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ORIGINAL PAPER

Fungal diversity and occurrence of mycotoxin producing fungi in tropical vineyards

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Abstract

Grapevine cultivars are distributed worldwide, nevertheless the fermentation of its grape berries renders distinct wine products that are highly associated to the local fungal community. Despite the symbiotic association between wine and the fungal metabolism, impacting both the *terroir* and mycotoxin production, few studies have explored the vineyard ecosystem fungal community using both molecular marker sequencing and mycotoxin production assessment. In this study, we investigated the fungal community of three grapevine cultivars (*Vitis vinifera* L.) in two tropical vineyards. Illumina MiSeq sequencing was performed on two biocompartments: grape berries (GB) and grapevine soil (GS); yielding a total of 578,495 fungal internal transcribed spacer 1 reads, which were used for taxonomic classification. GB and GS fungal communities were mainly constituted by Ascomycota phylum. GS harbors a significant richer and more diverse fungal community than GB. Among GB samples, Syrah grape berries exclusively shared fungal community included wine-associated yeasts (e.g. *Saccharomycopsis vini*) that may play key roles in wine *terroir*. Mycotoxin production assessment revealed the high potential of *Aspergillus* section *Flavi* and *Penicillium* section *Citrina* isolates to produce aflatoxin B1-B2 and citrinin, respectively. This is the first study to employ next-generation sequencing to investigate vineyard associated fungal community in Brazil. Our findings provide valuable insights on the available tools for fungal ecology assessment applied to food products emphasizing the coexistence between classical and molecular tools.

Keywords Fungal ecology · Next-generation sequencing · Terroir · Tropical vineyard · Wine

Introduction

Dry land is a high stress environment, especially for sessile photosynthetic organisms (de Vries and Archibald 2018). One of the major plant adaptations to cope with this challenge was the establishment of mutualistic associations with symbiotic microorganisms (Martin et al. 2017). The plant microbiome is a dynamic microbial community influenced by physical and chemical properties of surrounding soil (Xun et al. 2015), as well as by the host (Perez-Jaramillo et al. 2017). As major players in biochemical cycles, fungal communities are pivotal to plant development (Gadd 2006). Fossil records have shown that fungal association were determinant to plant terrestrial colonization and early forests development (Selosse et al. 2015).

Plant domestication was one of the most striking events of human history, ultimately defining modern society lifestyle (Martinez-Ainsworth and Tenaillon 2016). Grapevine (*Vitis vinifera* L.) is a globally economic and social relevant crop, which microbiome has been continuously explored revealing association to diseases (Hall et al. 2019) and resilience of pesticides effects on plant health (Perazzolli et al. 2014). Agricultural practices have been shown to influence the soil grapevine and grape berries microbiome to a different extent, while the first was deeply influenced the latter was

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only marginally affected (Chou et al. 2018). A similar pattern was observed through the comparison of the microbial assemblage of grapevine bark and grape berries (Vitulo et al. 2018).

The grapevine microbiome harbors distinct composition according to the tissue or biocompartment sampled (Morgan et al. 2017). One key assumption connecting microbiome from above- and below-ground biocompartments, reports grapevine soil as a reservoir of grape berries bacterial diversity (Zarraonaindia et al. 2015). The same type of samples and 16S bacterial sequencing revealed that geographical origin has a greater impact on bacterial community than cultivar (Mezzasalma et al. 2018). While bacterial role in wine-making is controversial, fungal part is not (Ferreira and Mendes-Faia 2020). *Saccharomyces cerevisiae* is the main microorganism involved in the alcoholic fermentation of grape must, nevertheless indigenous fungi can influence the early stages of the fermentation process affecting wine quality (Belda et al. 2017a). Fungal community inquire can uncover new autochthonous non-*Saccharomyces* fermenters which may be useful to the wine-making process. On the other hand, undesirable fungi can produce mycotoxins on grape products compromising the final quality and consumers health. Ochratoxin A (OTA) is a mycotoxin produced by several species of *Aspergillus* and *Penicillium* (Qi et al. 2016). *A. niger* and *A. carbonarius* are considered the main OTA producers in wine products in many countries (Paterson et al. 2018).

Tropical vineyards are located in the intertropical zone, throughout this region, Syrah is one of the largest produced grape cultivars (Kok 2014). Geographical origin and cultivar type is at the heart of winemaking, mainly due to the assumption that the land conveys unique features to the wine, creating peculiar sensory properties defining its *terroir* (Coggins et al. 2019). Thus, herein we employed next-generation sequencing along with isolation and polyphasic identification to investigate the fungal communities of grapevine soil (GS) and grape berries (GB) of three distinct cultivars from two tropical vineyards located in a semiarid climate zone. We also inquired differences in the fungal community assemblage at small scale distances, revealing wine-associated yeasts in Syrah grape berries.

Materials and methods

Vineyard sampling

Samples were obtained at harvest time from four vineyards located in São Francisco Valley region, at the latitude of 8º–9º S in northeastern, at the municipalities of Casa Nova – Bahia (V1) and Lagoa Grande – Pernambuco (V2), about 80 km apart (Fig. 1).

The Table 1 shows information regarding the geographical locations sampled in this study. This region has semiarid climatic conditions with high sunlight intensity (3000 h/year), low annual rainfall, absence of winter and availability of water for irrigation (Teixeira et al. 2013). The soils are classified as yellow eutrophic argisol/typical plintustalf (soil taxonomy alfisol) (Embrapa Solos 2006), usually with medium natural fertility. Rainfall historical data were obtained through the National Hydro-meteorological Network website (http://www.snirh.gov.br/hidroweb/), administered by the Water Agency of Brazil. Data were collected at the stations 940039 and 840015, located in the cities of Casa Nova and Lagoa Grande respectively. For these cities, the mean rainfall per year in the last 10 years was 24.30 mm and 30.1 mm respectively. The samples were acquired in 2017, one of the driest years of the last 10 years, with 10.7 mm mean rainfall in both cities. Regardless the year, the winter (July–September), is always the driest season, commonly with no rainfall at all.

For GB samples acquisition; a diagonal transect was drawn along the vineyard and three bunches of grapes from three equidistant plants were collected disregarding the ends. GB were acquired during the final stages of berries maturation (harvest season), in the July/August/September 2017 harvest. For GS samples, four bulk soil spots (100 g each) were collected at 10 cm deep and 20 cm distance radius of the plant selected for GB collection, employing a hand-auger. All samples were collected aseptically, stored in sterile plastic bags, transported to the laboratory within 2 h in ice boxes maintaining a temperature of 0–4 °C. Samples were stored for no longer than a month at − 80 °C.

DNA extraction

DNA from undamaged harvested grape berries was obtained under aseptic conditions in the laboratory. The DNA extraction was performed following the protocol described by Wilson (2001) with minor modifications. Briefly, GBs (30 g) were surface sterilized by rinsing with sterile water for 3 min, followed by 30% NaClO (sodium hypochlorite) for 3 min, and then 70% ethanol for 3 min. Next, GBs were crushed and re-suspended in 2.3 mL Tris EDTA buffer, followed by the addition of RNAse A (20 µg mL^{-1}) incubated at 37 °C for 15 min. After that, proteinase K (1 mg mL^{-1}), Sodium dodecyl sulfate, and 500 µL of glass beads were added. The mixture was vortexed for 2 min. A volume of 20 µL of a 10 mg mL^{-1} lysozyme solution was added, incubated again at 37 °C for 50 min. Then 400 µL of NaCl 5 M and 240 µL of Cetyl Trimethyl Ammonium Bromide (CTAB) were added and the mixture was incubated for 10 min at 65 °C, followed by phenol chloroform extractions and precipitation with isopropanol. The same was performed for GS samples starting from 3 g

Fig. 1 Vineyards (V1 and V2) geographical localization in the states of Bahia (light green) and Pernambuco (dark green) respectively. Other northeastern states are represented in light shaded green. The blue line represents the São Francisco river

Table 1 Vineyards localization and sample acquisition spots

Sample	Variety	Vineyard	Spots	Height (m)
S1	Syrah	Vineyard 1	1	419
			2	419
			3	419
S2	Syrah	Vineyard 2	1	370
			2	370
			3	370
T	Tempranillo	Vineyard 2	1	364
			2	364
			3	362
TN	Touriga Nacional	Vineyard 2	1	369
			2	372
			3	372

of sieved soil. The quantity and quality of extracted DNA were assessed by spectrophotometry (Eppendorf, Germany) and agarose gel (1%) electrophoresis, respectively.

High-throughput sequencing and fungal community analysis

Amplicons from GB and GS were sequenced using Illumina MiSeq platform yielding 578,495 paired-end reads. Raw fastq files can be accessed through Bioproject PRJNA686129. Raw reads were quality-filtered ($q > 30$) using Trimmomatic 0.39 (Bolger et al. 2014), then read pairs were merged and de-replicated using VSEARCH 2.10.4 (Rognes et al. 2016). De-replicated merged reads were denoised, filtered and operational taxonomic units (OTUs) were obtained clustering denoised sequences at 97% identity threshold using USEARCH 9.2.64 (Edgar 2010). Finally, OTU table was built mapping reads back at OTUs, and hybrid taxonomic classification was performed with the assist of the amptk taxonomy wrapper (Palmer et al. 2018) using UNITE 8.2 database (Nilsson et al. 2019). Alpha diversity estimators including richness (ACE, Chao1) and diversity (Shannon, Simpson) were calculated using phyloseq package 1.3.0 (McMurdie and Holmes 2013). Alpha diversity measures were compared between environments

(GB, GS) using non-parametric Kruskal–Wallis implemented in Vegan 2.5.6 (Oksanen et al. 2019). All analyses were performed under R 3.6 (R Core Team 2017).

Isolation and identification of *Aspergillus* spp. and *Penicillium* spp. from grape berries

To obtain fungi isolates, undamaged GBs (30 g) from each one of three collection points were surface sterilized by rinsing with sterile water for 3 min, followed by 30% NaClO (sodium hypochlorite) for 3 min, and then 70% ethanol for 3 min. The GBs were mixed with 225 mL of peptone water 0.1% and homogenized in a Stomacher (Metroterm, São Paulo, Brazil), paddle speed of 490/min for 2 min. Using aliquots of 0.1 mL from this mixture, serial dilutions (1:10 to 1:10,000) were spread plated onto plates containing Dichloran Rose Bengal Chloramphenicol media (DRBC) (Merck, Darmstadt, Germany) or 18% Dichloran Glycerol agar (DG-18) (Merck, Darmstadt, Germany). DRBC contained 5 g L^{-1} peptone, 10 g L^{-1} glucose, 1 g L^{-1} KH_2PO_4, 0.5 g L^{-1} $MgSO_4 \times 7H_2O$, 1 mL dichloran, rose bengal, 0.1 g L^{-1} chloramphenicol, and 15 g L^{-1} agar. DG-18 contained 5 g L^{-1} peptone, KH_2PO_4, 1 g L^{-1}, 0.5 g L^{-1} $MgSO_4 \times 7H_2O$, 1 mL dichloran, 0.1 g L^{-1} chloramphenicol, and 15 g L^{-1} agar, and 220 g L^{-1} glycerol. The plates were incubated at 25 °C for 7 days in a BOD type chamber.

The identification of fungi isolates was performed by transferring pure colonies from DRBC and DG-18 culture media to plates containing Czapek yeast extract agar (CYA) (Synth, São Paulo, Brazil) or malt extract agar medium (MEA) (Synth, São Paulo, Brazil). CYA contained 1 g L^{-1} KH_2PO_4, 5 g L^{-1} yeast extract, 15 g L^{-1} agar, 10 mL Czapec concentrate). MEA contained 20 g L^{-1} malt extract, 1 g L^{-1} peptone, 30 g L^{-1} glucose, 20 g L^{-1} agar). Macro and microscopic characteristics of *Aspergillus* and *Penicillium* colonies were assessed according to the literature (Houbraken et al. 2011; Pitt and Hocking 2009; Samson et al. 2010; Varga et al. 2011).

Mycotoxin production by fungi isolates

To determine whether fungi isolates were able to produce mycotoxins, a thin-layer chromatography was run according to Filtenborg and Frisvad (1980). First, isolates identified as *Aspergillus* section *Nigri* and *Penicillium* section *Citrina* were grown in CYA, while *Aspergillus* section *Flavi* was grown in YES medium, incubated for 10 days at 25 ºC. Yeast extract sucrose medium (YES) (Synth, São Paulo, Brazil) contained 20 g L^{-1} yeast extract, 150 g L^{-1} sucrose, 20 g L^{-1} agar, 0.1 g L^{-1} $ZnSO_4 \cdot 7H_2O$, 0.05 g L^{-1}, $CuSO_4 \cdot 5H_2O$. The purified grown isolates were tested for ochratoxin A (OTA), citrinin, aflatoxins B1, B2, G1, and G2; mycotoxin production was assessed using the plug agar method. After the incubation period, a radial plug agar (30 × 30 mm) was obtained from the center of the plate. The plug agar was placed on thin-layer chromatography plates (Merck-Silica Gel 60, 20 × 20). Mobile phase constituted of toluene, ethyl acetate, and formic acid 90% (60:30:10), along with the aforementioned mycotoxins standard solutions (Sigma-Aldrich, St. Louis, USA). The profile of secondary metabolites was evaluated using a CAMAG chromatovisor (UF-BETRACHTER) under 366 nm ultraviolet light. Microorganisms considered as mycotoxin producers exhibited retention factor and fluorescent colors alike to the toxin standards.

Results

Sequence analysis

Grape berries (GB) and respective grapevine soil (GS) sequencing yielded 578,495 sequences, an average of 72,311 per sample. These were clustered into 581 OTUs, which were filtered to remove singletons and non-Fungi OTUs, accounting 487 Fungi OTUs constituted by 536,030 sequences. The discovery rate was evidenced by plotting rarefaction curves, the plateaus observed in GB samples indicate that the major part of the fungal community was sequenced at 20,000 reads depth (Fig. S1—continuous curves). Whereas no GS sample achieved a plateau (Fig. S1—dashed curves). Significant differences in GB and GS fungal communities richness were observed as estimated by Chao1 ($p < 0.02$) and ACE ($p < 0.02$) (Fig. 2a, b). Significant differences in diversity was only detected by Shannon ($p < 0.02$), while Simpson ($p > 0.02$) failed to discard the null hypothesis (Fig. 2c, d). Tempranillo samples exhibited the lowest richness values both for GS and GB, while in other samples no distinct pattern could be observed.

Grape berries and grapevine soil fungal communities

The fungal taxonomic composition encompasses: 10 phyla, 27 classes, 57 orders, 88 families, 120 genera and 105 species. GS samples exhibited all 10 phyla, constituting a more diverse mycobiome than GB, that harbored only three phyla (Ascomycota, Basidiomycota and Mortierellomycota). Ascomycota phylum is the most prevalent both in GB and in GS samples (Fig. 3a), except for TN-GS sample from Vineyard 2, that exhibits Mortierellomycota as the most abundant phylum (Fig. 3b).

Next, we built bar plots to inspect the relative abundance of the fungal community across samples. The top 10 most abundant families bar plot shows different dominant taxa in GS samples, for S1-GS *Pleosporaceae* is the

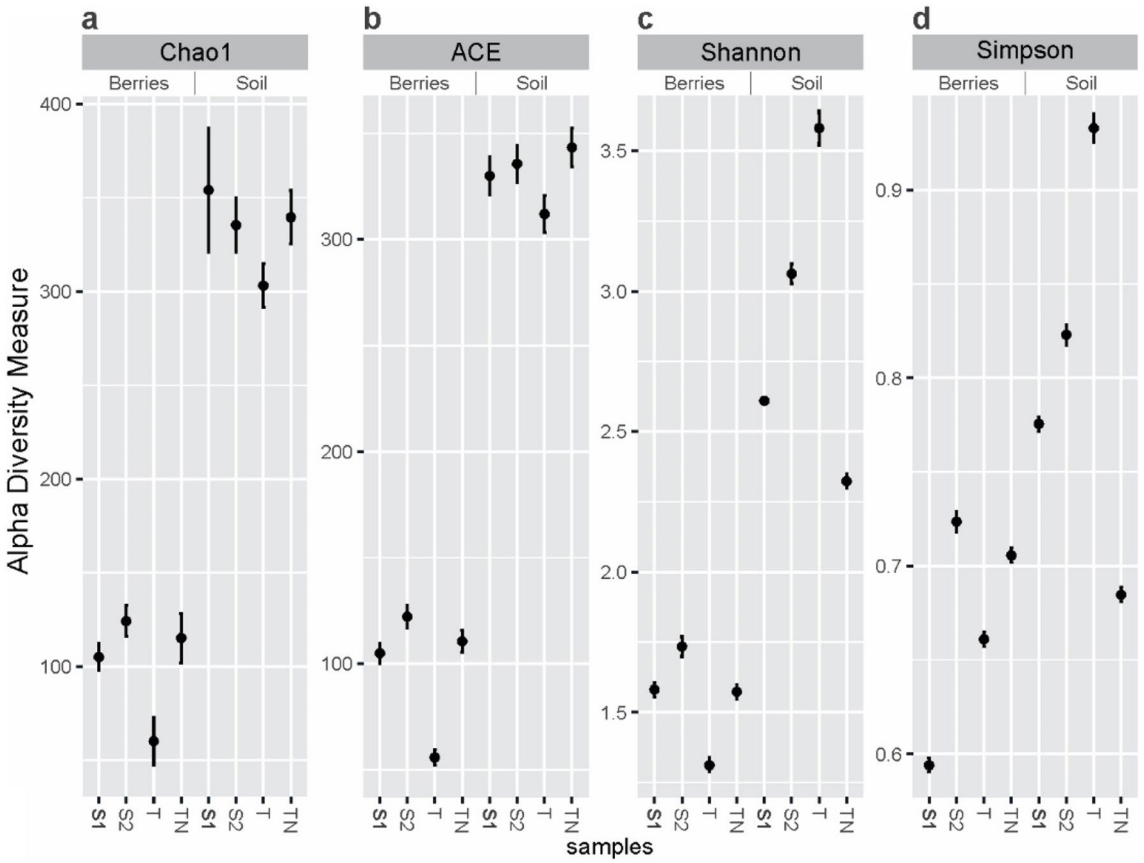

Fig. 2 Fungal richness and diversity indexes. **a** Chao1 richness index. **b** ACE—abundance based coverage estimator index. **c** Shannon diversity index. **d** Simpson diversity index. Bold-faced text accounts for V1 sample, while plain text for V2 samples. S, T and TN accounts for Syrah, Tempranillo and Touriga Nacional respectively

most abundant, while for TN-GS *Mortierellaceae* family represents the majority of the mycobiome, no clear patterns can be observed for other V2 samples (S2-GS and T-GS (Fig. 4a)). In GB, the most abundant families were *Mycosphaerellaceae* and *Aspergillaceae*, the only exception was the T-GB sample, which showed a sharp increase in *Aureobasidiaceae* abundance (Fig. 4a). Among GB *Alternaria*, *Aspergillus* and *Davidiella* were the most prevalent genera for all samples except T-GB that harbors *Aureobasidium* as the most abundant fungus, we further confirmed this taxon represents only one OTU classified as the yeast *Aureobasidium pullulans*. In contrast, for GS samples no clear pattern could be observed among samples (Fig. 4b). *Alternaria* and *Mortierella* were the most abundant genera in S1-GS and TN-GS, respectively.

In order to obtain an overview of OTUs distribution across all samples we built a heat map accounting the total diversity (Fig. S2), evidencing the distinct profile between GB and GS samples. Further, the heat map also indicates the distinction of T-GB community, compared to others GB samples even of the same vineyard (S2-GB and TN-GB).

OTUs distribution and the Syrah exclusively shared taxa

In order to investigate OTUs distribution across samples, Venn diagrams of all OTUs were built. In GB samples, S2 showed the highest number of unique OTUs (28), T exhibited the lowest (5), and 22 OTUs were shared by all samples (Fig. 5a). GS samples shared 129 OTUs, being S1, the only sample from V1, with the highest number of unique OTUs (52) (Fig. 5b). While the grape cultivar had no influence on shared OTUs among GS samples, Syrah GB samples shared the major fraction of their mycobiome (Fig. 5c). Additionally, these two Syrah samples exclusively share wine-associated yeasts namely: *Saccharomycopsis vini*, *Curvibasidium cygneicollum* and the genera *Wickerhamiella* and *Zopfiella*.

The high prevalence of OTUs belonging to mycotoxin producing genera (e.g. *Aspergillus*) among GB samples, led us to investigate the occurrence of mycotoxin producing

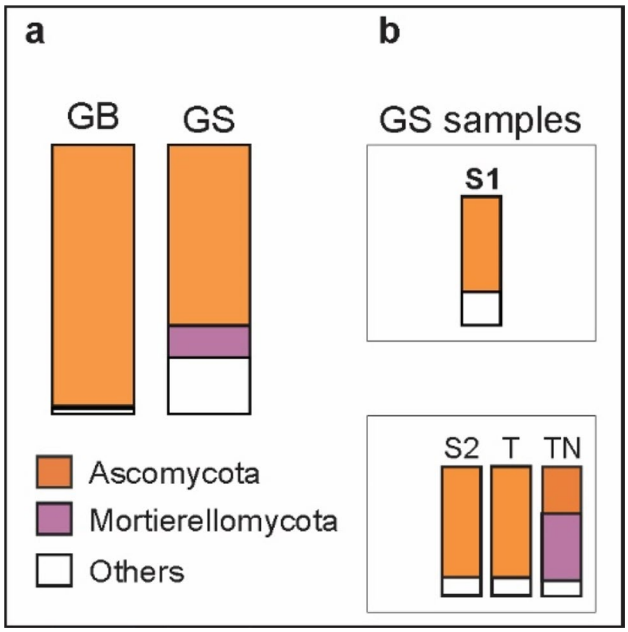

Fig. 3 **a** Total phylum diversity reported in grape berries (GB) and in grapevine soil (GS). **b** Phylum richness and diversity across GS samples. Bold-faced text accounts for V1 sample, while plain text for V2 samples. S, T and TN accounts for Syrah, Tempranillo and Touriga Nacional respectively

isolates on these samples. We have found that all *Aspergillus* section *Flavi* isolates were able to produce either aflatoxin B1 or B2, whereas the minor fraction of *Aspergillus* section *Nigri* isolates (12.9%) were ochratoxin A producers. At last, 60.7% of the *Penicillium* section *Citrina* isolates exhibited citrinin production (Table 2).

Discussion

This study is the first vineyard mycobiome sequencing in Brazil. Acquiring next-generation sequencing data from vineyards is not a straightforward procedure. Different grapevine biocompartments may exhibit remarkable differences, here we focused on obtaining a large number of reads per sample. The discovery rate was evidenced by plotting rarefaction curves, as no GS sample achieved a plateau, a deeper sequencing may be recommended for tropical vineyard soils. This profile has also been reported in other vineyards soils sequencing (Knight et al. 2020; Wei et al. 2018). Metabarcoding studies have revealed that phyllosphere and carposphere present biogeographic-specific traits that define the *terroir* properties (Bokulich et al. 2016; Knight et al. 2015). Zarraonaindia et al. (2015) analyzed shared bacterial OTUs among grapevine aerial tissues (leaves, grapes and flowers) and soil, revealing that the major fraction of grapevine bacterial microbiome originates in soil. Here we observed the same pattern, with a mean of 69.6 ± 5.3% of the berry mycobiome being also present in grapevine soil. As SFV is a semiarid region without, high variation in fungal communities are unexpected. In this context, we explored the differences in richness and diversity with special interest in OTUs associated to a particular cultivar.

Alpha-diversity estimators summarize the microbial community assemblage of an ecological niche with respect to its richness (i.e. number of OTUs), evenness (distribution of abundances of the OTUs), or both (Willis 2019). The higher diversity and richness of grapevine soil over other grapevine tissues have already been reported (Köberl et al. 2020; Wei et al. 2018), and are corroborated by our findings. However, considering only samples from the same vineyard (V2), we have showed that Tempranillo samples bear lowest richness in both GS and GB (Fig. 2a, b). While this profile is maintained for diversity estimators in T-GB sample, T-GS shows the higher values of Shannon and Simpson (Fig. 2c, d), evidencing that the correlation between GS and GB mycobiome is not straightforward. Soil mycobiome diversity is heavily linked to the environment and geographical location of the vineyard (Coller et al. 2019), a deeper approach on how spatial variation affected the grapevine's fungal community showed that few kilometers of distance introduced significant differentiation on these communities in Pinot Noir vineyards (Knight et al. 2020). In this sense, assuming that the grapevine cultivar has minimal impact on the surrounding bulk soil, the observed difference in GS fungal communities from the V2 can be related to the within vineyard variation.

The Ascomycete dominance in grape berries is well documented in different countries, such as in the USA (Bokulich et al. 2014), Spain (Wang et al. 2015) and China (Wei et al. 2018). Herein, the Ascomycota phylum was also the most prevalent for all GB and GS samples, being TN-GS sample the only exception, which harbored Mortierellomycota as the most abundant phylum (Fig. 3c). Despite this consensus, ITS sequencing of ascomycetes usually provides limited species information due to the little variation of this region within some groups of this phylum (Asemaninejad et al. 2016). To circumvent this, alternative amplicon targets have been suggested for fungal metabarcoding, such as the 18S ribosomal small subunit RNA (Banos et al. 2018) and the D1/D2 domain of the 26S ribosomal large subunit RNA (De Filippis et al. 2017). However, the ITS1 locus currently constitutes as the most promising target for fungal community assessment diversity (reviewed by Morgan et al. 2017). Regarding the Mortierellomycota phylum, the vast majority of reads were assigned to the *Mortierella alpina* species. Members of the *Mortierella* genus are saprobic in soil and can, occasionally, adopt endophytic or ectomycorrhizal lifestyle (Shelest and Voigt 2014). Also, *M. alpina* has been described as capable of performing benefic associations with *Crocus sativus*,

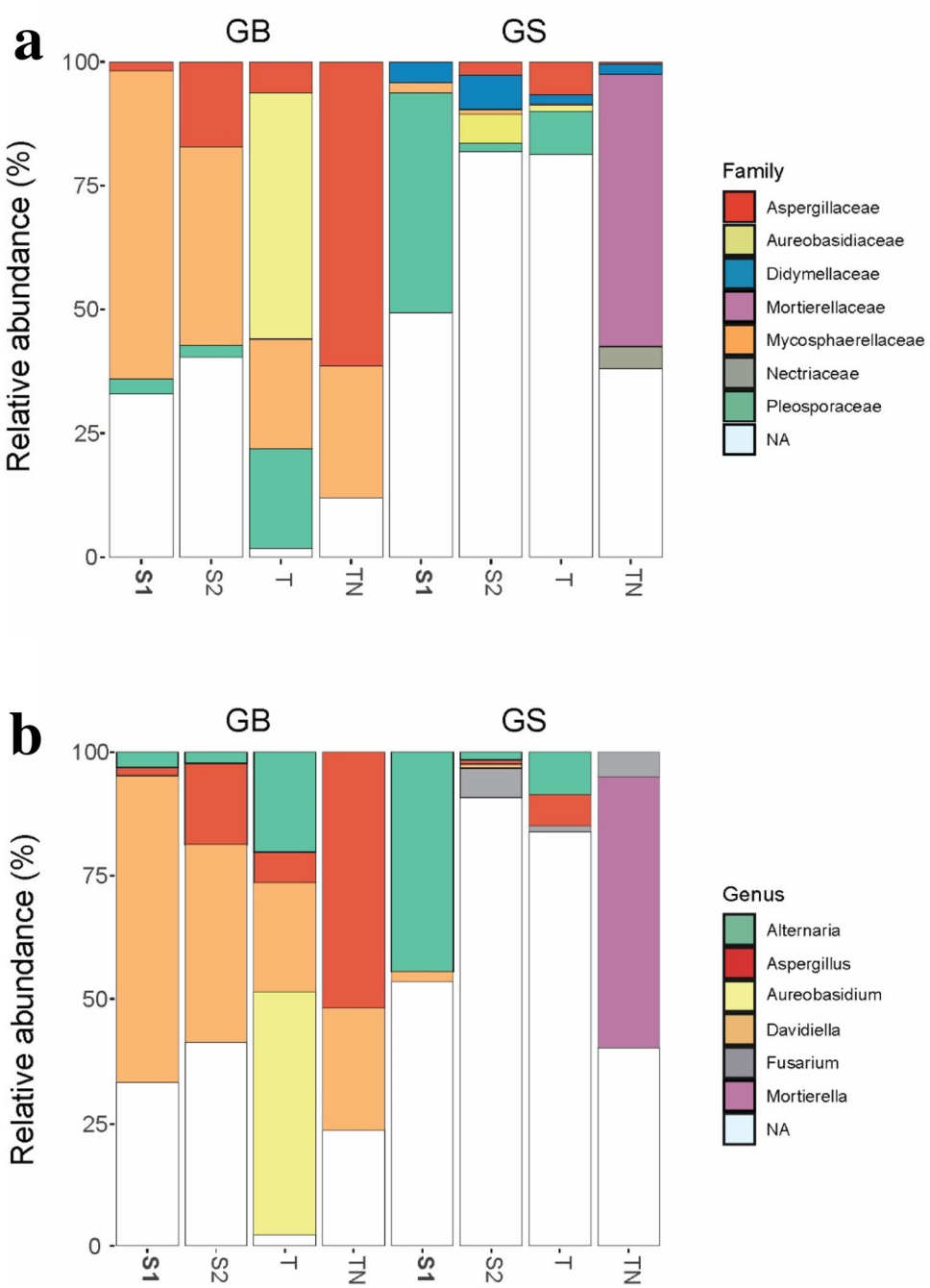

Fig. 4 Relative abundancies of vineyard mycobiomes. **a** Relative abundancy at family taxonomic rank; **b** Relative abundancy at family genus rank. White bars represent agglomerated less abundant OTUs. Bold-faced text accounts for V1 sample, while plain text for V2 samples. S, T and TN accounts for Syrah, Tempranillo and Touriga Nacional respectively

increasing tolerance to corm rot disease and enhancing apocarotenoids production (Wani et al. 2017).

Alternaria, *Aspergillus* and *Davidiella* are acknowledged as typical members of the vineyard environment (reviewed by Anagnostopoulos et al. 2019), and were the most abundant genera in GB samples. However, only Tempranillo GB exhibited *Aureobasidiaceae* as the most prevalent OTU. *Aureobasidium* high prevalence in Tempranillo leaves and grapes has also been documented in Texas High Plains, a semi-arid region in the US (Bougreau et al. 2019). The dimorphic ascomycetous black yeast *A. pullulans* has also been involved in the reduction of *Aspergillus carbonarius* (Dimakopoulou et al. 2008), this is partly corroborated by our data reported herein that shows *Aspergillus* reduction occurring concurrently with *Aureobasidium* increase for T-GB sample (Fig. 4b). Moreover, taxonomic affiliation achieved distinct outcomes in each environment, while it classified a mean of $78 \pm 21.2\%$ of the GB fungal community to genus, a mean of $63 \pm 17.1\%$ of GS reads were accounted to family. The low level of tropical soil taxonomic assignment mycobiome is related to the prevalence of developed countries as

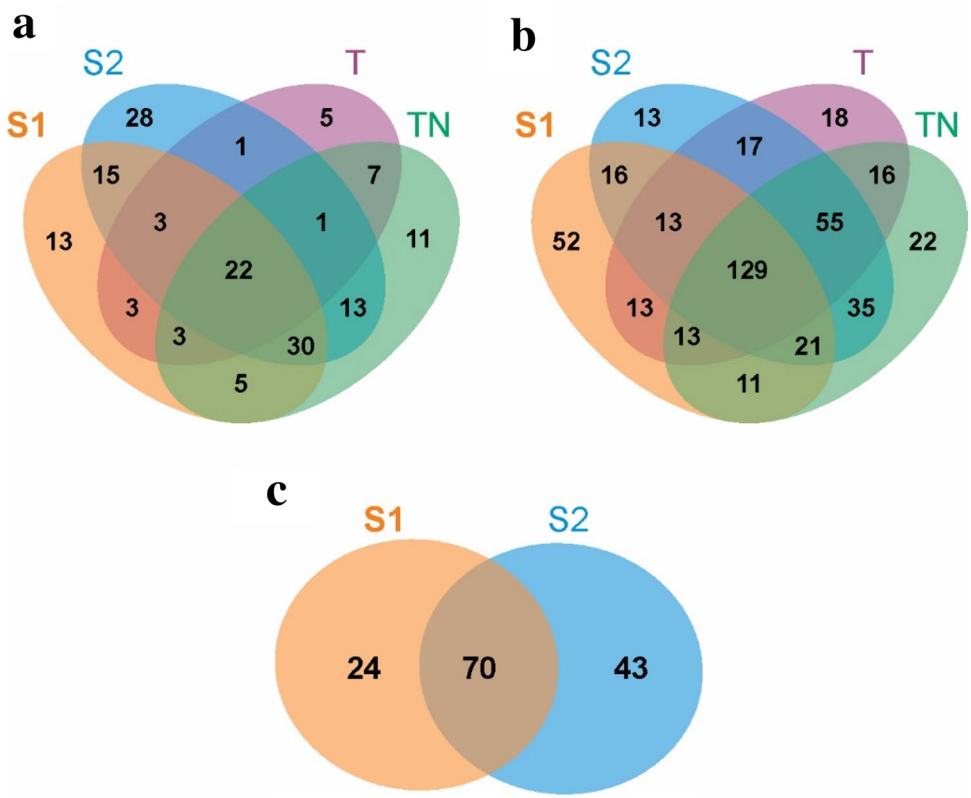

Fig. 5 Venn diagram of fungal community of: **a** Grape berries—GB; **b** Grapevine soil—GS (**b**); **c** Syrah GB samples. Bold-faced text accounts for V1 sample, while plain text for V2 samples. S, T and TN accounts for Syrah, Tempranillo and Touriga Nacional respectively

Table 2 Occurrence frequency (%) and toxigenic potential (%) of isolated and identified fungi from grape berries

Fungi isolates	S1	S2	T	TN	Mycotoxin production (%)
Aspergillus section *Flavi* (aflatoxin B1-B2)	38.46	11.58	43.88	3.57	100
Aspergillus section *Nigri* (ochratoxin A)	7.7	8.42	6.47	10.7	12.9
Penicillium sp. section *Citrina* (citrinin)	53.84	80	49.64	85.7	60.7

the main sequence contributors for UNITE database (Nilsson et al. 2019). In fact, a survey indicated Brazil along with other South American countries (e.g. Chile and Peru) among the most promising source for microbial isolation from soil due to high level of microbiome data classified as unknown phyla (Delgado-Baquerizo 2019).

The sample S1-GB shares a significant part of the fungal community with S2-GB from V2 (Fig. 5a, c). In contrast, this pattern is absent in GS samples of this cultivar, as S1-GS harbors a high number of unique OTUs (Fig. 5b). These opposite patterns of grape and soil Syrah samples, indicate that the vineyard soil mycobiome composition may be more influenced by geographical location rather than the cultivar, determining aspects as soil productivity and vine disease resistance (Belda et al. 2017b), thus enlarging the applications of fungal community determination in vineyards. Further, microbiome features can distinguish both locality and cultivar of grapes and soil, allowing the identification of both vineyard- and cultivar-associated signature OTUs (Mezzasalma et al. 2018).

We explored the exclusive Syrah's OTUs, detecting wine-associated yeasts such as *S. vini*, *C. cygneicollum* and the genus *Starmerella*. Yeasts are at the core of winemaking, because they carry out the transformation of sugar-rich musts into high quality wines. *S. vini* has been identified associated to grapes in different vineyards worldwide (Drumonde-Neves et al. 2016; Kachalkin et al. 2015; Kraková et al. 2012). While this fungus has been linked to rotten berries (Loureiro and Malfeito-Ferreira 2003), it was also reported to produce ethyl esters (e.g. ethyl butyrate and ethyl isovalerate) conferring fruity notes to fermented products (Gamero et al. 2016), this indicates this yeast as a promising target for isolation and complete identification. Another OTU exclusive of Syrah grapes was classified to the genus *Starmerella*, which was described to accommodate *Starmerella bombicola* isolated from the honey of

Bombus sp. (bumble-bee) (Rosa and Lachance 1998). In this genus, *Starmerella bacillaris* has been frequently associated to white and red wines in vineyards across the world, being frequently associated with overripe and botrytized grapes (reviewed by Englezos et al. 2017). Many interesting features have been described in *S. bacillaris* fermentation of grape must, highlighting the relevance of future studies to fully identify and isolate the *Starmerella* yeast herein reported. During winemaking, fermentation of sugars is mainly performed by *Saccharomyces cerevisiae*, which are inoculated after grape berry crushing (Albergaria and Arneborg 2016), while the dominance of the starter culture ensures fermentation stability and reproducibility, it deprives wines of the sensory complexity or distinctiveness that a spontaneous fermentation may offer (Nisiotou et al. 2019). In this context, there has been an increasing number of studies regarding the exploitation and utilization of non-*Saccharomyces* wine yeasts (Roudil et al. 2019). Indigenous yeast screening relies mainly in culture-based techniques which often overlooks less abundant taxa, thus sequencing may be an approach to perform a fast assessment of autochthonous yeasts diversity from vineyards.

Fungal amplicon sequencing is not suitable to provide high taxonomic resolution (i.e. at species level) (Creer et al. 2016). As our results have shown that a large proportion of the grape berries mycobiome was constituted by genera regarded as potentially mycotoxin producers (Fig. 4b), we performed fungi isolation and polyphasic identification coupled with thin-layer chromatography to detect mycotoxin producing isolates. *Aspergillus* section *Nigri* isolates showed small OTA production, which agrees with other surveys (Einloft et al. 2017; Rosa et al. 2002; Serra et al. 2006). On the other hand, *Penicillium* section *Citrina* high citrinin production, corroborate the findings of Freire et al. (2017). Black *Aspergillus* species grow at higher rates in temperatures ranging from 30 to 35 ºC, however OTA production is increased at lower temperatures (Hocking et al. 2007), therefore OTA production in grape berries of tropical vineyards before the harvest is unlikely.

High occurrence of *Aspergillus* section *Flavi* producing aflatoxin B1 was also reported in other vineyards in Lebanon (El Khoury et al. 2008) and Tunisia (Fredj et al. 2009). The impact of temperature, CO_2 concentration and water activity on the gene expression of the aflatoxin B1 biosynthetic genes *aflD* and *aflR*, revealed a strong stimulation of aflatoxin B1 production at 37 °C associated with smaller water activity and higher CO_2 concentration (Medina et al. 2015), raising concerns with regard to the climate changes and aflatoxin B1 production in food products.

As the terroir concept is linked to the local peculiarities and wine is a direct product of fungal metabolism, our findings may pave the way to more robust ecological queries in vineyards. Sharp differences occurred among the grapevine soil fungal communities and respective grape berries, but Ascomycota was the main prevalent phylum in both. Exclusive Syrah yeasts indicated the presence of wine-associated taxa, that may contribute to the early-stages of grape fermentation and be further exploited to characterize the wine *terroir* of this tropical semiarid region of Brazil. We also observed high mycotoxin production for some isolates, reinforcing the relevance of mycotoxin surveillance programs for wine products.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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