XIIIth International Terroir Congress
17–18 November 2020
Virtual Congress | Adelaide, Australia

MICROBIAL METAGENOMICS OF VINEYARD SOILS AND WINE *TERROIR*

Vadakattu Gupta[1], Rob Bramley[1], Paul Greenfield[2], Julian Yu[3], Markus Herderich[4]

[1]CSIRO Ag & Food, Urrbrae, SA, Australia
[2]CSIRO Energy, North Ryde, NSW, Australia
[3]Arizona State University, Arizona, USA
[4]AWRI, Urrbrae, SA, Australia

Corresponding author: Gupta.Vadakattu@csiro.au

Abstract

Aims: The aims of this study were to (i) characterize bacterial and fungal communities in selected Australian vineyard soils and (ii) determine if the soil microbiome composition and diversity varied between different zones within a vineyard.

Rationale: The soil on which vines are grown has been suggested to impart a unique quality to the grapes and wine due to the physiological responses of the vines to soil type, topography and climatic conditions, in addition to their viticultural management. The influence of bacteria and fungi in wine fermentation is well known but little is known about the effect of soil microbes, other than microbial pathogens, on grape composition or their role in vintage or site (*terroir*) impacts on grape composition.

Methods and Results: We investigated the potential relationships between soil microbiome composition measured using a metagenomic approach (16S rRNA and ITS region amplicon and metagenomic sequencing) and inherent spatial variation in grape metabolite composition, specifically the concentration of the 'impact aroma compound' rotundone in Shiraz grapes (*Vitis vinifera* L.) grown in vineyards in the Grampians region of Victoria and in the Adelaide Hills in South Australia. Results from the metagenomics analysis of surface soil samples collected from the previously identified 'rotundone zones' in a vineyard indicated marked differences in the genetic diversity and composition of the soil bacterial and fungal microbiomes of these zones. Soils from the high rotundone zone exhibited higher diversity of bacteria, but lower diversity of fungi, compared to the soils in the Low rotundone zone. In addition, the network analysis of the microbial community in the High rotundone zone soils appeared well structured, especially with respect to the bacterial community, compared to that in the Low rotundone zone soils. A few specific taxa/groups of microorganisms e.g. Acidobacteria-GP4 and GP7, Rhizobiales, Burkholdiales, Gaiellales, Alphaproteobacteria and the Nectriaceae and Tremellaceae families of fungi, were associated with the rotundone-based variation. Short-term mulching effects did not seem to mask the rotundone zone-based variation. Predictive functional profiling using 16S rRNA marker gene sequences, FAPROTAX-based analysis indicated differences in functional categories such as manganese oxidation, methylotrophy, methanotrophy, oxidation of sulfur compounds. These findings suggest that the observed taxonomic variation needs to be translated into functional aspects of soil microbiome before mechanistic links to rotundone concentrations can be established.

Conclusions: Distinct differences in soil bacterial and fungal community composition and structure in different zones within the same vineyard are associated with different propensities for grape berry rotundone concentration. Also, high rotundone zone soil exhibited a well-connected microbial community network by comparison with the Low rotundone zone soil.

Significance and impact of the Study: These findings of a systematic rotundone zone-based variation in soil microbiomes paves the way to bring together understanding of microbial ecology and viticultural management for improved grape composition and wine flavour (*terroir*).

Keywords: Rotundone, microbiome diversity, bacteria, fungi, grapes

Introduction

Soil habitat characteristics are known to modulate the diversity of the soil microbiome and the dynamics of plant-microbe interactions which play an important role in plant growth, abiotic and biotic stress tolerance, nutrition, productivity and product quality. The vineyard microbiome has been suggested to play specific roles in the productivity and disease resistance of the host plant. Also, the soil on which vines are grown has been suggested to impart a unique quality to the grapes and wine due to the physiological responses of the vines to soil type, topography and climatic conditions, in addition to their viticultural management (van Leeuwen and Seguin, 2006; van Leeuwen and de Rességuier, 2018; Bokulich *et al.,* 2016; Gupta *et al.,* 2019; Liu *et al.,* 2019). The influence of bacteria and fungi in wine fermentation is well known but little is known about the effect of soil microbes, other than microbial pathogens, on grape composition or their role in vintage or site (*terroir*) impacts on grape composition. The aims of this study were to (i) characterize bacterial and fungal communities in selected Australian vineyard soils and (ii) determine if the soil microbiome composition and diversity varied between different vineyard zones identified on the basis of the concentration rotundone in grape berries produced in them.

Materials and Methods

Surface soils (0-5 and 5-15 cm) were collected immediately prior to harvest from positions adjacent to selected geo-referenced vines from vineyards in the Grampians region of Victoria (Mount Langi Ghiran vineyard; 37^{o}S, 143^{o}E) and in the Adelaide Hills in South Australia (The Lane; 35°S, 138°E) during 2017 and 2018 vintage seasons. In the Grampians, individual soil sampling locations in each of the rotundone zones (Bramley *et al.,* 2017) were identified such that 7-10 samples from each zone were collected. A similar approach was followed at the Adelaide Hills site. Rotundone is an 'impact aroma compound' responsible for the peppery characteristic of some cool climate Shiraz (Wood *et al.,* 2008). We investigated the potential relationships between soil microbiome composition measured using a metagenomic approach (16S rRNA and ITS region amplicon and metagenomic sequencing) and inherent spatial variation in berry rotundone concentration. Full details of microbial composition, activity and soil physico-chemical properties are available in Gupta *et al.* (2019).

Results and Discussion

Soils from the high rotundone zone generally exhibited higher diversity of bacteria, but lower diversity of fungi, compared to the soils in the Low rotundone zones from the 2017 sampling in the Grampians region vineyard and from both the sites in 2018. Venn diagrams showed distinct and common bacterial and fungal OTUs among different rotundone zones (data not shown). Comparison of bacterial and fungal community composition from beta-diversity analysis (generated using the Bray–Curtis distance metric) showed significant dissimilarity between the rotundone zone samples and sites; for example, 2018 samples from both vineyards for Bacteria: ANOSIM Sites-Global R=0.341; P=0.01, Zones-Global R=0.946; P=0.01 and for fungi: ANOSIM Sites-Global R=0.341; P=0.01, Zones-Global R=0.946; P=0.01). PERMANOVA analysis showed that rotundone zone based variance explained 16.6% and 9.24% of variation (P=0.01) in bacterial community and 16.7 and 11.5% of variation in fungal community (P=0.01) in the 2017 and 2018 samples, respectively. Rotundone zone variation in the Grampians region vineyard was associated with a few specific taxa/groups of microorganisms e.g. Acidobacteria-GP4 and GP7, Rhizobiales, Burkholdiales, Gaiellales, Alphaproteobacteria and the Nectriaceae and Tremellaceae families of fungi, were associated with the rotundone-based variation in the 2017 samples (Figure 1). Network analysis of the microbial community in the High rotundone zone soils appeared well structured, especially with respect to the bacterial community, compared to that in the Low rotundone zone soils (data not shown). Well-connected microbial community networks are generally considered stable through the different seasons.

Additionally, results from the predictive functional profiling using 16S rRNA marker gene sequences from the Grampians region vineyard samples, FAPROTAX-based analysis indicated significant dissimilarity between rotundone zones (ANOSIM Zones-Global R=0.195; P=0.01; Figure 2A). Some of the functional categories showing significant differences included manganese oxidation, methylotrophy, methanotrophy, nitrate reduction, ureolysis, Fe-respiration, oxidation of sulfur compounds (Figure 2B). It is suggested that the majority of above-ground organ-associated taxa in grapevines originated in the soil, and their distribution reflected the influence of highly localized biogeographic factors and vineyard management (Zarraonaindia *et al.,* 2015). Also, distinct shifts in fungal communities coinciding with the developmental stage of veraison have been reported (Liu *et al.,* 2020). Therefore, it can be hypothesized that the high level of organization along with higher microbiome

diversity in the High rotundone zone soils would provide the vine plant with a stable microbial reservoir across varied seasonal environmental conditions influencing above-ground microbiome including berry microbiome. However, to effectively manipulate vineyard microbiome for a distinct wine terroir, a better understanding of specific functional consequences of the distinct microbiomes above and below ground is necessary.

Overall, the observation of a systematic rotundone zone-based variation in soil microbiomes paves the way to bring together understanding of microbial ecology and viticultural management for improved grape composition and wine flavour (*terroir*).

Acknowledgements

We are most grateful to the Rathbone Wine Group and in particular, Damien Sheehan (Mount Langi Ghiran), and to The Lane Vineyard (Michael Schreurs and Turon White) for allowing us access to their vineyards and to Damian Mowat (field sampling), Marcus Hicks (molecular analysis) and Stasia Kroker (microbiological analysis) for their technical assistance. The discussion with, and support, of Mark Krstic, Tracey Siebert, and Sheridan Barter (AWRI) are also gratefully acknowledged as is their work in the analysis of rotundone concentration. This project was funded by Wine Australia, CSIRO and AWRI.

References

Bramley, RGV., Siebert, TE., Herderich, MJ., Krstic, MP., 2017. Patterns of within-vineyard spatial variation in the 'pepper' compound rotundone are temporally stable from year to year. Australian Journal of Grape and Wine Research, 23: 42–47.

Gupta, VVSR., Bramley, RGV., Greenfield, P., Yu J., Herderich, MJ., 2019 Vineyard soil microbiome composition related to rotundone concentration in Australian cool climate 'peppery' Shiraz grapes. Frontiers in Microbiology, 10: 1607.

Liu, D., Zhang, P., Chen, D., Howell, KS., 2019. From the vineyard to the winery: how microbial ecology drives regional distinctiveness of wine. Frontiers in Microbiology, 10: 2679.

Liu, D., Chen, Q., Zhan, P., Chen, D., Howell, K., 2020. The fungal microbiome is an important component of vineyard ecosystems and correlates with regional distinctiveness of wine. mSphere, 5: e00534-20.

Louca, S., Parfrey, LW., Doebeli, M., 2016. Decoupling function and taxonomy in the global ocean microbiome. Science, 353 : 1272–1277.

van Leeuwen, C., de Rességuier, L., 2018. Major soil-related factors in terroir expression and vineyard siting. Elements, 14: 159–165.

van Leeuwen, C., Seguin, G., 2006. The Concept of terroir in viticulture. Journal of Wine Research, 17: 1–10.

Wood, C., Siebert, TE., Parker, M., Capone, DL., Elsey, GM., Pollnitz, AP., ..., 2008. From wine to pepper: rotundone, an obscure sesquiterpene, is a potent spicy aroma compound. Journal of Agriculture and Food Chemistry, 56: 3738–3744.

Zarraonaindia, I., Ownes, SM., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., ..., 2015. The soil microbiome influences grapevine-associated Microbiota. mBio, 6: e02527-14.

Appendix

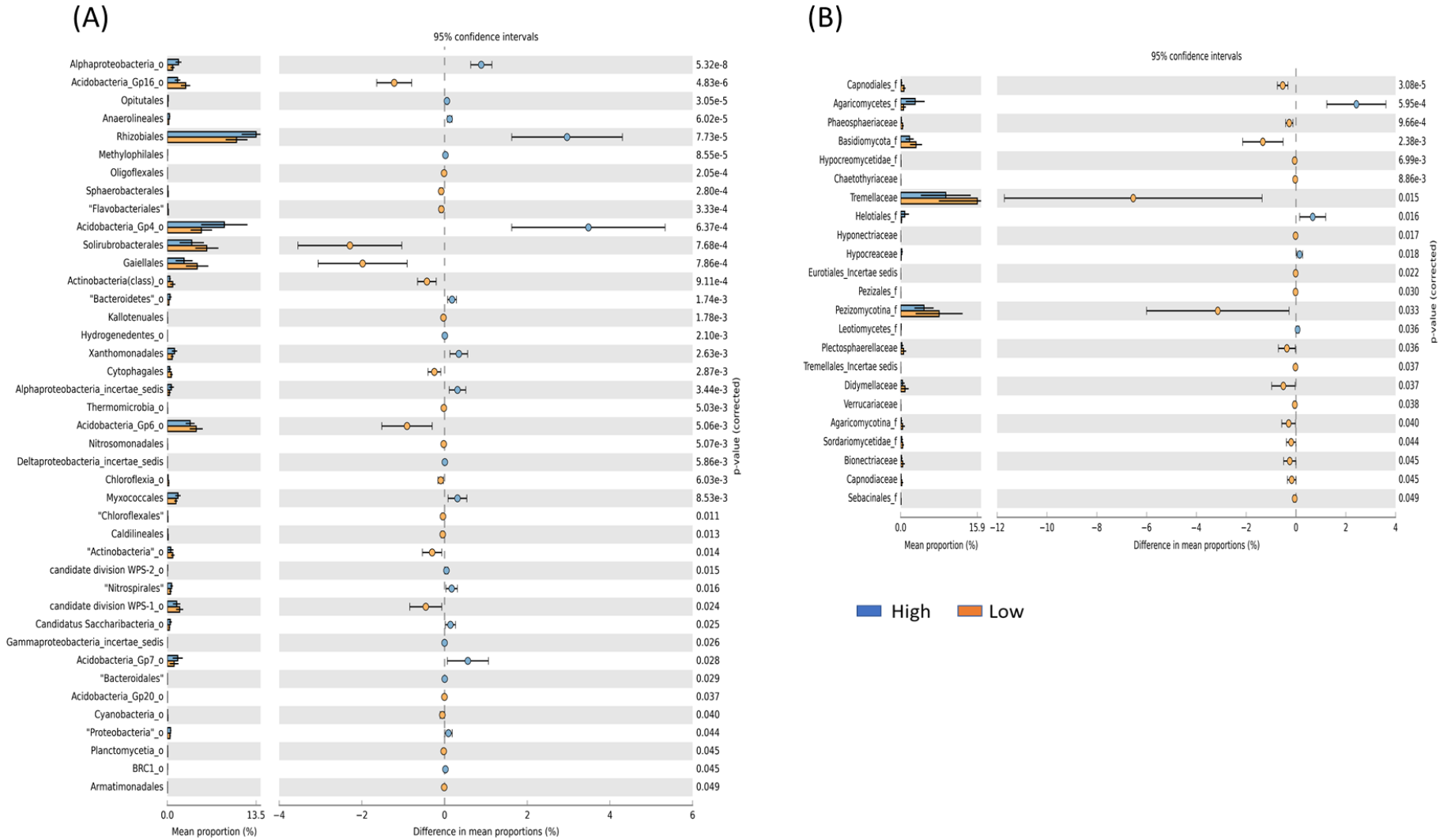

Figure 1: Differences in soil bacterial (A, at Order level) and fungal (B, at Family level) communities between the Low and High rotundone zones in a vineyard in the Grampians region of Victoria, from 2017 sampling. Specific members of bacterial and fungal communities varying between the zones were identified through analysis using STAMP software based on Welch's t-test (Gupta *et al.,* 2019).

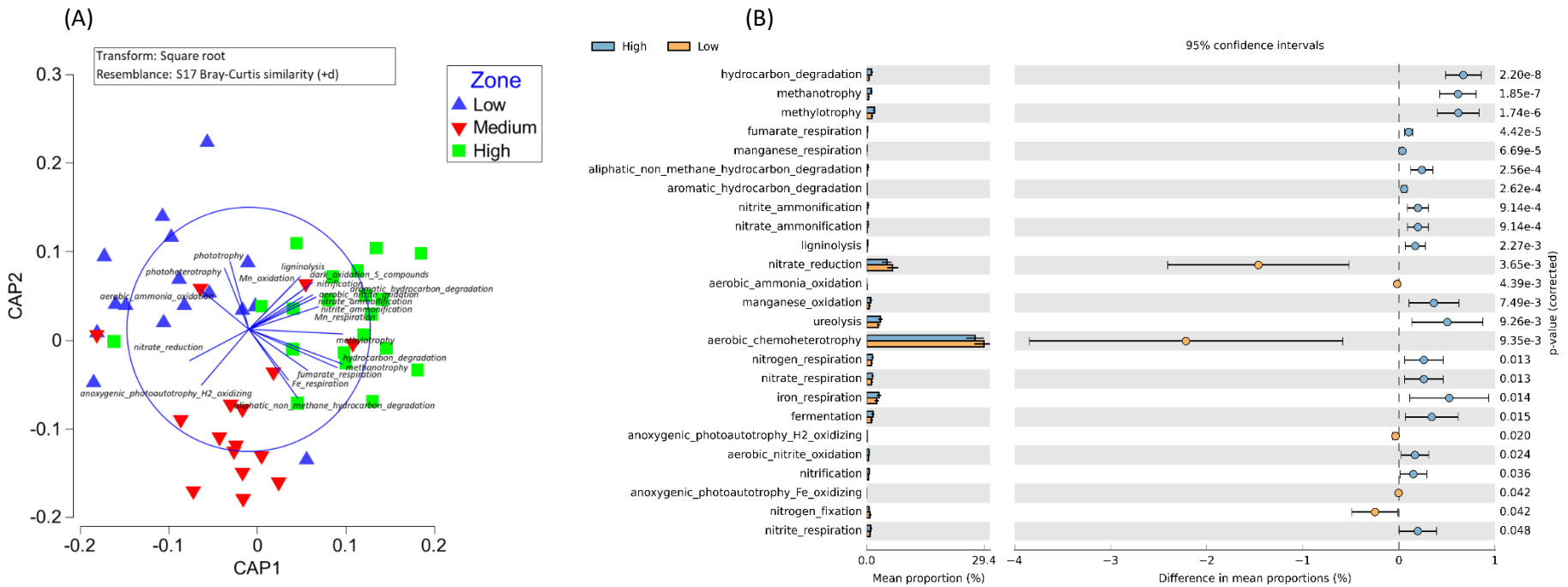

Figure 2: Relative abundances of metabolic and other ecologically relevant functional groups of soil bacterial community in the Low and High rotundone zones at the vineyards in the Grampians region of Victoria. (A) Canonical analysis of principle (CAP) ordination, constrained by zone; (B) relative abundances of metabolic and other ecologically relevant functions. Functional group data was derived from the bacterial (16S rRNA) OTUs for the 2017 samples using the FAPROTAX database (https://pages.uoregon.edu/slouca/LoucaLab/archive/FAPROTAX/lib/php/index.php; Louca *et al.*, 2016)