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Harnessing soil biological functions to improve grapevine management for a sustainable industry

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1. Abstract

This project evaluated the potential application of more precise measures of soil microbiology as a component of a wider soil health monitoring program developed by AGWA project 'Setting benchmarks and recommendations for management of soil health in Australian viticulture' (DPI1101). These new DNA based measures have provided unprecedented insights into the composition and function of microbial communities in vineyard soils of four wine growing regions; the Barossa Valley, McLaren Vale, Sunraysia and the Yarra Valley. This project has identified key relationships between these microbial and prevailing soil chemical features and to a lesser extent, vine performance parameters. It provides recommendations on the types of measures that could be integrated into existing minimum data sets as well and on a web based repository for capturing and interpreting this data as part of a long term, monitoring program.

In summary we have:

1. Contributed expert knowledge to selection of soil biological measures (community structure & function) for a minimum dataset of soil health
2. Contributed expert knowledge of existing soil quality monitoring programs to identify a user friendly web-based tool for data entry and interpretation that meets the needs of growers and is consistent with existing Australian frameworks (e.g. www.soilquality.org.au)
3. Applied a range of DNA-RNA based approaches for the assessment of soil microbial community structure and function on a subset of sites utilised in AGWA project 'Setting benchmarks and recommendations for management of soil health in Australian viticulture' (DPI1101).
4. Contributed biological information to web-based fact sheets that detail links between indicators of soil health, vine management and performance.

2. Executive Summary

Better management of soils for grape and wine production will deliver a broad range of benefits including increased grape production and quality, greater water and nutrient use by the vine, improved structure, disease suppression and remediation of pesticides. Approximately 80% of these benefits can be linked to soil functions that are mediated by the resident soil microbial communities comprising the bacteria, archaea and fungi. This study therefore supports the evidentiary concept of 'microbial terroir' as a determining factor in regional variation in soil quality *and* in wine quality.

'You can't manage what you can't measure'. This study investigates the potential to integrate more sophisticated measures of soil microbiology into the broad scale monitoring of soil quality in Australian winegrowing regions. Coupled with this it has demonstrated the adaptability of an existing web based monitoring tool (www.soilquality.org.au) to meet the needs of individual wine and grape growers who wish to assess i) how their current vineyard management practices influence a range of soil quality parameters, ii), how these parameters compare with that of their neighbours and peers and iii), what management practices can be used to improve soil health under vineyards.

A range of soil microbial properties were assessed using different approaches and all have delivered insights into the biogeography of the soil microbial communities present in soils in 4 different wine growing regions. The low resolution technology (e.g. TRFLP 16S & ITS) demonstrated that bacterial and fungal communities differ in composition with respect to regional (e.g. Barossa, McLaren Vale, Sunraysia and Yarra Valley) and local (mid vine, undervine and adjacent native vegetation) vineyard soil conditions. The separation based on region was largely attributable to the Yarra Valley and Sunraysia regions and to the contrasting pH (and interrelated measures such as Ex Ca and $CaCO_3$) and organic C levels in these regions. Locational differences in both fungal and bacterial communities were greatest between the undervine and native vegetation sites with the mid row location being intermediate. This again reflects not only land use differences (ie managed vineyard soil and unmanaged native vegetation soil) but specifically differences related to soil chemical characteristics as captured in the minimum data set of tests.

The other two high resolution DNA sequencing approaches that use either 16S & ITS amplicons to identify bacterial and fungal features or rely upon the whole metagenome provided broadly similar relationships to those observed in the TRFLP technology. That is, regional and locational factors shaped microbial community composition and function. Both amplicon and metagenomic sequencing technologies provided significantly more detail on the taxonomic and functional differences observed at regional and local levels. For example, fungal communities are largely comprised of the phyla, in order of diversity, the Ascomycota, Basidiomycota and Glomeromycota. The largest differences in communities of fungi were between the Yarra Valley and Sunraysia regions; Dothidiomycetes being significantly lower and Archaerhizomycetes being higher in the Yarra Valley compared to elsewhere. In these regions location in the vineyard was significant only for two groups of fungi. Bacterial communities were also strongly influenced by wine region and vineyard location, again mainly attributable to differences in Sunraysia and the Yarra Valley. Acidobacteria strongly defined communities in the Yarra Valley whereas Nitrospira was a distinguishing features in Sunraysia soils. Soil pH was a likely driver for these differences (low pH in the Yarra Valley and high pH in Sunraysia). Bacterial communities differed more extensively than fungi with respect to location in the vineyard with the most pronounced differences undervine and attributable mainly to Sunraysia and Yarra Valley regions. Functional categories also separated on region and location with the Yarra Valley supporting functions associated with decomposition and disease control and Sunraysia supporting functions associated with growth and survival. Soil pH and organic matter were major drivers. While further analyses of sequence data will tease out more subtle associations it is clear that these technologies provide unprecedented insight into microbial communities and the key soil chemical drivers.

In terms of selection of a measure for inclusion in www.soilquality.org.au, the low resolution method, based on TRFLP, may be sufficient to indicate the diversity that exists within the bacterial and fungal communities based on a set of established assumptions where the higher the microbial diversity the more resilient the soils. On the other hand a soil microbial DNA sequence based approach that provides information on discrete functions deemed to be critical to vineyard performance would be considered of high value. For example, the presence and extent of functions related to N and P mineralisation, disease control and breakdown of aromatic molecules (i.e. pesticides, humic substances) undervine might provide a good indicator of not only the resilience of the soil but also the health and performance of the vine.

Perhaps the most exciting prospect to emerge from this research is the establishment of the relationship between soil microbial communities, vine vigour and wine quality. This research has demonstrated the strong influence of region, location and vine vigour on specific groups of microbes. The significance of these relationships is yet to be fully elucidated but it is entirely possible that signature communities in soil will have a direct relationship on wine quality (ie 'Microbial terroir'). Knowledge of the chemical factors that influence or drive this relationship provides the means for manipulating these microbial communities to achieve the desired wine quality outcome.

Some challenges remain in terms of both sampling regimes and commercial availability of tests. Microbial communities are dynamic and therefore sensitive to sample handling. Stringency and standardised sampling regimes are required to preserve the integrity of the microbial communities at the point of sampling. Unfortunately the consequence of less stringency in sampling was seen for the SA wine growing regions where a significant number of samples did not yield sufficient high quality DNA to be useful for subsequent sequencing protocols. In terms of availability of testing facilities, even the standard biological tests chosen for the MDS proved to be difficult to get from commercial laboratories (refer to DPI1101). Labile carbon, potentially mineralizable nitrogen (PMN) and microbial biomass analyses are generally available on a semi-commercial basis only. For the more sophisticated tests, an existing DNA based testing platform, SARDI's PreDicta B platform could be utilised as is the case for the Grains industry soil quality monitoring program. An added advantage is access to a whole range of new measures of soil biota.

The project outputs were showcased at "Workshop 19: How to interpret soil health tests to assess vineyard performance" as part of the 15th Australian Wine Industry Technical Conference held in Sydney in July 2013. This workshop attracted a capacity crowd of 50 participants from all Australian wine regions and included several from overseas and there is demand for more.

The overarching recommendation arising from this project is to maintain the momentum and build on the foundations laid by this project and DPI1101.
Specific recommendations for AWGA consideration relate to the further development of microbial tests that will enable prediction of a healthy soil, a healthy vineyard and high quality wine by:

- Promoting an industry standard for soil biological sampling that ensures preservation of the microbial DNA at sampling and during transit
- Critically assessing the range of soil microbial parameters trialed in this study to select a minimum dataset of features/traits that can be directly related to specific vine performance criteria,
- Extending studies of soil microbial biogeography to establish linkages with microbial communities associated with vines (leaves and fruit); evidence to support the existence of 'microbial terroir' will inevitably lead to options for manipulation,
- Continuing to evaluate and interpret the microbial DNA sequence information to identify regional trends associated with soil chemistry and vineyard management.

Related to the web based soil quality monitoring tool, it is recommended that AGWA also consider:

- Identifying an industry specific web site curator independent of the current web design team,
- Identifying soil quality champions within the Grape and Wine industry to promote the adoption of the web based monitoring tool and to lead in the establishment of threshold values for the minimum dataset of measures,
- Identifying vineyard soil specialists to assist in the development of fact sheets that specifically identify management options to overcome soil constraints or to otherwise enhance wine production,
- Developing fact sheets that outline the importance of soil microbes in soil health, vine vigour and potentially wine quality.

3. Acknowledgements

This research was funded jointly by Australia's grape growers and winemakers through their investment body, the Australian Grape and Wine Association and the Victorian Department of Environment and Primary Industries (DEPI).

The project was a collaboration with team members from DEPI and University of Western Australia.

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4. Background

This project addresses one of the key strategies in the GWRDC's 5 year R&D plan, under Program 4, specifically 4a and 4b. Sustainable production (Program 4 – Sustainability of Industry, Environment and Communities) – Investigate and disseminate methods of improving sustainable viticulture practices. By world standards, Australia uses relatively advanced technologies for vineyard production, but still has the capacity to improve input use (water, pesticides, nutrients) and should be able to achieve this through greater knowledge of management on soil quality specifically related to biological functions.

This project will satisfy the strategic plan aims to improve environmental management systems to the satisfaction of producers as well as consumers.

The relationship between key soil quality indicators and vine performance is poorly resolved and the project 'Setting benchmarks and recommendations for management of soil health in Australian viticulture' has designed a minimum data set (MDS) of soil health measures aimed at improving knowledge and awareness of soil health related to vine health in four wine regions across Australia.

This closely aligned project, addresses recommendation two of AGWA's Soil Health Review (White, 2010) addressing the questions:
a) What are the links between soil biological function and vine performance? How are these influenced by soil type, environment and management practices?
b) What are the key biological indicators of soil quality and how can these best be measured and used in a benchmarking system?

It has capitalised on the background expertise being generated by GRDC Soil Biology Initiative - II regarding i) the potential utilisation of the existing web-based soil quality monitoring framework with decision support functions, ii) appropriate selection of biological indicators, iii) statistically robust sampling design to capture temporal and spatial variability within vineyards, and iv) the application of high resolution DNA/RNA based technologies (extraction, sequencing and assembly). This alignment will ensure significant cost-efficiencies.

In Australia, soils are valued as a resource for sustaining and improving plant based production enterprises. Some of the factors relevant to the grape and wine industry include:

- A desire to better relate soil quality to grape juice quality to achieve a more consistent product
- Increasing input (fertiliser N/P, lime, biocides) and energy costs, diminishing supply of P (peak P) (Vaccari, 2009)
- Questions of efficacy of 'alternative' products & services; fertilisers, soil ameliorants/biological additives and some commercial soil biological tests
- A need to address soil degradation: Soil health decline associated with loss of carbon, and increases in salinity and acidity is likely to be exacerbated further by climate variability (Campbell, 2008; Australia State of the Environment Report, 2006)
- A desire to improve the carbon content of agricultural soils generally and specifically to quantify the capacity of soils to store (and lose) carbon (Sanderman et al., 2010).

The role of the living component of soil, i.e. the soil biota and specifically the microscopic majority, has received little attention in the past with most efforts focussing on the physical and chemical attributes of soil such as pH, salinity, organic C, total and mineral N, total and available P and bulk density (Winder 2003; State of the Environment reporting, 2001 and 2006; www.environment.gov.au/soe). The recent logarithmic advances in genetic techniques (extraction and sequencing technologies), and in computational power have removed many of the constraints that previously existed when dealing with the most complex ecosystem on the planet (e.g. soils) giving unprecedented access to the unculturable majority (Chen *et al.* 2008; Keller and Zengler 2004; Xu 2006). DNA/RNA based technologies, including the 'omics' technologies such as metagenomics, metabolomics and transcriptomics, take a whole

community approach to identifying key microbial taxa and functions of interest. Global examples of the adoption of these for monitoring soil biological properties can be found at the following websites: www.earthmicrobiomeproject.com, www.ecn.ac.uk/targetedmonitoring, www.gissol.fr and www.neoninc.org. The data generated thus far highlights the key role of soil type and land-use with more subtle changes associated with management. Locally, the groundswell of interest from major stakeholders from RIRCs, LandCare, CMAs, and agribusiness, who value the potential of the soil biological asset to improve profitability without threatening sustainability provides compelling justification for investment (Heemskerk et al. 2010; www.dairyaustralia.com.au/Our-Dairy-Industry/Dairy-Moving-Forward).

5. Project Aims & Performance Targets

The overall aim of this project was to evaluate and apply medium and high resolution measures of soil microbial communities to align and potentially add value to the suite of soil quality indicator tests developed in AWGA project 'Setting benchmarks and recommendations for management of soil health in Australian viticulture' (DPI1101). The DNA-based microbial measures have been incorporated into multiparametric analyses to determine relationships with soil physico-chemical features and vineyard management.

The key objectives are;

1. To contribute expert knowledge in the selection of soil biological measures (community structure & function) as part of a minimum dataset of key indicator tests for vine health and juice quality.
2. To contribute expert knowledge of existing soil quality monitoring programs to identify a user friendly web-based tool for data entry and interpretation that meets the needs of growers and is consistent with existing Australian frameworks (e.g. www.soilquality.org.au)
3. To refine and apply a range of DNA-RNA based approaches for the assessment of soil community structure and function as a pilot subset of sites utilised in AGWA project 'Setting benchmarks and recommendations for management of soil health in Australian viticulture'.
4. To contribute biological information to web-based fact sheets that detail links between indicators of soil health, vine management and performance.

The following modifications were made to the outputs. Output I (year 3) was modified to exclude temporal analyses which also meant that the original output J (year 3) was no longer relevant and has been excluded. Output G (year 2) was modified from 'Soil biology & vine correlates established' to 'Vineyard management impacts on soil biological communities'. This reflected the difficulty in obtaining individual vine performance data at all sites sampled.

(NB for the sake of clarity, the lettering of outputs have been modified from the contracted outputs for the sake of continuity from year to year.

Year 1

A. Selection of standardised biological tests
B. A user friendly web-based tool for data entry and interpretation that meets the needs of growers and is consistent with existing Australian frameworks (e.g. www.soilquality.org.au)
C. Selection of sites based on soil type x management practices

Year 2

D. Sample collection coordination meeting with AGWA project 'Setting benchmarks and recommendations for management of soil health in Australian viticulture'
E. Soil collections undertaken for soil biological analyses
F. Data entry into web-based tool
G. Vineyard management impacts on soil biological communities
H. Articles for National newsletter published (The Australian Grape and Wine Grower, etc.)

Year 3

I. Selection of site for detailed spatial analyses through meeting with AGWA project 'Setting benchmarks and recommendations for management of soil health in Australian viticulture'
J. Data entry into web-based tool
K. Refereed articles published and final report submitted

6. Methods

Methods are outlined against each of the contracted outputs where appropriate. Note that original outputs C, D & E have been combined into output C (Site selection & sample collection for microbial and physico-chemical analyses), outputs J & D combined into output E (Data entry into web-based tool) and outputs H & K combined into output F (Delivery Activities).

A. Selection of standardised biological tests
B. A user friendly web-based tool for data entry and interpretation (e.g. www.soilquality.org.au)
C. Site selection & sample collection for microbial and physico-chemical analyses
D. Vineyard management impacts on soil biological communities. DNA extraction & sequencing
E. Detailed spatial analyses of Carr's Vineyard (Yarra Valley)
F. Data entry into web-based tool

A. Selection of standardised biological tests

The process for selecting biological tests is outlined in Section 6A. The project combined with DPI1101 contributing to a two day workshop held in Melbourne on 14-15 December 2011. The Agenda covered soil quality datasets already available in viticulture and other industries, the relevance of soil health programs to viticulture, and the selection of soil indicators for viticulture. The Workshop attendees were asked to consider what the industry expects as an outcome from measuring soil quality, what are the five most important soil functions to measure and why, what are the most useful soil indicators and what should industry be measuring? The responses were incorporated into the final selection of biological tests (Section 6 Results and Discussion).

B. A user friendly web-based tool for data entry and interpretation

A web designer and a data base curator were engaged through a sub contract agreement with University of Western Australia to oversee input of soils data and fact sheets into www.soilquality.org.au. A meeting was held at UWA to outline the expectations of the Grape and Wine industry (as identified in section A) and to ensure alignment with existing extension activities and materials (e.g. EntWine).

C. Site selection & sample collection for microbial & physico-chemical analyses

Sites were selected from four wine growing regions, the Barossa Valley, McLaren Vale, Sunraysia and the Yarra Valley as part of the wider monitoring being undertaken by DPI1101. More detailed descriptions can be found in the AGWA final report for DPI1101. Summary descriptions are provided in Section 6 Results/Discussion.

The timing of soil sampling in all regions was approximately 4 weeks post-harvest in order to sample when vineyard activities were less demanding than during the growing season and vintage. This coincides with a second flush of root growth (the first being at budburst) (Freeman and Smart 1976).

Three sampling locations were chosen at each vineyard: undervine, the midrow and an adjacent non-viticultural site for comparison within 50 m of the vineyard, preferably with native vegetation. This is referred to as the 'native' location throughout the report. Sampling locations were geo-referenced using a global positioning system (GPS) for repeat sampling. The surface horizon (0-15cm) was utilised for microbial measurements.

Soil cores were collected using a 5-7 cm diameter hand auger to minimise damage to roots. For the undervine surface samples, five cores were taken in line with the nearest dripper to the grapevine trunk but extending approximately 20 cm into the midrow (Figure 1). The five cores were composited and mixed in the field.

For midrow surface samples, five cores were taken directly in the centre of the midrow aligned with the undervine sampling (Figure 1). The five cores were composited and mixed in the field.

For the adjacent native vegetation site, five cores were also taken and composited.
A total of 93 vineyards were sampled. In some vineyards, more than one panel (up to 3) was sampled, whilst in others, only a single panel was sampled. Panels were treated as replicate samples. This informed subsequent selection of samples for high resolution microbial analyses.



Figure 1. Soil sampling from undervine and midrow, with native area in the background (DPI1102 technicians: Bernadette Carmody & David Pearce)

Soil samples were then divided for chemical analyses and biological analyses. A protocol was distributed to DPI1101 collaborators in SA who agreed to send samples on ice to the DPI1102 team. For the remaining two Victorian regions, Sunraysia and the Yarra Valley, our field technicians (pictured above) provided on site guidance in the appropriate collection of samples and vital metadata (for web based tool www.soilquality.org.au) as well as organising permits and overseeing Phylloxera quarantine guidelines in the Yarra Valley.

The soil samples were all analysed according to the indicator tests selected as the minimum data set for soil quality monitoring (as determined by DPI1101):

- biological indicator - microbial biomass (MB), labile organic C (LOC) & potentially mineralizable N (PMN)
- chemical indicators – pH (in $CaCl_2$ & water), electrical conductivity (EC), exchangeable cations, cation exchange capacity (CEC), exchangeable sodium percentage (ESP), total organic carbon, dissolved organic C (DOC) and Calcium carbonate ($CaCO_3$)
- physical indicators – aggregate stability (slaking and dispersion) , air dry soil consistency, water holding capacity (WHC)

The preferred method of analysing microbial biomass carbon, i.e. chloroform fumigation extraction (CFE), was not available as a commercial test. Instead, all surface soils were analysed by the substrate induced respiration (SIR) method available at CSBP. There is an assumption that SIR values can be multiplied by 30 to convert them to CFE values, depending on soil type (Anderson and Joergensen,1997). All of the Victorian samples were analysed by the DPI1102 project team using the CFE method for comparison with the SIR method.

Total nitrogen (Leco method) was added as it is provided as part of the tests required to calculate total organic carbon (Leco method). The additional biological indicators - potentially mineralisable nitrogen and labile carbon – were suggested at the expert workshop but not agreed upon by DPI1101, so they were included in the analyses of the Victorian soil samples only.

The physical assessments were performed in-house by the DPI1101 project team following the methodology described in Field *et al*. (1997).
The chemical analyses were performed on all surface and subsurface soil samples by CSBP Soil & Plant Analysis Laboratory, Bibra Lake Western Australia, with the exception of total organic carbon. This was derived from total carbon (Leco method) and calcium carbonate content using the formula: Total organic C = Total C – ($CaCO_3$ x 0.12). Exchangeable sodium percentage (ESP) was calculated as exchangeable Na / cation exchange capacity (CEC) X 100%.

Samples of approximately 50 g for microbial analyses were placed in falcon tubes and then immediately into ice or where appropriate into a portable fridge (4°C). These samples were subsampled to estimate gravimetric moisture (24 hours at 104°C) and stored at -80°C until they were extracted for DNA sequencing (See Section D).

D. Vineyard management impacts on soil biological communities. DNA extraction & sequencing.

A total of 326 soil samples were collected as part of a coordinated effort from undervine, midrow and adjacent non vineyard areas (eg native vegetation sites) in four southern Australian wine growing regions during the post- harvest period. Table 1 summarises the samples used for the various soil microbial analyses.

Table 1. A summary of the sites sampled in each region for the various DNA based microbial community analyses.

Region	No sites per region	TRFLP*		DNA Sequencing: Amplicons		DNA Sequencing: Metagenomic
		Bacteria (16S)	Fungi (ITS)	Bacteria (16S)	Fungi (ITS)	Whole genome shotgun
Barossa Valley	74	74	74	9	9	9
McLaren Vale	104	104	104	12	12	12
Sunraysia	76	76	76	37	37	30
Yarra Valley	72	72	72	43	43	43
Total samples	**326**	**326**	**326**	**101**	**101**	**94**

Terminal restriction fragment length polymorphism

i) Soil DNA extraction

DNA was extracted from each 0.4 g sample using the Mo Bio PowerLyser PowerSoil™ DNA extraction kit and following modified manufacturer's instructions. Extracted DNA was then quantified using a nanodrop™ instrument and then stored at -20°C prior to the following microbial community analyses. For many samples from the SA regions in particular, DNA extractions were repeated three times to improve yield and quality parameters. A low success rate was achieved as reflected in how many of these samples were taken through to DNA sequencing.

ii) TRFLP of 16S & ITS regions

Microbial community structure was assessed using terminal restriction fragment length polymorphism (TRFLP) analyses of bacterial 16S rRNA and fungal ribosomal ITS genes, with the primer pairs 63F/1087R (V1–V5 variable regions; Marchesi et al., 1998; Hauben et al., 1997) and ITS1F/ITS4R (ITS1, 5.8S, and ITS2 regions; Gardes and Bruns, 1993; White et al., 1990), respectively. Forward primers were labelled at the 5' end with 6-carboxyfluorescein fluorescent dye. Each 50 µL amplification reaction contained 20 ng of template DNA, 0.2 µM of each primer, 25 µL GoTaq master mix (Promega, Sydney, NSW, Australia) and 21 µL sterile H_20. After an initial denaturation step of 95°C for 5 min amplification proceeded for 30 cycles of 95°C for 30 s, 55°C (eubacteria) or 53°C (fungi) for 30 s, and 72°C for 1 min, with a final

extension step of 10 min at 72°C. PCR products were visualized on 1.4 % agarose gels with SYBR Safe DNA gel stain (Invitrogen, Mulgrave, Vic, Australia). Amplicons were purified using Millipore MultiScreen filter plates (Merck Pty. Ltd, Kilsyth, Victoria, Australia) and then 200 ng of purified amplicon was digested using restriction endonuclease MspI (Promega, Sydney, NSW, Australia) for 4 hours at 37°C. Restriction digests were cleaned using 0.01 volume glycogen (20 µg/µL; Invitrogen, Mulgrave, Vic, Australia), 0.1 volume sodium acetate (3M), and 2.5 volume ethanol (95%), followed by two sequential washes with 70% ethanol. Cleaned digests (2 µL) were resuspended in 10 µL formamide (Hi-Di™, Applied Biosystems, Mulgrave, Vic, Australia) with 0.25 µL 500 LIZ® size standard (Applied Biosystems, Mulgrave, Vic, Australia), and fragment analysis was performed on an ABI 3730xl DNA analyser (Applied Biosystems, Mulgrave, Vic, Australia).

TRFLP data was evaluated using GeneMapper™ software (v. 3.7; Applied Biosystems, Mulgrave, Vic, Australia), and profiles were trimmed to between 50 and 500 bp. After within-project normalization using the sum of signal algorithm, a bin width of 1.0 bp, maximum peak width of 1.5bp, and a minimum peak height of 50 rfu was used to determine the presence or absence of each TRF in each sample.

iii) Selection of sub-set of samples for sequence- based microbial community analyses

DNA samples were selected for further analysis based on several sets of criteria: The chosen samples 1), were shown by TRFLP analyses to have either highly similar or highly divergent community structures, 2) had sufficient DNA available to perform both amplicon-based sequencing and metagenomic sequencing on the same sample, 3) had DNA of sufficient quality for high-throughput sequencing, and 4) came from sites where replicate sampling had occurred (i.e. more than one mid-row or under-vine sample). From this process, 101 samples were selected for high resolution amplicon and metagenomic sequencing. Many of the samples from both SA regions were not of sufficient quality to be taken into these analyses and this had a significant impact on subsequent multivariate analyses.

iv) Amplicon sequencing of 16S & ITS regions

For amplicon sequencing, DNA was sent to the Ramaciotti Centre for Genomics. Each sample was sequenced to further examine both bacterial (16S region using 27f-519r primers) and fungal communities (ITS region using ITS1f-ITS4 primers). Samples were multiplexed into sequenced libraries of 96 samples using sample-barcodes, and then each library was run on an Illumina MiSeq using v3 2x300bp paired end chemistry.

Once sequencing was complete, sequences were analysed using a bioinformatics pipeline that we developed in-house, using currently available freeware scripts. Briefly, this involved the forward and reverse sequence (fastq) files being merged using the freeware program PEAR v0.9.3 (Zhang, et al., 2014). We trialled a number of merging programs including PandaSeq and one offered by the Ribosomal Database Project pipeline (RDP; http://rdp.cme.msu.edu/; Cole et al., 2014), but found that we obtained a better percentage of merged reads with PEAR, which is also designed to work with variable amplicon lengths, which is ideal for using with the variable length amplicons of the ITS1 region of fungi.

After merging an average of 95-97% reads were assembled for 16S sequences (average length of approx. 466 bp) over both studies, and an average of 57-66% were assembled for ITS1 reads (average length of approx. 545 bp). Merging also removing all sequences with non-defined nucleotides (N's). Assembled reads were then put through the UPARSE pipeline using the USEARCH v7 software (http://drive5.com/usearch/manual/uparse_pipeline.html; Edgar, 2013). This quality control step included quality filtering using a maximum estimated error of 1. For the fungal ITS1 reads, an extra step in the pipeline was involved: After quality filtering with USEARCH, filtered reads were put through the freeware ITSx (Bengtsson-Palme, et al., 2013), which is able to extract the ITS1 region from the sequence, removing any surrounding ribosomal sequence which can lead to very misleading results downstream if retained. This step also

detected those sequences which are fungal in origin, versus, for example, from plants, allowing us to select only the fungal reads.

The quality filtered reads were then rarefied to the number of reads of the smallest sample, and then reads were de-replicated using the UPARSE pipeline. Sequences were then sorted and singletons removed, clustered into operational taxonomic units (OTU's) and chimera checked to remove false sequences, which output all probable OTU's. The original merged reads, including singletons, were mapped back to this OTU list to determine the number of each individual OTU in each sample, creating an OTU matrix. The fasta sequences of each OTU were then submitted to RDP for taxonomic analysis using the Classifer software (Wang, et al., 2007). Either the Bacterial 16S or UNITE Fungal ITS training set was used for sequence comparison. The taxa information could then be merged with the OTU table for final analysis.

v) Metagenomic sequencing (whole genome shotgun sequencing)

For metagenomic sequencing, Illumina Nextera kits were used to construct metagenomic libraries for each soil total genomic DNA sample, and these libraries were pooled to run on an Illumina HiSeq machine (DEPI-Victoria) across 2 lanes. The generated fastq sequence files were uploaded to the MG-RAST website (metagenomics.anl.gov) for analysis (Meyer et al., 2008). Sequences loaded to this website go through the website's analysis pipeline, and in time will be available to the public, including other researchers, for use. The project folder on the web-server holds over 706 million reads, of 93 billion bp of sequence. Sequence reads that were assigned to subsystem categories, with the default BLAST threshold of 1e-10, a minimum identity cutoff of 60%, and a minimum read length of 15bp, was obtained from the MG-RAST web-server, and matrices of the relevant data created.

vi) Statistical analyses

Significant differences in microbial taxa between communities were examined by ANOVA, followed by post hoc tests to determine where significant differences occurred, using Genstat (v14.1.0.593; VSN International Ltd, UK).

Microbial community patterns were evaluated by nonmetric multidimensional scaling (NMDS) using Primer6 (v6.1.16; PRIMER-E Ltd, Ivybridge, United Kingdom). All data was transformed as appropriate to satisfy requirements for normality within the different statistical tests. The raw data from MG-RAST was first standardised by converting to percentages of assigned sequences per sample that were assigned to each category, and then log transformed before ordinations were performed. The overall significance of both region and location on microbial communities was assessed by Permutational MANOVA (Permanova+; v1.0.6 PRIMER-E Ltd, Ivybridge, United Kingdom). Biological analyses used Bray Curtis similarity resemblance matrices, with 999 permutations of residuals under a reduced model.

Distance based linear models (DistLM) were used to select the soil chemistry and function variables that best predicted microbial community structure and function (Selection criterion, AICc; Selection procedure, Stepwise), using the Primer6 and Permanova+ software package, (v6.1.16 and v1.0.6 respectively; PRIMER-E Ltd, Ivybridge, United Kingdom).

E. Carr's Vineyard Sampling

A vineyard site to test spatial variability in microbial communities was selected in the Yarra Valley. The site in Carr's Vineyard in Coldstream is described in Fig 2. Briefly, it is comprised of 196 individual sampling points of which A, C, E, G, I, K are undervine samples, with B, D, F, H, J, L being mid-row samples. Samples 1- 8 encompass low quality wine from higher vigour vines with 9-16 producing high quality wine from lower vigour vines. The definition of wine quality& vine vigour vines was based on previous data of individual vines within our study area as supported by airborne imagery of plant cell density (a measure plant biomass), obtained in 2011 (Viewpoint ™ technology) cross referenced with wine quality from individual vines within the site. This made it possible to differentiate the above sampling points and to then relate this to soil microbial community and chemical characteristics.

The area sampled is defined in Figure 2 by a grid labelled by rows A-L and horizontally from 1-16. Sampling commenced at row A, which coincides with row number 38, panel 2 as per vineyard labels and concluded on row L (or row number 43 as per vineyard row labels). The distance from A-L was approximately 18 m, and the distance from sample 1-16 was 90 m.

Sampling occurred 6 weeks post-harvest in 2014. Prior to soil collection, a permit was obtained because the site is in a Phylloxera zone. Twenty soil cores were taken from an area 1.4m^2 from depth 0-10cm, thoroughly homogenised and placed immediately on ice for transportation. Soils were then sieved and separated for chemical analyses (CSBP laboratories) and microbial analyses as previously outlined in section C. DNA extraction and sequencing was undertaken as outlined in section D.

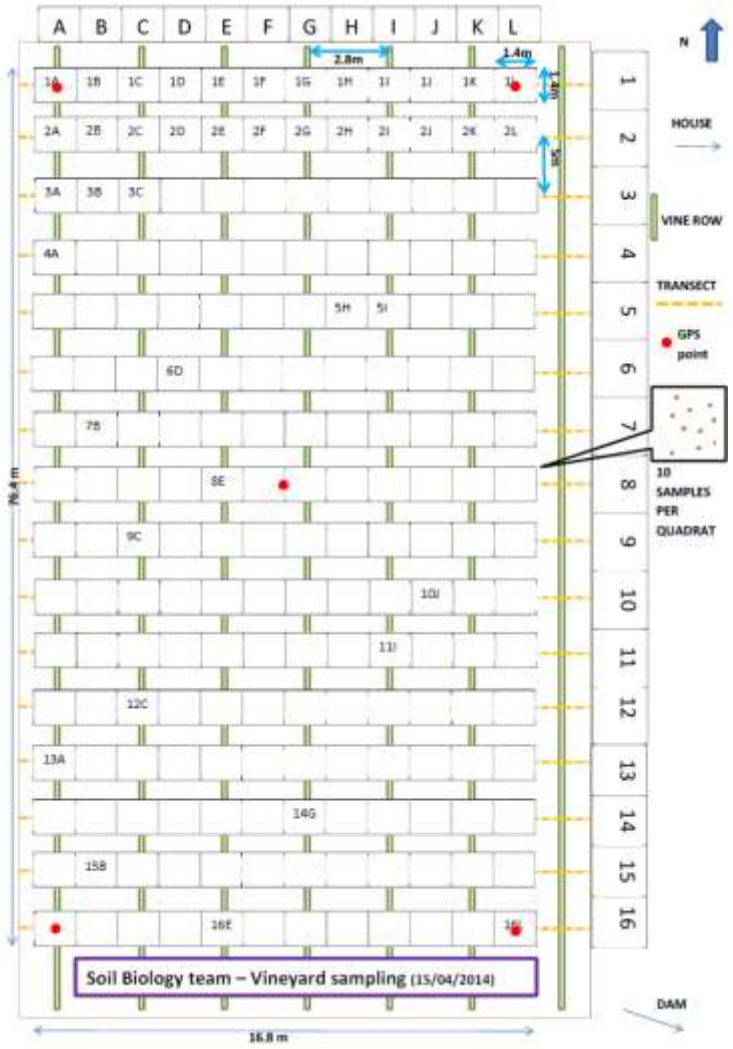


Figure 2. Sample layout in Carr's Vineyard for spatial sampling of microbial communities

F. Data entry into web-based tool

Spreadsheets of quality assured data from all 4 regional sites (Barossa valley, McLaren Vale, Sunraysia and Yarra Valley) were uploaded manually by the web designer. The web designer created a new website that runs alongside the existing website. This enables a large degree of flexibility to change parameters to suit individual industry needs. The existing operating system (eg Vista) has been upgraded to ensure ongoing support and greater privacy.

7. Results and Discussion

Results will be discussed against each of the contracted outputs where appropriate. Note that original outputs C, D & E have been combined into output C (Site selection & sample collection for microbial and physico-chemical analyses), outputs J & D combined into output E (Data entry into web-based tool) and outputs H & K combined into output F (Delivery Activities). The outputs addressed are:

A. Selection of standardised biological tests
B. A user friendly web-based tool for data entry and interpretation that meets the needs of growers and is consistent with existing Australian frameworks (e.g. www.soilquality.org.au)
C. Site selection & sample collection for microbial and physico-chemical analyses
D. Vineyard management impacts on soil biological communities
E. Detailed spatial analyses of Carr's Vineyard (Yarra Valley)
F. Data entry into web-based tool

A. Selection of standardised biological tests

This project provided significant oversight in the selection of a suite of biological measures to be included in a soil health monitoring program relevant for Australian grape and wine production soils. It drew upon prior knowledge developed globally (Black et al. 2008) and nationally through soil quality monitoring programs (GRDC Soil Biology Initiative and BioPlatforms Australia BASE project) and recommended the involvement of producers, advisers and technical experts to determine what tests to use and most importantly, the logical framework for selection of appropriate tests.

On 14-15 December 2011, a National Viticultural Industry Soil Quality Workshop was organised by project DPI1101 to identify a potential minimum set of indicators and tests for soil quality to provide decision support for grape growers for enhanced vine performance and overall system sustainability. The presentation also highlighted the role of soil microbial communities in critical soil functions and the vastly superior power of DNA-based technologies to resolve differences in these communities. Most importantly it highlighted how such biological data could be used to provide practical information to grape growers emphasising the importance of regionalising information to account for differences in soils and climate. Subsequent discussions with industry representatives, Cecil Camilleri (Yalumba), Bethany Collins (Treasury Wine Estate), Toby Bekkers (McLaren Vale) and Lexie McLymont/Dean Lanyon (GWRDC Five Sites project) enabled the establishment of a set of biological parameters that provided relevant information on soil functions that were considered of most importance to the grape and wine industry (Table 2).

Table 2. Summary of the biological tests chosen for potential inclusion in web site www.soilquality.org.au

Test	Method (Reference)	Indicates:
Microbial Biomass C	fumigation-extraction (Amato & Ladd, 1988)	Relative size of microbial community & potential for nutrient turnover
Potentially Mineralisable N (PMN)	Anaerobic Incubation, Method 7D2 (Rayment & Lyons 2010)	The amount of N in the plant available form
Labile C	(Weil et al. 2003, Method 6E1 Rayment & Lyons 2010)	The amount of C in the high turnover pool
Microbial community diversity	Terminal Restriction Fragment Length polymorphism (TRFLP)	Microbial community structure (bacteria & fungi)
Microbial community structure	Amplicon sequencing (16S & ITS)	Microbial community structure of all bacteria and fungi with taxa described to at least family level
Microbial community structure & function	Whole genome shot-gun sequencing 'Metagenomics'	Microbial community structure & function

B. A user friendly web-based tool for data entry and interpretation that meets the needs of growers and is consistent with existing Australian frameworks (e.g. www.soilquality.org.au)

This project compared and contrasted two existing soil health monitoring tools that could be adapted for monitoring the quality of Australian vineyard soils. In conjunction with DPI1101, the Cornell Soil Health model and that currently in use in Australia, www.soilquality.org.au were examined. The merits and drawbacks of each were presented to potential users (producers & consultants) at two industry attended meetings (DEPI Knoxfield Centre, Melbourne, December 2011 & the Plant Research Centre, Waite Institute, Adelaide, June 2012). It was concluded after the latter workshop that www.soilquality.org.au offered the flexibility and information required for monitoring individual wineries and for comparing soil condition in an industry and regionally relevant context. The traffic light approach (also part of the Cornell model), the ease of alignment with existing programs (e.g. ENTWINE) and the fact that this tool is curated as part of an existing industry investment appealed to producers and consultants.

C. Site selection & sample collection for physico-chemical & microbial analyses

Sites were selected from four wine growing regions as part of the wider monitoring being undertaken by DPI1101. A total of 93 vineyards were sampled. More detailed descriptions can be found in the AGWA final report for DPI1101. Summary descriptions are provided below.

i) Barossa Valley

One of the best known wine making regions in the world, located 60 km northeast of Adelaide in South Australia. With the oldest vines planted in 1843, the region contributes a significant proportion of Australian's premium grape and wine production.

Barossa has accounted for between 18 and 20 percent of Australian exports greater than $7.50 per litre in volume at a value worth $77.6M.

A total of 30 vineyard sites were identified through local knowledge and with the assistance of the Barossa Grape and Wine Association. A few weeks after harvest (late March – early April) 30 vineyards were sampled.

ii) McLaren Vale

Is located approximately 35 km south of Adelaide in South Australia. It is internationally renowned for the wines it produces.

A total of 31 vineyard locations were selected in 2013 for soil sampling. The vineyards were chosen, in consultation with the McLaren Vale Grape Wine and Tourism Association, to cover the range of soil types in the region, as identified in the 1:50,000 soil map for McLaren Vale obtained from Primary Industries and Regions, South Australia (PIRSA), as well as the range of viticultural management approaches (conventional, organic, etc.) used in the region. Details of the area of McLaren Vale planted to vines were obtained from the Phylloxera and Grape Industry Board of South Australia.

iii)Sunraysia

The Sunraysia or Murray Darling region is located along the Murray River in north west Victoria and western New South Wales and extends from the border of Victoria with South Australia in the west, to Balranald in New South Wales in the east. It is the second largest wine region in Australia and the biggest in Victoria, with more than 1000 growers and 25,000 hectares producing more than 400,000 tonnes annually.

A total of 20 vineyards were sampled in 2013.

iv) Yarra Valley

Victoria's Yarra Valley is located less than one hour's drive east of Melbourne with its western boundary being 45 kilometres east of Melbourne's CBD. The Yarra Valley was Victoria's first wine growing district with a history stretching back 170 years. Vines were first planted in 1838 and viticulture spread rapidly through the 1860s and 1870s. The Yarra Valley is now recognised as one of Australia's leading cool climate wine regions and the total area undervine is 2,500 ha producing 15,000 tonnes annually.

A total of 12 vineyards were sampled in 2013.

D. Vineyard management impacts on soil biological communities

Soil microbial communities in vineyards of 4 wine growing regions were measured using a low resolution method (TRFLP) and two high resolution DNA sequencing methods (16S & ITS amplicons; metagenomics).

Several statistical analyses have been applied as per the PRIMER™software program. Using Permutational MANOVA, it is possible to see the significance of factors (region and location) that influence community structures. Using a combination of statistical approaches such as non-metric multidimensional scaling (NMDS) and distance linear regression model (DistLM) it is possible to differentiate broad patterns of microbial communities associated with region and sampling location (mid-row, under-vine, or native) and to identify the edaphic factors (physico chemical factors) that influence these patterns (Appendix 5 i). Note that in the NMDS figures, the longer the vector line the more significant the relationship.

Fungal communities (ITS)
Using Permutational MANOVA, it is clear that the fungal communities differ significantly according to both region and location (Table 3). To evaluate the factors driving this, we need to look at each region individually.

Table 3. ITS fungal TRFLP: PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)
Region	3	61258	20419	11.448	0.001
Location	2	14605	7302.3	4.0942	0.001
Region x Location	6	22043	3673.8	2.0598	0.001

The structure of fungal communities was strongly influenced by regional factors as shown by the strong clustering of data points (Fig 3a). The overlap of SA regions, Barossa Valley and McLaren Vale indicate that communities are most similar between these sites.

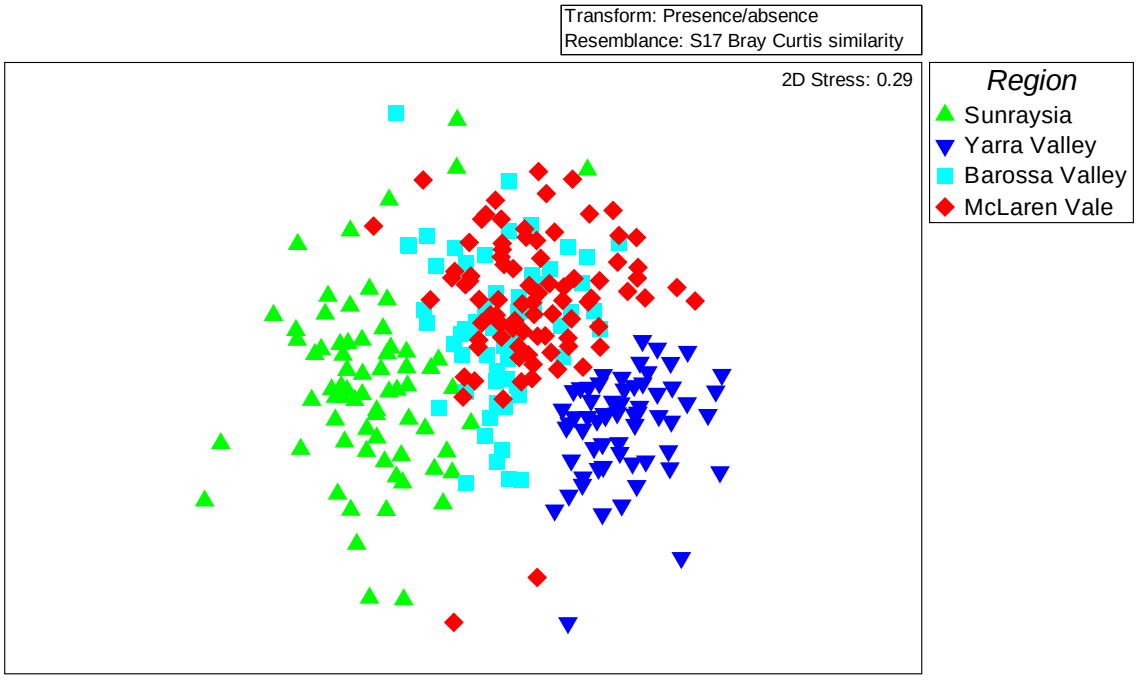


Figure 3a. Clustering of fungal communities by region

These regional differences in fungal communities may be attributed to lower levels of organic matter (including total N, total C, and PMN), combined with higher pH and higher concentrations of sodium (Na) and other cations (eg Ca and Mg) in the Sunraysia samples. Overall, pH followed by C factions (total and labile) are the primary factors driving the separation of Sunraysia fungal communities from the Barossa Valley, McLaren Vale, Yarra Valley fungal communities (Fig 3b).

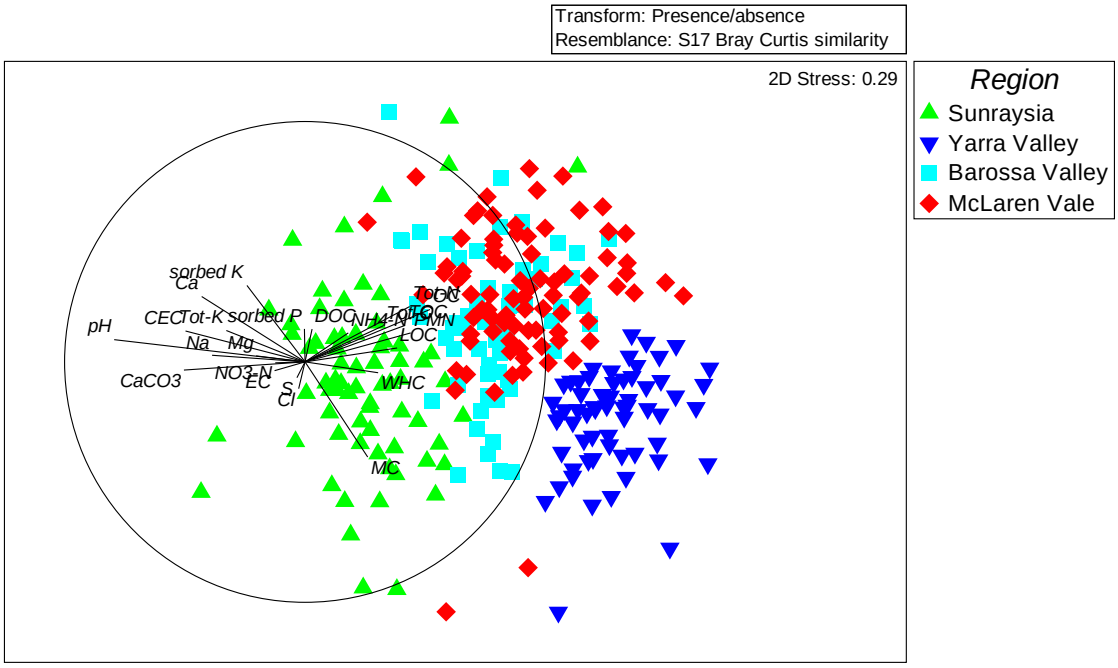


Figure 3b. Clustering of fungal communities by region showing influence of soil chemistry

The fungal communities are also influenced by sampling location within a region with fungal communities in soils under native vegetation being most different to those undervine. The communities in the midrow sampling point were somewhere in between (Figure 4a).

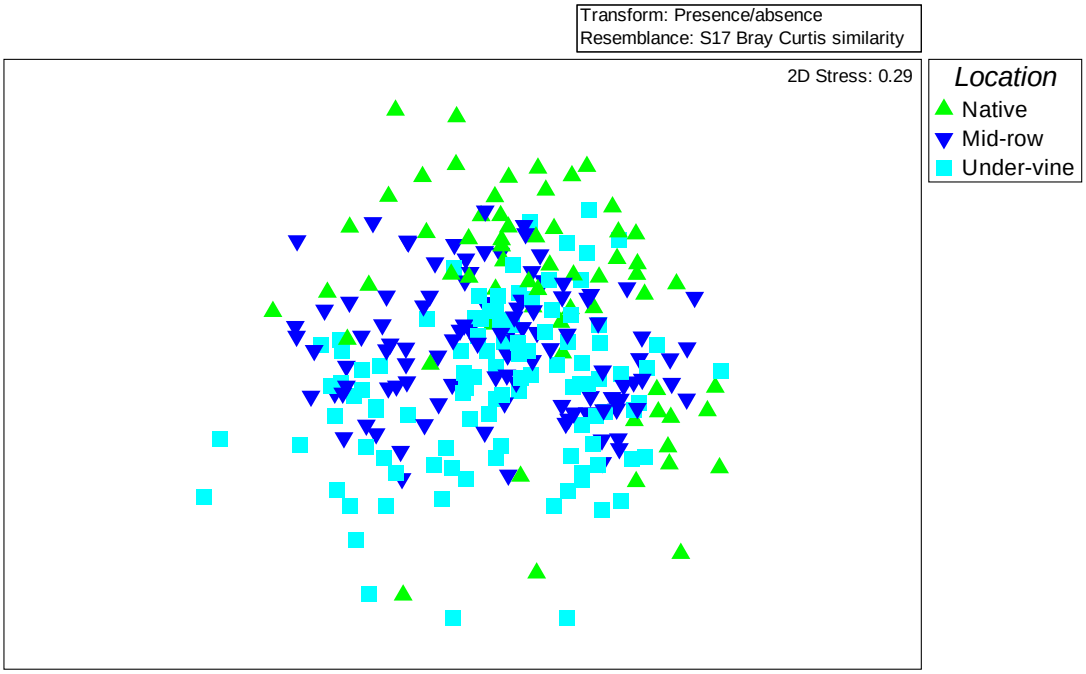


Figure 4a. Clustering of fungal communities by location

To evaluate the factor driving these locational differences, we examined each region individually.

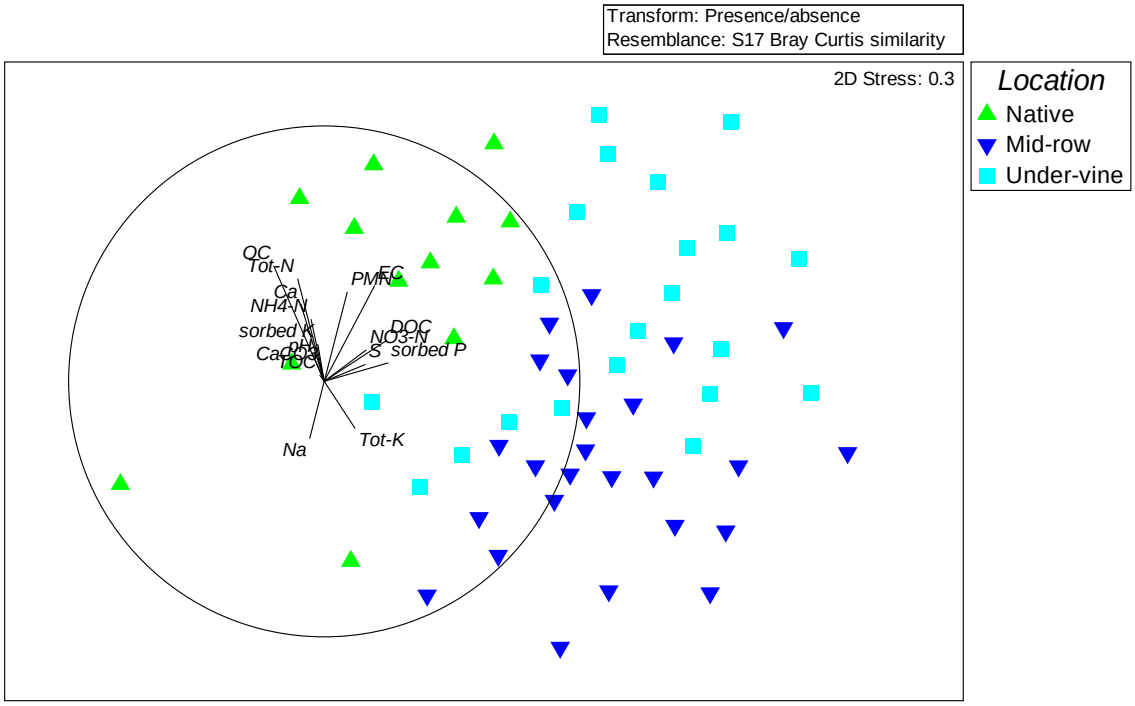


Figure 4b. Barossa Valley: Clustering of fungal communities showing influence of soil chemistry

In the Barossa Valley, sample location had a significant influence on the ITS fungal communities present (Fig 4b), with communities in the different sample locations being significantly different from each other when compared (Table 4). Although chemical variables such as Organic Carbon and total K appear to be associated with the patterns of distribution, we were not able to derive a DistLM model for fungal distribution at this site (no variable selected).

Table 4. ITS fungal TRFLP: PERMANOVA table of results for location

	Pseudo-F	P (perm)
Location	2.7952	0.001
Groups	**t**	**P (perm)**
Under-vine vs Mid-row	1.5075	0.001
Under-vine vs Native	1.6303	0.001
Mid-row vs Native	1.8824	0.001

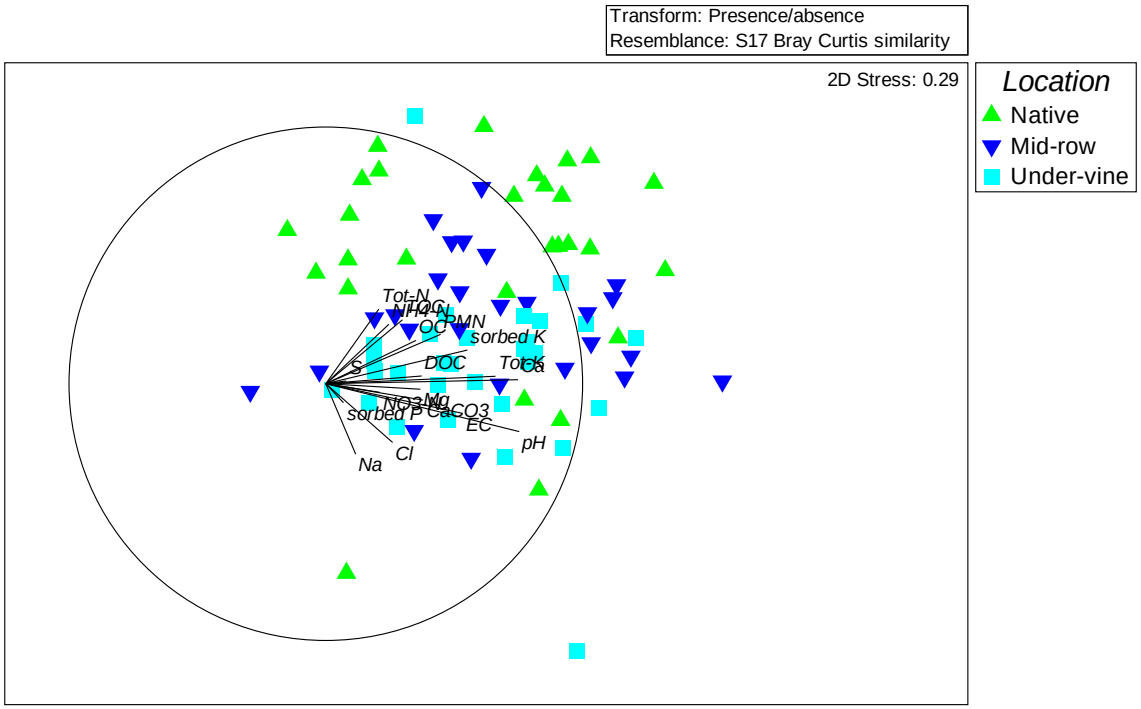


Figure 4c. McLarenVale: Clustering of fungal communities showing influence of soil chemistry

Sample location in the McLaren Vale region had a significant influence on the fungal communities (ITS-TRFLP) present (Fig 4c), with communities in the different sample locations being significantly different when compared (Table 5). For this fungal distribution pattern, the best fit DistLM model (R^2 = 0.114) included Ca, sorbed K, and total organic C (TOC; note that TOC is used as a proxy in this model for OC, total-N, total-C, as all variables are correlated with TOC at r > 0.9).

Table 5. ITS fungal TRFLP: PERMANOVA table of results for location

	Pseudo-F	P (perm)
Location	2.8172	0.001
Groups	**t**	**P (perm)**
Under-vine vs Mid-row	1.4091	0.002
Under-vine vs Native	1.9246	0.001
Mid-row vs Native	1.6399	0.001

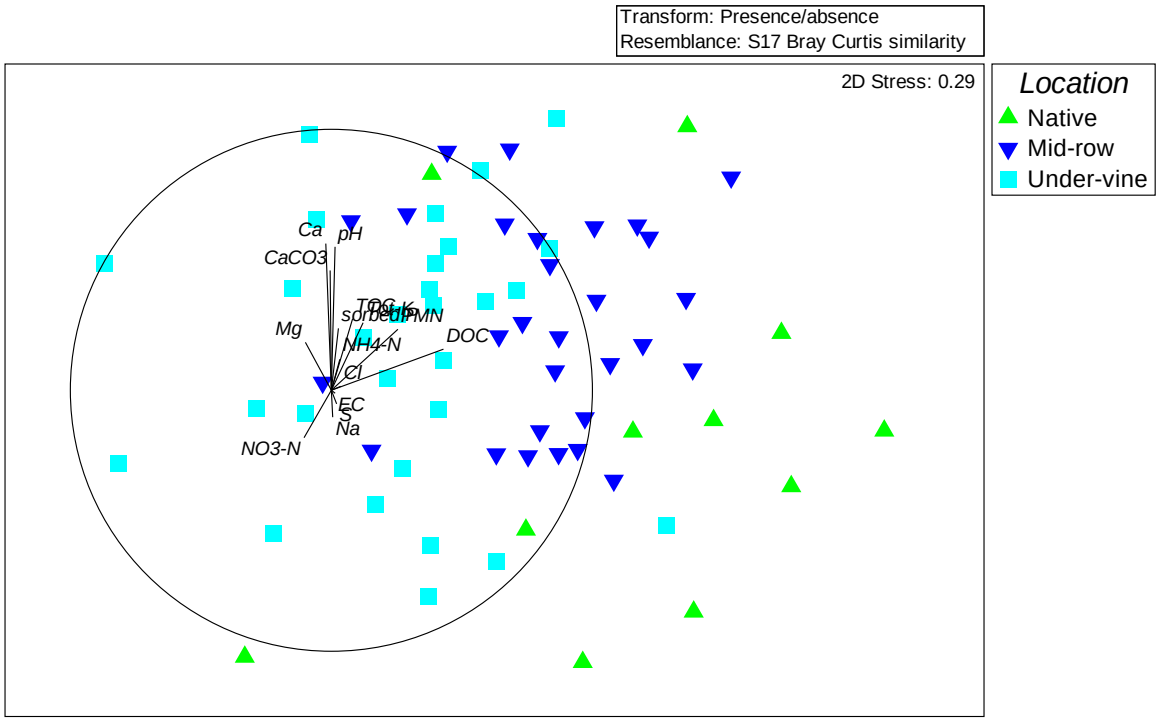


Figure 4d.Sunraysia: Clustering of fungal communities showing influence of soil chemistry

In the Sunraysia region, sample location had a significant influence on the fungal communities (ITS-TRFLP) present (Fig 4d), with communities in the different sample locations being significantly different when compared (Table 6). For this fungal distribution pattern, the best fit DistLM model (R^2 = 0.115) included Ca, total K, and dissolved organic C.

Table 6. ITS fungal TRFLP: PERMANOVA table of results for location

	Pseudo-F	P (perm)
Location	2.4043	0.001
Groups	**t**	**P (perm)**
Under-vine vs Mid-row	1.4014	0.002
Under-vine vs Native	1.72	0.001
Mid-row vs Native	1.563	0.001

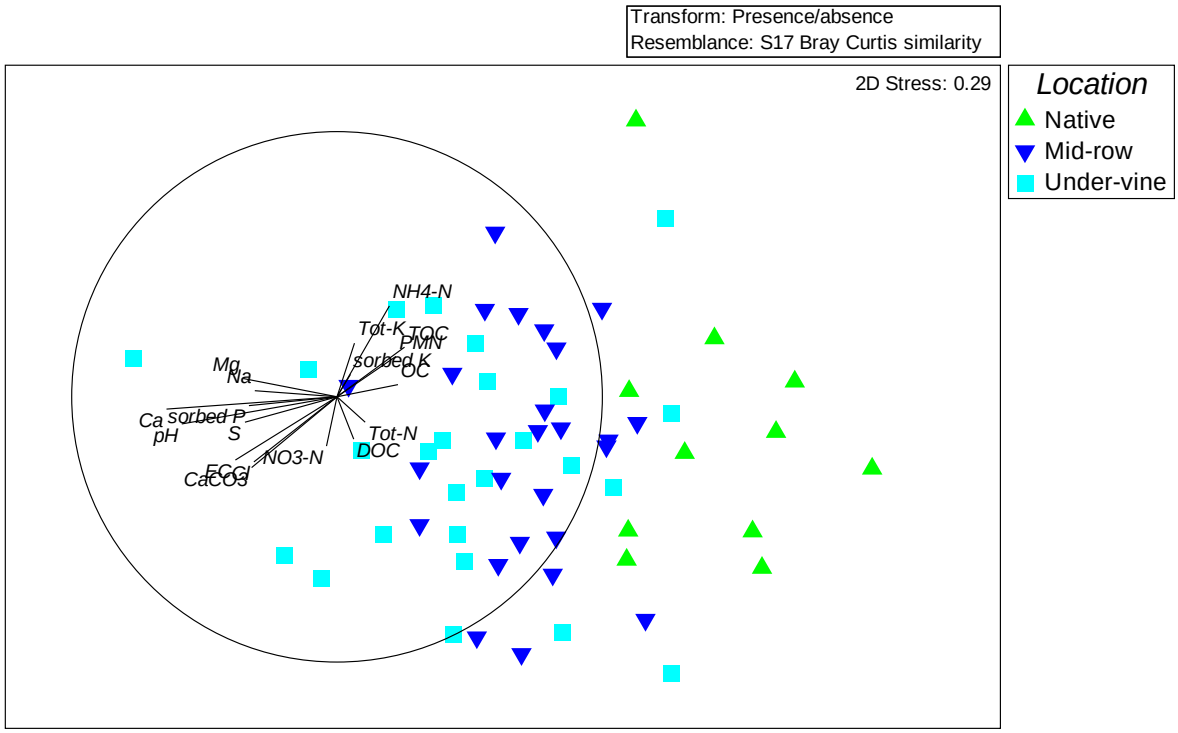


Figure 4e.Yarra Valley: Clustering of fungal communities showing influence of soil chemistry

In the Yarra Valley, sample location had a significant influence on the fungal communities (ITS-TRFLP) present (Fig 4e), with communities in the different sample locations being significantly different when compared (Table 7). For this fungal distribution pattern, the best fit DistLM model (R^2 < 0.100) included EC and Ca.

Table 7. ITS fungal TRFLP: PERMANOVA table of results for location

	Pseudo-F	P (perm)
Location	2.4834	0.001
Groups	**t**	**P (perm)**
Under-vine vs Mid-row	1.4038	0.001
Under-vine vs Native	1.6851	0.001
Mid-row vs Native	1.6797	0.001

Bacterial communities (16S)

Using Permutational MANOVA, it is clear that the 16S bacterial communities significantly differ according to both region and location in the vineyard (Table 8). In order to evaluate this, we need to look at each region individually.

Table 8. 16S Bacterial TRFLP: PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)
Region	3	94518	31506	26.048	0.001
Location	2	9665.5	4832.8	3.9956	0.001
Region x Location	6	17480	2913.3	2.4086	0.001

Like distribution patterns for fungal communities, bacterial communities are similar with strong clustering according to region and a clear separation between Sunraysia and the Yarra Valley with the two SA regions showing most overlap (Fig 5a).

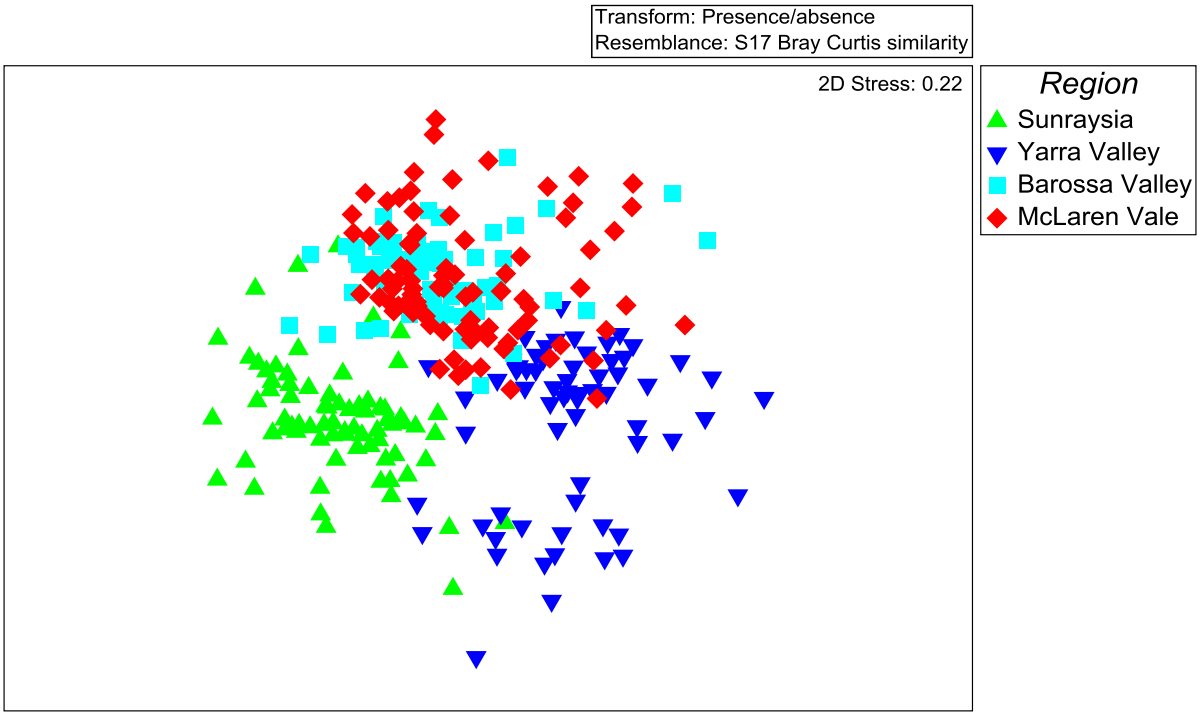


Figure 5a. Clustering of bacterial communities by region

Higher pH and $CaCO_3$ and lower levels of organic matter (including total N, total C, and PMN) are the primary factors driving the separation of Sunraysia bacterial communities from the Barossa Valley, McLaren Vale, and most of the Yarra Valley bacterial communities (Fig 5b).

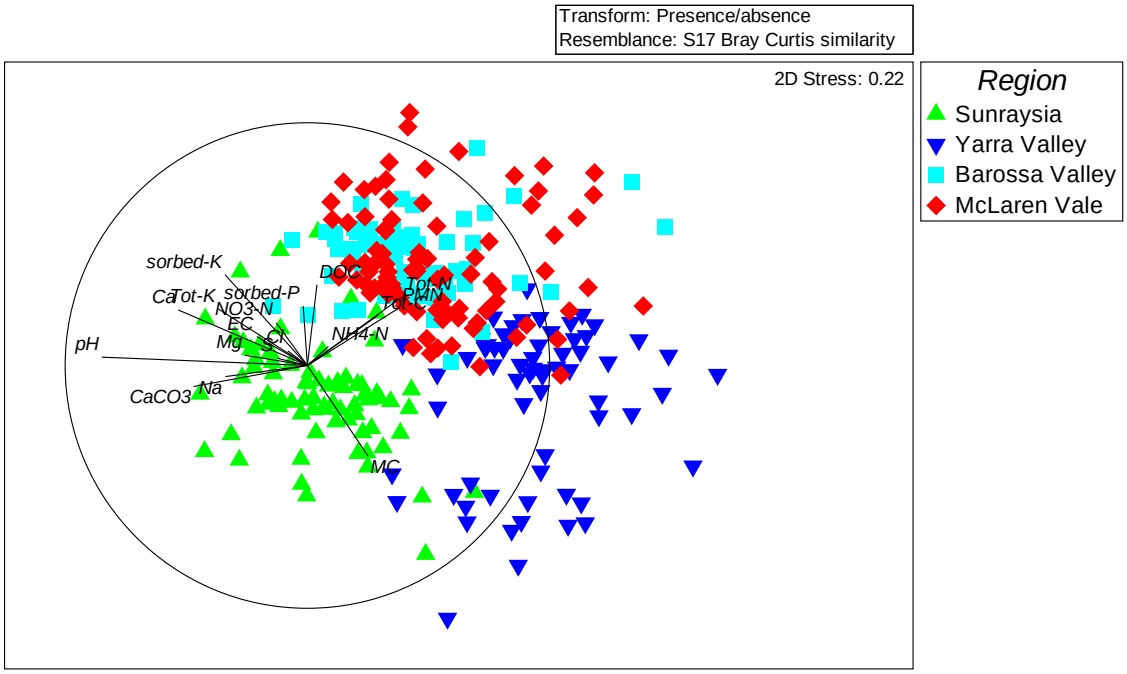


Figure 5b. Clustering of bacterial communities by region showing influence of soil chemistry

Using an NMDS analysis, it is not possible to differentiate any clear patterns associated with sample location (mid-row, under-vine, or native) on the bacterial community structure (Fig 6a). Instead, the overriding factor is regional soil characteristics.

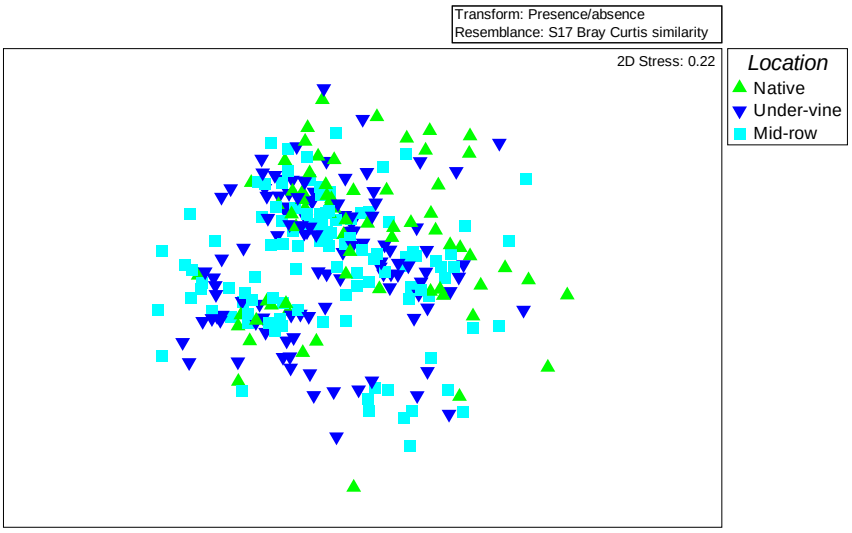


Figure 6a. Clustering of bacterial communities by location

To evaluate the factors driving these locational differences in bacterial communities, we therefore examined each region individually.

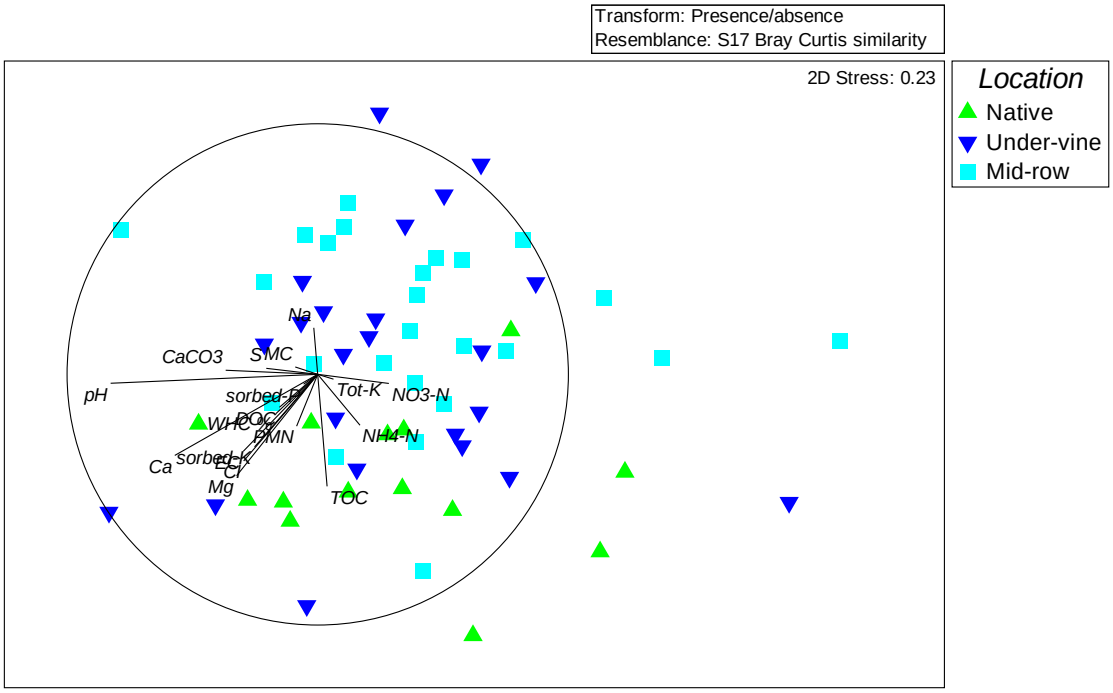


Figure 6b. Barossa Valley: Clustering of bacterial communities showing influence of soil chemistry

In the Barossa Valley, sample location had a significant influence on the 16S bacterial communities present (Fig 6b), with communities in the different sample locations being significantly different than each other (Table 9).

Table 9. 16S Bacterial TRFLP: PERMANOVA table of results for location

	Pseudo-F	P (perm)
Location	2.9712	0.001
Groups	**t**	**P (perm)**
Under-vine vs Mid-row	1.3666	0.018
Under-vine vs Native	1.8995	0.001
Mid-row vs Native	1.9385	0.001

The primary interactive soil chemical factors influencing this distribution (DistLM R^2 = 0.180) were the interrelated parameters of pH, Ca, and calcium carbonate levels, which primarily distinguished communities in the native soils from the managed soils.

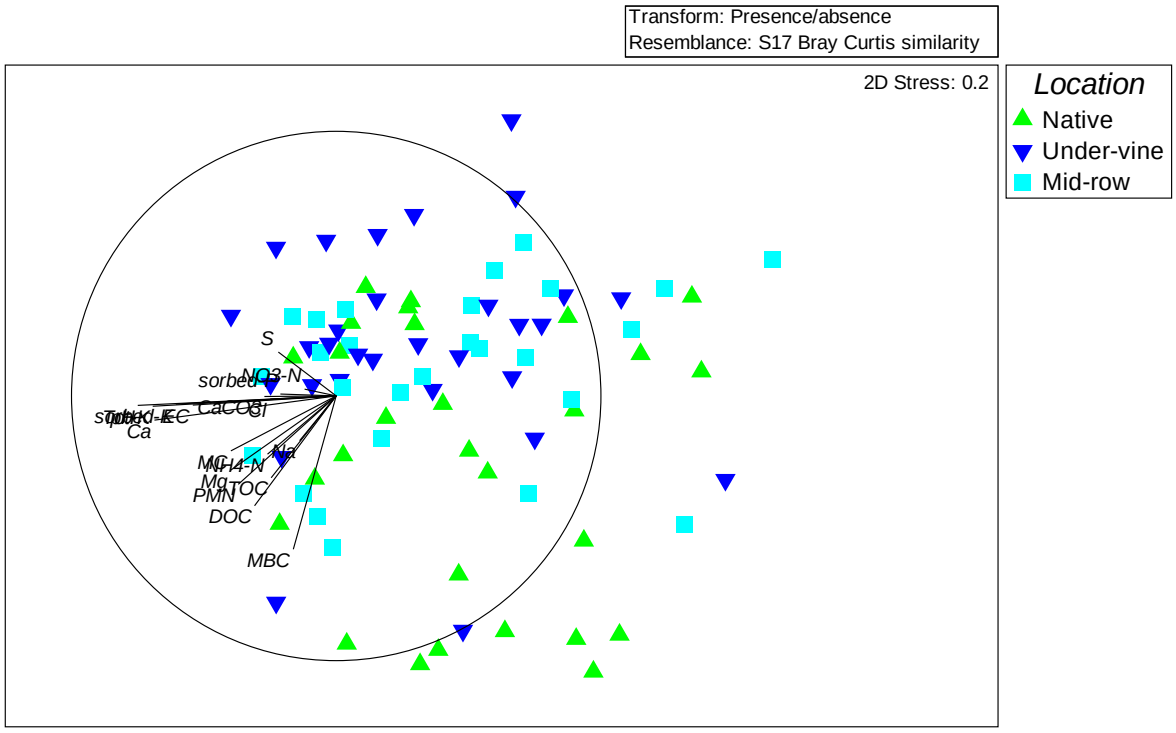


Figure 6c. McLaren Vale: Clustering of bacterial communities showing influence of soil chemistry

In the McLaren Vale, sample location had a significant influence on the 16S bacterial communities present (Fig 6c), with communities in the vineyards being significantly different than those in adjacent non-managed soils (Table 10).

Table 10. 16S Bacterial TRFLP: PERMANOVA table of results for location

	Pseudo-F	P (perm)
Location	2.8625	0.002
Groups	**t**	**P (perm)**
Under-vine vs Mid-row	1.0196	0.387
Under-vine vs Native	2.1309	0.001
Mid-row vs Native	1.6993	0.003

The primary interactive soil chemical factors influencing this distribution (DistLM R^2 = 0.315) were pH, total K, S, dissolved organic C, potentially mineralizable N, and total organic C (TOC; note that TOC is used as a proxy in this model for OC, total-N, total-C, as all variable are correlated with TOC at r > 0.9).

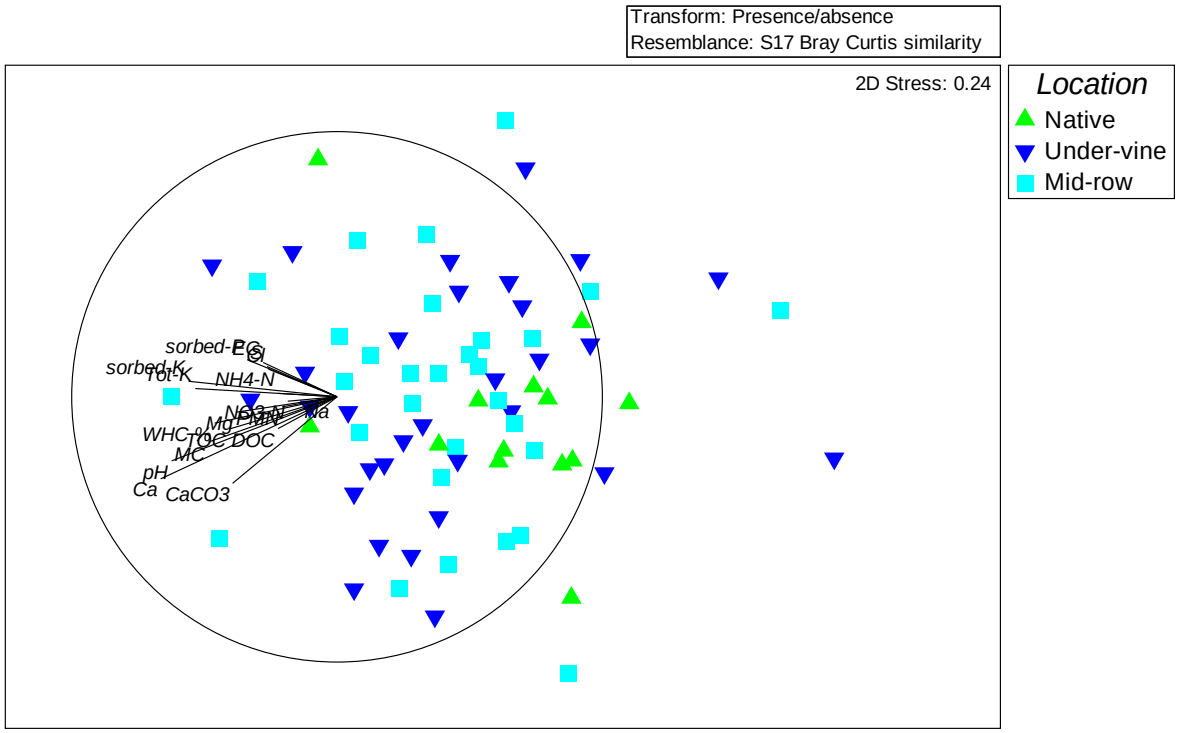


Figure 6d. Sunraysia: Clustering of bacterial communities showing influence of soil chemistry

In the Sunraysia region, although more difficult to distinguish by examining the 2D distribution pattern of the NMDS, the sample location still had a significant influence on the 16S bacterial communities present (Fig 6d), with under-vine communities being significantly different than mid-row or native communities (Table 11).

Table 11. 16S Bacterial TRFLP: PERMANOVA table of results for location

	Pseudo-F	P (perm)
Location	1.9153	0.003
Groups	**t**	**P (perm)**
Under-vine vs Mid-row	1.4216	0.011
Under-vine vs Native	1.5309	0.004
Mid-row vs Native	1.1474	0.157

The primary interactive soil chemical factors influencing this distribution (DistLM R^2 = 0.242) were pH, EC, Ca, total K, and dissolved organic C.

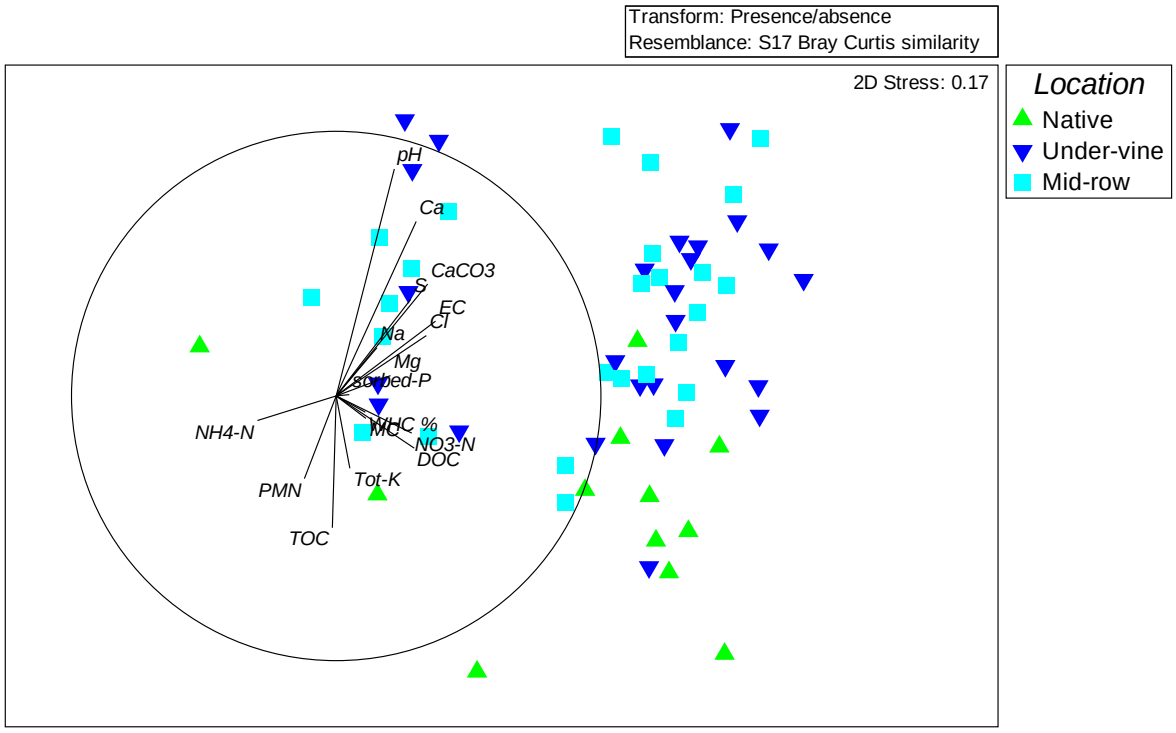


Figure 6e. Yarra Valley: Clustering of bacterial communities showing influence of soil chemistry

In the Yarra Valley, bacterial communities in soils under native vegetation were significantly different to those in the managed vineyard communities (Fig 6e) both undervine and midrow (Table 12).

Table 12. 16S Bacterial TRFLP: PERMANOVA table of results for location

	Pseudo-F	P (perm)
Location	3.4898	0.002
Groups	**t**	**P (perm)**
Under-vine vs Mid-row	1.2561	0.118
Under-vine vs Native	2.1406	0.001
Mid-row vs Native	2.2394	0.001

The primary interactive soil chemical factors influencing this distribution (DistLM R2 = 0.228) were pH, EC, and dissolved organic C.

i) Microbial communities in vineyards of 4 wine growing regions using a high resolution method (amplicon sequencing; ITS fungi & 16S bacteria)

Fungal communities (ITS amplicon sequencing)

Unlike TRFLP analyses, amplicon sequencing enables the taxonomic assignment of unknown DNA fragments from the fungal ITS region providing another vital layer of information. At the level of class or higher, regional factors influence the prevalence of all major phyla. Again as with the previous low resolution TRFLP analyses, the Barossa Valley and McLaren Vale soils show few significant difference in all fungal taxa listed in Table 13 (exception being Leotiomycetes). While Sunraysia soils are similar to the SA regional soils, the Yarra Valley shows significant differences in a number of fungal groups, particularly in the phylum Ascomycota and specifically, the classes, Dothideomycetes and Archaeorhizomycetes. The precise significance of these differences is yet to be resolved but Dothideomycetes are common plant residue decomposers and the latter, Archaeorhizomycetes, form beneficial associations with plant roots (Goodwin, 2013). Interestingly, Glomeromycetes, of which P-assimilating VAM fungi are a member, is higher in the Yarra Valley vineyards but only significantly so when compared to McLaren Vale vineyards.

Table 13. Percent abundance of the major fungal phyla or classes found in the different regions

Phyla	Class or higher	Barossa Valley	McLaren Vale	Sunraysia	Yarra Valley	Significance (*p* value)
Ascomycota	**Dothideomycetes**	**37.2 (12.6) a**	**39.6 (15.5) a**	**33.8 (8.7) a**	**17.9 (8.2) b**	***< 0.001***
	Sordariomycetes	**20.7 (5.8) ab**	**20.6 (6.0) ab**	**17.1 (6.9) b**	**24 (7.5) a**	***< 0.001***
	Leotiomycetes	**4.9 (5.5) a**	**1.1 (1.6) b**	**3.0 (3.8) ab**	**2.6 (1.7) ab**	***< 0.05***
	Eurotiomycetes	1.6 (1.3)	0.9 (0.8)	1.7 (1.4)	1.1 (0.9)	*ns*
	Pezizomycetes	0.2 (0.4)	0.4 (0.7)	0.3 (0.6)	0.6 (0.9)	*ns*
	Saccharomycetes	0.2 (0.4)	0.6 (1.3)	0.4 (1.0)	0.6 (2.0)	*ns*
	Archaeorhizomycetes	**0.0 (0.0) b**	**0.0 (0.1) b**	**0.1 (0.4) b**	**4.5 (5.9) a**	***< 0.001***
	Ascomycota (ID to phyla only)	**10.5 (8.4) ab**	**9.7 (7.8) ab**	**13.7 (10.9) a**	**3.5 (4.1) b**	***< 0.001***
Glomeromycota	**Glomeromycetes**	**5.3 (7.1) ab**	**2.3 (3.4) b**	**5.4 (6) ab**	**6.7 (4.1) a**	***< 0.05***
Basidiomycota	**Tremellomycetes**	**8.5 (7.2) ab**	**6.0 (4.2) b**	**6.7 (5.5) b**	**12.6 (6.9) a**	***< 0.001***
	Agaricomycetes	0.1 (0.2)	0.1 (0.1)	0.1 (0.3)	0.5 (0.9)	*ns*
	Microbotryomycetes	0.3 (0.3)	0.3 (0.5)	0.2 (0.2)	0.2 (0.5)	*ns*
Fungi Incertae sedis*	**Fungi Incertae sedis 10**	**0.5 (0.3) ab**	**0.5 (0.4) ab**	**0.4 (0.3) b**	**0.6 (0.3) a**	***< 0.05***
Mixed **	Sum other classes	0.2 (0.3)	0.2 (0.6)	0.1 (0.3)	*ns*	*ns*
Fungi (ID to Kingdom only)		0.7 (1.6)	2.3 (4.5)	1.1 (1.5)	0.9 (1.9)	*ns*
Unidentified sequences		**9 (3.4) b**	**15.9 (9.0) b**	**15.5 (12.3) b**	**23.7 (9.8) a**	***< 0.001***

* Formerly Zygomycota
**(<0.1% abundance each phyla or class)
Data are provided as means with standard deviations in parentheses. Barossa Valley: n = 9; McLaren Vale: n = 12; Sunraysia: n = 30; Yarra Valley: n= 43. Taxonomic groups with different letters are significantly different between regions at the given *p* value. ns = not significant

Using an NMDS analysis as we did with the low resolution TRFLP method, we can see how region selects for particular taxonomic groups of fungi. In the Yarra Valley there are at least five fungal orders that influence the clustering; the Hypocreales, Geoglassales (phylum Ascomycota), Auriculariales and Trichosporonales (phylum Basidiomycota) and Glomerales (Phylum Glomeromycota). In the other three regions it is more difficult to isolate any fungal orders that are closely associated with the clustering patterns (Fig 7a). The fungi identified in Yarra Valley are associated with higher C levels (e.g. microbial biomass C or MBC, TOC, OC), higher N levels (PMN & NH_4^+-N) and greater water holding capacity (Fig 7b).

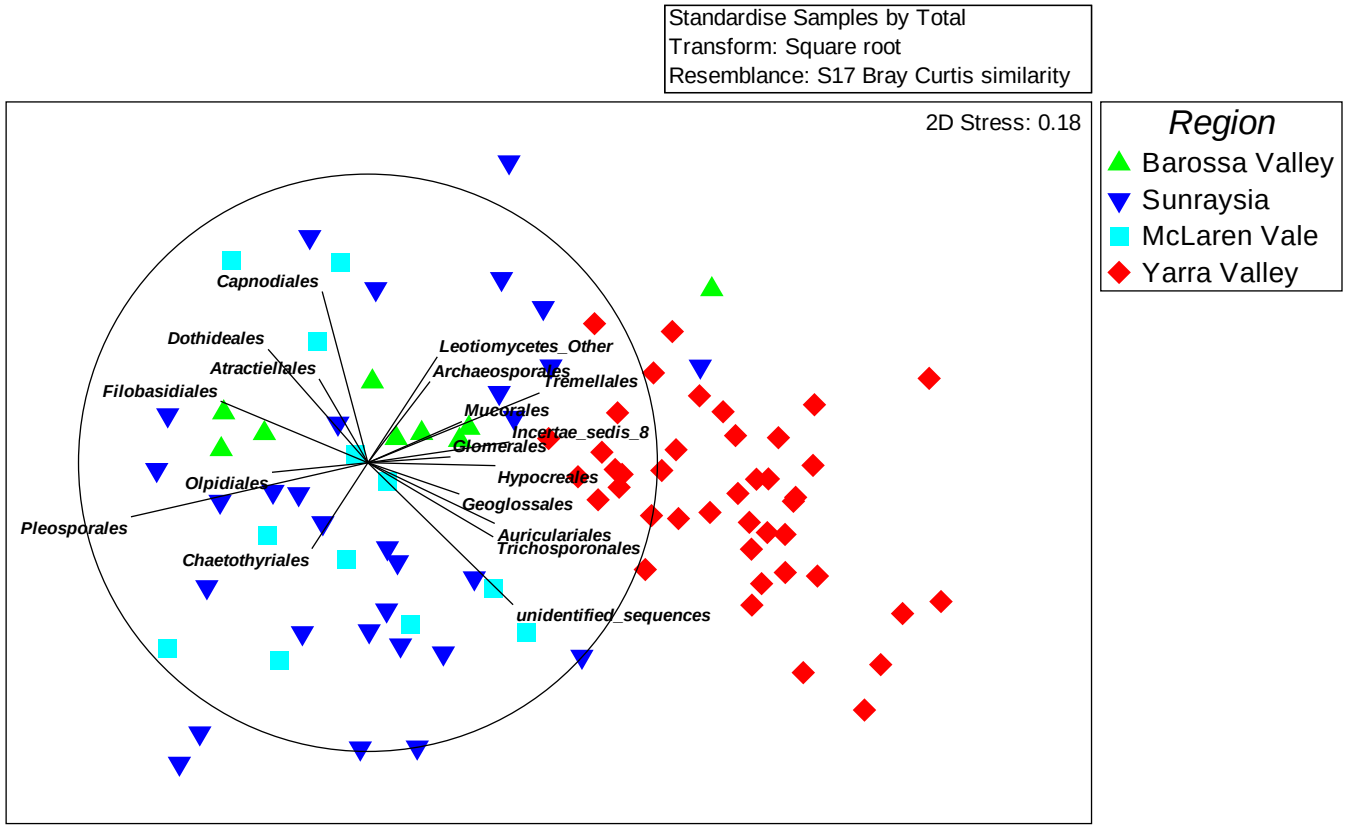


Figure 7a. Clustering of ITS fungal amplicons by region showing influence of fungal orders

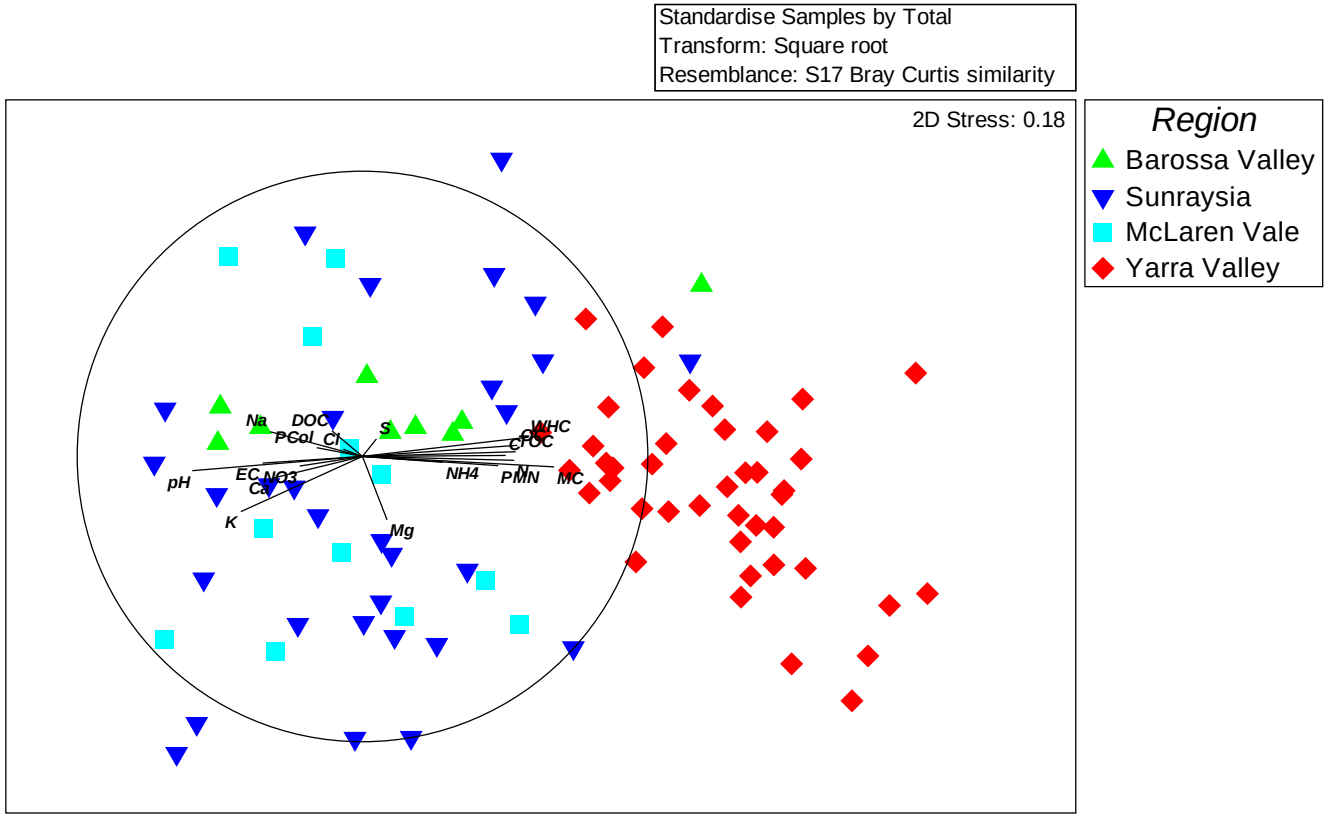


Figure 7b. Clustering of ITS fungal amplicons by region showing influence of chemistry

The effect of location in vineyard is less significant overall on fungal communities, with the exception of the Ascomycota, Eurotiomycetes and Archaeorhizomycetes and the Basidiomycota, Agaricomycetes (Table 14).

T**able 14. Percent abundance of the major fungal phyla or classes found in the different vineyard locations**

Phyla	Class or higher	Under-vine	Mid-row	Native	Significance (*p* value)
Ascomycota	Dothideomycetes	29.9 (11.7)	24.6 (13.8)	28.8 (15.3)	*ns*
	Sordariomycetes	22.1 (8.0)	20.2 (6.0)	20.5 (9.1)	*ns*
	Leotiomycetes	2.5 (3.0)	2.5 (1.7)	3.8 (4.8)	*ns*
	Eurotiomycetes	**1.5 (1.2) ab**	**0.9 (0.8) b**	**1.7 (1.4) a**	***< 0.05***
	Pezizomycetes	0.4 (0.8)	0.3 (0.6)	0.5 (1.0)	*ns*
	Saccharomycetes	0.4 (0.7)	0.8 (2.4)	0.2 (0.4)	*ns*
	Archaeorhizomycetes	**0.7 (2.1) b**	**3.9 (6.2) a**	**1.2 (2.9) ab**	***< 0.05***
	Ascomycota (ID to phyla only)	8.0 (8.0)	7.3 (8.9)	10.4 (10.3)	*ns*
Glomeromycota	Glomeromycetes	5.3 (4.4)	6.4 (5.8)	4.6 (5.0)	*ns*
Basidiomycota	Tremellomycetes	10.7 (7.3)	9.6 (6.9)	7.1 (5.0)	*ns*
	Agaricomycetes	**0.0 (0.1) b**	**0.3 (0.4) ab**	**0.6 (1.2) a**	***< 0.05***
	Microbotryomycetes	0.2 (0.5)	0.1 (0.2)	0.3 (0.5)	*ns*
Fungi Incertae sedis	Fungi Incertae sedis 10	0.5 (0.3)	0.6 (0.4)	0.5 (0.4)	*ns*
Mixed	Sum other classes	0.2 (0.7)	0.2 (0.5)	0.2 (0.5)	*ns*
Fungi (ID to Kingdom only)		1.5 (2.5)	0.6 (0.7)	1.3 (3.4)	*ns*
Unidentified sequences		16.0 (6.6)	21.7 (12.8)	18.4 (11.1)	*ns*

*** Formerly Zygomycota,**(<0.1% abundance each phyla or class)**
Data are provided as means with standard deviations in parentheses. Under-vine: n = 37; Mid-row: n = 37; Native: n = 20. Taxonomic groups with different letters are significantly different between locations at the given *p* value. ns = not significant

Location in vineyard within a regional context appears to have little influence on fungal communities at the Phylum level except for Glomeromycota and Fungi incertae sedis being more prevalent midrow compared to adjacent native vegetation site in the Yarra Valley and Sunraysia respectively (Table 15). It is worth noting however the very small sample size for this comparison in both SA regions.

Table 15. Percent abundance of the major fungal phyla or classes found in the different vineyard locations for each region

Phyla	Ascomycota	Basidiomycota	Chytridiomycota	Glomeromycota	Fungi Incertae sedis*	Unidentified	Number samples (n)
Barossa Valley							
Under-vine	84.3 (9.4)	7.6 (3.7)	0.1 (0.1)	1.0 (1.4)	0.3 (0.2)	6.7 (4.4)	3
Mid-row	76.2 (11.0)	10.9 (13.7)	0.0 (0.1)	6.5 (10.4)	0.5 (0.2)	5.9 (2.5)	3
Native	75.6 (9.9)	8.7 (4.2)	0.0 (0.0)	8.5 (6.8)	0.8 (0.3)	6.5 (2.8)	3
***p*-value**	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	
McLaren Vale							
Under-vine	81.1 (4.8)	4.3 (2.2)	0.0 (0.0)	4.5 (5.7)	0.6 (0.6)	9.4 (3.0)	4
Mid-row	69.8 (12.9)	9.8 (5.7)	0.0 (0.0)	1.6 (1.0)	0.3 (0.3)	18.5 (16.2)	4
Native	75.9 (16.6)	5.9 (2.9)	0.0 (0.0)	1.2 (0.9)	0.6 (0.4)	16.4 (14.3)	4
***p*-value**	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	
Sunraysia							
Under-vine	72.3 (9.9)	8.3 (7.0)	0.0 (0.0)	5.9 (5.5)	**0.4 (0.3) ab**	13.2 (7.2)	13
Mid-row	76.1 (12.0)	6.8 (4.4)	0.0 (0.0)	6.0 (7.5)	**0.5 (0.3) a**	10.5 (8.0)	11
Native	73.6 (7.4)	5.9 (5.1)	0.0 (0.0)	5.9 (7.4)	**0.2 (0.1) b**	14.4 (8.0)	6
***p*-value**	*ns*	*ns*	*ns*	*ns*	***< 0.05***	*ns*	
Yarra Valley							
Under-vine	60.3 (9.0)	16.1 (6.5)	0 (0.1)	**6.8 (4.5) ab**	0.5 (0.3)	16.2 (5.9)	17
Mid-row	57.2 (8.1)	12.5 (7.0)	0 (0.1)	**9.2 (4.0) a**	0.8 (0.4)	20.3 (7.6)	19
Native	65.3 (16.1)	11.8 (7.7)	0 (0.1)	**4.1 (1.9) b**	0.5 (0.3)	18.2 (9.6)	7
***p*-value**	*ns*	*ns*	*ns*	***<0.01***	*ns*	*ns*	

*** Formerly Zygomycota, ns = not significant**
Data are provided as means with standard deviations in parentheses. Taxonomic groups with different letters, within the same sub-column, are significantly different between locations at the given *p* value. ns = not significant

Bacterial communities (16S amplicon sequencing)

Again as in the low resolution TRFLP analyses of bacterial communities, we observe strong regional effects on communities at the bacterial phylum level (Table 16). The Yarra Valley soils tend to differentiate from other regional soils in in Beta Proteobacteria, Acidobacteria, Chloroflexi and Verrucomicrobia (generally higher) and in Alpha Proteobacteria and Actinobacteria (generally lower). The Barossa Valley and McLaren Vale soils are more abundant in Bacteroidetes and Sunraysia, strongly so for Nitrospira.

Table 16. Percent abundance of the major bacterial phyla or classes found in the different regions

Phylum	Barossa Valley	McLaren Vale	Sunraysia	Yarra Valley	Significance (*p* value)
Alpha-Proteobacteria	**16.6 (2.8) ab**	**18.5 (2.1) a**	**18.0 (3.1) a**	**14.9 (1.9) b**	**< 0.001**
Beta-Proteobacteria	**5.3 (0.7) ab**	**4.6 (0.9) b**	**4.3 (0.8) b**	**5.9 (1.7) a**	**< 0.001**
Gamma-Proteobacteria	4.2 (1.5)	4.0 (1.6)	3.3 (0.9)	3.5 (0.9)	ns
Delta-Proteobacteria	**1.7 (0.2) a**	**1.4 (0.3) ab**	**1.0 (0.3) b**	**1.6 (0.6) a**	**< 0.001**
Proteobacteria (unclassified)	**2.3 (0.3) a**	**2.1 (0.5) a**	**1.5 (0.3) b**	**2.1 (0.6) a**	**< 0.001**
Actinobacteria	**18.2 (2.7) ab**	**20.6 (3.2) a**	**21.5 (4.5) a**	**14.8 (3.5) b**	**< 0.001**
Acidobacteria	**10.8 (1.4) b**	**12.1 (4.7) b**	**11.9 (2.5) b**	**16.5 (2.9) a**	**< 0.001**
Bacteroidetes	**10.9 (3.0) a**	**9.5 (3.2) ab**	**6.4 (2.8) c**	**8.0 (2.8) bc**	**< 0.001**
Firmicutes	4.7 (3.2)	2.9 (1.0)	4.3 (1.8)	3.5 (2.0)	ns
Planctomycetes	**4.0 (0.8) a**	**4.0 (0.6) a**	**3.2 (0.8) b**	**3.6 (0.6) ab**	**< 0.01**
Chloroflexi	**2.1 (0.8) b**	**2.3 (0.5) b**	**3.3 (0.8) ab**	**4.0 (1.6) a**	**< 0.001**
Gemmatimonadetes	**1.2 (0.3) b**	**1.1 (0.4) b**	**2.1 (0.8) a**	**1.9 (0.7) a**	**< 0.001**
Nitrospira	**0.5 (0.3) b**	**0.5 (0.3) b**	**1.2 (0.5) a**	**0.3 (0.2) b**	**< 0.001**
Verrucomicrobia	**0.6 (0.3) ab**	**0.4 (0.2) bc**	**0.3 (0.2) c**	**0.8 (0.3) a**	**< 0.001**
Armatimonadetes	**0.5 (0.2) b**	**0.4 (0.2) b**	**0.7 (0.3) a**	**0.3 (0.1) b**	**< 0.001**
Cyanobacteria	0.9 (1.1)	0.4 (0.3)	0.9 (2.1)	1.1 (1.1)	ns
TM7	0.6 (0.5)	0.4 (0.3)	0.4 (0.3)	0.5 (0.3)	ns
Deinococcus-Thermus	**0.0 (0.0) b**	**0.0 (0.0) b**	**0.2 (0.2) a**	**0.0 (0.0) b**	**< 0.001**
unclassified	14.8 (1.7)	14.5 (1.7)	15.2 (2.6)	16.6 (2.0)	ns
Other phyla*	0.1 (0.1)	0.0 (0.1)	0.1 (0.1)	0.1 (0.2)	ns

***(<0.1% abundance each phyla or class)**
Data are provided as means with standard deviations in parentheses. Barossa Valley: n = 8; McLaren Vale: n = 12; Sunraysia: n = 30; Yarra Valley: n= 42. Taxonomic groups with different letters are significantly different between regions at the given *p* value.

NMDS analyses shows the strong influence of bacterial phyla, Acidobacteria, Verrucomicrobia, Proteobacteria, on the Yarra Valley clustering of samples and of Nitrospira, Deinococcus-Thermus and Actinobacteria on Sunraysia clustering. There is no strong association between bacterial phyla and the clustering of both SA regions (Fig 8a). At the next level of taxonomy, we revealed over 100 bacterial orders across all regions with specific groups, including the N-cycling Nitrosomonadales and the potential plant-growth promoting Rubrobacterales in higher more abundance in the Sunraysia soils. Other groups, including the bacteria that are known decomposers of organic matter (Clostridiales) and other putative carbon cyclers (Acidimicrobiales) are more dominant in Yarra Valley soils, where total organic matter is higher (Fig 8b).

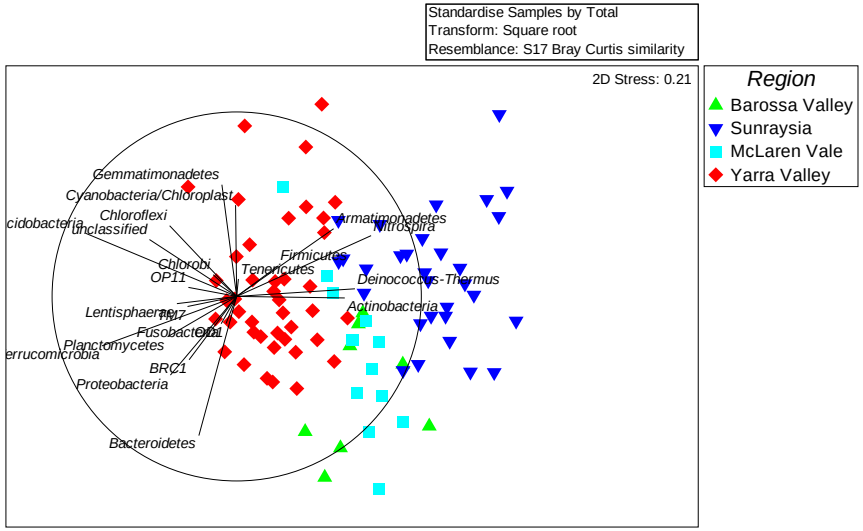


Figure 8a. Clustering of 16S Bacterial amplicons by region showing influence of bacterial phyla

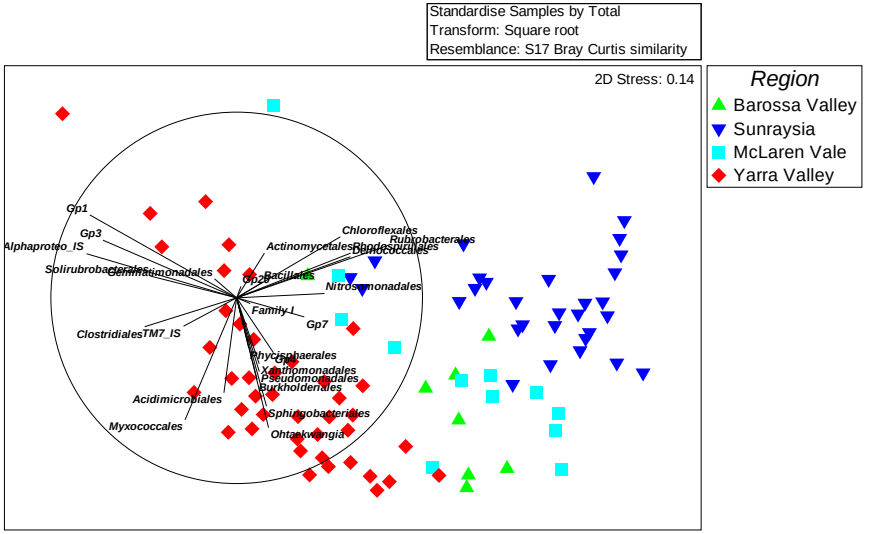


Figure 8b. Clustering of 16S Bacterial amplicons by region showing influence of bacterial order

Lower levels of organic matter (including total N, total C, and PMN), combined with concentrations of sodium (Na) and pH are the primary factors driving the separation of Sunraysia bacterial communities from the Barossa Valley, McLaren Vale, and most of the Yarra Valley bacterial communities (Fig 8c). This is highly similar to what was found using the TRFLP bacterial assays.

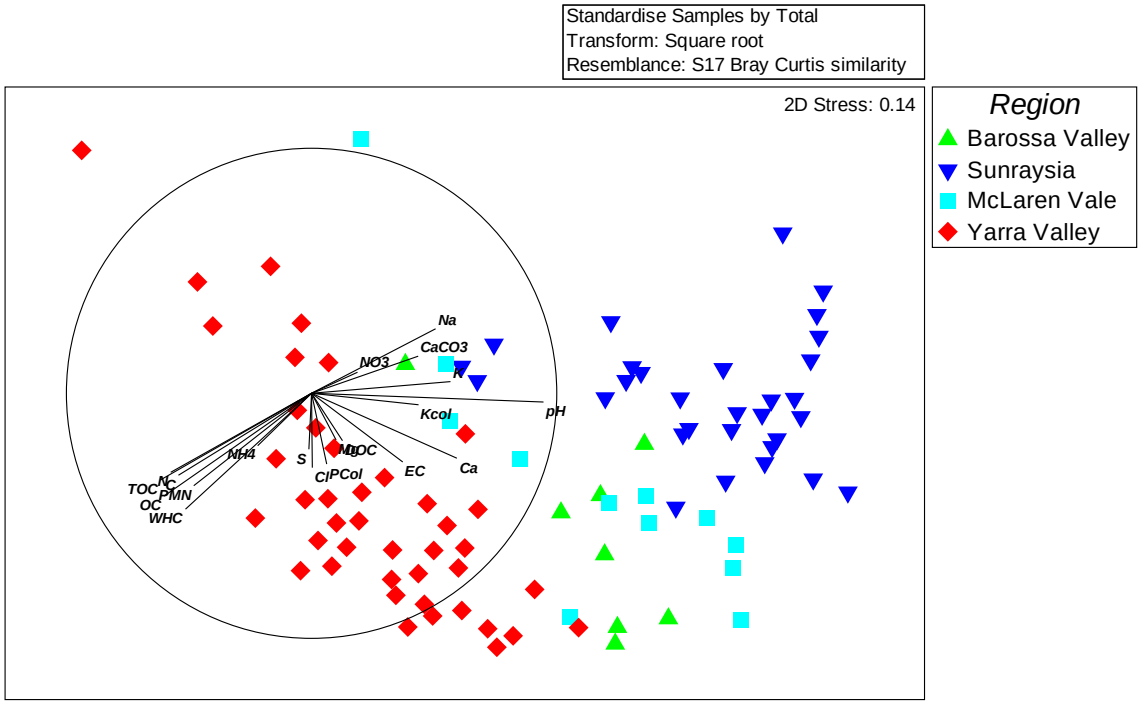


Figure 8c. Clustering of 16S Bacterial amplicons by region showing influence of chemistry

There are also strong overall vineyard locational influences on bacterial phyla (Table 17) being mainly reflective of the data from Sunraysia and Yarra Valley regions. Higher abundance of Beta, Delta and unclassified Proteobacteria were measured in the midrow and higher abundance of Firmicutes, Gemmatimonadetes and Cyanobacteria undervine. Soils under native vegetation are more similar to those in the midrow indicating the influence of vine roots and fertiliser and water delivery regimes on bacterial communities undervine.
These locational differences in bacterial communities are best illustrated by NMDS analyses which shows the influence of discrete bacterial orders and associated chemistry on the clustering of samples. However because of the low significance of phylum level abundance values from the Barossa Valley and McLaren Vale, a reflection perhaps on the relatively small number of suitable samples, these regions will not be displayed.

Table 17. Percent abundance of the major bacterial phyla or classes found in vineyard locations, irrespective of regions

	Under-vine	Mid-row	Native	Significance (*p* value)
Alpha-Proteobacteria	**15.8 (2.7) b**	**16.4 (2.4)ab**	**18.0 (3.6) a**	**< 0.05**
Beta-Proteobacteria	**5.0 (1.1) b**	**5.8 (1.6) a**	**4.3 (1.3) b**	**< 0.001**
Gamma-Proteobacteria	3.7 (0.9)	3.5 (0.9)	3.5 (1.6)	ns
Delta-Proteobacteria	**1.3 (0.5) b**	**1.6 (0.5) a**	**1.2 (0.5) b**	**< 0.01**
Proteobacteria (unclassified)	**1.8 (0.5) b**	**2.2 (0.6) a**	**1.7 (0.6) b**	**< 0.01**
Actinobacteria	**17.0 (3.7) b**	**17.0 (4.8) b**	**22.1 (5.0) a**	**< 0.001**
Acidobacteria	13.4 (3.4)	14.5 (4.2)	13.8 (3.7)	ns
Bacteroidetes	7.9 (3.5)	8.3 (2.2)	7.3 (3.8)	ns
Firmicutes	**4.7 (2.2) a**	**3.4 (1.8) b**	**2.8 (1.4) b**	**< 0.001**
Planctomycetes	3.5 (0.6)	3.5 (0.7)	3.8 (0.9)	ns
Chloroflexi	3.3 (1.3)	3.5 (1.0)	3.3 (2.0)	ns
Gemmatimonadetes	**2.2 (0.9) a**	**1.7 (0.6) b**	**1.3 (0.4) b**	**< 0.001**
Nitrospira	**0.8 (0.5) a**	**0.5 (0.4) b**	**0.6 (0.7) ab**	**< 0.05**
Verrucomicrobia	0.6 (0.3)	0.6 (0.4)	0.6 (0.4)	ns
Armatimonadetes	0.5 (0.2)	0.5 (0.2)	0.5 (0.5)	ns
Cyanobacteria	**1.5 (2.0) a**	**0.6 (0.5) b**	**0.5 (0.6) b**	**< 0.01**
TM7	**0.5 (0.3) a**	**0.5 (0.3) ab**	**0.3 (0.2) b**	**< 0.05**
Deinococcus-Thermus	0.1 (0.2)	0.1 (0.2)	0.0 (0.1)	ns
unclassified	**16.3 (2.1) a**	**15.8 (2.2) ab**	**14.4 (2.3) b**	**< 0.01**
Other phyla*	0.1 (0.2)	0.1 (0.1)	0.1 (0.1)	ns

***(<0.1% abundance each phyla or class)**
Data are provided as means with standard deviations in parentheses. Undervine: n = 37; Mid-row: n = 36; Native: n = 19. Taxonomic groups with different letters are significantly different between locations at the given *p* value.

Barossa Valley vineyard soils were less abundant in Gamma Proteobacteria compared to the adjacent native vegetation site and Delta Proteobacteria were more abundant undervine than midrow. Interestingly, Nitrospira were abundant undervine (Table 18).

Table 18. Percent abundance of the major bacterial phyla or classes found in vineyard locations in the Barossa Valley

% abundance of major bacterial groups	Sample location			Significance (*p* value)
	Under-vine	Mid-row	Native	
Alpha-Proteobacteria	15.4 (1.7)	16.6 (2.0)	17.3 (4.5)	ns
Beta-Proteobacteria	5.2 (1.5)	5.2 (0.6)	5.4 (0.6)	ns
Gamma-Proteobacteria	**3.4 (0.1) b**	**3.1 (0.9) b**	**5.9 (0.8) a**	**< 0.05**
Delta-Proteobacteria	**2.0 (0.1) a**	**1.5 (0.2) b**	**1.7 (0.2) ab**	**< 0.05**
Proteobacteria (unclassified)	2.6 (0.1)	2.1 (0.2)	2.4 (0.2)	ns
Actinobacteria	15.4 (2.8)	18.8 (2.2)	19.4 (2.6)	ns
Acidobacteria	10.5 (0.5)	10.5 (2.1)	11.3 (1.5)	ns
Bacteroidetes	10.0 (0.3)	10.6 (2.0)	11.8 (5.0)	ns
Firmicutes	7.2 (4.1)	5.8 (2.6)	2.0 (0.7)	ns
Planctomycetes	4.3 (0.7)	3.26 (0.7)	4.6 (0.2)	ns
Chloroflexi	2.8 (0.2)	2.5 (0.5)	1.3 (0.6)	ns
Gemmatimonadetes	1.2 (0.1)	1.37 (0.4)	0.97 (0.3)	ns
Nitrospira	**0.8 (0.4) a**	**0.5 (0.0) ab**	**0.17 (0.1) b**	**< 0.001**
Verrucomicrobia	0.4 (0.2)	0.5 (0.3)	0.96 (0.2)	ns
Armatimonadetes	0.5 (0.1)	0.6 (0.2)	0.3 (0.1)	ns
Cyanobacteria	1.7 (2.2)	1.2 (0.4)	0.0 (0.0)	ns
TM7	0.4 (0.3)	0.9 (0.8)	0.5 (0.3)	ns
Deinococcus-Thermus	0.0 (0.0)	0.0 (0.0)	0 (0.0)	ns
unclassified	16.1 (0.6)	14.8 (1.3)	14.0 (2.4)	ns
Other phyla*	0.2 (0.2)	0.1 (0.0)	0.1 (0.1)	ns

***(<0.1% abundance each phyla or class)**

Data are provided as means (Under-vine: n = 2; Mid-row and Native: n = 3) with standard deviations in parentheses. Taxonomic groups with different letters are significantly different between sampling location at the given *p* value.

For McLaren Vale soils, only one phylum, Planctomycetes, was less abundant undervine compared to the adjacent native vegetation site (Table 19).

Table 19. Percent abundance of the major bacterial phyla or classes found in vineyard locations in McLaren Vale

% abundance of major bacterial groups	Sample location			Significance (*p* value)
	Vine	Mid-row	Native	
Alpha-Proteobacteria	18.5 (0.6)	18.5 (1.0)	18.6 (3.9)	ns
Beta-Proteobacteria	5.2 (0.5)	4.1 (1.1)	4.6 (0.6)	ns
Gamma-Proteobacteria	4.0 (2.1)	3.3 (1.3)	4.7 (1.3)	ns
Delta-Proteobacteria	1.4 (0.3)	1.4 (0.5)	1.5 (0.3)	ns
Proteobacteria (unclassified)	2.1 (0.3)	2.1 (0.6)	2.1 (0.6)	ns
Actinobacteria	19.8 (2.2)	20.6 (1.8)	21.5 (5.3)	ns
Acidobacteria	11.0 (4.8)	13.6 (6.2)	11.7 (3.9)	ns
Bacteroidetes	11.1 (3.8)	8.6 (3.5)	8.9 (2.5)	ns
Firmicutes	3.1 (0.7)	2.7 (1.2)	3.0 (1.3)	ns
Planctomycetes	**3.6 (0.2) b**	**3.8 (0.5) ab**	**4.6 (0.5) a**	**< 0.05**
Chloroflexi	2.1 (0.5)	2.8 (0.4)	2.0 (0.5)	ns
Gemmatimonadetes	1.2 (0.5)	1.2 (0.4)	1.0 (0.4)	ns
Nitrospira	0.6 (0.3)	0.3 (0.2)	0.5 (0.3)	ns
Verrucomicrobia	0.3 (0.0)	0.4 (0.3)	0.6 (0.3)	ns
Armatimonadetes	0.4 (0.2)	0.6 (0.4)	0.3 (0.1)	ns
Cyanobacteria	0.6 (0.4)	0.5 (0.4)	0.2 (0.1)	ns
TM7	0.6 (0.4)	0.5 (0.4)	0.3 (0.2)	ns
Deinococcus-Thermus	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	ns
unclassified	14.5 (1.4)	15.2 (2.4)	13.8 (1.3)	ns
Other phyla*	0.1 (0.1)	0.0 (0.1)	0.0 (0.0)	ns

***(<0.1% abundance each phyla or class)**
Data are provided as means (n= 4 each) with standard deviations in parentheses. Taxonomic groups with different letters are significantly different between sampling location at the given *p* value.

For Sunraysia soils, both Beta and Gamma Proteobacteria were more abundant in vineyards compared to adjacent native vegetation. Actinobacteria and Armatimonadetes were less abundant in vineyard soils. Verrucomicrobia was more abundant undervine than midrow and in native vegetation soils (Table 20). Because of the greater significance in locational differences in bacterial communities, an NMDS analyses is warranted for this region to illustrate the influence of discrete bacterial orders and associated chemistry on the clustering of samples.

Table 20. Percent abundance of the major bacterial phyla or classes found in vineyard locations in Sunraysia

% abundance of major bacterial groups	Sample location			Significance (*p* value)
	Vine	Mid-row	Native	
Alpha-Proteobacteria	17.4 (2.7)	18.3 (3.0)	18.8 (4.2)	ns
Beta-Proteobacteria	**4.5 (0.6) a**	**4.5 (0.6) a**	**3.4 (0.7) b**	**< 0.01**
Gamma-Proteobacteria	**3.8 (0.5) a**	**3.4 (1.1) a**	**2.3 (0.5) b**	**< 0.001**
Delta-Proteobacteria	1.0 (0.3)	1.1 (0.2)	1.0 (0.3)	ns
Proteobacteria (unclassified)	1.5 (0.4)	1.5 (0.3)	1.3 (0.3)	ns
Actinobacteria	**19.4 (3.0) b**	**21.5 (4.3) b**	**26.6 (4.0) a**	**< 0.01**
Acidobacteria	12.1 (2.9)	11.4 (2.5)	12.3 (1.9)	ns
Bacteroidetes	6.8 (3.6)	6.4 (1.4)	5.5 (2.7)	ns
Firmicutes	4.7 (1.6)	4.5 (2.0)	3.1 (1.7)	ns
Planctomycetes	3.3 (0.7)	3.3 (0.9)	2.9 (0.7)	ns
Chloroflexi	**3.2 (0.6) ab**	**3.8 (0.7) a**	**2.9 (0.9) b**	**<0.05**
Gemmatimonadetes	2.4 (1.0)	2.0 (0.5)	1.6 (0.4)	ns
Nitrospira	1.3 (0.4)	1.1 (0.4)	1.4 (0.8)	ns
Verrucomicrobia	**0.4 (0.3) a**	**0.2 (0.1) b**	**0.1 (0.1) b**	**< 0.01**
Armatimonadetes	**0.6 (0.1) b**	**0.7 (0.2) b**	**1.1 (0.4) a**	**< 0.001**
Cyanobacteria	1.4 (2.9)	0.2 (0.2)	0.8 (0.8)	ns
TM7	**0.6 (0.4) a**	**0.3 (0.1) ab**	**0.2 (0.1) b**	**< 0.05**
Deinococcus-Thermus	0.2 (0.2)	0.3 (0.2)	0.1 (0.1)	ns
unclassified	15.3 (2.3)	15.5 (3.5)	14.6 (1.7)	ns
Other phyla*	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	ns

***(<0.1% abundance each phyla or class)**
Data are provided as means (Under-vine: n = 14; Mid-row: n = 10; Native: n = 6) with standard deviations in parentheses. Taxonomic groups with different letters are significantly different between sampling location at the given *p* value.

For the Sunraysia region, five bacterial orders are specifically associated with vineyards soils when contrasted with soils from nearby native vegetation sites (Fig 9a). Each of these groups have been associated with functions that are strongly implicated in soil health, vine performance and wine quality. For example, Verrucomicrobiales are indicative of higher nematode populations (nematode endosymbionts), Rhizobiales are associated with N fixation, Clostridiales are involved in organic matter breakdown, Desulfobacterales are strictly anaerobic and reduce soil sulfates and Ktedonobacterales are interestingly, a newly discovered group that utilise sugar and starch. Figure 9b highlights that pH, calcium carbonate, and dissolved organic carbon are primary factors (R^2 = 0.367) separating bacterial orders in the different locations.

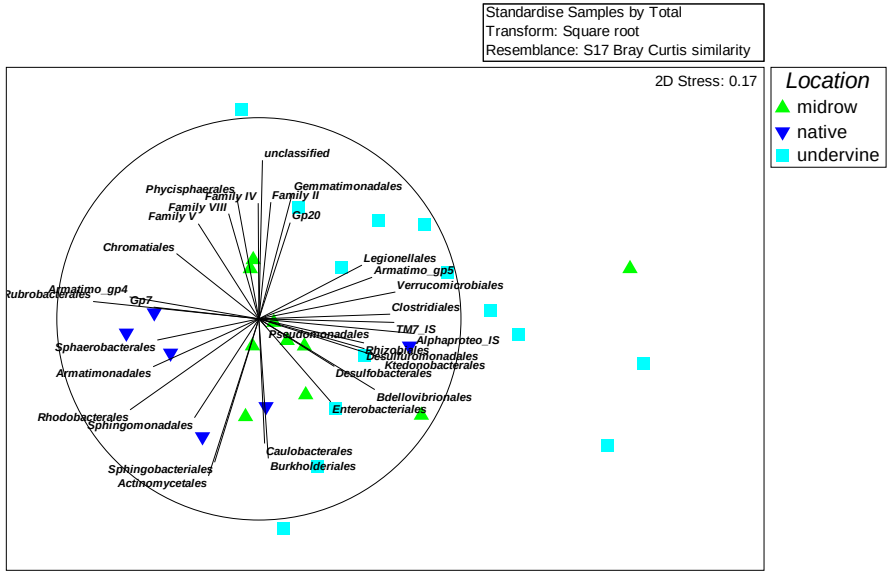


Figure 9a. Sunraysia Region: Clustering of 16S amplicons by location showing influence of bacterial orders

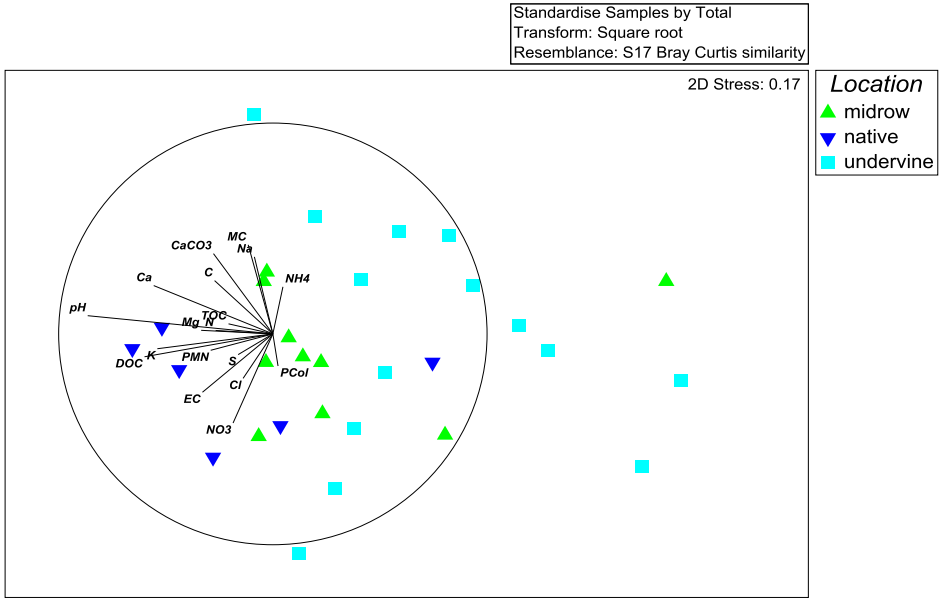


Figure 9b. Sunraysia Region: Clustering of 16S amplicons by location showing influence of chemistry

Higher abundance of Delta Proteobacteria, Bacteroidetes and Chloroflexi were detected in vineyards in the Yarra Valley, particularly in the midrow. This midrow location also had higher abundance of Beta and unclassified Proteobacteria. Gemmatimonadetes, Nitrospira and Cyanobacteria were all more abundant undervine (Table 21).

Table 21. Percent abundance of the major bacterial phyla or classes found in vineyard locations in the Yarra Valley

% abundance of major bacterial groups	Sample location			Significance (*p* value)
	Vine	Mid-row	Native	
Alpha-Proteobacteria	**13.9 (1.4) b**	**15.0 (1.1) b**	**17.3 (2.9) a**	**< 0.001**
Beta-Proteobacteria	**5.3 (1.4) b**	**7.0 (1.2) a**	**4.4 (1.9) b**	**< 0.001**
Gamma-Proteobacteria	3.7 (1.0)	3.6 (0.9)	2.7 (0.4)	ns
Delta-Proteobacteria	**1.5 (0.5) b**	**2.0 (0.4) a**	**0.9 (0.5) c**	**< 0.001**
Proteobacteria (unclassified)	**1.9 (0.5) b**	**2.5 (0.4) a**	**1.4 (0.7) b**	**< 0.001**
Actinobacteria	**14.6 (3.1) b**	**13.5 (2.6) b**	**19.4 (4.0) a**	**< 0.001**
Acidobacteria	15.3 (2.5)	17.0 (3.2)	18.0 (2.4)	ns
Bacteroidetes	**7.8 (3.2) ab**	**8.8 (1.8) a**	**5.7 (3.3) b**	**<0.05**
Firmicutes	**4.7 (2.5) a**	**2.6 (1.0) b**	**2.9 (1.6) ab**	**< 0.01**
Planctomycetes	3.6 (0.5)	3.6 (0.6)	3.7 (0.7)	ns
Chloroflexi	**3.7 (1.6) b**	**3.7 (1.3) b**	**5.5 (2.0) a**	**<0.05**
Gemmatimonadetes	**2.4 (0.7) a**	**1.7 (0.5) b**	**1.3 (0.4) b**	**< 0.001**
Nitrospira	**0.4 (0.2) a**	**0.2 (0.2) b**	**0.1 (0.1) b**	**< 0.001**
Verrucomicrobia	0.8 (0.3)	0.8 (0.3)	0.8 (0.4)	ns
Armatimonadetes	0.4 (0.2)	0.3 (0.1)	0.3 (0.1)	ns
Cyanobacteria	**1.8 (1.3) a**	**0.7 (0.5) b**	**0.5 (0.4) b**	**< 0.001**
TM7	0.5 (0.3)	0.5 (0.2)	0.4 (0.2)	ns
Deinococcus-Thermus	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	ns
unclassified	**17.6 (1.5) a**	**16.3 (1.4) ab**	**14.7 (3.4) b**	**< 0.01**
Other phyla*	0.1 (0.2)	0.1 (0.2)	0.0 (0.1)	ns

***(<0.1% abundance each phyla or class)**
Data are provided as means (Under-vine: n = 17; Mid-row: n = 19; Native: n = 6) with standard deviations in parentheses. Taxonomic groups with different letters are significantly different between sampling location at the given *p* value.

For the Yarra Valley region, bacterial orders are specifically associated with vineyard soils when contrasted with soils from nearby native vegetation sites (Fig 10a). The undervine sample distribution is influenced by higher abundances of Verrucomicrobiales, Rhodobacterales, Pseudomonadales, Bacillales, while the midrow have more Rhizobiales. The undervine bacteria are all known to possess significant plant growth promoting functions. As in Sunraysia, Verrucomicrobiales are indicative of higher nematode populations (nematode endosymbionts), Rhodobacterales are associated with aquatic environments whose functions are not well known and both Pseudomonadales and Bacillales are associated with plant growth promotion and bioremediation capabilities. The midrow abundance of the symbiotic N fixing group, Rhizobiales, may reflect the presence of pasture legumes at some sites.
Figure 10b highlights that the primary interactive soil chemical factors influencing this distribution (R^2 = 0.471) under vineyards were pH, EC, Colwell K, and potentially mineralizable N (PMN). This observation is in agreement with the low resolution TRFLP analyses of this region.

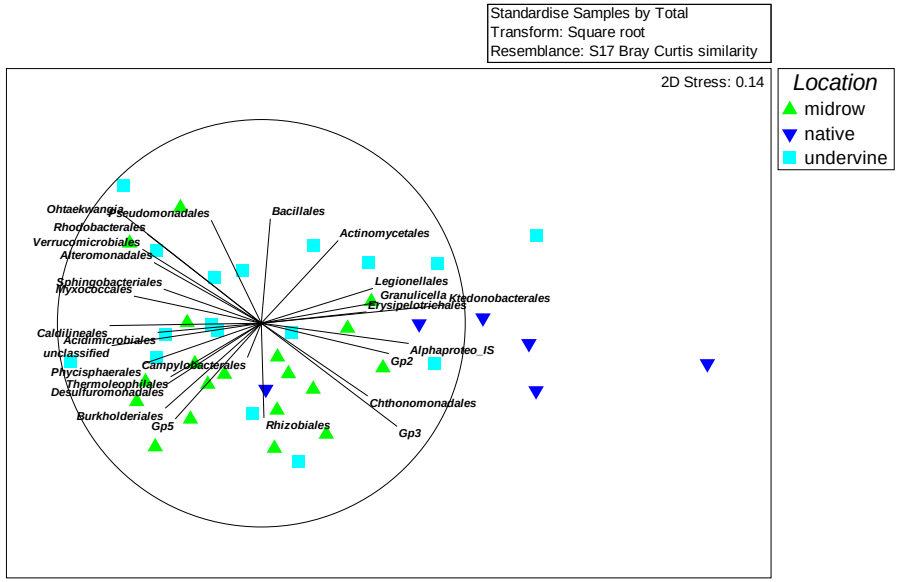


Figure 10a. Yarra Valley Region: Clustering of 16S amplicons by location showing influence of bacterial orders

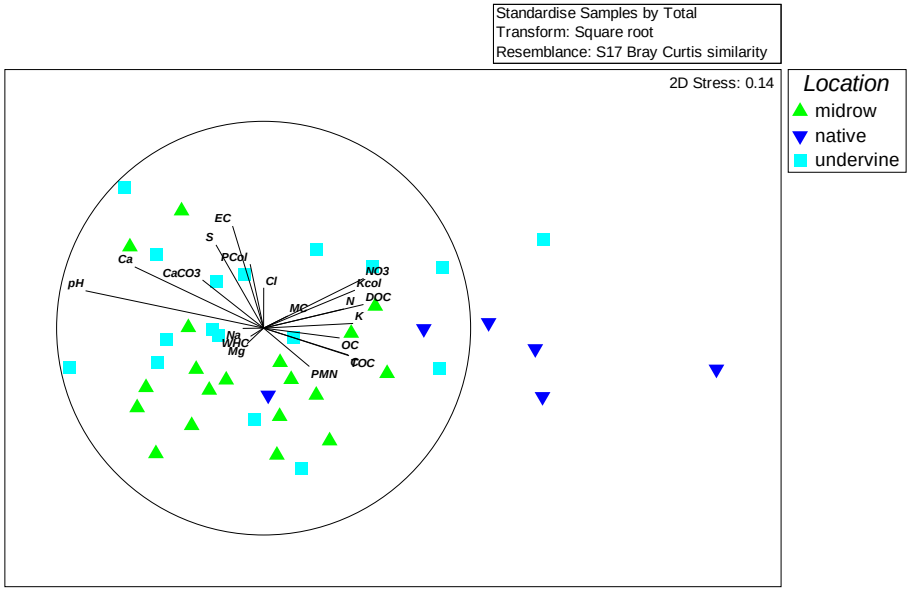


Figure 10b. Yarra Valley Region: Clustering of 16S amplicons by location showing influence of chemistry

ii) Soil microbial functions in vineyards of 4 wine growing regions using a high resolution method (metagenomics)

Previous analyses (TRFLP and 16S amplicon sequencing) provides for an assessment of the taxonomic description of soil microbial communities. The advantage of metagenomic analyses is that it enables description of the functional role of microbial communities. Table 22 summarises some of the key functions that have been measured in the soil microbial communities.

Table 22. List of Level 1 functional subsystem categories in MG-RAST

Amino Acids and Derivatives
Carbohydrates
Cell Division and Cell Cycle
Cell Wall and Capsule
Clustering-based subsystems
Cofactors, Vitamins, Prosthetic Groups, Pigments
DNA Metabolism
Dormancy and Sporulation
Fatty Acids, Lipids, and Isoprenoids
Iron acquisition and metabolism
Membrane Transport
Metabolism of Aromatic Compounds
Miscellaneous
Motility and Chemotaxis
Nitrogen Metabolism
Nucleosides and Nucleotides
Phages, Prophages, Transposable elements, Plasmids
Phosphorus Metabolism
Photosynthesis
Potassium metabolism
Protein Metabolism
RNA Metabolism
Regulation and Cell signalling
Respiration
Secondary Metabolism
Stress Response
Sulfur Metabolism
Virulence, Disease and Defense

Similar to previous NMDS analyses (e.g. 16S amplicon analyses), region has a strong influence on the clustering of functions and particularly so for Sunraysia and the Yarra Valley regions (Fig 11a). The two SA sites (McLaren Vale and the Barossa Valley) did not separate strongly, again likely to be attributable to the smaller sample size associated with poorer quality DNA obtained from these sites. There was no significant separation of functions related to location in vineyard (data not shown). This may have been due to the smaller sample size masking any effects or the use of the high level functions (level 1) which may not have provided the detail required to separate at the more sensitive location level.

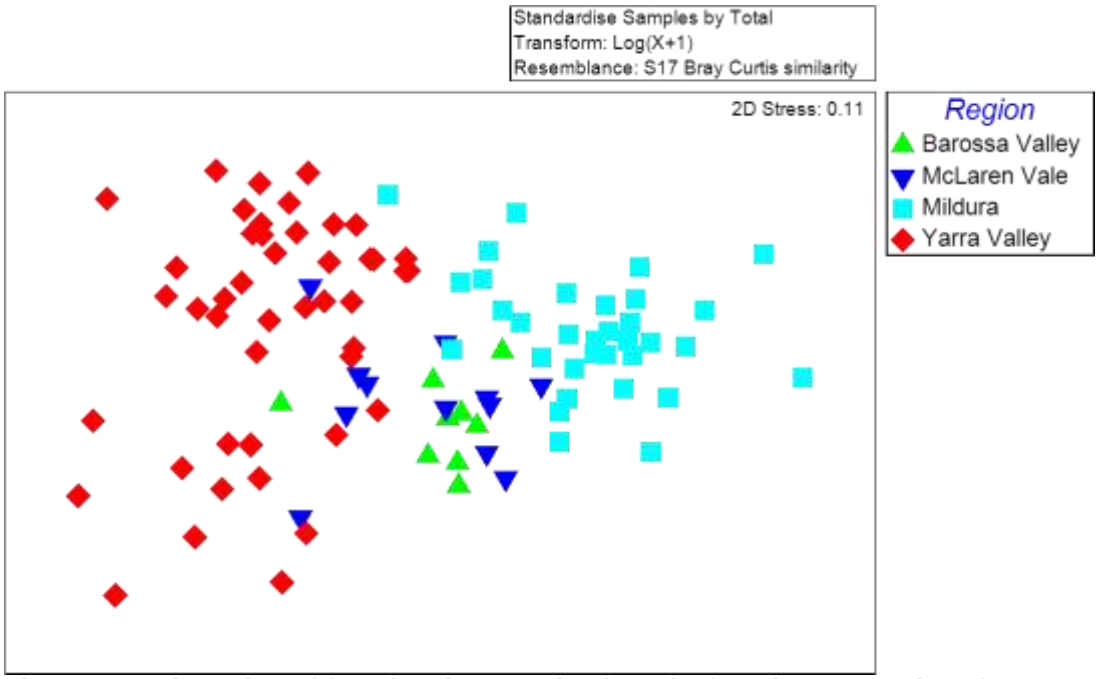


Figure 11a. Clustering of functional categories (Level 1 function categories of MG-RAST) by region

The specific functions that support the separation by region are demonstrated in Fig 11b. It is clear that functions related to the metabolism of aromatic compounds; virulence, disease and defense; and cofactors, vitamins, prosthetic groups & pigments are the 3 functional groups largely responsible for the separation of the sample regions. Interpreting this further, it would appear that there are more genes able to break down aromatic compounds in the Yarra Valley soils, which may be an indication that there are relatively higher amounts of humic acids in these soils, which is produced from the biodegradation of organic matter. This is supported by the higher total organic carbon (TOC) in the Yarra Valley. The only other significant variable that is separating functions by region is pH, with higher pH being a strong factor separating the Sunraysia samples (Fig 11c).

Again as in the amplicon sequencing of microbial communities, the effects of location in vineyard are only evident when the individual regions (e.g. of Sunraysia and Yarra Valley) are considered. In the Sunraysia region (Fig 12a), the native samples (N) can be seen to quite clearly separate from the vineyard samples, but there is less of a clear separation between the midrow and undervine samples. The same functions that separate regional samples also separate the Sunraysia vineyard locations (e.g. metabolism of aromatic compounds; virulence, disease and defense; and cofactors, vitamins, prosthetic Groups, pigments). Soil chemistry does not seem to explain the clustering of these functions in the various locations (Fig 12b).

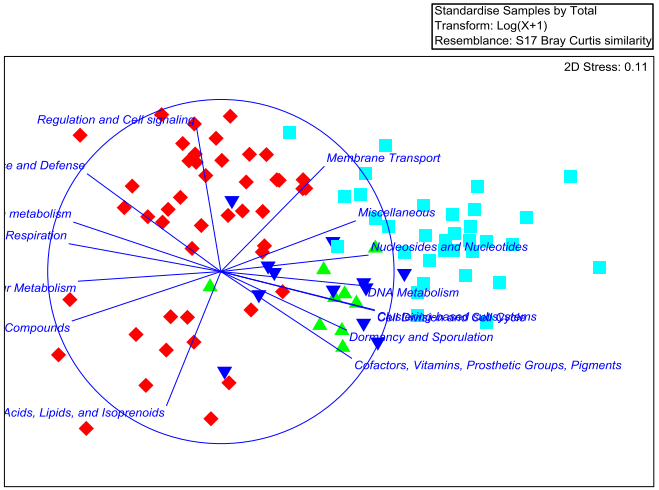


Figure 11b. Regional clustering of functional categories (Level 1 function in MG-RAST) showing the influence of specific functions.

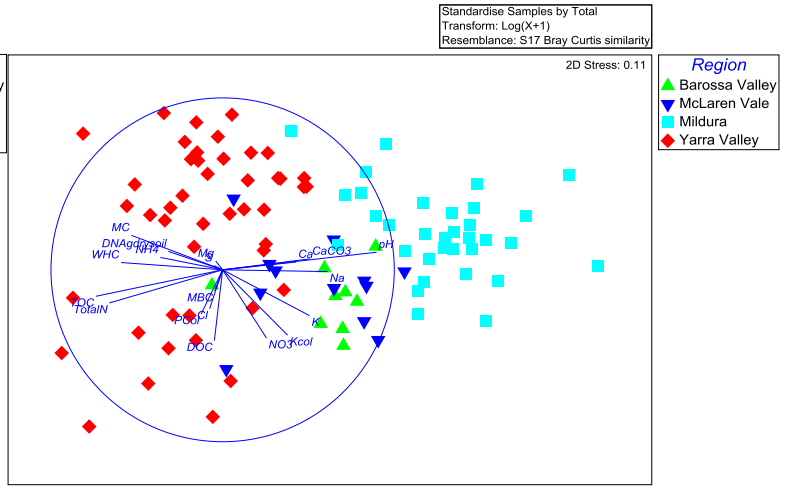


Figure 11c. Regional clustering of functional categories (Level 1 functions in MG-RAST) showing the influence of chemistry.

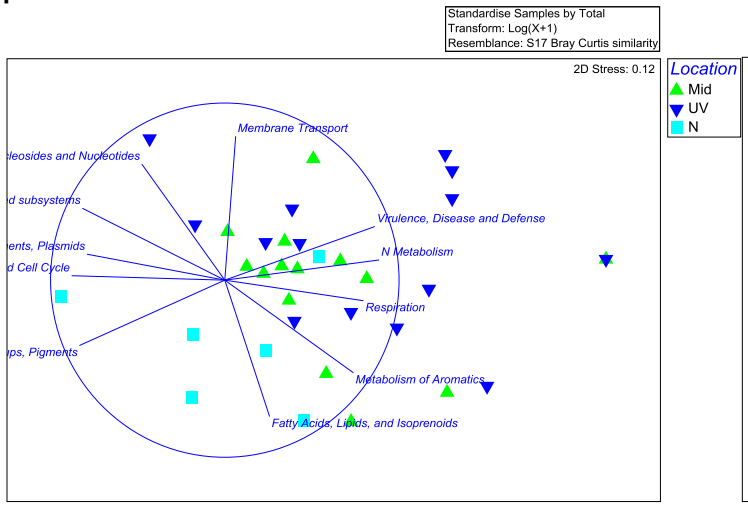


Figure 12a. Sunraysia Region: Clustering of functional categories showing the influence of specific functions.

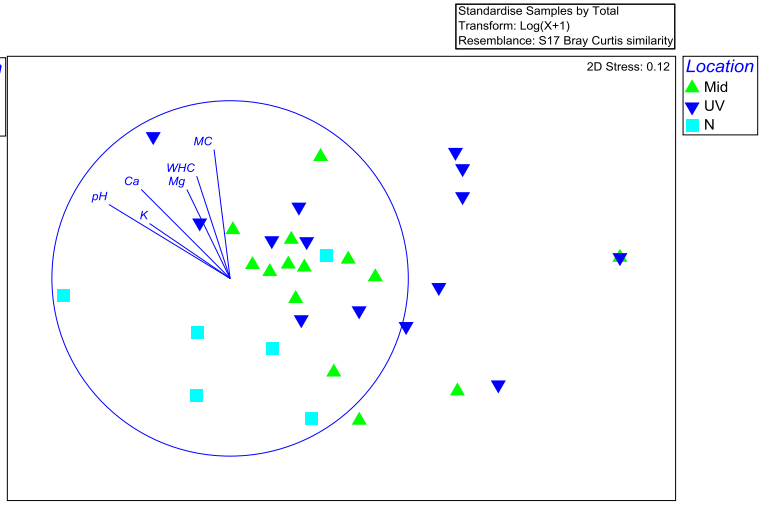


Figure 12b. Sunraysia Region: Clustering of functional categories showing the influence of chemistry.

In the Yarra Valley region (Fig 13a), the functions associated with the native samples (N) can be seen to separate from the midrow vineyard samples, but the undervine samples appear to show no particular clustering related to function except for cell division and cycling which could be depressed undervine due to the lower pH in this region (Fig 13b).

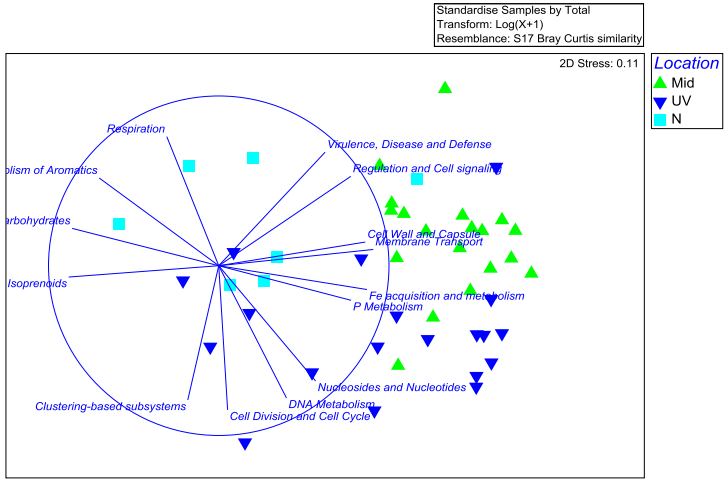


Figure 13a. Yarra Valley Region: Clustering of functional categories by location.

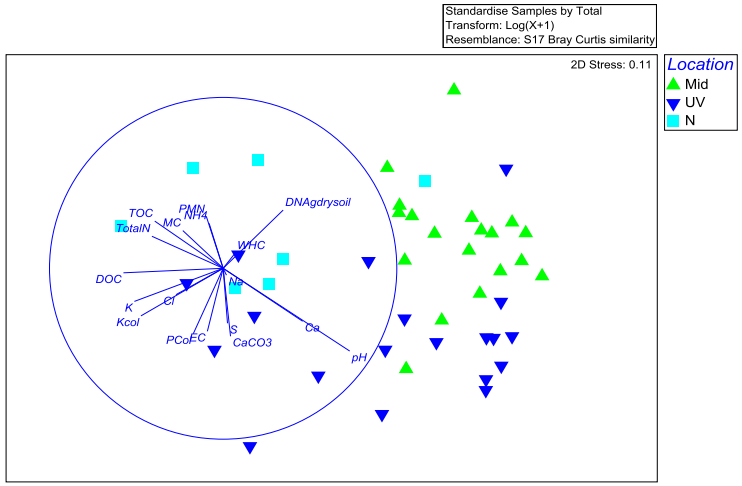


Figure 13b. Yarra Valley Region: Clustering of functional categories showing the influence of chemistry.

In general, level 1 microbial functions appear to be too high order to differentiate differences based on location in the vineyard. It may therefore be necessary to differentiate at a local functional level. This has not yet been attempted.

E. Detailed spatial analyses of Carr's Vineyard (Yarra Valley)



Carr's vineyard was selected to demonstrate the impact of local vineyard features on soil microbial communities (Fig 14). The microbial community analyses were based on 16S and ITS amplicon sequencing (see Section 5 Method D ii). Data was extracted from the OTU table containing the 192 samples generated after processing through an in-house bioinformatics pipeline. This data was then log-transformed and used for ordination by non-metric multidimensional scaling (NMDS) in the PRIMER™ software program.

Figure 14. Carr's Vineyard site

Bacterial communities (16S amplicon sequencing)

As seen previously in larger scale regional sampling, there is a strong effect of location in the vineyard with a clear separation of bacterial communities between undervine and midrow (Fig 15a). Unlike regional analyses, we were able to separate vine performance parameters at Carr's vineyard. Figure 15a indicates that there is a further separation in bacterial communities depending on whether the soil was derived from undervine or midrow of a low vigour vine that produced premium wines (e.g. Vinehigh/Midrowhigh in legend) or from undervine or midrow of a high vigour vine that produced lower quality wine (e.g. Vinelow/Midrowlow in legend).

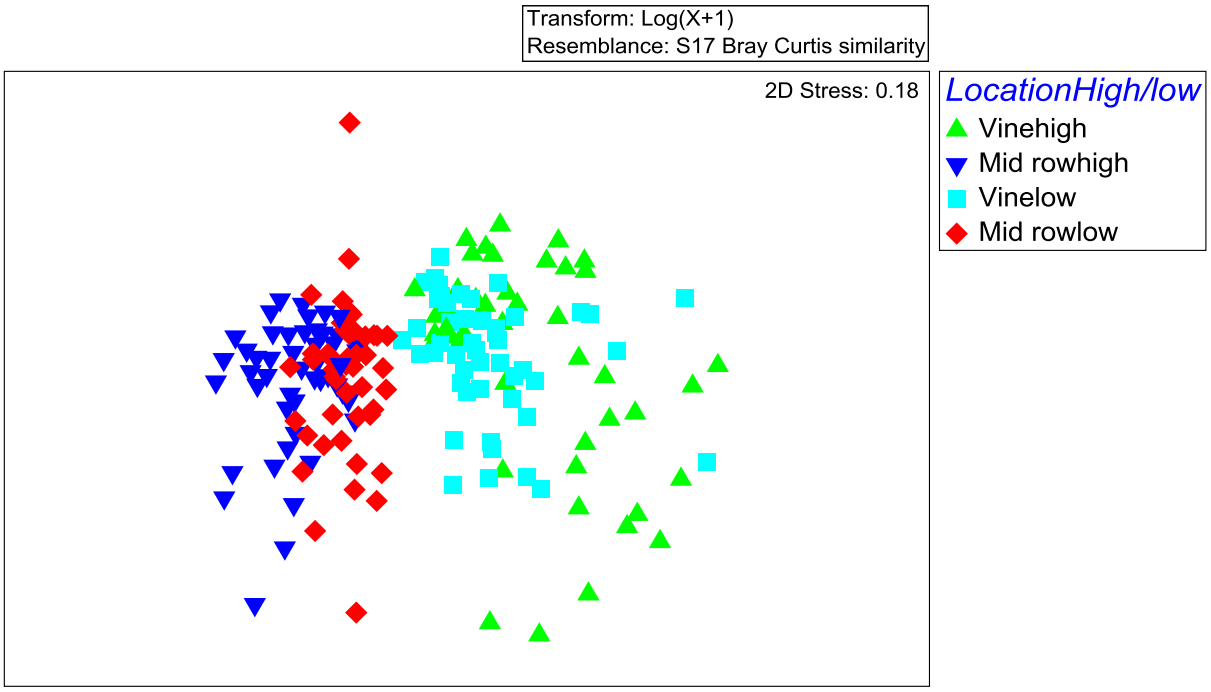


Figure 15a. Clustering of bacterial communities by location & vine performance parameters

Figure 15b identifies the bacterial phyla that are associated with these locations. Chloroflexi, Nitrospira, Gemmatimonadetes and Cyanobacteria are strongly associated with undervine samples. Planctomycetes and the candidate phyla WS3, which has no cultivated representatives, seem to be associated with midrow samples. High sodium levels and electrical conductivity (EC) levels undervine and organic C, total C and N and Manganese in midrow soils seem to be driving separation of bacterial communities at these locations (Fig 15c).

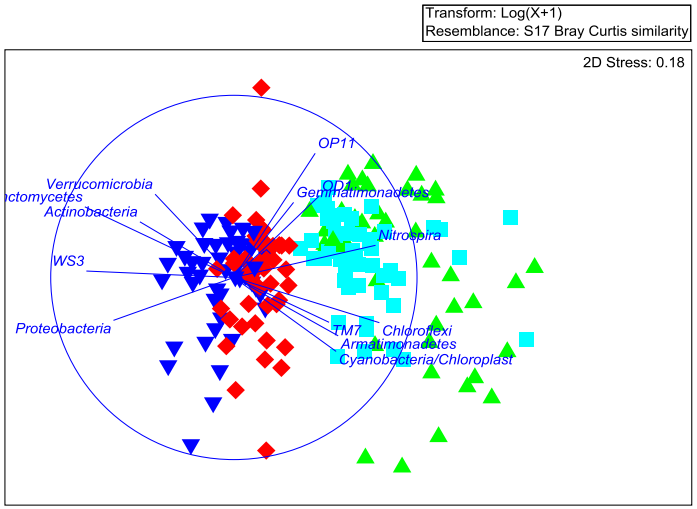


Figure 15b. Clustering of 16S amplicons by location in vineyard showing the influence of bacterial phyla.

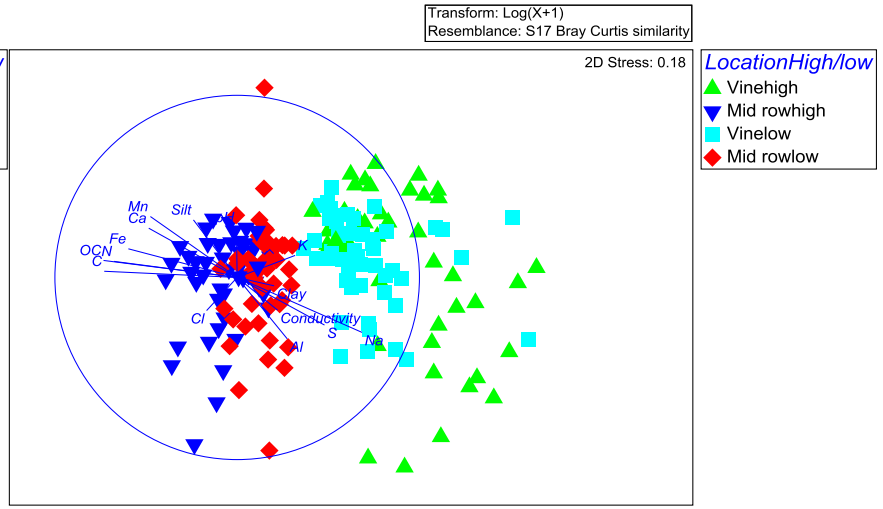


Figure 15c. Clustering of 16S amplicons by location in Carr's vineyard Carr's showing influence of chemistry.

If we focus on the undervine samples (Fig 16a) it appears that Actinobacteria and Chloroflexi influence the clustering of high quality wine/low vigour undervine samples while Firmicutes and WS3 vectors appear more likely to be influencing the separation of low quality wine/high vigour undervine samples. Sodium (Na) is strongly influencing separation of high quality wine/low vigour undervine samples with perhaps some influence of Chloride (Cl), Aluminium (Al), Sand and Sulphur (S). Calcium, total nitrogen and Organic C are influencing the clustering of low quality wine/high vigour undervine soil bacterial communities (Fig 16b). This is based on a step-wise DistLM that showed that the 9 chemical variables explained 42% of the differences in the bacterial community and Na on its own accounts for 15% of that variation (data not shown). The significance of the abundance of Actinobacteria and Chloroflexi of high quality wine/low vigour undervine samples is yet to be explained although it is known that Actinobacteria produce a wide range of metabolites that stimulate plant growth and reduce disease. Chloroflexi are capable of photosynthesis and are known to be halorespirers that can remove chlorides from pollutants and are not necessarily found in contaminated systems.

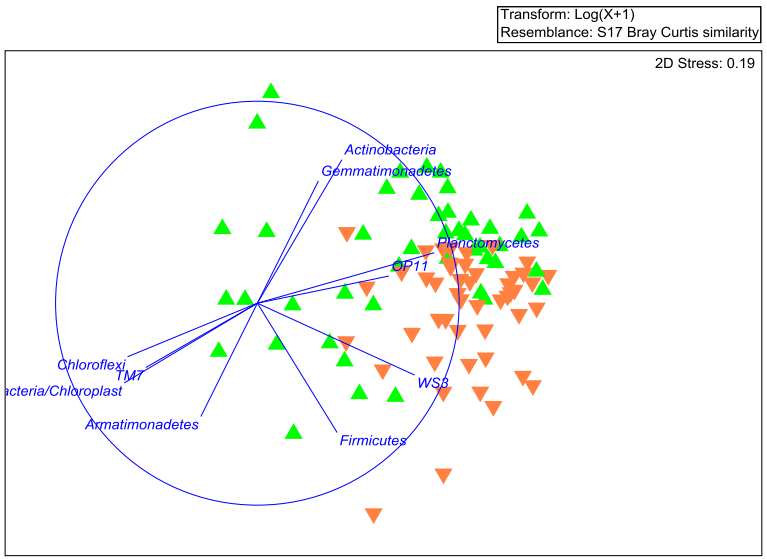


Figure 16a. Clustering of 16S amplicons undervine in vineyard showing the influence of bacterial phyla.

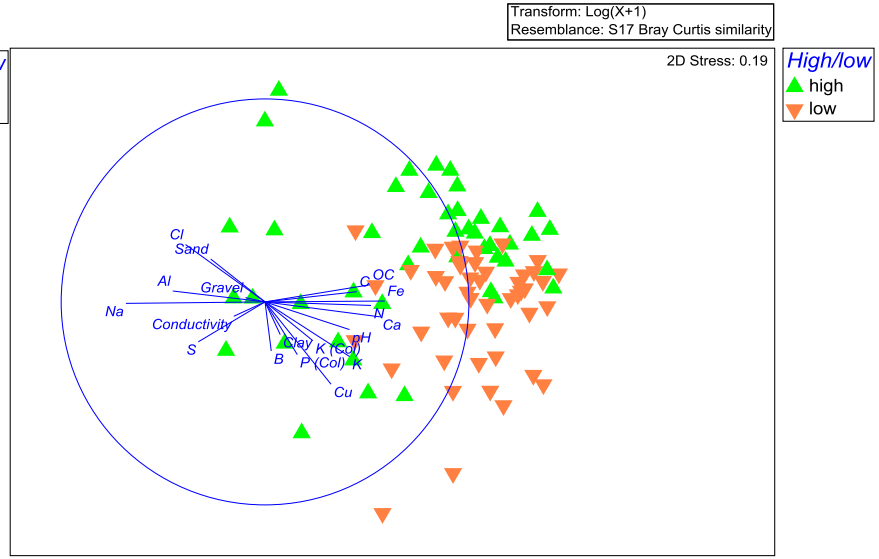


Figure 16b. Clustering of 16S amplicons undervine in Carr's vineyard Carr's showing influence of chemistry.

If we focus on the midrow samples, both the Nitrospira (strongly) and the Firmicutes appear to be driving the separation of the high and low quality clusters (Fig 17a). This coincides with a strong influence of Aluminium (Al), sand, Gravel and Chloride (Cl) on clustering of these communities in the high quality wine/low vigour undervine samples (Fig 17b). Again as in the undervine clustering pattern, a range of elements such as calcium, NO_3^--N and Organic C are influencing the clustering of soil bacterial phyla in low quality wine/high vigour soil bacterial communities.

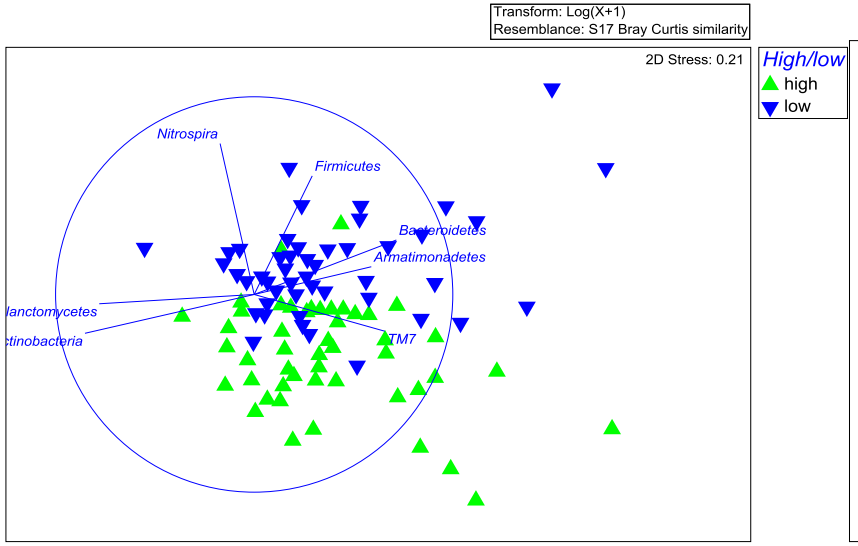


Figure 17a. Clustering of 16S amplicons midrow in Carr's vineyard showing the influence of bacterial phyla.

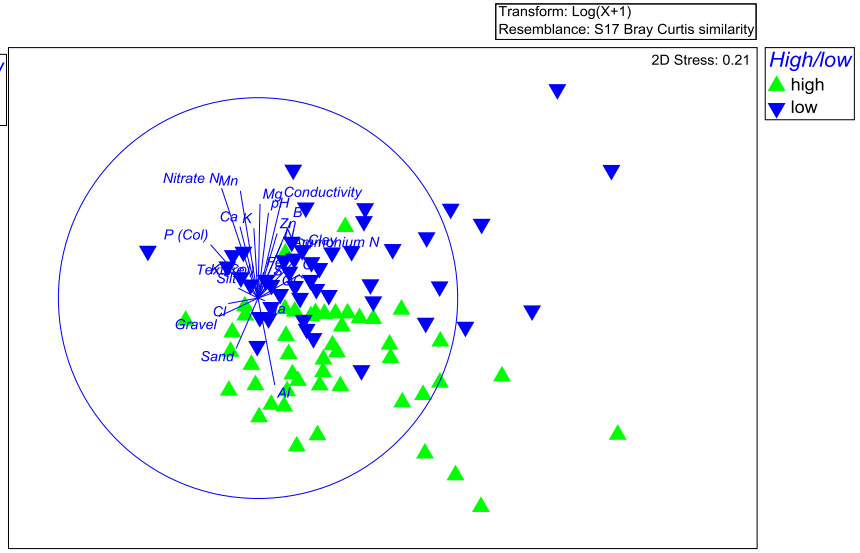


Figure 17b. Clustering of 16S amplicons midrow in Carr's vineyard showing influence of chemistry.

F. Data entry into web-based tool (www.soilquality.org.au)

A sub-contract with UWA, lead by Prof Daniel Murphy and supported by Andrew Wherret (web curator) and Jordan Brock (web designer) has supported the redesign of the existing web site (www.soilquality.org.au) to include a whole new website for the Australian Grape and Wine Industry (Fig 18).

Foundational information about the web site was provided by the originator Prof Daniel Murphy and the curator Andrew Wherret who both outlined the key features of the tool to scientists and key industry representatives. In turn industry representatives provided valuable feedback which were included in the design.

These features include:

- a meaningful dataset and sampling protocols with thresholds (to be) set by experts
- industry ownership, with own branding, curatability and data quality standards
- regional compartmentalisation of the data,
- data comparisons from one vineyard to another, where growers can view their own data against regional averages
- traffic lights indicating the significance of a specific parameter and the requirement for action
- calculators for assessing economics of specific management decisions to ameliorate specific soil based constraints
- fact sheets outlining the value of a specific soil parameter and existing knowledge

i) The AGWA Soil Quality website using the Barossa Valley region as an example of how data is displayed

The following screen shots step through the major features using the Barossa Valley as an example. Figure 18 provides a view of the front page. It is intended that this page will be more generic to represent the industries represented.



Figure 18. The front page of the web site with entry to AGWA data through selecting "Grape and Wine" and region of interest (eg Barossa Valley).

Under the 'Examine Wine and Grape Region, there is a list of all the regions where data has been added. At the moment, we can only display the Barossa Valley Region. Once you click on the Barossa Valley, you go to a regional map which depicts the individual sampling locations. Where

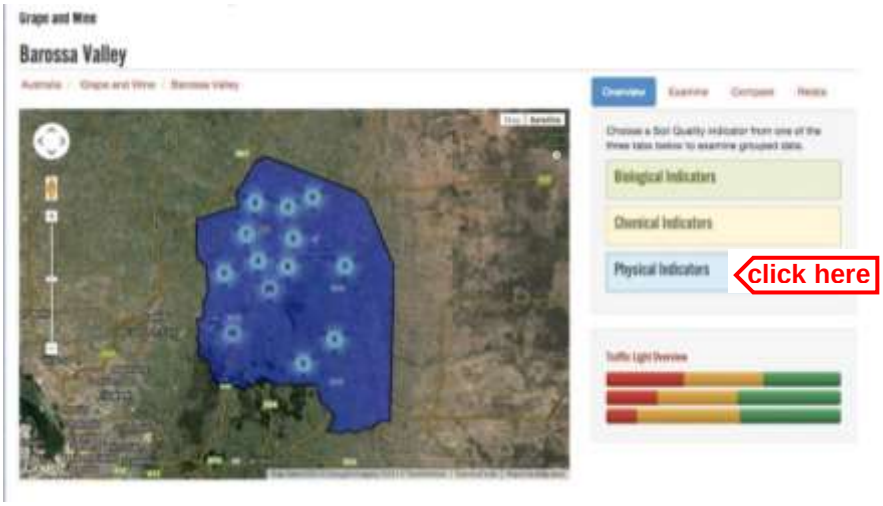


Figure 19. The Barossa Valley wine growing region displaying the sampling points where a minimum soil dataset has been uploaded.

there are several sites close together, they are added (Fig 19). Whilst there were more sites sampled than the total listed, strict stringency requirements meant that only 55 sites were uploaded. On the regional page display you will see that the minimum dataset is classified according to whether the data is biological, chemical or physical. Also displayed is the traffic light system which is a quick and easy way of identifying possible soil constraints to production and formulating management strategies to combat these issues.

For each soil quality indicator, a series of critical values have been applied relating to impacts on production and/or soil quality in general. For AGWA, it will become pertinent to gather together experts (industry & technical) to determine what these threshold values might look like for each region (or management unit). An indication of red implies a high risk of production impacts and the need to investigate management options urgently. An amber colour implies a moderate risk of production impacts and should be investigated further, while green implies low risk, however regular monitoring should continue.

Clicking on the indicator of interest (eg physical indicators) will allow you to look at the regional data as displayed in a bar chart of aggregate stability data (Fig 20). If the threshold ranges had been established, we would indicate with a traffic light graph under the x -axis which sites were considered low, medium, or high.



Figure 20. A bar chart illustrating the physical indicator of aggregate stability at 55 sites in the Barossa Valley wine growing region (0-15cm depth).

Figure 21 takes it a step further and splits into the different sampling locations (under vine, mid-row or native).You can see that for the aggregate stability range of 0-3 and 3-6, location in vineyard shows same patterns with undervine locations indicating a smaller percentage of sites have these values. Whereas a relatively higher proportion of sites undervine contribute in the higher ranges.



Figure 21. Distribution of aggregate stability values depending on location in vineyard (averaged across the 55 sites sampled in the Barossa Valley).

Figure 22 is the same as Fig 21 except it indicates pH values. From this you can see quickly that pH ($CaCl_2$) seems to fall within the acceptable range (5.5-7.5) and that the native vegeation soils contributes most strongly to this.



Figure 22. Distribution of pH ($CaCl_2$) values depending on location in vineyard (averaged across the 55 sites sampled in the Barossa Valley).

Perhaps one of the most popular features is the ability to compare the data from individual growers against the data stored in the database and obtained through the project (Fig 23). This can be done by clicking on the compare tab and either entering a code for your (from the curator) or by directly entering your own values.

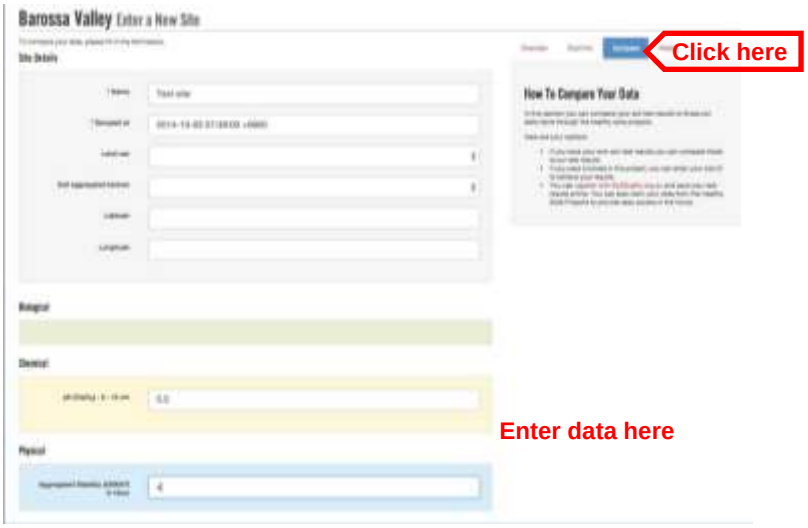


Figure 23. Data entry to enable comparison of individuals data against regional averages

A box and whisker plot with the midline depicts the average with lower and higher confidence limits and the dot represents your value (Fig 24). In this case the individuals value is less than the regional average which could indicate local differences in management that need closer monitoring.



Figure 24. Graphical representation of individual data compared to regional averages. In this example because there is only regional data, the state data will be the same.

Another feature worth mentioning is the 'relate' function which enables comparison of two parameters. This can be used to confirm the interelatedness of measurements (eg pH and Calcium carbonate measures) or a causative relationship that might be used in a predictive way (eg pH declines with microbial biomass).

ii) Ongoing requirements to establish the AGWA soil quality website.

This project has promoted the adoption of an existing web based soil quality monitoring tool and this report presents some of the key features that have been identified by industry representatives as relevant for grape and wine growers. However it is very much a work in progress and future development will require that the following considerations are addressed.

- Data – development of traffic lights. This involves developing consensus on critical values for each of the indicators that are presented. There are some values presented in one of the factsheets in DPI1101, but they appear as one value (therefore only two categories). It would be better to have red, amber and green categories. These may be different for different regions, but do give solid interpretive value to the user. While it is useful for the

user to compare themselves against others in a similar situation, what if all the data for that soil quality indicator is below par? The traffic light overview gives a snapshot of what the limiting soil quality indicators are in that region/system.

- Data – developing the temporal aspects of this monitoring tool. For some sites there are two sets of values from different sampling times. These data will need to be loaded on the website.
- Data – more is better. The more values there are, the more relevant the comparisons are. It comes down to having a critical mass of data that we can say reflects that specific region or soil type. It is therefore essential that awareness of this web site is heightened amongst advisers who can become regional champions for this tool (see last dot point).
- Factsheets – linkage from data through to information is useful to point the user toward management options. While DPI1101 has produced some useful fact sheets, more are required. The current web curator is prepared to provide external links to existing sites, however housing them within the site is much more user-friendly.
- Information – for each of the regions information has been pulled from the AGWA website, but more soil related text would improve this information.
- Identification soil quality champions within the Grape and Wine industry. This person understands the industry and has good knowledge of the soil related issue across the board. Any future development of the AGWA section of the website would be facilitated by them.

Summary recommendations that incorporate the above comments are provided below.

8. Outcomes, Conclusions & Recommendations

This study has assisted in laying the foundations for a soil quality monitoring program that integrates more sophisticated measures of soil biology in broad scale monitoring of Australian winegrowing regions. It builds upon the global recognition of the role of soil microbial communities in soil, grape and wine quality attributes and the recent emergence of DNA based technologies to vastly improve our understanding of the composition and functioning of soil microbial communities.

A range of soil microbial properties were assessed using these DNA based technologies. One is considered a low to medium resolution measure (e.g. TRFLP 16S & ITS) that indicates how bacterial and fungal communities differ in composition with respect to regional (e.g. Barossa, McLaren Vale, Sunraysia and Yarra Valley) and local (mid vine, undervine and adjacent remnant vegetation) vineyard factors. The two others are considered state of the art, high resolution DNA sequencing approaches that use either 16S & ITS amplicons to identify bacterial and fungal features to taxonomic level of order or rely upon the whole metagenome. This latter approach has the added feature of not only identifying who is present and abundant in the bacterial and fungal communities but what functions are being performed.

This study has also demonstrated how an existing web based monitoring tool (www.soilquality.org.au) can be adapted to meet the needs of individual wine and grape growers and advisers who wish to assess i) how their current vineyard management practices influence a range of soil quality parameters, ii), how these parameters compare with that of their neighbours and peers and iii), what management practices can be used to improve soil health under vineyards. Together with DPI1101 project, it has also contributed to the establishment of a standardized set of tests of soil quality and standardised protocols for comparing soils in the midrow, undervine and at an adjacent native site.

In terms of selection of a measure for inclusion into a web based tool, the low resolution method, based on TRFLP may be sufficient to indicate the diversity that exists within the bacterial and fungal communities based on a set of established assumptions where the higher the microbial diversity the more resilient the soils. On the other hand a soil microbial DNA sequence based approach that provides information on discrete functions deemed to be critical to vineyard performance would be considered high value measures. For example, the presence and extent of functions related to N and P mineralisation, disease control and breakdown of aromatic molecules (i.e. pesticides, humic substances) undervine might provide a good indicator of not only the resilience of the soil but also the health and performance of the vine. The ultimate selection of the most appropriate soil biological measure will require expert input into what are considered, based on emergent data, the soil biological functions that influence vine performance and what the regionally and locally appropriate threshold values should be for each of these.

Perhaps the most exciting prospect to emerge from this research is the establishment of the relationship between soil microbial communities, vine vigour and wine quality. This research has demonstrated the strong influence of region and location on specific groups of microbes. The significance of these relationships is yet to be fully elucidated but it is entirely possible that signature communities in soil will have a direct relationship on wine quality. Our observations support the notion of a non-random ‘microbial terroir’ to explain regional variation among wine grapes. Knowledge of the chemical factors that influence or drive this relationship provides the means for manipulating these microbial communities to achieve the desired wine quality outcome.

These soil biological measures rely upon stringent and standardised soil sampling regimes that preserve the integrity of the microbial communities as they exist at point of sampling. Unfortunately the consequence of less stringency in sampling was seen for the SA wine growing regions where a significant number of samples did not yield sufficient high quality DNA to be useful for subsequent sequencing protocols. An industry standard for soil biological sampling that ensures preservation of the microbial DNA at sampling and during transit will ensure regional benchmarking provides the growers with information that is practical and reliable for use in soil management strategies.

Even the standard biological tests chosen for the MDS proved to be difficult to get from commercial laboratories (refer to DPI1101). Labile carbon, potentially mineralizable nitrogen (PMN) and microbial biomass analyses are generally available on a semi-commercial basis but further effort needs to be put into the creation of demand for the biological test methods identified for the MDS. For the more sophisticated tests, an existing DNA based testing platform, SARDI's PreDicta B platform could be utilised for the recommended DNA based measures. This approach is in line with that of the Grains Research and Development corporation who are developing tests for nematode diversity and microbial community diversity thereby reducing handling errors.

The project outputs were showcased at "Workshop 19: How to interpret soil health tests to assess vineyard performance" as part of the 15th Australian Wine Industry Technical Conference held in Sydney in July 2013. This workshop attracted a capacity crowd of 50 participants from all Australian wine regions and included several from overseas and there is still significant opportunity to extend this information on the soil biological contribution to soil and wine quality more widely within the test regions and beyond.

The overarching recommendation arising from this project is to maintain the momentum and build on the foundations laid by this project and DPI1101.

Specific recommendations for AWGA consideration relate to the further development of microbial tests that will enable prediction of a healthy soil, a healthy vineyard and high quality wine by:

- Critically assessing the range of soil microbial parameters trialed in this study to select a minimum dataset of features/traits that can be directly related to specific vine performance criteria,
- Extending studies of soil microbial biogeography to establish linkages with microbial communities associated with vines (leaves and fruit); evidence to support the existence of 'microbial terroir' will inevitably lead to options for manipulation,
- Continuing to evaluate and interpret the microbial DNA sequence information to identify regional trends associated with soil chemistry and vineyard management.

Related to the web based soil quality monitoring tool, it is recommended that AGWA also consider

- Identifying an industry specific web site curator independent of the current web design team,
- Identifying soil quality champions within the Grape and Wine industry to promote the adoption of the web based monitoring tool and to lead in the establishment of threshold values for the minimum dataset of measures,
- Identifying vineyard soil specialists to assist in the development of fact sheets that specifically identify management options to overcome soil constraints or to otherwise enhance wine production,
- Developing fact sheets that outline the importance of soil microbes in soil health, vine vigour and potentially wine quality.

Appendix 1: Communication

More workshops are likely to occur in the coming few months now that the soil quality data is uploaded and the web site becomes more widely accessible.

The ongoing interpretation of the soil microbial community data will generate information that could be presented in field days and workshops.

7-8th August 2012: Key note address delivered by A/Prof Pauline Mele to the National Fodder Association Conference titled 'Sustainable and Productive Soil Systems – Significance of soil biota in maintaining productive soils'.

24 October 2012: Soil quality web-based monitoring and decision support tool workshop for the Grape and Wine industry, organised by A/Prof Pauline Mele as part of DPI1102, AgriBio, Bundoora. This workshop established the design features of a web based tool to assess soil quality in the Grape and Wine growing regions of southern Australia (www.soilquality.org.au). Several prominent industry representatives were in attendance and made valuable contributions to the development of a user-friendly web based tool.

4th April 2013: Meeting organised by A/Prof Pauline Mele with UWA subcontractors in Perth to discuss web design and layout. A staging area was established so that project team could visualise the draft AGWA layer.

14 July 2013. The project team lead by A/Prof Pauline Mele assisted in the delivery of information in "Workshop 19: How to interpret soil health tests to assess vineyard performance" as part of the 15th Australian Wine Industry Technical Conference held in Sydney, NSW. This workshop reached the full capacity of 50 participants from all Australian wine regions and included several from overseas. The presentation delivered was titled ''A web-based Soil Quality Monitoring Tool'.

October 2013, September 2014: Latrobe School of Life Sciences Lectures series in Soil Biology Monitoring: Demonstration of web based tool for monitoring vineyards, presented by A/Prof Pauline Mele.

August 2013, September 2014: University of Western Sydney Soil Biology Master Class presentations demonstrating the application of web based tools for assessing soil biological parameters delivered by A/Prof Pauline Mele.

5 November 2014: Workshop in the Mornington Peninsula organised by A/Prof Pauline Mele. Title of presentation 'Drilling down into the soil biological communities' (agenda attached below).

Soil Biology & Health Workshop

The workshop will demonstrate the utility of the soil quality web based tool (www.soilquality.org,au) for overcoming soil based constraints in the wine industry. Also an update of the microbial community analyses undertaken on samples collected will be included. DNA sequencing techniques have been run in order to characterise the composition and functions present in regional wine producing soils to determine what physical and chemical features shape these communities. Also some fairly simple modelling to try and predict how salinity might impact on soil functions.

Wednesday 5 November 2014: 10 – 12noon

Venue: Merricks General Wine Store

9.45am Tea/coffee on arrival

10 – 10.15am A monitoring system for soil health in vineyards
Dr Jacky Edwards (Project leader: AGWA National Soil health project)

10.15 – 10.45am Drilling down into the soil biological communities
- A/Prof Pauline Mele (Project leader: AGWA Soil biology in soil health)

10.45 – 11.45am Demonstrating the soil quality web based tool
www.soilquality.org.au
- Andrew Wherret (UWA & Living Farm Pty Ltd)

11.45am Discussion

RSVP names of those attending to admin@mpva.com.au by 24 October 2014 please

Australian Government
Australian Grape and Wine Authority

living farm

Appendix 2: Intellectual Property

Intellectual property is held by participating organisations.

Appendix 3: References

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Appendix 4: Staff

Name	*Title*	*Position in this project*	*Agency*
A/Prof Pauline Mele	Research Leader	Principal Investigator	DEPI Victoria
Dr Lori Phillips	Principal Research Scientist	Chief Investigator	DEPI Victoria
Bernadette Carmody	Technical Officer	Soil sampling/processing	DEPI Victoria
David Pearce	Technical Officer	Soil sampling/processing	DEPI Victoria
Clare Allen	Scientist	Soil processing (molecular biology)	DEPI-Victoria
Prof Daniel Murphy	Sub contract leader	Web site originator	UWA
Andrew Wherret	Technical specialist	Web site curator	Living Soils Pty Ltd
Jordan Brock	Technical specialist	Web Programmer	Consultant