RESOURCE ANNOUNCEMENT

Leaf Microbiome Data for European Cultivated Grapevine (*Vitis vinifera*) During Downy Mildew (*Plasmopara viticola*) Epidemics in Three Wine-Producing Regions in France

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Abstract

Grapevine downy mildew (*Plasmopara viticola*) is a major disease of European cultivated grapevine (*Vitis vinifera* L.) against which a large amount of synthetic pesticides are used. Developing microbial biocontrol of *P. viticola* could reduce the use of pesticides in viticulture and preserve human and environmental health. To achieve this goal, the ecological interactions that develop during infection between *P. viticola* and the vine foliar microbiome need to be explored. Here, we present metabarcoding datasets describing the bacterial and fungal communities of pairs of symptomatic and asymptomatic leaf samples collected during downy mildew epidemics in three major wine-producing regions of France. Fungal and bacterial communities were sequenced on a MiSeq Illumina platform, and the abundance of the oomycete *P. viticola* was quantified using qPCR. We provide the raw metabarcoding datasets, the amplicon sequence variant tables obtained after bioinformatic processing, the metadata describing sampling sites and tissue health conditions, and the code used for bioinformatic analysis. These datasets will enable microbiome comparison within pairs of symptomatic and asymptomatic samples collected at the same time on the same leaf. Such a comparison could help describe the ecological interactions between *P. viticola* and the grapevine foliar microbiome.

Downy mildew, caused by the oomycete *Plasmopara viticola*, is a major disease of European cultivated grapevine, *Vitis vinifera* L. (Fontaine et al. 2021). A current challenge in viticulture is to control downy mildew without using synthetic pesticides (Jacquet et al. 2022; Pertot et al. 2017). Exploring and exploiting the plant microbiome is one possible avenue to reach this objective (Busby et al. 2017; Toju et al. 2018). Fungal and bacterial communities associated with grapevine leaves have been extensively described using metabarcoding approaches over the last decade (Singh et al. 2018; Zarraonaindia et al. 2015), and experimental work suggests that these communities could contribute to grapevine resistance to downy mildew (Bruisson et al. 2019; Burruano et al. 2016; Ghule et al. 2018;

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Author contributions: C.V. and D.A.B. conceived and coordinated the project; F.D., I.D., and S.G. designed the sampling and collected samples and metadata; M.M., J.F.A., E.C., and E.G. developed protocols and performed the molecular biological work; D.B.-B. performed data analysis; D.B.-B. and C.V. wrote the manuscript; all authors edited the manuscript.

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The author(s) declare no conflict of interest.

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Keywords

bacteria, fungi, holobiont, metabarcoding, microbial community, microbiota, oomycete, pathobiome, phyllosphere, plant disease

Table 1. Sampling design[a]

Vineyard	Latitude	Longitude	Region	Grape variety	Sampling date
A	43.113225	2.095317	OC	Chasan	06/13/2018
B	43.506132	4.754621	OC	Chardonnay	06/14/2018
C	43.14223	3.13292	OC	Gamay	06/15/2018
D	44.791315	−0.578224	AQ	Merlot	06/26/2018
E	44.707248	0.244359	AQ	Cabernet Franc	07/27/2018
F	44.62885	−0.26322	AQ	Merlot	07/27/2018
G	49.017485	3.983795	CH	Chardonnay	07/11/2018
H	49.018104	3.980866	CH	Meunier	07/11/2018
I	49.063127	4.008855	CH	Pinot noir	07/16/2018

[a] Thirty pairs of symptomatic versus asymptomatic leaf samples were collected in nine vineyards (named A to I) belonging to three wine-growing regions in France (Occitanie [OC], Aquitaine [AQ], and Champagne [CH]) at the peak of infection by *Plasmopara viticola* in each vineyard. These vineyards were selected because they included untreated vines (at least one row) from which leaf samples were collected. GPS coordinates of the sampling sites are indicated in decimal degrees.

Musetti et al. 2006; Zanzotto et al. 2016). To continue exploring the ecological interactions between *P. viticola* and the grapevine leaf microbiome, we collected pairs of symptomatic versus asymptomatic leaf samples from untreated vineyards in three major French wine-producing regions and sequenced their fungal and bacterial communities. The present Resource Announcement provides the raw metabarcoding datasets, the amplicon sequence variant (ASV) tables obtained after bioinformatic processing, the metadata describing the sampling design, and the code used for bioinformatic analysis. Future analysis could use these data to identify microbial taxa enriched in asymptomatic samples and to infer microbial interaction networks (Nearing et al. 2022; Röttjers and Faust 2018).

Materials and Methods

Sampling. We collected 270 pairs of symptomatic (S) versus asymptomatic (A) foliar samples, corresponding to 9 vineyards × 30 vines, with each pair of samples taken from the same leaf. Grapevine leaves with sporulating mildew lesions were collected in nine vineyards (Table 1) that included control rows that were not treated with phytosanitary products. The whole sampling campaign lasted 1 month and a half (between June 13 and July 27, 2018), but all the samples of the same vineyard were collected on the same day. The sampling date (Table 1) varied among vineyards because it was chosen based on the development of downy mildew in each vineyard. The nine vineyards represented three major French wine-growing regions (Aquitaine [AQ], Champagne [CH], and Occitanie [OC]; Table 1). Therefore, the grapevine variety (Table 1) differed among vineyards, as varieties vary across regions.

In each vineyard, leaves were collected from 30 vines belonging to the untreated rows. One leaf per vine was collected using sterile gloves and placed in an individual plastic bag in a cooler until processing. Leaves were processed on the day of collection with sterilized tools in the sterile field of a MICROBIO electric burner (MSEI, France). From each leaf, we collected a symptomatic sample (S) and an asymptomatic (A) sample. Each sample was formed of two foliar disks of 12 mm diameter that were taken from either sporulating mildew lesions (S) or visually healthy tissue (A). During the sampling campaign, samples were kept in 2-ml autoclaved collection tubes stored in a box filled with silica gel. Screw caps of the tubes were left loose to allow the disks to dry. They were freeze-dried at the end of the sampling campaign.

DNA extraction. Total DNA was extracted with the DNeasy Plant Mini kit (Qiagen, France), with a slightly modified version of the protocol recommended by Kerdraon et al. (2019). Two autoclaved DNase-free inox 420C beads were added to each tube, and samples were ground at 1,500 rpm with the Geno/Grinder for 30 s, then 1 min and 1 min again, with manual shaking between each grinding step. Tubes were then centrifuged for 1 min at 6,000 × *g*. Leaf powder and 200 µl of buffer AP1 preheated to 60°C were mixed by vortexing the tubes for 30 s twice at 1,500 × *g* and centrifuging them for 1 min at 3,000 × *g*. Two hundred and fifty microliters of preheated buffer AP1 and 4.5 µl of RNase A were added to each tube and mixed by vortexing the tubes for 30 s twice at 1,500 × *g*. After 5 min of rest, 130 µl of buffer P3 was added to each tube, which was then mixed by gentle inversion for

15 s, incubated at −20°C for 10 min, and centrifuged for 1 min at 5,000 × *g*. The supernatant (450 μl) was transferred to a spin column and centrifuged for 2 min at 20,000 × *g*. The filtrate (200 μl) was transferred to a new tube, to which sodium acetate (200 μl, 3 M, pH 5) and cold 2-propanol (600 μl) were added. DNA was precipitated by incubation at −20°C for a minimum of 1 h and recovered by centrifugation (20 min, 13,000 × *g*). The pellet was washed twice with cold ethanol (70%), dried at 50°C for approximately 30 min, and dissolved in 100 μl of AE buffer. The negative extraction controls were represented by extraction reagents in an autoclaved 2-ml Eppendorf tube containing two autoclaved DNase-free inox 420C beads.

Fungal ITS amplification and quantification of *P. viticola*. The ITS1 region of the fungal ITS rDNA gene (Schoch et al. 2012) was amplified using primers ITS1F-ITS2 (Gardes and Bruns 1993; White et al. 1990). To avoid a two-stage PCR protocol, each primer contained the Illumina adaptor sequence and a tag (ITS1F: 5′-CAAGCAGAAGACGGCATA CGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTxxxxxxxxxxxxCTTGGTCATTT AGAGGAAGTAA-3′; ITS2: 5′-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTA CACGACGCTCTTCCGATCTxxxxxxxxxxxxGCTGCGTTCTTCATCGATGC-3′, where "x" is the 12-nucleotide tag). The PCR mixture (20 μl of final volume) consisted of 10 μl of 2× QIAGEN Multiplex PCR Master Mix (2× final), 2 μl each of the forward and reverse primers (0.1 μM final), 4 μl of water, 1 μl of 10 ng/μl BSA, and 1 μl of DNA template. PCR cycling reactions were conducted on a Veriti 96-well Thermal Cycler (Applied Biosystems) using the following conditions: initial denaturation at 95°C for 15 min, followed by 35 cycles at 94°C for 30 s, 57°C for 90 s, 72°C for 90 s, with a final extension of 72°C for 10 min. ITS1 amplification was confirmed by electrophoresis on a 2% agarose gel. Two marine fungi, *Candida oceani* and *Yamadazyma barbieri*, were used as positive controls as they were unlikely to be found in our samples. One positive control included 1 μl of 10 ng/μl DNA of *C. oceani* only, and the other included an equimolar mixture of both species. The negative PCR controls were represented by a PCR mix without any DNA template. Each PCR plate contained one negative extraction control, three negative PCR controls, one single-species positive control, and one two-species positive control. The abundance of *P. viticola* was quantified with real-time quantitative PCR targeting the ITS1 region (Supplementary Methods S1).

Bacterial 16S amplification. The V5-V6 region of the bacterial 16S rDNA gene was amplified using primers 799F-1115R (Chelius and Triplett 2001; Redford et al. 2010) to exclude chloroplastic DNA. To avoid a two-stage PCR protocol and reduce PCR biases, each primer contained the Illumina adaptor sequence, a tag, and a heterogeneity spacer, as described in Laforest-Lapointe et al. (2017) (799F: 5′-CAAGCAGAAGACGGCATACGAGATGTGACTG GAGTTCAGACGTGTGCTCTTCCGATCTxxxxxxxxxxxxHS-AACMGGATTAGATACCCKG-3′; 1115R: 5′-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCG ATCTxxxxxxxxxxxxHS-AGGGTTGCGCTCGTTG-3′, where HS represents a 0-7-base-pair heterogeneity spacer and "x" a 12-nucleotide tag). The PCR mixture (20 μl of final volume) consisted of 10 μl of 2× QIAGEN Multiplex PCR Master Mix (2× final), 2 μl each of the forward and reverse primers (0.1 μM final), 4 μl of water, 1 μl of 10 mg/ml BSA, and 1 μl of DNA template. PCR cycling reactions were conducted on a Veriti 96-well Thermal Cycler (Applied Biosystems) using the following conditions: initial denaturation at 95°C for 15 min, followed by 32 cycles at 94°C for 30 s, 53°C for 90 s, 72°C for 90 s, with a final extension of 72°C for 10 min. 16S amplification was confirmed by electrophoresis on a 2% agarose gel. Two marine bacteria, *Sulfitobacter pontiacus* and *Vibrio splendidus*, were used as positive controls as they were unlikely to be found in our samples. One positive control included 1 μl of 10 ng/μl DNA of *V. splendidus* only, and the other included an equimolar mixture of both species. The negative PCR controls were represented by a PCR mix without any DNA template. Each PCR plate contained one negative extraction control, three negative PCR controls, one single-species positive control, and one two-species positive control.

MiSeq sequencing. PCR products were purified, quantified (Quant-it dsDNA assay kit; Invitrogen), and used to constitute equimolar pools (Hamilton Microlab STAR robot). Mean fragment size was determined with a Tapestation instrument (Agilent Technologies). ITS and 16S amplicons were sequenced on one and a half and two runs of the Miseq Instrument (Illumina), respectively, with the reagent kit v2 (500 cycles). Sequence demultiplexing (with an exact index search) was performed at the PGTB sequencing facility (Genome Transcriptome Facility of Bordeaux, Pierroton, France) with DoubleTagDemultiplexer.

Bioinformatics. The MiSeq sequences were processed using the DADA2 pipeline v1.22.0 (Callahan et al. 2016) implemented in R (R Core Team 2020). Primers were identified

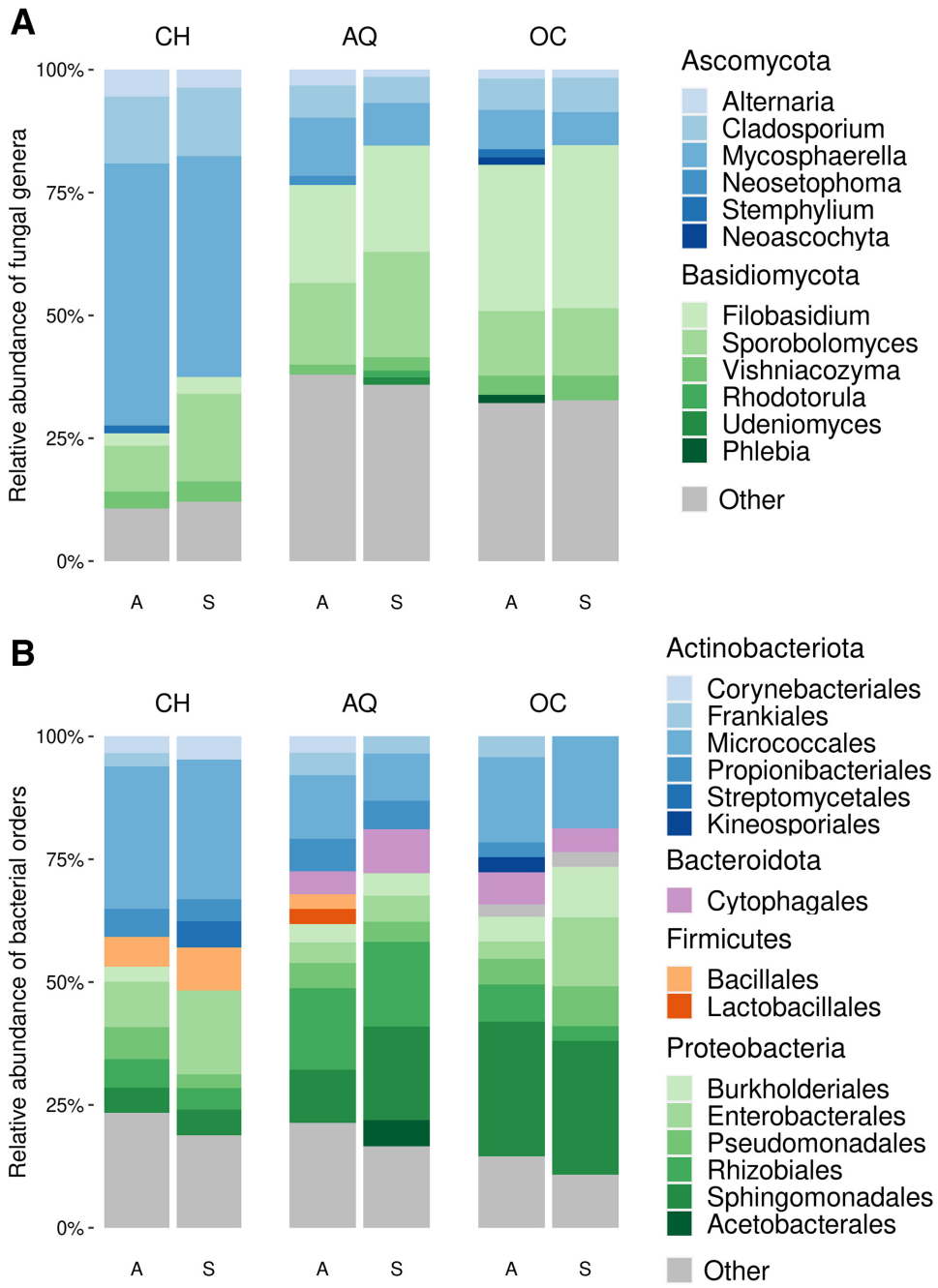

Fig. 1. Taxonomic barplots showing the relative abundance of **A,** fungal genera and **B,** bacterial orders in grapevine (*Vitis vinifera* L.) foliar samples that were either asymptomatic (A) or symptomatic (S) for downy mildew (*Plasmopara viticola*) for three major wine-producing regions of France (from North to South: Champagne [CH], Aquitaine [AQ], and Occitanie [OC]).

and removed using cutadapt v3.2 (Martin 2011), and the trimmed sequences were then parsed to the DADA2 algorithm to infer ASVs. Chimeras were removed using the *removeBimeraDenovo* function of DADA2. ASV taxonomic assignment was performed using an implementation of the naive Bayesian classifier (Wang et al. 2007) included in the DADA2 pipeline. The databases used for taxonomic assignment were SILVA v138.1 (Quast et al. 2012) and 'UNITE All Eukaryotes' v8.3 (Abarenkov et al. 2021) for 16S and ITS sequences, respectively. ASV tables, taxonomic assignments, and metadata were joined in phyloseq objects using the phyloseq bioconductor package v1.38.0 (McMurdie and Holmes 2013). To filter out possible contaminants, the combined method of the *isContaminant* function of the DECONTAM Bioconductor package v1.14.0 (Davis et al. 2018) was used, followed by the decontamination method described in Galan et al. (2016). Moreover, 16S ASVs identified as

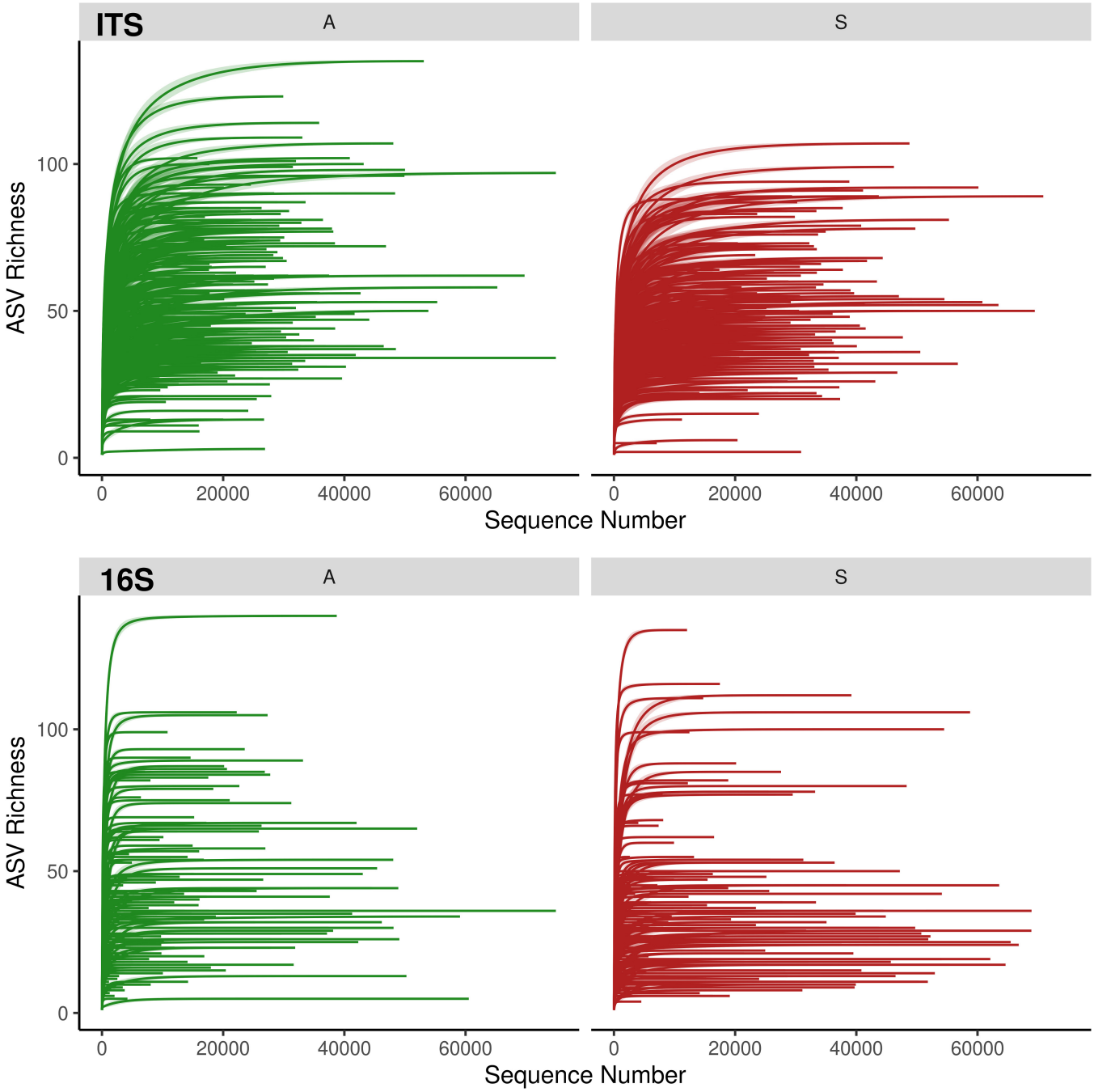

Fig. 2. Rarefaction curves showing the variation of amplicon sequence variant (ASV) richness as a function of the rarefaction threshold for the ITS and 16S metabarcoding datasets. Each curve corresponds to an asymptomatic (A, in green) or a symptomatic (S, in red) sample.

chloroplastic or mitochondrial with Metaxa2 v2.2.3 (Bengtsson-Palme et al. 2015), or according to their taxonomic assignment in the SILVA database, were removed. The remaining ASVs were clustered using the LULU algorithm (Frøslev et al. 2017) with default parameters. ASVs that could not be assigned to a bacterial or fungal phylum were removed. ASVs present in less than 1% of the samples were removed to make sure that the data were free of sequencing artifacts and low abundant contaminants (Cao et al. 2021). Finally, pairs of samples in which at least one of the samples had fewer than 1,000 sequences were removed.

Results

After filtering, the ASV table describing fungal communities was composed of 13,633,258 sequences distributed among 648 ASVs and 251 pairs of samples, with an average number of reads per sample of 27,158 (min: 3,565; max: 228,423; SD: 14,789). For bacteria, the

filtered ASV table was composed of 6,512,073 sequences distributed among 986 ASVs and 195 pairs of samples, with an average number of reads per sample of 16,698 (min: 1,105; max: 86,598; SD: 16,554). The taxonomic composition of communities and rarefaction curves are shown in Figure 1 and Figure 2, respectively.

Future Directions

This Resource Announcement provides two metabarcoding datasets describing the changes undergone by foliar fungal and bacterial communities during infection by downy mildew (*P. viticola*) in untreated grapevines (*V. vinifera* L.). These datasets will allow for community comparison within pairs of symptomatic and asymptomatic samples collected at the same time on the same leaf. Such a comparison could help unravel ecological interactions between *P. viticola* and the grapevine foliar microbiome.

Availability of Data and Materials

The sequence datasets have been deposited in NCBI SRA in bioproject PRJNA797225 and bioproject PRJNA797948. The biosample accession numbers are SAMN24973302 to SAMN24973835. Bioinformatic scripts, raw and filtered ASV tables in R phyloseq format, and tables showing variation in sequence counts during the bioinformatic process have been deposited in Recherche Data Gouv (https://doi.org/10.15454/2YDSBL).

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