Wine Australia for Australian Wine

THE UNIVERSITY of ADELAIDE

Under-vine cover cropping for healthy and productive soils



FINAL REPORT TO WINE AUSTRALIA

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Abstract

This project aimed to better understand the mechanisms behind under-vine cover crop and grape vine interactions in the vineyard. All cover crops reduced soil salinity at the Nuriootpa site, and did not induce water stress during hot conditions relative to the herbicide control. Cover crops that included *Medicago* sp. and annual grasses added nitrogen to the soil and did not incur yield reductions relative to the herbicide or straw mulch treatments. Overall this project has shown that in the Barossa and Langhorne Creek, under-vine cover crops (with good species selection) will perform as well or better than herbicide-based weed management.

In The Riverland, the trial demonstrated the effectiveness of Medic and Rye to generate significant under-vine biomass without use of additional water. After two years of data collection, most differences among treatments were not statistically significant, and further work is necessary to understand the full, long-term consequences of the trialled practices.

Executive Summary

Within vineyards, knowledge of under-vine management practices remains largely under-developed owing to the relative paucity of data. It generally requires considerable resources to remove weeds that can generate competition with the vine for soil moisture and nutrients. Current standard practice sees growers use regular herbicide applications to maintain a bare under-vine area, while very few growers allow any sort of plant growth in this space. Over time, this has been shown to deplete the soil of many important resources and can lead to soil compaction and hydrophobicity. An ideal agricultural system should be able to generate its desired product, while utilising the soil resources through the growing season, while replenishing them in the dormant season.

Across the 2019 and 2020 vintages, to test the effects of selectively growing a variety of grasses and pasture crops in the under-vine space, an initial trial was designed and implemented in two South Australian wine regions; The Barossa Valley (Nuriootpa), and Langhorne Creek. Simultaneously another trial in Tanunda explored the potential benefits of biofumigant crops on nematode abundance. Lastly, across the 2022 and 2023 vintages a fourth trial explored cover-cropping and other weed management in The Riverland. The goal of this extension was to test the effect of under-vine cover cropping and mechanical weeding in a semi-arid, extreme environment.

At the Barossa Valley and Langhorne Creek trial sites, a variety of industry-standard vineyard-floor management practices were selected for testing, these included an exclusive legume treatment; Medic (*Medicago*), one containing a combination of Medic and annual Ryegrass (*Lolium rigidum*), a combination of the vigorous grass; Fescue (*Festuca*) and the legume Strawberry Clover (*Trifolium*), the vigorous grass; Kasbah (cv. Cocksfoot, *Dactylis*) and the native species; Wallaby Grass (*Rytidosperma geniculata*). These under-vine cover crops were compared against both a straw mulch (*Triticale*) application and a herbicide-sprayed control (bare earth). Over two growing seasons, and building on the establishment of these trials four years prior, data was collected on the soil nutrients, soil moisture, soil hardness and soil microbiomes (including mycorrhizal fungi), vine nutrient status, canopy size, vine yield and berry chemistry.

By providing an active mulch layer on the soil surface, which would act as a weed suppressant, the treatment species selected were expected to have a number of broad beneficial effects;

- Reduced water evaporation;

- Improved soil carbon;
- Increased soil nitrogen (in leguminous cover crops); and
- Increased soil biodiversity.

In addition to these benefits, the ideal cover crop should also provide;

- No reduction in yield;
- No reduction in grape quality;
- No increase in watering; and
- No increased cost of management.

The third trial site, established in Tanunda, tested the effectiveness of biofumigant under-vine species to control nematode populations. After two seasons of attempts to establish biofumigant cover crops, greenhouse generated 'Nemcon' biomass was incorporated into soils at the trial site. No effects on nematode populations were observed, though due to the limitations in establishing the cover crops these results are inconclusive and may yield different results at sites with differing soils.

Finally, using soil from the Langhorne Creek trial site, impacts of the cover crops on soil arbuscular mycorrhizal fungi (AMF) was also investigated. Findings from this aspect of our project suggest that soil inoculum potential is unchanged by presence of cover crops vs herbicide control.

Overall, the outcomes generated from 2018 to 2020 at Nuriootpa and Langhorne Creek show that selection of species is critical to achieving the requirements of an under-vine cover crop. While the perennial grasses were included in the project because they were genetically programmed for summer dormancy, they continued to remain actively growing and competing with the vines throughout the season, and so were determined unsuitable for a role in the under-vine space. By contrast, the annual Medic and Ryegrass species, which have been bred as pasture plants to thrive in the Mediterranean environment, fitted into a functional role within the vineyard environment by providing all the benefits noted above. The contribution of the legumes to supply soil nitrogen, and the grasses to ameliorate soil structure, appears critical to enabling the balance to swing in favour of the provision of soil cover and its associated additional water use, compared to the maintenance of a bare under-vine strip.

At the Monash site in 2022 and 2023, the limited timespan of the trial yielded few significant differences among treatments, though trends similar to those in the earlier trials may be

present. Extension of this trial beyond 2023 is highly recommended as treatment effects on soil continues to accumulate.

Despite these positive findings, and the low cost of implementation, there remains considerable grower resistance to adopting these practices. It therefore requires a paradigm shift in growers' perceptions of the vineyard aesthetic. The "untidy" nature of an under-vine cover crop, as it cycles through maturity and senescence with minimal intervention, may not fit with traditionally-held perceptions of the archetypal vineyard. Just as it has taken, in some regions, many years for growers to stop cultivation of the mid-row, it will require a concerted effort to encourage them to move on from herbicide use and enable the ecosystem services that cover crops provide to perform the same function in vineyard floor management.

Background

In a previous project (UA 1303) funded by Wine Australia (WA hereafter), under-vine cover cropping was explored as an option to the management of the vine row. The rationale for this earlier work was based on the wine industries desire to maintain a clean, green image on the global stage, including the need to manage the vineyard soil in line with best management practice. The regular application of herbicides to the under-vine zone for weed prevention or control has been shown to have adverse consequences for soil carbon, which is directly correlated with soil quality. In addition, there are considerable issues with herbicide resistant weeds in vineyards and more recently concerns regarding the worker safety of all the knockdown herbicides used in viticulture. The main positive outcomes of UA 1303 were that in all regions (Riverland, Barossa, Langhorne Creek and Eden Valley) the growth of annual pasture legumes and grasses either as mono-crops or in combination generated yields and gross margins that were equivalent to or greater than the herbicide control. Positive benefits were also realized in soil quality, with organic carbon increasing at all bar the Waikerie site. The new project (reported on herein) was designed to build on this successful outcome. For widespread adoption of under-vine cover cropping to occur, a greater mechanistic understanding of the impacts of different under-vine management practices on vineyard systems is required. Two sites from the previous project (referred to as Nuriootpa and Langhorne Creek, hereafter) were the focus of more mechanistic work in the current project. The main emphasis was on the impacts of under-vine management on the soil (moisture, nutrients and 'health'), vines (yield, berry chemistry), and wine (small lot). The impact of the various cover cropping treatments on the inoculum potential of a specialised group of beneficial soil fungi that form symbiotic associations with most terrestrial plant species (including grape vines) was also studied at the two sites.

In addition to the continuation of two sites (Barossa and Langhorne Creek) an additional site (Tanunda, hereafter) was to be established to investigate the bio-fumigation potential of under-vine cover cropping. Soil borne nematodes are recognized to reduce yields by up to 40%. To overcome this suppression, vines can be grown on resistant and tolerant rootstocks, but options are also required for those vineyards already established on own roots that suffer nematode invasion of the root system. Chemical control with nematicides has occasionally been used, but is not recommended due to the toxicity of the nematicides and their cost. The other option is the growth of plants which generate chemicals with nematocidal properties. These bio-fumigants, which come from the brassica family, are grown in nematode infested

soils and then incorporated into the soil at maturity, where the nematocidal compounds are released. Some crop varieties have superior qualities based on the amount of active chemicals they produce, with several products on the market.

Previous work by other researchers (Rahman & Somers 2005, Penfold & Collins 2012) has shown their growth in the mid-row then transfer when flowering to the under-vine followed by burial to prevent the escape of volatile compounds, significantly reduced nematode populations. A new trial was therefore established in Tanunda to determine whether it may be possible to generate the same or better outcome using under-vine cover crops, including those with known nematocidal qualities.

Project Aims

The following project aims apply to sections 1-3 of the report.

Output	Target Date	Activities
Data from completed mycorrhizal inoculum potential glasshouse trial	31/12/2019	Harvest plants from mycorrhizal inoculum potential glasshouse experiment and assess mycorrhizal colonisation of roots.
Wine made using grapes from season 1. Feedback from winemakers (or a panel) to identify potential impacts of cover crops on wine quality	31/12/2019	Finish making wines using season 1 grapes from the most promising treatments (14 treatments in duplicate). Undertake basic chemical analysis of wines. Conduct wine tasting with local winemakers (or a panel) to obtain feedback regarding the impact of cover crops on wine quality, to ensure they do not compromise the final product.
Data on impacts of selected treatments on soil microbiomes and nematode communities (season 2)	31/03/2020	Collect soil for analysis of soil microbial communities on up to 40 experimental plots. Analyse of nematodes (SARDI diagnostics) at treatments where covercrops sown and nematodes are present.
Data from all sites on soil, berry, vine and cover crops (season 2)	31/05/2020	Measure cover crop performance (botanical assessment) on field sites. Collect and analyse petioles, berries, vine LAI and yield, and soil samples (as above) at established treatments at Nuriootpa, Langhorne Creek and Tanunda field sites.
Data for soil moisture and nutrient cycling (season 2)	31/05/2020	Monitor soil moisture and mineral N pools (i.e. nutrient cycling) over season (fortnightly). Analyse soil moisture and N data, statistics.
Final Report	30/10/2020	Analyse and interpret remaining data. Submit Final Report to Wine Australia.

Report Structure

For ease of interpretation, the results of this project are presented in three discrete Parts, as follows:

Part 1. Long term field trial results and discussion (Nuriootpa and Langhorne Creek sites).

Part 2. Cover crops and arbuscular mycorrhizal fungi inoculum potential (Nuriootpa and Langhorne Creek sites).

Part 3. Bio-fumigation trial (Tanunda site)

Part 4 (extension). Under-vine field trial results and discussion (Monash, SA Riverland).

This is then followed by overall conclusions and recommendations.

Departure from original application

Only minor departures from the original application occurred and are described below. All such departures were discussed with Wine Australia as they occurred;

The focus at the Tanunda site was to attempt a study on the effects of cover crops on nematode populations. During the first season, Nemcon establishment was patchy and poor, we believe likely due to residual herbicides in the soil, red-legged earth mites and hares. The other treatments established well, and we collected data as planned for those treatments (nematodes, soil properties, yields, vine etc, and mineral N and H_2O over the season). In the second season we attempted reestablishment of the Nemcon, but again this was unsuccessful. To help explain this we also attempted to establish Nemcon in an un-farmed soil from adjacent to the Tanunda site (i.e. where herbicides were not applied), and also at the Nuriootpa site, but these efforts were also unsuccessful. We tested the viability of the Nemcon seed in the glasshouse and it was fine, so the conclusion was that it is not a viable cover crop for these soils/systems. Nevertheless, in an attempt to answer the questions about impacts on Nemcon on nematodes (was it possible to establish it in the field) we grew Nemcon biomass in the glasshouse and incorporated it into the soil at the Tanunda site; samples were collected for nematode analysis. The other issue at the Tanunda site was that in Season 2 there were significant levels of die back in all treatments (minimal canopy and yields). We therefore measured a sub-set of key vine and soil properties (soil physicochemistry, cover crop botanical assessment, nematodes and basic harvest measures) to document this. We then redirected efforts and resources to the glasshouse trial above, and collection of additional data on temperature at the other sites, rather than more detailed

assessment (soil N and H_2O, and vine and berry properties, which could not be measured reliably) of the sick vines at the Tanunda site.

At the end of the 2020 season, as Covid-19 struck, we were not notified that pruning of our trial site at Langhorne Creek was to occur. As a result, we were unable to gather data on this for the second season. To attempt to compensate for this issue, we attempted to count cuts on the vine where canes were removed to get cane counts at least. This was completed, though due to the inaccuracy of this process, the data was not of use. Similarly, conducting additional tastings of wines made was not possible due to Covid-19; however, a complete set of wines have been retained.

Part 1 –Long term field trial

This section of the report describes the methods used, presents the results and discusses their implications for the long-term field trials based in Nuriootpa and Langhorne Creek. There were two main periods of data collection for these trials; October 2018 to March 2019 (vintage 2019 hereafter) and October 2019 to March 2020 (vintage 2020 hereafter).

Author participation

Dr Thomas Lines, Mr Chris Penfold, Dr Vinay Pagay, Dr Joseph Marks and Professor Timothy Cavagnaro

Methods – Nuriootpa and Langhorne Creek

Site locations, establishment and design

The field sites were located in Nuriootpa and Langhorne Creek. The sites were established as part of project UA1303. Full details of site establishment are presented in Penfold 2018.

Cover crop species were selected based on their growth habit (prostrate or upright), perennial or annually self-regenerating from their own seed bank, seed availability and cost and likely benefit to soil quality and vine productivity. All legumes were treated with the appropriate rhizobium inoculum immediately prior to sowing.

Sites were sown in autumn 2014 using a Taege® disc seeder, with one row sown within 20 cm of the vine trunk on each side of the vine row. The seed was covered by a chain followed by a press wheel. Seed produced by the sown cover crops was expected to germinate and fill the gap with plants in the following years.

Table 1: Seeding rates, plant species and cultivars used as under-vine cover crops according to site Nuriootpa (N) and Langhorne Creek (LC). Seeding occurred autumn 2014.

Code	Common name (cultivar)	Scientific name	Seeding rate (kg/ha)	Plant type	Site used
1	Straw mulch (Triticale)	*× Triticosecale*	50 t/ha	-	N, LC
2	Cocksfoot (cv. Kasbah)	*Dactylis glomerata*	8	Summer dormant perennial grass	N, LC
3	Wallaby grass	*Rytidosperma geniculata*	10	Native perennial grass	N, LC
4	Rats tail fescue (cv. Zorro)	*Vulpia myuros*	10	Annual self-regenerating grass	N, LC
5	Strand medic (cv. Angel); Barrel medic (cv. Sultan)	*Medicago littoralis* *Medicago truncatula*	20 20	Annual self-regenerating legumes	N, LC
6	Burr medic (cv. Cavalier); Button medic (cv. Bindaroo)	*Medicago polymorpha var brevispina* *Medicago orbicularis*	20 20	Annual self-regenerating legumes	N, LC
7	Spineless burr medic (cv. Scimitar); Annual ryegrass (cv. Safeguard)	*Medicago polymorpha* *Lolium rigidum*	20 30	Annual self-regenerating pasture legume and grass	N, LC
8	Strawberry clover Sheep fescue	*Trifolium fragiferum* *Festuca ovina*	20 3	Perennial legume and grass	N, LC
9	Gland clover (cv. Prima) Sub clover (cv. Mintaro)	*Trifolium glanduliferum* *Trifolium subterraneum ssp. brachycalycinum*	7 20	Annual self-regenerating pasture legumes	N was the only site sown with Prima
10	Herbicide control	*Glyphosate 540 g/L*	3 L/Ha	-	Added oxyflurofen (75ml/ha) when required

The field trials were established with a randomised complete block design. Each experimental treatment (Table 1) was replicated four times (i.e. four blocks). The field site layout for the Nuriootpa and Langhorne Creek trials is presented in Table 2 and Table 3 respectively. Cultivar grown at the Nuriootpa site was Shiraz, on its own roots. The cultivar grown at Langhorne Creek was Cabernet Sauvignon on its own roots.

Table 2: Nuriootpa treatment layout, established in 2014.

Row 2	Row 3	Row 4	Row 5	Row 6	Row 7	Row 8	Row 9
Block 1	Block 1	Block 2	Block 2	Block 3	Block 3	Block 4	Block 4
6	1	3	10	1	3	2	8
7	2	8	6	4	5	10	4
8	3	2	4	6	7	7	1
9	4	7	5	8	9	9	6
10	5	1	9	2	10	5	3

Table 3: Langhorne Creek treatment layout, established in 2014.

Block 1	Block 1	Block 1	Block 1	Block 1/2	Block 2	Block 2	Block 3	Block 3	Block 4	Block 4
Row 3	Row 4	Row 5	Row 6	Row 7	Row 8	Row 9	Row 10	Row 11	Row 12	Row 13
1	**3**	**5**	**7**	**10**	**7**	**4**	**10**	**3**	**8**	**2**
2	**4**	**6**	**8**	**3**	**9**	**6**	**6**	**8**	**4**	**10**
			9	**5**	**10**	**8**	**4**	**2**	**1**	**7**
					1	**2**	**5**	**7**	**6**	**9**
							9	**1**	**3**	**5**

Plant available nitrogen

Mineral N was measured in soils fortnightly from Oct 2018 to March 2019 (2019 vintage) and Oct 2019 to March 2020 (2020 vintage), as follows. Three cores to a depth of 10 cm were taken per plot and homogenised in a bag and returned to the laboratory for further processing and analysis. Approximately five grams of this soil was weighed and mixed with 25 mL of 2 M potassium chloride (KCl) for extraction of plant available N (nitrate and ammonium). Extractions were shaken at 150 rpm for 60 minutes before being centrifuged at 3000 g for five minutes and samples of the supernatant removed for freezing. A larger sample of the same soil (approx. 20 g) was weighed and dried at 105 ^{o}C for 48 hrs to determine gravimetric water content of the soil. Concentrations of NO_3^- (nitrate) and NH_4^+ (ammonium) were quantified colorimetrically as described by Miranda et al. (2001) and Forster (1995) respectively.

Soil physicochemistry

Soil collection for analysis was carried out in early October of both 2018 and 2019. At each plot three cores to 10 cm were taken and homogenised. These samples were air dried, sub

sampled and delivered to Australian Precision Agriculture Laboratory (APAL) for analysis (https://www.apal.com.au/SoilTesting.aspx).

The soil analytes quantified are presented in Table 4;

Table 4: Soil physicochemical properties quantified by APAL.

pH 1:5 water (pH units)	pH $CaCl_2$ (pH units)	Colwell Phosphorus (mg/kg)	Ca:Mg Ratio
K:Mg Ratio	ECR	CEC (cmol/kg)	Calcium (%)
Magnesium (%)	Potassium (%)	Sodium (%)	Salinity EC 1:5 (dS/m)
Iron (Fe) (mg/kg)	Manganese (Mn) (mg/kg)	Copper (Cu) (mg/kg)	Zinc (Zn) (mg/kg)
Dumas Total Nitrogen (%)	Dumas Total Carbon (%)		

Leaf blade analysis

In early December of both 2018 and 2019, two leaf blades were taken from three vines per plot. Petioles were removed and the leaves dried in an over at 40 degrees for at least 24 hours. Samples were then cooled in sealed bags containing hydroscopic silica gel before being transported to Australian Precision Ag Laboratory (APAL) for analysis (https://www.apal.com.au/PlantTesting.aspx)

The elements quantified in the leaf tissue analysis appear in Table 5;

Table 5: Leaf blade elemental nutrients quantified by APAL.

Aluminium (mg/kg)	Boron (mg/kg)	Calcium (%)
Chloride (%)	Copper (mg/kg)	Cobalt (mg/kg)
Iron (mg/kg)	Magnesium (%)	Manganese (mg/kg)
Molybdenum (mg/kg)	Nitrogen (%)	Phosphorous (%)
Potassium (%)	Sodium (%)	Sulphur (%)
Zinc (mg/kg)		

Stem water potential

Pressure bombing was used to measure water stress at each site three times (inflorescence, veraison and pre-harvest) throughout the 2019 and 2020 vintages. To two vines per plot, two leaves per vine were bagged with a non-light transmissible bag. These bags were left on for at least one hour before sampling. Measurements were taken within 1.5 hours of solar noon, on days when no significant cloud was present to reduce the photoactive radiation (PAR).

Leaf area index

In mid-December and late January, leaf area index (LAI) and T (ratio of transmitted to incident PAR) were measured to quantify differences in canopy size. The AccuPar ceptometer was used for the majority of readings, however if this piece of equipment was unavailable the viti-canopy app (De Bei et al. 2016).

Worm counts

In July 2020, 15 kg of soil was taken from each plot at Nuriootpa and Langhorne Creek trial sites. This soil was sifted by hand, worms collected, counted and weighed.

Cumulative temperature

Temperature loggers (96) were deployed to the Nuriootpa trial site in January 2020, before they were redeployed at Langhorne Creek shortly after (March, 2020). Loggers were placed inside plastic sun shades and affixed to the cordon, mid-trunk and base of two vines. Treatments measured were the herbicide control, straw mulch, Medic and Ryegrass and Fescue treatments.

Microbiomes

In July of 2018 and 2019, soil samples were taken and submitted for NextGen sequencing of the 16s Microbiome at Australian Genome Research Facility (AGRF). Soils were sampled and frozen (− 4°C) prior to DNA extraction and Illumina sequencing AGRF conducted DNA extractions, PCR amplification and sequencing on whole soil samples. In brief, DNA was extracted from 0.250 g of soil using a PowerSoil DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA, USA). Ribosomal RNA (16S) was analysed for bacterial communities using the forward and reverse primers 27F-519R (AGAGTTTG ATCMTGGCTCAG and GWATTACCGCGGCKGCTG; 16S). The following PCR conditions were used: an initial denaturation at 95 °C for 7 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 45 s and 72 °C for 60 s. A final extension was carried out at 72 °C for 7 min. Image analysis was performed in real time using MiSeq Control Softwarev2.6.2.1 and Real-Time Analysis v1.18.54. Sequence data were generated with the Illumina bcl2fastq 2.20.0.422 pipeline. Analysis of the sequence data was carried out using Qiime2 (version 2019.1). Analysis of the soil 16S microbiome revealed a total of 9,000 original taxonomic units (OTUs) in soil from July 2018, and 18,463 OTUs in soil from July 2019.

Soil penetration resistance

Penetration resistance was measured using a Rimik CP4011 cone penetrometer (Rimik Pty. Ltd., Toowoomba, Queensland) on 24-7-2019 when the soil moisture profile was at field capacity. Within each plot, 3 insertions to 600 mm depth were made, from which a mean resistance value was derived.

Pruning weights

At the end of the 2019 and 2020 seasons, after all leaves had dropped, pruning weights were taken at each site. Two vines per treatment were pruned and canes were counted and weighed. After the 2020 season, pruning weights were collected from Nuriootpa, however they were unable to be collected at Langhorne Creek, as the pruning contractor had carried out their work without our notification.

Soil moisture profiles

Soil moisture profiles were taken fortnightly throughout the 2019 and 2020 vintage seasons using the odyssey capacitance probe (see Figure 1) at 200 mm intervals down to 1000 mm. Capacitance probes work by measuring the di-electric constant of the soil, which changes with water content. To enable this, one access tube was installed within 300 mm of a vine and dripper in each plot.



Figure 1: Example of a capacitance probe inserted into access tube to a depth of one metre.

Harvest data collection

At the end of the 2019 and 2020 vintages, the research team was informed by the vineyard manager when harvest was taking place. Two to three vines per plot were selected and harvested. Bunches were counted and total yield per vine was weighed. Sub-samples of the fruit were collected and taken to the lab for individual bunch weights and berry weights. Three to four bunches were weighed individually, and subsequently 100 berries (25 per bunch) were weighed to determine average berry size.

Juice chemistry analysis

Sub-samples of grapes were crushed and used to measure the standard juice chemistry in the 2019 vintage which included pH, brix and titratable acid. A more thorough juice analysis was carried out during the second season. Berry samples for the 2020 vintage were submitted to the Australian Wine Research Institute (AWRI) for red wine analysis. The following metrics were quantified;

Table 6: Red wine metrics quantified by the AWRI in 2020 using grapes from both Nuriootpa and Langhorne Cree.

Alpha Amino Nitrogen (mg/L)	Titratable acid pH 7.0 (g/L)
Ammonia (mg/L)	Titratable acid pH 8.2 (g/L)
Brix (°Brix)	Total phenolics (a.u./g)
Free Anthocyanins (mg/g)	Total tannin (mg/g)
pH	Yeast assimilable nitrogen (mg/L)

Botanical analysis

Cover crop composition and dry weight was estimated twice per year using the Botanal methodology (Tothill et al. 1992). Briefly, a standard curve was generated by selecting sites within the vineyard of biomass ranging from 1-10 out of 10 for biomass. Quadrats were then cut and bagged for dry mass determination. The site was then walked by two operators with quadrats, estimating the biomass score (1-10) three times per plot. Species composition and bare earth percentage was noted. A standard curve generated using the dry masses of the samples and their relative score was used to generate a linear model, from which dry masses of individual species could be interpolated.

Winemaking

Small lot winemaking took place using grapes from the 2019 vintage using fruit from both Nuriootpa (shiraz) and Langhorne Creek (Cabernet Sauvignon). The protocol used by the AWRI-WIC for small lot wine making (pers comm) was followed over a number of months. Unfortunately, due to the extended and extremely hot ripening period, the Nuriootpa wine was deemed to be faulted and was not bottled.

Two replicates from each treatment were created by pooling grapes from two of the four blocks. Remnants from these replicates were then used to create a third ferment where the 'wild' endogenous yeast was allowed to colonise the vessel and carry out the primary fermentation. Thus, a total of 21 wines were produced and bottled and after a number of months. These wines were submitted to the National Amateur Wine Awards for a sensory analysis. Two wine makers at the AWRI also provided tasting notes on the 21 wines produced.

Data analysis

Prior to significance testing, each treatment variable was tested for normality and homoscedasticity using the Wilkes-Shapiro test. A linear mixed model was fitted using the R

package ASREML using *Treatment* as a fixed effect, and *Block* as random effects in the analysis. *Block* was included in our analysis to account for any underlying spatial heterogeneity of the vineyard sites. The significance of fixed effects (at $p< 0.05$) in all models was determined using approximate F-tests. All statistical analyses were conducted using R open source statistical software (version 3.6.2 in R-Studio version 1.2.1335) with additional agricultural data packages; "Biometrytraining" (Nielsen and Rogers, 2020), "dplyr", "agricolae" and "ggplot2".

Results

Data are presented below in a number of formats. Box plots, such as that in Figure 4 below represent a visual summary of the data. The median of the data is represented by the central horizontal line, while the boxes either side represent the upper and lower quartiles. The whiskers either side of the boxes represent the maximum and minimum values of the data. Any outliers appear as individual points. These figures also contain letters that indicate whether differences between the treatments were statistically significant; that is, two means followed by the same letter are not significantly different at the $p<0.05$ level. Other figures, such as that in Figure 2 present the data points, as well as a predicted mean (continuous line) and confidence intervals (grey area).

Due to the sheer quantity of data collected, we have focused this report on significant or noteworthy results. The majority of raw data is available in tables in Appendix 5. Further data on soil moisture profiles or mineral N availability can be provided upon request. All code used to perform graphical and statistical analysis is also available upon request.

A complete data set will be provided Wine Australia

Results - Nuriootpa

Soil Moisture Profile

Here data is presented for three time points for each treatment. In the 2019 vintage, at the first time point in October 2018 (Figure 2), moisture was depleted in the soil surface layers, though close to maximum recorded capacity (100%) at a depth of one metre. In early January of the same season, soil moisture from 40 cm to 80 cm has reduced for all treatments. Statistical analysis did not detect any significant effects among treatments.

In the 2020 season, trends similar to those of the previous season were observed. In late January 2020 a high rainfall event lead to a refill of soil moisture at shallower depths (0-40 cm). However, the soil in the 80-100 cm depth was still depleted of moisture compared to bud-burst at this time. Again, no clear treatment effects were observed. While soil moisture dynamics over depth and time are complex, there were no obvious negative impacts of under-vine cover crops compared to the herbicide or mulch treatments.

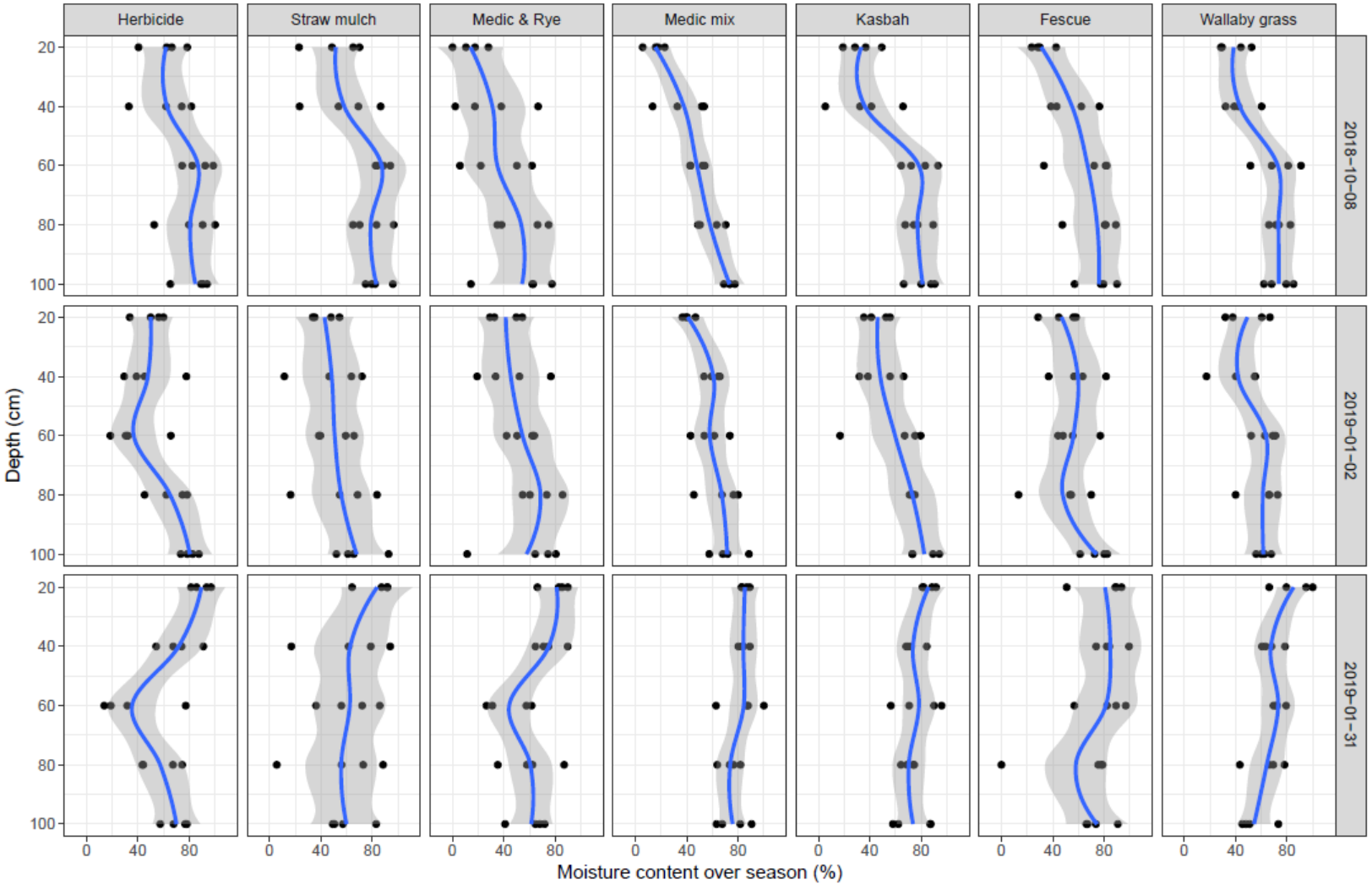


Figure 2 - Soil moisture profile, Nuriootpa, Vintage 2019

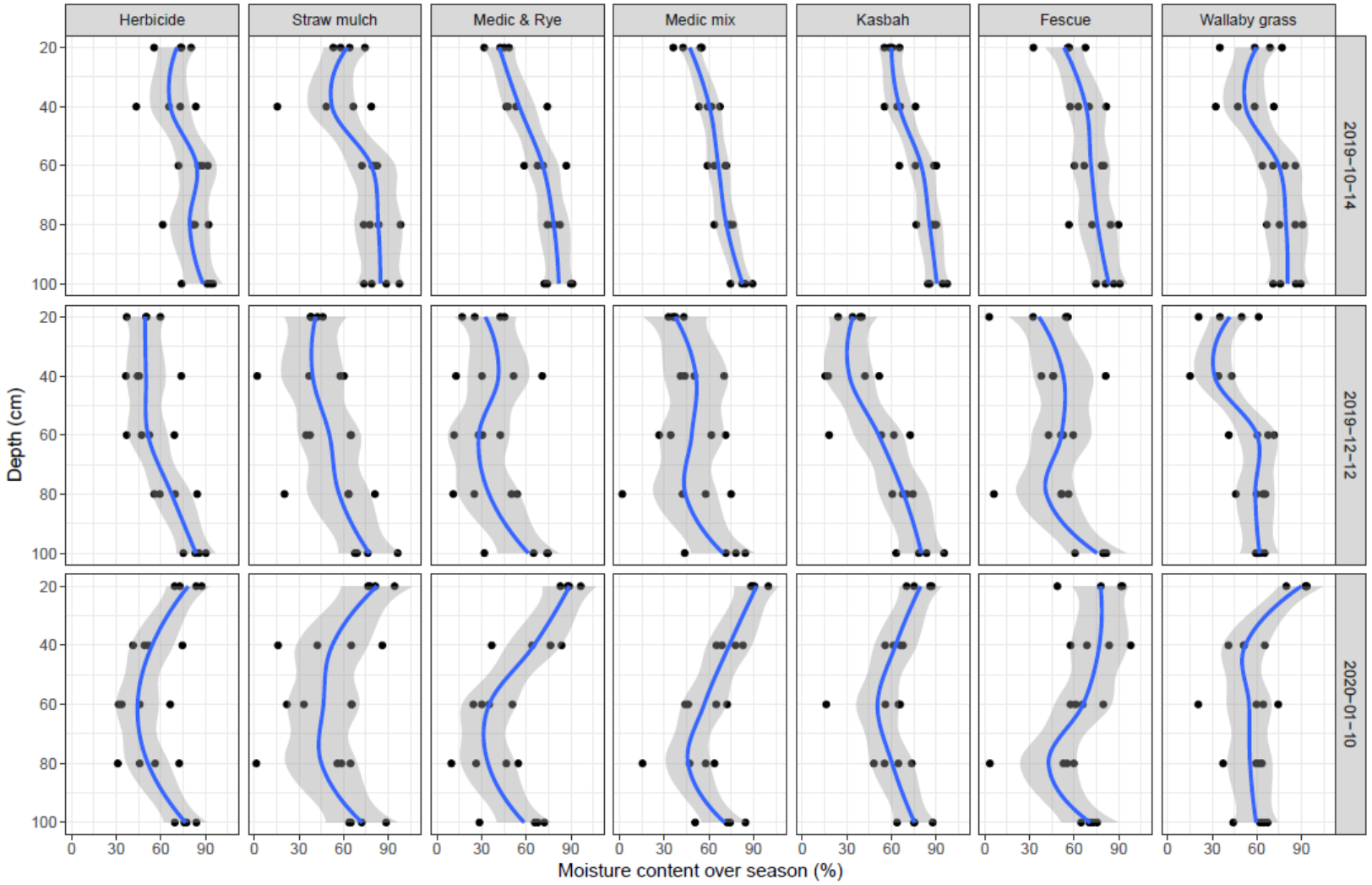


Figure 3 - Soil moisture profile, Nuriootpa, Vintage 2020

Soil physicochemical properties

The $pH_{(1:5\ soil:\ water)}$ of soils was close to neutral in all treatments. There were, however, some differences in soil pH among the experimental treatments. In particular, soil pH was significantly lower in the Medic treatment than the Kasbah treatment in 2019 (Figure 4), and most other treatments in 2020 (Figure 5). Generally, when compared to the herbicide control, the Medic mix reduced soil pH, and the grasses tended to increase it.

Standards suggest EC readings for salinity, measured as $EC_{(1:5\ soil:\ water)}$, should be below 0.24 dS/m (AWRI 2010, 2014) which is reached on the herbicide treatment in the dry 2020 year, when leaching of salts would have been restricted, and the bore water applied was known to carry excessive salts. Salinity was significantly higher in the herbicide control treatment than in all other treatments (Figure 6 and Figure 7). There was a strong correlation between soil sodium (Na) (see below) and salinity, suggesting that Na was the principal driver of the differences in soil salinity seen in this system.

Soil C concentrations at Nuriootpa ranged from 1.1 % to 1.7 %, which is within the typical range for vineyards in the region ("Barossa Valley - Grape and Wine Soil Quality" 2020). The concentration of carbon in the soil was lowest (albeit not significantly so) in the herbicide treatment in 2019 (Figure 8). However, in the 2020 samples, soil C was significantly lower in the herbicide treatment than the Medic Ryegrass mix, and marginally lower than all other treatments (Figure 9). That is, all sources of cover have generated higher concentration C in the soil when compared to the herbicide control, but the combination of Medic and Ryegrass has increased soil carbon by approximately 50%. It should be noted that this is a measure of C%, not stocks, which is the subject of a parallel project (PPA002643).

Soil N concentrations ranged between 0.1-0.2 % in 2019 (Figure 10) and 0.2-0.3 % in 2020 (Figure 11). No significant differences were observed between treatments.

Plant-available (Colwell) P was significantly lower for the Medic and Ryegrass and Medic mix treatments across both 2019 (Figure 12) and 2020 (Figure 13) vintages. The highest available P was in soils from both the herbicide control and Wallaby Grass treatment in 2020. Conversely, total N did not present any significant effects across 2019 or 2020 seasons, despite the presence of N fixing cover crops.

Some interesting patterns in soil cations were observed in this trial. Soil calcium (Ca) was significantly higher in the wallaby grass than the Medic mix in 2019 (Figure 14), with a similar trend remaining in 2020 (Figure 15). On the other hand, manganese was higher in the treatments where legumes were grown under-vine (Figure 16 and Figure 17). This finding was also consistent over the two years of sampling, likely due to reduce soil pH (see above). Soil potassium (K) was significantly higher in the straw mulch treatment than the Medic mixture in 2019 (Figure 18), as well as in 2020 (Figure 19). Soil K levels were significantly higher for the straw mulch treatment than the Medic mix in 2019 (Figure 18) and all other treatments in 2020 (Figure 19). Soil Na in 2019 (Figure 20) and 2020 (Figure 21) was significantly higher on the herbicide than other treatments. This is consistent with the salinity measurements (see above).

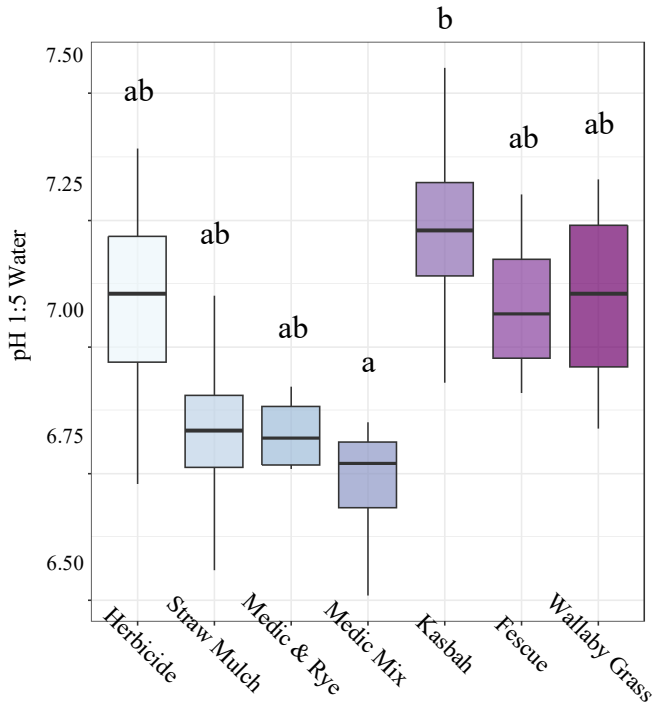


Figure 4 - Soil pH 1:5 Water, Nuriootpa, Vintage 2019

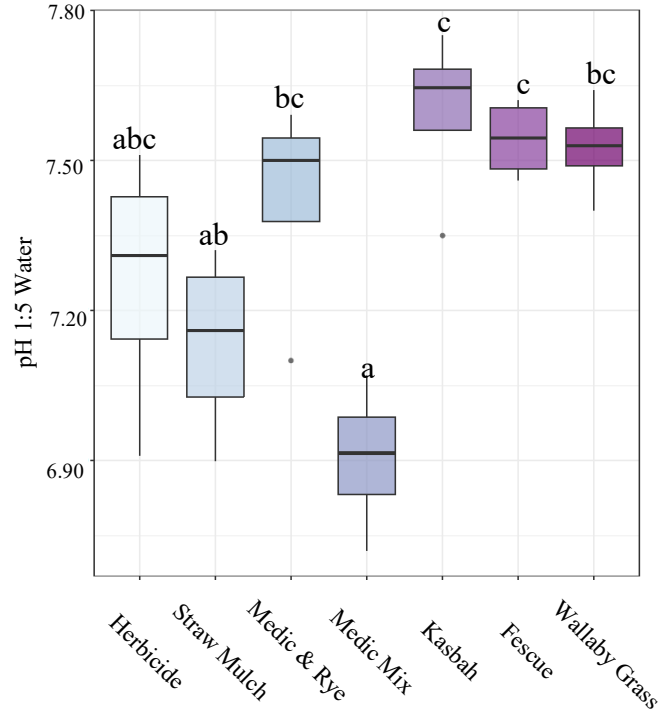


Figure 5 - Soil pH 1:5 Water, Nuriootpa, Vintage 2020

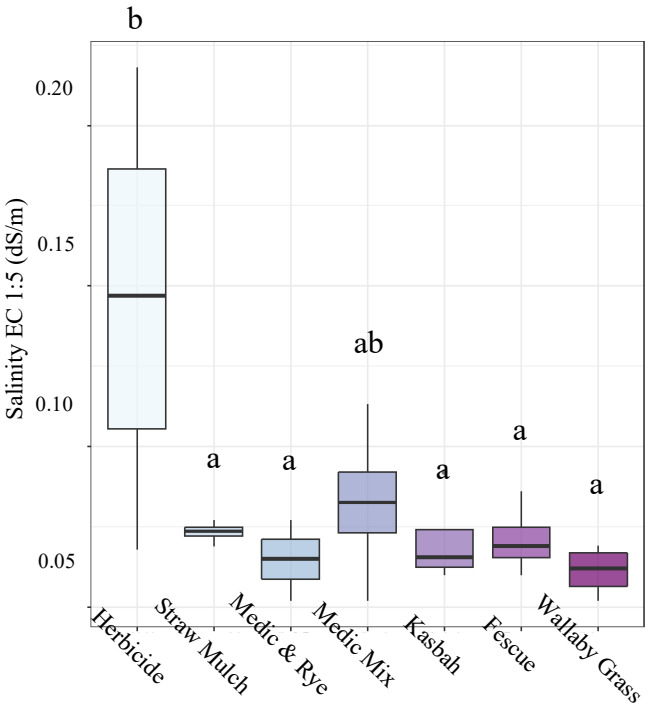


Figure 6 - Soil Salinity, Nuriootpa, Vintage 2019

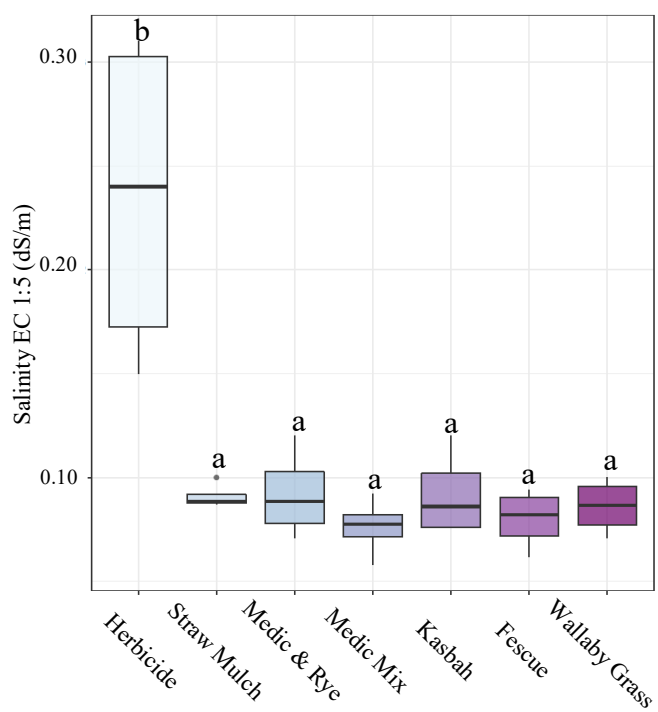


Figure 7 - Soil Salinity, Nuriootpa, Vintage 2020

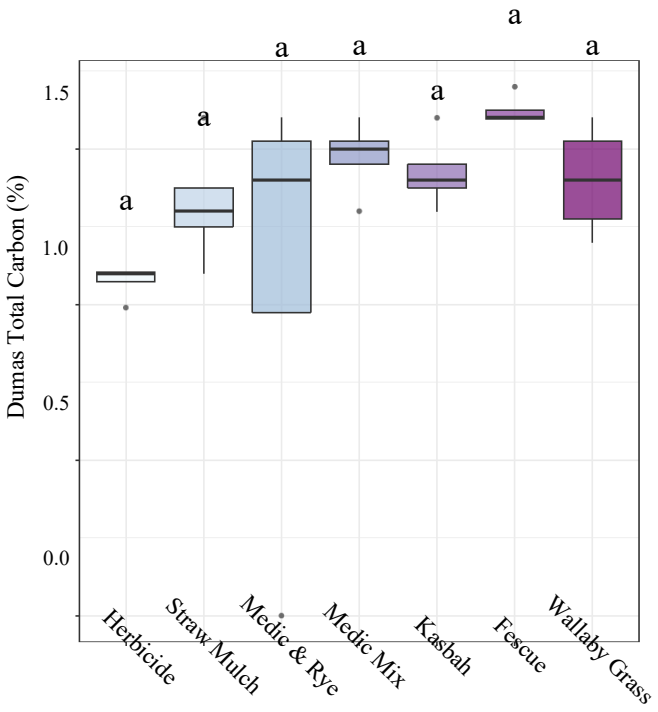


Figure 8 - Soil total carbon, Nuriootpa, Vintage 2019

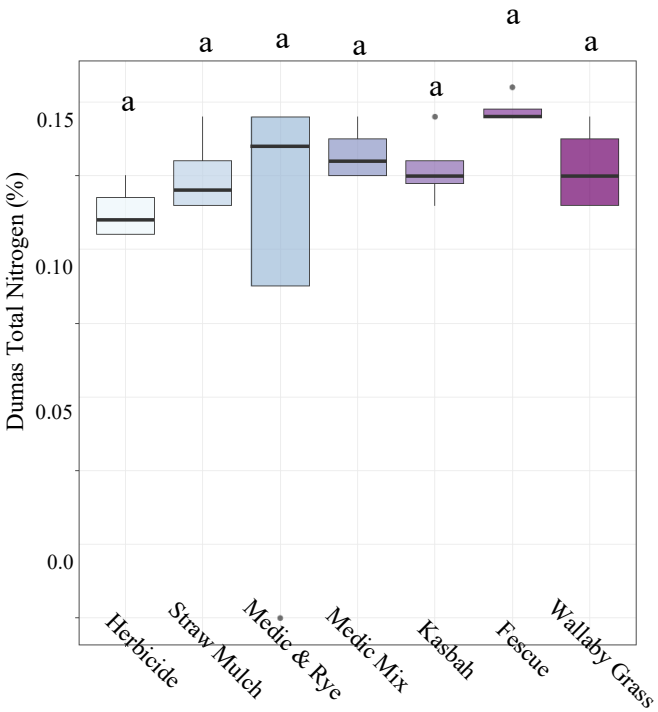


Figure 10 - Soil total nitrogen, Nuriootpa, Vintage 2019

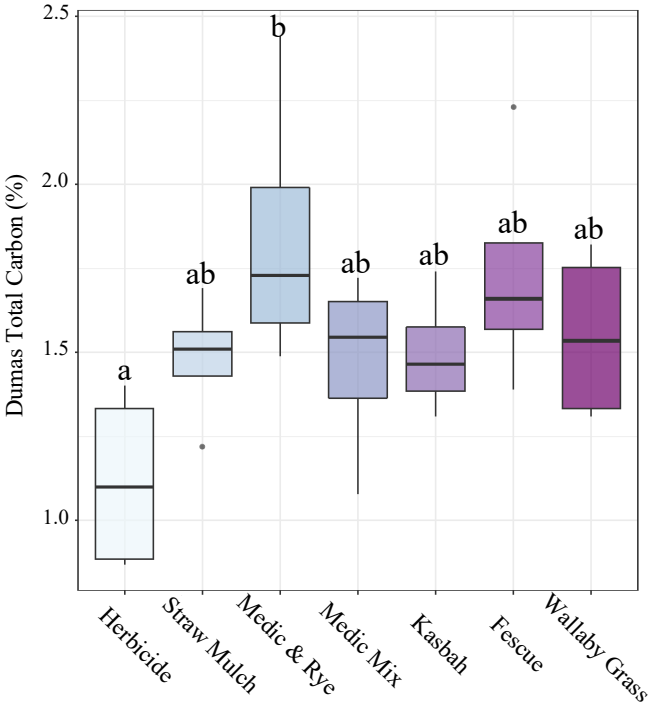


Figure 9 - Soil total carbon, Nuriootpa, Vintage 2020

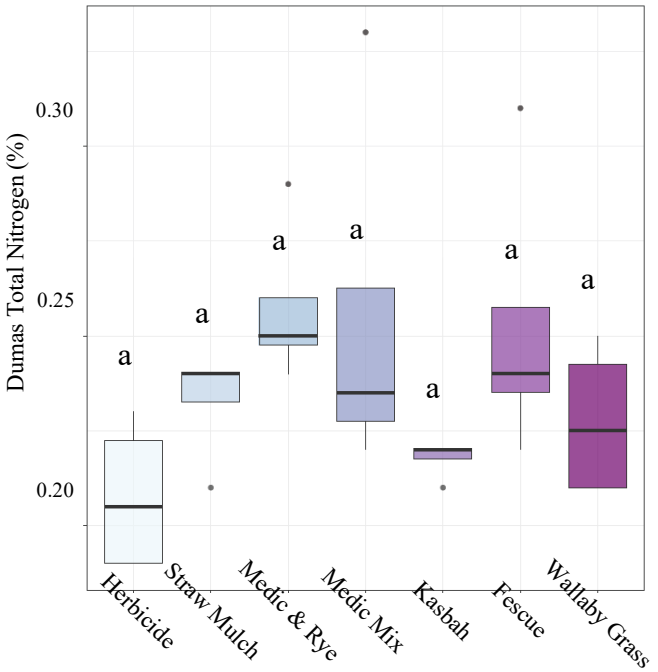


Figure 11 - Soil total nitrogen, Nuriootpa, Vintage 2020

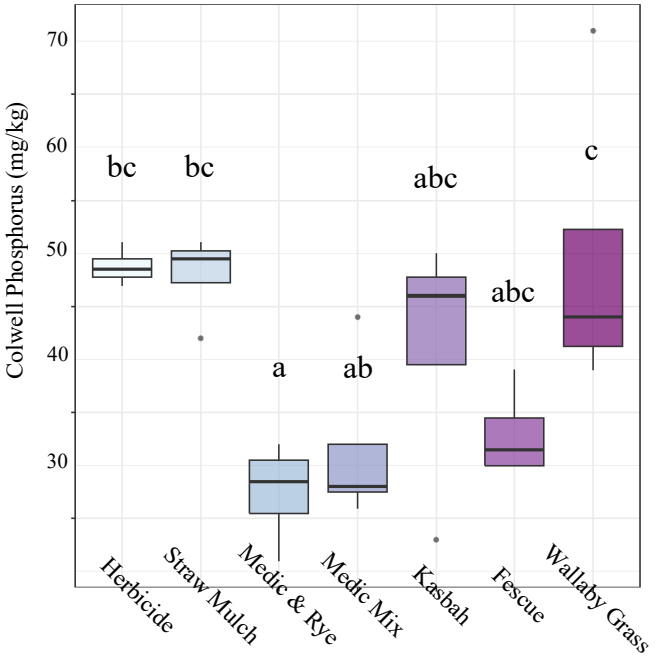


Figure 12 - Soil phosphorus, Nuriootpa, Vintage 2019

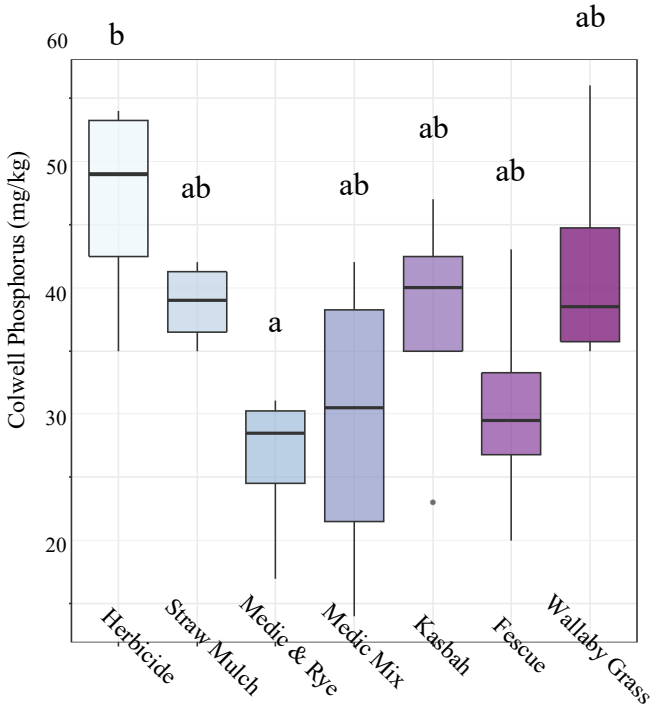


Figure 13 - Soil phosphorus, Nuriootpa, Vintage 2020

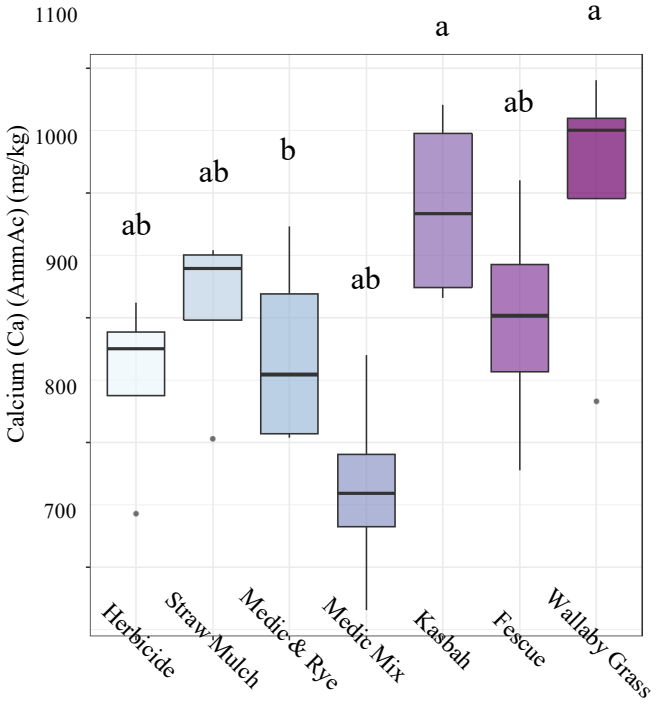


Figure 14 - Soil calcium, Nuriootpa, Vintage 2019

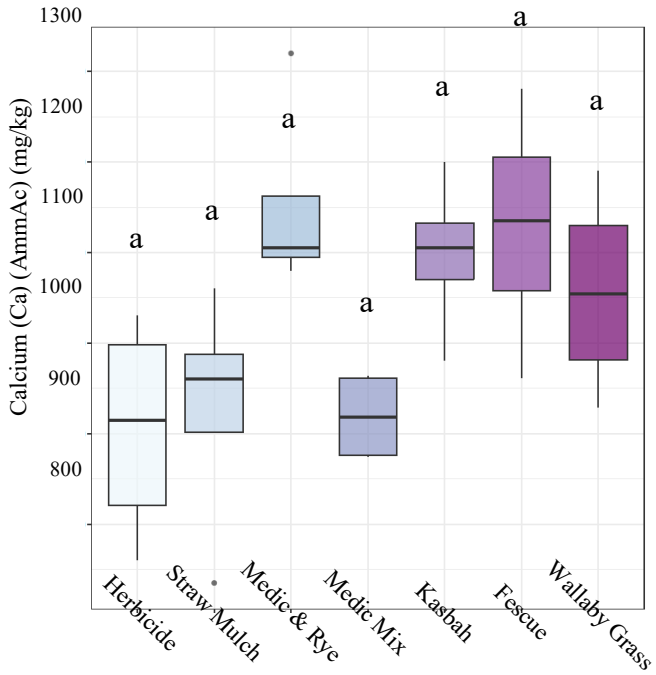


Figure 15 - Soil calcium, Nuriootpa, Vintage 2020

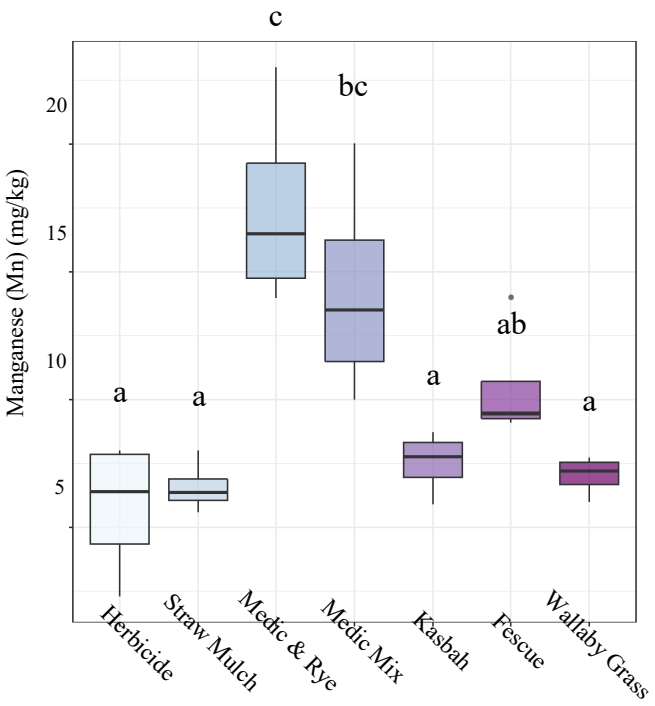


Figure 16 - Soil manganese, Nuriootpa, Vintage 2019

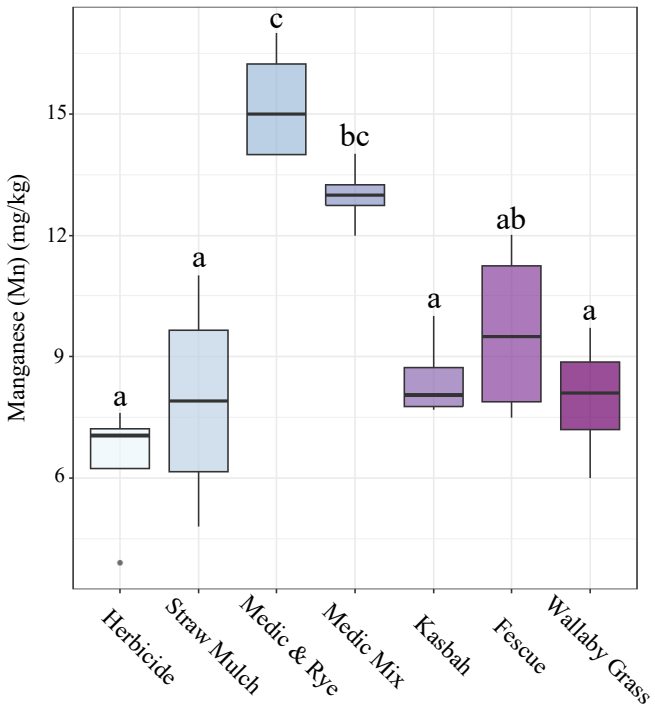


Figure 17 - Soil manganese, Nuriootpa, Vintage 2020

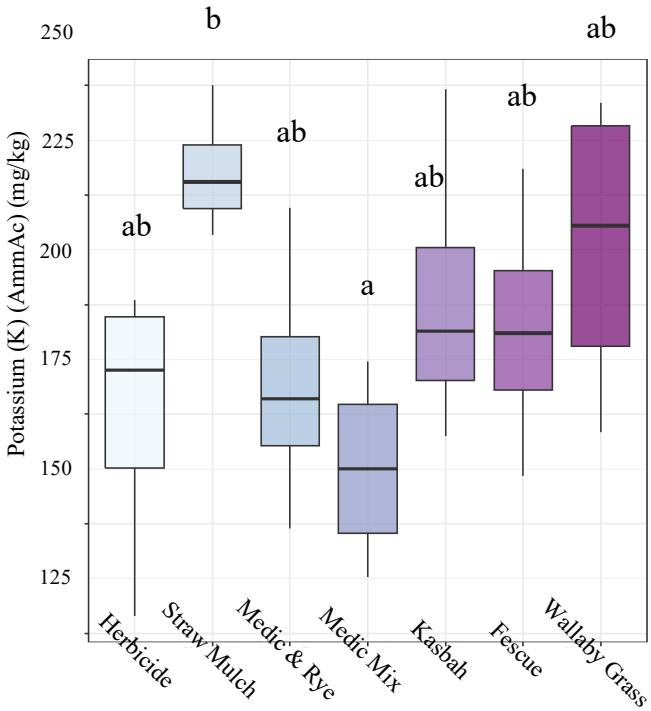


Figure 18 - Soil potassium, Nuriootpa, Vintage 2019

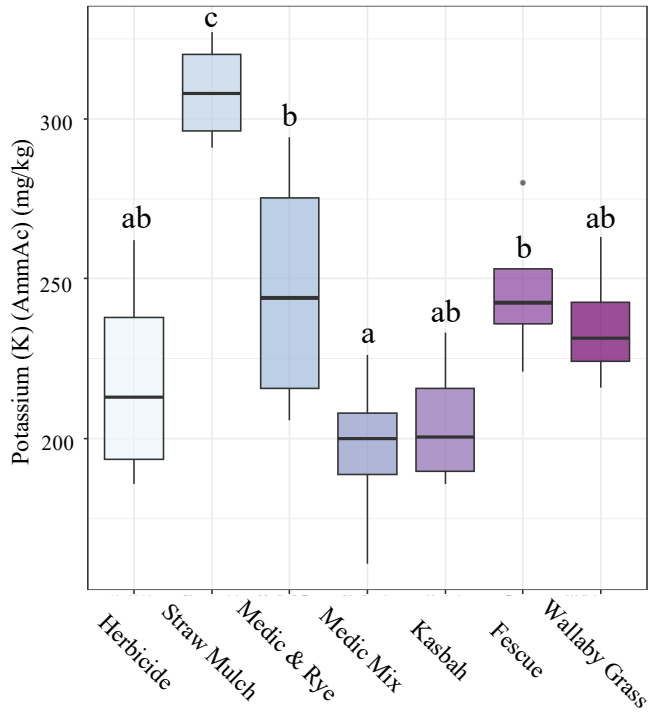


Figure 19 - Soil potassium, Nuriootpa, Vintage 2020

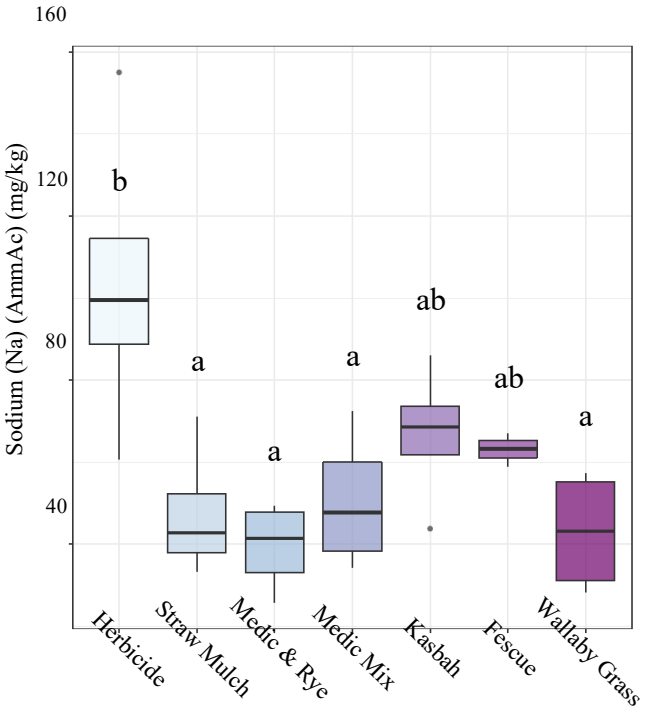


Figure 20 - Soil sodium, Nuriootpa, Vintage 2019

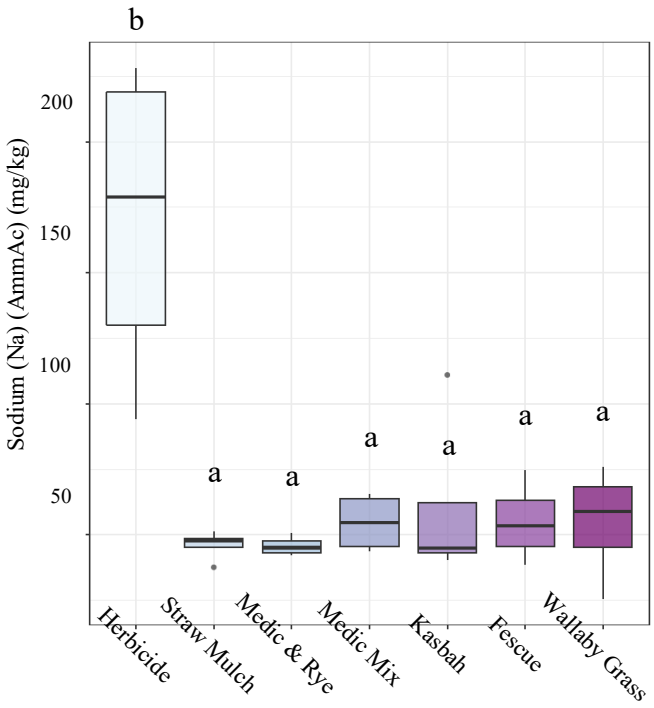


Figure 21 - Soil sodium, Nuriootpa, Vintage 2020

Soil mineral nitrogen

Nitrogen is recognised as the nutrient with the greatest influence on vine growth. It is also the nutrient that most often limits plant production globally. Soil NH_4^+ levels were generally low (< 10 mg/kg = ppm), likely due to continued plant uptake over the course of the growing season. There were also peaks in soil NH_4^+ over the growing season. Soil NO_3^- levels were generally less than 20 mg/kg, except when fertiliser was applied, or in the Medic treatments towards the end of the season as organic N was mineralised. N fertiliser was applied prior to budburst (5th Sep 2018), following flowering (30th Nov 2018) and post-harvest (10th Apr 2019) (see table in Appendix 5), and these fertigation events are noted by increased soil NH_4^+ levels at the first two events.



Figure 22: Soil ammonium at Nuriootpa across the 2019 vintage.



Figure 23: Soil mineral nitrate at Nuriootpa across the 2019 vintage.

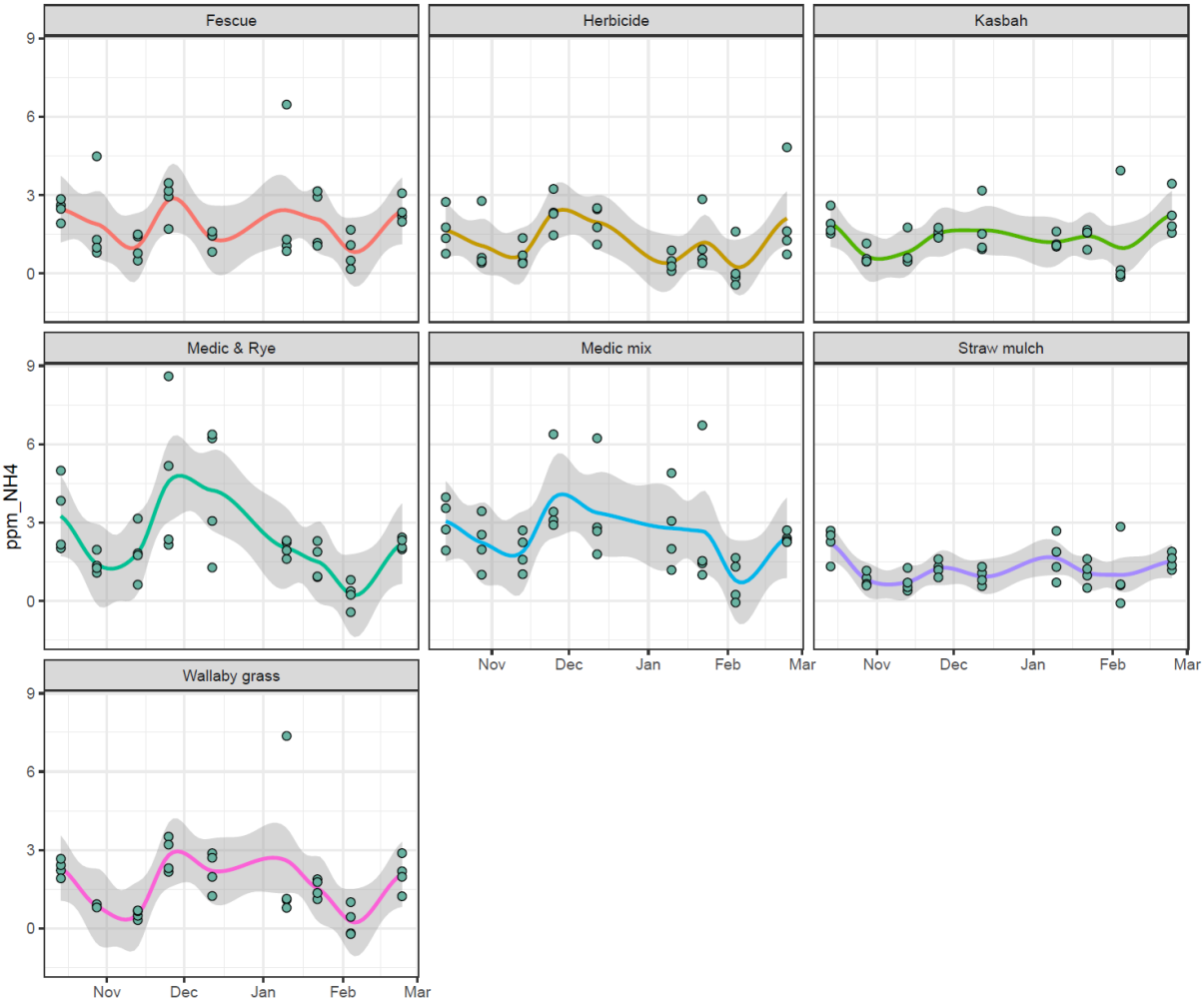


Figure 24: Soil mineral ammonium at Nuriootpa across the 2020 vintage.

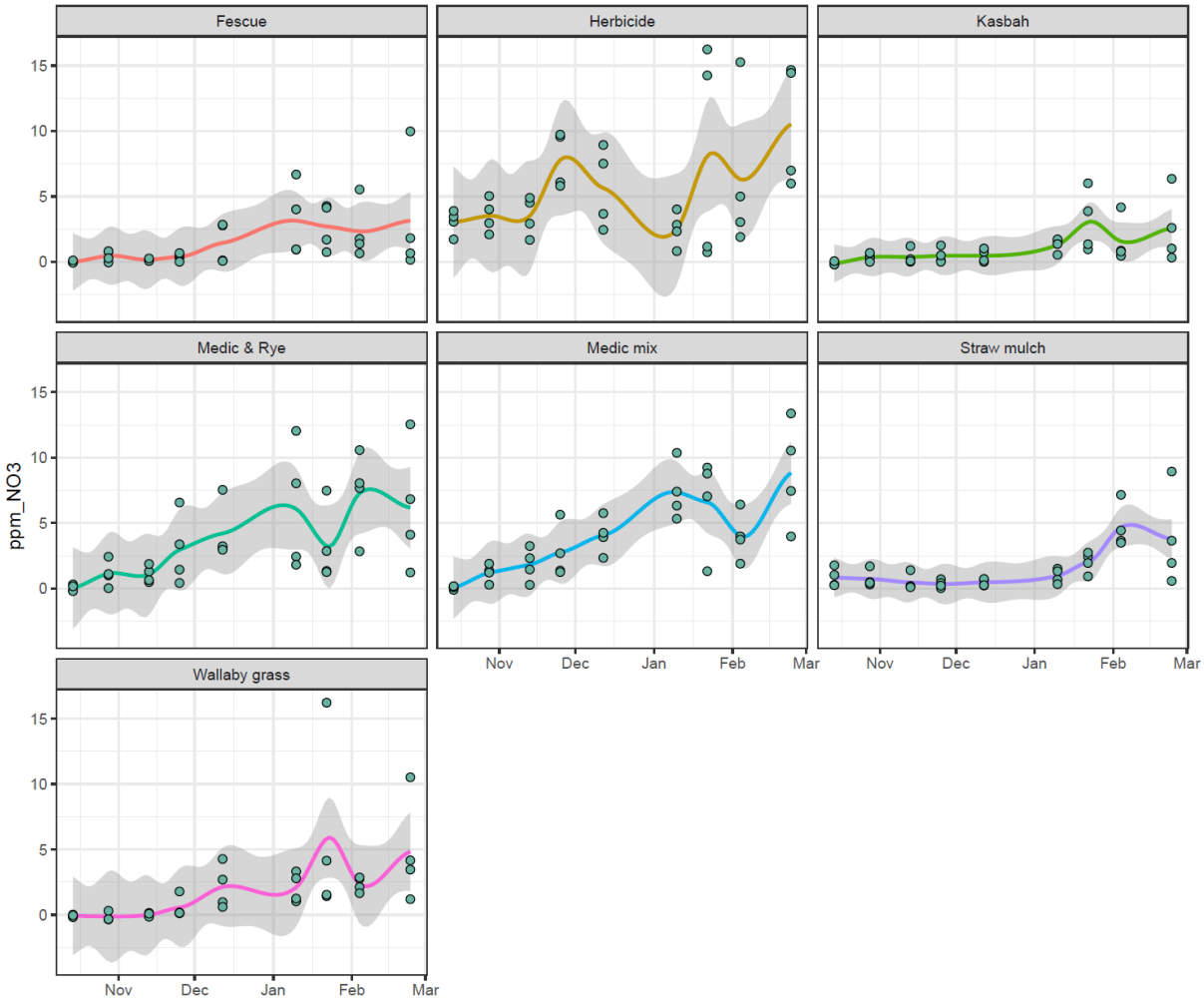


Figure 25: Soil mineral nitrate at Nuriootpa across the 2020 vintage.

Leaf blade analysis

In 2019, only the treatments of initial focus (herbicide, straw mulch, Medic and Ryegrass, Medic mix and Kasbah) were sent for leaf blade analysis. In light of some interesting results, it was decided to send samples for all treatments in 2020 for leaf blade analysis.

Leaf N levels of between 3-5 % are recognized as being desirable (Weir & Cresswell 1993). The competitive and perennial Kasbah treatment lead to significantly low leaf N in the vine relative to most other treatments in 2019 (Figure 32). Surprisingly leaf N was highest in the herbicide control in 2020 (Figure 33). This is inconsistent with the absence of treatment effects in total soil N (see above), nor available mineral N, where the only differences were elevated nitrates at the end of the season in the Medic treatments. Yeast available nitrogen (see below) was similarly independent of leaf N, and more correlated with the mineralised N results.

The B levels in leaf blades were within the acceptable range from 30 – 100 ppm (Figure 26 and Figure 27). Significantly higher boron in the Medic and Ryegrass treatment than the straw mulch treatment; this may be in part due to the reduced soil pH in the legume treatment (see above).

Cl is recognized as an essential element to plant growth, but can also be toxic at excessive levels and in conjunction with Na. The leaf Cl levels measured in 2020 vintage (Figure 29) were in the acceptable range of 0.1 - 0.8%, and there were no significant differences among treatments. A significant difference in leaf Cl levels in 2019 (Figure 28), with Medic treatments showing higher levels than the Kasbah, herbicide and mulch treatments, may be attributed to the mobile Cl infiltrating further into the soil profile and then being accessed by the vine roots.

Leaf manganese was significantly higher in the straw mulch treatment than the Kasbah treatment in the 2019 vintage (Figure 30). The same pattern was also observed in the next season (Figure 31) with Wallaby grass having the lowest leaf manganese concentrations. This was inconsistent with soil manganese levels (see above).

Leaf phosphorous (Figure 34 and Figure 35) is in concert with soil P, being lower on the Medic treatments, and according to established standards (0.25-0.4%) close to deficient on all treatments in 2020 (Figure 35).

The higher soil K levels of the straw treatment were reflected in the leaf blade as well. This was particularly the case for vintage 2019 (Figure 36), and less so for vintage 2020 (Figure 37). The levels noted were within the desirable range (Weir & Cresswell 1993) for this element.

Acceptable Na levels normally range from 0.05 to 0.1 % (Weir & Cresswell 1993), with 0.4% being above normal, so the level found across these treatments in the 2020 vintage is higher than desirable (Figure 39), though not in 2019 (Figure 38), possibly due to the higher quantity of winter rains in 2018.

Sulphur content in the leaves is higher for the straw treatment in both 2019 (Figure 40) and 2020 (Figure 41) vintages. Though this was not tested in the soil, it is most likely that high sulphur in the straw was the cause.

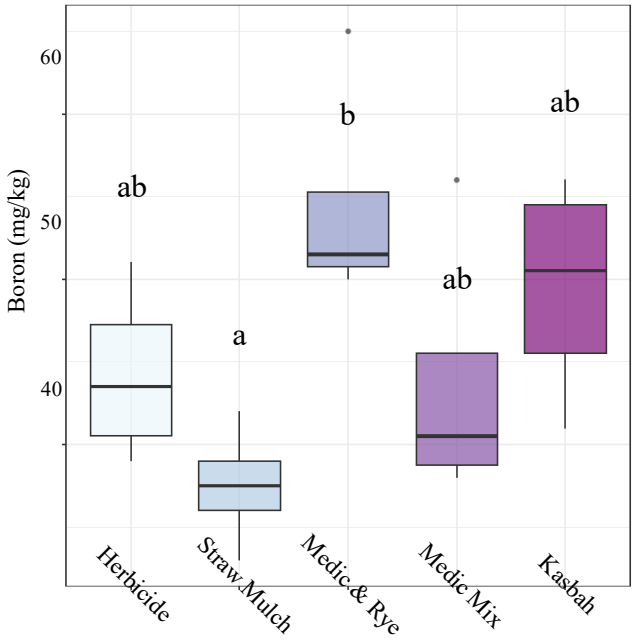


Figure 26 - Leaf boron, Nuriootpa, Vintage 2019

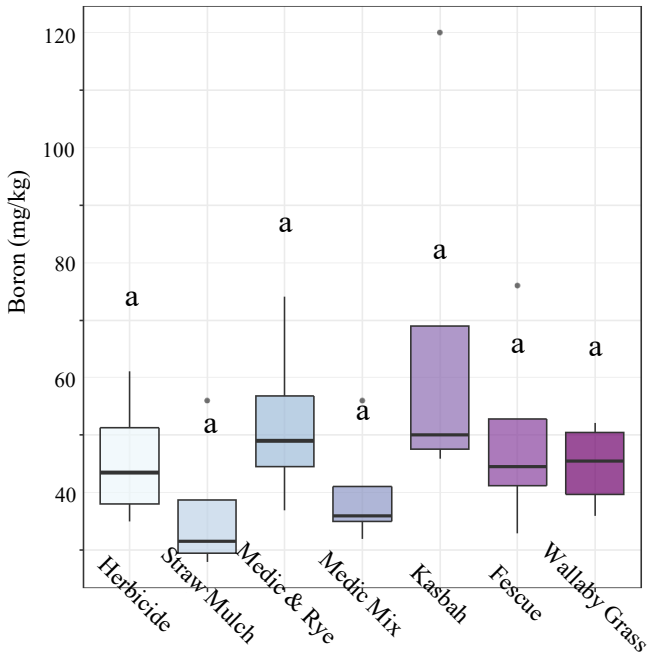


Figure 27 - Leaf boron, Nuriootpa, Vintage 2020

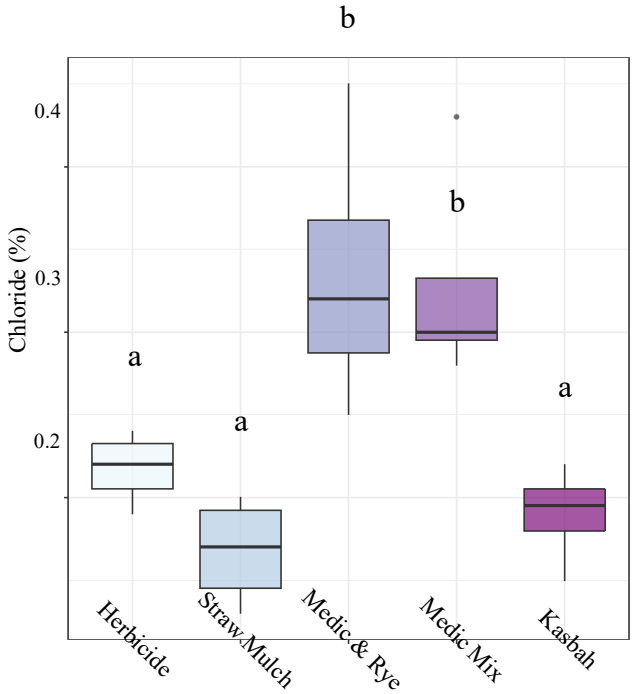


Figure 28 - Leaf chloride, Nuriootpa, Vintage 2019

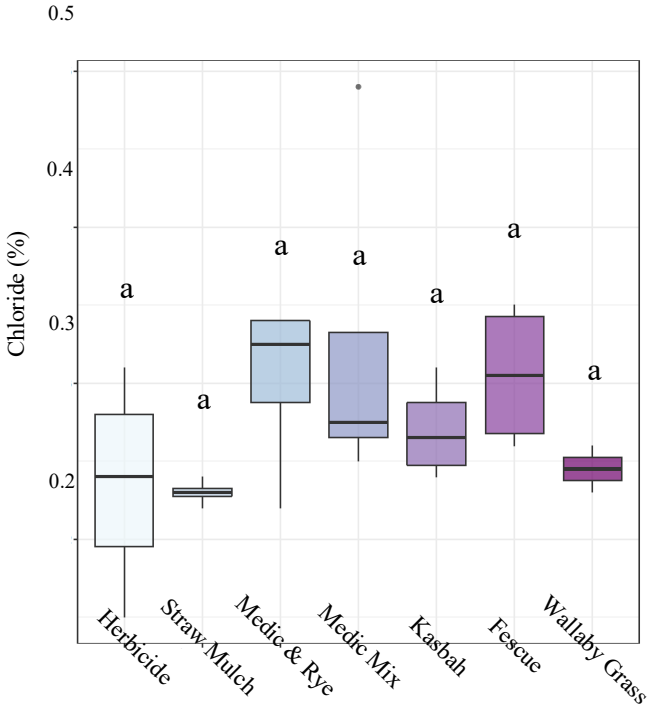


Figure 29 - Leaf chloride, Nuriootpa, Vintage 2020

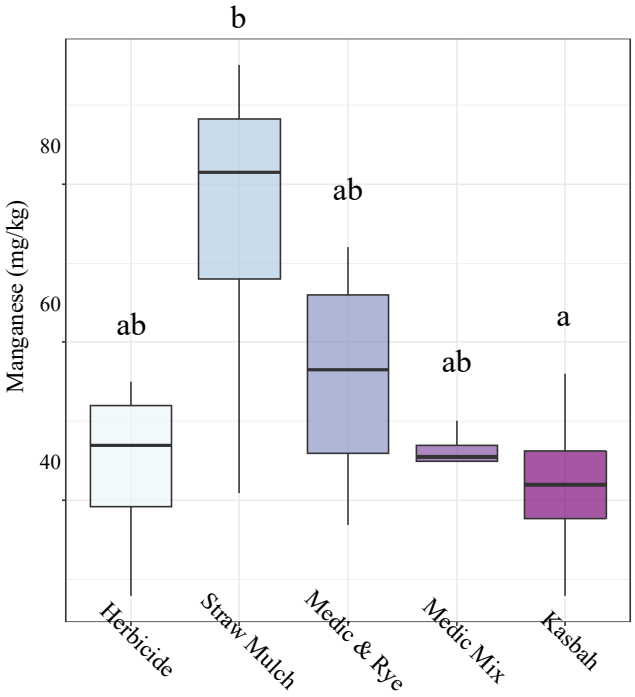


Figure 30 - Leaf manganese, Nuriootpa, Vintage 2019

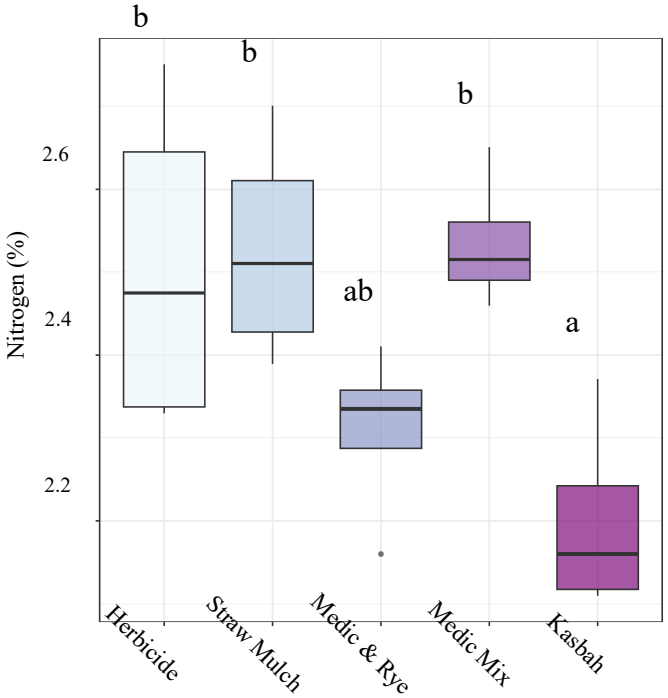


Figure 32 - Leaf nitrogen, Nuriootpa, Vintage 2019

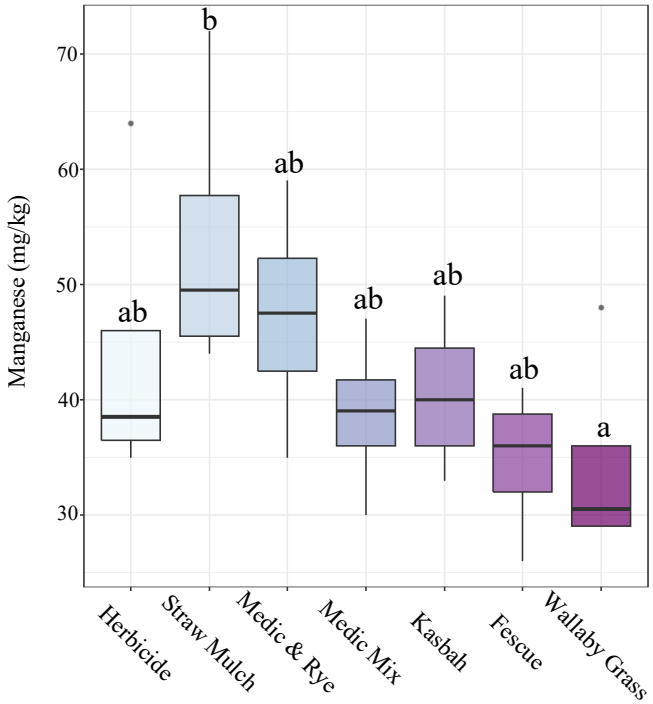


Figure 31 - Leaf manganese, Nuriootpa, Vintage 2020

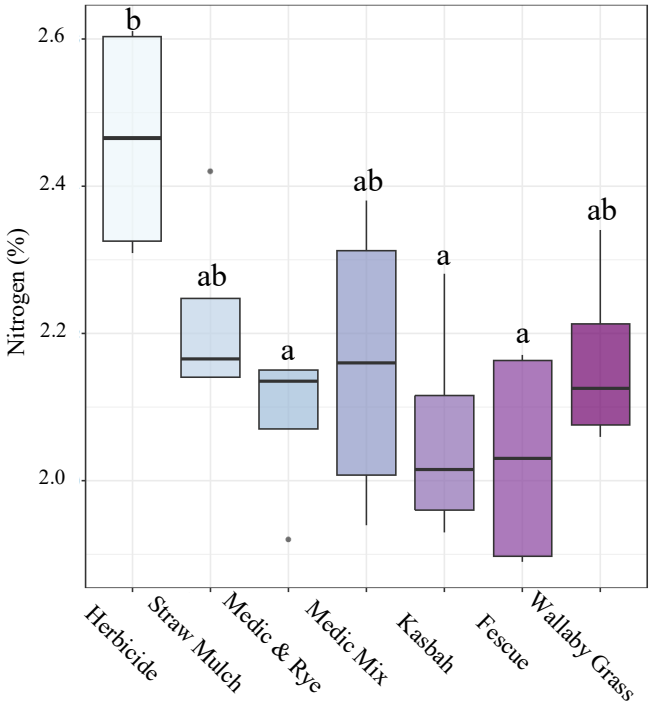


Figure 33 - Leaf nitrogen, Nuriootpa, Vintage 2020

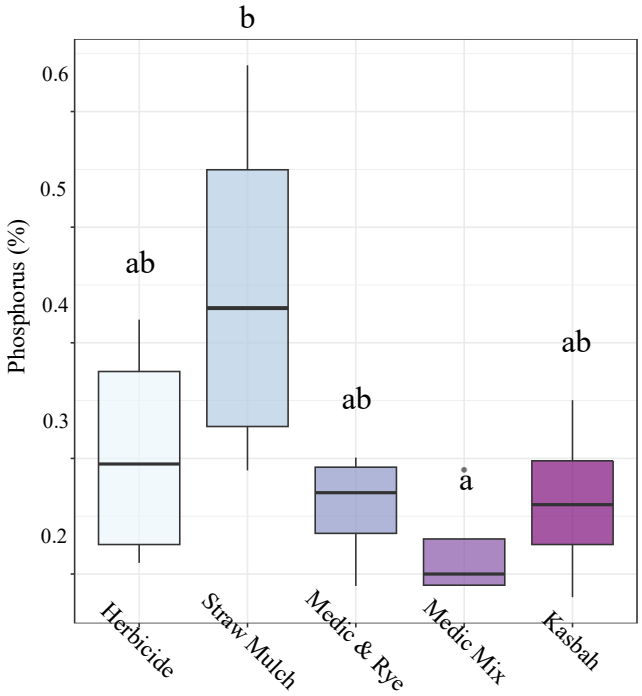


Figure 34 - Leaf phosphorus, Nuriootpa, Vintage 2019

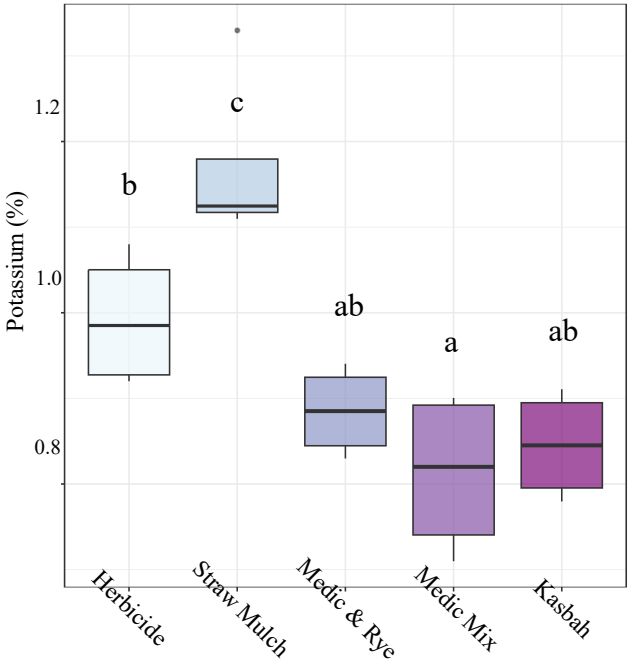


Figure 36 - Leaf potassium, Nuriootpa, Vintage 2019

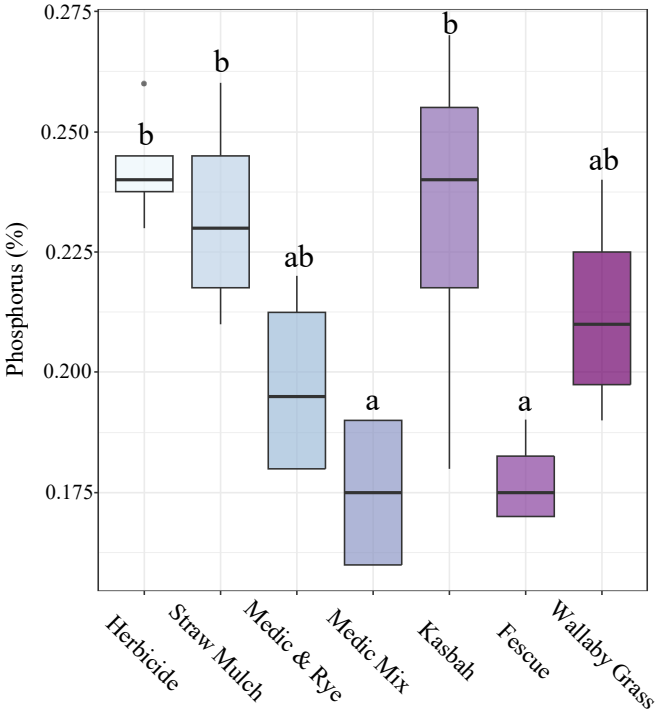


Figure 35 - Leaf phosphorus, Nuriootpa, Vintage 2020

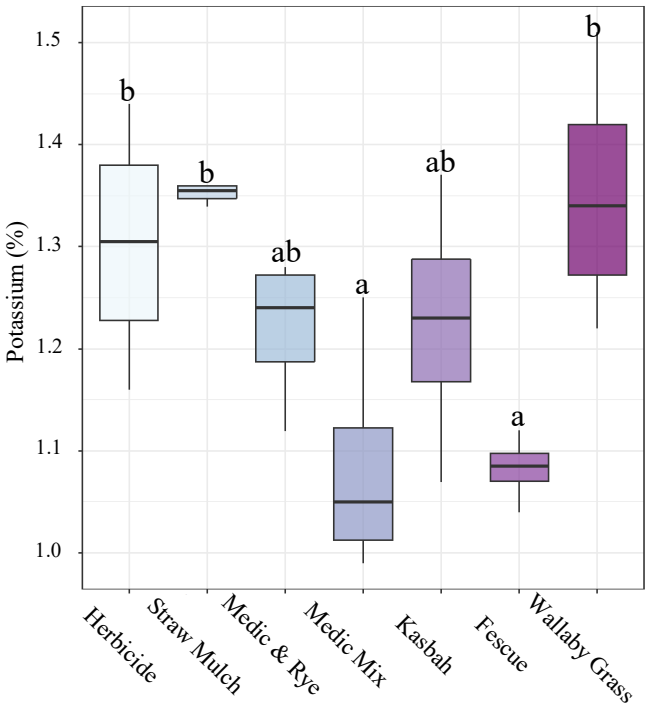


Figure 37 - Leaf potassium, Nuriootpa, Vintage 2020

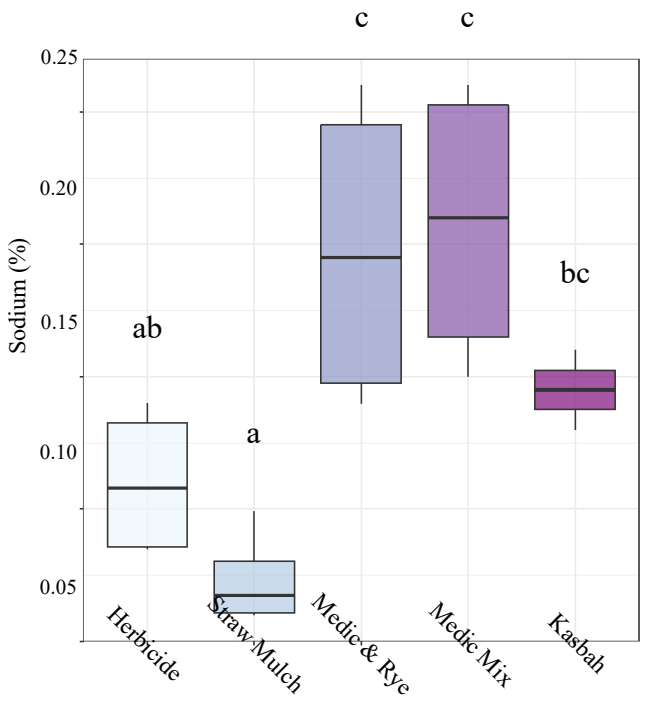


Figure 38 - Leaf sodium, Nuriootpa, Vintage 2019

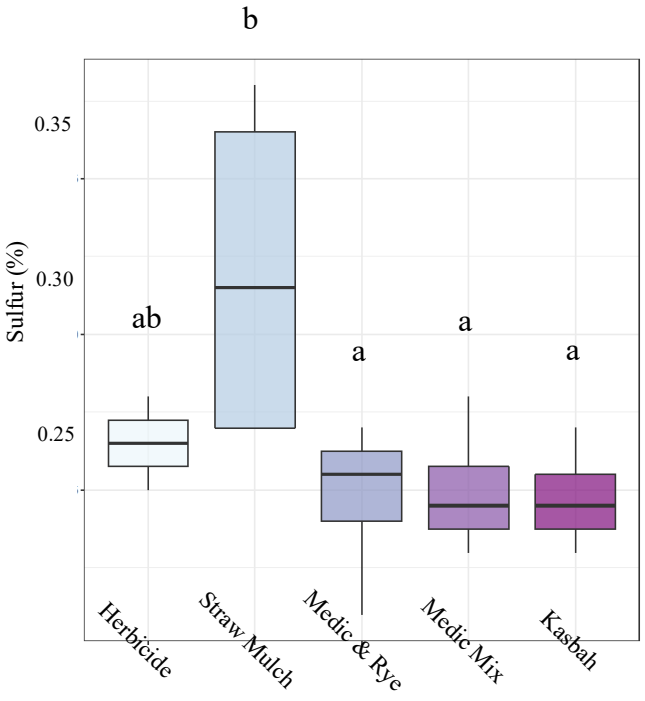


Figure 40 - Leaf sulfur, Nuriootpa, Vintage 2019

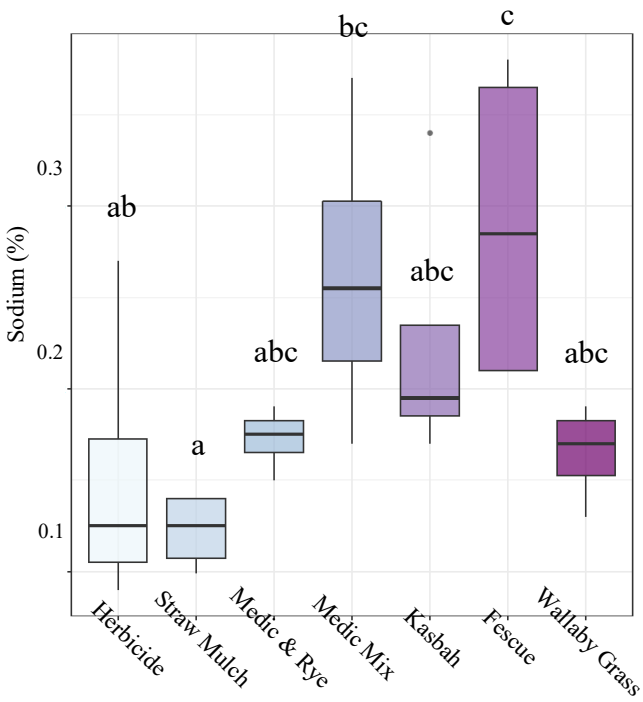


Figure 39 - Leaf sodium, Nuriootpa, Vintage 2020

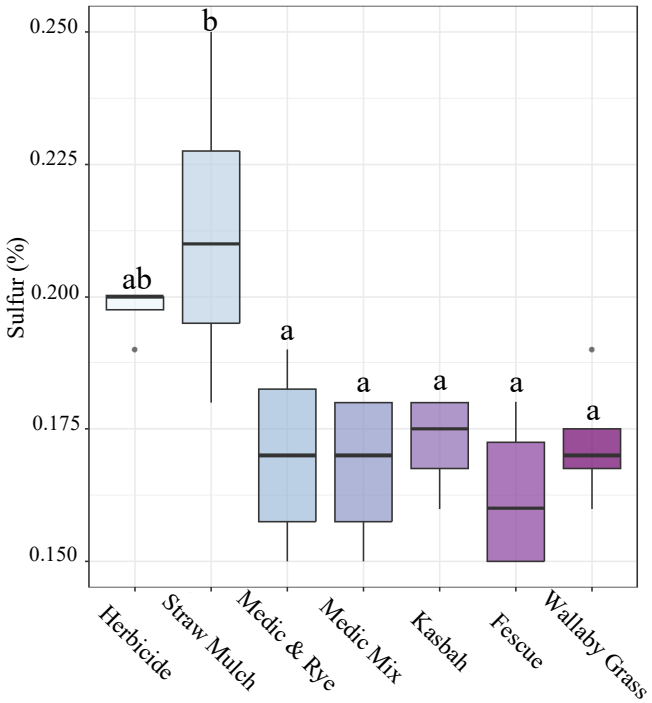


Figure 41 - Leaf sulfur, Nuriootpa, Vintage 2020.

Stem Water Potential

Stem water potential is commonly used as a measure of water stress. It is important to note that the more negative the value, the more water stressed the plant. Stem water potential was lowest in the herbicide control treatments across both seasons; that is, the vines were most water stressed in this treatment. In 2019 the difference between the herbicide control and the Kasbah treatment was significant (Figure 42), while in 2020 the significant difference was between the herbicide control and the Medic and Ryegrass treatments (Figure 43).

Soil penetration resistance

Soil penetration resistance is a measure of the pressure required to push a rod into the soil. More compact soil requires higher pressure. Soil resistance was significantly higher in the herbicide control than all other treatments at both 0-10cm (Figure 44) and 10-20cm (Figure 45) depths. Below this depth, there were no notable differences (data available on request).

Leaf Area Index

Leaf area index as determined by the Vitivisor app in 2020 (ceptometer not available) was higher for the straw mulch treatment than the perennial grasses of Kasbah cocksfoot and Fescue (Figure 47). In 2019, a similar trend was possible (Figure 46), however no significant differences were found.

Worms

Earthworms, which require readily available organic matter for their nutrition, were quantified in July 2019. There were large number of worms in the soil, with clear differences among treatments. Specifically, worms were least abundant in the herbicide control treatment and most abundant in the Medic and Ryegrass mix (Figure 48).

Cumulative temperature

Cumulative degree days (the total temperature experienced over time) at the soil surfaces and in the canopy were measured in the 2020 season using data loggers. This analysis revealed lower soil surface temperatures in the straw treatment compared to all other treatments tested (Figure 49). Maximum air temperature logged was 52.5 ^{o}C at the soil surface of the herbicide control on the 24th Feb 2020.

Microbiomes

Faith's phylogenetic diversity, a measure of the relative diversity among treatments was calculated using the NextGen sequencing results. The greater the value of this index, the greater the diversity. In this analysis we found the lowest levels of diversity in the herbicide treatment in both the 2019 (Figure 50) and 2020 (Figure 51) vintages compared to the other treatments included in this analysis. Taxa summary plots to order level can be seen in Figure 52.

Pruning Weights

Post season 2019 (Figure 53) and 2020 (Figure 54), cane weights were significantly higher in the straw mulch treatment than the Kasbah treatment. This is consistent with higher LAI readings (see above) and yield (see below) for these treatments.

Juice chemistry

In the 2019 vintage (Figure 55), no significant differences among treatments were observed in the juice pH. However, in the 2020 vintage (Figure 56), the Fescue treatment had a significantly higher juice pH than all others, except the Kasbah. The same effect could be seen at Langhorne Creek (see below), suggesting there is something treatment specific causing this. Extensive variables quantifying juice quality can be found below in Appendix 5.

Yield and Berry Metrics

Across both 2019 (Figure 58) and 2020 (Figure 59) vintages, there was no significant yield penalty for growing cover crops under vine. It should be noted that a damaging frost occurred at the Nuriootpa site at the beginning of the 2019 season, which would have interfered with these results.

In 2019 (Figure 60) there was no difference in bunch weight associated with growing cover crops under vines when compared to the herbicide control. In 2020 (Figure 61) straw mulch had a significantly higher bunch weight than the Kasbah treatment, while all other treatments fell somewhere in between.

Botanical analysis

To quantify the presence or absence of desired, (or undesired) species of plants in the under-vine space, a botanical analysis (or 'botanal') was carried out. Despite the 2018 winter season

being below average rainfall, there was still appreciable growth generated in some treatments over the winter and early spring periods, as shown in Figure 62. While included in the figure, the Zorro Fescue and cavalier/bindaroo medic/radish cover crops did not establish well, so the results shown are not reflective of the seed sown. This is also the reason these cover crops were not included in the study. The wallaby grass maintained a fairly uninvaded stand with some intrusion of Medic and annual grasses (mostly barley grass) occurring. The triticale mulch maintained a weed free status very effectively. In the sheep fescue/strawberry clover/bartolo clover treatment, sheep Fescue has become dominant, but is located primarily within 1 m of the dripper outlet. For the ryegrass/scimitar Medic combination, the proportion of Medic and Ryegrass has declined, and barley grass was recruited. While the integrity of the treatment had diminished, it continues to maintain a legume and annual grass component so the results will be reflective of their input. The Mintaro clover/Prima gland clover treatment in 2019 consisted mostly of barley grass with some broadleaf weeds. The two consecutive dry years impacted the establishment of the clover, but the barley grass was again recruited to fill any space. The control was maintained in a bare state by spraying with 3.5 L/ha of Alliance® herbicide (Paraquat® plus Amitrole®). This herbicide was used in preference to glyphosate due to Ryegrass resistance to glyphosate at this site.

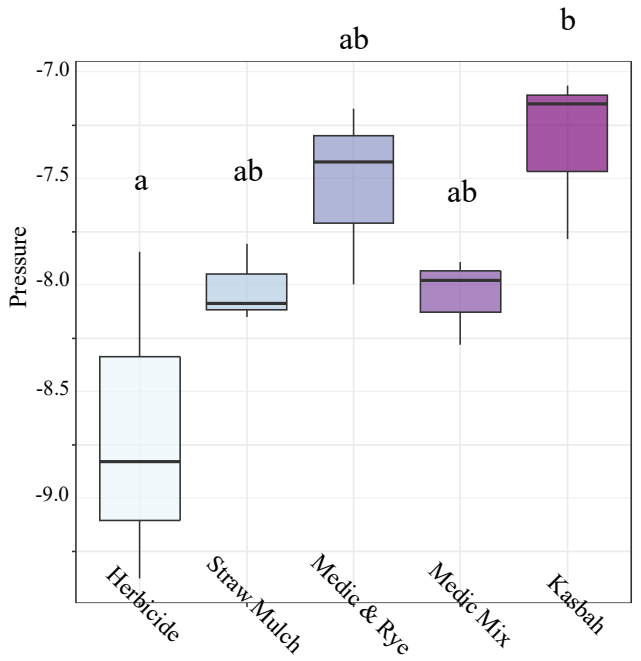


Figure 42 - Stem water potential 2019-01-31, Nuriootpa, Vintage 2019

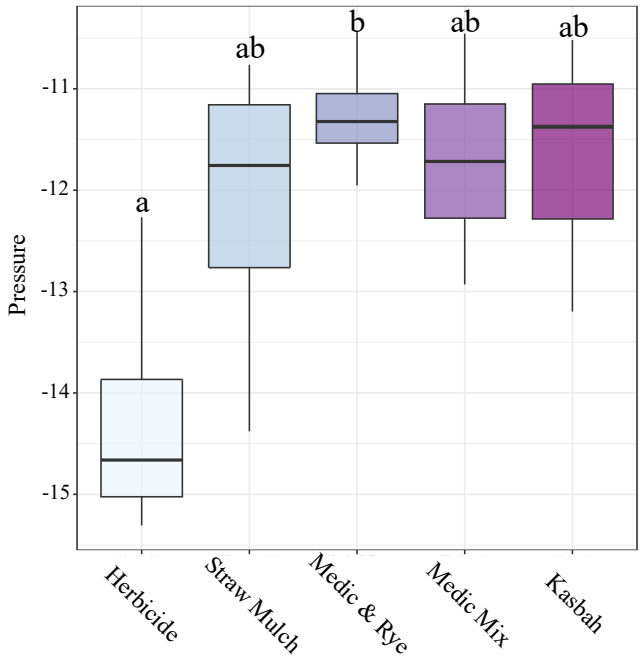


Figure 43 - Stem water potential 2020-03-11, Nuriootpa, Vintage 2020

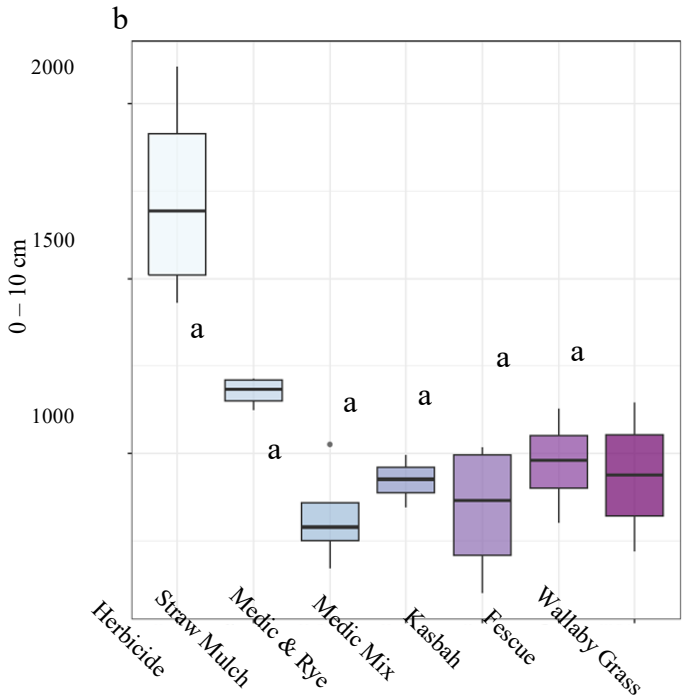


Figure 44 - Soil penetration resistance for 0-10 cm at Nuriootpa, 24-7-19

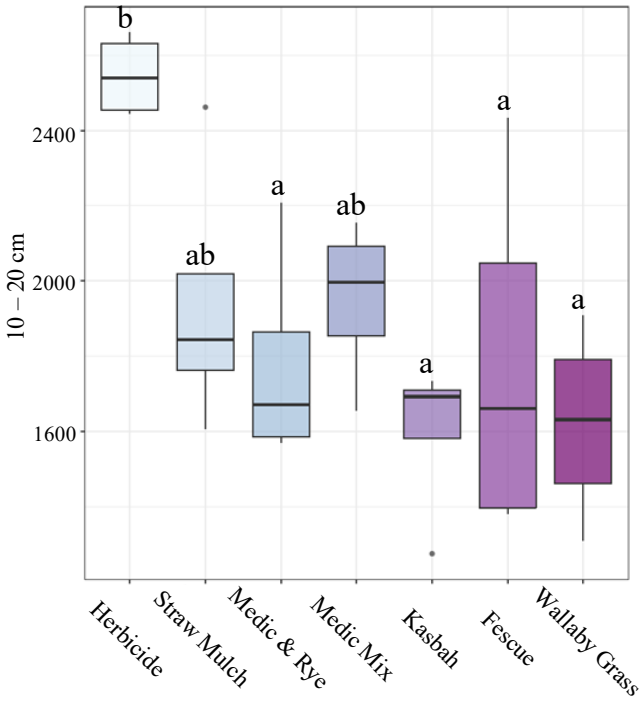


Figure 45 - Soil penetration resistance for 10-20 cm at Nuriootpa, 24-7-19

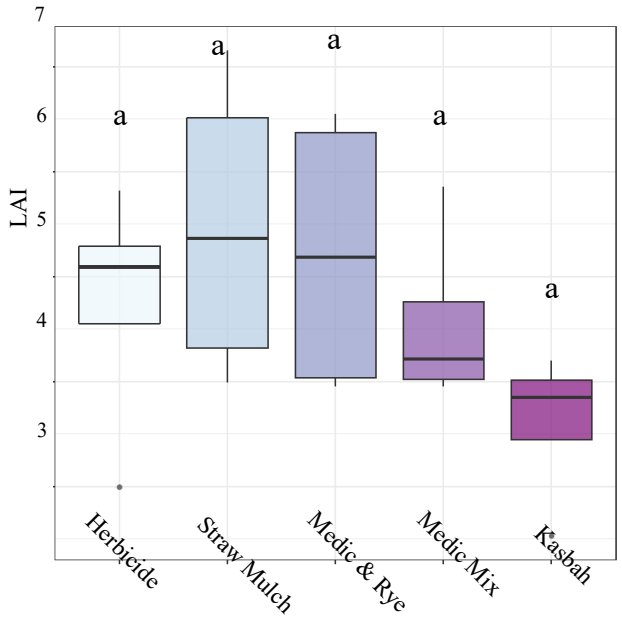


Figure 46: Leaf area index 2018-12-17, Nuriootpa, Vintage 2020

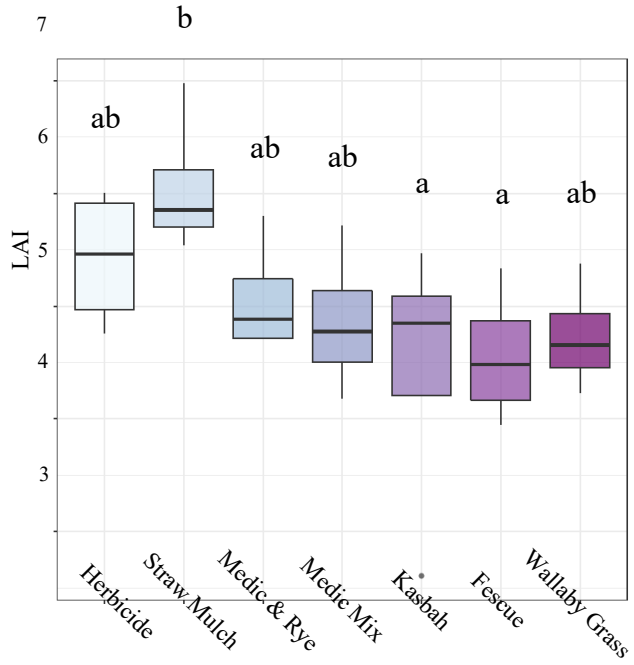


Figure 47 - Leaf area index 2020-02-04, Nuriootpa, Vintage 2020

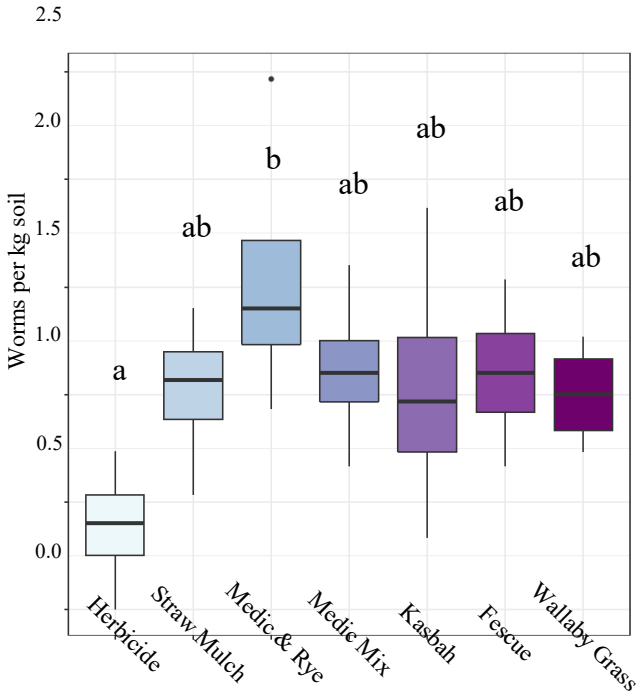


Figure 48 - Worms per kg soil, Nuriootpa, Vintage 2020

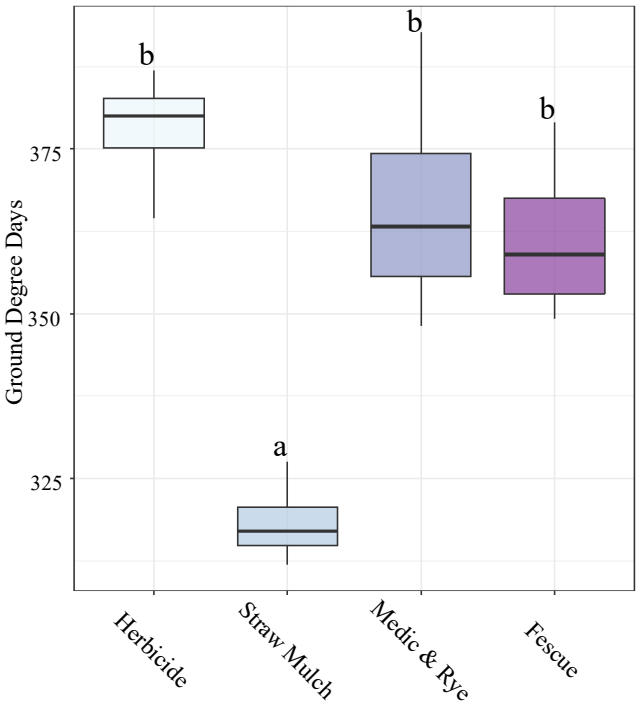


Figure 49 - Ground degree days, Nuriootpa, Vintage 2020

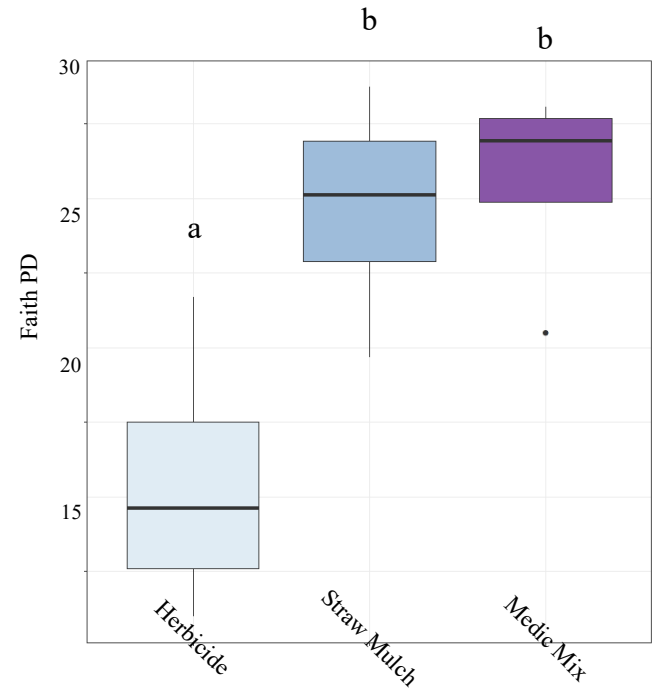


Figure 50 - Faith's phylogenetic diversity, Nuriootpa, Vintage 2019

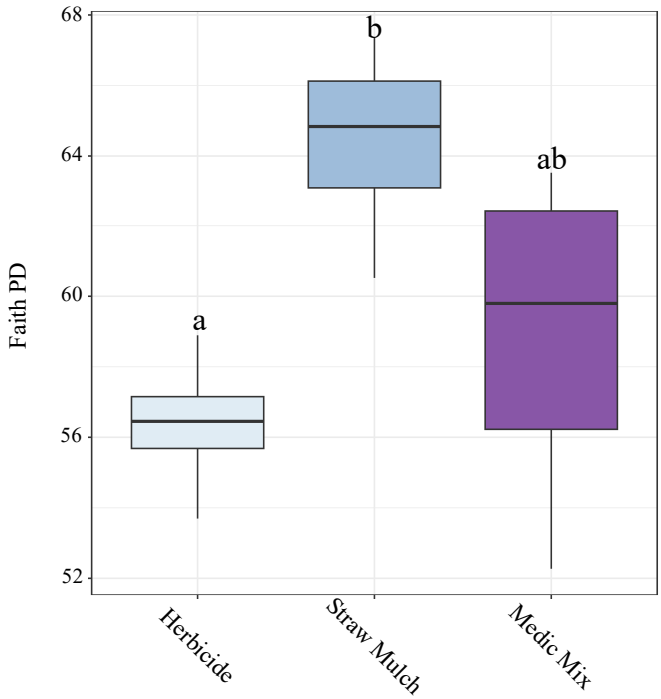


Figure 51 - Faith's phylogenetic diversity, Nuriootpa, Vintage 2020

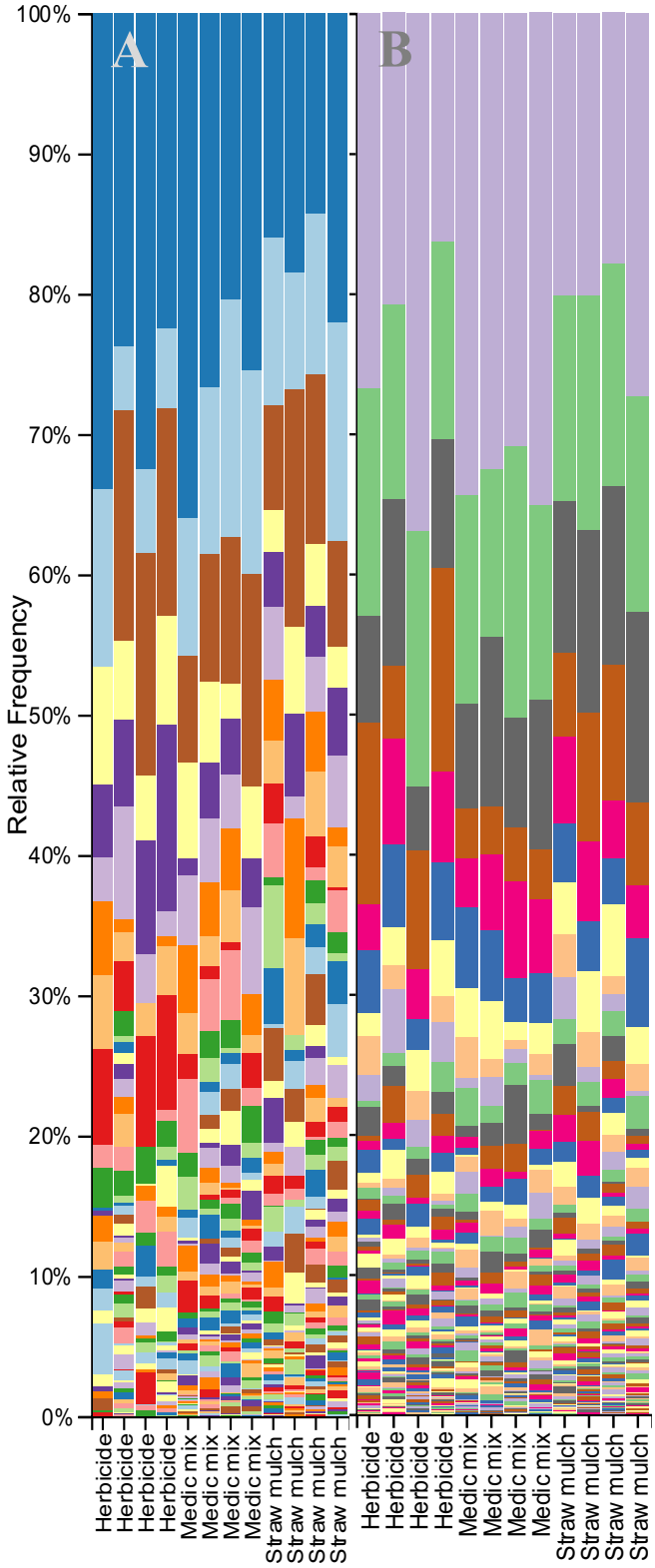


Figure 52: Nuriootpa taxa summary bar plots of relative frequency vs treatment (to order level). A) From July 2018, B) From July 2019.N. B. each colour bar represents a distinct taxon at the Order level.

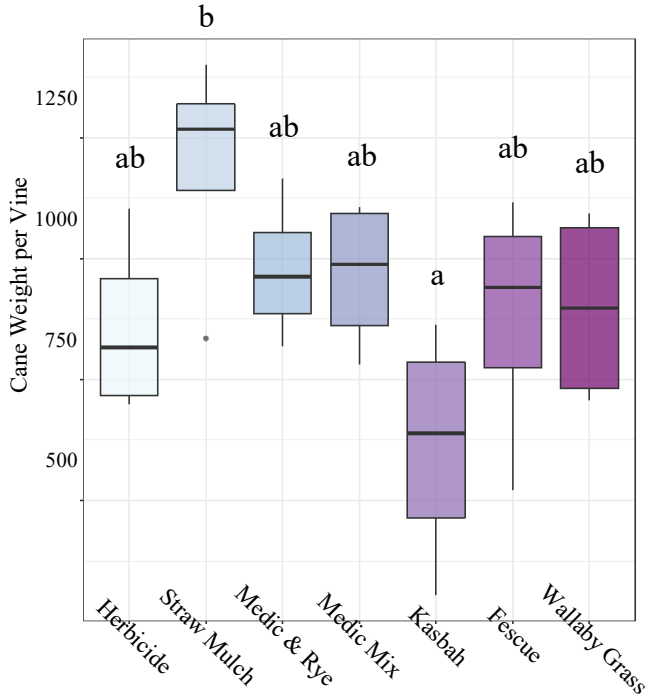


Figure 53 - Cane weight per vine, Nuriootpa, Vintage 2019

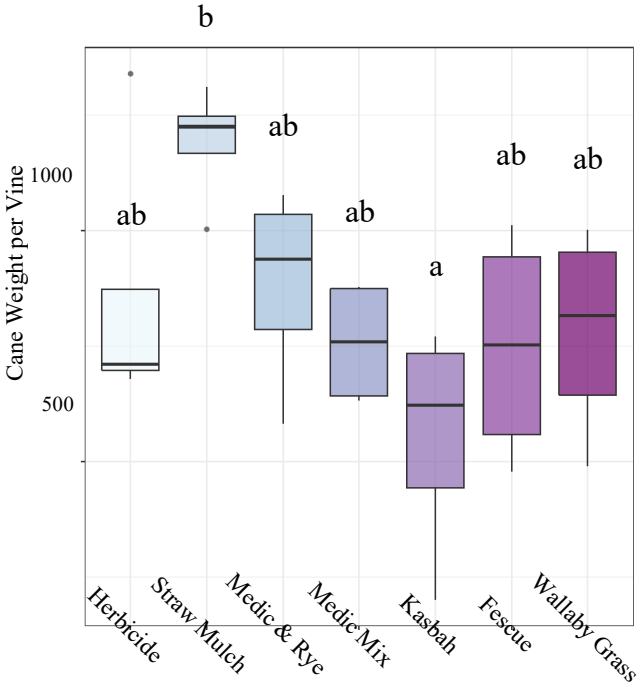


Figure 54 - Cane weight per vine, Nuriootpa, Vintage 2020

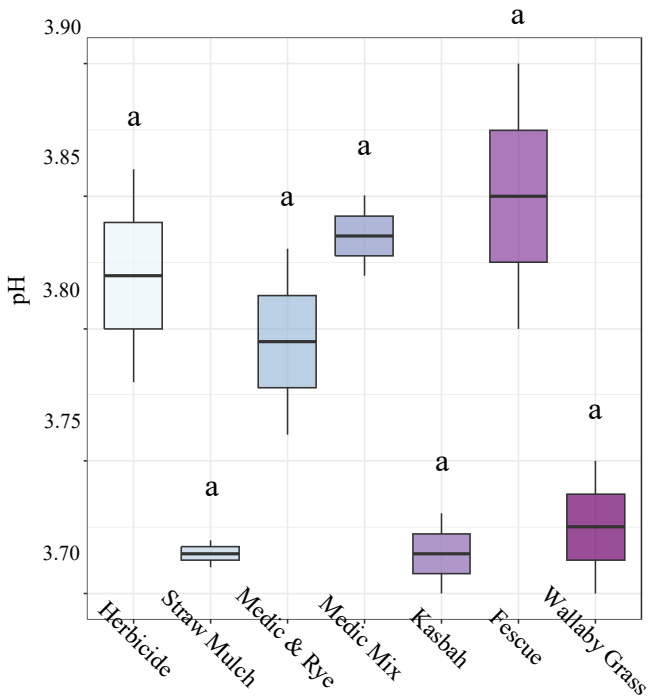


Figure 55 – Juice pH, Nuriootpa, Vintage 2019

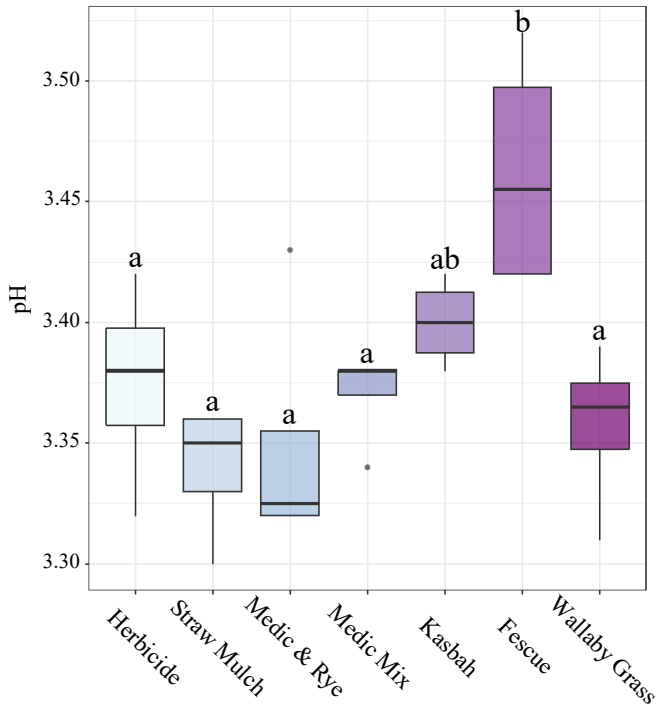


Figure 56 – Juice pH, Nuriootpa, Vintage 2020

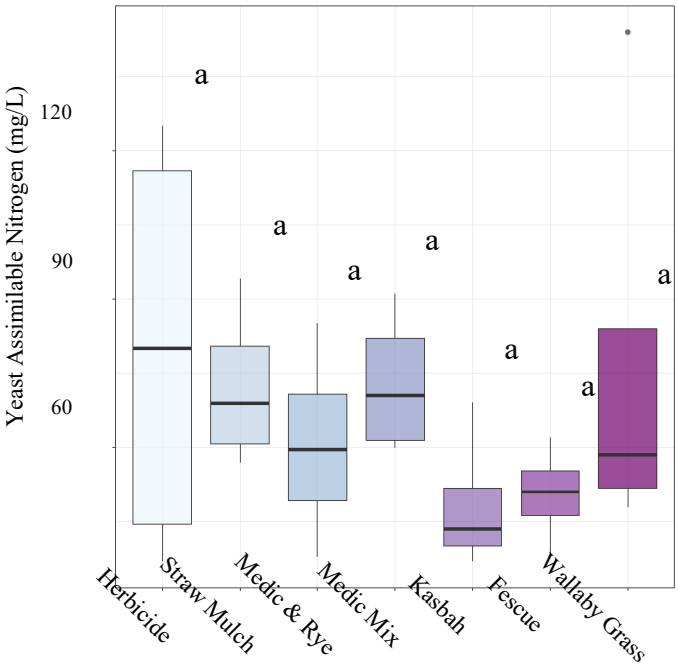


Figure 57 - Yeast assimilable nitrogen, Nuriootpa, Vintage 2020

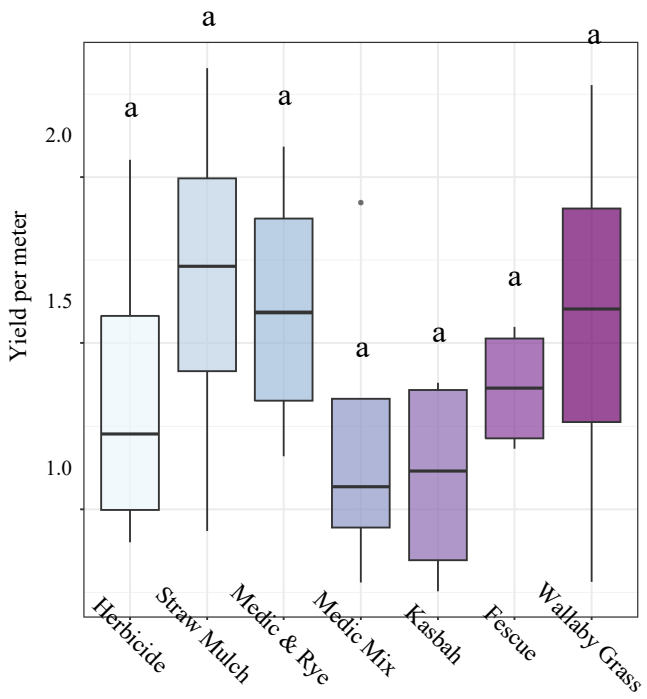


Figure 58: Yield (kg) per metre from Nuriootpa, 2019 vintage.

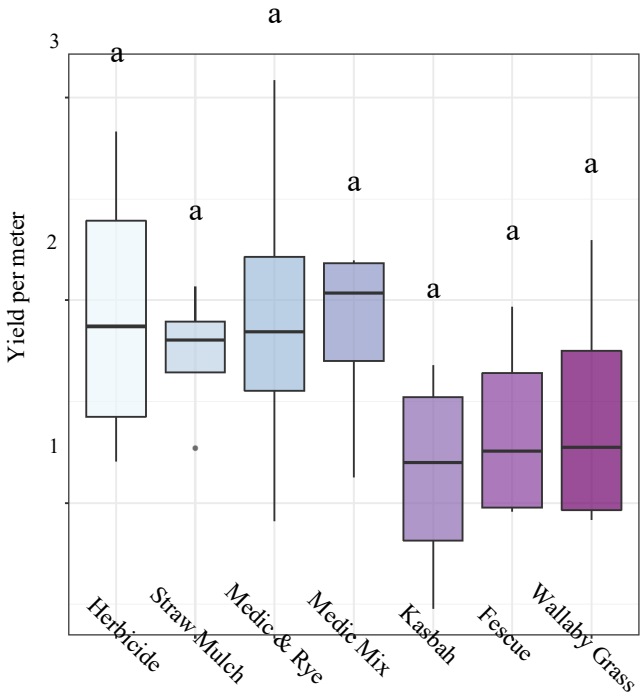


Figure 59: Yield (kg) per metre from Nuriootpa, 2020 vintage

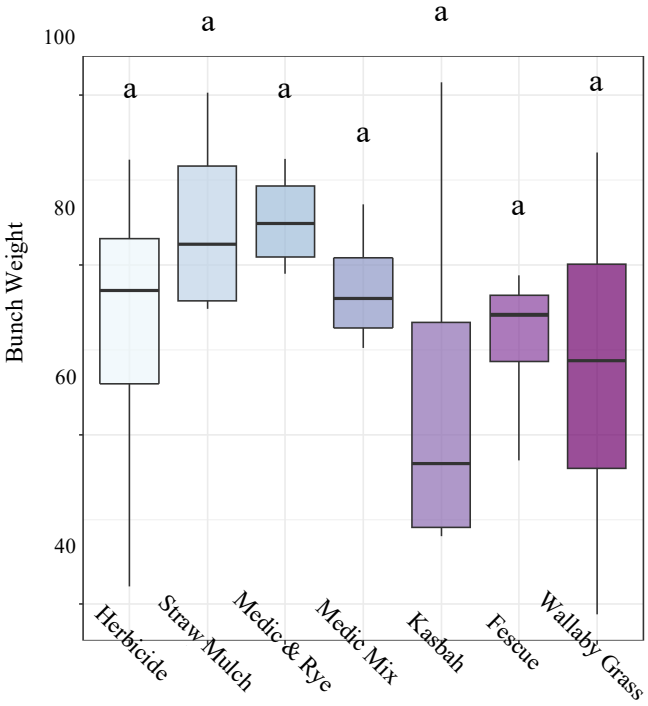


Figure 60 - Bunch Weight, Nuriootpa, Vintage 2019

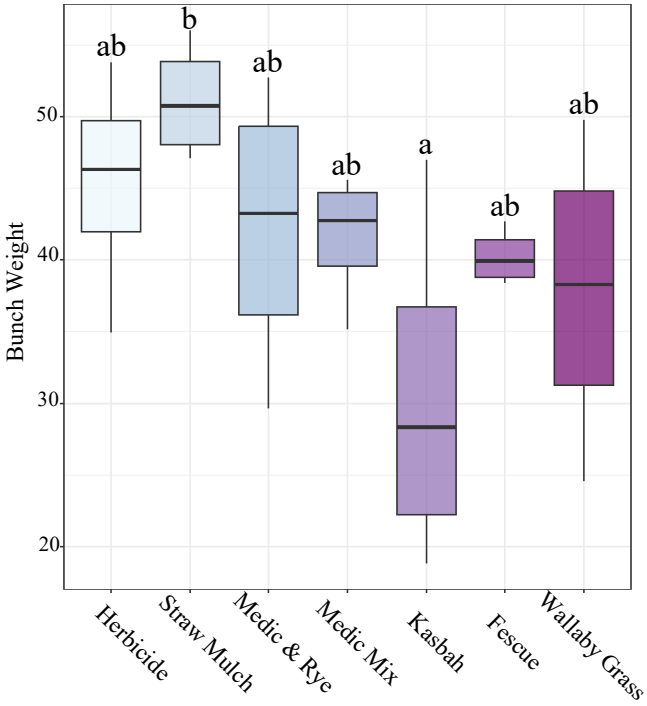


Figure 61 - Bunch Weight, Nuriootpa, Vintage 2020

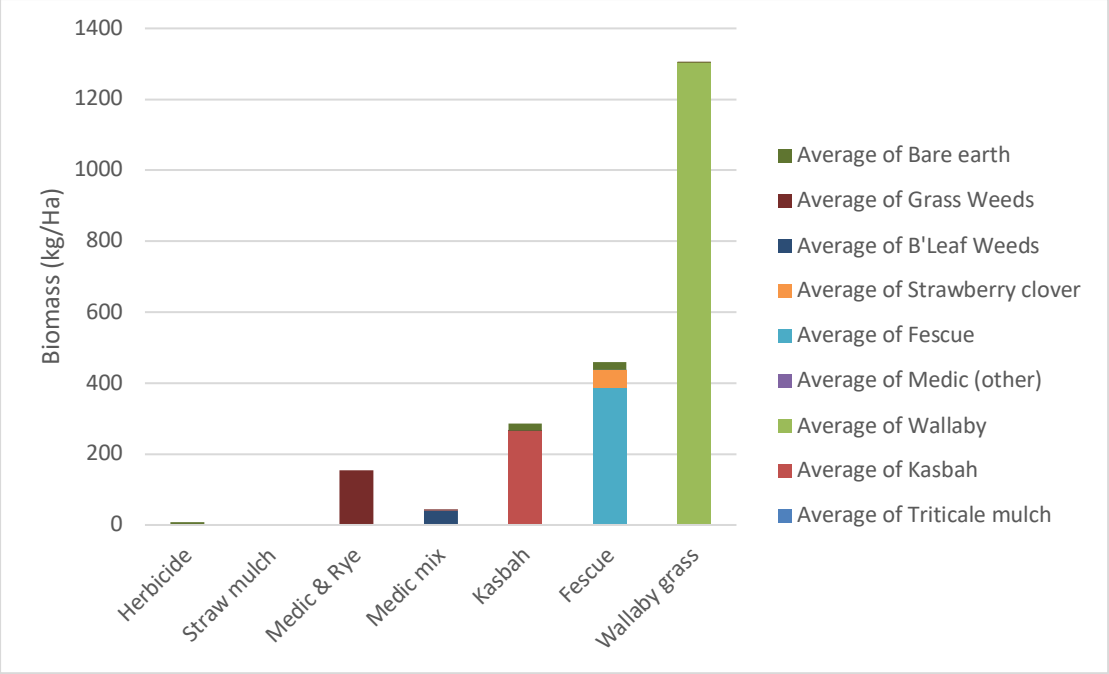


Figure 62: Botanical composition of under-vine cover crops, Nuriootpa, February, 2019.

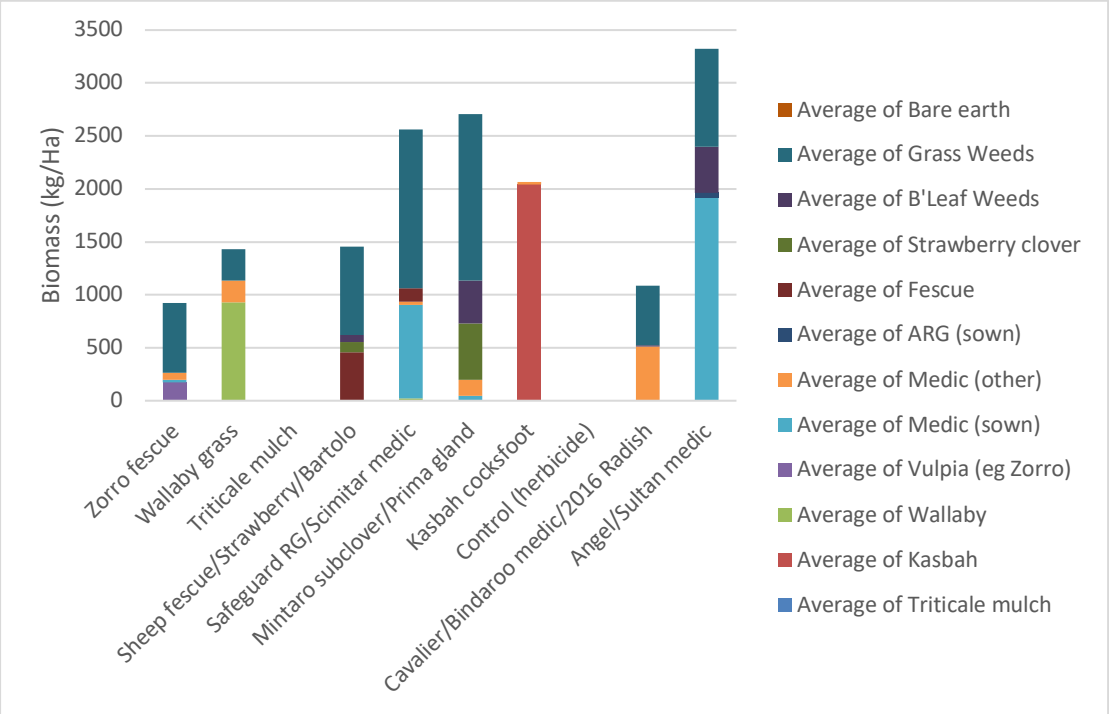


Figure 63: Botanical composition of under-vine cover crops, Nuriootpa, September, 2019

Wine making

Wines were made using the Shiraz grapes from each treatment from the Nuriootpa site. As mentioned in the methods section, due to the late and intense period of heat the two weeks before harvest, and with the professional advice of two winemakers, these wines were not bottled.

Results - Langhorne Creek

Soil Moisture Profile

Readings were taken fortnightly, three timepoints are given below, approximating budburst, flowering and harvest periods in both 2019 (Figure 64) and 2020 (Figure 65). Statistical analyses were carried out comparing each depth and each timepoint. No significant effects were detected. The irrigation regime at Langhorne Creek was intensive, preventing the soil in both 2019 and 2020 from decreasing in moisture greatly throughout the season. Only at harvest in 2020 is an obvious reduction in moisture visible at the 100 cm depth.

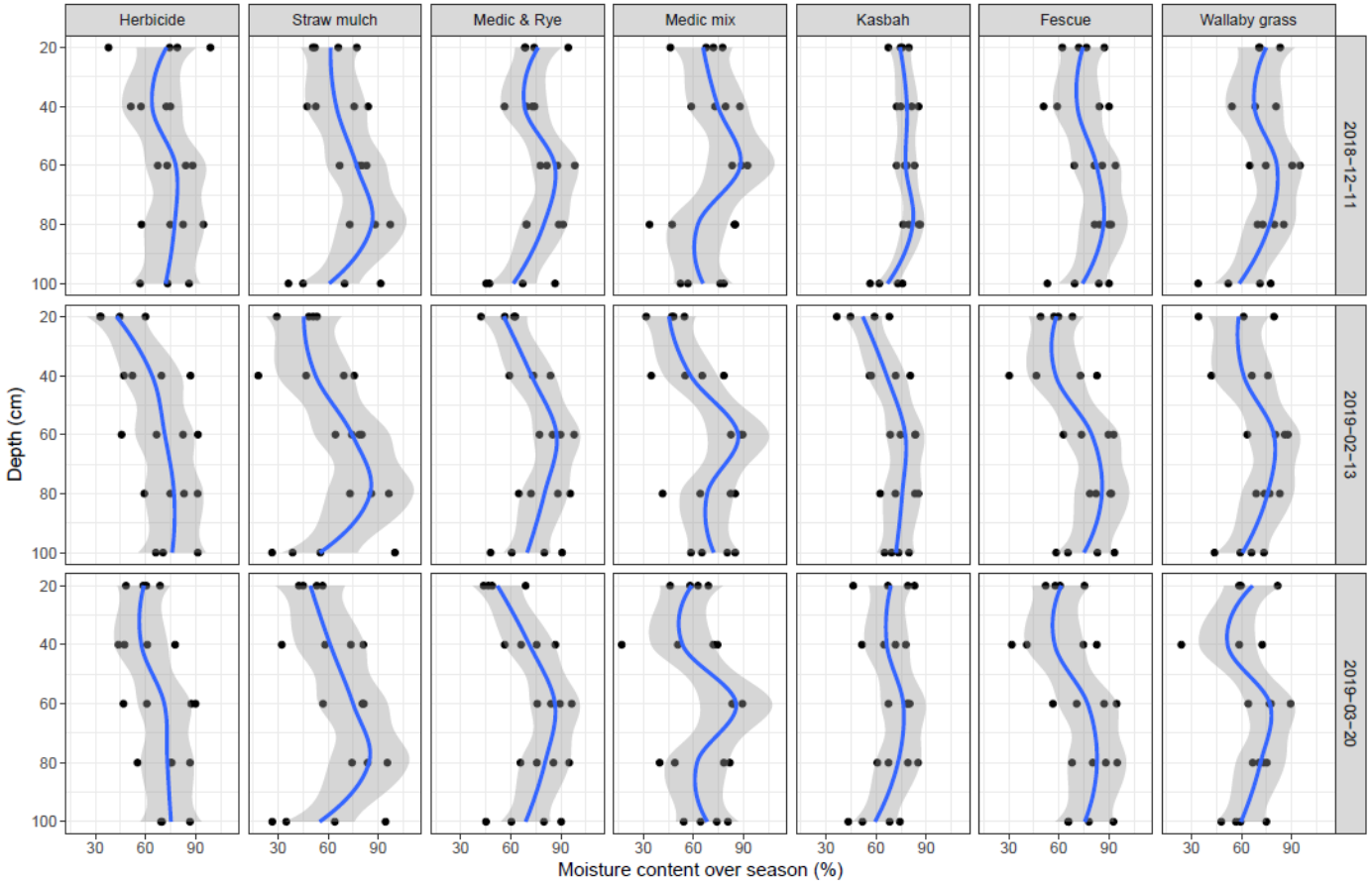


Figure 64 - Soil moisture profile, Langhorne Creek, Vintage 2019

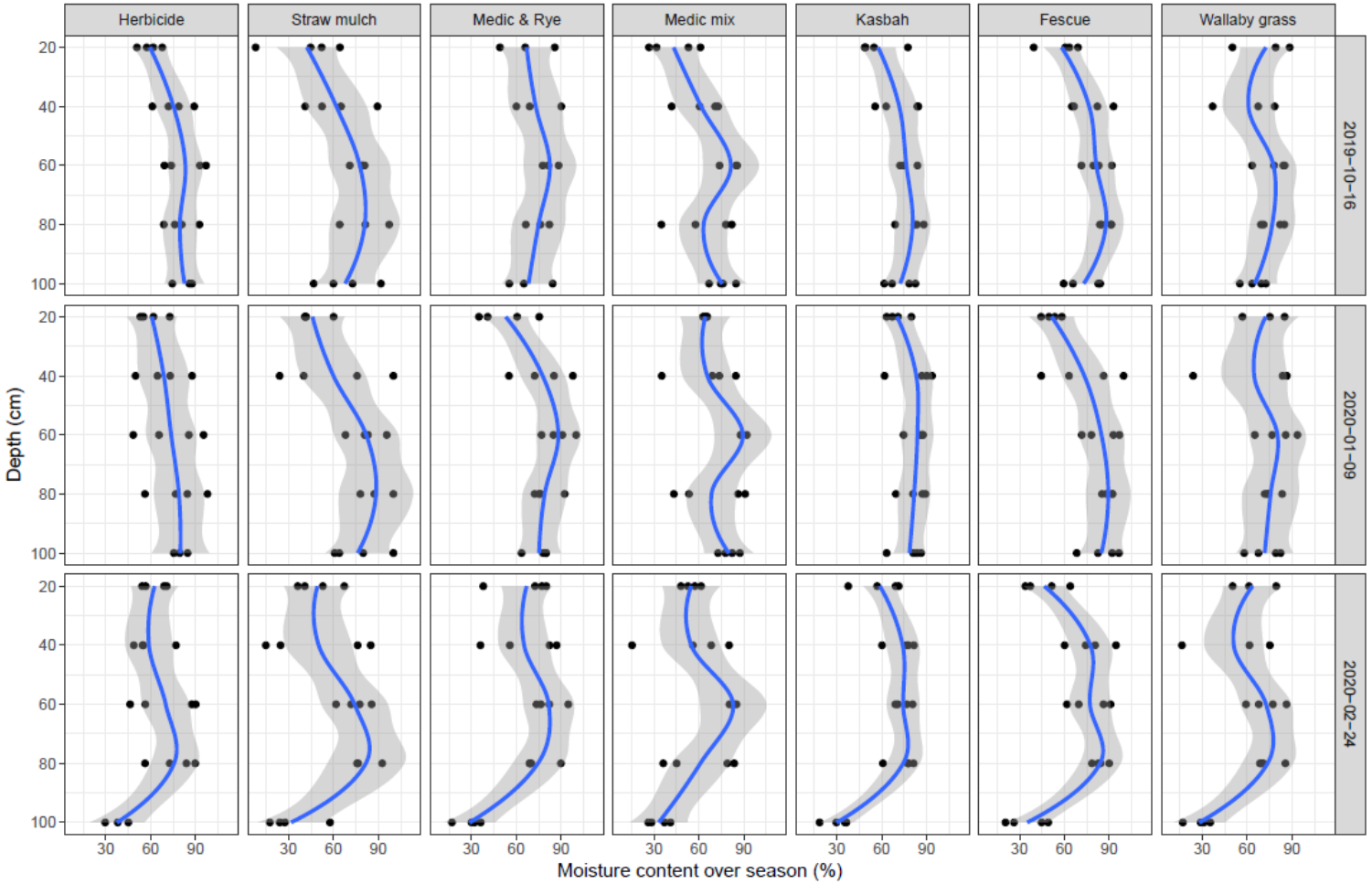


Figure 65 - Soil moisture profile, Langhorne Creek, Vintage 2020

Soil physicochemical properties

The soil $pH_{(1:5\ Soil:water)}$, measured in water was higher on the herbicide control than other treatments. With a pH in excess of 7.75 as shown on the herbicide control, the availability of nutrients such as iron, manganese, copper, zinc and boron may be reduced. The reason for the elevated pH on the control both in the 2019 (Figure 66) and 2020 vintage (Figure 67) may be due to excess sodium in the topsoil.

The standard (APAL, 2020) for soil Ca is in the range of 800-2000 mg/kg, which accounts for all treatments in both 2019 and 2020. Of interest is the lower amount found on the straw treatment. Marks (2020, pers comm) found straw mulch contained 0.55% Ca, so with over 100 t/ha of cereal straw used under-vine over 5 years approximately 550 kg / ha of Ca has been concentrated in the under-vine zone. With only 0.5 kg/t of fruit being exported from the site, it will be many years before the straw treatment is requiring further Ca additions. The results shown in figures 69 and 70 are therefore difficult to reconcile, as Ca was not being applied to the other treatments.

No significant differences between plant-available (Colwell) phosphorous were observed among treatments in 2019 (Figure 74). During the second season however, the Medic mix possessed significantly lower plant available P than the straw mulch (Figure 75).

Across both seasons, the herbicide control and straw mulch treatments had the lowest soil carbon, ranging from 1.1-1.3 %. This effect was significant in 2020 (Figure 71) where the Kasbah treatment had a soil carbon content of 2.2 % compared to the 1.1 % of the herbicide treatment.

Total soil N was significantly higher for the Medic and Ryegrass treatment when compared to the herbicide in 2019 (Figure 72). Active ground covers had significantly greater soil N than the herbicide and straw mulch in 2019. This was also seen in 2020 (Figure 73).

Recommended levels for soil manganese range from 1-10 mg/kg (APAL, 2020), which is exceeded by the Medic, Kasbah and Fescue treatments in both 2019 (Figure 78) and 2020 (Figure 79).

The recommended soil K is from 150 – 250 mg/kg, making most treatments in 2019 (Figure 80) except the Kasbah treatment, below the minimum required. A similar trend was observed in 2020 (Figure 81) where all treatments except herbicide were below the threshold. There were no significant differences among treatments in either year.

Soil Na levels were similar for all treatments at the start of the 2019 vintage (Figure 82). The herbicide treatment had significantly higher soil Na relative to the straw mulch in 2020 (Figure 83).

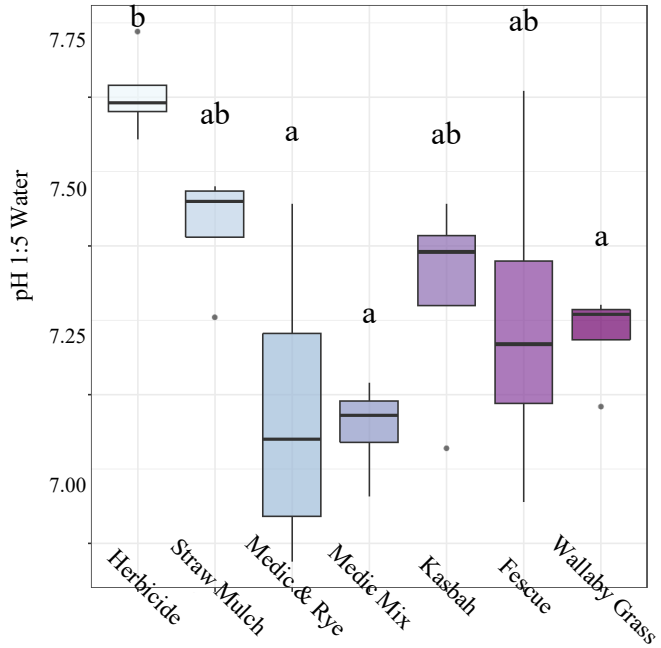


Figure 66 - Soil pH 1:5 Water, Langhorne Creek, Vintage 2019

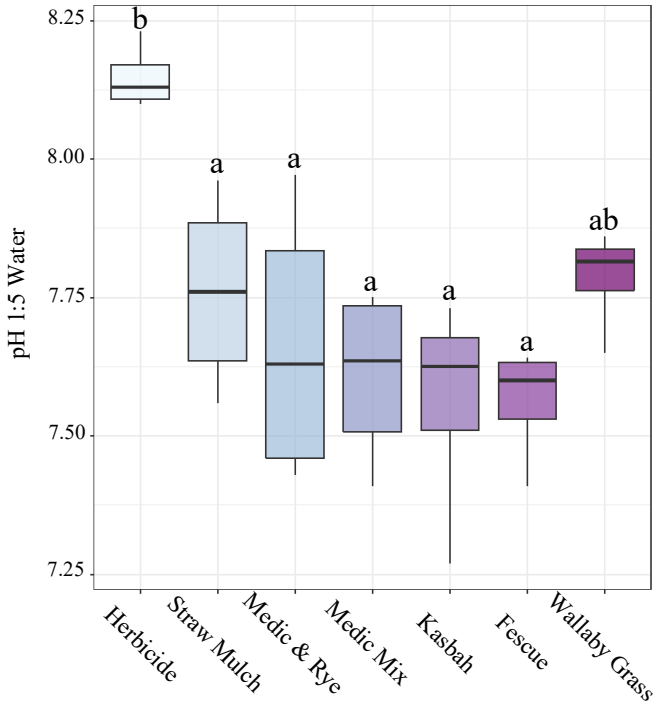


Figure 67 - Soil pH 1:5 Water, Langhorne Creek, Vintage 2020

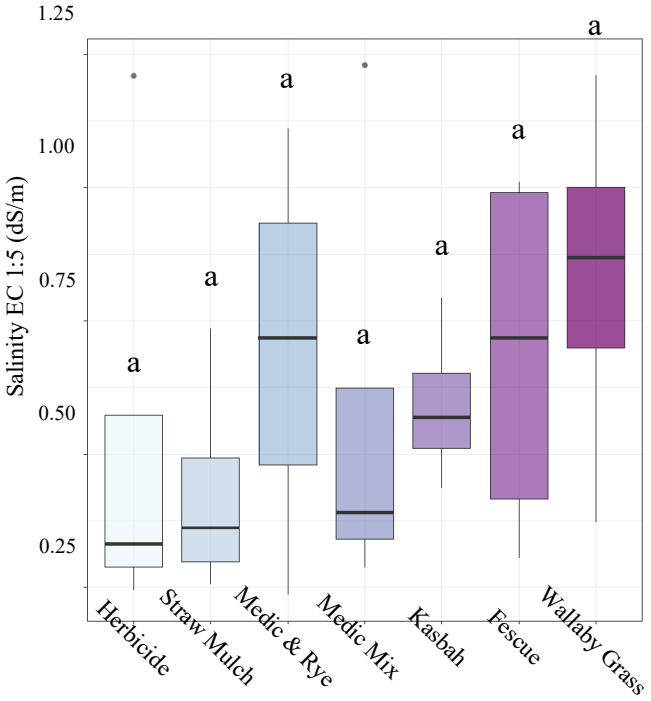


Figure 68 - Soil salinity, Langhorne Creek, Vintage 2019

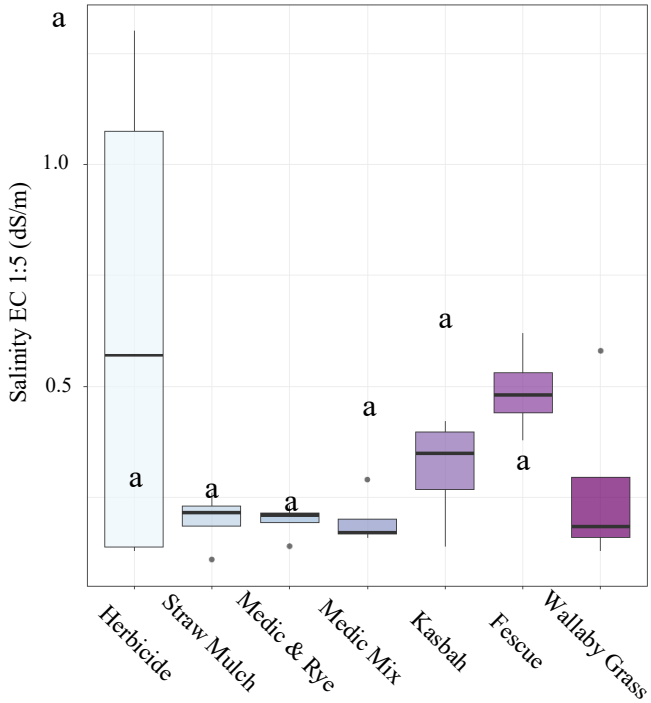


Figure 69 - Soil salinity, Langhorne Creek, Vintage 2020

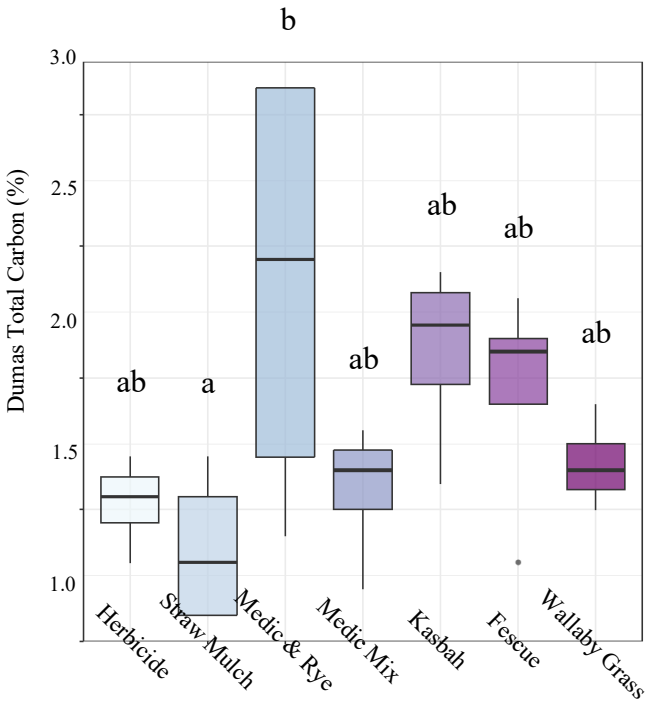


Figure 70 - Soil total carbon, Langhorne Creek, Vintage 2019

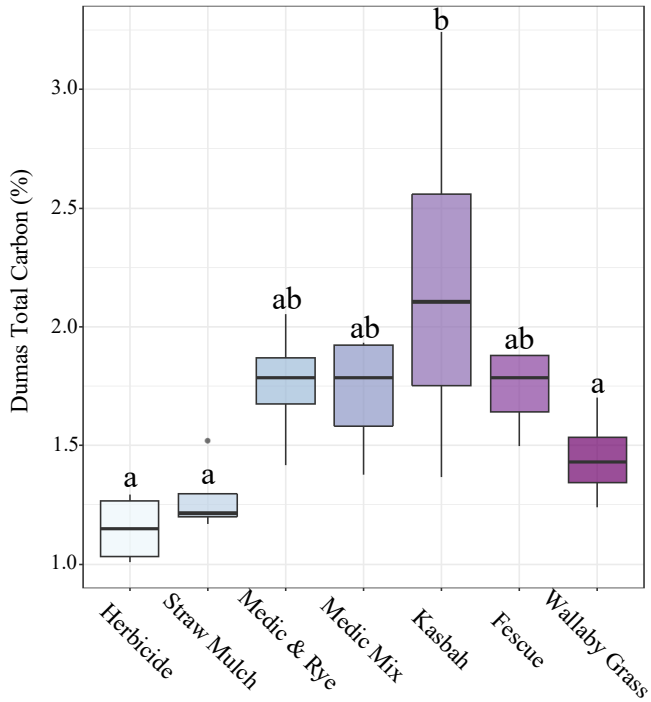


Figure 71 - Soil total carbon, Langhorne Creek, Vintage 2020

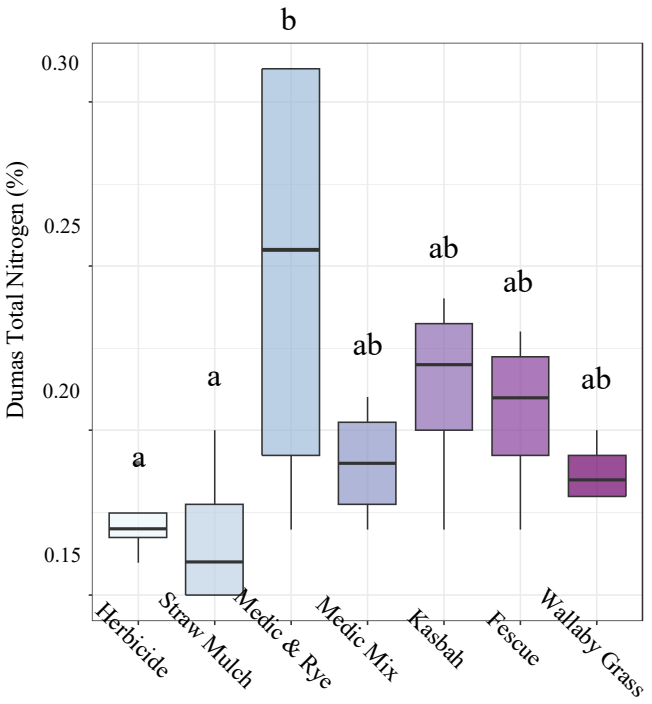


Figure 72 - Soil total nitrogen, Langhorne Creek, Vintage 2019

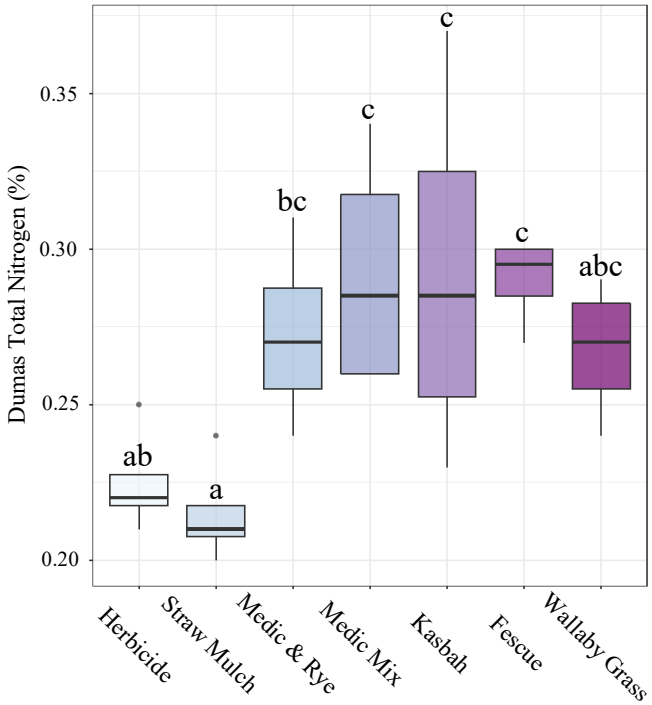


Figure 73 - Soil total nitrogen, Langhorne Creek, Vintage 2020

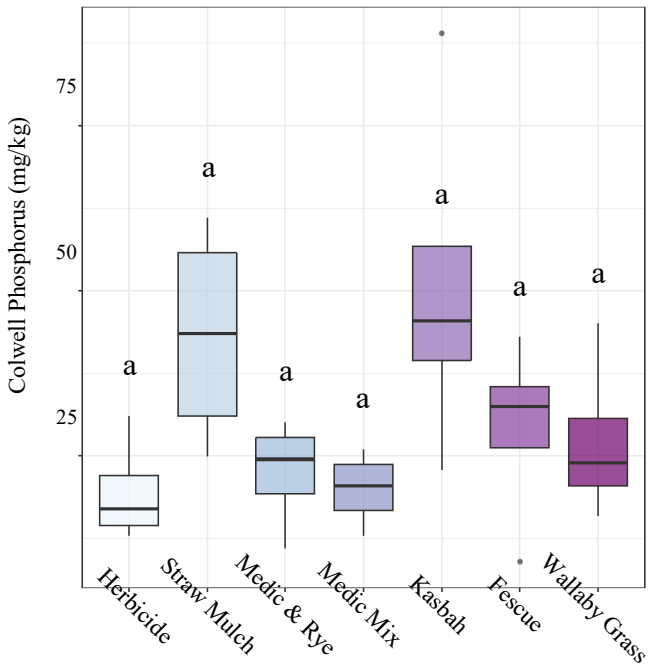


Figure 74 - Soil phosphorus, Langhorne Creek, Vintage 2019

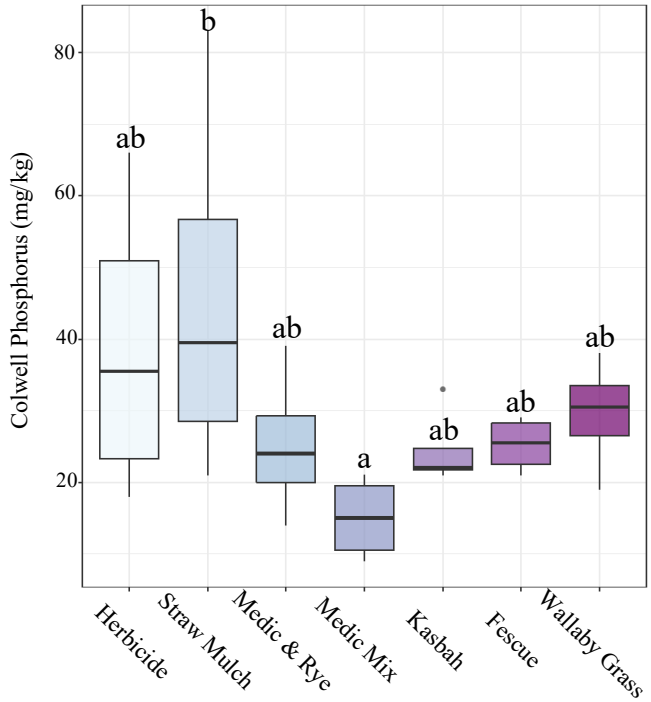


Figure 75 - Soil phosphorus, Langhorne Creek, Vintage 2020

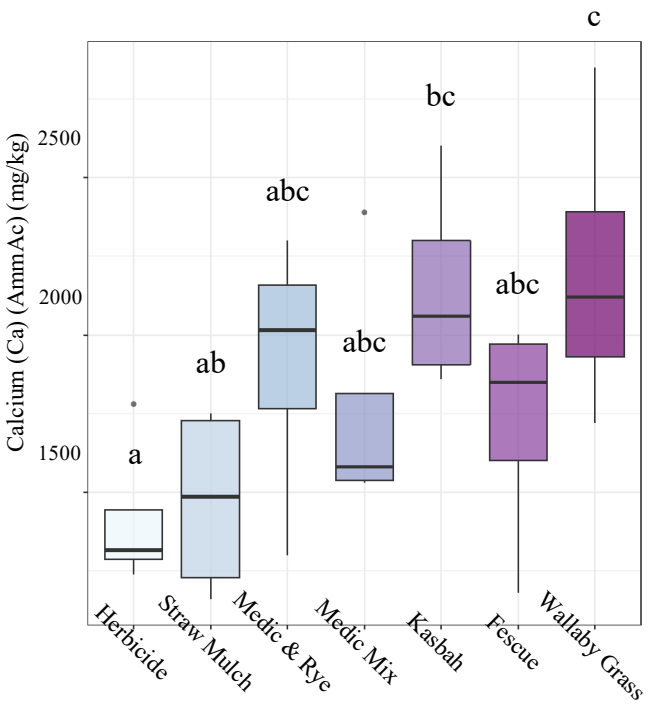


Figure 76 - Soil calcium, Langhorne Creek, Vintage 2019

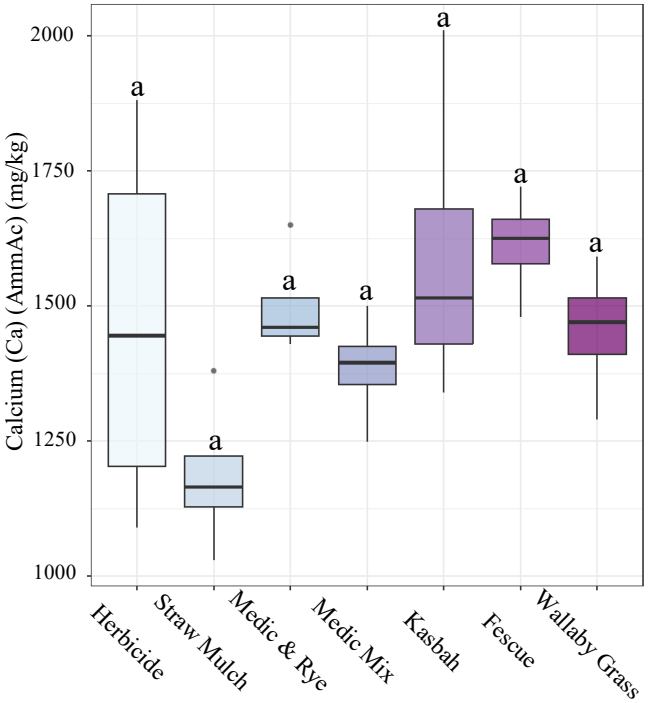


Figure 77 - Soil calcium, Langhorne Creek, Vintage 2020

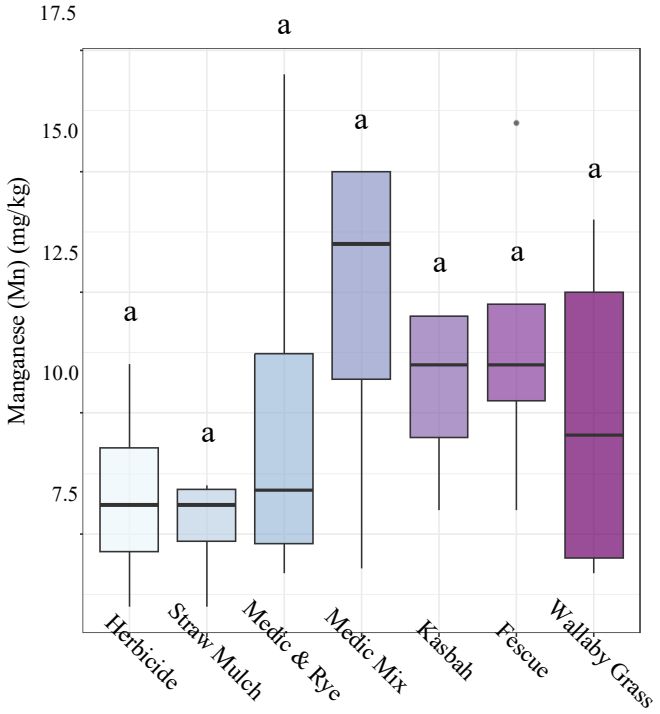


Figure 78 - Soil manganese, Langhorne Creek, Vintage 2019

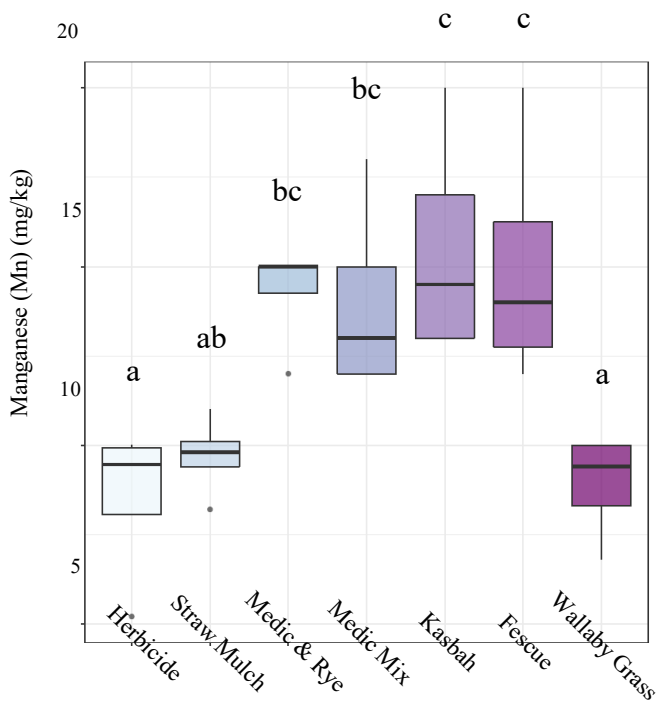


Figure 79 - Soil manganese, Langhorne Creek, Vintage 2020

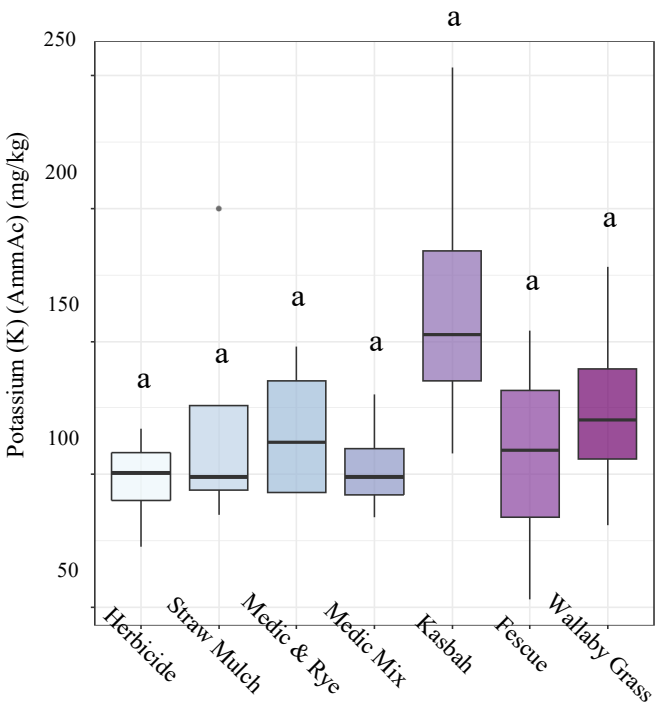


Figure 80 - Soil potassium, Langhorne Creek, Vintage 2019

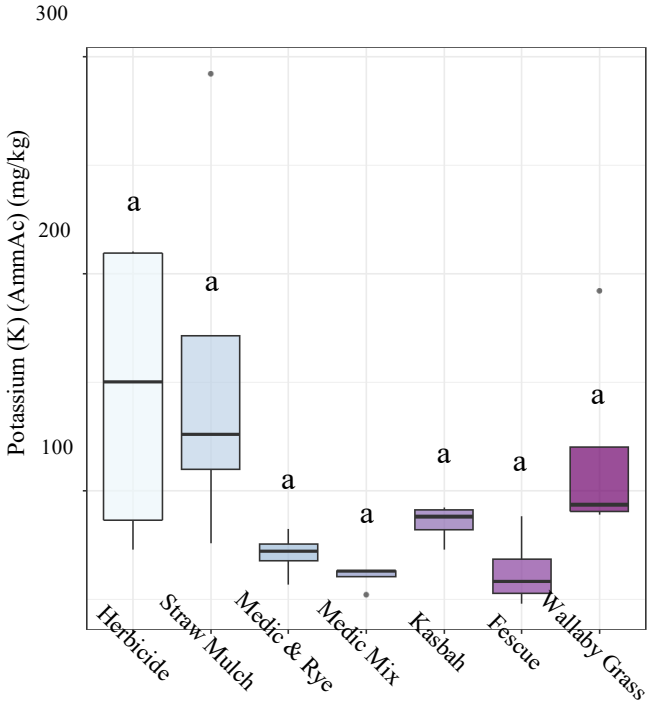


Figure 81 - Soil potassium, Langhorne Creek, Vintage 2020

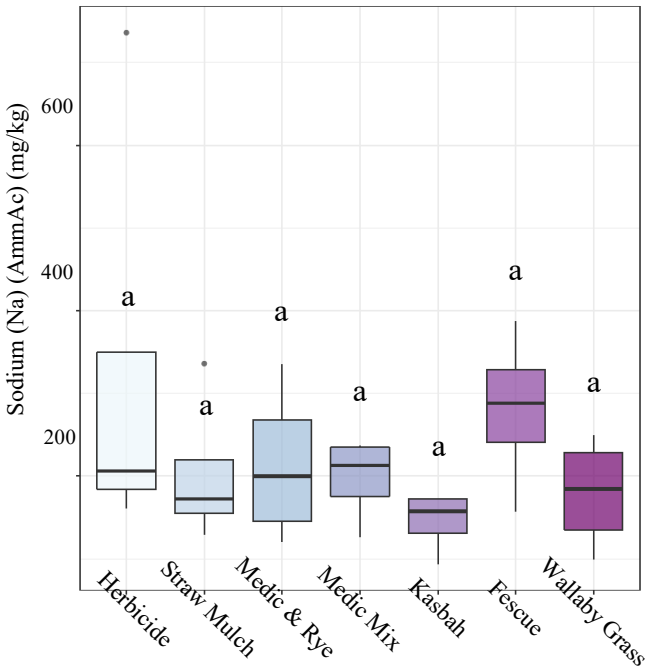


Figure 82 - Soil sodium, Langhorne Creek, Vintage 2019

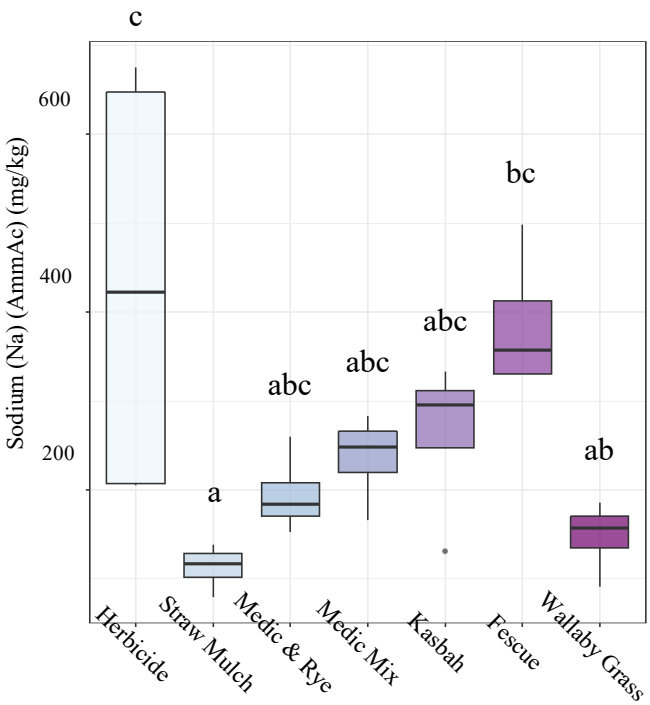


Figure 83 - Soil sodium, Langhorne Creek, Vintage 2020

Soil Mineral Nitrogen

Ammonium concentrations were generally low (<5ppm) across the seasons (Figure 84 and Figure 85) and among treatments, however within the expected standard of 1-10 ppm (APAL, 2020). There were clear increases in NH_4^+, following application of additional fertiliser in late October (pers comm.). The Medic treatments show limited benefits in soil N supply. A late spike in NO_3^- was visible in the Medic, Medic and Ryegrass and straw mulch treatments in 2019 (Figure 86), which was curiously not visible in the other treatments. At late November application of fertiliser was visible in all treatments but the Kasbah and Fescue treatments (Figure 87).

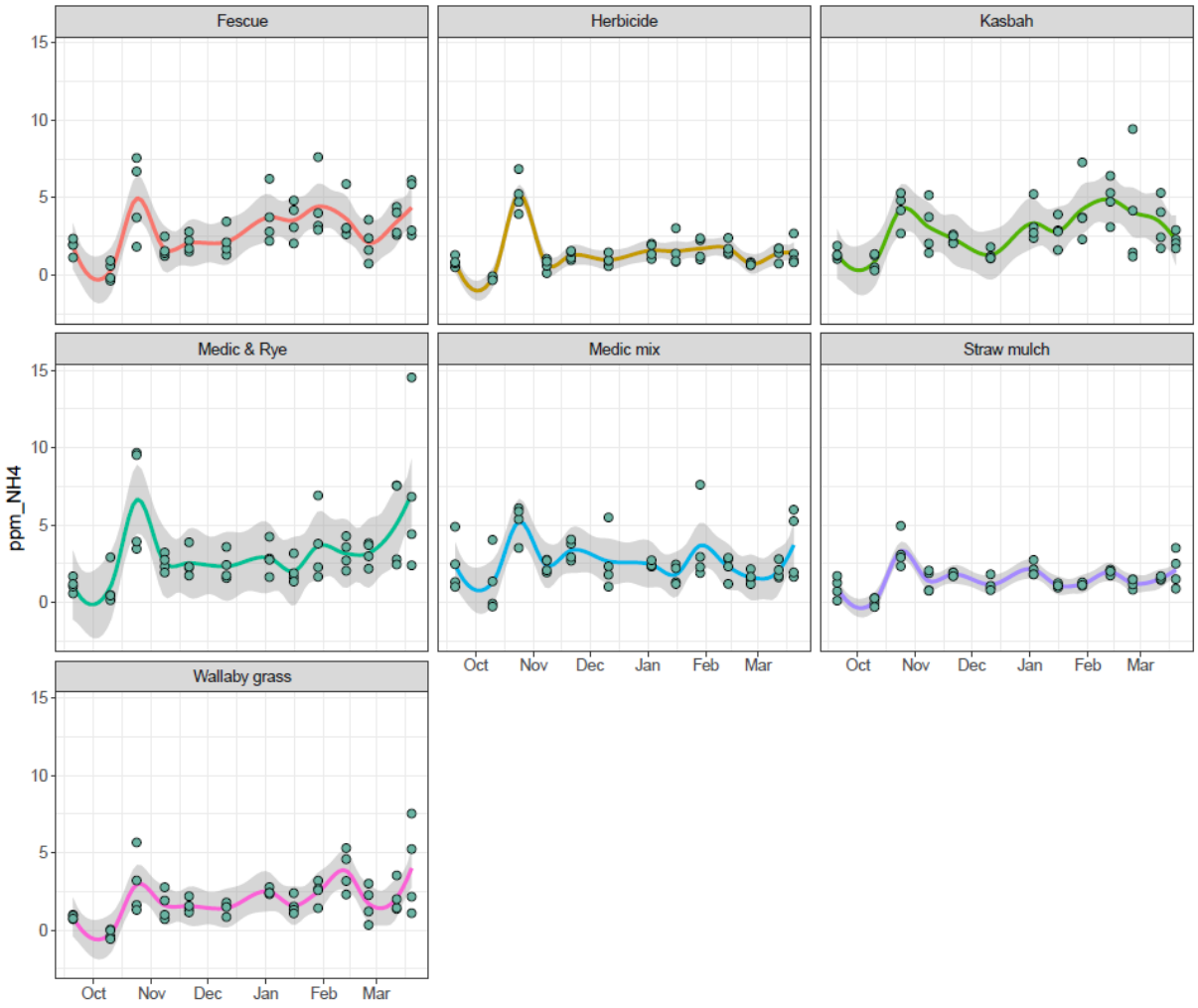


Figure 84 - Soil ammonium, Langhorne Creek, Vintage 2019



Figure 85 - Soil ammonium, Langhorne Creek, Vintage 2020

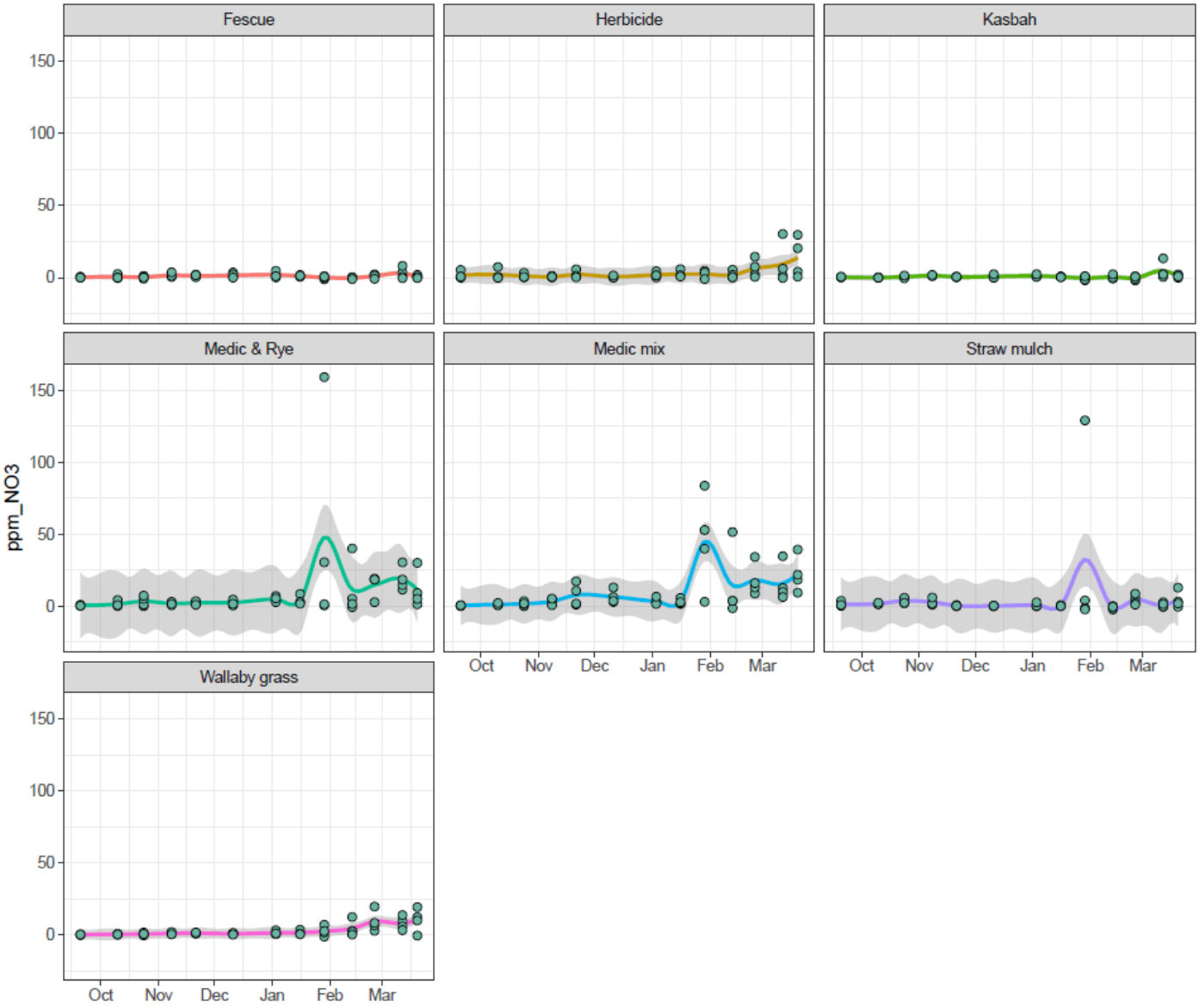


Figure 86 - Soil nitrate, Langhorne Creek, Vintage 2019



Figure 87 - Soil nitrate, Langhorne Creek, Vintage 2020

Leaf Blade Analysis

Leaf blade Ca levels were within the acceptable normal range of 1.2 to 2.5% (Weir & Cresswell 1993). In 2019 the concentration in the Medic treatment was lower than most all other treatments (Figure 88), and among the lowest in 2020 (Figure 89). In the 2020 season, Ca was highest in the Kasbah treatment.

The Cl levels in the leaf are all within acceptable levels of 0.1 to 0.8%. The two high water use cover crops (Kasbah and Fescue) show higher leaf Cl levels than the straw mulch. This is likely to be a function of the reduced soil moisture availability on the perennial grass treatments, while the smaller canopies they have produced may also have increased the concentration of Cl.

The leaf magnesium levels from 2019 (Figure 92) and 2020 (Figure 93) are well below the normal range of 0.3 to 0.6%. Leaf manganese was significantly higher for Kasbah than all other treatments in 2019 (Figure 94) while in 2020 no significant difference was observed (Figure 95).

The normal range for leaf N is from 3-5% (Weir & Cresswell 1993). In both 2019 (Figure 96) and 2020 (Figure 97) seasons, the Medic treatments provided the highest level of N, but the Medic growth was been poor compared to earlier years, so the results noted above are likely the residual N and mineralised N coming from the labile organic carbon.

While leaf P was variable among treatments, some patterns were apparent. For example, over the two years, leaf phosphorous was higher on the straw mulch treatment than the Medic mix (2019) (Figure 98) and the control and Fescue treatments in 2020 (Figure 99). The straw treatment is nearly in the normal range of 0.25 to 0.5%, while all other treatments are below normal but not deficient.

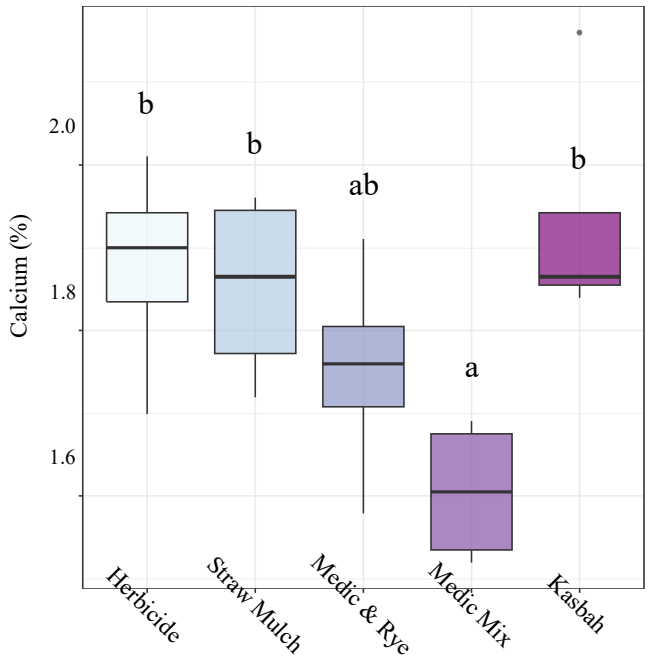


Figure 88 - Leaf calcium, Langhorne Creek, Vintage 2019

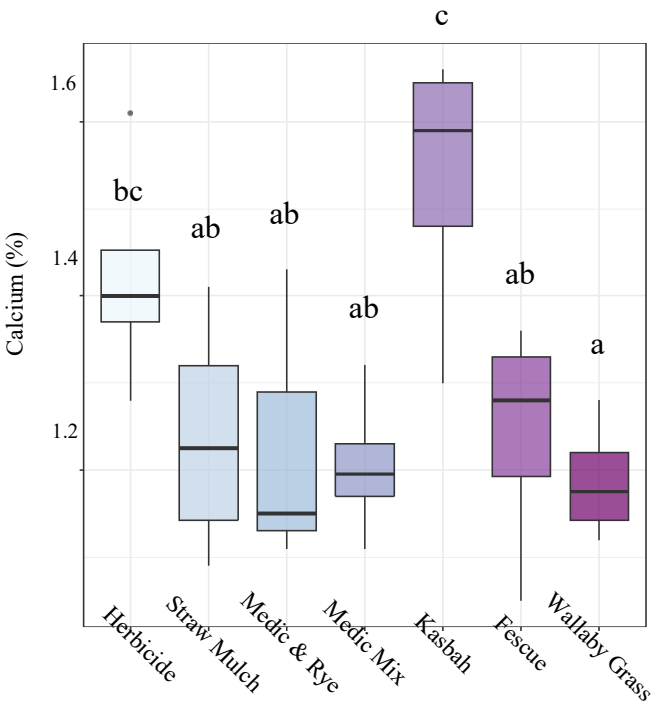


Figure 89 - Leaf calcium, Langhorne Creek, Vintage 2020

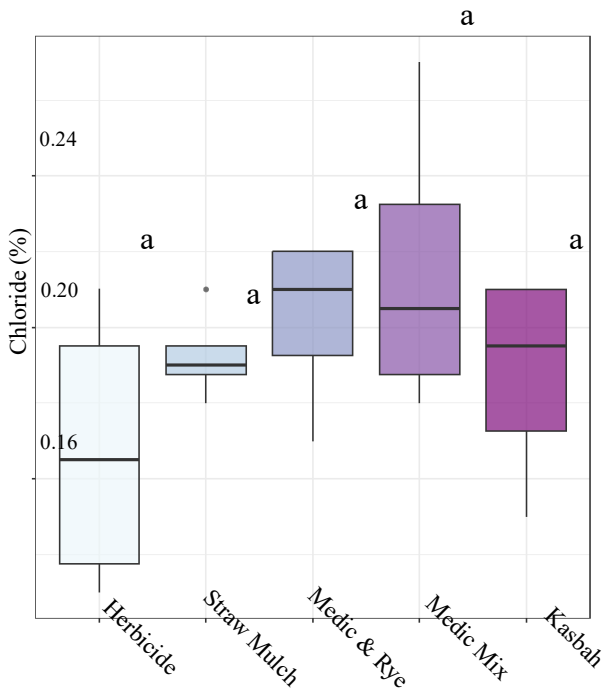


Figure 90 - Leaf chloride, Langhorne Creek, Vintage 2019

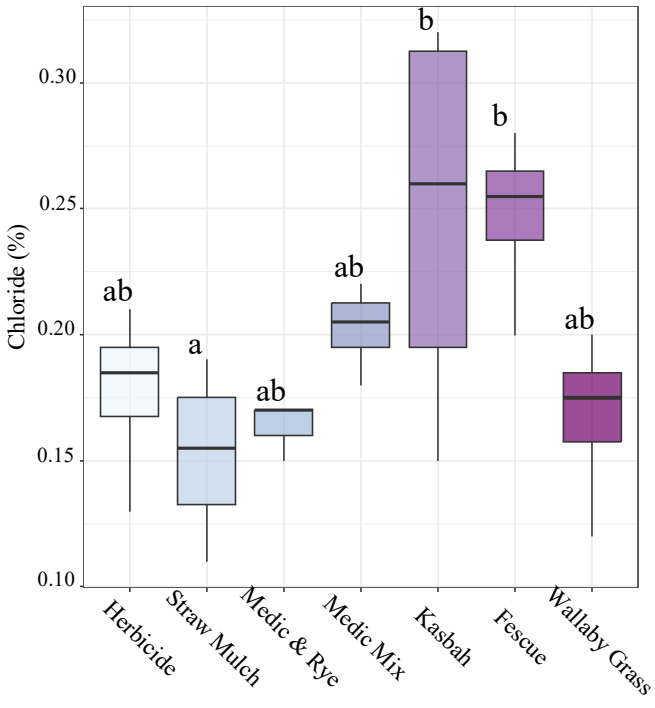


Figure 91 - Leaf chloride, Langhorne Creek, Vintage 2020

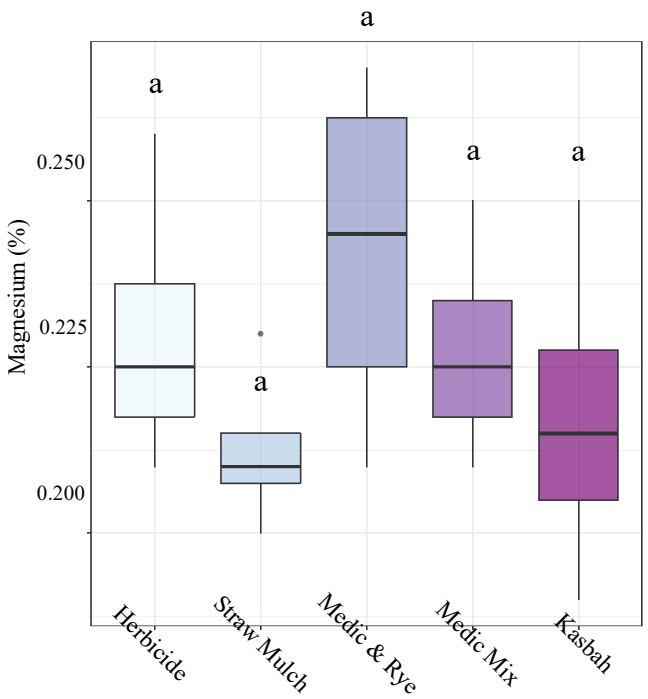


Figure 92 - Leaf magnesium, Langhorne Creek, Vintage 2019

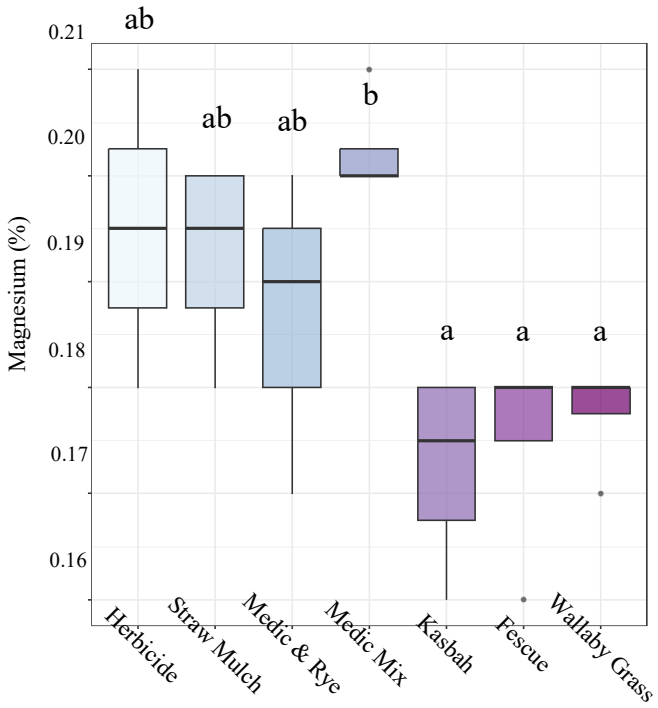


Figure 93 - Leaf magnesium, Langhorne Creek, 2020

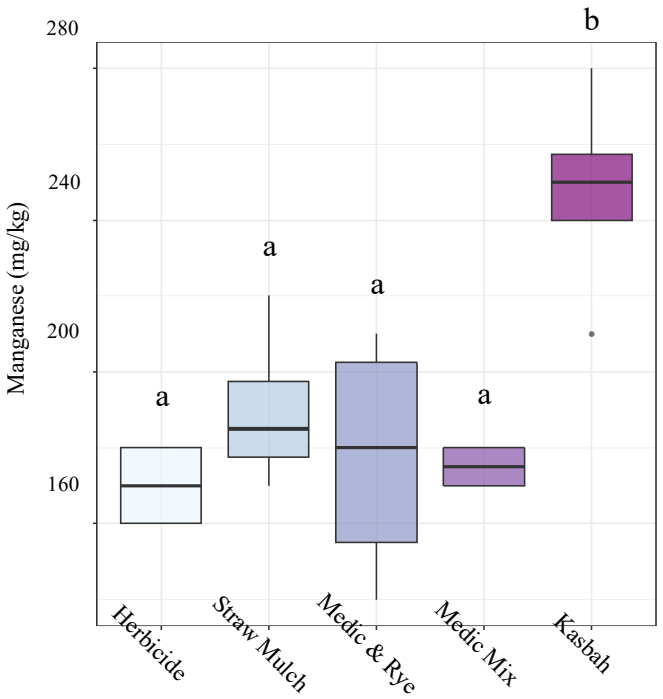


Figure 94 - Leaf manganese, Langhorne Creek, Vintage 2019

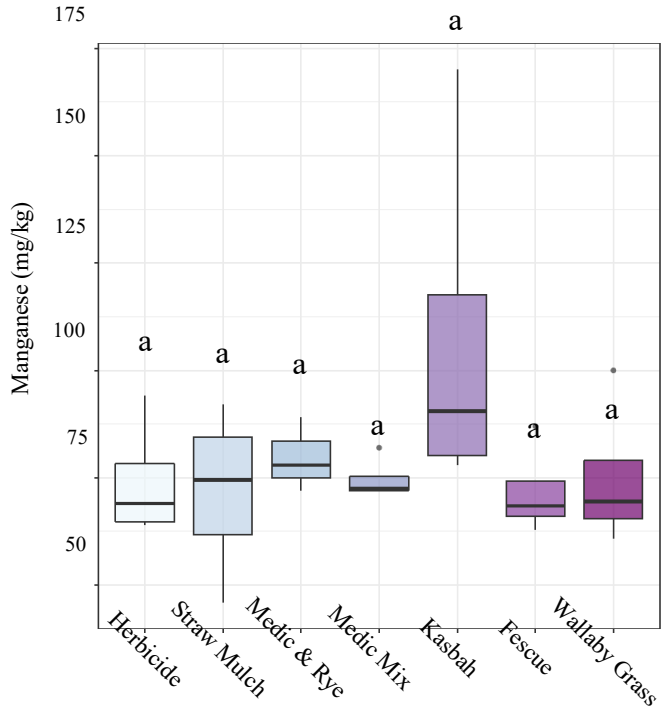


Figure 95 - Leaf manganese, Langhorne Creek, Vintage 2020

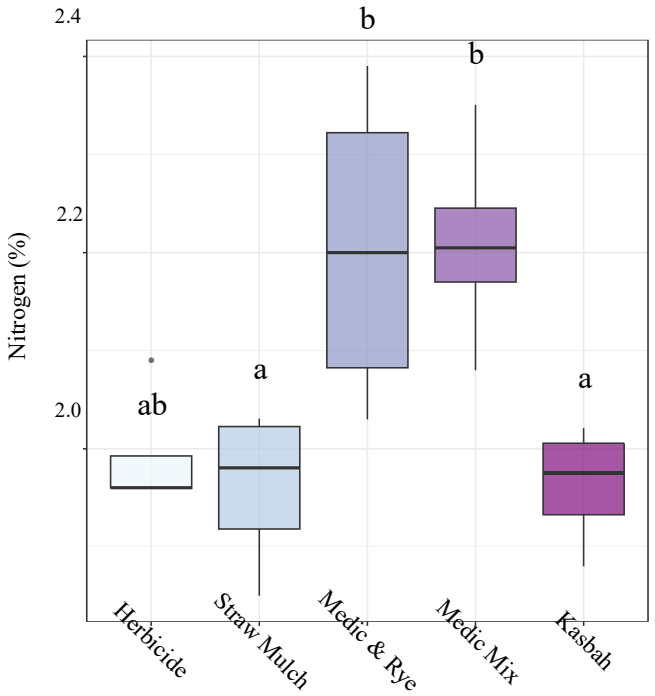


Figure 96 - Leaf nitrogen, Langhorne Creek, Vintage 2019

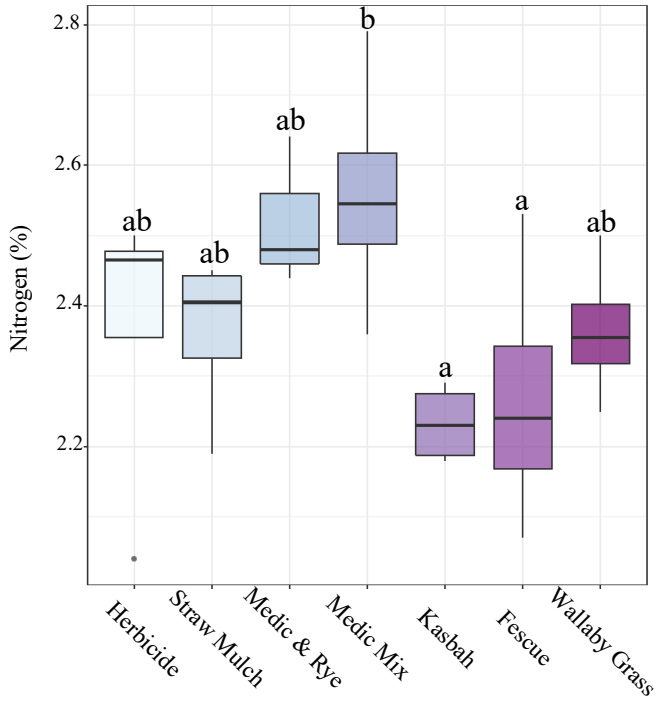


Figure 97 - Leaf nitrogen, Langhorne Creek, Vintage 2020

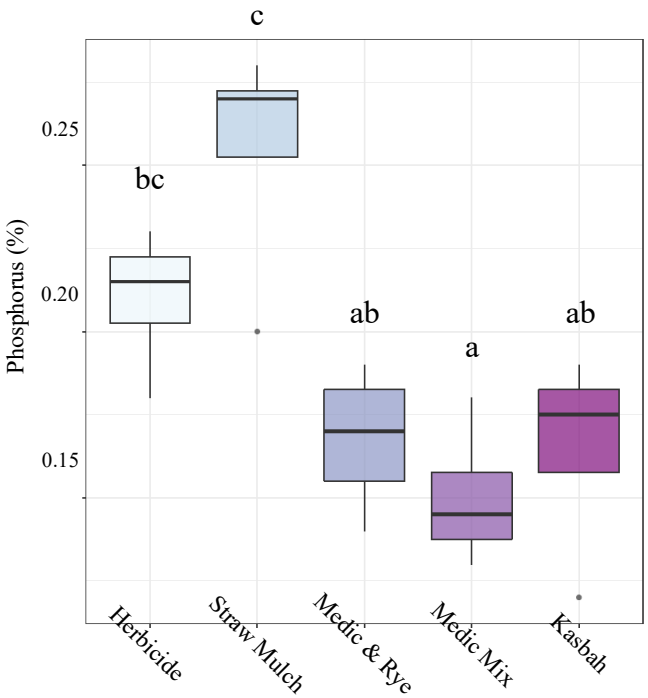


Figure 98 - Leaf phosphorus, Langhorne Creek, Vintage 2019

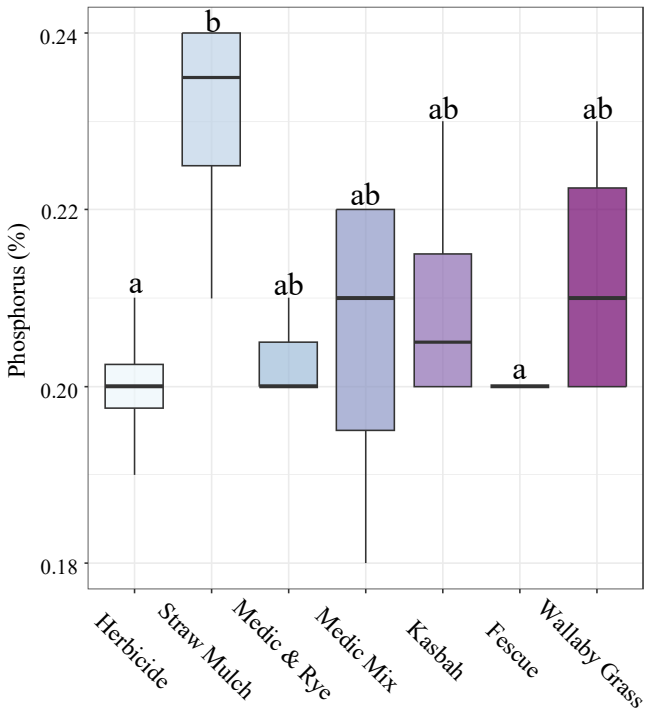


Figure 99 - Leaf phosphorus, Langhorne Creek, Vintage 2020

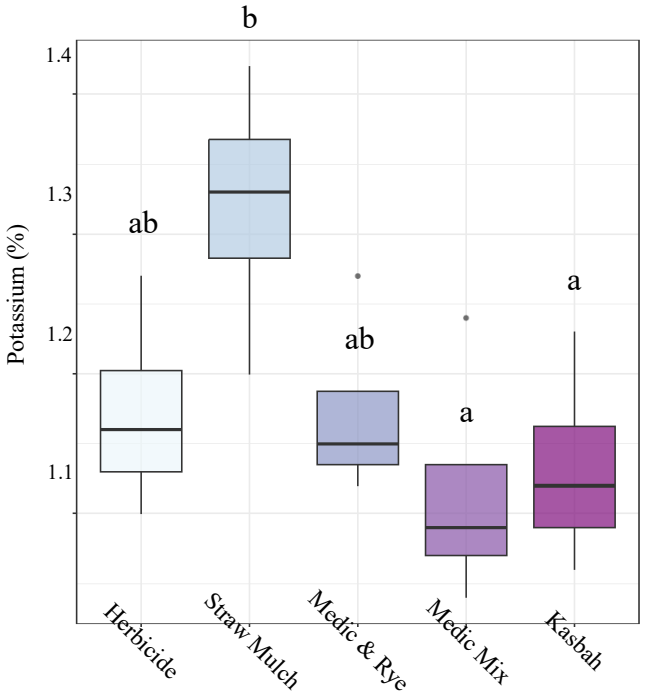


Figure 100 - Leaf potassium, Langhorne Creek, Vintage 2019

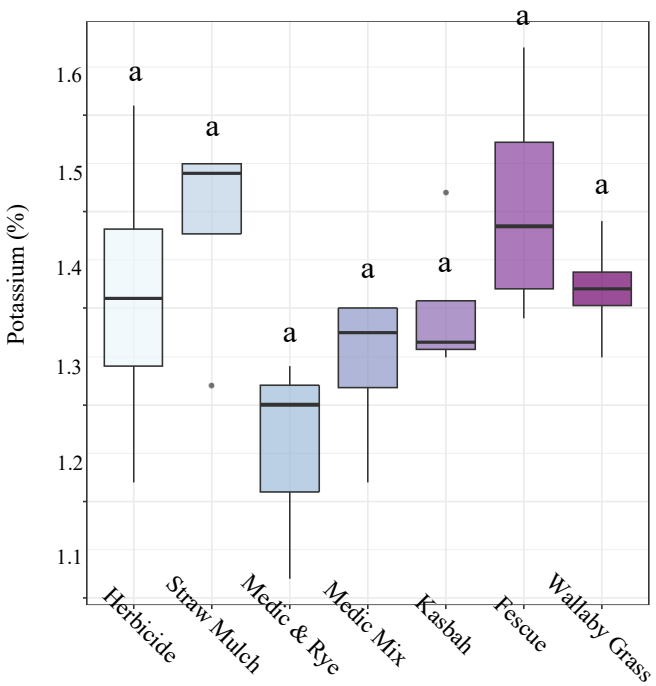


Figure 101 - Leaf potassium, Langhorne Creek, Vintage 2020

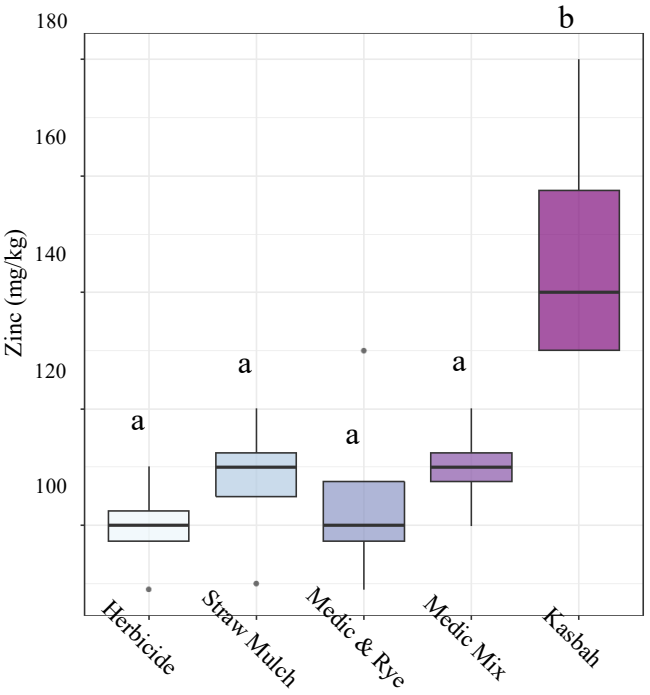


Figure 102 - Leaf zinc, Langhorne Creek, Vintage 2019

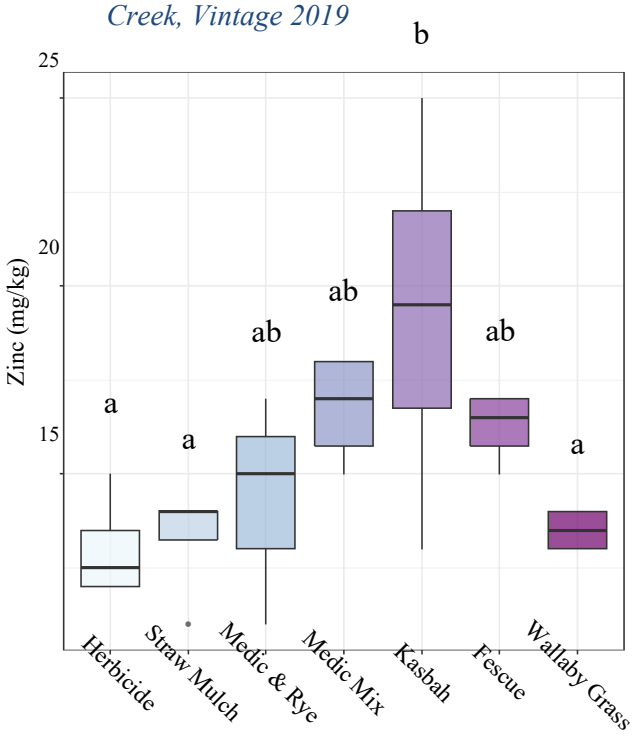


Figure 103 - Leaf zinc, Langhorne Creek, Vintage 2020

Leaf Area Index

The measurements of leaf area index for the 2020 (Figure 105) vintage show the impact of the two exotic perennial grasses on vine growth, whereby leaf area is significantly reduced on the Kasbah and Fescue treatments. For the 2019 season, differences in LAI were non-significant, though the Kasbah treatment still possessed the lowest LAI (Figure 104).

Worms

Worms were significantly more numerous in the Wallaby grass treatment than the herbicide treatment, which possessed the lowest concentration of worms (Figure 106). All other treatments ranged somewhere between these two, though variability meant significance was not reached.

Cumulative temperature

The cumulative temperature measured at the cordon was significantly higher for the Fescue treatment than the Medic, straw mulch and herbicide treatments (Figure 107). This trend is also detectable at the trunk (Figure 108) where the Fescue treatment has a significantly higher cumulative temperature than the Medic treatment, though not the straw or herbicide treatments.

Microbiomes

Faith's phylogenetic diversity, a measure of the relative diversity among treatments was calculated using the NextGen sequencing results. The greater the value of this index, the greater the diversity. No significant differences among levels of diversity were detected in both the 2019 (Figure 109) and 2020 (Figure 110) vintages. Taxa summary plots to order level can be seen in Figure 111.

Pruning Weights

In 2020 no pruning weights were measured at Langhorne creek due to early and unannounced pruning, prior to weights being taken. Though there were no significant differences in 2019, a trend, similar to that seen at Nuriootpa was present, whereby the Medic mix treatment has the highest per-vine pruning weight, while the Kasbah and Fescue possess the lowest.

Juice chemistry

Juice pH did not differ significantly in 2019 (Figure 112) but was significantly higher for the Fescue treatment in 2020 (Figure 113). Titratable acid (TA) was again, not significantly different in 2019 (Figure 114), but was significantly lower for the Fescue than most other treatments in 2020 (Figure 115).

Juice total tannins were highest for the Fescue and Kasbah treatments, while juice from the Medic mix treatment possessed a significantly lower tannin content (Figure 116).
Yeast assimilable nitrogen (YAN, Figure 117) was significantly higher for both the Medic mix and Medic and Ryegrass treatments relative to the herbicide and wallaby grass treatments. YAN is the sum of all available N in the juice, and its components, such as alpha amino N, and ammonia can be seen in appendix 5.

Yield and berry metrics

Individual bunch weights were highest for the wallaby grass treatment in 2019, though this was non-significant (Figure 118). In 2020 (Figure 119) the Fescue bunch weight was significantly lower than the herbicide, Medic mix, Medic and Ryegrass and straw mulch treatments.

Yields did not significantly differ in 2019 (Figure 120), while 2020 saw a significantly lower yield for the Fescue and Kasbah treatments. Importantly the Medic mix, Medic and Ryegrass and straw mulch treatments were not significantly different from the herbicide control (Figure 121).

Botanical Analysis

In 2019, the Fescue, Kasbah and wallaby grass maintained dominance, with very few other species present (Figure 122). Medic mix and Medic and Ryegrass treatments showed lower biomass relative to the 2020, but did not contain as many weeds.
In 2020, invasive grasses began to colonise the Medic treatments, however this did not diminish the Medic species' biomass, which was again approximately 1000 kg per hectare (Figure 123). Biomass for the grasses was lower than the previous year, though they maintained their monoculture composition closely.

Wine making

Wines were made using the Cabernet Sauvignon grapes from the Langhorne Creek field site. Two reps per treatment as well as one wild ferment were made. Half of the wines made using the commercial yeast, as well as the wild yeast wines were sent to the National Amateur Wine and Beer Show for a tasting analysis. The overall results can be seen in Table 7 below. Two winemakers from the AWRI also provided their time and provided a basic analysis of all the wines, as well as a comparison between the replicates. See Table 8 and Table 9.

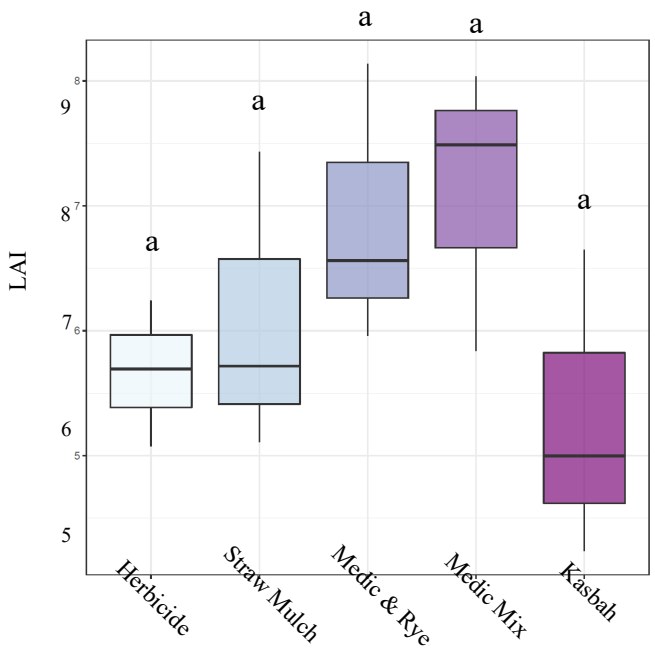


Figure 104 - Leaf area index 29-01-2019, Langhorne Creek, Vintage 2019

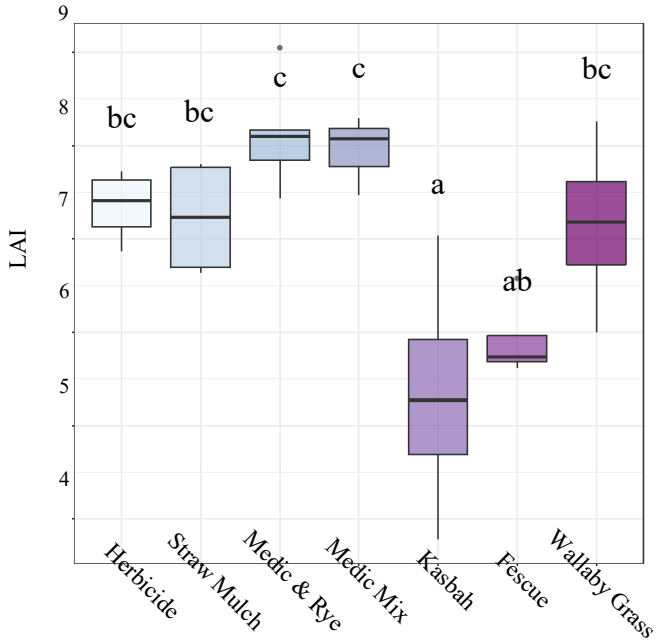


Figure 105 - Leaf area index 2020-01-28, Langhorne Creek, Vintage 2020

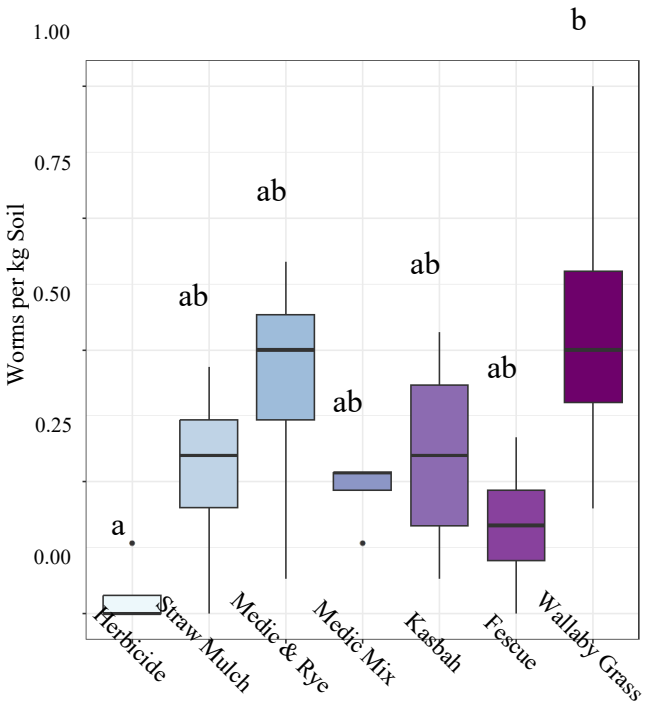


Figure 106 - Worms per kg soil, Langhorne Creek, Vintage 2020

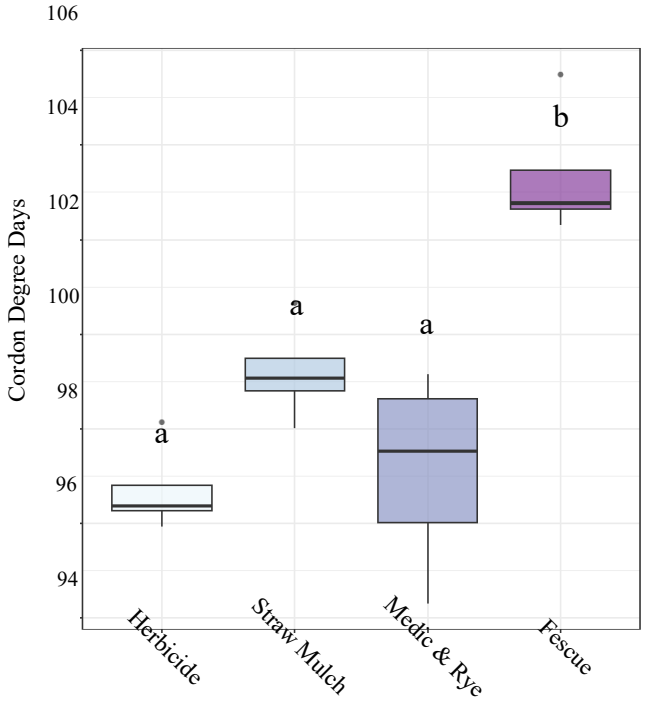


Figure 107 - Cordon degree days, Langhorne Creek, Vintage 2020

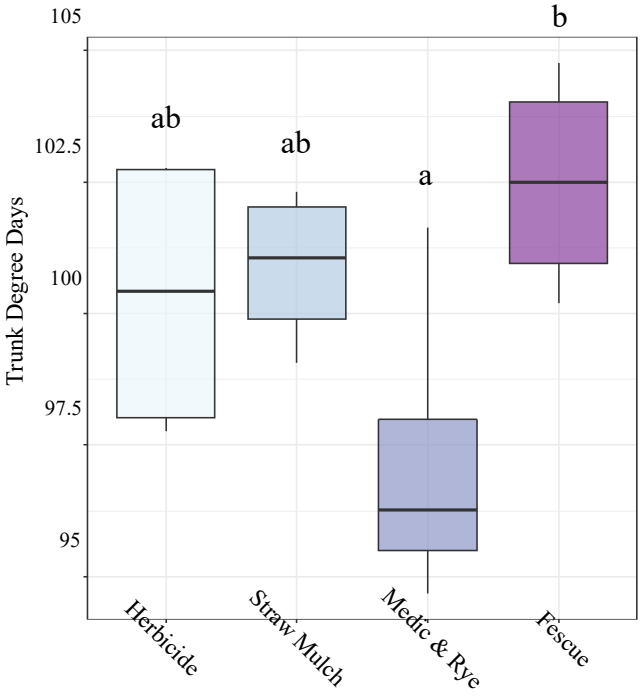


Figure 108 - Trunk degree days, Langhorne Creek, Vintage 2020

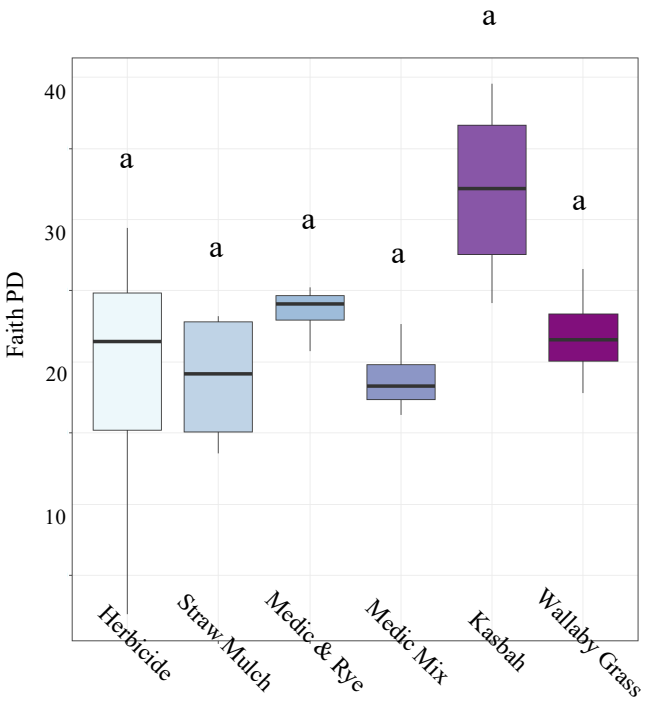


Figure 109 - Faith's phylogenetic diversity, Langhorne Creek, Vintage 2019

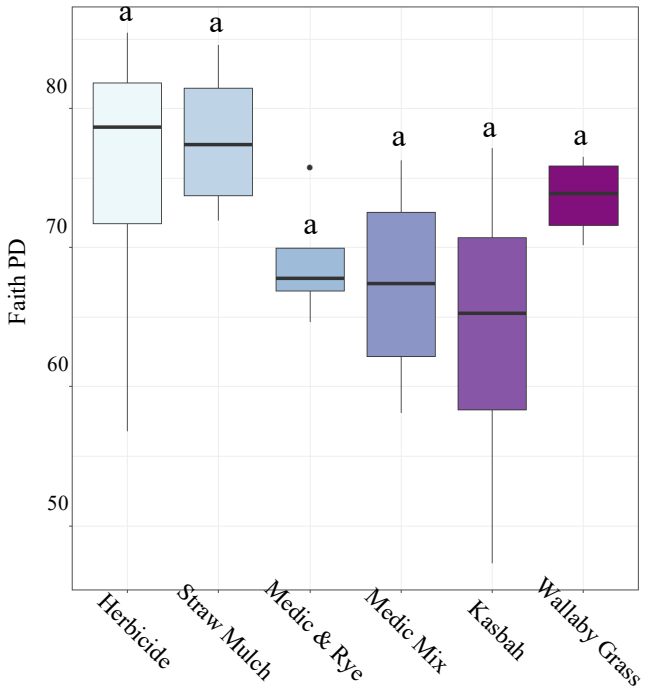


Figure 110 - Faith's phylogenetic diversity, Langhorne Creek, Vintage 2020

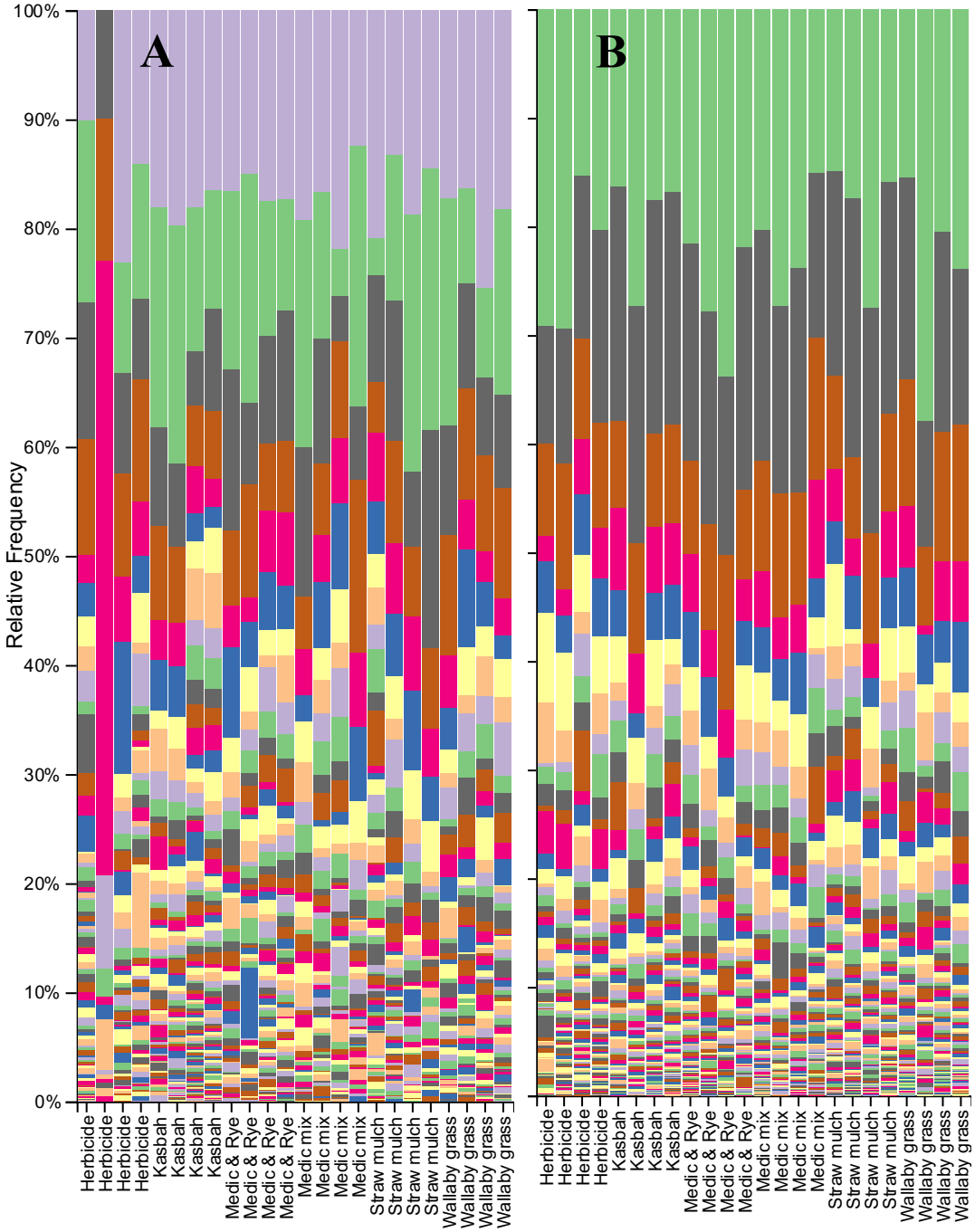


Figure 111: Langhorne Creek taxa summary bar plots of relative frequency vs treatment (to order level). A) From July 2018, B) From July 2019. N. B. each colour bar represents a distinct taxon at the Order level.

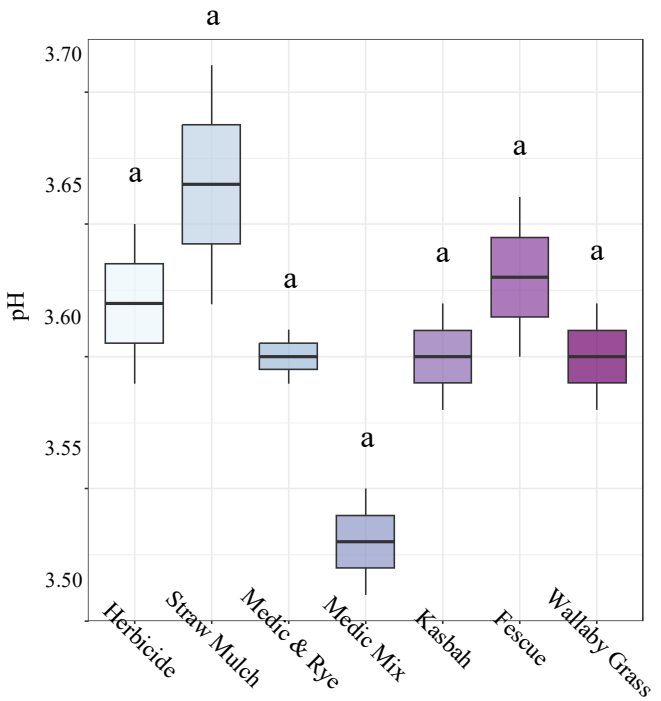


Figure 112 – Juice pH, Langhorne Creek, Vintage 2019

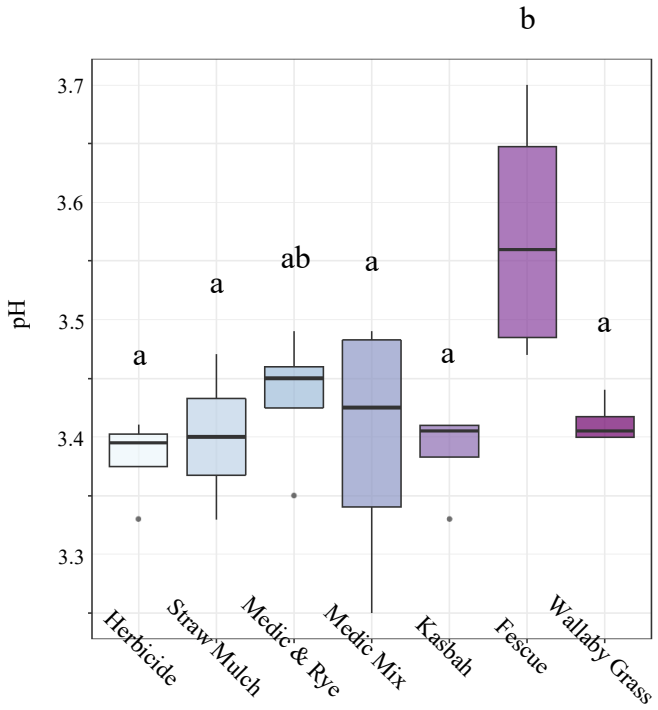


Figure 113 - Juice pH, Langhorne Creek, Vintage 2020

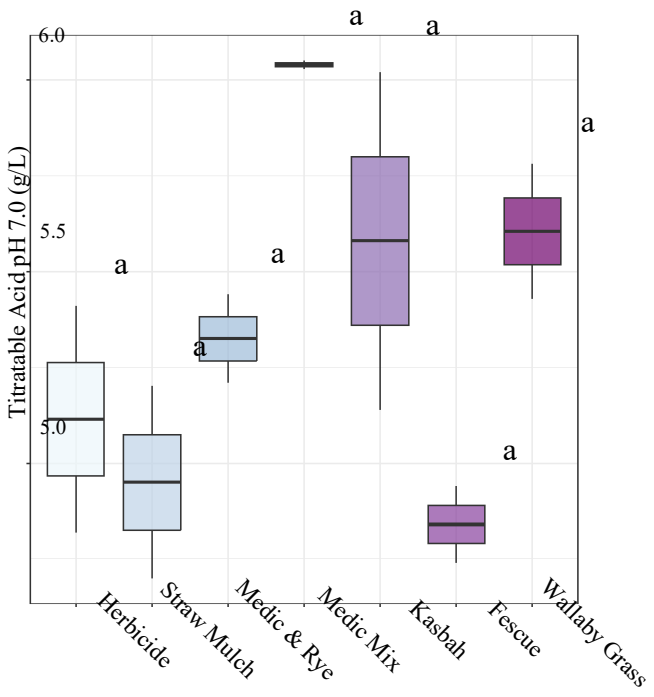


Figure 114 - Juice titratable acid, Langhorne Creek, Vintage 2019

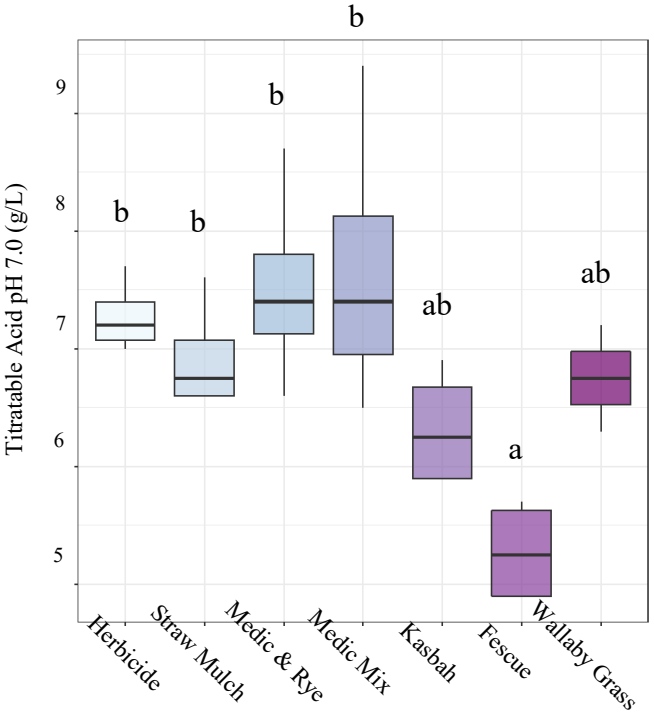


Figure 115 - Juice titratable acid (pH 7.0), Langhorne Creek, Vintage 2020

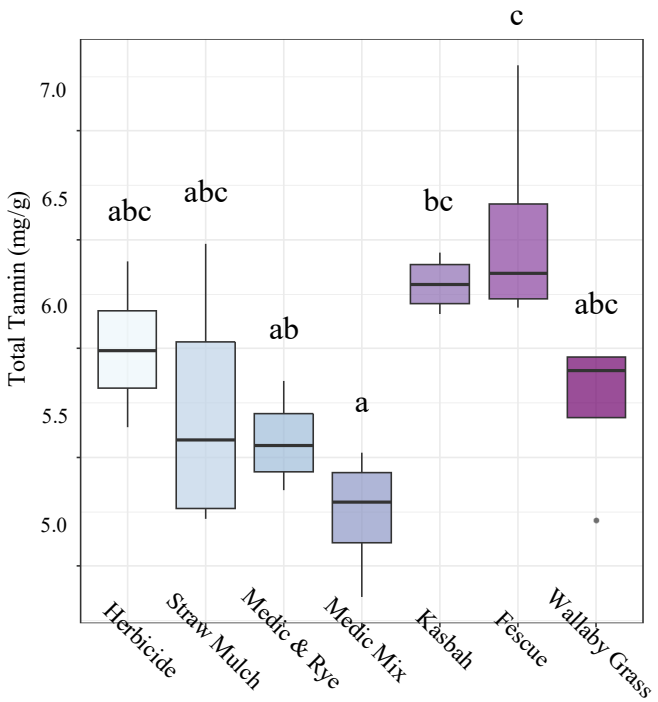


Figure 116 - Juice total tannin, Langhorne Creek, Vintage 2020

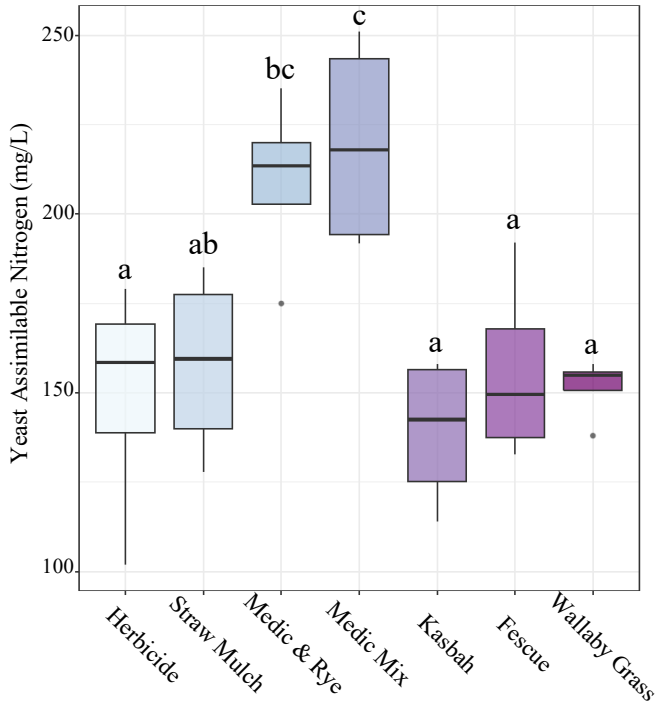


Figure 117 - Juice yeast assimilable nitrogen (YAN), Langhorne Creek, Vintage 2020

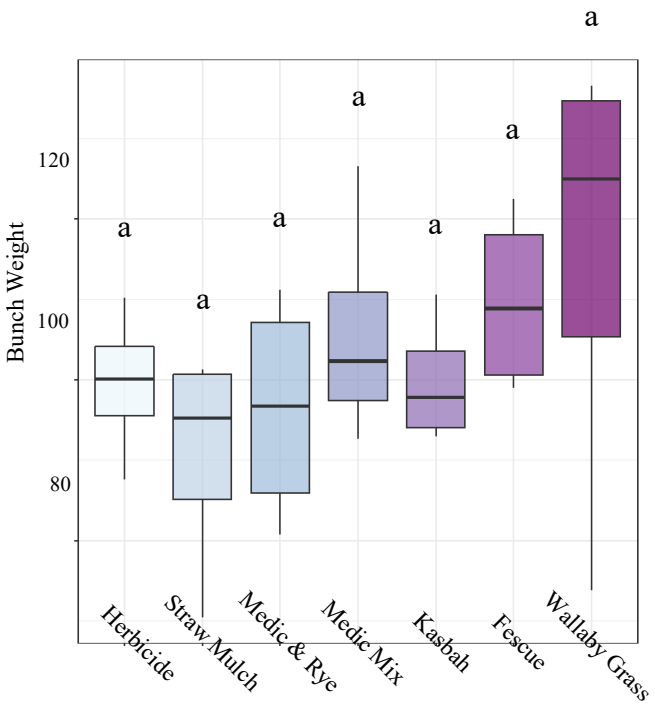


Figure 118 - Bunch Weight, Langhorne Creek, Vintage 2019

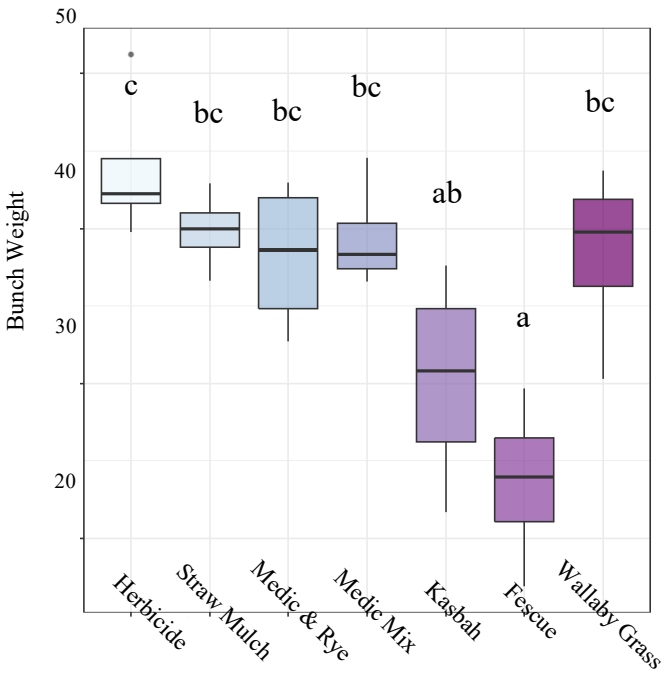


Figure 119 - Bunch Weight, Langhorne Creek, Vintage 2020

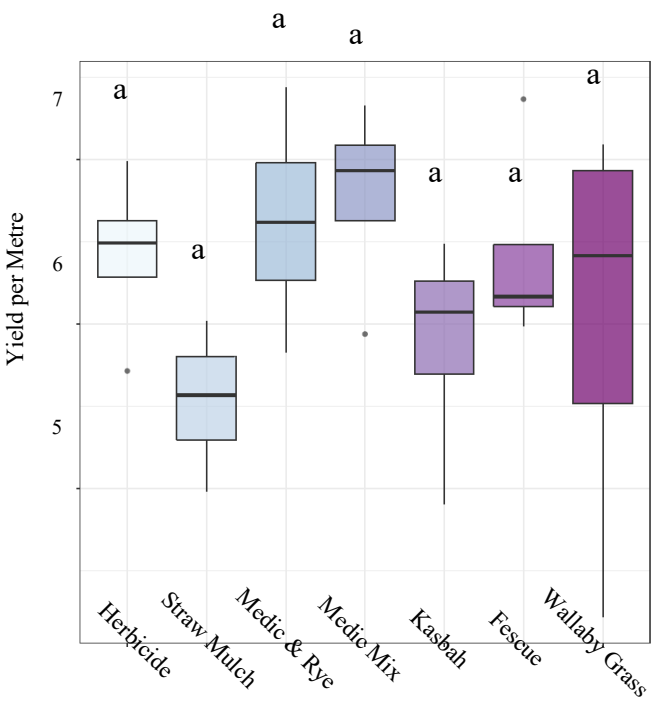


Figure 120 - Yield per metre, Langhorne Creek, Vintage 2019

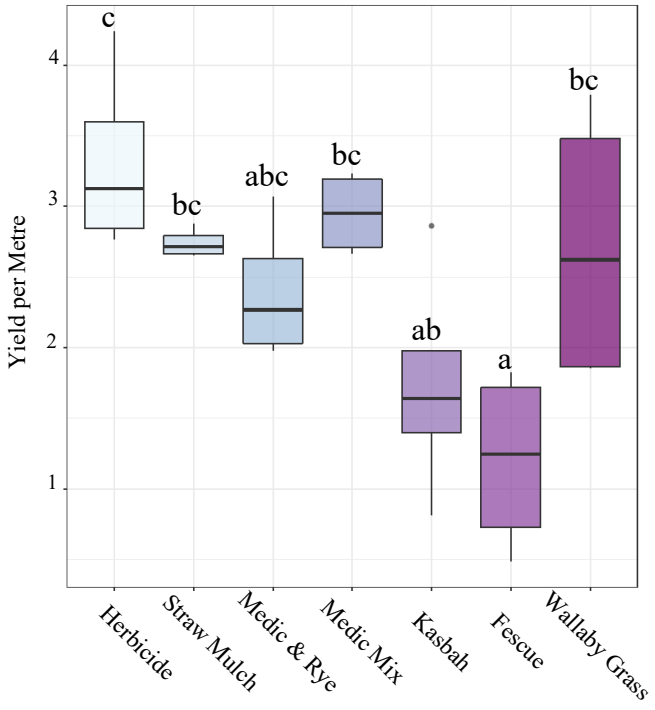


Figure 121 - Yield per metre, Langhorne Creek, Vintage 2020

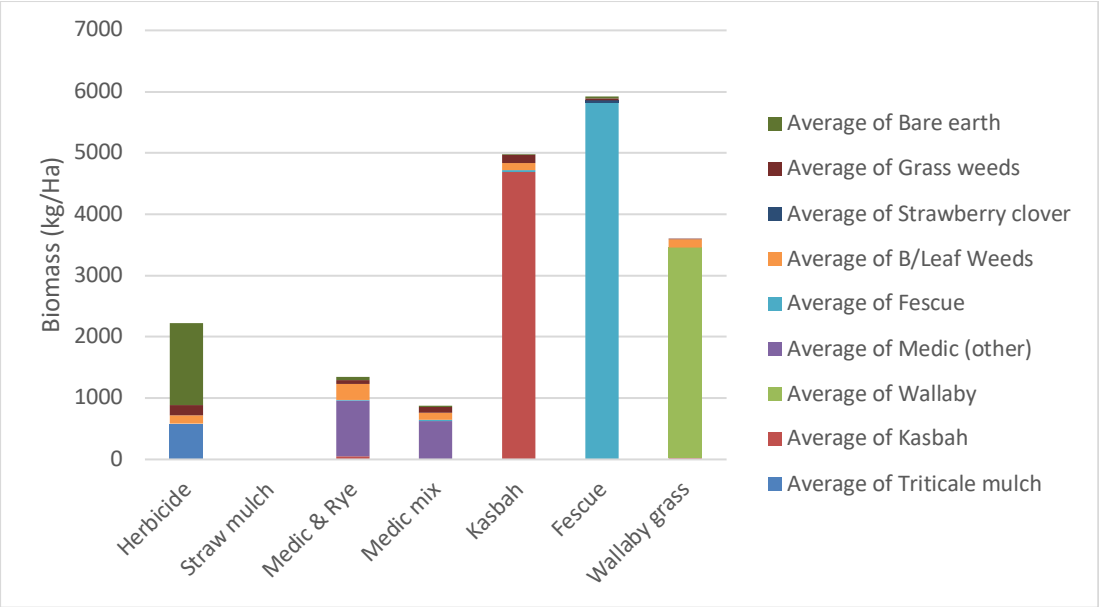


Figure 122: Botanical composition of under-vine cover crops, Langhorne Creek, February, 2019.

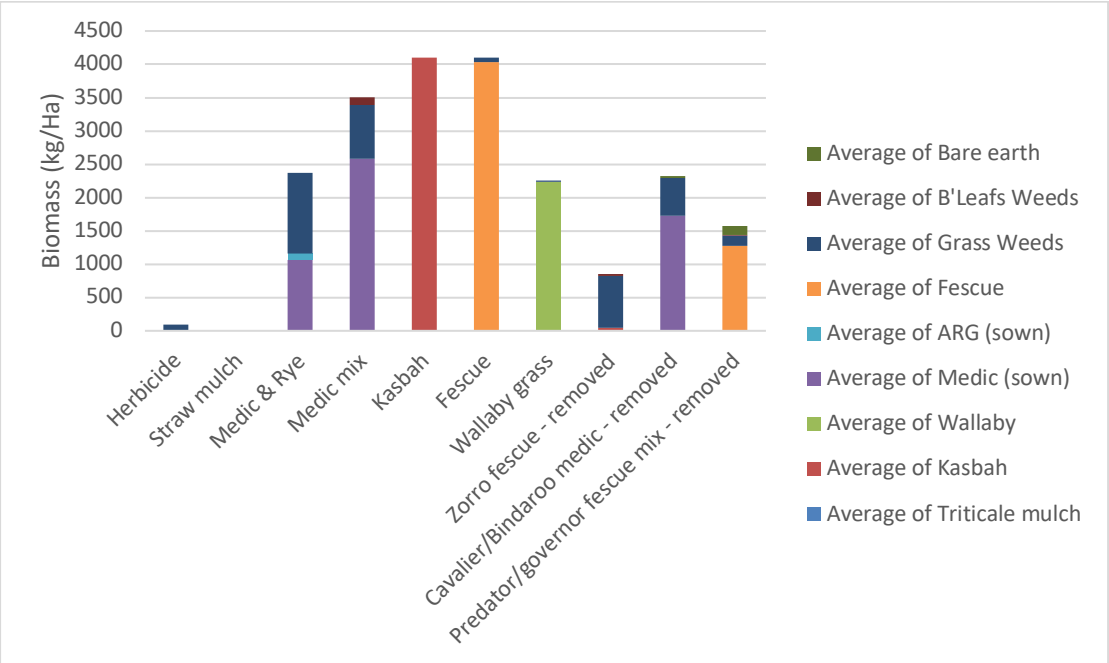


Figure 123: Botanical composition of under-vine cover crops, Langhorne Creek, September, 2019.

Table 7: Australian national amateur wine awards results.

Treatment	Yeast	Score out of 20	Medal
Straw mulch	Commercial	16	Bronze
Kasbah	Commercial	15	-
Wallaby grass	Commercial	15	-
Medic mix	Commercial	14	-
Medic & Rye	Commercial	15	-
Fescue	Commercial	13	-
Herbicide	Commercial	15.5	Bronze
Straw mulch	Wild yeast	16.5	Bronze
Kasbah	Wild yeast	15	-
Wallaby grass	Wild yeast	13	-
Medic mix	Wild yeast	16	Bronze
Medic & Rye	Wild yeast	17	Silver
Fescue	Wild yeast	15.5	Bronze
Herbicide	Wild yeast	12	-

Table 8: Tasting notes from taster 1, of all wines produced from Langhorne Creek after the 2019 vintage.

Tasting order	Treatment and rep	Colour and Condition	Nose	Palate	Score for Taint/Fault (0-9)
1	Herbicide B	Mid red with purple hue	Low intensity plum, savoury notes, dry grass, some herbal	Moderate fruit intensity, herbal, dry grass, straw character quite dominant Some length	
2	Herbicide A	"	Very similar nose to #1	Very similar palate. Slightly softer structure.	
3	Medic mix A	Light to mid-red with brick red hue	Moderate fruit intensity, red berries, cherries slightly lifted, some grass/herbal in the background	Low to moderate flavour, good acid, rhubarb acid and flavour, more fruit, drier palate. Ok length.	
4	Medic mix B	"	"	"	
5	Wallaby grass A	Mid red with purple hue	Low intensity fruit, liquorice, dark berries, seaweed	Good flavour and structure, good acid and tannin. Shows CAB character. OK length.	
6	Wallaby grass B	"	Low to moderate intensity, dark berries, some herbal tomato leaf	Similar palate but more dry grass, herbal, plummy, and a touch shorter	

7	Medic & Rye B	Mid red with brick red hue	Low intensity fruit, slight herbal, leafy notes, low red fruit	Low to moderate flavour, nothing too exciting, ok acid, lighter bodied, bit short.	
8	Medic & Rye A	"	"	" very similar palates	
9	Fescue A	Mid red with purple hue	Strong aldehyde and oxidation. Little fruit	Short, oxidised, aldehyde obvious	Aldehyde 7
10	Fescue B	Mid red with ruby hue	Low intensity, slight herbal, leafy notes, hints of oxidation.	Low intensity flavour, slight oxidation, short	
11	Straw mulch A	Mid red ruby hue (bright)	Low to medium intensity plums and dark fruits, herbal, dry grass in background	Good rich fruit with good acid and texture. Tannin is firm and gives length. Good CAB Character	
12	Straw mulch B	"	"	" But has some aldehyde too! Shame!	Aldehyde 2
13	Kasbah A	Mid red with ruby hue	Low to mid intensity leaf, herbal, grass, some red fruits	Moderate intensity flavour, some herbal character, ok acid. Some length. Touch hollow on back.	
14	Kasbah B	"	"	"	
15	Medic & Rye Wild yeast	Mid red, brick red hue (dull)	Ripe? Plum, partly raisin like, leafy	Follows to palate, ripe, porty, developed. Slightly bitter? Herbaceous finish	
16	Straw mulch Wild yeast	Mid red with brick red hue (dull)	Dull nose, porty, but herbaceous too, odd.	Fuller, low fruit flavour, lacks vibrancy, short.	
17	Medic mix Wild yeast	"	Meaty, beef fat nose, dirty nose	Slight minty flavour, but that about all, short.	
18	Kasbah Wild yeast	Mid red	Low to moderate intensity red fruits, ripe plum, grassy	Follows to palate, some vibrancy but falls away quickly. More texture and grip	
19	Herbicide Wild yeast	Poor colour, mid red with brick red hue	Dull, developed grassy, hay	Developed low fruit flavour, short	
20	Wallaby grass Wild yeast	Light to mid-red with brick red hue	Low fruit aroma, showing development.	Low flavour, short	
21	Fescue Wild yeast	Light to mid-red with brick red hue	Forward porty, aldehyde	Short, lean oxidised	

Table 9: Tasting notes from taster 2 of all wines produced from Langhorne Creek after the 2019 vintage.

Tasting order	Treatment	Colour and Condition	Nose	Palate	Score for Taint/Fault (0-9)
1	Herbicide B	Dark red/purple, dense	Medium aroma intensity, currants, floral, chocolate, herbal, slight reductive note	Medium body, low flavour concentration, low tannin	
2	Herbicide A	"	Similar to 1, slightly cleaner	Similar	
3	Medic mix A	Less dense, more red, brown tinge	Lifted, green and herbaceous, varietal, but not particularly fruity	Medium body, low flavour, higher acid, bitter to finish	
4	Medic mix B	Lighter/less dense than 3	Lower aroma intensity than 3, slightly reduced	Lighter in body to 3, similar bitter finish	
5	Wallaby grass A	Dense, dark red/purple. Denser than 1	Medium aroma intensity, currant, floral, pretty, varietal, blue fruit note	Med + body, some flavour concentration, subtle tannin, better balanced	
6	Wallaby grass B	"	Slightly more herbal, but similar.	Less concentrated than 5, similar tannin structure	
7	Medic & Rye B	Slightly more red than 1, darker	Similar to 6, ripe cabernet fruit with herbal note	Similar palate to 5 – some flavour concentration, subtle tannin	
8	Medic & Rye A	Similar	Similar	Similar	
9	Fescue A	Very dense, dark red, purple	Very aldehydic	DNT	7 – Aldehyde
10	Fescue B	Less purple than 9	Lower aromatic intensity, sulphur note (burnt match), touch of aldehyde	Broad, concentrated flavours, viscous	
11	Straw mulch A	Very similar to 1	Ripe cabernet aromas, cassis, blue fruits, touch of herbal	Savoury palate, almost salty, lower acid, very little tannin	
12	Straw mulch B	More purple	Aldehydic	DNT	5 – Aldehyde
13	Kasbah A	Similar to 1	Mix between ripe and herbal aromas	Medium body, savoury palate, but balanced	

14	Kasbah B	Bit more dense, touch more purple	Touch of aldehyde, ripe fruits, cassis	Broad, similar to 13	2 – Aldehyde
15	Medic & Rye Wild yeast	Lighter dark red, touch of brown	Herbal, slightly reductive, mustard seed, VA	Low flavour, sour, prickly acidity	3 – VA
16	Straw mulch Wild yeast		BIG VA	DNT	5 – VA
17	Medic mix Wild yeast	Lighter still	Very herbaceous, touch of volatility	Medium body, viscous, hard, sour acid	2 – VA
18	Kasbah Wild yeast	Darker than 17	Aldehyde, volatility	Broad, some flavour, sour acid	2 – ALD 2 – VA
19	Herbicide Wild yeast		Big VA	DNT	5 – VA
20	Wallaby grass Wild yeast		VA		
21	Fescue Wild yeast	Red/brown, light	Medium aroma intensity, blue fruits, herbal, red fruit	Thin attack, sour, lacking concentration, sour finish	

Discussion - Nuriootpa

Discussion of the data from Nuriootpa across the 2019 and 2020 vintages.

Water stress and soil moisture

Competition for water is frequently cited as the primary reason for using herbicide to prevent the growth of any plants in the under-vine space. Perhaps the key finding from this research is that, depending on the species, growing under-vine cover crops will not lead to water stress.

The straw mulch and Kasbah treatments are at two ends of the spectrum for water use. There were no obvious differences among soil moisture profiles of each treatment. That is, we found no clear negative impact of having a cover crop below the vine in terms of soil moisture. This absence of significant effect could be partly due to the variation induced by the rocky soils, however it is also possible that the under-vine space has little influence on soil moisture in comparison to the large mid-row area, as well as connectedness of adjacent rows and treatments. Differences in soil moisture may become more apparent in larger trials where there is less confounding influence. Despite this, the perennial Kasbah and Fescue treatments did lead to differences in canopy size, suggesting some level of interference with the development of the vines.

Despite not observing any differences via the capacitance probes, at Nuriootpa, the treatments did experience differing water stress as measured by the pressure bomb. Across both seasons the herbicide control experienced higher water stress than all other treatments. Although the herbicide control was similar in canopy size to the straw mulch and Medic treatments, a very low stem water potential implies that the canopy was at the limit of the water that could be supplied by its roots. According to Girona *et al.* (2006) midday leaf water potentials of -8 bar (-0.8 MPa) represents a well irrigated vine, -12 bar are moderately stressed vines and at -15 bar vines are in a severe stress situation. In 2020, the herbicide treatment was nearing the severe stress status, while the Medic and Ryegrass treatment was not moderately stressed. An excessively large (and unbalanced) canopy leaves the vine vulnerable to water stress on hot days, though the under-vine cover crop treatments were able to sustain the vines through these periods without stress. Taken together, the large canopy (i.e. more water demand) and

poorer soil water availability in the herbicide treatment (either due to evaporation or poorer root development), help explain this important result. It could thus be suggested that as a result, water applications could be reduced for these treatments.

Emerging from these figures is the reason the cover crops with annual Medics and grasses has performed as well if not better than the herbicide treatment. The re-introduction of annual plants including N-fixers may have led to improved root systems, as well as soils with their associated micro-biomes and earthworms, which when combined, have improved soil structure and decreased the soil strength in the top 20 cm, as shown by the penetrometer data (see below). With decreased soil strength, the vine root access to soil moisture and nutrients is increased, so the vine is not becoming moisture stressed in the same way that the herbicide treatment is.

Soil

The results of this trial demonstrate that the physical properties of the soil have undergone significant change. Soil pH can affect the availability and mobility of other critical nutrients; thus, it plays an important role in soil health. Salinity is a common problem in Australian vineyards (Stevens et al. 2011), in particular where bore or saline river water is used. Lanyon *et al*. (2004) note the importance of soil physical properties to vine performance. Variations in these properties underpin the benefits of the carefully selected cover crops over the use of herbicide.

Data presented here shows a reduction in salinity in all under-vine treatments using relative to the herbicide strip. This suggests that in soils of this type, if soil salinity is an issue, under-vine cover cropping may be a solution. The strong correlation between soil Na and salinity would suggest that Na is the principle driver behind the soil salinity shown above. With respect to soil hardness, Lanyon *et al*. (2004) state it is the combination of water supply as well as soil hardness (the critical penetration resistance) that limits root access to soil water creates vine water deficits. The non-limiting water range (NLWR) is characterised by the upper limit being controlled by soil macro-porosity and the lower limit by soil strength and water content. Vine root penetration is restricted beyond 2000 kPa (2 MPa) when measured at field capacity with a penetrometer.

When soil cover is provided either as straw mulch or cover crop, soil hardness in the 0-20 cm zone was below the 2000 kPa limit to root growth. The herbicide treatment has likely been losing water to evaporation and/or surface run off and preventing root access to the remaining water contained within its upper profile, effectively wasting that soil moisture. When looking to increase the resilience of viticultural production as rainfall diminishes and summers get hotter, it is critical that the soil is not just used for supporting the vines, but has best management practices employed to ensure it is functioning at its optimum for sustaining vine health and productivity.

Though not of concern in the Barossa Valley, changes to soil pH, specifically the reduction in pH of soil from the Medic treatments, play an important role in the way nutrients are consumed by the vine. The pH of soils from all treatments were close to neutral. Soil pH values in this range are not unusual for vineyards in the region ("Barossa Valley - Grape and Wine Soil Quality" 2020). As noted by Glendinning (2000), the zone around the legume root becomes more acidic (which helps increase the availability of bound nutrients such as phosphorous).

Soil carbon is frequently cited as a key indicator of soil health, increased soil biological activity, as well as helping to offset increasing concentrations of atmospheric CO_2 (Oliver et al. 2013, Riches et al. 2013). In this project, all sources of cover have generated a higher concentration of C in the soil compared to the herbicide control, with the highest relative increase in soil carbon approximately 50% for the Medic and Ryegrass treatment. This result is from 5 years of application of these treatments, and supports the findings of Ball *et al.* (2020). The combination of N from the legumes and the fibrous root system of the grasses generating below ground biomass with an associated microbial community are likely responsible for this increase in soil carbon. It is also worth noting that the abundance of earthworms was also high in the treatment (see above), which given their role in the soil C cycle, may help explain this result.

The European species of worms, which predominate in agricultural zones of Southern Australia, are mostly surface feeders, so they require organic matter accessible above ground. The maintenance of a bare soil surface by herbicide application removes the readily accessible food source for the worms, so numbers are not able to be sustained to the levels

that occur with other cover crop treatments, and in particular the Medic and Ryegrass combination. Earthworms are well recognized for their capacity to improve infiltration and cycle nutrients which are consolidated into their castings. The benefits of earthworms alone to the vineyard productivity cannot be quantified, but they do help improve soil health, as evidenced by numerous authors (Buckerfield & Webster 1996, Paoletti et al. 1998, Thomson & Hoffmann 2007). Gaupp-Berghausen *et al.* (2015) in a glasshouse experiment found glyphosate applied at below label rates reduced both the reproductive and casting capacity of earthworms. In this study, glyphosate was the primary herbicide used on the control treatment to prevent vegetation growth. The significant reduction in earthworms noted on the herbicide treatment is therefore likely to be a function of the direct effect of glyphosate on the worms, and the removal of their food source reducing the nutrients available for their growth and reproduction. It is a significant finding from this research, as it highlights the impact of weed removal in the under-vine zone.

The soils at the sites contained diverse (16S) communities of soil microbes (specifically bacteria and archaea). The soils were dominated by bacteria (compared to archaea) as is to be expected. Interestingly there were significant differences in microbial diversity among the treatments. This was reflected in a higher Faiths phylogenetic diversity index in the Medic and Ryegrass treatment than the herbicide control. It is likely that these differences are due to an indirect effect of the herbicide rather than a direct effect. That is, rather than the herbicide adversely impacting microbes, the effect was via a reduction of plant biomass and thence the quantify and diversity of plant biomass inputs into the soil. This finding is consistent with previous work summarised in a recent review of this topic (Rose et al. 2016). With increasing recognition of the soil microbiome in not only vine performance, but also terroir (Fabres et al. 2017), these findings may have implications that extend beyond soil health alone. It is also worth noting that another aspect of the soil microbiome was investigated in this project and is reported on in Part 2 of this report.

An under-vine botanical assessment was performed to monitor the state (i.e. persistence) of the original treatments. A desirable quality in under-vine cover crops is they will, in water limited environments, provide minimal competition with the vine for water. To achieve that requirement, they must be either winter-active annuals which senesce in spring and exist as a surface mulch thereafter, or they must be summer dormant perennial plants. The data shown

above, which was collected in February 2019, shows the green (and therefore transpiring) dry matter noted on each of the treatments at a time when no competition for scarce water is desirable. The triticale mulch has done an excellent job of precluding weed growth and the herbicide application to the control has also been very effective. On both the Angel/Sultan Medic treatment and the scimitar/ryegrass treatment there was some summer grass (mostly small witch grass [*Panicum capillare*] plants) growing through the Medic vine mulch, but at very low quantities of dry matter. By contrast, the perennials, namely Kasbah cocksfoot, Sheep fescue/strawberry clover and wallaby grass, all have considerably greater quantities of transpiring green leaf, which unless competition is required for constraining growth, is not desirable in vineyards. The reason for inclusion of the perennials in the project was the Kasbah and wallaby grass are both winter-spring active plants and summer dormant. They have not been grown in an irrigated vineyard scenario before, but it soon became apparent when provided with water over spring and summer, growth continued.

The two consecutive dry years impacted the establishment of the clover, but the barley grass has willingly filled the voids and again is utilising the soil N that has been fixed by the legume. Unless managed with selective herbicides, this succession of species will fill bare spaces, and the resources available will be used by plants better suited to the growing conditions presented at the time. Importantly, the legumes were able to set large amounts of seed in the first three years of the trial, and those seeds are lying in wait under the vines for the required conditions to break dormancy. When this occurs, the legumes will again dominate the biomass production with plenty of readily available N, and the cycle will start again. The same is the case for the Angel/Sultan Medic treatment, which in past years has displayed very strong Medic dominance. It is suggested that in this and the prior case, the legumes are acting as pioneer species due to the degraded state of the soil, having low organic matter (and hence mineralizable N) and high soil strength. After they have colonised for 2-3 years, the soil has returned to a better state of health which will support grass growth, which then dominates until the environmental conditions and soil N status dictate that legumes are again required.

Nutrients

Another justification for removing under-vine growth is to reduce competition for nutrients. This has been shown to be reasonable for the competitive grasses, and not true for other

treatments. In both years, the herbicide treatment has significantly higher leaf N than the Kasbah cocksfoot perennial grass. The grass also has high demands for N and is very effectively outcompeting the vines for N by utilising it for their own biomass production. Simultaneously, the vines in the herbicide treatment are accessing mineralised and fertigated N without competition from cover crops. All treatments were close to deficient for N in the leaf tissue, which is consistent with the low levels of plant available N measured throughout the seasons.

As the composition of the under-vine cover crops has changed due to the species succession, favouring grasses over the pasture legumes, the available soil N has declined, which likely impacted on the N availability to the vine. This suggests that some intervention may be required by the vineyard manager with these systems to help ensure the legume cover crops continue to provide the N required by the vines.

Leaf P was in concert with soil P, being lower on the Medic treatments, and according to established standards (0.25-0.4%) low on all treatments in 2020 (Oliver et al. 2013). Soil P levels were adequate however, suggesting a lack of availability for the vines. Of the available P, it is likely much of it has been accumulated in the Medic biomass above ground, and will only be available as that organic matter is mineralised over time. If the legume biomass is exported from the paddock or vineyard, then a net lowering of P will occur, but as in this case where the biomass remains *in situ* there should be no change to soil P. However, as these are recovering soils that had no previous organic matter decomposing on their surface, it is possible that the system is still in flux as it moves towards homeostasis, with a large proportion of P now deposited on the soil surface.

Soil Ca was higher in the wallaby grass than the Medic mix in 2019, with a similar trend remaining in 2020. The low relative Ca in the soil of the Medic mix treatment is possibly due to an increase in mobility of Ca by the reduced pH. In 2020 a lower population of legumes established on the Medic treatments which would have reduced the demand for Ca (see botanical analysis section). Although there are significant differences among treatments, they are of little concern, as the Ca content in the soil remains close to optimum levels. In a vineyard deficient of Ca, however, this should be considered.

Manganese levels were generally low in this soil compared to industry standards of 80 – 140 mg/kg of soil (Lanyon et al. 2004). Grapevines are not generally recognized as being very responsive to manganese, however it is of interest as it provides an understanding of how the influence of soil pH may affect uptake of less mobile metal cations. Manganese was higher in the treatments where legumes were grown under-vine. There are significant differences which are consistent over the two years of sampling. The availability of manganese in the soil increases with decreasing soil pH. That soil pH was lower in the Medic treatments possibly suggests manganese is being mobilised by the legumes. This result was not reflected in tissue analysis, where manganese levels were highest in the straw mulch treatment. Taken together, it is unlikely that the changes in manganese observed here are of concern, but it may be prudent to monitor this in sites where there are concerns. Leaf B (although not measured in the soil), was elevated in the Medic and Ryegrass treatments. This may be related to levels of B in the irrigation water.

According to Treeby *et al*., (2005) soil K levels in excess of 100 mg/kg are recognized as adequate for grape production, which is exceeded by all treatments in these results. Again, legumes have a high demand for K, with about seven times as much contained in the legume biomass (22 kg/t) as there is in grapes. Wheat straw, as used on the straw mulch treatment, contains approximately 10 Kg K/tonne of straw. Grapes export about 3 kg/tonne of fruit. With the straw applied at 55 t/ha, the 550 Kg of K contained in that application will be sufficient to supply about 18 grape harvests at 10 t/ha each. The significantly higher level of K on the straw treatment for both years of sampling is showing the grapevine removal from this treatment has not impacted soil K levels as much as other treatments.

Soil Na was higher for the herbicide than other treatments. Peacock and Christensen (2000) in Nicholas (2004) suggests Na levels below 690 mg/L will not create a problem in grapes. A mean of less than 200 mg/L on the herbicide treatment is well below the critical levels, but it is of considerable interest that very clearly a bare soil treatment generates higher soil Na. This may be a function of poorer soil porosity so the Na does not leach from the surface soil as effectively. It is also possibly a function of the lower soil organic matter on this treatment which may act as a chemical buffer for the Na on the other treatments.

Acceptable leaf Na levels normally range from 0.05 to 0.1 %, with 0.4% being above normal, so the level found across these treatments in the 2020 vintage is higher than desirable, though not in 2019, possibly due to the higher quantity of winter rains of 2018. An inverse relationship exists between soil and leaf Na levels, while leaf Cl and Na are obviously similar (see soil data above). As the only variable between treatments is the cover crop, it is suggested that as with Cl, the Na is not as accessible to the vine roots on the herbicide and straw mulch treatments, possibly due to changes in soil structure and infiltration characteristics, or due to the soil chemistry causing the salt to be more tightly bound to soil particles.

As mentioned above, the reduced soil pH seen in the Medic treatments potentially leads to mobilisation of cations, which may lead to higher rates of their uptake. Contra to this trend, the high soil manganese observed for the Medic treatments (see above) did not translate to a high leaf manganese content, while relatively high leaf manganese was observed in the straw mulch treatment. Desirable manganese levels for leaf blades are suggested to be from 40 – 150 mg/kg so the grass treatments in particular are at marginal levels. Deficiency of manganese is most likely to occur on sandy, calcareous soils which are not those found at this site. The higher soil K levels of the straw treatment have been reflected in the leaf blade as well. Particularly in vintage 2019 and less so in vintage 2020. The levels noted are in the desirable range for this element.

A difference in leaf Cl levels was seen in 2019, with Medic treatments showing higher levels, though this was not repeated in 2020. This is expressed as a dry mass percentage, and it is possible that xylem/phloem concentrations do not differ between treatments. Levels are less than toxic, however, like salinity, in a vineyard where Cl is an issue, this should be explored further.

Vines

By reducing evaporative losses from the soil surface and thereby maintaining soil moisture in the surface root zone, vines with Medic and annual grasses planted under-vine appear able to use applied water more efficiently than the perennial grass treatments. The latter remained actively growing over the summer, competing with the vine for moisture and nutrients and suppressing the production of leaves and their size.

At Nuriootpa significant differences in temperature were found at ground level, with the straw mulch treatment possessing a lower cumulative temperature than the other treatments tested. Despite the loggers also being placed under the senesced biomass (mulch) created by the cover crop treatments, only the straw was sufficiently dense to reduce the air temperature at the soil surface. This result may impact budburst, particularly in cool climate regions, by keeping the soil cooler, but there was no evidence at the Nuriootpa site of this effect. Reduced soil temperatures over summer are an advantage to the vines, as found by Yalumba Wines (AWRI 2016), where reduced energy and water usage plus increased fruit yields following straw application were other significant benefits derived from the practice.

The suppression of soil surface evaporation and weed growth by the straw mulch compared with the conversion of soil moisture into cover crop biomass had a dramatic effect on the pruning weights. The two seasons over which the trial was conducted were of below average rainfall, requiring additional irrigation to be provided. However, the extraction of water by the Kasbah provided an excellent example of the wastage in soil moisture that occurs with weeds competing for limiting resources. It therefore provides a cautionary tale of the need to select under-vine cover crops that are not vigorous at time of high water demand. Ideally a cover crop would grow quickly during the wet (rain-fed) months of the year, and the senesce in the warmer months to provide a mulch layer to reduce evaporation.

One of the most important factors that winemakers consider when grading fruit is juice pH. Differences were seen between treatments, most notable was the increase in pH of juice from the Fescue treatment. Although we cannot say with confidence the reason for this, it is possible that canopy size played a role. Often high soil K leads to high juice pH, but that is not consistent with the soil data from this treatment. The leaf blade Na levels were also high with the Fescue treatment, suggesting the potential for increased Na uptake on this treatment for reasons unknown (soil Na levels were not high) and then translocation through the vascular tissue to the fruit.

Along with other variables such as bunch number and berry weight, bunch weight is an important driver of vine yield. As noted by (Coombe & Dry 1992) bunch weight is influenced by macro- and micro-climate, management practices such as irrigation, nutrition status and

pruning severity along with the grape genetics. Not mentioned is plant competition, which in this case was provided notably by the Kasbah cocksfoot. Wallaby grass, also a perennial but not as vigorous in growth habit, also trended to lower bunch weights over both years. By contrast, the straw mulch treatment, in conserving water loss in two very dry seasons, had the highest bunch weights and yields for those two years. Also of note is the similarity in bunch weights between the herbicide control and the Medic, Medic and Ryegrass, wallaby grass and Fescue treatment in 2020.

Discussion - Langhorne Creek

Discussion of the data from Langhorne Creek across the 2019 and 2020 vintages.

Water stress and soil moisture

The pattern of water use appears to be consistent across both seasons. The operators at Langhorne Creek begin irrigation during winter, so the soil profile is at greater than 60 % moisture on all treatments to the measured depth of 100 cm, excepting the top 20 cm. Surprisingly the Kasbah, Fescue and wallaby grass treatments do not show a significant water deficit despite significant cover crop biomass production. This is likely due to the reduced vine canopy size which is compensating for the grass growth. At harvest time, all treatments show a reduction in soil moisture at 100 cm because irrigation to depth has ceased while there remains adequate soil moisture to maintain leaf and have the grapes mature.

There appears to be a close correlation between yield, LAI, and cover crop persistence through summer, but surprisingly the stem water potential shows no differences. It may be that the measurements were taken soon after a watering event, thereby reducing the moisture tension. It is also likely the vine is compensating for reduced soil moisture availability by reducing the canopy size, as shown by the LAI results, with a concomitant reduction in yield.

Soil

Soil at Langhorne Creek (a soft clay loam) differs from that at Nuriootpa (clay), and as mentioned, is irrigated relatively heavily. These differences impart major differences in the way the vines and soil interact.

The soil pH is higher on the herbicide control than other treatments. With a pH in excess of 7.75 as shown on the herbicide control, the availability of nutrients such as iron, manganese, copper, zinc and boron may be reduced. The reason for the elevated pH on the control is likely excess Na in the topsoil. The high Na levels are probably a function of lower organic carbon levels which will buffer the Na content. It is possibly also accumulating in the topsoil through excess moisture evaporation from soil with no mulch on the surface.

Baldock (2019) states that with the best soil management it often takes greater than five years to detect true changes in soil organic matter. In the case of this site, after five years of implementation, and no removal of the organic matter, there has been a 100% improvement in soil carbon by the Kasbah treatment, and 64 % with the Medic and Ryegrass treatment. This is a significant finding from this trial as it represents the improvements possible to one of the main drivers of soil health in a relatively short time period. The greatest increase in soil carbon concentration was made by the perennial grass (Kasbah) treatment.

As noted earlier, the Kasbah grass is marketed as being summer dormant, but under the conditions provided at this site, of considerable irrigation water application, the grass grew vigorously throughout the year. As found by Ball *et al.* (2020), the grass treatments increased organic carbon 22% more than the legume treatments, due to their greater fine root biomass. While Kasbah is not being advocated for as a desirable cover crop due to competition with the vines for water and nutrients, the treatment does display the capacity for dramatically changing soil carbon concentration in a relatively short period. Of more relevance to growers is the 64% increase in soil carbon generated by the Medic and annual Ryegrass treatments. Without compromising grape yields, this treatment has also provided enormous benefit to the soil, and as such is recommended as a favourable option for growers to consider.

It is also possible to put an economic value on this increase in soil carbon, based only on its fertiliser value, but without investigating carbon sequestration and the carbon trading market. In fertiliser value alone, a 1 % rise in soil carbon equates to about 1000 kg/ha of N in the top 10 cms of soil, which at approximately $1/kg, is $1000 dollars per hectare worth of N (Baldock 2019). Along with other ecosystem services that are provided by the cover crops (e.g. increased earthworm populations, soil cover provision and the habitat it provides), both monetary and environmental gains are attributed to the under-vine cover cropping.

Central to his project is recognition of the need to develop a management system for the under-vine zone that utilises soil biology to enhance the system sustainability. Earthworms are well recognized for their ability to aerate the soil and increase the available nutrition via their castes. With a requirement for quality organic matter to grow and reproduce, it is not surprising that with the removal of organic matter through herbicide application, the earthworm numbers trend towards being lower than all other cover crop treatments, and are less than the wallaby grass.

As mentioned previously, the soils at the sites contained diverse (16S) communities of soil microbes. Contra to the Nuriootpa results, Faith's phylogenetic diversity measure did not yield significant differences in microbial diversity among the treatments at Langhorne Creek. This is a surprising result and may be explained by the differences in soil types between the two sites. High levels of clay, and low irrigation rates in Nuriootpa are able to sustain varied microbial communities, while the sandier soils at Langhorne creek, combined with regular irrigation events, lead to more habitat-specific phylogenetic clusters of bacteria and archaea that are adapted to those conditions.

With over 3 MgL/ha of water applied through the drippers at Langhorne Creek, the water available for cover crop growth over the summer is considerable. It was not expected for the grasses (Kasbah cocksfoot, Fescue, and wallaby grass) to maintain growth over the summer period, but when supplied with regular irrigation they responded well. With nearly 5 t/ha of biomass production, considerable quantities of water have been used by the Kasbah and to a lesser degree the wallaby grass. The fescue/strawberry clover treatment was known to be active all year round, but potentially with lower stature its water use would not be high. At this site, when initially planted in 2014 the strawberry clover dominated, but after fixing large amounts of N over 3 years it senesced and the Fescue then became a sole cover crop. Its biomass production was significant, as shown above, but again it was at the expense of water availability to the vine. The herbicide control in this case shows bare soil, which is as it should be, and some straw which has blown in from an adjoining plot. Essentially it was a bare soil control though. The triticale mulch has again provided excellent weed control.

In contrast to the growth of the perennial grasses, the annual Medics and grasses have again behaved as expected, with only a few plants breaking dormancy and growing over summer, but not producing enough biomass to significantly affect water use or yields.

The negative impact of dry winters is not so great at the Langhorne Creek site, due to the higher quantity of water applied from an irrigation season which can start during winter if required. The perennial grasses (Kasbah, wallaby and Fescue) have all kept growing strongly, although the wallaby grass, which has not been bred for biomass production, has a lower output than the other two. In only its second year since (re)application, the triticale mulch continues to provide good weed control and cover of the soil to reduce evaporative losses. The annual Medics (cavalier, angel and scimitar) in conjunction with the annual grasses that have infiltrated the plots, have generated from 250 to 350 g / m^2 of biomass without any cost to the vines in terms of water loss, and when senesced by late spring, will add to the mulch on the soil surface as protection over the summer period. The Angel/Sultan treatment shows production of approximately 2.5 t/ha of Medic. Approximately 20 kg of N per tonne of dry matter is fixed by the rhizobial bacteria associated with the legumes, so in this case up to 50 kg/ha has been produced by the legumes. While this is only in the under-vine zone (about 1/3 of the vineyard floor) it is still a significant amount, as the vines only export 1 kg of N/tonne of fruit. What is not used by the vine will make a valuable contribution to the soil organic matter for access by the vine in latter years.

Nutrients

Soil P from the straw mulch treatment was high in both years of the trial when compared to the Medic treatment. As noted for the Nuriootpa site, the reason is twofold. Medics have a high requirement for phosphorous, driving the levels lower, and the straw treatments has imported about 60 kg/ha of phosphorous over the last five years, accounting for the differences noted. With Colwell P levels expected to be of at least 20 ppm, the quantities noted in both years are marginal on all treatments other than the straw, suggesting the need for regular leaf blade monitoring to ensure yield is not being compromised.

The lower total soil N noted above is likely a direct consequence of the lower soil organic C levels. With less soil organic matter available for mineralisation, lower soil N will result. As shown with C, there is an associated cost with employing management practices that result in

less soil N. Unless the lower N levels are compensated for, they will potentially manifest in compromised yields and potentially fruit quality.

The recommended soil K is from 150 – 250 mg/kg, making all treatments in one of the two years below the minimum required. There is no significant difference over the two years among treatments. Potassium is not bound in organic compounds unlike N and P, so it more readily leached from the straw than other elements. At the Langhorne Creek site however, despite the straw coming from the same source as that used at Nuriootpa, there is no apparent elevation in soil K resulting from the large amount of K contained in the applied straw.

While the straw mulch treatment has not led to an absolute rise in K levels compared to other treatments, it has had a significant effect on increasing K in relative terms compared to the other cations (the cation exchange percentage, see appendix 5). With normal levels being the range from 3 – 8 %, all treatments except the straw mulch are below the minimum, requiring tissue testing to ensure adequate amounts are being accessed by the vine.

Sodium is also a cation, so is assessed as a percentage of the clay complex along with K, Mg and Ca. Due to the comparatively high levels of K associated with the straw mulch treatment, the Na percentage is, in relative terms, lower for that treatment, but absolute levels may still be high. Recommended levels for Na are not greater than 6% exchangeable Na and 300 mg/kg absolute levels (APAL, 2019), which are exceeded by several treatments over the two years. Outstanding are the herbicide treatment and the exotic perennial grasses compared to the straw mulch treatment. The greater evaporation rate from the bare soil associated with the herbicide control is likely to be causing the elevated Na levels on that treatment. Other treatments which provided a mulch of dead material (straw and Medics) also have lower Na levels. The wallaby grass also showed low Na. Growth (and transpiration) of this native grass is low during the summer period, as it is a C3 grass. Potentially the fibrous root system enhances the leaching of Na and combines with the lower transpiration to enable lower soil Na.

The fertigationg events are readily noted in both 2019 and 2020. The NH_4^+ and NO_3^- not immediately utilised by the vines will then migrate down the soil profile with the wetting front. With close monitoring to prevent the leakage of water from below the root zone, as is

employed at this site, it is expected most of the applied N will be utilised by the vines. Based on 1 kg of N being required to produce a tonne of grapes (Glendinning 2000) the 18 units of N at 100 % fertilizer use efficiency could produce 18 t/ha, which is about the average yield for this site. Additional N, as noted earlier, will be sourced from mineralised organic matter. With increased soil organic matter levels associated with the Medic treatments, a greater pool of N will be available for the vines needs, and to add to the berries as amino acids and YAN, to support fermentation.

The Fescue provided the highest spike of NH_4^+ of all the treatments, though this is likely not representative of the treatment as a whole. Otherwise, the Medic treatments also show benefits in soil N supply, which is benefitting grape yield and YAN levels. By comparison, the sole species perennial grasses, straw mulch and herbicide control treatments are mostly unaffected by the fertiliser application. The levels of NH_4^+ are however within the expected standard of 1-10 ppm (APAL, 2020).

The fertigation treatment is again visible in the soil analysis for NO_3^-, with peaks obvious in the herbicide, Medic and wallaby grass treatments, where no cover crop is active. The Fescue and Kasbah treatments however are very active in spring and may have rapidly utilised the applied NO_3^- or allowed for rapid leaching of the nutrient to greater depths. By contrast, the NO_3^- applied to the non-active treatments has been allocated to the soil for uptake by the vine, which according to the data, occurred very quickly.

The Cl levels in the leaf are all within acceptable levels of 0.1 to 0.8%. The two high water use cover crops (Kasbah and Fescue) show higher leaf Cl levels than the straw mulch. This is likely to be a function of the reduced soil moisture availability on the perennial grass treatments, while the smaller canopies they have produced may also have increased the concentration of Cl. Chloride is considered an essential element for plant growth (Nicholas 2004), but along with Na it can be toxic leading to the burning of leaf margins, particularly in very hot weather. The levels noted in both years are well below those where toxicity may be considered a problem.

The leaf magnesium levels from 2019 and 2020 are well below the normal range of 0.3 to 0.6%. According to Nicholas (2004), magnesium deficiency in the vines can occur where

soils are very high in K, as the vines take up potassium in preference to magnesium. In this case, the soils at the measured depth of 0-10 cm were also low in K. Despite the low Mg levels, there were none of the deficiency symptoms described by Nicholas (2004) displayed on the vine leaves during either harvest. For these elements, it is more a case of making note that there were differences between treatments but none that would adversely affect vine performance.

The normal range for leaf N is 3-5%, which is not realized by any treatment over the two years. Of all the elements, N has the greatest impact on vine performance and can influence grape quality, either indirectly through canopy development, or directly through berry composition. In both 2018 and 2019 years, the Medics have provided the highest level of N, but the Medic growth has been poor compared to earlier years, so the results noted above are mostly the residual N and mineralised N coming from the labile organic carbon.

Over the two years, leaf P was higher on the straw mulch treatment than the Medic mix (2019) and the control and Fescue treatments in 2020. The straw treatment is nearly in the normal range of 0.25 to 0.4%, while all other treatments are below normal but not deficient. Without soluble P application, this nutrient has to be mineralised, and/or released from binding sites, in the soil to become available for plant uptake. It is suggested that the straw mulch is likely to provide better conditions for mineralisation to occur, through more stable soil moisture and temperature. The straw has provided extra phosphorous. Analysis of the straw shows only 0.06% P (Marks, pers. comm) is contained in the straw, but with an application rate to the under-vine zone of 50 t/ha, about 30 kg P/ha has been provided. This is in addition to 55 t/ha of straw having been applied in 2015, so over 60 kg of P/ha has been imported. With only 0.3 kg/t of phosphorous exported per tonne (Glendinning 2000), that addition via the straw will last about 11 years with an average yield of 18 t/ha in the absence of any further being provided. Release of P from binding sites in the soil can be impacted by soil pH, however no especially large impacts of treatments on soil pH were observed.

Marks (pers. comm) determined there was an average of 0.17% K in the wheat straw, which equates to 85 kg/ha that was applied with 50 t/ha of straw concentrated in the under-vine area. A similar amount was applied in 2015. Grapes export about 3 kg K /t of fruit harvested, or about 54 kg in an average 18 t/ha crop at this site. A significant difference was only shown

in 2019, with the straw treatment greater than the Medic mix. In both years the K levels were within the desirable range of 1 – 1.8%, and no significant differences were produced in the juice pH, which is a good outcome for all treatments.

Vines

Of the four treatments monitored for cordon temperature, the only one which maintained green growth over the summer period was the Fescue. Transpiring water to continue growing, the Fescue provided competition to the vine, resulting in reduced canopy development, as shown in the previous LAI figures. As a result of the smaller canopy, less light is intercepted so shading of the cordon wire zone is reduced, leading to a rise in temperature. Indirectly the choice of cover crop has therefore influenced to temperature of the fruiting zone, with potential implications for grape maturity and ultimately quality.

The trunk temperature shown above is in concert with the inverse of the LAI readings, whereby the Medic and Ryegrass treatments which display the largest canopy has the lowest recorded temperature on the trunk. It is therefore suggested the Medic and Ryegrass treatment has accrued less degree days than the Fescue treatment because the larger canopy is intersecting more light during the midday period so the trunk has a greater level of shading and therefore remains cooler. This finding is of considerable interest in a warming climate, and may be especially relevant in warmer regions.

Although there were no significant differences in pruning weights, a trend, similar to that seen at Nuriootpa was present, whereby the Medic mix treatment has the highest per-vine pruning weight, while the Kasbah and Fescue possess the lowest. This matches, as expected, with the LAI readings throughout the season and also correlates with higher yields.

The measurements of LAI show the impact of the two exotic perennial grasses on vine growth, whereby leaf area is significantly reduced on the Kasbah and Fescue treatments. Their competition for water and nutrients was significant compared to the two Medic treatments which had both senesced to provide a mulch on the soil surface and no competition. The LAI's are also in close accord with the bunch weights and yield for the 2020 season, suggesting the vines had compensated their reproduction in concert with their vegetative capacity.

The low bunch weights in 2020 on the perennial grass treatments are likely to be a function of several factors including soil moisture and nutrient availability. While the soil moisture curves suggest adequate moisture, there may have been times between the fortnightly sampling points that the vines were water stressed on the perennial grass treatments. It was also noted that these treatments also had low soil mineral N (NO_3^- and NH_4^+) levels, which combined with the above is the likely cause of lower bunch weights and ultimately yield.

The Langhorne Creek site does not suffer the physical soil constraints of the Nuriootpa site, in particular the sodic clays that have high soil strength which can restrict root activity. The clay loam at this site has no structural issues, so yields are going to be largely determined by soil moisture availability and potentially some physiological variables such as bud fertility due to light interception. Surprisingly, in 2019 there were no significant yield differences among treatments, but in 2020 the perennial grasses (Kasbah and Fescue) were significantly lower yielding treatments than the herbicide. All other treatments were not significantly different, again displaying that the growth of summer dormant (Medics and Ryegrass), or of low summer activity (wallaby grass), does not reduce yields in this region under the management regime that is used. The improved yield resulting from Medic-based cover crops noted at Nuriootpa was not observed at this site because the soil did not require amending in the same way as the Nuriootpa soils. The results are still very encouraging though, in that yield was not lost by enabling the cover crops, and money was saved by not using herbicides – a win-win outcome. Kasbah and Fescue are still performing strongly giving suppressed yield.

While the titratable acid levels are lower with the perennial grasses (which should correlate with a higher pH), the yields for these treatments are also lower. According to Iland *et al.* (2011), TA decreases due to a) a loss of malic acid from the cells, b) a loss of protons from the cell solution or c) dilution of the cell's components through water uptake. There were no significant differences in berry size (see appendix 5) across the treatments, so it can be assumed the difference noted is due to a loss of protons associated with an equivalent uptake of K which can be generated by more open canopies. This was not the case in this instance, as shown by the leaf area index, where depressed soil moisture availability to the vine has suppressed the canopy size on these treatments.

The total phenolics comprises non-flavonoids and flavonoids such as tannins, anthocyanins, flavanols and flavanols. There was a close correlation between total phenolics and anthocyanins and tannin levels noted in fruit from the 2020 vintage. The lowest yielding treatments have the highest level of phenolics. According to Iland *et al.* (2011) 100% of the anthocyanins and up to 49% of the tannins are derived from the grape skin and for total phenolics 6% in the flesh, 69% in the seed and 50 % in the skin for red grapes. Reducing berry size to increase the skin the flesh ratio through management of bunch numbers (e.g. minimal pruning or crop thinning) or increasing hang time will generally increase berry phenolic content. The cover crop competition for soil moisture on these treatments is likely to have concentrated the phenolics leading to the higher levels that were generated.

Yeast Available Nitrogen (YAN) is an important assessment for winemakers, as the quantity determines the nutrient availability for yeast during ferment, but in excess can have a detrimental impact, particularly on white wines, where protein instability may increase (Nicholas 2004). While correlations between soil, petiole and YAN are not always robust, there is consensus that applying N in a low N environment will increase vine productivity and may increase YAN's (Bell & Henschke 2005). They note however that research into N application and impact on vine and wine productivity and quality parameters does not include the impact of organic N such as produced through mineralisation of organic matter, because it is not readily manipulated or monitored. Organic N is however important in this study, where legumes have been used to improve soil health, soil organic matter content and to prevent yield loss from under-vine competition. According to these authors, the following levels apply to YAN's – low is < 100 mg N/L, moderate is from 250 – 350 and high is > 600 mg N/L. A world-wide survey suggests an average minimum limit for YAN's to ensure satisfactory completion of fermentation being 147 mg N/L. According to those standards, only the Medic treatments are nearing the moderate level for YANs, but all treatments would be expected to have musts that would complete fermentation. As to the impact of organic N on grape and wine quality, the review of Bell and Henschke (2005) and work such as Brunetto *et al.* (2013) are inconclusive as to the benefits or otherwise of higher levels of must N, or whether the N in the vineyard is provided from an organic or synthetic source.

Due to poor growth in the Medic treatments over the 2019 season, it is suggested that carryover benefits of N accumulation in the soil from previous seasons are likely. This is a desirable finding, suggesting that the increases in soil organic matter and potentially residual organic N from previous legume cover crops is now contributing to maintaining those desirable YAN levels into future seasons.

Wines made differed between treatments according to both AWRI experts, as well as the National Amateur Wine and Beer judges. The high juice pH, anthocyanins and phenolics in the Fescue treatment produced a dark wine with high levels of aldehyde faults, whereas the Medic mix and Medic and Ryegrass had fewer faults, however were also less flavoursome, potentially due to the lack of water stress throughout the season relative to the other treatments.

Part 2 – Cover crop and arbuscular mycorrhizal fungi inoculum potential

The following is a draft manuscript that is currently being submitted for peer review.

What lies beneath: herbicide, under-vine cover crop and mulch impacts on the inoculum potential of arbuscular mycorrhizal fungi.

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AMF; agroecosystem; *Vitis vinifera*; grapevine; vineyard floor management; sustainable viticulture.

Abstract

Purpose: Several studies have sought to quantify the influence of vineyard floor management on arbuscular mycorrhizal (AM) inoculum potential; however, few have investigated the effect of under-vine cover crops. To investigate this, seven treatments (five cover crops, one surface mulch and one herbicide-managed control) were established under-vine in a vineyard site at Langhorne Creek, South Australia in 2014. Following a four-year interval (2014-2018) and then a six-year interval (2014-2020) soils were sampled under-vine and two glasshouse trials were undertaken. The glasshouse trials aimed to test the hypothesis that cover crops planted directly under-vine increase the AM inoculum potential of leek (sp. *Allium porrum*) trap plants (Exp. 1) and medic (*Medicago truncatula*) and ryegrass (*Lolium rigidum*) (Exp. 2) when potted in soil sampled from under-vine treatments.

Method: After an eight-week growth period, leek plants were harvested and analysed for AM colonisation and root/shoot biomass to understand if a relationship could be established between the presence of cover crops and increased AM inoculum potential. Furthermore, prior to the second pot-growth experiment, a spore count was conducted on soil samples to test for differences in spore abundance.

Results: While some significant differences were observed in mean plant biomass, there were no significant differences in leek, medic or ryegrass colonisation by AMF. Additionally, there were no significant differences in the number of AM spores between treatments.

Conclusion: Results suggest that sources of AMF inoculum (spores mainly) remain present and viable in cover crop and bare earth treatments. This may be due to the encroachment of mid-row vegetation and perennial grapevines offering inoculum buffering.

Keywords:

Under-vine; cover crops; mulch, herbicide; inoculum potential; mycorrhiza.

Introduction

Sustainable viticulture is an increasingly important practice and one that encompasses multiple ecosystem processes and vineyard floor management practices. Sustainability in viticulture largely reflects a reversion to a more naturally-managed agroecosystem: decreasing the reliance on herbicide, pesticide and fertiliser inputs, while encouraging macro and micro biodiversity both above and below-ground (Celette *et al.* 2005; Guerra &

Steenwerth 2012). A steady adoption of sustainable viticultural practice has been driven, in part, by observable and quantifiable land degradation including but not limited to soil erosion, herbicide-resistance and a decline in biodiversity (Guerra & Steenwerth 2012; Wheeler & Crisp 2011). Cover-cropping is one such management practice aimed at enhancing soil-grapevine (sp. *Vitis vinifera*) interactions and the ecological intensification of the vineyard, while at the same time reducing the need for chemical inputs and invasive farming practices (Celette *et al.* 2005; Guerra & Steenwerth 2012; Peregrina *et al.* 2012). The use of cover crops in vineyards has a long-standing tradition, especially when sown in the mid-row. Anecdotal evidence has given way to mounting statistical data, showing positive influences of cover crops (species-specific) on vineyard soil and grapevine health. Such evidence has shown cover crops to influence nutrient cycling, water relations, pathogen-resistance, biodiversity, erosion-prevention, organic carbon content, and diversity of arbuscular mycorrhizal fungi (AMF) (Celette *et al.* 2005; Guerra & Steenwerth 2012; Monteiro & Lopes 2007). Although the weight of evidence has steadily increased for mid-row cover crop management, less experimental data has been accrued on *under-vine* management practice (Jordan *et al.* 2016). Sowing cover crops directly beneath grapevines may increase the effect of the ecological interaction both below-ground (roots) and above-ground (canopies), potentially exacerbating both positive and negative effects (Karl *et al.* 2016). One such targeted interaction that may be affected is the inoculum potential of AMF due to an increase in root biomass owing to the presence of cover crops.

Arbuscular mycorrhizal fungi are ubiquitous symbionts of approximately 80% of terrestrial plant species (Smith & Read 2008). The relationship is one of colonisation and exchange as arbuscular mycorrhizal (AM) hyphal networks infiltrate plant root cells, taking up plant-assimilated carbon in exchange for immobile nutrients such as phosphorus and zinc (Cavagnaro 2008; Smith & Read 2008). Grapevine (sp. *V. vinifera*) benefit from AM colonisation owing to their thicker root system and relative lack of fine root hairs (Holland *et al.* 2014; Radić *et al.* 2012). Moreover, soil health has been shown to benefit from increased hyphal networks through nutrient cycling; organic carbon content; soil structure and; water infiltration (Schreiner & Mihara 2009; Valentine *et al.* 2006). It is well-established that management practices, including soil cultivation and the inclusion of cover crops, can impact the formation of AM (Bowles *et al.* 2017). Several studies have focused on the relationship between cover crop presence and increased AM diversity, yet few have sought to quantify inoculum potential, especially under-vine (Baumgartner *et al.* 2010; Vukicevich *et al.* 2019).

The primary aim of this study was to determine the influence of five different species / combinations of cover crop, one straw mulch and one herbicide (bare earth) control on the inoculum potential of AMF under-vine. This aim was addressed in two separate experiments, using soil collected from a long-term field trial that has been established to study impacts of under-vine management options. In the first experiment, leek plants (sp. *Allium porrum*) were used as trap plants to test the hypothesis that leeks grown in soils sampled from under-vine cover crops would be significantly more colonised by AMF than those grown in non-cover crop soils (increased inoculum potential). To further understand inoculum potential between treatments, a second pot-growth experiment was undertaken, using medic (*Medicago truncatula*) and ryegrass (*Lolium rigidum*). In both experiments, the aims and hypotheses remained the same. Furthermore, it is hypothesised that if the inoculum potential (and therefore root colonisation) is significantly different between treatments then this will also present in plant biomass (correlating with root colonisation).

Materials and Methods

The trial site

Available to this project was a long-term field trial which comprised seven under-vine treatments (five cover crop combinations, a surface mulch and bare-earth control) (see Table 1 for detailed descriptions). The under-vine trial was located in a Cabernet Sauvignon block, located in Australia, at a site in the Langhorne Creek wine region (35.302”S, 138.952”E) and established in 2014. Vines at the site were 21-years-old at the time of sampling and are established on their own rootstock (Cab LC-10) and trained to a single cordon trellis. The vines are saw box pruned to 15-18 t ha^{-1} with the mid-row floor managed with a combination of slashing and heavy rolling; vine spacing is 1.8 m. The soil type is a Brown Sodosol to a depth of approximately one metre (Hall *et al.* 2009). The irrigation rate is approximately 3.5 ML ha^{-1} over a row-spacing of 2.5 m row^{-1} and dripper spacing of 0.6 m. Within this site, five cover crop treatments were sown under-vine using a towed mechanical no-till disc seeder, while the cereal straw mulch was hand-laid at a rate of 50 t ha^{-1}. The herbicide control was maintained using a combination of glyphosate (during vine dormancy) and glufosinate ammonium (when vines were active), applied thrice yearly.

Table 10. *List of seven experimental treatments with common name, species name and biological / ecological properties selected for experimental / industry-relevant significance. For the purpose of clarity, treatment names will henceforth be referred by their abbreviated code names, i.e. HC = Herbicide control; TM = Triticale mulch; M+R = Medic + ryegrass; M+M = Medic + medic (cultivar combination); KC = Kasbah Cocksfoot; F+C = Predator fescue + Strawberry clover and; WG = Wallaby grass.*

Treatment code	Common name	Species name	Properties
HC	Control (herbicide)	N/A	Traditional management practice, vegetation cover suppressed
TM	Triticale mulch	N/A	Soil stabilisation; temperature regulation and; soil moisture enhancement (Prosdocimi *et al.* 2016; Thomson & Hoffmann 2007)
M+R	Safeguard ryegrass/ Scimitar	*Lolium rigidum* / *Medicago polymorpha*	Annual grasses; fibrous roots for soil stabilisation; water infiltration potential; high N-uptake therefore grown in combination with medic (N-fixer)
M+M	Angel strand / Sultan barrel medic	*Medicago littoralis* / *Medicago truncatula*	N-fixers with low C:N ratio and useful for experimentation (Kumar & Goh 2003)
KC	Kasbah cocksfoot	*Dactylis glomerata*	Summer dormancy (can be suppressed if irrigated) and weed-suppression (ideal for dry climates) (Weston 1996)
F+C	Predator fescue / Strawberry clover	*Festuca* sp. / *Trifolium fragiferum*	Fescues can be overly vigorous – an advantage in wetter climates – and grown best in combination (Celette *et al.* 2005)
WG	Wallaby grass	*Rytidosperma caespitosa*	Native grass with adaptive traits to water-reduction; weed-suppression and; increased native microbial biodiversity (Mitchell 2003)

2018 SOIL NUTRIENT DATA	HC soil		TM soil		M+R soil		M+M soil		KC soil		F+C soil		WG soil	
	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)
Total N (%)	0.17	0.01	0.17	0.01	0.25	0.04	0.19	0.01	0.21	0.02	0.21	0.01	0.19	0.00
Plant-available (Colwell) P (mg kg^{-1})	19.5	4.03	43.2	8.51	22.5	4.2	20.0	2.86	50.8	13.8	29.3	7.2	27.3	6.3
Zn (mg kg^{-1})	4.2	0.6	4.2	0.62	5.2	0.9	4.6	0.8	4.8	0.5	3.8	0.8	4.3	0.4
2020 SOIL NUTRIENT DATA	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)
Total N (%)	0.19	0.01	0.20	0.01	0.22	0.02	0.22	0.01	0.20	0.02	0.21	0.00	0.20	0.01
Plant-available (Colwell) P (mg kg^{-1})	20.25	5.41	44.50	9.02	22.29.25	5.81	22.75	6.41	38.50	5.27	31.50	8.41	57.00	12.60
Zn (mg kg^{-1})	3.95	0.37	3.30	0.91	4.05	0.36	3.23	0.48	3.78	0.70	3.15	0.43	2.75	0.39

Table 11. Physicochemical properties of soils collected from beneath treatments of cover crops at the Langhorne Creek vineyard. N.B the experimental treatments at the site are described in Table 1. Values are Mean ± S.E.; n = 32 (see Table 1 for all treatment codes).

Experiment design

The experimental design was conducted using a randomised complete block design (RCBD), with each treatment replicated four times (n = 28). Spatial layout of vineyards assisted with a blocking design format, with nine vines accounting for three panels (per treatment). The mid-row was uniformly managed with minimal intervention. Cover crop treatments follow an annual growth cycle, flourishing in cooler, wetter months (May – September), while declining in warmer months to coincide with reduced recourse competition with grapevines during their flowering and fruiting season (October – January/February).

Sampling and processing

Soil sampling was conducted in October 2018 (experiment 1) and October 2020 (experiment 2) (austral spring) to coincide with grapevine bud burst. For each experimental plot, three soil samples were collected from a 0-10 cm soil layer using a stainless-steel core (10 cm diameter) and slide hammer. These three samples were then pooled and mixed at the individual plot level. Thus, there was one composite soil sample for each of the seven experimental treatments, in each of the four experimental blocks (i.e. four samples per treatment and 28 soil samples in total). All samples (n = 28 over two sites) were air-dried and then sieved to 2 mm with larger aggregates crushed to homogenise samples. Within the laboratory all soil samples were divided into three subsamples, except for the 2020 soil samples, which were divided into four. The first subsample was sent to the Australian Precision Agricultural Laboratory (APAL) in Hindmarsh, South Australia (https://www.apal.com.au, last accessed May 2020) for analysis of soil physicochemical properties (see Table 2). The second subsample was used to determine water-holding capacity (WHC) of the different soils, as described previously (Cavagnaro 2016). The third subsample was used in experiments 1 and 2, as follows.

Spore count

Three subsamples of each soil replicate (n = 28) were collected and the 53-250 µm fraction isolated by wet-sieving (Gerdemann & Nicolson 1963). Each subsample weighed 3.33 g, totalling 10 g per soil replicate. The content of the 53 µm sieve was collected and washed using a sugar-gradient centrifugation (Brundrett *et al.* 1996). After the second centrifugation, the spores were again collected on a 53 µm sieve and sugar residues washed off using tap water. Following, the spores were transferred to a nematode counting dish. The absolute

number of AMF spores per dish were counted under a dissection microscope at 80-120× magnification.

Exps. 1 and 2: glasshouse bioassay and harvest

The AMF inoculum potential of the soils was assessed in two separate experiments. Experiment 1: seedlings of leek (*A. porrum* L.) were grown in a glasshouse bioassay in October 2019. Experiment 2: seedlings of medic (*M. truncatula*) and ryegrass (*L. rigidum*) were grown (separately) in a glasshouse bioassay in October 2020. The following methodology accounts for both experiment 1 and 2: Leek plants were initially selected as the bioassay test plant species owing to its high affinity for forming AM (Torelli *et al.* 2000) and not being present in the plant communities at the field sites (negating potential home-field advantage). The use of medic and ryegrass were later selected (Exp. 2) to account for the possibility that leek affinity (Exp. 1) for AM symbiosis was such that treatment effects may have been masked. To do this, 500 g (oven-dry weight equivalent) was added to an 800 mL plastic free-draining pot and packed to a final bulk density of 1.3 g cm^{-3} (equivalent to field bulk density). Soil in each pot was then watered to 80% WHC (determined as described above) and left for one week to allow soil microbial biomass to re-equilibrate (Schimel *et al.* 2007). To each pot four pre-germinated seeds were planted. All seeds were surface sterilised by washing in a 4.125% NaOCl (sodium hypochlorite) solution, followed by rinsing with Reverse Osmosis (RO) water to remove traces of NaOCl and commercial fungicide. The seeds were the placed on moist filter paper in Petri dishes and incubated at 25°C in the dark for three to five days, when approximately 10 mm of the radical had emerged. Following sowing of the seeds, the pots were placed in a glasshouse on the Waite campus of the University of Adelaide and allowed to grow for a period of eight weeks. Plants were watered thrice weekly to 80% of WHC with RO water, and rotated on the glasshouse bench. Two weeks after sowing the number of seedlings was thinned to two per plant pot. Glasshouse daily temperatures ranged from 14°C (min) to 30.1°C (max) and daily recorded relative humidity (RH) ranged from 23.9% RH (min) to 21.1% RH (max).

After an eight-week growth period plants were destructively harvested, as follows. Plants were washed free of the soil in RO water. All fresh tissue (with a subsample of root tissue) was weighed and oven dried at 50°C and root dry weight (RDW) and shoot dry weight (SDW) was collected. A subsample of fresh root tissue was weighed for AMF root-colonisation analysis, using the gridline intersect method (Giovannetti & Mosse 1980). Roots

were washed in KOH (10% *W/V*) and stained with a solution of 50 mL 5% ink (black Schaeffer) to 50mL white vinegar, prior to microscopic analysis (SZ-STS Olympus microscope) of root-grid intersects (20× magnification).

Statistical analyses

Prior to significance testing, each treatment variable was tested for normality and homoscedasticity using the Wilkes-Shapiro test, and, if necessary, the data were transformed using either log, square root or an exponent function to achieve normality. Those variables that violated ANOVA assumptions of normality after transformations were analysed using a non-parametric approach (Friedman Test). Owing to the randomised complete block design (RCBD) of the field trials (and glasshouse trial), the data (for each site separately) was analysed using two-way ANOVA with *Treatment* and *Block* as factors in the analysis. *Block* was included in our analysis to account for any underlying spatial heterogeneity of the vineyard sites. Where two-way ANOVA revealed a significant difference ($p < 0.05$), Tukey HSD *post-hoc* analysis was used to determine pairwise comparisons between significantly different treatment means. As noted above, where the assumptions of normality could not be met, the data were analysed using a non-parametric (Friedman Test) approach analogous to a two-way ANOVA, with further *post-hoc* analysis used if testing showed significance. All statistical analyses were conducted using R open source statistical software (R version 3.6.3) (Team 2013) with additional agricultural data package, "agricolae" and visual output package, "ggplot2". Alpha was set at 0.05.

Results

Arbuscular mycorrhizal colonisation of leek roots (Exp. 1)

The inoculum potential of AMF was high in leeks grown across all soil treatments (Fig. 1), with the roots of all leek plants well-colonised by AMF. Across all treatments, the mean percentage of leek plant roots colonised by AMF were 66 ± 0.6%. There was no significant difference in AMF colonisation of the leek plants grown in soil collected from the various under-vine treatments. There were, however, significant ($p < 0.01$) differences in root length colonised (RLC) between treatments (Table 3). Specifically, RLC was significantly higher in leeks grown in treatments of KC and WG treatments, compared to the combined M+R treatment.

Arbuscular mycorrhizal colonisation of medic and ryegrass roots (Exp. 2)

The mean percentage of medic roots colonised by AMF were 78.9 ± 2.1%, while the mean percentage of ryegrass roots colonised by AMF were 20.6 ± 3.0% respectively ($p > 0.05$). The inoculum potential of AMF differed considerably between medic and ryegrass plants, which is to be expected, given their different affinities for symbiosis with AMF. Similarly, there were no significant differences in root length colonised (RLC) between treatments of medic or ryegrass ($p > 0.05$).

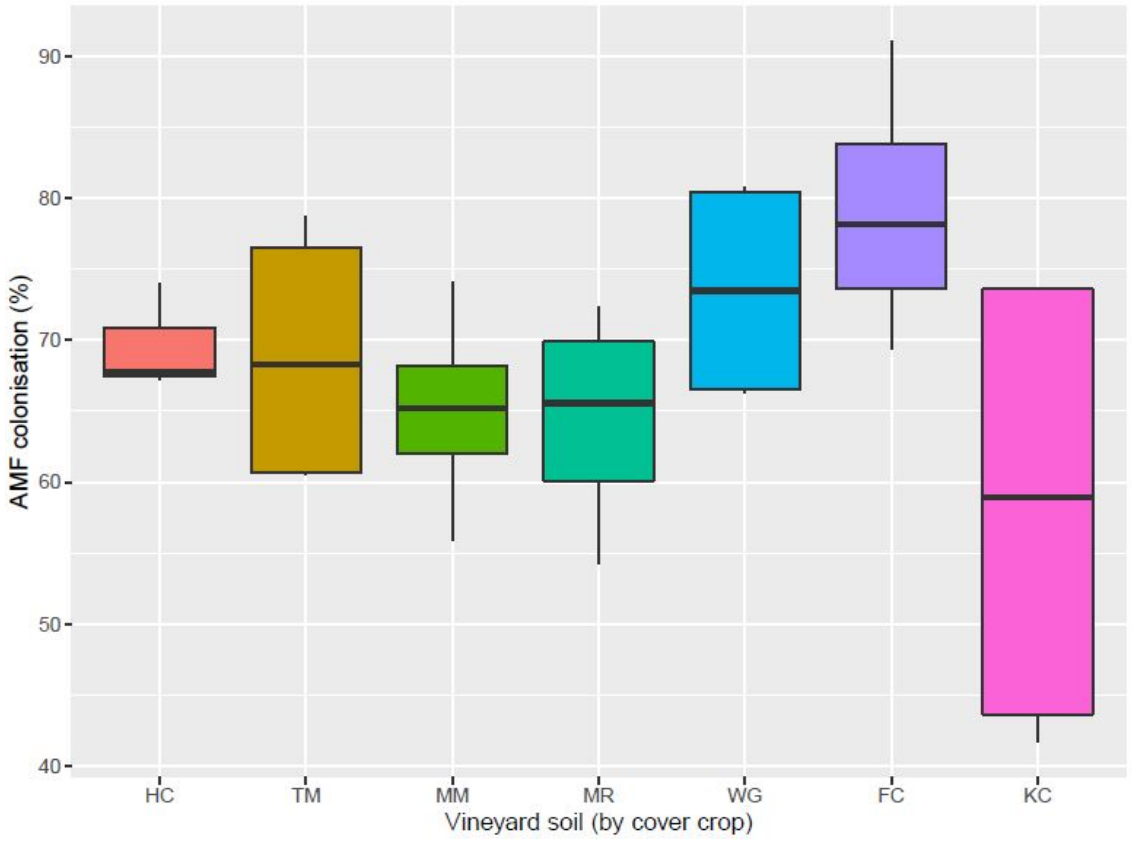


Figure 124. Boxplot showing percentage of leek roots colonised by AMF when grown in soils sampled from under-vine treatments at Langhorne Creek (Exp. 1). Treatments codes are as follows: HC = herbicide control; TM = Triticale mulch; MM = medic combination; MR = medic/ryegrass; WG = wallaby grass; FC = fescue/clover; and KC = Kasbah Cocksfoot. Boxplot shows median and interquartile range. Treatment means were non-significant (p > 0.05).

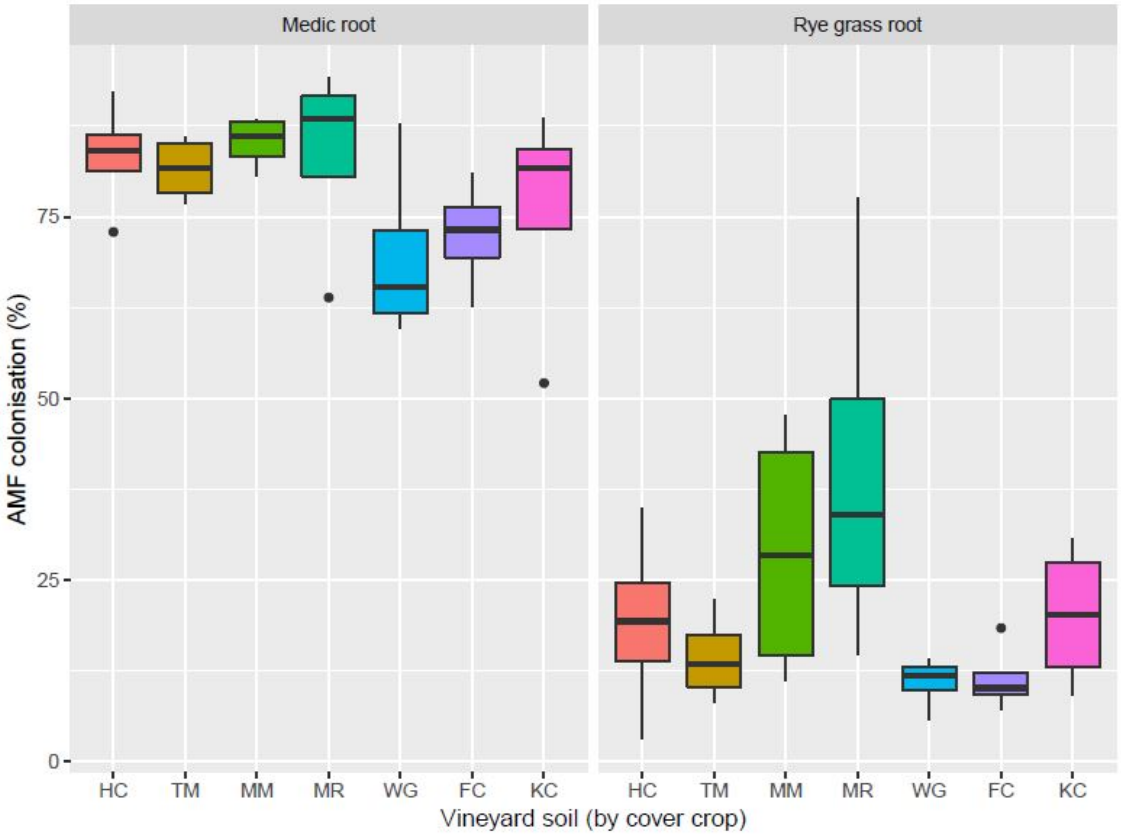


Figure 125. Boxplot showing percentage of medic and ryegrass roots colonised by AMF when grown in soils sampled from under-vine treatments at Langhorne Creek (Exp. 2). Treatments codes are as follows: HC = herbicide control; TM = Triticale mulch; MM = medic combination; MR = medic/ryegrass; WG = wallaby grass; FC = fescue/clover; and KC = Kasbah Cocksfoot. Boxplot shows median and interquartile range. Treatment means were non-significant ($p > 0.05$).

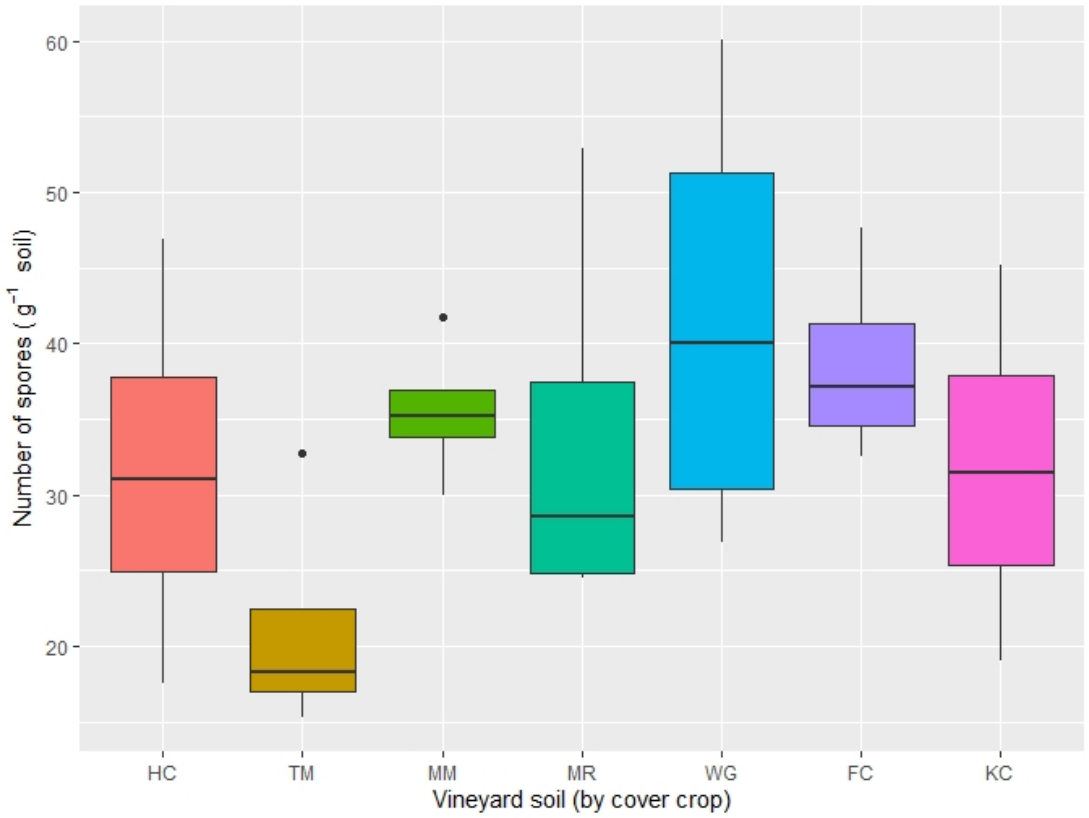


Figure 126. Boxplot showing number of AMF spores per gram of soil in treatments of soil sampled under-vine from Langhorne Creek (Exp. 2). Treatments codes are as follows: HC = herbicide control; TM = Triticale mulch; MM = medic combination; MR = medic/ryegrass; WG = wallaby grass; FC = fescue/clover; and KC = Kasbah Cocksfoot. Boxplot shows median and interquartile range. Treatment means were non-significant ($p > 0.05$).

Spore count data (Exp. 2)

Spore count analysis was conducted on 2020 soil samples (Fig. 2). Soil sampled from treatment WG showed the highest average spore count (41. ± 7.7 spores g^{-1} soil), while soil sampled from TM showed the lowest average spore count (21.2 ± 3.9 spores g^{-1} soil). The overall average spore count across all treatments was 33.5 ± 2.2 spores g^{-1} soil. Despite certain observable treatment differences (Fig. 2), these were non-significant ($p > 0.05$).

Plant root and shoot biomass (Exps. 1 and 2)

Leek total dry tissue weight (root dry weight plus shoot dry weight) was significantly different between treatments ($p < 0.01$) (Table 3). In both variables, leek plants grown in KC soil showed significantly higher total biomass than all other treatments, with those leeks grown in M+R soil presenting the lowest overall biomass. There were no significant

differences in leek biomass when grown in HC (control) soil vs any other under-vine treatment. Medic plants showed no significant differences in either root or shoot biomass across all treatments ($p > 0.05$). Ryegrass plants showed significant differences ($p < 0.03$) in total shoot weight, with ryegrass grown in treatments of TM soil presenting the greatest biomass vs those grown in HC soil.

Soil nutrient data (Exps. 1 and 2)

Soil nutrient concentrations were measured to test for total nitrogen, zinc and plant-available (Colwell) phosphorus. Soil sampled in 2018 (Exp. 1) showed the following mean values: total N = 0.20 ± 0.01%; total Colwell P = 30.36 ± 3.31 mg kg^{-1} and; total Zn = 4.43 ± 0.24 mg kg^{-1}. Soil sampled in 2020 (Exp. 2) showed the following mean values: total N = 0.20 ± 0.05%; total P = 34.82 ± 4.05 mg kg^{-1} and; total Zn = 3.46 ± 0.69 mg kg^{-1}. In soil sampled from all under-vine seven treatments there were no significant results in total N, Zn or P ($p > 0.05$). This was the case in soil sampled in 2018 (Exp. 1) and 2020 (Exp. 2).

Table 12. Plant biomass and arbuscular mycorrhizal colonisation data for leek, medic and ryegrass plants when grown in treatments of soil sampled under-vine at Langhorne Creek. N.B Significantly different treatment means are denoted by different letters (α = 0.05). Values are Mean ± S.E.; n = 32 (see Table 1 for all treatment codes).

PLANT BIOMASS & AMF DATA	HC		TM		M+R		M+M		KC		F+C		WG	
2018 LEEK DATA	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)
AM root colonisation (%)	69.64	2.20	68.95	4.82	64.42	4.01	65.07	3.75	58.30	8.86	79.19	4.64	73.50	4.05
Root length colonisation (cm)	418.7_{ab}	18.1	357.3_{ab}	67.8	115.5_{b}	37.6	386.1_{ab}	62.2	561.4_{a}	131.9	311.9_{ab}	68.6	492.2_{a}	95.9
Shoot total weight (g)	0.36_{ab}	0.16	0.38_{ab}	0.09	0.16_{b}	0.07	0.56_{ab}	0.12	0.80_{a}	0.16	0.37_{ab}	0.07	0.46_{ab}	0.06
Root total weight (g)	0.29_{ab}	0.10	0.29_{ab}	0.08	0.11_{b}	0.04	0.28_{ab}	0.04	0.55_{a}	0.07	0.22_{b}	0.04	0.42_{ab}	0.09
2020 MEDIC DATA	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)
AM root colonisation (%)	83.34	3.96	81.59	2.25	83.72	6.81	85.28	1.81	75.98	8.14	72.53	3.84	69.49	6.37
Root length colonisation (cm)	376.6	64.6	484.8	69.1	397.6	67.9	439.0	68.8	403.3	84.7	353.9	49.0	459.4	85.7
Shoot total weight (g)	0.61	0.16	1.84	0.15	1.03	0.16	1.33	0.22	0.95	0.30	1.14	0.19	1.23	0.38
Root total weight (g)	0.12	0.04	0.17	0.02	0.09	0.03	0.14	0.02	0.12	0.03	0.11	0.01	0.16	0.05
2020 RYEGRASSDATA	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)
AM root colonisation (%)	19.10	6.55	14.25	3.10	40.05	13.60	28.82	9.05	20.03	5.04	11.37	2.44	10.84	1.80
Root length colonisation (cm)	143.5	65.2	186.9	39.9	249.5	102.5	265.9	141.2	230.5	41.9	161.0	68.3	91.3	32.1
Shoot total weight (g)	0.37_{b}	0.14	1.09_{a}	0.05	0.70_{ab}	0.15	0.65_{ab}	0.21	0.78_{ab}	0.13	1.05_{ab}	0.18	0.55_{ab}	0.11
Root total weight (g)	0.28	0.13	1.05	0.37	0.35	0.11	0.53	0.20	0.72	0.10	0.80	0.17	0.50	0.25

Discussion & Conclusion

Exp. 1: leek trap plants and an initial hypotheses

In the sustained absence of under-vine vegetation (i.e. the herbicide and mulch treatments), we hypothesised that the AMF inoculum potential would be lower than where under-vine cover crops were present. However, this appears not to be the case. After an eight-week growth period, there were no significant differences in the percentage of roots colonised by AMF, which is suggestive of no significant differences in the inoculum potential of the under-vine soil treatments. Leek biomass (root, shoot and, subsequently, root length colonised) was significantly different, however, without evidence for differences in AM colonisation, this is likely a consequence of differences in nutrient contents of soils from the experimental plots, particularly in soil sampled treatments of KC (see Table 2). The initial hypothesis suggested that, after a four-year growth period, under-vine soils inhabited by cover crops would have an increased AMF inoculum potential compared with those managed with herbicide. Initial experimentation used leek (sp. *A. porrum*) as a suitable trap plant to avoid limiting results through possible home-field advantage interference, however in light of the above-mentioned results, two potential issues with initial hypothesis-testing: 1) *A. porrum* is a highly mycotrophic species (Smith & Read 2008) – a factor that could have inhibited treatment-based results owing to inherent plant-phenology and, 2) we did not conduct an initial spore count to test whether this fundamental source of inoculum differed between soil treatments prior to pot-growth experimentation. It became clear that these further questions needed to be explored prior to a definitive conclusion being asserted, and thus, a second experiment was conducted.

Exp. 2: mycotrophic vs non- mycotrophic trap plants

To further explore our initial hypothesis, a second line of inquiry was proposed, this time quantifying inoculum potential from the same treatment plots, however now using two different plants: medic and ryegrass (mycotrophic vs non-mycotrophic). This second experiment not only investigated inherent mycotrophic differences in root colonisation (medic vs ryegrass) but also allowed for temporal effects of inoculum deposition to become more pronounced. A spore count was also conducted on these soils, however, there were no significant differences in spore counts, thus answering one of the primary questions raised from Exp. 1. Similar to leek, medic is also highly mycotrophic (Smith & Read 2008), however, ryegrass is far less so and was thus chosen to mitigate any inherent plant phenology that might mask treatment-based differences (as seen in Exp. 1). Colonisation of medic roots

was higher than ryegrass roots, owing to the highly mycotrophic nature of medic (Smith & Read 2008). Despite differences in total colonisation (between trap plant species), there were no observable treatment effects (between the same trap plants grown in different soils). Although we are forced to reject our initial hypothesis, the differences in colonisation between trap plants provides insight into management practice. Inoculum potential is only *potential*, with hyphal networks only forming if a mycotrophic plant (and root system) is present. Therefore, it could be speculated that under-vine cover crops consisting of highly mycotrophic plants (e.g. medic) have the *potential* to become more highly colonised and thus support a greater abundance (network) of hyphal structures (Bowles *et al.* 2017).

Mycorrhizal spore inoculum is unaffected by herbicide treatment

Impacts of herbicide management on AMF vary considerably among studies and can, by nature, be both *direct* (deleterious or neutral impact on fungi) or *indirect* (removal of host plants leading to absence of AMF) (Rose *et al.* 2016). Druille *et al.* (2013) reported a negative correlation between rate of glyphosate application to soil, and spore viability and subsequent root colonisation. Moreover, they concluded that glyphosate became deleterious to AMF viability and root colonisation only when applied directly to the soil but not when applied to plant foliage (Druille *et al.* 2013). Moreover, while the literature suggests that AM hyphae may be prone to the detrimental effects of herbicides, less is mentioned on spore viability and capacity to buffer against herbicide leachate (Baumgartner *et al.* 2010). Based on results from a number of previous studies, even with twice the rate of conventional field application, several herbicide compounds showed little effect on inoculum potential (Mujica *et al.* 1999; Pasaribu *et al.* 2013). We can therefore conclude that herbicide-management under-vine may not necessarily result in a decrease in fungal inoculum potential. Indeed, these findings concur with our own, where soils from experiment 2 showed no significant differences in the number of spores between treatments. Herbicides may indirectly affect AMF inoculum potential merely due to the absence of host plants – a notable condition in cropping systems known as *long-fallow disorder* whereby the prolonged absence of host plants diminishes the ability of AM propagules to colonise subsequent crops (Thompson 1987). Although a well-regarded symptom of host-absence, *long-fallow disorder* may have been mitigated in our own field trial due to the buffering presence of grapevines and mid-row vegetation. Based on evidence from a meta-analysis conducted by Bowles *et al.* (2017), it could be predicted that maintaining under-vine cover would enhance AM inoculum potential; however, this was not the case. Several studies have concluded similar results, notably that

conducted by Baumgartner *et al.* (2010). We suggest several biotic and abiotic factors that may nullify under-vine cover crop influences on inoculum, with the most likely being the presence of mid-row vegetation and the grapevines themselves – both of which may provide their own source of AM inoculum, buffering against treatments where plants were absent (HC and TM).

Mid-row and grapevine inoculum, no-till practice and future studies

Although the experimental treatments considered here did not result in impacts on colonisation, there are other vineyard practices that may have influenced results. Within our trial it is likely that additional sources of AMF inoculum were present – buffered by vegetation in the mid-row and indeed the grapevines themselves. The relatively high AMF inoculum potential in soil denuded of under-vine vegetation could also be explained by the presence of grapevine roots (Holland *et al.* 2014; Radić *et al.* 2012; Schreiner & Mihara 2009; Valentine *et al.* 2006). The constant presence of a mycorrhizal-dependent species is a significant contributing factor to the replenishment of propagules and soil inoculum buffering capacity. Moreover, the mid-row may offer a source of AM inoculum, with a dense sward cover increasing AMF propagules from mid-row to under-vine. One study found hyphae – and spore propagules – extending some 30 cm (in the extreme) beyond the host root system (Harinikumar & Bagyaraj 1995). Given the width of the under-vine area is approximately 60 cm and is bordered either side by mid-row sward, it is reasonable to suggest mid-row impact on under-vine inoculum potential is influential. Further studies reached a similar conclusion, suggestive that cover crops (species-specific) increase fungal diversity and play pivotal roles in certain soil processes (Trouvelot *et al.* 2015; Vukicevich *et al.* 2019). Other authors suggest that while cover crops may increase fungal diversity, they appear to be a less dominant factor than the mere presence of perennial grapevines in the enhancement of mid-row inoculum potential (Baumgartner *et al.* 2010; Trouvelot *et al.* 2015).

One particularly pertinent variable that unites all treatments is the use of a *no-till* management practice. Evidence for tillage impacting negatively on AMF diversity and function is relatively consistent in literature, especially studies involving long-term field trials (Brito *et al.* 2012). A comparative analysis of tillage vs no tillage on a southwest German vineyard system found a marked decline in AMF spore density and fungal diversity in tillage treatments (Fritz & Bruno 2018). Where the literature appears inconsistent is in discussions surrounding specific propagule damage – spore vs hyphal fragments. Kabir (2005) highlights several key sources pertaining to AMF decline through tillage, however, through their own

study, suggested that while AMF spores may withstand tillage, hyphae fare poorly. Soils from our trial site saw a no-till practice (after treatments were established), leaving both hyphae and spores undisturbed. Sieving of soils prior to glasshouse experimentation may have impacted poorly on hyphal fragments, however, it is likely that fungal spores remained dormant (Smith & Read 2008). Incubation of soils prior to each planting was likely to activate AMF spores, enabling significant root colonisation throughout all treatments (Schimel *et al.* 2007). We did not directly test the effects of tillage vs no tillage on our treatments, and although vineyards remain relatively undisturbed during their working lives, changes in management practice and grapevine rootstock are important instances of potential disturbance. To further test the hypothesis that soil disturbance (or a lack thereof) is a dominant factor in AMF inoculum potential, we would propose a future, multifactorial trial that compared under-vine treatments in a till vs no-till study (i.e. herbicide till vs herbicide no till vs cover crop till vs cover crop no till).

To more accurately determine which vegetative feature is the dominant source of inoculum (if any), one could test the influence of mid-row vegetation on under-vine inoculum potential either by: 1) denuding the mid-row or, 2) inserting artificial barriers between the mid-row and under-vine; in each case eliminating mid-row influence such that only grapevines and under-vine cover crops are potential sources of inoculum. The direct effect of host presence appears pivotal to AM persistence via inoculation, however, it is also worth noting the indirect effects of disturbance, or in this case, a lack thereof.

Implications for under-vine management

The implications of these results in terms of future under-vine management practices are numerous, owing to the multifaceted influences of under-vine cover-cropping. Pertaining directly to AMF inoculum potential, the results appear uncertain as to the direct influence of different management practices. Sources of inoculum in the vineyard can be numerous, with mid-row and grapevine roots offering potential buffering capacity – aiding in the replenishment of AM spores. All of this aside, however, it should be noted that we are discussing inoculum *potential* – that is to say, the *potential* for plant-AM colonisation, which is high between all treatments, however, this is only possible in the presence of active plant roots. We have noted previously that increased AM hyphal networks are beneficial to soil fertility in numerous ways – more than simply nutrient acquisition for the focal crop (grapevines). Therefore, although AM inoculum (notably spores) may withstand herbicide application, they still require plant root systems in order to establish hyphal networks. Given

this, we may suggest that establishing under-vine cover is important to allowing these networks to form. Results from this study conclusively showed that not all plant roots are colonised to the same extent (e.g. medic vs ryegrass) and therefore, we may further suggest that planting a mycotrophic crop such as medic (*M. truncatula*) could be more beneficial to establishing hyphal networks than a less mycotrophic species, or for that matter, nothing at all.

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Part 3 - Bio-fumigation Trial

Author participation

Professor Timothy Cavagnaro, Dr Thomas Lines, Mr Chris Penfold and Dr Vinay Pagay.

Background

The incidence of root knot nematodes on own-rooted grapevines is widespread in Australia (Nicol et al. 1999), and the yield sacrificed due to their presence is reported at up to 32% (Stirling 1976), with losses to the Australian industry of $14 million dollars annually (Australia" 2020). The use of nematicides produced synthetically is no longer encouraged due to the potency, expense and non-selective nature of the products on soil organisms, plus work, health and safety concerns. There are presently no nematicides registered for use in Australian vineyards where grapes for wine will be used in exported products (Essling & Lord 2020).

Non-chemical options for their control include the use of resistant rootstocks and the encouragement of a diverse soil ecology which might naturally suppress the nematodes. Recent research (Abdel-Sattar et al. 2020) has investigated the use of mineral and biological alternatives to synthetic nematicides. They found the use of boric acid, applied at 25 gm/vine, with and without the addition of chitosan, generated similar yield improvement to the synthetic chemical treatment. It is proposed the benefits are derived partly by supplying nutritional benefit to the vines, as boron is critical to pollen germination, growth and thereby fruit set. As an acetylcholine esterase inhibitor in some insects, it is suggested this is a likely pathway for the nematicidal qualities exhibited.

Previous research in Australia (Rahman & Somers 2005) had identified the capacity of Brassica spp. to suppress root knot nematodes that infect vine roots, through the production of glucosinolates which are the hot, peppery substances found in many brassica species. Following mechanical desiccation, the myrosinase enzyme causes their breakdown to produce isothyocyanates (ITC's) which are antagonistic to nematode populations. To follow up on this work, with funding from Wine Australia, the capacity of under-vine cover crops to provide suppression of root knot nematodes (*Meloigodyne* spp.) was investigated.

Materials and Methods

It was necessary to quickly find a suitable site (due to project start date), so through the Barossa Grape and Wine Association, an email was sent out to its 550 members requesting access to a site with high nematode populations. As there were no responses to this request, an offer from Treasury Wine Estates was accepted, from which soil samples were taken and analysed by SARDI's Molecular Diagnostic Centre. The results showed nematode populations, predominantly consisting of *Meloidogyne areneria* and *M. javanica*, were present at the chosen site. While vine growth at this site was obviously stunted, possibly due to nematodes, there was little choice of site so a trial was proceeded with (in consultation with WA).

The treatments used were based on previous literature, seed availability and observations of under-vine cover crop growth from earlier trials. A commercially available tetraploid cross (*Brassica napus*, cv Nemcon) is recognised as providing high glucosinolate levels while also being agronomically suited to our soil and rainfall environment. Other species to be included in the trial were Medics, Ryegrass, clover, wheat and wallaby grass, all with potential for altering the soil biology in favour of beneficial soil biota.

A total of eight treatments were used, as shown below in Table 13.

Table 13: Bio-fumigant trial under vine species and their management.

Treatment No.	Treatment	Management
1	Herbicide control	Glyphosate or Basta
2	Nemcon radish	Slashed or rolled
3	Nemcon radish	Slashed or rolled, then buried
4	Triticale	Slashed or rolled
5	Safeguard Ryegrass	No intervention
6	Scimitar Medic and R/G	No intervention
7	Mawson sub clover	No intervention
8	Wallaby grass	No intervention

The trial was sown on 28/5/2018 using a Taege vineyard seeder, with one row sown each side of the vines within 20 cm of the vine trunk. The wallaby grass, with seed in a raw form, was sown by hand.

	ROWS CONTINUE					
	3 VINE BUFFER					
Panel 8		1	6	3	7	
Panel 7		6	7	4	1	
Panel 6		4	2	7	3	
Panel 5	BUFFER	8	3	5	6	BUFFER
Panel 4		7	8	1	5	
Panel 3		3	1	2	8	
Panel 2		5	4	6	2	
Panel 1		2	5	8	4	
	3 VINE BUFFER					
	ROW 1	ROW 2	ROW 3	ROW 4	ROW 5	ROW 6
	DIRT ROAD					

Figure 127: Biofumigation trial design, located at Treasury Wine Estates Hopelands Vineyard, Nuriootpa, SA.

Despite ensuring red-legged earth mite, which are major pests of emerging broadleaf plants, were controlled, the establishment of Medics, clover and Nemcon was poor throughout the trial site. Following conversation with the vineyard manager (Laurie Ryan) it then became apparent that they had sprayed the herbicide Stomp (Pendimethalin 330) at 9 L/ha the previous spring. Label recommendations suggest not planting green manure crops (including some Medicago and Brassica spp.) for 12 months after application. While the trial was sown more than 12 months after this application, there may have been a legacy effect. The Ryegrass and triticale were however unaffected, but the very dry season did impact on their growth.

In September 2018, soil moisture monitoring access tubes were inserted in each of the 32 plots. Soil moisture and N were sampled fortnightly over the growing season. Vine water potential was measured using midday pressure bombing, and the site was harvested on the 13th of March. Unfortunately, variability across the site was so great that no significant results were obtained (data not shown).

A minimum of six samples of soil from each treatment, with a total mass of at least 200 g, was taken from 0 to 10 cm and the replicates bulked then subjected to analysis by APAL for their chemical properties. Leaf petioles/blades were sampled at flowering in 2018 and analysed for their nutrient status by APAL laboratories (See Part 1 methods).

In May 2019, the Medic, clover and Nemcon treatments were re-sown, but again the Nemcon failed to establish. To determine if this was due to the seeding process, soil from the site was used to grow Medic, Ryegrass and Nemcon seed in a glasshouse at Roseworthy. While the Ryegrass and Medic provided good establishment the Nemcon again failed (seed viability was not a problem). It was therefore suspected that the very high rate of Pendimethalin (Stomp) application in 2017, coupled with dry seasons restricting the bio-degradation of the herbicide, again prevented the growth of Nemcon in the second year.

Given this unfortunate response from the leading contender for biofumigation (Nemcon), project resources were redirected (in consultation with WA) to the establishment of another a small plot trial was initiated. A stand of Nemcon plants were grown in the glasshouse at the Waite Institute, harvested and shredded into small pieces, then buried in 0.1 m2 plots under-vine at the Tanunda site on the weed free control plots. Application rates for the Nemcon were calculated at the equivalent of 10 t/ha, similar to growing 5 t/ha in the mid-row and side-throwing under-vine in a concentrated zone. The material was buried due to the volatile nature of the glucosinolates, requiring coverage with soil to prevent loss of the active ingredients. Soil was sampled three times (days 0, 1, 5 and 12) over the next fortnight to assess the impact on nematode numbers of the Nemcon foliage.

Seasonal conditions

As noted in the 2020 vintage report for the Barossa (Howell & Rose 2020), weather conditions in 2019 generated yields at least 50% below the 10-year average. The 2019 calendar year was the driest on record at the Nuriootpa Research Station. The main reason,

aside from nematodes, for the low yield at the Hopelands vineyard is believed to be the combination of strong winds in early November, cold nights in mid-November and extremely hot weather in late November, including a 42 ºC day on November 20.

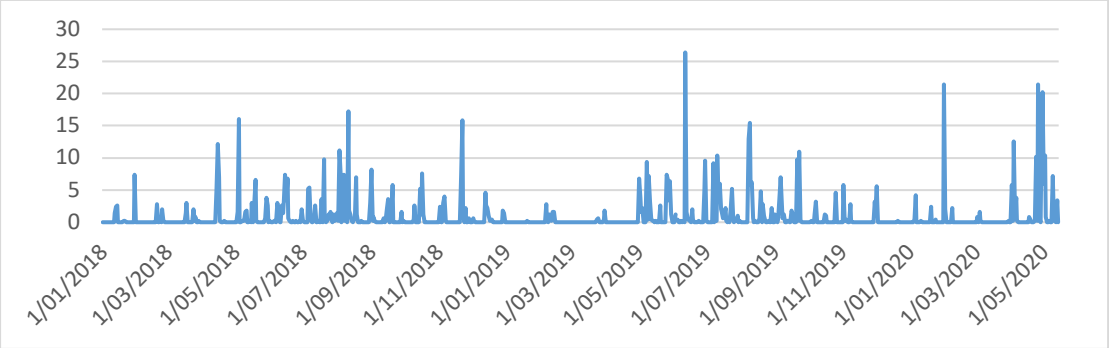


Figure 128: Rainfall received over the trial period was below average, with 106 and 109 mm received respectively for the 2019 and 2020 growing seasons.

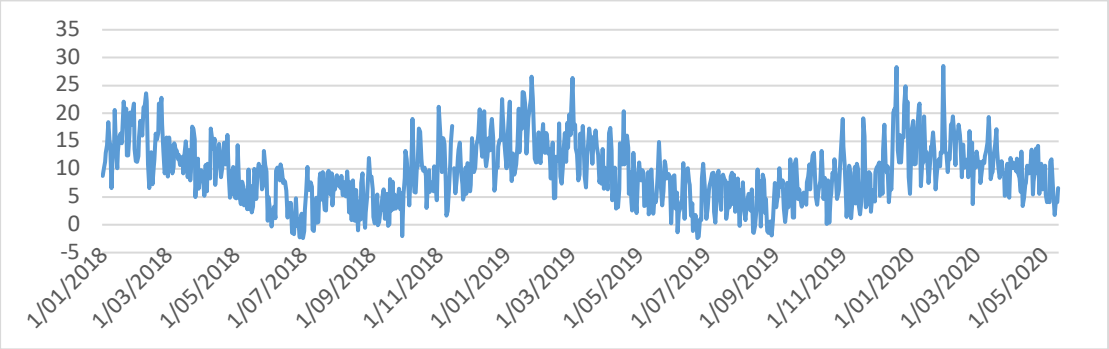


Figure 129: Minimum temperatures showed some below zero events in both the 2019 and 2020 seasons.

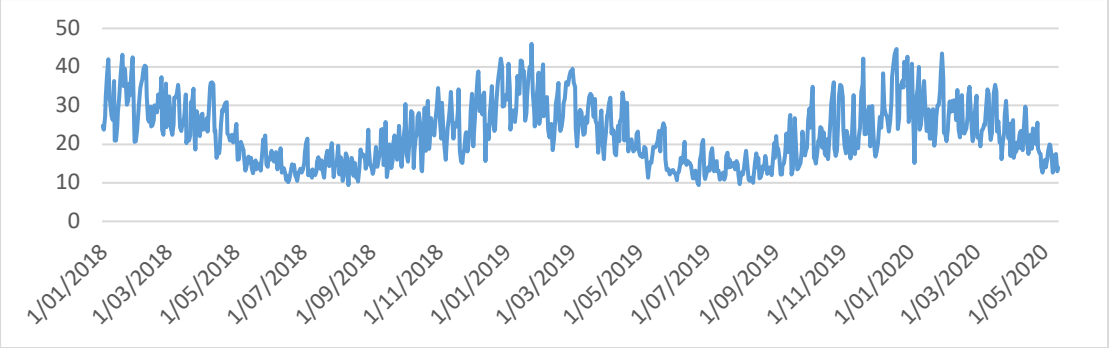


Figure 130: Maximum temperatures showed hotter events in spring 2019 than 2018, which affected flowering and ultimately yields.

From the perspective of establishing and generating results from a cover cropping trial, the environmental conditions of low rainfall and heat events at flowering compromised the cover crop growth and severely affected vine yield, preventing the acquisition of meaningful production data.

Routine sampling on soil and moisture profiles were performed on the site in 2019. Equivalent sampling was not performed in 2020, with resources redirected to the small plot trial and to the other main project sites.

Results & Discussion

Botanical composition

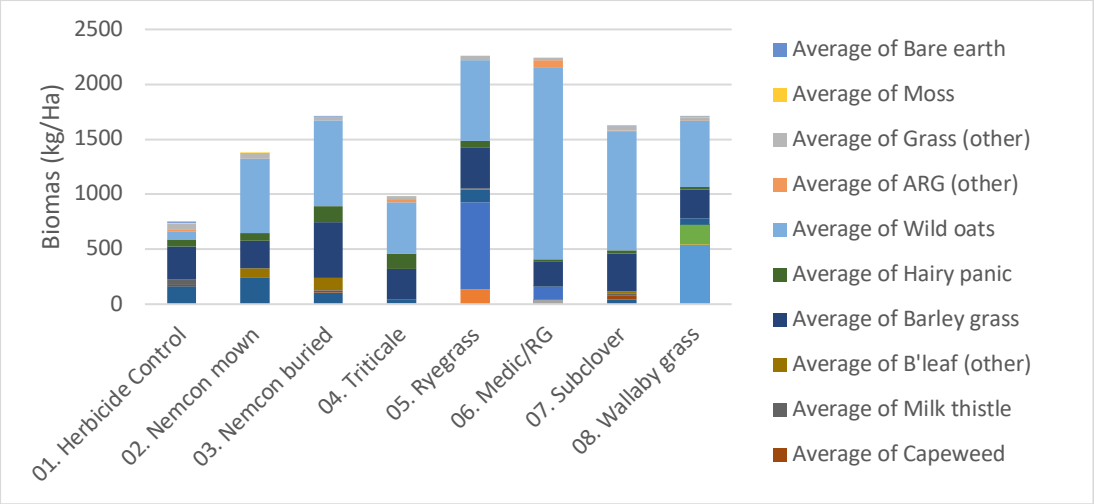


Figure 131: An assessment of the botanical composition and dry matter (kgs) of treatments following their germination – 26-5-2020.

As shown in Figure 123, wild oat was the dominant plant on the trial site. The data was acquired prior to attending to the weed issues apparent by using a line trimmer which effectively reduced the wild oats biomass and allowed the preferred species to perform. While all treatments are shown here, there had been no Nemcon growing on those treatments, as it failed to germinate in soils which seemed hostile to its growth. The triticale at this stage was a mulch on the soil surface from the previous season. The Ryegrass, Medic and wallaby grass had all re-established albeit at compromised levels of biomass due to the wild oat and barley grass competition.

The total amount of biomass produced of up to 2.5 t / ha is high for so early in the season, reflecting the seasons early break with a substantial rainfall event, and the inherent fertility of the site. In relation to the vines, it would also suggest there are no nutrient limitations to their growth.

If wild oats and barley grass are considered as weeds, and in this case the wild oats, which grew into the grape canopy during the previous spring, was exhibiting weedy behaviour, then in a commercial sense they would need controlling. This could have been conducted with an under-vine mower in spring 2019, or where herbicides were accessible, then a selective grass killer would have been suitable. This would also have left behind the herbicide resistant Ryegrass which was not so dominant and of lower stature than the wild oats.

This trial site has displayed the difficulties with accessing locations where their history can cause unexpected consequences in the trial which can impact results dramatically.

Soil physicochemistry

Soil pH (Figure 132), showed a difference of 0.26 units between the lowest (clover, Medic and triticale) and the highest (herbicide, Ryegrass) treatments. The lower pH expressed with the legumes is likely due to their deposition of H^+ ions into the soil during the process of N fixation. The Ryegrass treatment is instead utilising N ions in its growth as NH_4^+ or NO_3^-, leading to a possible slight increase in soil pH. In slightly alkaline soils as noted at the trial site, a lowering of pH via the legumes is not a concern, with some elements becoming more available to the vines as the pH gets closer to neutral.

Soil salinity (Figure 133), as determined via Na, electrical conductivity (EC) and total dissolved salts (TDS) measures, showed significant differences in the latter two measures. The herbicide treatment had higher EC and TDS than the Medic and clover, but this was not due to Na content in the soil. Instead magnesium was higher on the herbicide treatment, as it had not been removed in growing foliage and had possibly not leached with improved infiltration associated with the cover crop treatments. It is also possible the herbicide treatment had lost moisture from evaporation, concentrating the salts remaining in the upper soil profile.

Soil Plant-available phosphorous (Figure 134) appeared lower on the Medic/Ryegrass, clover and triticale treatments than the herbicide control. Legumes have a high demand for phosphorous as a critical element in the N fixation process, which is likely to have produced this finding. The triticale also grew well, and as a cereal also requires adequate P for optimal growth. The P levels shown below are very high however, which is likely a result of the application of compost to the under-vine prior to the establishment of this trial.

Soil carbon concentrations did not change significantly over the short term of the trial. Given the two years of below average rainfall, which the cover crops are reliant on for their winter activity, and consequently compromised biomass production over that time, it is not surprising that carbon levels had not increased over that time. With the intimate linkage between soil carbon and N, there was also no shift in soil N.

Of the trace elements, iron (Fe) and magnesium were lower on the Medic/clover than the herbicide and Ryegrass treatments and the Fe was very low across all treatments according to recognized standards. Leaf yellowing which is symptomatic of Fe deficiency (lime induced chlorosis) was not however evident, suggesting there were other issues that were preventing the growth in these vines. Other soil nutrients (manganese and zinc) were also very low, and these in combination have potentially played a role in the very poor yields that were experienced over both years of this trial.

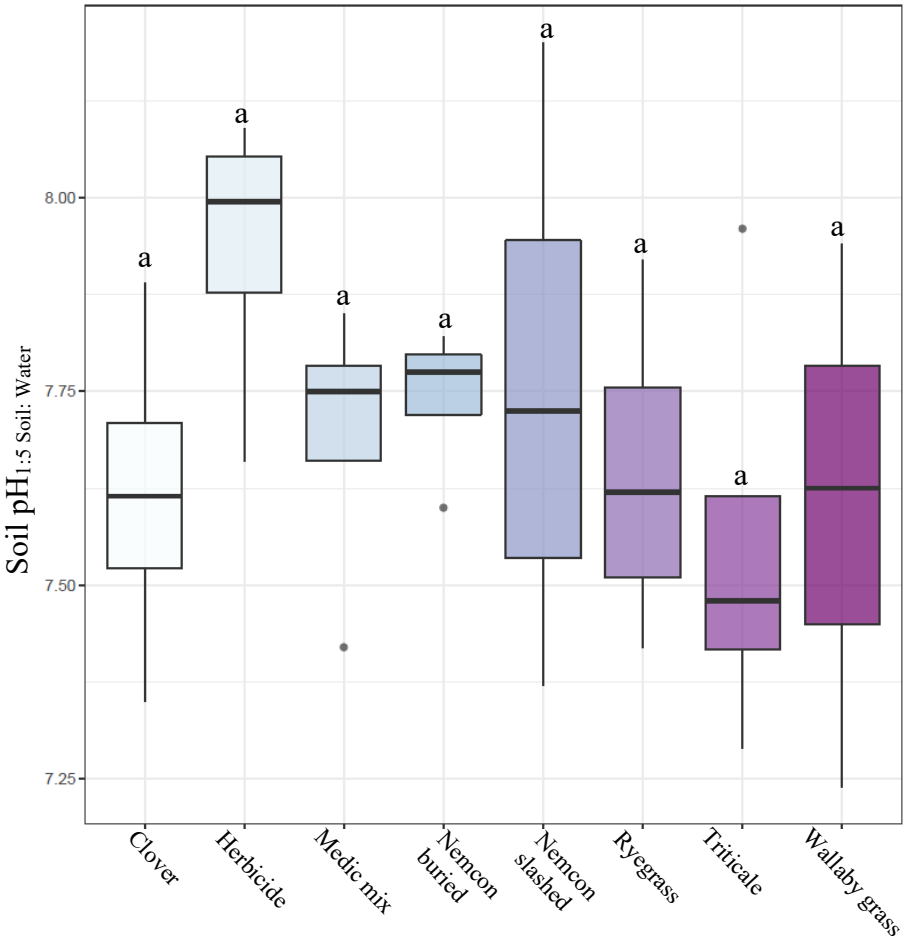


Figure 132: Soil pH Soil pH 1:5 Water, Tanunda, Vintage 2020

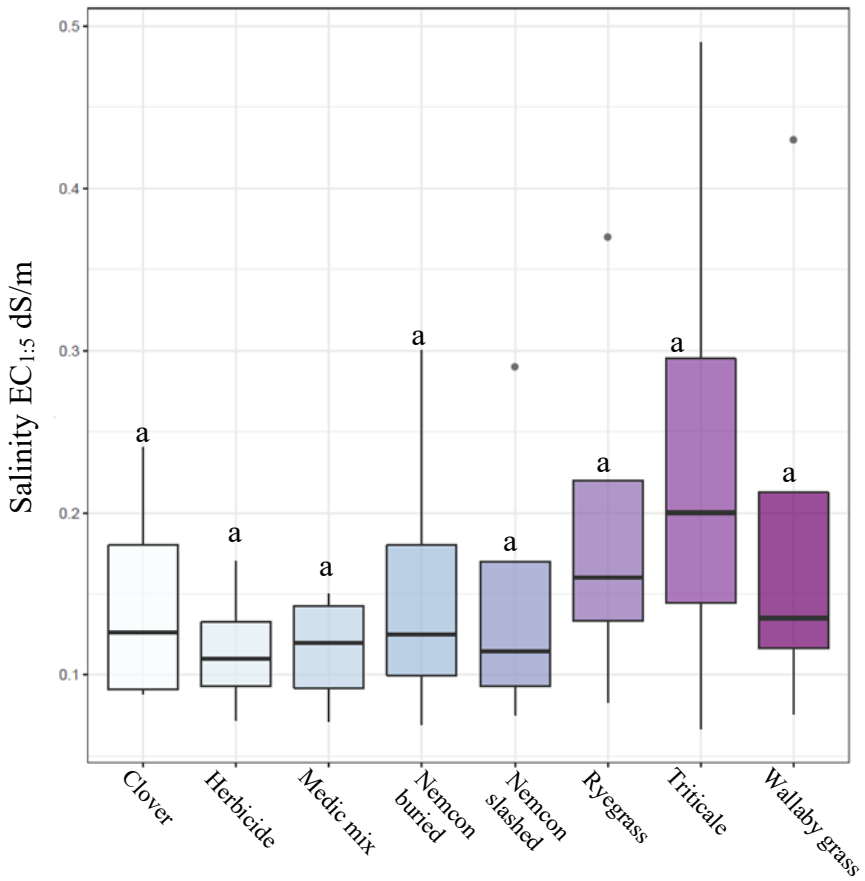


Figure 133: Salinity as determined by measuring electrical conductivity.

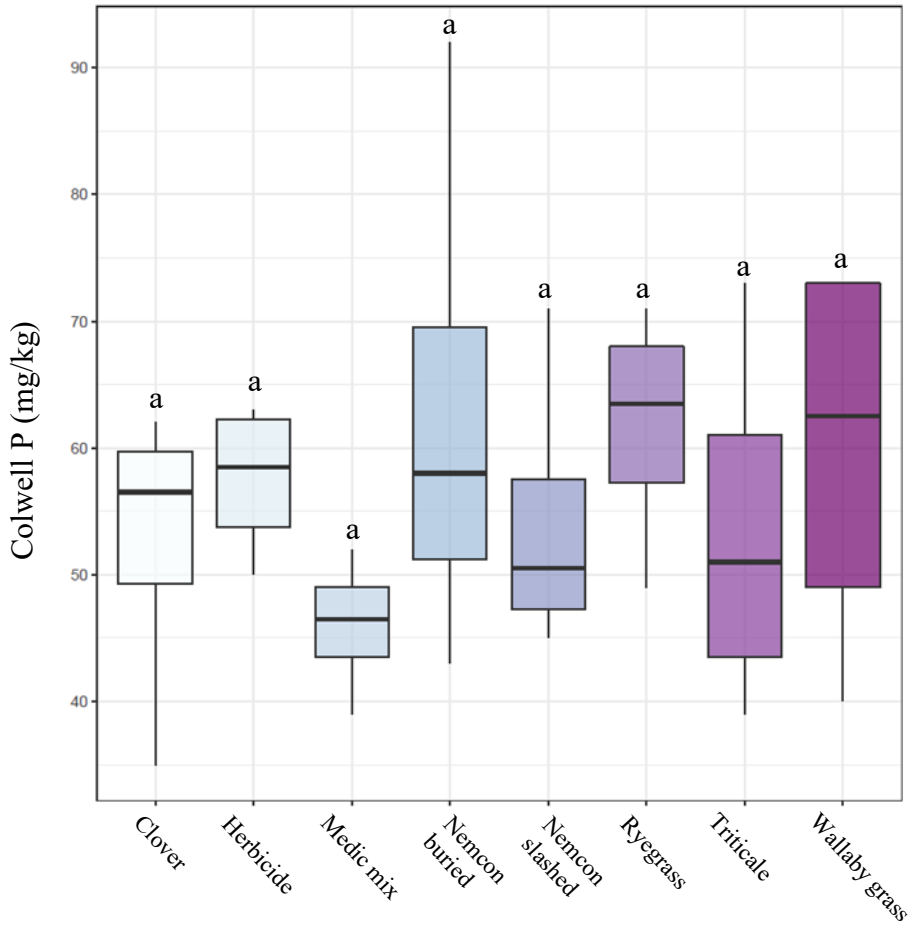


Figure 134: Available soil P.

Tissue chemistry

Leaf blades were used rather than petioles to measure vine nutrient status as they have been determined to provide more accurate information on the vine response to N and to forecast the likely YAN's in the must (Schreiner & Scagel 2017).

Analysis showed differences between treatments for boron (B), chloride (Cl), aluminium (Al) and potassium (K). Desirable levels of B are necessary due to its potential impacts on fruit set and ultimately yields (Abdel-Sattar et al, 2020). According to the standards for leaf blade at flowering (the sampling time used in this case) the levels noted at this site were acceptable and should not have impacted yield. A possible explanation for the increased uptake on the Ryegrass treatment is that both B and Cl have accumulated in the subsoil which is the source of soil moisture for the vines on this treatment because the Ryegrass has utilised the water in the A horizon more so than the herbicide and wallaby grass. A greater concentration of K on the herbicide compared to the Ryegrass treatment is again likely to be related to soil moisture availability. K in the surface soil of the Ryegrass treatment would have been less available to the vines as the Ryegrass had dried the upper soil profile.

Of the remaining elements analysed, N, K, Ca, Mg, Na, Cu, Zn and Mn were all within the desired range for optimal growth. Only phosphorous was low (0.17 compared with 0.25 – 0.4 being desired), which brings into question the vines capacity for P uptake given the soil P levels were in excess of requirements.



Figure 135: Triticale prior to mowing, 2019.



Figure 136: Medic and Ryegrass, 2019.



Figure 137: Mawson sub-clover.

Yield

In 2019 the yields were very low, with no significant differences between treatments. High variability across the site and between individual vines compromised the potential for observance of treatment differences. This may be in part due to nematode infestation at the site.

The 2020 season saw very poor fruit set across the Barossa and many other regions. The Tanunda trial site was no exception. Of the 64 vines assessed for prospective yield on the eleventh of March, 2020, only one small bunch was found. The balance of the block on which the trial was located provided similar yield results, with most of it not harvested. It was therefore impossible to generate conclusions for the treatment effects on yield.

Soil moisture:

Fortnightly readings of soil moisture over the 2019 growing season are shown below (Figure 138) as three snapshots during that time. While the Ryegrass in particular appears to have depleted soil moisture at 60 cm at the final sampling (harvest 13-3-2019) when compared to the control and wallaby grass, there were no significant differences for any of the treatments, due to the large variability in the data. It is also exacerbated by the poor vine performance at this site, whereby the vine response to cover crop treatment was minimal, so demands on soil moisture were consistent across the treatments. Annual Ryegrass is primarily active during winter and spring, and after setting seed it senesces to form a mulch on the soil surface. The apparent reduction in soil moisture shown on the Ryegrass treatment in March is therefore unexpected, but maybe a function of the vine roots becoming more active as a result of improved soil structure resulting from the Ryegrass growth.

Wallaby grass is also a C3 grass, meaning it is genetically programmed for winter and spring activity. This cover crop also grew very well, and went dormant over the summer period, which stopped water uptake, and this is reflected in Figure 138.

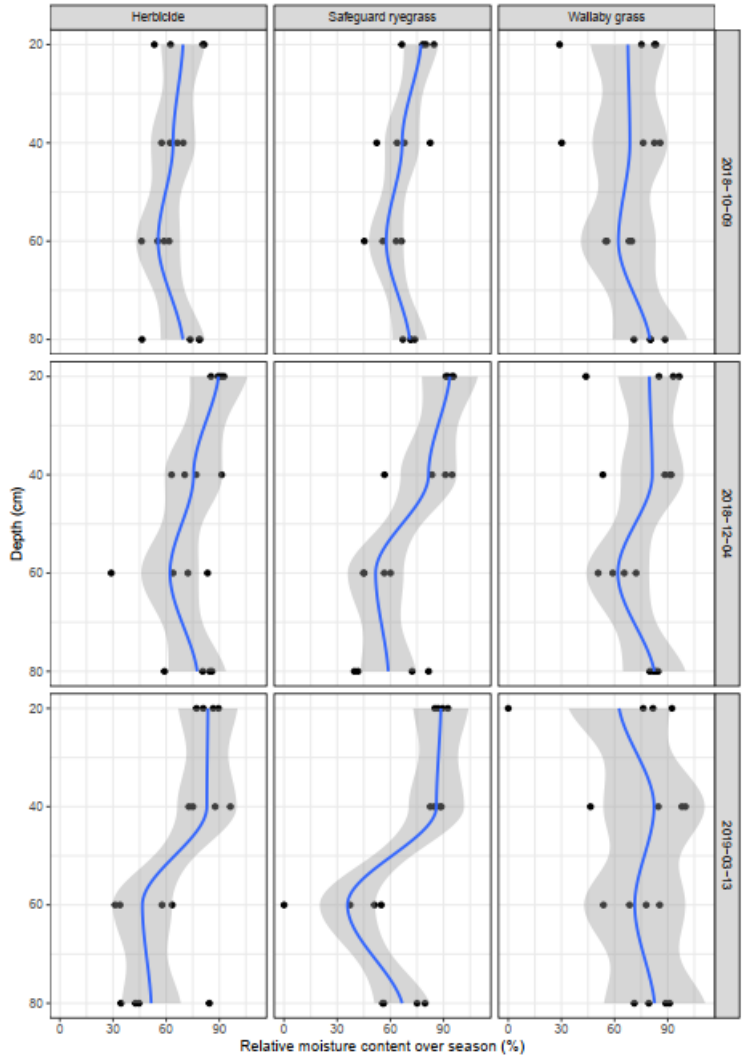


Figure 138: The relative soil moisture content for 3 treatments (herbicide, Ryegrass and wallaby grass) over 3 time points for the 2019 growing season.

Mineral Nitrogen

NH_4^+ is generated naturally through the mineralization of organic matter to inorganic (mineral) N. This process speeds up in spring due to the combination of soil warming and moisture availability, enabling a spike in soil microbial activity. This leads to the "spring flush" in plant growth. All treatments displayed similar spikes in NH_4^+ being released in the October-November period, but again there was considerable variability in the data preventing significant differences to be evident. The release of NH_4^+ is mostly coming from the labile soil organic matter, or that which is readily mineralized by the soil microbes, and certainly in this first year is unlikely to be influenced by the cover crop.

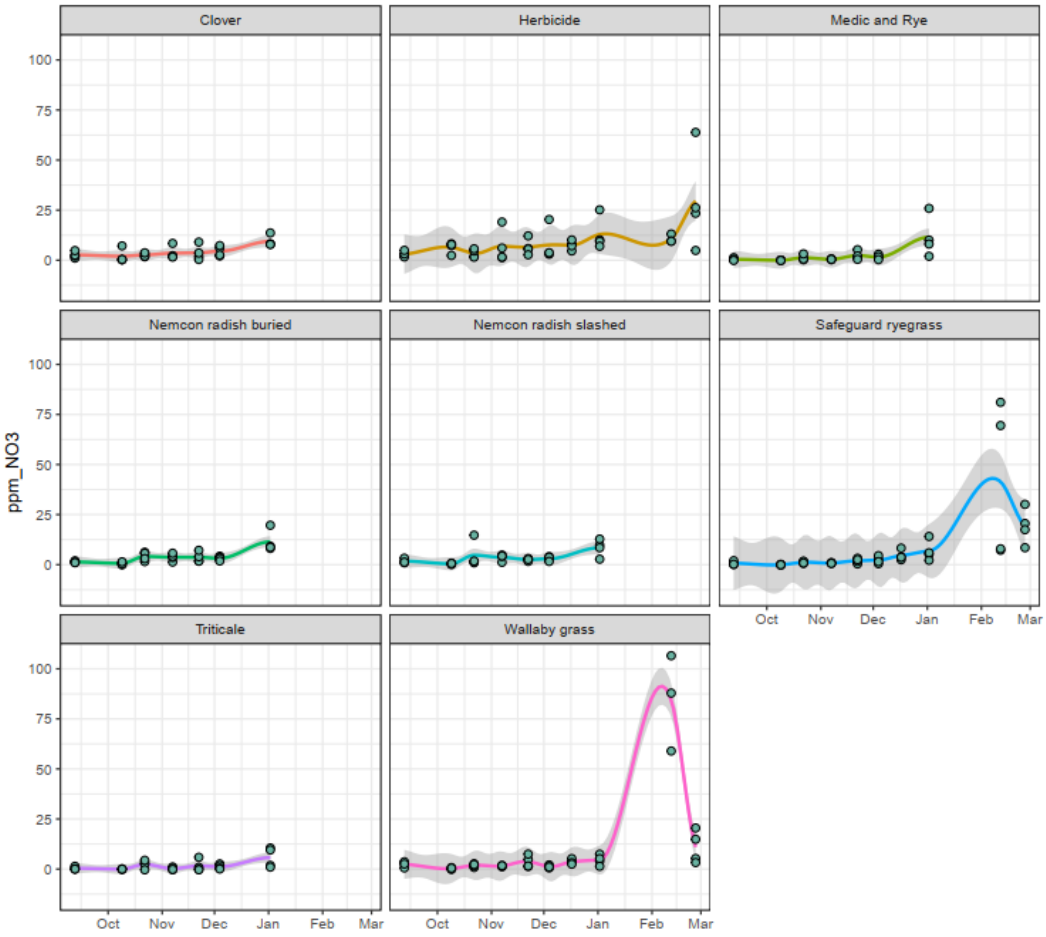


Figure 139: Soil mineral nitrate as extracted from soil samples collected across the 2019 vintage.

Following mineralisation of soil organic matter to produce NH_4^+, the next phase of the N cycle is nitrification to produce NO_3^-. Like NH_4^+, NO_3^- is highly mobile in the soil and readily taken up in solution by plants. Soil nitrate levels are low across all treatments for the growing season until February, when large spikes occur in the wallaby grass and to a lesser extent in the dormant Ryegrass treatments. The herbicide treatment did not respond to the same degree, but was somewhat higher in NO_3^- with no competition from cover crop growth. The spike was due to an application through the dripline of Easy N (42.5% N) at 10 L / Ha (4.25 Kg/Ha) on December 19, with a post-harvest application also applied on March 3, 2020.

Nematode populations:

Despite high rates of application of partially macerated Nemcon leaf and stem material which was immediately buried, there was no significant effect on nematode populations from day

one to day 12 after burial (Figure 140). Morra & Kirkegaard (2002) found the release of the isothiocyanates (ITC) was greatest immediately after cutting and incorporation, and it was enhanced with rain, with the benefit significantly reduced after 72 hours. They also noted that the greater the destruction and maceration of the plant tissue, the greater the release of ITC's. Other researchers (Bending & Lincoln 1999) found the maximum ITC levels in a sandy loam soil at 96 hours after incorporation. Morra and Kirkegaard (2002) therefore concluded that most of the ITC would be released after 4 days, though this will depend on soil physical and chemical characteristics, temperature and moisture. Even with ideal conditions for the ITC release, including fine maceration of plant tissue prior to its burial, followed by maintaining the soil in a moist state, the chemical release beyond 1% of active ingredient is difficult to achieve; such conditions were not experienced in this trial, with the soil drying quickly in summer despite the plots being located under drippers. The leaves were roughly chopped as would be expected of a side throw mower.

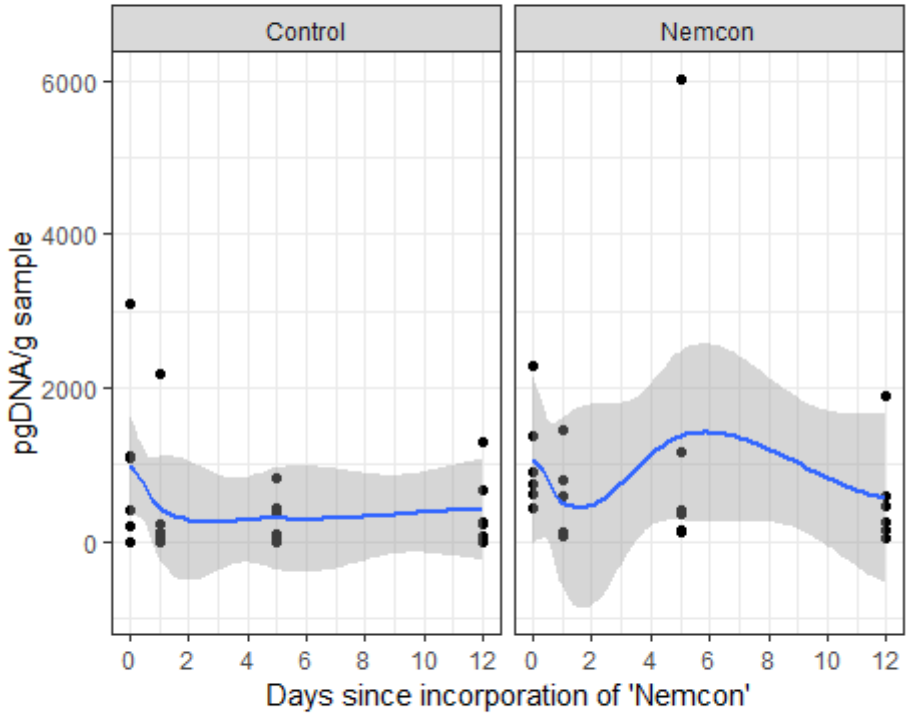


Figure 140: Nematode DNA concentration (pgDNA per gram of soil) after in-situ nemcon brassica incorporation (right), or only soil surface disturbance (left).

In May 2020, the main trial site was sampled to determine if simply growing cover crops would be sufficient to impact on nematode numbers, even if they are not recognized for biofumigation qualities. Again, no significant response from the range of cover crops when

compared to the control was apparent (see Figure 141). It is encouraging though that there is no apparent increase in nematode populations generated from the growth of any of the cover crops under-vine, which may have been a concern to some operators. While there were no significant differences, there was a trend for reduced numbers where a cover crop was grown. This is in line with basic ecological principles of increasing biodiversity reducing the likely incidence of spikes in pest numbers. We have shown on the other two trial sites using NGS that microbial biodiversity is significantly greater where cover crops are grown compared to the bare herbicide control. It is therefore suggested there is the potential for nematode suppression through more easily managed cover crops and changing the soil microbial population dynamics away from pathogenic species to beneficials, but unfortunately this conclusion could not be drawn from the relatively short time frame of this trial.

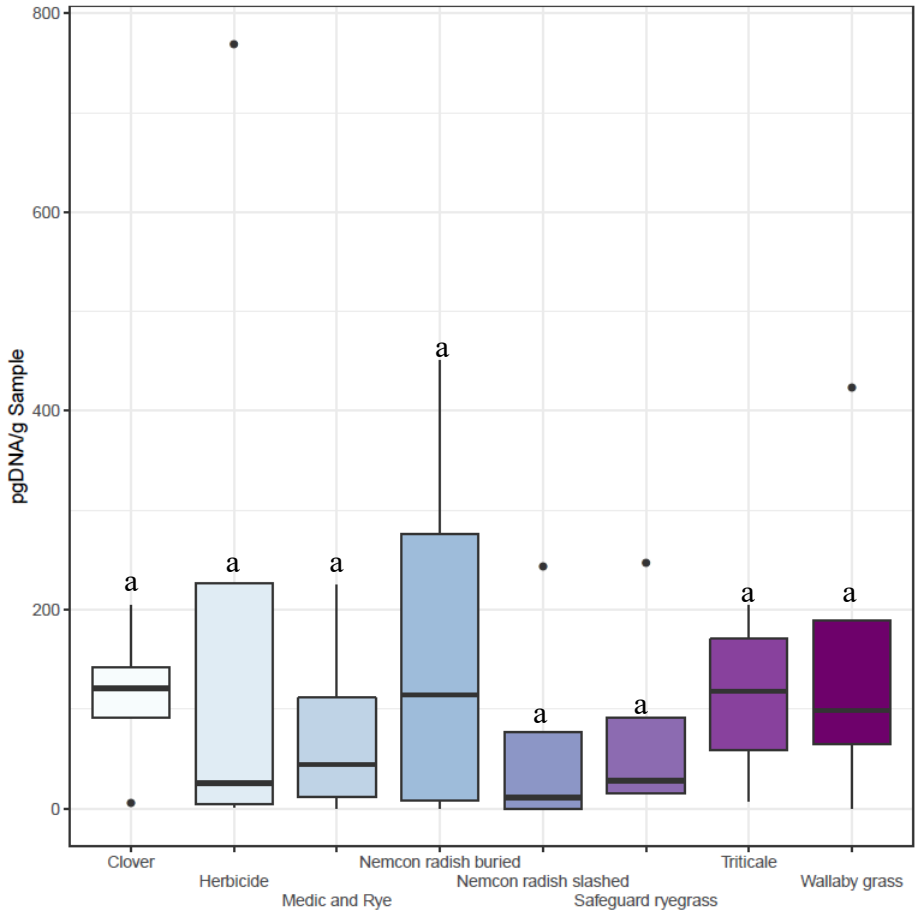


Figure 141: Nematode populations following two years of under-vine cover crop.

Observations and Conclusions:

From an agronomic perspective, this trial was valuable in providing lessons for the implementation of under-vine cover cropping. Points of note were;

- Prior to sowing, the site was reasonably clean of weeds in the under-vine zone, and a light dressing of compost had been applied in previous years to try to improve vine vigour. However, herbicide resistant Ryegrass is a considerable problem over that and many other vineyards, so high rates (9 L/ha) of Pendimethlan (Stomp) herbicide were used in 2017 for its control along with summer weeds. The herbicides biodegradation was inhibited by a dry summer and autumn, which was not accounted for in the selection of the site. The cover crop seed was sown at 1 cm deep, which was most likely in the band of herbicide residue, which prevented the germination and establishment of the susceptible broadleaf species – Medics, clovers and the Nemcon mustard.

  Growers wishing to implement under-vine cover crops need to plan in advance their residual herbicide usage, to ensure there are no residues in the soil that could inhibit seed germination.
- All the grass cover crops (Ryegrass, triticale) were quite tolerant of the herbicide residues and established well. In the following season, Medics and clover were sown and the establishment was very successful. The sowing of Medics into the Ryegrass mulch worked very well, as the seeding was early, prior to Ryegrass germination, so little competition was given to the Medics. This outcome was a valuable lesson for growers who might already have Ryegrass under-vine, in that it does not have to be removed prior to balancing the sward composition with a pasture legume, so long as the seeding is done early and with a suitable implement.
- Nematodes. Given the difficulties in managing Nemcon in order to bury it post mulching, both of which are hard to accomplish under-vine, is it better to plant cover crops under-vine to redress the soil biology from pathogen to beneficial species. Despite concerted efforts to make the biofumigation aspect of this trial work, the Nemcon did not grow well in the under-vine environment at this site. To establish successfully, it will require a soil free of antagonistic chemical residues, and of weeds. This may be found at some sites, but those conditions are going to be met more readily in the mid-row. To gain the maximum value from the biofumigant, it is also a requirement, based on past trials, that the biomass of the crop be macerated to release the active chemicals (isothiocyanates) and buried, so they are captured in the soil.

Again, this will be more readily achieved with the crop grown in the mid-row. Ideally a shallow trench would be dug alongside the vine into which the mown and macerated crop is side-thrown and soil thrown on top to seal the volatile chemicals below ground. This process would be very difficult to duplicate with crop grown under-vine. While not providing statistically significant results, the trend shown in the cover crop trial for less nematodes where the cover crops were grown is encouraging. Given the importance of nematodes to the grape industry, it is therefore recommended that a well-constructed trial investigating cover cropping and alternate treatments such as those investigated by Abdel-Sattar (2020) be investigated at a site known to have nematode issues, but also homogenous so variability is minimised. We are confident that the development of a readily implemented, low cost system of nematode control would be greatly appreciated by the grape growing community.

Part 4 – Monash

This section reports on extension work conducted in the South Australian Riverland winegrowing region, near the community of Monash.

Author participation

Dr Thomas Lines, Dr Joseph Marks, Dr Vinay Pagay and Professor Timothy Cavagnaro.

Background

In a previous project (UA 1303) funded by Wine Australia (WA hereafter), under-vine cover cropping was explored as an option to the management of the vine row. The rationale for this earlier work was based on the wine industries desire to maintain a clean, green image on the global stage, including the need to manage the vineyard soil in line with best management practice. The success of the initial project led to a longer-term extension of the trial sites, with a specific emphasis on the Nuriootpa and Langhorne Creek trials. From 2018-2022, extensive research was conducted on these sites, with the primary aim to investigate the role of seven under-vine treatments (five cover crop combinations, a straw mulch and herbicide-managed bare earth control) on both the soil health and, importantly, on the grapevines themselves. Important results from this second phase of experimentation found that treatments of medic (Medic Mix and Medic with Ryegrass) did not impose a yield penalty. Moreover, evidence from both previous studies showed that under-vine soils planted with a cover crop were able to increase their organic carbon stocks by up to 23% versus a traditional herbicide practice. Moreover, treatments of Medic and Ryegrass lead to an overall increase in mineral N, owing to rhizobial root colonisation of leguminous Medic. In all, it was concluded that specificity of cover crop selection was critical, especially in typically warm, dry climates.

The success of these initial trials has paved the way for this third iteration of under-vine (and mid-row combination) trials – this time with a sole emphasis on the South Australian Riverland wine region. A vineyard trial site was established in 2021 near Monash in South Australia. This new project was established to test the effects of four under-vine treatments (native species, Medic and Ryegrass, mechanical weeder and herbicide control) managed in combination with the mid-row practices (no intervention, side-thrown biomass and native species). The new trial has reduced the number of cover crop species to highlight both native mixes and the Medic and Ryegrass combination that showed the most significantly influential results from the previous studies. Furthermore, the new trial has sought to test the combinative application of secondary, mid-row management strategies to function in tandem with those primary under-vine practices. The importance of this trial is highlighted by its refined approach to the trial design (specific cover crops and management practices) and by its location; the Riverland, where more than 30% of Australia's grapevines are grown and

where hot, dry summers, and autumnal frost, encourage new strategies for vineyard floor management.

Materials and Methods

Site location, establishment and design



Figure 142. The field site was located near Monash, in the South Australian Riverland.

Cover crop species were selected based on their growth habit (prostrate or upright), perennial or annually self-regenerating from their own seed bank, seed availability and cost and likely benefit to soil quality and vine productivity. All legumes were treated with the appropriate rhizobium inoculum immediately prior to sowing. In contrast to our previous trials at Nuriootpa and Langhorne Creek (see Parts 1-3, herein), treatments at the Monash trial site were further selected based on management practice as well as ground cover. Thus, combinations of treatments were factored together in combination to test their effects on various soil and grapevine properties. Treatments of cover crops were established, as well as treatments of herbicide and these were further combined with both mechanical weed treatments and mid-row treatments, thus testing the effects of side-thrown mid-row cover.

Sites were sown in autumn 2021 using a hand pushed Jang JP-1 seeder, with one row sown within 20 cm of the vine trunk on each side of the vine row. Where seed was sown in the mid-row, the same hand-pushed seeder was used to plant five rows of seed either side of the target vine plot. For native seeds, Seeding Natives was contracted to plant seed using their

proprietary seeder. Native seeds were sown under-vine by hand with a rake (due to their ‘fluffy’, low density nature, normal seeding methods are not effective with native grass seed. Seed produced by the sown cover crops was expected to germinate and fill the gap with plants in the following years.



Figure 143: Clemens knife weeder (top) and multi-clean (bottom) used as mech weeder 1 and 2 respectively.

To investigate the effects of different mechanical weeding methods on soil health and vine productivity, two mechanical weeding methods were employed. “Mech weeder 1” was a

knife cultivator that weeds the under-vine by pushing a blade at shallow depth, along the vine row. “Mech weeder 2” was a string weeder (or whipper-snipper) based system for killing weeds in the under-vine. Due to limitations of supply of this equipment, alternative but similar systems were used in year two.

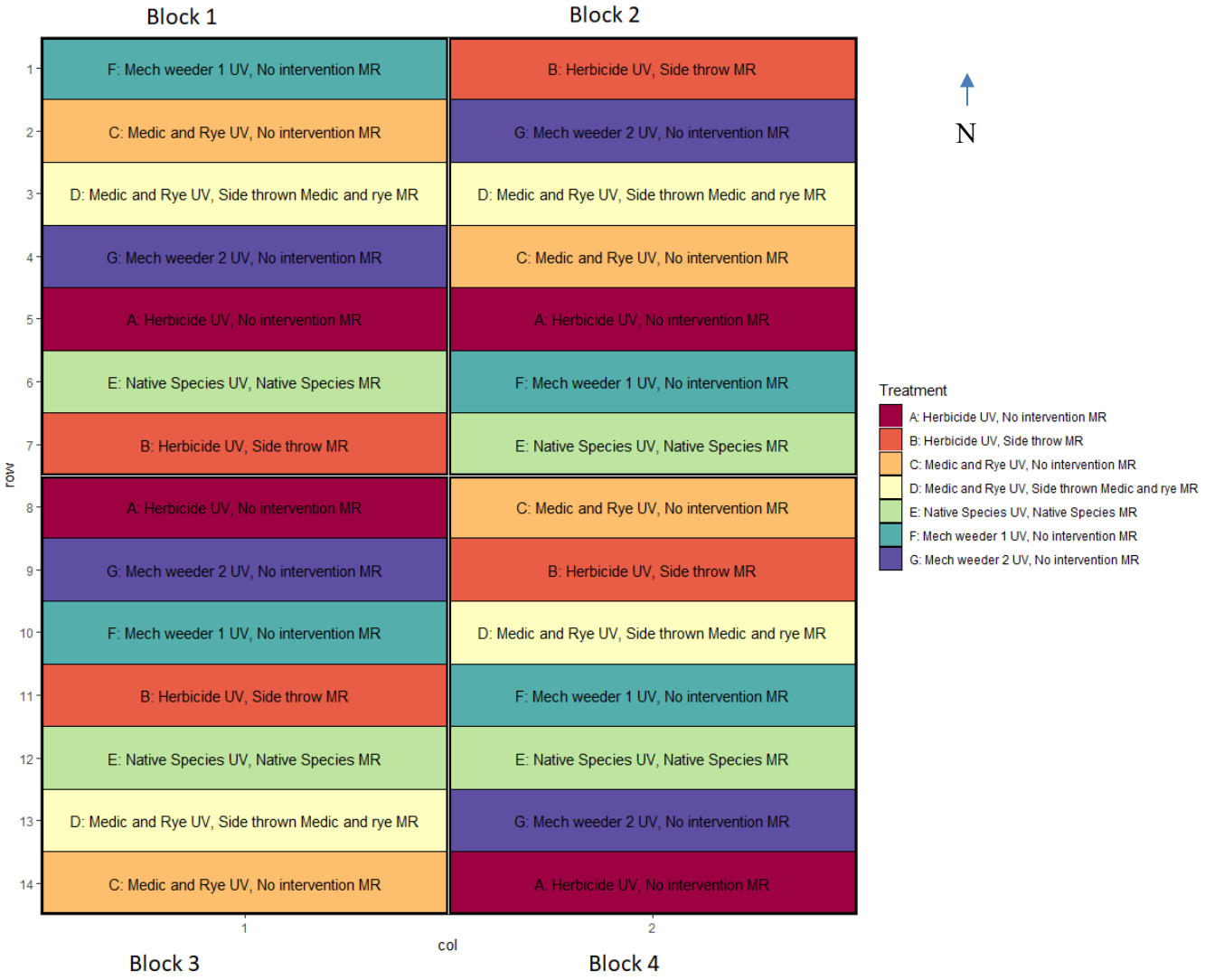


Figure 144. Diagram showing Monash trial design with seven treatments randomly allocated to four blocks in a randomised complete block design.

Soil physicochemistry

Soil collection for analysis was carried out at harvest in 2022 and 2023. At each plot three cores to a depth of 10 cm were taken and homogenised. These samples were air dried, sub-sampled and delivered to Australian Precision Agriculture Laboratory (APAL) for analysis (https://www.apal.com.au/SoilTesting.aspx).

Table 14. Soil physicochemical properties quantified by APAL.

pH 1:5 water (pH units)	pH $CaCl_2$ (pH units)	Plant-avaliable (Colwell) Phosphorus (mg/kg)	Ca:Mg Ratio
K:Mg Ratio	ECR	CEC (cmol/kg)	Calcium (%)
Magnesium (%)	Potassium (%)	Sodium (%)	Salinity EC 1:5 (dS/m)
Iron (Fe) (mg/kg)	Manganese (Mn) (mg/kg)	Copper (Cu) (mg/kg)	Zinc (Zn) (mg/kg)
Dumas Total Nitrogen (%)	Dumas Total Carbon (%)		

Leaf area index

In mid-December and late January, leaf area index (LAI) and T (ratio of transmitted to incident PAR) were measured to quantify differences in canopy size. The AccuPar LP-80 ceptometer was used to take these readings.

Cumulative temperature

Temperature loggers (96) were deployed across the Monash vineyard trial site during the 2021/22 and 2022/23 growing seasons. Loggers were placed inside plastic sunshades and affixed to the lower cordon (~1.4 m), and base (at soil surface) of two vines of all treatments.



Figure 145. Photograph of temperature logger encased in plastic sunshade attached to vine cordon.

Microbiomes

In July of 2022, soil samples were taken and submitted for NextGen sequencing of the 16s and ITS Microbiome at Australian Genome Research Facility (AGRF). Soils were sampled and frozen (− 4°C) prior to DNA extraction and Illumina sequencing AGRF conducted DNA extractions, PCR amplification and sequencing on whole soil samples. In brief, DNA was extracted from 0.250 g of soil using a PowerSoil DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA, USA). Ribosomal RNA (16S) was analysed for bacterial and archaeal communities using the forward and reverse primers 27F-519R (AGAGTTTG ATCMTGGCTCAG and GWATTACCGCGGCKGCTG; 16S). Internally transcribed spacer sequence data was analysed for fungal community composition and amplified using the ITS1-ITS2 forward and reverse primers (ITS1: CTTGGTCATTTAGAGGAAGTAA, ITS2: GCTGCGTTCTTCATCGATGC). The following PCR conditions were used: an initial denaturation at 95 °C for 7 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 45 s and 72 °C for 60 s. A final extension was carried out at 72 °C for 7 min. Image analysis was performed in real time using MiSeq Control Softwarev2.6.2.1 and Real-Time Analysis v1.18.54. Sequence data were generated with the Illumina bcl2fastq 2.20.0.422 pipeline. Analysis of the sequence data was carried out using Qiime2 (version 2023.2). Analysis of the soil 16S and ITS microbiomes revealed a total of 20,600 and 4,211 original taxonomic units (OTUs) respectively in soil from September 2022.

Soil moisture profiles

Soil moisture profiles were taken at the major phenotypic timepoints throughout the 2022-2023 seasons using an Odyssey capacitance probe that measures moisture, at 20 cm intervals, down to a depth of 1 m. Capacitance probes work by measuring the di-electric constant of the soil, which changes with water content. In each plot one access tube was installed within 40 cm of a vine and dripper in each plot.

Harvest data collection

At the end of the 2022 and 2023 vintages, the research team was informed by the vineyard manager when harvest was taking place. Each treatment was harvested using the following approach: one metre of fruit was harvested from each treatment plot, with all bunches harvested from the area between the two cordons. Bunches were counted and total yield per vine was weighed. Sub-samples of the fruit were collected and taken to the lab for individual

bunch weights and berry weights. Three to four bunches were weighed individually, and subsequently 100 berries (25 per bunch) were weighed to determine average berry size.

Juice chemistry analysis

A comprehensive juice analysis was carried out at the end of each season. Berry samples for the were submitted to Affinity Labs for red grape analysis for quantification of the following properties;

- Alpha amino nitrogen
- Ammonia
- Brix
- Free anthocyanins
- pH
- Titratable acid
- Total phenolics
- Total tannin
- Yeast assimilable nitrogen

Data analysis

A linear mixed model was fitted using the R package ASReml-R using *Treatment* as a fixed effect, and *Block* as random effects in the analysis. *Block* was included in our analysis to account for any underlying spatial heterogeneity of the vineyard sites. Prior to significance testing, each treatment variable was tested for normality and homoscedasticity using the Wilkes-Shapiro test and visualisations of the residuals plots. The significance of fixed effects (at $p< 0.05$) in all models was determined using approximate F-tests. All other statistical analyses were conducted using R open source statistical software (version 3.6.2 in R-Studio version 1.2.1335) with additional agricultural data packages; "Biometrytraining" (Nielsen and Rogers, 2020), "dplyr", "agricolae" and "ggplot2".

Results

Soil Moisture Profile

Soil moisture probes were deployed to detect differences in soil moisture at five depth points between at treatments. Measurements were taken during the 2022 and 2023 growing seasons. Data points reveal a high degree of variation across depth intervals, with no significant differences detected between treatments means during either growing season.

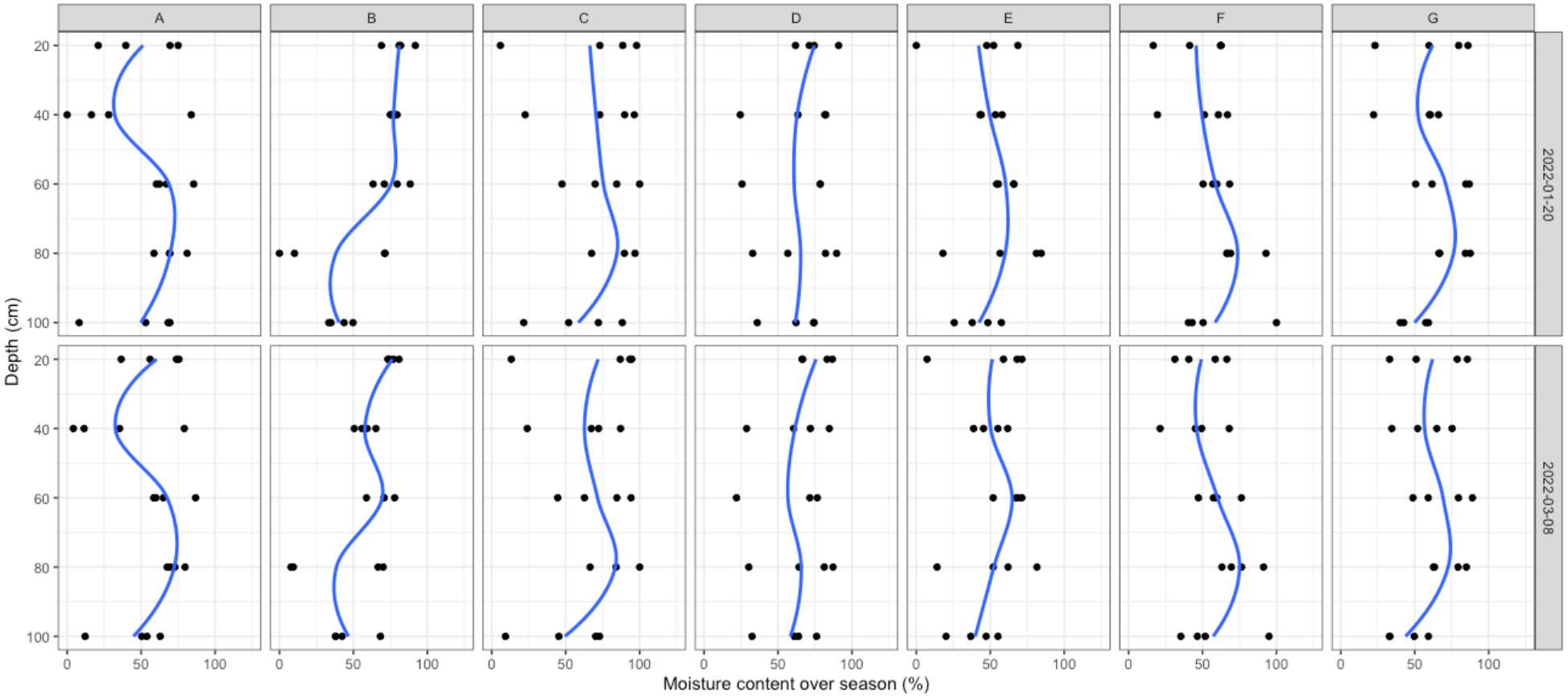


Figure 146. Plot displaying soil moisture at five depth intervals captured at two timepoints over the 2022 growing season.

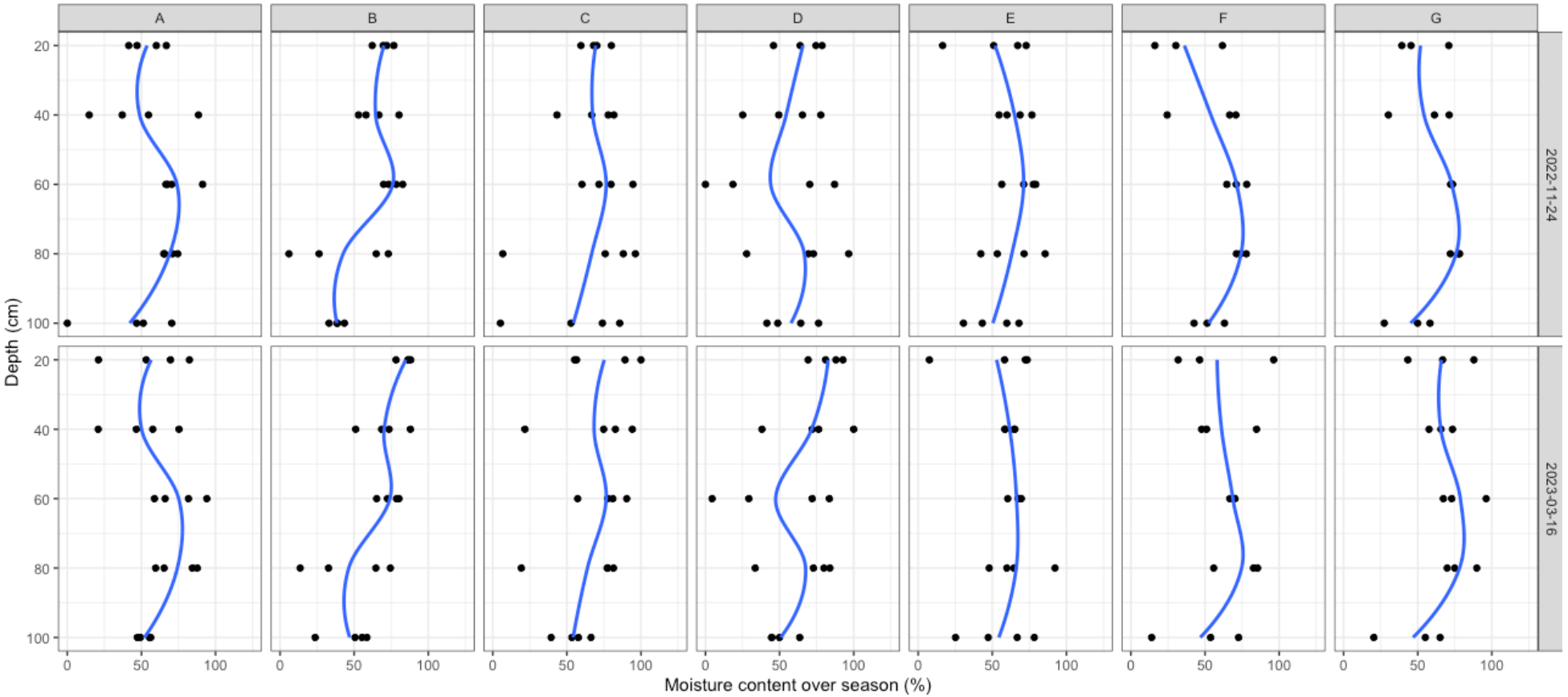


Figure 147. Plot displaying soil moisture at five depth intervals captured at two timepoints over the 2023 growing season.

Soil physicochemical properties

A number of soil physicochemical properties were measured across both growing seasons to determine if vineyard floor treatments may have had an effect. Soil pH (1:5 water) was tested, with no significant differences found in soils sampled from beneath the seven established treatments during the 2022 growing season. Although no significant differences were observed ($\alpha = 0.05$), there were some differences in treatment means. The highest mean pH was observed in treatment E (native species UV, native species MR), while the lowest mean pH was observed in treatments D (medic and rye UV, side throw medic and rye MR) and F (mech weeder 1 UV, no intervention MR). Similar trends were observed in data from 2023, with highest mean pH associated with treatment E, while the lowest was associated with treatment F (Figure 148 & Figure 149).

Soil salinity was also measured using $EC_{1\ soil:5\ water}$ (dS/m) as a means of measuring differences in salinity between treatments. Although some minor differences were observed, there were no significant differences ($\alpha = 0.05$) in soil salinity from the 2022 sample data. Additionally, salinity data from the 2023 growing season revealed no significant differences between treatment means. Although no significance was detected at the alpha cut-off, treatment E was observed to have a mean EC of 0.41 dS/m – nearly twice that of other treatment means (Figure 150 & Figure 151).

Total soil C was analysed between treatments for the 2022 growing season, with C as a percent of soil mass ranging from 1.3% in treatment G (mech weeder 2 UV, no intervention MR) to 1.6% in treatment B (herbicide UV, side throw MR) and treatment E. Data from harvest, 2023, showed total C ranging from 1.03% in treatment F to 1.58% in treatment E.

Both plant-available (Colwell) P and total nitrogen (N) were compared among treatments. Soil sampled during harvest, 2022, revealed no significant differences between plant-available (Colwell P) or total N. Despite a lack of statistical significance, plant-available (Colwell P) measurements were observably variable between treatments (Figure 161), with treatment C (medic and rye UV, no intervention MR) representing the lowest reading (72 mg/kg) and treatment E showing the highest (94 mg/kg). A lack of statistical significance is likely a result of high variation within sample treatments. Total N was also non-significant, with soil treatments observed to have highly similar total N levels. Similar results were observed in data from vintage 2023, with both total P and total N non-significant between

treatment means. Plant-available (Colwell) P, although not significantly different between treatments, was found to be highest in treatment B (herbicide UV, side throw MR, 311.5 mg/kg) and lowest in treatment D (medic and rye UV, side throw medic and rye MR, 272.5 mg/kg).

Total calcium (Ca), magnesium (Mg) and potassium (K) were also measured during the 2022 growing season. Total Ca appeared to show differences between treatments, with treatment E observed to have a total average Ca concentration of 4,925 mg/kg, while treatment D was reported to be only 2,385 mg/kg. Despite observable differences in mean Ca, statistical analysis did not reveal statistically significant differences among any of the treatments. Both Mg and K concentrations were also non-significant. Samples from vintage 2023 showed similar results for magnesium and potassium concentrations – both were non-significant between treatments. However, calcium concentrations were shown to be statistically different, with treatment E (native species UV, native species MR) presenting the highest mean concentration at 5,020 mg/kg and treatment F (mech weeder 1 UV, no intervention MR) presenting the lowest at 2,317.5 mg/kg.

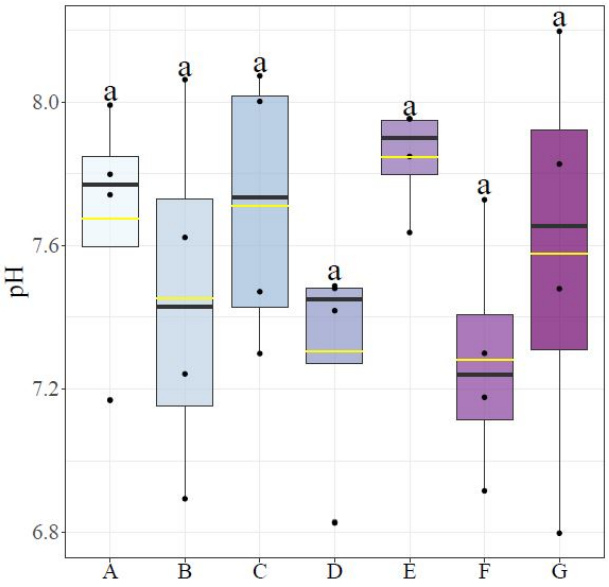


Figure 148. Boxplot displaying median values for soil pH from the 2022 growing season with yellow lines indicating mean values.

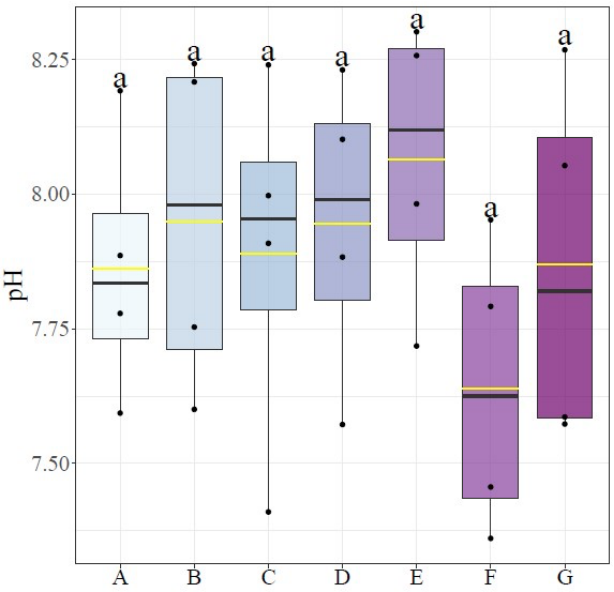


Figure 149. Boxplot displaying median values for soil pH from the 2023 growing season with yellow lines indicating mean values.

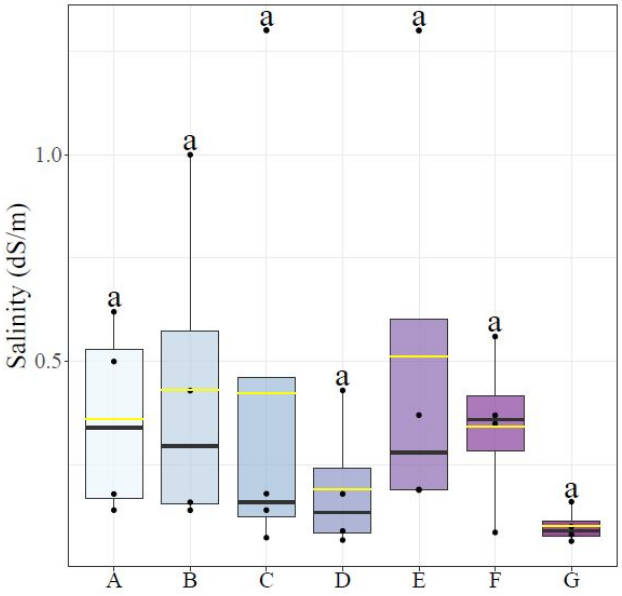


Figure 150. Boxplot displaying median values for soil salinity from the 2022 growing season with yellow lines indicating mean values.

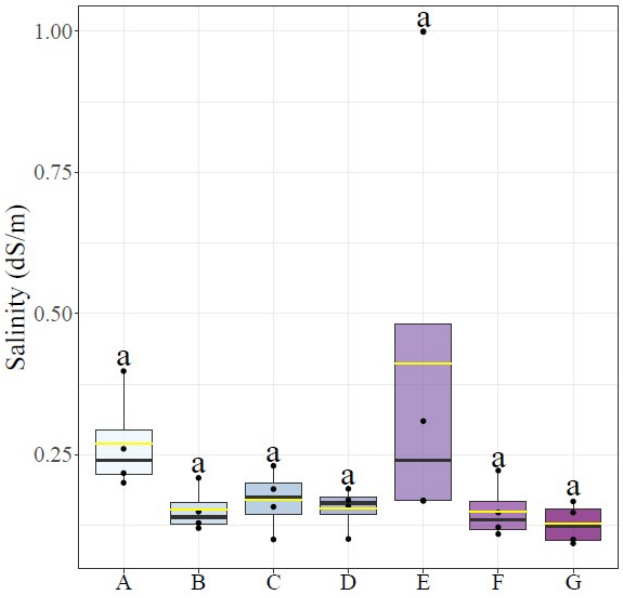


Figure 151. Boxplot displaying median values for soil salinity from the 2023 growing season with yellow lines indicating mean values.

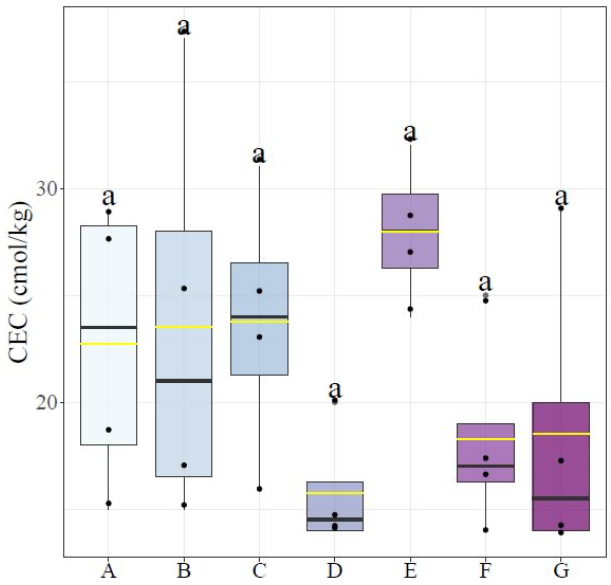


Figure 152. Boxplot displaying median values for soil cation exchange capacity from the 2022 growing season with yellow lines indicating mean values.

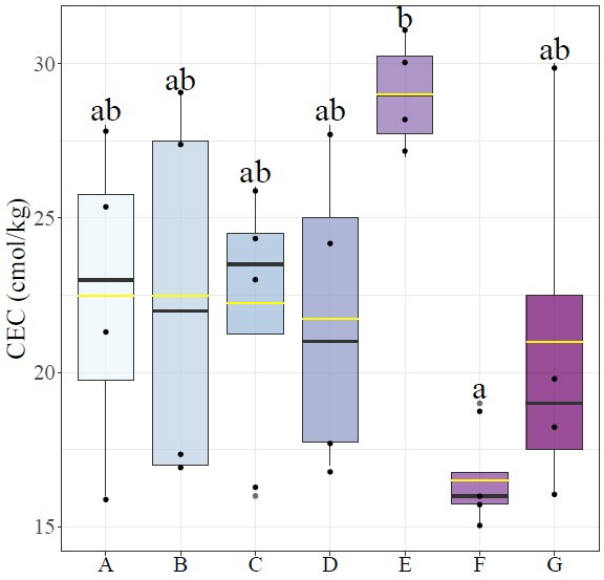


Figure 153. Boxplot displaying median values for soil cation exchange capacity from the 2023 growing season with yellow lines indicating mean values. Different letters denote significant differences in mean values between treatments.

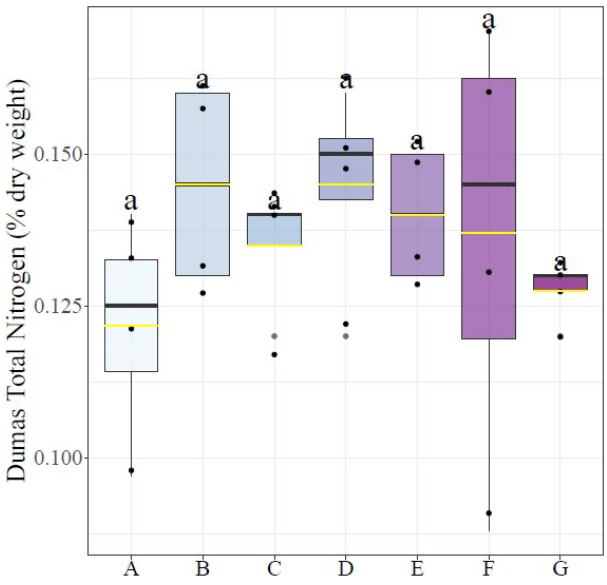


Figure 154. Boxplot displaying median values for soil total nitrogen concentration from the 2022 growing season with yellow lines indicating mean values.

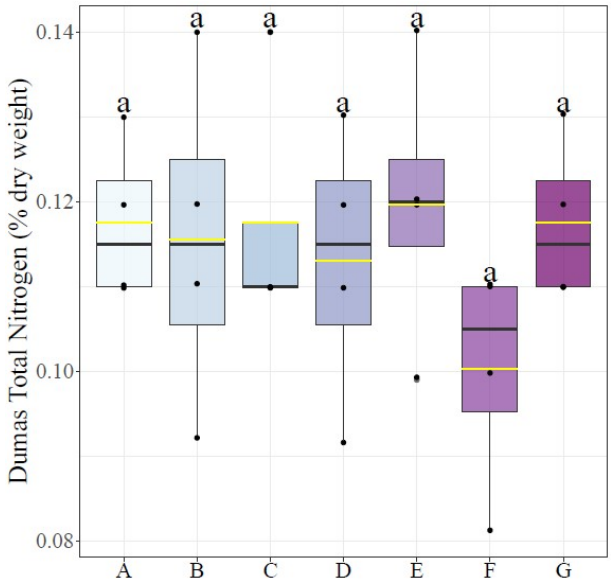


Figure 155. Boxplot displaying median values for soil total nitrogen concentration from the 2023 growing season with yellow lines indicating mean values.

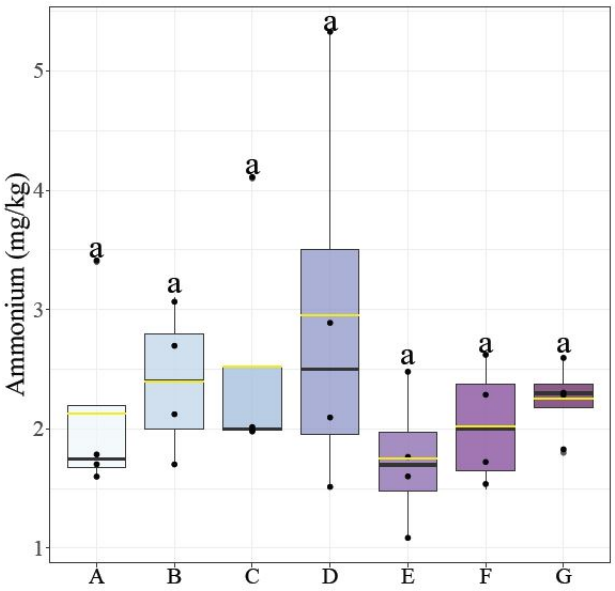


Figure 159. Boxplot displaying median values for soil ammonium concentration from the 2022 growing season with yellow lines indicating mean values.

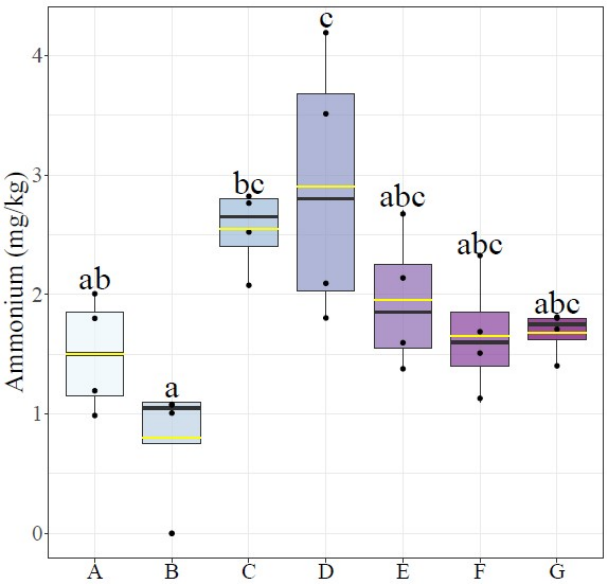


Figure 158. Boxplot displaying median values for soil ammonium concentration from the 2023 growing season with yellow lines indicating mean values. Different letters denote significant differences in mean values between treatments.

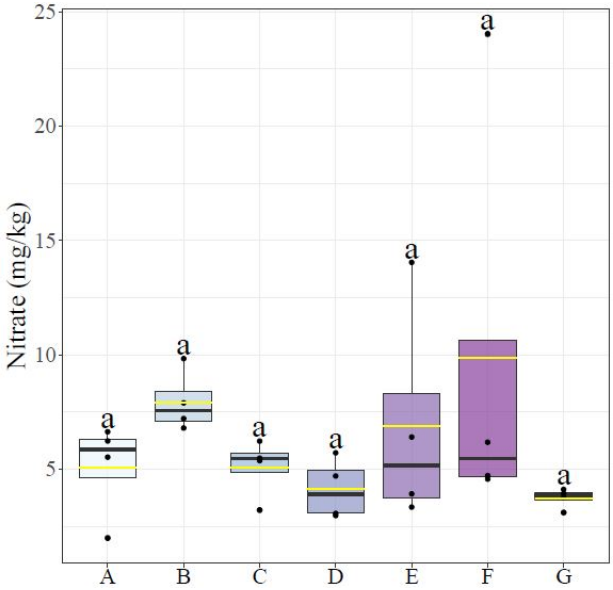


Figure 156. Boxplot displaying median values for soil nitrate concentration from the 2022 growing season with yellow lines indicating mean values.

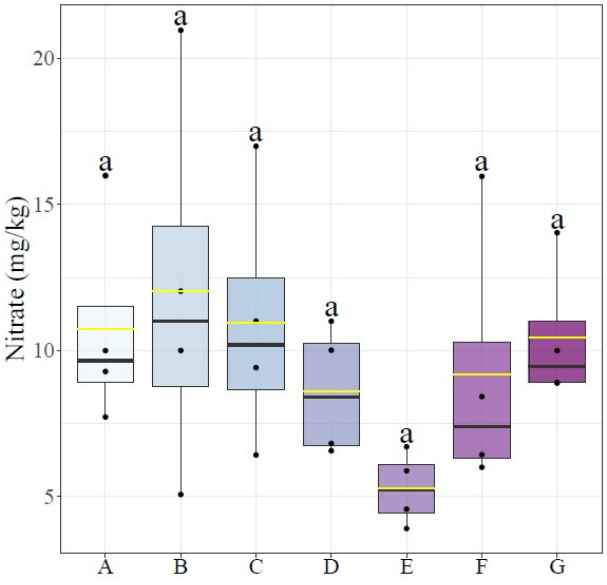


Figure 157. Boxplot displaying median values for soil ammonium concentration from the 2023 growing season with yellow lines indicating mean values.

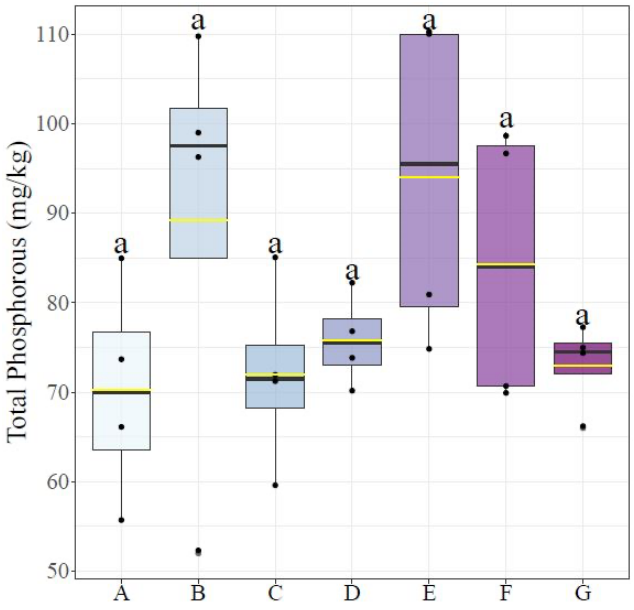


Figure 161. Boxplot displaying median values for soil phosphorus concentration from the 2022 growing season with yellow lines indicating mean values.

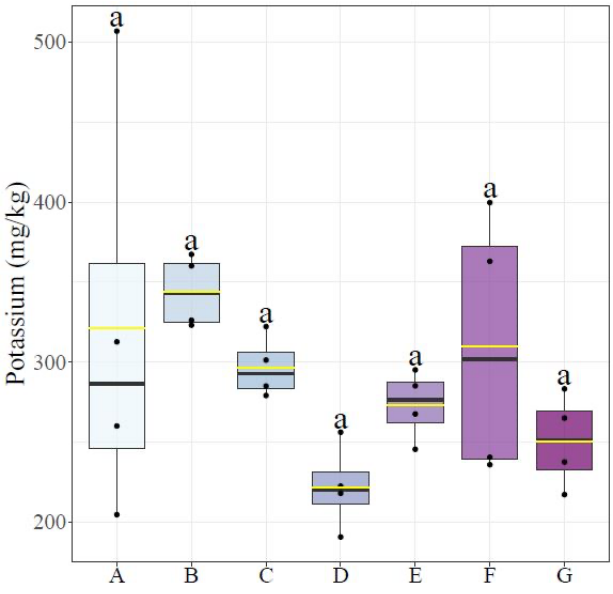


Figure 160. Boxplot displaying median values for soil potassium concentration from the 2022 growing season with yellow lines indicating mean values.

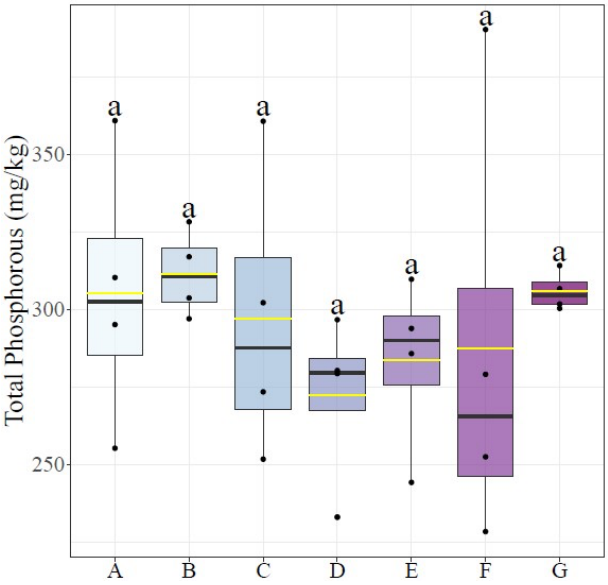


Figure 162. Boxplot displaying median values for soil phosphorus concentration from the 2023 growing season with yellow lines indicating mean values.

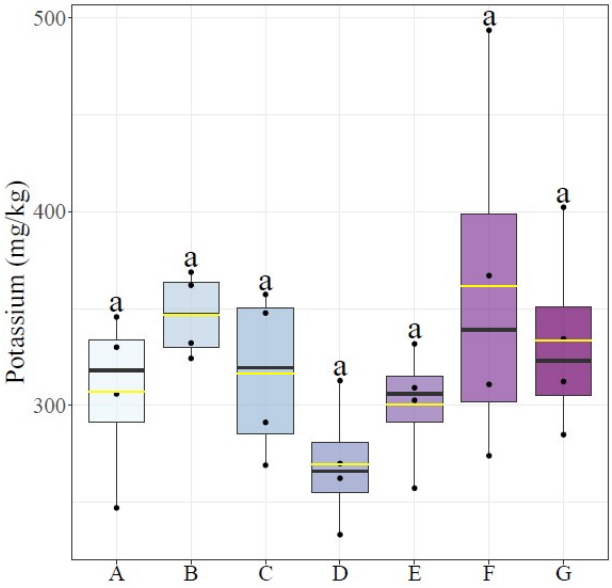


Figure 163. Boxplot displaying median values for soil potassium concentration from the 2023 growing season with yellow lines indicating mean values.

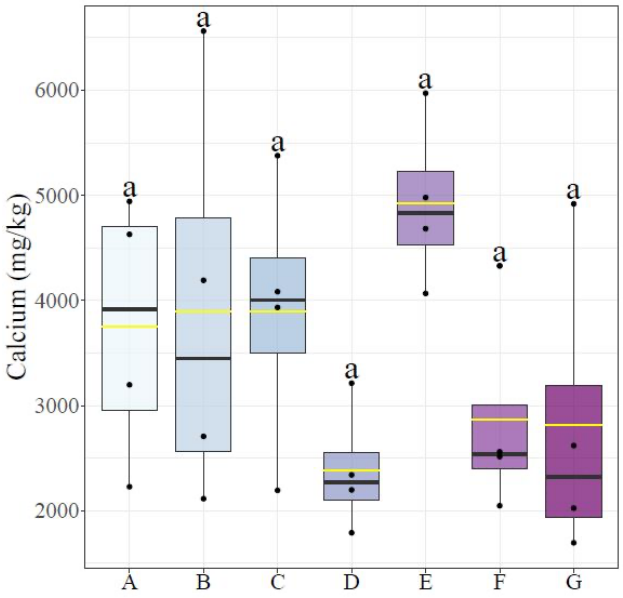


Figure 164. Boxplot displaying median values for soil calcium concentration from the 2022 growing season with yellow lines indicating mean values.

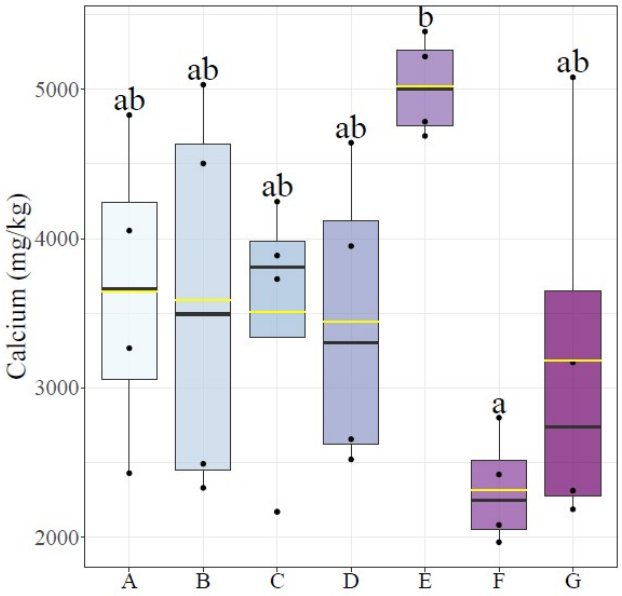


Figure 165. Boxplot displaying median values for soil calcium concentration from the 2023 growing season with yellow lines indicating mean values. Different letters denote significant differences in mean values between treatments.

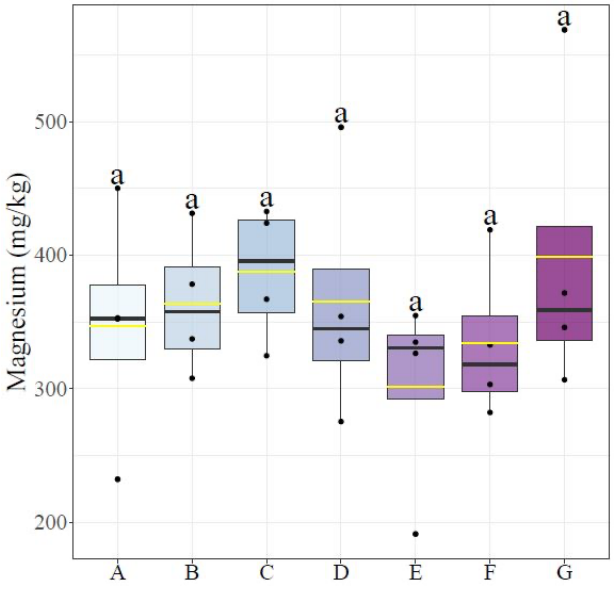


Figure 166. Boxplot displaying median values for soil magnesium concentration from the 2022 growing season with yellow lines indicating mean values.

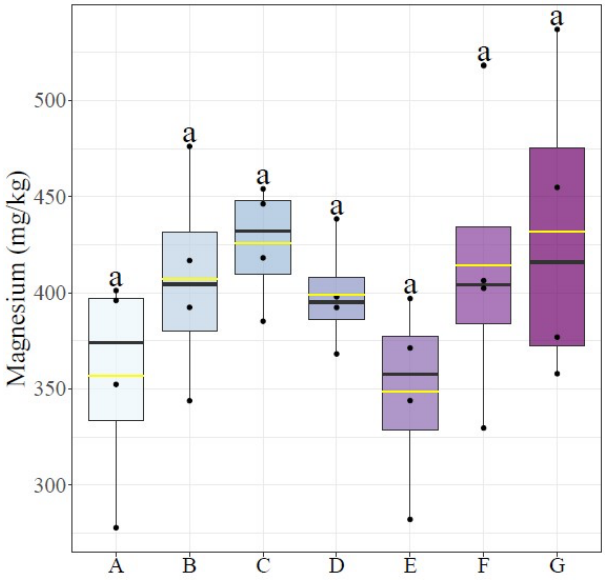


Figure 167. Boxplot displaying median values for soil magnesium concentration from the 2023 growing season with yellow lines indicating mean values.

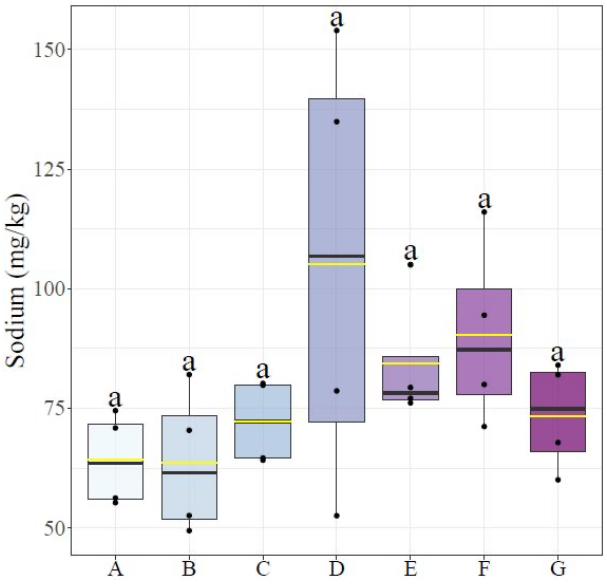


Figure 168. Boxplot displaying median values for soil sodium concentration from the 2022 growing season with yellow lines indicating mean values.

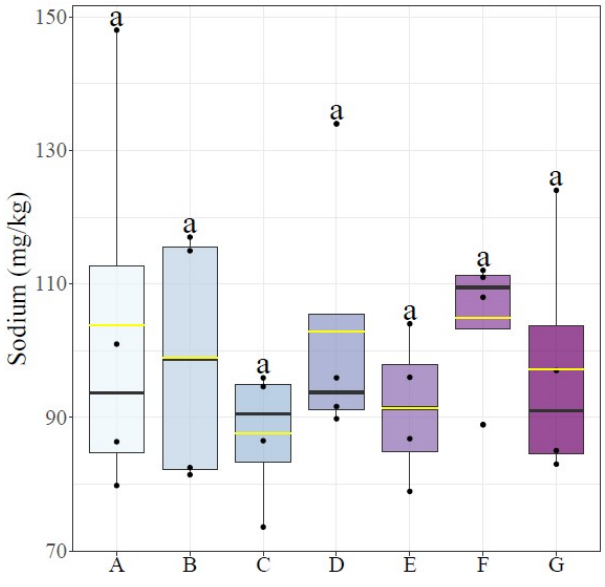


Figure 169. Boxplot displaying median values for soil sodium concentration from the 2023 growing season with yellow lines indicating mean values.

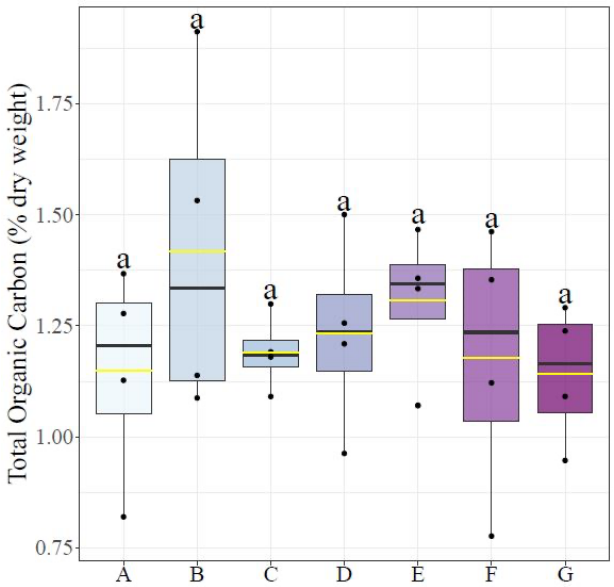


Figure 170. Boxplot displaying median values for soil organic carbon concentration from the 2022 growing season with yellow lines indicating mean values.

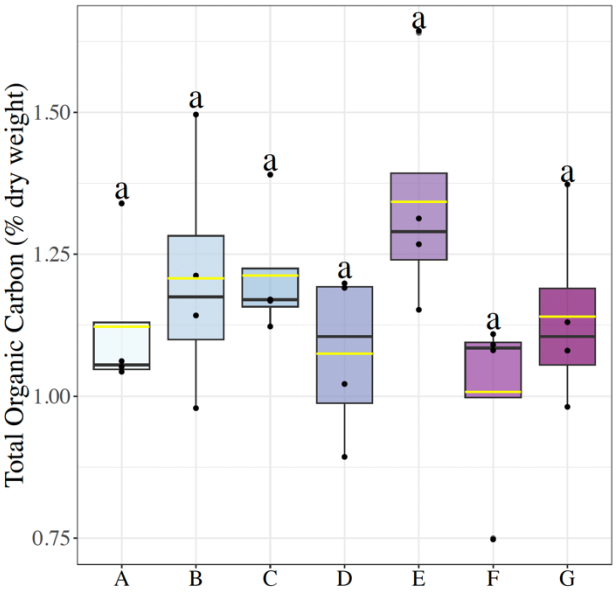


Figure 171. Boxplot displaying median values for soil organic carbon concentration from the 2023 growing season with yellow lines indicating mean values.

Soil penetration resistance

Soil penetration resistance was measured during the 2021/2022 and 2022/2023 growing seasons, in an effort to detect potential differences in soil strength in the top 30 cm, stratified 0-10 cm and 10-30 cm respectively. Despite a lack of statistical significance ($p > 0.05$) between treatments, within each growing season there were noticeable differences in soil strength. Data from the 2021/2022 growing season did not demonstrate any significant differences among treatments. At depth 0-10 cm showed treatments E (native species UV, native species MR) and G (mech weeder 2 UV, no intervention MR) at 1,196 and 1,154 kPa of pressure respectively. At the same depth, treatments C (medic and rye UV, no intervention MR) and F (mech weeder 1 UV, no intervention MR) presented the lowest soil penetration resistance at 661 and 691 kPa respectively. At 10-30 cm depth, treatments G (mech weeder 2 UV, no intervention MR) and E (native species UV, native species MR) presented the greatest resistance (1,872 and 2,109 respectively).

Data from August 2022 and March 2023 (Figure 172 & Figure 177) were also statistically non-significant, yet with the observable difference that these data showed increased soil penetration resistance overall as compared to August 2021. Where the lowest soil resistance in August 2021 at 0-10 cm was 661 kPa, the lowest values for August 2022 and March 2023 at this depth were 1,340 kPa and 2061 kPa respectively. There was less of a distinct contrast in soil resistance between seasons at depth 10-30 cm.

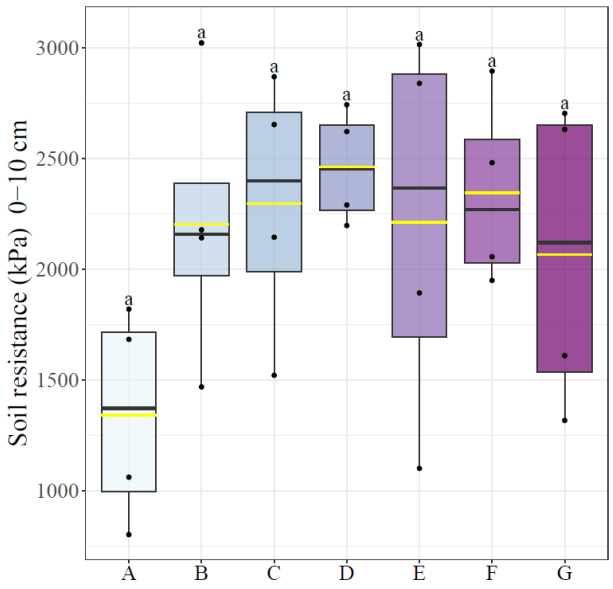


Figure 172. Boxplot displaying median values for soil penetration resistance at depth 0-10 cm from August 2022 with yellow lines indicating mean values.

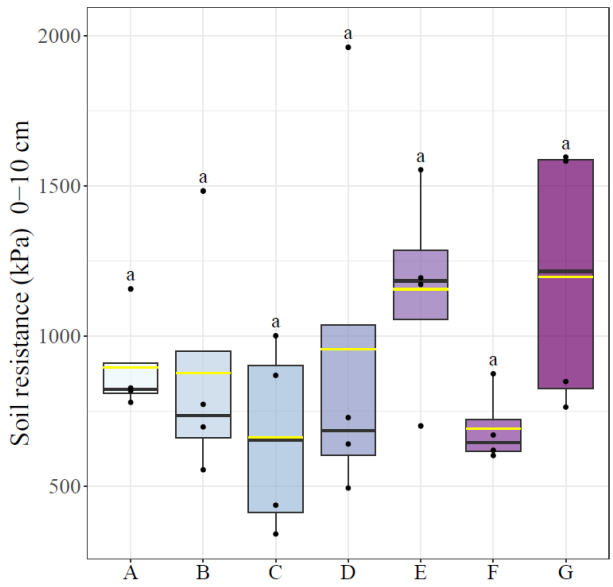


Figure 173. Boxplot displaying median values for soil penetration resistance at depth 0-10 cm from March 2023 with yellow lines indicating mean values.

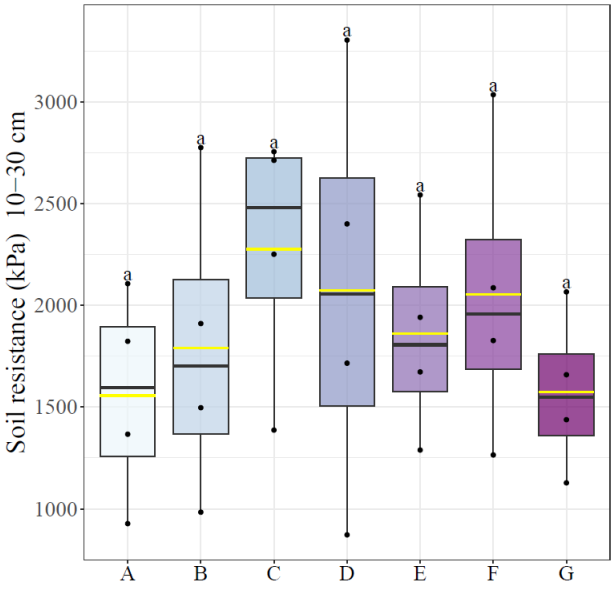


Figure 174. Boxplot displaying median values for soil penetration resistance at depth 10-30 cm from August 2022 with yellow lines indicating mean values.

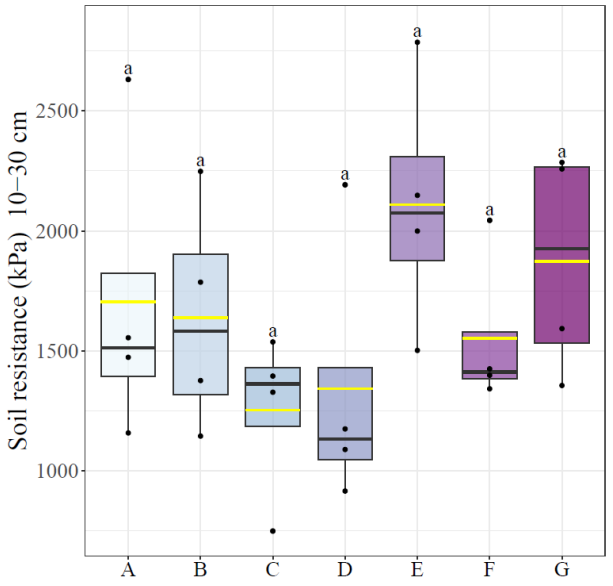


Figure 175. Boxplot displaying median values for soil penetration resistance at depth 10-30 cm from March 2023 with yellow lines indicating mean values.

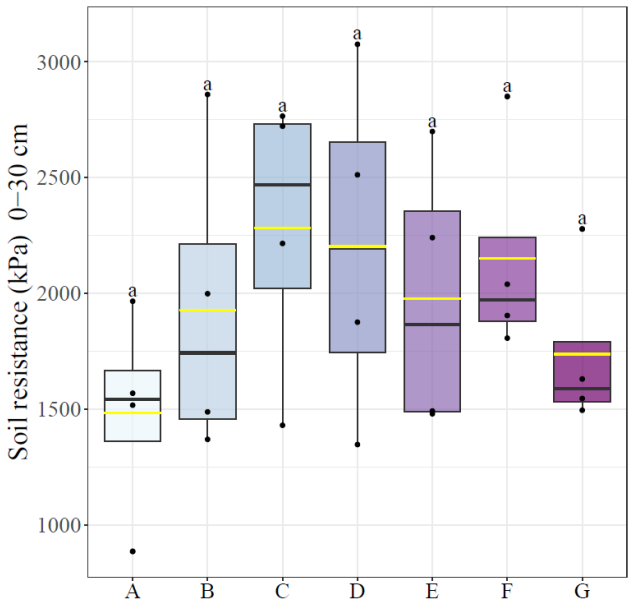


Figure 176. Boxplot displaying median values for soil penetration resistance at depth 0-30 cm from August 2022 with yellow lines indicating mean values.

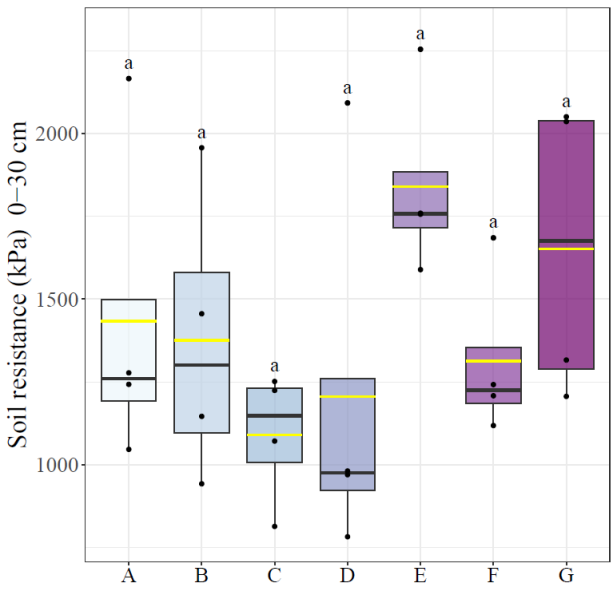


Figure 177. Boxplot displaying median values for soil penetration resistance at depth 0-30 cm from March 2023 with yellow lines indicating mean values.

Leaf Area Index

Leaf area index (LAI) was determined using a ceptometer for the 2022 and 2023 growing seasons at Monash. Data from the 2022 growing season was non-significant between treatment means. Differences between predicted treatment means were extremely minimal, with variation low. Although LAI data collected during the 2023 growing season was also statistically non-significant, there was an overall stronger separation between treatment means. The highest LAI reading was associated with treatment A (7.94), while the lowest was associated with treatment E (6.66).

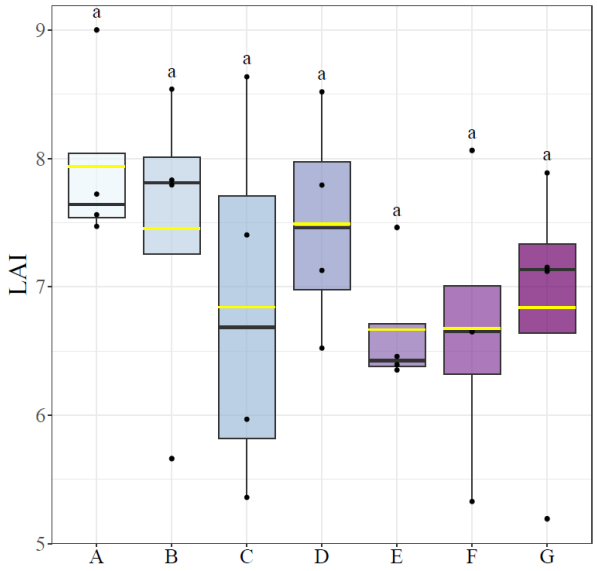


Figure 178. Boxplot displaying median values for leaf area index from harvest 2022 with yellow lines indicating mean values.

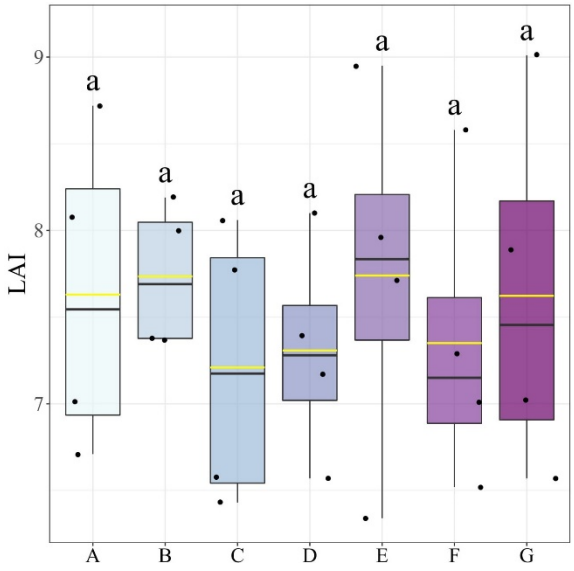


Figure 179. Boxplot displaying median values for leaf area index from the 2023 growing season with yellow lines indicating mean values.

Cumulative temperature

Air temperature was measured across both the 2022 and 2023 growing seasons between all treatments. During both seasons, cumulative air temperature was not observed to fluctuate significantly between treatments.

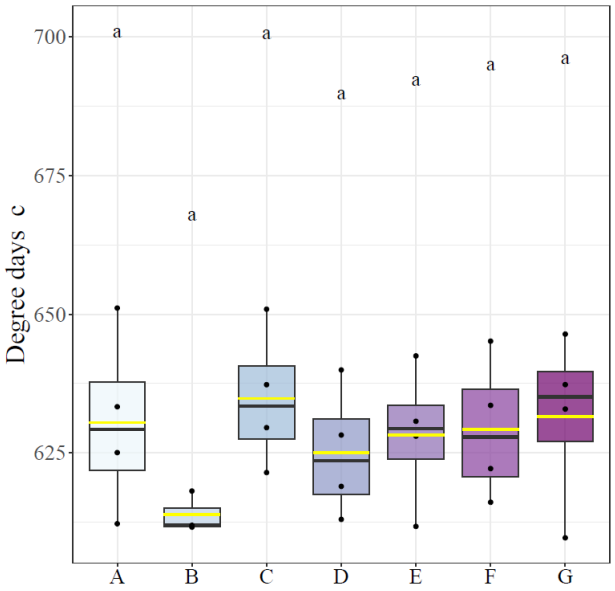


Figure 180. Boxplot displaying median values for cumulative air temperature during the 2022 growing season with yellow lines indicating mean values.

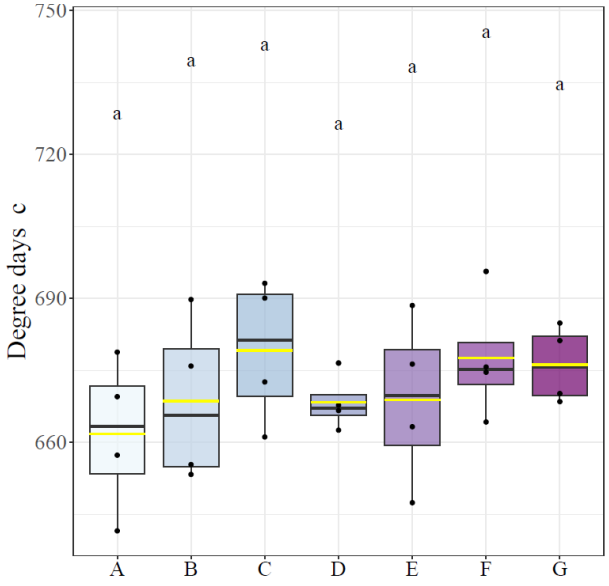


Figure 181. Boxplot displaying median values for cumulative air temperature during the 2023 growing season with yellow lines indicating mean values.

Microbiomes

Bacterial and fungal diversity were measured between treatments, with several key metrics employed. Faith's phylogenetic diversity, a measure of the relative diversity among treatments was calculated using the NextGen sequencing results. The greater the value of this index, the greater the diversity. No significant differences among levels of diversity were detected in either bacterial (16S) or fungal (ITS) diversity (Figure 182 and Figure 184). Additionally, both Shannon's diversity and Evenness metrics revealed no significant differences in microbial diversity between varying management practices (treatments) during the 2022/23 growing season (Figure 183, Figure 186 and Figure 187).

Taxa summary plots at the family taxonomic level (Figs 185 and Figure 190) for both bacteria and fungi visualise the diversity of families present in the soils sampled from Monash in the 2022/2023 growing season. An analysis of the taxa did not reveal any significant differences between treatments for presence or absence of specific taxa.

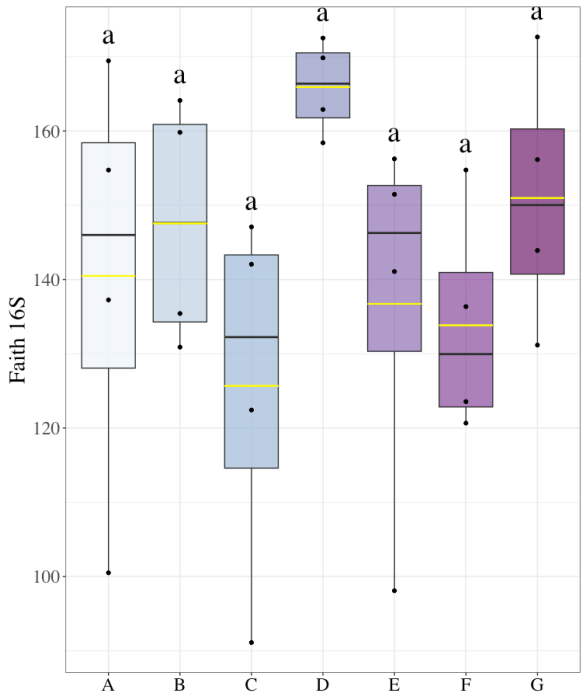


Figure 182. Boxplot representing Faith's diversity metric to assess significant differences in bacterial communities in soil sampled from different treatments.

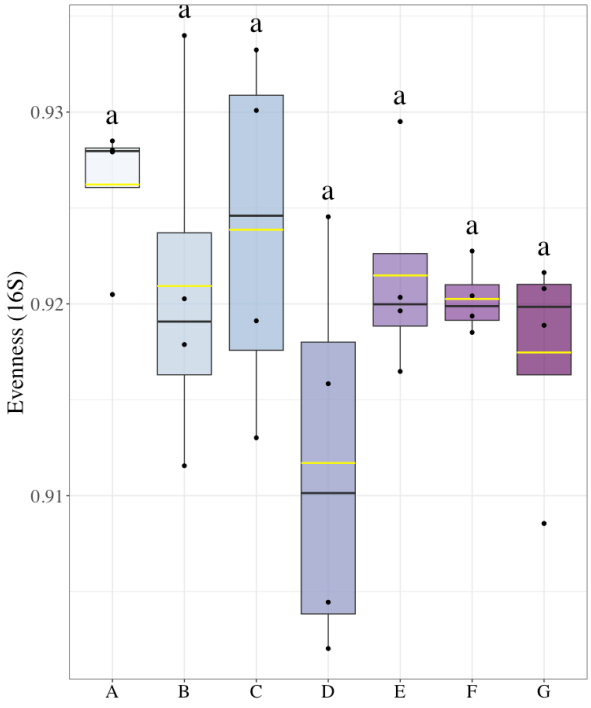


Figure 183. Boxplot representing evenness as metric to assess significant differences in bacterial communities in soil sampled from different treatments.

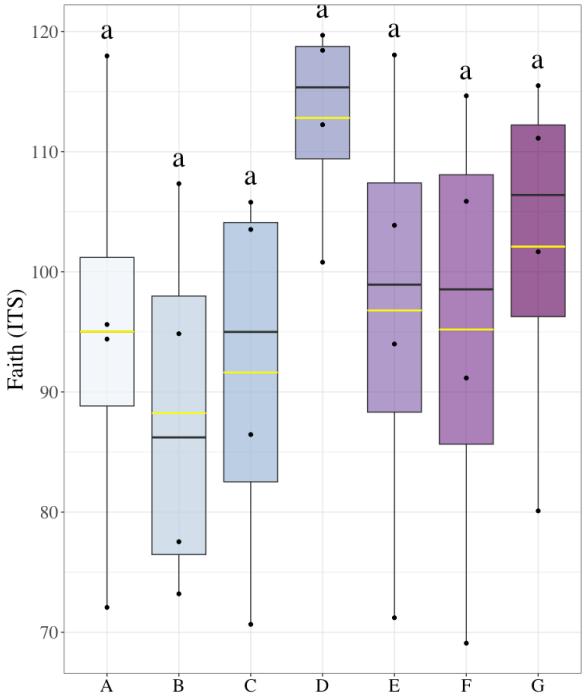


Figure 184. Boxplot representing Faith's diversity metric to assess significant differences in fungal communities in soil sampled from different treatments.

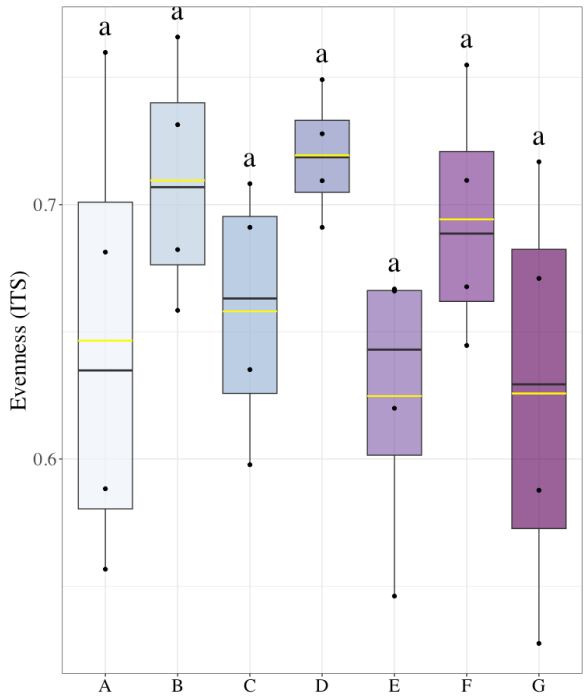


Figure 185: Boxplot representing evenness as metric to assess differences in bacterial communities in soil sampled under-vine for different treatments.

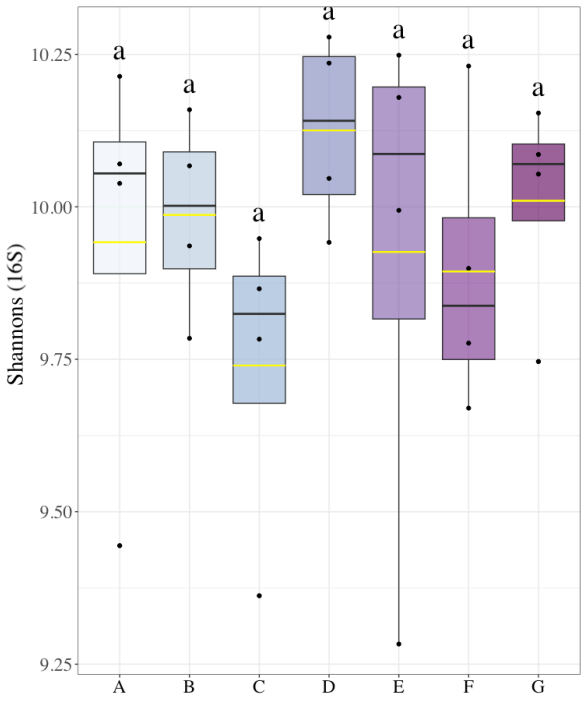


Figure 186. Boxplot representing Shannon's diversity metric to assess significant differences in bacterial communities in soil sampled from different treatments.

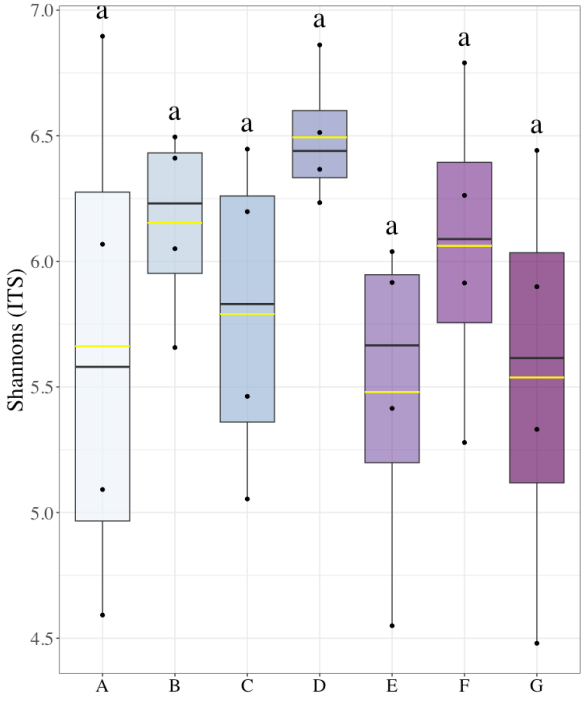


Figure 187 Boxplot representing Shannon's diversity metric to assess significant differences in fungal communities in soil sampled from different treatments.

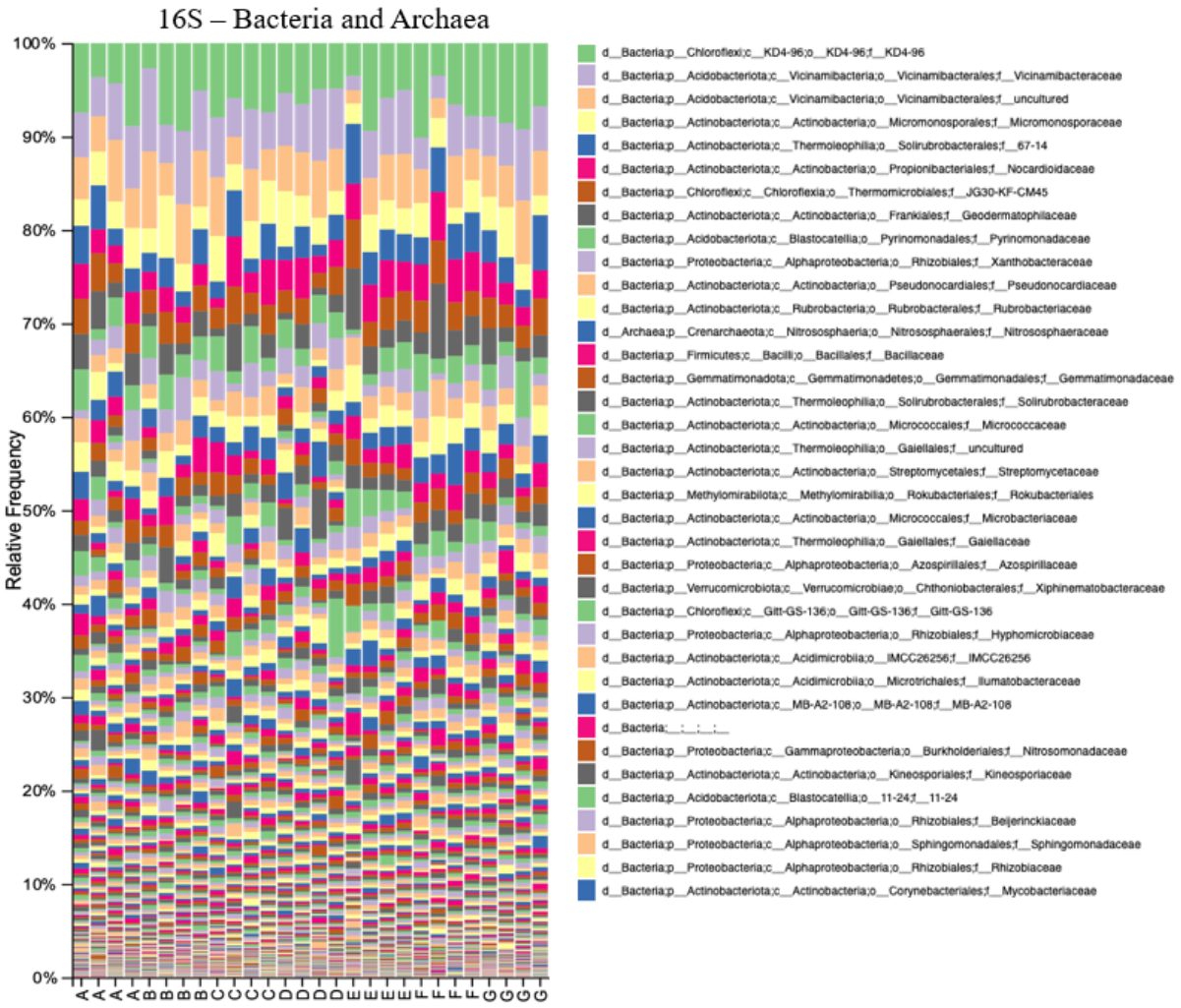


Figure 188: Taxa bar plots of identified bacterial families as assigned using qiime2 using the green genes database.

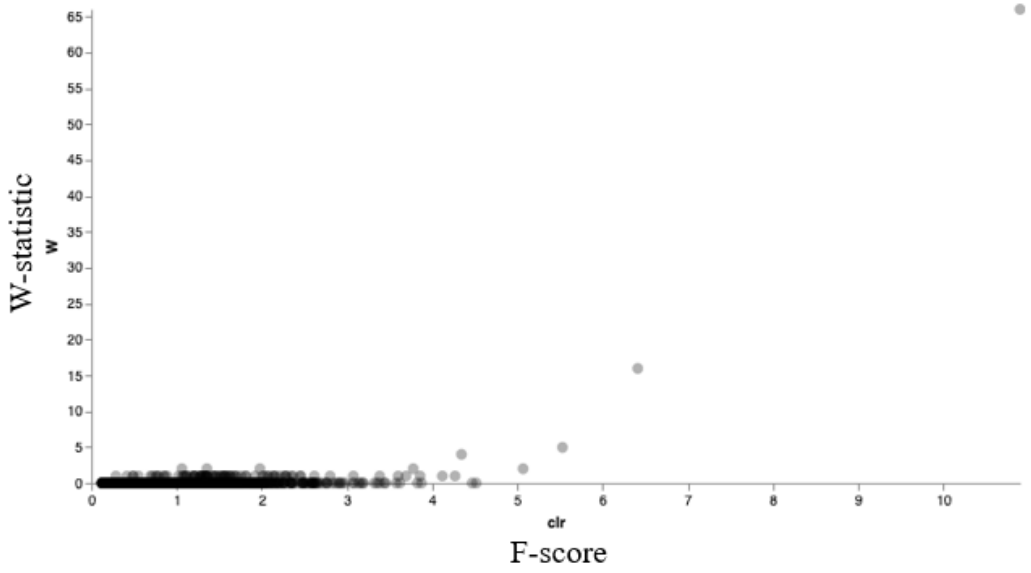


Figure 189: Grayscale volcano plot illustrating the results of a Qiime composition ANCOM analysis in Qiime2 to identify treatment-associated bacterial genera exhibiting statistically significant differences. Each data point corresponds to a genus. Genera that lie to the top-right differ more based on treatment than



Figure 190: Taxa bar plots of identified fungal families as assigned using qiime2 using the UniteITS database

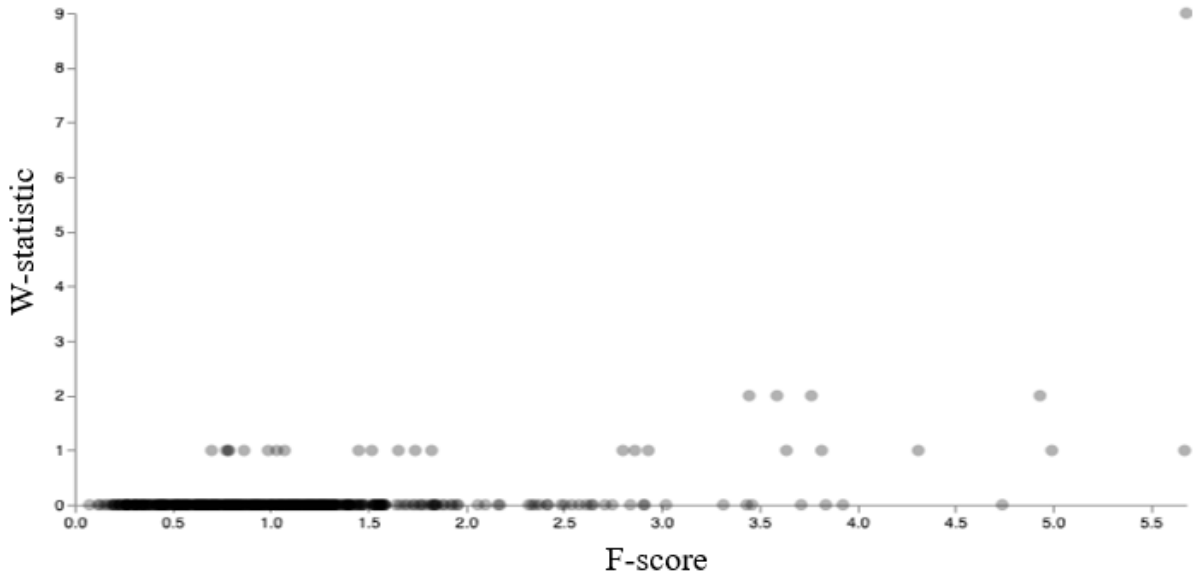


Figure 191: Grayscale volcano plot illustrating the results of a Qiime composition ANCOM analysis in Qiime2 to identify treatment-associated fungal genera exhibiting statistically significant differences. Each data point corresponds to a genus. Genera that lie to the top-right differ more based on treatment than others.

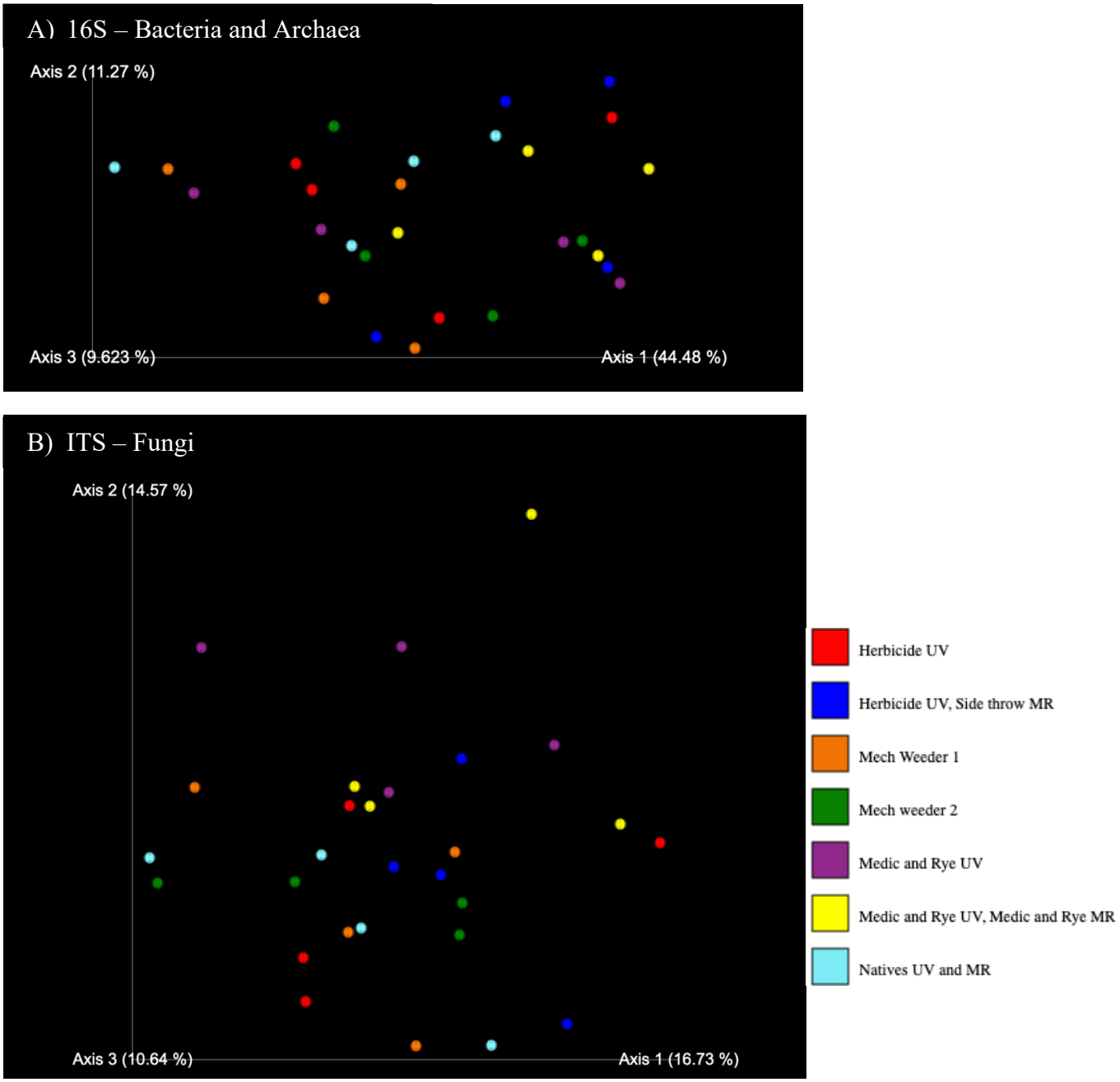


Figure 192: (A) Bacterial and Archaeal, and (B) Fungal beta diversity as characterised by weighted-unifrac analysis. Points closer together indicate microbial communities are more similar.

Juice chemistry

Grape juice chemistry was analysed post-harvest for the growing seasons 2022 and 2023. The key metrics used for analysis were Alpha Amino Nitrogen, Ammonia, Brix, free anthocyanins, pH, total phenolics and yeast assimilable nitrogen (YANS). Although berry juice chemistry metrics were non-significant for the 2022 growing season, there were some observable differences. Total phenolics, for example, were lowest in treatment A (126.4 absorbance units) and highest in treatment G (143.5 absorbance units). Yeast assimilable nitrogen (YANS) was lowest in treatment F (49.8 mg/L) and highest in treatment G (60.5 mg/L) (Figure 202, Figure 204, Figure 207, Figure 208).

Data collected from the 2023 harvest showed significant differences in both brix and free anthocyanins (Figure 198, Figure 200). Brix was lowest in treatment F (24.9) and highest in treatment E (26.1). Free anthocyanins were lowest in treatment D (1.0) and highest in all other treatments. Moreover, where YANS was highest in treatment G during the 2022 (Figure 207) harvest, during the 2023 harvest YANS was highest in treatment D (116.3). Treatment G showed YANS increasing from 60.5 during 2022 to 86.5 during 2023 (Figure 208).

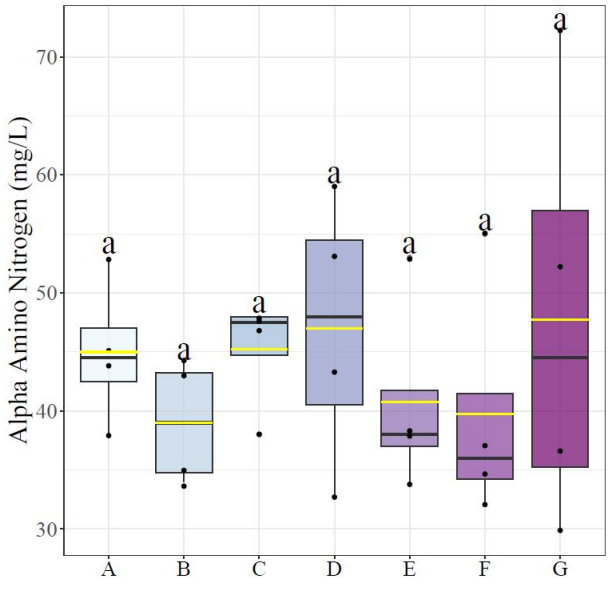


Figure 193. Boxplot displaying median values for berry alpha amino nitrogen concentration in vintage 2022, with yellow lines indicating mean values.

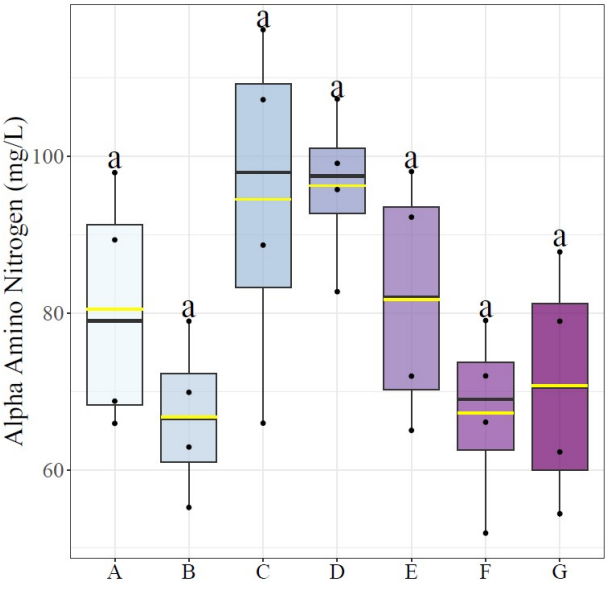


Figure 194. Boxplot displaying median values for berry alpha amino nitrogen concentration in vintage 2023, with yellow lines indicating mean values.

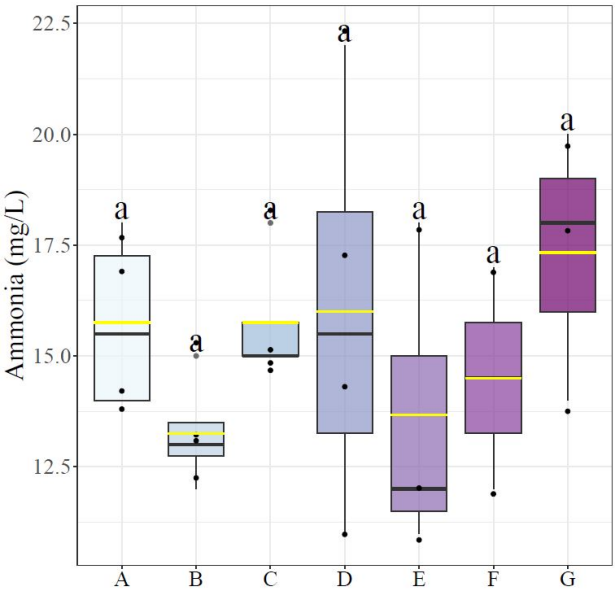


Figure 195. Boxplot displaying median values for berry ammonia concentration in vintage 2022, with yellow lines indicating mean values.

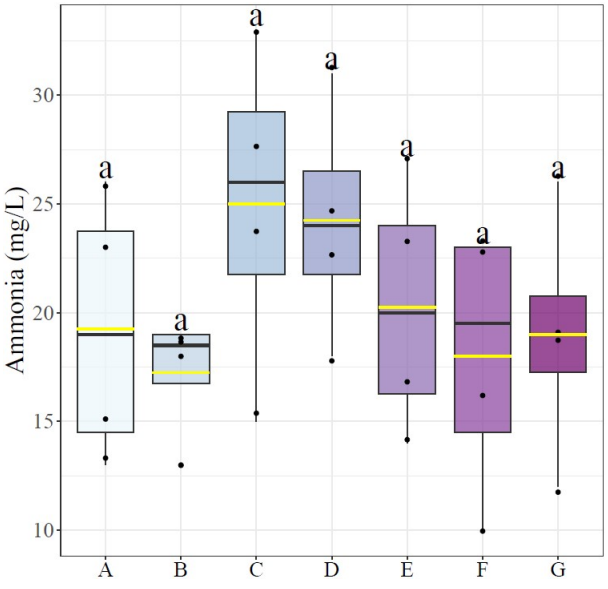


Figure 196. Boxplot displaying median values for berry ammonia concentration in vintage 2023, with yellow lines indicating mean values.

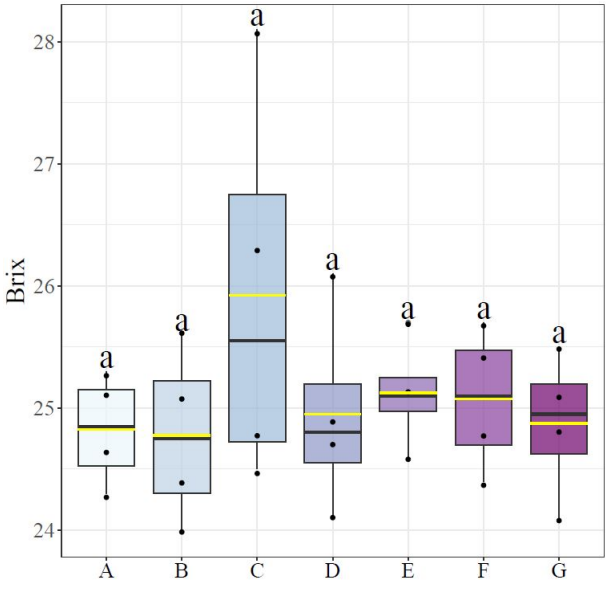


Figure 197. Boxplot displaying median values for berry brix in vintage 2022, with yellow lines indicating mean values.

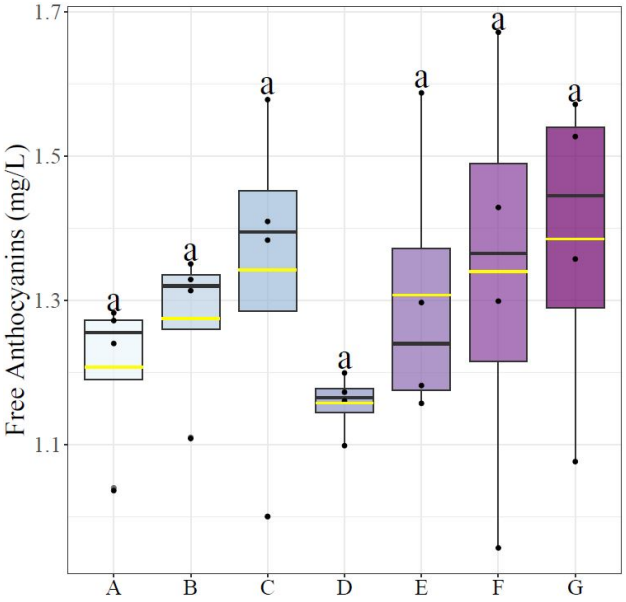


Figure 199. Boxplot displaying median values for berry free anthocyanin concentration in vintage 2022, with yellow lines indicating mean values.

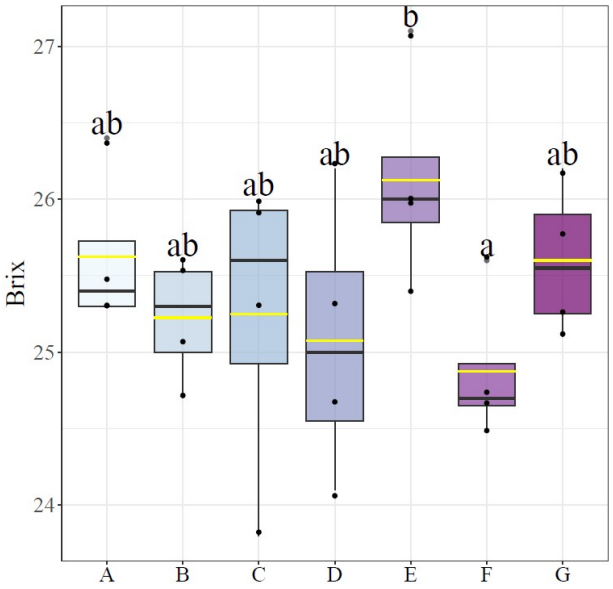


Figure 198. Boxplot displaying median values for berry brix concentration in vintage 2023, with yellow lines indicating mean values. Different letters denote significantly different treatment means.

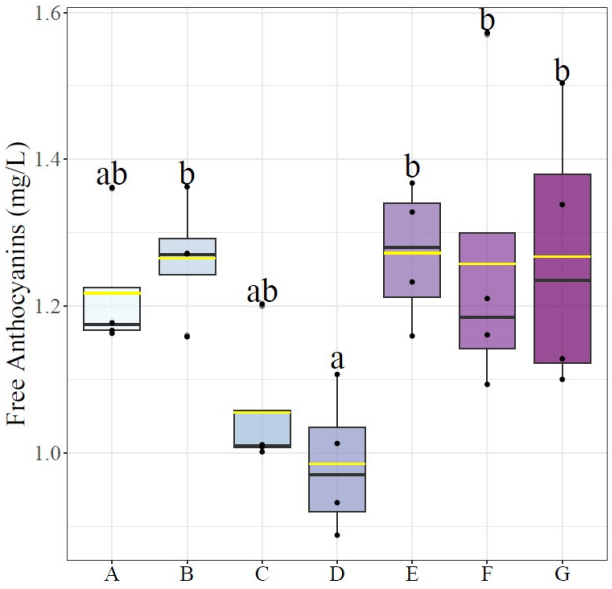


Figure 200. Boxplot displaying median values for berry free anthocyanin concentration in vintage 2023, with yellow lines indicating mean values. Different letters denote significantly different treatment means.

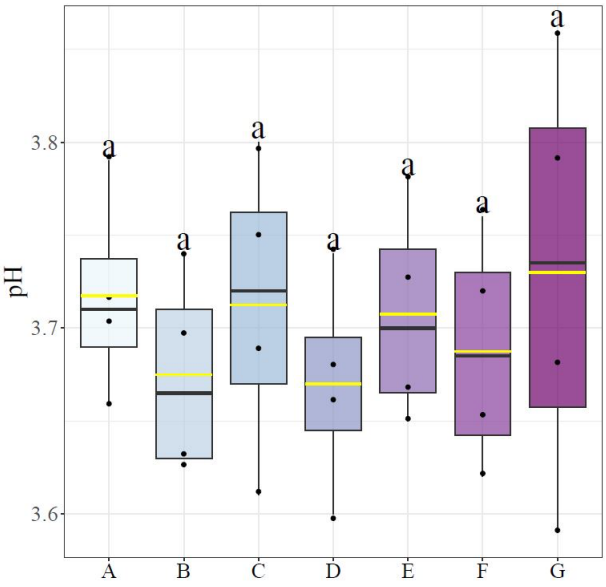


Figure 201. Boxplot displaying median values for berry pH in vintage 2022, with yellow lines indicating mean values.

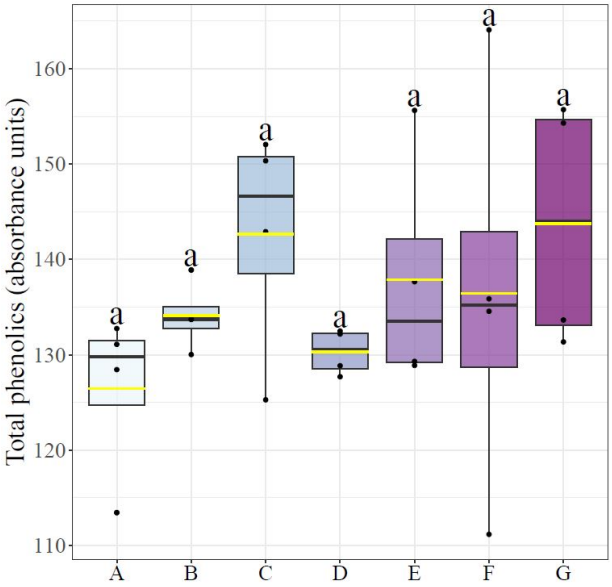


Figure 202. Boxplot displaying median values for berry total phenolics in vintage 2022, with yellow lines indicating mean values.

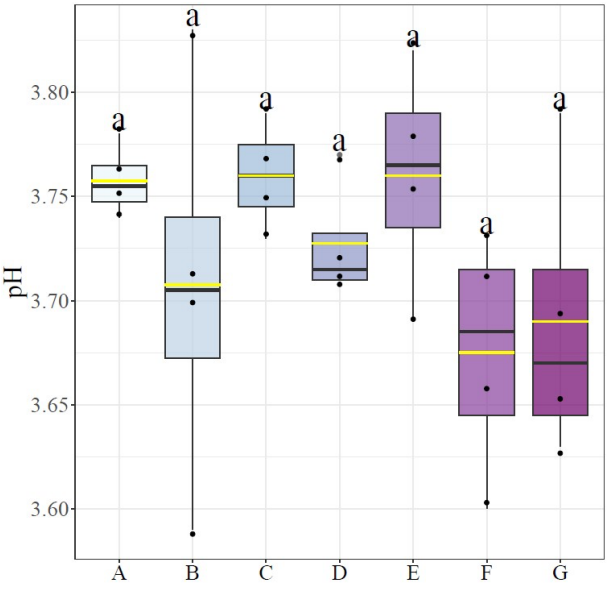


Figure 203. Boxplot displaying median values for berry pH concentration in vintage 2023, with yellow lines indicating mean values.

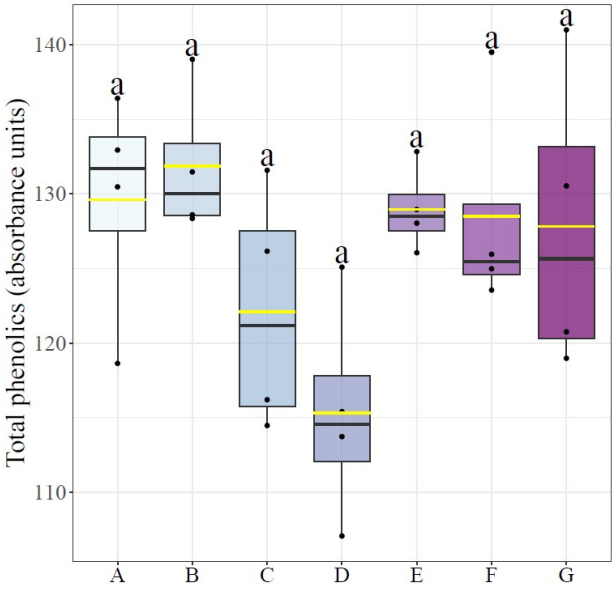


Figure 204. Boxplot displaying median values for berry total phenolics concentration in vintage 2023, with yellow lines indicating mean values.

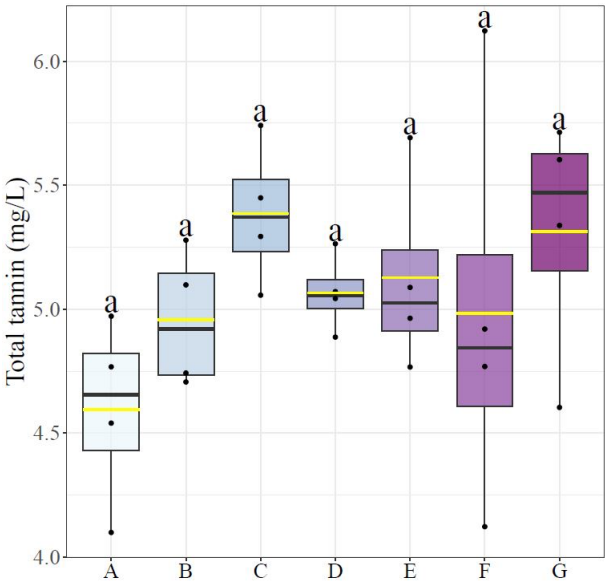


Figure 205. Boxplot displaying median values for berry total tannin concentration in vintage 2022, with yellow lines indicating mean values.

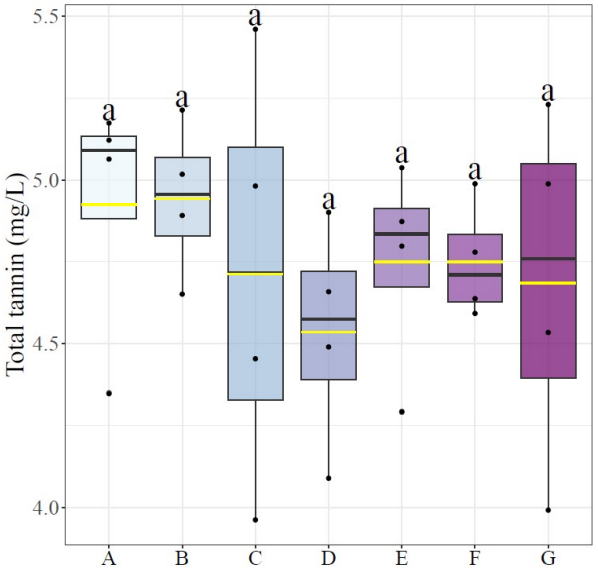


Figure 206. Boxplot displaying median values for berry total tannin concentration in vintage 2023, with yellow lines indicating mean values.

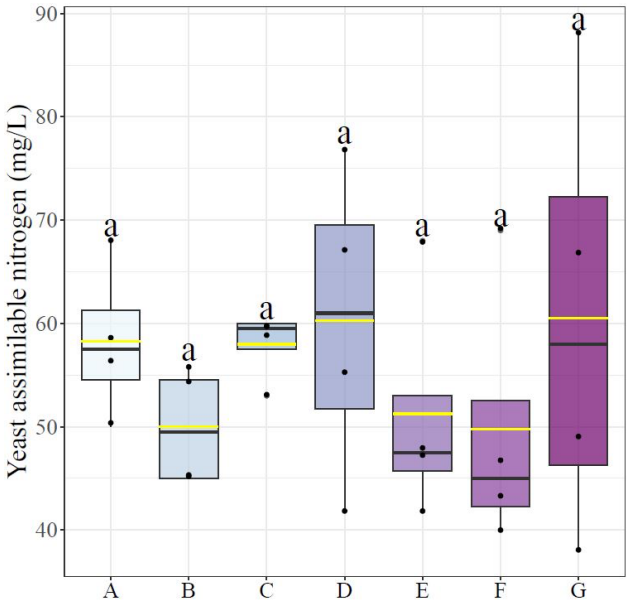


Figure 207. Boxplot displaying median values for berry yeast assimilable nitrogen concentration in vintage 2022, with yellow lines indicating mean values.

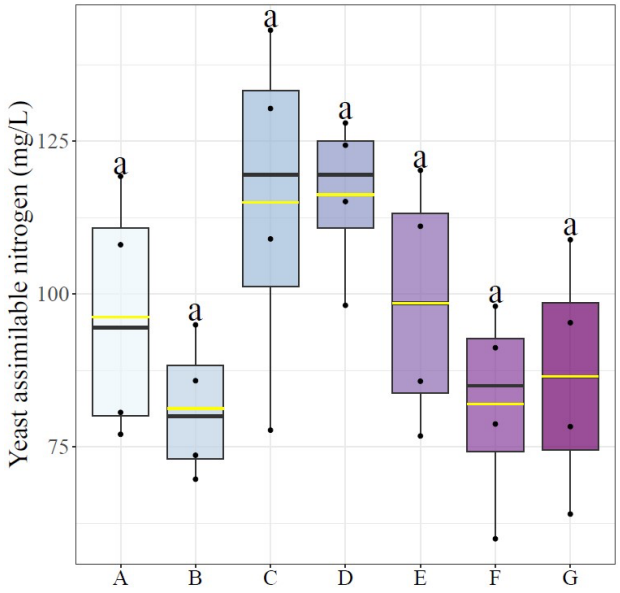


Figure 208. Boxplot displaying median values for berry yeast assimilable nitrogen concentration in vintage 2023, with yellow lines indicating mean values.

Yield and Berry Metrics

During the 2022 harvest, grape yield and bunch weight were measured to determine if vineyard floor practices had an effect (Figure 209, Figure 211). In each case we found no significant differences in either yield or bunch weight. Although there were no significant differences in yield response to any of the treatments, predicted means did vary from 9.09 kg per metre for treatment F (mech weeder 1 UV, no intervention MR) to 12.29 kg per metre for treatment D (medic and rye UV, side throw medic and rye MR). The predicted means for bunch weight were more tightly grouped, showing less treatment variation.

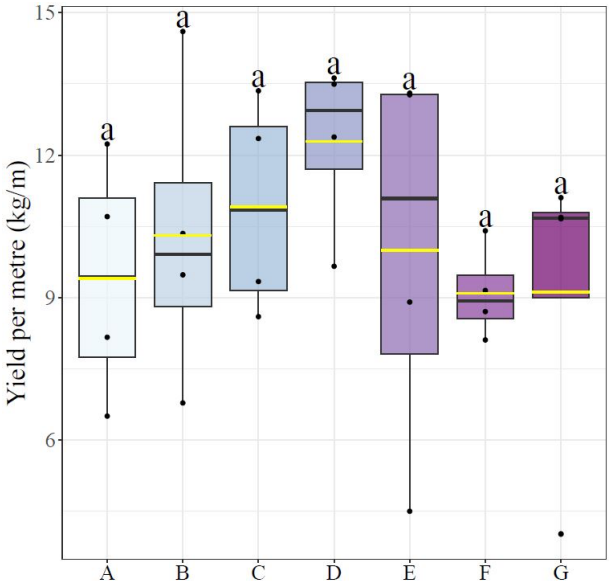


Figure 209: Boxplot displaying median values for fruit yield per metre during the 2022 harvest with yellow lines indicating mean values.

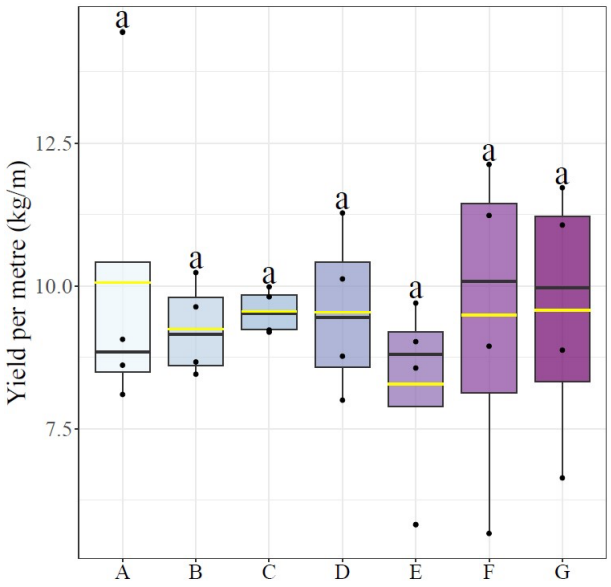


Figure 210. Boxplot displaying median values for berry fruit per metre during the 2023 harvest with yellow lines indicating mean values.

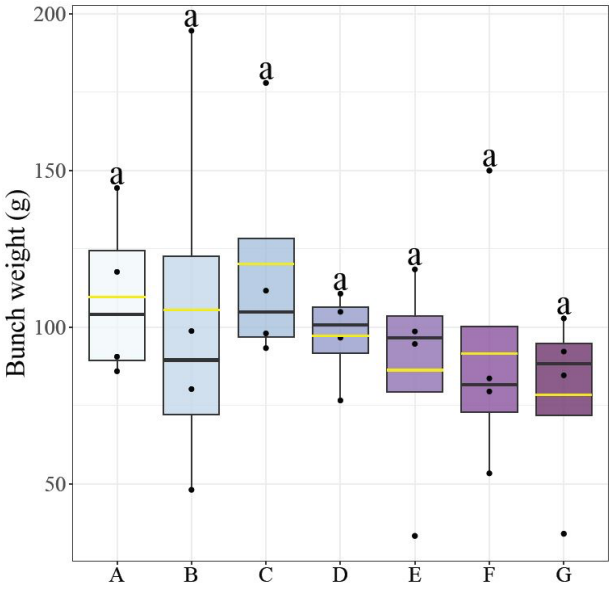


Figure 211. Boxplot displaying median values for berry bunch weight during the 2022 harvest with yellow lines indicating mean values.

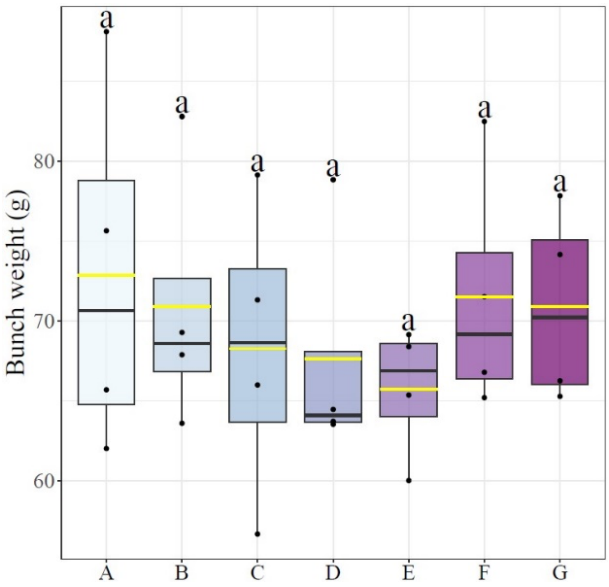


Figure 212: Boxplot displaying median values for berry bunch weight during the 2023 harvest with yellow lines indicating mean values.

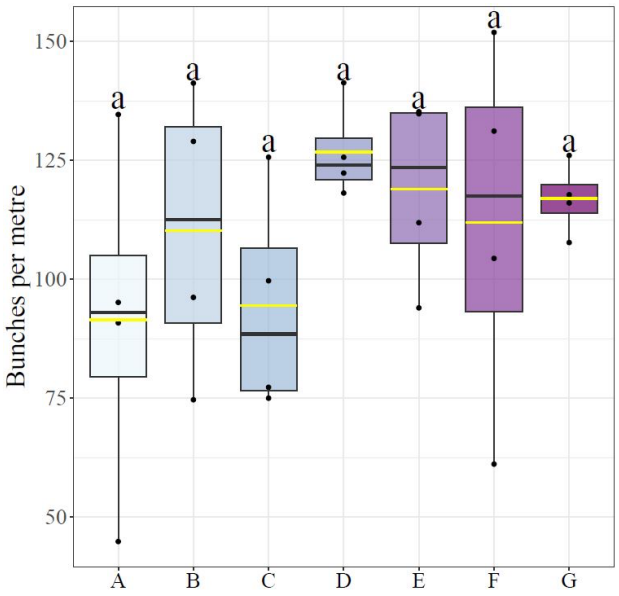


Figure 213. Boxplot displaying median values for berry bunches per metre during the 2022 harvest with yellow lines indicating mean values.

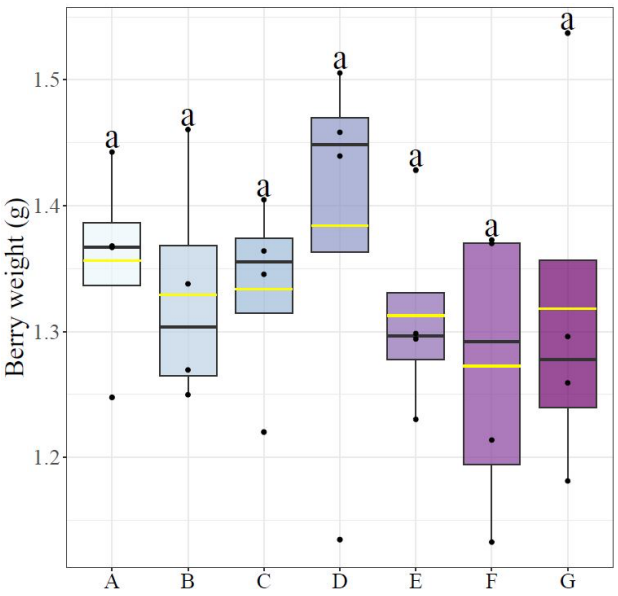


Figure 215. Boxplot displaying median values for berry weight during the 2022 harvest with yellow lines indicating mean values.

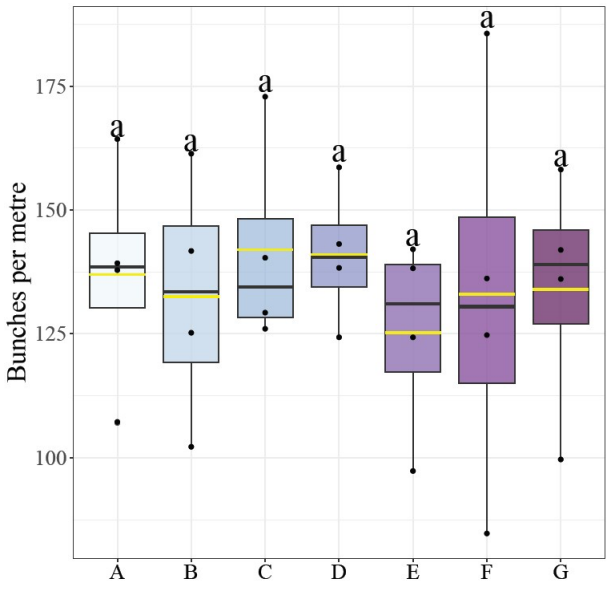


Figure 214. Boxplot displaying median values for berry bunches per metre during the 2023 harvest with yellow lines indicating mean values.

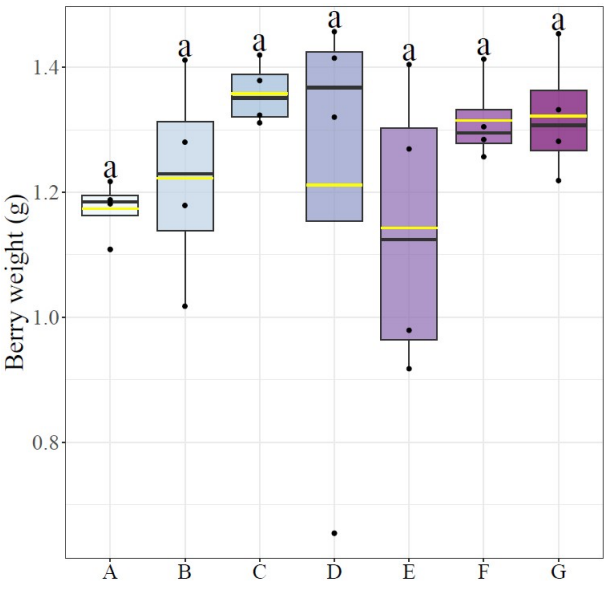


Figure 216. Boxplot displaying median values for berry bunches per metre during the 2023 harvest with yellow lines indicating mean values.

Discussion

Soil physicochemical properties

Two years post trial establishment saw observable – albeit subtle – differences in soil physicochemical properties between treatments. This is not unexpected given that sampling occurred relatively soon after trial establishment, and based on our work in Nuriootpa and Langhorne Creek, we would expect strong treatment effects to be observed after a greater period of time. Nevertheless, and indeed promisingly, although largely not statistically significant, certain differences between data from 2022 vs 2023 were evident. Treatments during the 2023 growing season showed an overall increase in soil pH (7.5 in 2022 vs 7.9 in 2023). Soil pH influences the availability of certain nutrients, with higher pH limiting the availability of nutrients such as Fe, Mn, Cu, Zn and B. Conversely, slightly elevated pH may increase the availability of some nutrients such as K, S, Ca and Mg. Although differences in pH were subtle – and unlikely to be treatment-related after two years – we may expect to see increased differences in pH between soils harbouring root systems (i.e. cover crops) vs herbicide. As the trial continues, rhizospheric pH levels may decreases slightly (compared to the herbicide-managed under-vines) owing to increases in soil organic carbon which acts as a buffering agent against pH fluctuation.

Cation exchangeability is a crucial factor in supporting soil fertility through the increase in relative abundance of binding sites and thus the ability for soil to hold essential nutrients for plant availability. Cation exchange capacity (CEC) showed significant treatment differences in the 2023 growing season, yet not during the 2022 growing season – a result indicative of treatment-based influence on soil physicochemistry. On average, the overall CEC of soils increased from 21.5 cmol/kg in 2022 to 22.2 cmol/kg in 2023. Treatment-based influences on CEC are most likely indirect – a result of cover crop biomass increasing and retaining soil organic carbon. The cation exchange capacity of soils can be directly correlated with soil organic matter (or soil organic carbon) content (Ramos *et al.* 2018). Across both 2022 and 2023 seasons, under-vine CEC was highest for the native grass treatment. In 2023, this effect was significant relative to the knife cultivated mechanical weeding practice. The perennial nature of native grasses lends them to having a rapid and noticeable effect on soil physicochemistry. Persistent root growth and exudation, in concert with fungal decomposers and increased organic carbon concentrations,(Rahman *et al.* 2021) are likely to be responsible for this change in CEC.

Organic carbon concentrations measured during the 2022 growing season did not reveal any significant differences between treatments. However, during the 2023 growing season, organic carbon increased under certain treatments, with treatment E (native species UV and MR) having significantly higher organic carbon concentrations than treatment F (mech weeder 1 UV). The presence of cover crops have been shown to both increase and retain soil organic carbon (SOC) in vineyards (Ruiz-Colmenero *et al.* 2013).Two independent studies conducted in South Australian vineyards aimed to quantify SOC stocks and concentrations under treatments of cover crop combinations, a straw mulch and a herbicide-managed control (Ball *et al.* 2020; Marks *et al.* 2022). After five years of continual under-vine management, treatments planted with cover crops showed a 22.7% increase in SOC stocks as compared to the herbicide control (Marks *et al.* 2022). In both cases, combinations of grass and legume provided high biomass output leading to increased root biomass and increased C translocation (Ball *et al.* 2020). Moreover, it is well established that physical disturbance of soil (in this case mechanical weeding) can result in a decrease in soil C concentrations (Rahman *et al.* 2021), which the early results presented here appear to support. While early results from the Monash trial appear to show treatment-based effects, Baldock (2019) suggests that management-based trials often take greater than five years to show repeatable differences in organic carbon.

Berry production and juice chemistry

How cover crops affect grapevine fruit production (yield, bunch count, berry size, etc.) is highly variable and influenced by several factors such as climate, seasonality, grapevine varietal and cover crop type (Guerra & Steenwerth 2012); see also Parts 1 and 2 herein. Although after two years we were unable to discern any significant differences in grapevine fruit production, we may expect to see certain trends emerging given more time. Under this hypothesis – and, as previous studies have shown – we expect to see reduced vigour and yield with perennial ground covers (Guerra & Steenwerth 2012; Ruiz-Colmenero *et al.* 2013). Cover crop type, as well as climatic conditions, are essential factors, with highly vigorous crops such as fescue (in previous chapters) reducing vine vigour and yield as compared to less vigorous crops such as ryegrass and Medicago. The decision on which cover crop to employ should be regarded carefully, with decisions shaped by climatic conditions, desired outcomes and grapevine varietal. Another interpretation of this is that no adverse impacts on yield were observed, which is of particular importance in this region. Nevertheless, long-term

data, spanning years with different climatic conditions, are required before firmer conclusions can be drawn.

Despite grapevine yield and other fruit production metrics presenting a lack of statistical significance, we were able to discern some significant differences in berry juice chemistry. Notably, we observed significant treatment-based differences in both brix and free anthocyanins in data collected during the 2023 growing season but not 2022. Brix levels from 2023 were highest in grapes harvested from treatment E (native species UV and MR) and were lowest in treatment F (mech weeder 1 UV, no intervention MR). There appears to be a general trend of increasing brix in all treatments in 2023 as compared to 2022. This could simply be a result of vintage variation, with increased temperature and light hours producing greater berry sugar. Given a distinct and visible treatment effect, we might surmise that cover crops are having an effect on grape sugar content. Previous trial sites have provided evidence for this change, where the more aggressive/perennial cover crop species caused higher sugar content in the vines, while the Medicago containing under-vine led to slightly less sugar (given the same irrigation). Literature from previous studies agrees with this hypothesis, showing that although vintage variation is prominent in dictating brix content, vineyard floor management practice also has an effect. A study in the United States found significantly higher brix concentration in berries sampled from treatments with under-vine cover crops (Hickey *et al.* 2016). Their findings accord well with a review by (Guerra & Steenwerth 2012) who suggest that competition for water reduces berry size, leading to higher concentrations of sugar.

Moreover, and by the same mechanism, reduced grapevine canopy size may lead to greater light penetration, reduced shading and therefore increased brix in cover crop treatments (Guerra & Steenwerth 2012). Within the same review, the authors further reported on cover-cropped grapevines producing fruit with increased soluble solids and phenolics (Guerra & Steenwerth 2012). Although these metrics were not significantly different in our own study, we were able to discern significantly higher free anthocyanins in berries sampled from cover crop treatments – notably in treatments B, E, F and G. Anthocyanin content decreased with the medic and rye treatments in 2023. This was a significant effect for treatment D (medic and rye both under-vine and mid-row). Similarly, phenolics were lowest for treatment C and D in 2023. There are a number of hypotheses for these changes, as we'd seen similar effects in the previous trials. One proposed hypothesis is the effect of the cover crop on berry size.

With leguminous under-vine treatments, yeast assimilable nitrogen increased (Figure 117). This is likely due to the nitrogen itself, or improved access to water we cannot say. Its effect would be to dilute anthocyanins and phenolics in the resultant juice, as the skin: liquid ratio decreases.

Despite an overall lack of statistical significance in berry chemistry, treatment-based trends that we anticipated could be emerging. Vitally important to winemakers, yeast assimilable nitrogen was highest for treatments C and D (medic and rye under-vine for both) in 2023. This seemed to be driven by alpha-amino nitrogen in the juice. According to the AWRI, the minimum YAN requirement for red wine is approximately 100 mg/L. Of all the treatments, only the medic and rye averaged greater than this threshold, meaning that winemakers may not need to add artificial nitrogen sources to ferments with this cover crop treatment.

Importantly, here we see the direct effect of different cover crops leading to significantly different effects in fruit, after only two seasons. These effects could have large impacts on the characteristics and quality of the wine produced. With higher sugar content in berries from the native grass treatment, or with lower anthocyanin and phenolics in the medic and rye treatments, growers deciding to manage their under-vine space with cover crops should be prepared for changes to their fruit, and will need methods to allow for this. In the case of medic and rye leading to bigger berry size, higher YAN and lower anthocyanins and phenolics, one approach might be to reduce water application. However, this hypothesis has not been tested. Similarly, for perennial grasses, something often termed a competitive effect is inescapable. Reduced canopy size and resultant yield decrease was observed at Langhorne Creek, Nuriootpa (see sections 1 and 2 herein) and its early stages are observable at Monash. While caution should be taken when making generalisations, there is a growing body of evidence from these three distinctively different regions that significant juice effects are induced by ground cover selection. Further work is necessary to determine exactly how individual cover crop species should be managed to optimise quality.

Soil microbiome

There was a surprising lack of statistical significance to emerge in the microbiome analysis of under-vine soils. High biomass ground cover, senescing and decomposing for two years would almost certainly lead to substantial changes in microbial community when compared to bare earth. Despite this, treatments demonstrated no statistical difference when comparing

metrics of alpha diversity, including Faith's phylogenetic diversity, Shannon's diversity or Pielou's evenness. Overall values for Faith's diversity metric (a measure of evolutionary diversity) were high, when compared to our earlier studies. Lack of difference between treatments for both Shannon's diversity and evenness suggested that treatments did not influence microbial composition substantially, and trends appear absent for both bacterial and Archaeal and fungal datasets.

Comparisons among treatment populations can be carried out directly with analysis of beta diversity. Two samples with completely different histories and microbial functions can have the same alpha diversity, yet vastly differing beta diversity. In this trial, we saw no trends in beta diversity, suggesting all samples, even those from the same treatment are different.

Upon examination of the taxa present in the soil samples, the presence of toxic freshwater cyanobacteria and other aquatic species was revealed. This is not entirely unusual in highly irrigated vineyards, but given the extreme flooding that occurred during the 2022/2023 season, in combination with the algal blooms and filtration issues faced by most growers, we believe it is highly likely that non-soil-based species were pumped directly into the vineyard through the irrigation system. This may have led to high levels of microbial contamination throughout the trial site, vastly increasing the diversity for all treatments, and masking any potential any treatment specific results.

One of the aquatic microbes identified was a cyanobacterium from the genera *Microcystis*. This specific genus produces compounds called microcystins, which are known to be toxic to people and livestock. They have also been shown to have toxic effects on broadacre species by limiting yields, germination, and photosynthesis (Zerrifi *et al.* 2019). The toxins can also accumulate in the fruit of tomato plants. This is an important finding, and one very few viticulturists will be considering through times of flood.

Riverland Recommendations

It is acknowledged that two years of data do not adequately portray the influences of cover crops on a perennial cropping system such as viticulture. However, some promising trends are emerging, and it is therefore recommended that the trial be continued so the impacts of treatments on these systems can play out to a point where more concrete management recommendations can be made.

Overall Recommendations

Nuriootpa and Langhorne Creek (Part 1)

- Vines with the Medic mix or Medic and Ryegrass treatments under-vine did not impose a yield penalty, even in extremely low yielding years such as the 2019 and 2020 vintages. This evidence alone suggests that with correct species selection, under-vine management without the use herbicide is a feasible practice.
- Water stress: Vines with the Medic mix or Medic and Ryegrass treatments were less water stressed than the herbicide control. They also had similar or larger canopy sizes. This suggests that not only are the cover crops improving the soil in some way, but that they are helping the vines maintain better water balance. This is further evidence that counters the traditional hypothesis that under-vine cover crops will compete with grapevines for water and stunt the vines.
- Nutrient use: Under-vine cover crops did not lead to nutrient deficient vines as a result of competition for nutrients in the soil. The levels of N were enriched by the leguminous Medics. The Medic treatments, however, did lead to reductions of P in the soil. As their biomass was not exported from the site, we expect that the consumed available P has now been stored in the Medic biomass in an organic form, which will return to the soil as it breaks down and becomes mineralized. Thus, it is only a temporary reduction in soil P, but is an important consideration in establishing the cover crop.
- An ideal cover crop would grow quickly during the wet (rain-fed) months of the year, and then senesce in the warmer months to provide a mulch layer to reduce evaporation. The Kasbah and Fescue treatments sustained growth at both sites throughout the summer season. Despite no visible increase in water stress or reduction in soil moisture, the canopies of vines treated with these cover crops were significantly reduced. This led to reduced yields and high pH juice. Thus, we would not recommend the use of these species in a climate with limiting rainfall.
- At Nuriootpa, all treatments lead to a reduction in salinity, relative to the herbicide control. Though salinity was not a problem at this site, these findings suggest that in soils of this type, if soil salinity is an issue, under-vine cover cropping may be a viable solution.
- A 64% increase in soil carbon concentration was generated by the Medic and Ryegrass treatment. Without compromising grape yields, this treatment has also

provided enormous benefit to the soil, and as such is recommended for growers to use in similar environments.

- The application of straw mulch remains an effective way of keeping soils cool and reducing soil hardness. It remains, however, the most expensive option.

Arbuscular Mycorrhizal Fungi Experiments (Part 2)

- Soil inoculum potential appeared unchanged by the presence of cover crops vs a herbicide-managed control.

Biofumigant Trial - Tanunda (Part 3)

- Establishing nemcon 'bio-fumigant' brassicas proved to be extremely difficult at our trial sites in the Barossa Valley. Literature suggests that if successful establishment of Nemcon occurs, this method for nematode control should be effective. We would recommend that anyone interested in attempting this, run a small trial before making an investment in large quantities of seed.

Monash Trial (Part 4)

- Medic and annual rye are an effective under-vine cover crop in this warm inland region. Sufficient biomass can be generated under-vine through the provision of early season irrigation, for production of a deep litter layer. In drier years where water is more costly and irrigation starts later, this may not be possible.
- Native grass can be established under-vine but may leave space for establishment of invasive species such as fleabane.

Overall

- Species selection for under-vine cover cropping is critical to achieving the desired effects.
- Medic and annual rye can be effective at producing an active mulch layer without leading to reductions in yield.
- Selection of perennial ground covers (particularly large grasses such as Fescue and Kasbah) for under-vine cover crops can lead to reduction in canopy size and yield. In The Riverland, data is limited on native grasses due to the short period of the trial.

Appendix 1: Communication

Over the course of this project there was much interest from the sector, and as a result many presentations and outreach activities were delivered, as follows:
In 2018 and 2019 Chris Penfold presented to viticulture students at the Waite on vineyard floor management. Since 2020 Thomas Lines has taken over this role.
2021-2023.

In 2023;

- Thomas Lines and Joseph Marks created two informative videos;
    - one on soil organic carbon and under-vine management https://youtu.be/hqKl6og5Wxg
    - one on the results from the Monash trial: https://youtu.be/ELKY3tOdr9U
- Scheduled webinar for August, 2023 – Thomas Lines & Timothy Cavagnaro
- Thomas Lines spoke on behalf of Timothy Cavagnaro at the ASVO CO23 conference.

In 2022;

- Thomas Lines created one informative video on cover cropping in the under-vine area of vineyards: https://youtu.be/AFfdauWUWKs
- Created two informative flyers on *Clarifying Carbon* (see Appendix 6).
- Thomas Lines arranged, chaired and presented a workshop at the Australian Wine Industry Technical Conference. Tim Cavagnaro and Joseph Marks also presented at this workshop.
- Timothy Cavagnaro presented at a separate workshop at AWITC on soil C organised by AWRI.
- Timothy Cavagnaro presented at two Viticulture Master Classes.

In 2021;

- Thomas Lines spoke at Longview cellar door
- Thomas Lines presented at the Riverland and The Hunter Valley AWRI roadshow.

In 2020;

- Chris Penfold and Thomas Lines each gave presentations at the AWRI roadshow in Langhorne Creek.

- Timothy Cavagnaro, Thomas Lines and Chris Penfold spoke and Longview Cellar door to growers and winemakers of the Adelaide Hills and McLaren Vale wine regions.
- Chris Penfold, Thomas Lines and Timothy Cavagnaro, presented an AWRI webinar which set records for registrations and attendance.
- Chris Penfold was going to provide the keynote address at the Australasian Symposium for Greening Vineyards in NZ in July though this was cancelled due to Covid-19. Chris Penfold had talks in Tasmania and Victoria booked though this was cancelled due to Covid-19.
- Chris Penfold presented at Macclesfield.
- Chris Penfold produced a fact sheet on cover cropping which is on the WA website, and two articles for the Grape grower & Winemaker on results from the previous 3-year project and a retrospective look at vineyard floor management.

In 2019;

- Thomas Lines presented prelim findings from this project to growers from the Limestone Coast Grape and Wine Council
- Chris Penfold presented at two workshops at the AWITC – Enhancing biodiversity in Vineyards and Weed management.
- Chris Penfold delivered talks in the Yarra Valley on under-vine management in October.
- In June 2019 attended the GiESCO conference in Greece.
- In July Chris Penfold spoke to Clare grapegrowers and landowners at a deer management evening about under-vine cover cropping.

In 2018;

- Thomas Lines and Timothy Cavagnaro spoke on the 'Country Hour' for Radio National. This was also developed into a large web article.
- Chris Penfold spoke at the Improving Soil Health for Riverland Vineyards workshop in December.
- An AWRI webinar and a roadshow presentation at Loxton on cover cropping in May (Chris Penfold).
- An Under-vine cover cropping and pruning workshop organised by Wine Tasmania in May (Chris Penfold).
- In July a webinar for Landmark growers and agronomists and a presentation to growers while staying in the Margaret River region (Chris Penfold).

Appendix 2: Intellectual Property

No intellectual property has arisen from this research.

Appendix 3: References

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Appendix 4: Staff

University of Adelaide staff employed, or who played a direct role in this project were; Chris Penfold (Part 1-3), Dr Thomas Lines (Part 1-4), Prof. Timothy Cavagnaro (in-kind), Dr Vinay Pagay (in-kind); Dr Joseph Marks (Parts 1, 2 & 4).

Appendix 5: Cover crops and carbon stocks

Below is the abstract of a published manuscript entitled: "Cover crops and carbon stocks: How under-vine management influences SOC inputs and turnover in two vineyards". The manuscript is published in *Science of the Total Environment* and was authored by Joseph Marks, Thomas Lines, Chris Penfold and Timothy Cavagnaro.
https://doi.org/10.1016/j.scitotenv.2022.154800

Abstract

There is a growing awareness surrounding the importance of maintaining and increasing soil organic carbon (SOC, henceforth) stocks in vineyard systems. Increasing SOC positively influences numerous soil properties and has the added advantage of removing atmospheric CO2, thereby helping to mitigate the effects of climate change. Cover crops have long been used to influence soil properties in vineyard mid-rows, including increasing SOC content. Few studies, however, have quantified cover crop influence on SOC stocks and composition in the under-vine area, owing to a general reluctance to adopt under-vine cover crop management. This research aims to quantify SOC stocks and dissolved organic carbon (DOC) in soils from four treatments of under-vine management practice including two cover crop combinations, a straw mulch and herbicide-managed control across two vineyard sites established in 2014. We sampled soils under-vine to depths of 0–30 cm (stratified; 0–10 cm and 10–30 cm) and quantified both SOC concentrations and bulk density to ascertain SOC stocks. Further to this, we quantified water extractable organic carbon (WEOC) as a measure of the labile carbon stock, and measured heterotrophic respiration in a laboratory incubation as an indication of SOC turnover. We found that cover crop-managed soil under-vine sequesters up to 23% more soil organic carbon (SOC) as the traditional, herbicide practice over a five-year period of growth. Microbial activity increased by more than double in cover crop soils, owing to an increase in DOC and that there is evidence for more resistant C in cover crop soils. These results suggest that cover crop management under-vine is a viable solution to increase SOC stocks within vineyard systems. Taken together, the results of this study indicate that a shift from bare earth to cover crops in the under-vine region has the potential to sequester carbon in vineyard soils.

Appendix 6: Effects of cover crops on canopy temperature

Introduction

The following is adapted from the Honours Thesis written by Benjamin Hayward, supervised by Dr Thomas Lines and Prof. Timothy Cavagnaro.

Introduction

Effective management of vine canopy temperature is crucial for regulating vine productivity and viability in semi-arid viticultural regions where water may be limited. This management is achieved through the control of soil temperature and moisture, significantly influenced by vineyard floor management (Guerra & Steenwerth, 2016). With projections of a warming and drying climate in many of the world's major winegrowing regions, this is a cause for significant concern within the industry.

Soil and canopy temperature and moisture are interrelated, as water movement responds to thermal exchange processes such as sunlight reflection and evapotranspiration occurring primarily at the soil and canopy surfaces (Hillel, 1998; Keller, 2020). Direct sunlight radiation on grapevine canopy surfaces causes heat loading, regulated by vines through radiation emission, convection heat loss, and evaporative cooling via transpiration (Keller, 2020). The transpiration rate increases with soil water potential gradient and stomatal conductance, driven by increased soil water availability. Conversely, soil water deficits reduce vine transpiration, raising canopy temperatures. Transpiration decreases above 30°C, and vine stress occurs at ambient temperatures above 35°C (Hochberg et al., 2015). Therefore, temperature is a major determinant of vine performance and stress.

Vineyard floor management involves regulating the soil surface in the under-vine and mid-row sections to optimise vine performance and yield by influencing water and nutrient uptake and soil physicochemical processes such as nitrogen mineralisation (Guerra & Steenwerth, 2016). This is often achieved by creating a bare earth surface through cultivation and herbicide application, aiming to eliminate weeds and improve soil nutrient and water content (Guerra & Steenwerth, 2016; Bavougian & Read, 2018).

Bare earth management can result in higher soil temperatures due to low surface albedo and high heat absorption (Coombe & Dry, 1992). This leads to increased evaporation and reduced

vine water availability, causing water stress in limited water conditions at ambient air temperatures above 35°C. Vine water stress negatively impacts fruit quality and vine vitality, with fruit quality generally associated with high total soluble sugars (Morlat & Jacquet, 2003; Tesic et al., 2007).

Due to the potential negative impacts of bare earth management, there is growing interest in mulching and cover cropping among viticulturists globally (Fourie & Freitag, 2010; Fourie, 2011; Gattullo et al., 2020). Mulching involves applying biomass to the vineyard floor to suppress weed growth and retain soil moisture, reducing evaporation and increasing vine water potential gradient (Guerra & Steenwerth, 2016). Cover cropping, which includes monocultures or polycultures of annual and perennial monocots and dicots, is integrated into the vineyard mid-row or under-vine soil system to suppress weeds and interact with grapevine nutrient and water uptake (White & Krstic, 2019). However, there are reservations about cover crops competing with grapevines for water and nutrients. Studies have found decreased vine vigour due to competitive grass cover crops (Morlat & Jacquet, 2003; Guerra & Steenwerth, 2016). Nonetheless, evidence suggests that cover crops with selected traits can effectively manage canopy growth. In high rainfall regions, grasses control excessive vigour, while grasses coupled with legumes can control vigour and provide nitrogen (White, 2015; Guerra & Steenwerth, 2016). Reduced soil water potential gradient leads to smaller canopies, allowing vines in water-limited environments to maintain water and nutrient uptake and avoid stress.

Most studies on cover crops in vineyards focus on mid-row effects rather than under-vine areas (Tesic et al., 2007; Nguyen et al., 2013; Bavougian & Read, 2018). The under-vine area is crucial due to root accessibility to water and nutrients and proximity to cover crop roots for vigour control. In contrast, mid-rows often have compacted soils and higher solar exposure, reducing vine root distribution and potentially diminishing the efficacy of vineyard floor management (White, 2015). Data on these variables can be collected via proximal (at the site) or remote (at a distance) methods. Studies on vineyard floor management's impact on canopy temperature have primarily used proximal sensing methods to measure volumetric water content, thermal infrared canopy leaf temperature, vine water potential, stomatal conductance, and biomass production (Tesic et al., 2007; Bavougian & Read, 2018).

Proximal sensing of soil moisture by Bavougian and Read (2018) recorded higher soil moisture in Marquette vines in the under-vine area compared to herbicide-treated vines in

Nebraska, USA. In contrast, Tesic, Keller, and Hutton (2007) observed decreased mid-row soil moisture in Chardonnay vines with grass cover in Wagga Wagga and Tumbarumba, NSW, resulting in reduced canopy vigour and higher quality yields. These findings suggest that under-vine cover crops can manage vine water availability and canopy density, thus controlling vine stress.

Recent years have seen the effective use of remote sensing in irrigation management via thermal infrared imagery and vegetation indices. Espinoza et al. (2017) differentiated between varied irrigation rates of Cabernet Sauvignon in Washington State, USA, using thermal infrared imagery obtained via UAV. The study found a negative correlation between canopy temperature and stomatal conductance and a positive correlation between NDVI and stomatal conductance, indicating that low irrigation rates increased vine water stress. Matese et al. (2018) observed increased grapevine water stress with deficit irrigation, measured through proximal and remote sensing of temperature in Vermentino, Cagnulari, and Cabernet Sauvignon vines in North-Eastern Sardinia, Italy.

These studies suggest that remote sensing can effectively quantify vineyard water use through thermal infrared imagery, vegetation indices, and proximal temperature sensing. Given the focus on mid-row management, measuring canopy temperature is an effective method for determining grapevine stress responses to water and heat in vineyard floor management. Therefore, it was hypothesised that combining remote sensing and vineyard floor management would effectively determine the impact of under-vine management on canopy temperature.

The study aimed to use proximal sensing of vine canopy air temperature and thermal infrared imagery of leaf surface temperature and NDVI indices obtained through remote sensing to measure the effects of under-vine management practices in vineyards within a semi-arid climate. Specifically, the study aimed to measure canopy temperature effects from under-vine treatments through proximal and remote sensing of temperature and NDVI.

In this addendum, "proximal sensing" refers to vine cordon air temperature, while "remote sensing" refers to vine canopy surface temperature.

Materials and Methods

Trial sites were as previously described for Nuriootpa and Langhorne Creek (LC).

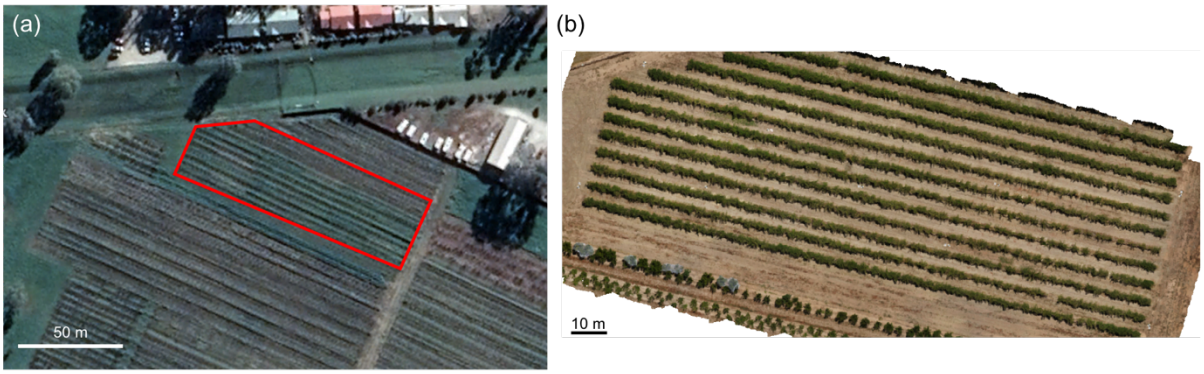


Figure 217. Satellite imagery outlining the experiment design perimeter at Nuriootpa (captured 8-2020) (a). RGB imagery captured during remote sensing of Nuriootpa (captured 10-2-21:1426 -1435h) (b).

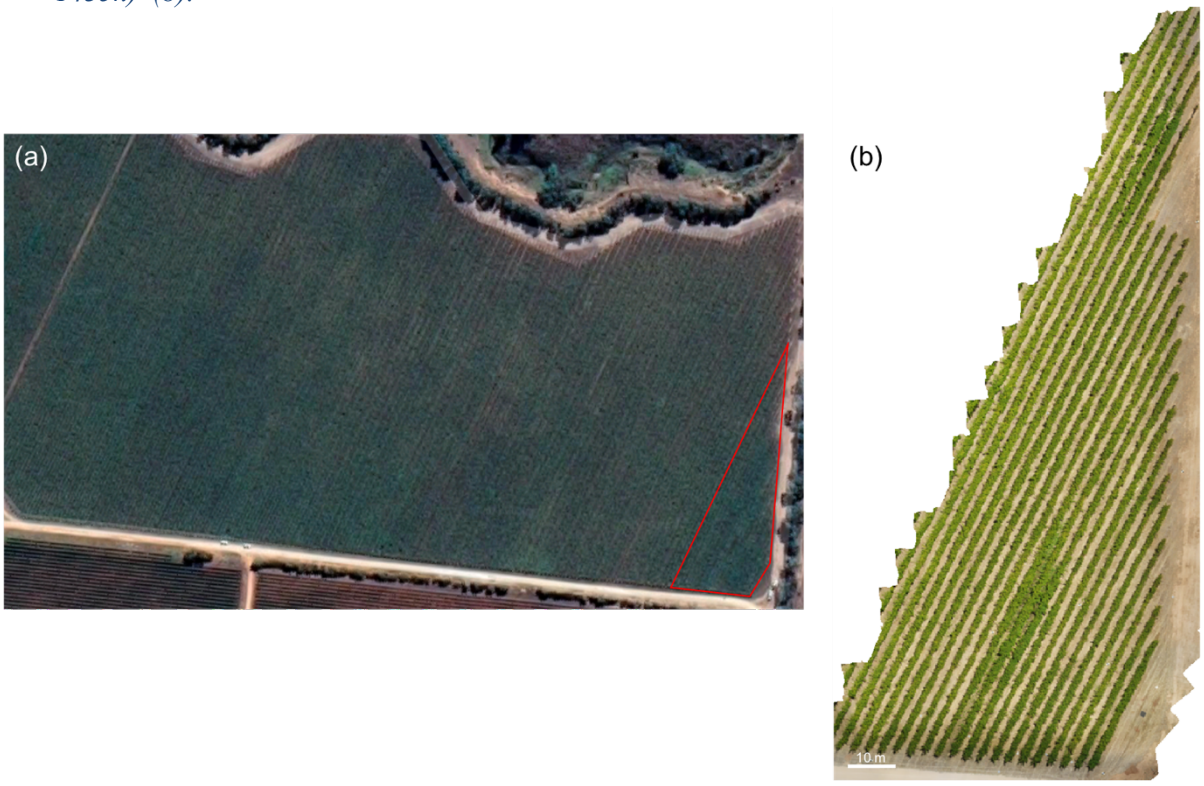


Figure 218. (a) Satellite imagery outlining the experiment design perimeter at Langhorne Creek (captured 3-2020). (b) RGB imagery captured during remote sensing of Langhorne Creek (captured 22-1-21: 1412 – 14:25h) (b).

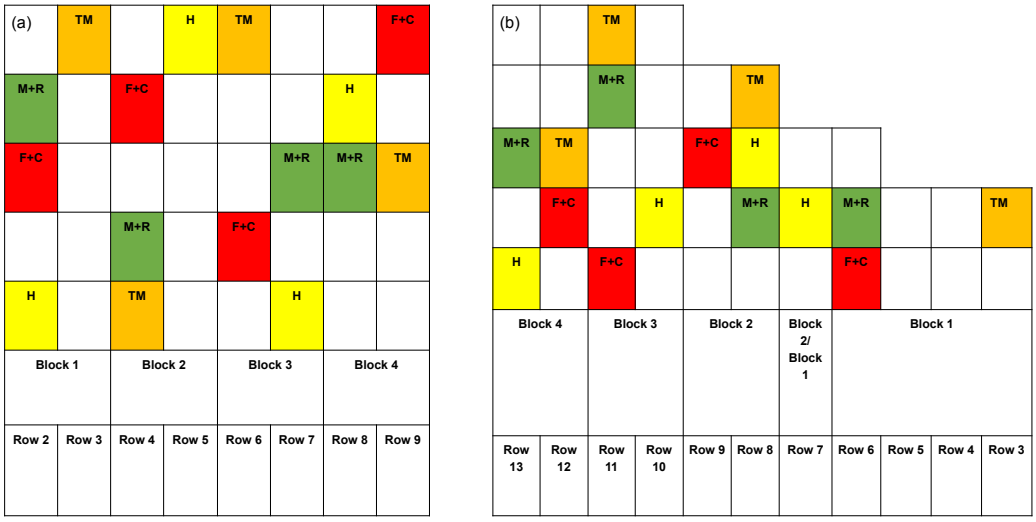


Figure 219. Experimental design RCBD trial layout at (a) Nuriootpa and (b) Langhorne Creek. Nuriootpa is positioned in an east – west configuration from bottom up. Langhorne Creek is positioned in a south – north configuration from bottom up. The treatments were herbicide (H), triticale mulch (TM), fescue and clover (F+C), medic and rye (M+R).

Proximal sensing

The proximal sensing of temperature within the vineyard was achieved using iButton data loggers (Thermochron, USA). These were configured to log temperature hourly. Each data logger was enclosed within a casing that protected the sensor from direct sun radiation, and allowed for the attachment to vines.

In Nuriootpa, 16 loggers were attached to vine cordons in a configuration that was hoped to account for spatial variability for the remote sensing UAV flight. In Langhorne Creek, 48

loggers were distributed in accordance with the RCBD layout, with loggers in triplicate for each treatment plot. The location of each data logger was recorded using GPS and identified in the remote sensing imagery (via the use of flags) where they were used as ground control points for remote sensing data processing. The recording of proximal data occurred simultaneously with the remote sensing during UAV flight.

Remote sensing

The remote sensing of both sites was achieved by using a DJI M210 RTK UAV (unmanned aerial vehicle). The UAV was equipped with three sensors. These sensors were: Red Green Blue sensor (RGB, Zenmuse X7 35 mm lens, 23.5 x 12.5 mm sensor, 24 megapixel camera, 3.0 mm pixel resolution sensor), Multispectral sensor (Micasense RedEdge MX – blue, green, red, red edge and near infrared bands processes to reflectance using a calibration panel and downwelling sensor, 2.0 cm pixel resolution. Only NDVI derived from NIR and R bands was used) and thermal infrared sensor (Zenmuse XT2, 640 x 512, 30 Hz Radiometric with 13 mm lens. 3.0 cm pixel resolution). In the data collection, the imagery was all georectified to ground control using Propeller Aeropoints placed throughout the vineyard floor, providing up to 2 cm spatial accuracy.

The flight route planning was completed with the DJI pilot software, which was operated by a qualified drone pilot from URAF (unmanned research aircraft facility). At Langhorne Creek, the remote sensing was completed on 22-1-21 between 1412 – 1425h (EL 34-36) and for Nuriootpa on 10-2-21 between 1426 -1435h (EL 35-36).

The mean ambient air temperature data recorded at nearby weather stations of Nuriootpa PIRSA for Nuriootpa and Strathalbyn Racecourse for Langhorne Creek was obtained from the Bureau of Meteorology and used in comparison with the proximal and remote sensing of canopy temperature at both sites.

Image processing

URAF captured and processed the data in Agisoft Metashape Pro 1.6 for RGB imagery and Pix4D Mapper 4.5 for multispectral imagery and thermal infrared imagery. The processed data was presented as orthorectified mosaics, which was analysed using the software QGIS 2.2.

QGIS

Within QGIS, polygons of 0.2 x 0.7 m dimensions were aligned with individual vines within the treatments in the imagery from both sites. These specific dimensions were used to ensure continuity between thin and wide canopies, where 0.2 m width avoided spatial interference from the ground. All imagery was georectified by URAF, therefore the locations of the treatments were marked out for remote sensing for later data extraction. A polygon was assigned to each plot of each treatment of interest.

From the multispectral orthorectified mosaic, an NDVI raster layer was generated through the QGIS raster calculator. This layer was generated using the NDVI equation (Equation 1).

Equation 1. $$NDVI = \frac{NIR-Red}{NIR+Red}$$

Where:

NDVI = Normalised Difference Vegetation Index

NIR = Near Infrared Band

Red = Red Band

Zonal statistics were run to capture the statistical data from each polygon within each thermal infrared and NDVI raster layer. The treatments were marked by polygons within the orthorectified mosaics, where they were used to extract data from specific pixels within the polygons within the thermal IR and NDVI imagery.

Langhorne Creek was broken into west and east sides, where the proximal sensing of temperature dataset was used in reference to the treatment locations in the site (Figure 220a). The remote sensing of temperature was used at datapoints that were not influenced by under vine treatments, therefore additional polygons were generated in order to extract data specific to these points (Figure 220b).

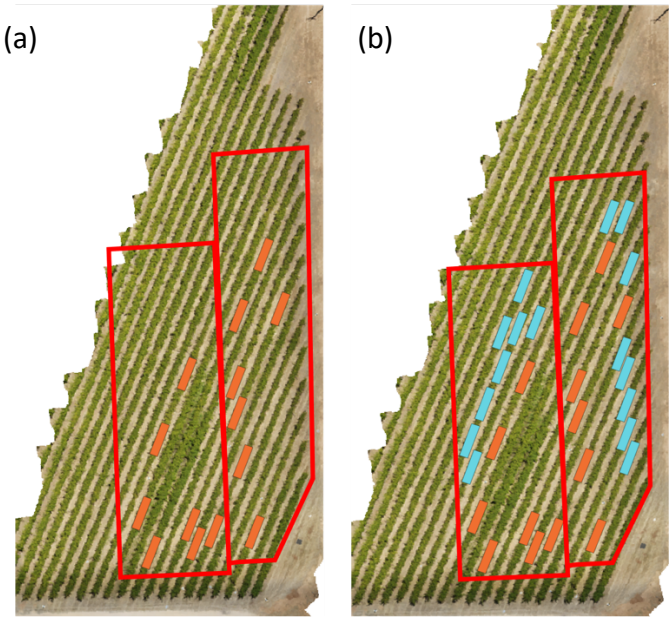


Figure 220. Comparison between western and eastern sides of site with treatment datapoints marked by orange polygons (a). Comparison between western and eastern sides of site with untreated vine datapoints marked by blue polygons in reference to the treated vines. No treatment datapoints were used in statistical analysis (b). Treatments within the irrigation leak were excluded from the dataset.

Statistical analysis

Statistical analyses of linear mixed models were carried out in R 4.1.1 (R Core Team 2021) using asreml-R, dplyr and biometry training packages (Butler 2021, Wickham et al. 2021, Nielsen et al. 2021) . Within the proximal sensing dataset of Nuriootpa, two loggers fell to the ground during temperature measurement, therefore were excluded from the statistical analysis.

Within all measurements in Langhorne Creek, triticale mulch block 4 and fescue and clover block 3 were excluded from the statistical analysis, as they were located within the irrigation leak of the site.

Results

Comparison between proximal and remote methods of temperature data capture

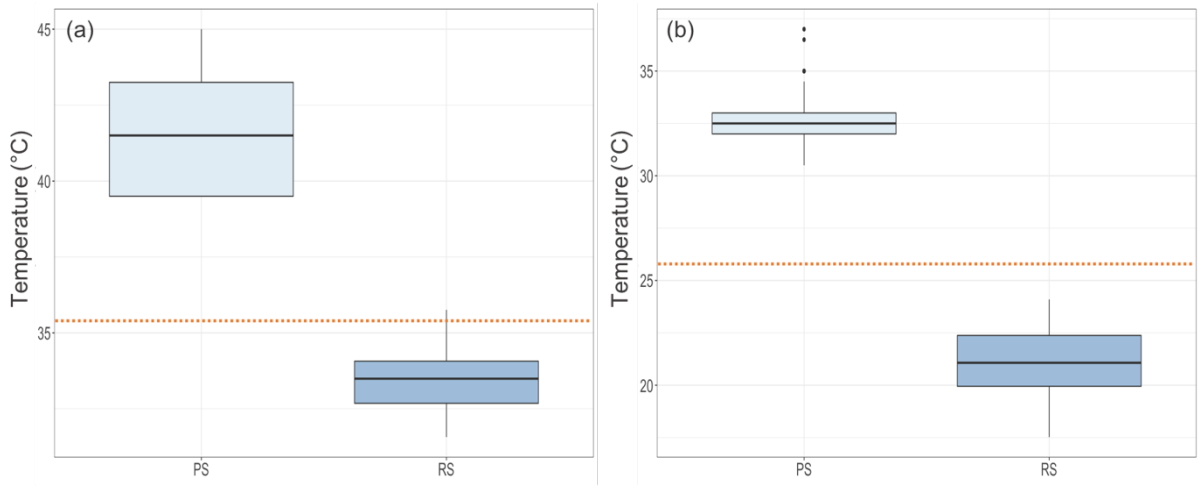


Figure 221. Mean vine canopy temperature measured at (a) Nuriootpa ($p=1.90^{-24}$, $n_{PS}=8$, $n_{RS}=48$) and (b) Langhorne Creek ($p=1.17^{-52}$, $n_{PS}=42$, $n_{RS}=42$) through proximal sensing (PS) and remote sensing (RS), based on one way ANOVA (Tukey's Test). Both methods of temperature measurement were completed simultaneously. The dashed orange lines represent the mean ambient air temperature recorded at weather stations within close proximity to both vineyards.

Throughout the course of UAV flight and proximal sensing, the mean ambient air temperature measured at a nearby weather station for Nuriootpa (a) was 35.30°C (10-2-2021) (Bureau of Meteorology 2021). This was 6.23°C lower than the mean vine canopy temperature measured through proximal sensing at 41.53°C. Furthermore, the weather station data was 1.71°C higher than the mean vine canopy temperature measured in remote sensing at 33.59°C.

The mean ambient air temperature measured at a nearby weather station in Langhorne Creek (b) was 25.80°C (22-1-2021) (Bureau of Meteorology 2021). This was 7.10°C lower than the mean vine canopy temperature measured through proximal sensing at 32.88°C. The weather station data was 4.56°C higher than the mean vine canopy temperature measured in remote sensing at 21.24°C.

Overall, at Nuriootpa there was a 7.94°C difference in measurement of temperature between proximal sensing and remote sensing, with proximal sensing being higher than the ambient air temperature and remote sensing being lower than the ambient air temperature.

At Langhorne Creek, there was a 11.64°C difference in measurement of temperature between proximal sensing and remote sensing, with proximal sensing being higher than ambient air temperature and remote sensing being lower than the ambient air temperature.

Proximal sensing of canopy temperature at Nuriootpa and Langhorne Creek

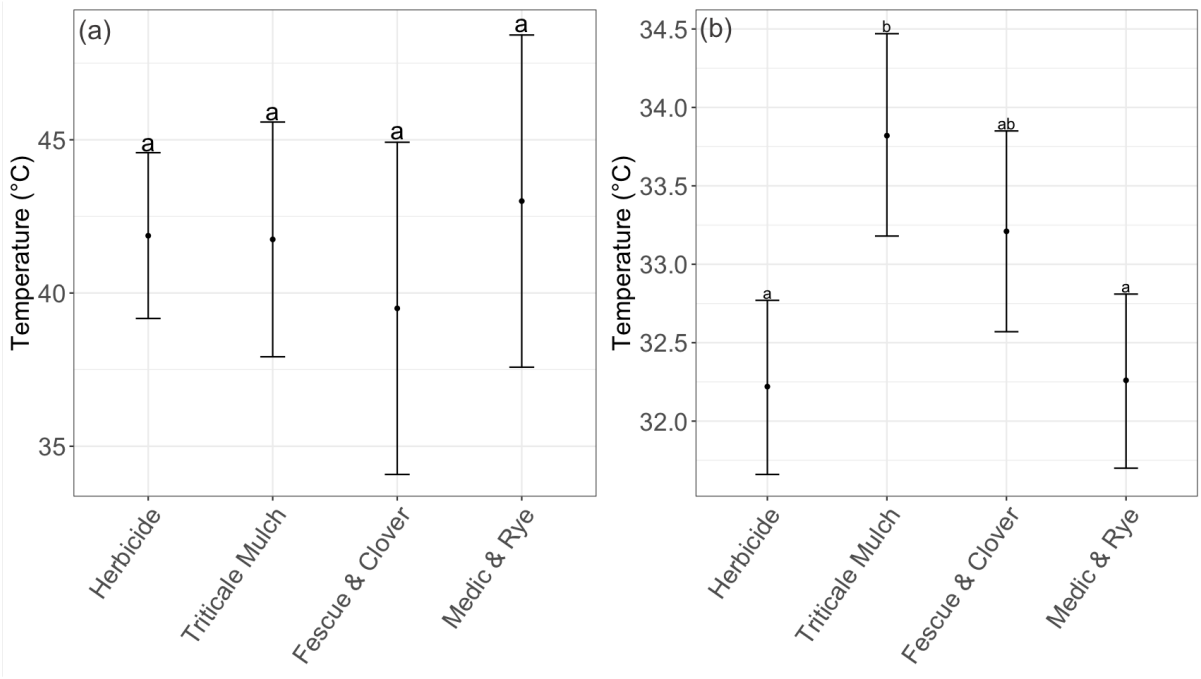


Figure 222. Vine canopy temperature measured at (a) Nuriootpa (n_{PS}=8) (10-2-2021) and (b) Langhorne Creek (n_{PS}=42) (22-1-2021) at cordon height through proximal sensing of vines subject to under vine management treatments of herbicide (bare earth), triticale mulch, fescue and clover and medic and rye. The temperature dataset presented was measured simultaneously during the UAV flights at both sites. Values represent the predicted mean based on linear mixed models. Means followed by the same letter are not sig diff at the P<0.05 level (Tukey's test).

There were no significant differences in vine canopy temperature between any of the treatments at Nuriootpa (Figure 222a). In contrast, at Langhorne Creek triticale mulch recorded the highest mean temperature of 33.94°C, which was significant in comparison to

herbicide and medic and rye at mean temperatures of 32.17°C and 32.21°C respectively (Figure 222b). There was no significant difference between triticale mulch and fescue and clover, where the latter produced a mean temperature of 33.22°C. Additionally, no significant difference was observed between herbicide and fescue and clover and medic and rye.

Remote sensing of canopy temperature at Nuriootpa and Langhorne Creek

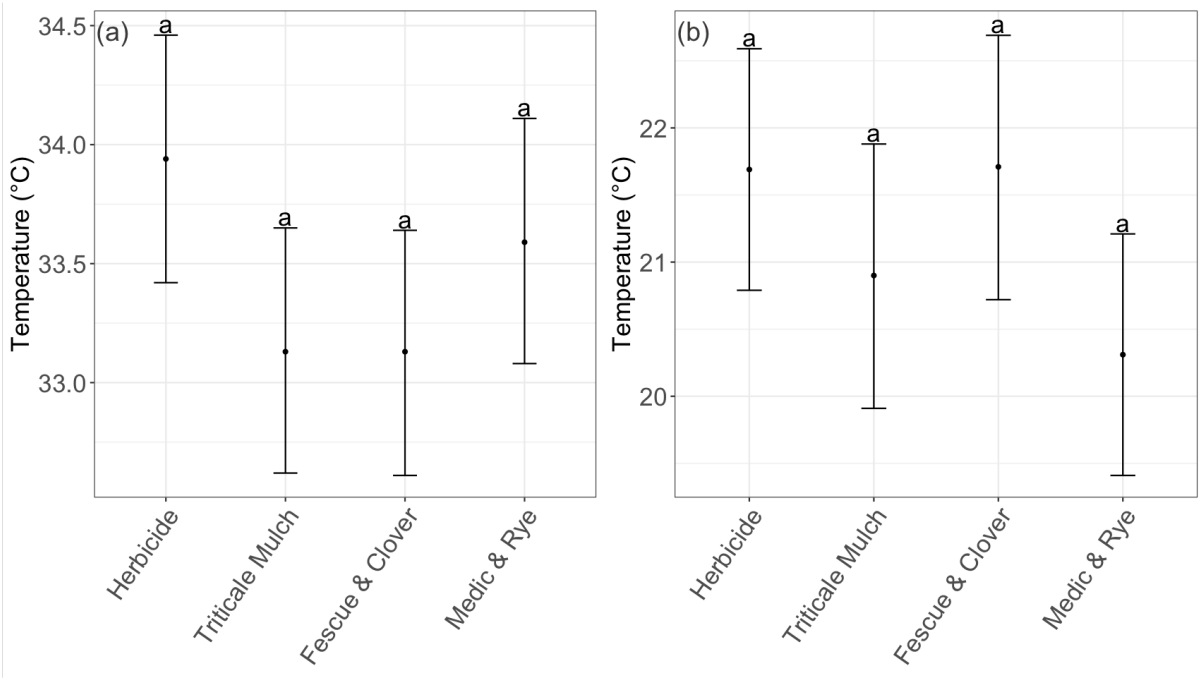


Figure 223. Vine canopy temperature measured at (a) Nuriootpa (n_{PS}=48) (10-2-2021) and (b) Langhorne Creek (n_{PS}=42) (22-1-2021) from an aerial point of view through thermal infrared imagery from remote sensing of vines subject to under vine management treatments of herbicide (bare earth), triticale mulch, fescue and clover and medic and rye. The temperature dataset presented was recorded using a UAV at both sites. Values represent the predicted mean based on linear mixed models.

Measurements of vine canopy temperature through remote sensing at Nuriootpa (Figure 223a), no significant difference was observed between the treatments. Similarly, at Langhorne Creek, no significant difference was observed between the treatments (Figure 223b).

Proximal and remote sensing comparison between western and eastern sides of Langhorne Creek

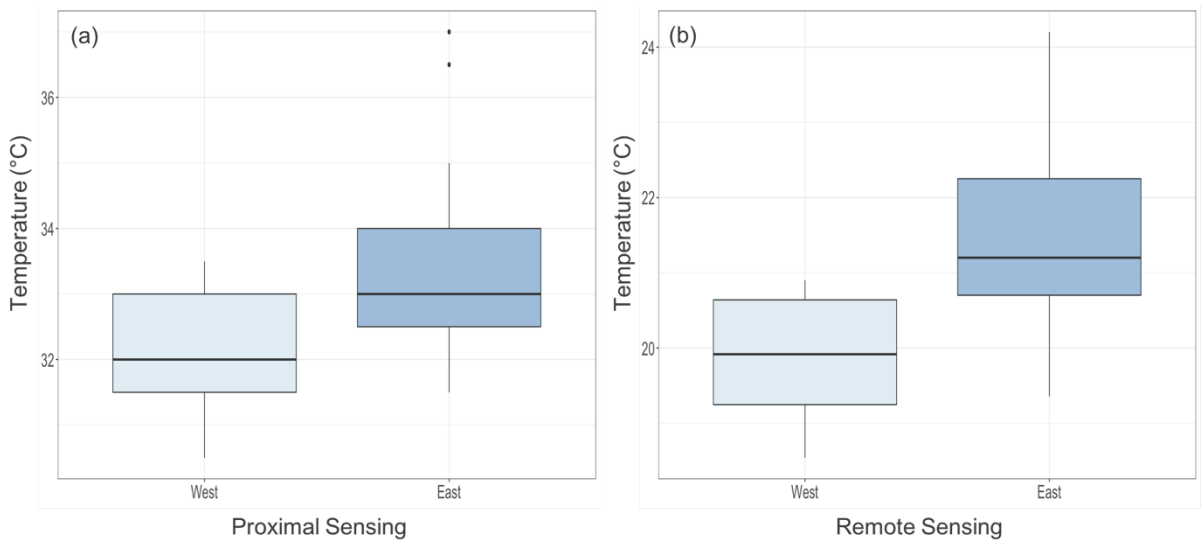


Figure 224. Vine canopy temperature measured at Langhorne Creek (22-1-2021) at (a) cordon height through proximal sensing ($p=0.0004$, $n_{West}=7$, $n_{East}=7$)) and (b) at aerial point of view through remote sensing of vines unaffected by the experiment design ($p=0.02$, $n_{West}=8$, $n_{East}=8$). All treatments located within proximity to the irrigation leak were excluded from the dataset. Values represent median, quartiles and outliers. Values were determined based on one way ANOVA (Tukey's Test).

Within Langhorne Creek the western and eastern sides of the vineyard were determined to produce a significant difference in mean canopy temperature at cordon height through proximal sensing (Figure 224a). The mean temperature was 32.07°C in the western side of the vineyard, whilst 33.50°C in the eastern side. Similarly, in remote sensing a significant difference in mean canopy temperature between the western and eastern sides of the site were observed. The mean temperature was 19.87°C in the west and 21.58°C in the east (Figure 224b).

Remote sensing NDVI dataset between both sites

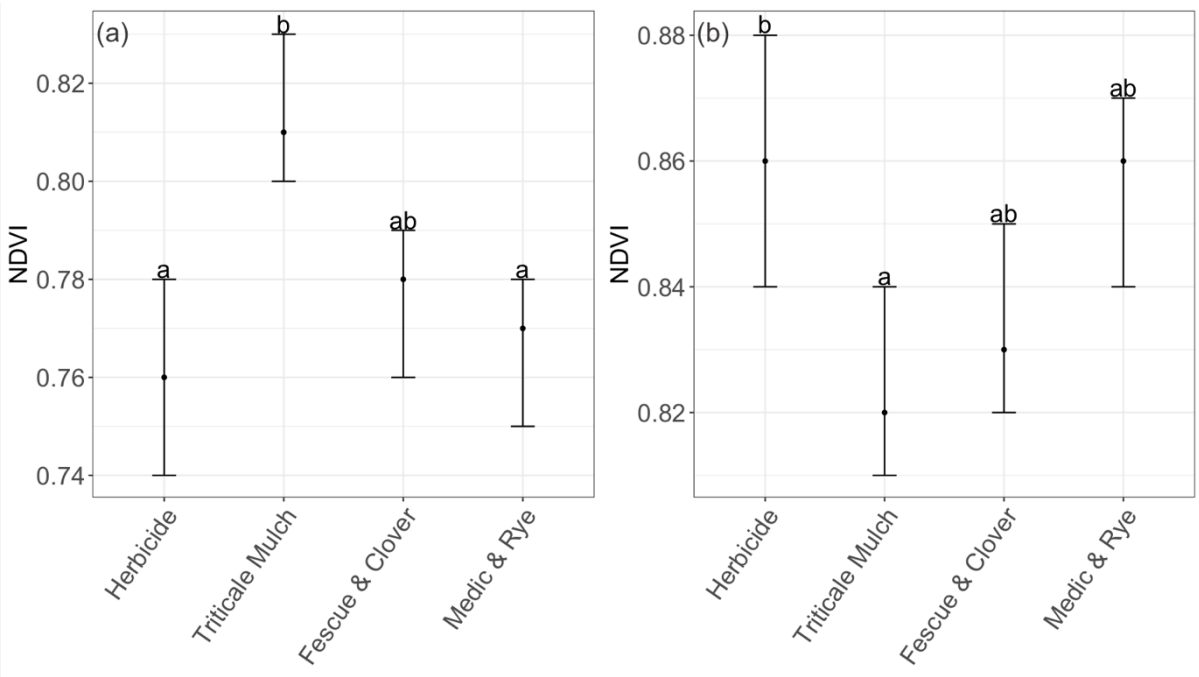


Figure 225. Vine NDVI measured at (a) Nuriootpa (n_{PS}=48) and (b) Langhorne Creek (n_{PS}=42) from an aerial point of view through remote sensing of vines subject to under vine management treatments of herbicide (bare earth), triticale mulch, fescue and clover and medic and rye. Values represent the predicted mean based on linear mixed models. Means followed by the same letter are not significantly different at the P<0.05 level (Tukey's test).

Based on the measurement of NDVI through remote sensing at Nuriootpa (Figure 225a), no significant difference was observed between herbicide and the cover crop treatments of fescue and clover and medic and rye. Triticale mulch recorded the highest NDVI at 0.81, which was significantly different to herbicide and medic and rye at NDVI of 0.76 and 0.77 respectively. No significant difference occurred between triticale mulch and fescue and clover where the latter produced an NDVI of 0.78.

At Langhorne Creek (Figure 225b), no significant difference was recorded between herbicide, fescue and clover and medic and rye. Furthermore, no significant difference was observed between triticale mulch, fescue and clover and medic and rye. However, herbicide at 0.86 was significantly different to triticale mulch at 0.82.

Discussion

Impacts of under vine treatments on canopy temperature in Nuriootpa

There was no impact of under vine treatments on canopy temperature in Nuriootpa, regardless of the measurement method (Figures 6 and 7). This suggests that all under vine management systems had an equal influence on vine canopy temperature. Optimal grapevine growth occurs between 25°C and 35°C before stress responses are induced (Hochberg et al., 2015). Temperatures above 40°C can seriously damage vine metabolic functions and stress response capacities. A study by Hochberg et al. (2015) comparing Shiraz and Cabernet Sauvignon vines found that Shiraz is more physiologically and metabolically sensitive to temperatures above 35°C, with reduced net assimilation rate, dark respiration, and water use efficiency. These responses were attributed to heat stress, where increased non-photochemical quenching suggested energy dissipation through heat. As indicated earlier, it is unlikely that vines in Nuriootpa experienced temperatures exceeding 40°C, as suggested by the proximal sensing data (Figure 5). Therefore, the similar mean canopy temperatures obtained through remote sensing (33.59°C) and weather station data (35.3°C) (Figure 5) suggest that the vines were experiencing early symptoms of water stress.

The canopy temperatures from proximal and remote sensing in Nuriootpa indicate no apparent vine water stress at the time of measurement (Figures 6 and 7). However, as mentioned earlier, early signs of stress may have been present. Thus, using temperature measurements in this context may have been ineffective for analysing the seasonal effects of under vine treatments, as real-time temperature may not represent the overall vineyard response.

Studies by Zuniga Espinoza et al. (2017) found thermal infrared imagery better suited for analysing short-term water stress effects in Cabernet Sauvignon. Differences in NDVI in Nuriootpa (Figure 9) suggest that under vine treatments had a more significant impact on the long-term grapevine water stress response. Zulini et al. (2005) observed decreased leaf pigment development with prolonged water stress in Riesling, while Zuniga Espinoza et al. (2017) found decreased GNDVI with increased water stress. GNDVI, an index of chlorophyll, increases with water and nitrogen uptake (Helman et al., 2018). NDVI also increases with these factors, making it more effective in determining the accumulated water stress response in grapevines compared to thermal data.

The herbicide treatment at Nuriootpa did not differ from fescue and clover and medic and rye in NDVI effects (Figure 9). NDVI measures the absorbance of red light and reflectance of near-infrared light in plant cells, indicating canopy "greenness." Higher NDVI values suggest greater leaf density, influenced by water and nutrient uptake. Thus, NDVI is highly useful for determining water concentration within the under vine network throughout the growing season (Drissi et al., 2009; Zuniga Espinoza et al., 2017).

Based on the similar NDVI between herbicide, fescue and clover, and medic and rye treatments, it is speculated that these treatments experienced extended water stress and responded by maintaining lower canopy density throughout the growing season (Figure 9). Bare earth soils are prone to higher evaporation rates, leading to higher soil temperatures and reduced vine water availability, thus decreasing water and nutrient uptake (Coombe & Dry, 2004; Steenwerth & Guerra, 2012; White, 2015). Consequently, vegetative growth is reduced, resulting in lower canopy density. Similarly, vines with grass under vine cover crops may have lower canopy density due to competition for water and nutrients (Steenwerth & Guerra, 2012). Vine roots must expand to greater soil depths, where subsurface water and nutrient availability is minimal due to cover crops (Guerra & Steenwerth, 2016). Consequently, low NDVI may indicate vine stress in herbicide and cover crop treatments.

Previous studies on mulch in vineyard floor management consistently show lower soil temperatures (Fourie & Freitag, 2010; Guerra & Steenwerth, 2016; Bavougian & Read, 2018). Mulch application effectively shades and reflects heat, reducing soil drying and maintaining soil moisture. By preserving vine-available water, triticale mulch allows for continued water and nutrient uptake, maintaining a higher water potential gradient and avoiding water stress. This results in a higher vine canopy density (Fourie & Freitag, 2010). This is consistent with the higher NDVI observed in the triticale mulch treatment at Nuriootpa (Figure 9).

Impacts of under vine treatments on canopy temperature at Langhorne Creek

The results for Langhorne Creek differed from those at Nuriootpa. At Langhorne Creek, no clear relationship was established between proximal and remote sensing (Figure 5). The temperature range obtained from proximal sensing appeared exaggerated (Figure 5), and no

significant differences between treatments were observed using remote sensing (Figure 7). These findings contradict the hypothesis that under vine treatments would significantly influence vineyard temperature.

Unlike the mean proximal sensing temperature of 32.88°C, the ambient air temperature of 25.8°C suggests that vines would not experience water stress, typically induced at temperatures above 35°C (Hochberg et al., 2015). This is consistent with the mean temperature obtained from remote sensing at 21.24°C (Figure 5). The lack of significant differences between treatments is likely due to water variability within the vineyard (Figure 8).

Thermal data analysis revealed that treatments in the eastern half of the vineyard produced higher canopy temperatures in proximal sensing. This finding was supported by remote sensing, where untreated vines on the eastern side recorded higher canopy temperatures than those on the western side (Figure 8). It is probable that the western side received more water due to an irrigation leak. This situation highlights the value of remote sensing, which provides an instantaneous summary of a site, allowing for specific responses to spatial variability within the vineyard.

Proximal sensing of herbicide, fescue and clover, and medic and rye treatments indicated relatively low ambient canopy temperatures across all treatments (Figure 6). Similarly, NDVI measurements for these treatments showed comparable results (Figure 9). Hunter et al. (2021) observed that Shiraz vines in South Africa with north-south oriented rows had higher sun exposure compared to east-west oriented rows, due to the sun's movement casting shadows throughout the vineyard for most of the day. When vineyard floor shading from the canopy accounts for 40% or more, soil evaporation diminishes to 10-20%, with the remaining water primarily lost through canopy transpiration (White, 2015). This effect is particularly significant with drip irrigation, leading to greater canopy development and higher transpiration rates (Iland et al., 2011).

Lower ambient canopy temperatures in the herbicide treatment likely resulted from canopy shading, reducing soil water loss through evaporation. Additionally, a lower bare earth surface albedo from shading would lead to less light reflectance into the canopy. Similarly, in the cover crop treatments, lower ambient canopy temperatures in proximal sensing likely resulted from reduced floor reflectance due to a lower albedo. With supplementary irrigation, the competitive nature of fescue and rye roots with vine roots would be minimal, allowing efficient vine water and nutrient uptake and robust canopy growth. Higher NDVI values in herbicide, fescue and clover, and medic and rye treatments likely indicate a long-term response to vineyard water availability (Zulini et al., 2005; Zuniga Espinoza et al., 2017).

Triticale mulch showed similar significance to fescue and clover and medic and rye in NDVI, but differed from herbicide (Figure 9). Triticale mulch had an NDVI comparable to the cover crops, where irrigation regulated vine water availability, rendering moisture retention from mulch unnecessary. The cover crop treatments did not compete significantly with vine roots for water and nutrients, allowing reasonable canopy uptake and vine evaporative cooling, resulting in higher leaf density in vines treated with triticale mulch and cover crops. However, triticale mulch differed significantly from herbicide in proximal sensing and NDVI, likely due to the experimental design, with triticale mulch primarily located on the eastern side of the vineyard (Figure 8). Herbicide was mainly on the western side, where greater water availability likely led to higher vine canopy density compared to triticale mulch.

Lower water availability in triticale mulch treatments may have resulted in reduced vine canopy density relative to herbicide.

Conclusion

In this study it was found that differences exist between proximal sensing of vine cordons, and remote sensing of canopy surfaces. No significant differences between under vine management methods on canopy temperature were observed in remote sensing at both sites and in proximal sensing at Nuriootpa. Through NDVI both sites suggested that canopy stress response was impacted by vine water and nutrient uptake which was influenced by the relationship between the under vine treatments and the availability of water. Implications for under vine management suggest that the selection of treatment should be decided based on climatic environment such as high rainfall or semi- arid and management practices such as irrigation and row orientation. Furthermore, implications for remote sensing in under vine management suggest that spatial variability is easily recorded, allowing for tailored vineyard management.

Future study should focus on the measurement of canopy temperature in under vine treated vines in extreme conditions, where the present study was recorded in relatively mild conditions in comparison to previous years. Furthermore, measuring canopy temperature in yield management could prove effective, where variable change in canopy stress is easily detectable.

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Appendix 7: Clarifying Carbon – information flyers



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Clarifying carbon: plants, soils, and the carbon cycle?

With so much interest in the potential to sequester carbon in vineyard soils, it is important to understand how carbon makes its way into the soil. With this knowledge, steps can be taken to maximise the amount of carbon that enters, and is retained in, the soil.

> **"The good news is, if you are growing plants, be them vines, cover crops or other vegetation, you are already in the business of capturing carbon from the atmosphere and storing it in your soil!"**

What is Carbon?

On the surface, this is a relatively simple question; Carbon is the sixth element on the Periodic Table. Perhaps a better question is: In what chemical 'forms' do we find carbon? Carbon is found in many different compounds, which are often referred to as 'forms' of carbon. An atom of Carbon, be it in a glucose molecule in a berry, or a cellulose molecule in the trunk of a vine, is still a Carbon atom. However, these two 'forms' of Carbon behave very differently in the environment. So, from a Carbon sequestration perspective, it is not only the amount of Carbon that matters, but also the 'form'. Carbon can be found in both organic and inorganic forms; here we focus on organic Carbon.

How do plants capture Carbon?

Photosynthesis is a complex, and arguably *the* most important, biological process. Using the energy of the sun, through a series of complex yet elegant biochemical reactions, plants capture CO_2 from the atmosphere, and convert it into stored energy in the form of carbohydrates. Once the Carbon is captured by plants it can then be used to power their growth and development. Likewise, it also provides a source of energy for the organisms that eat and decompose plant materials. As such, photosynthesis provides the energy for most all life on Earth, and lies at the heart of the global Carbon cycle.

Labile Carbon

- refers to forms of soil organic carbon that have rapid turnover times; that is, they can be readily oxidised, leading to release of CO2 form the soil to the atmosphere. Examples include glucose, fructose and other simple sugars.

Recalcitrant Carbon

- refers to soil organic Carbon that is resistant to decomposition. Recalcitrant carbon can take many years (even millennia) to decompose. Examples include cellulose, lignin and charcoal.

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How does carbon enter the soil?

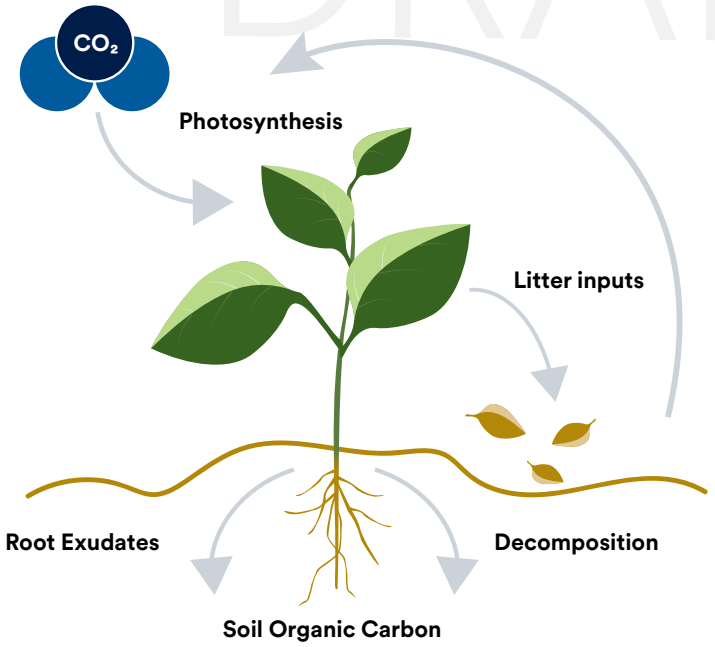
Carbon in plants can enter the soil through a variety of pathways. Plants roots exude Carbon rich compounds into the rhizosphere – that soil in contact with plant roots. Once in the rhizosphere, these exudates can be consumed by soil microbes. Plant tissues entering the soil, be it via the death and decomposition of roots, or the fall of plant litter onto the soil surface, represent another pathway for Carbon to enter the soil. Soil invertebrates play an important role in breaking plant materials into smaller fragments and moving them into the soil profile, where they can be colonised and further decomposed by microbes.



What happens to plant derived carbon in the soil?

Once in the soil, many organisms go to work on plant residues, to extract the energy they contain. This process of decomposition results in the loss of some Carbon to the atmosphere (via respiration), but it also sees Carbon being assimilated into the bodies of those organisms. As a result, Carbon can move into progressively more stable forms of Carbon that can be retained, or sequestered, in the soil. As noted above, the tougher and more recalcitrant the forms of Carbon, the longer they will be retained in the soil. Moreover, the better protected these Carbon containing materials are, for example if they are encased in soil aggregates, the less likely they are to be lost to the atmosphere.

Carbon Pathways into Soil



What can we do to sequester carbon in the soil?

To build Carbon in soil we need to:

Maximise Carbon inputs: increasing plant cover (e.g. cover crops) means more photosynthesis, and therefore, more Carbon captured from the atmosphere. Adding Carbon rich materials (e.g. compost) to the soil can also help increase soil Carbon.

Minimise soil disturbance: cultivation of the soil can expose previously protected Carbon to potential loss. It also disturbs soil biota and breaks up their delicate networks in the soil, thereby reducing their ability to help process and store Carbon.

The global Carbon cycle.

Soil may represent that largest terrestrial sink for Carbon on the planet, but this is only a small part of the Carbon story. While there are steps we can take to sequester Carbon in the soil, prevention is better than cure. That is, the potential to sequester Carbon in the soil should not be overstated, and it is important to view Carbon sequestration as part of a wider solution that seeks to minimise our Carbon footprint in other ways, such as reduced fuel use, and more efficient use of resources (e.g. fertiliser management). That said, aside from potentially helping to off-set Carbon emissions, increasing levels of Carbon in soil provides many other co-benefits, but that is a story for another day...

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Clarifying Carbon: What are Carbon Stocks?

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There is a lot of talk about sequestering carbon in vineyard soils, and for good reason. Building carbon in soil can improve soil health, help achieve carbon neutrality, and open-up opportunities to participate in carbon farming. Central to the sequestration of carbon in soils is measuring carbon stocks, which raises the question:

What are carbon stocks and how are they measured?

What is a Carbon Stock?

In simplest terms, a carbon stock is the amount of carbon within a known volume of soil, and is typically reported as tonnes of C per hectare. To calculate a carbon stock you need to know two things: the first is the concentration of carbon in a soil sample, and the second is the bulk density of the soil sample. While carbon stocks can be measured at any depth, they are typically taken from 0-30 cm soil layer where most organic carbon is found.

Carbon and soil

Soil carbon comes in many 'forms', that is, carbon containing molecules or materials. At the broadest level, soil carbon can be divided into either organic or inorganic, carbon. Inorganic carbon is typically derived from rock containing calcium carbonates, such as those in the Limestone Coast region of South Australia. Organic carbon is derived from once-living material – typically plants and microbes. While inorganic carbon is important, it is the organic carbon pool that we are trying to grow when sequestering carbon in agricultural soils.

Did you know?

A carbon credit is equivalent to one tonne of CO_2 which, because of the presence of oxygen, doesn't require one tonne of carbon to be produced.

1 tonne C makes 3.67 tonnes of CO_2.

In other words, every tonne of carbon you sequester is worth 3.67 carbon credits!



Limestone in a Coonawarra soil

Photo: Wine Australia

Soil organic carbon (SOC), soil organic matter (SOM) and total C.

The terms SOC and SOM are often used interchangeably, however, they are not exactly the same thing. Where SOC refers to the amount (or concentration) of carbon present in an organic form in the soil, SOM refers to the amount of organic matter, of which about 58% is carbon. Total C, another commonly used term, refers to the total amount of carbon, be it in an organic or inorganic form. When it comes to increasing carbon stocks, it is SOC that we are interested in.

Measuring carbon stocks

Carbon stocks are a combination of two measurements: carbon concentration and bulk density.

1. Measuring carbon concentrations requires a specific method of processing. A soil sample must be air-dried and sieved to collect a sample of soil particles less than 2 mm in diameter.
2. Once soil processing is completed, a subsample can be used to measure the carbon concentration. The most common method in use is dry combustion, which involves heating sample to very high temperatures, and measuring the CO_2 released by infrared analysis. If the soil contains inorganic carbon, this first must be removed using acid.
3. These analyses are generally conducted at a commercial laboratory, with results presented as SOC with units of %, parts per million, or mg C per kg soil. Many routine soil tests include a measure of SOC, but this is only half of the information needed to calculate a carbon stock.
4. Measuring bulk density is a simple, yet precise procedure. There are several methods, however, the most common requires the careful collection of a undisturbed soil core using a metal open-ended core of known volume. The soil sample is weighed immediately and then dried at 105°C and re-weighed (dry) to account for soil moisture.



In-tact Soil Core

Photo: Joseph Marks

The first step to growing soil carbon stocks is measuring baseline levels. Once your baseline is established, you can consider how to grow your carbon stocks, but it all starts with a good understanding and measure of soil carbon stocks.

Bulk density can then be calculated using the dry soil weight and the volume of the core, as follows:

Bulk density (g/cm^3) = dry soil weight (g) / soil volume (cm^3)

When calculating bulk density, it is important to account for the mass and volume of rocks (gravel content) within the sample core, and where soils have been compacted between measures of carbon stocks, a correction for soil compaction may also need to be considered.

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