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Exploring the microbiota of resistant varieties of grapes in organic farming

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Context and objectives

Grape varieties resistant to mildew and powdery mildew make it possible to reduce the use of phytosanitary products both in conventional and organic farming systems and as such can provide a solution to health and environmental concerns associated with fungicide use.

The aim of this project is to explore the microbiota of resistant grape varieties in comparison to conventional *Vitis vinifera* cultivars in various viticulture systems (conventional, organic and absence of treatment) in France and to determine the impact of the microbial communities on the fermentation kinetics with indigenous yeasts.



Experimental devices

- Three INRAE agricultural devices in Gruissan (Pech Rouge), Bordeaux and Marseillan (Vassal), and a private vineyard in Minervois.
- Four resistant hybrid cultivars: Floréal, G5, Artaban, G14.
- Five traditional cultivars: Muscat, Grenache, Syrah, Carignan, Merlot.
- Two vintages: 2021 and 2022



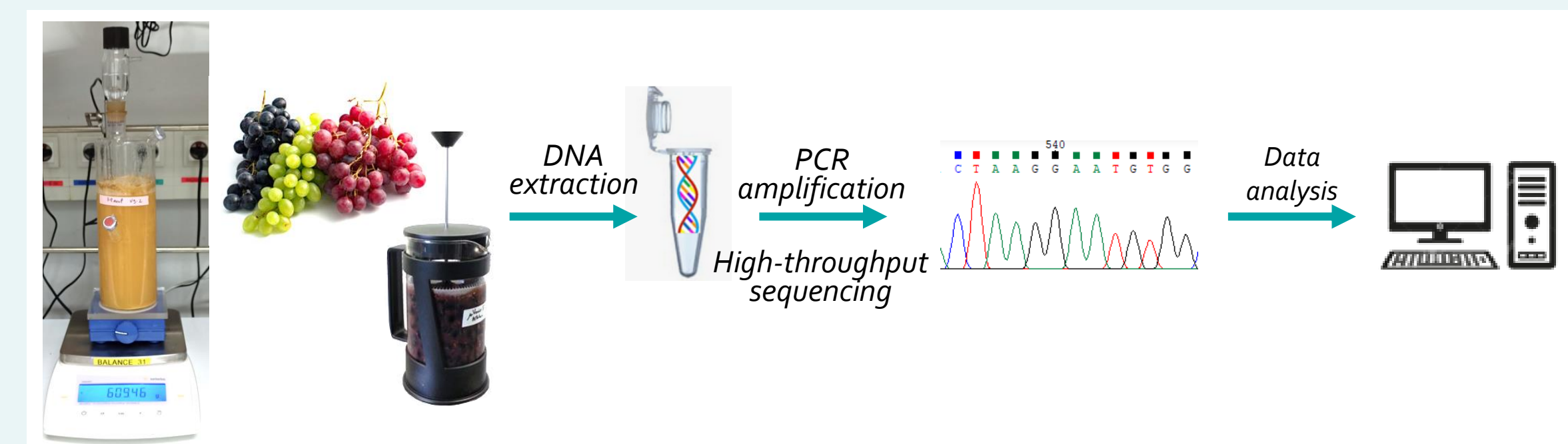
Sample collection and fermentations

Spontaneous fermentations were done at scale 1 L (white grapes) or 1 kg (red grapes) in triplicates. Samples for the microbiota analysis were taken from the surface of berries and at 2 stages of fermentation: at T0 and after 10 g/L of CO₂ released (T1).

Methodology

High-throughput amplicon sequencing approach

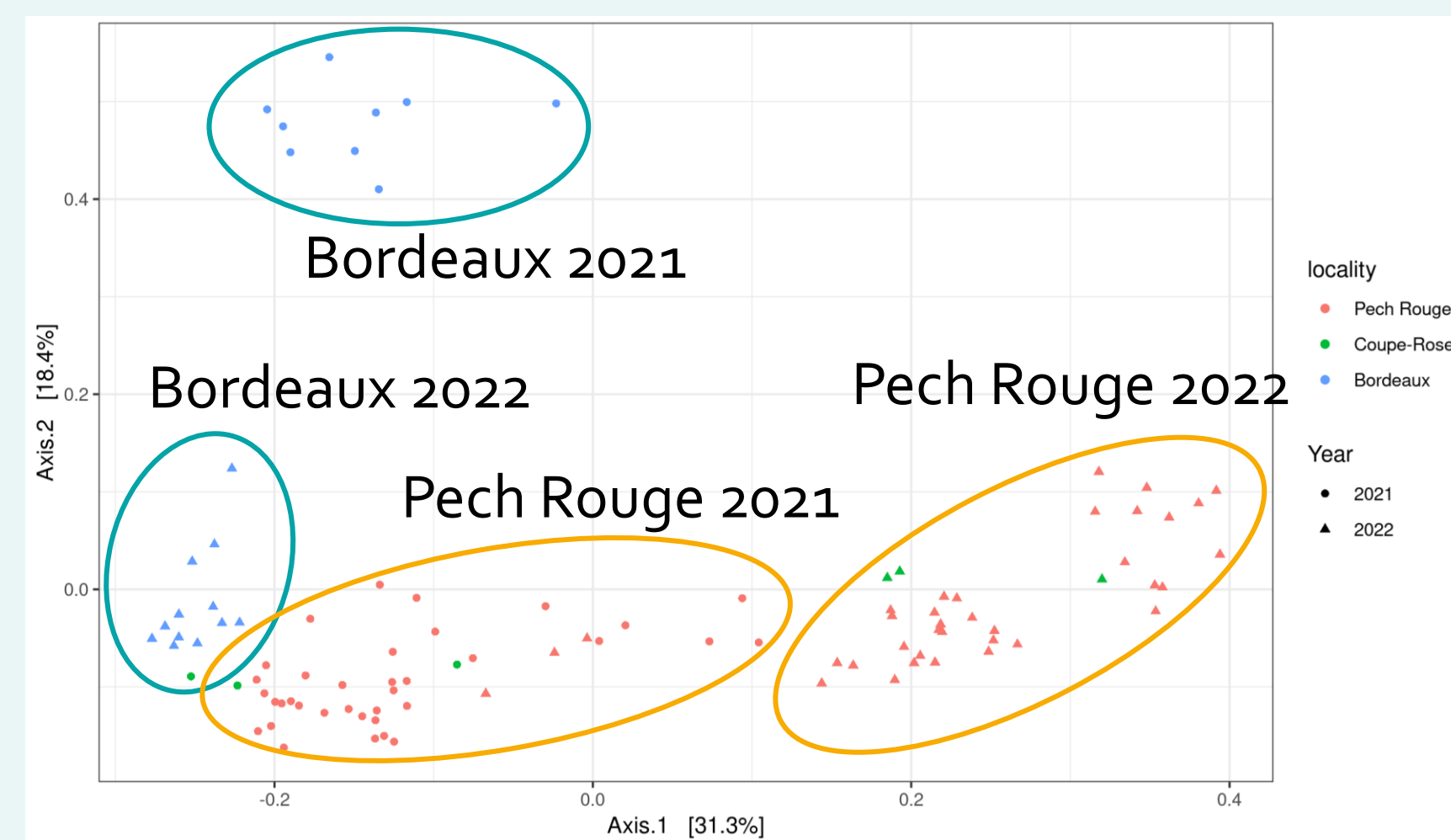
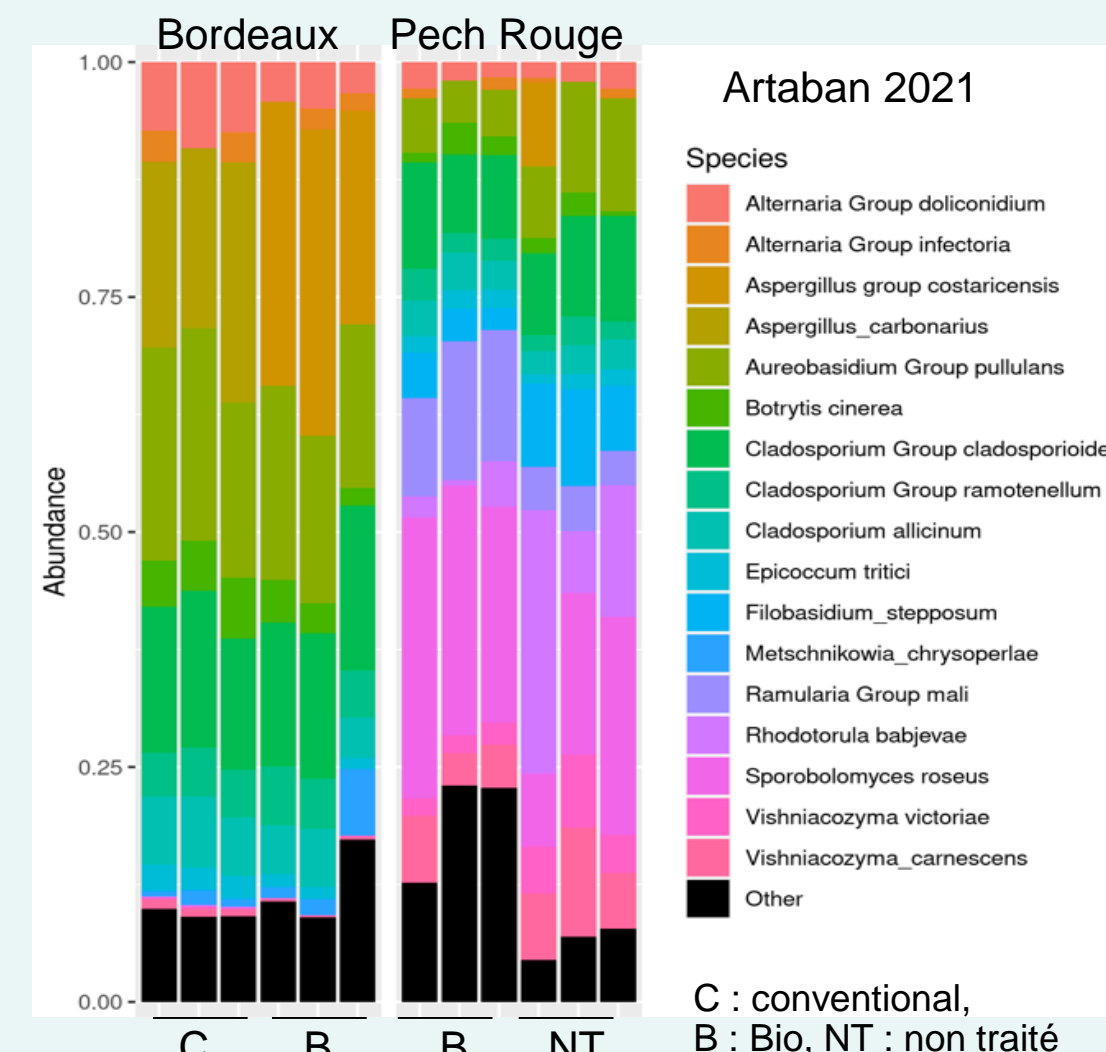
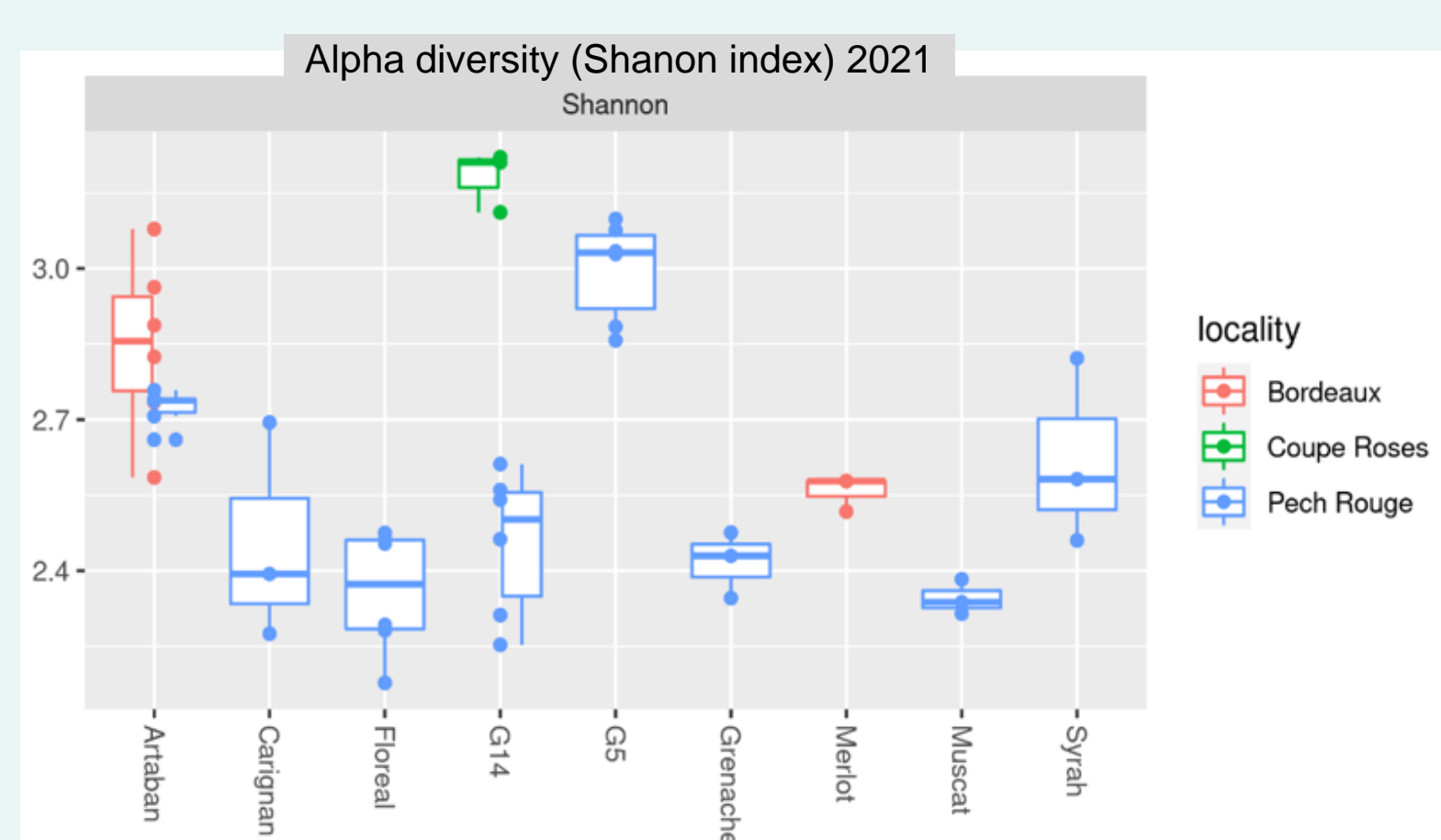
DNA from cell pellets was used to amplify the ITS1 region of the rRNA gene (Fungi). The amplicons were sequenced with the Illumina MiSeq sequencing system (2x 300 bp). The data (30,000 to 165,000 reads per sample) were analyzed with a FROGS/Dada2 strategy.



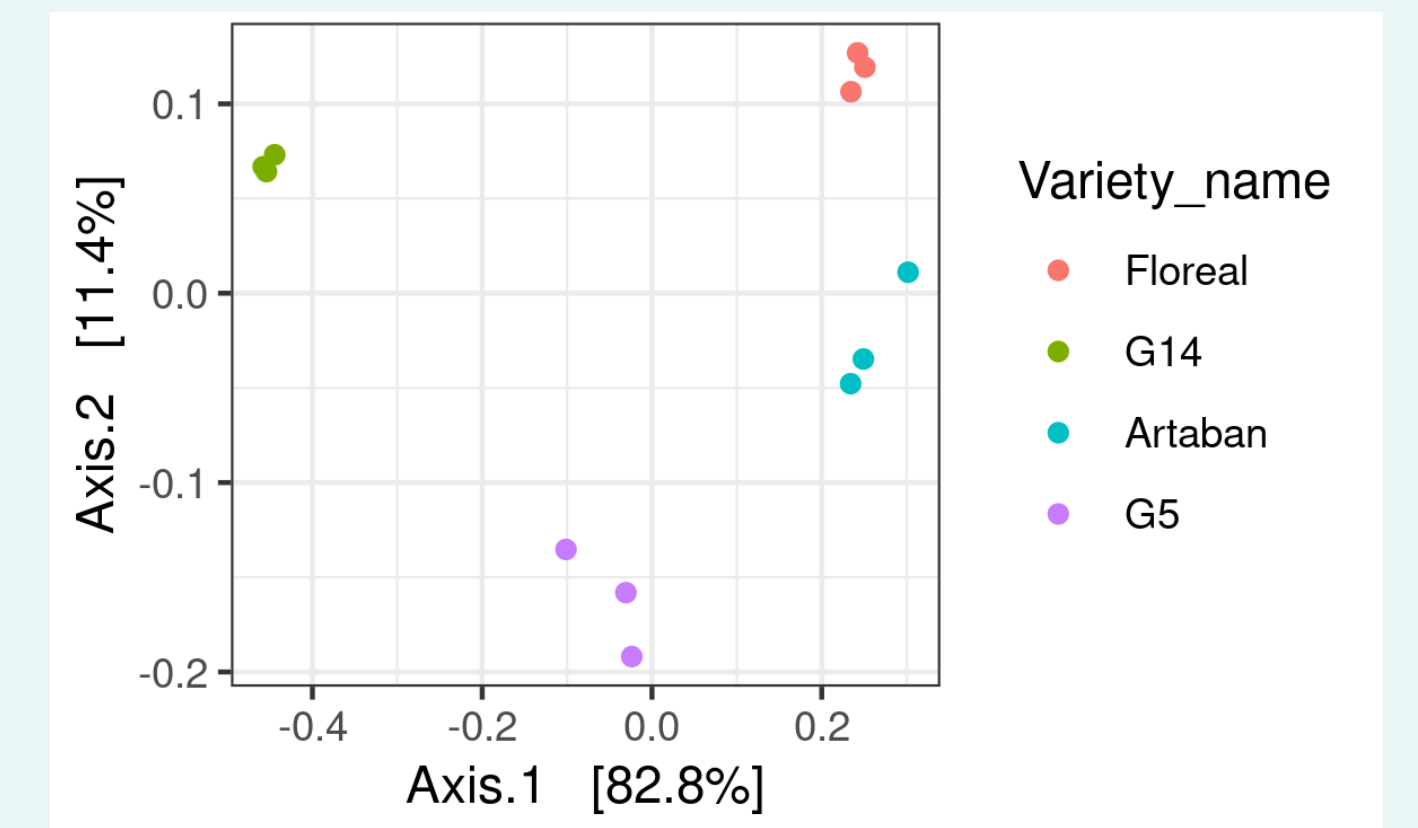
Main results

Diversity of berry microbiota: a strong impact of region and vintage

- ✓ Great difference in yeast diversity depending on the varieties and areas/regions (from 125 to more than 200 species per modality).
- ✓ Very few fermentative yeasts but many filamentous fungi including pathogenic species: powdery mildew exclusively present on non-resistant grape varieties.
- ✓ Region and vintage are the main factors of diversity, probably due to abiotic factors (climate and soil). Cultural practices did not show a significant effect.
- ✓ The variety effect is significant in 2021 at Pech Rouge and Vassal.

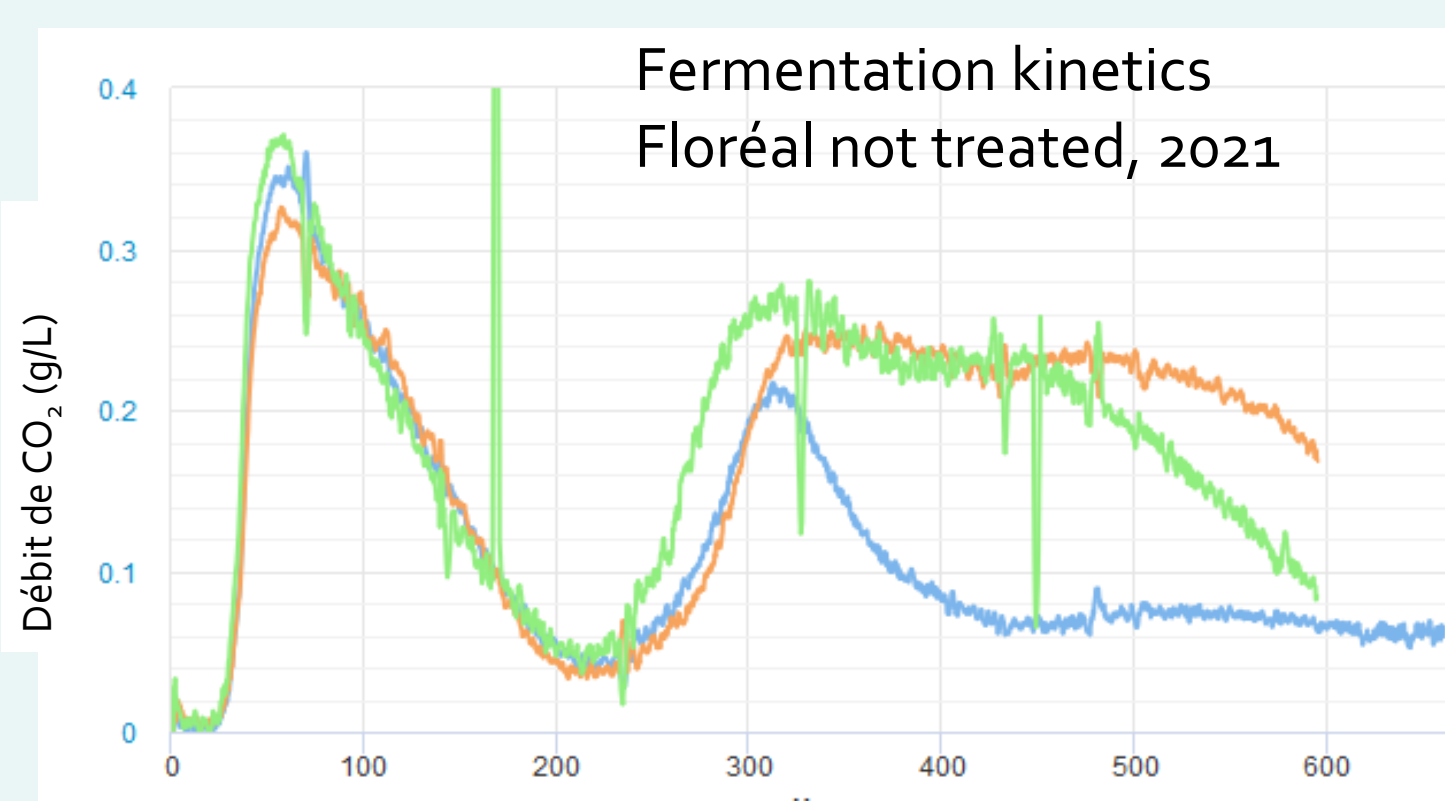


A significant effect of variety at Vassal

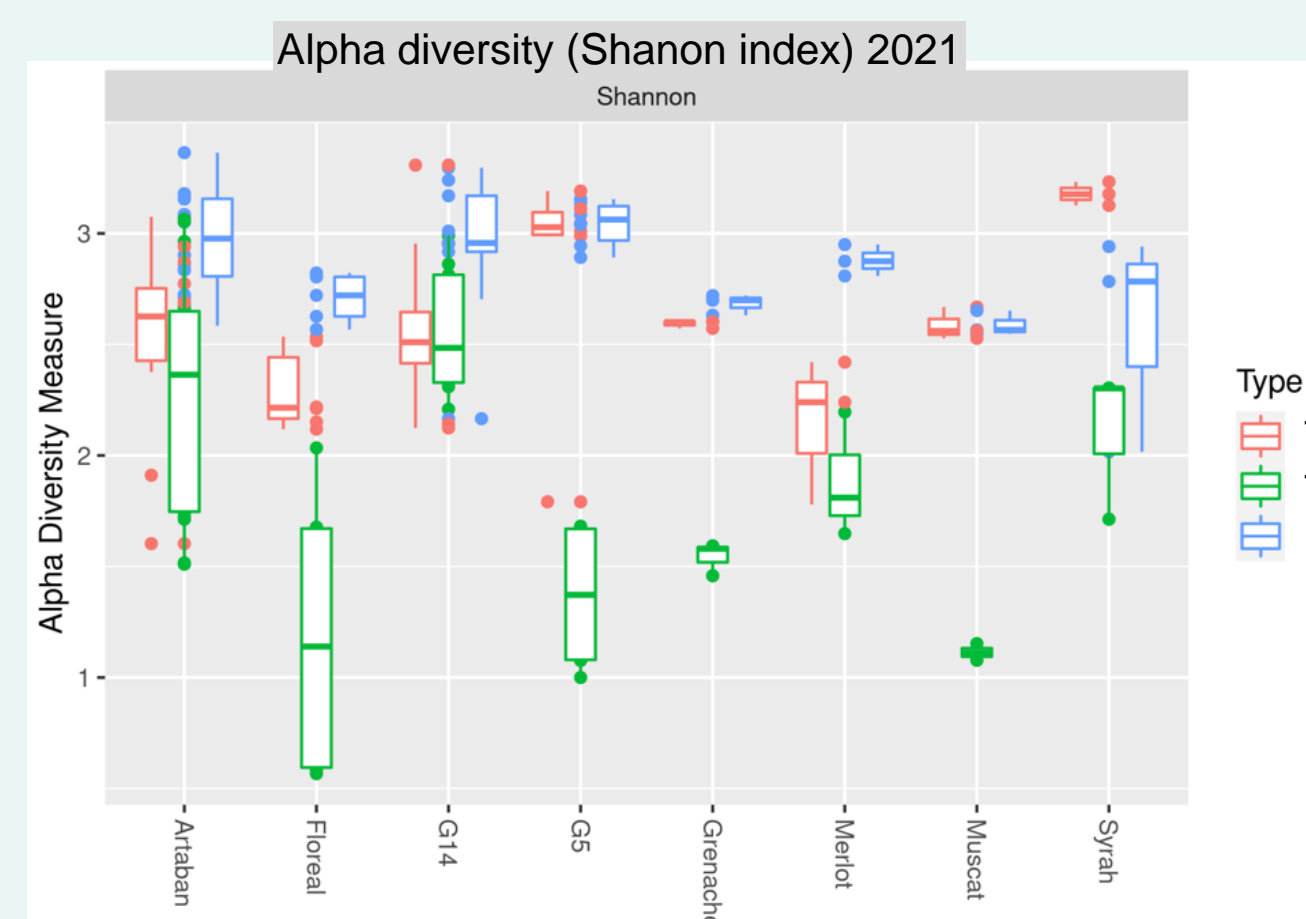


Capricious spontaneous fermentations

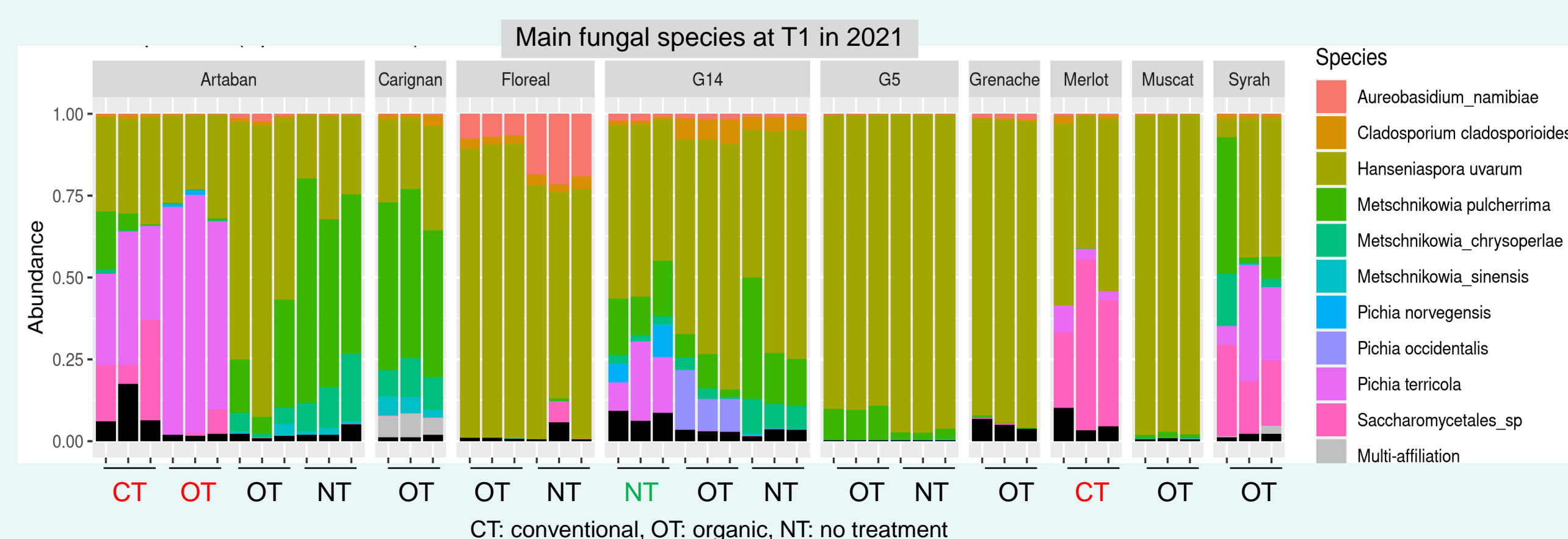
- 48 microvinifications in 2021 and 63 in 2022.
- Good repeatability of triplicates up to 250 h, short lag phase and Vmax quickly reached but the CO₂ flow is low → non-*Saccharomyces* yeasts
- After 250 h, the kinetics is random with either a restart of the fermentation associated with *S. cerevisiae*, or a sluggish and incomplete fermentation. → relevance of the scale 1L/1kg?
- Variability of kinetics depends on varieties and treatments.



Diversity of the grape must microbiota



- Loss of diversity during fermentation due to the growth of fermentative yeasts.
- Strong effect of the region at T1 (10 g/L of CO₂ released), with great differences concerning the main yeast species → impact on fermentation kinetics and on the organoleptic quality of wines.



Conclusion and Perspectives

Ongoing research focuses on a broader set of resistant cultivars in order to:

- ✓ understand the variety effect and the factors that modulate microbiota variation. → A wider set of varieties, grown in several areas. More factors tested (vineyards, vintage, variety, treatment, maturity, agro-ecological environment)
- ✓ Investigate whether the composition of the berry skins (polyphenols, polysaccharides, proteins) is a structuring factor in the variation of the microbiota.

