Teasing apart *terroir*: the influence of management style on native yeast communities within Oregon wineries and vineyards

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Abstract

Newer sequencing technologies have allowed for the addition of microbes to the story of terroir. The same environmental factors that influence the phenotypic expression of a crop also shape the composition of the microbial communities found on that crop. For fermented goods, such as wine, that microbial community ultimately influences the organoleptic properties of the final product that is delivered to customers. Recent studies have begun to study the biogeography of wine-associated microbes within different growing regions, finding that communities are distinct across landscapes. Despite this new knowledge, there are still many questions about what factors drive these differences. To collect our data we took soil, bark, leaf, and grape samples from within each vineyard from five different vines of pinot noir. We also collected must and a 10º brix sample from each winery. Using these samples, we performed 18S amplicon sequencing to identify the yeast present. We then used metabolomics to characterize the organoleptic compounds present in the bottled wine from the blocks the year that we sampled. We are actively in the process of analysing our data from this study.

Introduction

An important aspect of wine, both commercially and scientifically, is the concept of *terroir*. *Terroir* refers to the environmental factors that contribute to the phenotype of a crop, including climate, topography, soil characteristics, and processing methods. While the concept *terroir* can be applied to any crop plant, it possesses a special position at the core of wine's identity. Recently, the concept has expanded to include the influence of microbial communities upon winemaking (Belda *et al.* 2015, Bokulich *et al.* 2016). Microbes can affect the winemaking process from start to finish. Within the vineyard mutualistic fungi and bacteria in the soil can alter nutrient availability, increase tolerance to stressors such as drought, and lower rates of pathogenic infections (Belda *et al.* 2015). Microbes on and within the plant can further change its response to stressors and complete with pathogen microbes for space. Beyond the vineyard, the entire process of fermentation is driven primarily by microbes. While most modern wineries rely on commercial yeasts, primarily *Saccharomyces cerevisiae*, many are beginning to adopt spontaneous fermentation methods (sometimes referred to as "natural" wines) or some combination of the two. Spontaneous fermentation involves allowing inoculation of must with microbes from the environment. There is considerable diversity in wine associated microbes, and that diversity can produce a wide range of compounds that influence wine characteristics (Jolly *et al.* 2014, Rossouw & Bauer 2016). The source of these "wild" microbes is thought to be a combination of the plant itself, airborne dispersal, equipment, and staff (Bokulich *et al.* 2016). Despite this assumption, few studies have sought to quantify the relative contribution of each of these sources to the characteristics of the fermentation or final product. Additionally, parsing apart the influence of different aspects of *terroir* upon microbial communities in vineyards is mostly unexplored.

In the state of Oregon, USA there are nearly 1000 wineries across 21 American Viticultural Areas (AVAs). This encompasses a wide range of climates in a gradient from hot and dry in the south to cold and wet in the north. There also exists a wide variety of management styles including biodynamic, sustainable, and conventional. Biodynamic and sustainable vineyards require standards to be met prior to certification. Biodynamic vineyards

are certified through the Demeter International standard while sustainable vineyards are certified through LIVE, which is a Pacific Northwest organization that bases its program on international Integrated Production standards. This makes Oregon a perfect natural laboratory to test for the simultaneous influences of climate and management on *terroir* and microbes in vineyards and wineries. We set out to answer three main questions: 1) How does management style (i.e., conventional vs. sustainable/biodynamic) alter microbial communities in vineyards independent of climate? 2) What is the relative contribution of different sources (soil, bark, leaf, and grape) to wine fermentation communities? 3) Are detectable differences in fermentation communities correlated with differences in metabolite diversity?

Materials and methods

We sampled 15 vineyards across eight AVAs. Each AVA contained a pair of vineyards, one conventional and one sustainable/biodynamic, with one exception (Table 1). At each vineyard we randomly selected five Pinot noir or Syrah vines contained within the same block. From each vine we took a 10 cm x 2.5 cm soil sample near the base, a bark sample, one leaf, and one cluster of grapes during harvest season. Additionally, we obtained 100mL samples of must at crush, fermenting must near 0°Bx, and finished wine from the barrel. For two vineyards we obtained additional samples at approximately 10°Bx.

To process leaf and bark samples for DNA extraction we froze them using liquid nitrogen and crushed them using a sterilized mortar and pestle. Soil samples were hand homogenized prior to extraction. For must and wine samples we centrifuged 18mL at 13,000g for 5 minutes and removed the supernatant. We then rinsed the pellet with phosphate buffered saline (PBS) followed by centrifuging at 13,000g for 5 minutes and removal of the supernatant. This rinsing step was repeated three time. We performed DNA extraction immediately following the last rinse.

DNA was extracted from soil and wine samples using Qiagen PowerSoil Kits, while DNA was extracted from leaf, bark, and grape samples using Qiagen PowerPlant Kits. To characterize the microbial communities of our samples we performed 16S (bacteria), 18S (yeast), and ITS (fungi) amplicon sequencing. Additionally, we performed quantitative PCR (qPCR or RT-PCR) on must and wine samples to approximate true abundance.

Must and wine samples were also sent off to the West Coast Metabolomics Center at University of California Davis for identification and quantification of metabolites related to organoleptic properties.

Results and discussion

At the time of this abstract being written, we have not received our amplicon sequencing data. We have, however, received our metabolomics data and have finished the qPCR portion of our study. Based on initial analyses we have found that time point has the largest effect on metabolite composition (Fig. 1). This is not surprising, as the fermentation process rapidly alters precursor compounds found in grapes and introduces microbially derived metabolites. This is further supported by the increase in active yeast cells as fermentation progresses based on our qPCR data (Fig. 2). Despite this, there are several metabolites that are significantly different between management styles (conventional or sustainable/biodynamic) regardless of the geographic location or varietal of grape (Table 1). Even more metabolites show this pattern when considering the time points separately from one another (i.e., 40 metabolites at timepoint 2 or ~0°Bx).

Our next steps will be to use sequencing data to explore correlations between the presence and abundance of microbial species with the presence and abundance of metabolites (Morton *et al.* 2009). This will allow us to better understand if the differences we see in metabolite profiles are due to changes in microbial communities, or another measured factor. Some modern studies have clearly demonstrated that differences in microbial communities can be tied to differences in wine characteristics (Belda *et al.* 2015, Bokulich *et al.* 2016), but differences can still be attributed to a variety of other factors such as climate and topography. Due to management having measurable influences on microbial communities within vineyards (Agrabati *et al*. 2019), and variation in microbial responses to disturbances such as fungicides (Allison & Martiny 2008, Shade *et al.* 2012), it is reasonable to expect management to influence the final characteristics of wine.

Conclusion

Based on our initial round of data there are differences in metabolite profiles between wines due to management styles independent of other factors. Our other questions will be answered once we are able to analyze our metabolite and qPCR data in combination with microbial sequencing data from our samples. The decisions

involved in managing a vineyard are based on priorities of the owners and manager, as well as the environment in which their vines are established. Studies like our can be used by industry members to make informed decisions on how to manage their land and vines.

Table 1. Metabolites with significant differences between vineyard management styles in ten wines from Oregon, USA.

Metabolite	[1]S stdev	[2]C stdev	S mean	C mean	Fold Change	t-value	p-value	KEGG
1661	0.46	0.61	3595.28	2999.86	1.20	-2.43	0.017	N/A
121482	0.46	0.31	2847.14	3239.86	0.88	2.43	0.017	N/A
395982	0.51	0.35	1834.16	2101.95	0.87	2.35	0.020	N/A
390400	1.11	1.64	5137.63	4128.20	1.24	-2.16	0.033	N/A
1-monostearin	0.76	0.63	1596.80	2000.29	0.80	2.11	0.037	D01947
418093	1.17	0.95	3640.78	1955.75	1.86	-2.07	0.041	N/A
134712	0.52	0.26	628.80	681.96	0.92	2.06	0.042	N/A
21709	0.91	0.90	3195.27	2255.84	1.42	-2.05	0.043	N/A
beta-alanine	0.89	0.75	1706.94	2101.88	0.81	2.03	0.044	C00099
125811	0.89	0.90	3081.80	4055.07	0.76	2.01	0.046	N/A
glycerol-alpha-phosphate	0.59	0.49	770.91	888.82	0.87	1.87	0.065	C03189

[1]S = Sustainable/biodynamic management
[2]C = Conventional management
Metabolite name or number and fold change of metabolites that are significantly different or nearly significant between management styles across all time points, American Viticultural Areas, and grape varietals.

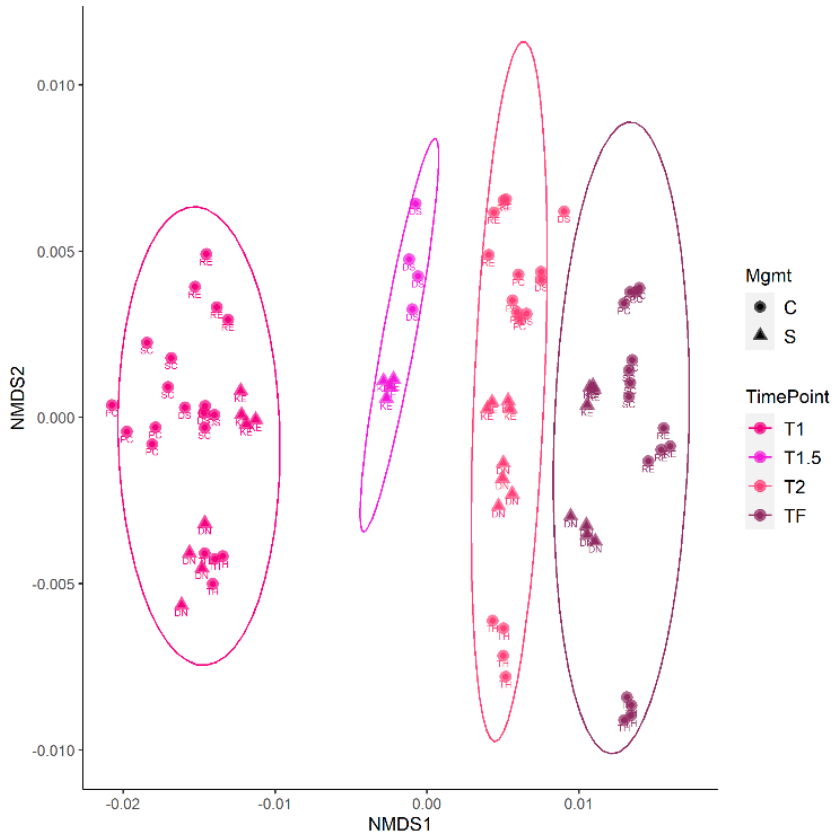

Figure 1. Non-metric multidimensional scaling ordination of metabolites from ten wines in Oregon, USA.
Dissimilarity was calculated using Euclidean distances between wine metabolite samples. Time points are based on degrees brix; T1 ~ 20°Bx, T1.5 ~ 10°Bx, T2 ~ 0°Bx, TF is bottled wine following barrel ageing. Samples are identified using their two-letter vineyard code and shape indicates management style; C = conventional, S = sustainable/biodynamic. Circles indicate 95% confidence intervals.

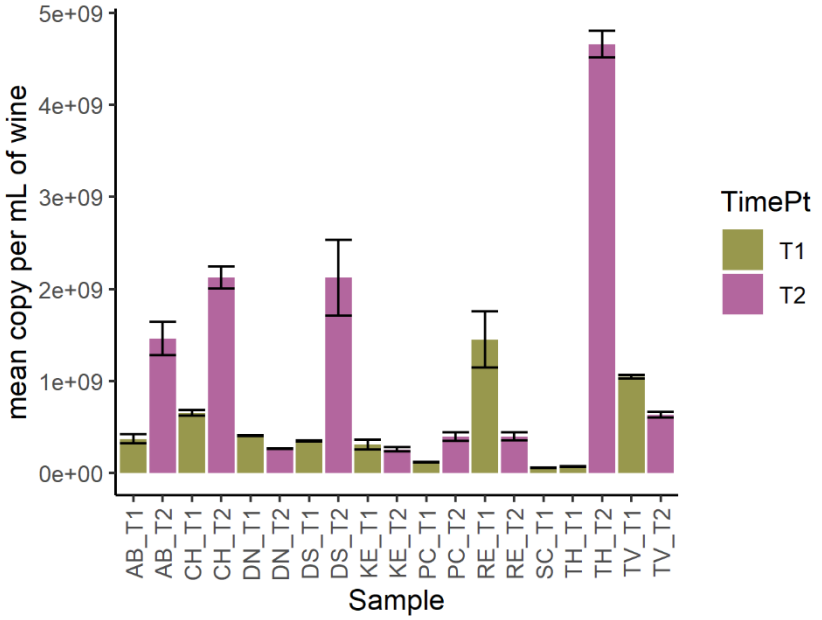

Figure 2. Mean copy of 18S amplicon per mL of wine from ten wines in Oregon, USA.
18S amplicon concentrations can be used as a proxy for yeast cell abundance. Time points are based on degrees brix; T1 ~ 20°Bx, T1.5 ~ 10°Bx, T2 ~ 0°Bx, TF is bottled wine following barrel ageing. Samples are identified using their two-letter vineyard code. Error bars represent standard deviation between three replicates of each sample.

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