Geological Characteristics of Close Geographical Related Vineyards Influence Microbiomes of Two Soil Depth Profiles With Potential Impacts on Wine Quality

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Research

Keywords: itis vinifera, soil microbiome, 16S and ITS barcoding, holobiont, microbial terroir

Posted Date: August 25th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-64048/v1

Abstract

Background The role of soil microbiomes in agriculture is now becoming more and more important, leading to definition of plants as complex organisms formed by the plant itself plus the microbes inhabiting its tissues, the surface of every organ and the ones living adhered or in proximity of the roots. In this context it is important to define the factors able to influence the composition of such microbial communities. In addition, as already demonstrated, the microbial community associated to a specific soil is able to predetermine the health status of the crops in the future. For this reason, we decided to investigate how the soil geological characteristics can influence the microbial community associated to the close geographical related vineyard.

Results We decided to analyse a calcarenitic and a marly-limestone soil which are both typical of the viticulture in the Menfi area (Sicily, Italy). Moreover, since vineyard is a semi-anthropic environment we decided to investigate both the top (10-20 cm) and deep (120 cm) soil layers as anthropic influenced and almost-undisturbed soil respectively. Interestingly we observed that, despite the close geographical proximity, the soil microbiomes were slightly different and that in addition, the soil geological characteristics are able to influence the root distribution and the accumulation of both pathogen- and symbiont-related genera. Furthermore, sensory profiles of the Grillo wines obtained from the two different soils confirmed the tight link between soil origin and wine traits.

Conclusions In contrast to what observed in other works we pointed out that the geological characteristics of soils can have an important influence on soil microbial composition and assemblage in close geographical related vineyards, with a potential effect on wine features. Furthermore, defining the microbial composition of agricultural soils it's a crucial step to achieve a more sustainable agriculture and viticulture. In this optic, the observed link between the geological characteristics and the microbiome profiles need to be taken into account when considering a soil for the successful establishment low chemical input agriculture practices.

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

Background

Soil microbial biomass has crucial roles in Earth's biogeochemical cycles in both natural and human managed ecosystems [1]. Despite the challenges of living organism survival in such environment, if we consider viruses, bacteria and fungi we can estimate that each gram of soil can contain up to millions of individual microorganisms [2]. These organisms play key roles in nutrient cycling, soil fertility, soil carbon sequestration and show also both direct and indirect effects on the plants and animals health [1-3]. In this respect, some microorganisms evolved the ability to associate with plants forming mutualistic symbioses [e.g. arbuscular mycorrhizal fungi (AMF) or rhizobacteria]. Furthermore, the importance of interactions between plants and microorganisms was also described as an additive ecological function able to be a major trait in extending the plant's ability to adapt to many environmental stressful conditions [4]. Since this wide importance of microbes on plant health, they are seen as a reservoir of additional genes and functions for their host, and the plants whit their interacting microbes are defined as the so-called holobiont entity [5].

Many important crops that comprise the same or very similar genotypes, including grapevine, display differential geographic phenotypes in terms of morphological and sensorial signatures which are generally described as the *terroir* [6]. In specific, viticulturists have been selectively growing vine cultivars from local wild *Vitis vinifera* subsp. *sylvestris* varieties which presented differences among grape size and shape, berry colour, flavour, yield and many other phenotypic aspects [7]. The most interesting individuals were then multiplied by vegetative propagation for years, during which genetic and somatic modifications can spontaneously occur. Those events gave rise to an intra-varietal variability associated with phenotypic and biochemical variation which led to the description of grapevine clones [8]. Despite this very detailed characterization, the same grape clones (which are still vegetative propagated) can show differences among phenotypic characteristics and biochemical traits when growth in different environment, confirming that the plant genome is not the only player able to shape the phenotype. In specific, if we look at the final product (i.e. wine), it is well known that indigenous yeasts and bacteria inhabiting the berries surface can have a wide impact on flavour and aroma of typical wines [9-12]. In this optic, on their journey from the vineyard to the winery, grapes are transformed into wine through microbial biochemical processes, with unquestionable consequences for wine quality parameters.

Indeed, thanks to the above mentioned importance of microbes associated to grape plants and their impacts on wine characteristics, several studies tried to address which are the main factors driving the microbial *terroir* composition of the holobiont. Among all the parameters, one of the most important is the geographical location. It was already demonstrated that exist a clear delineations

among local natural population of yeasts inhabiting berries [13, 14]. Similarly, a study which took in consideration the geographic distance, analysed its impact on both fungal and bacterial communities [15]. The latter work clearly displayed that spatial processes play an important role in structuring the biogeographic pattern of grape-associated fungal communities but do not influence the bacterial ones. Interestingly, another main factor influencing the microbial *terroir* is the soil. It was demonstrated that, the majority of organ-associated microbes reflected the ones found in the surrounding soil, and their distribution were in turn influenced by the highly localized biogeographic factors and vineyard management [16]. The strict relationship among geographical location and microbiome structure was also recently reported among the Italian famous wine region of Trentino [17]. In such work the authors suggested a general inverse correlation between the geographical location and the microbiome structure.

As further level of complexity we need to mention that *Vitis vinifera* cultivars are commonly grown using rootstocks. Rootstocks are used because of their ability to cope some biotic or abiotic factors, such as phylloxera [18], nematodes [19], salinity [20], water limitation [21] and playing also a role in the growth-defence trade-off balance [22]. It was already demonstrated that the microbiome of the plant surrounding soil is strongly influenced by rootstock genotype [23]. In specific the latter work demonstrated that the genotype of the grape root system is able to select and recruit microbes that will then colonize the aboveground organs influencing both fruit and wine qualities.

For all the above mentioned reasons we decided to study the microbial composition of bulk soil associated to the root of grapevine cultivar Grillo in a limited geographical area to determine which are the most important player able to shape the wine tipicity. We selected two different soil types which lead to the production of the same Grillo wine but with different organoleptic features [24] and we analysed the soil chemical and microbial compositions including the wines organoleptic profile. Furthermore, to understand the anthropic-mediated impact on the microbial communities we analysed the soil at 15 cm and at 1.2 meter in depth.

Methods

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 is located in Contrada Finocchio (37°37'06.0"N 12°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°35'02.7"N 13°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 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 Menfi area the summers are warm, muggy and dry and the winters are long, cold, windy, and partly cloudy defining a harsh Mediterranean environment. Over the course of the year the rainfall are about 490 mm and the temperature typically varies 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 and were subject to standard cultural practices (soil, nutrition, irrigation, canopy and pest management) routinely used in the Menfi area. The training system was Guyot consisted of one fruit cane of 8/10 buds and its total length was about 0.6-0.8 m per vine. Vine spacing was 2.5 m × 0.9 m (intra row and inter vines) equal to 4.444 vines per hectare and the fruit cane was trained 0.7 m above ground with one pairs of surmounting catch wires for a canopy wall extending about 1.5 m above the fruit cane.

Grapevine root development

As the root systems of grapevines are capable to reach a large volume of soil exploration, influenced mostly by soil conditions, the classical profile wall method was chosen as the most appropriate one to determine root distribution and density [25, 26]. In February 2016, during the dormant period, six vines per vineyards with similar scion circumferences were selected. For each two vines, a trench of approximately 1.20 m deep was dug parallel to the vine row, first at 1.00 m and then at 0.40 m distances from the vine trunk. At each distance, roots were counted by using a 1.2 m high and 2.0 m wide grid system placed against the profile wall, the grid was divided in sub-grid block with a size of 0.2 m x 0.2 m, two vines for each trench have been considered. Roots were plotted in five depths (0-20 cm, 20-40 cm, 40-60cm, 60-80 cm, 80-100 cm) and were classified into three root diameter classes according to size: Ø < 1.0 mm = fine roots; Ø 1.0-3.0 mm = medium roots and Ø > 3.0 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 according to [27]. A high index reflects more thin roots relative to medium and thick roots as a result of more favourable soil conditions.

Wines profiles

Using a standard protocol [28], Settesoli winery provided a separate vinification of the grapes coming from the two soils (about 7,000 kg of grapes per vinification). The tasting analysis was carried out in Settesoli using the internal trained panel test made up of 13 oenologists (8 males, 5 females). Demographic aspects were recorded at the beginning of the first session and no information about the nature of the study was provided in order to reduce bias. Twenty-five mL aliquots of each wine at 20 ± 1°C were poured into wineglasses coded with a random three-digit number and covered to avoid dispersion of volatiles. Wines were then presented during four evaluation sessions using a randomized design with three replicates for each wine. For the quantitative evaluation of the intensity of attributes (visual, olfactory, gustatory and retro-olfactory) a questionnaire providing discrete scale responses with intervals from 1 to 9 has been used.

Soil DNA isolation and sequencing

Total nucleic acids were obtained as previously reported from 1 g of soil [29, 30]. 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 [31]. The primer ITS3-ITS4 were used to amplify the highly variable spacer ITS2 of the rDNA fungal operon [32] by Macrogen, Inc. (South Korea). Sequencing of both bacterial and fungal libraries were done with the MiSeq Illumina apparatus.

Metaphylogenomic analyses, taxonomic distributions

PrinSeq v0.20.4 [33] was used for a first strict quality control on raw data that were then processed in Qiime 2 [34]. A specific pipeline was used for fungal analysis: retained reads were then used to identify the start and stop sites for the ITS region using the hidden Markov models (HMMs) [35], created for fungi and 17 other groups of eukaryotes. Briefly, the software allows to distinguish true sequences from sequencing errors. In order to distinguish true sequences from those containing errors, sequences have been sorted by abundance and then clustered in a greedy fashion at a threshold percentage of identity (97%). Trimmed sequences are then analysed with DADA2 [36], which models and corrects Illumina-sequenced amplicon errors. Sequence variants are then taxonomically classified through the UNITE database (we selected the reference database built on a dynamic use of clustering thresholds) [37]. For graphic representation, only genera with an average relative abundance higher than the settled 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 [38] trained to the full length 16S database retrieved from the curated NCBI database.

Statistical analyses

Statistical analysis for microbiome data was performed with R (Version 3.4.4) using phyloseq (version 1.24.0) to import, store and analyse data [39]. To convert data from phyloseq, the official extension phyloseq to_deseq2 was used [40]. Fungal and bacterial communities were used to evaluate beta-diversity deriving from the Bray-Curtis distance matrix (complete dataset were considered at the OTU level clustered with a cut-off threshold of 97% identity). The matrix was further used as input to run a non-parametric multivariate analysis (PERMANOVA) (p values were corrected with sequential Bonferroni significance) and non-metric

multidimensional scaling (NMDS) was calculated among both the fungal and bacterial diversity in the samples using PAST [41]. Statistical analysis for the determination of significant differences between roots number was carried out using Student-Newman-Keuls test ($p \leq 0.05$) Statistica version 8 (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. [28].

Results

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 each level varies from few centimeters up to 80-100 centimeters. Sometimes are present intercalations of nummulitic and resedimented biocalcarenites. The above described lithological complex is ascribed to the lower portion of the so called Ragusa Formation, particularly to the Leonardo Member. The general characteristics of the succession are strongly influenced by the predominant presence of the compact limestones or of the more or less marly levels containing 20 to 60% clay in addition to other constituents. The permeability degree of the lithological complex is generally scarce due to the presence of very low permeable marly levels; however, the locally presence of limestone with high secondary permeability, due to fissuration induced by tectonic processes, allows a moderate drainage of the groundwater and the formation of small aquifers with local importance.

Eastward of Menfi town, where Contrada Bertolino and the second vineyard are located, largely outcrops a middle-upper Pliocene terrigenous succession locally known as Marnoso-arenacea del Belice formation. It consists, from bottom, of several teens of meters of fine to medium sandstones; the sandy particles are mainly rounded quartz grains, but levels of resedimented biocalcarenites also occurred. They upwards go to sandstones and coastal calcarenites and conglomerates. The substratum in the area is formed of up to 50 meters of thick package of hemipelagic shales and marls, and brownish siltstones with interbedded siltstones and calcarenite mudstones. The Marnoso-arenacea del Belice formation underlie the quaternary marine deposits mostly represented by lower Pleistocene yellowish partially cemented calcarenites and biocalcarenites alternating to thick beds of biocalcirudites, with thin intercalation of marls levels, conglomerates lens and calcareous sands and gravels. The total thickness of the quaternary complex varies from few meters, as in the interest area, up to few tens meters. The natural porosity characteristics of the above mentioned lithological complexes, specially of the calcarenites levels and of the small cemented sands, allows a good drainage and circulation of the groundwater partially confined by marly and loam/clay grained thin bedded intercalation with a low permeability.

Soil chemical analyses

The average values of physical and chemical characteristics of the two soils are reported in Supplementary Table 1. The pH resulted alkaline, but in an optimum pH range for plant nutrient uptake [42].

Root system distribution and development

Table 1 reports the roots density at two different distances from the vine trunk (0.40 and 1.00 m) dividing the roots in three diameter classes and the average number of roots/m^2 found in the two soils along the dug trench. In those soil conditions, we found that the total roots number of the C soil was higher compared to ML one at 0.40 m distance from vine trunk, while the mean roots density did not vary significantly at 1.00 m distance from vine trunk. Considering the root size we found thinner roots (Ø < 1 mm) in ML soil at 1.00 m from the vine trunk, and more abundant thickest roots (Ø 1 - 3 mm and Ø > 3 mm) for both distances (0.4 and 1 m) in C soil were the sandy texture allowed to a more roots diffusion (Table 1). We found some significant differences: an average ratio of 7.1 versus 3.8 at 0.4 m from vine trunk ($p < 0.05$) and 10.9 versus 4.3 at 1 m from vine trunk ($p < 0.05$) for ML and C, respectively (Supplementary Table 2).

For what concerns the vertical distribution, in C soil the major number of roots (62 roots / 0.04 m^2) was located between 0.40 and 0.60 m of soil depth and at 0.40 m distance from vine trunk. Indeed, for ML soil, we observed the major number of roots at the same depth (0.40-0.60 m) but at 1.00 m distance from vine trunk (48 roots / 0.2 m^2).

Bacterial community diversity and composition

The bacterial community was analyzed 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 that in both soil types a significant difference between superficial and deep microbial composition occur (Tab. 2). Interestingly both the indices suggest that calcarenite superficial (CS) display the same diversity of deep marly limestone (MLD).

The complete bacterial community composition for each sample type at the family level is reported in Supplementary Tab. 3 and statistical results of pairwise comparisons are reported in Supplementary Tab. 4. Here, to simplify, we are describing results for the families which represent at least the 2% of the bacterial community (Fig. 1a). Regarding the calcarenite soil we can observe that the composition of superficial (CS) and deep (CD) samples is quite different as also reported by the statistical analysis in Supplementary Tab. 4 and in the PCoA analysis (Supplementary Fig. 1). It is worth nothing that the CD sample displays the higher number of taxa and the most different composition if compared with all the other samples. In detail, the superficial sample is richer in *Nitrososphaeraceae* and *Bacillaceae* whereas *Sphingobacteriaceae* and *Comamonadaceae* are more abundant in the 120 cm depth sample (Supplementary Tab. 4). Interestingly, pairwise comparison of superficial calcarenite and marly limestone samples revealed that at the family level only three showed significant differences in abundance. Among the three families, *Bacillaceae* and *Nitrososphaeraceae* are more abundant in CS sample than in MLS one. Regarding the ML soil we observed 21 families more abundant in superficial (MLS) sample and 28 families that are more represented in 120 cm depth (MLD) sample. Among the differentially abundant families *Acidobacteriaceae*, *Sphingomonadaceae*, *Gemmatimonadaceae*, *Nitrospiraceae*, *Acidimicrobiaceae*, *Verrucomicrobia* subdivision 3 are over-represented in MLS sample whereas *Bacillaceae*, *Nitrososphaeraceae*, *Streptomycetaceae* and *Sphingobacteriaceae* are over-represented in MLD sample. We then also compared the two deep soil types observing the wider number of differential abundant families. The families over-represented in MLD sample are 36, among them *Nitrososphaeraceae*, *Bacillaceae*, *Hyphomicrobiaceae*, *Rhodospirillaceae*, *Chitinophagaceae* and *Sinobacteraceae* which belong to the top selected families representing about the 60% of microbial composition. In parallel, 32 families are more abundant in CD: among them *Sphingobacteriaceae*, *Comamonadaceae*, *Pseudomonadaceae*, *Nitrospiraceae*, *Acidobacteriaceae*, *Sphingomonadaceae* and *Cytophagaceae*.

Regarding the bacterial composition at the genus level (Supplementary Tab. 5) we decided to retain only taxa representing at least the 2% of the overall community (Fig. 1b). Pairwise comparisons were done to underlay the significant differences among sample types (Supplementary Tab. 6). First, we started with CS and CD samples. There are 8 genera which are more present in the CS samples, whereas 21 genera are more abundant in CD samples. Among genera more present in CD soil we found *Pedobacter*, *Flavobacterium*, *Variovorax* and *Pseudomonas*. When comparing the CS and MLS soils we observed only few differences: 4 genera are more abundant in CS soil, among them the *Flavobacterium* genus, and only one is more present in MLS soil. Then, we compared MLS to MLD observing a high number of differentially abundant genera: 37 are more present in MLS whereas 50 are more present in MLD. Among genera more abundant in MLS we observed *Acidobacterium*, *Sphingomonas*, *Nordella*, *Stenotrophobacter*, *Nitrospira* and *Vicinamibacter*. In parallel, among the genera more abundant in MLD we found *Flavobacterium*, *Pedobacter* and *Steroidobacter*. Finally we also compared the genera of MLD and CD soils. As reported for the families, also for genera we found in this comparison the widest number of differentially abundant genera: 61 are more abundant in MLD samples, whereas 62 are more present in the CD soil. Among them *Steroidobacter* and *Thiobacter* are more present in MLD and *Pedobacter*, *Flavobacterium*, *Variovorax*, *Acidobacterium*, *Nitrospira*, *Nordella*, *Vicinamibacter*, *Sphingomonas* and *Stenotrophobacter* are more abundant in CD samples.

Fungal community diversity and composition

As for the bacterial also for the fungal community we analyzed 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 a significant difference between superficial and deep microbial composition occur (Tab. 3). The difference is more marked in calcarenite than in marly limestone soils.

The complete fungal community composition for each sample type at the family level is reported in Supplementary Tab. 5 and statistical results of pairwise comparisons are reported in Supplementary Tab. 6. Here, to simplify, we are describing results for the families which represent at least the 2% of the bacterial community (Fig. 2a). As a first general observation we can note that at least the family level, the composition of both CD and MLD soils is almost the same. There is only one family (*Phaffomycetaceae*, which

is not in the representative group above the 2%) that shows a differential abundance as reported in Supplementary Tab. 6. When comparing the CS and CD soils we observed 4 families more representative of CS samples and 6 more abundant in CD samples. Among families more present in CD samples, we observed *Botryosphaeriaceae*, *Togniniaceae* and *Chaetomiaceae* which are in the group above the 2% threshold. Then we compared the CS and MLS samples: 8 families were more abundant in CS samples (among them the *Clodosporiaceae*) and 13 were more abundant in MLS soil. Among the latter we found *Dermataceae* and *Togniniaceae*. Finally, we also compared MLS and MLD samples: 13 are the families more abundant in MLS (among them the *Dermataceae* family) and 10 families are more present in MLD ones. Among the last group *Botryosphaeriaceae* and *Chaetomiaceae* are above the 2% threshold.

The complete fungal community composition for each sample type at the genus level is reported in Supplementary Tab. 9 and statistical results of pairwise comparisons are reported in Supplementary Tab. 10. Here, to simplify, we are describing results for the families which represent at least the 2% of the bacterial community (Fig. 2b). Similarly to what observed for the family level, also for the genus level minimal differences occurred between the CD and MLD soils. In specific we observed only one genus more abundant in CD (*Stemphylium*) and one more abundant in MLD (*Cyberlindnera*), both accounting for less than the selected threshold. When comparing CS and CD samples we observed 18 genera more abundant in CS samples (among them only *Dactylonectria* is above the 2% threshold) and 6 genera more abundant in CD samples. Among the latter we found *Neofusicoccum*, *Camarosporium*, *Phaeoacremonium* and *Humicola*. Comparing then the composition of two different superficial soils (CS vs MLS) we found the wider number of differentially abundant genera (54 in total), 25 more present in CS and 29 more present in MLS. Looking at the genera above the threshold, *Chaetomium* is more abundant in CS whereas *Laetinaevia* and *Phaeoacremonium* are more present in the MLS soil. The last pairwise comparison regards the MLS and MLD samples. The number of genera more present in MLS samples is 28 (*Laetinaevia* and *Dactylonectria* are above the 2% threshold) whereas 19 are more abundant in MLD (*Camarosporium* and *Neofusicoccum* are above the threshold).

Since specific fungal genera can have an important impact on plant development we decided also to focus on pathogenic (Fig. 3) and mycorrhizal (Fig. 4) fungi. Looking at the pathogenic ones, we can observe that in both soils *Neofusicoccum* species are more abundant in the 120 cm depth samples than in superficial ones. On the contrary *Ilyonectria* is more abundant in both soil types in the superficial layer. In addition, in calcarenite soil we observed a significant accumulation of *Phaeoacremonium* genus in the CD samples. Moreover, the genus *Cadophora* is more abundant in superficial samples of marly limestone (MLS) soil than in deep (MLD) ones. Regarding the mycorrhiza, in both soils the *Glomus* genus is more abundant in the 120 cm depth samples. In the case of *Rhizophagus* genus, it shows an opposite pattern, being more abundant in CD than CS and MLS than MLD samples respectively. Finally, the genus *Funneliformis* was detected only in the MLS sample.

Community structure

Community structure is always represented by two independent factors: the diversity and the complexity of taxa present in each sample. Diversity indices (Taxa, Shannon, Simpson and Evenness), representing species richness and evenness, were calculated for both bacterial and fungi in CS, CD, MLS and MLD as reported in Tab. 2 and Tab. 3 respectively. In addition, after the bioinformatics classification of amplicons for both the 16S, ITS and the two communities analyzed together, we reduced the dataset of each biological replicate to a bi-dimensional scaling using a Bray-Curtis distance matrix and plotting the results in corresponding non-metric multidimensional scaling (NMDS), as reported in Supplementary Fig. 2. A cluster heatmap of 16S and ITS communities considered together is reported in Supplementary Fig. 3. Moreover, co-occurrence analyses of 16S, ITS and the two communities analyzed together are reported in Supplementary Fig. 4, 5 and 6. Statistical analyses of co-occurrence relationship are reported in Supplementary Tab. 11-12-13.

Wine sensory analyses

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

Discussion

The aim of our work was 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 which in turn impact on the wine tipicity. Furthermore, we also considered that the vineyard is an anthropic environment and for this reason we analyzed both the superficial (anthropic disturbed) and deep soils (almost undisturbed) 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 to grapevine plants is the geographical region in which they are growing [17, 43, 44]. On the other hand, it is also well known that soil microbes strongly influence the microbial composition of their hosts, playing and important role in shaping the vine-associated microorganisms and the microbial community structures [30, 45]. For these reasons we decided to analyze two geographical closely related soils with different geological characteristics: a calcarenite and a marly limestone soil respectively.

A first interesting observation come from the root ratio between thin versus medium and thick roots (rooting index): ML soil displayed a much higher rooting indexes than C soil for both distances (0.4 m and 1 m) from the vine. In consequence we can propose that ML soil conditions were more favorable for a wide and diffuse soil root exploration. Furthermore, it is interesting to observe that in the vertical root profile C soil displayed the highest roots concentration in the 0–0.60 m soil depth for both trunk distances (140 roots/m^2 in total) suggesting an impairment in colonizing the deep soil. In ML soil the roots were able to explore a more wider soil area (from 0 to 0.8 m depth) since no physical resistance occurred but with a lower root density, especially considering the closest trunk distance (0.40 m). In addition, for both soil types in the deeper layers (0.80-1.0 m), the roots density decreased significantly.

As a first general consideration, we can observe that, as previously reported by other papers [16, 17], the superficial layer display quite similar bacterial features with some minor differences between the two geographical related sites. Interestingly this pattern seems to be completely unattended for the deep samples: the bacterial analysis showed that there are 123 genera which significantly accumulate with differences in the two soil types. This result is quite interesting since, almost the majority of paper always look at the superficial layer which is highly perturbed by human activities, agricultural residues and climate features. Interestingly, this pattern seem to be completely opposed in for the fungal analyses: the two superficial soil types displayed 55 genera which significantly accumulate with differences whereas the deep layers significantly differ for only 2 fungal genera.

Interestingly, it is worth noting that the wines produced by the two vineyards display different organoleptic characteristics: the wine from the C field showed a higher score in terms of appreciation for its complexity in terms of olfactory sensory and pleasantness, while the wine form ML soil display different olfactory scents. Accordingly, plant microbiomes are able to widely impact on the wine features, and this aspect is well described by the microbial *terroir* concept [6, 12, 46]. Looking at our results we can speculate that, despite the chemical composition of the two soil types is quite different and probably able to impact on the organoleptic qualities of wines, also the different soil microbial composition can have an effect on quality features in light of the soil-plant *continuum*. For example, presences of different mycorrhizal species between the two soil types can results in different physiological and biochemical modulation of the plant metabolism. Mycorrhizal fungi are known to play crucial ecological services, as for example the enhancement of the plant nutritional status (both for water and minerals) and the induction of the priming state triggering growth-defence tradeoffs responses [47, 48]. In this light, it is also well known that some mycorrhizal species can impact differently the plant behaviors [49], thus in the present case the presence of different mycorrhizal species can impact the grape behavior inducing physiological and biochemical adjustment between the two selected sites.

Nowadays the main factors which are considered as driver for the plant biochemistry responses are: i) the ecology of plant-associated microbes, ii) the viticulture management and iii) the environmental conditions. The impact(s) on grape metabolomics and wine flavor is likely due to complex interactions between them that need further investigations to be dissected. In this respect, a recent work [50] clearly demonstrated that fungal and bacterial communities analyzed in different soils were associated to diverse levels of rotundone concentration in grape berries. In detail, the authors demonstrated that the microbial structure and the well-connected soil bacteria community co-occurrence networks were linked to the high rotundone areas in respect to lower ones. Additionally, a recent work highlighted how the soil fungal communities of Pinot noir cv. played the principal role in shaping wine aroma profiles and regional distinctiveness more than soil or climatic features [51]. These findings further support the strong connection between soil microbial communities and plant performances, including fruit production yield and quality characteristics.

Since the microbial *continuum* between soil and plants can have also detrimental effects, favouring the colonization by fungal pathogens [30, 45] we decided to have a focus on wood pathogens, which can penetrate through trunk wounds (especially the ones

caused by agricultural practices and that are close to the soil surface) and through the root system, causing then plant illness [52–55]. In C soil we observed that two wood pathogen genera (*Neofusicoccum* and *Phaeocremonium*) result significantly over-represented in the 120 cm depth sample (both more than 4 times more abundant in the deep soils than in the superficial one), whereas in the ML soil only *Neofusicoccum* displayed to be over-represented in the 120 cm depth soil (about 3.5 times more abundant than in superficial sample). This result in quite interesting since, as previously reported, accumulation of fungal pathogens in vineyard occur i) after long term cultivation [45] and ii) where infected plants are present [30]. On the contrary, *Ilyonectria* genus, which is associated to black foot disease [56], result more present in both soil types in the superficial samples, representing an issue for the plant health status and for the young plants which are routinely used as replacement for died and/or compromised vines [57]. Interestingly in the analysed vineyards we didn't observed any sign of esca or black foot diseases, suggesting that the complexity of interaction among plants-pathogens-environment lead to no symptoms development.

Additionally, some genera of plant symbionts were detected for both fungi and bacteria. We first focused on mycorrhizal fungi, that have a relevant impact due to the involvement in complex interactions with plants [47], able to induce physiological changes such as the resistance induction against biotic [49, 58, 59] and abiotic [60–62] stresses which fall into the so called mycorrhiza induced resistance (MIR) [63–65]. In specific, the *Glomus* genus was found to be more abundant in both CD and MLD soils, suggesting that the higher proportion of fine roots found in the deep layers are the ones more actives in recruiting microbial symbionts. It is worth noting that, *Glomus* species are often associated to an enhanced tolerance to salinity and water deficit stresses [66–68], suggesting that this genus can play important roles for the vines adaptation to the semi-arid Mediterranean areas. In parallel, *Funnelliformis* and *Rhizophagus* genera, which are well-known plant symbionts (Chitarra et al., 2016), are significantly over-represented in the MLS soils if compared to MLD and C soil samples. This result is consistent with the root distribution, where in the ML vineyard we observed wide roots diffusion up to 80 cm form the surface and then a strong reduction in root density. Moreover, we also observed a significant difference for the fine roots (< 3 mm of diameter) which are more abundant in ML than in C soil, allowing the establishment of a higher number of symbioses.

Flavobacteriaceae, which comprise among others the *Flavobacterium* genus, it's an ubiquitous family of bacteria, commonly found in agricultural soils which is often associate to positive features such as the ability to degrade pesticides [69, 70] and the ability to induce resistance against fungal and bacterial pathogens in several crops [71, 72]. Interestingly, in our work we observed an over-representation of such family and genus in both the CD and MLD soils, suggesting that also this group of microbes can play important role in protecting plants against pathogens. Noteworthy, the *Bacillus* genus, which also encompasses several species with beneficial effects on plants [73, 74], was found to be differentially represented in the two soil types. For the C soil we observed a significant accumulation of such genus in the superficial samples, whereas for the ML soil it over-accumulates in the deep layer. This result suggests different soils propensities to host this genus that deserves further studies to be elucidated.

Our work is also aimed to enrich the knowledge about soil microbiome since it's a frontier research field. Crop losses due to plant pathogens are an ever-increasing threat for agricultural productions. While food demands increase, there is a compelling need to reduce the use of environmentally harmful pesticides and agrochemicals [75]. To initiate a more sustainable agriculture, manipulation of microbiomes associated to plants and soils has been suggested as a reliable alternative for impairing pathogens development and to obtain suppressive conditions [1, 76, 77]. However, we still need to unshed how the complex interactions among plants, pathogens, and soil features determine the development of plant diseases under field conditions. In this respect, the researcher provisions about all the drivers involved in determine the assembly of the host-associated microbiome such as the genetic background of the pathogen and the host and the presence of biotic and abiotic stresses are decisive to decipher the outcome of plant-pathogen interactions [78]. Among all the field drivers, it was recently demonstrated that the initial soil microbiome composition is able to predetermine if plants are able to survive or succumb to diseases [79]. Taking in consideration all these data, the descriptions of soil microbiomes are of pivotal importance to develop new agricultural strategies and to achieve the needs of a more sustainable viticulture.

Conclusions

In this study we pointed out that the geological characteristics of soil can have an impact on both plant roots development and soil microbial composition and assemblage in the different soil layers with a final effect on wine features (summarized in Fig. 6). In specific since the grapevine is regarded as one of the most impacting crop species in term of environmental sustainability, the microbial composition of soil can play pivotal role in enhancing the ability of vine to cope biotic and abiotic stresses. The ability to

exploit the native microbial diversity of soils will became one of the most important agricultural practices in the next future, and for this reason the description of microbial species associated to the semi-arid Mediterranean environment become essential. Furthermore, the soil features-mediated root distribution play also important role for the plant water availability, since 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 take into account when considering a soil for the successful establishment of a vineyard, especially if it is located into semi-arid regions where the water availability is limited and its use in agriculture play a fundamental role in defining the sustainability. Further studies to link the soil microbial composition and the wines features are still ongoing.

Declarations

Ethics approval and 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.

Competing Interests

The authors declare that they have no competing interests.

Funding

Part of the work was supported by Settesoli winery S.C.A. Menfi (AG) Sicily (IT) and by the BIOPROME in the frame of the DiBio project founded by the Italian Ministry of Agriculture.

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.

Acknowledgements

The Figure 6 was created with BioRender.com.

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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 ($p < 0.05$).

		Root density			**Root density**
Distance from vine trunk	Treatment	(number of roots/m^2 profile wall/root size)			(number of roots/m2 profile wall)
		Ø < 1 mm	Ø 1–3 mm	Ø > 3 mm	
	ML	81	26 b	15 b	121 b
0.4 m	**C**	72	57 a	34 a	164 a
	Significance	ns	**	**	**
	ML	123 a	30	14 b	168 a
1 m	**C**	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..

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

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.

	CS				CD				MLS				MLD			
	Av.		SD		Av.		SD		Av.		SD		Av.		SD	
Taxa	122.67	±	3.21	c	99.00	±	0.00	a	119.33	±	2.31	c	105.00	±	1.73	b
Simpson	0.86	±	0.01	a	0.92	±	0.00	c	0.86	±	0.00	a	0.90	±	0.00	b
Shannon	2.77	±	0.06	ab	3.02	±	0.01	c	2.66	±	0.09	a	2.93	±	0.04	bc
Evenness	0.13	±	0.01	a	0.21	±	0.00	c	0.12	±	0.01	a	0.18	±	0.00	b

Figures

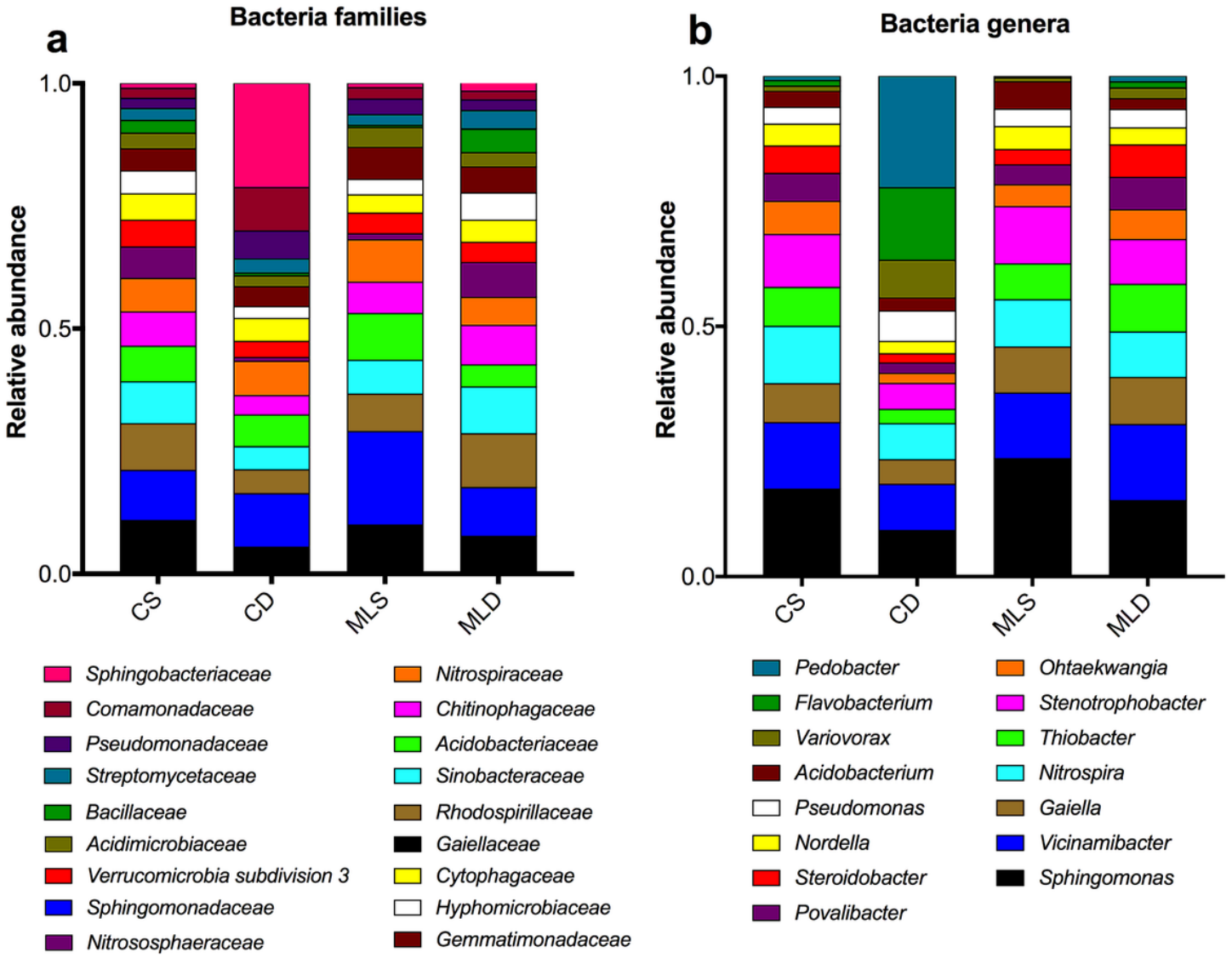

Figure 1

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: marly-limestone 120 cm deep. Only orders or genera representing at least the 1% over

the total number of classified amplicons were retained.

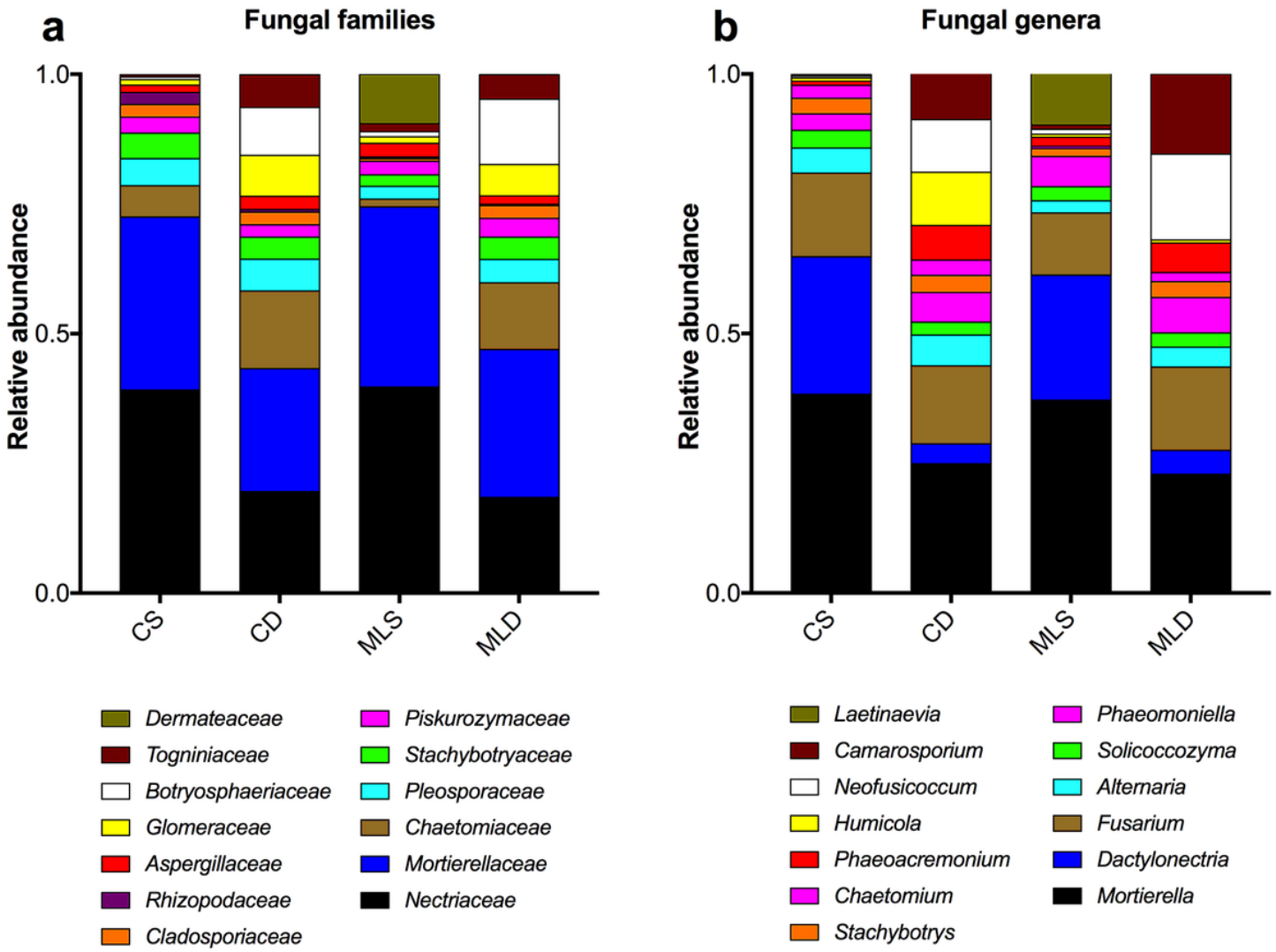

Figure 2

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: marly-limestone 120 cm deep. Only orders or genera representing at least the 1% over the total number of classified amplicons were retained.

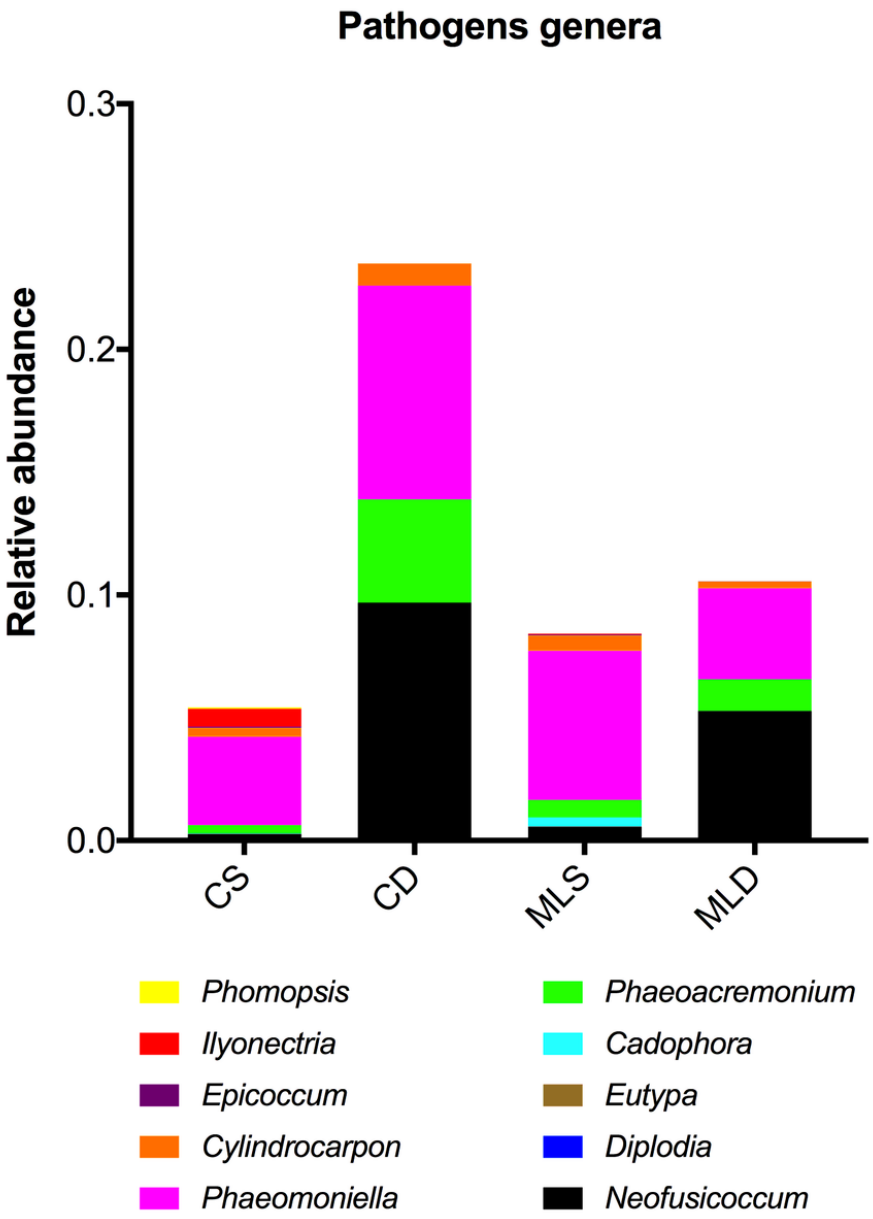

Figure 3

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.

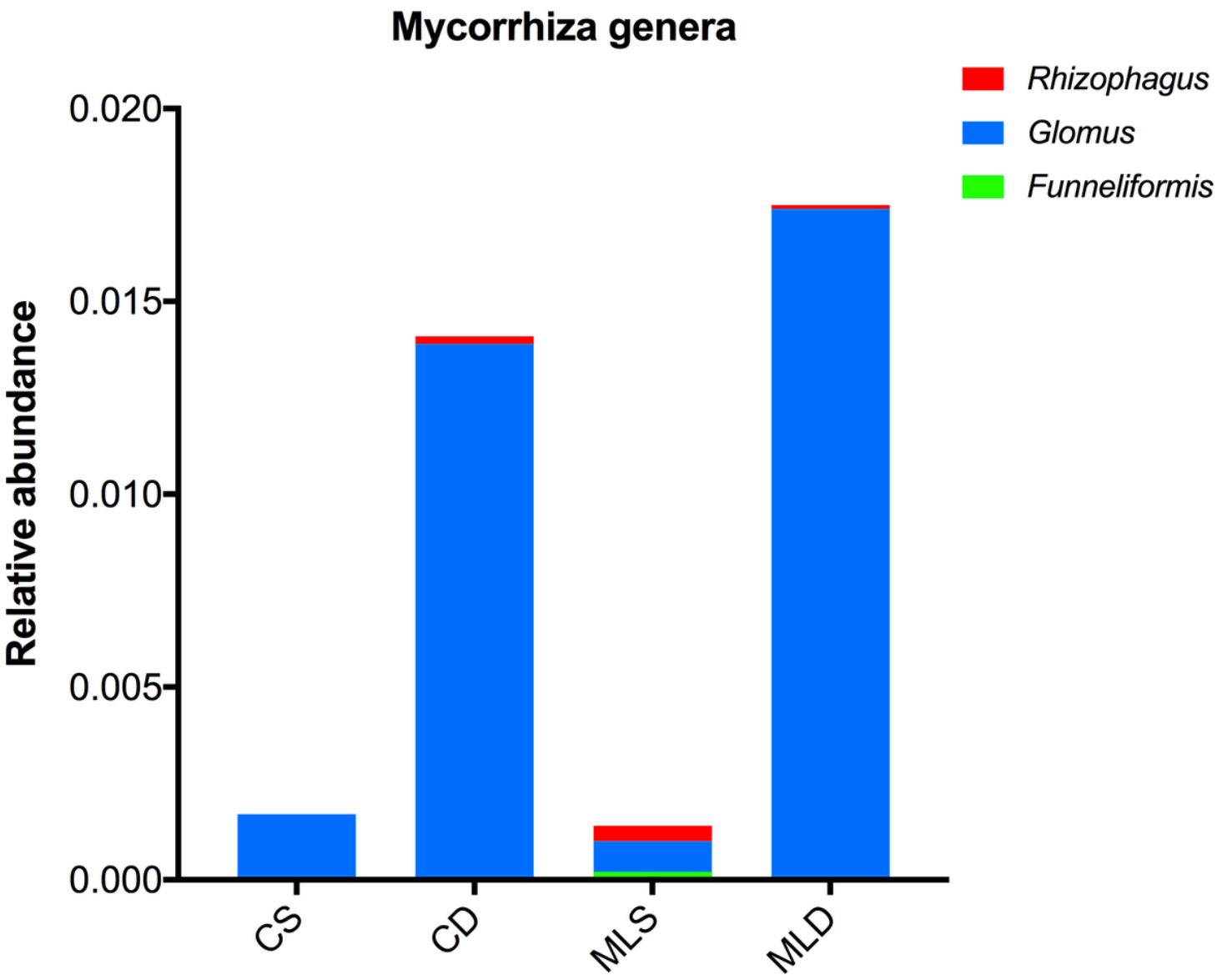

Figure 4

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.

Figure 5

Average wine quality features determined by the tasting profile. Asterisks denote significant differences according to ANOVA, * = $p < 0.05$ and ** = $p < 0.01$.

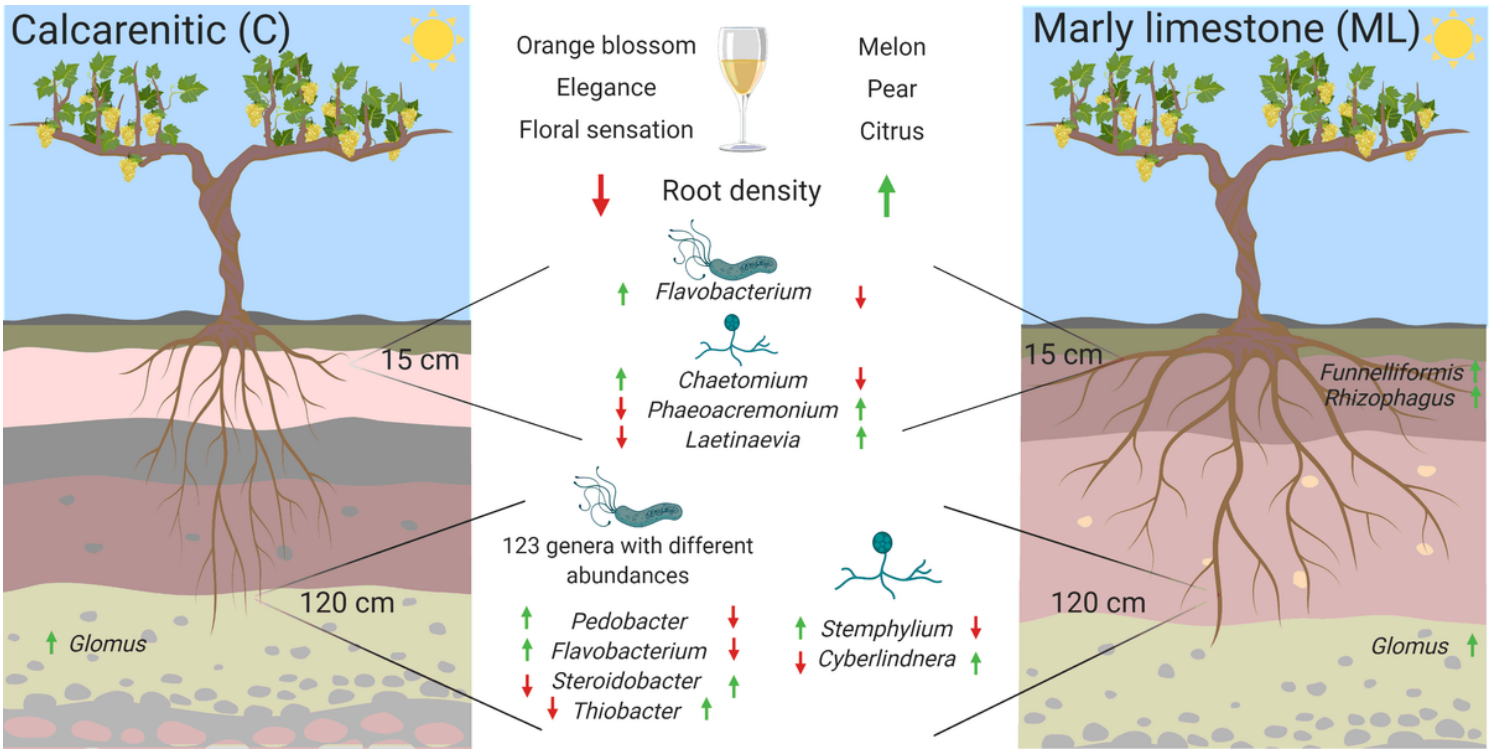

Figure 6

Summary of the main differences in terms of wine features, root index and microbiome composition observed between the two soil types. In the centre top part differences among aromas and root index are highlighted. Downstream, in the centre of the figure are reported the microbial differences for both bacterial and fungi occurring between C and ML at 15 cm and 120 cm depth from the soil surface. Mycorrhizal genera are reported in C or ML where they are more abundant in respect to the other samples (either soil type or layer). Green up-facing arrows means a higher score/value whereas red down-facing arrows means lower score/value (left arrows refer to C soil whereas right arrows refer to ML soil).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SUPPLEMENTARYFIGURELEGENDS.docx
- SupplementaryTables111213Cooccurence.xls
- SupplementaryTables.docx
- SupplementaryFig6Cooccurrence16S.tif
- SupplementaryFig5CooccurrencITS16S.tif
- SupplementaryFig4CooccurrencITS.tif
- SupplementaryFig3Clusterheatmap.pdf
- SupplementaryFig2NMDS.pptx
- SupplementaryFig1PCoABacteria.pptx