Do we need to consider grape phyllosphere microbiome in breeding schemes ?

Patrice This, Prashant Singh, Sylvain Santoni, Jean-Pierre Péros

Patrice This, Prashant Singh, Sylvain Santoni, Jean-Pierre Péros

Do we need to consider grape phyllosphere microbiome in breeding schemes?

Affiliation
UMR AGAP Institut, Univ Montpellier, CIRAD, INRAE, Institut Agro, F-34398 Montpellier, France

Correspondence
Patrice This*: patrice.this@inrae.fr, Prashant Singh: singh.prashant.iitkgp@gmail.com, Sylvain Santoni: sylvain.santoni@ inrae.fr, Jean-Pierre Péros: jean-pierre.peros@ inrae.fr

Summary

The aerial surface of the plant (phyllosphere) is the habitat of complex microbial communities. These communities may have profound effects on host plant health and its performance traits.

When breeding new cultivars, *i.e*. the aerial component of a grape plant, one can simply ignore the phyllosphere in breeding schemes if its composition is mainly dependent on the environment. It is considered an important component if the genotype is the main driver of the phyllosphere composition. In order to answer this question, we have analysed several factors influencing the structure of the phyllosphere microbial community. Using amplicon sequencing of the 16S rRNA gene and of the internal transcribed spacer (ITS), we explored the microbial diversity at genus level for both bacteria and fungi present in the phyllosphere of leaves and grape berries. We analysed it on different grape taxonomic level (between five *Vitis* species or a set of *Vitis vinifera* cultivars chosen to represent the three genetic pools of the species), for different years and on five commercially important varieties of *Vitis vinifera* that were sampled from three different French terroirs. Our results indicated the presence of complex microbial diversity and assemblages in the phyllosphere. A significant effect of several factors (organ, grape species, growing year and terroir) on taxa abundance was observed with varying degrees of effect. At a given location, genotypes have an impact on microbial assemblage in the phyllosphere of leaf and berries, most pronounced on fruits but the effect of terroir was much stronger than the cultivar identity when the leaf phyllosphere of five grapevine varieties grown in different agro-climatic zones was compared. Limitations of the study as well as implied consequences of this work will be discussed.

Keywords

Biotic interactions, Phyllosphere microbiome, amplicon sequencing, extended ideotype

Introduction

Microbiome is the collective name for the ecological communities that live on, in or near an organism, including pathogenic, commensal and symbiotic partners. The 'Plant Microbiome' for its part, consists of microbial communities in three compartments: the rhizosphere, corresponding to the zone of soil immediately surrounding the roots, the endosphere composed of the micro-organisms present inside the plant tissues and the phyllosphere corresponding to the aerial surface of a plant (stem, leaf, flower, fruit), and by extension the microorganisms present on the surface of the plant.

The plant microbiome, which mostly consists of bacteria and fungi, are involved in major functions such as nitrogen fixation (Jones 1970), carbon sequestration (Bringel and Couée, 2015), degradation of pesticides and organic pollutants (Brandl *et al.*, 2001; Bulgarelli *et al.*, 2013) and plant resistance to biotic and abiotic stresses (Vandenkoornhuyse *et al.*, 2015), but also diseases.

In grape, the microbiome on berries may also have an impact on wine fermentation (Bokulich *et al.*, 2013). It could provide solutions in the future, in order to maintain grapevine cultivation in a context of climate change (Gambetta *et al.*, 2020) and pesticide-free agriculture (Jacquet *et al.*, 2022; Pertot *et al.*, 2017). The grape microbiome evolves during the lifetime of the vine, from the grafting stage in the nursery through to decline in the vineyard, and also on a larger scale, through the domestication (Fournier *et al.*, 2022).

The phyllosphere is rather less extensively studied as compared to the rhizosphere and endosphere (Vorholt, 2012). Due to the limited nutrient availability and because the phyllosphere is directly in contact with the atmosphere and fluctuating climatic conditions, it is a dynamic and stressful habitat for its microbial colonizers.

Until now, the microbiome has not been considered as an important component in breeding schemes. We could in particular take into account the capabilities of the genotype to

Submitted/accepted for publication: April 3, 2023/June 12, 2023

recruit specific microbial communities as an important trait to improve. But does the grape genotype have an important role as driver of microbiome composition?

Several work in grape have suggested that microbiome differs from one organ to the other (Zarraonaindia *et al.*, 2015), that the environmental conditions at different geographic locations (Bokulich *et al.*, 2013; Gao *et al.* 2019), the growing seasons (Guzzon *et al.*, 2021; Marzano *et al.*, 2016) and the soil composition (Zarraonaindia *et al.*, 2015) are important drivers of the microbiome. Similarly, farming system (Castrillo *et al.*, 2019; Vega-Avila *et al.*, 2015) and more specifically copper treatments (Martins *et al.*, 2012), watering and tillage (Vink *et al.* 2021), the age of the plant or the organ (Berlanas *et al.*, 2019; Ji *et al.*, 2019; Martins *et al.*, 2012) and post-harvest treatments (Salvetti *et al.*, 2016) influence microbiome composition. In a given location, several studies have also identified an effect of the varieties (Awad *et al.*, 2020; Bao *et al.* 2022; Marasco *et al.*, 2018; Zhang *et al.*, 2019; Zhang *et al.*, 2020) but on different components of the microbiome. Analyses considering different levels of variation and enabling comparison of different drivers, on the same microbiome components were however sorely needed.

We thus analysed grape phyllosphere, both bacterial and fungal communities, through a metabarcoding approach, taking into account several levels of variation: the genetic identity of the scion (analysing diversity at different botanical levels), the organ (analysing both leaf and berry phyllosphere) and the environment (analysing phyllosphere during 2 years, at spring and harvest and for five cultivars analysing leaves harvested in 3 agro-climatic zones in France).

Material and Methods

Samples

Twenty fully developed asymptomatic leaves from *Vitis vinifera, V.riparia, V. pentagona, Muscadinia rotundifolia and Parthenocissus* were collected from the repository of Institut Agro Montpellier in southern France (Mediterranean) in the Spring season (mid of May 2017 and 2018, before fungicide spraying) by Singh *et al.* (2019). These species represent quite well separated branches on a phylogenetic tree (Fig. 1).

Similarly, leaf samples from five commercially important varieties (Cabernet-Sauvignon, Chardonnay, Syrah, Grenache, Sauvignon blanc) were taken from three different French geographic locations, (INRA field stations from Bordeaux, Montpellier, and Colmar) representing the three agro-climate zones in the mid of spring season (before spraying of fungicides) by Singh *et al.* (2018b).

Finally, five cultivars from each of the three main genetic pools, which were selected (Table 1) to maximize the distance between genetic pools were analysed (Singh *et al.* 2018b). Leaf samples were taken at Vassal INRAE Experimental Unit (Marseillan-Plage, France near Montpellier) from four to five plants of each cultivar in the spring season (mid of May 2017, before spraying of fungicides) and harvesting season (September 2017). Berries were also collected from eleven of these varieties during the harvest season.

Processing of the samples and data analysis

Microbial communities from the surfaces of the organs were washed and DNA was extracted according to the procedures of Singh *et al.* (2018b).

Unrooted tree of 15 species of vitacea based on sequenes at 229 genes (Wen et al, 2013)

Fig. 1: Phylogenetic unrooted tree of 15 species of *Vitaceae* based on sequences at 229 genes (Wen *et al.*, 2013) and position of the species sampled in the study (photo credit: INRAE domain of Vassal)

Table 1: Identity of the 15 grapevine cultivars sampled grouped in the three genetic pools

	Genetic Pool		
	WW	**WE**	**TE**
Cultivars of Vitis *vinifera*	Donzelinho	Basicata	Ichkimar
	Petit Verdot	Alba Imputotato	Khoussaïné blanc
	Camaraou Noir	Gros Bourgogne	Sourkhak Biley
	Courbu	Koilliniatico	Abouhu
	Savagnin Blanc	Negru Vertos	Dabouki

To access bacterial communities, the V4 region of the 16S ribosomal gene was amplified using primers 515F and 806R (Caporaso *et al.* 2011). Fungal community diversity and abundance were accessed using modified ITS9 and ITS4 primers targeting the ITS2 region (Lundberg *et al.* 2013). PCR products were then analysed by sequencing using 2 × 250 bp MiSeq v2 sequencing (Illumina Inc., San Diego, CA, USA).

Paired-end sequence reads from 16S and ITS sequences were filtered, trimmed and processed with the dada2 v1.8 (R Bioconductor package). A core Divisive Amplicon Denoising Algorithm (DADA) was performed on these filtered files and amplicon sequence variants (or OTUs) were inferred after chimeras removal. Taxonomy was assigned to bacterial and fungal OTU sequences using the RDP classifier and UNITE data base. Alpha and β-diversity estimates were obtained using phyloseq package. PCoA ordination was performed on variance stabilised log-transformed data using the Bray-Curtis dissimilarity matrix and visualised by using their base functions in the phyloseq package. For more details on the data analysis, see Singh *et al.* (2018b and 2019).

Results

Effect of the organs

Comparison of the phyllosphere of leaves and berries (Fig. 2), revealed significantly different Chao1 estimates of α- diversity for both bacterial and fungal communities (ANOVA, for16S-data: Chao1, P = 0.007; for ITS data: Chao1, P = 4.53e-08). The distinction between leave and berry phyllosphere was also clearly observed on the PCoa analysis (Figure 2 C and D): total bacterial and fungal communities for leaves were well separated from those of berries (PERMANOVA; for 16S data: at F = 45.384, R2 = 4.121, P = 0.001; for ITS data: at F = 48.306, R2 = 2.539, P = 0.001)

Effects of year and genetics

In a second experiment, we analysed the phyllosphere of leaves for the five species *Vitis vinifera, V.riparia, V. pentag-*

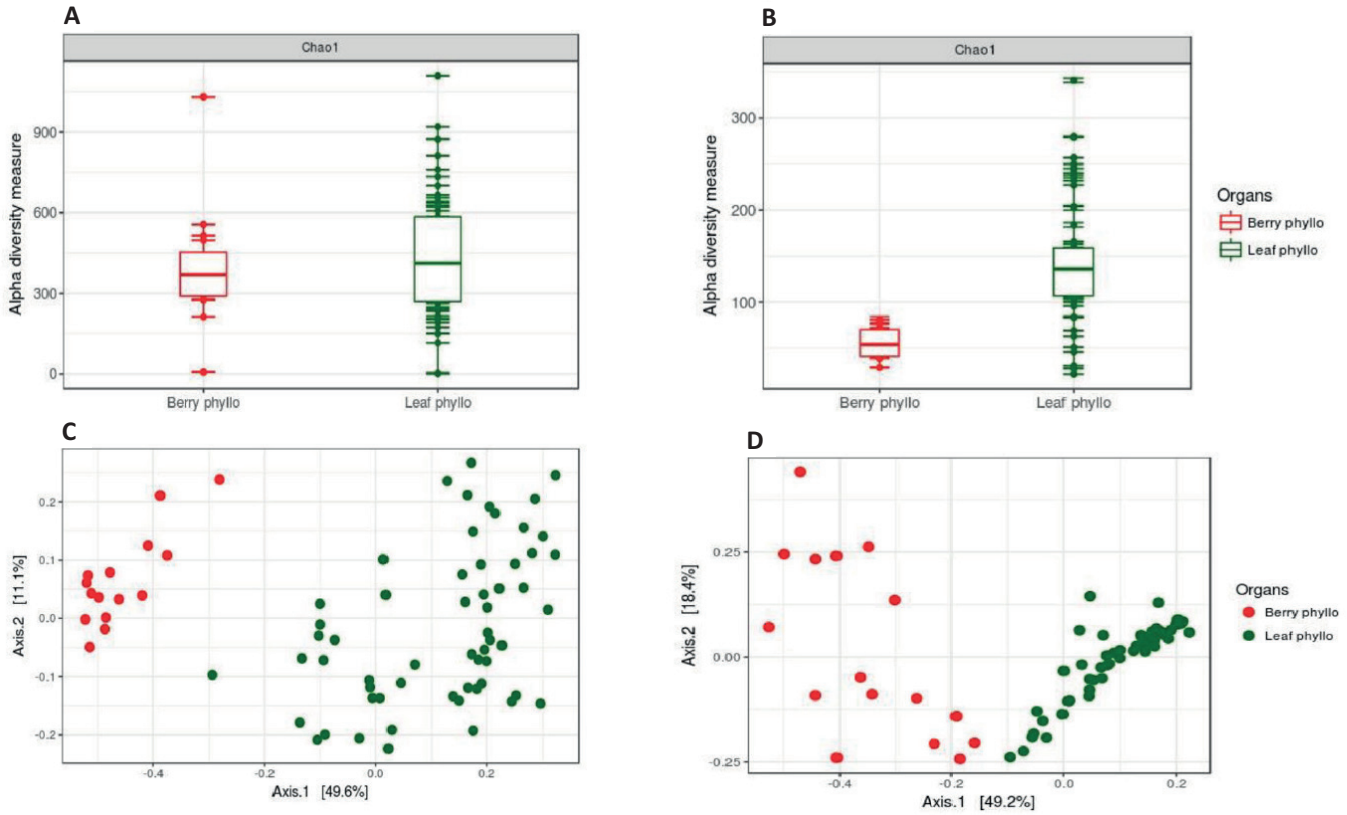

Fig. 2: Chao1 estimates of α- diversity for (A) bacterial and (B) fungal data-sets for both the organ types. PCoA plots using Bray-Curtis distance between samples for (C) bacterial and (D) fungal data-sets as per leaf and berry samples based on Bray-Curtis distance matrices, explaining >60% variations with first two axes (taxa with variance < 1e-05 were trimmed). From Singh *et al.*, 2018b.

ona, Muscadinia rotundifolia and Parthenocissus during two consecutive years (2017 and 2018).

Samples for fungal communities from the five species were clearly differentiated by the PCoA analysis on microbial abundance data according to the year (Fig. 3 B). This differentiation was not as clear for bacterial communities (Fig. 3 A). In a given year, a lower but significant impact of grape species in shaping phyllosphere microbiome, especially the fungal microbiome, could also be observed, even if distance of the PCoA did not coincide with genetic distances.

Effects of environment and genetics

In a third experiment, five commercially important varieties, 'Cabernet-Sauvignon', 'Chardonnay', 'Syrah', 'Grenache', 'Sauvignon blanc', were sampled in three different French agro-climate zones: Oceanic, Continental and Mediterranean.

The analysis of the leaf phyllosphere from each cultivar in the 3 zones were then compared: the PCoA on the Bray-Curtis dissimilarity matrix (Figs. 4 A and B) revealed a clear differentiation of the samples according to the agro-climatic zones rather than the identity of the cultivars (PERMANOVA for 16S data: F = 12.98, p = 0.001; for ITS data: F = 6.094, p = 0.001). The diversity estimates also indicated very significant differences in OTU richness (Figs 4 C and D) between the three regions (ANOVA for 16S data: F = 25.73, p = 3.11 10^{-7}; for ITS data: at F = 26.329, p = 2.5 10^{-7}).

In addition, different relative abundance of few genera were observed among the three agro-climate zones (Figs. 4 E and F).

Conclusion

Many published papers identified the effect of varieties on microbiome composition (Awad *et al.*, 2020; Bao *et al.* 2022; Marasco *et al.*, 2018; Zhang *et al.*, 2019; Zhang *et al.*, 2020). Many papers also detected a greater effect of environment on microbiome than genotype (Bagheri *et al.*, 2019; Belessi *et al.*, 2022; Berlanas *et al.*, 2019; Bokulich *et al.*, 2013; Kioroglou *et al.*, 2019; Mezzasalma *et al.*, 2018; Portillo *et al.*, 2016). These papers however did not necessarily compare the same component of the plant microbiome, nor the same level of genetic diversity. For these reasons, we designed several experiments analysing the phyllosphere using the same metabarcoding methods and comparing different botanical levels.

The experiments described in the presentation clearly demonstrated on one hand that the organ has a strong effect on the microbial communities that inhabit its surface. It is probably due to difference in nutrient availability but also of microclimate around the fruits differing from the harsher climatic condition of leaves.

On the other hand, the comparison of the effect of both year and environment *versus* genetic identity of the scion revealed that the main driver of phyllosphere microbiome composition is the environment. Comparison of *V. vinifera* cultivars in the three different agro-climatic zones suggested that there is not only a difference in taxonomic compositions, but each agro-climate zone has a unique microbial signature. Comparing a larger diversity of *V. vinifera* cultivars, Singh *et al.* (2018a) confirmed the small effect of genotype and found only two bacterial taxons (genus *Gemmatimonas* and *Hymenobacter*), and one fungal taxon (genus *Penicillium*) differentially abundant between *V. vinifera* cultivars from different genetic pools.

At a given year, a lower but significant impact of more distant genetic material could however be observed.

In conclusion, since the grape genotype does not have an important role as driver of microbiome composition, particularly in *V. vinifera*, one can consider that breeding cultivars that may be used in very different environments may not need to take into account the global composition of the phyllosphere in breeding schemes. It does not mean that microbiome may not be important, in particular in order to maintain grapevine

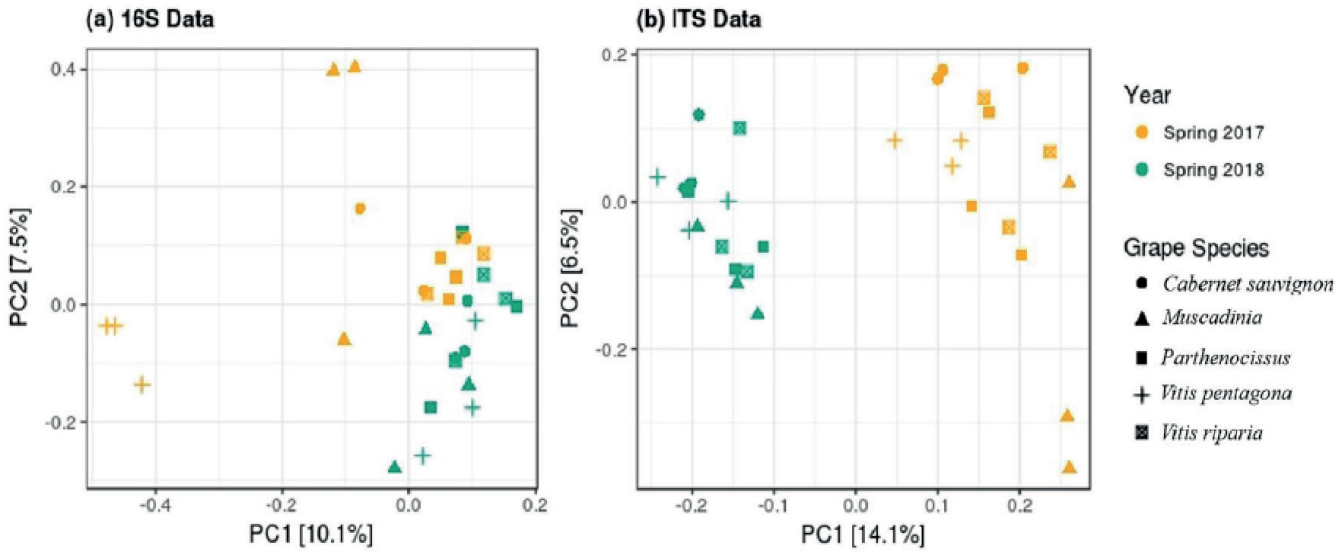

Fig. 3: PCoA ordinations of (a) bacterial and (b) fungal communities derived from leaf phyllosphere of 5 different species at two growing years, using Bray-Curtis distance matrix. Both the axis explains ~20% of variations. The shape represents grape species (N = 30). From Singh *et al.*, 2019.

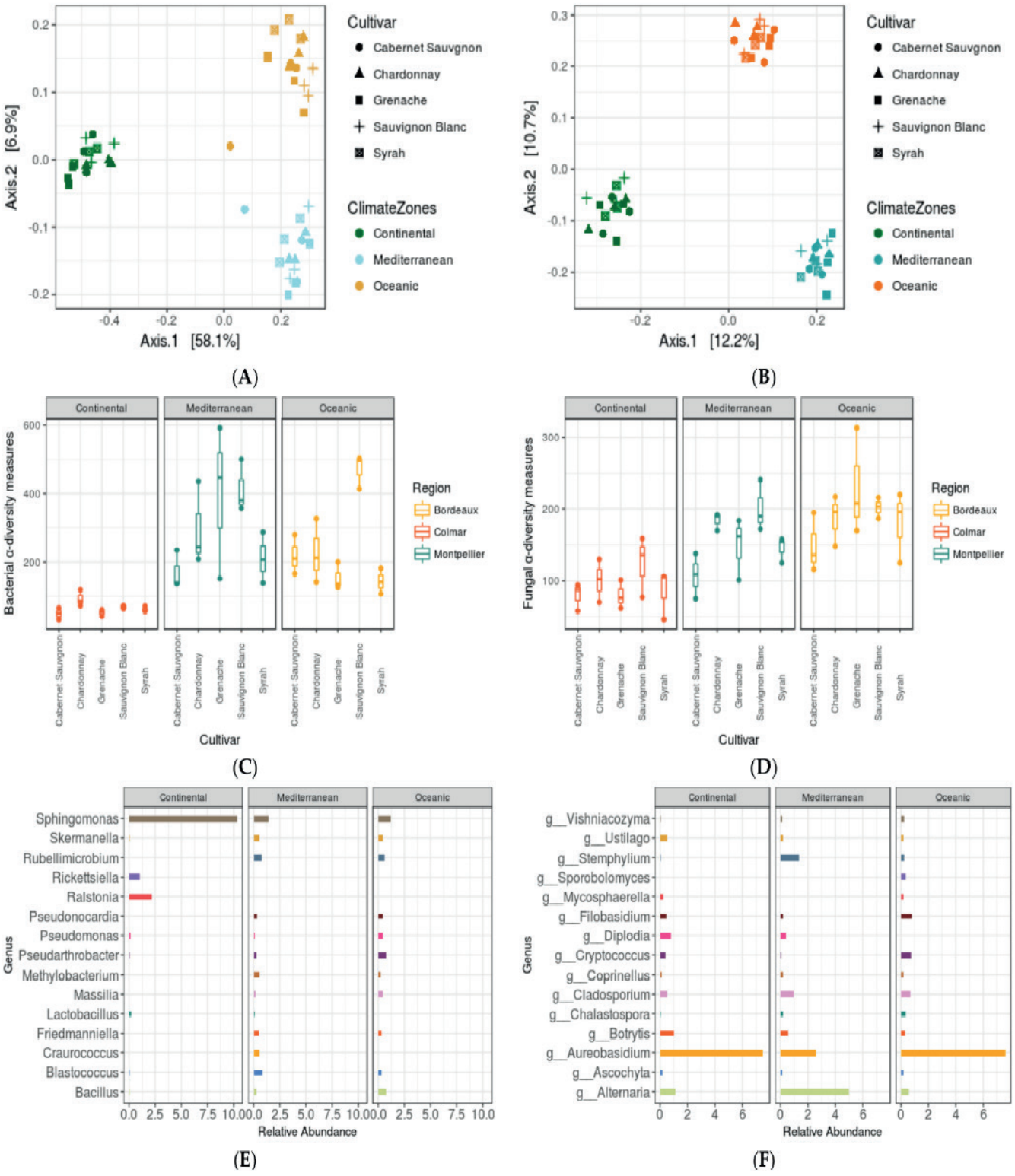

Fig. 4: PCoA plots displaying strong (A) bacterial and (B) fungal compositional dissimilarity among agro-climate zones and observed (C) bacterial and (D) fungal α- diversity measures of each variety (X-axis) grouped in three agro-climate zones and relative abundance plot for (E) bacterial and (F) fungal genera displaying differential abundance of few genera among three agro-climate zones (or region). n = 45. From Singh *et al.*, 2018b.

cultivation in a context of climate change and pesticide-free agriculture, and it would be interesting to investigate more deeply to identify specific taxons differentiating the microbial communities on specific cultivars. Similar analysis should be done for the other components of the plant microbiome, the endosphere and the rhizosphere.

Conflicts of interest

The authors declare that they do not have any conflicts of interest.

References

Awad, M., Giannopoulos, G., Mylona, P., Polidoros, A., 2020: Genotype may influence bacterial diversity in bark and bud of *Vitis vinifera* cultivars grown under the same environment. Applied Sciences 10 (23), 8405. DOI: 10.3390/app10238405.

Bagheri, B., Philipp, C., Horacek, M., Bauer, F., Setati, M., 2019: Microbial diversity in grape musts from Austrian and South African grape varieties and regions. BIO Web of Conferences 12, 02028. DOI: 10.1051/bioconf/20191202028.

Bao, L., Sun, B., Wei, Y., Xu, N., Zhang, S., Gu, L., Bai, Z. 2022: Grape cultivar features differentiate the grape rhizosphere microbiota. Plants 11 (9), 1111. DOI: 10.3390/plants11091111.

Belessi, C., Chalvantzi, I., Marmaras, I., Nisiotou, A., 2022: The effect of vine variety and vintage on wine yeast community structure of grapes and ferments. Journal of Applied Microbiology 132 (5), 3672–3684. DOI: 10.1111/jam.15471.

Berlanas, C., Berbegal, M., Elena, G., Laidani, M., Cibriain, J., Sagues, A., Gramaje, D., 2019: The fungal and bacterial rhizosphere microbiome associated with grapevine rootstock genotypes in mature and young vineyards. Frontiers in Microbiology 10, 1142. DOI: 10.3389/fmicb.2019.01142.

Bokulich, N.A., Thorngate, J.H., Richardson, P.M., Mills, D.A., 2013: Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. PNAS 111, E139-E148. DOI: 10.1073//pnas.1317377110.

Brandl, M.T., Quinones, B., Lindow, S.E., 2001: Heterogeneous transcription of an indoleacetic acid biosynthetic gene in *Erwinia herbicola* on plant surfaces. PNAS 98 (6), 3454–3459. DOI: 10.1073/pnas.061014498.

Bringel, F., Couée, I., 2015: Pivotal roles of phyllospheremicroorganisms at the interface between plant functioning and atmospheric trace gas dynamics. Frontiers in Microbiology 6, 486. DOI: 10.3389/fmicb.2015.00486.

Bulgarelli, D., Spaepen, S.S., Themaat, E. V.L., Shulze-Lefert, P., 2013: Structure and functions of the bacterial microbiota of plants. Annual Revue Plant Biology 64, 807-838. DOI: 10.1146/annurev-arplant-050312-120106.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011: Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. PNAS 108 (Suppl. 1), 4516–4522. DOI: 10.1073/pnas.1000080107.

Castrillo, D., Rabunal, E., Neira, N., Blanco, P., 2019: Yeast Diversity on Grapes from Galicia, NW Spain: Biogeographical Patterns and the Influence of the Farming System. Oeno One 53 (3), 573-587. DOI: 10.20870/oeno-one.2019.53.3.2379.

Fournier, P., Pellan, L., Barroso-Bergadà, D., Bohan, D. A., Candresse, T., Delmotte, F., Dufour, M.-C., Lauvergeat, V., Le Marrec, C., Marais, A., Martins, G., Masneuf-Pomarède, I., Rey, P., Sherman, D., This, P., Frioux, C., Labarthe, S., Vacher, C., 2022: Chapter Two – The Functional Microbiome of Grapevine throughout Plant Evolutionary History and Lifetime. In: Bohan, D. A., Dumbrell, A. (Eds.): Advances in Ecological Research, vol. 67, 27-99. Academic Press. DOI: 10.1016/bs.aecr.2022.09.001.

Gao, F., Chen, J., Xiao, J., Cheng, W., Zheng, X., Wang, B., Shi, X., 2019: Microbial community composition on grape surface controlled by geographical factors of different wine regions in Xinjiang, China. Food Research International 122, 348-360. DOI: 10.1016/j.foodres.2019.04.029.

Gambetta, G.A., Herrera, J.C., Dayer, S., Feng, Q., Hochberg, U., Castellarin, S.D., 2020: The physiology of drought stress in grapevine: towards an integrative definition of drought tolerance. Journal Experimental Botany 71, 4658-4676. DOI: 10.1093/jxb/eraa245.

Guzzon, R., Bertoldi, D., Roman, T., Zanzotti, R., Franciosi, E., 2021: Spatial and seasonal structure of bacterial communities within alpine vineyards: Trentino as a case study. Microbial Ecology 85, 108-120. DOI: 10.1007/s00248-021-01948-9.

Jacquet, F., Jeuffroy, M.H., Jouan, J., Le Cadre, E., Litrico, I., Malausa, T., Reboud, X., Huyghe, C., 2022: Pesticide-free agriculture as a new paradigm for research. Agronomical Sustainable Development 42, 8. DOI: 10.1007/s13593-021-00742-8.

Ji, W., Han, K., Cai, Y., Mu, Y., Zhao, L., Zhang, M., Hou, C., Gao, M., Zhao, Q., 2019: Characterization of rhizosphere bacterial community and berry quality of Hutai No.8 (*Vitis vinifera* L.) with different ages, and their relations. Journal of the Science of Food and Agriculture 99 (10), 4532-4539. DOI: 10.1002/jsfa.9690.

Jones, K., 1970: Nitrogen fixation in phyllosphere of Douglas Fir, *Pseudotsuga douglasii*. Annals of Botany 34, 239-244. DOI: 10.1093/oxfordjournals.aob.a084358.

Kioroglou, D., Kraeva-Deloire, E., Schmidtke, L., Mas, A., Portillo, M., 2019: Geographical origin has a greater impact on grape berry fungal community than grape variety and maturation state. Microorganisms 7 (12), 669. DOI: 10.3390/microorganisms7120669.

Lundberg, D.S., Yourstone, S., Mieczkowski, P., Jones, C.D., Dangl, J.L., 2013: Practical innovations for high-throughput amplicon sequencing. Nature Methods 10, 999-1002.

Marasco, R., Rolli, E., Fusi, M., Michoud, G., Daffonchio, D., 2018: Grapevine Rootstocks Shape Underground Bacterial Microbiome and Networking but Not Potential Functionality. Microbiome 6. https://doi.org/10.1186/s40168-017-0391-2.

Martins, G., Miot-Sertier, C., Lauga, B., Claisse, O., Lonvaud-Funel, A., Soulas, G., Masneuf-Pomarède, I., 2012: Grape berry bacterial microbiota: impact of the ripening process and the farming system. International Journal of Food Microbiology 158 (2), 93-100. DOI: 10.1016/j.ijfoodmicro.2012.06.013.

Marzano, M., Fosso, B., Manzari, C., Grieco, F., Intranuovo, M., Cozzi, G., Mule, G., Scioscia, G., Valiente, G., Tullo, A., Sbisa, E., Pesole, G., Santamaria, M., 2016: Complexity and dynamics of the winemaking bacterial communities in berries, musts, and wines from Apulian grape cultivars through time and space. Plos One 11 (6). DOI: 10.1371/journal.pone.0157383.

Mezzasalma, V., Sandionigi, A., Guzzetti, L., Galimberti, A., Grando, M., Tardaguila, J., Labra, M., 2018: Geographical and cultivar features differentiate grape microbiota in Northern Italy and Spain vineyards. Frontiers in Microbiology 9, 946. DOI: 10.3389/fmicb.2018.00946.

Pertot, I., Caffi, T., Rossi, V., Mugnai, L., Hoffmann, C., Grando, M.S., Gary, C., Lafond, D., Duso, C., Thiery, D., Mazzoni, V., Anfora, G., 2017: A critical review of plant protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. Crop Protection 97, 70-84. DOI: 10.1016/j.cropro.2016.11.025.

Portillo, M., Franques, J., Araque, I., Reguant, C., Bordons, A., 2016: Bacterial diversity of Grenache and Carignan grape surface from different vineyards at Priorat wine region (Catalonia, Spain). International Journal of Food Microbiology 219, 56-63. DOI: 10.1016/j.ijfoodmicro.2015.12.002.

Salvetti, E., Campanaro, S., Campedelli, I., Fracchetti, F., Gobbi, A., Tornielli, G., Torriani, S., Felis, G., 2016: Whole-metagenome-sequencing-based community profiles of *Vitis vinifera* L. cv. Corvina berries withered in two post-harvest conditions. Frontiers in Microbiology 7, 937. DOI: 10.3389/fmicb.2016.00937.

Singh, P., Gobbi, A., Santoni, S., Hansen, L.H., This, P., Peros, J.P., 2018: Assessing the impact of plant genetic diversity in shaping the microbial community structure of *Vitis vinifera* phyllosphere in the Mediterranean. Frontiers in Life Science 11 (1), 35-46, DOI: 10.1080/21553769.2018.1552628.

Singh, P., Santoni, S., This, P., Peros, J.P., 2018: Genotype-environment interaction shapes the microbial assemblage in grapevine's phyllosphere and carposphere: an NGS approach. Microorganisms 6 (4), 96. DOI: 10.3390/microorganisms6040096.

Singh, P., Santoni, S., Weber, A., This, P., Peros, J.P., 2019: Understanding the phyllosphere microbiome assemblage in grape species (Vitaceae) with amplicon sequence data structures. Scientific Reports 9, 14294. DOI: 10.1038/s41598-019-50839-0.

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., Dufresne, A., 2015: The importance of the microbiome of the plant holobiont. New Phytologist 206, 1196-1206. DOI: 10.1111/nph.13312.

Vega-Avila, A., Gumiere, T., Andrade, P., Lima-Perim, J., Durrer, A., Baigori, M., Vazquez, F., Andreote, F., 2015: Bacterial communities in the rhizosphere of *Vitis vinifera* L. cultivated under distinct agricultural practices in Argentina. Antonie Van Leeuwenhoek 107 (2) 575-588. DOI: 10.1007/s10482-014-0353-7.

Vink, S., Chrysargyris, A., Tzortzakis, N., Salles, J., 2021: Bacterial community dynamics varies with soil management and irrigation practices in grapevines (*Vitis vinifera* L.). Applied Soil Ecology 158, 103807. DOI: 10.1016/j.apsoil.2020.103807.

Vorholt, J.A., 2012: Microbial life in the phyllosphere. Nature Reviews Microbiology 10, 828-840.

Wen, J., Xiong, Z., Nie, Z., Mao, L., Zhu, Y., Kan, X., Ickert-Bond, S., Gerrath, J., Zimmer, E., Fang, X., 2013: Transcriptome Sequences Resolve Deep Relationships of the Grape Family. *PLOS ONE*, *8* (9). https://doi.org/10.1371/journal.pone.0074394.

Zhang, J., Wang, E., Singh, R., Guo, C., Shang, Y., Chen, J., Liu, C., 2019: Grape berry surface bacterial microbiome: Impact from the varieties and clones in the same vineyard from Central China. Journal of Applied Microbiology 126 (1), 204-214. DOI: 10.1111/jam.14124.

Zhang, S., Wang, Y., Chen, X., Cui, B., Bai, Z., Zhuang, G., 2020: Variety features differentiate microbiota in the grape leaves. Canadian Journal of Microbiology 66 (11), 653-663. DOI: 10.1139/cjm-2019-0551.

Zarraonaindia, I., Owens, S., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., Bokulich, N., Mills, D., Martin, G., Taghavi, S., van der Lelie, D., Gilbert, J., 2015: The soil microbiome influences grapevine-associated microbiota. mBio, 6 (2), e02527-14. DOI: 10.1128/mBio.02527-14.