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Small scale fungal community differentiation in a vineyard system

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ABSTRACT

Microbes influence the quality of agricultural commodities and contribute to their distinctive sensorial attributes. Increasingly studies have demonstrated not only differential geographic patterns in microbial communities and populations, but that these contribute to valuable regionally distinct agricultural product identities, the most well-known example being wine. However, little is understood about microbial geographic patterns at scales of less than 100 km. For wine, single vineyards are the smallest (and most valuable) scale at which wine is asserted to differ; however, it is unknown whether microbes play any role in agricultural produce differentiation at this scale. Here we investigate whether vineyard fungal communities and yeast populations driving the spontaneous fermentation of fruit from these same vineyards are differentiated using metagenomics and population genetics. Significant differentiation of fungal communities was revealed between four Central Otago (New Zealand) Pinot Noir vineyard sites. However, there was no vineyard demarcation between fermenting populations of *S. cerevisiae*. Overall, this provides evidence that vineyard microbiomes potentially contribute to vineyard specific attributes in wine. Understanding the scale at which microbial communities are differentiated, and how these communities influence food product attributes has direct economic implications for industry and could inform sustainable management practices that maintain and enhance microbial diversity.

1. Introduction

Traditionally geography is believed to have little impact on the distribution of microbes due to their large population sizes and seemingly limitless dispersal abilities (Finlay, 2002; Martiny et al., 2006; O'Malley, 2008). This is encapsulated in the Baas-Becking hypothesis: 'everything is everywhere – the environment selects' (Baas-Becking, 1934) and there is now a growing body of evidence for biogeographic differentiation between both microbial communities and populations (Bokulich et al., 2014; Hanson et al., 2012; Knight and Goddard, 2015; Martiny et al., 2006; Meyer et al., 2018; Morrison-Whittle and Goddard, 2015). Differences in microbial communities have been described between different environmental niches and geographic regions on large scales over 100s of kilometres, but few address the nature of these patterns at smaller scales. We still have little understanding of forces that drive differential microbial community assemblage patterns in space, but the little data and analyses available suggest that both natural selection and neutral processes play a role, with perhaps natural selection being the more important (Morrison-Whittle and Goddard, 2015).

Microbes play key roles in the production of quality agricultural commodities destined for human consumption. They contribute both positively in their nutrient cycling roles, negatively in their potential to cause livestock and crop diseases, and by directly transforming crops to economically and socially important commodities such as bread, wine and beer (Barata et al., 2012; Berg et al., 2014; Philippot et al., 2013; Whipps, 2001). In viticulture, pathogenic fungi potentially alter fruit composition and quality by affecting the concentration of odourless precursors in the fruit (Barata et al., 2012; Thibon et al., 2011, 2009), but little is known about microbial-vine rhizosphere interactions and how they might influence fruit composition and quality. More is understood regarding microbial contributions to wine chemical and sensorial properties during the fermentation process, where, in spontaneous ferments, many species interact to produce the final product (Fleet, 2003). While these diverse species all contribute to wine aroma and flavour, the fermentation process is driven by diverse populations of *Saccharomyces cerevisiae* (Fleet, 2003; Goddard, 2008; Howell et al., 2006).

Geographic variances in food and beverage sensorial properties have important economic and consumer preference consequences (van Leeuwen and Seguin, 2006). These geographic differences are encapsulated in the concept of *terroir*, which is arguably most well known in wine. Only recently however, have microbes been shown to contribute to this geographic variation in agricultural produce (Knight

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et al., 2015). Different species and strains of yeast are known to produce different flavour and aroma compounds during fermentation (Anfang et al., 2009; Bagheri et al., 2018; Dubourdieu et al., 2006; Howell et al., 2004). In addition, geographic differentiation in microbial communities associated with vines and wines has been demonstrated worldwide for both bacterial and fungal communities, as well as populations of the main fermentative yeast species *Saccharomyces cerevisiae* (Bokulich et al., 2014; Gayevskiy and Goddard, 2012; Knight and Goddard, 2015; Miura et al., 2017; Morrison-Whittle and Goddard, 2018, 2015; Taylor et al., 2014). When combined, there is thus potential for these differential microbial communities and populations to contribute to regionally distinct aromas and flavours in wine. This has been experimentally demonstrated during fermentation using *S. cerevisiae* in New Zealand where geographically structured regional populations were shown to produce regionally distinct chemical properties in Sauvignon Blanc (Knight et al., 2015; Knight and Goddard, 2015); however little is known about whether regionally differentiated fungal communities contribute to regional differences in fruit composition and quality.

Within New Zealand there is now evidence from multiple studies showing geography, at least on the scale of 100s of kilometres, is a driver of fungal community differentiation in vineyards and wine fermentation (Gayevskiy and Goddard, 2012; Morrison-Whittle and Goddard, 2018, 2015; Taylor et al., 2014). Differences between Chardonnay vineyards have been recorded using both culturing and next-generation sequencing approaches (Gayevskiy and Goddard, 2012; Taylor et al., 2014). In Sauvignon Blanc vineyards, geography accounts for 7% of fungal community differentiation in the vineyard (Morrison-Whittle and Goddard, 2015) and regional differentiation was observed between the fungal communities in crushed juice and early ferment (Morrison-Whittle and Goddard, 2018). However, it is widely asserted by viticulturists and winemakers world-wide that wines from different vineyards within a geographic region also exhibit distinct sensorial characteristics, termed by some as micro-*terroir* (Jung, 2014). Whether fungal communities are differentiated at these smaller scales, and thus potentially contribute to these site-to-site sensorial differences in wine, is not well understood. The few studies that do refer to differentiation between adjacent vineyard sites are confounded by other factors of interest such as management practice (Setati et al., 2012); however there is evidence of increased fungal community differentiation by increasing geographic distance over 35 km on grape bunches and leaves in Carmenere vineyards in Chile (Miura et al., 2017). Physical characteristics of the soil, particularly soil organic carbon, have been shown to affect fungal community composition at sub-vineyard scales in a single vineyard in southwest China and highlight the importance of small scale variations in environmental conditions on microbial community composition (Liang et al., 2019). Population differentiation in *S. cerevisiae* is evident at regional (Gayevskiy and Goddard, 2012; Knight and Goddard, 2015) and global scales (Liti et al., 2009; Wang et al., 2012); however there is evidence of gene-flow at scales smaller than 100 km (Hyma and Fay, 2013; Knight and Goddard, 2015). In contrast, small but statistically significant differences between *S. cerevisiae* populations residing in different vineyards has been demonstrated on smaller scales of within 10 km in the southwest France (Börlin et al., 2016) and within 1 km in Canada (Martiniuk et al., 2016). Combined, there is emerging evidence suggesting there could be a microbial component to anecdotal vineyard differences in wine attributes described by winemakers. Here we investigate whether differentiation in fungal communities and *S. cerevisiae* populations exist at smaller within-region scales with robust replication and molecular genetic techniques to further our understanding of how microbial communities and populations vary at finer scales, and whether this has the potential to contribute to finer-scale differences in wine.

Central Otago, New Zealand, represents the southernmost wine-growing region in the world located at 45° south and the sub-region of Bannockburn occupies one of the warmest, driest sites in this region. The microbial communities and populations in Central Otago display the greatest divergence from other regions in New Zealand (Knight and Goddard, 2015; Taylor et al., 2014). Pinot Noir is the dominant grape variety grown in this region, expressing distinctive site-to-site characteristics. Mt. Difficulty own a number of vineyards in Bannockburn, and the view of the winemaker is that four of these vineyards have the potential to produce different wines, and are often produced as single vineyard wines. Here we test if fungal differentiation exists at the vineyard scale by focusing on these four neighbouring but distinctive vineyards. We test 1) whether the fungal communities in the vineyard soil (where they may affect fruit composition and quality) differs between the vineyard sites using next-generation sequencing and community composition analyses; and 2) whether the *S. cerevisiae* populations in the spontaneous ferment of fruit from these vineyards (where they are driving the fermentation and producing different chemical and sensorial properties in the wine) differs.

2. Material and methods

2.1. Vineyard sites

Four vineyard sites located on the south bank of the Kawarau River in Bannockburn, Central Otago, New Zealand managed by Mt. Difficulty Wines were sampled during the 2013 harvest (Fig. 1). Long Gully (7.62 ha, orientation 20°), Manson's Farm (4.09 ha, orientation 54°), Pipeclay Terrace (6.83 ha, orientation 10°) and Target Gully (4.22 ha, orientation 7°) represent vineyards that currently, or have previously produced single vineyard Pinot Noir wine as they are consistently recognised by the winemaker and consumers to potentially produce wines with distinct sensory properties. The maximum distance between any of the vineyard sites is approximately 1 km and all vineyards are within a 2 km radius of one another. All vineyards were managed using the same viticultural practices and the same synthetic applications. Therefore, any effects of vineyard management could be considered minimal. Additional information on the planting at each site can be found in Supplementary Table 1 and general soil data for the sites is available in Supplementary Table 2.

2.2. Vineyard fungal community sampling, molecular methods and analysis

The fungal community present at each vineyard site was estimated from environmental DNA extracted from soil samples (Thomsen and Willerslev, 2015). Soil samples were collected at véraison, approximately six to eight weeks before harvest. Within each vineyard site, topsoil samples were collected from six evenly distributed vines. At each of the six vines, six individual topsoil samples were taken radially from around the base of each vine, totalling 36 soil samples per vineyard. Samples were immediately placed on ice and transported to the University of Auckland where they were frozen at −20 °C prior to analysis.

A composite soil sample was prepared from 1 g of soil from each of the six samples taken radially around each vine and mixed thoroughly. DNA was extracted independently from each of these six composite soil samples per vineyard resulting in six DNA extractions per vineyard site. DNA extraction was performed using the Zymo Research Soil Microbe DNA MiniPrep™ kit (Irvine, CA, USA). The DNA concentration of each sample was measured in triplicate using a Nanodrop® spectrophotometer and necessary dilutions were made to all samples to give a final DNA concentration of 10 ng/μl.

A 600 bp portion of the D1/D2 region of the 26S ribosomal RNA locus was amplified using the fungal specific primers NL1 and NL4 (Kurtzman and Robnett, 2003). This region allows for accurate downstream fungal identification via sequence alignment (Taylor et al., 2014). Multiplex identifiers were added to the forward primers to allow for bioinformatic sample discrimination. Four samples failed to amplify reducing the total number of samples to 20. AmpureXP beads were used to clean the PCR products and their quality was confirmed using an

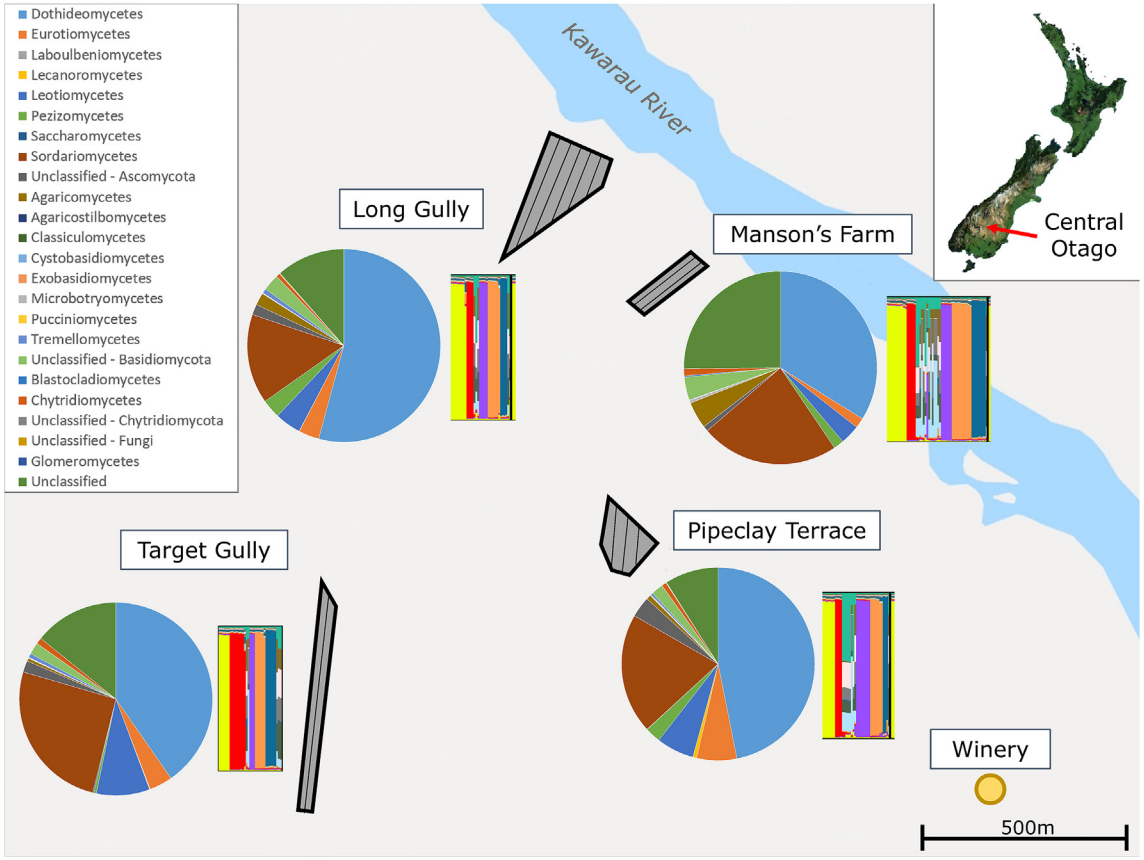

Fig. 1. Map of the study sites in Bannockburn, Central Otago, New Zealand. The four vineyard sites sampled are outlined in black with the vineyard orientation indicated with thin black lines. The location of the winery is indicated in yellow. The pie charts next to each vineyard site represent the proportion of reads assigned to each of the fungal classes indicated in the key. The DISTRUCT plots beside each site depicts the ancestry profiles of the *S. cerevisiae* genotypes analysed from each of the vineyard wines. Each vertical line represents one individual and the colours represents the proportion of ancestry assigned to each of the inferred populations from the InStruct analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Agilent 2100 Bioanalyzer High Sensitivity DNA® kits (Santa Clara, CA, USA). PCR products were pooled in equimolar amounts and sequenced using the Roche 454 GS Junior system at the University of Auckland's Centre for Genomics and Proteomics.

Processing of the sequencing data was performed using Mothur v.1.30 (Schloss et al., 2009). Primer sequences, reads less than 200 bp in length, low quality reads and homopolymer errors were identified and removed using the *PyroNoise* algorithm (Quince et al., 2011). Reads were aligned to the SILVA eukaryotic sequence reference database (Pruesse et al., 2007; Quast et al., 2013). PCR chimeras were removed using the *uchime* algorithm (Edgar et al., 2011). A distance matrix was generated using the command 'dist.seqs', and reads were clustered into operational taxonomic units (OTUs) with a 98% pairwise similarity score. This cut-off point was used based on approximate empirical delineations between species within the Ascomycota and Basidiomycota at the D1/D2 26S region (Kurtzman and Robnett, 1998; Romanelli et al., 2010). Taxonomic classification was performed by comparing a representative sequence from each OTU to the SILVA Fungi LSU training database. All OTUs were classified at kingdom, phylum, class, order, family and genus levels. The raw counts of reads assigned to each OTU were converted into proportions for each sample to standardise for the variation in reads per sample (McMurdie and Holmes, 2014). The sequence data is available in the Short Read Archieve under accession number PRJNA587013.

For statistical analyses, OTUs were considered to approximate species and were not collapsed based on their taxonomic classifications. Rarefaction curves for each sample were calculated using the 'rarecurve' command in the R package '*vegan*' (Oksanen et al., 2018). Variations in the vineyard fungal communities observed from the soil analysis were investigated over a number of different levels and using different tools. All analyses were performed in the statistical software R: version 3.4.1 (R Core Team, 2017). The absolute number of species (absolute species richness) was calculated for each sample and differences between vineyards were investigated using ANOVA. Differences between vineyard sites were further tested for by adding an additional level of complexity that accounts for the types of species present in each sample (relative species richness). This was done using binary Jaccard dissimilarities in a PERMANOVA as this limits the calculation of Jaccard dissimilarities to presence/absence of OTUs or species. To then add the additional dimension of the abundances of species (as inferred by the number of reads for each OTU) and thus evaluate the community composition, non-binary Jaccard dissimilarities were used in a PERMANOVA again testing for differences between vineyard sites. All PERMANOVA analyses were implemented with the 'adonis' command in the R package '*vegan*' (Oksanen et al., 2018). A diagrammatic explanation of the terms absolute species richness, relative species richness and community composition can be viewed in Morrison-Whittle and Goddard (2018). In addition to these analyses, to test the null hypothesis that the fungal community composition was randomly distributed across the four vineyard sites, additive diversity partitioning and hierarchical null model testing was implemented (Anderson et al., 2011; Crist et al., 2003). Metrics of both species richness (S) to test the presence/absence of each OTU and Shannon's index to account for the abundance of OTUs as inferred from read counts were used and the analyses were implemented using the 'adipart' command also in the R package '*vegan*'. Community differences between vineyard sites were

visualised using Constrained Correspondence Analysis (CCA) implemented in the R package '*vegan*'. CCA allows for partitioning of the variance into that explained by the vineyard site and that which is residual. The plot produced rotates the data to best visualise the variation explained by vineyard site.

2.3. Ferment Saccharomyces cerevisiae population sampling, molecular methods and analysis

Fruit from the four vineyard sites was commercially hand-harvested, processed and spontaneously fermented (i.e. no commercial starter yeast were added) in replicate single vineyard batches. There were variable numbers of ferments performed per vineyard site due to differences in their physical size resulting in four ferments from Long Gully, two from Manson's Farm, five from Pipeclay Terrace and three from Target Gully. Fruit was picked at approximately 25 °Brix and pH 3.2. Sulfur dioxide (SO_2) additions were made at a rate of 50 mg/L. Ferment samples were taken at approximately 5 °Brix as assessed through daily hydrometer measurements which corresponds to when *S. cerevisiae* abundance is likely greatest (Goddard, 2008). Samples of 50 mL were taken from each of the single vineyard tanks immediately after the morning punch-down, correlating with when the tank is likely the most homogenous. The sampling valve on each tank was washed with 70% ethanol prior to opening to avoid microbial contamination. The yeast cells in each sample were pelleted by centrifugation at 3500*g* for 4 min immediately after sample collection. The supernatant was discarded and the pellet was refrigerated and sent to the University of Auckland for processing.

The yeast pellets from each tank were plated on YPD agar with chloramphenicol (1% (w/v) yeast extract, 2% (w/v) peptone, 2% (w/v) glucose, 1.5% (w/v) agar and 200 mg/L chloramphenicol (Gayevskiy and Goddard, 2012)) in serial dilutions and incubated at 25 °C for two days. A total of 93 individual isolates were selected for each vineyard site from across the replicate tanks and were stored in 15% glycerol at −80 °C until further analysis.

Genomic DNA was extracted from colonies using a 1.25 mg/mL Zymolyase solution dissolved in 1.2 M sorbitol and 0.1 M KH_2PO_4 at pH 7.2 and treated with EMA to bind unwanted DNA fragments (Rueckert and Morgan, 2007). Species discrimination between *S. cerevisiae* and *S. uvarum* was performed using a multiplex PCR reaction and species-specific primers (de Melo Pereira et al., 2010). Genotyping of the *S. cerevisiae* isolates by 10 unlinked microsatellite loci was performed using capillary electrophoresis on an ABI3130XL (Applied Biosystems®, Life Technologies, Victoria, Australia) as described in Richards et al. (2009).

The resulting alleles were identified using the Microsatellite Plugin available in Geneious Version 6.1.6 (https://www.geneious.com), and allele sizes were binned to control for errors due to plus-A effects and run to run variation. Identical genotypes from the same vineyard site were conservatively considered to be a result of clonal expansion and these we collapsed to a single count in the final dataset. Genotype matching and estimates of inbreeding were calculated using GenAlEx (Genetic Analyses in Excel) version 6.5 (Peakall and Smouse, 2012, 2006). HP-Rare was used to estimate allelic richness of the populations at each vineyard site by rarefaction (Kalinowski, 2005). Population structure was investigated using a Bayesian clustering algorithm implemented in InStruct (Gao et al., 2007). This was deemed to be the most appropriate clustering method for this dataset as it does not assume Hardy-Weinberg Equilibrium and accounts for inbreeding which is expected due to the nature of *S. cerevisiae*'s replication and reproduction (Knight and Goddard, 2015). Three chains of one million MCMC iterations were performed for K = 1–15, each with a burn-in of 10,000 iterations. The Gelman-Rubin statistic was used to confirm the convergence of the MCMC chains (Gelman and Rubin, 1992) and the resulting ancestry profiles were visualised using DISTRUCT (Rosenberg, 2004). ObStruct was used to statistically test whether resulting ancestry profiles were structured by vineyard site (Gayevskiy et al., 2014).

3. Results

3.1. Vineyard fungal communities

A total of 55,197 good quality DNA sequence reads were obtained from the 20 composite soil samples collected across all vineyard sites (Supplementary Table 3). A total of 918 > 97% identity operational taxonomic units (OTUs, which approximate species) were distinguished and taxonomic assignment revealed 5 Phyla, 22 classes, 44 orders, 71 families and 89 genera. At the Phylum level, 52% of these comprised Ascomycota and 7.4% comprised Basidiomycota. The remaining OTUs were designated either Blastocladiomycota, Chytridiomycota or Glomeromycota (5.4%), or were unable to be classified (35.2%). Under the assumption that the number of reads approximates abundance, then Ascomycota remains dominant with 79% of total reads followed by Basidiomycota with 5.2%. These estimates are in line with previous metagenomic fungal community diversity from Central Otago vineyard soils (Morrison-Whittle and Goddard, 2015). The rarefaction curves for each sample can be viewed in Supplementary Figs. S1–S4. Further analyses of community composition are all performed on the OTUs, not the taxonomic classifications.

The distribution of taxonomic classes identified at each of the sites is shown in Fig. 1. Species richness for each site, as measured by the total number of OTUs identified, was greatest at Pipeclay Terrace with 577, followed by Manson's Farm with 484, Long Gully with 466 and Target Gully with 379 but species richness does not significantly differ among vineyards (ANOVA: $F_{3,16} = 1.32$, $P = 0.30$). When the types of species present are accounted for (relative species richness), highly significant differences were observed between vineyard sites (PERMANOVA with binary Jaccard dissimilarities: $R^2 = 0.236$, $P = 1 \times 10^{-4}$), as is reflected by the differential pie-chart portions in Fig. 1. These differences between vineyard sites are also evident when the abundances of species are considered (PERMANOVA with non-binary Jaccard dissimilarities: $R^2 = 0.244$, $P = 1 \times 10^{-4}$). Overall, approximately 25% of the variance in species types and abundances is explained by the vineyards the communities derived from (an average R^2 of 0.24). The striking difference between the fungal communities at each of the vineyard sites is evident when the community data are visualised in a CCA plot (Fig. 2). Pairwise PERMANOVAs taking into account the abundances of OTUs show there are significant differences ($P > 0.03$) between all vineyards except Long Gully and Target Gully, and Pipeclay Terrace and Target Gully; three of the Target Gulley replicates were lost reducing the power to differentiate this vineyard (Table 1; Fig. 2).

We used an alternate method of analyses (additive diversity partitioning and hierarchical null model testing) accounting for both species richness and abundance (as measured by Shannon's index) to test for vineyard specific fungal communities, and these are in-line with inferences from PERMANOVA analyses (Table 2). For species richness, the observed values for alpha diversity or variation within each locus (α_1) and within each vineyard (α_2), and beta diversity or variation between the loci (β_1) are significantly lower than the simulated values under the null hypothesis; however, the observed beta diversity or variation between the vineyards (β_2) is higher than simulated. This shows the communities within vineyards, but not necessarily within loci, are more similar to each other than they are between vineyards, supporting different fungal communities between vineyards ($P < 10^{-4}$). The same is true when the abundance of these species is accounted for, except the beta diversity or variation between loci (β_1) is also higher than the simulated value.

3.2. Ferment Saccharomyces cerevisiae populations

Of the 372 isolates tested, 358 (96.2%) were positively identified by PCR as *S. cerevisiae* and 14 (3.8%) were identified as *S. uvarum*. Twelve

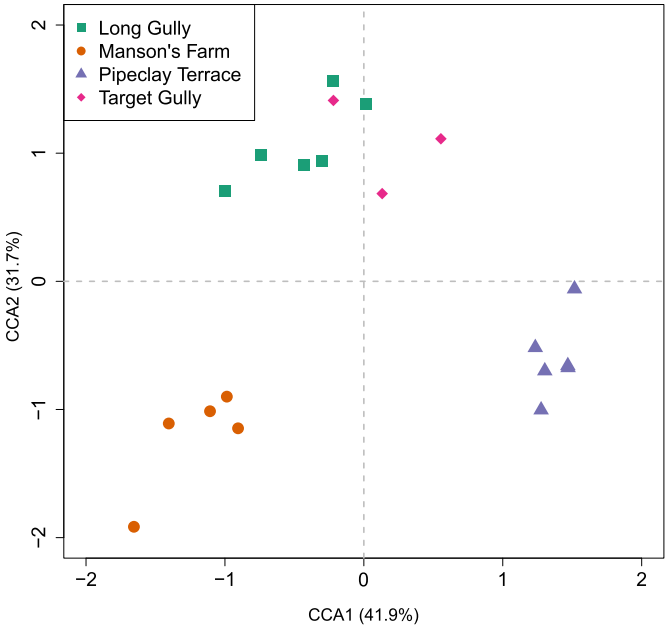

Fig. 2. CCA plot showing the variation in the fungal communities from each of the vineyard soil samples analysed. The shape and colour of the sample points indicate the vineyard site sampled as described by the legend. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

of the *S. uvarum* isolates were from the Pipeclay Terrace wines and two were from the Target Gully wines. From the 358 *S. cerevisiae* isolates, 337 genotypes were successfully determined; however, one of the 10 microsatellite loci (YOR267C) repeatedly failed to amplify and was subsequently removed from analyses. Therefore, the following analyses were performed on nine microsatellite loci. None of the genotypes identified matched those of commonly used commercial strains in New Zealand (Richards et al., 2009). Identical genotypes from each vineyard site were collapsed to one representative to conservatively account for clonal expansion during fermentation. This resulted in a total of 167 *S. cerevisiae* isolates for analysis; 35 from Long Gully, 57 from Manson's Farm, 39 from Pipeclay Terrace and 36 from Target Gully (Supplementary Table 4). This level of within ferment *S. cerevisiae* diversity is similar to other estimates derived from commercial ferments in New Zealand (Gayevskiy and Goddard, 2012; Goddard et al., 2010; Knight and Goddard, 2015).

A total of 82 alleles were identified across all loci with between 3 and 17 different alleles at each locus. Consistent with Knight and Goddard (2015), YFR028C and YML091C reported the greatest number of alleles with 14 and 17 respectively. Allelic richness did not differ between the sites with all estimates falling within one standard deviation of each other (Table 3). No loci conformed to Hardy-Weinberg equilibrium ($P = 0.001–0.04$) suggesting a high degree of inbreeding within this *S. cerevisiae* population (Table 3). This is expected given *S. cerevisiae*'s life cycle and is in-line with other New Zealand spontaneous ferment populations (Gayevskiy and Goddard, 2012; Goddard et al., 2010; Knight and Goddard, 2015). InStruct (Gao et al., 2007) was employed to analyse these data due to the inbred nature of these populations and analyses suggest the optimal number of subpopulations (K) were 11 given the data. The ancestry profiles for the isolates do not appear to cluster by vineyard site (Fig. 1) and ObStruct (Gayevskiy et al., 2014) analyses show there is no correlation between inferred population structure and vineyard site ($R^2 = 0.01$, $P = 0.72$).

Table 2
Pairwise PERMANOVA results between vineyard sites. All *P*-values have been corrected for multiple comparisons using the Benjamini & Hochberg correction for false discovery rates (Benjamini and Hochberg, 1995).

Vineyard sites		R^2	*P*-value
Long Gully	Manson's Farm	0.16	0.0315*
Long Gully	Pipeclay Terrace	0.16	0.027*
Long Gully	Target Gully	0.15	0.1692
Manson's Farm	Pipeclay Terrace	0.21	0.018*
Manson's Farm	Target Gully	0.22	0.0315*
Pipeclay Terrace	Target Gully	0.13	0.321

4. Discussion

There is increasing evidence for geographic differentiation of microbial communities and populations at > 100 km regional scales (Bokulich et al., 2014; Gayevskiy and Goddard, 2012; Knight and Goddard, 2015; Martiny et al., 2006; Morrison-Whittle and Goddard, 2015; Taylor et al., 2014), but we do not understand the scale at which such patterns hold (Meyer et al., 2018; Miura et al., 2017). Here we demonstrate differentiation between vineyard soil fungal communities but not between populations of grape must *S. cerevisiae* within a 2-km radius in Central Otago, New Zealand, providing evidence of fungal geographic differentiation at sub-regional scales.

Previous work using metagenomics and *S. cerevisiae* population genetics show Central Otago to harbour the most differentiated fungal communities and *S. cerevisiae* populations associated with vineyards and wine from the New Zealand regions analysed to date (Knight and Goddard, 2015; Taylor et al., 2014). Absolute species richness, as measured by the number of OTUs, for all four sites combined (918 OTUs) is comparable to previous reports of soil fungal diversity from the Central Otago region generally (845 OTUs) (Morrison-Whittle and Goddard, 2015). PERMANOVA analyses, additive diversity partitioning and hierarchical null model testing converge to show there are differences between the vineyard fungal communities at each of the four Central Otago vineyard sites. Previous research in arid Australia investigating microbial diversity on a gradient from $1\text{-}10^{10}$ m^2 indicates that microbial eukaryote communities show high local diversity but moderate regional diversity, with geographical distance being a better predictor of diversity than land use (Green et al., 2004). The authors argue ascomycetes are responding to small scale changes in soil chemistry, water and resource concentrations rather than geomorphic land system classifiers (Green et al., 2004). This is supported by evidence from a vineyard in China that shows the effect of soil organic carbon on the diversity of soil fungal communities (Liang et al., 2019). Miura et al. (2017) report a clear distance-decay relationship for fungal

Table 1
Results from the additive diversity and null model testing of the vineyard communities using both richness and abundance indices (9999 permutations).

		α_1 (within vine)	α_2 (within vineyard)	β_1 (between vines)	β_2 (between vineyards)	γ
RICHNESS (*S*)	Observed	189.6	476.5	287.0	441.5	918.0
	Simulated	341.0	664.6	323.6	253.4	918.0
	P-value	1×10^{-4}	1×10^{-4}	1×10^{-4}	1×10^{-4}	1
ABUNDANCE (SHANNON'S INDEX)	Observed	3.52	4.06	0.54	0.33	4.38
	Simulated	4.25	4.35	0.09	0.03	4.38
	P-value	1×10^{-4}	1×10^{-4}	1×10^{-4}	1×10^{-4}	1

Table 3
Summary statistics for the *S. cerevisiae* populations isolated from each vineyard site.

Region	Number of individuals[a]	Site specific genotypes	Mean allelic richness[b]	Inbreeding Coefficient F_{IS}
Long Gully	35	18 (51.4%)	6.32 ± 2.63	0.399
Manson's Farm	57	37 (64.9%)	7.26 ± 2.89	0.387
Pipeclay Terrace	39	23 (59.0%)	5.77 ± 1.91	0.458
Target Gully	36	13 (36.1%)	7.59 ± 2.91	0.463

[a] The number of individuals included in the final analysis after the collapsing of identical individuals due to clonal expansion within vineyard sites.
[b] Calculated for each locus independently using HP-Rare and based on 68 genes. The mean ± 1 standard deviation are reported.

communities using six vineyard sites in Chile across a range of approximately 35 km suggesting spatial factors could influence site specific microbial communities and by extension the site's terroir. On a global scale, climatic, edaphic and floristic variables have been shown to be the strongest predictors of fungal diversity, again demonstrating the importance of the environment on fungal communities (Tedersoo et al., 2014). Given the small scale and controlled environments (Pinot Noir vineyards managed by the same company in the same way) evaluated here, climatic and floristic variables are less likely to be strong drivers of fungal community differentiation; however it is possible the fungal communities are responding to differences in local soil types and nutrient availability, and these difference are in-line with the inference that natural selection is the dominant force defining fungal community assemblage in New Zealand vineyards (Morrison-Whittle and Goddard, 2015). Site level soil analyses were available from the growers at the vineyard site level (Supplementary Table 2) but no soil composition data was measured for the specific soil samples analysed in this study. While some variation in the parameters measured between the sites is seen in the site level data, whether these differences directly affect the fungal biodiversity measured at the sites was not able to be explicitly tested; however, differences in soil composition between the sites would comprise part of the potential difference that may define why fungi differ between sites. Regardless of the drivers of these differences, there is potential that different fungal communities at the sites differentially affect fruit and wine quality (Bokulich et al., 2014; Gilbert et al., 2014).

While we see differences between the overall fungal communities between vineyard sites, there is no spatial differentiation of populations of one species in this community (*S. cerevisiae*) isolated from spontaneous ferments of fruit derived from these vineyards. There is evidence for *S. cerevisiae* population differentiation at distances of over 100 km (Gayevskiy and Goddard, 2012; Knight and Goddard, 2015) and global scales (Liti et al., 2009; Wang et al., 2012); however, there is evidence for reasonable gene-flow among regional populations in New Zealand. Importantly, Knight and Goddard (2015) detected no *S. cerevisiae* population differentiation between native and agricultural habitats less than 100 km apart in multiple New Zealand regions including Central Otago, and the inference here is again in-line with this: *S. cerevisiae* populations are homogenised below ~100 km. In addition, a genome wide population study across distances less than 17 km in the USA provided evidence for *S. cerevisiae* gene-flow (Hyma and Fay, 2013). *S. cerevisiae* is sessile, but it has been associated with numerous potential vectors. On small scales insects are likely vectors and *S. cerevisiae* is known to be associated with bees, wasps and fruit flies (Goddard et al., 2010; Reuter et al., 2007; Stefanini et al., 2012), and *S. cerevisiae* has been shown to disperse within vineyards (Buser et al., 2014). *S. cerevisiae* has also been associated with birds which may provide a vector for larger distances (Francesca et al., 2012). The movement of people and their associated agricultural articles has also been inferred to move *S. cerevisiae* at small (Knight and Goddard, 2015) and global scales (Fay and Benavides, 2005; Legras et al., 2007). Given the evidence of *S. cerevisiae* movement across multiple scales by a number of potential vectors, it is perhaps not surprising we find no differentiation between *S. cerevisiae* populations in these vineyards given their close proximity.

Alternatively, it is possible there are vineyard-specific populations, but that fruit processing in the winery has obscured this. Fruit from all sites was processed at the same winery located within 2 km of the vineyard sites (Fig. 1). However, tanks were decontaminated and cleaned before being filled with fruit. High-throughput sequencing of the fungal ITS region from surface samples of a USA pilot-winery indicates surfaces may harbour large populations of *S. cerevisiae* and other yeasts prior to harvest, but the majority of the surface communities before and after harvest comprised organisms with no known link to wine fermentations (Bokulich et al., 2013). In addition, Bokulich et al. (2013) were unable to evaluate *S. cerevisiae* population diversity as they sequenced the ITS region. Other studies examining winery resident *S. cerevisiae* strains have reported that winery resident populations are important sources of yeasts in uninoculated fermentations (Blanco et al., 2011; Ciani et al., 2004; Santamaría et al., 2008). With the potential for different yeast species brought in from the vineyard to establish and populate resident winery microbiota, the opportunity for the same strains to be introduced to successive ferments and even successive vintages is conceivable (Bokulich et al., 2013).

Understanding how microbial communities and populations vary at different scales has direct implications for the quality and sensorial properties of agricultural products. Previous research has experimentally demonstrated that different *S. cerevisiae* populations present in different New Zealand regions can contribute regionally distinct chemical properties to Sauvignon Blanc wine (Knight et al., 2015). While no differences in *S. cerevisiae* populations were observed at the finer geographic scale examined here, differences were observed in the vineyard fungal communities. The potential of microbes to influence the regionally distinct style of a wine goes beyond the one dimensional effect of regionally differentiated fermenting strains of *S. cerevisiae*. Other yeast species are present during fermentation and are known to contribute and interact to produce different sensorial properties in wine (Anfang et al., 2009; Comitini et al., 2011; Jolly et al., 2014; Rossouw and Bauer, 2016). Additionally, microbial communities in the vineyard can affect vine and fruit quality through their roles in nutrient cycling, disease and potentially crop development (Barata et al., 2012; Berg et al., 2014; Philippot et al., 2013; Whipps, 2001). As the vineyard sites in this study comprised different fungal communities it is reasonable to suggest that these differences may interact with small scale soil and climate variances and potentially contribute to the unique sensory properties of these single vineyard wines. Controlled experiments must be conducted to formally assess whether different vineyard microbial communities modulate wine sensory characteristics. More generally, having a better understanding of the scale at which microbial communities are differentiated, and the effects these communities have on agricultural systems, has direct economic implications for the food and beverage industry and could inform sustainable management practices that maintain and enhance microbial diversity and thus food and beverage quality.

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Declaration of competing interest

None.

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Appendix A. Supplementary data

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