Microbial terroir – current research round-up

The following notes and images formed the basis of a presentation on microbial terroir (a new entry in the latest, fourth edition of the Oxford Companion to Wine) given by Jancis at Pinot Noir NZ in Wellington last week. I have simply added a few snippets of additional text to my original notes to make it read more easily but otherwise retained the note-like presentation. I've also reproduced those slides that included images. We are publishing it here in the hope that others might be interested in this topic and also to provide – for those who were at the event – full references to any papers and research cited. A big thank you to all the researchers who kindly sent me details of their current and recent research projects. I would like to emphasise that these are just examples of the sort of research going on around the world today; it is not a comprehensive list of such projects.

For background on the MICROWINE research network (examples below), see Microbiologists – wine's new heroes.

Introduction

The following quotation from 'Regional microbial signatures positively correlate with differential wine phenotypes: evidence for a microbial aspect to terroir' by Sarah Knight, Steffen Klaere, Bruno Fedrizzi & Matthew R Goddard, an open-access Scientific Report on www.nature.com from 2015, sets the scene:

‘Wine … arguably displays the strongest geographic signatures of all agricultural products and thus is a superb model to evaluate the degree to which there might be a microbial aspect to terroir. However, even for wine, the drivers of terroir remain largely untested.’

Knight *et al*’s paper summarises the work done in the Goddard Lab, Auckland University, on yeasts but I chose the quotation because it encapsulates our current lack of knowledge and the need for further research. Sarah Knight is based at the University of Auckland whereas Mat Goddard is back in the UK but still has a 25% time commitment to the work in Auckland.

What is microbial terroir?

Microbial terroir is all about the influence of site-specific fungi and bacteria on the flavour of wine.

- Fungi and bacteria on grape skins, including yeasts, may contribute to the link between a specific vineyard site and the flavour of the wine produced from that vineyard.
- Bacteria and fungi in a soil’s organic matter may bridge the gap between vineyard geology and soil type on the one hand and the distinctive flavour of a specific wine on the other.
- Are winery microbes part of microbial terroir? That probably depends on your definition of terroir.

EXAMPLES OF RECENT AND CURRENT RESEARCH

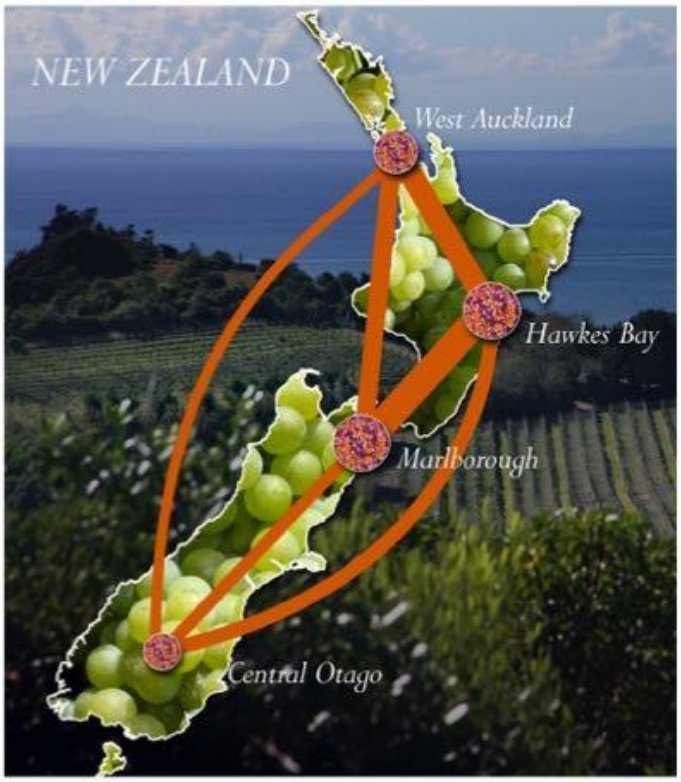
1 The Goddard Lab, Auckland: Fungal communities on Chardonnay

- Fungal communities on Chardonnay grapes in four regions of NZ were examined.
- DNA sequencing technology revealed 200 different species.
- Significant differences in major areas.
- How are these differences expressed in wine composition?

Source: Michael W. Taylor, Peter Tsai, Nicole Anfang, Howard A. Ross and Matthew R. Goddard, ‘Pyrosequencing reveals regional differences in fruit-associated fungal communities’, *Environmental Microbiology* 16/9 (2014)

Chardonnay fungal communities

Differences in Chardonnay fungal communities in different regions of NZ. The size of the circles is proportional to the fungal diversity in each region. The thickness of the lines represents how similar these communities are: the thicker, the more similar. (Mat Goddard)



2 Goddard Lab – yeasts in the vineyard

- Research showed unique yeast populations in different areas of NZ.
- There were regional differences among species of *Saccharomyces cerevisiae* populations found in spontaneous ferments.
- Subsequent research suggested a link between these regionally distinct yeasts and the volatile compounds found in the wine (created by the yeast metabolism, ie metabolites).

Source: Gayevskiy, R., and Goddard, M. R., 'Geographic delineations of yeast communities and populations associated with vines and wines in New Zealand', *ISME Journal*, 6 (2012), 1281–90. doi: 10.1038/ismej.2011.195. [*ISME Journal = Multidisciplinary Journal of Microbial Ecology*]

According to Knight *et al*:

'Microbes, predominantly fungi, may significantly affect the "phenotype" of wine firstly by affecting grapevine and fruit health and development, and thus quality, and secondly by manipulating wine flavor, aroma and style due to their actions during fermentation. During alcoholic fermentation fungi including *Saccharomyces cerevisiae*, the primary yeast involved in wine fermentation, not only convert sugars into ethanol but also produce an array

of secondary metabolites, including volatile compounds, that are important to wine aroma and flavor. While grape-derived compounds may provide varietal distinctions, at least yeast-derived acids, alcohols, carbonyl compounds, phenols, esters, sulfur compounds and monoterpenoids all significantly contribute to wine quality and aroma.'

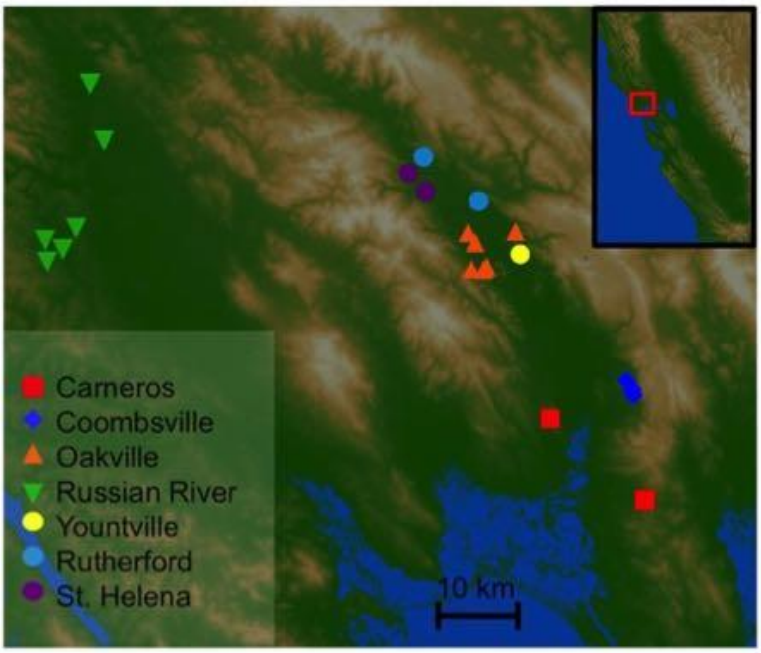
3 Goddard Lab – microbes in the vineyard and surrounding environment

- Ongoing research into how the microbes in vineyards and ferments relate to those in the surrounding environment and at the specificity of these in different areas of NZ.
- 'Without giving the result away overly, it is apparent that the connections between vineyards and surrounding native forests is significant and bespoke to regions.' (Goddard, personal communication)

4 Mills Lab UC Davis – microbial contribution to regional wine characteristics

- Both grape microbiota and compounds found in the wines distinguish viticultural areas and vineyards within Napa and Sonoma Counties.
- The bacterial and fungal consortia of wine fermentations, composed from vineyard and winery sources, correlate with the chemical composition of the finished wines.

Map of sampling sites across Napa and Sonoma Counties.



Nicholas A. Bokulich et al. mBio 2016; doi:10.1128/mBio.00631-16

Source: Nick Bokulich, Thomas S. Collins, Chad Masarweh, Greg Allen, Hildegarde Heymann, Susan E. Ebeler, David Mills, 'Associations among wine grape microbiome, metabolome, and fermentation behavior suggest microbial contribution to regional wine characteristics', mBio, the open-access online journal published by the American Society for Microbiology

As the authors note in this paper, there are also potential economic benefits to the application of this research: ‘In addition to enriching our understanding of how growing region and wine properties interact, this may provide further economic incentive for agricultural and enological practices that maintain regional microbial biodiversity.’

5 Steenwerth Lab, UC Davis

Steenwerth Lab, UC Davis

(a) Effects of vineyard management on soil bacteria

- Soil resources varied with respect to management practices.
- These soil resources also correspond to shifts in microbial community structure.
- Community structure differs among biodynamic, organic and conventional systems.
- Cover crops and tillage strongly influence microbial community structure.
- Effects of management practice also vary with winegrowing region and soil type.

Source: Kayla N. Burns, Nicholas A. Bokulich, Dario Cantu, Rachel F. Greenhut, Daniel A. Kluepfel, A. Toby O'Geen, Sarah L. Strauss, Kerri L. Steenwerth, 'Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: Differentiation by vineyard management', *Soil Biology and Biochemistry,* 103 (December 2016)

In the conclusion to the abstract of this paper, the authors note: 'This work allows for subsequent assessments of interrelationships of vineyard

management, microbial biodiversity and their combined influence on soil quality, vine health, and berry quality.'

(b) Effects of geographic features on soil bacteria

- Wine-growing regions, or AVAs, were distinguished by soil resources pools.
- Soil bacterial community structure reflects distinctions in soil resources.
- Distinctions increased with increasing geographic distance.
- Microbial communities represent links in the soil environment to wine *terroir*.

Source: Kayla N. Burnsa, Daniel A. Kluepfelb, Sarah L. Straussb, Nicholas A. Bokulicha, Dario Cantu, Kerri L. Steenwerth, 'Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: Differentiation by geographic features', *Soil Biology and Biochemistry* 91 (2015)

The authors note at the end of the abstract: 'We reason that AVA, climate, and topography each affect soil microbial communities through their suite of impacts on soil properties. The identification of distinctive soil microbial communities associated with a given AVA lends support to the idea that soil microbial communities form a key in linking wine terroir back to the biotic components of the soil environment, suggesting that the relationship between soil microbial communities and wine *terroir* should be examined further.'

6 Joint research by Kerri Steenwerth of USDA and Dario Cantù and Ron Runnebaum of UC Davis

The emphasis is mine:

- A five-year project with a long-term goal to connect climatic, edaphic [soil], and microbial characteristics of site to the chemical and sensory attributes of a wine across multiple vintages.
- **Central hypothesis: physical and chemical characteristics of the site (climate, soil) directly structure the site microbiome. Then, in concert with the climatic and edaphic site characteristics, the microbiome partly mediates berry development and metabolism through its impact on nutrient acquisition and berry metabolism. The effects of the climate, soil and microbiome will be evident in the chemical and sensorial characteristics of the finished product.**

- Initial experiments in 2015: Jackson Family Wines gave access to Pinot Noir wine grapes of the same clone from fifteen vineyards along a nearly 900 mile (1400 km) longitudinal transect (sea-level to nearly 1500 ft [>400 m] elevation), spanning coastal regions from Santa Rita Hills in southern California to Willamette Valley in Oregon.
- Next experiments will be more tightly controlled and run over multiple vintages: ten of these vineyards contain the identical combination of rootstock clone and scion clone but diverse set of soils and climates. They will look at both environmental attributes and cultural practices.
- Wine fermentation over multiple vintages will be tightly controlled using highly automated UC Davis fermentors.

7 Bordeaux ISVV – high genetic diversity and long–term persistence of yeast strains in Sauternes

Isabelle Masneuf-Pomarade (Bordeaux ISVV and Bordeaux Sciences Agro) explained the research:

- A three-year study in Bordeaux focusing on vineyard- and winery-associated *S cerevisiae* population, the impact of the appellation and of the farming system (conventional versus organic) and the link between vineyard and cellar *S cerevisiae* population.

- Research published (*Applied and Environmental Microbiology*, 2016) shows that in Sauternes there is no specific *S cerevsiae* community associated with a given wine estate; exchange occurs between estates.

Post scriptum: Michael Brajkovich MW worked closely with Mat Goddard and Velimir Gayesvsky during their research on yeasts unique to New Zealand and to Brajkovich's own Kumeu River estate (see reference above). He commented after this presentation that the reason there was no difference in yeast populations between estates in the results of this Sauternes study was probably because grapes are kept so long on the vine that during this extended period insects have time to carry yeast between different vineyards.

8 MICROWINE

As explained in Microbiologists – wine's new heroes, MICROWINE is an EU-funded network of 15 PhDs around the world:

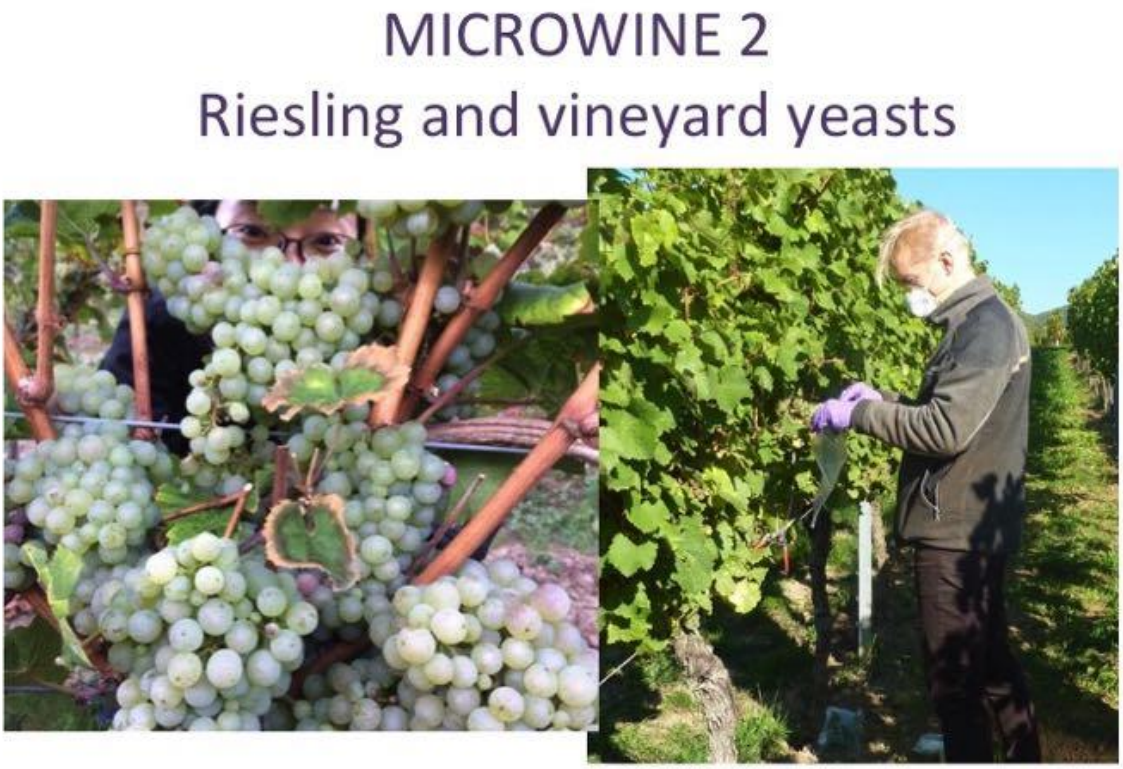
- A Marie Curie training network around the world with a hub in Copenhagen.
- Using DNA sequencing of microbial genetic material and powerful computation analyses to study the action of microbes from plant protection and nutrition, through to wine fermentation process.

Microwine 1: Contribution of wine microorganisms to the aroma composition of wine and its sensory impact

- Inês Pereira Biscaia de Oliveira (above), University of Zaragoza, Spain, working with Vicente Ferreira, leading researcher in wine aroma.
- Understanding the role of yeast characteristics on wine aroma composition and sensory properties.
- Initial experiments study the effect of different strains of cultured *S cerevisiae* and the presence of elements such as Zinc and SO2 in model wine.
- Results reveal oxidation reactions during fermentation and the influence of zinc on fruity and sweet aromas.

Microferments, Zaragoza

Microwine 2: Riesling and vineyard yeasts – the dynamics of microbiomes in the winery



- Kimmo Sirén and Sarah Mak, Neustadt, Pfalz (pictured above).
- To identify and profile the different origins of the microbiome, mainly yeast, conducting spontaneous fermentation and to follow their succession during the winemaking process.
- To understand the interplay between vineyards and winery flora, and relate spontaneous fermentations to aroma formation and sensory analysis.

Microwine 3: Soil and grape microbes and their relationship to wine

- Student Alex Gobbi, Aarhus: ‘a deep investigation about the microbial community harboured in different vineyards might be the key to characterise a typical microbial fingerprint for a specific area; this could be a driving force for the development of specific wine traits attributable to the major concept of terroir.
- Also looking at the impact of vineyard management.

9 Nigel Greening, Felton Road, Central Otago – mycorrhizal fungi

Nigel Greening, Felton Road

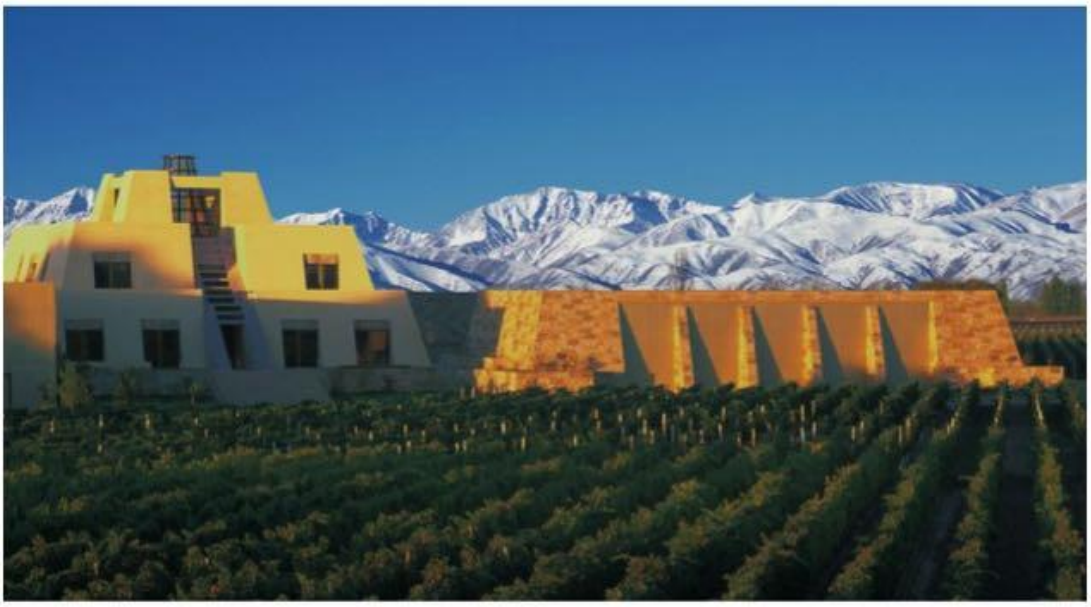
Mycorrhizal fungi

Mycorrhizal fungi: small symbiotic fungi that work in union with vine roots and play a critical role in the vines' ability to extract nutrients from the soil.

- Felton Road mass-selection cuttings will be planted at Riversun nurseries in soils that contain mycorrhiza from the vineyards the cuttings were taken from.
- Nigel Greening: 'Will this make a difference? Who knows? But the more we learn about these things the more we realise how careful we may need to be to protect our "somewhereness".'

10 Catena Institute, Mendoza

Catena Institute, Mendoza

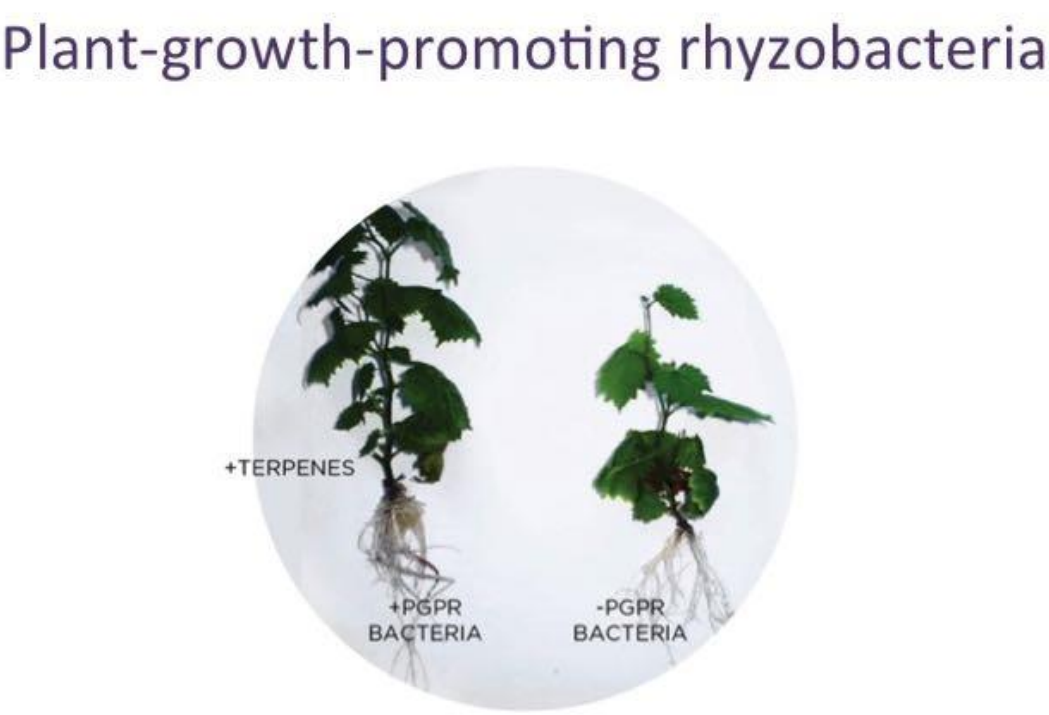


In partnership with:

- UC Davis
- UNCUYO (National University of Cuyo)
- CONICET (Argentina's National Council of Scientific and Technical Research)
- IBAM (Instituto de Biología Agrícola de Mendoza)
- MICROWINE

(a) Plant-growth-promoting rhyzobacteria

This research looks at bacteria – as opposed to fungi – that colonise plant roots and have a symbiotic relationship with the plant.

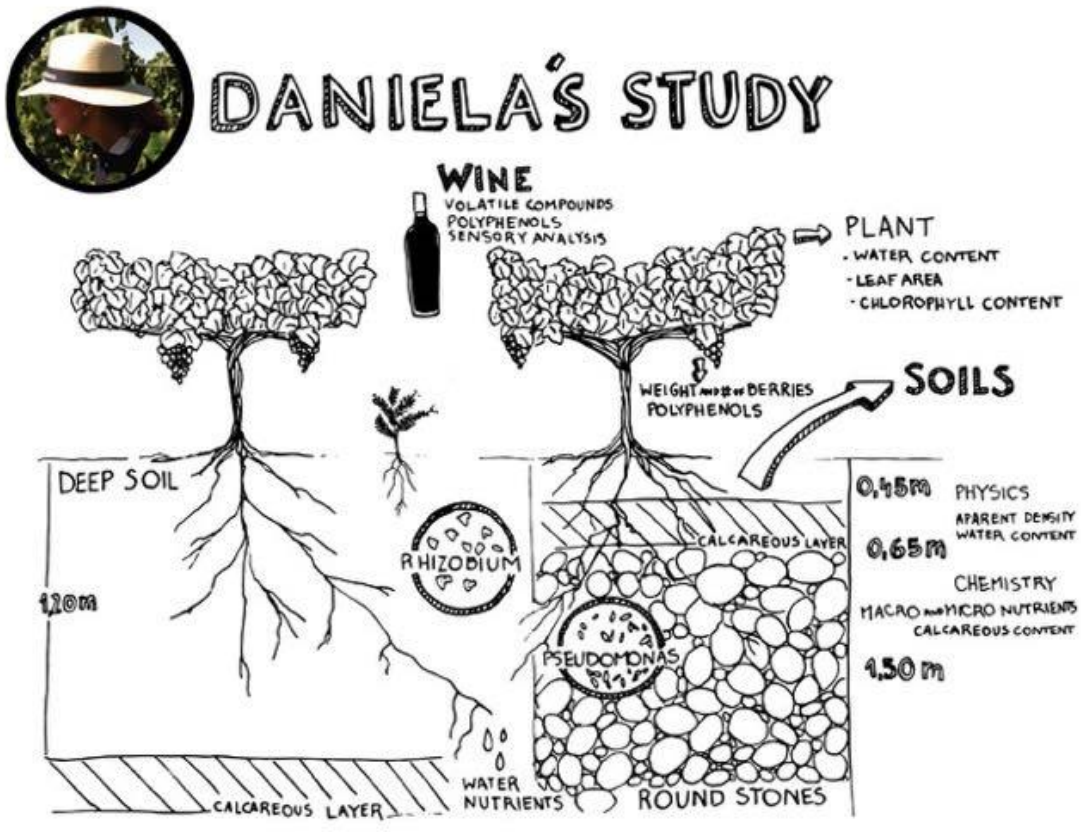


- Two PGPR bacteria isolated in our Adrianna Vineyard at almost 5,000 feet elevation.
- The PGPR-inoculated Malbec plants had greater natural abscisic acid synthesis (which protects against water stress) and terpene synthesis in the leaves. Inoculated plants had diminished plant water loss rate.
- PGPR play a role in helping our Malbec vines survive the stress of our high-elevation desert climate (the terpenes help the plant withstand better the greater UV-radiation that exists at high altitude).

(b) Phylloxera

- Aggressive strains of phylloxera are found in both in Mendoza vineyards at higher and lower elevations.
- Sandy soils are not immune to phylloxera in Argentina.
- However, phylloxera has been in Argentina for at least a century and does not seem to be spreading. 99% of vineyards are own-rooted.
- We hypothesize that microbial populations may play a role in stopping the spread of phylloxera in Argentina.
- Plans at Catena to work to continue this research with MICROWINE (see above)

(c) Daniela Mezzatesta's PhD soil study



Daniela Mezzatesta is doing her PhD on the impact of soil on the growth, yields and quality of Malbec wines in different high-elevation soils. This includes the implications for soil microbiology.

- Two adjacent vineyards identical in elevation, microclimate and vine age – but one has deep sandy soil, the other stony soil.
- Investigating the influence of the soil types on all aspects:

1. Soil characteristics: eg water retention, temperature, micronutrient content, oxygen content, microbial population etc.
2. Vine characteristics: eg length of shoots, yields, root morphology, solids content in grapes, polyphenolic profile, terpene and volatile compound content.
3. Wine characteristics: polyphenolic content, volatile terpenes.

11 Microblitz project – soil samples, University of Western Australia

I included this project at the end just as an example of the growing interest in this topic, not just for viticulture and winemaking but more widely to increase the sustainability of agricultural land.

- A citizen science project where crowd-sourced science is building a state-wide map detailing the biodiversity and health of our environment using DNA sequencing to identify the biodiversity of microbes in our soils.
- Aim: to create a baseline map that can be shared and used to monitor, manage and protect WA’s precious environment.