



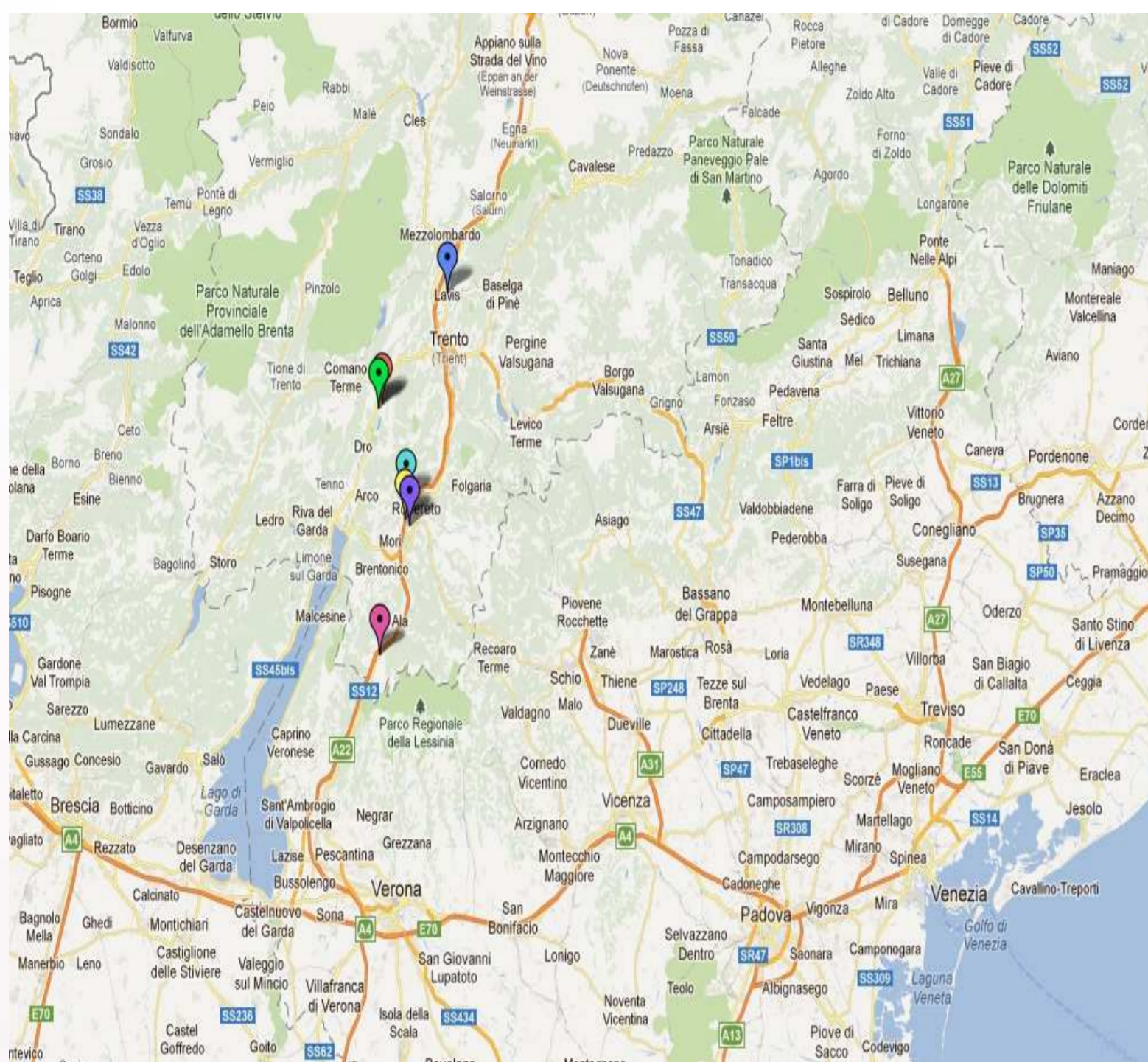
# Grapevine bacterial endophytes control multiple grapevine pathogens

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## ABSTRACT

A collection of bacterial endophytes isolated from wild and domesticated grapevine was characterised for 30 features, arranged in five major groups: plant growth promotion (PGP), antibiotic resistance (AB), secretion of enzymes (ENZ), quorum sensing (QS) and biocontrol (BICO). Many bacterial strains showed a high biocontrol potential against known grapevine pathogens *in vitro* (*Botrytis cinerea*, *Botryosphaeria dothidea*, *Botryosphaeria obtusa*, *Phaeoacremonium aleophilum*, *Phaeoacremonium aleophilum*) and *in vivo* (*B. cinerea* and *Plasmopara viticola*). Interestingly, effective biocontrol agents were classified into several, often distantly related genera. By comparing bacterial strains from wild and cultivated grapevines, we show that the strains with the best biocontrol activity against multiple pathogens were mostly isolated from wild grapevine. Conversely, strains from domestic grapevines were generally more efficient in the *in vitro* biocontrol of *P. chlamydospora*. We also discuss here the other groups of analysed features, their distribution and possible ecological significance.



### Sample names and their characterization:

- we sampled domesticated grapevine (*V. vinifera* subsp. *vinifera*) from five locations and a total of 20 vineyards in the same county (Trentino, Northern Italy). In each location four vineyards were sampled, representing each of the four treatments: organic and integrated pest management (IPM) and cultivars Chardonnay and Merlot.
- we sampled wild grapevines from *V. vinifera* subsp. *sylvestris* plants obtained from different areas of Italy. Plants from North (11), Centre (22), South (18) and Insular (5) Italy were used for this study.

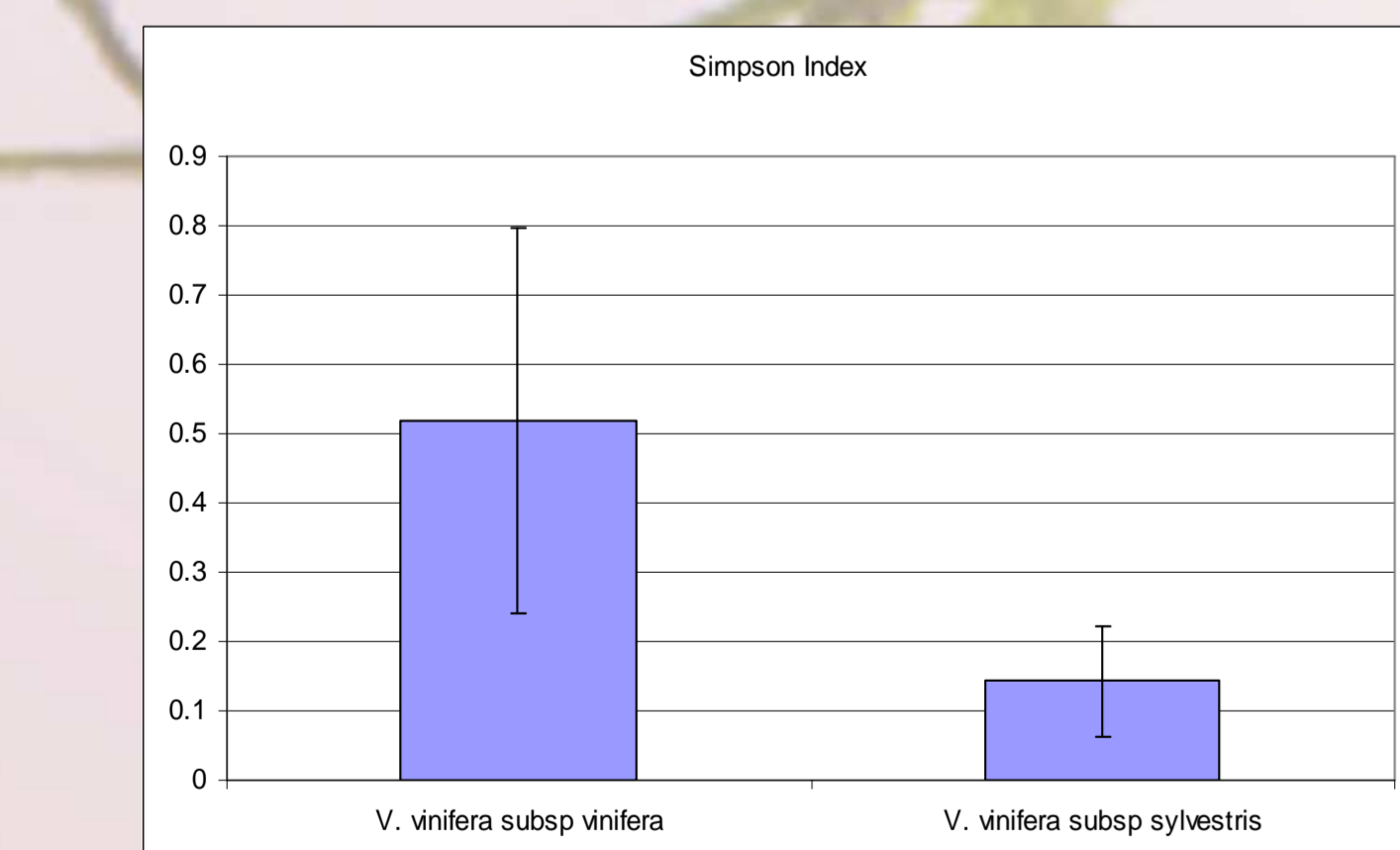
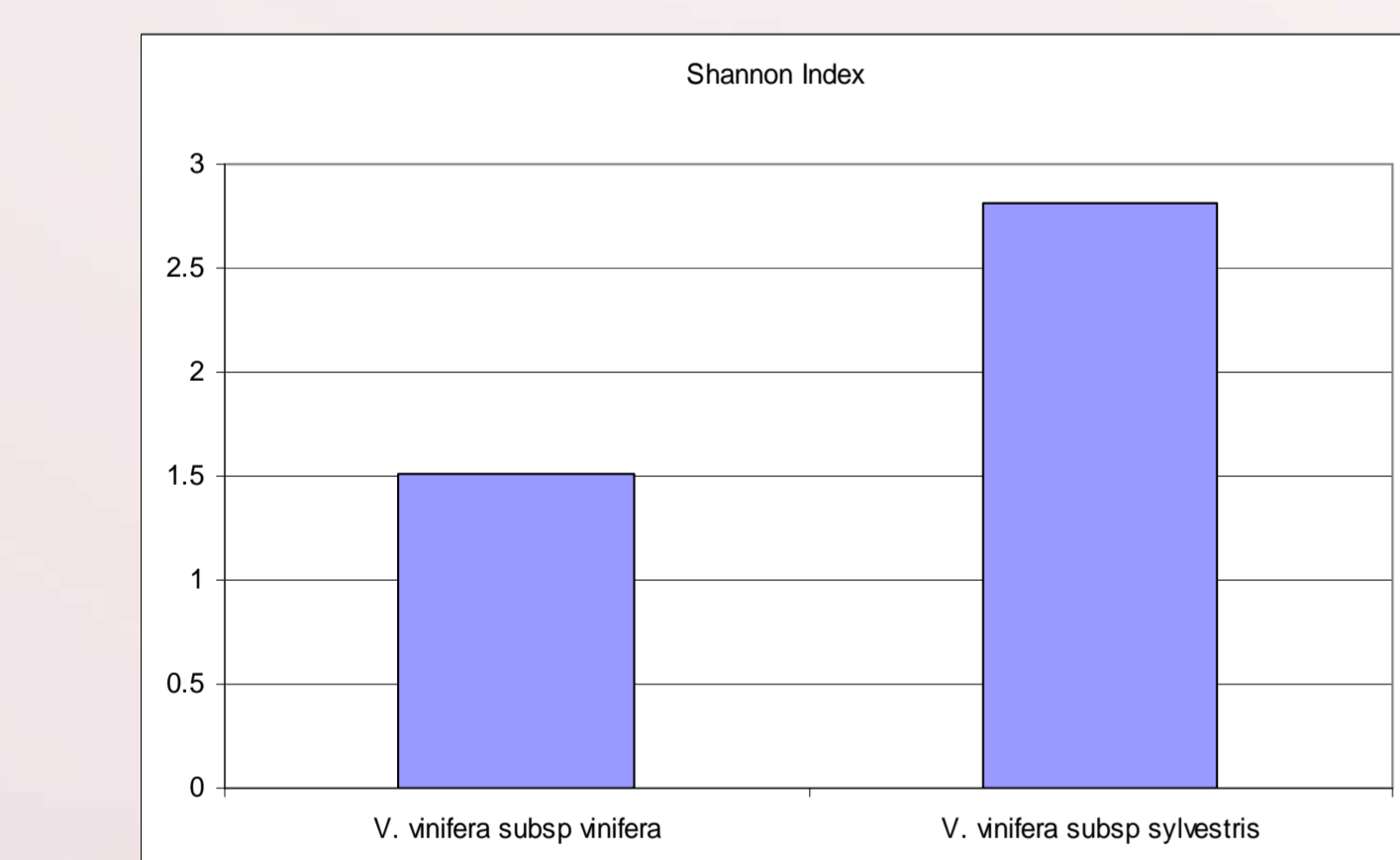
### Identification of endophytic bacteria.

All bacterial isolates were identified by sequencing the 16S rDNA region. We considered isolates assigned to the same species and isolated from the same plant not to be unique. Of hundreds of endophytic bacteria, we selected 160 "unique" isolates. Unique isolates were grouped into 28 genera.

The isolates from **cultivated grapevines**, were grouped in only **7 genera**, while isolates from **wild grapevines** were grouped in **26 genera**. Only bacteria belonging to the genera *Erwinia* and *Rhodotorula* were isolated only from domesticated grapevines.

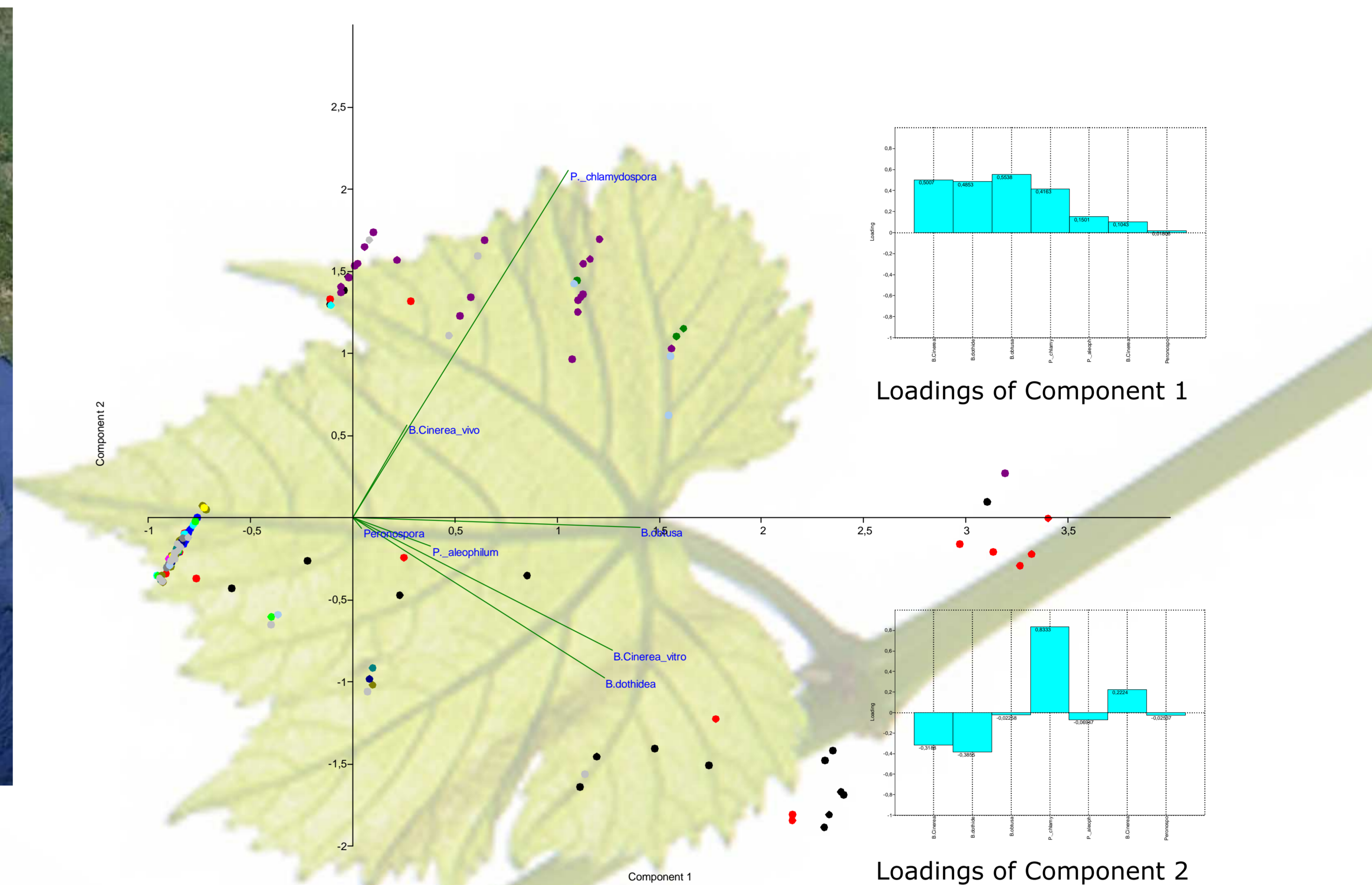
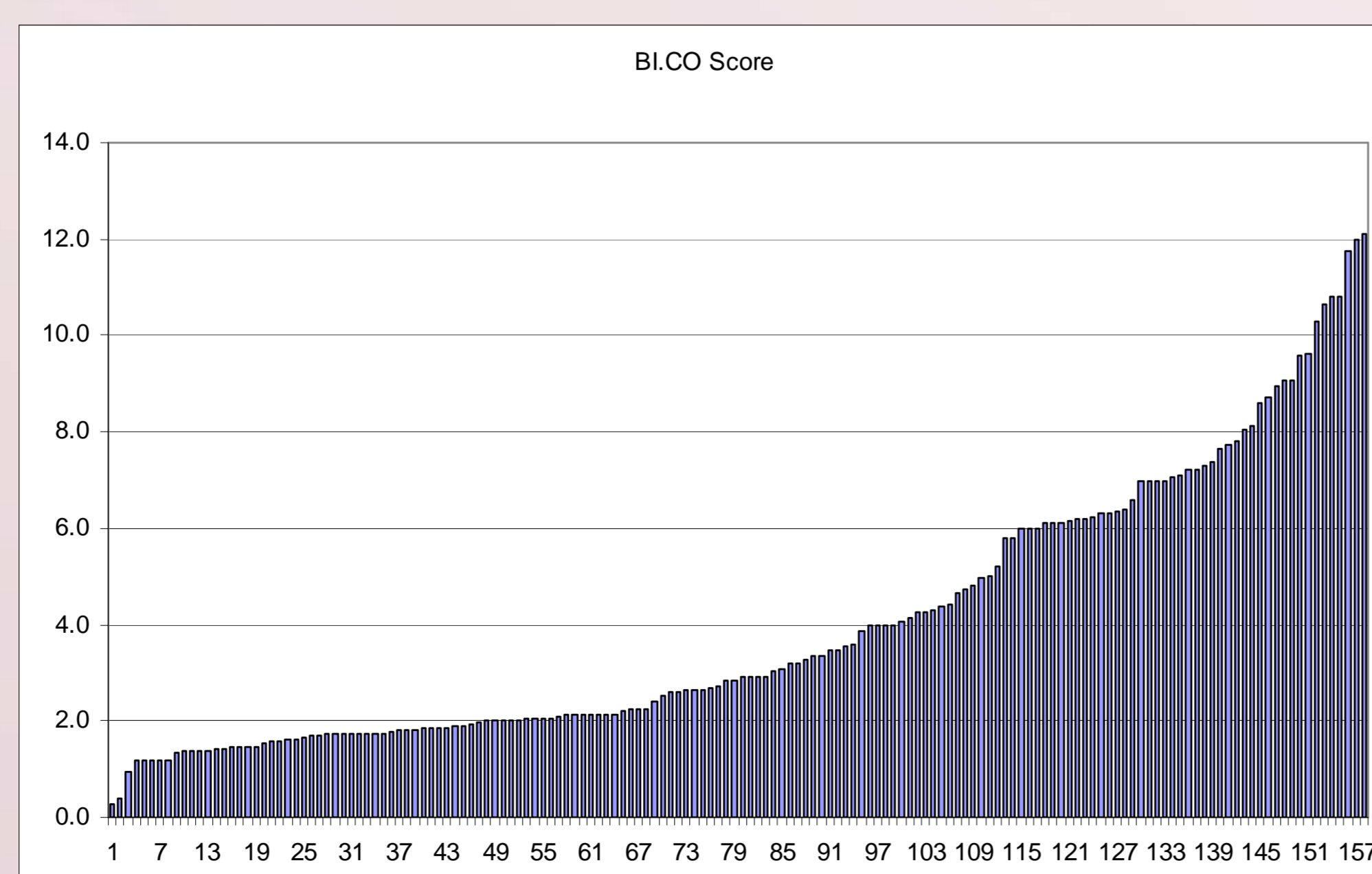
The analysis of diversity indices for the two sets of isolates strongly suggest a higher species diversity in the endosphere of wild grapevines. This is in agreement with the observation of a much higher number of taxa in wild grapevines.

The Simpson index also suggests a higher degree of taxonomic concentration in endophytic microbial communities of domesticated grapevines. Approximation bars associated with Simpson indices do not overlap, hinting to significantly different communities in the two types of grapevines.



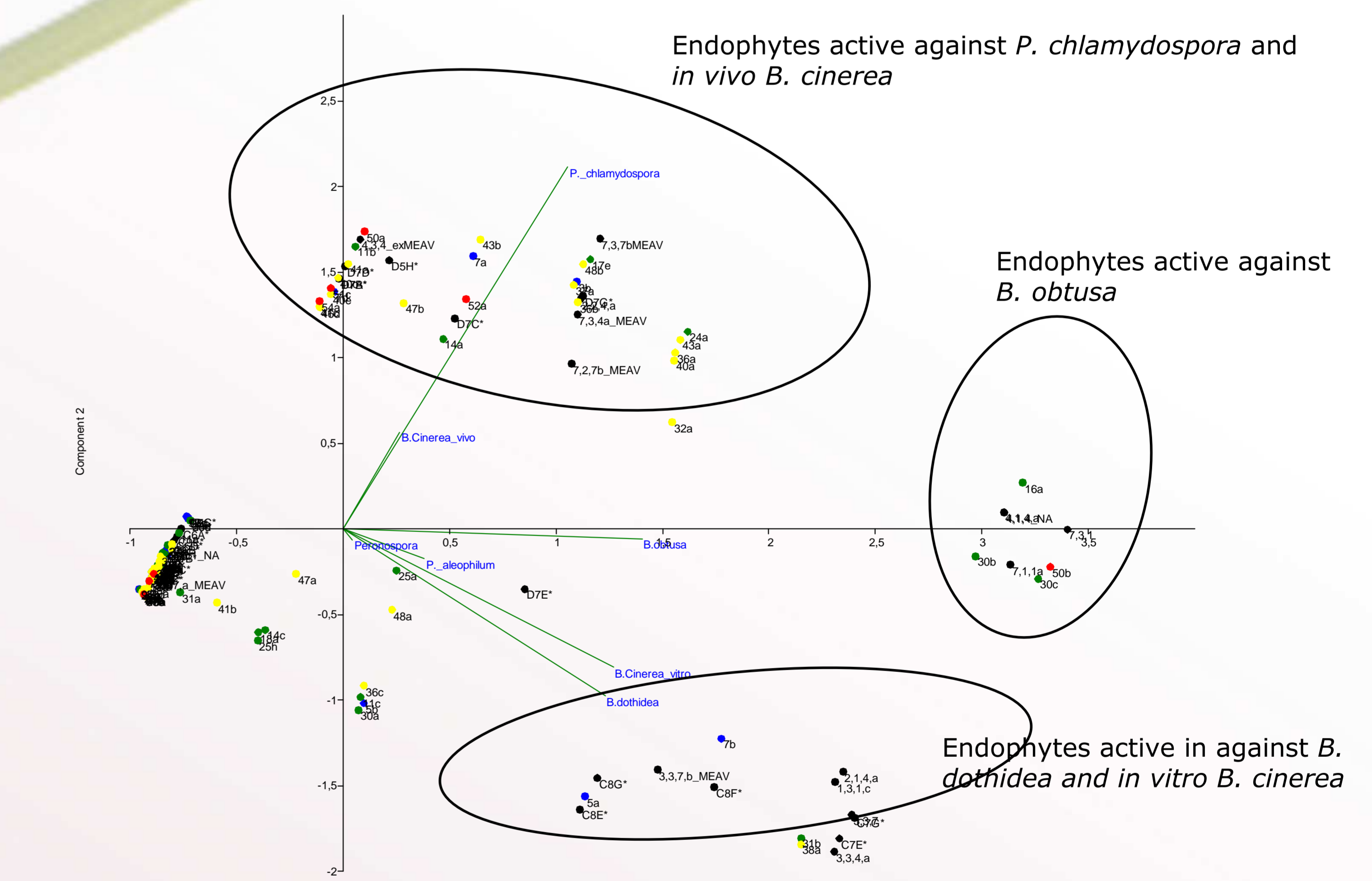
### Biocontrol performance score of all 160 tested strains.

The score is calculated as the sum of seven individual biocontrol tests: two *in vivo* tests, and five *in vitro* tests. Each test score ranges from zero to two. We set an arbitrary value of eight to select "high biocontrol potential" (HBP) endophytes. Sixteen strains (10%) were scored as HBP microorganisms.



### Principal Component Analysis (PCA) scatter plot obtained from biocontrol data of the 160 bacterial endophytic strains analysed here.

Colour legenda: Black = *Pseudomonas*; Red = *Bacillus*; Purple = *Pantoea*; Light blue = *Enterobacter*; Grey = *Leclercia*



**Principal Component Analysis (PCA) scatter plot.** The same scatter plot is shown here. We highlight the performance of isolates from domesticated (Black dots) and wild (Blue, Red, Yellow and Green, by geographic origin) grapevines. Interestingly, strong biocontrol agents were found, that were able to control the full range of grapevine pathogens analysed.

### Conclusions

Endophytic microbial communities in wild and domesticated grapevines seem to be different. Wild grapevine plants harbour a much more diverse array of taxa.

The most abundant genera of culturable bacterial endophytes were *Bacillus*, *Pseudomonas*, *Pantoea* and *Sphingomonas*. In cultivated grapevines, *Pseudomonas*, *Pantoea* and *Curtobacterium* were prevalent. In wild grapevine, *Bacillus* and *Sphingomonas* were more represented.

Surprisingly, despite the much lower microbial diversity in domesticated grapevines, bacterial endophytes from *V. vinifera* subsp. *vinifera* have a high biocontrol activity against all groups of tested grapevine pathogens.

This may suggest that the domestication process has impacted heavily on the biodiversity of bacterial endophytes, but bacteria conferring protecting plants against pathogens have been conserved.