CHARACTERIZATION OF THE OLIVE XYLEM MICROBIOME COMMUNITY COMPOSITION BY METABARCODING GREATLY DEPENDS ON THE MATRIX USED TO EXTRACT DNA AND 16S UNIVERSAL BACTERIAL PCR PRIMERS

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ABSTRACT

Understanding of xylem sap microbiome is becoming of relevant importance for plant health as it could include microbes that may protect against xylem-limited pathogens, such as *Xylella fastidiosa*, and support key biological processes. Furthermore, the negative pressure, low oxygen and nutrient content of the xylem sap make it an unique and unexplored microbial environment. In this study, we evaluated the differences obtained in the characterization of the xylem microbiome composition when using xylem sap extracted (**Fig. a**) from xylem vessels using a Scholander pressure chamber (**Fig. b**) or when using macerated fine chips obtained from xylem tissues (**Fig. c**) from 10-year old (**Fig. d**) or 1-year old (seedlings) olive trees (**Fig. e**). We also compared four different PCR primer pairs targeting 16S rRNA for their efficacy to avoid co-amplification of mitochondria and chloroplast 16S rRNA, as this suppose an important drawback in metabarcoding studies. PCR primers tested included 799F/1062 (PCR1, V5-V6), 799F/1115 (PCR2, V5-V6), 967/1391 (PCR3, V6-V8) and 799F/1193 (PCR4, V5-V7) (**Fig. f, g**). Illumina paired-end sequence quality control and chimeric filtering was performed with DADA2 using QIIME2. Taxonomy affiliation into OTUs at 99% was based on Silva reference database. The highest mitochondria and chloroplast amplification was obtained when using xylem chips and 799F/1062 primers (PCR1) (77.7%) and 967/1391 primers (PCR3) (99.6%) primers (**Fig. 1**). On the contrary, 799F/1115 primers (PCR2) and 799F/1193 primers (PCR4) showed the lowest mitochondria (<6.76%) and chloroplasts (<0.02%) amplification, and the highest number of OTUs identified, 245 and 247, respectively (**Fig. 1**). Interestingly, only 81/236 and 27/240 OTUs or 66/144 and 21/149 genera were shared between xylem sap or wood shavings after amplification with 799F/1115 primers (PCR2) or 799F/1193 primers (PCR4), respectively (**Fig. 5**). The most abundant bacterial genera (>50% of reads) included *Anoxybacillus*, *Cutibacterium*, *Methylobacterium*, *Pseudomonas*, *Rathayibacter*, *Sphingomonas and Spirosoma*; however, their relative importance varied depending of the matrix and primer pairs used. These results will help to optimize analysis of xylem microbiome community composition and more importantly to understand its driving and modifying factors.

OBJECTIVE

To evaluate the effect of primer pair combinations, type of xylem sap extraction procedure and olive plant age in assessing the structure and diversity of bacterial community composition of olive xylem sap.

WORKFLOW

1. Plant sampling, xylem sap extraction and DNA extraction → **2. Library sequencing preparation** → **3. Bioinformatic and statistical analysis**

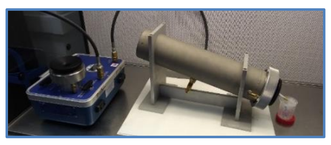

Fig. b. Scholander pressure chamber

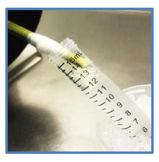

Fig. a Xylem sap extraction

Fig. c. Woody chips extraction

Fig. d. 10 years old olive tree

Fig. e. 1 year old seedling

Fig. f. DNA extraction

PCR name	Primer Forward	Primer Reverse
PCR1	799F	1062R
PCR2	799F	1115R
PCR3	967F	1391R
PCR4	799F	1193R

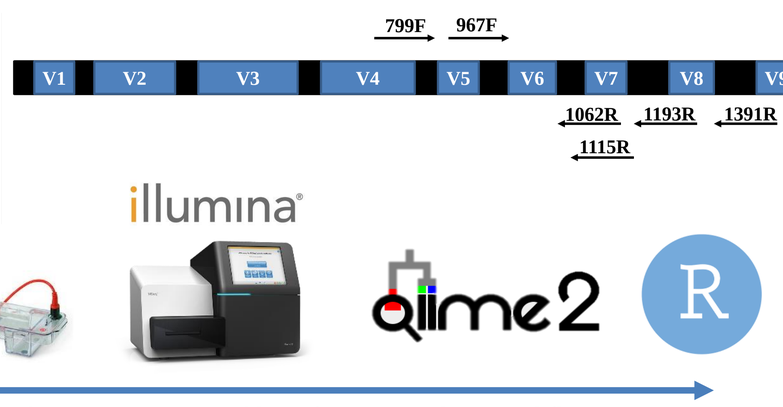

Fig. g. Library sequencing and bioinformatic analysis workflow

RESULTS

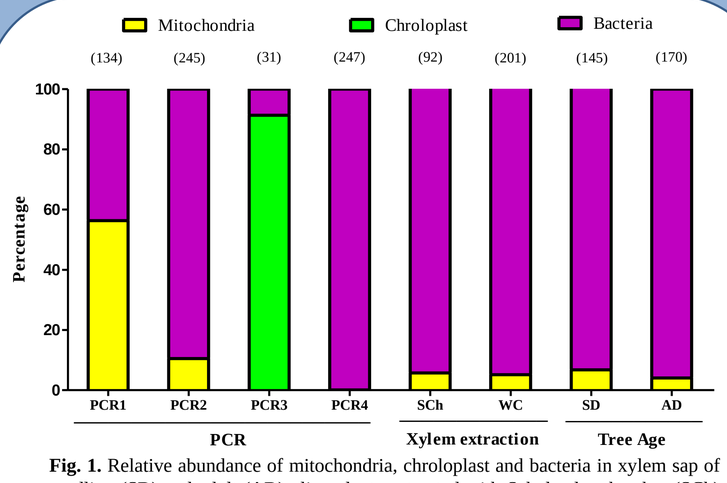

Fig. 1. Relative abundance of mitochondria, chroloplast and bacteria in xylem sap of seedling (SD) and adult (AD) olive plants extracted with Scholander chamber (SCh) or woody chips (WC) as determined by using four PCR primers combinations. Number of observed OTUs in brackets.

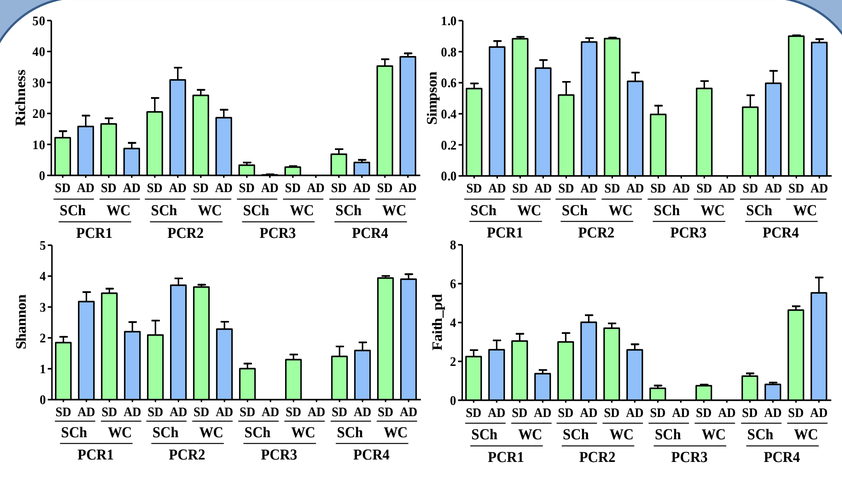

Fig. 2. Richness, Simpson, Shannon and Faith_pd diversity indices at genus taxonomic level according to the xylem sap extraction method [Scholander chamber (SCh) or woody chips (WC)] and plant age [Seedlings (SD) or adult (AD) olive plant] using different PCR primers pairs.

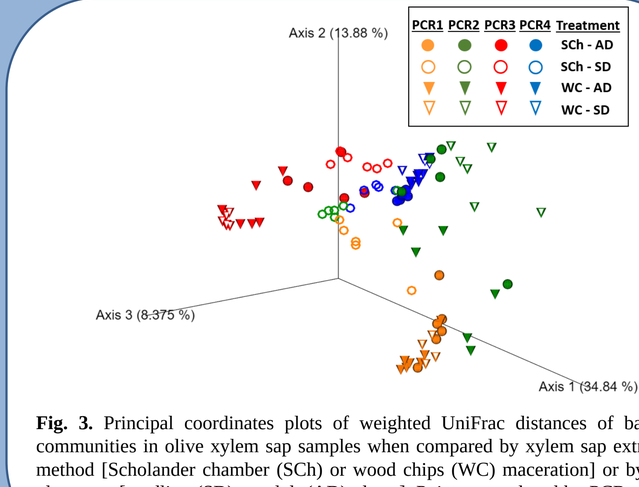

Fig. 3. Principal coordinates plots of weighted UniFrac distances of bacterial communities in olive xylem sap samples when compared by xylem sap extraction method [Scholander chamber (SCh) or wood chips (WC) maceration] or by olive plant age [seedling (SD) or adult (AD) plants]. Points are colored by PCR primers combinations and shaped by xylem sap extraction method and olive plant age.

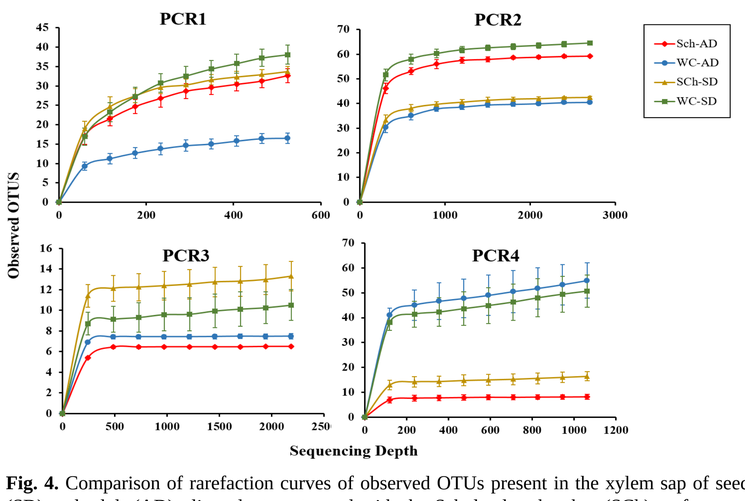

Fig. 4. Comparison of rarefaction curves of observed OTUs present in the xylem sap of seedling (SD) and adult (AD) olive plants extracted with the Scholander chamber (SCh) or from woody chips macerates (WC) using four PCR primers combinations. Error bars represent standard derivation of three independent tree replicates. Data were rarified to minimum sequences.

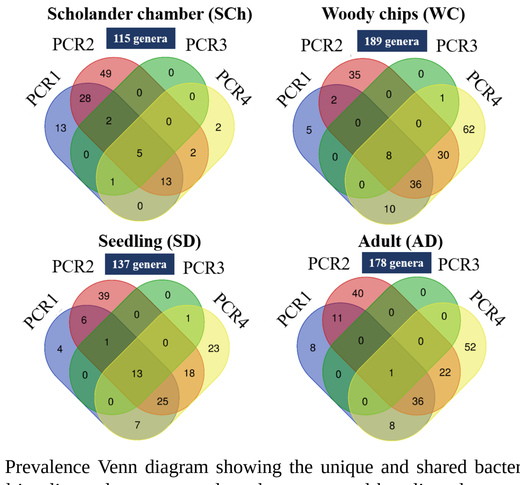

Fig. 5. Prevalence Venn diagram showing the unique and shared bacterial genera obtained in olive xylem sap samples when compared by olive plant age [seedling (SD) and adult (AD) plant] or by xylem sap extraction method [Scholander chamber (SCh) or wood chips (WC) maceration] using different PCR primers pairs.

CONCLUSIONS

- **Selection of adequate primer pairs combinations is essential to avoid co-amplification of mitochondria and chloroplast 16S rRNA when using plant material (Fig. 1).**
- **There was a great bias in assessing the bacterial community composition mainly depending on the primer pair combination. This was demonstrated by the differences in and alpha (Fig. 2, 4) and beta diversity measures (Fig.3) and the bacterial OTUs identified (Fig. 5).**
- **Main phylogenetic distances among bacterial communities were due to PCR primer combination followed by xylem sap extraction method and olive plant age (Fig. 3).**
- **Highest number of unique bacteria genera were obtained when using 799F/1193 primers (PCR4) and xylem sap extracted from woody chips (62) and from adult plants (52) (Fig. 5).**

ACKNOWLEDGMENTS

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