CENTENARY OF THE OIV
45th WORLD CONGRESS OF VINE AND WINE
14-18 OCTOBER 2024 FRANCE-DIJON
OIV
Available on IVES Conference Series vine & wine
DOI : https://doi.org/10.58233/7SaZ5oYR

Arbuscular mycorrhizal fungi as biomarkers of vineyard yield in Champagne

Lucile Pellan[1], Paola Fournier[1], Marc Brevot[2], Emilie Chancerel[3], Erwan Guichoux[3], François Delmotte[1], Laurence Mercier[2] and Corinne Vacher[1]

[1] INRAE, Bordeaux Sciences Agro, ISVV, SAVE, Villenave-d'Ornon, France
[2] Centre de Recherche Robert-Jean de Vogüé, Moët-Hennessy, Oiry, France
[3] INRAE, Univ Bordeaux, BioGeCo, 33610 Cestas, France

Abstract. Grapevines are colonized by a multitude of microorganisms (fungi, bacteria, oomycetes), mainly through roots in contact with the soil microbial reservoir. Some microorganisms cause disease and others have positive or negative effects on the plant (protection against pathogens, resistance to abiotic stress, improved nutrition). In addition to their functional roles, microorganisms respond rapidly to environmental changes (climate, cultivation practices), which could make them candidate biomarkers of the performance of the viticultural ecosystem. The aim of this study is (i) to test whether a positive relationship exists between microbial diversity and vineyard yield, (ii) to identify potential microorganisms that are biomarkers of high and stable yields over time, and (iii) to test whether arbuscular mycorrhizal fungi (AMF) are over-represented among these biomarkers. Vine plots with long-term contrasted yields were selected in Champagne based on multi-year records of yield. We sampled the vine roots and characterized the microbiota using metabarcoding approaches targeting bacterial and fungal communities. Our analyses revealed no relationship between microbial taxonomic diversity and grape yield. Using differential abundance analyses and supervised machine learning algorithms, we identified microbial biomarkers of high versus low yield in the root compartment. We identified 58 fungal and oomycete biomarkers, among which there were several AMFs. These AMFs were all biomarkers of high-yielding plots, suggesting a direct role in vine growth and productivity. These microbial biomarkers of stable and high yields will enable the development of diagnostic programs for the functioning of viticultural ecosystems.

1. Introduction

Cropping systems worldwide are increasingly exposed to various disturbances from local to global scales, that can negatively affect yields, particularly within the current context of global climate change [1]. Grape production, in particular, faces challenges related to the development of resistant varieties within protected designations of origin (PDOs) [2]. It relies heavily on pesticides to control pathogens [3], [4], despite the growing political and societal demand to reduce the use of chemical pesticide and fertilizers because of their detrimental effects on human health and the environment [5]. These combined challenges have led to issues such as lower yields, uprooting of plants (dieback) [6], [7], and reshaping landscape of viticulture. The year 2022 marks a new threshold, with the lowest global production since 1961 according to an analysis based on twenty-nine countries accounting for 94% of global production in 2022 [8]. This situation highlights the need for innovative strategies to sustain and revitalize viticultural landscapes.

The study of vineyard microbiota is an emerging field with the potential to improve vine adaptation to climate change and prevent pathogenic infections [9]. In particular, harnessing the functional microbiota could contribute to this transition. Plants and their associated microorganisms form a holobiont - a co-evolved community that includes bacterial, archaeal, and various eukaryotic species such as fungi. The health of the holobiont relies on beneficial interactions between the host and its microbiota, while microbial dysbioses are often associated with disease [10]. Plants are colonized by a multitude of microorganisms mainly through roots. The enrichment of bacterial and fungal endophytic and rhizospheric microorganisms through plant roots is not a random process, but rather a targeted one. Current models suggest that soil microorganisms serve as a reservoir [11], with microbes being attracted to roots by nutrients such as

carbohydrates and amino acids, in addition to specific plant secondary metabolites [12], [13]. Furthermore, plant defense-signaling also influences this selective recruitment process.

The grapevine microbiota are implicated in a variety of functional roles in viticultural agrosystems [14]. Some have a negative effect on the plant, such as the pathogens *Plasmopara viticola* and *Erysiphe necator*, which are responsible for two major diseases, downy and powdery mildew respectively. These pathogens cause significant yield losses depending on the severity of the attacks [15], [16], [17]. Conversely, other microorganisms have beneficial effects on the plant [18], particularly microorganisms antagonistic to pathogens, such as *Bacillus subtilis* or *Pythium oligandrum*. These microorganisms act either directly—through mechanisms like antibiosis, parasitism, or niche competition—or indirectly by inducing resistance, thereby limiting the development of bio-aggressors and enhancing the plant's resistance to biotic stress factors [19], [20], [21]. Plant growth promoter also colonize grapevines and are primarly localized in the roots, such as arbuscular mycorrhizal fungi (AMF, ex: *Rhizophagus irregularis*). These symbiotic organisms improve the grapevine hydric and mineral nutrition, resistance against abiotic stresses, and promote general plant fitness [22], [23]. Overall, the beneficial properties of these various microorganisms may even overlap and consequently contribute to increasing crop yield [24], [25], [26], [27].

The ability of complex microbial communities to resist pathogen invasion and enhance plant growth and productivity is closely linked to their diversity [28], [29]. A loss of key microbial species within these communities can engender disease outbreaks or result in sub-optimal plant yields [30]. Microbial communities with higher phylogenetic and taxonomic diversity not only produce a broader range of molecules that promote plant growth and health (such as biocontrol substances, phytohormones, and soil structuring compounds), but also support beneficial ecological processes such as microbial redundancy, complementarity and synergy [31]. In addition to these functional roles, microbiota respond rapidly to environmental changes (climate, cultivation practices), making them promising candidate biomarkers for assessing the performance of viticultural agrosystems [32], [33].

The aim of this study is to (i) determine whether a positive relationship exists between microbial diversity and vineyard plot yield, (ii) identify microorganisms that are root biomarkers of long-time high and stable yields over time, and (iii) assess whether arbuscular mycorrhizal fungi (AMF) are over-represented among these biomarkers. To test these hypotheses, we sampled vine roots from 20 plots in the Epernay region (Champagne, France) in June 2022. These plots, selected based on yield data collected over the last ten years, comprised 10 pairs with contrasted yields. We specifically sampled the roots, which serve as the entry point for microorganisms colonizing the vine and the preferred habitat for growth stimulators. This approach allowed us to capture the microbiota potentially involved in vine physiology and yield development, particularly those present in the most productive plot of each pair.

2. Material and methods

2.1. Selection of study sites based on annual yield database

The vineyard plots were selected from a database maintained and provided by the *Centre de recherche Robert-Jean de Vogüé* (Moët-Hennessy, Oiry, FRANCE). This database contains records of approximately 2000 vineyard plots in the Grand Est region of France, including details of plot characteristics, vine characteristics, vineyard management practices and yield history from 2009 to 2020. Based on this yield survey, we identified pairs of vineyard plots with contrasted yields (defined by the ability of the plot to produce above-average yields consistently over monitored years). To ensure fair comparisons, the plots were grouped into homogeneous categories based on geographical area, grape variety, rootstock, age range (production state, between 20 and 40 years), management and pruning method. Within these homogeneous groups, plots were each considered as good or bad yielding if their annual yield was either higher (high yield plot) or lower (low yield plot) than the homogeneous group's average annual yield for at least 80% of the years monitored. If several plots met these criteria, the plot pair was formed by combining the high yield and low yield plots separated by the shortest geographical distance. This selection process resulted in the selection of 10 pairs of plots.

2.2. Sampling design and sample processing

All biological samples were harvested in a single campaign spread over 9 days (June 28 to July 6 2022), corresponding to phenological stage of grapevine BBCH 73-75 (beginning of grape bunch formation). In every selected plot, a grid consisting of 6 sampling points distributed over two rows in the center of the plot were identified. In each of these six areas, we collected root composite samples from three adjacent visually healthy vines which were representative of the plot's general condition. At the base of vine trunk, the top 5-10 cm of soil was removed and the undersoil was carefully excavated to a depth of 25 cm to expose the roots of the vines. About 5 growing root fragments were harvested with disinfected tools from each vine plant. Samples were maintained on ice until arrival at the laboratory and were stored at -80°C.

Roots samples were taken one by one from -80°C freezer, and crushed by hand into their sampling bag to fragment the tissues. In a microbiological safety cabinet, the samples were cut into 1cm pieces, following which they were transferred to sterile tubes and freeze-dried, before grinding with steel beads in TissueLyser II (QIAGEN) : 4 rounds of 2 min 30 sec at 25 Hz interspersed with a 30 sec pause.

2.3. DNA extraction and metabarcoding

DNA from root samples were extracted with a CTAB chloroform/isoamyl alcohol protocol (250 mg of grounded and freeze-dried sample input) [34]. Amplification, library preparation and sequencing were carried out at the Genome Transcriptome Platform of Bordeaux (https://pgtb.fr/). First PCRs were performed with 5 µl of template DNA, 2 µl of each primer concentrated to 3µM, 4 µl of HOT FIREPol® MultiPlex Mix 10mM MgCl2 concentrated to 5X, and ultrapure water to 20 µl. The primer pair: 799F (5'-AACMGGATTAGATACCCKG-3') / 1115r (5'-AGGGTTGCGCTCGTTG-3') [35] was specifically chosen for the amplification of bacterial sequences because it excludes chloroplast DNA. The PCR conditions comprised an initial denaturation step at 95°C for 15 min, 30 cycles of denaturation at 95°C for 30 sec, annealing at 47°C for 30 sec, and elongation at 72°C for 30 min, followed by a final extension step at 72°C for 5 min. Oomycete and fungal sequences were amplified using the primer pair : ITS1catta (5′-ACCWGCGGARGGATCATTA-3′) / ITS2ngs (5′-TTYRCKRCGTTCTTCATCG-3′) specific for the ITS regions of fungi and oomycetes [36]. The PCR conditions comprised an initial denaturation step at 95°C for 15 min, 35 cycles of denaturation at 95°C for 30 sec, annealing at 48°C for 30 s, and elongation at 72°C for 30 min, followed by a final extension step at 72°C for 5 min. DNA amplicon size and purity were evaluated with 1% agarose gel electrophoresis. Amplicons were purified to eliminate primer and primer dimers and then submitted to a second PCR for the Illumina specific sequence adaptator ligation and index (Nextera-XT combinatorial index). Sequencing was performed on a NextSeq 2000 system (Illumina) using P1 reagents and 301:10:10:301 cycles.

2.4. Sequencing data processing

Sequencing data processing was carried out with FROGS pipeline v4.1.0 [37] on the Galaxy France server (https://metabarcoding.usegalaxy.fr/) with separate analyses for each barcode region (16S, ITS). Sequencing data processing included dereplication, sequences clustering, removing chimera and filtration step, which results in the clustering of sequences into ASV, Amplicon Sequence Variant. Taxonomic assignments of 16S and ITS short reads were conducted using SILVA 138.1 [38] and UNITE Fungi 8.3 [39] reference databases, respectively. The assignments were performed with both the RDPClassifier [40] and BLASTn+ algorithms [41]. Finally, we used the *metabaR* v1.0.0 R package to decontaminate the datasets [42].

2.5. Statistics

All statistical analyses were performed using R v4.2.3 (R Core Team, 2023). Microbial community analyses were conducted using the R *phyloseq* package v1.42.0 [43] and figures were generated using the *ggplot2* package v3.5.1 [44]. To investigate whether the diversity of soil, root microbial assemblages was higher in vineyard plots with a high yield (H1), the α-diversity of microbial population was assessed through Inverse Simpson Index for each sample. To investigate if the yield levels were associated with a higher abundance of specific taxa, as biomarker taxa of high and low yield, we used a set of three complementary methods. First, we performed a differential abundance analysis with the *Maaslin 2* package v1.12.0 [45]. Second, we conducted an indicator species analysis using the *IndicSpecies* package v1.7.14 [46]. Finally, a Random Forest (RF) analysis was performed to identify the most important predictor ASVs of vineyard yield as biomarkers ASVs. To this end, we used the *microranger* package [47], which incorporates RF classification functions derived from the *ranger* package [48], specifically designed for microbial community classification.

3. Results and discussion

3.1. Ten pairs of vineyard plots (with low vs high yield) were selected according to the annual yield database

Following the plot selection process, a total of twenty plots forming ten pairs of plots with contrasted yields (each pair comprising one high-yield plot and one low-yield plot) were selected to establish the experimental design. This specific experimental set-up was developed to study the effect of plot yield while minimizing potential confounding factors (Fournier and Pellan et al. 2022). Plot yield levels were determined by a long-term study over several years (2009-2019), and we selected pairs of plots with contrasted yields that were otherwise as similar as possible. Samples were collected at the start of grape cluster formation to highlight the microbiota present and potentially involved in this yield-forming phase. For each pair, the annual yield of the high-yield plot was always higher than that of the low-yield plot. These pairs of plots covered 9 geographical areas of the Champagne region, spread over three production zones (Vallée de la Marne, Montagne de Reims and Côte des blancs): Avize, Aÿ, Bouzy, Cramant, Hautvillers, Le Mesnil sur Oger, Oger, Romont and Vertus, with 1 pair per zone, except in Aÿ where two pairs were selected. The ten pairs of plots were planted with the same rootstock (41B), they were all in full production stage (between 20 and 40 years since plantation) and their management style was similar (conventional viticulture, HVE/Sustainable Viticulture certification) alongside minimized intra-pair plot distance. Six pairs out of ten were planted with Chardonnay variety (Chablis pruning method), while the remaining four were planted with Pinot Noir variety (Cordon pruning method).

3.2. Microbial diversity is not higher in grapevine plots with high yield

After sequencing and processing data of samples collected from the pairs of plots with contrasted yields, the final fungal dataset comprised 9,331,270 reads grouped into 473 ASVs, while the final bacterial dataset comprised 25,598,44 reads grouped into 1,097 ASVs. For bacteria, 19 phyla were identified in the root tissues. The bacterial

community was dominated by Actinobacteria (38.7%), Proteobacteria (35.4%), and Chloroflexi (10.5%) (Figure 1), a composition similar to that reported in other studies characterizing the bacterial communities in grapevine roots [49], [50], [51]. In terms of fungal diversity, 10 phyla were detected in the root habitat, with Ascomycota representing the predominant phylum (92.4%), followed by Basidiomycota with 4% of the identified sequences. This is also consistent with previous studies on the fungal microbiota of grapevine roots [49], [52]. Interestingly, Glomeromycota ranked third, accounting for 2.6% of the sequences, and included arbuscular mycorrhizal fungi, which play a crucial role in water and nutrient absorption in grapevines [23]. We also detected the presence of oomycetes represented by the phylum Stramenopila and accounting for 0.35% of the relative abundance.

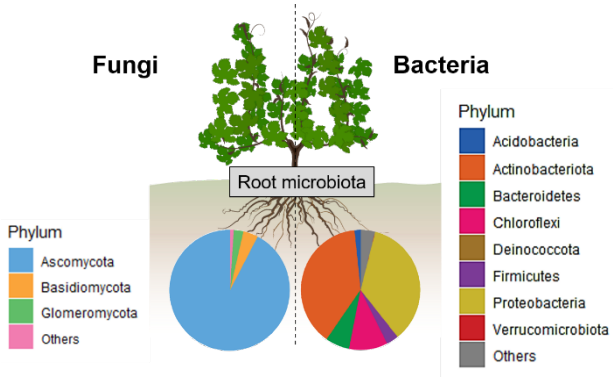

Figure 1. Microbial community profiles of grapevine roots. Each part of circular diagram represents the average proportion of sequences assigned to each phylum for all plots, based on the analysis of ITS and 16S metabarcoding for fungi and bacteria respectively. Figure displays only phyla that account for more than 1% of sequences; all other phyla are grouped in the "Others" category (Aphelidiomycota, Basidiobolomycota, Blastocladiomycota, Mucoromycota, Olpidiomycota, Rozellomycota for fungal phyla and Bdellovibrionota, Desulfobacterota, Entotheonellaeota, Gemmatimonadota, Halanaerobiaeota Methylomirabilota, Myxococcota, Nitrospirota, Patescibacteria, Planctomycetota, Spirochaetota for bacterial phyla).

In contrast with our hypothesis, we did not find any differences in diversity between the microbial assemblages associated with high-yield plots and those associated with low-yield plots for both bacterial and fungal communities (Figure 2). These findings suggest that strengthening specific key microbial taxa have a more significant impact on grapevine health and yield performance than the broader microbial diversity.

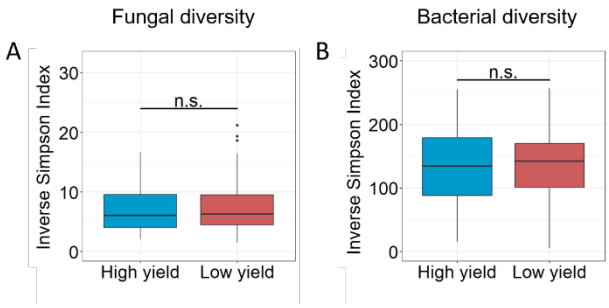

Figure 2. Diversity of microbial communities as a function of vineyard yield. Fungal (A) and bacterial (B) diversity index (Inverse Simpson Index) from high or low yield plot are presented. Pairwise Wilcoxon test was performed to identify statistical differences between diversity index from high or low yield plots (p-value > 0.05 : n.s.).

3.3. Some root fungal species are biomarkers of vineyard yield.

Our analyses identified 58 fungal ASVs that were significantly more abundant in plots with either low or high yields, as determined by at least one of three different analytical methods. The use of combined integrated approaches, which included differential abundance analysis, indicator species analysis, and machine learning methods, enabled a more precise identification of biomarkers from metabarcoding data [53].

In grapevine roots, some fungal ASVs were consistently more abundant in plots with high yield, including fungi belonging to the *Didymella* genus and the *Glomeraceae* family (Figure 3). *Glomeraceae* are a family of arbuscular mycorrhizal fungi that play a crucial role in water and nutrient absorption in grapevines [23]. Conversely, some ASVs were significantly more abundant in plots with low yield, such as the plant pathogenic oomycete *Pythium ultimum* and fungi from the *Laburnicola* genus and *Helotiales* order. *Pythium ultimum* is associated with damping-off and root rot diseases in hundreds of diverse plant hosts, including corn, soybean, wheat, potato, tomato, and perennial plants such as apple trees [54], [55], [56]. This pathogen has also been identified in the root microbiota of grapevines, particularly in studies focusing on vines in decline [57] or those affected by root rot [58], [59]. Its preferential presence in low-yield plots may lead to root system damage, reducing nutrient absorption capacity and partially explaining the decreased yield potential.

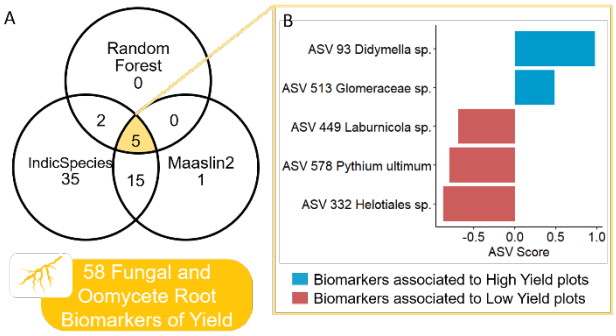

Figure 3. Root fungal and oomycete biomarkers of vineyard yield. A. Root fungal and oomycete ASV biomarkers were selected using three complementary methods: indicator species (Association coefficient with $p < 0.05$), differential abundance (Maaslin2 coefficient with $q < 0.1$) and random forest (Gini index with $p < 0.1$) analyses. This resulted in a final set of 58 ASVs. B. Root fungal and oomycete ASV biomarkers identified with the three different methods and associated ASV score.

3.4. Among fungal biomarkers of yield, arbuscular mycorrhizal fungi are associated with high yield.

Among the 58 fungal biomarkers associated with yield, 39 were associated to high yield and 19 to low yield (Figure 4). Within the high-yield biomarkers, arbuscular mycorrhizal fungi (Glomeraceae family) accounted for 8 of the 25 ASVs with an identified lifestyle (taxonomic affiliation sufficient to find a match in the FugalTraits database if the lifestyle of these fungi are known). By

contrast, AMFs were not found among the fungal biomarkers of low-yield plots (Figure 4).

AMFs that were biomarkers of high yields primarily belonged to the genus *Glomus*, with two ASVs identified as *Rhizophagus irregularis*. The AMF group are the most prevalent mycorrhizae associated with grapevine roots, with *Glomus* and *Rhizophagus* being among the most frequently recovered genera in grapevine root studies [60], [61]. These symbiotic fungi are actively recruited by grapevine roots during water and nutrient stress and are well-known for their plant growth-promoting capabilities [62]. Indeed, these fungi enhance soil exploration by proliferating extra-radicular hyphae, which play a crucial role in improving the uptake and transport of water from the bulk soil to the vine [63]. This mycelial network also provides phosphorus and nitrogen to plant root cells [64] and improves overall soil structure, reducing erosion [65]. In a study using non-targeted metabolomics and targeted transcriptomics, AMFs demonstrated their ability to reprogram primary metabolism, in line with the concept of nutrient exchanges between plants and mycorrhizal fungi. This includes a strong induction of Pathogenesis Related (PR) protein expression in roots and slight increased defense hormone levels in leaves [66]. Another study showed that pre-mycorrhizal inoculation of grapevines alters the expression of several *Plasmopara viticola* effectors, thus reducing downy mildew symptoms [67]. These findings suggest that, in addition to promoting vine growth and resistance to abiotic stress, AMFs may also confer enhanced resistance to grapevine pathogens and could play a significant role in the yield differences observed in the study plots.

Among the biomarkers associated with high yield, we also identified a higher number of saprotrophic fungi, particularly those from soil and litter environments. Saprotrophic fungi play a crucial role in ecosystem functions, including carbon and nitrogen cycling [68], [69], metal complexation, and the release of organic acids and solubilizing enzymes. These processes can alter soil minerals and make nutrients such as potassium, phosphate, magnesium, calcium, and iron bio-available to plants. Additionally, they contribute to soil particle rearrangement, which can improve water retention [70] and resistance to soil erosion [71]. The enhanced availability of water and nutrients may influence soil fertility and, consequently, the elaboration of yield. In a study by Ning et al. (2021) [72], the saprotrophic fungal communities were found to be strongly associated with a multifactorial soil fertility index, which is central to soil health and quality in agroecosystems.

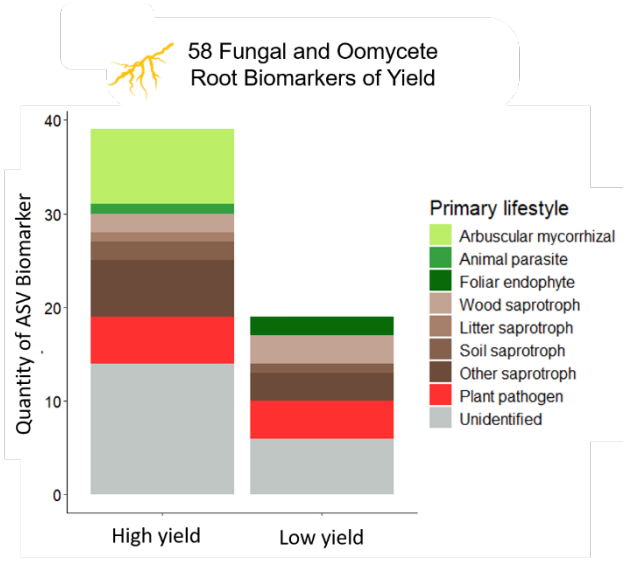

Figure 4. Primary lifestyle of all significant root fungi identified as biomarkers of high and low yield plots. The primary lifestyle of fungal biomarkers were inferred based on the FungalTraits database. Lifestyles associated with beneficial functions for the plant are in greens colors, lifestyles associated with indirect functions are in browns and lifestyles associated to nefast functions for the plant are in red.

4. Perspectives

After establishing a relationship between the presence of AMFs (among others) in the grapevine root compartment and improved long-term yield, we plan to sequence the extracted DNA using AMF-specific primers to better identify the AMF species associated with high yield. The choice of primer set is known to introduce taxonomic biases, particularly for Glomeromycota strains [49]. Therefore, we hypothesize that some species in this family may not have been detected due to primer limitations. To enhance taxonomic affiliation, we will re-sequence the root DNA using long-read technology to cover the entire 16S RNA sequence, which will enhance taxonomic resolution [73]. Additionally, we will conduct microbial community analyses of the soil compartment as a reservoir in the same plots, combined with soil physicochemical analyses, to determine the influence of soil type on microbiota distribution and better control of confounding factors [70], [74]. Finally, we will assess the predictive power of these microbial biomarkers using random forest algorithms [47], [53], [75]. Ultimately, we aim to develop a high-throughput detection tool for these biomarkers in vineyards using a microfluidic chip [76].

5. Funding

This work is funded by the Fondation Université Bordeaux and the French National Research Agency (ANR) under the grant 20-PCPA-0010 (VITAE project).

6. References

1. 1. E. A. Martin, B. Feit, F. Requier, H. Friberg, and M. Jonsson, Adv. Ecol. Res., **60**, 59–123, (2019)

2. 2. R. Töpfer and O. Trapp, Theor. Appl. Genet., **135**, 3947–3960, (2022)
3. 3. A. Nasehi, M. Nasr Esfahani, A. Nasr Esfahani, L. Mohammadbagheri, M. J. Yazdi, and M. Mohammadi, Ecol. Genet. Genomics, **29**, (2023)
4. 4. Agreste, (2019). [Online]. Available: https://agreste.agriculture.gouv.fr/agreste-web/download/publication/publie/Chd2407/cd2024-7_PK-GC2021-IFT.pdf
5. 5. N. Dhankhar and J. Kumar, Mater. Today Proc., (2023)
6. 6. F. Bernizzoni, M. Gatti, S. Civardi, and S. Poni, Am. J. Enol. Vitic., **60**, 339–348, (2009)
7. 7. K. Bou Nader, M. Stoll, D. Rauhut, C. D. Patz, R. Jung, O. Loehnertz, H. R. Schultz, G. Hilbert, C. Renaud, J. P. Roby, S. Delrot, and E. Gomès, Eur. J. Agron., **104**, 1–12, (2019)
8. 8. OIV, (2022). [Online]. Available: https://www.oiv.int/sites/default/files/documents/OIV_State_of_the_world_Vine_and_Wine_sector_in_2022_2.pdf
9. 9. R. Darriaut, V. Lailheugue, I. Masneuf-Pomarède, E. Marguerit, G. Martins, S. Compant, P. Ballestra, S. Upton, N. Ollat, and V. Lauvergeat, Hoticulture Res., **9**, (2022)
10. 10. F. Mesny, S. Hacquard, and B. P. Thomma, EMBO Rep., **24**, (2023)
11. 11. G. Santoyo, J. Adv. Res., **40**, 45–58, (2022)
12. 12. C. E. Prescott, S. J. Grayston, H. S. Helmisaari, E. Kaštovská, C. Körner, H. Lambers, I. C. Meier, P. Millard, and I. Ostonen, Trends Ecol. Evol., **35**, 1110–1118, (2020)
13. 13. V. Vives-Peris, C. de Ollas, A. Gómez-Cadenas, and R. M. Pérez-Clemente, Plant Cell Reports 2019 391, **39**, 3–17, (2019)
14. 14. P. Fournier, L. Pellan, D. Barroso-Bergadà, D. A. Bohan, T. Candresse, F. Delmotte, M. C. Dufour, V. Lauvergeat, C. Le Marrec, A. Marais, G. Martins, I. Masneuf-Pomarède, P. Rey, D. Sherman, P. This, C. Frioux, S. Labarthe, and C. Vacher, Adv. Ecol. Res., **67**, 27–99, (2022)
15. 15. F. Fabre, L. Delbac, C. Poeydebat, and M. Zaffaroni, bioRxiv, 1–31, (2024)
16. 16. M. Jermini, P. Blaise, and C. Gessler, Vitis, **2**, 77–85, (2010).
17. 17. S. Savary, L. Willocquet, S. J. Pethybridge, P. Esker, N. McRoberts, and A. Nelson, Nat. Ecol. Evol. 2019 33, **3**, 430–439, (2019)
18. 18. G. Armijo, R. Schlechter, M. Agurto, D. Muñoz, C. Nuñez, and P. Arce-Johnson, Front. Plant Sci., **7**, (2016) Accessed: Aug. 05, 2022. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fpls.2016.00382
19. 19. R. S. Jayawardena, W. Purahong, W. Zhang, T. Wubet, X. H. Li, M. Liu, W. Zhao, K. D. Hyde, J. H. Liu, and J. Yan, *Biodiversity of fungi on Vitis vinifera L. revealed by traditional and high-resolution culture-independent approaches*, **90**. Springer Netherlands, 2018.
20. 20. O. A. Boiu-Sicuia, R. C. Toma, C. F. Diguță, F. Matei, and C. P. Cornea, Plants 2023, Vol. 12, Page 2553, **12**, 2553, (2023)
21. 21. P. Jeandet, P. Trotel-Aziz, C. Jacquard, C. Clément, C. Mohan, I. Morkunas, H. Khan, and A. Aziz, Agronomy, **13**, 2225, (2023)
22. 22. P. A. Noceto, P. Bettenfeld, R. Boussageon, M. Hériché, A. Sportes, D. van Tuinen, P. E. Courty, and D. Wipf, Mycorrhiza, **31**, 655–669, (2021)
23. 23. S. Trouvelot, L. Bonneau, D. Redecker, D. van Tuinen, M. Adrian, and D. Wipf, Agron. Sustain. Dev., **35**, 1449–1467, (2015)
24. 24. M. Karoglan, T. Radić, M. Anić, Ž. Andabaka, D. Stupić, I. Tomaz, J. Mesić, T. Karažija, M. Petek, B. Lazarević, M. Poljak, and M. Osrečak, Agric. 2021, Vol. 11, Page 615, **11**, 615, (2021)
25. 25. A. Raio and G. Puopolo, World J. Microbiol. Biotechnol., **37**, 1–8, (2021)
26. 26. S. Zhang, A. Lehmann, W. Zheng, Z. You, and M. C. Rillig, New Phytol., **222**, 543–555, (2019)
27. 27. A. Nawaz, M. Shahbaz, M. Asadullah, A. Imran, M. U. Marghoob, M. Imtiaz, and F. Mubeen, Front. Microbiol., **11**, (2020)
28. 28. C. A. Mallon, J. D. Van Elsas, and J. F. Salles, Trends Microbiol., **23**, 719–729, (2015)
29. 29. G. Berg, M. Köberl, D. Rybakova, H. Müller, R. Grosch, and K. Smalla, FEMS Microbiol. Ecol., **93**, 50, (2017)
30. 30. M. J. Blaser, Cleve. Clin. J. Med., **85**, 928–930, (2018)
31. 31. M. Saleem, J. Hu, and A. Jousset, Annu. Rev. Ecol. Evol. Syst, **50**, 145–168, (2019)
32. 32. D. A. Nikitin, M. V. Semenov, T. I. Chernov, N. A. Ksenofontova, A. D. Zhelezova, E. A. Ivanova, N. B. Khitrov, and A. L. Stepanov, Eurasian Soil Sci., **55**, 221–234, (2022)
33. 33. F. Ma, C. Wang, Y. Zhang, J. Chen, R. Xie, and Z. Sun, Int. J. Environ. Res. Public Health, **19**, 13888, (2022)
34. 34. E. Bruez, J. Vallance, J. Gerbore, P. Lecomte, J.-P. Da Costa, L. Guerin-Dubrana, and P. Rey, PLoS One, **9**, (2014)
35. 35. Chelius, M.K. and E. W. Triplett, Microb. Ecol., **41**, 252–263, (2001).

36. 36.F. H. Behrens and M. Fischer, Phytobiomes J., 1–25, (2022)
37. 37.F. Escudié, L. Auer, M. Bernard, M. Mariadassou, L. Cauquil, K. Vidal, S. Maman, G. Hernandez-Raquet, S. Combes, and G. Pascal, Bioinformatics, **34**, 1287–1294, (2018)
38. 38.C. Quast, E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glöckner, Nucleic Acids Res., **41**, 590–596, (2013)
39. 39.R. H. Nilsson, K. H. Larsson, A. F. S. Taylor, J. Bengtsson-Palme, T. S. Jeppesen, D. Schigel, P. Kennedy, K. Picard, F. O. Glöckner, L. Tedersoo, I. Saar, U. Kõljalg, and K. Abarenkov, Nucleic Acids Res., **47**, 259–264, (2019)
40. 40.Q. Wang, G. M. Garrity, J. M. Tiedje, and J. R. Cole, Appl. Environ. Microbiol., **73**, 5261–5267, (2007)
41. 41.C. Camacho, G. Coulouris, V. Avagyan, N. Ma, J. Papadopoulos, K. Bealer, and T. L. Madden, BMC Bioinformatics, **10**, 1–9, (2009)
42. 42.L. Zinger, C. Lionnet, A. S. Benoiston, J. Donald, C. Mercier, and F. Boyer, Methods Ecol. Evol., **12**, 586–592, (2021)
43. 43.P. J. McMurdie and S. Holmes, PLoS One, **8**, (2013)
44. 44.H. Wickham, *ggplot2: Elegant Graphics for Data Analysis.* Springer-Verlag New York, 2016. Accessed: Sep. 05, 2024. [Online]. Available: https://ggplot2.tidyverse.org
45. 45.H. Mallick *et al.*, PLOS Comput. Biol., **17**, (2021)
46. 46.M. De Cáceres, P. Legendre, and M. Moretti, Oikos, **119**, 1674–1684, (2010)
47. 47.M. C. Cambon, M. Trillat, I. Lesur-Kupin, R. Burlett, E. Chancerel, E. Guichoux, L. Piouceau, B. Castagneyrol, G. Le Provost, S. Robin, Y. Ritter, I. Van Halder, S. Delzon, D. A. Bohan, and C. Vacher, Mol. Ecol., **32**, 5944–5958, (2023)
48. 48.M. N. Wright and A. Ziegler, J. Stat. Softw., **77**, (2015)
49. 49.P. Bettenfeld, J. i Canals, L. Jacquens, O. Fernandez, F. Fontaine, E. van Schaik, P.-E. Courty, and S. Trouvelot, J. Adv. Res., (2021)
50. 50.G. Novello, E. Gamalero, E. Bona, L. Boatti, F. Mignone, N. Massa, P. Cesaro, G. Lingua, and G. Berta, Front. Microbiol., **8**, 1–11, (2017)
51. 51.L. Dries, S. Bussotti, C. Pozzi, R. Kunz, S. Schnell, O. Löhnertz, and A. Vortkamp, Microorganisms, **9**, 1–12, (2021)
52. 52.D. Liu and K. Howell, Environ. Microbiol., **23**, 1842–1857, (2021)
53. 53.S. Lutz, N. Bodenhausen, J. Hess, A. Valzano-Held, J. Waelchli, G. Deslandes-Hérold, K. Schlaeppi, and M. G. A. van der Heijden, Nat. Microbiol., **8**, 2277–2289, (2023)
54. 54.M. Salman and R. Abuamsha, BioControl, **57**, 711–718, (2012)
55. 55.A. E. Dorrance, S. A. Berry, P. E. Bowen, and P. E. Lipps, Plant Heal. Prog., **5**, (2004)
56. 56.Y. Zhu, J. Shao, Z. Zhou, and R. E. Davis, Hortic. Res., **6**, (2019)
57. 57.C. F. J. Spies, M. Mazzola, and A. McLeod, Eur. J. Plant Pathol., **131**, 103–119, (2011)
58. 58.L. Chiarappa, Phytopathology, **49**, 510–519, (1959).
59. 59.W. D. Gubler, K. Baumgartner, G. T. Browne, A. Eskalen, S. Rooney Latham, E. Petit, and L. A. Bayramian, Australas. Plant Pathol., **33**, 157–165, (2004)
60. 60.R. Darriaut, L. Antonielli, G. Martins, P. Ballestra, P. Vivin, E. Marguerit, B. Mitter, I. Masneuf-Pomarède, S. Compant, N. Ollat, and V. Lauvergeat, Front. Microbiol., **13**, 1–21, (2022)
61. 61.D. Liu and K. Howell, Environ. Microbiol., **23**, 1842–1857, (2021)
62. 62.M. J. Carbone, S. Alaniz, P. Mondino, M. Gelabert, D. Tekielska, R. Bujanda, and D. Gramaje, J. Fungi, **7**, (2021)
63. 63.R. Biasi, E. Brunori, S. Vanino, A. Bernardini, A. Catalani, R. Farina, A. Bruno, and G. Chilosi, Agric., **13**, 1051, (2023)
64. 64.G. C. Popescu, Curr. Trends Nat. Sci., **5**, 107–116, (2016).
65. 65.G. W. T. Wilson, C. W. Rice, M. C. Rillig, A. Springer, and D. C. Hartnett, Ecol. Lett., **12**, 452–461, (2009)
66. 66.M. L. Goddard, L. Belval, I. R. Martin, L. Roth, H. Laloue, L. Deglène-Benbrahim, L. Valat, C. Bertsch, and J. Chong, Front. Plant Sci., **12**, (2021)
67. 67.A. Cruz-Silva, A. Figueiredo, and M. Sebastiana, Sustainability, **13**, 1226, (2021)
68. 68.S. Fontaine, C. Henault, A. Aamor, N. Bdioui, J. M. G. Bloor, V. Maire, B. Mary, S. Revaillot, and P. A. Maron, Soil Biol. Biochem., **43**, 86–96, (2011)
69. 69.A. Van der Wal, T. D. Geydan, T. W. Kuyper, and W. De Boer, FEMS Microbiol. Rev., **37**, 477–494, (2013)
70. 70.L. Philippot, C. Chenu, A. Kappler, M. C. Rillig, and N. Fierer, Nat. Rev. Microbiol., **22**, 226–239, (2023)
71. 71.M. H. Beare, S. Hu, D. C. Coleman, and P. F. Hendrix, Appl. Soil Ecol., **5**, 211–219, (1997)

72. 72.Q. Ning, L. Chen, C. Zhang, D. Ma, D. Li, X. Han, Z. Cai, S. Huang, and J. Zhang, Appl. Soil Ecol., **159**, 103843, (2021)

73. 73.L. Tedersoo, M. Bahram, L. Zinger, H. Nilsson, P. Kennedy, T. Yang, S. Anslan, and V. Mikryukov, Authorea Prepr., 1–33, (2021).

74. 74.W. Islam, A. Noman, H. Naveed, Z. Huang, and H. Y. H. Chen, Environ. Sci. Pollut. Res. 2020 2733, **27**, 41225–41247, (2020)

75. 75.L. Anand, T. Gentimis, A. B. Downie, and C. M. R. Lopez, bioRxiv, (2024)

76. 76.M. C. Dufour, N. Magnin, B. Dumas, S. Vergnes, and M. F. Corio-Costet, BMC Genomics, **17**, (2016)