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# Characterization of Indigenous Microbial Communities in Vineyards Employing Different Agronomic Practices: The Importance of Trunk Bark as a Source of Microbial Biodiversity

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**Abstract:** Microbiomes are essential to viticulture and winemaking since various fungi and bacteria can exert positive and negative effects on grape health and wine quality. The current work evaluates the communities of culturable fungi and bacteria associated with Corvina vines derived from two vineyards from a similar terroir (within the Valpolicella DOC area, Italy) but on which different management practices were employed: organic and conventional farming. Samples of bark and grapes were collected in four spatial points for each vineyard. Populations of bark-associated microorganisms were monitored during ripening season (at veraison and at harvest time), and results were integrated with data from grape-associated microorganisms, sampled right before harvest. Culturable populations of fungi and bacteria were determined by plate counting on WL and PCA culture media. For fungi, biodiversity was also assayed on all samples through molecular methods, by ITS-RFLP analysis. Although this does not represent a comprehensive evaluation of the microbiome, since culturable and countable microorganisms only represent a portion of microbial biodiversity, our results emphasize the importance of vine trunk bark, not only as an interesting habitat to be characterized for monitoring microbial biodiversity in vineyards but also as a potential source of microbial viable species for further isolation.

**Keywords:** microbial terroir; *Vitis vinifera*; microbiota; bark; grape



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## 1. Introduction

Microbiomes are essential to viticulture and winemaking since diverse fungi and bacteria can exert positive and negative effects on grape health and wine quality. Vineyards, indeed, provide an appropriate environment for growth of different types of microorganisms. [1,2] As wine is a fermented natural product, the vineyard also serves as a key point of entry for quality-modulating microbiota, particularly in wine fermentations that are conducted without the addition of exogenous yeasts [2]. Thus, the sources and persistence of wine-relevant microbiota in vineyards critically impact its quality. Moreover, differences in microbiota within and between vineyards may contribute to regional wine characteristics [2]. Indeed, in recent years, several studies introduced the concept of "microbial *terroir*", since the commensal microbial flora that coexists with the grapevine may be one of the factors that influence the traits traditionally associated with the terroir notion [3–6].

Previous studies showed that the surface of grape berries represents a natural source of microorganisms that have various impacts on wine quality [6–8]. Nevertheless, grape bunches, the primary substrate of winemaking, are ephemeral like all fruits, and thus cannot be a stable habitat for microbes [9]. Soil microorganisms are able to colonize parts of the plant above the ground, including leaves and fruits, to reach grape berries [10]. Therefore, soils have repeatedly been argued to be the primary reservoir for microbiota on plant surfaces aboveground, including in grapevines [2,10,11]. Despite their importance,

the diversity of epiphytic microbiota on aboveground grapevine compartments remains inadequately described, as is the role of different plant parts together with vineyard soil in microbial colonization of berries [12]. In particular, among other vine parts that might share their microbiota with grape skin, such as roots, leaves and flowers, bark has received attention only in a few research papers [9,11,12]. The importance of vine trunk bark has been proposed only recently, not only as a potential source of inoculum for grapes but also as an interesting habitat to be characterized for monitoring microbial biodiversity in vineyards and the impact of agronomic management on it [9,12,13]. Indeed, this niche is stable over seasons, relatively rich in nutrients, and harbors a rich epiphytic microbiota, as bark has been shown to host a greater microbial diversity and species richness than grapes and leaves [9]. Therefore, its exploration allows much more biodiversity to be unveiled than the sole grape berry characterization. Moreover, recent findings suggest that geographic and anthropogenic factors (such as agronomic management) impact both the bark and grape microbiome, but to a different extent [12].

The many recent studies that have focused on deciphering the grape- and grapevine-associated microbiome (reviewed in [2,8,14]), to determine how viticultural practices could potentially influence these communities and their dynamics [8,9,12,15–18], are mainly based on new technologies for massive species identification that were recently developed [8].

At the same time, it is also worth noting that grape-must microorganisms, particularly important in spontaneous fermentations, enter from the vineyard [2]. Therefore, in parallel with the continuously growing research based on high throughput genomic techniques (NGS), studies based on culture-dependent methods, are still performed and considered valuable for deciphering the viable, culturable fraction of microorganisms [7,11,19–24]. The latter, indeed, contains the microbial species that can access subsequent winemaking stages, and therefore represents the first step into the study of the interesting positive effects on flavor diversity and wine quality potentially obtainable by using "friendly" native yeasts that allow the microbial terroir flora to participate and contribute during fermentation [25,26].

In this study, samples of bark, a non-ephemeral part of the vine, were analyzed together with grape berries with the aim of unveiling the composition of their culturable epiphytic microbial communities. Moreover, the impact of different agronomical practices on the composition of both grape and bark culturable microbiota was investigated, for the first time considering both eukaryotes and prokaryotes. For microbiological analysis, therefore, a culture-dependent approach was applied, coupled with molecular characterization. In particular, populations of bark-associated viable microorganisms were monitored during the ripening season (at veraison and at harvest time), and results were integrated with those obtained about grape-associated microorganisms, sampled right before harvest. The study was conducted in Valpolicella DOC, a renowned winegrowing area of northern Italy, whose terroir has been object of previous studies due to the great interest of consumers in the arising wines [27,28].

## 2. Materials and Methods

### *2.1. Bacteria and Yeasts/Fungi Isolation*

Twenty-four samples of grapes and bark were collected aseptically from grapevines in vineyards from the Valpolicella DOC viticultural area (45°28′33.3″ N 11°03′49.2″ E), Veneto, Italy, during the 2015 growing season, transported on ice and directly processed. The local grape variety was Corvina, according to regional product specifications [28]. Two vineyards differing in agronomic management were studied; details of management practices were gathered through interviews with agronomic consultants and vineyard managers: Briefly, in the organic vineyard, pest management was achieved only through copper/sulphur-based products (which were also used in the conventional vineyard in comparable amounts), and no herbicide was used (for more details, please see the Acknowledgements Section and the Supplementary Table S1). The two vineyards were chosen to be as homogeneous as possible in terms of rootstock (Kober 5BB), training system (Guyot),

and vine age, to avoid these factors becoming confounding variables in the results, as previously shown [29]. For each vineyard, two plots were considered and sampled; all the plots are situated within a radius of 500 m (as shown in Supplementary Figure S1), this guaranteeing homogeneity of climate (including precipitation) and similarity for soils, as previously described for this area [27,30]. At each vineyard, four samples were collected (two per plot) in distal points of different rows and processed independently. Therefore, 8 samples of grape berries (500 g each approx.), collected in September, 6–8 days before harvest, represented two agronomic management practices (organic and conventional). Sixteen samples of grapevine bark (20 g each approximately) represented two farming systems as described above and two time-points: samples were collected in July (at veraison) and September (a few days before harvest, together with grape berries). Fifty grams of berries, randomly picked from each 500 g sample, were placed in a sterile 500 mL flask containing 100 mL solution of phosphate-buffered saline (PBS) at pH 7.4 in order to wash them and release all the epiphytic microorganisms from the surface and minimize tissue lysis to avoid endophyte release as much as possible. This step was processed at 23 °C for 3 h with slow shaking. An amount of 2 g of bark, randomly picked from each 20 g sample, was placed in a in a sterile 100 mL flask containing 20 mL solution of PBS at pH 7.4 in order to wash the bark and release all the microorganisms from the surface. Decimal saline dilutions were plated on Wallerstein Laboratory (WL) nutrient agar for yeast and fungal growth (Oxoid, Basingstoke, UK) supplemented with 10 mg/L chloramphenicol to inhibit bacterial growth, according to [31] and Plate Count Agar (PCA) medium (Oxoid) for bacterial growth. Colony forming units (CFU) were evaluated by counting colonies on plates displaying 30–300 colonies (appropriate dilution); the final calculation considered initial dilution rate (berries or bark to PBS). Plates were incubated in aerobiosis at 25 °C for 48 to 72 h. All plating assays were conducted in triplicate (3 independent samples for each flask were collected, and serial dilutions thereof were plated). Therefore, each condition was represented by the following 12 repetitions: 2 plots × 2 sampling points per plot (biological) × triplicate (technical).

About 10 yeast/fungal colonies were selected for molecular identification from every WL sample and subjected to direct colony PCR [32]. Based on WL colony observation, the selected colonies were picked for encompassing the most biodiversity and, at the same time, for being representative of the relative abundances of colony morphologies, as previously proposed [33].

### *2.2. Molecular Characterization of Yeasts and Fungi*

The eukaryotic isolates from WL medium were identified by PCR-RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers (ITS), performed according to [34] with some modifications [33]. For ITS region primers, ITS1 and ITS4 were used to amplify a region of the rDNA repeat unit, which includes two non-coding regions, designated as the internal transcribed spacers (ITS1 and ITS2), the 3′ part of the 18S, the 5′ portion of the 26S, and the entire 5.8S rDNA genes. A 3 µL aliquot of cell suspension, prepared as described above, was heated at 94 °C for 2.5 min and then subjected to PCR amplification using 35 cycles with initial denaturation at 94 °C for 30 s, annealing at 53.5 °C for 30 s, and extension at 72 °C for 30 s. The restriction reactions were performed at 37 °C for 2 h in a 15 µL mixture containing 5 U of HinfI or HaeIII enzyme (Fermentas International Inc., Burlington, ON, Canada) set up according to manufacturer's instructions. Restriction fragments were run on 2% (*w*/*v*) agarose gel containing 0.1 µg/mL of Gel-Red TM (Biotium, Hayward, CA, USA). Bands were visualized by UV trans-illumination, digital images were acquired with the EDAS290 capturing system (Kodak, Rochester, NY, USA), and restriction profiles were analyzed using BioNumerics (Applied Maths, Sint-Martens-Latem, Belgium) software. Amplification products were checked for purity by agarose gel electrophoresis and then subjected to sequencing. DNA sequencing (Sanger reaction with fluorescent nucleotides, run and analyzed by capillary electrophoresis) was performed by BMR-genomics (http://www.bmr-genomics.it/, accessed on 5 May 2021). FASTA files were extracted

for alignment from chromatograms through Chromas software (Technelysium Pty Ltd., Brisbane, Australia, ver. 2.6.6), after quality trimming (double-check on double peaks, low quality filtering on 3′ as proposed by Chromas automatic optimization). Strains were identified by comparison of the ITS profiles with the Yeast-id genome database [35] and confirmed by sequence alignment of sequencing results (FASTA) with sequences available at the NCBI database (GenBank) using the standard nucleotide_nucleotide homology search Basic Local Alignment Search Tool (BLAST, [36]): Ref_seq database was selected, and results were obtained filtering out uncultured samples (search was limited to records that excluded: uncultured/environmental sample sequences), query coverage was set to >97%, and all the best performing results got scores of identity >99.7% (E-value < $10^{-146}$). Afterwards, direct submission of sequence data to GenBank was performed. GenBank accession number(s) provided by NCBI for nucleotide sequence(s): MW958226–MW958252 (see Table 1 for specific entries).

**Table 1.** Molecular characterization of fungal species.

| ITS Profile | Scientific Name—BLAST Identification | Query Coverage | E Value | Percent Ident | Acc. Length | NCBI Assigned Accession Number |
|---|---|---|---|---|---|---|
| C-L____ | *Naganishia globosa* | 100% | $7.00 \times 10^{-176}$ | 100.00% | 376 | MW958239 |
| F2____ | *Hanseniaspora uvarum* | 97% | 0.0 | 100.00% | 703 | MW958230 |
| H____ | *Cladosporium ramotenellum* | 99% | 0.0 | 98.69% | 510 | MW958231 |
| I____ | *Cladosporium cladosporioides* | 100% | 0.0 | 100.00% | 552 | MW958233 |
| J____ | *Aureobasidium pullulans* | 100% | 0.0 | 100.00% | 597 | MW958235 |
| J2____ | *Zygosaccharomyces rouxii* | 97% | 0.0 | 100.00% | 644 | MW958236 |
| M____ | *Aureobasidium pullulans* | 100% | 0.0 | 99.81% | 597 | MW958240 |
| O2____ | *Filobasidium magnum* | 100% | 0.0 | 100.00% | 582 | MW958243 |
| V2____ | *Starmerella bacillaris* | 100% | 0.0 | 100.00% | 546 | MW958251 |
| X____ | *Paraconiothyrium sp.* | 98% | $7.00 \times 10^{-146}$ | 100.00% | 520 | MW958252 |

### 2.3. Statistical Analysis

- **Culture data.** The statistical significance of the differences between bacterial and fungal counts in samples from the different ecosystems was tested by one way ANOVA followed by Tukey's honestly significant difference test (Tukey's HSD).
- **ITS-RFLP results analysis.** Shannon–Wiener (H') and Simpson's (S) biodiversity indices were calculated following standard matrixes [37], as previously described [11], entered into a Microsoft Excel dedicated sheet, and validated through Student's *t* test (test.t), using the two-tailed distribution and two-sample unequal variance (heteroscedastic). Complete data containing profiles distribution and biodiversity indices calculation can be found in Supplementary Material (Table S3).

## 3. Results and Discussion

### 3.1. Dynamics of Microbial Populations

Both yeast/fungi and bacteria epiphytic populations resulted in between $10^3$ and $10^5$ CFU/g in bark and between $10^4$ and $10^5$ in grapes, which is consistent with data in the literature [7]. At veraison, bark hosted a balanced population between yeasts/fungi and bacteria (Figure 1), whereas at harvest time bacteria were significantly more numerous than yeasts and fungi in this part of the vine (Figure 2). Conversely, in terms of orders of magnitude, yeasts were balancing bacteria on grapes surface, this being consistent with previous findings: bacterial populations are usually mainly represented by species belonging to *Proteobacteria* and *Firmicutes* phyla and are lower than those of yeasts in sound grapes [7,12,14,38]; the exception is related to damage since acetic acid bacteria are stimulated by berry damage. Moreover, a significant difference was found between grape

and bark samples concerning the extent of fungal populations (Figure 2), possibly due to an increase in nutrient availability on grape skin upon ripening, as previously proposed [7].

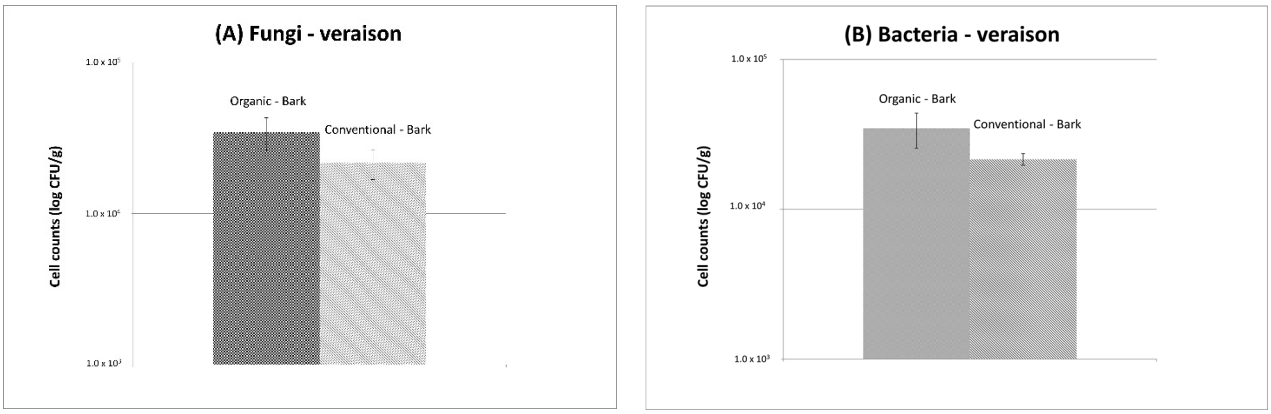


**Figure 1.** Microbial populations in bark samples at veraison. (**A**) Yeast/fungal populations determined on WL agar; (**B**) Bacterial populations determined by plate counts on PCA agar. Vertical bars show standard deviation (each condition represents four biological samples that were diluted and plated in triplicate).

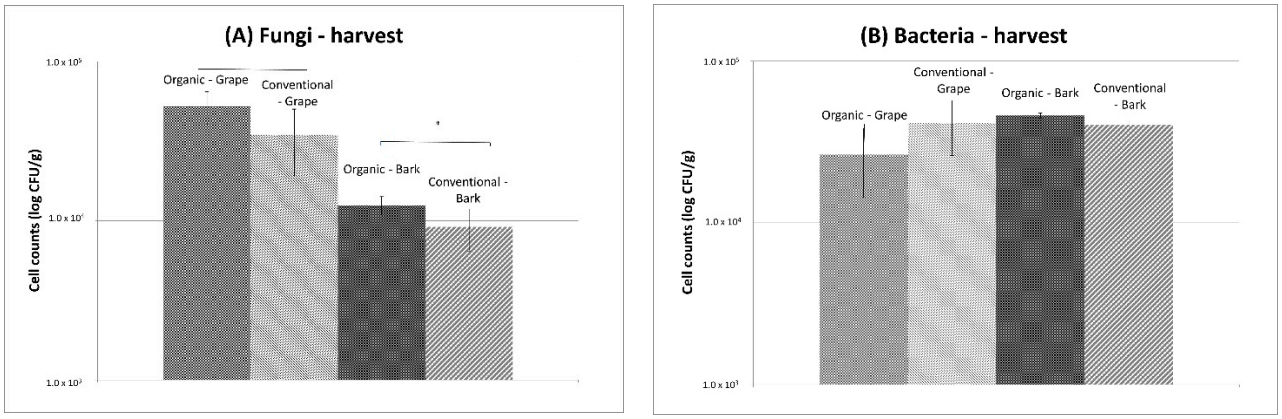


**Figure 2.** Yeast and bacteria population in grape and bark samples a few days before harvest. (**A**) Yeast/fungal populations determined on WL agar; (**B**) Bacterial populations determined by plate counts on PCA agar. Vertical bars show standard deviation (each condition represents four biological samples thta were diluted and plated in triplicate). * shows significant difference between groups ($p < 0.05$).

Furthermore, the proportion between viable bacterial populations on grape and bark (at harvest) confirms differences lower than 0.5 logs, with the slightly higher amount recovered on bark (Figure 2), which were previously described [11].

Concerning the impact of agronomic practices, although there were no statistically significant differences, results show a similar trend for both yeast and bacteria associated with bark at veraison (Figure 1), which can suggest relatively more important populations in the organic vineyard, and the same was observed for yeasts in different samples at harvest time (Figure 2). Bacteria at harvest did not show a coherent trend (Figure 2). It is important to remark that most of the treatments employed specifically in the conventional vineyard, including herbicide, were spread before veraison; thus, their impact (if any) could be more visible at veraison than at harvest (for details, see Supplementary Table S1). Moreover, in the conventional vineyard, a fungicide (having a possible impact on yeast/fungi rather than bacteria, [15]) was used for protecting grapes against *Botrytis* attack a few days before harvest.

### *3.2. Fungal Community Composition Analyzed by ITS-RFLP*

A total of 62 colonies, randomly collected from WL plates, equally distributed by type of sample and time of sampling, were examined by ITS-RFLP region analysis in order to investigate the biodiversity of the whole yeast population [33,34]. An example of electrophoretic patterns representing restriction fragments is shown in Figure 3. Photos of bands visualization were acquired and restriction profiles were analyzed using BioNumerics software. Based on a computerized analysis of ITS restriction electrophoretic patterns, the isolates showing identical or similar patterns were grouped into the same profile. A total of 10 different profiles were recognized.

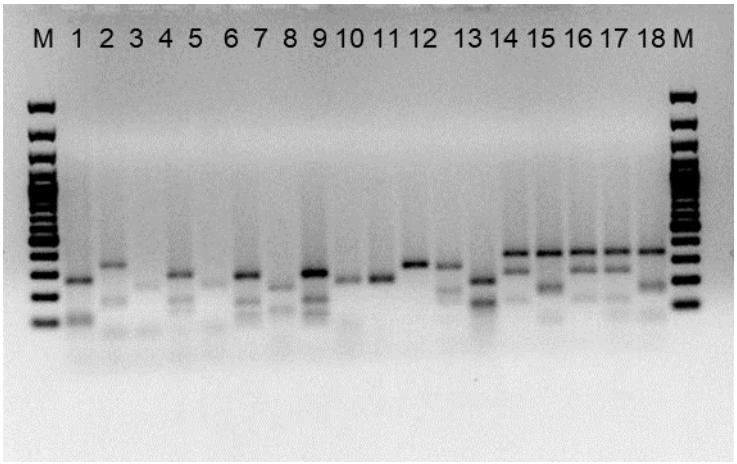


**Figure 3.** Agarose gel electrophoresis of HinfI restriction profiles (18 colonies out of 62). M: molecular marker, 100 bp ladder (Fermentas).

After a search for restriction profiles in the Yeast-id database [35] for having a first species assessment and allowing for dereplication (data not shown), at least one sample for each ITS-cluster was amplified and sequenced for confirmation of species identification as described in Materials and Methods. In particular, a nucleotide BLAST search [36] on sequence results gave the most probable taxa identification.

Alignments allowed a thorough taxonomic identification: species level was reached with good confidence level (identity scores above 98%) in all but one the profiles, whereas one of the detected profiles could be identified only at genus level. Taxa including *Naganishia globose, Cladosporium spp. (C. ramotenellium, C. cladosporoides), Aureobasidium pullulans, Filobasidium magnum*, *Hanseniaspora uvarum*, *Zygosaccharomyces rouxii, Starmerella bacillaris,* and *Paraconiothyrium* sp. Were detected, being the species belonging to the yeast-like fungus *Aureobasidium*, the most abundant in the whole dataset. In this study, two different restriction profiles were associated, after sequencing, with *Aureobasidium pullulans* species: Hypothesizing that this might be due to differences at strain level (as previously observed [39]), we decided to keep both entries, although both belonged to the same species. All the above mentioned yeast and fungal genera were previously detected in vineyards and winery-related environments [7,33,40–42]. In particular *Filobasidium, Naganishia,* and *Paraconiothyrium* have been found in soil, bark, leaf, and occasionally in grape or grape juice [14,43,44]; *Aureobasidium* and *Cladosporium* are considered ubiquitous [14,22]. *Starmerella, Zygosaccharomyces*, and *Hanseniaspora* are usually found in grape and fermenting juices and in grape marc [14,22,44]; these genera have been found both in sound and damaged grapes in the case of diseases, thus none of them can be specifically associated either with a good or poor sanitary state of grape berries [7].

### *3.3. Comparison of Fungal Distribution and Biodiversity in Different Habitats and Vineyards*

Concerning the distribution of the detected profiles among habitats (grapevine parts), grape berries showed lower ITS-based yeast genetic diversity than bark, harboring only

six different profiles compared with eight. Four profiles were found only in bark samples, whereas only two were specific to grape and four were shared (Figure 4). As expected based on the literature [44], all the species traditionally associated with grape berries (*Hanseniaspora uvarum*, *Zygosaccharomyces rouxii*, *Starmerella bacillaris*, *Aureobasidium pullulans)* were recovered in grape samples, with *Starmerella* and *Aureobasidium* also present in bark. In previous research, when samples from different vine sub-compartments were compared, bark communities showed the greatest overlap with grape [11,12] or fermenting juice [9] communities, with a portion ranging from 30% to 56% of species in common; therefore, our results corroborate the observation of a partial but substantial overlap between habitats (50% of bark taxa). This confirmation is important since scientific papers about epiphytic grapevine–bark microbiome are still few [9,11,12]; therefore, the overlap between grape and bark epiphytic microbial communities in different vineyards and situations is still hardly predictable. Indeed, although both trunk-bark and berry surfaces are exposed to the same environment (thus, to similar microorganisms), these two plant compartments offer different nutrients, so the microorganisms able to survive on each can be very specific.

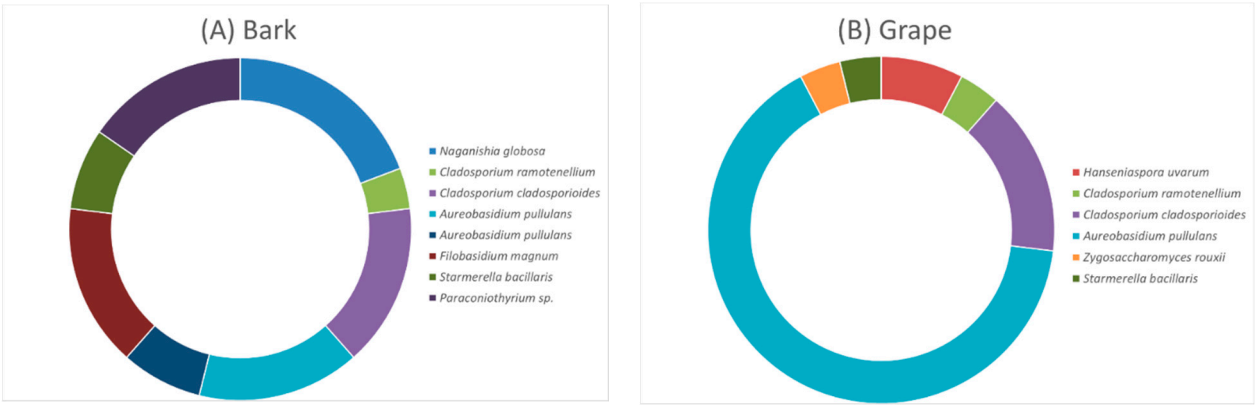


**Figure 4.** Distribution of fungal profiles recovered from all samples (organic and conventional vineyard) at different sampling times, plotted according to sample source: (**A**) Bark (30 sequences); (**B**) Grape (22 sequences).

Regarding the impact of farming system on the repartition of yeast/fungal profiles, results of ITS restriction patterns and sequence analysis are shown in Figure 5. None of the profiles were found only in the conventional vineyard, three were specific for the organic vineyard, and seven were shared. The three taxa specific to the organic vineyard encompassed two bark-associated species (*Naganishia globosa*, *Filobasidium magnum*) and one grape-associated species (*Hanseniaspora uvarum).* When comparing these findings with previous studies, some differences may arise—for instance, with results from Grangeteau et al. [15]. Although recovered taxa are quite compatible in terms of phyla, their repartition between organic and conventional vineyards is different. One possible explanation is that the amount of copper/sulphate was, in our case, similar between the two vineyards (see Supplementary Materials for further details), whereas this was not the case for the Grangeteau and coworkers' study, where copper was supposed to exert a selective pressure, as the authors also demonstrated with a further study [45].

Viable eukaryotic biodiversity was also calculated, in terms of Shannon–Wiener (H') and Simpson's (S) diversity indices for communities [37]; results are reported in Table 2.

Biodiversity was higher in bark samples (H' = 1.99, S = 0.86) than in grape samples (H' = 1.14, S = 0.54). Indeed, not only was the number of different profiles higher in the former than in the latter, but also in grape a single genetic profile (of six detected) accounted for 65% of the community diversity, whereas in bark the most represented profile (of eight detected) only reached 20%, as shown in Figure 5. Differences in biodiversity are perfectly in line with alpha diversity results obtained in the few available previous observations comparing microbial communities of bark and grapes, based either on cultural [11] or NGS [12,13] methods.

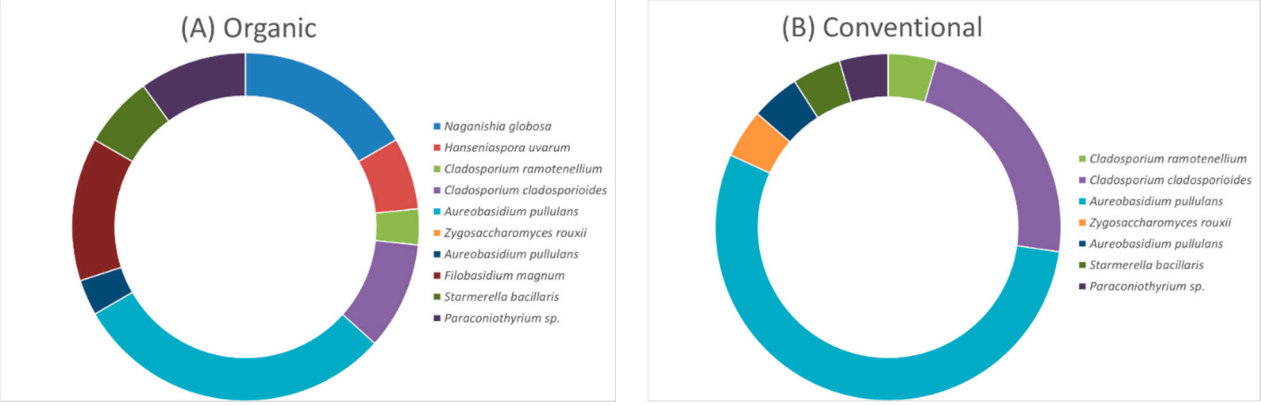


**Figure 5.** Distribution of fungal profiles recovered from all samples (grape and bark) at different sampling times, plotted according to vineyard agronomic management: (**A**) Organic (26 sequences); (**B**) Conventional (26 sequences).

**Table 2.** Biodiversity indices calculated on individuals belonging to different genetic profiles within each group.

| | Shannon–Wiener's (H') | | Simpson's (S) | |
|---|---|---|---|---|
| Sample source | | | | |
| BARK | 1.99 | ** | 0.86 | * |
| GRAPE | 1.14 | | 0.54 | |
| Vineyard agronomic management | | | | |
| ORGANIC | 1.98 | * | 0.83 | ns |
| CONVENTIONAL | 1.37 | | 0.64 | |

Significance level of differences between groups calculated via *t*-test: $p < 0.05$ *; $p < 0.01$ **.

Biodiversity was greater in the organic farmed vineyard (H' = 1.98, S = 0.83) than in the conventional one (H' = 1.37, S = 0.64), but in this case, statistical significance was lower and, therefore, does not allow sharp affirmations. Nevertheless, it is worth noting that in the conventional vineyard, a single genetic profile (of 7 detected) accounted for 55% of the community diversity, whereas in the organic one the most represented profile (of 10 detected) only reached 30%, as shown in Figure 5. This is possibly because differential patterns of fungal diversity might exist between various habitats in organically and conventionally managed vineyards, and there are several factors that could plausibly be driving these differences. Indeed, other studies have reported that a number of specific human interventions can affect microbial diversity in specific habitats of commercially managed ecosystems [11–13,23,46]. For instance, results by Martins and co-workers [23] showed a significant impact of both the farming system and the maturity stage on the epiphytic yeast and yeast-like community of grape berry. Shifts in the microbial community were related to changes in the composition of the grape–berry surface, particularly the occurrence of residues from pesticide treatments. The cultivable fungal population also varied significantly depending on the farming system [46]. In two different vineyards, the microorganism counts were significantly higher for organically than for conventionally farmed grapes, which is always observed (although not being statistically significant) in our results as well. A comparison of organic versus conventional vineyards in two wine producing areas, considering various spatial and temporal parameters, allowed the authors to hypothesize that fungicide treatments were the main factor responsible for the differences in microbial population densities and community differences [46].

Although no correlations can be inferred between the impact of farming system and specific yeast species (no statistically significant differences between abundances for the single taxa), it is worth remarking that the most abundant profile in both vineyards was the same (J) and is ascribed to the ubiquitous yeast-like fungus *Aerobasidium pullulans*, an inter-

esting species recently re-evaluated for its properties as being possibly related to biocontrol features to be exploited [47]. This microorganism is also the dominant one in grape samples (Figure 4), whereas the slightly major microorganism in bark samples is *Naganishia globose*, a eukaryote recently described as a typical member of the vineyard aboveground and soil environment in both culture-dependent [22,48] and culture-independent [49] studies.

## 4. Conclusions

Beyond results about epiphytic populations size that confirm data in the literature [7], findings about fungal communities' characterization confirm, from a "culturable" perspective, the recent results obtained on bark through NGS [12], and strengthen the importance of grape bark as a source of microbial biodiversity, putting forward its potential interest also for "culturomics".

Viable eukaryotic biodiversity was higher in bark compared with grape and was slightly higher in the organic vineyard compared with the conventional one. Moreover, in the two vineyards, fungal communities showed slightly different genera and species compositions, although no correlations can be inferred between the impact of farming system and specific yeast species (no statistically significant differences between abundances for the single taxa), and our data do not allow phylogenetic evaluation of communities.

In this study, grapevine trunk bark was confirmed to harbor significantly greater species richness than fruit, as previously observed both for bacteria [11,12] and for yeast/fungi [13]. Moreover, the portion of overlap within the fungal community between the two habitats was partial and at a previously observed extent [11,12]. Overall, our results emphasize the importance of vine trunk bark, not only as an interesting habitat to be characterized for monitoring microbial biodiversity in vineyards but also as a potential source of microbial viable species for isolation, in terms of culturable yeasts and yeast-like fungi, which may represent the most technologically relevant fraction of the whole community, being a potential inoculum for grapes. Finally, our results also confirm that, in grapevines, plant compartments exert a stronger influence on biodiversity indices than agronomic management [12,13] or site [50], as niche effects exert selective pressure within sites [2].

Although this is not a full picture of the situation, since culturable and countable microorganisms only represent a small portion of microbial biodiversity and a single-vintage sampling cannot rule-out a possible vintage effect [15], results also indicate that sustainable management in vineyards can affect the qualitative composition of aboveground microbial communities, as previously shown by other studies. Therefore, we believe that further investigation encompassing trunk bark as a sample source, coupling both culture-dependent and high-throughput molecular methods (NGS), performed on several subsequent vintages, would be an interesting in-depth analysis.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/agronomy11091752/s1, Figure S1: Map (satellite view) of the vineyard objects of this study; the red point represents the GPS coordinates explicated in the Materials and Methods section, Table S1: Vineyards agronomic management details, Table S2: Biodiversity indices calculations, Table S3: ITS profiles distribution.

**Author Contributions:** Conceptualization, T.N.; methodology, T.N. and F.G.; validation, T.N., F.G. and D.T.; investigation, T.N. and F.G.; resources, D.T.; writing—original draft preparation, T.N.; writing—review and editing, F.G. and D.T. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** All sequence data supporting reported results can be found in the NCBI publicly accessible repository: the dataset presented in this study is openly available in the Genbank Nucleotide Database (https://www.ncbi.nlm.nih.gov/nuccore/MW958230, accessed on 5 May 2021) with accession numbers MW958226–MW958252. Sequencing standards from BMR Genomics are openly available at https://www.bmr-genomics.it/ (accessed on 5 May 2021).

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**Conflicts of Interest:** The authors declare no conflict of interest.

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