

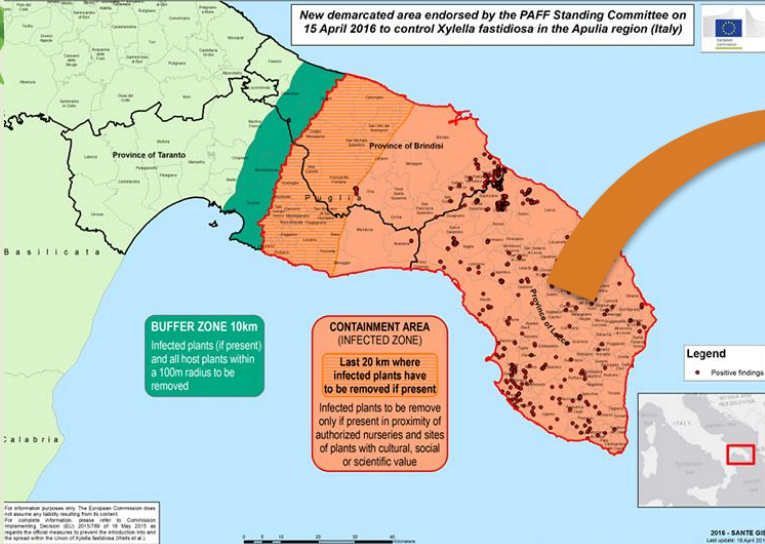


Understanding the olive microbiome of susceptible and resistant cultivars for sustainable biocontrol

Baptista P¹, Cameirão C¹, Giampetruzzi A³, Morelli M⁴, Abou Kubaa R⁴, Altamura G⁴, D'Attoma G⁴, Pereira JA¹, Lino Neto T², Sisto A⁴, De Bellis P⁴, Saldarelli P⁴



New demarcated area endorsed by the PAFF Standing Committee on 15 April 2016 to control *Xylella fastidiosa* in the Apulia region (Italy)

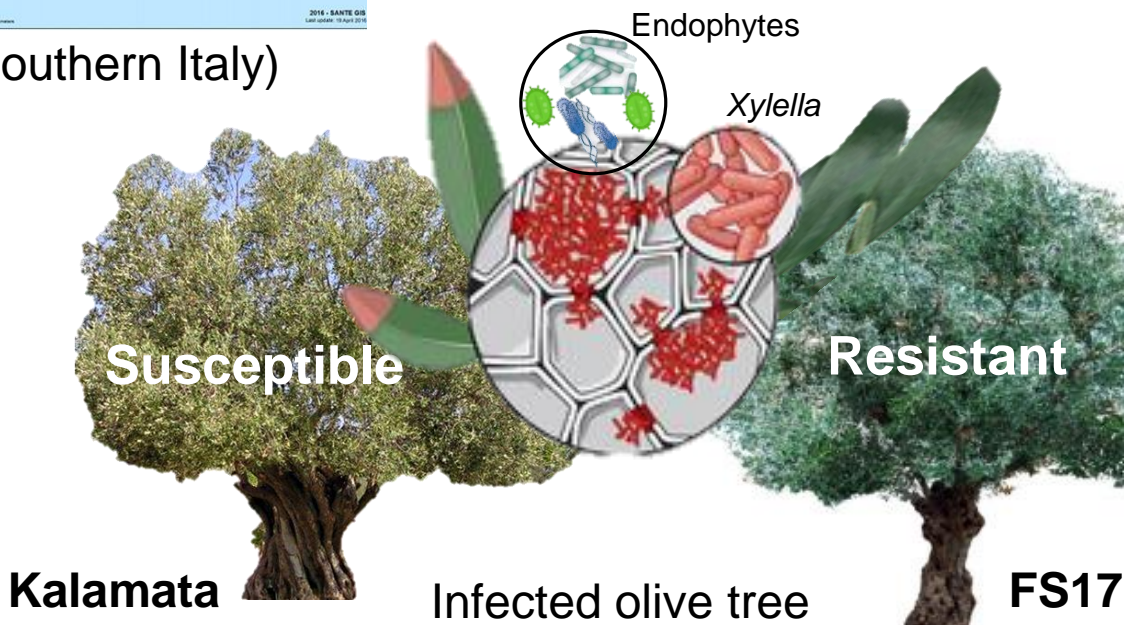


Olive Quick Decline Syndrome (OQDS)



Apulian Region (southern Italy)

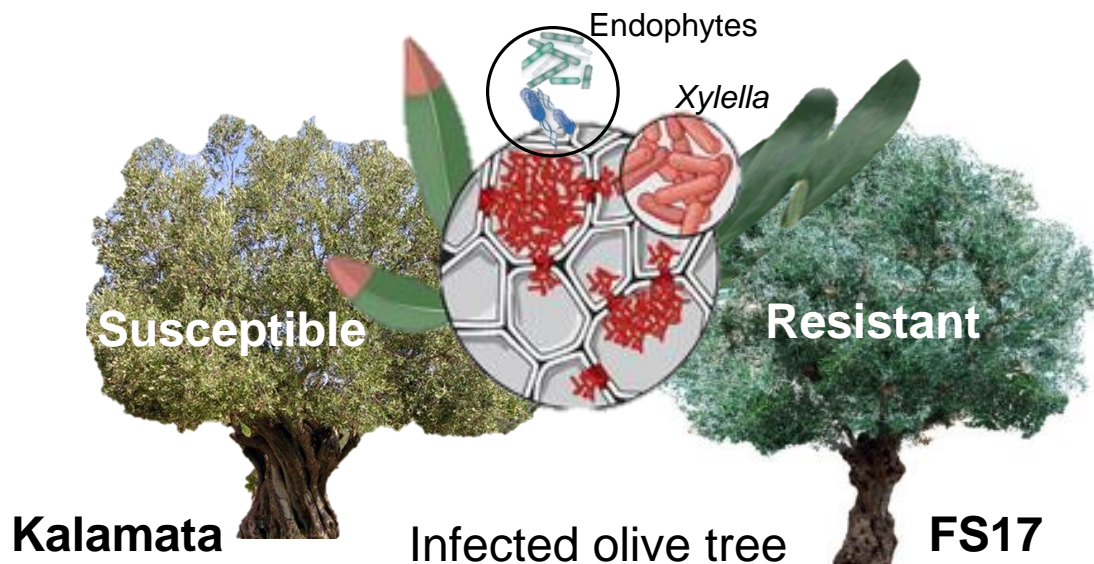
Framework



Does the endophytic microbiota play any role in disease resistance?



Aims



- 1 Evaluate the dynamics of endophytes and *Xylella* communities in susceptible and resistant olive cultivars
- 2 Evaluate the role of endophytic communities in the expression of OQDS symptoms
- 3 Identify potential endophytes with antagonistic activity against *Xylella*



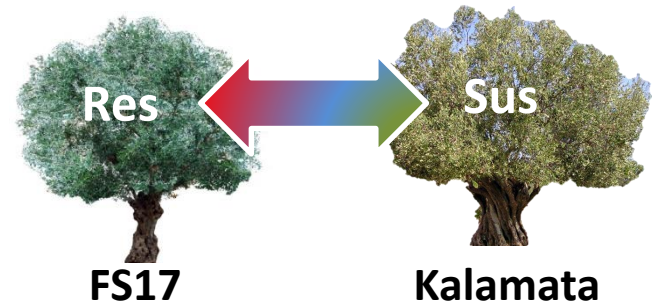
Xylem vessel

Approach

Olive twigs collection



Collection in Sannicola (outbreak area in Italy)



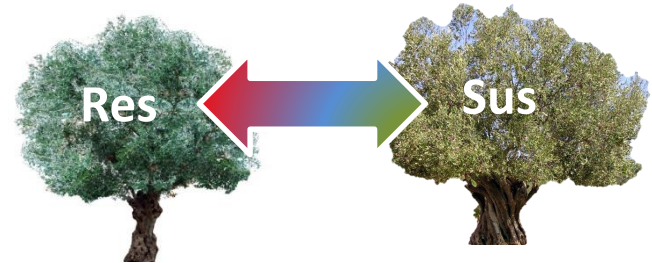
April 2017 and November 2018

DNA extraction from xylem wood shavings

Xylella abundance



qPCR



12 trees FS17 (6 High; 6 Low)

12 trees Kalamata (6 High; 6 Low)

Microbiome

DNA shotgun sequencing

Illumina

Bacteria

Fungi

Bacteria

Fungi

NextSeq 500 with 2x150bp reads

400 M read pairs/ 120 Gb raw data

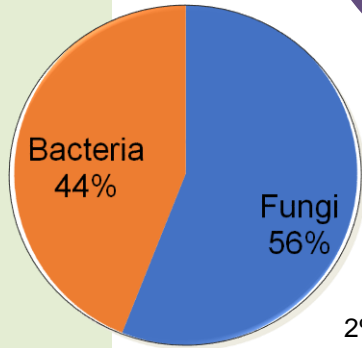


V3-V4 region

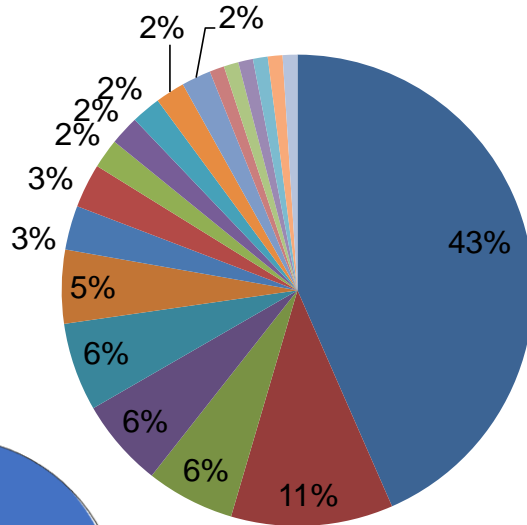


ITS1 region

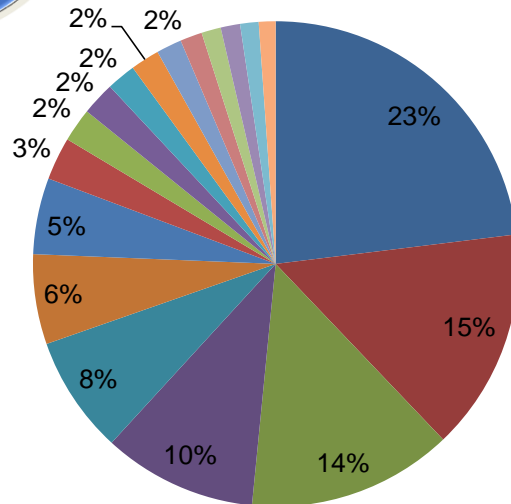
Overall microbial community



Bacteria



Fungi



- Xylella
- Asaia
- Others
- Methylobacterium
- Friedmanniella
- Pseudomonas
- Sphingomonas
- Streptomyces
- Klebsiella
- Mycobacterium
- Bacillus
- Staphylococcus
- Mycoplasma
- Hymenobacter
- Bradyrhizobium
- Burkholderia
- Corynebacterium
- Lactobacillus
- Streptococcus

- Wallemia
- Mitosporidium
- Others
- Lobosporangium
- Colletotrichum
- Phycomyces
- Paracoccidioides
- Aspergillus
- Blastomyces
- Lodderomyces
- Puccinia
- Coccidioides
- Histoplasma
- Trichophyton
- Neurospora
- Thermothelomyces
- Marssonina
- Ascoidea

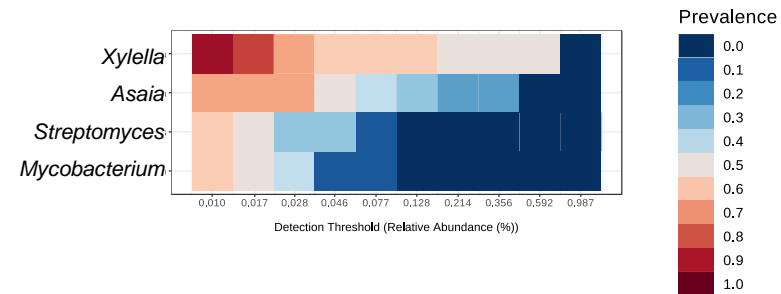
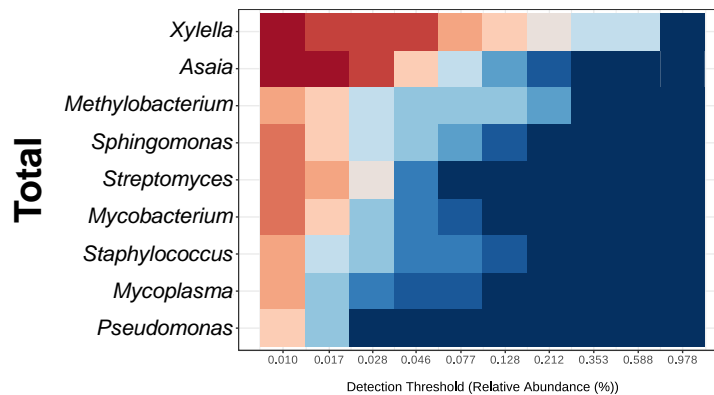
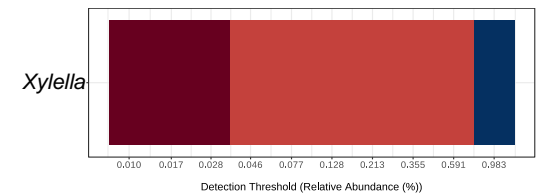
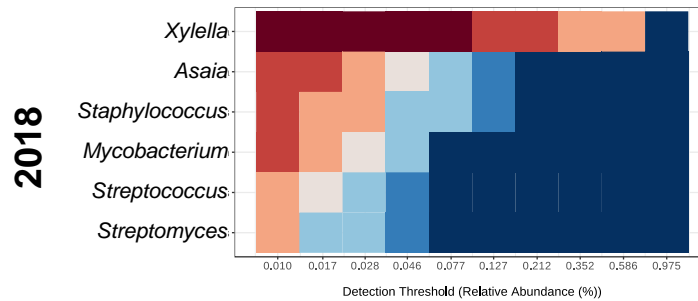
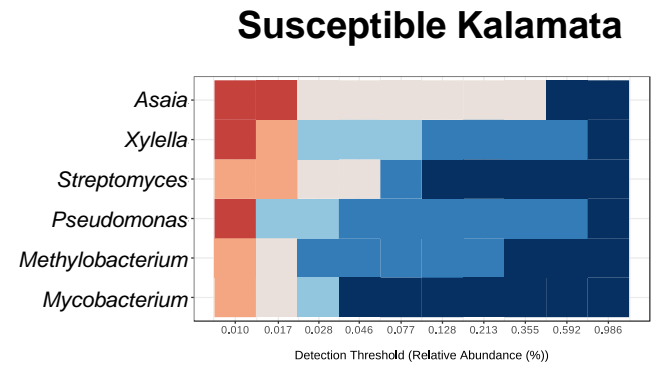
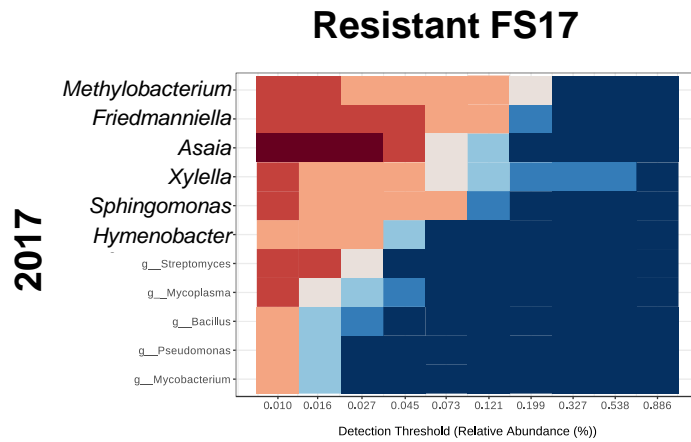
Proteobacteria (75%)
Actinobacteria (13%)



Ascomycota (44%)
Basidiomycota (25%)

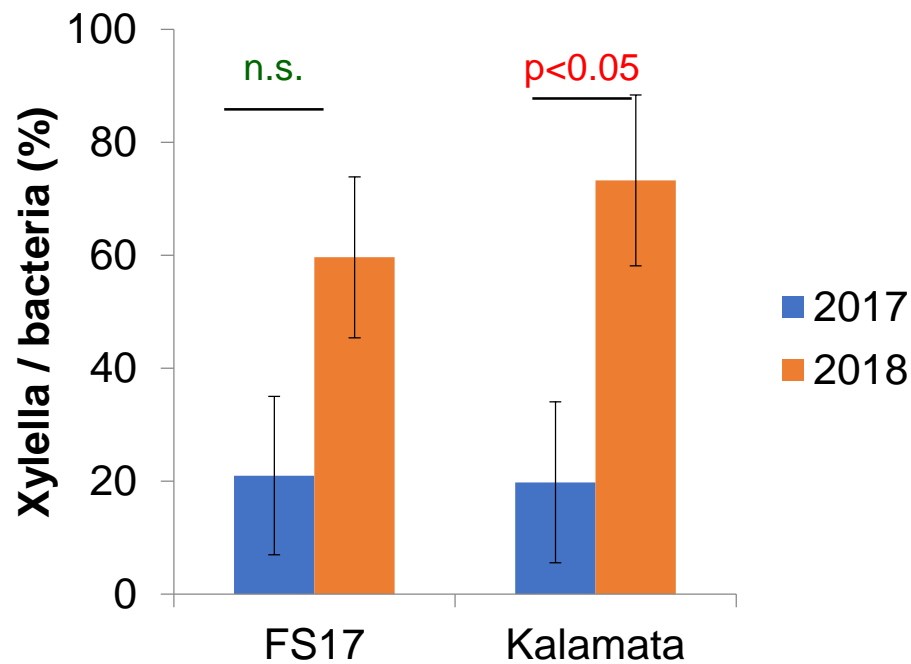


Core bacterial community



Genera having a 50% prevalence among samples and 0.01% abundance

Core bacterial community



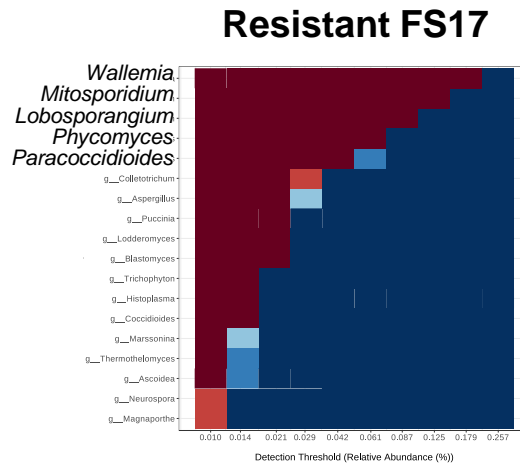
Average of *Xylella* abundance over the total Bacteria

Xylella tends to totally occupy the internal tissues

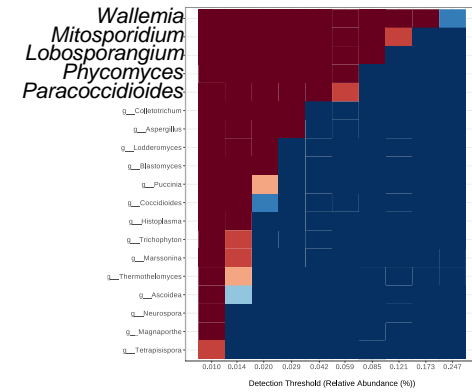
This was particularly obvious in the cv Kalamata

Core fungal community

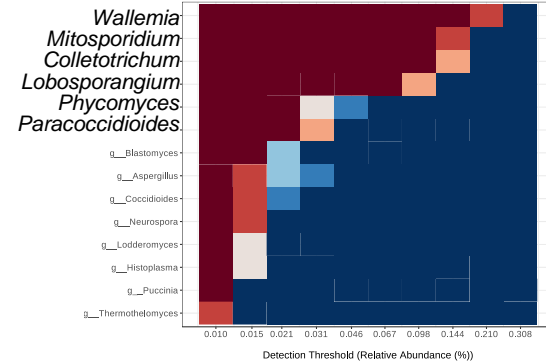
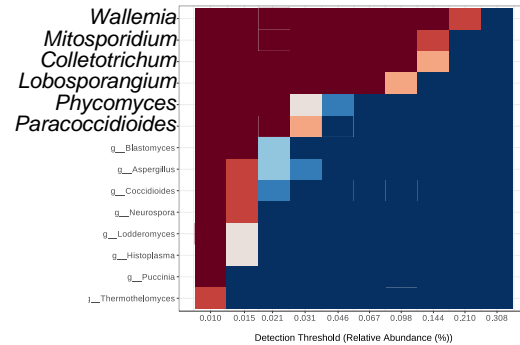
2017



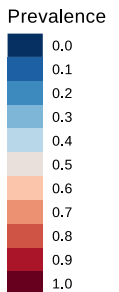
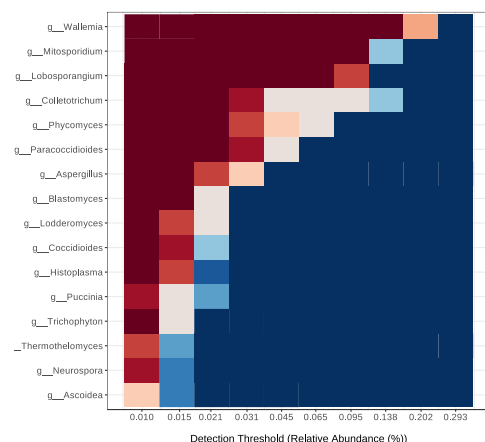
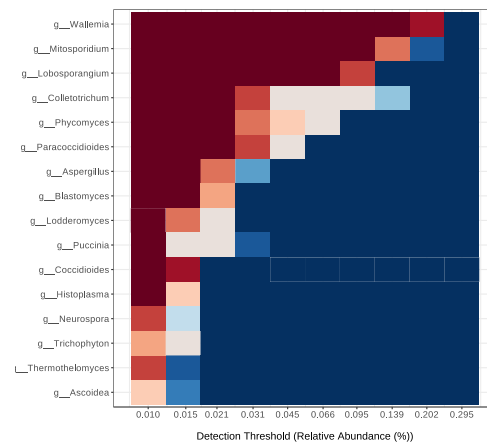
Susceptible Kalamata



2018

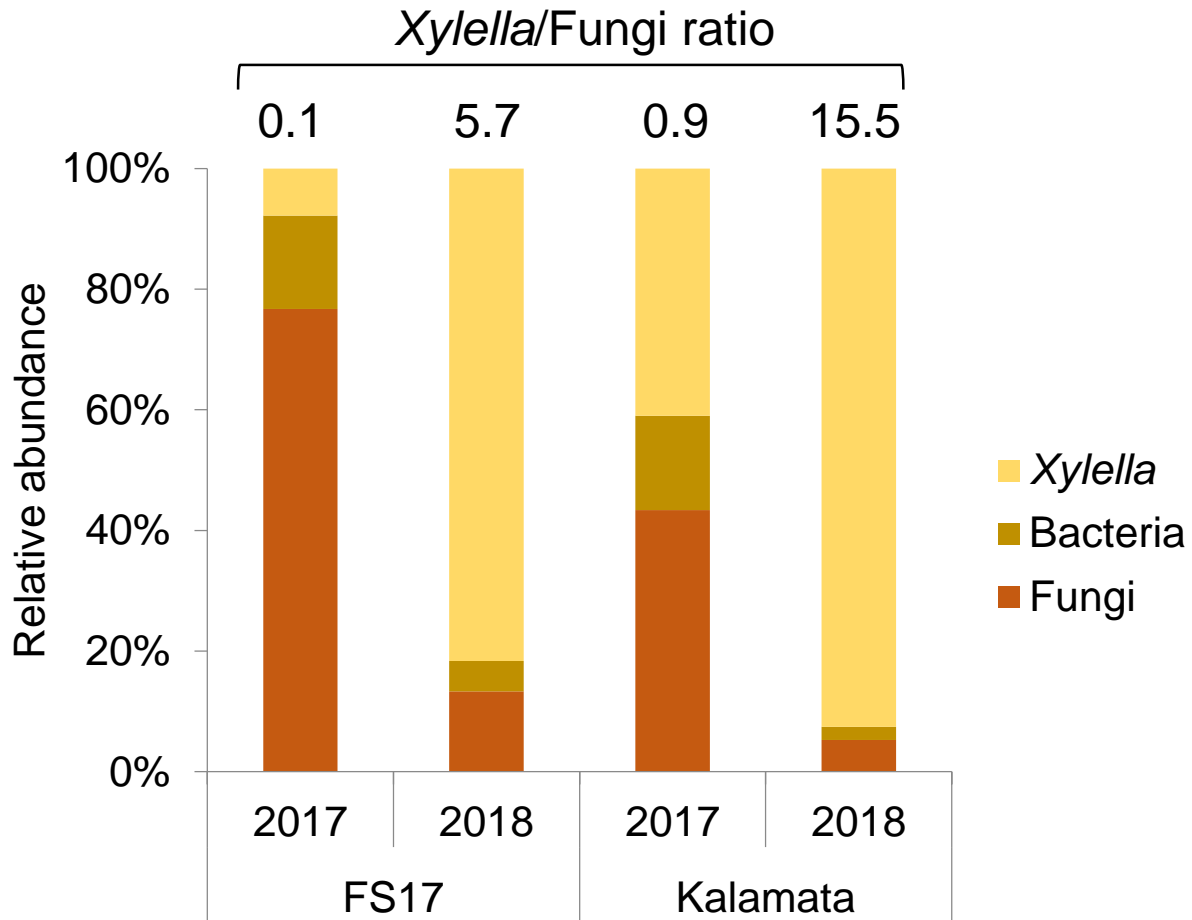


Total



Genera having a 50% prevalence among samples and 0.01% abundance

Core fungal community

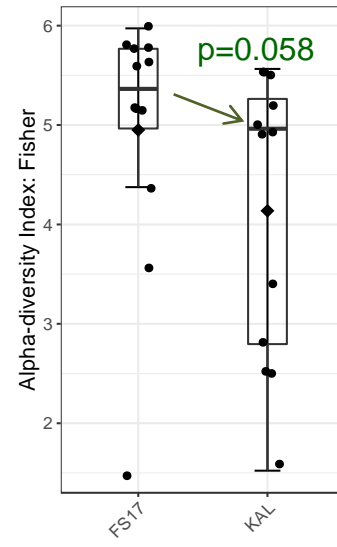
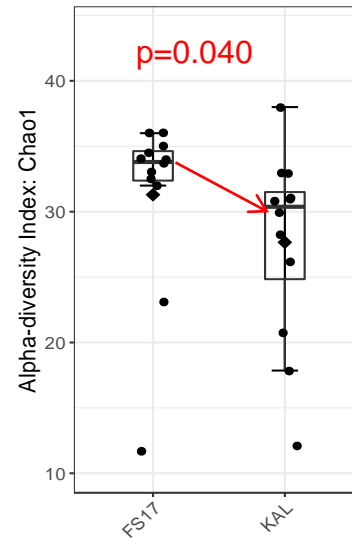
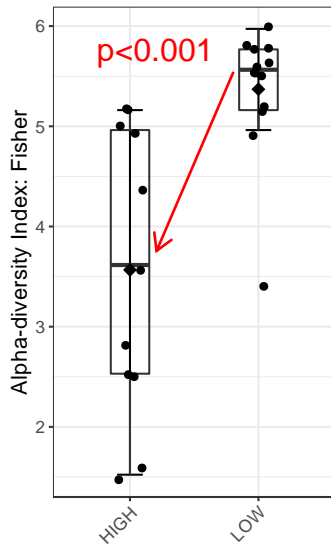
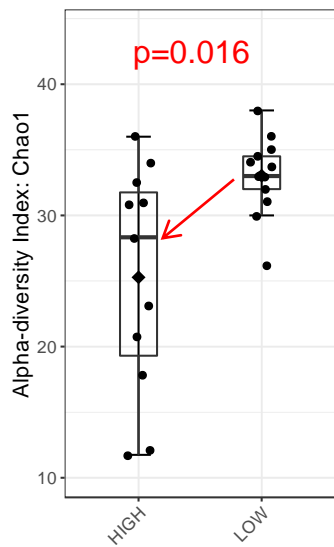


Comparison of *Xylella* abundance over the total Fungi

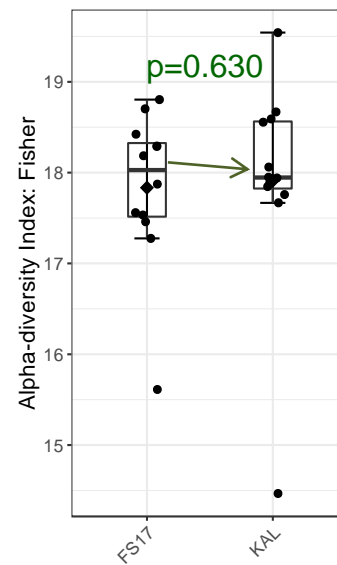
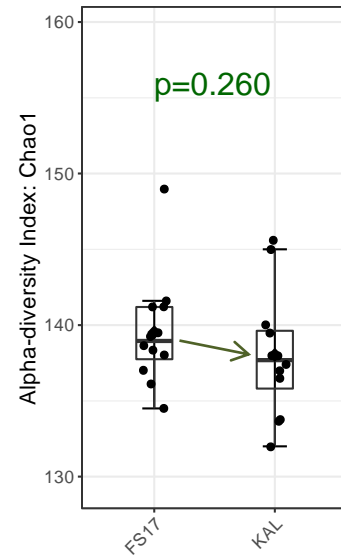
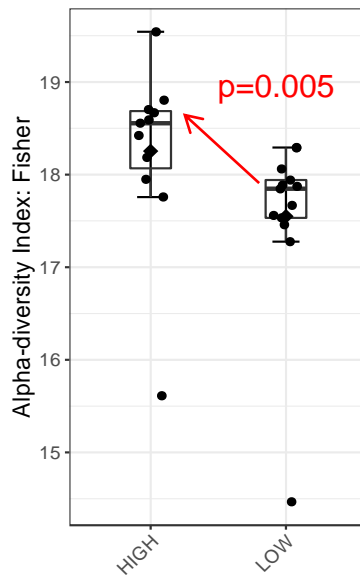
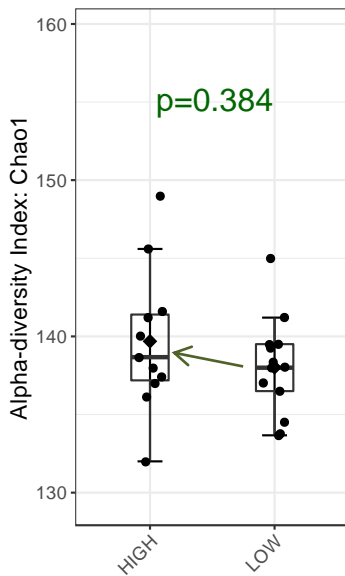
The increase in *Xylella* abundance in the second year inverts the *Xylella*/Fungi ratio, especially in Kalamata

Overall microbial diversity

Bacteria

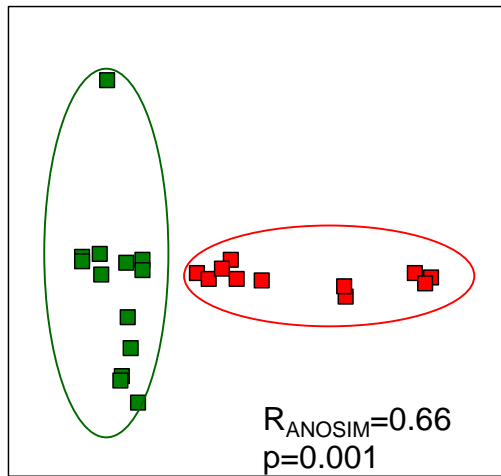


Fungi

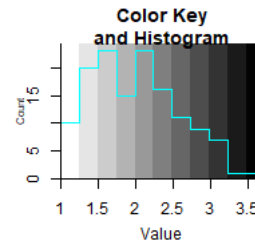


Overall microbial composition

Bacteria



■ Low
■ High

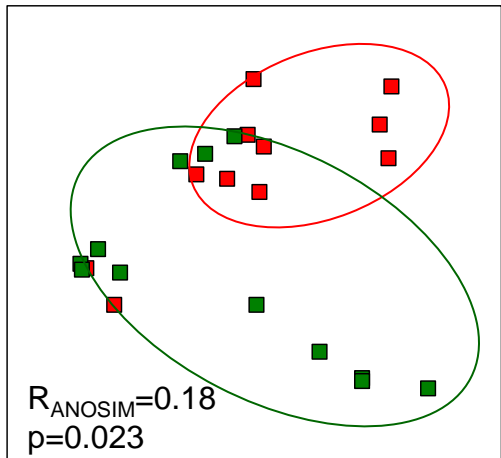


Non-metric multidimensional scale plot

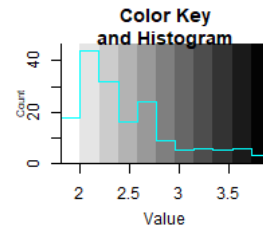


FS17 Kal FS17 Kal
Low High

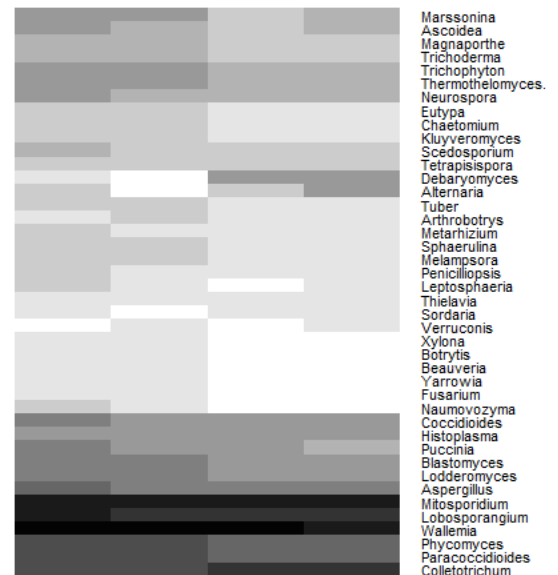
Fungi



■ Low
■ High

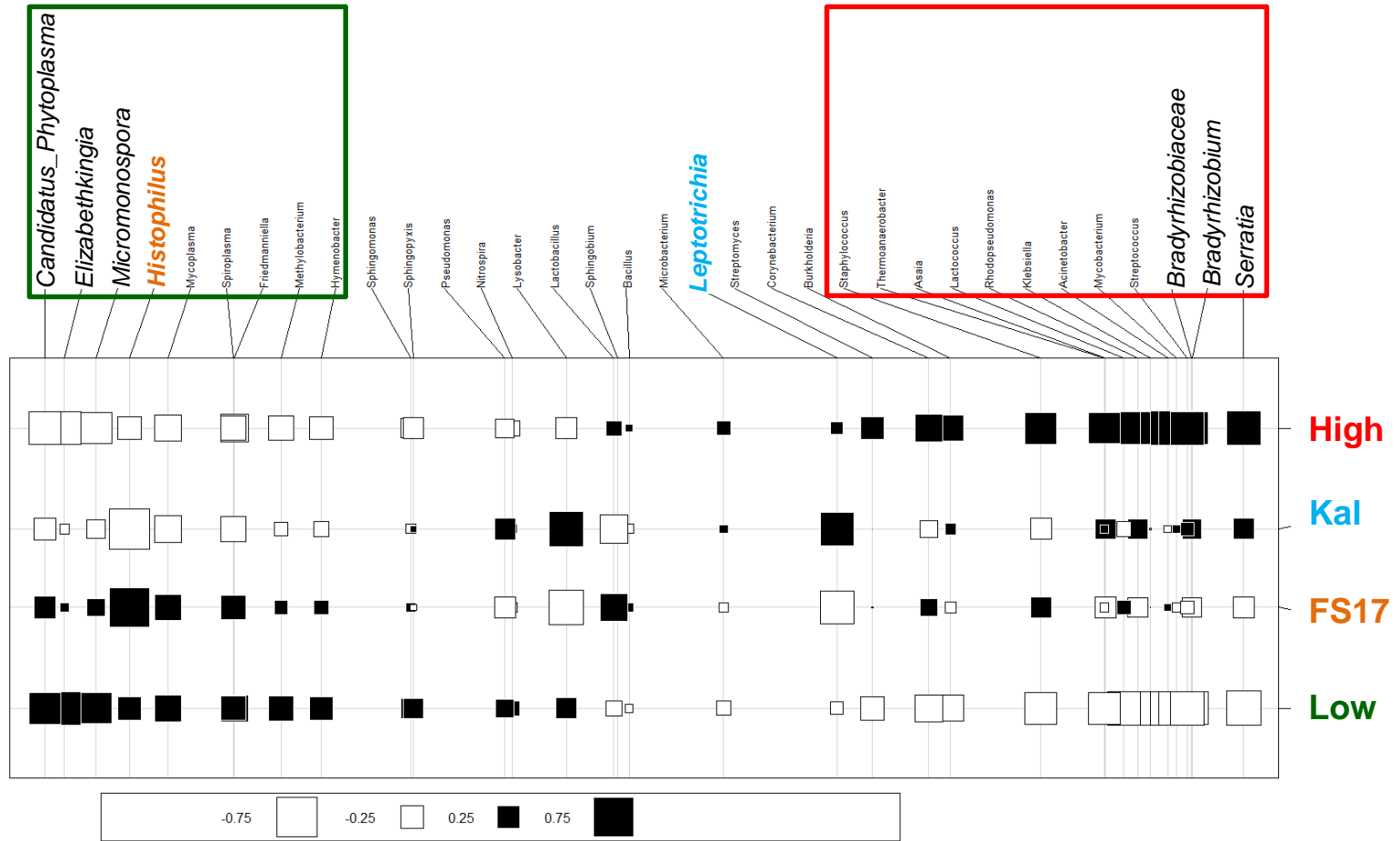


Non-metric multidimensional scale plot



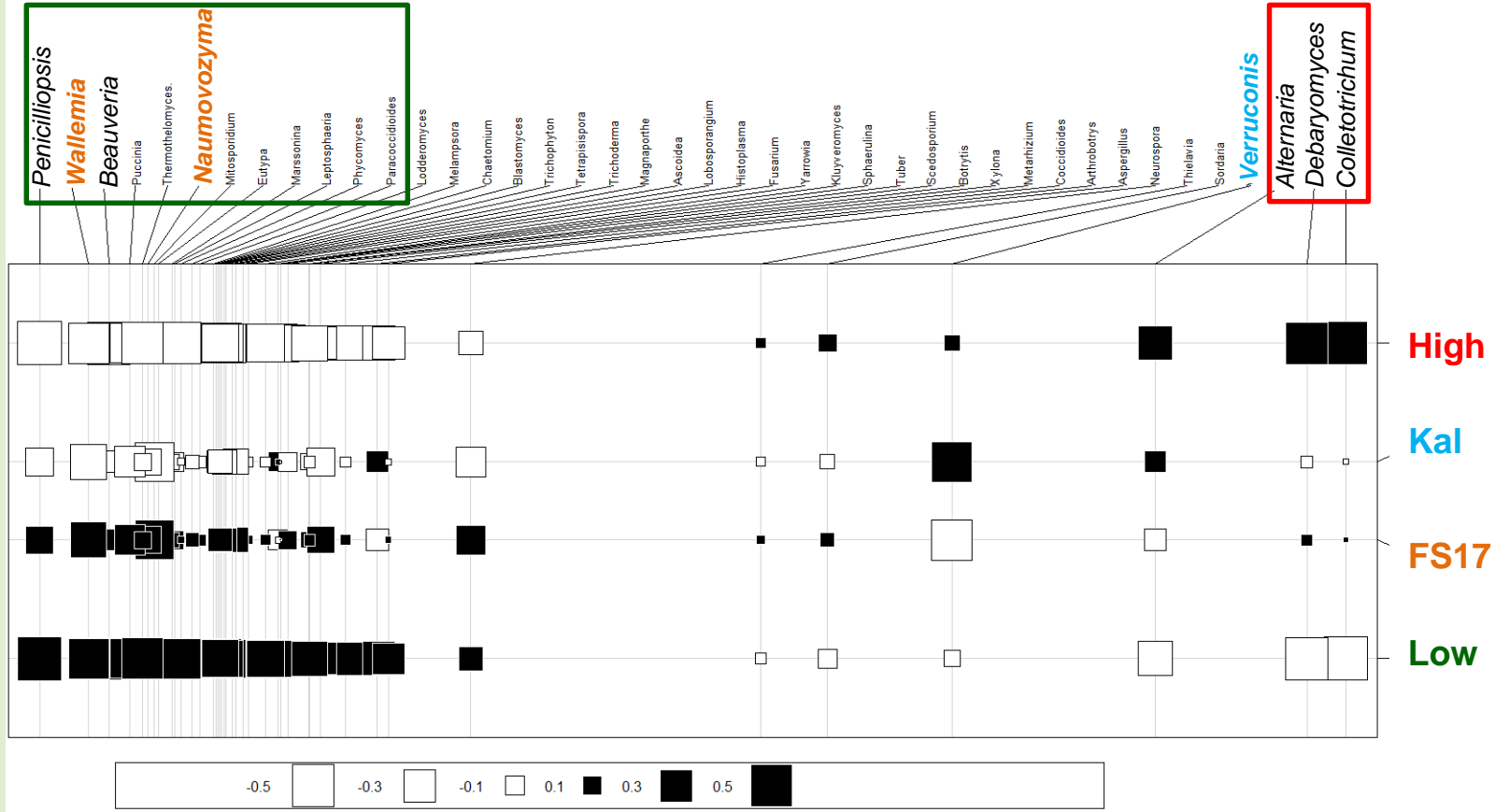
FS17 Kal FS17 Kal
Low High

Endophytic bacteria-Xylella relationship

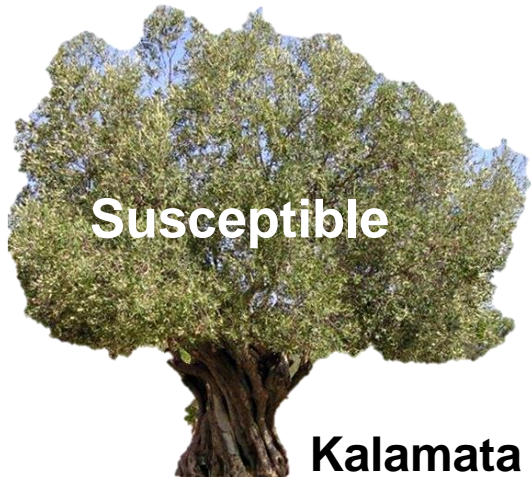


Co-inertia factorial map of bacterial communities, presenting positive (■) and negative (□) relationships with cultivars and *Xylella* abundance

Endophytic fungi-Xylella relationship



Co-inertia factorial map of fungal communities, presenting positive (■) and negative (□) relationships with cultivars and *Xylella* abundance



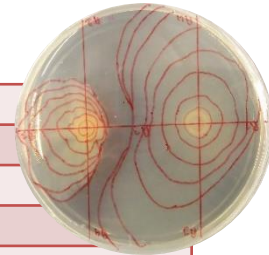
- Methylobacterium pseudosasicola*
- Curtobacterium ammoniigenes*
- Curtobacterium citreum*
- Sphingomonas aerolata*
- Novosphingobium soli*
- Methylobacterium pseudosasicola*
- Sphingomonas aquatilis*
- Sphingomonas insulae*
- Sphingomonas aquatilis*
- Sphingomonas panacis*
- Sphingomonas aerolata*
- Methylobacterium pseudosasicola*
- Micrococcus yunnanensis*



None of the isolates tested showed antagonistic effect against *X. fastidiosa*



- Sphingomonas aerolata*
- Methylobacterium sp.*
- Methylobacterium phyllostachyos*
- Curtobacterium ammoniigenes*
- Methylobacterium pseudosasicola*
- Sphingomonas sp.*
- Agrococcus jenensis*
- Sphingomonas aquatilis*
- Methylobacterium phyllostachyos*
- Methylobacterium pseudosasicola*
- Methylobacterium sp.*
- Sphingomonas sp.*
- Sphingomonas aquatilis*



Xylella dominates the bacterial endophyte community of both cultivars, and becomes the unique genus of the core bacterial community of the susceptible cv. Kalamata, in the 2nd year

This increase in *Xylella* abundance changed drastically the diversity and composition of bacterial community, particularly in the susceptible cv. Kalamata

These changes might be due to the presence of *Xylella* that probably encourage the establishment of other bacterial species

Specific bacterial /fungal genera were associated to the high or low abundance of *Xylella* in each cultivar, and their ability to trigger disease should be studied in the future

Acknowledgments



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