant in MLD. *Rhizophagus* shows an opposite pattern: it is more abundant in 369 deep samples (CD and MLD) than superficial (CS and MLS) samples. Finally, we detected 370 Funneliformis only in MLS.

371

372 *Community structure*

373 Community structure is always represented by two independent factors: the diversity and the 374 complexity of taxa present in each sample. Diversity indices (Taxa, Shannon, Simpson and Evenness), 375 which represent species richness and evenness, were calculated for both bacteria (Table 2) and fungi 376 (Table 3) in CS, CD, MLS and MLD. In addition, after the bioinformatics classification of amplicons 377 for both the 16S, ITS and the two communities analysed together, we reduced the dataset of each 378 biological replicate to a bidimensional scaling using a Bray-Curtis distance matrix and plotting the 379 results in corresponding non-metric multidimensional scaling (NMDS), as reported in Supplementary 380 Fig. 3. A cluster heatmap of 16S and ITS communities considered together is reported in Supplementary

Fig. 4. Moreover, co-occurrence analyses of 16S, ITS and the two communities analysed together are reported in Supplementary Figs. 5, 6 and 7. Statistical analyses of co-occurrence relationship are reported in Supplementary Tables 11, 12 and 13.

384

385 Wine sensory analyses

Berry and must features are reported in Table 4. Considering that the two vineyards have the same row and interrow spacing, there was higher productivity in the vineyard grown in C soil. On the other hand, the must chemical composition signalled a richer sugar content where the yield was lower (ML soil), combined with a lower level in total acidity. There were no differences when considering must YAN (yeast assimilable nitrogen).

The tasting results are reported in Fig. 5. The results show a good differentiation between the two wines. Regarding the olfactory scents, the wine obtained from grapes grown in C soil resulted was richer and generally more interesting, with higher scores for orange blossom and elegance, but also in terms of pleasantness and floral retro olfactory sensations. The wine obtained from grapes grown in ML soil was richer in ripe fruit notes such as melon, pear and citrus. In the mouth, there were no differences for sapidity, body and acidity.

397

398 Discussion

We aimed to investigate the possibility that not only the geographical distance, but also the soil geological characteristics are involved in the definition of the microbial composition. That factor can, together with the physiochemical characteristic of soil, impact wine typicity. Furthermore, we also considered that the vineyard is an anthropic environment; hence, we analysed both the superficial (anthropic disturbed) and deep (almost undisturbed) soils to understand how the microbial composition shifts along the profile. It is well known that one of the most important factors influencing the microbial composition associated with grapevine plants is the geographical region in which they are growing 406 (Berendsen et al. 2012; Mezzasalma et al. 2018; Coller et al. 2019). On the other hand, it is also well 407 known that soil microbes strongly influence the microbial composition of their hosts, playing an 408 important role in shaping the vine-associated microorganisms and the microbial community structures 409 (Manici et al. 2017; Nerva et al. 2019). For these reasons, we decided to analyse two close 410 geographically related soils with different geological characteristics: calcarenite and marly limestone 411 soil.

412 An interesting observation is the root ratio between thin versus medium and thick roots (rooting 413 index): ML soil displayed much higher rooting indexes than C soil for both distances (0.4 and 1 m) from 414 the vine. Based on this finding, we propose that ML soil conditions are more favourable for wide and 415 diffuse soil root exploration. Furthermore, it is interesting to observe that in the vertical root profile, C 416 soil displayed the highest root concentration in the 0–0.60-m soil depth for both trunk distances (140 417 roots/m² in total), suggesting an impairment in colonising the deep soil. In ML soil, the roots were able 418 to explore a wider soil area (from 0-0.8 m) because there was no physical resistance and a lower root 419 density, especially considering the closer trunk distance (0.40 m). In addition, for both soil types in the 420 deeper layers (0.80–1.0 m), the root density decreased significantly. Considering the deeper soil level, 421 both soils have the same humidity in spring, but as the seasons progress, the differences between soils 422 are more and more evident. This different behaviour of soil to retain humidity markedly influences the 423 root distribution and density. Indeed, water scarcity boosts root growth and diffusion, phenomena that 424 our data confirmed, namely more medium roots close to the vine trunk and thicker roots far from the 425 vine trunk in C soil.

As a first, general consideration of the bacterial community, we found that, as previously reported by other researchers (Zarraonaindia et al. 2015; Coller et al. 2019), the superficial layer displays quite similar bacterial features, with some minor differences between the two geographically related sites. Interestingly, this pattern seems to disappear completely in the deep samples: the bacterial analysis showed 123 differentially abundant genera between the two soil types. This result is quite interesting because the majority of papers have always looked at the superficial layer, which is highly perturbed by human activities, agricultural residues and climatic features. Of note, this pattern seems to be the
complete opposite for the fungal community: the two superficial soil types displayed 55 differentially
abundant genera, but there were only two differentially abundant genera for the deep soil types.

435 It is worth noting that the wines produced by the two vineyards displayed different organoleptic 436 characteristics: the wine produced from grapes grown in C soil showed a higher score in terms of 437 appreciation for its complexity in terms of olfactory sensory and pleasantness, while the wine from 438 grapes grown in ML soil displayed different olfactory scents. Furthermore, the yield and quality of 439 berries showed minor differences, with a significantly higher sugar content in wine from grapes grown 440 in ML soil and a significantly higher acidity level for must for wine from grapes grown in C soil. 441 Accordingly, plant microbiomes can widely impact wine features, and this aspect is well described by 442 the microbial terroir concept (Van Leeuwen and Seguin 2006; Gilbert et al. 2014; Knight et al. 2015). 443 Based on our results, we can speculate that, although the chemical composition of the two soil types is 444 quite different and impacts the organoleptic qualities of wines, the different soil microbial composition 445 can play a part by affecting quality features in light of the soil-plant continuum. For example, the 446 presence of different mycorrhizal species between the two soil types can result in different physiological 447 and biochemical modulation of the plant metabolism. Mycorrhizal fungi are known to play crucial 448 ecological services, for example, the enhancement of the plant nutritional status (both for water and 449 minerals) and the induction of the priming state triggering growth-defence tradeoff responses (Balestrini 450 and Lumini 2018; Alagna et al. 2020). From this perspective, it is also well known that some 451 mycorrhizal species can differentially impact plant behaviours (Volpe et al. 2018). Thus, in this 452 experiment, the presence of different mycorrhizal species can impact the grape behaviour, inducing 453 physiological and biochemical adjustment between the two selected sites.

The main factors that are considered to drive plant biochemical responses are: i) the ecology of plant-associated microbes, ii) viticulture management and iii) environmental conditions. The impact(s) on grape metabolomics and wine flavour is likely due to complex interactions between them that need further investigation to be dissected. In this respect, a recent work (Vadakattu et al. 2019) clearly 458 demonstrated that fungal and bacterial communities analysed in different soils are associated with 459 diverse levels of rotundone concentration in grape berries. In detail, the authors demonstrated that the 460 microbial structure and the well-connected soil bacterial community co-occurrence networks were 461 linked to the high rotundone areas with respect to lower ones. In addition, a recent work highlighted 462 how the soil fungal communities of Pinot noir cv. played the principal role in shaping wine aroma 463 profiles and regional distinctiveness more than soil or climatic features (Liu et al. 2020). These findings 464 further support the strong connection between soil microbial communities and plant performance, 465 including fruit production yield and quality characteristics.

466 The microbial continuum between soil and plants can have also detrimental effects, favouring 467 colonisation by fungal pathogens (Manici et al. 2017; Nerva et al. 2019). Therefore, we decided to 468 examine wood pathogens, which can penetrate through trunk wounds (especially the ones caused by 469 agricultural practices and that are close to the soil surface) and through the root system, causing plant 470 illness (Whiteman et al. 2003; Giménez-Jaime et al. 2006; Aroca et al. 2010; Gramaje and Armengol 471 2011). In C soil, two wood pathogen genera (Neofusicoccum and Phaeoacremonium) are significantly 472 overrepresented in the 120-cm depth sample (both > 4 times more abundant in the deep soils than in the 473 superficial one), whereas in ML soil, only Neofusicoccum is overrepresented in the 120-cm depth soil 474 (about 3.5 times more abundant than in superficial sample). This result is quite interesting because, as 475 previously reported, the accumulation of fungal pathogens in vineyards occurs i) after long term 476 cultivation (Manici et al. 2017) and ii) where infected plants are present (Nerva et al. 2019). On the 477 contrary, the Ilvonectria genus, which is associated with black foot disease (Cabrala et al. 2012), is more 478 abundant in the superficial samples of both soil types; this phenomenon represents an issue for the plant 479 health status and for young plants, which are routinely used as replacement for died and/or compromised 480 vines (Agustí-Brisach et al. 2014). Interestingly, in the analysed vineyards, we did not observe any sign 481 of esca or black foot diseases, suggesting that the complexity of the plant-pathogen-environment 482 interaction does not lead to symptom development.

483 We also detected some genera of fungal and bacterial plant symbionts. We first focused on 484 mycorrhizal fungi, which have a relevant impact due to the involvement in complex interactions with 485 plants (Balestrini and Lumini 2018). They are able to induce physiological changes such as the 486 resistance induction against biotic (Pozo et al. 2013; Volpe et al. 2018; Alagna et al. 2020) and abiotic 487 (Balestrini et al. 2017; Balestrini et al. 2018; Mannino et al. 2020) stresses, which fall into the so-called 488 mycorrhiza induced resistance (MIR) (Pozo and Azcón-Aguilar 2007; Jung et al. 2012; Cameron et al. 489 2013). The Glomus genus is more abundant in both CD and MLD, suggesting that the higher proportion 490 of fine roots found in the deep layers are the ones more active in recruiting microbial symbionts. It is 491 worth noting that Glomus species are often associated with an enhanced tolerance to salinity and water 492 deficit stresses (Fileccia et al. 2017; Harshavardhan and Kumar 2018; Zhang et al. 2018), suggesting 493 that this genus can play important roles for vine adaptation to the semi-arid Mediterranean areas. In 494 parallel, Funneliformis and Rhizophagus genera, which are well-known plant symbionts (Chitarra et al. 495 2017), are significantly overrepresented in the MLS compared with MLD and C samples. This result is 496 consistent with the root distribution, where in the ML vineyard we observed wide root diffusion up to 497 80 cm form the surface and then a strong reduction in root density. Moreover, we also observed a 498 significant difference for the fine roots (< 3 mm diameter), which are more abundant in ML than in C 499 soil, allowing the establishment of more symbioses.

500 Flavobacteriaceae, which comprises the Flavobacterium genus (among others), is a ubiquitous 501 family of bacteria, commonly found in agricultural soils, and is often associated with positive features 502 such as the ability to degrade pesticides (Navarisseri et al. 2015; Parte et al. 2017) and the ability to 503 induce resistance against fungal and bacterial pathogens in several crops (Kolton et al. 2016; Kwak et 504 al. 2018). We observed an overrepresentation of the above-mentioned family and genus in both CD and 505 MLD, suggesting that this group of microbes can also play important role in protecting plants against 506 pathogens. Of note, the Bacillus genus, which also encompasses several species that exert beneficial 507 effects on plants (Fendrihan et al. 2016; Shafi et al. 2017), is differentially represented in the two soil 508 types. For C soil, we observed a significant accumulation of such genus in the superficial samples, whereas for ML soil, it overaccumulates in the deep layer. This result suggests different soil propensities
to host this genus, and this eventuality deserves further investigation.

511 We also aimed to enrich the knowledge about the soil microbiome because it is a frontier 512 research field. Crop losses due to plant pathogens are an ever-increasing threat for agricultural 513 production. While food demands increase, there is a compelling need to reduce the use of 514 environmentally harmful pesticides and agrochemicals (Pennock et al. 2015). To initiate more 515 sustainable agriculture, manipulation of microbiomes associated with plants and soils has been 516 suggested as a reliable alternative for impairing pathogens development and to obtain suppressive 517 conditions (Raaijmakers and Mazzola 2016; Fierer 2017; Trivedi et al. 2020). However, we still need 518 to unravel how the complex interactions among plant, pathogen and soil features determine the 519 development of plant diseases under field conditions. In this respect, researcher provisions about all the 520 drivers involved in determine the assembly of the host-associated microbiome, such as the genetic 521 background of the pathogen and the host and the presence of biotic and abiotic stresses, are decisive to 522 decipher the outcome of plant-pathogen interactions (Kwak et al. 2018). Among all the field drivers, it 523 was recently demonstrated that the initial soil microbiome composition can predetermine whether plants 524 are able to survive or succumb to diseases (Wei et al. 2019). Considering these data, the descriptions of 525 soil microbiomes are of pivotal importance to develop new agricultural strategies and to achieve the 526 needs of more sustainable viticulture.

527

528 Conclusions

In this study, we have highlighted that the geological characteristics of soil can impact both plant root development and soil microbial composition and assemblage in the different soil layers with a final effect on wine features (summarised in Fig. 6). The grapevine is regarded as one of the most impactful crop species in terms of environmental sustainability, and thus the soil microbial composition can play a pivotal role in enhancing the ability of vines to cope with biotic and abiotic stresses. The ability to exploit the native microbial diversity of soils will became one of the most important agricultural 535 practices in the future; hence, describing the microbial species associated to the semi-arid Mediterranean 536 environment has become essential. Furthermore, the soil-feature-mediated root distribution play also 537 important role for the plant water availability, because the possibility to explore the deep layers increase the possibility to exploit tasks of soil where the water is more available. This is an additional detail to 538 539 take into account when considering a soil for the successful establishment of a vineyard, especially if it 540 is located in semi-arid regions where water availability is limited and its use in agriculture plays a 541 fundamental role in defining the sustainability. To date, it is still impossible, at least in a natural 542 environment, to define the contribute of the single feature (soil characteristics, microbial composition, 543 physiochemical parameters, etc.) on the plant performances and for this reason we can only suppose 544 that the differences observed between the two vineyards are due to the combination of such interacting 545 parameters. Due to the potential biotechnological application of microbes in agriculture, additional 546 studies to link the soil microbial composition and the wines features are still ongoing.

Declarations

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Conflicts of Interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The SRA accession numbers of the NGS reported in this paper are deposited in NCBI under the BioProject PRJNA655455; BioSample SAMN15735114 and SAMN15735115; SRA accession SRR12436974 and SRR12436973.

Code availability

Not applicable.

Author contributions

L.N. and W.C. designed the experimental system, carried out the wet lab experiments, analysed data and wrote the manuscript draft. D.T., L.N. and W.C. worked out vine roots system. D.T. analysed wines sensory description. D.T., A.G, L.M. and G.G. helped to design the experiments, contributed to the writing and carefully revised the manuscript.

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- 740

741 **FIGURE LEGENDS**

Figure 1. Relative abundances of bacterial families (A) and genera (B) among soil samples. CS: calcarenite superficial; CD: calcarenite 120 cm deep; MLS: marly-limestone superficial; MLD: marlylimestone 120 cm deep. Only orders or genera representing at least the 1% over the total number of classified amplicons were retained (n=3).

Figure 2. Relative abundances of fungal families (A) and genera (B) among soil samples. CS: calcarenite superficial; CD: calcarenite 120 cm deep; MLS: marly-limestone superficial; MLD: marly-limestone 120 cm deep. Only orders or genera representing at least the 1% over the total number of classified amplicons were retained (n=3).

- Figure 3. Relative abundances of the main trunk pathogens among soil samples. CS: calcarenite
 superficial; CD: calcarenite 120 cm deep; MLS: marly-limestone superficial; MLD: marly-limestone
 120 cm deep (n=3).
- Figure 4. Relative abundances of the main mycorrhizal fungi soil samples. CS: calcarenite superficial;
 CD: calcarenite 120 cm deep; MLS: marly-limestone superficial; MLD: marly-limestone 120 cm deep
 (n=3).
- Figure 5. Average wine quality features determined by the tasting profile. Asterisks denote significant differences according to ANOVA, * = p < 0.05 and ** = p < 0.01.
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763 Figure 6. Summary of the main differences in terms of wine features, root index and microbiome 764 composition observed between the two soil types. In the centre top part differences among aromas and 765 root index are highlighted. Downstream, in the centre of the figure are reported the microbial differences 766 for both bacterial and fungi occurring between C and ML at 15 cm and 120 cm depth from the soil 767 surface. Mycorrhizal genera are reported in C or ML where they are more abundant in respect to the 768 other samples (either soil type or layer). Green up-facing arrows means a higher score/value whereas 769 red down-facing arrows means lower score/value (left arrows refer to C soil whereas right arrows refer 770 to ML soil).

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773 SUPPLEMENTARY FIGURE LEGENDS

- Figure S1. PCoA representing the four soil samples analysed for 16S sequences (n=3).
- **Figure S2.** NMDS algorithm based on Bray-Curtis distances matrixes were used to reduce into a bidimensional scaling data obtained for bacteria (A), fungi (B) and the overall microbial community (C) (n=3).
- Figure S3. Cluster heatmap built using data of both 16S and ITS amplicon sequencing to highlight
 relationship among samples.
- 780 Figure S4. Significant co-occurrence network of bacterial communities and interactions among OTUs
- in soil samples. The nodes are sized according to degree of connection (Supplementary Table 11). Green
 lines indicate that there are co-occurrence interactions and red lines indicate mutualistic exclusions
 (n=3).
- **Figure S5.** Significant co-occurrence network of fungal communities and interactions among OTUs in soil samples. The nodes are sized according to degree of connection (Supplementary Table 12). Green lines indicate that there are co-occurrence interactions and red lines indicate mutualistic exclusions (n=3).
- **Figure S6.** Significant co-occurrence network of both bacterial and fungal communities considered together and interactions among OTUs in soil samples. The nodes are sized according to degree of

- connection (Supplementary Table 13). Green lines indicate that there are co-occurrence interactions and
- 791 red lines indicate mutualistic exclusions (n=3).

TABLES

Table 1. Root density of small (< 1 mm), medium (1 - 3 mm) and permanent (> 3 mm) roots for marly limestone (ML) and calcarenite (C) soils at 0.4 and 1 meter from the trunk. Asterisks indicate statistical significance as attested by Student-Newman-Keuls test (p < 0.05) (n=3).

	_		Root density			
Distance from vine	Treatment	(number of	(number of roots/m2			
trunk	Troutmont	Ø < 1 mm	Ø 1 - 3 mm	$\emptyset > 3 \text{ mm}$	profile wall)	
	ML	81	26 b	15 b	121 b	
0.4 m	С	72	57 a	34 a	164 a	
	Significance	ns	**	**	**	
	ML	123 a	30	14 b	168 a	
1 m	С	85 b	36	28 a	149 b	
	Significance	*	ns	**	*	

Table 2. Richness estimators and diversity indices for bacterial (16S) communities sampled in the four different soil types. Statistical ANOVA was

 conducted to detect significant differences, different letter in each row means significant differences according to Tukey's HSD test (n=3).

	CS				CD				MLS				MLD			
	Av.		SD		Av.		SD		Av.		SD		Av.		SD	
Таха	133.33	±	0.575	b	137.667	±	0.577	С	131.667	±	0.577	а	132.000	±	0.000	а
Simpson	0.970	±	0.002	а	0.955	±	0.002	а	0.964	±	0.002	b	0.974	±	0.001	С
Shannon	3.985	±	0.038	С	3.727	±	0.018	а	3.900	±	0.033	b	4.062	±	0.032	с
Evenness	0.404	±	0.017	b	0.302	±	0.006	а	0.375	±	0.012	b	0.440	±	0.014	С

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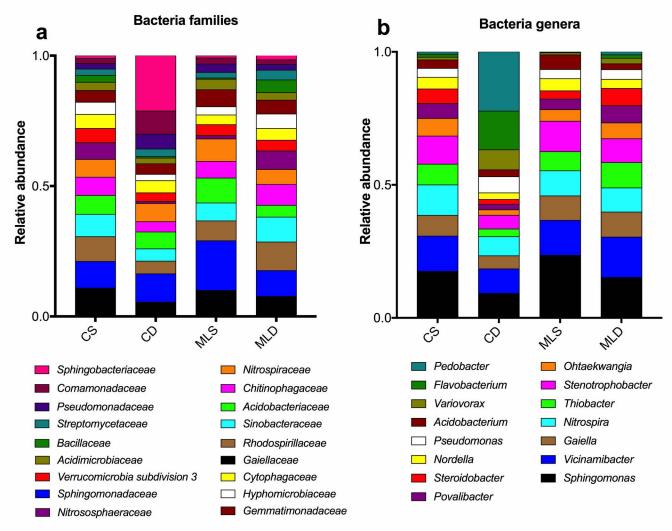
Table 3. Richness estimators and diversity indices for fungal (ITS) ommunities sampled in the four different soil types. Statistical ANOVA was
 conducted to detect significant differences, different letter in each row means significant differences according to Tukey's HSD test (n=3).

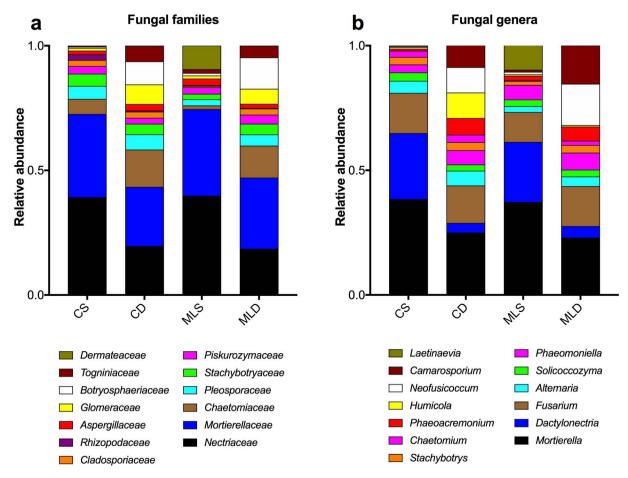
	CS	5		CD)		MLS			ML	D	
	Av.	SD		Av.	SD		Av.	SD		Av.	SD	
Таха	122.67 ±	3.21	С	99.00 ±	0.00	а	119.33 ±	2.31	С	105.00 ±	1.73	b
Simpson	0.86 ±	0.01	а	0.92 ±	0.00	С	0.86 ±	0.00	а	0.90 ±	0.00	b
Shannon	2.77 ±	0.06	ab	3.02 ±	0.01	С	2.66 ±	0.09	а	2.93 ±	0.04	bc
Evenness	0.13 ±	0.01	а	0.21 ±	0.00	С	0.12 ±	0.01	а	0.18 ±	0.00	b

Table 4. Single plant productivity and grape chemical composition for the two vineyards analysed in 2017.

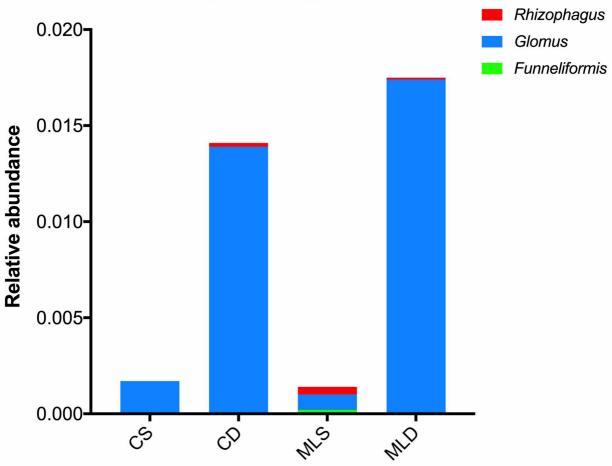
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		С				ML			
	Av.		St.Dev	A١	1.		St.Dev		
Vine yield (kg)	3,4	±	0,57	2	,5	±	0,40	*	
Bunch weight (g)	197	±	54,00	1	34	±	46,10		
Berry weight (g)	3,04	±	0,09	2,	13	±	0,11	**	
Winter wood (vine/kg)	0,87	±	0,33	0,	67	±	0,17		
Sugars (°Babo)	16,6	±	0,70	18	,1	±	0,65	*	
Total Acidity (g/L)	7,37	±	0,33	6	,8	±	0,21	*	
рН	3,08	±	0,15	3,	13	±	0,12		
Malic Acid (g/L)	2,43	±	0,21	1	,8	±	0,24	**	
Tartaric Acid (g/L)	8,27	±	0,35	7	,1	±	0,41	*	
Yeast assimilable nitrogen (YAN)	244	±	23,00	2	19	±	19,00		

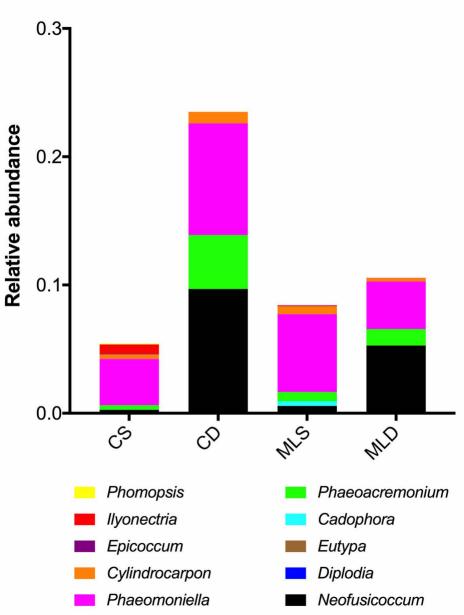


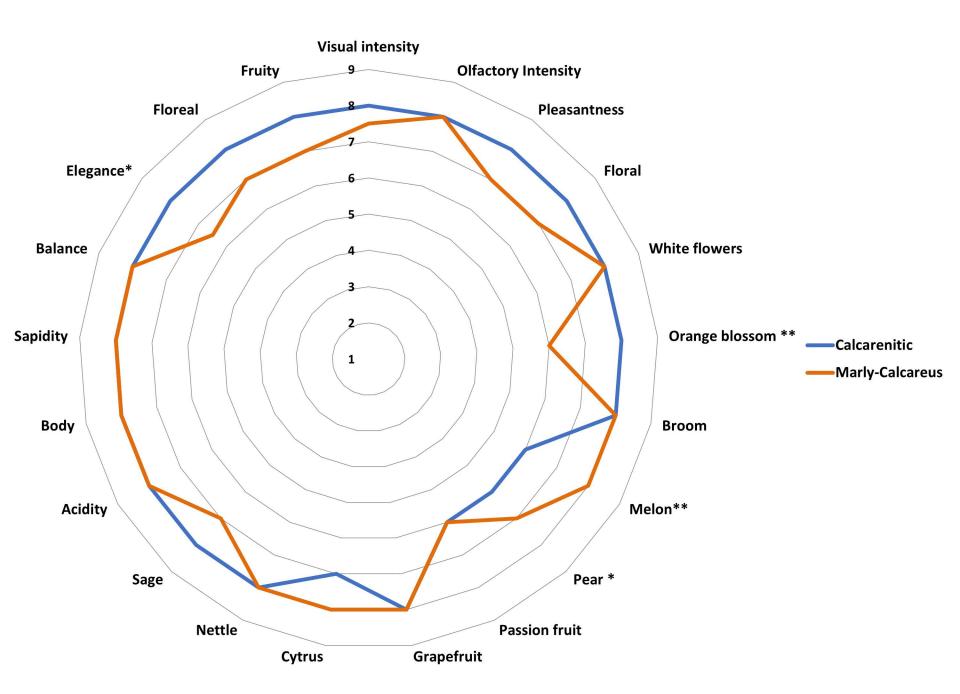


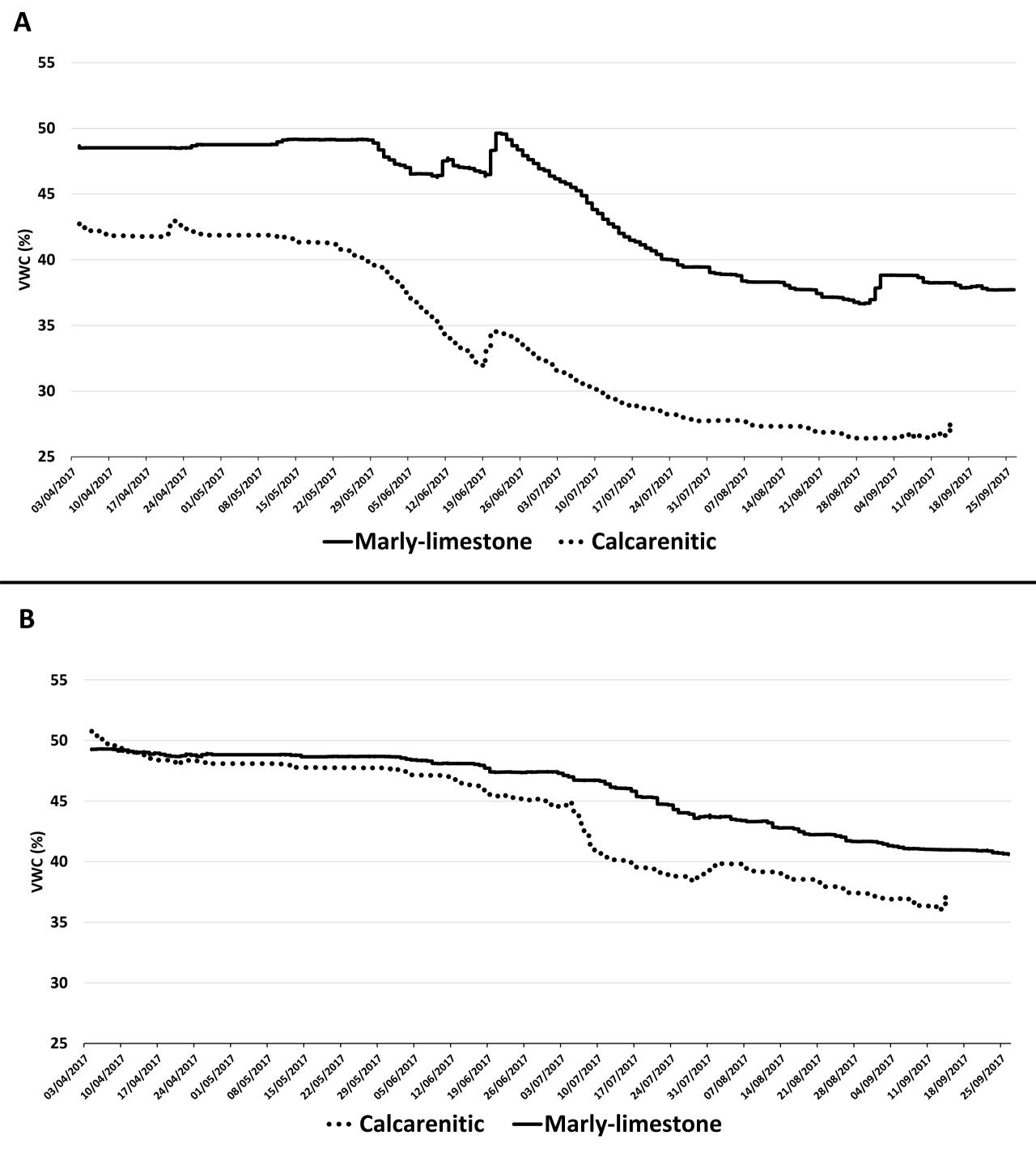
Mycorrhiza genera

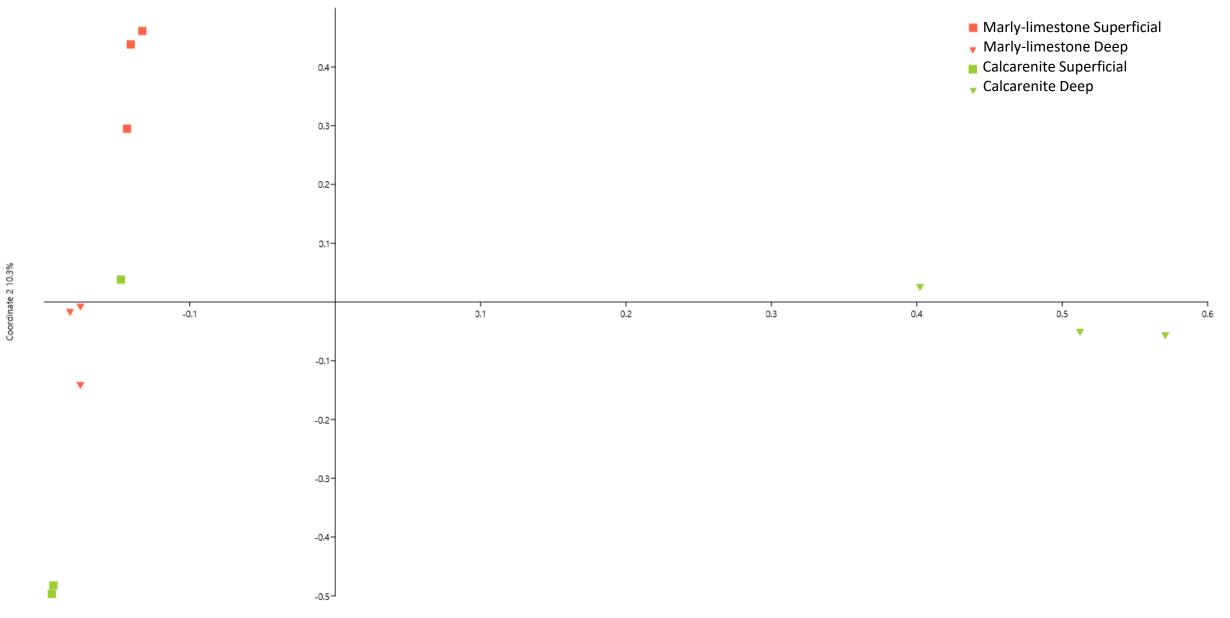


Pathogens genera

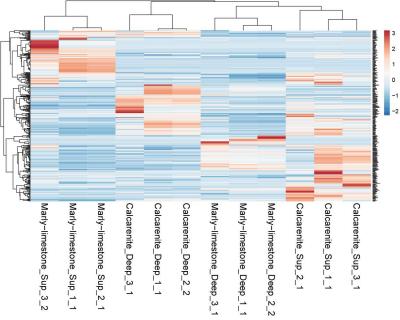


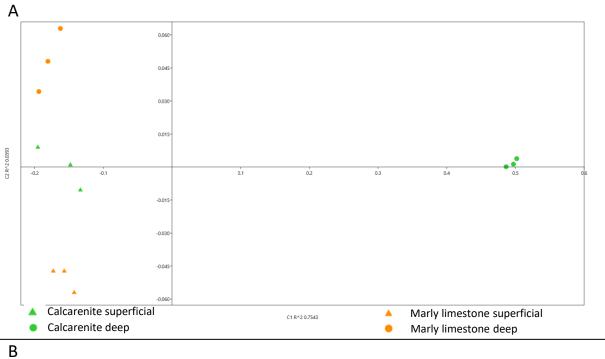


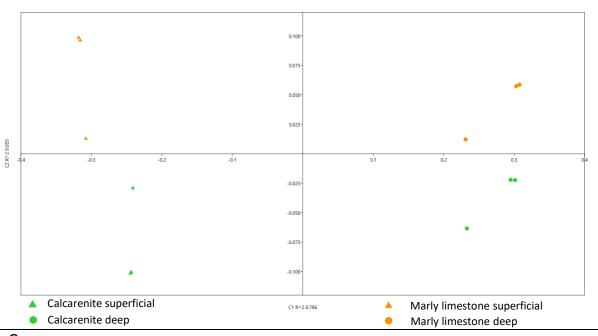


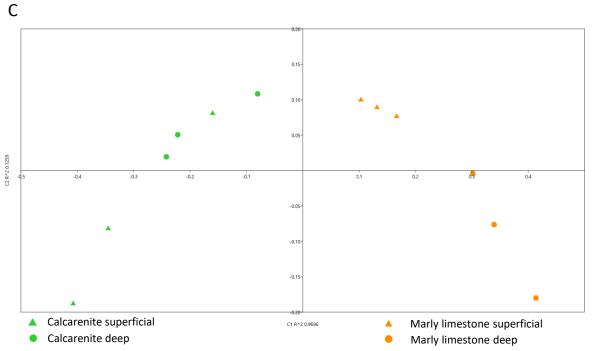


Coordinate 1 75.9%









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Piscinipacter
                                aculum
                           Chizothecium
                        errimicrobium
                            olderi
                     Gongronella
                Actinomadura
                    etobacter
               Acin
                 Mobacillus
        Rhodocytophaga
         Methy ophilus
      Methylopacterium
       Rhodopirellula
      Virgisporangium
      Desu
            uromonas
       Ru
            bacter
      Blas
            coccus
    Pseudonocardia
      Sker
           manella
      Geminicoccus
      Chitinophaga
Candidatus-Nitrosocosmicus
                                                              leola
      Nitrososphaera
      Thermo
            oleophilum
      Hyphomicrobium
        Parvit
              erribacter
   Candidatus-Nitrosoarchaeum
           Pedomicrobium
              Ca
             Ophic
                 KO
               Sphir
                   Candidatus-Udaeobacter
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Pedobacter Catelliglobosispora ostridium errimonas IODACIE Chthoniobacter Sphingobacterium Denitratisoma bacterium htaekwangia Soft Rhodoplanes Lacibacterium

Desulf<mark>ovibrio</mark>

