Characterisation of fungal communities of developmental stages in table grape grown in the northern region of South Africa

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Abstract

Aims: To determine fungal communities that characterise table grapes during berry development.

Methods and Results: Two agro-ecologically different table grape commercial farms (site A and B) were used in this study. Samples were collected at full bloom, pea size and mature stages, from three positions (inside centre, eastern and western peripheral-ends) per site. Total DNA extraction, Illumina sequencing and analysis of 18 pooled samples for fungal diversity targeting ITS1-2 generated a total of 2035933 high quality sequences. The phylum *Ascomycota* (77.0%) and *Basidiomycota* (23.0%) were the most dominant, while the genera, *Alternaria* (33.1%) and *Cladosporium* (24.2%) were the overall dominant postharvest decay causing fungi throughout the developmental stages. Inside centre of site A were more diverse at full bloom (3.82) than those at the peripheral-ends (<3.8), while at site B, the peripheral-

ends showed better diversity, particularly the eastern part at both full bloom (3.3) and pea size (3.7).

Conclusion: Fungal population diversity varies with different phenological table grape growth stages and is further influenced by site and vine position within a specific vineyard.

Significance and Impact of the Study: The information on fungal diversity and succession in table grapes during preharvest growth stages is critical in the development of a more targeted control strategy, to improve postharvest quality of table grapes.

Key words: diseases, diversity, fungi, postharvest, yeasts.

Introduction

Table grape (*Vitis vinifera*. L) is ranked one of the most economically important deciduous fruit in South Africa. Of the total area under deciduous fruit production, 32% is table grapes (Hortgro, 2016). The grape industry is export oriented, having more than two thirds of the total production aimed for export markets (SATI, 2016). Like any other plant, the phyllosphere of table grape harbours diverse microbiota including fungi. Phyllosphere fungi, can either be phytopathogenic (Ottesen *et al*. 2013; de Oliveira *et al*. 2014; Rodríguez-Gálvez *et al*. 2015), neutral or beneficial agents (Sanzani *et al*. 2014) to fruit yield and quality. Field phytopathogenic fungi on table grape surfaces can potentially remain on the harvested bunch going into the packhouse and subsequent packing and cold chain distribution influencing the final product quality at the end of the supply chain. This may impact the microbial dynamics after harvest and contribute to fruit deterioration and eventual postharvest losses. Table grapes are susceptible to a wide range of phytopathogenic fungi, including *Botrytis* (Youssef *et al*. 2014; Youssef *et al*. 2015), *Penicillium* (de Oliveira *et al*. 2014) and *Erysphe* (Armijo *et al*. 2016) species, which compromise physiological processes, quality and vigour during fruit

development, and after harvest. Such may result in substantial yield losses and decay at the market end.

Among other factors, biotic (e.g. vineyard age, cultivar, season) and abiotic factors (e.g. geographic location, temperature, rainfall, chemical sprays etc.) contribute significantly to the microecosystem in vineyards (Comitini and Ciani 2008; Bokulich *et al*. 2014; Karlsson *et al*. 2014). Eukaryotic communities of wine grapes have previously been shown to be geographically delineated and can be specific to particular regions (Gayevskiy and Goddard 2012). Moreover, chemical sprays in vineyards have been reported to cause a sharp decline in eukaryotic biodiversity during ripening of grape vines, which negatively interfere with the phytopathogens/phytoprotectors microbiome balance (Pinto *et al*. 2014).

Although a lot of work has been done on grape (*Vitis vinifera* L.) microbiomes, most of the researchers focussed on wine grapes (Garijo *et al*. 2011; Gayevskiy and Goddard 2012; Bokulich *et al*. 2014), with little done on microbial diversity in table grapes at the preharvest stage to better understand phytopathogen dominance and prevalence. This study characterises fungal communities of table grapes in different biogeographical sites of South Africa based on Illumina Next Generation Sequencing.

The objectives of this study were to determine: (i) changes in composition of fungal communities at berry developmental stages (full bloom, pea size and commercial mature stage) ('phenological stage' effect); (ii) fungal diversity at different positions within the vineyard ('vine position' effect) and (iii) potential influence of agro-ecological location ('site' effect) on the fungal communities. Information generated from this study is of value towards development of improved postharvest disease management strategies at preharvest.

Materials and Methods

Site and sample collection

Table grape samples (cv. Crimson Seedless) representing three phenological stages (full bloom, pea size and commercial maturity) at preharvest were collected from two commercial farms (site A and B) in the northern production region situated in the north eastern part of South Africa. The farms are located in two geographically different locations, more than 100 km apart. Site A is situated at an elevation of 899 m above sea level, whereas site B is at 1123 m. Site B, generally receives higher annual rainfall (350-700 mm) than site A (150-350 mm) under a normal rainfall pattern (Weather Burro, Pretoria). At both sites, the samples were taken from 11 year old Y-trellises vineyards under netting and drip irrigation. The farms are GlobalGAP certified for over 10 years. Near-by crops found on site were citrus, water melon, natural forests and pasture grasses. Site B also reared beef cattle in feedlots. Samples were collected from three vines located on the eastern and western peripheral-end and inside centre within the vineyard for each site per developmental stage. For each vine three samples were collected from each sampling point and these were analysed for viable growth and isolation of cultures (results not shown) and the three samples per sampling point were then pooled for further analysis. All samples were detached aseptically from vine with disposable sterile gloves, placed in sterile brown paper bags and transported in cooler boxes with ice packs. All samples were processed within 24 hrs of sampling.

Isolation of microorganisms from table grape material

Microorganisms from all samples (n = 18) were isolated as previously described by Gomba *et al*. (2016), with slight modifications. A 25 g sample from each vine was placed in a beaker with sterile peptone buffered water (PBW) (Biolab, Merck, Johnnesburg, South Africa) supplemented with 0.025% Tween80 (Associated Chemical Enterprises, Johannesburg),

sonicated for 5 min. in an ultrasonic water bath (Labotec, Johannebsurg) to dislodge microbes from plant surfaces. The microorganisms in wash were then concentrated onto 0.45 µm cellulose nitrate filters (Sartorius Stedim Biotec, Goettingen, Germany) using vacuum filtration. Filters were stored at 4^{o}C for at most a week prior to DNA extraction.

DNA extraction, PCR amplification and sequencing

Total community DNA was extracted from the filter using the fungal / bacterial Zymo Research kit (ZymoResearch, USA), following supplier's protocol. The DNA concentration in each sample was determined using Qubit® Fluorometer (dsDNA HS (High Sensitivity Assay Kit) (Lifescience Technology, Johannesburg) prior to further analysis. The fungal ITS region was amplified using ITS1 (TCCGTAGGTGAACCTGCGG) and ITS2 (GCTGCGTTCTTCATCGATGC) (White *et al.* 1990). Amplification was done in a 28 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA), with initial heating at 94°C for 3 min, followed by 28 cycles of 94°C for 30 s of denaturing, annealing at 53°C for 40 s and extension at 72°C for 1 min, after which a final elongation step at 72°C for 5 min was performed. The PCR products were verified on 2% agarose gel to determine the success of amplification and the relative intensity of bands and were further purified using calibrated Ampure XP beads. The purified PCR product was used to prepare Illumina DNA library. Sequencing was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq following the manufacturer's guidelines.

Data analysis and taxa classification

Sequence data were processed using MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA). The sequences from the Illumina were joined and depleted of bar codes. Sequences with ambiguous base calls and those < 150bp (Porras-Alfaro *et al*. 2014; Gu *et al*.

2017) were discarded. Noises, singletons and chimeras were removed from sequences. Generated operational taxonomy units (OTUs) were defined by clustering at a similarity threshold of 97%. Final OTUs were used for taxonomic assignment using BLASTn against a curated database derived from RDPII and NCBI (www.ncbi.nlm.nih.gov, http://rdp.cme.msu.edu). Diversity indices (Shannon, Chao1 and Observed richness) (Abdelfattah *et al*. 2016b) for each sample were calculated in 'Visualization and Analysis of Microbial Populations Structure (VAMPS)' (http://vamps.mbl.edu). Normalization of the OTU table was done with rarefaction to an even depth of 1000 sequences in order to maintain homogeneity of samples. The indices were then calculated from the rarefied OTU table. Krona plots (Ondov and Phillippy 2011) were used to show taxonomic probability mass. To determine uniqueness of detected OTUs between phenological stages and sites, Venny 2.1 (http://bioinfogp.cnb.csic.es/tools/venny/index.html) was used.

Statistical analysis

The data was subjected to General Linear Models procedure of Statistical Analysis Systems (SAS) version 9.4 (Institute Inc., Carry NC, USA) to determine differences in microbiome diversity between sites, phenological stages and vine positions within a vineyard. A heatmap with data matrix was constructed based on fungal family prevalence's representing at least 3% of the relative abundance at family level per sample, using default parameters in ClustVis (http://biit.cs.ut.ee/clustvis/).

Results

Sequence data

A total of 2035933 reads were obtained and assigned to 1212 fungal OTUs after paired-end alignments, quality filtering, and deletion of chimeric, singletons, and plant sequences. After

collapsing biological replicates, samples showed a varying number of reads per sample ranging between 61941 and 113966, while the number of OTUs was between 250 and 537. Within the phenological stages, the pea size stage had the highest number of OTUs observed for both site A (859) and site B (798). On average, the pea size had 43.9% of the total OTUs followed by full bloom (30.1%) and mature stages (26.0%), respectively. When vine field sampling positions were considered, the western peripheral-end of site A had the lowest overall observed number of OTUs (179), whereas at site B, the eastern peripheral-end had the least (190) (Table 1).

Observed richness and fungal diversity indices

Regardless of sample position, the pea size stage had the highest observed species richness and consisted of 250 and 283 observed taxa, for site A and B, respectively, at an even depth of 1000 sequences (Table 1). Sample position showed the highest richness (121) in commercial mature samples collected at the western peripheral end of site B. Conversely, site A revealed the richest samples (91) at the central inside position of the vineyard. The Shannon-Weaver diversity index indicated low variation (2.09) in the level of diversity among the pea size samples of site A along the eastern peripheral end and high diversity (3.71) on the western peripheral end in commercial mature samples (Table 1). In site B, the Shannon-Weaver index increased with the change in phenological development, while at site A, a sharp decline by 0.82 from full bloom (3.32) to pea size (2.50) was noted (results not shown).

Overview of table grape fungal communities at preharvest stages

The phylum *Ascomycota* was the most abundant in all samples irrespective of sampling position and phenological stage, accounting for 77% of total population (Fig. 1). This was

Table 1. Analysis summary and alpha diversity results of fungal communities from two commercial vineyards (site A and B) at full bloom, pea size and commercial maturity stages of table grapes.

Sample source[a]	Total reads	Total OTUs	OUTs 1000[b]	Shannon-Weaver Diversity	Chao1
Site A					
Full bloom (FB)					
EPe	100217	189	68	2.8	71.85
Ci	88628	187	91	3.82	91.74
WPe	61941	161	73	3.34	74.14
Overall FB			118	3.62	126.68
Pea size (PS)					
EPe	98348	345	78	2.09	147.14
Ci	113966	282	91	2.32	129.03
WPe	108721	232	81	3.08	121.5
Overall PS			107	2.83	223.04
Commercial maturity (CM)					
EPe	111790	146	70	3.7	70.5
Ci	106892	145	64	3.68	64
WPe	140310	145	61	3.71	61.5
Overall CM			128	4.11	136
Site B					
Full bloom					
Epe	155621	214	97	3.3	110.88
Ci	149414	165	65	3.16	65.5
WPe	140454	221	86	3.04	110.5
Overall FB			121	3.32	175.02
Pea size					
Epe	126981	185	90	3.74	91.33
Ci	131035	303	82	3.55	108.89
WPe	141676	310	111	3.3	149.35
Overall PS			127	3.89	225
Commercial maturity (CM)					
Epe	94606	170	78	3.15	90.46
Ci	69940	139	69	2.54	71.94
WPe	95393	236	121	3.68	134.52
Overall CM			135	3.52	197.16

[a] Samples were from the eastern peripheral-end (EPe) and central inside (Ci) and western peripheral-end (WPe) positions in the vineyard. [b]An even depth of 1000 sequences was used to determine OTUs.

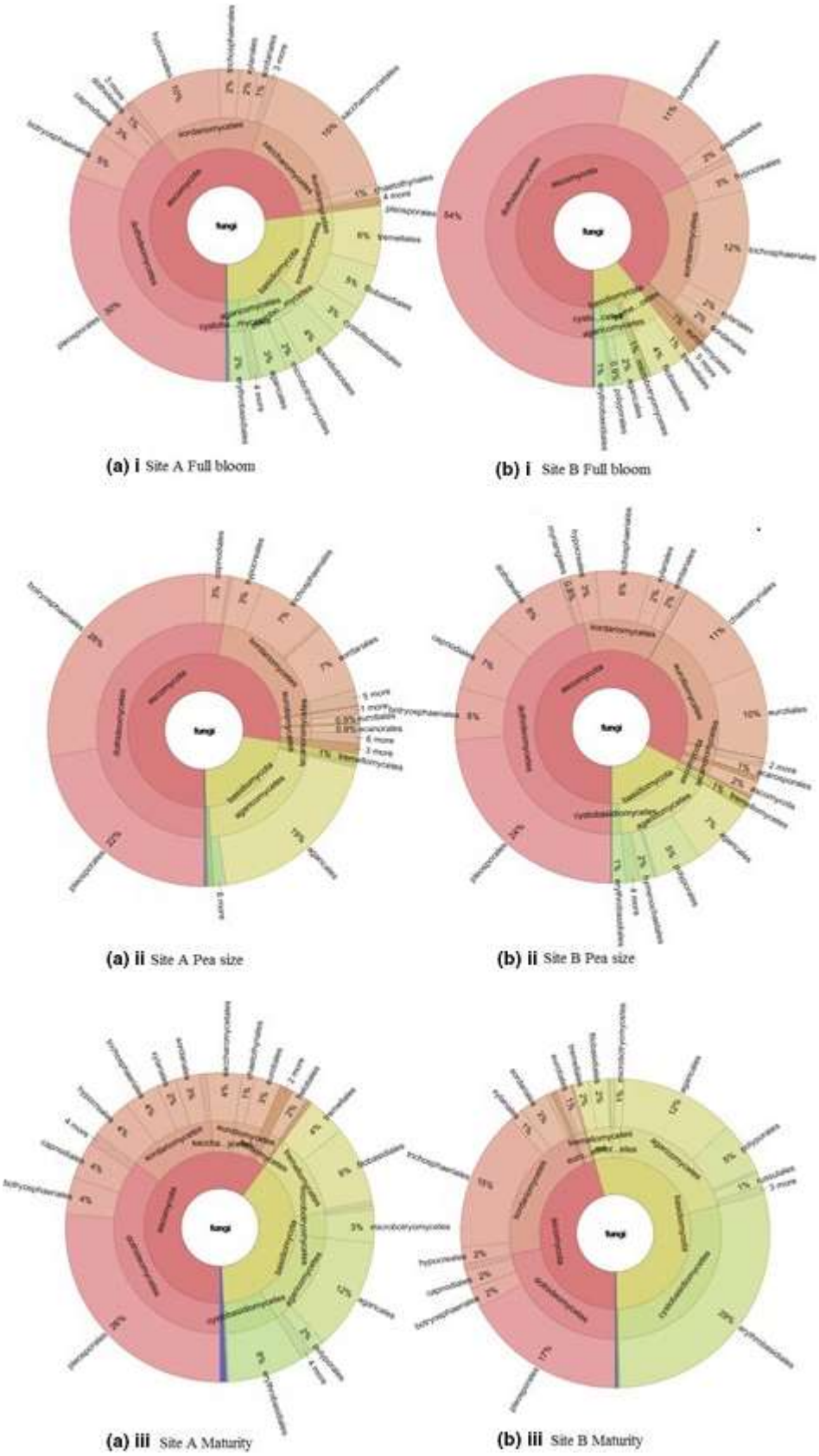

Figure 1. Overall relative abundance of fungal phyla (interior circles), classes (second exterior circle) and orders (third exterior circle) in site a (left) and b (right) at full bloom (ai and bi), pea size (aii and bii) and commercial mature stages (aiii and biii).

followed by the *Basidiomycota* (23.0%). Other phyla identified included *Blastocladomycota*, *Glomeromycota*, *Cryptomycota* and *Chytridiomycota*, but at very low frequencies (<0.2%). The *Ascomycota* were mainly represented by the member classes of *Dothideomycetes* (48.7%), *Sordariomycetes* (17.0%), *Eurotiomycetes* (6.5%), *Lecanoromycetes* (1.6.0%), *Saccharomycetes* (1.0%), *Ascomycota* (0.9%), *Leotiomycetes* (0.7%), *Pezizomycotina* (0.1%), *Orbilomycetes* (0.02%) and *Arthoniomycetes* (0.01%). The *Basidiomycota* were dominated by the classes *Agaricomycetes* (16.6%), *Cystobasidiomycetes* (3.29%), *Tremellomycetes* (2.3%), *Microbotryomycetes* (0.81%), *Agaricostilbomycetes* (0.07%), *Basidiomycota* (0.01%) and *Wallemiomycetes* (0.02%). *Pleosporales* was the dominant order (25.1%) followed by *Botryosphaeriales* (17%) and others.

A shift in the most abundant orders was observed between the stages (Fig.1). *Pleosporales* (25.1%), reduced by 11.1% and 27.1% from full bloom to pea size in site A and B, respectively. However, from pea size to harvest, an increase of 7.8% was noted in site A, while site B showed a decline of 13.4%. On another note, *Botryosphaeriales* (17.0%) was more dominant in pea size stage of site A (17.9%) than site B (6.3%). Unlike the *Pleosporales*, this order increased from full bloom to pea size at site A, by 12.7%, while from pea size to harvest, a reduction of 13.9% was observed.

Fungal diversity between sites and sampling positions at peripheral-end and inside centre of the vineyard

In site B, a shift in the fungal diversity was noted from full bloom to mature stage. *Ascomycota* decreased with phenological changes, both on vines at peripheral end and inside centre of the vineyard. At the peripheral-end *Ascomycota* decreased by 33% from full bloom to mature stage, whereas in the inside part of the vineyard, *Ascomycota* decreased by 3.5-folds (Fig. 2). On the contrary, the phylum *Basidiomycota* improved with changes in

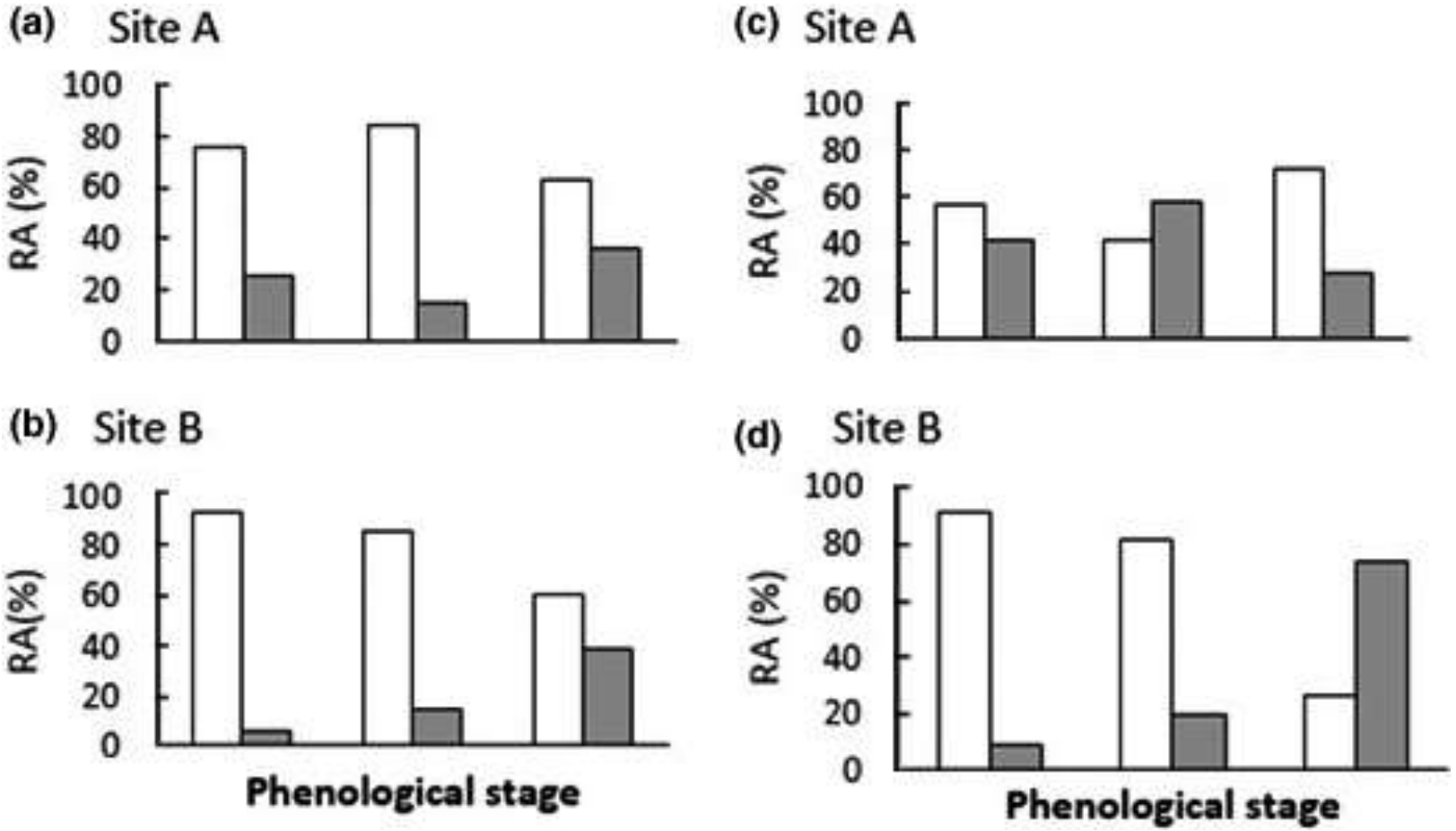

Figure 2. Relative abundance (RA) of the two predominant phyla (Ascomycota (■) and Basidiomycota (□)) at peripheral end (eastern and western peripheral end combined) (a) and (b) and inside centre (c) and (d) of the vineyard in site A and B, for full bloom (FB), pea size (PS) and commercial mature stages (CM).

phenological stage at both peripheral-end and inside central position of the vineyard. While in site A, an inconsistent shift in predominant phyla from full bloom to mature stage was noted between the peripheral and inside part of the vineyard.

Composition and diversity of fungal microbiota in table grapes phenological stages

Several unique taxa were exclusively detected in all phenological stages, as indicated by the Venn diagrams (Fig. 3). In site A, 17, 136 and 10 taxa were detected exclusively to full bloom, pea size and mature stage samples, respectively (Fig. 3a). Whereas in site B, 23, 93 and 35 were only detected in the respective stages (Fig. 3b). Of the total taxa detected from both sites, 138 (33%) were common taxa to all phenological stages (Fig. 3c). The families *Hypocreomycetidae* and *Hysteriaceae* were unique to site A at full boom stage, while *Debaryomycetaceae* and *Parmeliaceae* were exclusive to site B only.

At genus level, a significant shift in fungal microbiota was observed from full bloom to mature stage. Out of 251 detected genera only five (*Cystofilobasidium, Kwoniella, Hypocrea, Pyronema and Xylaria*) were shared between full bloom and mature stage (results not shown). Pea size and mature stages had the most common genera (10.0%), followed by full bloom and pea size (9.2%). *Alternaria*, *Cladosporium*, *Rhodotorula*, *Aureobasidium*, *Cryptococcus, Sporobolomyces, Epicoccum* were the most abundantly shared genera between the three phenological stages. These shared genera were in the phyla, *Ascomycota* and *Basidiomycota*.

Furthermore, a change in relative abundance of the dominant genera was revealed from one phenological stage to the other. (Fig. 4). Overall, the genera, *Alternaria* was dominant at full bloom (43.3%) and decreased as the berry developed to pea size (30.9%) and commercial mature stages (18.3%), respectively. Contrary, the *Rhodotorula* genera increased by 29.4% relative abundance from full bloom to commercial mature stage. On another note,

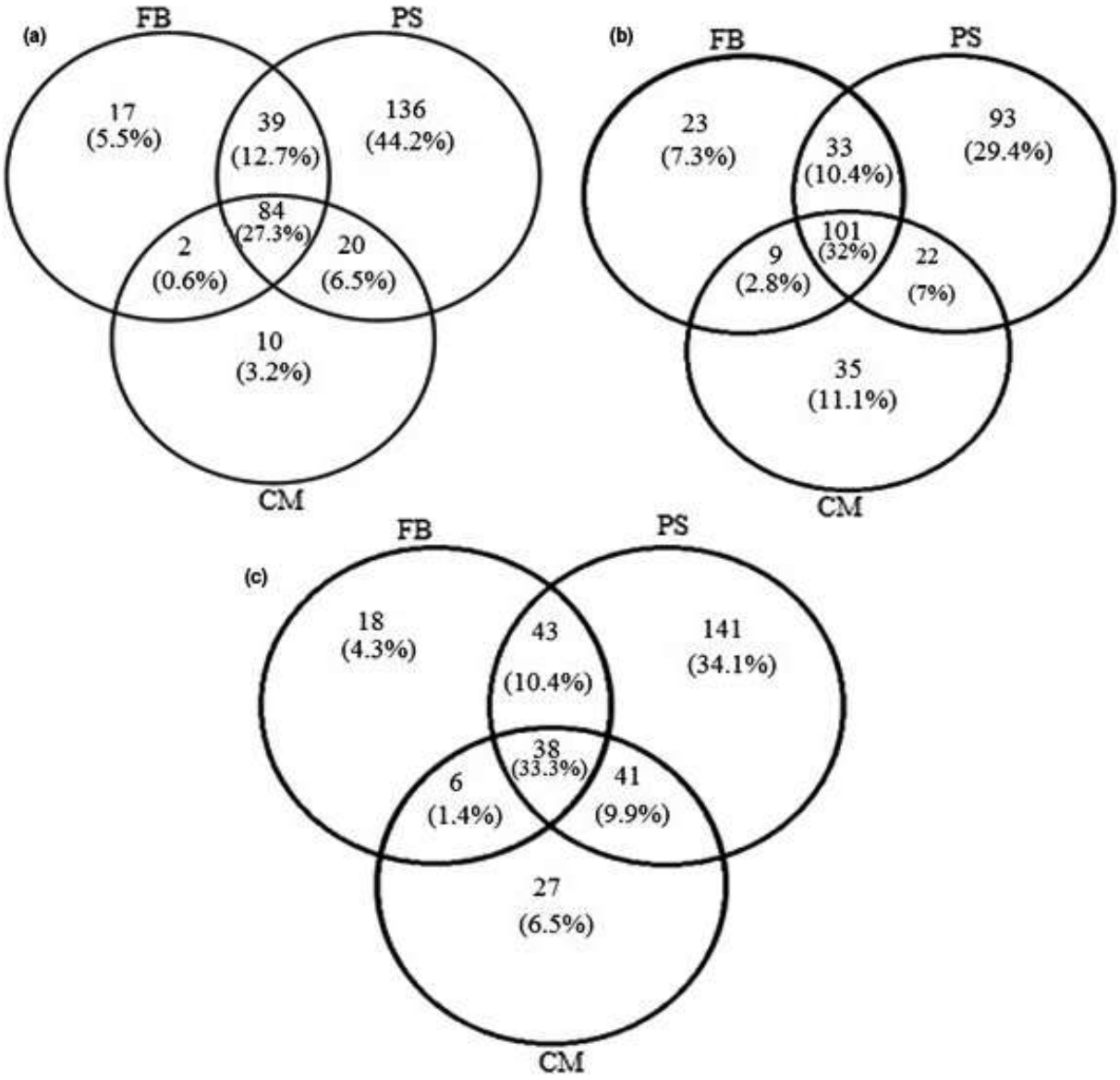

Figure 3. Venn diagram showing the distribution of unique and shared OTUs within the three phenological stages (full bloom (FB), pea size (PS) and commercial maturity (CM)) in the two sampling sites independently (a) and (b). An overall distribution of the unique and shared OTUs is shown in 'c'. The number of OTUs in each phenological stage is indicated in the respective circles.

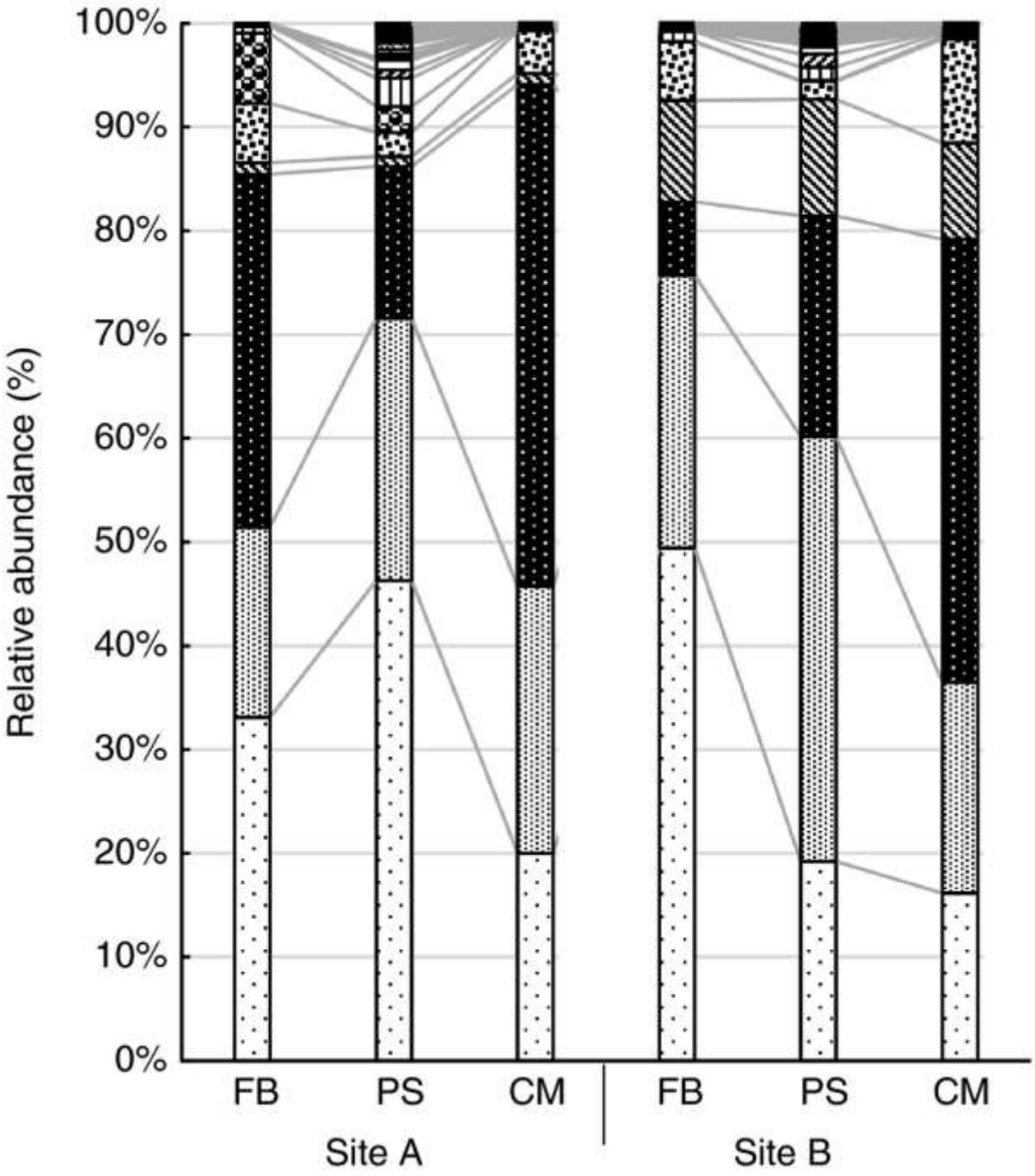

Figure 4. Relative abundance of predominant fungal genera detected at full bloom (FB), pea size (PS) and commercial maturity (CM) stages of table grapes in the two sites investigated. (■) *Phaeococcomyces*; (■) *Flavodon*; (■) *Preussia*; (■)*Paraconiothyrium*; (■) *Sydowia*; (■) *Sclerotinia*; (■) *Bartalinia*; (■) *Coniosporium*; (■) *Aspergillus*; (■) *Gelasinospora*; (■) *Bullera*; (■) *Periconia*; (■) *Coprinellus*; (■) *Peniophora*; (■) *Lambertella*; (■) *Curvularia*; (■) *Filobasidium*; (■) *Sporidiobolus*; (■) *Nigrospora*; (■) *Exserohilum*; (■) *Disciseda*; (■) *Bulleromyces*; (■) *Lycoperdon*; (■) *Bipolaris*; (■) *Phoma*; (■) *Fusarium*; (■) *Bagnisiella*; (■) *Acarospora*; (■) *Leptosphaerulina*; (■) *Epicoccum*; (■) *Sporobolomyces*; (■) *Cryptococcus*; (■) *Aureobasidium*; (■) *Rhodotorula*; (■) *Cladosporium*; (■) *Alternaria*.

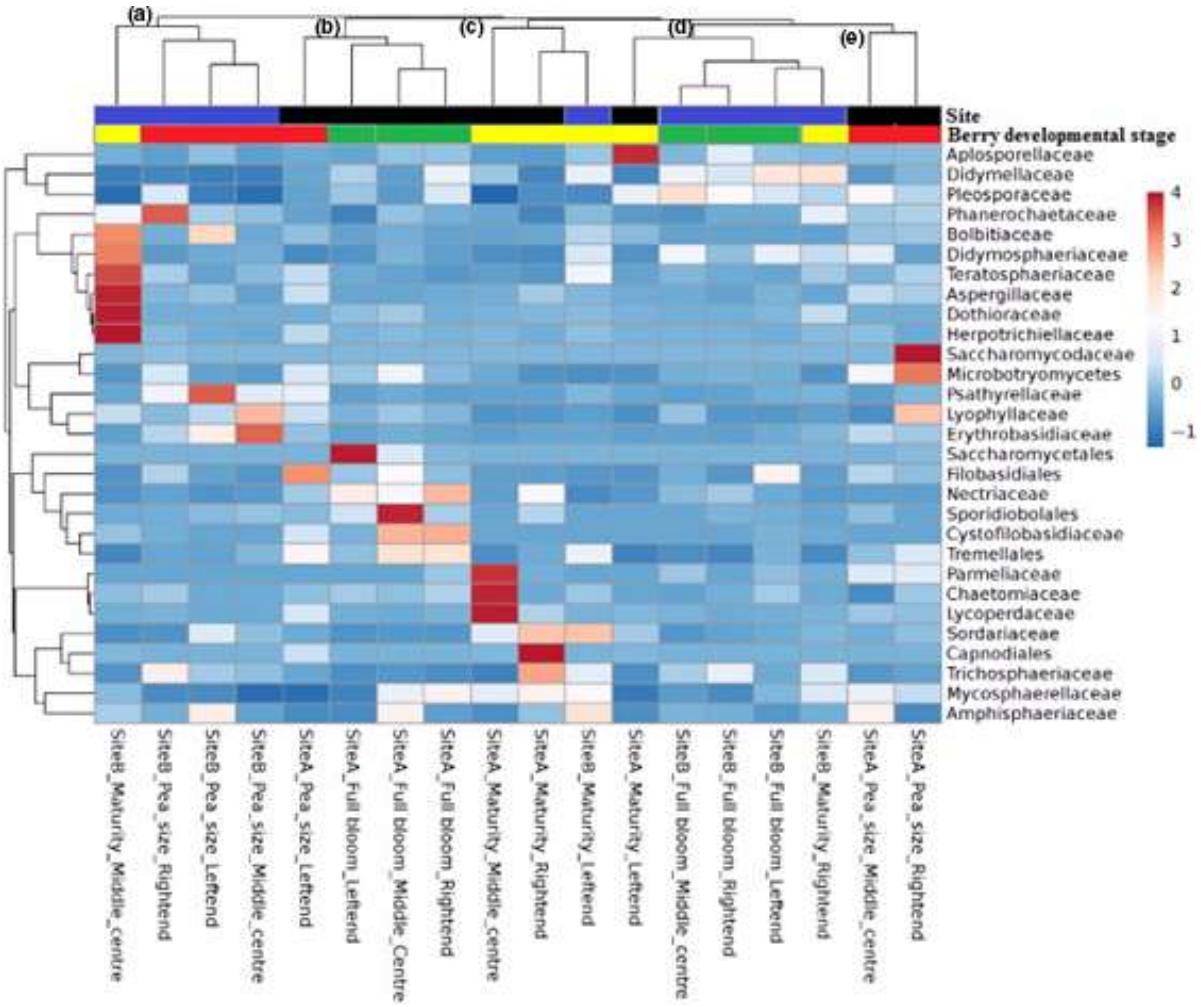

Figure 5. Heatmap representation of fungal communities in table grape berry developmental stages (■full bloom, ■pea size and ■maturity) from two agro-ecologically different sites (■site A and ■site B). The 'rightend' in each site represent samples from the western peripheral end position, while the 'leftend' is for samples from the eastern peripheral end, and middle centre is for central inside position.

Cladosporium was highly abundant (33.5%) at pea size compared to other stages, where it remained at similar dominance levels (23%). *Alternaria*, the most prevalent postharvest fungi detected was highly dominant at pea size (46%) in site A and at full boom (48%) in site B (Fig. 4).

Heatmap and clustering analysis

The heatmap analysis (Fig. 5) clustered full bloom samples uniquely by their developmental stage with respect to their different sources in clusters B and D. However, cluster B included a pea stage sample from the same site while the other two site A pea size samples were in E. On the other hand, cluster D included two samples from harvest stage for both sites although that for site A was in its own subgroup. In cluster A, all pea size stage samples from site B were grouped, but included one sample from the mature stage although it was clustered away in its own subcluster. Although samples clustered mainly by their sources with respect to developmental stages, cluster C included two maturity stage samples from different sites. All clusters had either samples from one developmental stage or from consecutive stages but none had samples from all three stages nor the first (full bloom) and last (maturity) stages together.

Discussion

Findings from this study revealed diverse fungal communities that characterise the reproductive phase of table grapes, from full bloom to maturity. Moreover, different agro-ecological environments showed significant fungal community variation with respect to developmental stage and positions within the vineyard. The major findings of this study were the detection of diverse fungi and significantly different population abundances within different developmental stages (full bloom, pea size and maturity), vine position in the

vineyard and between different agro-ecological sites. Studies looking at the effect of production environments were reported mainly in wine grapes (Schmid *et al*. 2011; Martins *et al*. 2014; Perazzolli *et al*. 2014).Wine grapes differ to table grapes in terms of physiological and morphological attributes. Wine grapes are generally smaller in size, with higher sugar and acid levels compared to table grapes. Table grapes have a thicker pulp and a thinner skin than wine grapes, as they are intended for fresh produce market. Hence, such data is more applicable to wine quality processing, however, not much information is available on the microbiota of table grapes with relevance to their effect on postharvest pathogens. Here, we report the first study on table grapes fungal microbiome during berry development phase, and its relevance to postharvest pathogens.

Variation in fungal diversity between different sampling positions (central inside, eastern and western peripheral ends) within a vineyard was highly significant for both sites A and B, with respect to all three developmental stages. A number of factors are likely explanations to the observed differences. During berry development, table grapes under-go various physiological and biochemical changes (Conde *et al*. 2007), that may affect the diversity of the fungal communities inhabiting each micro-environment within a developmental stage. The full bloom stage is characterised by a higher ecological interaction with other organisms, such as pollinating insects, whereas the berry stages are defined mainly by physiochemical changes, such as increase in levels of phenolic compounds at pea size (Conde *et al*. 2007; Zhang *et al*. 2016) and accumulation of sugars at the mature stage (Wu *et al*. 2015).

The vine position effect on table grape microbiota was further described by Shannon-Weaver diversity index. As expected, the Shannon-Weaver diversity index revealed that, samples at the inside centre of the vineyard were richer than samples at the peripheral-end of the vineyard. This could be explained by variability in temperature, humidity and UV-light

within a block or vineyard, as highlighted in previous studies (Sternad Lemut *et al*. 2015; Sui *et al*. 2015). On another note, environmental conditions, such as solar radiation interfere with the production, germination and survival of conidia, which affect the life cycle of the pathogen. This interference with the life cycle, limits the fungal population size and further dispersal (Gilberto et al., 2015). Moreover, due to limited air circulation within vines at the inside centre, compared to the peripheral-ends, a micro-climate may prevail which could favour a wider diversity of fungal populations and reduce fungicide efficacy.

Sunlight exposure is generally high on vines at the peripheral-end compared to the inside central vines due to canopy effect, this could result to a sterilizing effect on the bunch surfaces caused by UV radiation from sunlight, hence affecting the microbial composition and population. Previous researchers have also found that canopy micro-climate conditions (air circulation, temperature and humidity) in grapevine (Sternad Lemut *et al*. 2015) influence incidence of microbial infection in grapes. Furthermore, relative humidity in apples surfaces (Lahlali *et al*. 2008) enhance the population of antagonistic yeasts. This could relate to the variability observed in the selected vine positions of the study. This microbiome variability may have an influence on the pathogenic populations which could affect the postharvest product.

Similar to previous work in common deciduous fruit including, strawberries (Abdelfattah *et al*. 2016a), wine grapes (Tailor *et al*. 2014) apples (Abdelfattah *et al*. 2016b) and pears (Volschenk *et al*. 2016), the phyla *Ascomycota* and *Basidiomycota* were dominant irrespective of phenological stage. *Ascomycota* was the highest detected phylum possibly due to its morphological structure forming an ascus made of a thick layer (Mycelial and Systematics, 2013), which might give protection against adverse extreme conditions. However, a shift in their abundance at order level from full bloom to mature stage was noted. Fungal communities found at full bloom predominately consisted of the order

Botryosphaeriales, particularly *Aplosporellaceae*, and order *Pleosporales* (e.g *Pleosporaceae* and *Didymellaceae*). The abundance of the order *Botryosphaeriales* doubled at pea size but further reduce to 2% at maturity, while *Pleosporales* reduced by at least two-folds at pea size and mature stage. *Agaricales* and *Erythrobasidiales* were prevalent at the mature stage. This order *Botryosphaeriales*, include saprophytic, endophytic and pathogenic species which affect a wide range of hosts. This may enhance their survival and reproductive mechanism, which subsequently contribute to their high incidence.

The genus *Rhodotorula*, was the most abundant yeast detected on table grape at all phenological stages examined in the study. This was represented by two species (*Rhodotorula glutinis*, and *Rhodotorula nothofagi*) and other non-identified taxa. On cherry fruit surfaces, the *R. glutinis* is known for its antifungal properties against postharvest decay (Tian *et al*. 2004). The abundance of *Rhodoturula* genus was influenced by the changes in development during the berry reproductive cycle. Initially, at full bloom the population of *Rhodoturula* was low (16.5%) and drastically increased to 45.9% at the mature stage, contrary to that of a preharvest wine grape study by Renouf *et al*. (2005). In their study, this genus was highest at veraison compared to berry set and harvest stage of wine grapes (Renouf *et al*. 2005). Other workers also reported its abundant occurrence in winery-resident microbiota (Bokulich *et al*. 2013), although its role in wine fermentation was not clearly defined.

Cryptococcus, a potential biocontrol agent against postharvest pathogens (Liu *et al*. 2013) was the second most abundant yeast colonizer found predominately in all stages, as previously found in other fruit material (Renouf *et al*. 2005; Tailor *et al*. 2014; Abdelfattah *et al*. 2016a; Abdelfattah *et al*. 2016b). The full bloom and mature stages had a higher population of this genus compared to pea size.

Yeast-like fungus, *Aureobasidium pullulans* were also detected in this study. *A. pullulans* have been isolated from fruit surfaces and used as antagonists against postharvest pathogens (*Penicillium expansum* (Oro *et al*. 2016), *Botrytis cinerea* (Sánchez *et al*. 2012)) in several pome fruits (Sánchez *et al*. 2012; Zhang *et al*. 2012). This yeast-like fungi is also associated with plant growth promoting attributes, through the production of phytohormones, such as auxins (Fu et al. 2016). Compared with other microbiome on grapes phyllosphere studies (Renouf *et al*. 2005), the overall population of *A. pullulans* revealed in this study was lower (<10%). A higher population of *A. pullulans* was detected at full bloom (43332) and pea size (44397) stages in site B, whereas in site A, the opposite was observed in the two respective stages.

Fungal populations associated with table grape postharvest decay were characterised by the prevalence of mainly *Alternaria* and *Cladosporium*, while other fungi showed a very low relative abundance. *Alternaria alternata* dominated the postharvest pathogen profile, however, this genus expressed a decrease as the *Rhodotorula* yeasts increased with berry development (from full bloom to maturity), possibly due to the inhibitory effect of this yeast against postharvest decay pathogens, as earlier reported on other fruit surfaces (Tian *et al*. 2004). These dominant fungal genera (*Alternaria*, *Cladosporium* and *Rhodotorula*) were also the most commonly isolated from the plating of serial dilutions of the grape washing (unpublished data). Interestingly, *Botrytis cinerea*, a widely known postharvest decay pathogen in grapes (Sanzani *et al*. 2012; Gubler *et al*. 2013; Saito *et al*. 2013), was not detected in our study, possibly due to its low isolation frequency (Rivera *et al*. 2013), and elevation effect (Powelson, 1959; Pardatscher and Schweigkofler, 2009). Other researchers have also shown similar findings using 454 pyrosequencing (Sylla *et al*. 2013) in strawberries at preharvest. This also reveals that a healthy microbiome composition should keep the pathogenic populations in low or even undetectable levels.

In conclusion, this study provides deeper knowledge of fungal community dynamics associated with table grapes in the field and how they change during berry development. Part of the identified taxa were previously reported in grape-associated fungi, particularly in wine grapes, but others were detected for the first time or could not be classified. The unclassified fungi detected in this study prove that classification of grape associated fungi is far from being completely documented. This present research will provide baseline information for future work targeting specific fungal populations associated with postharvest losses in table grapes. More knowledge on the compositional variation in table grape fungal microbiota, as influenced by different cultivars, packaging material, handling and management strategies along supply chains from field to consumer are needed.

Future studies looking at preharvest management practices that could enhance the natural abundance of potential biocontrol communities, such as the genus *Rhodotorula*, *Cryoptococcus* and yeast-like fungus, *Aureobasidium pullulans* should be considered, in order to reduce populations of postharvest pathogens. This will intern be valuable in developing a biocontrol strategy integrated with existing spray programmes and may result in reduced sprays and lower pesticide costs and reduce postharvest losses due to decay. Furthermore, the knowledge gathered in this study can be useful in planning and application of phytosanitary control procedures.

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Conflict of Interest

There was no conflict of interest.

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