# Fungal diversity during fermentation correlates with thiol concentration in wine

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## Abstract

**Background and Aims:** Agricultural products deriving from the same genotypic clone often have different physical and sensory properties that influence their overall quality and value. Microorganisms may play key roles throughout the production of many crops, affecting plant and fruit health and modifying plant materials to produce socially and economically important commodities. Following this idea, we investigated whether fungal diversity both prior to and during fermentation was correlated with the concentration of three volatile thiols important to Sauvignon Blanc aroma and flavour.

**Methods and Results:** We used molecular and metagenomics approaches to quantify yeast populations and GC/MS to quantify thiols and analysed these using random forest statistical approaches. The species of *Saccharomyces* yeasts present at the end of fermentation are significantly correlated with the concentration of 4-mercapto-4-methylpentan-2-one, while several other fungal species present in the must, which are known to be associated with vine and fruit health, are also correlated with thiol concentration.

**Conclusions:** These data highlight the relationship between the presence of *Saccharomyces uvarum* and the production of 4-mercapto-4-methylpentan-2-one, while some members of the fungal community correlate with thiol concentration generally. Thus, components of the fungal community may potentially affect the accumulation of odourless precursors in grape via pathogenic effects during fruit ripening, but further research is required to confirm such speculation.

**Significance of the Study:** This work emphasises the need for a better understanding of the interactions between microbial populations and agricultural products, and has implications for the management of fungal diversity and disease in these systems.

*Keywords: fungi metagenomics,* Saccharomyces

## Introduction

Microorganisms play key roles in the production of quality agricultural goods, affecting plant and fruit health and converting plant materials into socially and economically important commodities (Whipps 2001, Fleet 2006, Peiffer et al. 2013, Philippot et al. 2013). During the processing of plant materials such as grapes for winemaking, different species and strains of yeast are known to produce variable concentration of volatile compounds that affect the sensory properties of the final products (Howell et al. 2004, Viana et al. 2008, Anfang et al. 2009, Zott et al. 2011). In wine, many of these yeast-derived aromas and flavours produced during fermentation result from the conversion of odourless precursors in the grape must (Darriet et al. 1995, Tominaga et al. 1998, Swiegers and Pretorius 2005, Dubourdieu et al. 2006). Pathogenic fungi present in vines and grapes may also potentially alter the concentration of odourless precursors in the grapes of infected vines and bunches (Thibon et al. 2009, 2011, Barata et al. 2012) and thus may potentially affect the final flavour and aroma of a wine. Volatile thiols are a class of compounds produced from aromaless precursors by microorganisms, and these tend to positively contribute to wine styles (Coetzee and du Toit 2012). Here we investigate the relationship between the concentration of three volatile thiols in wine and fungal species (including yeast) diversity in grape juice and during fermentation to reveal the effect of microbial species diversity on one sensory aspect of wine.

Spontaneous fermentation of grape juice to wine is completed by a succession of indigenous yeasts that occur naturally on grapes, which are transferred to the grape must (Pretorius 2000, Xufre et al. 2006). During the early stages of a spontaneous fermentation, a diversity of yeast species is observed with *Saccharomyces* species being rare (Pretorius 2000, Xufre et al. 2006, Goddard 2008). As the fermentation progresses, *Saccharomyces* species outcompete other microorganisms by engineering the ecosystem through the preferential fermentation of sugars to create a toxic hot-anaerobic-alcoholic environment (Goddard 2008). Through this fermentative process, *Saccharomyces* species also produce a wide range of metabolites that have a positive influence on wine sensory attributes including volatile thiols (Lambrechts and Pretorius 2000, Swiegers et al. 2006, 2009). Typically, *Saccharomyces cerevisiae* is responsible for wine fermentation, although the presence of *Saccharomyces uvarum* has also been widely reported (Torriani et al. 1999, Naumov et al. 2000, Demuyter et al. 2004, Masneuf-Pomarède et al. 2010, Zhang et al. 2010). Studies show that these two species of *Saccharomyces* produce different levels of volatile compounds during wine fermentation (Murat et al. 2001, Masneuf et al. 2002, Dubourdieu et al. 2006, Masneuf-Pomarède et al. 2010). Other species of the

*Saccharomyces sensu stricto* species complex are rarely reported associated with vineyards and wine ferments (Naumova et al. 2005, Sicard and Legras 2011).

Non-*Saccharomyces* species present in the early stages of fermentation also contribute desirable sensory properties and complexity to the wine (Romano et al. 2003a, Clemente-Jimenez et al. 2005, Ciani et al. 2006, Hernández-Orte et al. 2008, Anfang et al. 2009, Comitini et al. 2011, Gobbi et al. 2013), although negative effects have also been reported (Comitini et al. 2011). Much work has evaluated the contribution of particular non-*Saccharomyces* yeasts to wine composition, often using co-inoculation trials with *S. cerevisiae*, and different species have been shown to contribute different attributes (Ciani and Maccarelli 1998, Romano et al. 2003b, Clemente-Jimenez et al. 2005, Hernández-Orte et al. 2008, Ciani et al. 2010, Comitini et al. 2011). While non-*Saccharomyces* yeasts appear to play a role in the final flavour and aroma of a wine, *Saccharomyces* species are required to complete the fermentation with different species and strains interacting to produce unique flavour and aroma profiles in the finished wine (Howell et al. 2006, Anfang et al. 2009, Sadoudi et al. 2012). Both fungal communities and *S. cerevisiae* populations have been shown to vary with geographic region (Bokulich et al. 2014, Taylor et al. 2014, Knight and Goddard 2015) affording the potential for microorganisms to contribute to regionally distinct wine phenotypes (Knight et al. 2015, Bokulich et al. 2016).

Sauvignon Blanc is a major contributor to the New Zealand (NZ) wine industry comprising 86% of wine exports (New Zealand Winegrowers 2016). The volatile thiols 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercapto-hexan-1-ol (3MH) and 3-mercapto-hexan-1-ol acetate (3MHA) are important in Sauvignon Blanc aroma and flavour and are typically described as having box tree, passionfruit, black currant bud, broom, grapefruit and guava characteristics (Dubourdieu et al. 2006, Swiegers et al. 2009, Coetzee and du Toit 2012). These compounds are highly potent with a low sensory detection threshold and are made by yeast during fermentation from odourless precursors in the grape must (Darriet et al. 1995, Tominaga et al. 1998, Dubourdieu et al. 2006, Coetzee and du Toit 2012).

Here we spontaneously ferment commercially derived *Vitis vinifera* cv. Sauvignon Blanc grape juice sourced from several geographic regions in NZ, and we correlate the resulting concentration of thiols with fungal diversity in these juices and ferments. Fungi present at the start and *Saccharomyces* species present at the end of fermentation were quantified as well as the final concentration of 3MH, 3MHA and 4MMP.

## Materials and methods

### Sample collection and fermentation

Sauvignon Blanc juice was collected from 37 vineyards across NZ, comprising six vineyards from each of Hawke's Bay, Martinborough, Nelson, the Awatere Valley, Central Otago and the Wairau Valley (and an extra sample derived from the Wairau Valley); samples were taken from the tank after pressing (see Figure S1 for locations). These samples were couriered on ice to The University of Auckland. A 50 mL sample of each juice was centrifuged at 1811× *g* for 5 min to pellet microorganisms. The supernatant was discarded and the cells frozen at −20°C for further community sequencing analyses detailed below. Each juice was

spontaneously fermented at 15°C in 10 L volumes. Sauvignon Blanc is typically fermented at a lower temperature in NZ and thus 15°C was chosen to reflect industry practice. As spontaneous fermentation progresses, the yeast community becomes dominated by *Saccharomyces* species, most commonly reported as *S. cerevisiae* and *S. uvarum* (Torriani et al. 1999, Naumov et al. 2000, Demuyter et al. 2004, Masneuf-Pomarède et al. 2010, Zhang et al. 2010). However, data show that the formation of the thiols, 3MH and 3MHA, occurs early in the fermentation (Harsch et al. 2013). Thus after 21 days, 50 mL samples were taken to characterise the *Saccharomyces* populations and the concentration of thiols in the wine. These samples were again centrifuged at 1811× *g* for 5 min and frozen at −20°C for later chemical analysis.

### Fungal community analysis of the juice

The composition of the fungal community in the initial juice samples was quantified using Roche 454 next generation sequencing technology (Margulies et al. 2005). Total DNA was extracted from the juice samples using the Zymo Research Soil Microbe DNA MiniPrep kit (Zymo Research Group, Irvine, CA, USA). A 600 bp fragment of the D1/D2 26S ribosomal RNA locus, known to provide a good signal for fungal community differentiation (Taylor et al. 2014), was amplified using the fungal specific primers NL1 and NL4 (Kurtzman and Robnett 2003). Distinct multiplex identifiers were added to the primers to bioinformatically distinguish between samples. AmpureXP beads (Beckman Coulter, Indianapolis, IN, USA) were used to clean the polymerase chain reaction (PCR) products and remove primer dimers with the final quality confirmed using Agilent DNA1000 chips (Agilent, Santa Clara, CA, USA). The samples were multiplexed and unidirectionally sequenced using a Roche 454 GS Junior sequencer (Roche, Basel, Switzerland) at The University of Auckland.

Post-processing of DNA sequencing data was performed using Mothur version 1.30 (Schloss et al. 2009). Low quality and erroneous sequences were removed starting with primers, low quality reads and reads smaller than 200 bp. Subsequently, homopolymer errors as identified using the PyroNoise algorithm (Quince et al. 2009) and finally PCR chimeras identified using the UCHIME algorithm (Edgar et al. 2011) were removed. Individual sample identifiers were assigned to the remaining reads and the data were merged for further analysis. To further account for potential error in the dataset, unique sequences were compared to a fungal reference database and those not assigned to fungi were removed. The remaining sequences were clustered into groups or operational taxonomic units (OTUs) sharing more than 98% identity. Multiple species of Ascomycota and Basidiomycota (fungi) have empirically been shown to differ by less than 2% at the 26S rDNA gene (Kurtzman and Robnett 2003, Romanelli et al. 2010); thus these groups are considered to approximate species. To taxonomically identify each OTU, the 'classify.seqs' command in Mothur was used. This script selects a representative sequence for each OTU (i.e. a sequence that had a minimum distance to other sequences in the same cluster). These sequences were compared to a fungal taxonomic database and classified to all levels including genus level and above. Consensus sequences with less than a 70% match at any taxonomic level were listed as unclassified.

As further quality control, any OTU that had less than five reads total, or was present in only one sample, was

conservatively removed. The raw counts of reads assigned to each OTU were converted into a proportion for each sample to standardise for the variation in reads per sample (McMurdie and Holmes 2014). The raw sequence data for each sample are available in GenBank in the Sequence Read Archive (SRA) under accession number SRP110972.

### *Saccharomyces species analysis at the end of fermentation*
Yeasts from the 21st day of spontaneous fermentation were identified using culture-based methods and molecular identification, but not metagenomics analysis as *Saccharomyces* species are expected to dominate the community at this time (Goddard 2008). Samples were plated in serial dilutions on yeast extract peptone dextrose (YPD) agar (1% yeast extract, 2% peptone, 2% glucose with 50 µg/mL chloramphenicol to retard bacterial growth) and incubated at 28°C for 2 days. Colonies observed on these plates were all round, smooth and cream in colour, typical of *Saccharomyces* species. Thus, 94 individual colonies from each sample were isolated for molecular identification. Genomic DNA was extracted with a 1.25 mg/mL zymolyase solution dissolved in 1.2 mol/L sorbitol and 0.1 mol/L $KH_2PO_4$ at pH 7.2 and treated with ethidium monoazide to bind unwanted DNA fragments (Rueckert and Morgan 2007). *Saccharomyces cerevisiae* and *S. uvarum* are the most commonly reported *Saccharomyces* species from wine fermentations, while other members of the *sensu stricto* complex are more commonly associated with natural environments (Naumova et al. 2005, Sicard and Legras 2011). A multiplex PCR was performed with primers that can identify *S. cerevisiae*, *S. uvarum* and *S. pastorianus* (de Melo Pereira et al. 2010). Of the identified isolates, the proportion of *S. uvarum* in each sample was calculated. It is important to note that as only two *Saccharomyces* species were identified in the fermentation samples, the inverse of the proportion of *S. uvarum* is the proportion of *S. cerevisiae*.

### *Thiol analysis*
The Day 21 wine samples (50 mL) were sent to Hill Laboratories Limited, Hamilton, NZ, for quantitative chemical analysis of the volatile thiols 3MH, 3MHA and 4MMP. Chemical compounds were extracted from the wine samples using solid phase microextraction (SPME) and quantified in extracts using GC/MS.

### *Statistical analysis*
We used a combination of approaches to investigate the relationship between the microbial community of the samples (the explanatory variables) and final concentration of thiols (the response variables). The concentration of thiols was log transformed to account for exponential behaviour of the measurements. Regional variation of thiol potential was identified as a potential confounder (Lund et al. 2009, Benkwitz et al. 2012), and thus the origin of juices was included in models to account for this variation. Random forest analysis (Breiman 2001, Cutler et al. 2007) was employed to identify the covariates most important in explaining the variation in thiol concentration. A random forest analysis generates bootstrapped regression trees based on the explanatory variables, and uses the averages of these trees to estimate the relative importance of each of the variables in explaining the response (Cutler et al. 2007). To assess the importance of a variable, the average prediction error is used, which assesses how useful a variable is to determine the value of the response. It is measured by the

mean squared error and is computed using a permutation approach (Hastie et al. 2009). The importance of all explanatory variables was ranked, and the top ranked variables were further investigated using conditional inference trees (Horthorn et al. 2006). For a partition to be generated in a conditional inference tree, a statistically significant difference is required, minimising bias and overfitting (Horthorn et al. 2006). All analyses were performed in R version 3.2.1 (R Core Team 2015) using the packages 'randomForest' (Liaw and Wiener 2002) and 'party' (Horthorn et al. 2006).

To investigate further the effect of the *Saccharomyces* species influencing the fermentation, Pearson's product-moment correlations were calculated between the proportions of *S. uvarum* as a total of all *Saccharomyces* isolates found in the Day 21 wine samples and the concentration of thiols in these samples.

## Results
The 454-sequencing of the initial juice samples resulted in a total of 29 253 quality reads after processing. In 11 samples, the proportion of OTU002 was high (Dataset S1). This OTU was identified as the genus *Saccharomyces*, which should be rare prior to fermentation (Goddard 2008). A high proportion of *Saccharomyces* suggests that fermentation had started in these samples, possibly due to a large proportion of damaged berries (Mortimer and Polsinelli 1999) or variance in transit time to the laboratory. We do not have any juice composition data to determine whether the high proportion of *Saccharomyces* in these samples was because of damaged fruit or the start of fermentation; thus we conservatively removed samples for which the *Saccharomyces* abundance exceeded 10%, and this reduced the dataset to 26 samples. After removing OTUs that had less than five reads and were present only in one sample (as explained above), the number of quality reads for each sample ranged from 149 to 1372 and 88 OTUs were defined in total (Dataset S2).

At the end of fermentation, two species of *Saccharomyces* were identified: *S. cerevisiae* and *S. uvarum*. Isolates from some samples could not be identified due to PCR failure and were scored as unknown (Dataset S1). Failed samples were randomly distributed among samples, and over 70 isolates from each sample were positively identified, and used to calculate the proportion of each species. *Saccharomyces cerevisiae* and *S. uvarum* varied in proportion across the Day 21 samples: eight samples reported no *S. uvarum*, and one was entirely *S. uvarum* (see Dataset S1).

A wide range of concentration was observed for the three volatile thiols quantified (Dataset S1). One sample from Martinborough (MARLI) reported a concentration of both 3MH and 3MHA below the detection threshold of the analysis (10 ng/L), and 4MMP was low at just 16 ng/L. As two of the three response variables were unable to be quantified, this sample was removed from further analyses, resulting in a total of 25 samples. Of these remaining samples, the highest concentration of 3MH was 10 100 ng/L, of 3MHA was 2745 ng/L and of 4MMP was 291 ng/L (Dataset S2). All thiols exhibited a skewed distribution with many smaller values and a long tail of few, larger values. We therefore used the log of these concentration values for analysis to avoid larger values biasing the analyses. 4-Mercapto-4-methylpentan-2-one (4MMP) was unable to be quantified in four samples as it was below the 10 ng/L detection threshold of the analysis. For analytical purposes, the concentration for these samples was imputed as a random number between zero and ten (the detection limit of the assay). This approach

permits the inclusion of these samples in analysis while preventing undue influence on the model. The final dataset can be seen in Dataset S2.

Yeasts modulate the balance between 3MH and 3MHA through the production of an alcohol acetyltransferase that converts 3MH to 3MHA, and an esterase enzyme that reverses this reaction (Swiegers et al. 2006). These two volatile thiols thus exist in an equilibrium and should be correlated with one another as previously reported by Masneuf-Pomarède et al. (2006). Pearson's product-moment correlation coefficient shows 3MH and 3MHA as significantly positively correlated ($r = 0.72$, $t_{34} = 6.10$, $P = 6.37 \times 10^{-7}$). As the concentration of these compounds is not independent, we analysed the molar sum of 3MH and 3MHA to investigate their total production as well as the ratio of the two to test if yeast species affects their equilibrium.

### Effect of species diversity on the concentration of 4MMP
Random forest analysis revealed that 44% of the observed variation in 4MMP concentration may be explained by region, proportion of *S. uvarum* in the Day 21 samples, and the proportion of the 88 OTUs identified in the juice. The proportion of *S. uvarum* at the end of fermentation and the region of the juice was sourced from stand out as the two most important variables explaining the concentration of 4MMP in the Day 21 wine samples (Figure 1). OTU0011 and OTU0046 were ranked next with similar importance scores, and the importance of subsequent variables reduces (Figure 1).

Taxonomic identification suggests that OTU0011 corresponds to the genus *Alternaria* and OTU0046 corresponds to the genus *Penicillium*. Partial dependence plots indicate that 4MMP concentration is highest in the Wairau and Awatere Valleys, which are located in the Marlborough region, and in Martinborough (Figure 2). The relationship between the proportion of *S. uvarum* and the concentration of 4MMP is not linear but suggests that a higher proportion of *S. uvarum* results in a higher concentration of 4MMP (Figure 2). Subsequent conditional inference tree analysis using these same
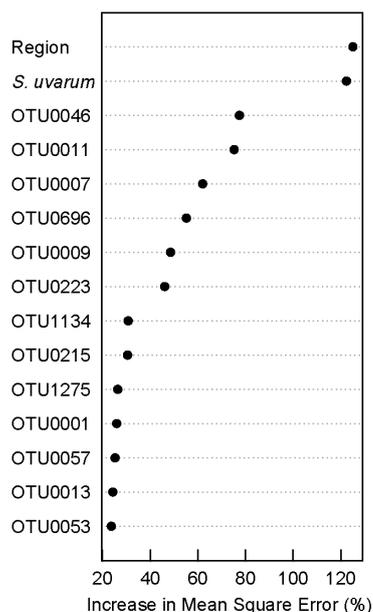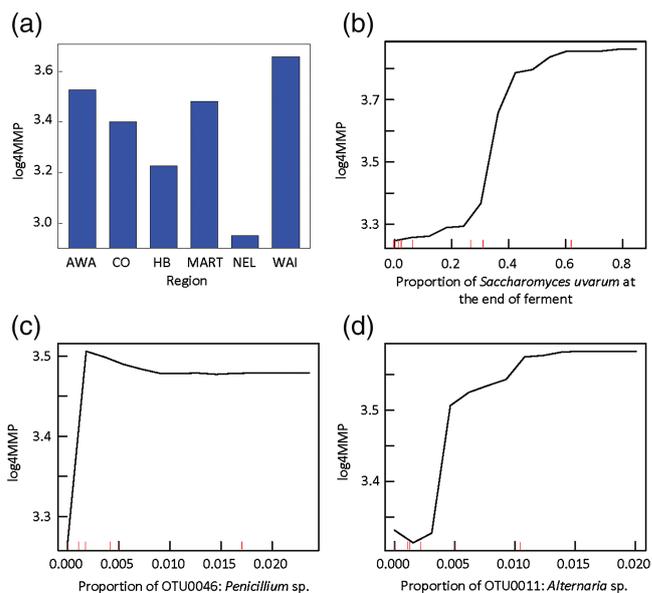


**Figure 2.** 4-Mercapto-4-methylpentan-2-one (4MMP) partial dependence plots for the top four variables: (a) region; (b) *Saccharomyces uvarum* at the end of fermentation; (c) proportion of OTU0046 to *Penicillium* sp. and (d) proportion of OTU0011 to *Alternaria* sp. identified in the random forest analysis while holding the other variables constant. The tick marks (|) on the *x*-axis of the panels (b)–(d) represent the deciles of the training data and thus reflect the spread of the data. AWA, Awatere Valley; CO, Central Otago; HB, Hawke's Bay; MART, Martinborough; NEL, Nelson; WAI, Wairau Valley. The location of each of the regions is shown in Figure S1.

four variables resulted in only two terminal nodes explained by the proportion of *S. uvarum* at the end of fermentation (Figure 3). Ferments with a proportion of *S. uvarum* less than 11% produced a significantly lower concentration of 4MMP compared to that of ferments with a proportion higher than 11%.

### Effect of species diversity on 3MH and 3MHA
As the production of 3MH and 3MHA are linked, we first investigated whether any variables could explain the molar sum of 3MH and 3MHA. Random forest analysis returned a best model in which the predicted molar sum was negatively correlated with the observed molar sum: meaning we are unable to identify any significant effect on the molar sum of 3MH and 3MHA.

Different species and strains of yeast may affect the equilibrium between 3MH and 3MHA during fermentation (Swiegers et al. 2005, Coetzee and du Toit 2012). Therefore, we also tested the ratio of 3MH to 3MHA in the wine produced. A random forest analysis explained 7.6% of the total variance, with geographic region reported as the most important factor (Figure 4). This was followed by the proportion of *S. uvarum* at the end of fermentation, then OTU0008 and OTU0053. Taxonomic assignments suggest that OTU0008 corresponds to an unclassified genus of the Sclerotiniaceae family and OTU0053 corresponds to an unclassified species of the Didymellaceae family (Table 1). No significant splits were identified using conditional inference tree analysis.

### A closer look at Saccharomyces species diversity at the end of fermentation
The analyses above show that the proportion of *S. uvarum* at the end of fermentation correlates with the concentration of thiols. We performed additional analyses with all 36 samples testing the effect of *Saccharomyces* species composition on



**Figure 1.** Variable importance as a measure of the percent increase in mean square error when that variable is removed from the random forest analysis for 4-mercapto-4-methylpentan-2-one (4MMP). The taxonomic identification of the OTUs is listed in Table 1. OTUs, operational taxonomic units; *S. uvarum*, *Saccharomyces uvarum*.
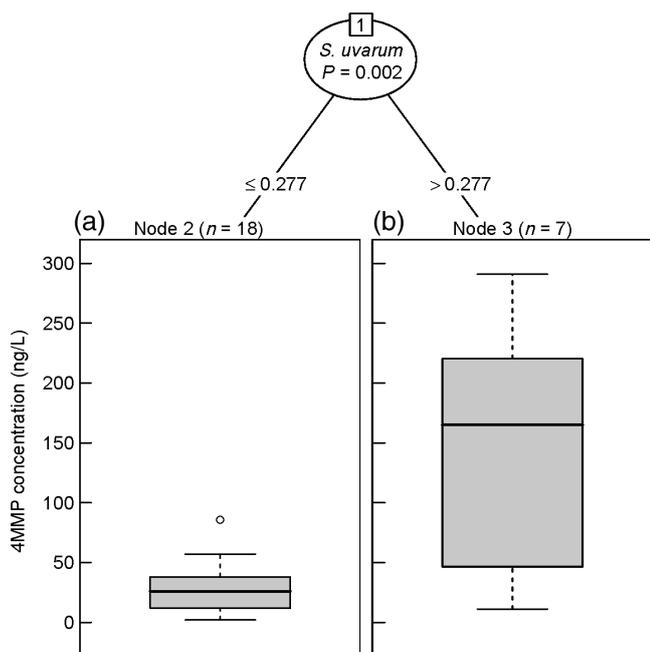
**Figure 3.** Conditional inference tree explaining the concentration of 4-mercapto-4-methylpentan-2-one (4MMP), performed using the 25 wine samples in Dataset S2. Only three nodes were identified in this analysis, with the proportion of *Saccharomyces uvarum* at the end of fermentation being identified as the only explanatory variable that significantly affects 4MMP concentration. (a) The 18 wine samples with a proportion of *S. uvarum* at the end of fermentation less than 0.277 have a significantly lower concentration of 4MMP compared to that of (b) the seven wine samples with a proportion of *S. uvarum* higher than 0.277 (*P* = 0.002).

the concentration of thiols. The proportion of *S. uvarum* is significantly positively correlated with the concentration of 4MMP (*r* = 0.607, $t_{34}$ = 4.46, *P* = 8.55 × 10$^{-5}$). The molar sum of 3MH and 3MHA or the ratio of 3MH and 3MHA are not significantly correlated with the proportion of *S. uvarum* at the end of fermentation (*r* = 0.285, $t_{34}$ = 1.74, *P* = 0.092 and *r* = 0.24, $t_{34}$ = 1.44, *P* = 0.16).
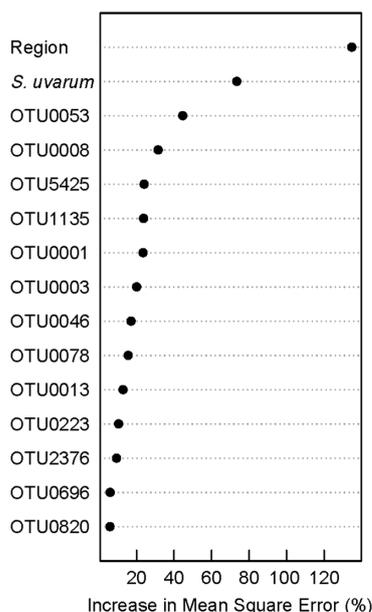


**Figure 4.** Variable importance as a measure of the percent increase in mean square error when that variable is removed from the random forest analysis for the ratio of 3-mercapto-hexan-1-ol (3MH) and 3-mercapto-hexan-1-ol acetate (3MHA). The taxonomic identification of the OTUs is listed in Table 1. OTUs, operational taxonomic units; *S. uvarum*, *Saccharomyces uvarum*.

**Table 1.** The taxonomic identification of the operational taxonomic units identified and presented in the random forest analyses.

| OTU code | Genus identification |
|---|---|
| OTU0001 | *Columnosphaeria* |
| OTU0003 | *Cladosporium* |
| OTU0007 | *Torulaspora* |
| OTU0008 | Unclassified (Family: Sclerotiniaceae) |
| OTU0009 | *Davidiella* |
| OTU0011 | *Alternaria* |
| OTU0013 | *Hanseniaspora* |
| OTU0046 | *Penicillium* |
| OTU0053 | Unclassified (Family: Didymellaceae) |
| OTU0057 | *Columnosphaeria* |
| OTU0078 | *Wickerhamomyces* |
| OTU0215 | Unclassified (Phylum: Ascomycota) |
| OTU0223 | Unclassified (Kingdom: Fungi) |
| OTU0696 | Unclassified (Phylum: Ascomycota) |
| OTU0820 | Unclassified (Phylum: Ascomycota) |
| OTU1134 | Unclassified (Order: Dothideales) |
| OTU1135 | *Phaeodothis* |
| OTU1275 | Unclassified (Phylum: Ascomycota) |
| OTU2376 | *Malassezia* |
| OTU5425 | Unclassified (Phylum: Ascomycota) |

OTU, operational taxonomic unit.

## Discussion

Microorganisms are vital for the production of quality agricultural commodities, affecting product quality throughout the development process (Whipps 2001, Fleet 2006, Peiffer et al. 2013, Philippot et al. 2013). For wine we attempt to elucidate which fungal species modulate the production of three important volatile thiols in Sauvignon Blanc. We show that different proportions of *Saccharomyces* species fermenting the juice, and differences in the fungal community in the starting juice, significantly correlate with the concentration of thiols in the wine, recapitulating the importance of microorganisms in the production of quality agricultural commodities.

*Saccharomyces* species are responsible for completing fermentation and different species and strains have been shown to produce different metabolites important to the final aroma and flavour of a wine (Masneuf et al. 2002, Howell et al. 2004, Dubourdieu et al. 2006). Here we provide evidence that a higher proportion of *S. uvarum* correlates with a higher concentration of 4MMP. This is consistent with reports that *S. uvarum* produces a higher concentration of 4MMP compared to *S. cerevisiae*, and this difference may be attributed to variation in the IRC7 gene (Masneuf et al. 2002, Dubourdieu et al. 2006, Roncoroni et al. 2011). The gene IRC7 is necessary for 4MMP production by yeast and while it is largely functional in *S. uvarum*, it is often not in *S. cerevisiae* due to a 38 bp deletion; therefore, the presence of a functional IRC7 gene may be responsible for a higher concentration of 4MMP observed when a higher proportion of *S. uvarum* was recorded (Masneuf et al. 2002, Dubourdieu et al. 2006, Roncoroni et al. 2011). This and previous studies show that *S. uvarum* is found throughout NZ (Zhang et al. 2010). The NZ Sauvignon Blanc is known for its fruity flavours and high concentration of thiols (Lund et al. 2009). Thus, perhaps the cooler climate combined with the cooler Sauvignon Blanc fermentation temperature employed by the NZ wine industry provides *S. uvarum* with an opportunity to more greatly partake in fermentation and thus contribute to a higher concentration of 4MMP typical of NZ Sauvignon Blanc.

The relationship between the community of fungi in juice prior to fermentation and the thiol concentration in wine is less clear. However, the random forest analyses do show a positive correlation between some members of the fungal community and the concentration of volatile thiols. Although some apparently benign fungal species have been shown to interact with various *S. cerevisiae* strains during fermentation to affect aroma production (Ciani et al. 2006, Viana et al. 2008, Anfang et al. 2009, Comitini et al. 2011), the species of fungi identified in this analysis as potentially affecting thiol concentration in wine are largely associated with plant and fruit diseases. *Alternaria* species on grapes are typically associated with grape bunch rot (Lorenzini and Zapparoli 2014) and the family Sclerotiniaceae contains a range of plant pathogens including *Botrytis cinerea*, which cause noble and bunch rot on grapes. Noble rot can affect the metabolism of the vine itself, altering the composition of the grape berries and thus affecting key aroma and flavour compounds in the wine (Thibon et al. 2009, 2011, Blanco-Ulate et al. 2015). Indeed, infection of healthy grapes by *B. cinerea* resulted in wines with a higher concentration of volatiles, particularly 3MH (Tominaga et al. 2006). Finally, the family Didymellaceae consists of a range of plant pathogens including many representatives of the genus *Phoma*, a known cause of leaf and stem spots (Aveskamp et al. 2008, Zhang et al. 2009). The data presented here suggest that pathogenic fungal species may be able to modulate the flavour and aroma of wine, here specifically the concentration of thiols, possibly by their effect on vine and fruit health, and the resulting impact on must composition. This is not surprising as previous research has shown that different types of grape rot and disease have different effects on the chemical composition and thus sensory properties of the wine (Barata et al. 2012). The three volatile thiols examined in this work are synthesised by yeast from odourless precursors in the juice; therefore, if these fungal species affect the accumulation of precursors in the grape, they could alter the potential for thiol production during fermentation. Alternatively, these vine and fruit pathogens may directly interact with fermenting yeasts to affect volatile composition in wine. The results presented in this study are by no means conclusive and simply highlight a correlation between species abundance and the concentration of three volatile thiols; however, it raises many interesting questions regarding the role of fungal communities throughout the entirety of the grape growing processes, and further research into these relationships could prove to be extremely relevant to industry and help inform vineyard management decisions.

It is important to acknowledge that it is possible the correlation of fungal community in the juice and the thiols obtained from wine samples resulted from the differential ripeness of grapes. Aroma compounds accumulate in grapes throughout the ripening process (González-Barreiro et al. 2015), and if fungal species diversity also changes during fruit ripening, the patterns observed in this analysis may reflect the ripeness of the grapes at harvest, rather than any effects imparted by the fungal community itself. Grape berries have been shown to harbour different fungal communities at different stages of their development with unripe berries reported to have a predominance of *Rhodotorula*, *Cryptococcus* and *Candida* species, along with the yeast-like fungus *Aureobasidium pullulans*, whereas ripe berries additionally harbour *Hanseniaspora* and *Metschnikowia* species (Fleet 2003). Statistical analysis suggests that differences in the fungal communities are apparent between grapes at the start of berry ripening and those that are overripe; however, at stages potentially experienced around harvest, no consistent differences in fungal diversity have been detected (Martins et al. 2014). As all the samples in this study were commercial juices collected at harvest, it could be assumed that the fruit was at a similar stage of ripeness and thus the likelihood of this effect confounding our results is not large.

## Conclusions
The results presented here provide further evidence towards the contribution of *S. uvarum* to 4MMP production during fermentation, highlighting the importance of the fermenting population of yeast on the final aroma and flavour of wine. There are also indications that fungi in juice associated with vine and fruit disease may influence the aroma potential of a wine. As this is a correlative study focused on a limited number of volatile thiols, further investigation into the mechanisms of these affects is required; however, these findings do recapitulate the importance of an integrated approach to the study of agricultural phenotypes and quality characteristics and emphasise the need for a better understanding of the interaction of microbial ecology in these systems.

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## Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website: http://onlinelibrary.wiley.com/doi/10.1111/ajgw.12304/abstract.

**Figure S1.** The location of the six regions where samples were collected from 37 vineyards. All samples were sent to The University of Auckland for fermentation and processing.

**Dataset S1.** Results from all samples analysed including thiol concentration of the wine and species diversity.

**Dataset S2.** The cleaned dataset that analysis was performed on. Ambiguous samples and low incidence OTUs were removed as described in the results.