Research Article

The Microbial Terroir of the Nemea Zone Agiorgitiko cv.: A First Metataxonomic Approach

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Received 24 August 2022; Revised 10 January 2023; Accepted 10 March 2023; Published 17 March 2023

Academic Editor: Serge Delrot

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Vitis vinifera L. cv. Agiorgitiko is one of the most popular indigenous wine grape varieties in Greece, cultivated almost exclusively in the Nemea Protected Designation of Origin (PDO) zone. Here, the microbiota of soil, grapes, and wine, during controlled (CF) and spontaneous (SF) fermentations of Agiorgitiko cv. from three vineyards in the PDO Nemea zone were explored, using both classical microbiological analysis and metataxonomics to get evidence about the microbial terroir of the PDO Nemea zone. The classical microbiological analysis revealed higher total mesophilic counts in soil, while in both grapes and wine samples, yeasts prevailed. Lactic acid bacteria and acetic acid bacteria counts were lower in grapes compared to wine and soil. Metataxonomic analysis revealed that, regarding yeasts/fungi, genera Fusarium, Sarea, and Alternaria dominated in soil; Aureobasidium, Cladosporium, and Penicillium in grapes; Saccharomyces in wine during CF; and Hanseniaspora and Saccharomyces in wine during SF. Regarding bacteria, genera Skermanella, Acidobacterium, and Ohtaekwangia dominated in soil, Sphingomonas, Micrococcus, and Rubrobacter in grapes, while Tatumella, Alcanivorax, and Komagateibacter in wine during both CF and SF. Finally, the factors that significantly influence the microbiota of soil, grapes, and wine samples were assessed, and potential microbial biomarkers were identified for the first time in a Greek grape variety.

1. Introduction

Vitis vinifera L. is an economically and culturally important crop that has been cultivated since ancient times. In Greece, Vitis vinifera L. cv. Agiorgitiko is the most cultivated indigenous red grape variety, located almost exclusively in the Nemea Protected Designation of Origin (PDO) zone, Peloponnese, Southern Greece. The wine region of Nemea has a Mediterranean climate, which is characterized by mild cold winters and warm, dry summers. The major part of the zone lies in the South Central Corinthia district, while a few smaller parts are located in the regions of Sikyonia and Stymfalia. Informally, the zone is divided into three subzones on an altitude basis, to the mountainous (600–850 m), the semi-mountainous (350–600 m), and the lowland (250–350 m) subzones. Thus, the zone is characterized by variations both in terrain and climate conditions, which impact the maturation process, the microbial ecology, and the physicochemical parameters of the grapes [1].

Winemaking is a complex biochemical process involving not only the interaction between yeasts and bacteria [2] but also by many other factors that shape the final product [3]. The influence of microorganisms on winemaking begins in the vineyard and expands through the fermentation process. Must spontaneous fermentation has been employed for centuries in winemaking. However, in the early 1980s, well-
defined starter cultures had begun to emerge, and since then they have been extensively used, thus improving standardization and effectiveness of the fermentation [4]. The use of Saccharomyces cerevisiae as starter culture is the most widespread practice in winemaking due to its remarkable sugar consumption rate and ethanol production coupled with a high alcohol tolerance [5, 6].

Until recently, the microbial diversity of grapes and wine has been explored mostly by culture-dependent techniques [7], which, however, often fail to detect microorganisms, which are either stressed, damaged, or nonculturable [8]. During the last decades, the limitations of the conventional methods have led to the development of high-throughput sequencing (HTS)-based approaches to study the structure of microbial communities through culture-independent metagenomics approaches. These approaches allow the identification of all microorganisms within an ecosystem through metataxonomics or metagenomics.

Metagenomics analysis has become a useful tool in food microbiology, especially by using amplicon-based sequencing [9]. However, the application of metagenomics in the assessment of grapevine and wine fermentation microbiota is relatively recent, and, so far, studies have shown that grape and wine microbiota are driven by cultivar, climate, environmental conditions, viticultural farming practices, as well as fermentation processes during wine making [6, 10]. Even though Agiorgitiko cv. is among the major red wine varieties cultivated in Greece, currently, only culture-dependent approaches have been employed to fingerprint its microbial diversity [11–14].

The aim of the present study was to explore the microbiota of soil, grapes/musts, and their corresponding wines produced, both by controlled and spontaneous fermentations, for three selected vineyards in the PDO Nemea zone using both classical microbiological analysis and metataxonomics to get evidence about the microbial terroir of the PDO Nemea zone.

2. Materials and Methods

2.1. Site Description and Sample Collection. Soil and grape samples of the Agiorgitiko variety were collected during the 2018 harvest period from three different vineyards, located in the (A) Asprokambos (ID 9; latitude, 37°54′23.04″ B; longitude, 22°33′39.27″ A), (B) Central Nemea (ID 72; latitude, 37°50′3.30″ B; longitude, 22°38′16.12″ A) and (C) Koutsi (ID 36; latitude, 37°51′21.50″ B; longitude, 22°40′8.80″ A) areas of the Nemea PDO zone Figure 1. Vineyard A lies at an altitude of 802 m belonging to the mountainous subzone of Nemea, while vineyards B and C belong to the lowlands subzone, lying at an altitude of 274 and 286 m, respectively.

Healthy and undamaged grapes as well as the respective soil samples were collected in triplicate at three time points during grape ripening, i.e., before veraison (July), at veraison (August), and at harvest (September; at 21–23 Brix). Representative grape berries (200 g) from the center area of each vineyard, in order to avoid possible contamination from neighboring crops, were collected using gloves and placed in sterile plastic bags. Soil samples (200 g) were collected approximately 20 cm away from the respective grapevine trunk at a depth of about 10–15 cm from the surface, after removing leaves and small stones. Sampling points and samples’ code numbers are presented in Table 1. All samples were transferred under refrigeration (4°C) and analyzed immediately (classical microbiological analysis) immediately or stored at −80°C (amplicon sequencing).

2.2. Fermentation Process. At harvest (21–23 Brix), 25 kg of grapes were collected from the selected vineyards where the samplings occurred, transferred to the laboratory, and stored at 4°C overnight. The next day, stems and leaves were manually removed from the grape berries using gloves to avoid contamination, and 4 kg of undamaged berries were pooled together in a sterile plastic bucket and hand-crushed using gloves again. Subsequently, the must and crushed grapes were transferred into sterile plastic barrels.

For each vineyard, triplicate spontaneous (SF) and controlled fermentations (CF) were performed as follows: Grape pomace for both types of fermentation were sulphited by adding 50 mg L⁻¹ of potassium metabisulphite. After 1 h, 40 mg L⁻¹ pectolytic enzymes (Safizym® Col Plus, Fermentis, France) were added. In the case of the CF, 1 h after the enzyme addition, the must was inoculated with 250 mg L⁻¹ of the SafEno™ HD S135 commercial yeast.
of organics and inorganics nitrogen (ratio 1:1; Springferm, Fermentis: Diammonium Phosphate) were added, while the same amount of nitrogen was also added in the middle of the CF. Neither commercial yeast culture nor nitrogen source were added to the SF, and the procedure was left to evolve spontaneously by the indigenous grape microbiota. Both types of fermentations were performed at 22°C, with the grape pomace being stirred daily to facilitate contact between the solid and liquid phases. At the end of the alcoholic fermentation, both types of wine were supplemented with 50 mg·L⁻¹ of potassium metabisulphite. Wine samples were collected at four different fermentation stages (Table 1) and further analyzed.

- Yeast and fungal growth, at 30°C and 37°C, respectively, for 72 h under anaerobic conditions (double layer agar); (3) acetic acid bacteria (AAB) on a medium containing 1% w/v glucose (PanReac AppliChem, Darmstadt, Germany), 0.8% w/v yeast extract (Biokar), 1.5% w/v bacteriological peptone (Sigma-Aldrich, St. Louis, US) and 1.5% w/v agar (Condalab) supplemented with 0.1% v/v cycloheximide and 0.05% v/v penicillin (50 mg·mL⁻¹; Biochrom GmbH, Berlin, Germany) to prevent the growth of Gram positive bacteria at 30°C for 72 h under microaerophilic conditions; and (4) total mesophilic counts, on nutrient agar (Condalab) supplemented with 0.1% v/v cycloheximide at 30°C for 48 h under microaerophilic conditions. Results were expressed as the mean ± standard deviation (SD) log CFU g⁻¹ (grapes and soil) or log CFU mL⁻¹ (wines) of three biological replicates per sample.

### Table 1: Sampling points and codes of the soil, grapes, and wine samples examined.

<table>
<thead>
<tr>
<th>Sampling point</th>
<th>Soil (S)</th>
<th>Grapes (G)</th>
<th>Controlled Fermentation (CF)</th>
<th>Spontaneous Fermentation (SF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before veraison</td>
<td>S1</td>
<td>G1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Veraison</td>
<td>S2</td>
<td>G2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Harvest</td>
<td>S3</td>
<td>G3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Must in SF/inoculated must in CF</td>
<td>—</td>
<td>—</td>
<td>CF1</td>
<td>SF1</td>
</tr>
<tr>
<td>Early fermentation</td>
<td>—</td>
<td>—</td>
<td>CF2</td>
<td>SF2</td>
</tr>
<tr>
<td>Midfermentation</td>
<td>—</td>
<td>—</td>
<td>CF3</td>
<td>SF3</td>
</tr>
<tr>
<td>End of fermentation</td>
<td>—</td>
<td>—</td>
<td>CF4</td>
<td>SF4</td>
</tr>
</tbody>
</table>

### 2.3. Physicochemical Analysis.

Fermentations were monitored daily by measuring sugar concentration (Brix) using a Brix digital refractometer (Way-15, Abbe Refractometer, England). Below a Brix threshold value of 8, the specific gravity was determined using a hydrometer until a threshold of 0.990–0.992. Afterwards, the residual sugars (g·L⁻¹) were estimated enzymatically, and the fermentations were considered complete when the residual sugars were <2 g·L⁻¹.

### 2.4. Microbiological Analysis.

Soil (S1–S3) and grape (G1–G3) samples, as well as must and wine samples collected at specific time points during fermentations (CF1–CF4 and SF1–SF4), were subjected to classical microbiological analysis. Soil and grape samples (10 g) were transferred aseptically to sterile stomacher bags with 90 mL of sterile Ringer’s solution (Biokar Diagnostics, Beauvais, France) and homogenized in a stomacher (Lab-Blender 400, Seward Ltd., London, UK) for 120 s at room temperature, to avoid potential microbial contamination, e.g., by hand-crushing. Soil homogenate, must-like suspension from the crushed grapes and wine samples were serially diluted in Ringer’s solution and either 1 mL or 0.1 mL of the dilutions were poured or spread on selective growth media to enumerate the following microbial groups, which are of technological importance: (1) yeasts and molds on yeast extract glucose chloramphenicol (YGC) agar plates (Condalab, Madrid, Spain), at 25°C for 48 h under microaerophilic conditions; (2) mesophilic and thermophilic lactic acid bacteria (LAB) on De Man, Rogosa and Sharpe (MRS) agar (Condalab) supplemented with 0.1% v/v cycloheximide (50 mg·mL⁻¹; Merck, Darmstadt, Germany) to suppress yeast and fungal growth, at 30°C and 37°C, respectively, for 72 h under anaerobic conditions (double layer agar); (3) acetic acid bacteria (AAB) on a medium containing 1% w/v glucose (PanReac AppliChem, Darmstadt, Germany), 0.8% w/v yeast extract (Biokar), 1.5% w/v bacteriological peptone (Sigma-Aldrich, St. Louis, US) and 1.5% w/v agar (Condalab) supplemented with 0.1% v/v cycloheximide and 0.05% v/v penicillin (50 mg·mL⁻¹; Biochrom GmbH, Berlin, Germany) to prevent the growth of Gram positive bacteria at 30°C for 72 h under microaerophilic conditions; and (4) total mesophilic counts, on nutrient agar (Condalab) supplemented with 0.1% v/v cycloheximide at 30°C for 48 h under microaerophilic conditions. Results were expressed as the mean ± standard deviation (SD) log CFU g⁻¹ (grapes and soil) or log CFU mL⁻¹ (wines) of three biological replicates per sample.

### 2.5. Total DNA Extraction and Amplicon-Based Sequencing.

The grape and wine samples were initially washed according to [15] and microbial DNA was extracted using (DNeasy®PowerFood® Microbial Kit; Qiagen, Hilden, Germany), whereas for soil samples DNeasy® PowerSoil® Pro Kit (Qiagen) was applied. DNA elution and storage, as well as determination of DNA concentration and quality were performed accordingly [16]. Yeast/fungal and bacterial diversity was evaluated by amplicon sequencing. Primer pairs ITS1F-ITS2R and 27F-519R were used for the amplification of the ITS1-ITS2 yeasts/fungal DNA region and the V1–V3 hypervariable region of the bacterial 16S rRNA gene, respectively [17]. A paired-end sequencing (2 × 301 bp) was performed on the Illumina MiSeq sequencing platform at Molecular Research (Mr. DNA, Shallowater, Texas, USA), generating 5,018,007 ITS and 3,815,667 16S sequences.

Raw sequencing data are deposited at the European Nucleotide Archive (ENA) under the study PRJEB50321.

### 2.6. Bioinformatics and Statistical Analysis.

Quality control of raw fastq sequences was performed according to Papademas et al. [17]. In brief, the barcodes and primers were removed, paired-end sequences were merged and afterwards, short sequences (<200 bp), sequences with ambiguous base calls, as well as chimeras were removed, resulting in 2,518,538 ITS and 1,908,255 16S sequences. Operational taxonomic units (OTUs) formation and their taxonomic assignments were performed accordingly [16].
Yeast/fungal and bacterial diversity analysis was evaluated in R v.3.6.3 using phyloseq [18], ggplot2 [19], and several custom packages [20]. The intrasamples diversity estimation (alpha-diversity) was calculated using the observed and inverse Simpson index values. For analyzing the bacterial and yeast/fungal community diversity (betadiversity) a multidimensional scaling (MDS) principle coordinates analysis (PCoA) based on Bray–Curtis distance metrics and a hierarchical clustering dendrogram combining single as a clustering algorithm and Bray–Curtis as a distance measure were performed on the OTUs taxonomically assigned at the genus level. All these plots were generated in R using ggplot2 package. Furthermore, hierarchical clustering dendrograms combining ward as the clustering algorithm and Euclidean as the distance measure, heatmap and partial least squares discriminant analysis (PLS-DA) were conducted using metaboanalyst 5.0 [21]. In addition, permutational multivariate analysis of variance (PERMANOVA) was performed with adonis2 in the vegan package v.2.6-4 [22] using Bray–Curtis distances and 10,000 permutations. Moreover, microbial fingerprints of grapes were visualized by the Krona tool [23] and lastly, linear discriminant analysis (LDA) effect size (LEfSe) was performed on the most abundant OTUs, i.e., OTUs with ≥50 reads, to detect key yeast/fungi and bacteria taxa as well as potential biomarkers [24].

Multiple sample comparison and intrasamples diversity estimation (alpha-diversity) was performed accordingly [25]. Values of $P < 0.05$ were considered to be statistically significant.

3. Results and Discussion

3.1. Microbiological Analysis. Results obtained through the classical microbiological analysis for soil and grape samples as well as the results of microbiological and physicochemical analyses of must and wine samples during CF and SF for vineyards A (VA), B (VB) and C (VC) are presented in Supplementary Table 1.

3.1.1. Microbial Counts of Soil Samples. As it was expected [26], soil was a richer microbial ecosystem than grapes with higher counts for all microbial groups examined. Total mesophilic microbiota and yeast counts in the soil samples of VA and VB had a declining trend, while, on the contrary, in VC a slight increase was observed. Both mesophilic and thermophilic LAB population in all three vineyards decreased from the time point before veraison to harvest, with the exception of the thermophilic LAB of VC. The highest decrease was observed again in VA and VB samples, for both microbial groups. Finally, AAB counts in the soil samples of all three vineyards slightly increased at harvest. Although soil could be regarded as a natural reservoir of the grapevine associated microbiota, very few studies have analyzed the soil microbiota via classical microbiological analysis. When Corneo et al. [27] collected soil samples in the Trentino region, Northern Italy, where Chardonnay cv. is the prevailing cultivate, they found that although sampling time significantly affected the yeast and bacteria counts, sampling site did not, as in our study.

3.1.2. Microbial Counts of Grape Samples During Ripening. In grapes, counts of the total mesophilic microbiota decreased during ripening in all three vineyards, while in contrast, yeast population increased. During ripening, more nutrients are available on the surface of the grapes, the acidity decreases, and the microenvironment of grapes is under less stress at harvest, creating a favorable environment for yeast growth [28, 29]. In agreement to our results, several other studies on different grape cultivars have reported an increase in yeasts in grapes during ripening [2, 29–33]. On the other hand, when Chalvantzi et al. [11] examined yeast population of grapes at harvest from four different PDO viticultural regions of Greece, they reported lower counts in the grapes from the Nemea region (with no cultivar names given) than that found in our study. An increase trend was also observed in the case of LAB in our study, as their growth is also related to the increased availability of nutrients at harvest. Our results were in agreement with those of other studies [13, 29]. Finally, apart from the grapes at harvest in VB, AAB were not detected in any of the other grape samples. This was somehow unexpected, as AAB are considered to be one of the most common microbial groups in grapes [29].

3.1.3. Microbial Counts of Must and Wine Samples. In the case of fermentations, the results showed that the microbial evolution had similar trends in all samples no matter vineyard and controlled or spontaneous fermentation, with very few exceptions though. Total mesophilic counts either highly decreased or were eliminated at the end of both CF and SF in all three vineyards, except of CF of VB, where counts slightly increased. As expected, yeast counts increased during CF for all vineyards due to the addition of the commercial yeast culture S. cerevisiae/S. bayanus. A similar trend in yeast population has been reported by Pateraki et al. [14]. Mesophilic and thermophilic LAB counts differed among the three vineyards analyzed in our study at the beginning of both CF and SF. In the SF must samples, LAB counts are in agreement with those reported by Krieger-Weber et al. [34]. During both SF and CF, a declining trend was observed for LAB counts. The elimination of LAB in most of the samples at the end of the fermentation was most probably due to the accumulating ethanol, the lack of nutrients, or the competition with other microbial groups [13]. Finally, AAB counts were quite high in the must samples of both CF and SF but either decreased or were eliminated at the end of the fermentation as they are sensitive to alcohol [35].

3.2. Metataxonomic Analysis

3.2.1. Alpha-Diversity Analysis. In total, 42 samples were analyzed, i.e., nine soil samples (S1A–S3A; S1B–S3B and S1C–S3C), nine grape samples (G1A–G3A; G1B–G3B and...
In order to assess the resemblance of the microbial complexity among the three vineyards, the alpha-diversity was estimated on the basis of the different sample source, i.e., soil, grapes and wines during CF and SF. The observed and inverse Simpson indices were used to evaluate the community richness and diversity (both richness and evenness), respectively. Regarding yeast/fungal communities of soil and grapes, there were no differences among the three vineyards (Supplementary Figures S1A and S1B). On the other hand, a significant difference ($P < 0.05$) was observed between the richness (Observed index) of VA and VC for both CF and SF, as well as between the diversity (inverse Simpson index) of VA and VC during CF (Supplementary Figures S1C and S1D). Similarly, no significant differences were observed in bacteria communities of soil and grapes as well as wine samples during CF among the three vineyards (Supplementary Figures S2A–S2C). A significant difference ($P < 0.05$) was only observed between the diversity (inverse Simpson index) of wine samples during SF in VC with the other two vineyards (VA and VB) (Supplementary Figure S2D).

### 3.2.2. Yeasts/Fungal Composition

Both yeasts/fungal and bacterial microbiota of all samples analyzed were assessed up to the genus level for a more accurate identification, due to the high-level similarity between closely related taxa. This is important in the case of yeasts/fungi, as cut-off equivalents for genus or species level are quite difficult [36].

#### (1) ITS Amplicon Data of Soil Samples

Similar to the classical microbiological analysis, the results of the amplicon sequencing confirmed the higher biodiversity of soil than that of grapes and wine samples of both CF and SF for all three vineyards. Ascomycota was the dominant phylum in all but one soil sample, followed by Basidiomycota and Chytridiomycota (Supplementary Figure S3A). The above-mentioned phyla were mainly represented by the families Sclerotiniaceae, Mortierellaceae, Netriaceae, Pyrenomataceae, Cordycipitaceae, Lecanoromycetes, and Entolomataceae (Figure 2(a)). At the genus level, VA had the highest diversity among the three vineyards. Entoloma, Botrytis and Myrothecium were the predominant in VA, Sarea, Fusarium, and Isaria in VB, and Pseudoaurelia, Fusarium, and Pseudaurelia in VC (Figure 2(b)).

To further explore the degree of diversity among the soil samples, a hierarchical clustering and an MDS/PCoA plot were generated based on OTUs that were taxonomically assigned at the genus level (Figures 2(c) and 2(d)). Two main groups were observed, one formed solely by S1A due to its different microbial fingerprint, as mentioned above, and one by the rest of the soil samples. Subclusters revealed that VB samples grouped together, with S1B and S2B being more similar compared to S3B. This was also the case with the VC samples, as S1C and S2C grouped together in a separate cluster. Finally, S2A and S3A grouped together with the VC samples in the same cluster.

#### (2) ITS Amplicon Data of Grapes, Must and Wine Samples

The yeast/fungal microbiota of grapes was characterized for all vineyards by a high level of the Ascomycota phylum and to a lesser extent of Basidiomycota, mainly at the time point before veraison (Supplementary Figure S3A). The dominant families found in all vineyards were Aureobasidiales, Cladosporaceae, Pleosporaceae, and Aspergillaceae (Figure 3(a)). At the genus level, the most abundant genera in the three vineyards were *Aureobasidium* and/or *Cladosporium*, followed by *Penicillum* and/or *Alternaria* (Figure 3(b)). Nevertheless, the relative abundances varied along the stage of grape ripening and across the three vineyards. In addition, wine fermentation-related genera, such as *Saccharomyces* and *Hanseniaspora*, were detected in grape microbiota at low relative abundance (<2.0%) in all samples of VA (G1A–G3A) and at the time point of harvest for VB (G3B) and VC (G3C). It is also worth mentioning that many of the genera detected in the soil were also found in the grapes, either at higher (e.g., *Alternaria*, *Aureobasidium*, and *Cladosporium*) or lower relative abundances (e.g., *Cryptococcus*, *Entoloma*, *Sarea*, *Isaria*, and *Psathyrella*).

As it was expected, the yeast/fungal communities during wine making were less diverse than in soil or grapes, no matter if they were CF or SF [43]. Ascomycota dominated during both types of fermentation in all three vineyards (Supplementary Figure S3B). As expected, in CFs, the family *Saccharomycetaeae*, represented by the genus *Saccharomyces*, was the predominant one due to the inoculation of must with the commercial yeast culture *S. cerevisiae*/*S. bayanus* (Figures 3(a) and 3(b)). Compared to CFs, must and wine samples during SF were dominated by both *Saccharomycodaceae* and *Saccharomycetaeae* families (Figure 3(a)). At the genus level, *Hanseniaspora* was the fungi are very common inhabitants of soil, as they have mechanisms by which they can cope with the harsh conditions prevailing in this ecosystem [37]. Therefore, the soil fungi biodiversity is quite high compared to the yeast diversity [30], which was also confirmed in our study. Among the most abundant genera that have been previously identified in vineyards’ soil are *Fusarium*, *Entoloma*, *Aureobasidium*, *Giberella*, *Penicillium*, *Sporobolomyces*, and *Aspergillus* [26, 38, 39], which belong to filamentous fungi and are in accordance with our results. Regarding wine fermentation-related yeasts, i.e., *Saccharomyces* and *Hanseniaspora*, it has been established that they are present at low abundances in the vineyards’ soil [40]. It should be mentioned that even though the family *Saccharomycetaceae* had a low relative abundance in the soil samples analyzed in our study as well, it was among the 35 most abundant families identified. Interestingly, in soil samples collected in a vineyard in Ribera del Duero, Spain, *Saccharomycetaceae* was the most abundant family in the summer season [41]. Moreover, the identification of the genus *Psathyrella* in all three vineyards (VA, VB, and VC) examined in the present study, despite the low relative abundances (<11.3%), was somehow unexpected, as it has been previously associated with other ecosystems, such as grassland and wood [42], but not with vineyards’ soil.
The predominant one in SF1A, SF1B, and SF1C samples. However, during SF, the relative abundance of *Saccharomyces* increased, and at the end of SF the genus became the most abundant, due to its high adaptation to the harsh fermentation conditions [28, 44]. Finally, genera associated with the grape microbiota, e.g., *Cladosporium*, *Sclerotinia*, *Lachancea*, and *Metschnikowia*, were mainly detected in SF1A, SF1B, and SF1C samples, although at low relative abundances.

The hierarchical clustering and PCoA analysis of all grapes, must, and wine samples revealed two main groups, one containing all grape samples and one with all must and wine samples from both CF and SF (Figures 3(c) and 3(d)). Subclusters revealed that G1A grouped separately from the rest of the grape samples, wine samples from CF along with SF4C formed a cluster due to the high relative abundance of the genus *Saccharomyces*, and SF1A and SF1B grouped together due to the high relative abundance of *Hanseniaspora*. In addition, SF2A was grouped separately as it was characterized by the equivalent presence of *Hanseniaspora* and *Saccharomyces*, SF1C remained unclustered, as its microbial fingerprint was the most diverse among all must and wine samples.
wine samples, and lastly, the rest of the SF samples were grouped together.

Similar to our results, several other studies on different grape varieties have reported the dominance of yeast-like fungal or filamentous fungal genera (e.g., *Aureobasidium*, *Alternaria*, *Cladosporium*, and *Cryptococcus*) compared to the yeast ones (e.g., *Hanseniaspora* and *Saccharomyces*) [39, 45–51]. It should also be noted that the genus *Paathryella* was found both in soil and grape samples in our study, reinforcing the belief that soil is a major reservoir of microorganisms for the grapevine [52–54]. Indeed, when Morrison-Whittle et al. [55] examined the microbiota of soil, bark, and mature grapes from 12 Sauvignon Blanc vineyards in the Marlborough region, South Island, New Zealand, they showed that several fungal taxa were shared not only between soil and grapes but also with the must. Furthermore, Mezzasalma et al. [56] examined grape samples of *Vitis vinifera* L. cv. Grenache at harvest and reported the genera *Aureobasidium*, *Alternaria*, and *Hanseniaspora* among the most abundant ones, while in our study *Hanseniaspora* was identified at low relative abundance. As in the case of soil, the genera *Saccharomyces* and *Hanseniaspora* are not frequently detected in grapes at high abundances [30]. Many studies have shown that the microbial genera of grapes are eliminated in early fermentation and replaced by the most common wine fermentation-related microorganisms [48, 57, 58]. At the beginning of SF, the non- *Saccharomyces* yeasts, such as *Hanseniaspora* (*Kloeckera*), *Candida*, and *Metschnikowia* usually dominate the yeast/fungal microbiota [59]. However, these yeasts have a low fermentative activity and a low SO₂ resistance, and at the same time, they cannot survive until the end of the fermentation [2]. In our study, *Hanseniaspora*, which has been reported to contribute to wine quality [2], was the genus that dominated the must samples of all three vineyards. However, as in other studies [49, 56, 60], the relative abundance of *Hanseniaspora*

**Figure 3:** Composition plots of the relative abundances of the 20 most abundant yeast/fungal OTUs taxonomically assigned at the family (a) and genus (b) level in grapes and wine samples of VA (G1A–G3A; CF1A–CF4A; SF1A–SF4A), VB (G1B–G3B; CF1B–CF4B; SF1B–SF4B), and VC (G1C–G3C; CF1C–CF4C; SF1C–SF4C). Samples from each vineyard are presented together in subpanels. Hierarchical clustering (c) and principal coordinates analysis (PCoA) (d) of yeast/fungal communities at the genus level in grapes and wine samples of VA (G1A–G3A; CF1A–CF4A; SF1A–SF4A), VB (G1B–G3B; CF1B–CF4B; SF1B–SF4B), and VC (G1C–G3C; CF1C–CF4C; SF1C–SF4C).
decreased at the end of fermentation but was not eliminated, while the abundance of Saccharomyces increased. On the contrary, the yeast/fungal microbiota during CF is more stable compared to SF, as Saccharomyces dominates from the beginning to the end of the fermentation [61].

3.2.3. Bacterial Composition. Compared to yeasts/fungi, bacterial microbiota was more diverse. In all ecosystems (soil, grapes, wine), proteobacteria, acidobacteria, and firmicutes were the predominant phyla, represented by several families and genera.

(1) 16S Amplicon Data of Soil Samples. Regarding soil, the bacterial microbiota was quite similar in all samples no matter the vineyard of origin. Among the phyla identified, Proteobacteria and Actinobacteria were the predominant ones (Supplementary Figure S4A). At the family level, Rhodospirillaceae, Planctomycetaceae, Sphingomonadaceae, Acidobacteriaceae, Cytophagaceae, Methylobacteriaceae, Psuedomonadaceae, and Chitinophagaceae were the most abundantly identified in soil samples across all three vineyards (Figure 4(a)). At the genus level, Skermanella was the predominant one in S1A and S1C (Figure 4(b)). In the case of VB, the genus Acidibacterium was the predominant one in all sampling points, whereas the same genus also dominated S2C as well as all samples at harvest (S3A, S3B, S3C). Exceptionally, in S2A the most abundant one was Ohtaekwangia. The subdominant microbiota of the soil samples consisted of the genera Pseudomonas, Gemmatimonas, Sphingomonas, Pirellula, Zavarzinella, Arthrobacter and Niastella with varying abundances depending on the sample. The relatively low abundances of the taxa identified indicate the high bacterial diversity of this ecosystem.

Even though the bacterial composition seemed to be quite similar among the soil samples of the three vineyards, the hierarchical clustering and PCoA plot based on the 16S data at the genus level revealed three main clusters (Figures 4(c) and 4(d)). The first was formed by S3A and S3B, the second by only S3C, and the third by the rest of the soil samples. This clustering indicates that the bacteria microbiota of soil samples at the first two sampling points (before veraison and at veraison) was more similar compared to that of the third sampling point (at harvest). This could be explained as soil is under less stress at harvest, as agrochemical applications, i.e., the use of fertilizers, liming and acidifying agents, soil conditioners and pesticides, have been stopped several weeks ago [29]. Interestingly, sub-clusters revealed that S1B and S2B as well as S1C and S2C grouped together, as also happened in the ITS clustering (Figure 2(c)).

As in the case of yeast/fungi, soil hosts a great number of bacteria as well. The microbial composition in vineyard soil depends on many factors, such as soil texture, nitrogen content, phosphorus content, carbon to nitrogen ratio, water content and pH, vinicultural area and fertilizer applications, which can alter the relative abundances of bacteria taxa [62–64]. In our study, the results revealed the dominance of the phyla Proteobacteria, Acidobacteria, Bacteroidetes and Gemmatimonadetes in all three vineyards no matter the vineyard of origin. Several studies have shown that these bacterial phyla, along with Verrucomicrobia, Planctomycetes, Actinobacteria, Chloroflexi and Firmicutes, are the most common ones in soil vineyard [54, 62, 63, 65]. At the family level, Rhodospirillaceae, Planctomycetaceae, Sphingomonadaceae, Acidobacteriaceae, Cytophagaceae, Methylobacteriaceae, Pseudomonadaceae and Chitinophagaceae, which were mainly identified in all three vineyards in our study at different relative abundance, have been previously reported as part of the soil microbiota [26, 54, 62].

(2) 16S Amplicon Data of Grapes, Must and Wine Samples. In grapes, the phylum Proteobacteria was the predominant one, followed by Actinobacteria and Firmicutes (Supplementary Figure S4B). Proteobacteria was mostly represented by the families Pseudomonadaceae, Sphingomonaceae and Rhodospirillaceae, while Firmicutes and Actinobacteria by the families Leuconostocaceae, Staphylococaceae and Rubrobacteraceae, respectively (Figure 5(a)). Among the three vineyards, VA had the most stable bacterial profile during grape ripening. Focusing on the most abundant genera, as in the case of yeast/fungal microbiota, a similar distribution pattern to that observed at the family level was revealed, as one or two genera corresponded to all reads assigned to a certain family. Thus, Micrococcus and Rubrobacter-dominated samples G1B and G1C, respectively, while Skermanella dominated both G2B and G2C (Figure 5(b)). The family Leuconostocaceae that dominated harvest samples of both VB and VC vineyards was represented by the genus Oenococcus. Other genera detected in the grapes of all three vineyards were Microbacterium, Lactobacillus, Propionibacterium, Alcanivorax, Staphylococcus, Tatumella at different abundances depending on the sample.

The bacteria microbiota of must and wine samples consisted of the same phyla as those found in the grapes (Supplementary Figure S4B). Interestingly though, in the SF1C sample, the phylum Terenicies was the most abundant one, while it was detected in the respective grape and soil samples with relative abundance only <1.0%. It is also worth mentioning that this phylum was also identified in samples CF1C, CF2C, CF3C and CF1B, while in the other samples it was either not detected or its relative abundance was <1.3%. Among the families identified, Enterobacteriaceae, Alcanivoraceae, Acetobacteraceae, Propionibacteriaceae, Mycoplasmaceae and Leuconostocaceae were the predominant ones with different abundances among the must and wine samples of the three vineyards (Figure 5(a)). Regarding SF, a higher consistency was observed at the family level both among the sampling points of each vineyard and the three vineyards examined. The families Enterobacteriaceae and Acetobacteriaceae were the predominant ones in all samples, except of SF4A and SF1C, where Sphingomonadaceae and Mycoplasmaceae dominated, respectively (Figure 5(a)). Within Enterobacteriaceae, the genus Tatumella dominated mostly in the SF of all three vineyards as well as in the CF2B and CF3B samples (Figure 5(b)). More specifically, in VA, the genus Tatumella was the predominant one until the mid-fermentation
(SF3A), while at the end of the fermentation (SF4A) Sphingomonas dominated. On the contrary, in the CF of the same vineyard, Sphingomonas was detected at relative low abundance, while the genus Alcanivorax was the predominant one throughout fermentation. The genus Tatumella in VB dominated the SF1B and SF2B as well as CF1B and CF2B samples (Figure 5(b)). Both in CF and SF of VB, the genus Komagataeibacter of the family Acetobacteraceae dominated at the end of fermentation (CF4B and SF4B), followed by the genus Propionibacterium. This was in line with the classical microbiological results, as VB was the only vineyard where AAB population was not eliminated in CF.

In the case of VC, the genus Mycoplasma dominated both SF1C and CF1C samples, while in SF2C – SF4C samples Tatumella was the most abundant. Regarding CF of VC, the most abundant genera in CF2C and CF3C were Bacillus, Oenococcus and Tatumella. In CF4C, the aforementioned genera were found at relatively low abundances (0.6–11.9%), while the genus Propionibacterium was the predominant one.

The higher biodiversity of bacteria taxa compared to the yeasts/fungi ones was also reflected in the hierarchical clustering and PCoA plot of all grape, must and wine samples analyzed (Figures 5(c) and 5(d)). In contrast to the ITS clustering (Figure 3(d)), grape samples were not grouped together in one main cluster. As it was expected, 16S clustering was also different compared to the ITS one for the must and wine samples, due to the lower yeast/fungi biodiversity especially in the case of wine samples, in which Saccharomyces and Hanseniaspora were the dominant genera identified. The main groups revealed by 16S clustering were formed by G1B, G1C, G2B and G2C.
Figure 5: Composition plots of the relative abundances of the 30 and 35 most abundant bacterial OTUs taxonomically assigned at the family (a) and genus (b) level, respectively, in grapes and wine samples of VA (G1A–G3A; CF1A–CF4A; SF1A–SF4A), VB (G1B–G3B; CF1B–CF4B; SF1B–SF4B) and VC (G1C–G3C; CF1C–CF4C; SF1C–SF4C). Samples from each vineyard are presented together in sub-panels. Hierarchical clustering (c) and principal coordinates analysis (PCoA) (d) of bacterial communities at the genus level in all grapes and CF panels. Hierarchical clustering (c) and principal coordinates analysis (PCoA) (d) of bacterial communities at the genus level in all grapes and wine samples (G1A–G3A; CF1A–CF4A; SF1A–SF4A; G1B–G3B; CF1B–CF4B; SF1B–SF4B; G1C–G3C; CF1C–CF4C; SF1C–SF4C).

that were grouped separately from the rest of the other grape (as well as must and wine) samples. In addition, G1A, G2A and G3A grouped together, with G2A and G3A formed a subcluster, as also occurred with the ITS clustering (Figure 3(c)). G3C sample grouped together with CF3C, CF1C, CF2C and SF1C, indicating a similar bacteria microbiota among the grapes at harvest, the wine samples during CF (up to the mid-fermentation) and the must in SF for VC. The rest of the wine samples during SF (i.e., SF2C–SF4C) grouped together, due to the dominance of the genus Tatumella. Another cluster was formed by the wine samples of VA during CF (CF1A–CF4A), while, on the contrary, the majority of VA samples during SF (SF1A–SF3A) were grouped with the respective ones of VB during CF (CF1B–CF3B) as well as SF1B and SF3B.

Contrary to the findings for the yeast/fungal community in grapes, bacterial composition showed many differences among the three vineyards and among the sampling points for VB and VC. Lactobacillales, Bacillales, Enterobacteriales, Pseudomonadales, Actinomycetales and Rhodospirillales have been reported both in our study and in the literature as the bacteria phyla found at high abundances in grapes [56, 58]. Focusing on the genera identified, Micrococcus, Rubrobacter, Skermanella, Microbacterium, Alcanivorax, Staphylocoecus, Tatumella, Propionibacterium and Bacillus were the most abundant ones. Some of these genera have been previously associated with wine grapes microbiota.
3.2.4. Impact of Different Factors on Shaping the Yeasts/Fungi and Bacterial Microbiota of All Samples Analyzed.

PERMANOVA analysis was performed to assess the significance of different factors on shaping the yeasts/fungi and bacterial microbiota of the samples analyzed. Starting with the whole dataset, all factors individually, i.e., sampling point (Before veraison, veraison, harvest, must in SF, inoculated must in CF, early fermentation, midfermentation and end of fermentation), fermentation (CF and SF), vineyard (A, B, and C) and ecosystem (soil, grapes, and wine), and the interaction fermentation: Vineyard significantly affected both yeasts/fungi and bacterial communities (Supplementary Table S2). All these factors explained approximately 61% and 58% of the total variation for yeasts/fungi and bacterial microbiota, respectively. Afterwards, the analysis was conducted on different types of samples, as the ecosystem was found to be a significant factor ($P = 0.0001$), as mentioned before. PERMANOVA analysis performed on the soil samples (Supplementary Table S3) revealed that only sampling point was significant ($R^2_{\text{soil/fungi}} = 0.32$, $P = 0.019$; $R^2_{\text{bacteria}} = 0.56$, $P = 0.017$). On the contrary, vineyard was found to significantly affect the microbial composition of grape samples, ($P_{\text{yeasts/fungi}} = 0.018$; $P_{\text{bacteria}} = 0.017$) explaining almost 50% of distribution among the samples for both yeasts/fungi and bacteria (Supplementary Table S4). Regarding the wine-associated yeasts/fungi microbiota, all factors individually, i.e., sampling point, fermentation, and vineyard, were significant ($P = 0.0001$; Supplementary Table S5). Fermentation and vineyard were the main factors, each explaining over 25% of the total variation, followed by sampling point ($R^2 = 0.11$). Some interactions were also significant, i.e., sampling point: vineyard and fermentation: vineyard ($P = 0.0001$), accounted together for approximately 36% of the variation. On the contrary, fermentation and vineyard were the main factors ($P = 0.0001$) impacting on the bacteria communities of wine samples, followed by Sampling point ($P = 0.026$). All these factors explained almost 60% of the total variation. Finally, fermentation: vineyard was the only interaction that significantly affected the bacterial communities of wine samples ($P = 0.0001/R^2 = 0.15$).

PERMANOVA has been performed in several studies to assess the factors that are important in shaping the microbiota. The type of samples has been found to be one of the major explanatory variables in many studies, which is in line with our results [54, 55, 72–74]. In addition, biogeography has been widely studied in recent years, and according to many studies, apart from other factors, geographical region seems to significantly influence the microbiota [11, 67, 74–81]. In our study, geographical region was an important factor only in the case of grapes, while it did not appear to significantly affect the microbiota (both yeasts/fungi and bacteria) of soil samples. However, as only three vineyards were selected and analyzed from one vintage in our study, additional work is required to understand the wine-related terroir in the PDO Nemea zone.

3.2.5. Microbial Biomarkers Associated with the Geographical Region. As PERMANOVA results indicated that the vineyard factor was significant for the microbiota of grape samples, we decided to perform further analyses to identify microbial biomarkers associated with the geographical region of each vineyard. According to the Venn diagram, from the total of 949 yeast/fungi OTUs that were identified in all grape samples (i.e., 275, 509, and 569 OTUs for GA, GB, and GC, respectively), grapes from the closely located lowland subzones VB and VC share 338 OTUs (Figure 6(a)). On the
other hand, grapes from the remote mountainous sun-zone VA shares only 66 OTUs with those of VB, and, interestingly, none with those of VC, indicating differences in the microbial communities’ structure of GA compared to GB and GC samples. Of note, these 66 shared OTUs between GA and GB were only found in G3B and not in G1B and G2B.

Figure 6: Venn diagram of the unique and shared yeasts/fungi (a) and bacteria (c) OTUs found in at least one grape sample during ripening (G1, G2, G3) per vineyard (VA, VB, VC). Hierarchical clustering dendrogram of yeasts/fungi (b) and bacteria (d) OTUs identified in all grapes analyzed. Samples are colored according to the vineyard, i.e., red for Asprokambos (A; ID 9), green for central Nemea (B; ID 72) and blue for Koutsi (C; ID 36).
samples. Based on the hierarchical clustering dendrogram, in which we did not group the grape samples per vineyard, G3B formed a cluster with G2A and G3A, and G3C with G1A (Figure 6(b)). However, it should be mentioned that although G3C and G1A do not share OTUs, they are clustered together due to the absence of reads in the same OTUs. Krona plots of the grape yeast/fungi microbiota also highlighted their differences up to the species level, both during grape ripening and among the three vineyards (Supplementary Figure S5). Furthermore, LEfSe analysis was performed on the most abundant OTUs, i.e., OTUs with \( \geq 50 \) reads to determine significantly abundant common taxa among GA, GB, and GC (LDA score \( > 3.6 \)) and potential microbial biomarkers (Supplementary Figure S6A). Of note, GB had the most biomarkers, i.e., six, including taxa up to Cladosporium cladosporioides, Sporobolomyces sp., and Cryptococcus sp. (Figure 7(a)). It should be mentioned that C. cladosporioides has been linked to the development of Cladosporium rot in grapevines [82], which is a common disease of red wine grape cultivars, especially of Cabernet Sauvignon cv., in Chile, as C. cladosporioides is favored by the delayed harvest of Cabernet Sauvignon cv. grapes [83, 84]. Regarding GA samples, Penicillium sp. was found to be a potential biomarker according to the LEfSe analysis, while it is interesting that none of the GC OTUs were significantly abundant.

Similar to the yeast/fungi Venn diagram, from the total of 1,350 bacteria OTUs found in all grape samples, GB and GC shared 516 while GA and GB only 94 OTUs (Figure 6(c)). The differentiation of the GA microbiota compared to that of GB and GC during grape ripening was evident in the Krona plots (Supplementary Figure S7) and in the hierarchical clustering dendrogram, as the mountainous subzone GA samples grouped separately from the lowland subzones GB and GC (Figure 6(d)). PLS-DA analysis also supported the discrimination of GA samples (Figure 8(a)), and, thus, variable importance in projection (VIP) scores of PLS-DA were used to estimate the influence of the geographical region on bacteria OTUs. Based on the VIP scores, nine OTUs were considered as highly influential (Figure 8(b)). In details, OTU_4 (Pseudomonas sp.), OTU_6 (Sphingomonas sp.), OTU_15 (Sediminibacterium salmonense), OTU_16 (Sphingomonas sp.), OTU_14 (Microbacterium trichothecenolyticum), and OTU_17 (Comamonas sp.) were highly associated with the GA samples; OTU_4657 (Micrococcus luteus) and OTU_4915 (Lactobacillus delbrueckii) with GB and OTU_4597 (Rubrobacter xylanophilus) with GC samples. In addition, the LEfSe analysis was performed once again on the most abundant OTUs, highlighting Alphaproteobacteria, Bacteroidetes, and Proteobacteria as well as Propionibacteriaceae and Propionibacterium as the most significantly abundant taxa (LDA score \( > 4.8 \)) for GA and GC, respectively (Supplementary Figure S6B). Of them, Alphaproteobacteria (GA), Propionibacteriaceae (GC), and Propionibacterium (GC) can be considered as potential biomarkers, while none of the GB OTUs was distinctive (Figure 7(b)).

One of the first and most significant studies regarding the microbial biogeography of wine grapes was performed by Bokulich et al. [15], demonstrating that region, climatic factors, grape cultivar, and vintage nonrandomly shape the bacteria and yeasts/fungi communities of wine grapes. A few years later, the same group analyzed 777 Chardonnay and Cabernet Sauvignon must and wine fermentation samples to see whether cultivar, vineyard, and viticultural area has an impact on the bacterial and fungal communities [57]. The authors demonstrated that the viticultural areas were distinguished based on the must microbiota, and, more specifically, Chardonnay exhibited

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**Figure 7:** Linear discriminant analysis (LDA) effect size (LEfSe) taxonomic cladograms of the most abundant OTUs. Significantly discriminant yeasts/fungi (a) and bacteria (b) taxa that can be used as potential biomarkers for the grapes are colored based on the vineyard. A yellow-colored node corresponds to a nonsignificantly differentiated OTU.
higher microbial composition differentiation than Cabernet Sauvignon. A microbial biogeography study has been also performed by Pinto et al. [48]. The authors found that compared to the yeasts/fungi microbiota, bacteria communities appeared to be more influenced by the geographical region, which was in accordance with our results. In addition, region-associated microbial signatures have been also found by Mezzasalma et al. [80] in grape and wine samples of the Cannonau cv. from four Sardinian regions. Furthermore, regional patterns regarding both yeasts/fungi and bacteria microbiota have been found by Liu et al. [78] in grape and wine samples, with a more distinct trend being observed in the case of yeasts/fungi. Finally, Kamilari et al. [85] identified potential yeasts/fungi and bacteria biomarkers in must and wine fermentation samples of two grape cultivars from five viticultural areas in Cyprus using LEfSe analysis. The authors found that the biomarkers were associated with each cultivar and geographical region, throughout the fermentation process.

4. Conclusions

A combination of classical microbiological analysis and metat Paxonomy was used for the first time to evaluate the microbial community structure of soil, grapes, and wine from three Agiorgitiko cv. vineyards in the PDO Nemea zone in Greece, with the ultimate goal being to collect pieces of evidence about the microbial terroir of the zone. The classical microbiological analysis highlighted that soil and wine had higher microbial counts than grapes; however, only few significant differences were observed among the three vineyards when comparing samples of the same ecosystem, i.e., soil, grapes, and wine. The metat Paxonomy analysis revealed that, in general, bacterial communities were more diverse than those of yeasts/fungi, in particular in the case of soil. Furthermore, the geographical region factor was found to significantly influence the microbiota of grapes and wine samples, but not that of soil samples. However, additional experiments are needed and are in progress, including more Agiorgitiko cv. vineyards from the PDO Nemea zone and samplings from different vintages and climate conditions, to assess the hypothesis about a uniform microbial terroir in the zone.

Data Availability

Raw sequencing data are deposited at the European Nucleotide Archive (ENA) under the study PRJEB50321.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

This work was supported by the European Regional Development Fund of the European Union and the Greek National Funds through the Operational Program Competitiveness, Entrepreneurship, and Innovation, under the call RESEARCH—CREATE—INNOVATE (project code: T1EDK-04202).
Supplementary Materials

Supplementary Table S1. Mean ± SD of Brix (Brix) and reducing sugars (g L⁻¹) of must and wine samples and microbial counts (log CFU g⁻¹ or ml⁻¹) of all samples of VA, VB, and VC examined. Supplementary Table S2. PERMANOVA on whole dataset based on the ITS and 16S data using adonis2, Bray–Curtis distances and 10,000 permutations. Significance codes: 0 "***" 0.001 "**" 0.01 "*" 0.05 "." 0.1 " " 1. Supplementary Table S3. PERMANOVA on soil dataset based on the ITS and 16S data using adonis2, Bray–Curtis distances, and 10,000 permutations. Significance codes: 0 "****" 0.001 "***" 0.01 "**" 0.05 "." 0.1 " " 1. Supplementary Table S4. PERMANOVA on grapes dataset based on the ITS and 16S data using adonis2, Bray–Curtis distances, and 10,000 permutations. Significance codes: 0 "****" 0.001 "***" 0.01 "**" 0.05 "." 0.1 " " 1. Supplementary Table S5. PERMANOVA on must and wine dataset based on the ITS and 16S data using adonis2, Bray–Curtis distances, and 10,000 permutations. Significance codes: 0 "****" 0.001 "***" 0.01 "**" 0.05 "." 0.1 " " 1. Supplementary Figure S1. Alpha-diversity boxplots for yeast/fungal communities identified in soil (A), grapes (B) and wine samples during CF (C) and SF (D). An asterisk (*) denotes statistically significant difference (P < 0.05). Supplementary Figure S2. Alpha-diversity boxplots for bacterial communities identified in soil (A), grapes (B) and wine samples during CF (C) and SF (D). An asterisk (*) denotes statistically significant difference (P < 0.05). Supplementary Figure S3. Composition plots of the relative abundances of the 10 most abundant yeast/fungal OTUs taxonomically assigned at the phylum level in soil samples (A) of VA (S1A–S3A), VB (S1B–S3B) and VC (S1C–S3C) and in grapes and wine samples (B) of VA (G1A–G3A; CF1A–CF4A; SF1A–SF4A), VB (G1B–G3B; CF1B–CF4B; SF1B–SF4B) and VC (G1C–G3C; CF1C–CF4C; SF1C–SF4C). Samples from each vineyard are presented together in subpanels. Supplementary Figure S4. Composition plots of the relative abundances of the 20 most abundant bacterial OTUs taxonomically assigned at the phylum level in soil samples (A) of VA (S1A–S3A), VB (S1B–S3B) and VC (S1C–S3C) and in grapes and wine samples (B) of VA (G1A–G3A; CF1A–CF4A; SF1A–SF4A), VB (G1B–G3B; CF1B–CF4B; SF1B–SF4B) and VC (G1C–G3C; CF1C–CF4C; SF1C–SF4C). Samples from each vineyard are presented together in subpanels. Supplementary Figure S5. Krona plots illustrating the yeasts/fungi microbiota of grape samples during ripening (G1, G2, and G3) per vineyard (VA, VB, and VC). Supplementary Figure S6. Linear discriminant analysis (LDA) of the most abundant OTUs. Significantly discriminant yeasts/fungi (A) and bacteria (B) taxa for the grape samples are colored based on the vineyard. Supplementary Figure S7. Krona plots illustrating the bacteria microbiota of grape samples during ripening (G1, G2, and G3) per vineyard (VA, VB, and VC). (Supplementary Materials)

References


