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Effects of agriculture management on fungal phyllosphere diversity in vineyards and its relationship with native forests

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Agriculture is one of the main drivers of land conversion and agriculture practices can impact on microbial diversity. Here we characterized the phyllosphere fungal diversity associated with Carmenere grapevines under conventional and organic agricultural management. We also explored the fungal diversity present in the adjacent sclerophyllous forests to explore the potential role of native forest on phyllosphere in vineyards. After conducting deep amplicon sequencing, no significant differences in fungal diversity indices and community structure were detected between organic and conventional vineyards, suggesting that the phyllosphere microorganisms of grapevines are highly resilient to agricultural treatments. On the other hand, we found a high proportion of shared fungal OTUs between vineyards and native forests. In addition, both habitats had similar levels of fungal diversity despite forest samples were derived from multiple plant species. In contrast, the community structure was different between habitats. Nevertheless, the native forest had more unidentified genera and OTUs unique to this habitat than did the vineyards. *Cladosporium*, *Aureobasidium,* and *Endoconidioma* were more abundant in vineyards, whereas *Davidiella*, *Didymella*, and *Erysiphie* were more abundant in forests. Overall, this study argues that a better understanding of the relationship native forests and agroecosystems is needed for maintaining and enhancing ecosystem services provided by natural ecosystems. Finally, knowledge of microbial communities living in the Chilean Mediterranean biome is needed for appropriate conservation management of these biomes and their classification as biodiversity hotspots.

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Running title: Fungal diversity on vineyards

Abstract

Agriculture is one of the main drivers of land conversion and agriculture practices can impact on microbial diversity. Here we characterized the phyllosphere fungal diversity associated with Carmenere grapevines under conventional and organic agricultural management. We also explored the fungal diversity present in the adjacent sclerophyllous forests to explore the potential role of native forest on phyllosphere in vineyards. After conducting deep amplicon sequencing, no significant differences in fungal diversity indices and community structure were detected between organic and conventional vineyards, suggesting that the phyllosphere microorganisms of grapevines are highly resilient to agricultural treatments. On the other hand, we found a high proportion of shared fungal OTUs between vineyards and native forests. In addition, both habitats had similar levels of fungal diversity despite forest samples were derived from multiple plant species. In contrast, the community structure was different between habitats. Nevertheless, the native forest had more unidentified genera and OTUs unique to this habitat than did the vineyards. *Cladosporium*, *Aureobasidium,* and *Endoconidioma* were more abundant in vineyards, whereas *Davidiella*, *Didymella*, and *Erysiphie* were more abundant in forests. Overall, this study argues that a better understanding of the relationship native forests and agroecosystems is needed for maintaining and enhancing ecosystem services provided by natural ecosystems. Finally, knowledge of microbial communities living in the Chilean Mediterranean biome is needed for appropriate conservation management of these biomes and their classification as biodiversity hotspots.

Keywords: amplicon sequencing, Chile, ecosystem services, Mediterranean biome, organic management, yeast.

INTRODUCTION

Land conversion is one of the most important drivers of habitat loss, changing the biophysical conditions of natural ecosystems and affecting the ecosystem functioning of these habitats (Vitousek et al. 1997, Griffits & Philippot 2013). One of the main drivers of land conversion is agriculture, which has transformed forests and grasslands into arable land for food production (Fielder et al. 2008). Agriculture involves management practices and the addition of chemical or organic products to improve growth plant, increase plant biomass, eliminate crop pests, and reduce weed competence (Fiedler et al. 2008). These practices can be classified into two main categories: conventional or organic management (Coll et al 2011). Conventional agriculture involves the application of inorganic fertilizer (e.g. nitrogen and phosphorous) to improve crop production, and synthetic insecticides and herbicides are used to control pests and weeds. Conversely, organic agriculture employs organic fertilizers (e.g. compost, humus), biological control to manage pests, and tillage or grass-cutting to manage weeds.

Habitat conversion and agricultural management have profound effects on the physical and biological properties of agroecosystems. For example, water and soil quality, microbial community structure, invertebrate abundance, and bird species richness have all been shown to be affected by agriculture management and habitat conversion (Coll et al. 2011; García-Orenes et al. 2013). In light of this, organic farming has been proposed as a potential agricultural practice to increase biodiversity in farmlands (Hole et al. 2005; Chamberlain et al. 2010). On the other hand, Gabriel et al. (2010) report that organic farms have positive effects on the diversity of plants, bumblebees, and butterflies, but not necessarily on hoverflies and birds. Agricultural

practices can also affect the diversity of microorganisms in vineyards and agroecosystems (Bevivino et al. 2014; Castañeda et al. 2015; García-Orenes et al. 2013). For instance, the application of organic matter (e.g. oat straw) increases fungal abundance in managed soils and results in a microbial community structure similar to that found in forest soils (García-Orenes et al. 2013). Agricultural management can also influence the bacterial microbiota associated with the surfaces of leaves, fruits, and vegetables, otherwise known as the phyllosphere (i.e. the microbial habitat found on the above-ground surface of plants) (Ottensen et al. 2009). Indeed, Karlsson et al. (2014) has found significant effects of fungicide use on the fungal evenness and richness of wheat phyllosphere, but fungicide use was not found to effect wheat fungal pathogens. The effect of vineyard management on fungal soil community structure has also been reported, where organic and biodynamic vineyards support higher fungal diversity than conventional vineyards (Bagheri et al. 2005; Setati et al. 2012). However, the effects of agricultural practices on phyllosphere microbiota have only recently been studied, and it is yet not well known how the presence and abundance of key microorganisms affect food or wine production (but see Perazzolli et al. 2014; Morrison-Whittle et al. 2017).

Winemaking relies on the microbial contribution of bacteria and yeasts from grapevine growth to wine fermentation (Fleet 2003; Mills et al. 2008;). In particular, yeasts play important roles in alcoholic fermentation, consuming sugar and producing ethanol, and also contribute to the sensorial features of wine (Fleet 2003; Renouf et al. 2005). Most wineries around the world employ commercial yeasts (e.g. *Saccharomyces cerevisiae*) to control fermentation, but recent evidence indicates that grape microbiota plays an important role in both spontaneous and inoculated fermentation, contributing to the taste and flavor of wine (Ganga & Martínez 2004; Renouf et al. 2005). Recently, it has been shown that the diversity of grape microbiota is

92 associated with local environmental conditions, suggesting that microbial *terroir* does
93 influencing the organoleptic features of wine (Bokulich et al. 2014; 2016).
94 Reduced habitat heterogeneity as a consequence of habitat conversion could also
95 influence the fungal diversity of vineyards. It has been reported that plant diversity is linked to
96 soil fungal diversity because a higher number of plant hosts increases the availability of potential
97 fungi-host interactions (Chung et al. 2007; Holland et al. 2016). However, despite that some
98 studies have explored the differences in soil microbiota between vineyards and surrounding
99 vegetation (Orgiazzi et al. 2012; Holland et al. 2016), there is little knowledge about how the
100 phyllosphere differs between managed and unmanaged habitats. Furthermore, native vegetation
101 can be a suitable habitat for fermenting yeasts, which can be widely found in fruits and leaves
102 (Kurtzman et al. 2011). For example, Saccharomyces fungi have been found growing on the
103 ground and on trees (Libkind et al. 2011; Sampaio and Gonçalves 2008). Additionally,
104 fermenting yeasts can be found in honeydew, a sugary fluid excreted by aphids feeding on trees
105 (Serjeant et al. 2008). Overall, the high availability of substrates and hosts in native forests
106 compared to in vineyards should lead to increased fungal diversity in forests.
107 In the present study, we characterized the fungal diversity of Carménère grapes subjected
108 to different agricultural practices, and we also compared this diversity to that found in
109 surrounding sclerophyllous forests. For this, we undertook amplicon sequencing and estimated
110 the diversity, community structure, and fungal composition of several organic and conventional
111 vineyards in central Chile. We focused on Carménère grapes because they suffered a phylloxera
112 infestation in 1867 in Europe that greatly reduced the area cultivated with this grape, and
113 currently the largest Carménère cultivar is found in Chile (Mondaca & Hinrichsen 2007). Despite
114 its historic and enological value, microbiota associated with Carménère grapes has not been

explored until now (but see Miura et al. 2017 for geographical patterns in bacterial diversity). At the same time, the fungal diversity of the phyllosphere of Chilean sclerophyllous forests is completely unknown. This biome is considered as a biodiversity hotspot because it is threatened by diverse human activities while it harbors high plant endemism; thus, it is a priority for conservation management (Myers et al. 2000). However, knowledge of the fungal communities of this biome is scarce, and studies employing genomics could provide valuable information for conservation strategies in these areas (Heilmann-Clausen et al. 2014)

MATERIALS AND METHODS

Sampling

Samples were collected during 2014 from six vineyards (three vineyards employing conventional management and three vineyards employing organic management) and surrounding sclerophyllous forest. All vineyards and forests are located in the Colchagua Valley, Chile (34°15'S-34°50'S; 70°15'W-72°00'W), and samples were taken during the last week before the Carménère harvest (April in the Southern Hemisphere).

In each vineyard, three plots containing Carménère cultivars and located close to sclerophyllous forest were chosen. Within each plot, undamaged grape berries and grape leaves were collected from three grapevines located close to the border with the forest and from another three grapevines located 30 m toward the center of the vineyard plot. In the forests, leaves were collected from three trees at the border with the vineyard and from four trees located 30 m toward the center of the forest plot. If at the sampling point there was more than one tree species, the sample was composed equally of all species. Common native tree species in the Chilean sclerophyllous forest were litre (*Lithrea caustica*), boldo (*Peumus boldus*), peumo (*Cryptocarya*

alba), quillay (*Quillaja saponaria*) and espino (*Acacia caven*). Fruits from forest trees were almost absent during the autumn, and they were not included in the sampling. All of the samples were collected using surgical gloves and sterilized scissors. Upon collection, the samples were stored in sterilized hermetic plastic bags and maintained on dry ice until arrival at the laboratory at the Universidad Austral de Chile (Valdivia, Chile). At the laboratory, the samples were stored at –20ºC until DNA extraction.

DNA extraction and Illumina sequencing

The grape and leaf samples were transferred to 50 ml tubes and suspended in 200 ml of a 0.9% NaCl– 0.02% Tween20 solution. Each tube was shaken for 2 h at 100 rpm in a RS-60 multirotator (BioSan, Latvia) at 100 rpm and at room temperature. The wash solutions were filtered using sterilized gauze to eliminated large pieces of plant tissue. Then, the solutions were centrifuged for 5 min at 1500 rpm. The supernatant was transferred to new 50 ml tubes and centrifuged for 20 min at 7500 rpm. Genomic DNA was extracted from the resulting pellets using a PowerSoil DNA isolation kit (MoBio Laboratories, United States) following the manufacturer's instructions. After extraction, DNA was quantified employing a fluorescence method with a Quan-iT PicoGreen dsDNA kit (Invitrogen, United States). All samples (grape berries, grape leaves, and forest leaves) were processed following the same protocol.

To characterize fungal diversity, two genomic regions were amplified: ITS2 and D2/LSU regions. To amplify these genomic regions, we chose the following primers according to Pinto et al. (2014): ITS2-F (5' – GCATCGATGAAGAACGC – 3') and ITS2-R (5' – CCTCCGCTTATTGATATGC – 3'); and D2-F (5' – AAGMACTTTGRAAAGAGAG – 3') and D2-R (5' – GGTCCGTGTTTCAAGACG – 3'). These pairs of primers have been described as

161 complementary for the identification of fungi associated with vineyards (Pinto et al. 2014).
162 Amplicon sequencing was performed using 250-bp paired-end sequencing on an Illumina MiSeq
163 sequencer (Illumina, United States) following the manufacturer's instructions of the
164 Metagenomic Sequencing Library Preparation protocol.
165
166 **Data analysis**
167 Using FastQC (Andrews 2010), quality of sequences was checked. Then, raw sequences
168 were quality filtered for a Q-value higher than 26 and for sequences longer than 150 bp using the
169 script Reads_Quality_Length_distribution.pl (Balint et al. 2014). Forward and reverse filtered
170 sequences were paired using Pandaseq with a minimum overlap of 5 bp (Masella et al. 2012).
171 After this, each paired-end sequence file was split into two different files using Fqgrep
172 (https://github.com/indraniel/fqgrep) with each file only contained sequences starting with the
173 ITS or D2 primer sequences. Overall, a total of 108 fastq files were produced (54 samples × 2
174 amplicons). These files were then converted into fasta files, merged into one single file, and
175 primer sequences were trimmed.
176 Quality filtered and trimmed sequences were analyzed using QIIME v1.9.1 (Caporaso et
177 al. 2010). Using uclust, operation taxonomic units (OTUs) were clustered with the pick-open-
178 reference-otus script with 97% identity levels (Edgar 2010) with a percentage of failure
179 sequences of 10%. The taxonomic assignment of OTUs picked from the ITS reads was
180 performed using BLAST against the UNITE fungal database version 7 (Abarenkov et al. 2010).
181 The assignment of OTUs picked from the D2 reads was performed using BLAST against the 28S
182 LSU RDP database version 7 (Liu et al. 2012). In contrast to ITS, the D2/LSU region is suitable
183 for phylogenetic analysis (Porter & Golding 2012). Then, using PyNAST (Caporaso et al. 2010),

D2 sequences were aligned against a template alignment from the 28S LSU RDP database. With the aligned sequences, a phylogenetic tree was constructed using FastTree (Price et al. 2010). For both amplicons, we removed OTUs matching non-fungal sequences and with total abundances less than 0.001% in the final OTU table.

All downstream analyses were performed in R using the DESeq2 (Love et al. 2014), phyloseq (McMurdie & Holmes 2013), and vegan packages (Oksanen et al. 2011). To standardize the number of sequences among samples, samples were rarefied to the sample with the lowest number of sequences. Then, we estimated OTU richness, Shannon diversity, and Pielou evenness for each sample. We also estimated the phylogenetic diversity (Faith's PD) for the D2 amplicon. We compared the diversity indices between agricultural management (conventional and organic management) and between plant tissues (grape berries and grape leaves) using two-way ANOVAs. We also used a one-way ANOVA to compare diversity indices between habitats (grape leaves and forest leaves).

To compare the fungal community structure between agriculture managements and plant tissue we conducted a two-ways PERMANOVA using the adonis function of the Vegan R package. We also conducted a one-way PERMANOVA to compare the community structure between vineyards and native forests. This analysis was based on the ITS2 Bray Curtis distance matrix and the D2 normalized weighted-Unifrac matrix. UniFrac incorporates the phylogenetic relatedness between OTUs found in a sample (Lazupone & Knight 2005). These distance matrices were also used as inputs for the non-metric dimensional scaling (NMDS). Finally, we also compared OTU abundances across categorical effects using an ANOVA. *P*-values were corrected by the Benjamini-Hochberg (False Discovery Rate; FDR) procedure for multiple comparisons; this was done using QIIME and DESeq2. Finally, the core microbiome of each

sample was estimated using QIIME, and the resulting OTU lists were used to construct Venn diagrams and visualize the number of OTUs exclusive and shared between different types of samples. The Venn diagrams were plotted with Venny 2.1 (Oliveros 2015).

RESULTS

For ITS2, a total of 3,606,629 raw sequences were analyzed with the QIIME pipeline. After processing with QIIME, we obtained 3,567,599 sequences that clustered into 897 OTUs (97% sequence similarity), and the samples were rarified at 19,450 sequences (SFig. 1A). For D2, a total of 4,135,846 raw sequences were analyzed with the QIIME pipeline. After processing with QIIME, 4,115,319 sequences clustered into 642 OTUs (97% sequence similarity), and the samples were rarified at 26,350 sequences (SFig. 1B-C).

Fungal diversity within vineyards: management and plant tissue effects

We compared the relative abundances of 897 OTUs between types of agricultural management (organic and conventional). From this, the relative abundances of only 16 OTUs differed between management types (FDR: $p < 0.05$ for all cases; STable 1). Of these 16 OTUs, 4 OTUs were more abundant in conventional vineyards and12 OTUs were significantly more abundant in vineyards with organic management (Fig. 1A). From the 616 OTUs (78.7%) found in 90% of the samples, only 30 OTUs were found in all vineyard samples (i.e. core vineyard microbiome). Furthermore, 69 OTUs (8.8%) were exclusively found in conventional vineyards, and 98 OTUs (12.5%) were only found in organic vineyards (Fig. 1C,E). Regarding taxon abundance, we found that only the relative abundance of the *Dothioraceae* family was significantly different between types of agricultural management (conventional = 3.9%; organic

= 10.5%; FDR *P*-value = 0.02; STable 2). Within this family, the abundance of *Aureobasidium* was higher abundances in organic (9.9%) than in conventional vineyards (3.6%), and these differences were marginally significant after corrections for multiple comparisons (FDR *P*-value = 0.057).

We also compared the OTU relative abundances between grape berries and grape leaves (Fig. 1B), and we found that the abundances of 21 OTUs were more abundant in grape berries, while 68 OTUs were significantly higher in grape leaves (FDR: $p < 0.05$ for all cases; STable 3). Shared OTUs between grape berries and grape leaves were 557 (70.8%), whereas 25 OTUs (3.2%) were only found in grape berries and 205 OTUs (26%) were only found in grape leaves (Fig 1D,E). Comparing genus abundances, we found that 55 genera were significantly different between plant tissues, but only *Davidiella* was relatively more abundant in grape berries than in grape leaves (FDR: $p = 0.004$; STable 4).

The values of all diversity indices (OTU richness, Shannon diversity, phylogenetic diversity, and Pielou evenness) were not significant different between agriculture management types (Fig. 2, Table 1). In contrast, diversity indices were significantly lower for grape berries than for grape leaves (Fig. 2, Table 1). Additionally, no significant interactions were found between plant tissue and management for the diversity indices estimated (Fig. 2; Table 1). Regarding the fungal community structure estimated using ITS2, the effects of plant tissue (PERMANOVA: $F_{1,32} = 4.58$, $P < 0.0001$) and agricultural management (PERMANOVA: $F_{1,32} = 1.77$, $P = 0.014$) were significant, but no significant interaction between plant tissue and agricultural management was detected (PERMANOVA: $F_{1,32} = 1.05$, $P = 0.30$). There was clear separation between grape leaves and grape berries along the NMDS1 axis, while the agricultural management was separated along axis NMDS2 (Fig. 3A). For D2, there was a significant effect

253 of plant tissue on the community structure (PERMANOVA: $F_{1,32} = 4.20$, $P = 0.003$; Fig. 3B).
254 However, the effect of agricultural management on community structure was not significant
255 when the community structure was estimated using D2 (PERMANOVA: $F_{1,32} = 2.10$, $P = 0.13$;
256 Fig. 3B). Finally, no significant interaction was found between the effects of plant tissue and
257 agricultural management on the community structure (PERMANOVA: $F_{1,32} = 0.72$, $P = 0.54$).
258
259 **Fungal diversity comparison between vineyards and native forest**
260 We compared OTU relative abundances between forest leaves and grape leaves and we
261 found 351 OTUs that differed in terms of their relative abundances (FDR: adjusted $p < 0.05$ for
262 all cases; STable 5). Of these, 227 OTUs were significantly more abundant in forests, and 124
263 OTUs were more abundant in vineyards (Fig. 4A). We also explored the presence of OTUs
264 found in at least 90% of the samples: 692 OTUs (79.7%) were present both in forest and in
265 vineyard samples, but only 44 OTUs were found in all of the 54 samples. These 44 OTUs
266 represent the core microbiome across habitats. Additionally, 106 OTUs (12.2%) were
267 exclusively found in the forest samples, and 70 OTUs (8.1%) were only found in vineyard
268 samples (Fig. 4B). Fungal OTUs found in the present study were classified into 203 genera, and
269 the most abundant were *Davidiella* (vineyards = 35.6%; forest = 17.7%; FDR *P*-value < 0.0001),
270 *Cladosporium* (vineyards = 21.6%; forest = 25.8%; FDR *P*-value = 0.20), *Aureobasidium*
271 (vineyards = 6.9%; forest = 14.3%; FDR *P*-value = 0.0498), *Endoconidioma* (vineyards = 2.6%;
272 forest = 8.5%; FDR *P*-value < 0.0001), *Didymella* (vineyards = 6.0%; forest = 1.6%; FDR *P*-
273 value < 0.0001), *Erysiphie* (vineyards = 5.6%; forest = 0.03%; FDR *P*-value = 0.025). In total,
274 we found that the abundances of 60 genera were significantly different after FDR correction for
275 multiple comparisons. Of these, the relative abundances of 34 genera were significantly higher in

forest samples, and 26 genera were more abundant in vineyard samples (Fig. 4C; STable 6). We also searched for yeasts involved in winemaking (i.e. Saccharomycetales), but the relative abundances of these yeasts were extremely low in all samples (< 0.01%). Yeasts belonging to the genera *Hanseniaspora* and *Saccharomyces* were more abundant in vineyards than in forest samples. Despite this, these differences in abundances were not significant between habitats with the exception of *Metschnikowia* that had a significantly higher relative abundance in the vineyard samples (FDR *P*-value < 0.0001) (STable 6).

Contrary to our expectations, the diversity indices (OTU richness, Shannon diversity, phylogenetic diversity, and Pielou evenness) were not significantly different between the forest and vineyard samples (Table 2: analyses performed for ITS2 and D2). On the other hand, we found that habitats exhibited significantly different community structure regardless of the distance matrix employed: Bray-Curtis matrix (PERMANOVA: $F_{1,34} = 6.94$, $R^2 = 0.17$; $P < 0.0001$) or the weighted-Unifrac matrix (PERMANOVA: $F_{2,34} = 42.49$, $P < 0.0001$). Furthermore, separate clusters were found for the forest and vineyard samples in the NMDS plot (Fig. 5). The forest samples showed higher variability in the multidimensional space than the vineyard samples. This suggests that the high plant diversity of the forest could induce higher variability in the fungal assemblage.

Discussion

Effect of agriculture managements and plant tissues on fungal communities

We found that grape leaves had higher microbial diversity and more unique OTUs than grape berries. On the other hand, and has been shown in other studies, the abundance of *Davidiella* was extremely high in grape berries and grape leaves (Bokulich et al. 2014; Piao et al.

299 2015; Pinto et al. 2015; Taylor et al. 2014) It is possible that sugar compounds produced by
300 grape berries can constrain the number of fungal species capable of inhabiting this type of habitat
301 thus explaining the lower diversity found here. Indeed, we collected mature grape berries, which
302 have higher sugar concentrations than growing grape berries (Mane et al. 2017).

303 While conventional agriculture is a major contributor to greenhouse gas emissions,
304 biodiversity loss, agrochemical pollution, and soil degradation, organic farming systems are
305 considered as more sustainable agricultural practices (Reganold et al. 2016). Moreover, several
306 studies have shown that insecticides and fungicides have negative effects on biodiversity, and
307 insecticides also reduce the efficacy of biological control in farming systems (Gabriel et al. 2010;
308 Geiger et al. 2010; Bevivino et al. 2014). In spite of this, here microbial diversity did not differ
309 between different management practices, regardless of if diversity was estimated using ITS2 or
310 D2. This being said, we found significant effects of agriculture management on fungal
311 community structure as determined with ITS2. However, analysis using D2 showed that the
312 agriculture management did not have an effect on fungal community structure. Given that D2
313 sequences provide the phylogenetic relationships within the fungal communities, we are more
314 confident in supporting the lack of an effect of agriculture management on fungal communities.
315 This finding agrees with previous studies which have shown that fungal communities present in
316 grapevines are not affected by biocontrol agents or synthetic fungicides (Kecskeméti et al. 2016;
317 Perazzolli et al. 2014). Therefore, previous findings and our results suggest that microorganisms
318 of grapevine phyllosphere are highly resilient to agricultural treatments.

319 In terms of taxa abundance, we found that only the genus *Aureobasidium*, family
320 Dothioraceae, exhibited higher abundance in organic vineyards compared to conventional
321 vineyards ($P = 0.057$). Interestingly, the abundance of *Aureobasidium* was also high in the forest

samples. Previous studies have also found *Aureobasidium* enriched in organically managed grapevines (Grube et al. 2011; Martins et al. 2014). This increased abundance of *Aureobasidium* in organic vineyards compared to that found in conventional vineyards could be explained because this genus is capable of tolerating copper and sulphur pesticides, which are permitted in organic management (Gadd & de Rome 1988; Killham et al. 1981). On the other hand, the application of Kresoxim-methyl in conventional vineyards has been shown to reduce *Aureobasidium* abundance in Japanese pear leaves (Chung et al. 2013).

Fungal communities in native forests and vineyards

We found a high proportion of shared fungal OTUs between vineyards and native forests (79.7%), indicating that most of the taxonomic units were present in both habitats and supporting the idea that "everything is everywhere, but environment selects" (Baas Becking 1934). Additionally, the diversity indices were similar between forests and vineyards. Despite this, community structure was different between habitats which may have been driven by differences in the composition and relative abundances of OTUs. Indeed, the most abundant OTUs in the vineyard samples included the genera *Davidiella*, *Didymella* and *Erysiphie*. In contrast, *Aureobasidium* and *Endoconidioma* were the most abundant genera in the forest samples. *Davidiella* and *Aureobasidium* have been previously reported as highly abundant in vineyards (Bokulich et al. 2014; Setati et al. 2015), suggesting that these taxa represent the core microbiome of vineyards regardless of grape variety (Cabernet, Carménère, Chardonnay, Merlot, Zinfandel) and geographic region (Chile, Italy, South Africa, United States) (Bokulich et al. 2014; Pancher et al. 2012; Setati et al. 2015).

In both forests and vineyards, we also found some OTUs identified as wine fermenting yeasts (e.g. *Hanseniaspora*, *Metschnikowia*, *Saccharomyces*), yet the relative abundance of these yeasts was very low in both habitats. This finding is consistent with previous studies showing that *S. cerevisiae* is rarely found on healthy grape berries (Barata et al. 2012) but is highly abundant in grape musts when alcoholic fermentation has begun (Bagheri et al. 2015). However, it has been reported that native forests near vineyards are sources of fermenting yeasts. Specifically, fermenting yeasts have been found on *Nothofagus* (Southern beeches) in North Patagonia (Libkind et al. 2011) and New Zealand (Serjeant et al. 2008) and also on *Quercus* (oaks) in Portugal (Sampaio and Gonçalves 2008). Furthermore, other study has also shown that the dispersal of foliar fungi is not locally limited; specifically, the composition of airborne fungal communities does not differ between vineyard and forest patches (Fort et al. 2016). From this it can be seen that forest-derived microorganisms can influence vineyard microbiota and vice versa. In a previous study, we have shown that the greater the geographic distance between vineyards, the more different is the fungal community structure associated with grape berries (Miura et al. 2017). This finding implies that local- or landscape-scale factors such as geographical barriers and variation in ecological niches could affect the spatial patterns of the microbial communities of the grapevine phyllosphere. This being said, further studies are needed to quantify the relative contribution of native forests to vineyard microbiota.

Regarding microbial diversity, host taxonomic identity has been shown to be an important driver of phyllosphere microbial community composition (Kembel & Mueller 2014; Whipps et al. 2008) Thus, different plant communities should have different abundances of microbes and their microbial communities should differ. Despite this, we found that both habitats had similar levels of alpha diversity despite that forest samples were collected from multiple plant species. This is

inconsistent with our hypothesis and with the results of another study showing higher fungal OTU richness on forest leaves compared to grape leaves (Fort et al. 2016). Thus, here was have found that both forests and vineyards contain almost the same species (80% of OTUs were shared between habitats). A possible explanation for this could be due to the unique characteristics of sclerophyllous trees which have leaves with thick and hard cuticles that can act as a barrier for invasive microorganisms (Bringel & Couée 2015; Yeats & Rose 2013). Also, these species produce aromatic volatile compounds with antimicrobial effects (Velásquez & Montenegro 2017). Nevertheless, the phyllosphere community structure was significantly different between vineyards and native forests. These differences were mainly due to differences in the abundance of some OTUs. For example, the relative abundance of *Erysiphe*, a genus which contains many plant pathogen species that cause powdery mildew (Kassemeyer & Berkelmann-Löhnertz 2009), was higher in vineyard compared to in forest samples. In contrast, OTUs identified as *Aureobasidium* were more abundant in forest than in vineyard samples. This genus has been shown to be capable of copper-detoxification (Gadd & de Rome 1988) and is also a biocontrol agent for grapevine diseases (Compant & Mathieu 2016).

Conclusion

Interestingly, even though the forest samples consisted of multiple plant species, the fungal alpha-diversity of these native sclerophyllous tree leaves was similar to that of grape leaves. Nonetheless, beta-diversity differed greatly between native forests and vineyards. We argue that the characteristics of sclerophyllous tree leaves might pose strong selective pressure on fungal assemblages, and this could be an important factor influencing the landscape-specific diversity of phyllosphere fungal communities in Chilean Mediterranean ecosystems.

Controversially, our results also demonstrate that management practices do not affect the composition and structure of the fungal communities associated with grapevines. This can be explained by the high resilience of fungi to chemical treatments employed by conventional vineyards, but also by the buffer effect provided by surrounding native forest. Therefore, native forests could greatly contribution to vineyard phyllosphere microbial communities and are clearly worthy of further investigation to determine the extent to which these natural ecosystems act as reservoirs of microbial diversity. Overall, this study argues that a better understanding of the relationship between native forests and agroecosystems is needed for maintaining and enhancing ecosystem services provided by natural ecosystems. Specifically, knowledge about fungal diversity can be used to identify fermenting yeasts valuable for the wine industry, and further this information can be used to better evaluate the ecosystem services provided this native ecosystem.

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414

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418

419 **Competing Interests**

420 The authors declare that they have no competing interests.

421

422 **Author Contribution**

423 Luis E. Castañeda conceived and designed the experiments, analyzed the data, wrote the paper,
424 prepared the figures and tables, and reviewed drafts of the paper.
425 Toshiko Miura wrote the manuscript and reviewed drafts of the paper.
426 Roland Sánchez conceived and designed the experiments, analyzed the data, performed the
427 experiments, and reviewed drafts of the paper.
428 Olga Barbosa conceived and designed the experiments, contributed reagents/materials/analysis
429 tools, and reviewed drafts of the paper.

430

431 **Field Study Permissions**

432 The owners of vineyards, whose properties include native forest patches, granted all necessary
433 permits to access to the sampling sites in Colchagua Valley: Apaltagua, Emiliana, Lapostolle,
434 Montes, Veramonte and Viu Manent vineyards.

435

436 **Data availability**

437 FASTA, BIOM, metadata and OUT table files are deposited at Figshare

438 https://figshare.com/s/68dc39d98231cecd1a2b

439

440 **References**

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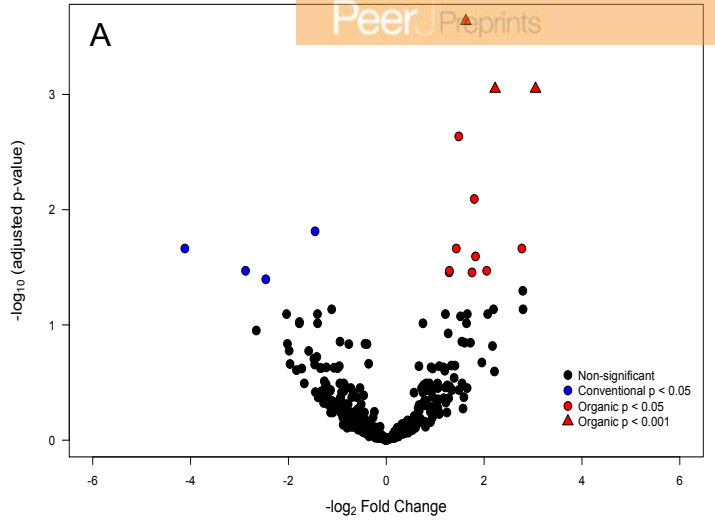
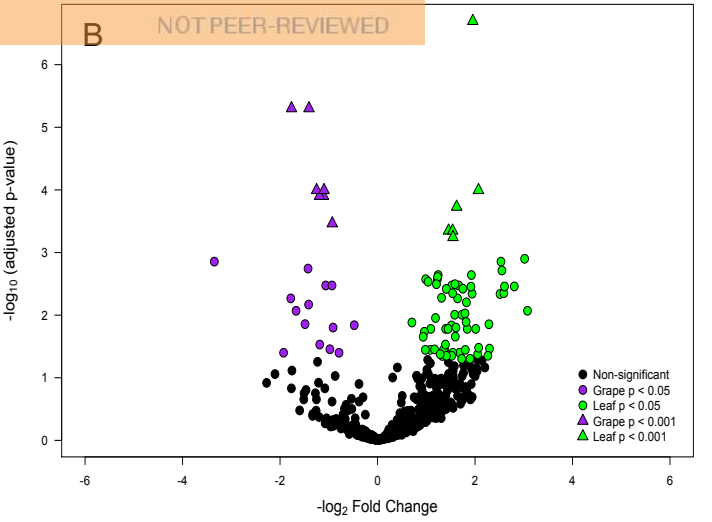
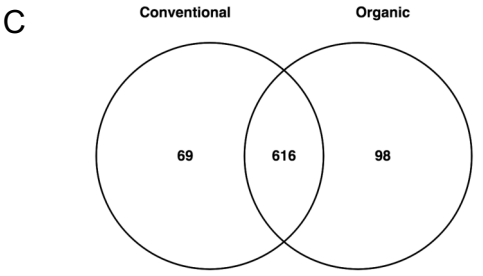
Figure 1(on next page)

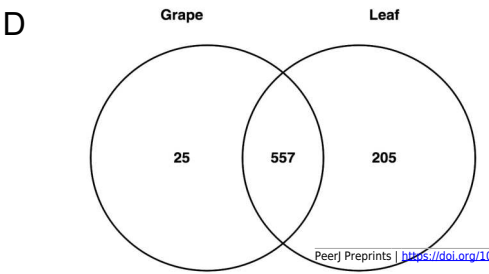
Fungal operational taxonomic units (OTUs) associated with agriculture management and plant tissue found in Carmenere vineyards.

(A) Volcano plot showing OTUs that were significantly more abundant in conventional vineyards (blue circles: $P < 0.05$) and OTUs that were significantly more abundant in organic vineyards (red circles: $P < 0.05$, red triangles: $P < 0.001$). Black circles represent OTUs that did not significantly differ between agriculture management types. Each point represents an individual OTU, the x-axis indicates the fold change of abundance, and the y-axis indicates the adjusted *P*-values (i.e. after FDR correction). (B) Volcano plot showing OTUs that were significantly more abundant in grape berries (purple circles: $P < 0.05$, purple triangles: $P < 0.001$) and OTUs that were significantly more abundant in grape leaves (green circles: $P < 0.05$, green triangles: $P < 0.001$). Black circles represent OTUs that did not significantly differ between agriculture management types. Each point represents an individual OTU, the x-axis indicates the fold change of abundance, and the y-axis indicates the adjusted *P*-values (i.e. after FDR correction). (C) Venn diagram showing OTUs exclusive to conventional and organic vineyards and OTUs shared between agriculture management types. (D) Venn diagram showing OTUs exclusive to grape berries and grape leaves and OTUs shared between plant tissues. (E) Venn diagram showing exclusive and shared OTUs for grape berries (Org-grape) and grape leaves (Org-leaf) from organic vineyards, and for grape berries (Conv-grape) and grape leaves (Conv-leaf) from conventional vineyards.









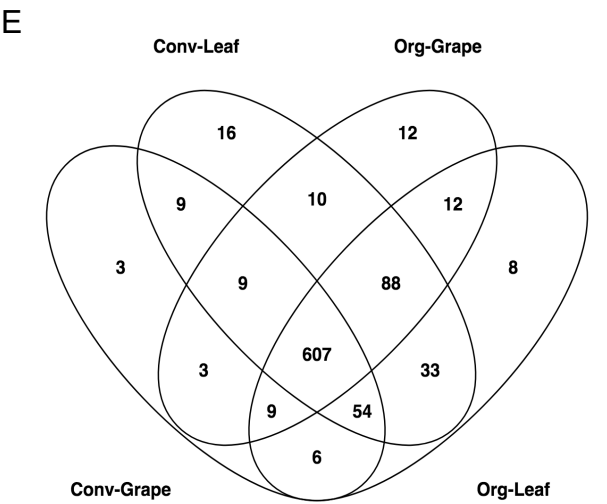


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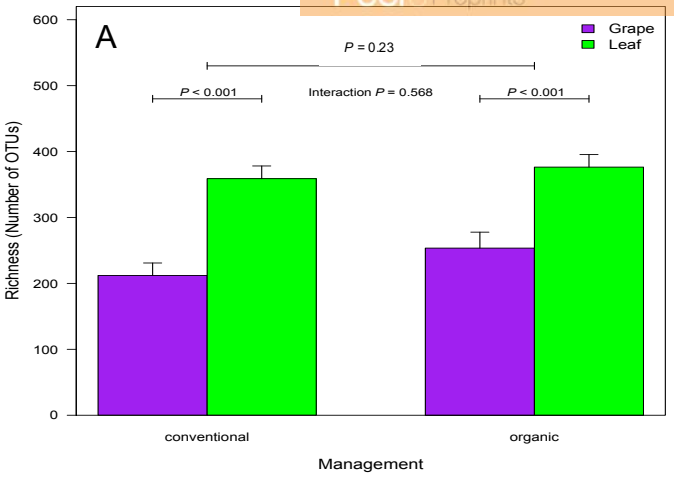
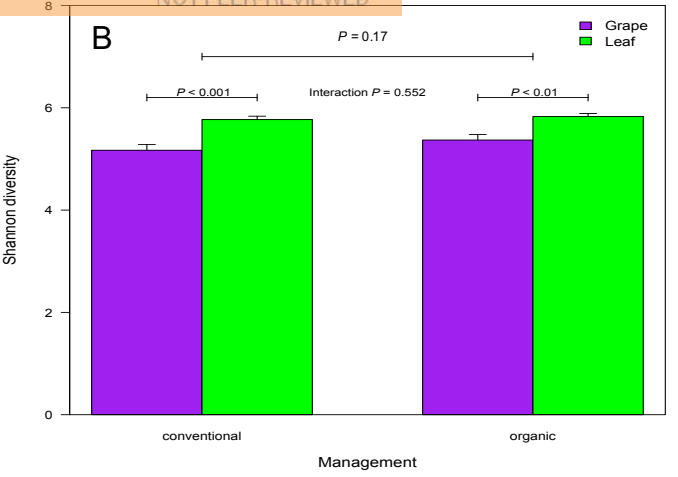
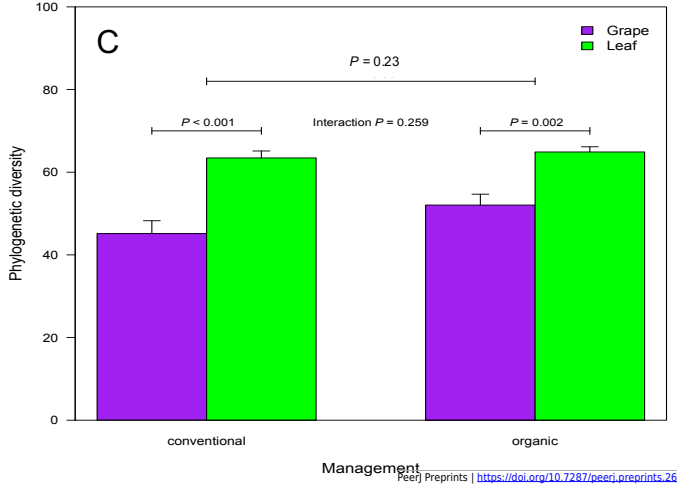
Figure 2(on next page)

Fungal diversity indices associated with agriculture management and plant tissue found in Carmenere vineyards

(A) Richness (number of OTUs), (B) Shannon diversity, (C) Phylogenetic diversity, and (D) Pielou evenness estimated for grape berries and grape leaves from organic vineyards and for grape berries and grape leaves from conventional vineyards. *P*-values were estimated from two-way ANOVAs using agriculture management and plant tissue as main effects, and from the interaction between these effects.







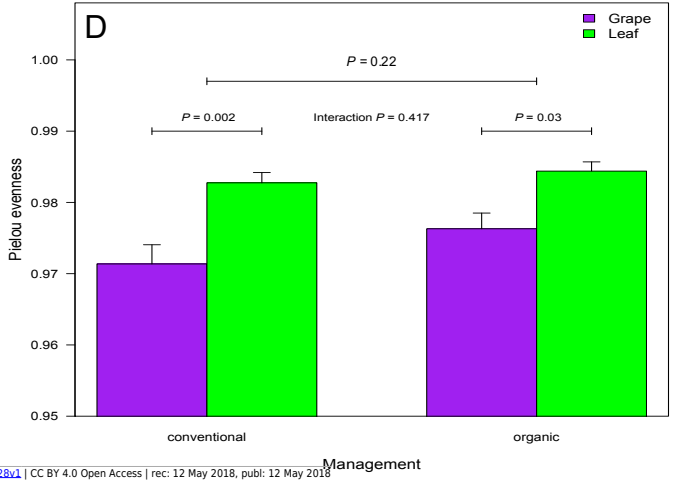


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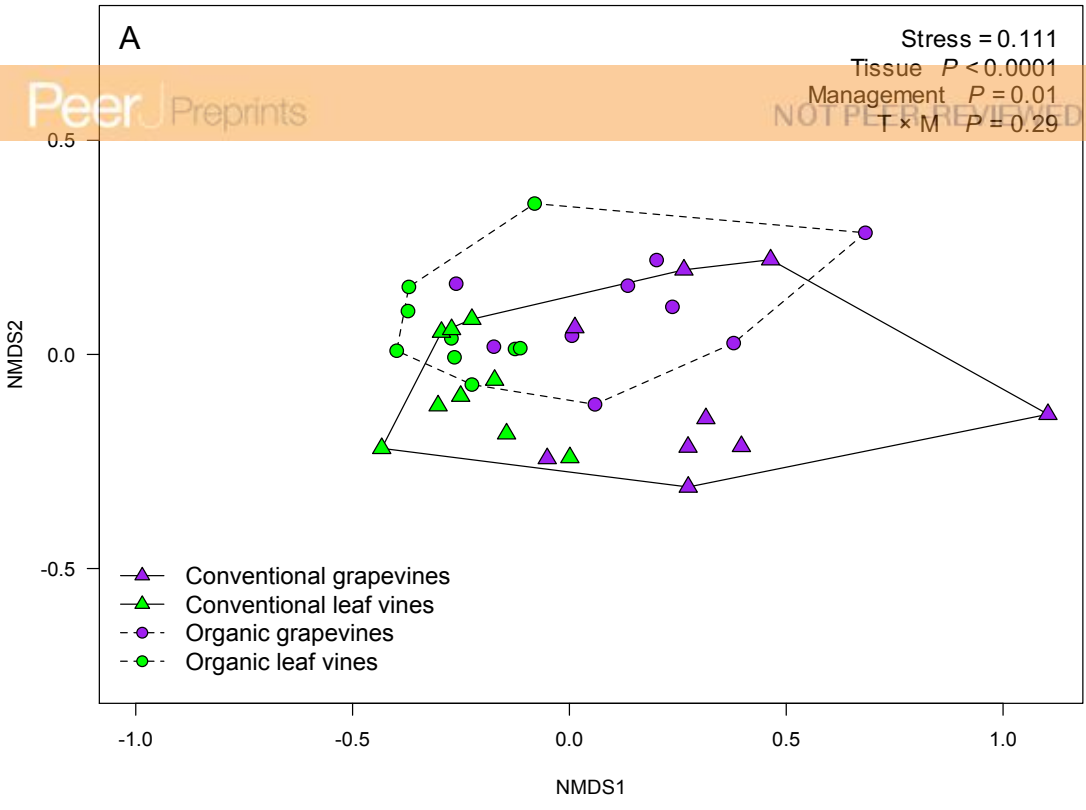
Figure 3(on next page)

Fungal community structure associated with agriculture management and plant tissue found in Carmenere vineyards

Non-metric multidimentional scaling (NMDS) plot of fungal communities sampled from grape berries and grape leaves of organic vineyards, and from grape berries and grape leaves of conventional vineyards. (A) ITS2 and (B) D2 amplicon sequencing was used to characterized communities. *P*-values indicate significant differences (or not) between plant tissues, agriculture management types, and the interaction between these main effects.



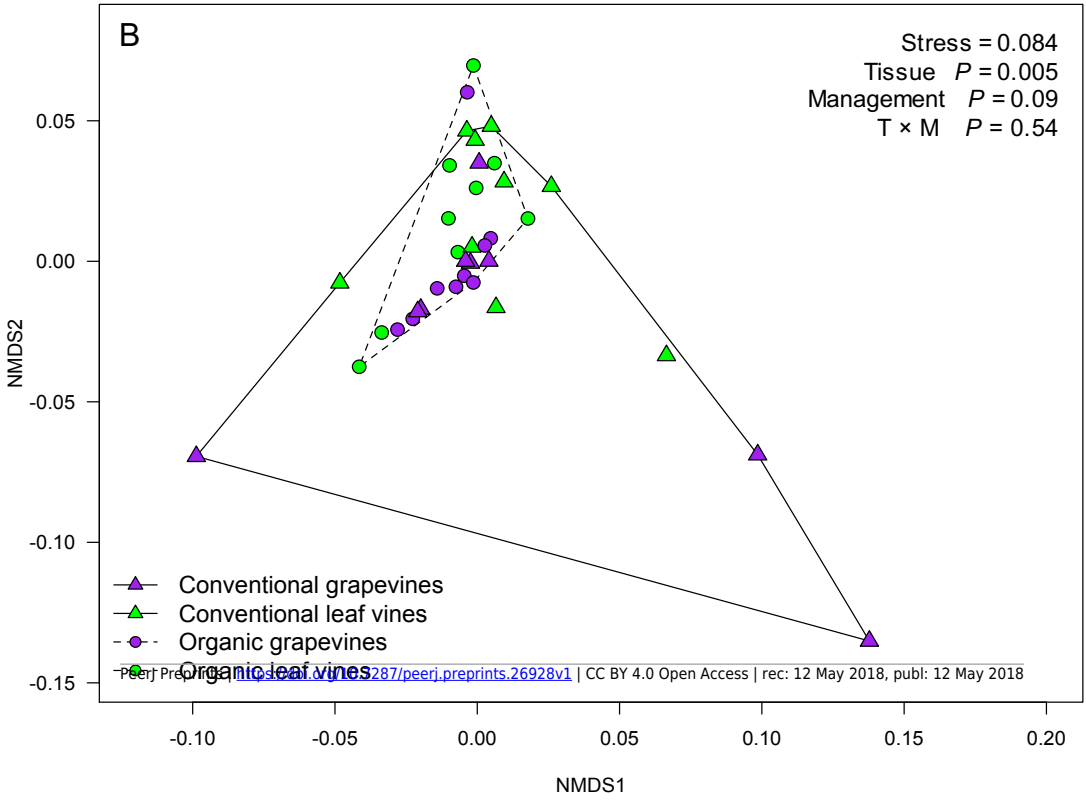


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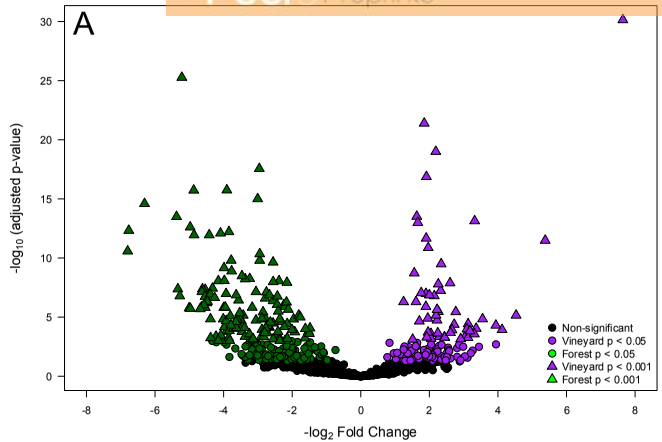
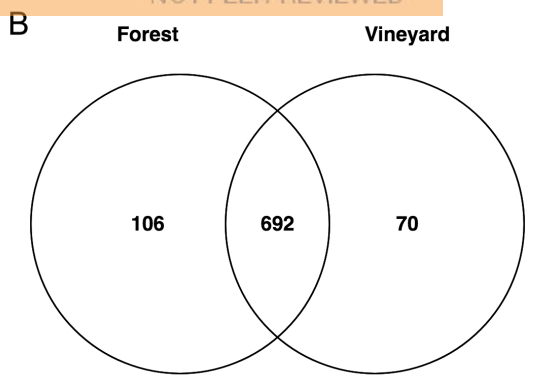
Figure 4(on next page)

Fungal operational taxonomic units (OTUs) associated found in Carmenere vineyard and native forests

(A) Volcano plot showing OTUs that were significantly more abundant in leaves sampled from native trees (dark green circles: $P < 0.05$, dark green triangles: $P < 0.001$) and OTUs that were significantly more abundant in leaves sampled from grapevines (dark purple circles: $P < 0.05$, dark purple triangles: $P < 0.001$). Black circles represent OTUs that were non-significantly more abundant in either habitat. Each point represents an individual OTU, the x-axis indicates the fold change of abundance, and the y-axis indicates the adjusted *P*-values (i.e. after FDR correction). (B) Venn diagram showing exclusive OTUs for forests, vineyards, and OTUs shared between habitats. (C) Relative abundances of fungal genera found in forest and vineyard samples. This plot excludes OTUs with abundances less than 0.01%.





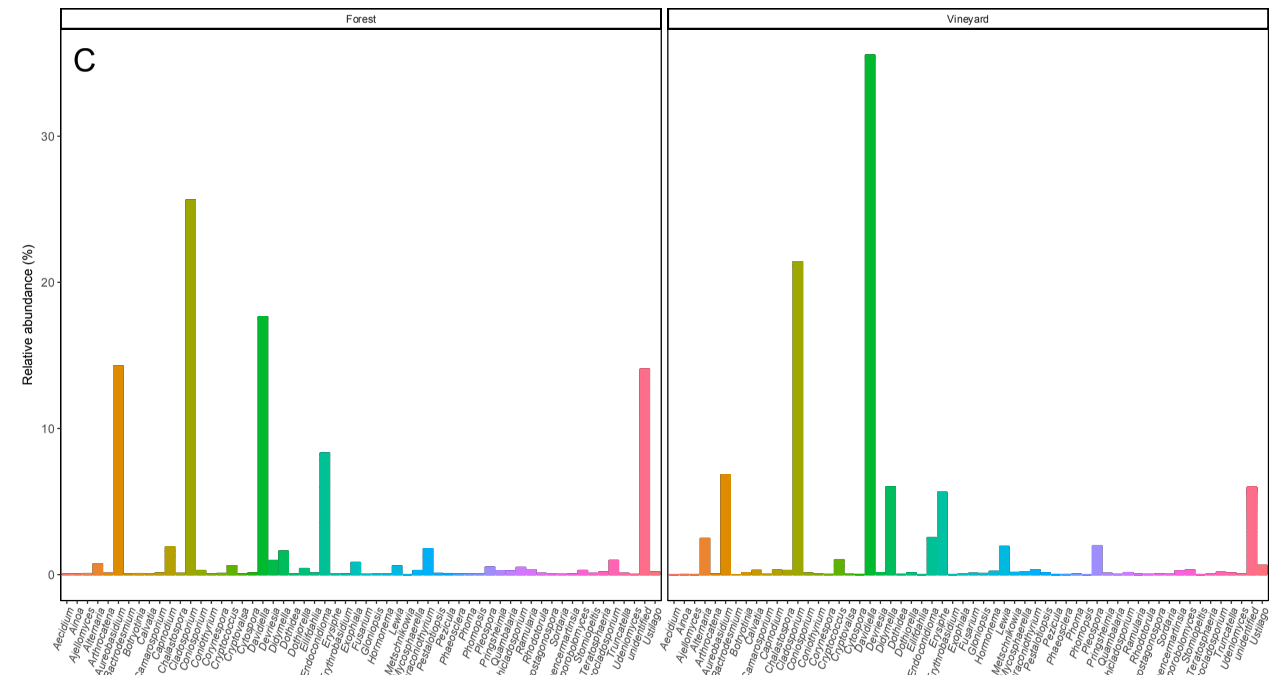


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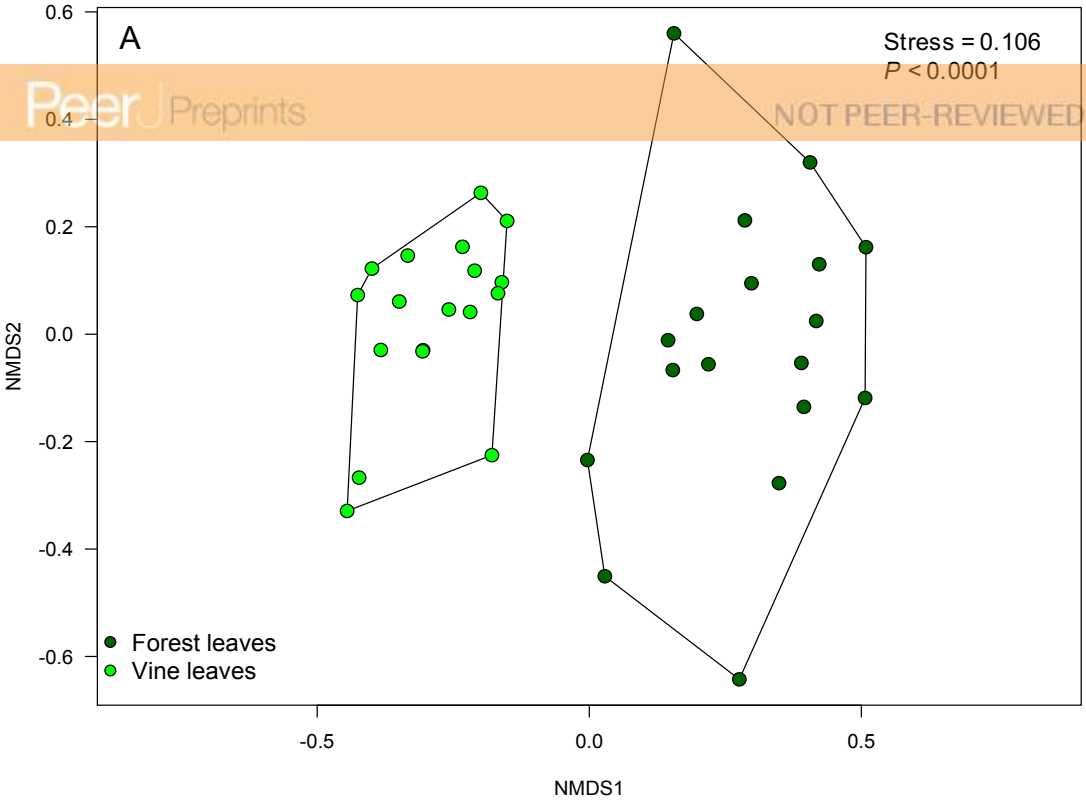
Figure 5(on next page)

Fungal community structure associated found in Carmenere vineyard and native forests

Non-metric multidimentional scaling (NMDS) plot of fungal communities inhabiting forests and vineyards. Community structure was assessed using (A) ITS2 and (B) D2 amplicons. *P*-values indicated significant differences between the structure of the fungal communities inhabiting grape leaves and native tree leaves.



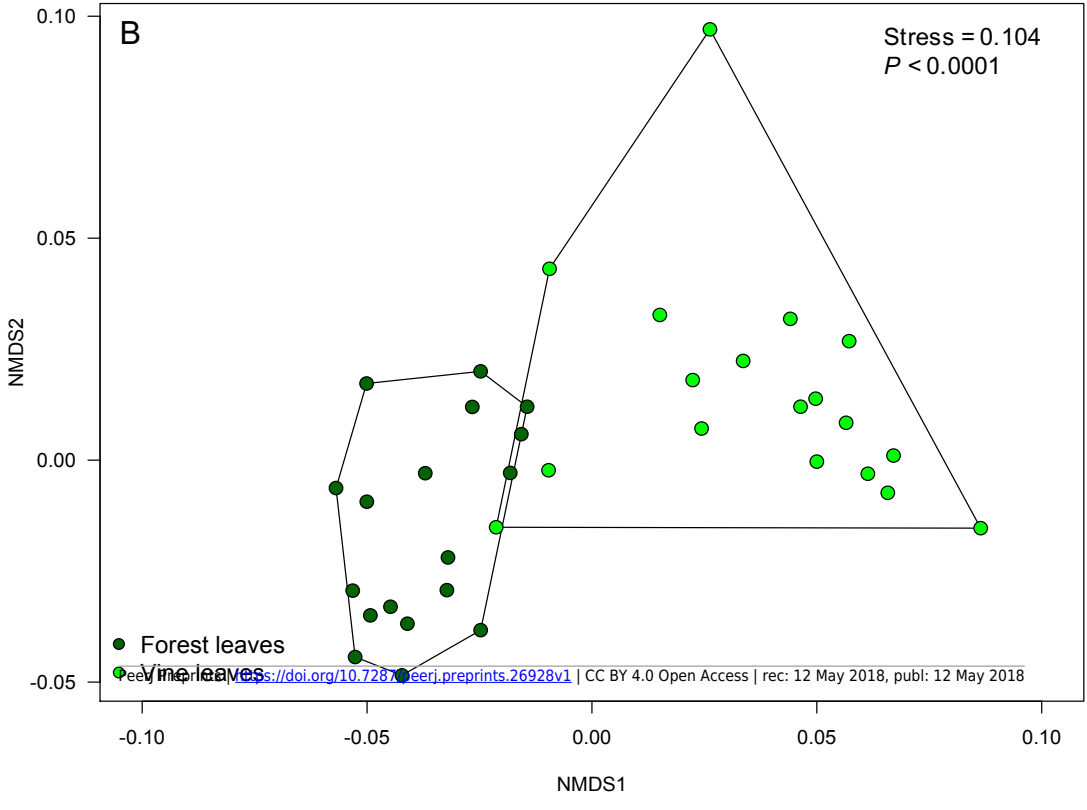


Table 1(on next page)

Fungal diversity indices associated with agriculture management and plant tissue found in Carmenere vineyards

Diversity indices (mean ± standard error) for fungal communities sampled from grape berries and grape leaves collected in conventional and organic vineyards. The indices, with the exception of phylogenetic diversity, were estimated using ITS2 and D2 amplicons. The last three columns show the F-values and their significances for the effects of plant tissue (grape berries versus grape leaves), agriculture management (conventional versus organic management), and the interaction between these effects. NS means $P > 0.05$, * means $P < 0.05$, and *** means $P < 0.001$.

Diversity index	Amplicon	Conventional grape berry	Conventional grape leaf	Organic grape berry	Organic grape leaf	Tissue $F_{1,32}$	Management $F_{1,4}$	Interaction $F_{1,32}$
OTU richness	ITS2	212.1 ± 18.9	358.9 ± 19.3	253.6 ± 24.2	376.4 ± 19.0	43.5^{***}	2.08^{NS}	0.34^{NS}
	D2	168 ± 8.1	276 ± 14.8	189.6 ± 15.5	289.2 ± 13.0	55.4^{***}	1.52^{NS}	0.09^{NS}
Shannon diversity	ITS2	5.17 ± 0.11	5.77 ± 0.07	5.37 ± 0.11	5.83 ± 0.06	35.3^{***}	2.11^{NS}	0.63^{NS}
	D2	4.98 ± 0.05	5.51 ± 0.06	5.09 ± 5.57	5.57 ± 0.05	55.1^{***}	1.54^{NS}	0.17^{NS}
Phylogenetic diversity	D2	48.1 ± 2.8	63.5 ± 1.7	52.1 ± 2.6	64.9 ± 1.3	42.9^{***}	1.50^{NS}	0.34^{NS}
Pielou evenness	ITS2	0.971 ± 0.003	0.983 ± 0.001	0.976 ± 0.002	0.984 ± 0.001	23.9^{***}	2.73^{NS}	0.68^{NS}
	D2	0.969 ± 0.003	0.982 ± 0.001	0.975 ± 0.002	0.984 ± 0.001	31.4^{***}	1.55^{NS}	0.20^{NS}

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Table 2 (on next page)

Fungal diversity indices associated found in Carmenere vineyard and native forests

Diversity indices (mean ± standard error) for fungal communities sampled from forest and grape leaves. The indices, with the exception of phylogenetic diversity, were estimated using ITS2 and D2 amplicons. The last column shows the F-values and their significances for the effect of habitat (native forest versus vineyards). NS means $P > 0.05$.

Diversity index	Amplicon	Forest leaves	Grape leaves	$F_{1,34}$
OTU richness	ITS2	379.8 ± 13.8	367.7 ± 13.3	0.40NS
	D2	303.1 ± 11.6	282.6 ± 9.7	0.18NS
Shannon diversity	ITS2	5.77 ± 0.04	5.74 ± 0.04	0.34NS
	D2	5.55 ± 0.04	5.47 ± 0.04	0.20NS
Phylogenetic diversity	D2	66.5 ± 1.05	64.2 ± 1.03	0.12NS
Pielou evenness	ITS2	0.974 ± 0.001	0.974 ± 0.001	0.15NS
	D2	0.973 ± 0.001	0.971 ± 0.001	0.12NS