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Agronomic tools to support a whole system approach to reducing water risk in rain dependent and supplementary irrigated viticulture



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1. Abstract

The question of vineyard water management in Australia is often associated with irrigation, but in many cooler and intermediate climate regions, rainfall will make the greater contribution to the seasonal water requirement. This project investigated the extent of management control over this rainfall derived water, with a focus on factors that influence grapevine root distribution, ground cover competition and the possibilities to improve drought resilience without increasing reliance on irrigation. Through a combination of field based studies with contrasting established vineyards in the Orange Wine Region, and water balance modelling to simulate potential drought adaptation strategies, it was shown that there is still significant scope within existing water budgets to adapt to future increases in water demand. A requirement, and topic for future research, may be the development of flexible floor management strategies to follow rainfall extremes, and approaches to encourage root exploration for a greater share of available soil water capacity when new vineyards are established.

2. Executive summary

This project examined the extent of management control over water use in rain dependent viticulture with the aim of identifying strategies to improve drought resilience of current and future vineyards. It was undertaken against a backdrop of increasing use of permanent ground cover, and a need to better understand how the benefits of ground cover can be balanced against increased competition in water limited environments. A field-based component of the project, using sites in commercially operated vineyards, addressed the question of cover crop water use traits and the impact of irrigation, rainfall and soil water competition on grapevine root distribution. This was complemented by additional field and controlled environment studies of individual cover crop species. A water balance modelling component, undertaken in collaboration with the Hochschule Geisenheim University (HGU) in Germany, then used this information to simulate soil water dynamic responses to varied ground cover management practices. By extending these simulations using longer term historical climate data, possible adaptation strategies were then compared based on the risk of exceeding soil water stress thresholds at different stages through the season.

The project built on a two year lead-in study funded through the former National Wine and Grape Industry Centre, where soil water dynamics and grapevine water status were monitored at three Shiraz vineyards in the Orange wine region through the 2019/20 and 2020/21 seasons. Soil water content was measured with a manual capacitance probe using 1.5-1.6 m deep access tubes, and plant water status with a pressure chamber to record stem water potential. No treatments were applied at the vineyards, but there was a contrast of water availability with one rain-fed site and two irrigated sites. There was also a contrast in floor management, with one vineyard having full permanent plant ground cover and two having permanent ground cover in the mid-row only with herbicide used in the undervine area. Support provided by Wine Australia through CSU 1702-9 allowed the continuation of these measurements through the 2021/22 and 2022/23 seasons. The new project also supported the upgrade of site instrumentation, and a detailed study of grapevine root distribution. This component of the work used soil coring in combination with next generation DNA sequencing to produce transect maps of root length density from the vine row to mid-row. It allowed the root depth of each plant species found in the vineyards to be determined and linked to water use characteristics of the leaves. Microbial profiling of the same root samples provided further insight into the impact of floor management, irrigation and soil depth on root function.

The first soil moisture measurements in winter 2019 showed that the profiles had been significantly depleted during the preceding seasons to at least a depth of 1.6 m, meaning that the vineyards entered the third and final season of the drought with soils at well below field capacity. The water that was available was used by late spring at two of the three vineyards, and plant water potential measurements showed that the vines were in severe water stress by the end of December. Irrigation volumes at the third vineyard site were just sufficient to avoid the severe water stress threshold, but at all three vineyards the impacts of water stress were clear with reduced shoot growth and berry weights, low yeast assimilable nitrogen and rapid increases in juice sugar concentrations. The water deficits were only relieved by late summer storms and then a return to more average autumn and winter rainfall. Three subsequent La Niña events, following the declaration of the first in late September 2020, then shifted water availability to the other extreme.

Field capacity was estimated at each site from periods of stable water content during winter of the subsequent seasons with the aim of establishing the total amount of water that could be stored within the rootzone at each vineyard. For the rain-fed vineyard, where the soil was most suited to the use of capacitance probes, the total profile sum was estimated at 250 mm. Soil shrinkage with clay layers around the access tubes at the other two vineyards prevented an accurate lower limit being

established, and therefore a full profile sum could not be determined with confidence. However, values of at least 300 mm would be expected given the soil types and root depth at these vineyards. On a volumetric basis this is equivalent to 2.5 to 3 ML/ha of plant available stored water in the profile when full, which is well beyond the capacity of most vineyards in the region to compensate for with irrigation in the event of low winter rainfall and drought.

The root transect mapping showed that grapevine roots were present to the depth of the soil moisture monitoring, but the roots of a number of ground cover species where able to match this. Perennial grasses were the dominate group at all of the vineyards, with the roots of two introduced pasture species, *Festuca arundinacea* and *Paspalum dilatatum*, and the native *Bothriochloa macra* at the warmer site, consistently found between 1.3 and 1.6 m. Roots of the perennial herb *Plantago lanceolata* could also reach this depth, and even short lived annual roots of *Avena barbata* and *Trifolium subterranean* were found to at least 1 m deep, before the grapevines reached bud-break. Most of the other species present also appeared to occupy at least the top 1 m of the profile. These findings suggest that in the case of established ground cover, the full sum of water potentially available to grapevine roots is also accessible by the other plants. As a number of the annual and perennial species identified are active months before the grapevines develop a full canopy, they can also utilise this water before the grapevine roots have sufficient transpiration-driven water demand to compete.

For lateral root exploration, it was found that grapevine roots did not occupy the full volume of soil under the mid-row. As the proportion of irrigation relative to rainfall derived water increased, grapevine roots were mostly aligned with the vine row leaving the soil under the mid-row dominated by the roots of other plants. Where water was only supplied by rainfall there were more roots in the mid-row compared to the vine row, but these were deeper in the profile. It appeared that the most extensive exploration of grapevine roots occurred with the combination of some irrigation, but then higher rainfall, to encourage exploration of the mid-row. Even then, the top soil was dominated by the roots of the ground cover species. The root length density on the 0-10 cm depth increment was over 20 cm/cm^3, providing an extremely competitive environment for vine roots, and well above the values thought to be needed to extract all the plant available water from the surrounding soil.

The possibility of planting cover crop species that use less water is often discussed, but the findings of this project suggest that this would be difficult to achieve in practice in higher rainfall regions. In general, all species that had green leaves had equal or higher rates of transpiration than grapevine leaves, and as a mixed population, could access the same soil water and for more of the season. For example, in addition to deep roots, many of the C3 annual and perennial grasses commence growth through winter and early spring and use a significant amount of the stored soil water early in the season. The C4 grasses and perennial C3 grasses then have the collective capacity to respond more rapidly to summer rainfall, or maintain green cover on the vineyard floor after the grapevine canopy has senesced in autumn. A cover crop plot trial on the Charles Sturt University Orange campus also demonstrated the challenges of trying to establish a specific species, and have it persist in the presence of a seed bank or neighbouring population of plants that are more competitive.

Some of the benefits of retaining permanent ground cover, in addition to soil physical and chemical attributes include their lower maintenance, improved trafficability in the mid-row, suppression of less desirable weeds, and reducing vigour in wetter seasons. The preliminary results of the microbial profiling from the current study also suggest the presence of ground cover causes a relative shift towards more favourable bacterial and fungal taxa. For example, the families *Bradyrhizobiaceae* and *Rhizobiaceae*, which include many symbiotic nitrogen fixing species, the family *Rhodospirillaceae* and genera *Burkholderia* and *Azospirillum* which include free-living

nitrogen fixing species that can associate with grasses. Both of the families were more abundant in roots under permanent ground cover than where herbicides were used at the trial sites, potentially providing a source of nitrogen for the vine roots below. Conversely, the fungal family *Nectriaceae*, which contains a number of root pathogens was associated with roots at all sites and all depths, but was more abundant where there were more grapevine roots. Where there was a side-by-side comparison of long term permanent mid-row ground cover with bare soil under the vine row, soil carbon was also slightly higher in the mid-row. All of these factors have the potential to enhance the long term sustainability of a vineyard, so the key question appears to be the development of agronomic practices that retain as many of the benefits of ground cover as possible while having the flexibility to move soil water balance in favour of the grapevines during dry seasons or drought.

In the first step toward developing such strategies, the HGU water balance model was successfully converted to the southern hemisphere and validated against the measured soil water data from the rain-fed trial site. Modelled data for the other two vineyards were then used to calculate the relative shares of grapevine transpiration, cover crop transpiration and soil evaporation for the study period. Simulations for the rain-fed vineyard were also run using SILO interpolated weather data back to 1889, to compare the conditions of the recent drought with historical droughts in the long term climate record. The results of the modelling show that the relative share of grapevine water use reaches about one third at the maximum canopy extent, but ranged between 14 and 20% on a whole season basis due to the earlier growth of the ground cover plants and relatively wide row spacing that allows more radiation to reach the vineyard floor than is intercepted by the vine canopy. For the simulation run back to 1889, the 2017 to 2019 drought appeared to be the only period in the historical climate record where severe soil water deficits extended for three full consecutive years.

A set of four simulations was then run from 1970, comparing contrasting percentages of vineyard ground cover, shallower cover roots and narrower row spacing with the current vineyard parameters. The impacts of these practice changes were summarised as the number of days below mild and severe water stress thresholds, which were defined as when the percentage of available soil water fell below 40% and 10% respectively. None of the simulated adaption practices would have made a difference to the severity of the 2017 to 2019 drought, but having no ground cover or 50% ground cover, reduced the average risk of severe water deficit in the bud-break to veraison period from 19% to between 4 and 5%. This is where the most impact on yield would be expected. Reducing the row spacing from 3 m to 2 m did not reduce total water use as it exchanged light interception by ground cover plants with light interception by the grapevine canopy. However, yield relative to water use would have potentially been increased by a third with the increase in planting density. Simulating a shallow cover crop root system, although potentially difficult to achieve in practice, suggested an intermediate reduction in water risk where grapevine roots do not have to compete in the whole profile for water.

For existing vineyards, the work has highlighted that the water competition from cover crops is significant, and that there may be less scope to control this through species selection or existing management practices than is generally appreciated. In a higher rainfall region like Orange, this may not be a problem in all seasons, and irrigation would usually be sufficient to buffer shorter periods of drought. However, in the event of longer term dry periods that reduce irrigation storage, ground cover plants can rapidly deplete soil water in spring that could otherwise be used by the vines. For these situations, future work could focus on methods to more effectively reduce water competition on a temporary basis. If there is a permanent decline in water availability relative to water demand with climate change, permanent adaptations may be required. This could include the approach of using mid-row ground cover in every second row or alternating mid-rows of permanent perennial

cover with a mid-row managed on annual basis according to water availability.

For future vineyards, there is more scope for adaptive changes to drought and rainfall variability, including the selection of new sites with higher water holding capacity that allows more buffering from high rainfall seasons to low, differing slope and aspect, and the ability to vary row spacing, orientation and canopy architecture to modify radiation interception. However, even for existing sites that are being replanted, there could be significant gains in storage capacity by managing the early seasons after planting to increase root depth and lateral spread. This could include the way irrigation is managed and cover crops are introduced, in the first few years following planting. The project results also suggest that if soil moisture monitoring is used, some measurements could be made away from the volume of soil wetted by irrigation, where they are not influenced by short term irrigation events that have minimal impact on total available soil water. This would track to longer term rainfall driven trends in soil water availability necessary for advanced drought planning, ideally with deep profile probes that can provide a volumetric water sum.

As a concluding point, there is still an important question as to what rainfall scenarios need to be prepared for in the future. While the 2017 to 2019 drought appeared to be the most severe on record, and tested irrigation supplies to the limit, the vines did recover in the next season. Is the priority for future research the possibility of another three-year drought or worse, a gradual long-term increase in water demand relative to water availability, or strategies that consider both? The study findings suggest that current practices could be adapted to a gradual rise in water demand without the need for more water, but possibly not for a drought that extended beyond three years without increased irrigation security. Similar to the climate risk studies undertaken with the HGU water balance model in Germany, further research could run management simulations forward in time with downscaled climate projection data. Understanding how future water demand will change compared to historic conditions could provide a clearer understanding as to how much existing practices would need to change in order to adapt to future water supply scenarios.

3. Background

The capacity to adapt to increasing rainfall variability and drought is a critical question, with Australian wine regions expected to become warmer and more arid (Remenyi *et al.*, 2019). The availability of irrigation will be a key factor in adapting to these changes, and a significant amount of research has already been undertaken on irrigation techniques, efficiency, and scheduling tools for warmer and hot climate regions. However, vineyards in cooler climate regions have received less attention regarding water despite being more immediately dependent on rainfall and lacking the buffering capacity of the dedicated irrigation schemes to carry forward significant volumes of water from high rainfall seasons. Even for vineyards with relatively secure irrigation water, the amount of water is typically not sufficient to mitigate the effects of compounding seasons of rainfall deficit. This raises a question of how much management control there is over water availability when the amounts of irrigation are small compared the larger rainfall driven trends in soil water content. Cool and intermediate climate vineyards are also more likely to have permanent ground cover, which brings additional questions about species selection and how to the manage competition for water in dry seasons. Both of these points are important if future drought risk in rain dependent viticulture is to be addressed without increasing reliance on irrigation.

Research into drought impacts on cool climate vineyards commenced prior to the start of the current project with a lead-in study in the Orange wine region that followed soil and plant water dynamics at three Shiraz vineyards through 2019/20 and 2020/21 growing seasons. This work was funded through the former National Wine and Grape Industry Centre, and was prompted by the significant rainfall deficits that had developed since the end of the last El Niño event in 2016. The three vineyards were instrumented from mid-2019 to monitor soil moisture in the vine row and mid-row with manual capacitance probe readings, and accompanied by regular stem water potential measurements, as well as growth and phenology monitoring. An initial round of soil coring was also undertaken at each site in October 2021, and molecular methods developed to allow future mapping of grapevine root distribution in cover cropped vineyards. The results of this work showed that quantifying plant available water in the whole rootzone, as opposed to just the volume of soil wetted by irrigation, may provide a much more complete understanding of the seasonal water situation. It also suggested that irrigation and floor management can have a greater impact on grapevine root distribution than commonly appreciated, and that research into over-cropping strategies would also need to look at the below ground responses to understand the longer term impacts on vineyard performance.

In the objectives proposed for the new project, the aim was to build on this lead-study and to draw on the combined experience of rain-fed and irrigated viticulture in developing strategies for more drought resilient vineyards. Firstly by giving more consideration to management of the rainfall derived water, and then understanding how limited irrigation can be used to best effect when volumes are small relative to the larger rainfall and evapotranspiration-driven changes in seasonal water balance. Where grapevines are traditionally grown without irrigation, the importance of this rainfall-evapotranspiration relationship is implicit in many viticultural practices. These include encouraging the development of more extensive root systems when vineyards are planted, low density plantings with small canopies and no ground cover in traditional dry grown regions. In more temperate regions that experience intermittent drought, floor management practices might be varied in response to seasonal water availability, or the proportion of ground cover varied to balance the benefits and risks associated with bare soil in vineyards (e.g. Griesser *et al*., 2022). As a general observation, the need to retain a range of floor management options seems to be more accepted internationally than in Australia, which in turn may be a reflection of the widespread use of irrigation.

To complement the field based studies, an additional objective of the new project was to compare the measured soil water values with the output of a European vineyard water balance model from the Hochschule Geisenheim University in Germany (*Hofmann et al.*, 2014). This model, which has been validated in the field, provides a good compromise between simpler crop coefficient approaches and more detailed functional structural models. It has sufficient complexity to account for factors such as canopy architecture and porosity, cover crop interactions, and slope and aspect, but is efficient enough to be run at a regional scale with large climate datasets (Hofmann *et al.*, 2021). Recently a server-based version has been producing a daily 10-day advanced soil water forecast for growers, as well comparing the current water situation to historical seasons[1]. This could have similar potential applications for the Australian wine industry from supporting shorter term management decisions through to assessing longer term climate risk and adaptation strategies.

Drawing together the lead-in study findings, and ideas for a combined measurement and modelling approach for responding to seasonal rainfall variability, six objectives were proposed as follows:

1. Collect a two-season dataset of vineyard soil water dynamics, grapevine transpiration and point measurements of mid-row water use from already established trial sites in the Orange region. In collaboration with HGU, use this information to test and validate their vineyard water balance model for the prediction of grapevine water requirements under Australian viticultural conditions.
2. Refine and continue the development of molecular techniques to allow the depth and relative root abundance of cover crop species and / or resident vegetation to be assessed from soil cores. At the Orange trial sites, characterise grapevine root distribution, the overlap with the rootzones of other species, and the relative share of plant available soil water.
3. Using a combination of field and pot-based experiments, determine the extent of inherent variation in water use traits of selected cover crop species and their transpiration response to the onset of water stress.
4. With the validated water balance model and parameters relating to root distribution, floor management and canopy size realistically varied, re-run historical seasons for each vineyard in progressive iterations and determine the potential effect on vine water status and soil water dynamics. Test how the measured variation in cover crop water use traits would translate differences in vineyard water use and influence the share of soil water available to grapevines through the season.
5. Based on measured winter recharge of the full rootzone, seasonal rainfall forecasts and available irrigation, propose strategies for the use of canopy and vineyard floor management practices in existing vineyards that can be used to minimise the impact of within and between season rainfall variability on vineyard productivity and fruit composition.
6. Based on improved understanding of functional water use traits of potential cover crop species, their influence on grapevine root distribution, and results from the companion project on ecosystems services, propose longer term strategies for the establishment of vineyards that have more natural water use efficiency and 'built-in' drought resilience.

The work undertaken in relation to these objectives is presented and discussed under corresponding headings from section 6.1 to 6.6. A project variation extended the final reporting date to March 2024, and added an objective to extend the molecular based root study to include microbial profiling, which has been added to the root distribution section. An additional objective was to conduct a final meeting to present the results of the water balance modelling.

[1] https://rebschutz.hs-geisenheim.de/wetterstationen/wasserhaushalt.php

4. Project Aims

Output	Target Date	Activities
Detailed operational and management plan developed to coordinate research activities between collaborating organisations and industry partners.	31/08/2021	Project planning meeting at the Orange campus of CSU with project participants, collaborators and advisors attending in person or remotely. The objective will be to familiarise the project group with the lead-in work undertaken from 2019 to 2021 and then to collectively plan the new components of the project in detail.
A replicated plot trial established to compare water use traits and establish crop coefficients of a selection of commonly used and contrasting native and introduced cover crop species.	31/10/2021	A field study established outdoors at the CSU Orange campus horticulture centre with replicated plots of selected cover crop species (approximately 2 x 2 m plots and 6 replicates). Based on initial discussions these are likely to include *Rytidosperma sp, Bothriochloa macra*, *Microlaena stipoides*, *Lobularia maritima*, *Trifolium resupinatum*, *Trifolium subterraneum*, *Festuca arundinacea*, *Plantago lancolata* and *Lolium perenne*. Except for *L. maritima* and *T. resupinatum*, which are included as a link to the companion project on ecosystem services, all other species are present at least of the three existing vineyard sites and have traits of interest for further investigation. A final decision will be made on the species selected for the cover crop plot and pot trials during the project planning meeting. Access tubes installed to 1.6 m in each plot for measurement of soil water dynamics with existing manual capacitance probe. Weather station installed in immediate proximity to record rainfall and parameters for calculating reference crop potential evapotranspiration.
Instrumentation upgrade of three existing soil moisture monitoring sites in Shiraz vineyards in the Orange region completed. Capacity to automatically record grapevine water potential and transpiration obtained. Capacity to measure cover crop evapotranspiration at key periods during the season obtained. Selected data accessible online for all project participants.	31/10/2021	Installation of six grainer type sap-flow gauges and three 'FloraPulse' stem water potential sensors at each of three trial sites for validating values predicted from vineyard water balance model. Two of existing weather stations upgraded for remote access to match current capacity of third station and linked. Adaptation of existing multiplexed CO_2 / H_2O gas exchange system as previously constructed at the NWGIC (Smith *et al.* 2019, BMC Plant Biology 19:1-15) for targeted measurements of cover evapotranspiration (photosynthesis also obtained) in the undervine or mid-row area.
Industry informed of project commencement and results from lead-in research.	31/12/2021	Industry journal article prepared describing project objectives and presenting results from the lead-in pilot study of vineyard water dynamics and root distribution at the Orange trial sites.
A pot experiment established for later dry down study to determine possible variation in isohydric v anisohydric behaviour in cover crop species.	31/12/2021	Using the same cover crop species as selected from the plot study above, establish individual plants in large pots for later dry down study. Annuals grown from seed, slower growing perennials (eg. *Bothriochloa macra*) transplanted from vineyard trial sites and allowed to re-establish in pots.
A set of root samples collected from each of the three Orange field trial sites to characterise the relative distribution of grapevine and cover crop roots. Relative	31/01/2022	Soil cores collected at three distances from vine row to mid-row to a depth of 1.6 m. Where practical with commercial operations and soil conditions, soil pits will also follow to assess structural root

share of plant available water quantified.		distribution in transect across and along the row. Roots washed from cores and scanned to determine root length density. Roots frozen for later molecular analysis for species identification, with selected roots stored individually from the deeper soil core samples.
The first season of soil water dynamics, whole vine transpiration, water status and weather data collected from three trial sites.	31/05/2022	Volumetric soil water content made fortnightly through winter and weekly through the growing season with a manual capacitance probe to 1.6 m in the vine row (4 tubes) and 1 m in the mid-row (4 tubes). Whole vine transpiration recording with automated sap-flow system together with stem water potential dynamics and weather parameters needed for water balance calculations. Supporting phenology, shoot growth, berry composition, yield, bud-fruitfulness and pruning weight data also collected. Carbohydrate and nutrient reserves at harvest and dormancy determined with rapid ATR-FTIR based method to inform later management strategy development.
A preliminary comparison of modelled versus measured soil and vineyard water use dynamics made with the first season of field data. First designs of whole vineyard water management strategies proposed.	30/06/2022	Project meeting at the Orange campus of CSU with project participants, collaborators and advisors attending in person. Separate funding would be sought to contribute to visit of HGU collaborators to Australia. Water balance model converted for use in southern hemisphere and run using measured weather data, cover crop root distribution information and starting soil water conditions at each vineyard. Capacity to modify vineyard water balance discussed in relation to practical aspects of floor management, canopy manipulations and irrigation practices. Preliminary strategies proposed for further development in season 2 of the project.

Financial Year Two

Output	Target Date	Activities
A set of root samples from contrasting vineyard systems for additional method validation and preliminary assessment of wider variation in root distribution.	31/12/2022	Targeted soil coring undertaken at selected vineyards in other NSW regions for climate and floor management contrast to the main Orange field sites.
A data set showing the presence or absence of grapevine roots at each depth increment to 1.6 m from the vine row to the mid-row. A qualitative assessment of relative abundance of grapevine DNA relative to the abundance of other individual root species DNA obtained. Relative share of the rootzone defined and streamlined method for future root distribution surveys proposed.	31/01/2023	DNA extractions performed on all collected root samples from the soil coring, and sample library stored at -78 °C for ongoing analysis. Presence of absence of *Vitis vinifera* roots confirmed with species specific PCR and / or qPCR primers developed at the NWGIC. Selected samples from upper rootzone (0 to 80 cm) sent for next generation sequencing for assessment of relative proportion of grapevine and cover crop roots, and selected individual root samples from lower root-zone (80 to 160 cm) to identify depth limit of cover crop water competition.
A data set showing the stomatal conductance response of cover crop species to water stress and characterised in relation to isohydric or anisohydric like behaviour.	31/03/2023	Using the cover crop species established in pots in the previous season undertake dry-down studies to assess the stomatal response to the progressive onset of water stress. Determine relationship between stomatal conductance as measured with a porometer or gas exchange system and fraction of transpirable soil water as measurement gravimetrically. Some targeted analysis of other

		physiological parameters such as leaf water potential, ABA, 13C, or other leaf metabolites to support publication of work in higher impact scientific journal.
A data set showing the seasonal dynamics of crop coefficients of a selection of contrasting cover crop species.	31/05/2023	Volumetric soil water content measured at approximately weekly intervals from establishment to establish crop coefficients of cover crop species in the replicated plot study. Soil coring undertaken in Oct / Nov 2022 to determine root length density and distribution down the profile.
The second season of soil water dynamics, whole vine transpiration, water status and weather data collected from three trial sites.	31/05/2023	Volumetric soil water content made fortnightly through winter and weekly through the growing season with a manual capacitance probe to 1.6 m in the vine row (4 tubes) and 1 m in the mid-row (4 tubes). Whole vine transpiration recording with automated sap-flow system together with stem water potential dynamics and weather parameters needed for water balance calculations. Supporting phenology, shoot growth, berry composition, yield, bud-fruitfulness and pruning weight data also collected. Carbohydrate and nutrient reserves at harvest and dormancy determined with existing rapid ATR-FTIR based method to inform later management strategy development.
Final management strategies proposed for responding to different seasonal water supply scenarios based on known winter recharge of the full-rootzone, three- and six-month rainfall projections and likely irrigation availability.	30/06/2023	Water balance model and output in the form of predicted daily vine water use, cover crop water use, evaporation, and soil water dynamics compared with measured values. With the measured soil water dynamics from the two seasons of lead-in work and then two seasons of newly collected data as a reference, run simulations with the real weather and irrigation data to test the impact on seasonal vine water status of varying other manageable factors. Test capacity to more deliberate direct soil moisture through desired windows at key reproductive development stages to maximise fruit quality and minimise impacts of water stress on yield in the following season.
Combined project achievements and industry implications disseminated via final report to Wine Australia, industry journal article and peer reviewed Publications.	30/06/2023	Project findings compiled, final report completed, and detailed industry article published. Joint scientific publication prepared describing water balance model validation, the results of the soil coring and molecular root ID study and the cover crop plot study.

Financial Year Three – contract variation

Output	Target Date	Activities
Final management strategies proposed for responding to different seasonal water supply scenarios based on known winter recharge of the full rootzone, three- and six month rainfall projections and likely irrigation availability. Combined project achievements and industry implications disseminated via	31/03/2024	Water balance model and output in the form of predicted daily vine water use, cover crop water use, evaporation, and soil water dynamics compared with measured values. With the measured soil water dynamics from the two seasons of lead-in work and then two seasons of newly collected data as a reference, run simulations with the real weather and irrigation data to test the impact on seasonal vine water status of varying other manageable factors. Test capacity to more deliberate direct soil moisture through desired windows at key reproductive

final report to Wine Australia, industry journal article and peer reviewed publications.		development stages to maximise fruit quality and minimise impacts of water stress on yield in the following season. Project findings compiled, final report completed, and detailed industry article published. Joint scientific publication prepared describing water balance model validation, the results of the soil coring and molecular root ID study and the cover crop plot study.
Data that provides insight to the functional role of vine and cover crop roots in relation to competition for water.	31/03/2024	Contract microbial profiling of DNA samples extracted from roots found in soil cores described.
Final Report	31/03/2024	Preparation of Final Report.

5. Materials and Methods

5.1 Field monitoring sites

5.1.1 Vineyard location and management overview

Water use and weather monitoring sites were established within blocks of mature own-rooted Shiraz at See Saw Wines Balmoral Vineyard (33°16'12.84"S, 148°59'54.66"E), Ross Hill Wines Ross Hill Vineyard (33°13'45.19"S, 149° 2'40.84"E), and Angullong Wines Angullong vineyard (33°32'48.48"S, 148°54'7.45"E) in mid-2019. These are subsequently referred to as Sites BA, RH and AN. The monitoring sites were located approximately 50 m into each block and spanned a section of 12 adjacent vines in one row where all subsequent soil and plant-based measurements were undertaken. No changes were made to the commercial management practices. The vineyards represented three of the four main soil types in the region, as well as a contrast in temperature, rainfall and evapotranspiration associated with the difference in altitude (Table 1).

Table 1: Brief summary of trial site soil and climatic conditions.

		Soil details – (0-30 cm, row and mid-row average)						Climate		
Site	Altitude (m)	Soil type	pH(H_2O)	EC 1:5 µS/cm	CEC mmol/kg	Colwell P mg/g	TN %	Rain (mm)	ETo (mm)	GST (°C)
BA	880	Ferrosol	6.85	51.4	6.1	7.5	0.134	900	1118	16.5
RH	755	Dermosol	6.23	67.5	7.8	21.4	0.130	866	1147	17.1
AN	610	Vertosol	6.41	85.4	24	13.9	0.132	750	1213	18.5

Site BA is organically managed and certified, and has a permanent mid-row and undervine ground cover. Site RH and AN also both have permanent mid-row ground cover, but are conventionally managed with herbicide used in the undervine area approximately twice per season. At all vineyards the vines were spur pruned, cordon trained and planted at a vine and row spacing of 2 m and 3 m respectively. Site BA and RH have a VSP trellis with rows running approximately north-south. At Site AN, the rows run approximately east-west and the canopy is lifted on the southern side and allowed to sprawl on the northern side. Site BA has irrigation installed but the block was not irrigated during the four years of this study and has had minimal irrigation historically. At the RH and AN sites, both are drip irrigated, with RH irrigating with a combination of dam and bore water with an approximate water availability of 0.2 to 0.5 ML/ha depending on season. The Site AN vineyard is irrigated with a combination of high security water originating from the Carcoar dam, and supplemented by surface dams on the property and bore water with a total water availability of approximately 1 to 1.5 ML/ha.



Figure 1. From left to right. Site BA, RH and AN in mid-summer 2021.

5.1.2 Weather stations

In Spring 2020 a weather station was installed at the end of the instrumentation row at Sites BA and RH, and about 60 m to the south-west at Site AN. These stations consisted of a Campbell Scientific

CR1000 datalogger with a tipping bucket rain gauge, a screened air temperature and relative humidity sensor (Campbell Scientific EE181), a digital thermopile pyranometer (Campbell Scientific CS320) and an anemometer (Synchrotac 710-1980). A mechanical pressure switch (Honeywell 5000 series 76056) was connected to the drip line in late 2021 to record irrigation events during the 2021/22 and 2022/23 seasons at Site RH and AN.

5.1.3 Soil water monitoring

Through winter and early Spring in 2019, four access tubes for manual volumetric soil water content measurements with a capacitance probe (Diviner 2000, Sentek Technologies, Stepney, SA) were installed at approximately equal spacing along the section of 12 vines to be used for the plant based measurements. These were installed manually using a metal cutting edge on the end of the access tube to a target depth of ~ 1.8 m to allow reading to the full 1.6 m of the capacitance probe after installation of the sealing bung. Some tubes could only be installed for 1.5 m readings due to the hard soil conditions at depth. The tubes were positioned slightly outside the line of the cordon, with two on one side of the row and two on the other side. None were positioned directly under a dripper, but two of the four tubes at each of the irrigated sites were partially in the wetted soil volume. At Site BA, tubes were also installed in Spring 2019 to a depth of 1 m in the mid-row adjacent to each of the deeper vine row tubes, with two tubes down one mid-row on one side of the 12 vines and two tubes down the mid-row on the other side. Mid-row tubes positioned in the same way were added at Site RH and AN in winter 2020. From installation in 2019 through to the end of June 2023, the vine row tubes were read at approximately weekly intervals during the four growing seasons and two to four weekly intervals through later autumn and winter. The default diviner calibration equation was used at all sites and the reference point set to make the first reading at the centre of the 0-10 cm depth increment, with subsequent measurements every 10 cm from that point.



Figure 2. Installation of access tubes for Sentek Diviner readings in winter 2019.

In spring 2021, as part of the instrumentation upgrade for the new project, a second logger station was installed within the row with the manual access tubes and an automated 80 cm capacitance probe (EP100G-08, Entelechy Pty Ltd, Golden Grove, SA) was installed directly under a dripper at each site. The combination of measurements then gave a representation of the larger rootzone with the deeper manual access tube readings, the high frequency measurements in the irrigated soil volume (where irrigation was used), and then inputs from irrigation or rainfall from the weather station records.

5.1.4 Plant water status monitoring and δ13C

Stem water potential readings were made at each site at approximately two weekly intervals at each vineyard for the four seasons spanned by the study. These commenced around flowering and were made mid-afternoon (~14:00-15:00 hrs) on six exposed mature leaves selected randomly along the 12

vines which had been enclosed in opaque reflective foil bags for approximately 45 minutes before reading. Pre-dawn leaf water potential measurements were made on a few occasions to provide later comparison with predictions from the water balance model. For automated measurement of water potential, two microtensiometers (FloraPulse, Davis, CA) on one of the 12 vines at each site and connected to the same CR1000 data logger used for the automated soil moisture probe.

Just before pruning each winter, the basal two nodes of one cane from each of the 12 monitoring vines were collected, and analysis replicates formed by bulking the samples from alternating vines. These were oven dried at 60 ^{o}C and ground to 0.12 mm and δ13C isotopic analysis performed on Elementar Isoprime mass spectrometer at the ANU Stable Isotope Laboratory in Canberra. Additional analysis on the same samples was also undertaken to determine total N concentrations.

5.1.5 Whole vine water use

In late spring 2021, Granier-type sap flow sensors (PS-GP3.5, PlantSensors, Darwin, NT) were installed on six of the 12 monitoring vines at each vineyard. These were run from a constant current power supply (PS-PS12) from the same manufacturer, which was in turn powered by a combination of a 100 AMPH deep cycle battery and two 40 W solar panels mounted at the end of the measurement row. The full length of the trunk was wrapped in 10 mm thick aluminium foil-faced polyethylene insulation, and the probe voltage differential recorded at 15 minute intervals from the same logger as the soil probe and water potential sensors. Standard calculations for Granier-type sensors were used to convert these to sap-flow rates and daily water use sums.



Figure 3. Field instrumentation for water use and environmental monitoring.

5.1.6 Phenology, canopy growth, berry development and yield

Phenology assessments were made at every field visit with modified E-L stages recorded for 20 random buds or shoots across the 12 measurement vines. The shoot length of 20 random shoots was also recorded at each visit until the first trimming in early summer. At intervals through the season the approximate width and height of the canopy, as well as visual light porosity estimate were recorded to provide an input for the canopy light interception calculations in the HGU water balance model. Photos were also taken at each visit for later descriptions of mid-row activity and timing of mowing or other management passes. Berry diameter was measured on a random 20 berries from shortly after fruit-set through to harvest, and 50 berry samples collected from approximately E-L 31 at two weekly intervals until harvest. Two additional 50 berry samples were collected from the corresponding vine in the two neighbouring rows to provide replicates for subsequent juice analysis. These were weighed fresh on the day of collection before being frozen for later analysis. After thawing, the samples were hand pressed and soluble solids recorded with a digital refractometer. Alpha-amino nitrogen (NOPA), ammonia, tartaric and malic acid concentrations were determined enzymatically using a Thermo Scientific Arena analyser. The berry skins were separated, blotted to absorb free juice and frozen and ground at -80 ^{o}C for later colour analysis, although this analysis is still in progress.

5.2 Mapping grapevine and cover crop root distribution

5.2.1 Plant reference collection

Commencing with the lead-in study in 2020 and continuing into 2021, individual plants representing the main perennial species present across the vineyard floor of the three monitoring sites were dug up with roots intact then potted and transferred to a glasshouse. Annual species were grown from seeds collected from the vineyard. These plants provided reference samples for the subsequent development of DNA-based root identification methodology. For the vineyard plants that could not be readily identified, intact specimens were collected at flowering, pressed and dried, and sent for identification at the Australian National Herbarium. Some plants remain to be confirmed to species level with herbarium specimens at the time of writing this report, and are listed based on results from DNA sequencing. The collection consisted of 30 different species, including *Vitis vinifera*, and additional individual plants where the same species was collected from more than one site.

5.2.2 DNA sequencing for plant species identification.

Method development work undertaken in the lead-in study determined that the primer pair ITS-An5(18S Forward–CCTTATCATTTAGAGGAAGGAG) and ITS-An4 (26S Reverse–CCGCTTATTGATATGCTTAAA) developed by Cheng *et al.* (2016) were suitable to differentiate the plant species in these vineyards by Sanger sequencing. This primer pair was designed to amplify the ITS1 and ITS2 region of the angiosperm rDNA with expected PCR product of ~ 700 bp. DNA was extracted from individual fresh leaf samples from the reference plant collection using the DNeasy Plant Pro Kit (Qiagen, Germany) following the manufacturer's protocol, and used for PCR with the above primers and protocol described by Cheng *et al.* (2016). All PCR products were sent to the Australian Genome Research Facility (AGRF) for two-way Sanger sequencing using the ITS-An5 and ITS-An4 primers. All DNA sequences generated were compared with the sequences from the GenBank database using Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequencing had a two-fold purpose of providing a library of reference sequences and complementing the herbarium identification to provide an accurate picture of the plant species present in each vineyard.

5.2.3 Soil coring and root sample collection

In summer 2022, transect soil coring was undertaken at Site AN on 13 January, at Site RH on 1 February and at Site AN on 7 February. Soil cores were collected to a depth of 1.6 m directly under the cordon and then at 50 cm intervals out to the centre of the mid-row providing a transect of four cores. This transect was replicated in four locations surrounding, but not directly within, the area of 12 monitoring vines to avoid interference with the soil moisture readings. Where the full depth of 1.6 m was reached, these soil cores were divided into nine depth increments of 0-10, 10-20, 20-30, 30-40, 40-60, 60-80, 80-100, 100-130 and 130-160 cm. Within the lowest depth increment of each core, the visual presence or absence of roots was also recorded at a higher resolution of 10 cm so that the lowest depth of roots at each vineyard could be summarized.

The first 100 cm depth was cored with a 47 mm diameter tube with a 43 mm diameter cutting tip, and then continuing the same hole after the wider core was removed, from 100 to 160 cm depth with a 35 mm diameter tube and 32 mm diameter cutting tip. These samples were stored in a cool room at 4 ^{o}C and within the seven day period following coring at each site, the roots were manually washed from each sample through a combination of 2 mm and 1 mm sieves after soaking and dispersing the soil in buckets of water. As each set of nine root samples from each core was completed, the roots from each were floated in clear plastic trays and scanned at 400 DPI for preliminary image analysis and determination of root length density (WinRHIZO 2022, Regent Instruments INC. Canada). The roots were then transferred to 5 mL plastic vials and frozen at -20 ^{o}C before being freeze dried and ground to a fine powder using a Tissue Lyser (Qiagen, USA) and stored at -80 ^{o}C. Any structural grapevine roots were removed after scanning so the subsequent molecular analysis could be

undertaken on the fine root fraction. The weight of the fine root fraction was determined at grinding, and the structural roots after oven drying to estimate total root biomass.



Figure 4. Transect soil coring at Angullong vineyard, 13 January 2022.

5.2.4 DNA sequencing for plant species identification

Root species identification was undertaken for the samples collected at all depth increments from the vine row and centre of mid-row positions from each transect with Next Generation Sequencing (NGS). This represented half of the samples collected, with the aim to provide the maximum contrast between the varying undervine management practices and the permanent mid-row ground cover at each vineyard. For the NGS, the ITS-An3 (5.8S Forward -YGACTCTCGGCAACGGATA) from Cheng *et al.* (2016) was paired with the ITS-An4 reverse primer used for the Sanger sequencing to generate an expected PCR product of 400 bp. DNA was extracted from sub-samples of homogenised freeze-dried roots (10-50 mg) using the DNeasy Plant Pro kit following the manufacturer's protocol. All root DNA samples were quantified by fluorometry (Quantus, Promega, USA) and quality-tested by PCR using the ITS-An3 and ITS-An4 primers prior to submission for NGS. These were sent to the Australian Genomics Research Facility (AGRF) Melbourne Profiling Service for customised NGS on the Illumina MiSeq platform and subsequent bioinformatics.

5.2.5 Root microbial profiling

ITS and 16S microbial profiling was undertaken by NGS on a subset of the same root DNA extracts used for the plant species identification for the purpose of providing further functional insight into the roots under contrasting floor management practices, irrigation, soil type and soil depth. The increments chosen were the 10-20, 30-40, 60-80 and 100-130 cm for both the vine row and mid-row, avoiding the top 10 cm increment which contained some non-living plant material debris. Where sufficient DNA remained of the original extracts, the vine row 130-160 cm depth increment was also included for analysis. In most cases there was sufficient DNA for all four replicates of the paired row vs. mid-row comparison, and two to four replicates of the 130-160 cm samples for the vine row. These were sent to AGRF (Brisbane laboratory) for analysis on the Illumina NextSeq 2000 Sequencing System and bioinformatics. As these samples had been washed by hand with low pressure water to remove soil, but not sterilised, the results represent a combined measure of rhizoplane and endosphere microbial diversity. All PCR amplifications and high throughput sequencing were performed by AGRF, with V3-V4 primers for bacterial 16S rDNA (forward – CCTAYGGGRBGCASCAG, reverse

GGACTACNNGGGTATCTAAT), and ITS1F-ITS2 for fungal ITS (forward- CTTGGTCATTTAGAGGAAGTAA, reverse GCTGCGTTCTTCATCGATGC).

After high throughput sequencing, downstream bioinformatics analysis was performed by AGRF. The paired end reads were processed using AGRF's divpro pipeline, which includes quality control, filtering, trimming, denoising and taxomic classification on Amplicon Sequence Variants (ASV). Further processing using Cutadapt (Martin 2011). The processed data were then imported into QIIME2 (Bolyen *et al.* 2016) for taxonomic classification of each ASV using SILVA and Greengenes for 16S and UNITE for ITS.

5.3 Site soil characterisation

A final round of soil coring was undertaken in late May 2023 at each site to collect two sets of samples to assist with further site characterisation. For the first set of samples, four replicates of paired cores from directly under the cordon and the centre of the mid-row were taken with 47 mm diameter tube with a 43 mm diameter cutting tip. These were collected to a depth of 30 cm and analysed for total soil carbon, total N, total P (Colwell), pH and electrical conductivity. Prior to analysis these were oven dried to allow calculation of bulk density.

For the second set of samples, three soil cores were collected along the vine row at each vineyard to a depth of 1.6 m using a 35 mm diameter tube and 32 mm diameter cutting tip. The cores were divided into 10 cm depth increments to match the profile readings made by the manual soil moisture probe. The weight of each increment of the three replicates was recorded before and after drying to determine volumetric water content at the time of sampling, and bulk density. Although not undertaken at the time of writing this report, the intent is to undertake particle size analysis for each depth for later calculation of water holding capacity using pedotransfer functions.

5.4 Water use traits of ground cover plant species

5.4.1 Replicated cover crop plot trial

A cover crop trial was marked out in spring 2021 at the CSU campus in Orange with six replicates of 10 plots in a randomised block design. These were cultivated mechanically and then again manually prior to planting. Each plot was 2 x 2 m, with 0.5 m separating plots within rows and 1 m between replicate rows. At the centre of each plot a 1.2 m deep access tube was installed for manual soil moisture readings with the same capacitance probe used for the field sites.



Figure 5. Establishment and planting of the cover crop trial at the CSU campus in Orange in spring 2021, and view of trial in February 2022.

Through spring and early summer, seven different plant species were established from a combination of seeding or transfer and division of mature plants from one of the collaborating vineyards. The seeded plants were *Microlaena stipoides* (Burra weeping grass) and *Rytidosperma caespitosum* (Evans Wallaby grass) which were first growing in seedling trays before being transplanted a density of 100 plants/m^2, and *Lobularia maritima* spp. *Benthamii*, the fescue and Italian ryegrass hybrid *Festulolium braunii* (cv. Perun) and *Plantago lanceolata* (cv. Ranger) which were sown directly into the cultivated plots. The Perun and Ranger seeds were supplied free of charge from the S&W Seed Company and were sown at the upper seeding rate recommended for high rainfall locations. The transplanted species were *Paspalum dilatatium* and *Bothriochloa macra,* which were planted at a density of 25 plants/m^2. An additional treatment was kept mowed to ground level as a comparison and the two remaining sets of six plots were not used.

Soil moisture readings commenced in late spring 2022 and continued into early autumn in 2023. Crop coefficients for this period were calculated used FAO56 ET_o gridded data from SILO (Jeffrey *et al.*, 2001), and rainfall recorded with a manual rain gauge at the trial site.

5.4.2 Replicated plant species pot trials

To characterise individual plant water use traits through dry-down comparisons, a replicated pot trial was established in spring 2022 with eight different plant selections (Table 2). These were either grown from seed planted 12 months previously in a glasshouse in Orange or dug up from the vineyard trial sites. Both groups of plants transferred to a glasshouse on the CSU Wagga Wagga campus where they replanted in 10 L pots made from 115 mm diameter x 600 mm lengths of PVC pipe filled with a commercial potting mix blend (Australian Native Landscapes, Orange, NSW). The plants were allowed to grow for several months with non-limiting water and nutrition to reach a size consistent with mature plants at the vineyard sites and/or the replicated plot trial.

Table 2. Summary of glasshouse dry-down experiments

Species	Source	Comment
Experiment 1	January 10 to January 31 2023.	
Microlaena stipoides	Seed cv Burra	Native C3 grass, small population at one trial site
Trifolium subterraneum	Seed cv Dalsa	Introduced legume, present at all vineyards
Plantago lanceolata	Seed cv Ranger	Commercial cultivar of common vineyard species
Festulolium braunii	Seed cv Perun	Introduced C3 grass for comparison
Experiment 2	February 3 to February 20 2023	
Plantago lanceolata	vineyard	Introduced biennial herb, naturalised in NSW
Paspalum dilatatum	vineyard	Introduced C4 pasture species, present at all sites
Bothriochloa macra	vineyard	Native C4 grass, found at all vineyard trial sites
Festuca arundinacea	vineyard	Introduced C3, mid-row species at two trial sites

The first dry-down experiment started with the seed-grown group of plants on 10 January 2023, after freely drained weight (i.e. pot weight at soil field capacity) of each pot had been established during the preceding three days. The pots were weighed 24 hours after the start of the experiment, and the control treatment only watered back to field capacity. Coarse gravel was used to cover the top of the soil to reduce evaporation losses. The daily measurements and re-watering of the controls were continued until there was no weight change evident for the same species in the non-watered treatment. This required more time for the species with lower leaf area. The same procedure was then repeated for the second experiment, which started on 3 February 2023.

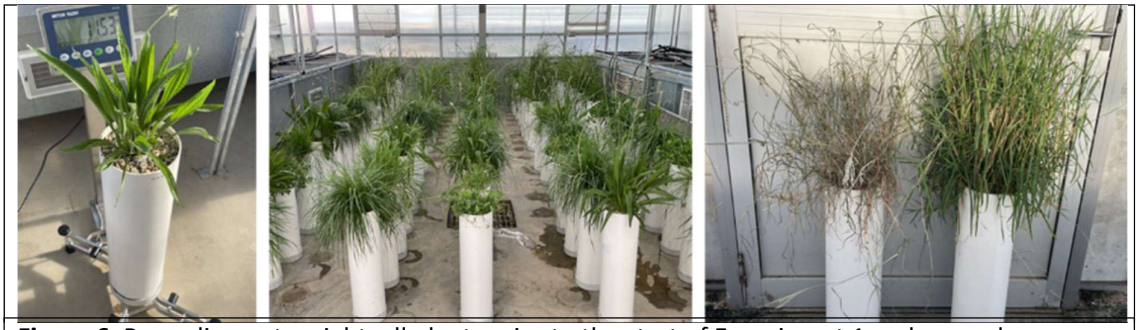


Figure 6. Recording pot weight, all plants prior to the start of Experiment 1 and example comparison of *Bothriochloa macra* plants at the end of the dry-down period in Experiment 2.

At the end of each experiment, all of the plants were destructively harvested to measure shoot dry biomass. An estimate of total leaf area was made from the weight of a leaf sub-sample that was scanned with a leaf area meter prior to drying. With available resources it was not possible to wash all the soil from the pots to record root biomass. However, visual observations of root growth were made after sliding the plants out of the pots, and a sub-sample of roots were taken for subsequent image analysis using the same method as for the field based soil coring.

5.4.3. Stomatal conductance and leaf gas exchange survey of vineyard

During the 2022/23 season, surveys of leaf-level water use traits of mid-row and undervine plant species were undertaken at the three vineyard trial sites, as an alternative to gas exchange chambers originally planned for the project. One set of measurements was made at each vineyard at the end of November, and then two more through summer in December and February. Stomatal conductance measurements were made using an LI-600 porometer (LiCor Biosciences, Lincoln, NE, USA) on any plant species with sufficiently wide leaves, and with paired measurement of the abaxial and adaxial surface. Measurements of grapevine leaf stomata conductance were also made at the same time. Where leaf width allowed and plant height from the ground allowed, additional sets of gas exchange measurements were also made with an Li-6400XT portable photosynthesis system (LiCor Biosciences).



Figure 7 The LI-600 porometer being used for rapid survey of stomatal conductance of ground cover species (left), and the Li-6400XT system for more detailed assessment of leaf gas exchange traits.

5.5 Water balance modelling

The modelling component of the project was undertaken using the water balance model described by Hofmann *et al*. (2014), which was validated under production conditions similar to those of the Orange region and can simulate seasonal water use and partitioning from the scale of single vineyards to entire regions (Hofmann *et al*., 2021). The initial objective was the adaptation of the model to run

southern hemisphere simulations, and then to validate the model by comparison with the measured plant and soil data from the Orange trial sites. The second objective was to test how variations in vineyard management practice or vineyard design change vineyard water use, and the share of water use between the vines, ground cover plant species and evaporation. In the shorter term for the benefit of existing vineyards, but also for the assessment of water risk under future climate scenarios

The model runs on a daily time step, with temperature, humidity, wind speed, solar radiation and rainfall. Water inputs from irrigation can also be included, which in the current version of the model are added as equivalent rainfall. Starting parameters that can also be varied are the amount of plant available soil water, row spacing and orientation, canopy architecture and density, and the relative share of cover crop or bare soil on the vineyard floor. Root competition for water between the vine and ground cover plants and bare soil can also be approximated by varying the relative share of available soil water. Reference crop evapotranspiration is partitioned between the grapevine canopy, ground cover plants and bare soil on the vineyard floor by a radiation interception model that calculates absorption, transmission and scatter of photons from statistical relationships determined by numerical ray tracing simulations (Monte Carlo method). Feedback of soil water content on the water use of both vines and ground cover plants is incorporated in the model with a correction factor that progressively reduces transpiration if the soil dries past 40% of the total available water. This approximates the change from readily to deficit available water where stomatal conductance begins to reduce to conserve water.

For the adaption of the model to the southern hemisphere, the elements of the radiation model components calculating the position of the sun with regard to the slope and aspect of the vineyard were modified and checked. The model was also modified for a half year shift of the vegetation period (calculation of growing degree days, development of cover crops and other parts), and were adapted to run with SILO weather data. For the modelling work undertaken to this point, the simulations have mainly focussed on Site BA with runs for both the current vineyard and hypothetical changes to row spacing, percentage of ground cover and root depth of the ground cover plants (Table 3). With a VSP canopy and a history of minimal irrigation, it is similar to vineyards the model was originally developed for, and allowed a focus on checking output for the components of the model that had been converted for the southern hemisphere. As discussed in the following sections, the soil type allowed the most accurate assessment of available soil water of the three sites with the capacitance probe. Simulations for Site RH and AN were run for the current vineyard parameters.

Table 3: Summary of water balance model simulations from 1970 to December 2023 for Site BA

Simulation	Description
100% ground cover	Model run with current vineyard parameters of 100% plant ground cover, 3 m row spacing, 250 mm of plant available water in the rootzone and roots of the ground cover plants matching the depth of the grapevines (represented as sharing the full amount of soil water).
50% ground cover	Current vineyard parameters but with 50% plant ground cover simulating alternate rows of bare ground.
Bare ground	Current vineyard parameters but with 0% ground cover, simulating bare ground at evaporation only component of water loss from the vineyard floor.
2 m row spacing	Current vineyard parameters with full ground cover, but with row spacing reduced from 3 m to 2 m.
Shallow cover crop roots	Current vineyard parameters, but shallower rooting ground cover plants represented by reducing the share of available soil water to 150 mm.

6. Results and discussion

6.1 Vineyard water use and model validation data

6.1.1 Study period climatic conditions

Climatic conditions during the four seasons of the study spanned severe drought in the 2019/20 season and then three consecutive La Niña events in the 2020/21, 2021/22 and 2022/23 seasons. The study was also preceded by significant rainfall deficits in 2017/18 and 2018/19 (Figure 1). This meant that irrigation availability and soil water were already very limited as the 2019/20 season commenced, with the three dry years now referred to as the 2017 to 2019 drought.

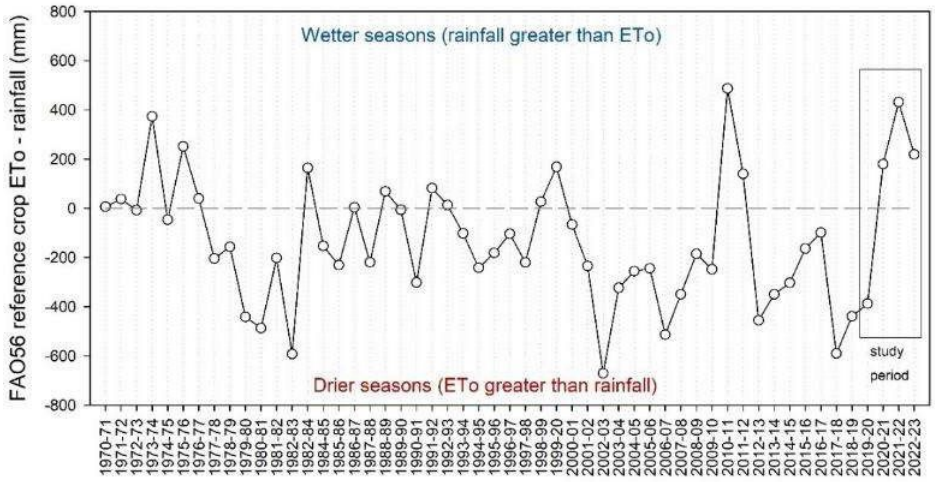


Figure 8. Seasonal water balance (FAO56 ETo – rainfall) calculated from interpolated SILO data for the current BOM Orange Airport AWS.

The subsequent La Niña events then delivered record cumulative rainfall, and this was the first time since the first vineyards of the modern region were established in the early 1980s that three very dry seasons had been followed by three seasons of above average rainfall. While this did not impact the original project objectives around drought adaptation, it highlighted the point that climate adaptation strategies need to consider both rainfall extremes. It also highlighted the importance of the duration of drought relative to wetter seasons, and the capacity of soil and irrigation storage capacity in a region to buffer the extremes in water availability.

Table 4. Seasonally aligned 12 month rainfall, evapotranspiration irrigation sums for the three monitoring sites. Rainfall for Site BA and Site AN are from the BOM Borenore (Lynden-Brae) and Orange (Angullong) stations respectively, ETo and Site RH rainfall are from interpolated SILO data.

	Site BA			**Site RH**			**Site AN**		
Season	Rain	ETo	Irrigation	Rain	ETo	Irrigation	Rain	ETo	Irrigation
2019/20	575	1210	-	612	1222	51 mm	634	1275	137 mm
2020/21	1007	1040	-	989	1052	-	882	1107	-
2021/22	1180	925	-	1156	938	10 mm	1007	985	70 mm
2022/23	1162	986	-	1105	1001	22 mm	1122	1053	116 mm

The capacity of all vineyards to compensate for dry conditions in 2019/20 with irrigation was limited (Table 4). At Site BA there was no irrigation water available for the 2019/20 season. Site RH received 0.5 ML/ha between August 2019 and February 2020, and Site AN, 1.4 ML/ha. At an equivalent of 50 and 140 mm respectively, this was well below what would have been needed to meet the large rainfall deficits developed in the preceding three seasons. The impact on the canopy size and ground cover plants was pronounced compared to the subsequent seasons (Figures 1 and 9).



Figure 9. From left to right. Site BA, RH and AN in early to mid-January 2020.

Site RH and AN were both irrigated in the three following seasons, with Site RH requiring some irrigation through later summer and autumn and Site AN from late December or early January. Detailed irrigation information was collected for the 2021/22 and 2022/23 seasons for these sites as reported in the following sections. Irrigation was not required or used at Site BA following the end of the drought.

6.1.2 Soil and vine water dynamics

6.1.2.1 Site BA

The vineyard at Site BA was located on soil with a depth of approximately 1.8 m above decomposed rock, and was visually dry at this depth when the access tubes were installed in July 2019. As the lower limit of water extraction and field capacity for each depth were established over the subsequent seasons, the retrospective calculation of plant available water at each depth showed that some water was present to 80 cm, but confirmed the visual observations that there was limited soil water below that depth (Figure 10a). This suggests complete drying of the profile to the measurement depth had occurred through the 2018/19 season, or progressively over the two preceding seasons. The water that was in the profile was depleted rapidly in spring, and reached the lower limit of water extraction in January 2020. Field capacity, or the drained upper limit, was estimated from values obtained in winter 2021 and 2022. Summing the plant available water between these dry and wet points at each 10 cm depth increment provided an estimate of total plant available water holding capacity of 250 mm, or an equivalent of 2.5 ML/ha (Figure 10a). Some roots were observed below the measurement depth to close to 1.8 m when the access tubes were installed, suggesting that this is still a conservative estimate, and that some additional soil water could be available deeper in the profile.

The soil water measurements showed a clear seasonal pattern of recharge and depletion that extended to the full depth of the probe readings. In rain-free periods during the season where the soil was able to dry down from a relatively full profile, or at least from within the readily available water range, there was progressive extraction of water that strongly suggests the presence of roots to the depth of measurements. Water depletion rates reached approximately 1 mm per day in the upper soil layers, declining down to 0.2 mm per day at 1.5 m (Figure 10b). The mid-row readings that were paired with the vine-row readings to a depth of 1 m followed a pattern of soil water dynamics that was almost identical. Given that this vineyard was not irrigated, this indicates cycles of uniform wetting due to rainfall followed by uniform rates of soil water extraction across the

vineyard. For soil water extraction to be uniform across the vineyard it would in turn indicate uniform transfer of the transpiration driven water demand to root uptake, particularly below the 10 to 20 cm increments where the impact of evaporation would be small.

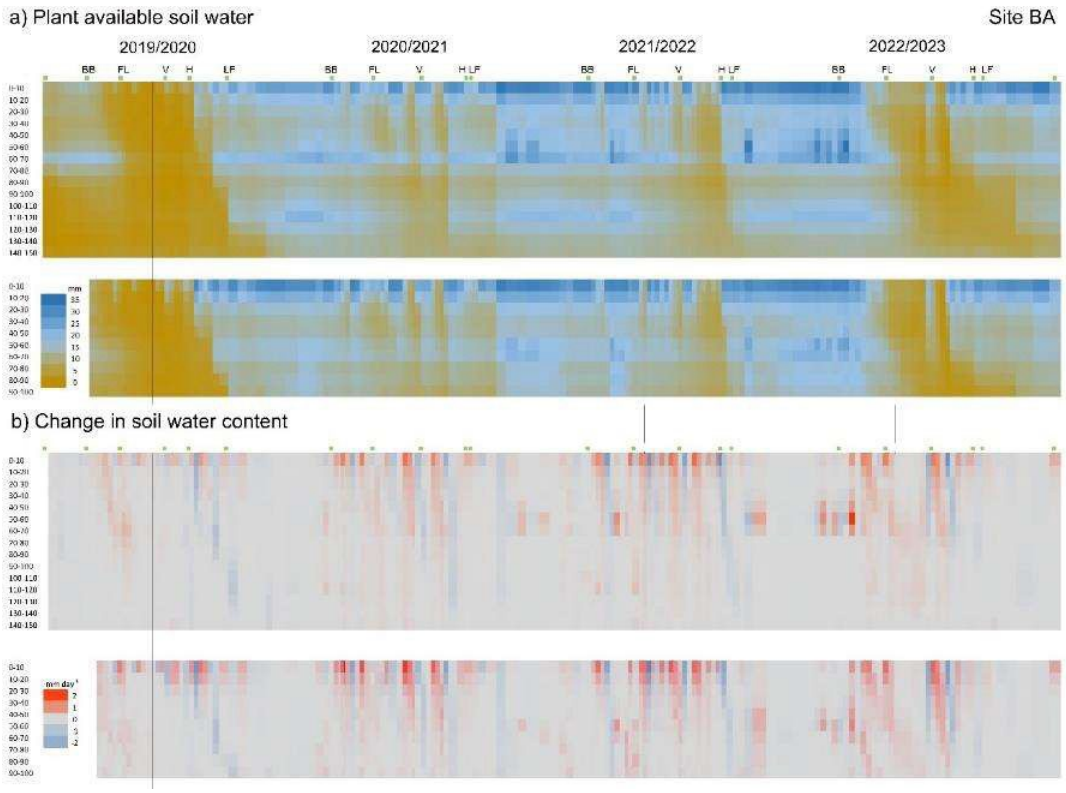


Figure 10. Summary of soil water dynamics at Site BA showing plant available water per 10 cm depth increment (a) and average rate of change between measurement dates (b). BB, FL, V, H and LF indication E-L 4, 19, 35, 38 and 43 respectively.

Ideally, pre-dawn water potential would be used throughout the study rather than just at a few time points, as this is closely linked to the average water availability in the rootzone in rain-fed vineyards, as the plant water content reaches equilibrium with the soil overnight (Williams and Araujo 2002). Stem water potential introduces the additional influence of daily variation in climatic conditions, as well as possible differences in stomatal responses between varieties. However, the stem water potential followed the available soil water values reasonably closely, and provided a definitive reference measurement of plant water status to compare between measurement dates and vineyards.

In spring 2019, the vines were already in moderate water stress at flowering and reached the severe water stress threshold of – 1.4 MPa by 20 December (Figure 11b). Visually, the plants on the vineyard floor were showing the effects of water stress by 15 November, and had completely senesced by the end of November. The vines remained severely stressed for approximately six weeks until rainfall through late February and March began re-filling the soil profile. In the 2020/21 and 2021/22 seasons the vines mostly remained in the no or mild stress range with just one measurement in early 2021 in the moderate stress range during a period of warm and dry weather. The spring of 2022 was extremely wet, but then the profile dried rapidly with drier conditions through summer with one measurement

on 7 February passing the strong water stress range of – 1.0 MPa. This was the only time in the three later seasons of the study that irrigation might have been considered at this vineyard, but even then, the need was alleviated by rainfall shortly after.

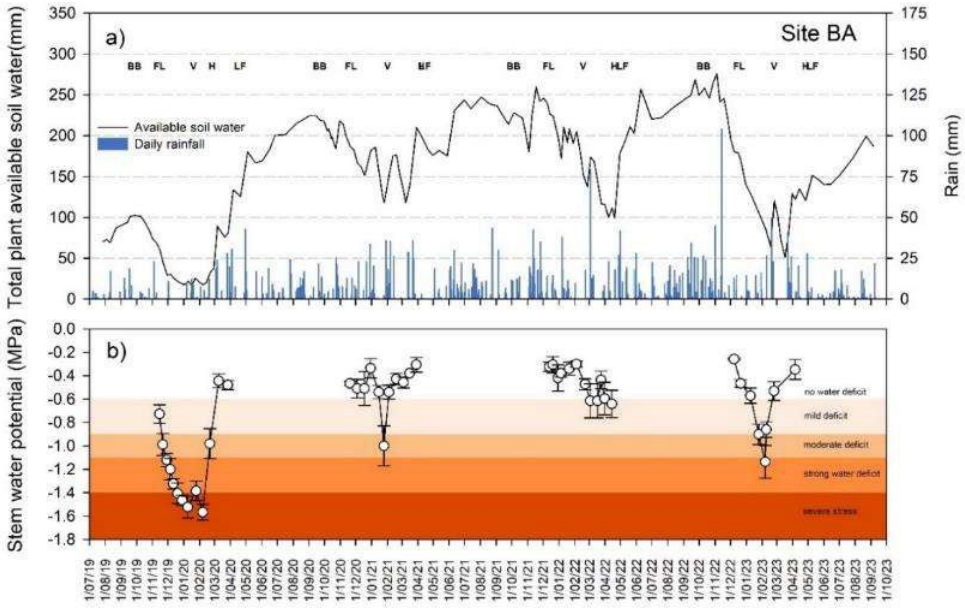


Figure 11. Seasonal pattern of total plant available soil water (a) and stem water potential (b) at Site BA.

6.1.2.2 Site RH

The vineyard at Site RH was only located 6.2 km to the NE of Site BA but on a soil derived from much older volcanic parent material and had slightly lower rainfall. At the location of the monitoring site the soil depth was 1.8 to 2 m based on the depth of rock that was encountered when installing the access tubes. A clay layer was evident from about 0.8 to 1.3 m when the tubes were installed in mid-September 2019 and visually appeared to be holding some moisture. Above and below this layer the soil was extremely dry, and like Site BA, the presence of roots in auger samples from access tube installation suggest this had also been dried by plant water uptake. The seasonal water dynamics and pattern of water extraction with depth also followed a similar pattern to Site BA, with the depth and rates of water extraction both comparable (Figure 12a). However, there was a significant difference from Site BA as instead of the 2019/20 season establishing the lower limit of extraction as with Site BA, progressively drier lower limits were reached in each subsequent season at Site RH.

The reason for the decrease in the lower limit of water extraction is not clear at this point, but the apparent gain was mostly associated with the changing lower limits of extraction in the heavier clay layers in the mid-profile. With the retrospective determination of plant available water, based on the lower measured soil content in later seasons, some water had apparently remained available in the profile at the peak of the drought. A possibility considered was that root growth and function had been reduced during successive dry seasons leading into 2019/20, and then progressively recovered through the following wet seasons. However, at the end of the final study season, between June and July in 2023, there was an apparent gain of 155 mm of soil water content whereas only 44 mm of rain fell during the same period. This suggests a more likely explanation that there had been some shrinkage of the clay from around the tube at these depths through summer and autumn and then

swelling again back into contact after the rain. As discussed below, this effect was even more pronounced on the heavy clay soils at Site AN. Preferential flow down the outside of the tube could also have occurred, and highlights a difficultly in quantifying plant available water by direct measurement with capacitance probes that are sensitive to air gaps around the sensors. With this difference in lower limit, the estimated total plant available water content of 220 mm from the first season increased to around 350 mm by the fourth seasons when the lowest dry point of autumn 2023 was included. The stem water potential measurements at the same time points showed that the vine water status was similar despite the large apparent difference in water remaining in the profile.

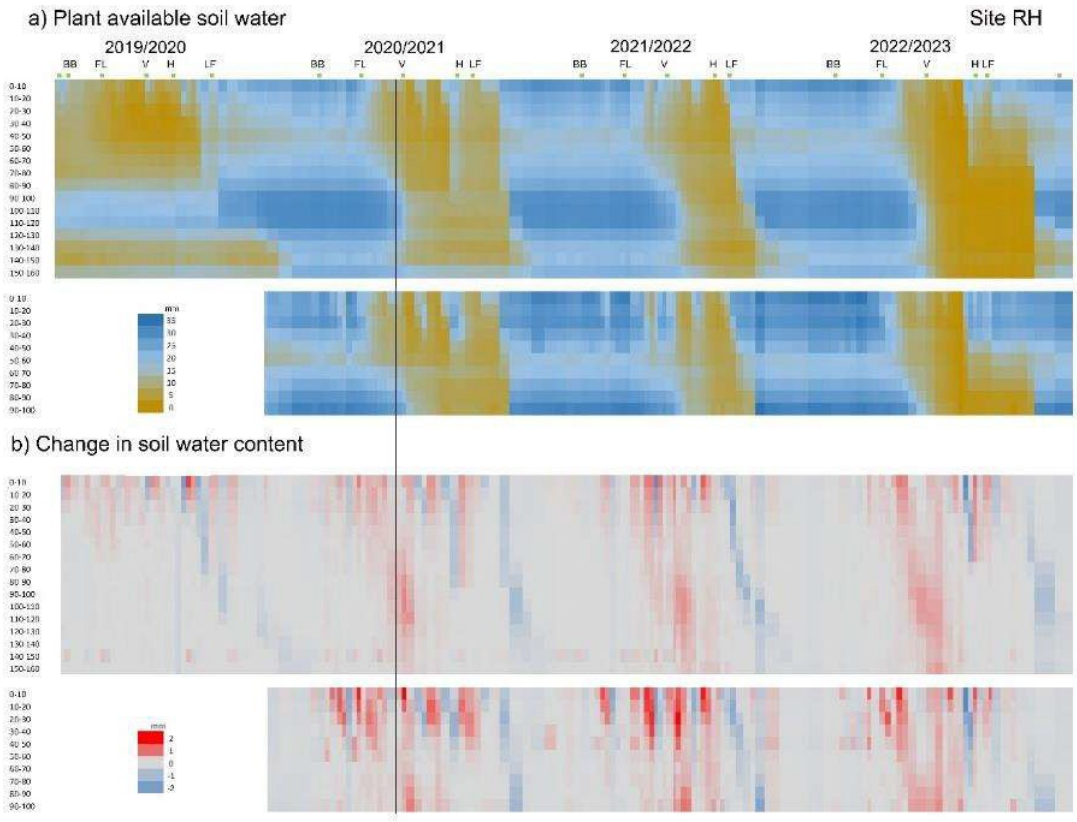


Figure 12. Summary of soil water dynamics at Balmoral vineyard showing plant available water per 10 cm depth increment (a) and average rate of change between measurement dates (b). BB, FL, V, H and LF indication E-L 4, 19, 35, 38 and 43 respectively.

For the mid-row, the tubes were not installed at Site RH until winter 2020 and so missed the opportunity to record the dry points of the 2019/20 season. However, the measurements across the three subsequent seasons confirmed the observations at Site BA that the seasonal pattern of water replenishment and extraction from the mid-row was similar to the first 1 m of the vine row tubes. The main seasonal trends in soil water content were driven by rainfall and evapotranspiration, with irrigation events not evident in the averaged values across the four tubes. This would partly reflect the position of the tubes, with two of the four away from the wetter zone under the drippers, and also the lower frequency of the manual readings. The volumes of individual irrigation events were also very small compared to the total water holding capacity of the soil.

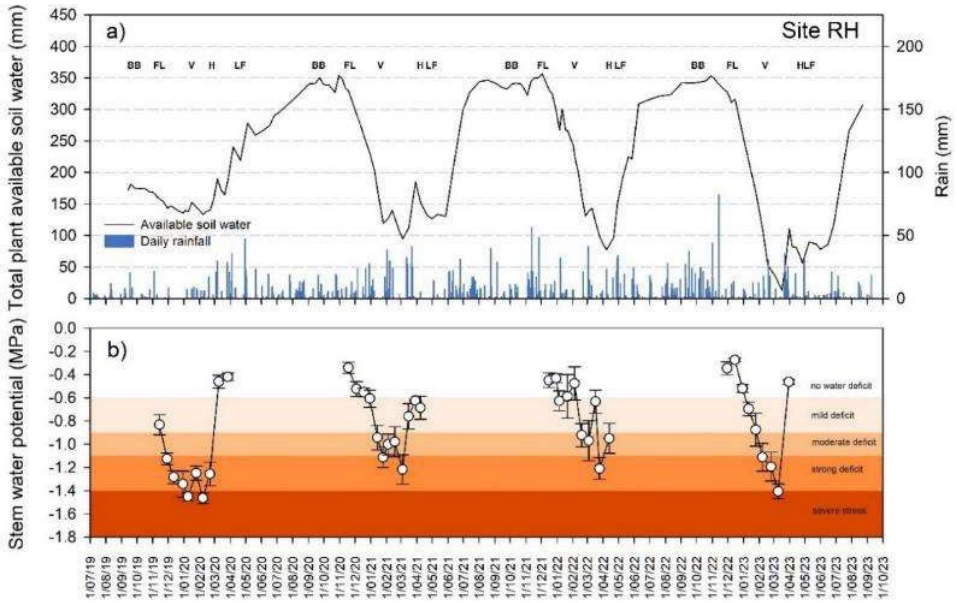


Figure 13. Seasonal pattern of total plant available soil water (a) and stem water potential (b) at Site RH.

6.1.2.3 Site AN

The vineyard at Site AN was located 32 km to the SSW of Site 1 on a cracking vertosol that transitioned to a lighter gravel containing horizon at approximately 1.3 to 1.6 m deep. The seasonal drying and wetting patterns were still clearly evident in the soil water measurements, but the shrink and swell cycles of the clay caused a number of challenges with the capacitance probe and access tubes. In the mid-row, large deep cracks developed down the centre of the mid-row during the driest summer periods, which intersected with three of the four mid-row tubes. Only the measurements for the fourth tube are shown in Figure 6.7a, and even then the variability down the profile suggests issues with soil contact around the tubes at some depths.

In the undervine area there were many smaller cracks distributed more uniformly across the soil surface instead of single larger cracks. However, the total profile sum of ~450 mm (Figure 14a) is still high compared to the upper range of ~ 350 mm reported for other vertosol soils in Australia (Verburg *et al.* 2017), suggesting that air gaps in the proximity of the tubes were still present. Soil volume changes also caused a seasonal rise and fall of about 5 cm. For the vine-row tubes, which appeared to be fixed in position by the gravel-containing horizon deeper in the profile, the soil would then rise and fall through the season relative to the access tube. This meant that the first reading in the 0-10 cm increment was partly above ground when the soil was very dry, reducing the apparent lower limits for the tubes further if this increment was included in the profile sums.

As with Site RH, stem water potential proved to be a more reliable parameter than soil moisture monitoring for characterising differences in water availability across seasons. Particularly for the maximum seasonal water deficits where the soil water content did not correlate well with the lowest stem water potential readings. Site AN had a higher demand for water due to lower rainfall and higher ET demand, which was able to be met at least partly by irrigation. This resulted in the most stable season-to-season stem water potential thresholds of the three vineyards, with sufficient irrigation to mostly avoid sustained periods of severe water deficits in 2019/20 (Figure 15b), with sufficient ET demand to develop a water deficit despite the higher rainfall in the subsequent three seasons.

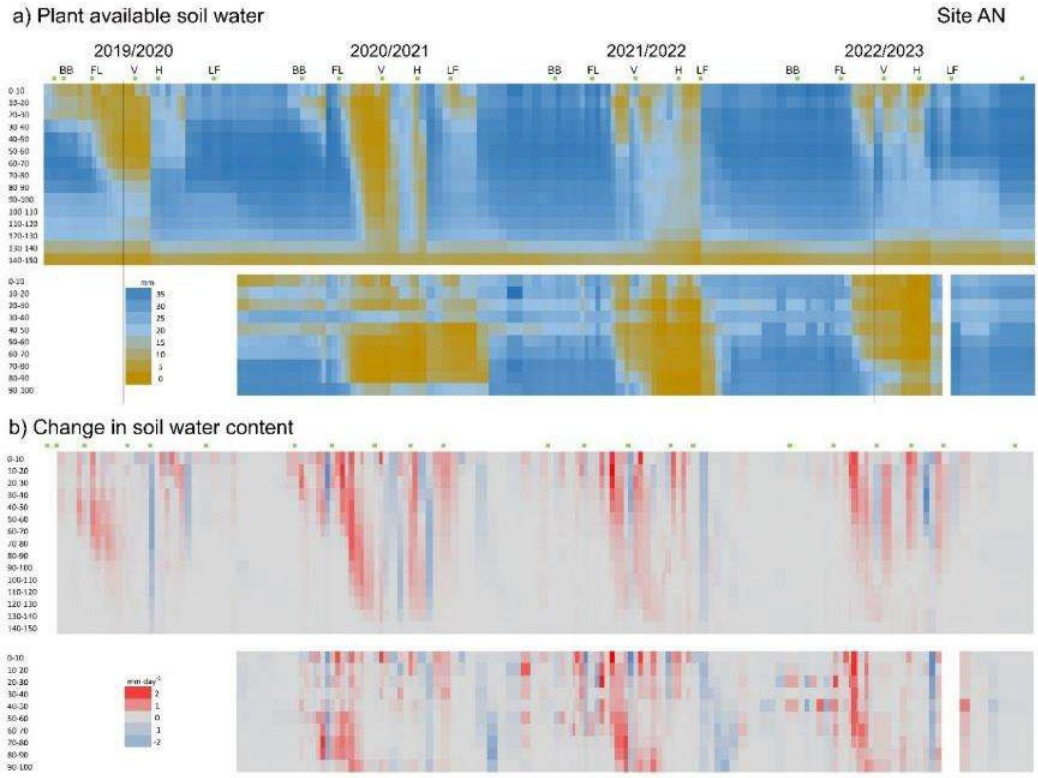


Figure 14. Summary of soil water dynamics at Balmoral vineyard showing plant available water per 10 cm depth increment (a) and average rate of change between measurement dates (b). BB, FL, V, H and LF indication E-L 4, 19, 35, 38 and 43 respectively.

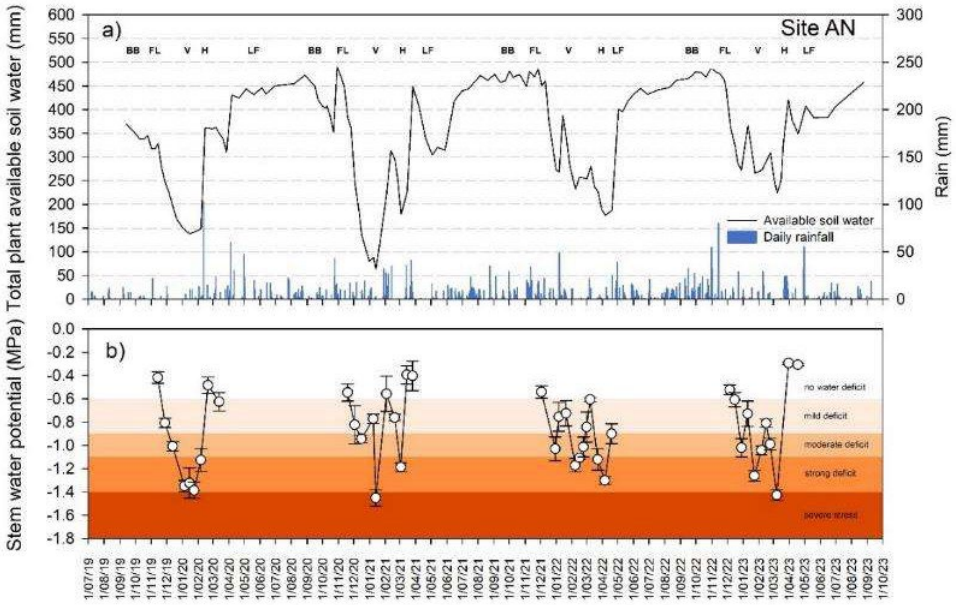


Figure 15. Seasonal pattern of total plant available soil water (a) and stem water potential (b) at Site RH.

6.1.2.4 Total available water and profile distribution

Acknowledging the inability to establish accurately the lower limit of extraction at two of the sites, values of total plant available water of 250 to 350 mm are not inconsistent with upper ranges reported to depths of 1.5 to 1.6 m for these soil types with other crops (APSoil database). However, they are higher than some other values reported for Australian vineyard soils in warmer climates (e.g. up to 140 mm, Phogat *et al.*, 2022). They are also above the 200 mm threshold that has been described as very high or excessive for vineyard soils (Cass 2002), although the vineyards in this study were not overly vigorous. The reason for the lower values often appears related to sub-soil constraints that limit or are expected to limit the depth of the root system, and that total available soil water values have mostly been derived from physical properties rather than from measurement of water extraction by plants in the vineyard (e.g. Myburgh *et al*., 1996). For vineyards in the current study, we have also collected soil cores for this purpose, with particle size analysis planned to provide an independent estimate of the profile sum. However, one advantage of the soil moisture monitoring approach to determining the profile sum, is that it can indicate the depth of root activity where these might not be evident in soil pits or soil samples collected for the purpose of physical analysis.

At Site BA, the first 80 cm of the profile held just over 60% of the total available water, meaning there was still 40% or ~100 mm of water below this depth. If the roots extended past the measurement depth to 1.8 m, which appeared to be the case when the tubes were installed, then this would be closer to 140 mm or half the total available water assuming the same water holding capacity of the lowest depths read with the capacitance probe. At 100 and 120 cm, which would be the longest soil moisture probes usually installed in commercial vineyards, the available soil water below these depths would have been approximately 78 and 42 mm, respectively. These are values comparable to 0.5 to 1.5 ML/ha of irrigation that would be typically available to growers across the Orange wine region. As Site RH, the situation appeared to be similar, with a clear seasonal trend at the 150-160 cm depth increment that suggested the lower depth of water extraction had not been reached. Site AN was the only vineyard where the tubes did seem to be installed to the lower depth of root activity, although there was still a seasonal fluctuation of ~6 mm that might be attributable root water uptake.

One important practical implication of knowing the total available water capacity of the rootzone, which in turn requires knowledge of root depth, is that it quantifies the maximum storage capacity of the vineyard soil. While growers will have reasonably accurate knowledge of dam storage capacity and / or volumes of irrigation available from external water sources, there is less certainty about the volume of soil storage capacity. If this is underestimated, then the capacity to compensate for rainfall deficits and drought with irrigation may be overestimated. If it is known, then ability to meet the expected seasonal water demand can be assessed more accurately early in the season, and decisions around budgeting or priorities made with a more complete picture of the water situation.

6.1.3 δ13C integration of seasonal vine water status

The relationship between carbon isotope composition of grape juice and vine water status through the ripening period has been well established (Gudillère *et al.*, 2002, van Leeuwen *et al.*, 2009), with water deficit decreasing the photosynthetic discrimination against ^{13}C which is then reflected in the isotope ratio of photosynthate translocated from the leaves to the berries. This provides an integrated measure of vine water status during the ripening period that allows retrospective comparison of seasonal or spatial differences in water availability.

For the carbon isotope composition of the lower two nodes of dormant canes in the current study, which are easier to sample and process than grape sugars, a relationship between mean seasonal stem water potential and carbon isotope discrimination was also observed (Figure 16a). Under severe water deficit there was less discrimination against ^{13}C compared to seasons of higher water availability, leaving the cane tissues at the end of the season in the dry seasons with a higher ratio of ^{13}C to ^{12}C.

Excluding the spring and autumn stem water potential measurements from the mean strengthened the relationship (Figure 16b). This was mostly due to the autumn measurements, where the water status of the vines had usually recovered, but the shoots were past the stage where much new carbon incorporation as growth or stored carbohydrates would be expected.

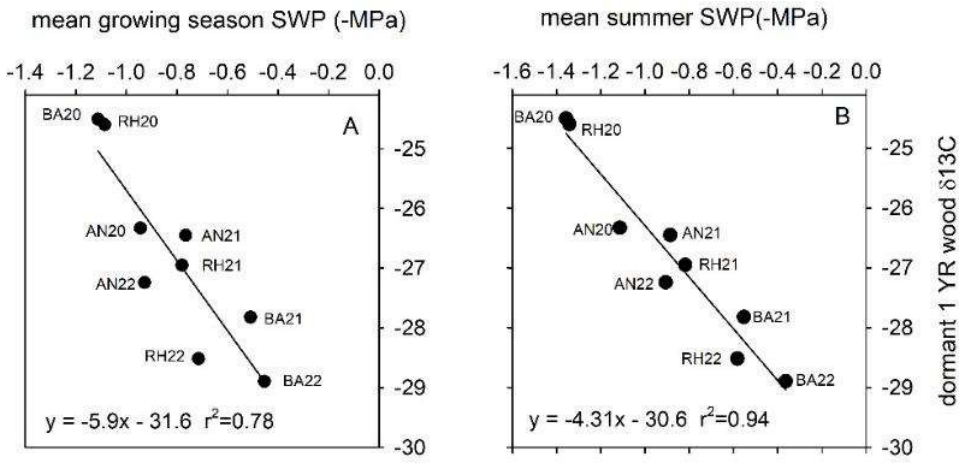


Figure 16. Relationship between carbon isotope discrimination (δ13C) of the basal two nodes (spurs) of dormant canes and the mean stem water potential for that season.

Site BA had the largest season to season change in δ13C, decreasing from -24.51 ‰ to -28.90 ‰ as the vines recovered from the dry conditions in 2019/20 through the next two La Niña seasons. Site AN had the least variation in δ13C, which suggests that the irrigation was able to moderate the effects of the drought in the first season, but there was still sufficient water demand with the higher ET and lower rainfall at lower altitude to run a deficit irrigation regime despite the higher rainfall in the wetter seasons. Site RH had slightly more negative δ13C than BA in 2019/20, which might reflect a measurable impact of the small amount of supplementary irrigation available. Like AN, lower rainfall and higher ET, moderated the impact of the wetter seasons. Spur samples were also collected in the 2022/23 season but have not been analysed at the time of writing.

6.1.4 Automated water potential measurements

The original objective of the microtensiometer installation was to provide more detail on the diurnal and seasonal time course of grapevine water status than could be obtained with the manual pressure chamber readings. In particular, pre-dawn water potential where the vine water status and soil water content should be in equilibrium. At Site RH and AN, the sensors initially performed well, but appeared to lose hydraulic continuity with the trunk, and then the sensor outputs drifted from the earlier clear diurnal time course. When the sensors were removed at the end of the 2022/23 season, although well past the point where the last meaningful data was obtained, there was significant gelatinised sap build-up around the sensor chip that had displaced the mating compound. With a relatively wet spring in 2021 it is possible that bleeding phloem or xylem sap had caused a loss of hydraulic connection with the trunk wood. At Site BA, despite the logging system and installation being identical to the other sites, no meaningful trunk water potential data were obtained during the study. Troubleshooting in the field, including full wiring checks, earthing upgrade to prevent any possible ground loops, and confirming the mechanical fixing of the sensors themselves, could not resolve the issue. The main

information provided by these sensors was therefore the diurnal time course in the weeks immediately following installation at Site RH and AN (Figure 17).

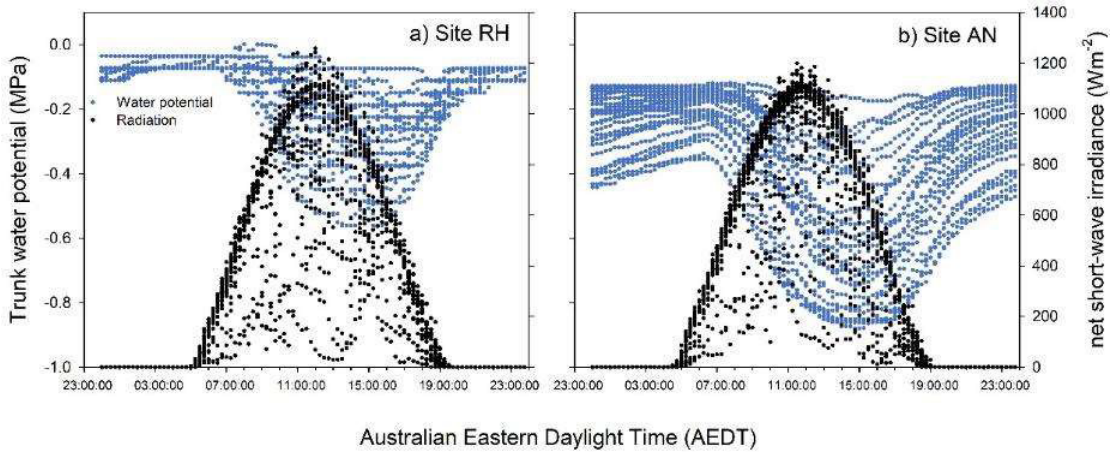


Figure 17. Microtensiometer readings made during summer of the 2021/22 season shown relative to solar radiation measurements recorded at the corresponding trial site weather station. Dates for Site RH 20/12/21 to 12/01/22, and Site AN 09/12/21 to 5/01/22.

At Site RH, trunk water potential declined to ~ -0.5 MPa during the day compared to a stem water potential low of -0.63 MPa recorded on 5 January with the pressure chamber. This was in the mild deficit range, and values returned to close to zero overnight. For Site AN, trunk water potential reached values between -0.8 and -0.9 MPa compared to a low of -0.10 MPa with the pressure chamber on 31 December. On the days with of higher water stress, the trunk water potential did not fully recover overnight. Despite not providing a longer term record of trunk water potential, the information obtained was still very valuable as it confirmed that the manual stem water potential measurements, which were made mid-afternoon, corresponded to the time of day when the vines had the largest water deficit. This in turn was consistent with visual observations of the canopy appearing more water stressed in mid-afternoon than at solar noon, and a recent study showing stem water potential values are still declining in the middle of the day (Tian and Schreiner 2021).

6.1.5 Automated transpiration measurement

The grainer-type sapflow gauges installed at the sites also had some technical issues, but still produced significant high quality periods of measurements over the 2021/22 and 2022/23 seasons for comparison with the modelled calculations of transpiration, and responses to rainfall and irrigation. Some of these issues were related to temporary loss of battery charge during extended periods of overcast weather, and others due to normal challenges expected with long wiring runs and instrumentation installed for extended periods in commercially operated vineyards.

To take a period from early in the installation as an example, Figure 18 shows the mean sapflow data for six vines and the corresponding reference crop evapotranspiration (ETo) from the respective trial weather stations from 9 - 18 January 2022. It highlights the similarity of transpiration across the three vineyards, and by extension, the similarity of the driving atmospheric water demand as indicated by the similar daily ETo time course. Site BA and RH, with the same row orientation and VSP canopies, followed each other very closely, including a morning peak commonly observed with Grainer-type gauges. Site AN had lower sapflow rates, possibility due to being more water stressed at the time, but otherwise closely tracked the other sites and daily change in ETo.

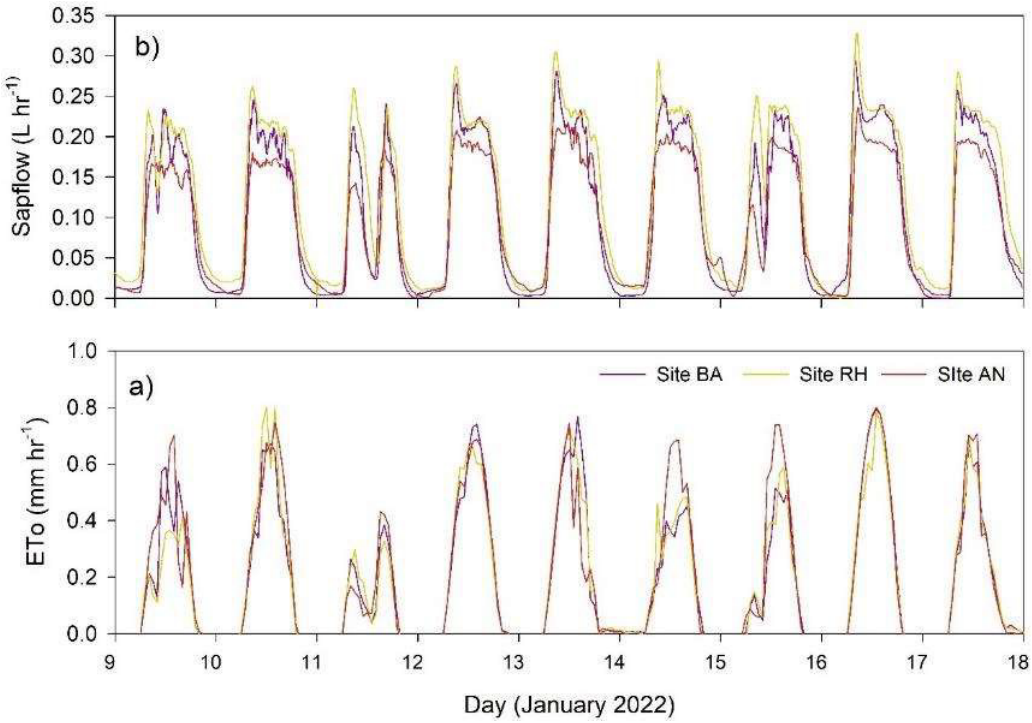


Figure 18. Example period of sapflow measurements from January 2022 (a) and comparison with hourly ETo calculations (b) made at the trial site weather stations.

On a per vine basis, the average daily water use for the period was 2.5 L, 3.0 L and 2.3 L at Site BA, RH and AN, respectively. The canopy at Site RH was slight wider and denser than BA, which may account for the higher transpiration rate between these two otherwise similar vineyards. Converted to mm per day based on the 1666 vines per hectare planting density, the total water usage for the period represented between 8 - 12% of the ET_O sum for the period, or between 12 - 16% of the ETo sum if converted to a vineyard water use estimate using a standard (non-stressed) crop coefficient of 0.7 (FAO56, Allen *et al*., 1998). As discussed later, these values are low relative to the literature.

6.1.6 Automated soil moisture probes

The soil water measurements in the project were undertaken manually as this allowed more replication than would have been possible with automated full profile probes, and the measurements at one to four week intervals were sufficient to track the broader seasonal trends and provide the validation measurements for the water balance modelling. The pressure chamber measurements, shoot growth, phenology and berry sampling all needed to be undertaken at the sites anyway. However, with the wider recording intervals, the manual soil moisture measurements did not capture the higher temporal resolution information that would be obtained with automated probes. A single 80 cm probe was therefore installed under a dripper at each site in spring 2021 to allow a comparison with the more common commercial practice.

At Site BA, which was not irrigated during the study period, the soil moisture readings for the automated probe tracked rainfall events and provided comparable information to the manually read tubes for the first 80 cm (Figure 19a). However, across the two seasons there were times where the soil water content below 80 cm could differ when the water content of the upper 80 cm was similar.

This would be expected if roots are present below the depth of the automated probe, but highlights the value of deeper soil moisture monitoring for a more complete understanding of water availability. At Site RH the automated probe readings also tracked the main trends in the manually read probes, but reached a similar dry point in 2022 and 2023 compared to the first 80 cm of the manual tubes which were drier in 2023 than 2022 (Figure 19b). Possibly this was due to the difference in installation method, with slurry of mixed soil around the automated probe reducing the previously discussed issues with soil shrinkage around the manually read access tubes, or variation in the radius of measurement with the two probe types.

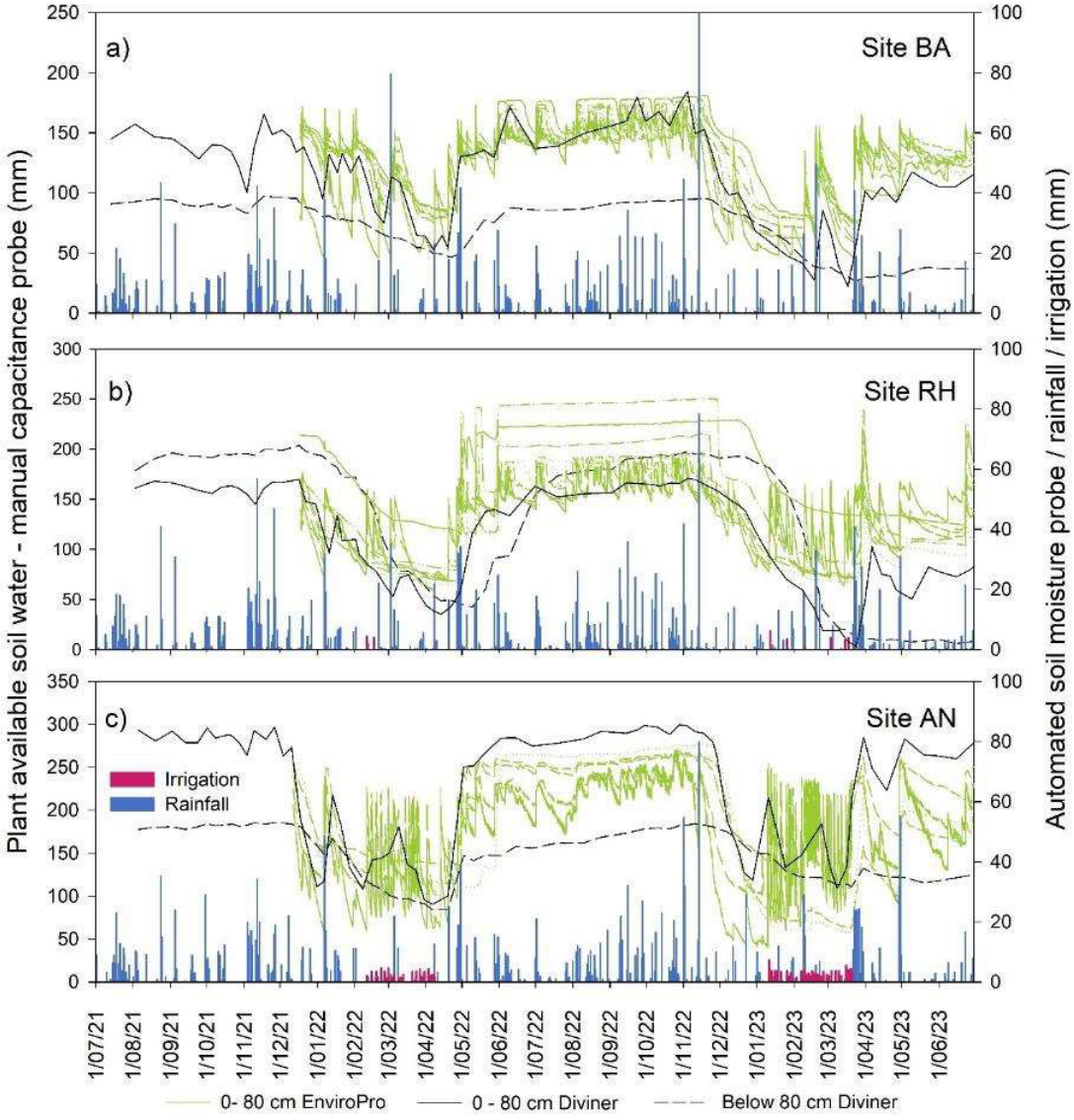


Figure 19. Comparison of the manual and automated soil moisture probe readings in relation to irrigation and rainfall in the 2021/22 and 2022/23 seasons. Irrigation volumes are converted to a mm equivalent for comparison with rainfall events.

At Site AN, which received small frequent irrigations as the soil profile dried leading to the end of the year, there was a pronounced difference in the high frequency wetting and drying cycles under the dripper compared to the averaged soil water content along the vine row (Figure 19c). This would be a

very similar situation to many drip irrigated vineyards in Australia, with the vines transitioning from water sourced from the whole rootzone following winter to a smaller irrigation supplied soil volume under each dripper. In this case, from a zone about 20 cm wide reaching a depth of about 40 cm with each irrigation event. For Site RH the dry down of profile took longer, with strong or severe stem water potentials only reached late in summer or earlier autumn when some irrigation was applied. Although not the focus of this study, this does raise a question how the transition of water uptake from the whole rootzone to just the area under the drippers influences nutrient uptake.

6.1.7 Spring shoot growth

Shoot growth was monitored from shortly after bud-break in each season, until growth either ceased due to water stress in 2019/20, or the first trimming in the later three seasons (Figure 20). At Site BA, shoot growth had stopped by 21 November when 10% of the available soil water was remaining and the stem water potential reached -1.1 MPa in the strong to severe stress range. At Site RH growth ceased approximately one week later on 6 December and between stem water values of -1.1 to 1.3 MPa based on the measurement dates a week either side of the shoot measurement. Shoot growth had stopped by the same date at Site AN, although with stem water potential at approximately -0.9 MPa. In the subsequent seasons, active shoot growth continued until canopies filled the trellis by late spring or earlier summer and were trimmed.

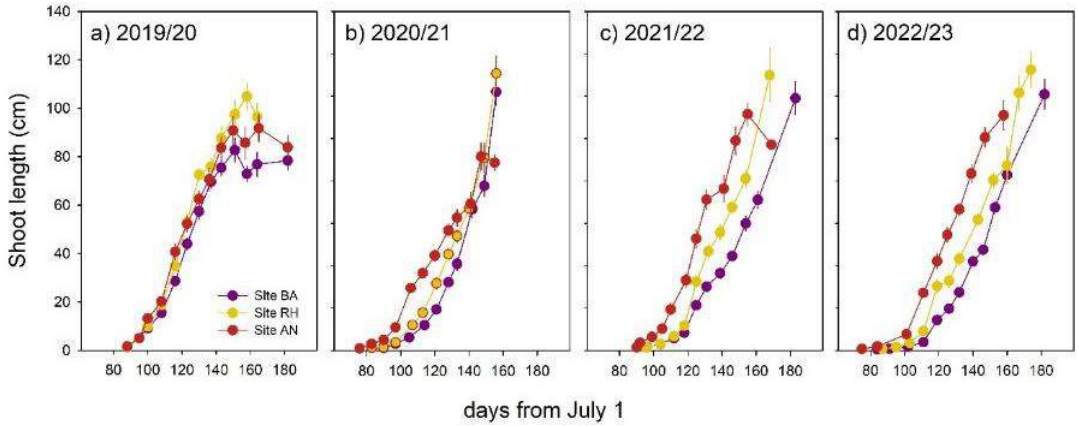


Figure 20. Summary of shoot length measurements from ~ E-L4 through to the cessation of shoot growth or first trimming. n=20, error bars ± SE Mean.

6.1.8 Berry growth and composition.

The contrast between dry conditions of 2019/20 and the subsequent wetter seasons was particularly evident for berry growth (Figure 21). In 2019/20 berry weights at Site BA just reached 0.4 g, whereas weights between 1.5 and 2 g were reached over the next three seasons. Berry weights at Sites RH and AN appeared to benefit from the limited irrigation in the first season, with average weights at harvest reaching 0.72 and 1 g, respectively. This restriction of berry growth caused a rapid increase in sugar on a Brix basis, with Sites BA and RH several months ahead of their harvest dates in later seasons.

In the onset of sugar accumulation at veraison, there was a progression from Site AN to RH to AN that reflected in the gradient in temperature from the lower altitude site to the highest. This was also seen clearly in the organic acids (Figure 22), with the total reflecting similar trends in both malic and tartaric acid (not shown). Site RH had the largest berries on average for the three vineyards, reaching 2 g before losing some weight leading into harvest. These berries accumulated sugar proportionally higher rate so that there was no delay in sugar concentration relative to the other vineyards.

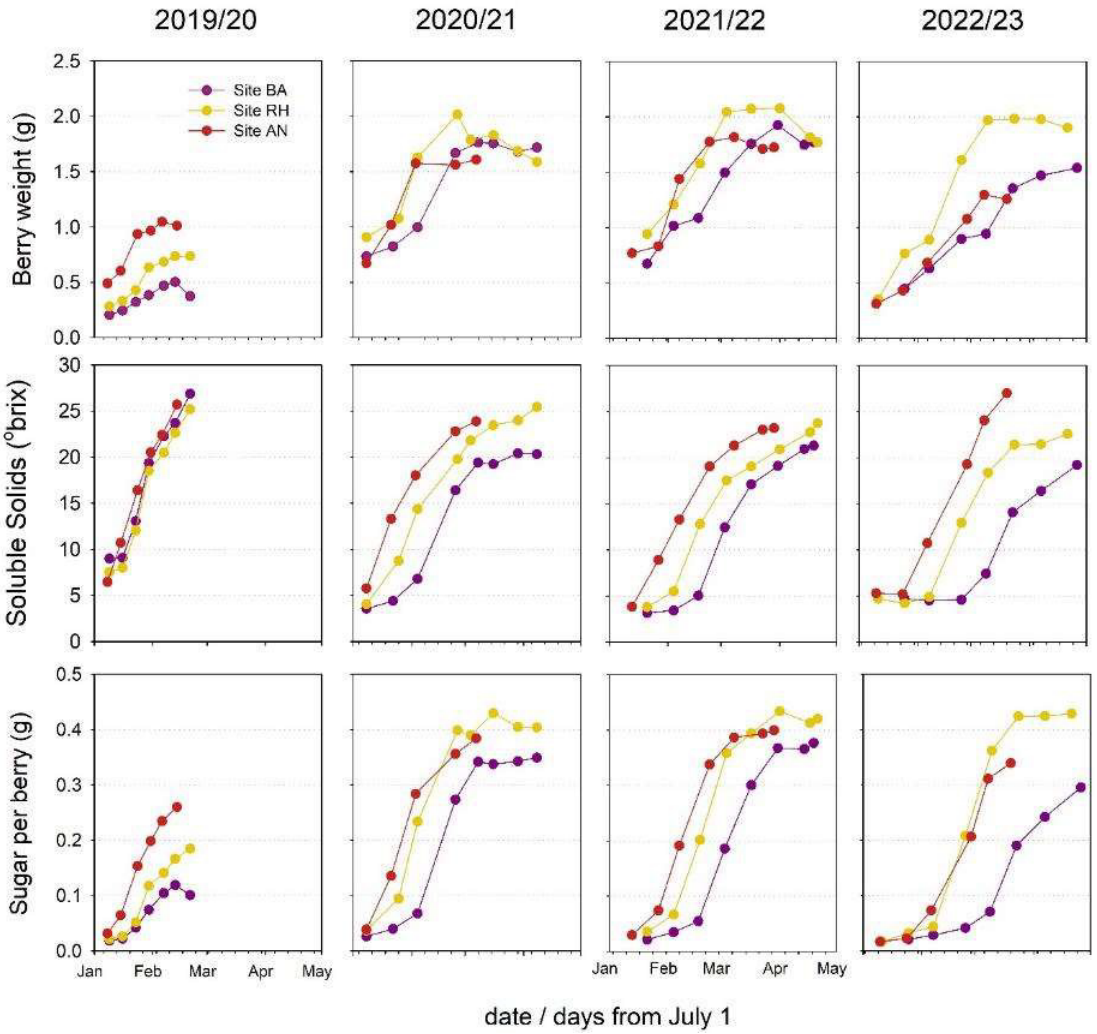


Figure 21. Summary of berry fresh weight, soluble sugar content and sugar per berry for the four seasons of the study.

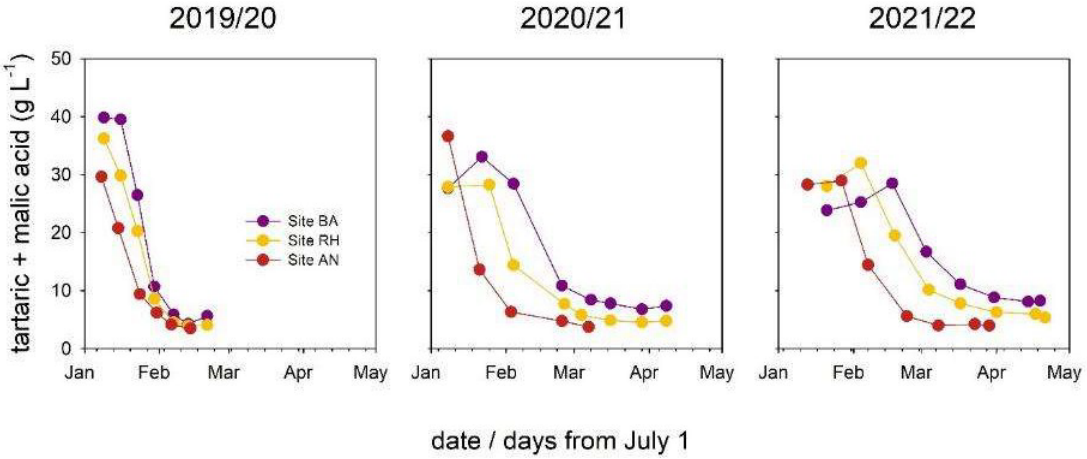


Figure 22. Summary of juice acid concentrations. Tartaric acid analysis for 2022/23 has not yet been undertaken for the 2022/23 season.

Analysis of ammonia and alpha amino nitrogen was undertaken for all berry sampling dates corresponding to Figure 21, with the results for the last sampling date in each season summarised in Table 5. Petiole sampling was not undertaken, but total N analysis was conducted on the dormant wood samples used for the δ13C measurements to provide additional indicator of vine N status.

The yeast assimilable nitrogen (YAN) concentrations were mostly over the minimum 100 mg/L recommended for red wine fermentations[2] except for Site BA in 2020, and Site AN in 2020 and 2022. Site RH had the consistently highest YAN concentrations, with the relative differences between vineyards already evident at the earlier sampling dates around veraison (not shown). The reason for this difference was not clear, and no nitrogen fertiliser was applied at Site RH. There was no correlation between the total N content of the dormant cane wood at pruning, which indicates N status from stored reserves, either comparing juice and wood with the same season, or wood with YAN in the following season. Site RH did have slightly higher wood N concentrations on average, but this was small relative to the site differences in YAN. There was also no difference in soil N in the top 30 cm from the last coring at the end of the 2022/23 season (Table 1).

Table 5 Berry N composition at harvest (or closest berry sampling date) and total N concentration of the basal two-nodes from the canes at dormancy.

		Juice		Dormant cane
	Ammonia	**NOPA**	**YAN**	
Site BA		N mg/L		Total N (%)
21/02/2020	13.4	67.7	78.8	0.62
8/04/2021	26.7	94.8	116.8	0.59
19/04/2022	37.2	86.5	117.2	0.61
26/04/2023	7.6	111.9	118.1	0.48
Site RH				
21/02/2020	28.3	110.7	134.0	0.64
8/04/2021	49.3	103.7	144.3	0.64
21/04/2022	60.2	107.2	156.8	0.65
21/04/2023	52.5	288.9	332.1	0.55
Site AN				
14/02/2020	9.0	38.9	46.3	0.58
7/03/2021	34.5	115.4	143.8	0.58
29/03/2022	20.5	57.1	74.0	0.60
20/03/2023	18.5	139.6	154.9	0.48

Yield parameters were recorded for the second two seasons of the study, and ranged between 1.4 and 3.3 kg per vine, or approximately 2.4 and 5.4 t/ha (Table 6). Pruning weights ranged between 1.2 and 3.4 kg per vine, which is a larger difference than suggested with the shoot growth rates at the start of the season. At Site RH, which had the highest pruning weights, the canes were thicker, had more laterals, and continued active growth along trellis wires more often than the other VSP canopy at Site BA. Considering the total N measurements made on the lower nodes of these canes, the total N content of the canopy at Site RH was potentially significantly higher than the other vineyards due to the difference in size. This could provide some explanation for the higher YAN values at this site, which are not evident in the concentration-based comparison of dormant wood between sites.

[2] https://www.awri.com.au/industry_support/winemaking_resources/wine_fermentation/yan/

Table 6. Yield and pruning weight parameters for the second two seasons of the study.

	Yield (kg/vine)	Pruning weight (kg)	PW Ratio	Canes per vine	Cane weight (g)	Bunches per vine	Bunch weight
			2022				
Site BA	1.43	1.53	0.9	30	51	20	73
Site RH	2.55	2.43	1.1	27	90	23	111
Site AN	2.83	1.41	1.4	31	46	22	128
			2023				
Site BA	3.22	1.34	2.4	37	36	41	79
Site RH	3.27	3.41	1.0	40	86	33	100
Site AN	3.05	1.17	2.6	27	43	32	96

Based on pruning weight ratios, all sites were below the commonly cited optimum crop load range of 5 to 10 kg of fruit per kg of pruning weight (Bravdo *et al*., 1985), and therefore could have potentially carried more fruit. Site BA did not fully ripen in the 2022/23, but this was due to a cooler season, late veraison and lower temperature at the end of the season. Bud-dissections undertaken at the end of each season showed that these low yields were primarily due to low bud-fruitfulness, and in turn primary bud necrosis, or dead or completely missing buds (Figure 23). Although the missing buds at the end of 2021 appeared to be associated with mite damage to shoots in spring 2020, bud-necrosis was high irrespective of wet or dry seasons, shoot vigour or training system.

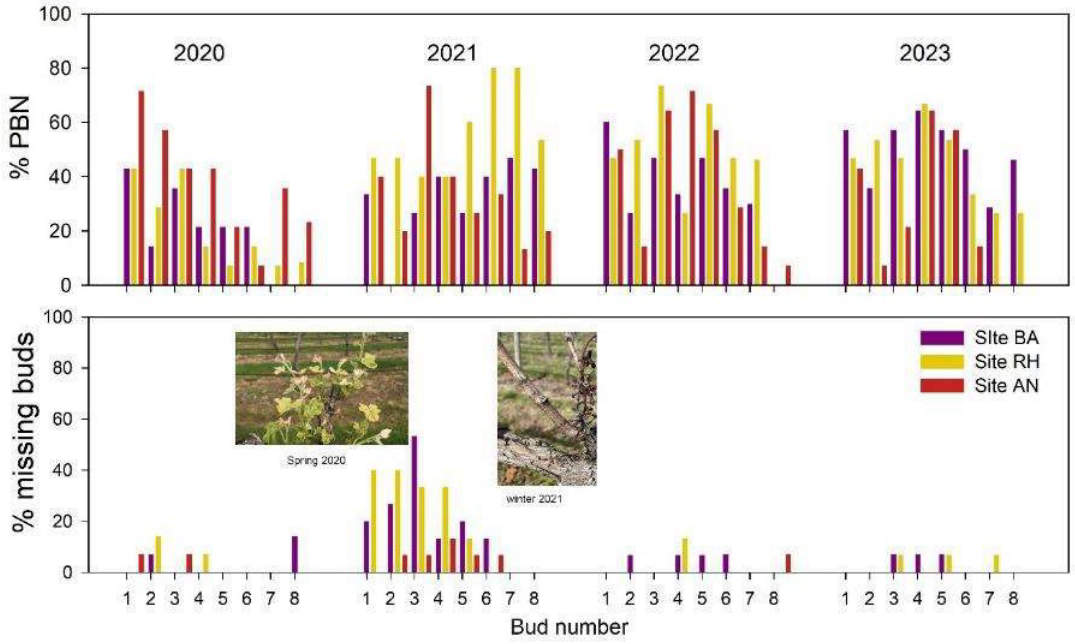


Figure 23. Primary bud-necrosis (a) and missing buds (b) for the first eight buds of dormant canes across the four winters of the study period. Values are the mean of ~14 shoots.

To summarise the above ground growth and fruit composition measurements, the study was characterised by strong impacts of water stress in the 2019/20 season, followed by three relatively

similar seasons of adequate (or more than adequate) water availability. Phenology, shoot growth and the berry ripening and composition clearly reflected the gradient in temperature from the highest altitude Site BA at 880 m down to Site AN at 610 m, although significantly compressed in 2019/20, and there were no major management changes during the four seasons. The canopy development measurements, including canopy width and porosity estimates that are not shown, were primarily collected for parameterising the radiation interception model. The pruning weight data also provided some above ground biomass information to complement the results from the root system assessments. The water balance model does not include any assimilation or yield components, but the berry growth data collected here could be used to develop relationships between soil or plant water status given the range of climatic conditions spanned during the study.

6.2 Mapping root distribution in cover cropped vineyards

6.2.1 Plant species identification and reference DNA sequencing

The trial sites were all located in mature vineyards that had well established permanent mid-row plant cover. Alternating mid-rows of *F. arundinacea* had been sown earlier in the life of the vineyard at Site BA, and in every mid-row at Site AN. The other plant species present, along with those at Site RH, appeared to be combination of pasture species reflecting earlier land use for grazing, local native grass species and a wide range of naturalised introduced species (Figure 24). Site BA also had permanent undervine cover following transition to organic management, consisting of species that had spread across from the mid-row. Slashing or mowing was used at all sites, including the undervine area at Site BA, and Site BA and RH used additional intensive grazing with sheep over winter.



Figure 24. Example images of the range of plant species found across the vineyard floor at the three trial sites.

At the time of writing, 29 species have been identified through Sanger sequencing and verification with herbarium samples where the identity was not clear (Table 7). These represent the majority of the species found across the three vineyards, with a number of species being common to all. Perennial grasses dominated the identified plants with *F. arundinacea* (BA, AN), *L. perenne* (BA), *B. macra* (AN) *P. dilatatum* (BA, and to a lesser extent AN and RH) representing the main green leaf area on the vineyard floor during the growing season. During late winter and spring, there was a

transient leaf area contribution from annual grasses, including *B. hordeaceus* (BA, RH) and *A. barbata* (BA). Among other native species, some plants of *R. caespitosum* were present at each vineyard, as well as *M. stipoides* at Site BA. With a combination of annual and perennial grasses, as well as C3 and C4 photosynthetic pathways, the period of active green ground cover could extend at least a month either side of grapevine bud-break and leaf fall at the sites with sufficient rainfall.

Table 7. Summary of plant species found on the vineyard floor across the study trial sites.

Scientific name	Common names	Comment
Grasses		
Avena barbata	Bearded Oat	naturalised, C3, annual
Bothriochloa macra	Red Grass	native, C4, perennial
Bromus catharticus	Prairie Grass	naturalised, C3, perennial
Bromus hordeaceus	Soft brome	naturalised, C3, annual
Carex inversa	Common sedge, knob sedge	naturalised, C3, annual
Chloris truncata	Windmill Grass	native, C4, perennial
Dactylis glomerata	Cocksfoot	naturalised, C3, perennial
Digitaria Sanguinalis	Summer Grass	naturalised, C4, annual
Eleusine tristachya	Goose Grass, Crabgrass	naturalised, C4, perennial
Festuca arundinacea	Tall Fescue	naturalised, C3, perennial
Lolium perenne	Perennial Ryegrass	naturalised, C3, perennial
Microlaena Stipoides	Weeping Grass	native, C3, perennial
Panicum effusum	Hairy Panic	native, C4, perennial
Paspalum dilatatum	Paspalum	naturalised, C4, annual
Rytidosperma caespitosum	Ringed Wallaby Grass	native, C3, perennial
Setaria parviflora	Slender or pale Pigeon-grass	naturalised, C4, perennial
Setaria verticillata	Whorled Pigeon Grass	naturalised, C4, annual
Vulpia bromoides	Squirrel tail fescue, Silver Grass	naturalised, C3, annual
Dicots		
Hirschfeldia incana	Hairy Brassica, Buchan weed	naturalised, C3, annual/biennial
Conyza sumatrensis	Fleabane, Tall Fleabane	naturalised, annual
Hypochaeris radicata	Catsear, Flatweed	naturalised, annual or perennial
Malva parviflora	Small-flowered mallow	naturalised, annual or perennial
Medicago polymorpha	Burr Medic	naturalised, annual
Modiola caroliniana	Red Flowered Mallow	naturalised, annual or perennial
Plantago lanceolata	Lamb's Tongues, Plantain	naturalised, C3, perennial
Polygonum aviculare	Wireweed	naturalised, C3, annual or biennial
Trifolium arvense	Hares-foot clover	naturalised, C3, annual
Trifolium campestre	Hop trefoil, Hop clover	naturalised, C3, annual
Trifolium subterraneum	Sub-clover	naturalised, C3, annual
Vitis vinifera	cv. Shiraz	naturalised, C3, perennial

Amongst the dicots, *T. subterraneum* was the species with the most recognised agronomic value amongst a range of mostly common naturalised weeds, and represented the main legume component at Site BA and RH. Some plants were also found at Site AN, but appeared to decline in number through subsequent seasons of the study. At Site AN, *T. arvense* and *T. campestre* were also present, although they were short lived and appeared quite sensitive to water deficit as the mid-row soil dried. The perennial herb *P. lanceolata* represented the main non-grass leaf area through the season at two vineyards (BA, RH). The other species listed in Table 6.6, which are all common across the region, were found in smaller numbers.

6.2.2 Spring 2020 soil coring – single root sequencing and mid-row root length density

The main reason for undertaking the soil coring in spring 2020 was to collect root samples that could be used to validate molecular methods for species identification, and then to provide separate estimates of grapevine and non-grapevine root length density (RLD) for each depth increment using the molecular identification to confirm visual identification.

Criteria used to select probable vine roots prior to sequencing were based on darker colour due to lignification, larger diameter and branching characteristics (Figure 25). These criteria have been largely confirmed by the sequencing undertaken to date with no roots initially assessed as grapevine subsequently found to be another species. In identifying the other root species, either from comparison with sequences from the leaves of the reference plants, or from GenBank for some species not in the collection, it also provided an initial insight into the early competition for soil water.



Figure 25 Example images of individual roots, ITS2 based species identification and depth of collection from Spring 2020 soil coring. **(a)** *Vitis vinifera* 80-90 cm **(b)** *Vitis vinifera* 90-100 cm **(c)** *Vitis vinifera* 90-100 cm **(d)** *Trifolium subterraneum* 90-100 cm **(e)** *Paspalum dilatatum* 90-100 cm **(f)** *Festuca arundinacea* 70-80 cm **(g)** *Trifolium subterraneum* 80-90 cm **(h)** *Phalaris aquatica* 90-100 cm **(i)** Avena *barbata* 90-100 cm.

For the example roots shown in Figure 25, three *V. vinifera* roots are compared to those of *T. subterraneum* and *A. barbata* from Site BA, *P. aquatica* from Site RH and *P. dilatatum* and *F. arundinacea* from Site AN. The presence of annual species roots at 90-100 cm at Site BA, where the

vines were just past bud-break when the coring was undertaken, demonstrates a capacity of relatively short lived annuals to attain access a significant proportion of the plant available soil water before the vines have established a canopy. Although there are further samples in storage to be sent for sequencing, none of the light coloured roots sequenced to date have been identified as *Vitis vinifera.*

To determine grapevine root length density, the total root length from each was first recorded for the scan of each depth increment. Adobe Photoshop with manual image editing was then used to produce a second version of each scan with non-vine roots removed according. This was done with the visual criteria above, but could be undertaken with machine learning based image processing in the future.

The distribution of roots with depth was similar at all sites, with the highest RLD in the 0-10 cm increment, and then decreasing exponentially with depth (Figure 26). The 0-10 cm increment RLD of 8.5 cm/cm^3 at Site AN is comparable to values obtained by coring of mixed grazing pastures in NSW (Lodge and Murphy 2006). However, the values of 21 and 16 cm/cm^3 for Site BA and RH are high relative to most reports in the literature for crop or pastures species.

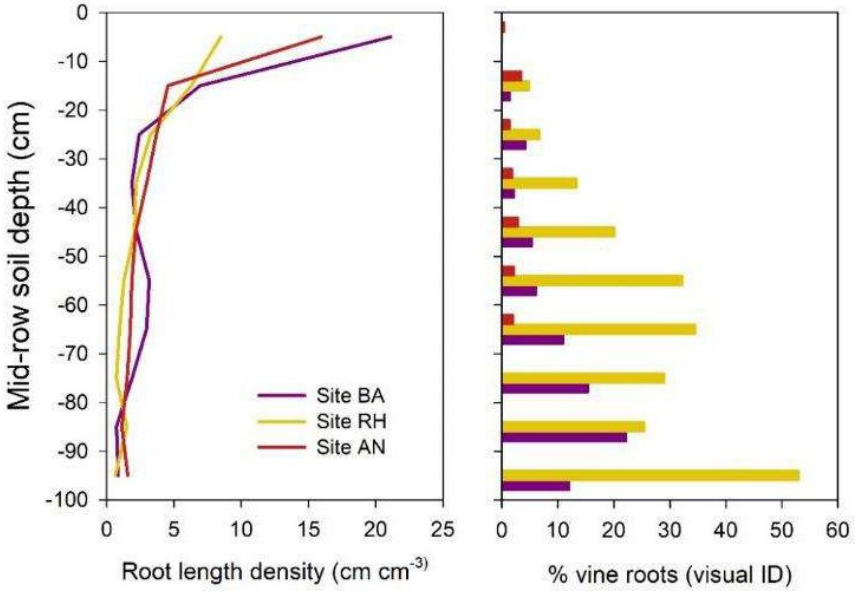


Figure 26. Mid-row **r**oot length density and relative proportion of grapevine roots by depth for the October 2020 soil coring.

At Sites BA and RH, the proportion of grapevine roots increased with depth, with none visually evident in the top 20 cm, and only a small percentage in the top 30 cm. Although grapevine roots did not represent the majority of root length at any depth, they increased in percentage down the profile. Site RH had more grapevine root exploration of the mid-row than BA. Visually, the grapevine roots at this depth were thicker than any of the cover crop roots, and would probably have represented a higher proportion of the total root biomass if this had been recorded. At Site AN, very few grapevine roots could be visually observed at any depth.

In studies across a range of species a RLD of 1 cm/cm^3 has been used as a threshold for extraction of plant available soil water (e.g. White *et al.*, 2015; Zhang *et al.*, 2020), and in turn based on earlier calculations of 0.5 to ~ 1.5 cm/cm^3 (Passioura 1983). Below this value, water extraction is related to

RLD, but above this value there appears to be no additional benefit of extra root length. The RLD of sites was there well above the value needed to extract all the soil water, and only fell below the threshold for the two lower depths at Site BA and three of the four lower depths at Site RH.

6.2.3 Transect root distribution mapping

For the transect coring undertaken in 2022, the same relative pattern of grapevine and non-grapevine roots in the mid-row cores was evident, with the proportion of larger diameter and lignified grapevine observed to increase with depth. Due to the large number of images, manual processing to provide separate vine and non-vine RLD values has not yet been undertaken. However, a preliminary processing pass for total root length and average root diameter has been made for transect maps of total RLD and comparison with the results from the 2020 sampling.

For the mid-row core position at Site BA, the distribution of RLD down the profile had changed little from the coring undertaken 28 months previously (Figure 27). At Site RH, RLD had increased by 2022 in the top 10 cm, but remained the same as the 2020 sampling down to 100 cm. This change in root length density at the surface could reflect the difference in the size of the ground cover plants, which were closely grazed coming out of winter in 2020, but actively growing and not recently mown in 2022. Site AN was the only site with a change in RLD between dates. The reason for this is not clear, but the relative distribution down the profile was remained similar.

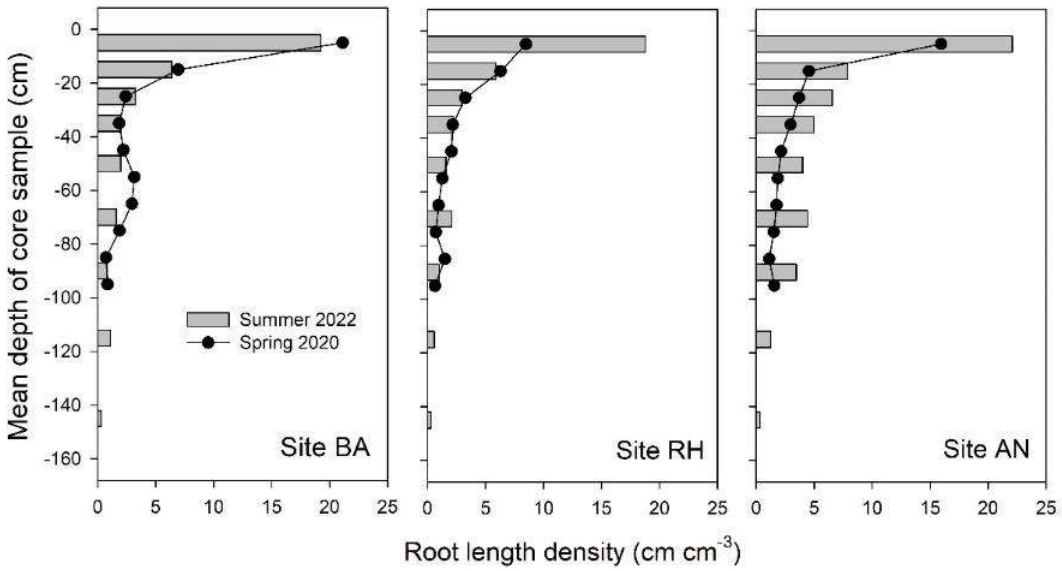


Figure 27. Comparison of root length density values obtained from the mid-row soil coring to 1 m in spring 2020, and to 1.6 m in summer 2022.

The 2022 coring also confirmed the observations during access tube installation in 2019 that roots did extend to the lower depth of soil moisture monitoring, with roots recorded by visual assessments to the lowest depth of 150 to 160 cm in most of the cores as they were collected. In the transect RLD profiles there was a consistent pattern across sites with high RLD under the mid-row ground cover at 100 and 150 cm from the vine row, and lower RLD under the vine row (Figure 28). This was irrespective of undervine management practice, with RLD higher in the vine row at Site AN where herbicide was used than at Site BA that had 100% ground cover. The RLD at 50 cm was intermediate to the vine row and 100 cm position. The distribution of roots down and across the soil profile was very similar at Sites BA and RH, with a gradient of high RLD immediately under the mid-row that decreased vertically and diagonally towards the lowest vine row depth. Many of the vine row and deeper RLD values were less than 1 cm/cm^3 at these two sites, suggesting a significant volume of soil where water

extraction rates may have been limited by the density of roots (Passioura 1983).

For Site BA, if an assumption is made that the soil water holding capacity remains the same from vine row to mid-row, then about 140 mm of the estimated total profile sum of 250 mm is in deeper soil layers where extraction may be limited by low RLD. If the proportion of grapevine roots is higher at these depths, they may at least be able to compete for a greater share of this water. In the very high RLD zones under then ground cover plants, which also lose water to evaporation, grapevine roots would appear to have little chance of competing for water. The spatial and temporal differences between the soil moisture readings and RLD measurements limit the ability to make a direct comparison between the rate of water uptake and length of roots present at each depth increment. However, in the upper soil layers there was a large difference between RLD in the vine row compared to mid-row, whereas the rate of water extraction was similar in both.

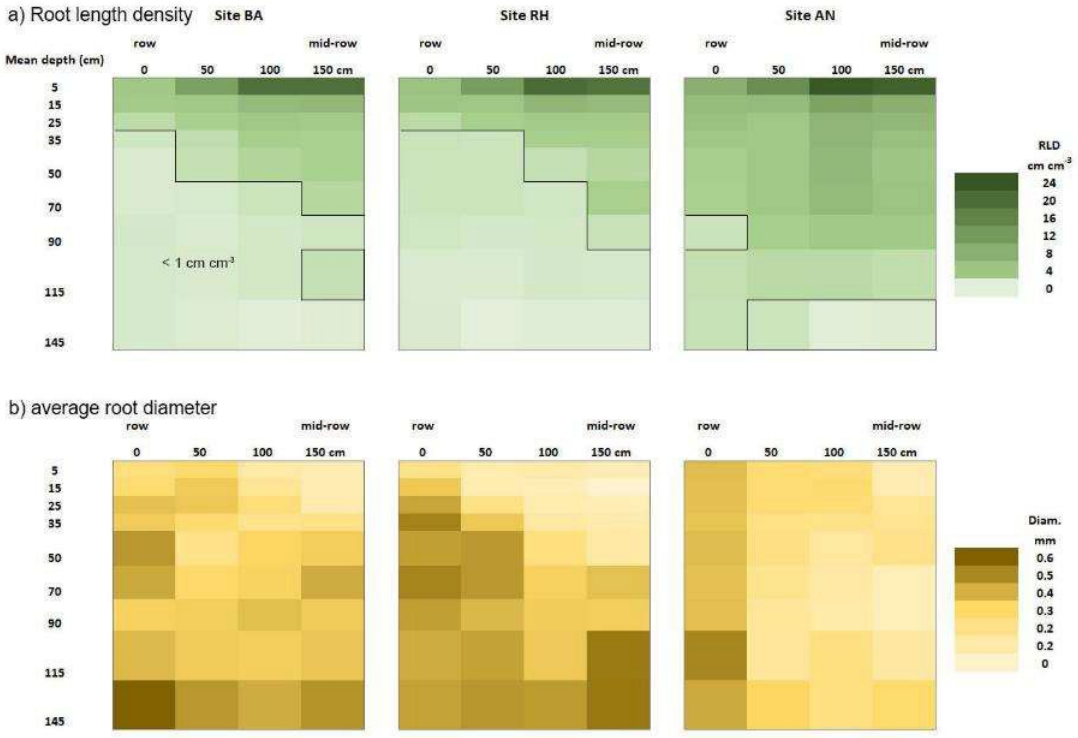


Figure 28. Preliminary summary of root length density (a) and average root diameter (b), obtained from transect coring from the vine row to centre of mid-row in January and February 2022.

At Site AN there was a clear visual alignment of darker grapevine roots with the vine row (Figure 29), and few obvious vine roots in the mid-row cores. There were also fewer locations with RLD values below 1 cm/cm^3, with either high vine RLD down the profile under the vine row, or high RLD of the mid-row ground cover continuing deeper than seen at the other sites. This matches the observation for the mid-row cores from AN in the earlier 2020 sampling where few grapevine roots were observed in the mid-row. A possible reason for this root distribution pattern is that the higher irrigation at this vineyard, and possibly the lack of competing ground cover, has aligned the root distribution with the vine row. As the mid-row includes the winter active *F. arundinacea*, this may also be an interactive effect of earlier drying of the mid-row reducing the opportunity for later vine root growth into the mid-row. Wheel compaction, or an interaction of wheel compaction with earlier soil drying may also create a barrier to grapevine roots crossing directly through surface soil layers.

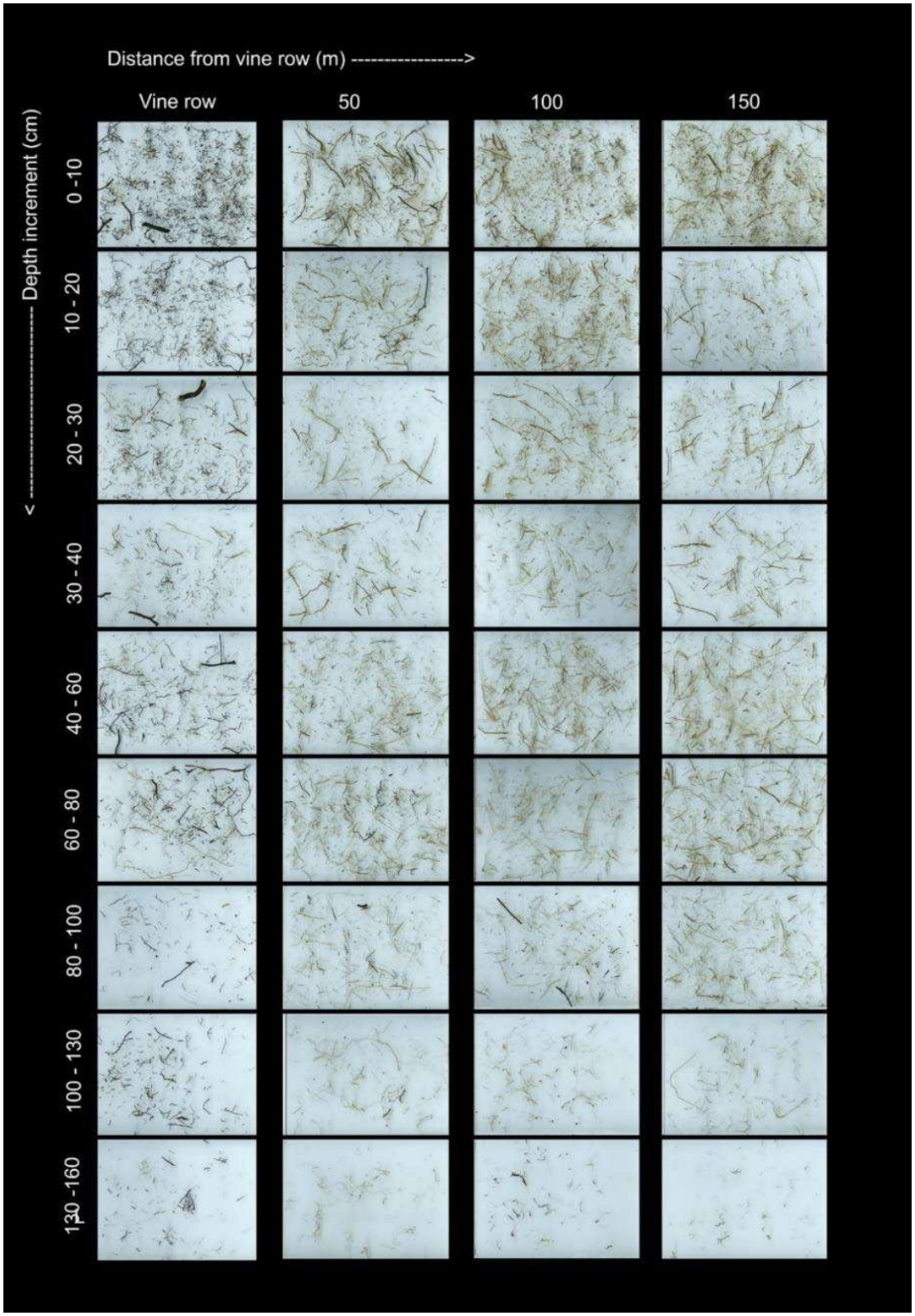


Figure 29. Example of one of the four transects from Site AN, showing the scanned root images arranged by distance from the vine row and depth.

6.2.4. Root growth and soil structure

During the processing of samples from the two rounds of soil coring, some observations of core samples were made as the cores were broken into smaller pieces prior to washing (Figure 30). Although not an in-depth study, these observations provided some further insight into how effectively the roots of grapevines and other species can access water in the profile.

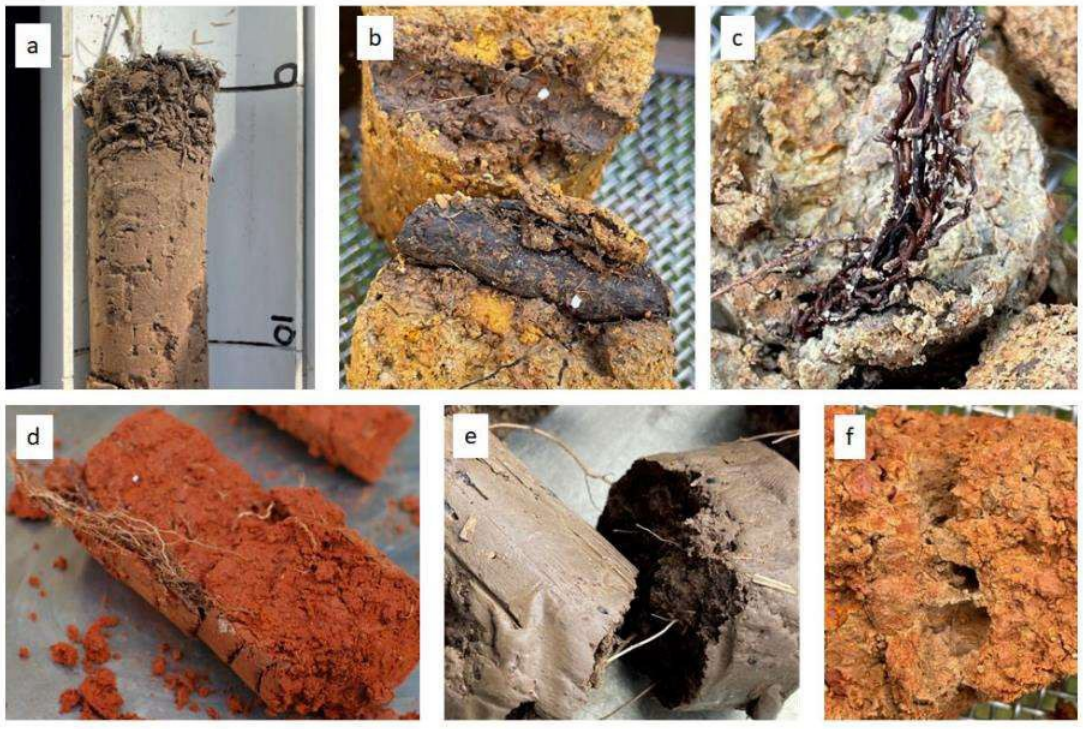


Figure 30. Example images of roots in core samples found prior to washing from Site BA (a,c,d,f), Site RH (b), and Site AN (e).

In the 0-10 cm increment there were large numbers of roots distributed evenly through the core sample, and this was similar to 30 or 40 cm. Progressing further down the profile a number of examples were found where roots had followed biopores or other lower resistance pathways, and where multiple roots followed the same path. Some of these appeared to be of the same species, while others were clearly difference species. In other cases, small roots followed larger structural grapevine roots. At Site AN, roots did not appear to have difficultly growing through clay where there were no obvious pores. Some macropores were also observed without roots. The RLD values calculated for these sites are average for each core samples, but these observations show that these roots are not necessarily uniformly distributed deeper in the profile. Relating RLD values to water uptake potential may therefore provide an over estimate of the extraction within that soil layer. However, such pores may also enable deeper root growth.

From the 1.6 m soil cores collected from the vine rows in Autumn 2023, bulk density and oven dried water content were recorded for each of the 10 cm depth increments (Figure 31). The main purpose of this coring was to collect samples for particle size analysis, and with the initial bulk density measurement, allow a calculation of volumetric water content. Detailed photographs were also taken of each depth increment to assist with a full soil characterisation. Both of these are still in progress. At all vineyards the bulk density was lowest for the surface 0-10 cm, ranging from 1.1 to 1.3 g/cm^3, and increasing through the 20 to 40 cm depths. At Site BA and RH, the bulk density was then

relatively constant down to the depth of 1.6 m. At Site AN the bulk density increased after 1.2 m, and at 1.5 m approached a value of 2 g/cm^3 which might be expected to limit to root growth. However, roots were observed at this depth, and the soil water content measurements show a contribution to the total available water (Figure 14). For bulk density values at the other sites, there was also no evidence from transect soil coring data that these were sufficient to limit root growth.

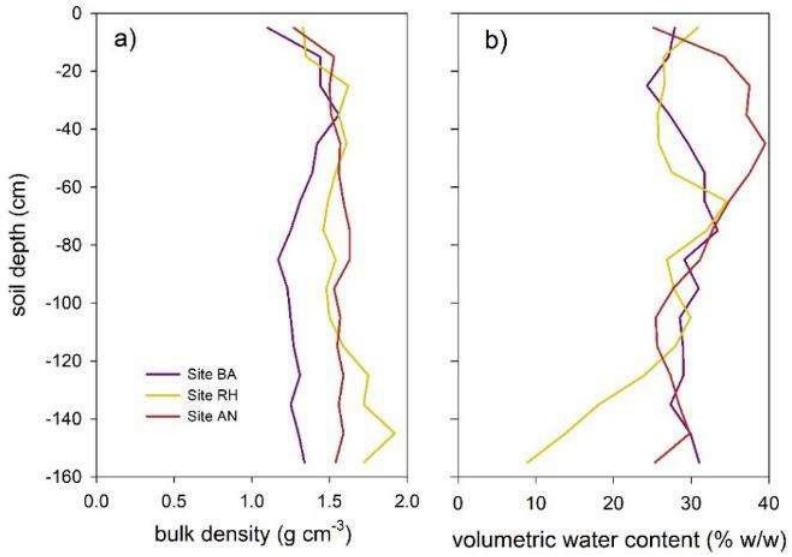


Figure 31. 2023 vine row coring summary showing bulk density (a) and volumetric water content (b) on 24 May (Site BA), 30 May (Site RH), and 31 May (Site AN).

When the coring was undertaken in late May 2023, Site BA and RH had only received enough rainfall to begin recharging the upper part of the profile. For Site BA, down to about 80 cm, and at Site RH, down to about 70 cm. Below this depth, the soil was still as dry (based on the capacitance probe readings) as the lower limits reached through March and April where stem water potential measurements had reached the strong and severe water stress ranges. While water remaining in these layers may have all been too tightly bound for roots to extract, the oven dry values show a surprisingly high residual water content. With the low RLD values at these depths, and roots not necessarily uniformly distributed through the soil, this raises a future research question about how much extractable water is being left behind by grapevine roots. Potentially, during extended drought, a progressive loss of this more tightly held water and a loss of some buffering that may be available when soil is first dried down from a full profile.

6.2.5 Plant root species identification by next generation sequencing

The sequencing undertaken on the MiSeq platform ITS-An5 and ITS-An4 angiosperm primers of Cheng *et al.* (2016) was successful in identifying root species that matched the plant species previously identified in each vineyard. As a preliminary summary of a large amount of data, Figures 32-34 show the relative abundance of OTUs for each sample within species, and corrected for core volume differences within the nine depth increments. The relative abundance of DNA between species could vary for number of reasons other than differences in root biomass, including growth stage of the roots and DNA extractability, so the most confident comparison between species is presence of absence at a given position. At this stage the bioinformatics summary is also based on publicly available NCBI database sequences rather than the reference samples collected from the vineyard.

At Site BA (Figure 32), grapevine DNA was identified in all depth increments in the vine row and mid-row cores, although in relatively higher abundance in the mid-row. The only exception was the 0-10 cm increment in the mid-row where almost no *Vitis* DNA was found.

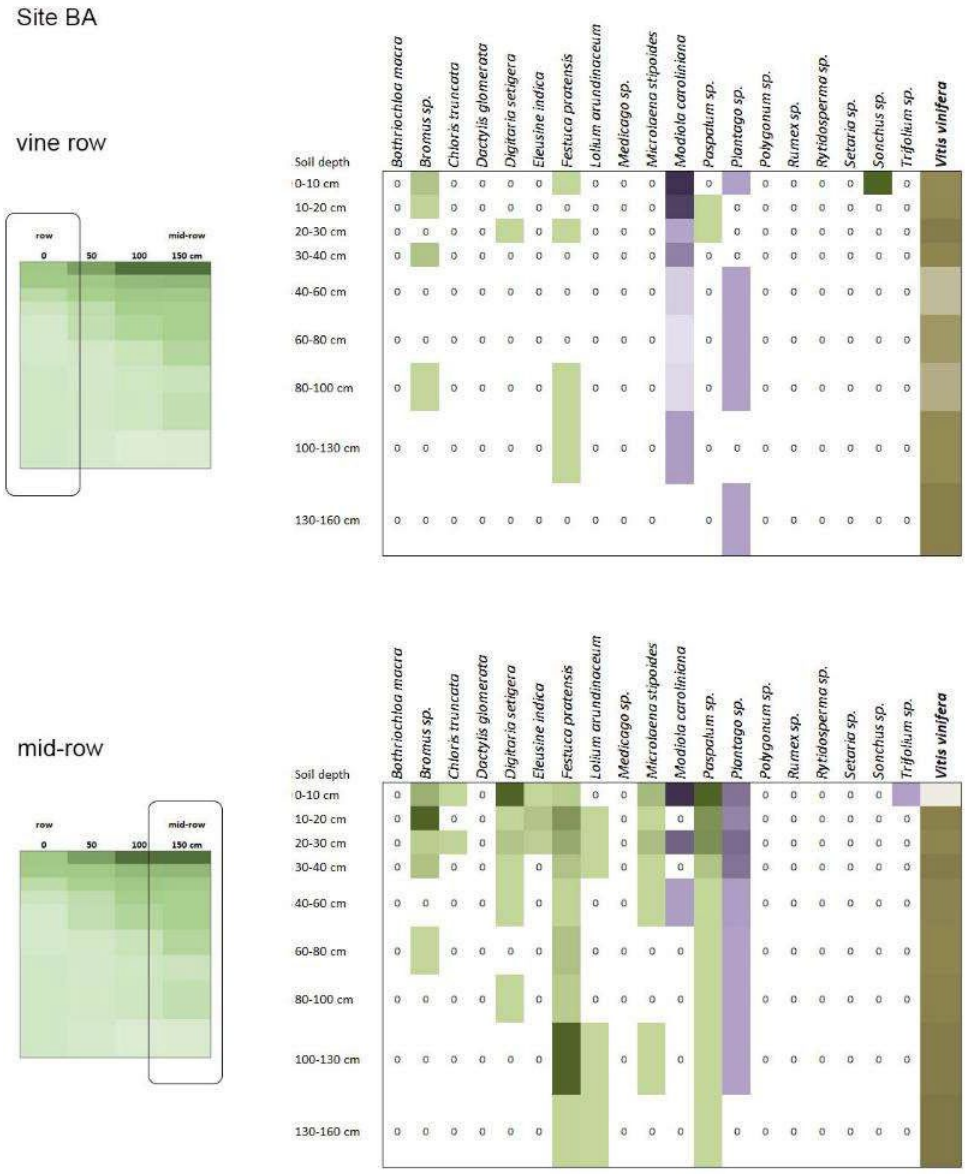


Figure 32. Summary of relative abundance data for root species DNA in the vine row and mid-row depth increments at Site BA. Monocot, dicot and grapevine roots indicated by green, purple and brown colour scales respectively. Lower abundance species are not shown.

For the ground cover roots, most of the species identified were perennial grasses. Roots from *Lolium arundinaceum* (syn. *F. arundinacea*) and P. *dilatatum* were found to the lowest depth increment, as well as *Festuca pratensis* which was not one of the plants identified in the vineyard. Possibly *F. pratensis* is being identified as *F. arundinacea* with the shorter NGS read lengths, but further sequencing may be required from the vineyard to confirm this. The main non-grass DNA from the

ground cover plants came from *P. lanceolata* and reached the 100 to 130 cm depth increment. The other species were *M. caroliniana*, which was more abundant under the vine row, and *T. subterraneum* where DNA was found just at 0-10 cm. In the vine row, DNA from *M. caroliniana* was found from the surface to the 130 to 160 cm depth despite being a relatively small plant above ground.

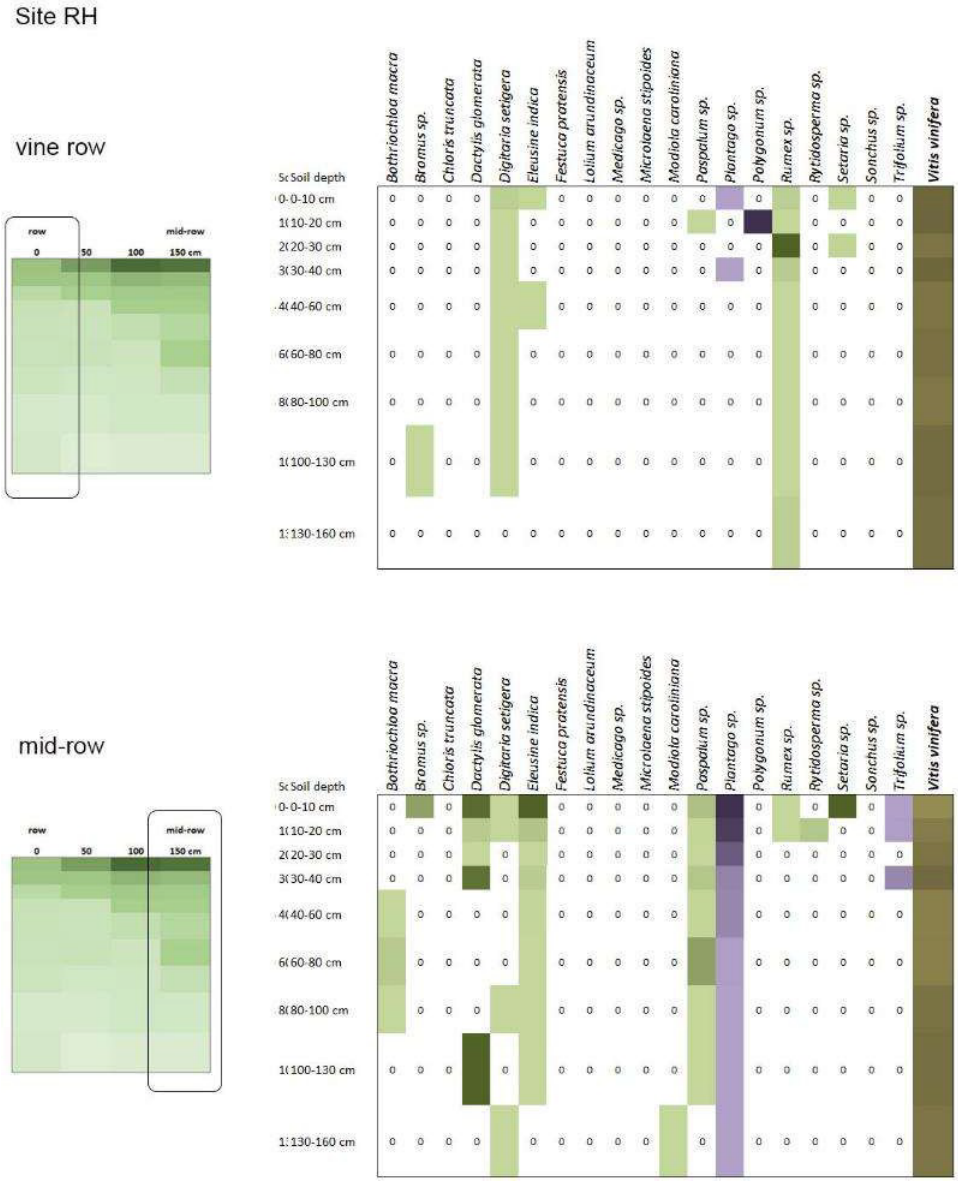


Figure 33 Summary of relative abundance data for root species DNA in the vine row and mid-row depth increments at Site RH. Monocot, dicot and grapevine roots indicated by green, purple and brown colour scales respectively. Species with low DNA abundance across all sites are not shown.

At Site RH, grapevine DNA was also present in all depth increments of the vine and mid-row tubes, although with similar relative abundance compared to Site BA, which had more grapevine DNA in the mid-row (Figure 33). For the ground cover species in the mid-row, *P. dilatatum* and *P. lanceolata* had roots between 100 and 160 cm. No *L. arundinaceum* was present at the site, but between the native

B. macra, and introduced *E. indica*, *D. setigera* and *D. glomerata*, the full profile was covered by the roots of other grass species.

Site AN showed the most distinct difference between the vine row and mid-row cores, with abundant grapevine DNA in all depth increments under the vine row, but relatively few other species (Figure 34). Although not providing a clear species match with the NGS, *P. aviculare* was present in some locations under the vine row and was probably the species identified from the root samples. Although not present under the vine row, *L. arundinaceum* plants dominated along the edge of the mid-row where the DNA results suggest they could grow roots laterally to access water from the irrigation. Some DNA from *B. macra*, which grew further into the mid-row, was also present.

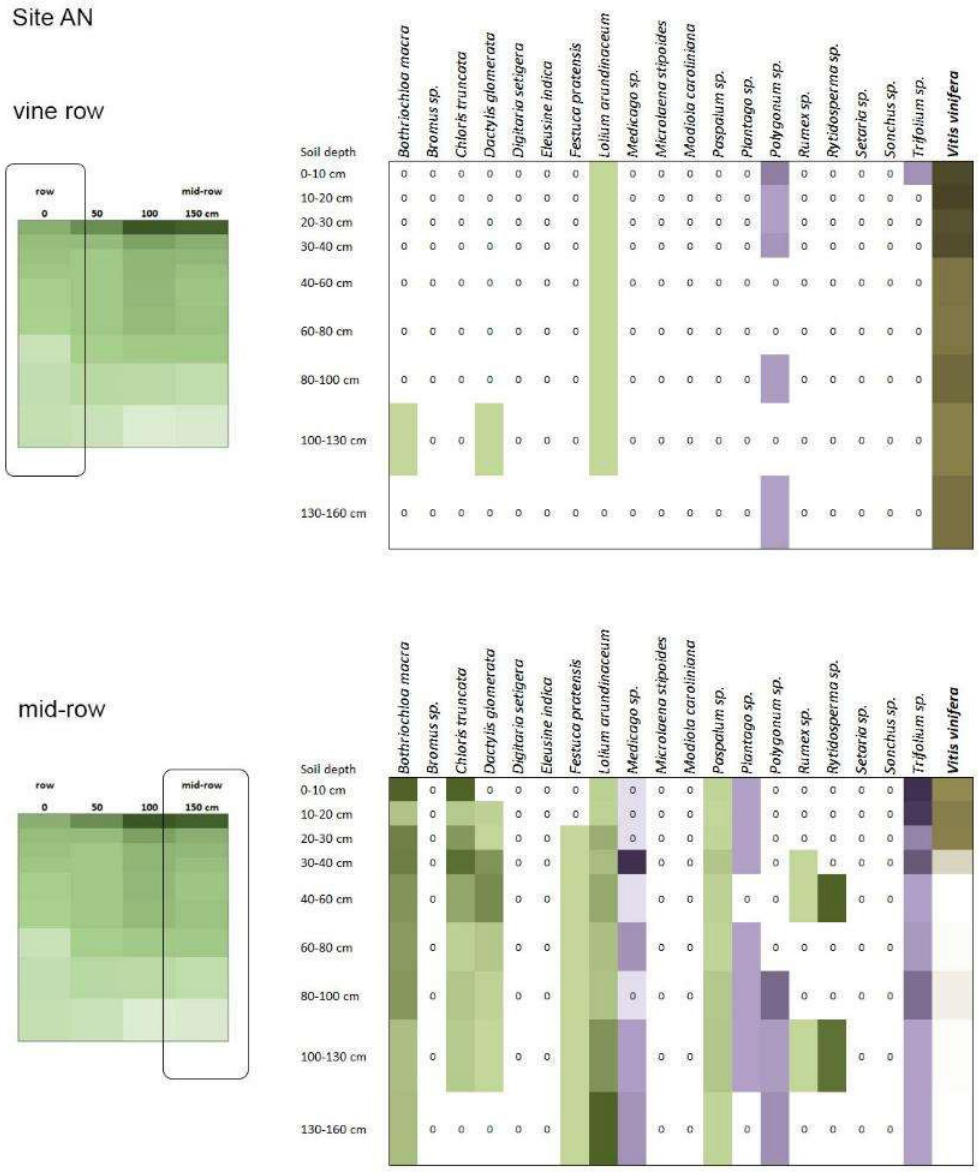


Figure 34 Summary of relative abundance data for root species DNA in the vine row and mid-row depth increments at Site AN. Monocot, dicot and grapevine roots indicated by green, purple and brown colour scales respectively. Species with low DNA abundance across all sites are not shown.

Grapevine DNA was only found in higher amounts in the upper 30 cm of the mid-row, but was essentially absent below this depth. This matches the observation from the earlier coring undertaken in 2020. In contrast to grapevine DNA, there was abundant DNA from multiple grass species that reached the full depth of the sampling. The main component of the mid-row cover was *L. arundinaceum, B. macra*, and in some places *P. dilatatum* and the DNA results suggest they also dominated the soil under the mid-row. Roots of several other perennial grasses were identified. Amongst the dicots, the main species present were the legumes *T. subterraneum*, *T. striatum* and *M. polymorpha,* although they did not appear very abundant at the surface at this time of year. Some DNA from *P. lanceolata* was also identified, but it is not clear where this had come from, as there were no plants of this species in the immediate vicinity at the time of sampling. Again, this could be a result of a mis-classification given the shorter NGS read length, and some further work is required to compare sequences from the NGS with the longer Sanger sequencing reads from the reference collection.

One final point regarding the difference between the visually classified roots and the molecular results is that any new white grapevine roots would be missed with the visual classification, and could explain the presence of grapevine DNA in the surface layers where there were few lignified grapevine roots. This may have minimal implications for water uptake given the overwhelming dominance of ground cover roots, but it could be important for nutrient uptake if it gives access to the more fertile surface layers or access to nitrogen that may not make it past the ground cover roots to deeper soil layers.

6.2.6 Microbial profiling

The sequencing and bioinformatics for the microbial profiling have only just been completed at the time of writing so the results presented here are a very preliminary and high level summary of taxa differences in relation to site, floor management practice and soil depth. For 16S, taxonomic classifications have also just been presented for the Greengenes database. A complete set of 36 samples for both ITS and 16S were sequenced successfully for Site BA. For Site RH, 32 samples for ITS and 34 16S were sequenced successfully, and 33 for both regions for Site AN. At the family level, over 200 fungal taxa were identified across the three sites, and over 100 bacterial taxa. Phylogenetic trees for both are presented in Appendix A and B.

The bacterial taxa present were similar within the family and genus levels across the three sites. Differences between sites were the result of a shift in relative abundance of these taxa. Figure 35 shows the combined data for the three vineyards, with the width of each graph segment reflecting the proportion of the 103 samples where each taxon was found, and the bars within each segment, the absolute abundance on a percentage basis. The three smaller plots show the same data on an individual vineyard basis.

The most abundant taxa were from the orders *Nostocales*, the cyanobacteria, and *Acidimicrobiales* but were mostly not identified to further (Appendix 5a). At the family level the most abundant taxa across the three sites were the *Bradyrhizobiaceae*, *Hyphomicrobiales*, *Rhodospirillaceae*, *Ktedonobacteraceae* and *Rhizobiaceae*.

Within the *Bradyrhizobiaceae* the main genus present was *Bradyrhizobium*, which is most commonly associated with symbiotic nitrogen fixation in soybean, a plant species not present in these vineyards, but also one of the dominant taxa in soils across the globe (Delgado-Baquerizo *et al.,* 2018). The genus has also been reported to be found with the associative nitrogen fixing bacteria *Hyphomicrobiales* (Sarao *et al.*, 2024), which is consistent with the results from the present study where this family was also found in high abundance in association with roots. The *Rhodospirillaceae* includes the genus *Azospirillum*, which was present here in small numbers, and in turn includes growth promoting free-

living nitrogen fixing species of found in association with grasses (Steenhoudt and Vanderleyden 2000). There is very little information regarding the role of *Ktedonobacteraceae* in soils, and even less with roots, but they are globally distributed, have been identified in vineyards and in association with grasslands (e.g. Hendgen *et al.,* 2018, Gschwend *et al*., 2021). The fifth most abundant family was the *Rhizobiaceae*, which includes species that form symbiotic nitrogen fixing associations with legume roots, and the most commonly mentioned group when the benefits of cover crops are discussed. However, from a very preliminary comparison these were not always associated with the presence of root DNA from legume species, and there were few root nodules obvious in the scanned images.

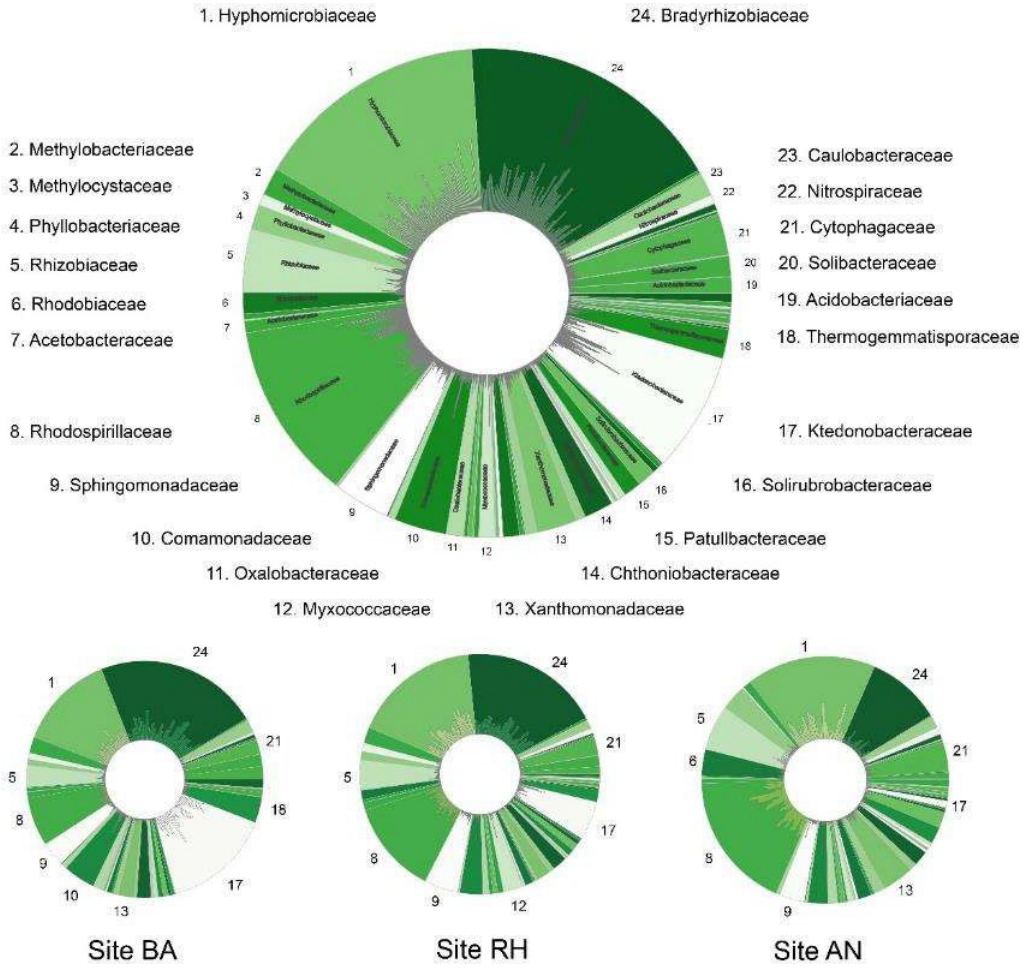


Figure 35 Proportion of bacterial families for the whole data set (top), and separated for the individual sites (bottom). Taxa not identified to family level are omitted.

The principal coordinates analysis (PCoA) of the combined data set showed a separation between soil under permanent ground cover, and soil with either intermittently bare (Site RH) or bare (Site AN) soil under the vine row (Figure 36). This was predominantly associated with a greater abundance of *Bradyrhizobiaceae*, and to a lesser extent *Rhizobiaceae*, *Sphingomonadaceae* and *Comamonadaceae.* The latter two families are both diverse and found across a range of habitats. Within the *Sphingomonadaceae*, the main genus present was *Sphingomonas.* These are generally regarded as

beneficial bacteria, which can occur as endophytes in root tissue and include species that promote plant growth and suppress pathogens (Glaeser and Kämpfer 2014). Within the *Comamonadaceae*, the contribution to the vine-row and mid-row separation was primarily due to differences in the genera *Methylobacterium* and *Methyloligellaceae*, which are plant associated bacteria that utilise methanol from root exudates (Macey *et al.,* 2020).

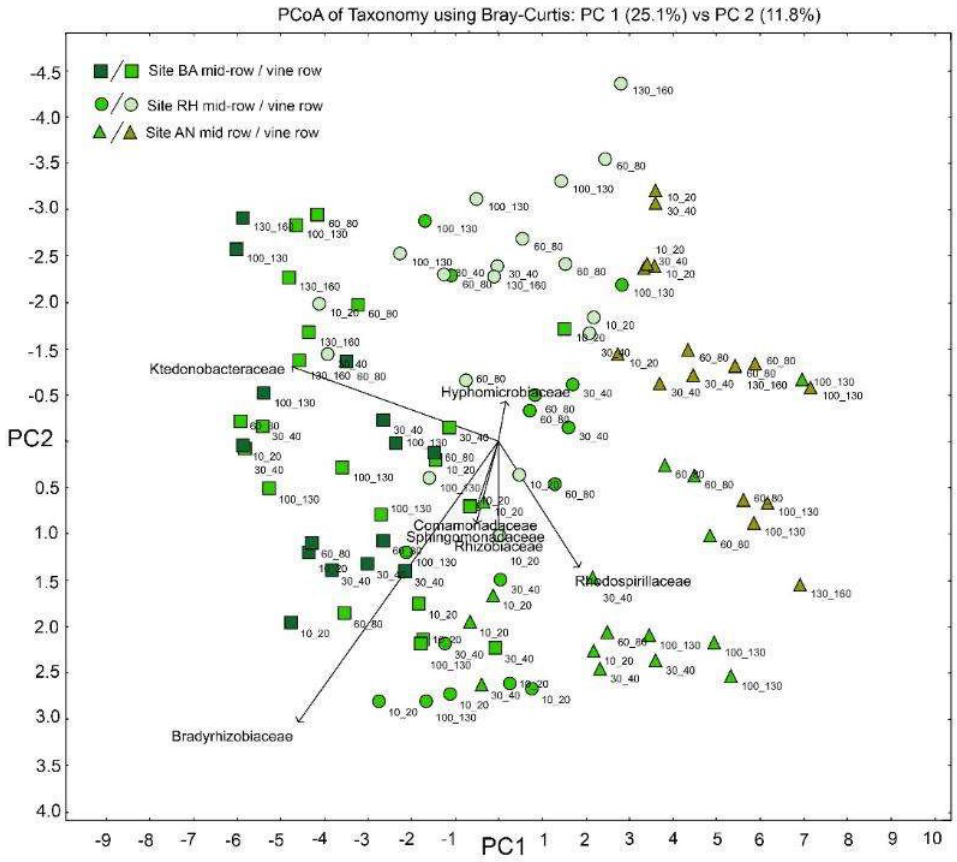


Figure 36. Principal coordinates analysis for family level root bacterial diversity for site and ground cover at the coring position. The labels show the depth of each sample.

For the fungal diversity, the families *Didymellaceae* and *Didymosphaeriaceae* were two of the most abundant taxa across the three sites (Figure 37), both of which belong to the *Pleosporales* order. Both have also been identified on grape berries (Lorenzini *et al.*, 2016). Within the *Didymellaceae*, the predominant genera across the sites were *Epicoccum*, *Phoma*, and *Didymella,* which contain potential plant pathogens. With the *Didymosphaeriaceae* family the two main genera identified were *Paracamarosporium* and *Montagnula.* There is more information in the literature on the taxonomy of this family than their role in association with plants, but it includes shoot pathogens and saprobes. Another abundant taxon was the *Nectriaceae*, with the three main genera *Ilyonectria, Dactylonectria Fusarium.* These are commonly associated with grapevines around the world and contain potential root pathogens, including some associated with young vine decline. In the present study they were identified at all sites with no apparent impact on above ground growth, but the distribution did follow the higher abundance of grapevine roots rather than the roots of ground cover plants.

Amongst some of the beneficial taxa, one abundant family was the *Cortinariaceae,* which contains *ectomycorrhizal* fungi associated with many plants and contribute to nitrogen access and carbon cycling (Liimatainen *et al.*, 2022). Although in lower abundance, roots at all sites were associated with species of the arbuscular mycorrhizal family *Glomeraceae,* which can be particularly important for phosphorus uptake, and are thought to be important for grapevines to compensate for a relative coarse root system. Site RH had the highest abundance of *Glomeraceace*, but they were present at all sites and to all depths in both the vine row and mid-row samples.

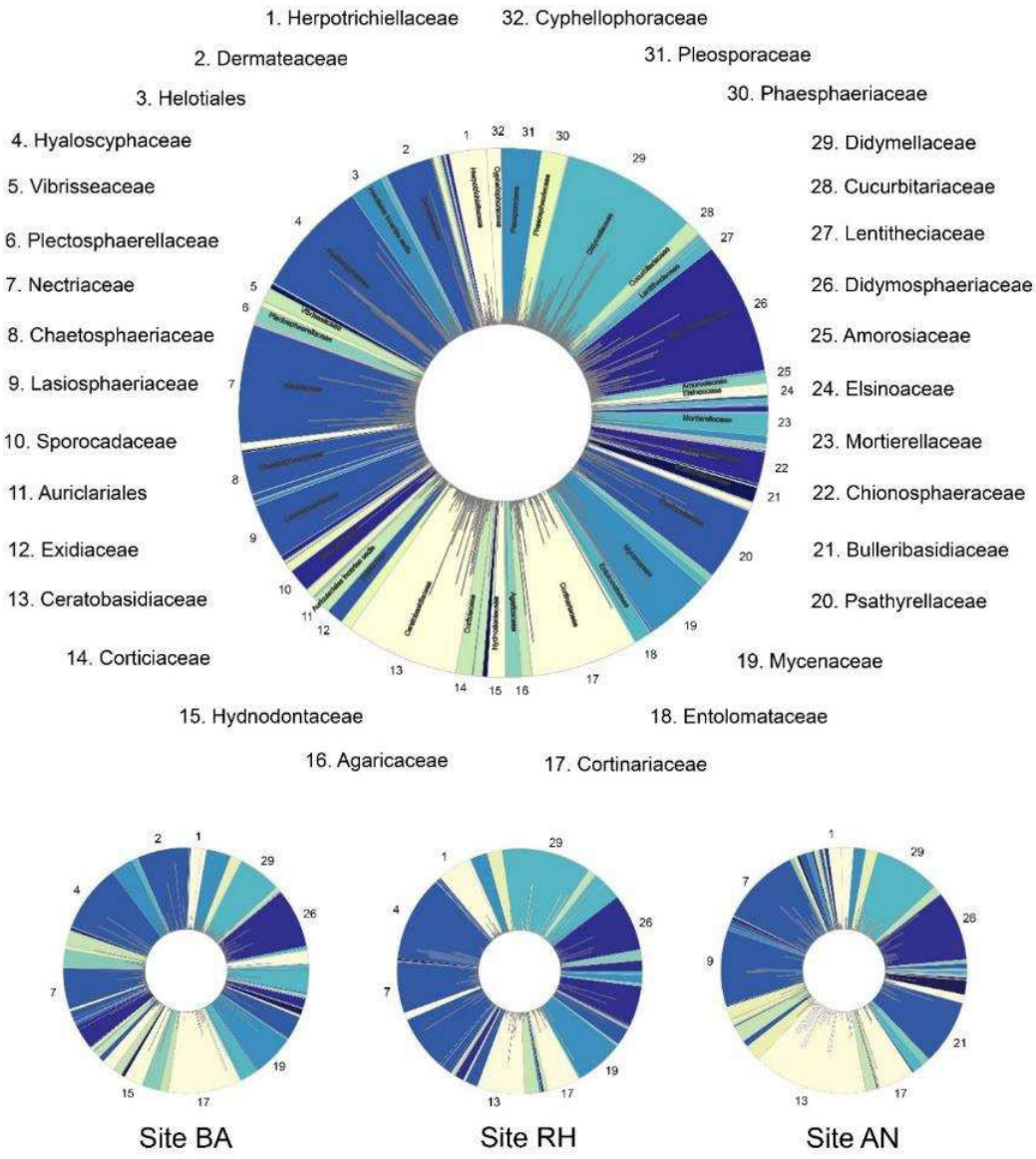


Figure 37. Proportion of fungal families for the whole data set (top), and separated for the individual sites (bottom). Taxa not identified to family level are omitted.

For other individual site comparisons, grouping was associated at all sites with differences in *Didymellaceae* and *Didymosphaeriaceae* which are described previously, the *Hyaloscyphaceae* which are generally saprobes, at Site BA and RH (Figure 38). At Site AN, the *Lasiosphaeriaceae* and

Psathyrella, are both saprobes from the phyllum *Ascomycota*, separated samples from the vine row and mid-row. At Site RH, there was also some separation between sampling positions with saprobes from the *Chaetospaeriaceae*, and *Chionosphaeraceae*, of which there is very little information in the literature.

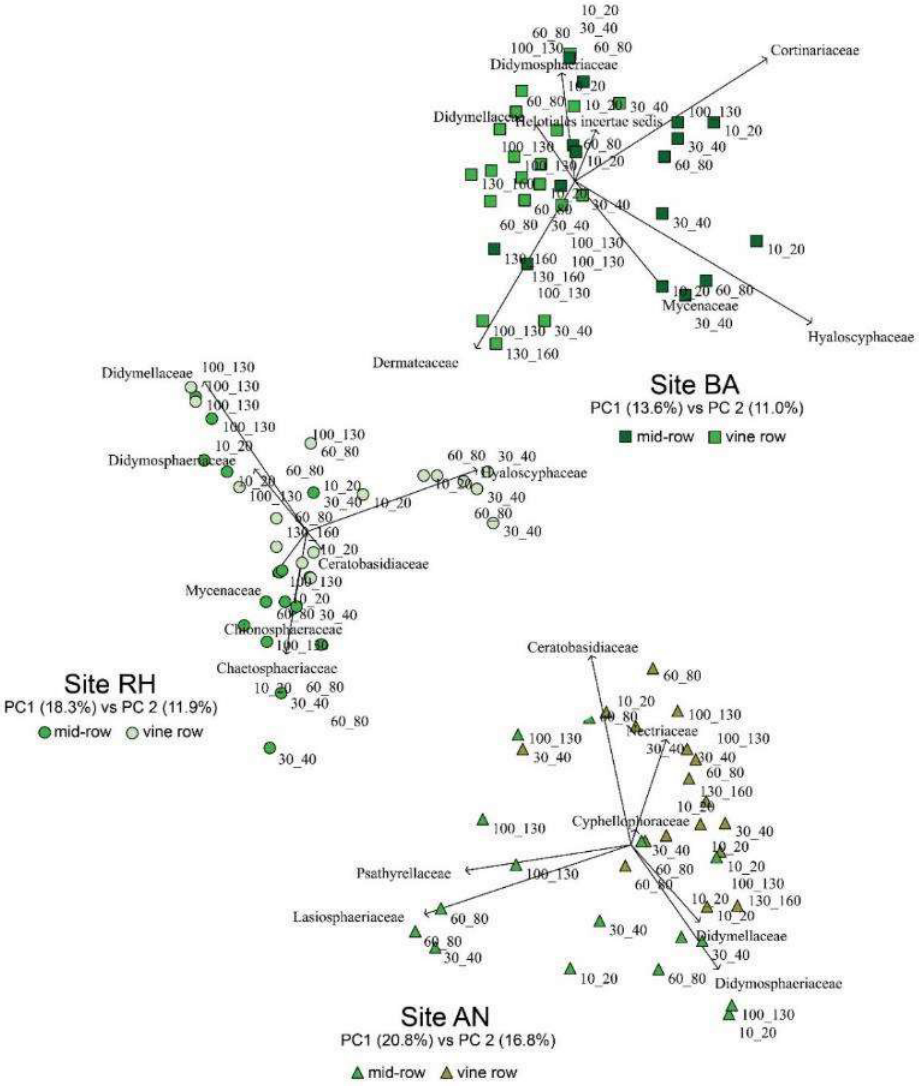


Figure 38. Principal coordinates analysis for family level root bacterial diversity for each site and core position. Labels show the depth of each sample.

For future analysis, the combination of plant species data and root traits with this information will provide a much better understanding of the influence of ground cover management on both bacterial and fungal diversity. In general it is clear that there is an effect, and one that appears to be beneficial in increasing taxa associated with nutrient uptake and cycling. The extent to which these benefit grapevines in relation to the other species making up the vineyard ground cover, and how their associations can be exploited to improve grapevine productivity, could be a key question for further research into sustainable low-input management systems.

6.3 Water use traits of ground cover species

6.3.1 Stomatal conductance survey

During the second half of December 2022, a stomatal conductance survey was undertaken across the three trial sites using a LiCor LI-600 porometer. Some additional measurements were made on the cover crop trial at the CSU Campus. About 380 leaves were measured on the upper and lower surface to compare grapevine leaves with those of the ground cover species (Figure 39). There was no criterion for selection of the ground cover species other than the leaves being green and wide enough for the porometer leaf chamber.

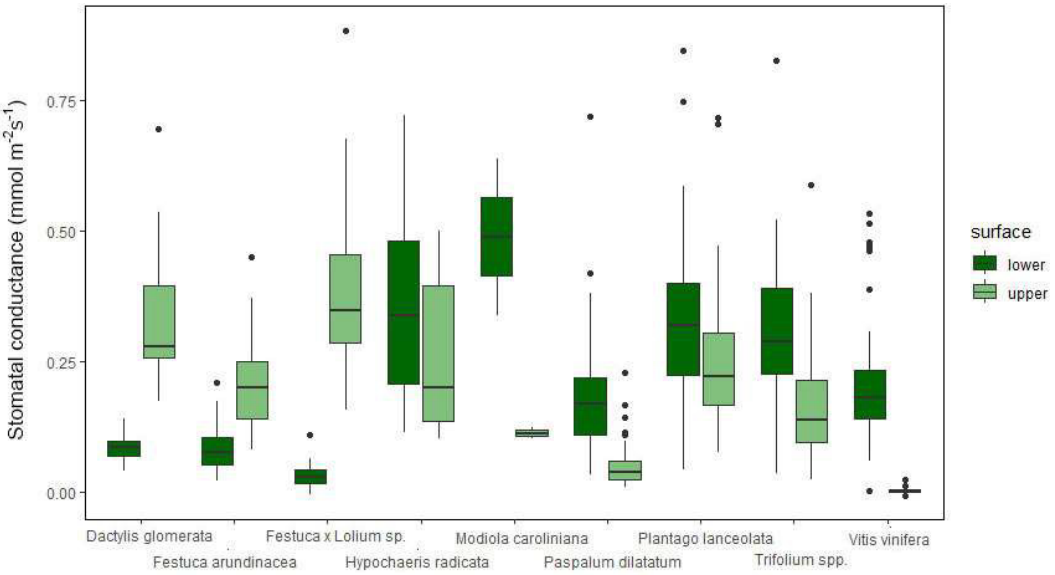


Figure 39. Summary of stomatal conductance measurements made from 14-30 December 2022 on the upper and lower leaf surface of actively transpiring ground cover plant species.

Grapevine leaves had the lowest average stomatal conductance, with the ground cover dicots having the highest values, and the grasses intermediate. While the stomatal ratio varied between them, all of the plants in the study except the grapevines had stomata on both the upper and lower leaf surface. Grapevines only have stomata on the lower leaf surface and this was very evident in the readings with only slight drift in the measurements giving some spread around zero for the upper surface. In the grapevine canopy, where the non-transpiring upper leaf surfaces are mostly facing outwards, water vapour loss will be into the canopy. This contrasts with the ground cover plants, where water is lost from both sides of the leaves.

6.3.2 Ground cover species leaf gas exchange and WUE

Extending the survey to include gas exchange measurements reduced the number of species that could be surveyed to six, due to the larger leaf chamber of the infrared gas analyser. Except for the grapevine, leaves of the other species were placed side-by-side to fill the area of the 6 cm^2 of the chamber, or the leaf area was adjusted in the analyser software where there were still some gaps.

The measurements showed grapevine leaves had the lowest transpiration of the six species at 3.4 mmol H_2O $m^{-2}s^{-1}$ followed by *P. dilatatum* at 3.7 mmol H_2O $m^{-2}s^{-1}$ and the other species between 5.5 and 7 mmol H_2O $m^{-2}s^{-1}$ (Figure 40). These values are consistent with the relative difference in stomatal conductance recorded for these species with the porometer. For photosynthesis rates, the average for the grapevine leaves was 8.7 µmol CO_2 $m^{-2}s^{-1}$ compared to 20 µmol CO_2 $m^{-2}s^{-1}$ and a range of 15.5

to 16.9 µmol CO_2 $m^{-2}s^{-1}$ for the four other species. With high photosynthesis rates and lower transpiration *P. dilatatum* had significantly higher instantaneous water use efficiency (A/E) that the other five species.

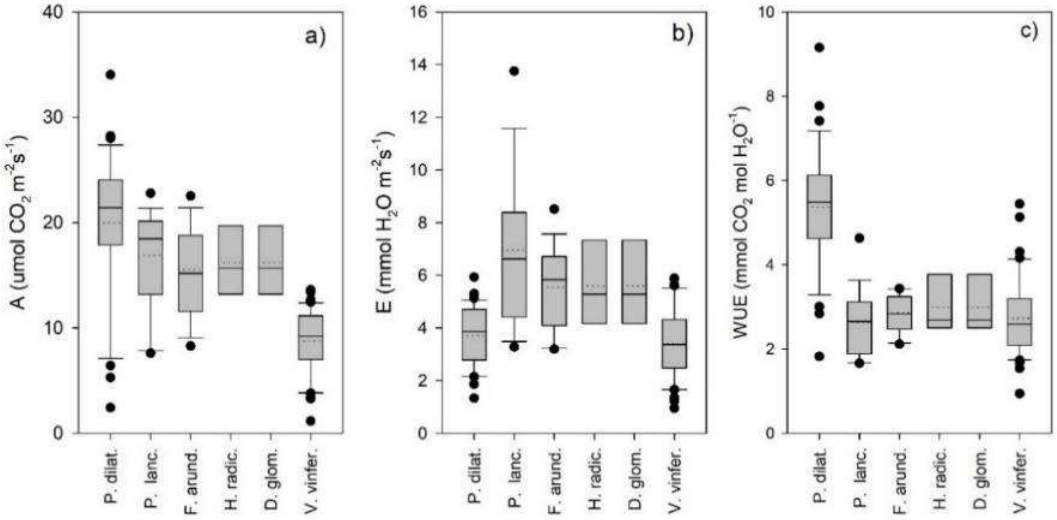


Figure 40. Summary of single leaf gas exchange measurements made for *P. dilatatum* (n=33), *P. lanceolata* (n=18), *L. arundinaceum* (n=16), *H. radicata* (n=3), D. glomerata (n=3) and *V. vinfera* cv. Shiraz (n=43) on over nine measurements dates between 24 November 2022 and 17 February 2023.

The relative differences in gas exchange would shift in favour of the grapevine leaves as the mid-row dried later in the season and irrigation created a larger gradient in favour of vine-row roots. For the earlier measurements in November, the grapevine leaves were also younger compared the leaves of some of the C3 plants which commenced growth in late winter or early spring. However, these measurements provide a snapshot of the inherent differences in gas exchange traits when the plants are growing with adequate soil water.

6.3.3 Ground cover species field plot trial

The field plot trial established on the CSU campus was undertaken with the objective of comparing water use of some of the main species already found in the trial site vineyards (Figure 39). Some of the perennial grasses were transplanted directly from the vineyard given the short study duration, and others sown from commercial seed. One additional species, *L. Maritima*, was included as a flowering plant that can be used to provide support for beneficial insects. The trial was established in spring 2021 with ten 2 x 2 m treatment plots replicated six times, with diviner access tubes in the centre of each; water use measurements were taken through the 2022/23 season. Seven of the treatments were planted, one was kept as a mown control, and two were not used.

The overall experience from the trial was that it was very difficult to establish and maintain plots of a single plant species for very long, particularly in a high rainfall environment on soil with a significant seed bank, and with *Pennisetum clandestinum* (Kikuyu) having been well established in the trial area previously. If an attempt was made to establish a different species in existing vineyards in a similar situation, this experience suggests that it would require significant time, cultivation and / or herbicide use and high seeding rates. Some plots also needed to be netted after seeding to keep out wild ducks and other birds, and there were also problems with sheep and cattle getting through the farm fences. This slowed the initial establishment, but did provide some insight into grazing preferences once the plots had matured.



Figure 41 Photographs showing the mown control (a) and *Paspalum dilatatum* (b) in early March, and *Plantago. lanceolata* (c,d) and *Festulolium braunii* (e,f) in early December 2023 compared the same day as the other pictures.

The two most successful treatments in terms of establishment and persistence were the commercial *P. lanceolata* selection (cv. Ranger, Figure 41c&d) and the fescue and Italian ryegrass hybrid *F. braunii* (cv. Perun, Figure 41e&f). The vineyard-collected *P. dilatatum* (Figure 37b) established very well in the planting season, but *P. clandestinum* and other grasses had grown into the plot by the second season. However, these three treatments had the highest proportion of the intended species, so the decision was made just to focus on these for the soil moisture measurements. The mown control represented a more severe version of the main method of mid-row management in the region.

These soil water measurements showed no difference in the rate of water extraction between species or with the mown control (Figure 42a). Based on very approximate calculations of water use relative to reference crop evapotranspiration (from SILO gridded data) and manually recorded rainfall, the mean crop coefficients ranged from approximately 0.2 to 1.0 with the highest values after rainfall (Figure 42b). Although this was only a small, short term comparison, it did not suggest any difference in water use traits between these species, or that mowing was effective in slowing transpiration if green leaf area was retained under the height of mowing.

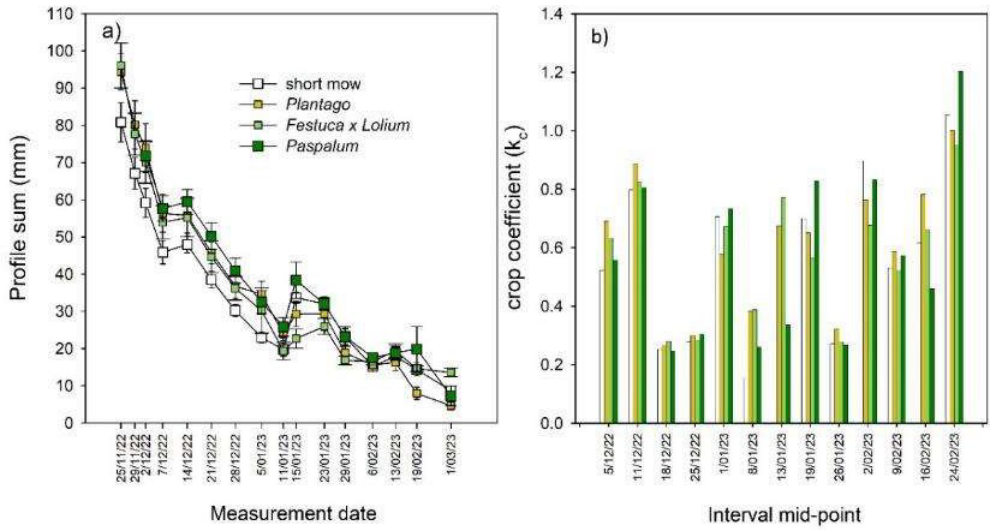


Figure 42. Change in soil water content under each plot as summed from values above the lower reading for each depth increment (a), and water use relative to ETo (b).

Amongst the other species, *L. Maritima* was easy to seed and established and flowered well in the 2021/22 season. However, by the next summer it had reduced to a few plants amongst the species that had been present prior to the trial being established. For native grass species, the commercially sourced *R. caespitosum* established poorly and the plots were quickly dominated by other species and reduced to just to a few isolated plants (Figure 43a). This may have been partly related to seed contamination from another native grass, *Lachnagrostis filiformis*, which was not discovered until flowering and meant that many of the propagated plants were probably not the intended species.



Figure 43. *Rytidosperma caespitosum* (a), *Microlaena stipoides* (b) *Bothriochloa macra* (c)

The other C3 native, *M. stipoides* showed more promise and has been suggested as a good candidate for use in the undervine area due to its shade tolerance, ability to spread laterally from rhizomes, and larger seeds (M. Mitchell, personal communication). In above ground growth it was slower than the introduced species, and therefore was not included in the water use comparison. However, a visual assessment of the plots in the 2023/24 season found it had established well with the additional 12 months (Figure 43b). The C4 native *B. macra* also persisted, but did not grow sufficiently during the study period to fill the treatment plots. However, this species is well adapted to dry conditions, and its ability to form the significant component of a permanent mid-row was clearly demonstrated at

Site AN. Side-by-side with the other main C4 grass, *P. dilatatum*, *B. macra* is less aggressive and starts growth later in the season, and could be the preferred choice if a species is needed to provide competition with summer active weeds in wet seasons.

6.3.4 Ground cover species pot trials

6.3.4.1 Experiment 1

The first dry-down experiment started with the re-watering weights either too close or slightly above field capacity, meaning they sometimes drained. This was partly due to time constraints, and partly due to hot weather in the lead-in period and trying to get an accurate upper limit weight when the plant was rapidly transpiring water from the top of the pot while water was still draining from the soil at ground level. With warm weather, the dry-down treatment also moved out of the readily available water range before the further leaf growth of the control treatment reduced the drainage problem. Consequently, relative transpiration values were very variable and have not been presented.



Figure 44. Example plants for the control and dry-down treatments of *Microlaena stipoides* (a), *Trifolium subterraneum* (b), *Plantago lanceolata* cv. Ranger (c) and *Festulolium braunii* (d) on 31 January 2022.

Despite these challenges, a simple comparison between water use can be made from the control treatment water use measurements from later in the experiment (Table 8). These showed similar water use rates on a leaf area basis, consistent with the earlier comparisons of gas exchange parameters between C3 grasses and dicot leaves. From sub-sampled roots when the plants were destructively harvested, the average root diameter was 0.28 mm and did not differ significantly between the four species.

Table 8. Water use rate per unit leaf area of plants in Experiment 1.

Species	Shoot dry weight (g)	Leaf area (m^2)	Average water use ($L/m^2/d$)
Microlaena stipoides	30.6	0.242	1.33
Trifolium subterraneum	30.2	0.514	1.03
Plantago lanceolata	48.0	0.793	0.95
Festulolium braunii	56.1	0.621	1.08

6.3.4.2 Experiment 2

For the second dry-down experiment, weather conditions were cooler at the start of the experiment and more time was taken to determine a weight as close as possible to field capacity. This enabled more accurate relative transpiration rates to be established, and also gave a period of a few days before the dry-down treatment depleted the readily available water.



Figure 45. Example plants for the control and dry-down treatments of *Paspalum dilatatum* (a), *Bothriochloa macra* (b), *Plantago lanceolata* (c) and *Festuca arundinacea* (d) on 20 February 2022.

Comparing the average water use of the control treatment as shown for Experiment 1, *P. dilatatum* and *B. macra* both had lower rates of water use than *F. arundinacea* and *P. lanceolata* (Table 9). This consistent with the higher WUE of the C4 compared to C3 photosynthetic pathway, and for *P. dilatatum,* consistent with the single leaf measurements made in the field. Some gas exchange measurements were undertaken at the end of the experiment to compare *P. dilatatum* and *B. macra*, and the WUEi values were 4.9 and 5.1 µmol CO_2 / mol H_2O, respectively. This compares to a value of 2.7 µmol CO_2 / mol H_2O for the grapevine leaves. From sub-sampled at the destructive harvested, the average root diameter was 0.29 mm and did not differ significantly between the four species, or from Experiment 1.

Table 9. Water use rate per unit leaf area of plants in Experiment 2.

Species	Shoot dry weight (g)	Leaf area (m^2)	Average water use ($L/m^2/d$)
Festuca arundinacea	33.4	0.397	1.19
Bothriochloa macra	85.1	0.655	0.70
Plantago lanceolata	25.7	0.272	1.18
Paspalum dilatatum	61.6	0.354	0.82

From previous studies with grapevines and other plant species, transpiration starts to slow when approximately 60% of the plant available water has been used (e.g. Patakas *et al.*, 2005). The fraction of transpirable water (FTSW) is a term used to describe what fraction of the total is remaining, which means transpiration would begin to slow at an FTSW of 0.4. This is approximately what was observed in Experiment 2 (Figure 46a), with transpiration slowing to zero through as the lower 40% of available soil water was used. It took more time for *P. dilatatum* and *B. macra* to reach this point due to lower water use, but the relative response was the same.

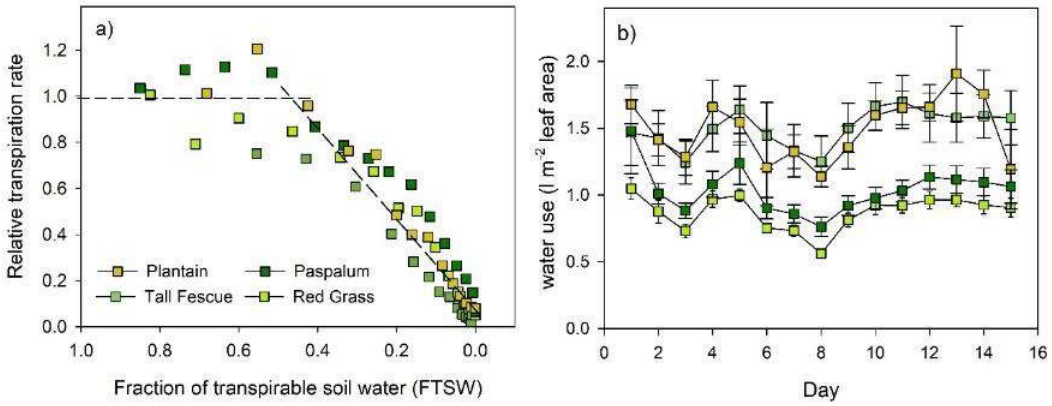


Figure 46. Relative transpiration in relation to soil water content (a) and absolute water use of the control treatment during Experiment 2.

6.3.5 Managing ground cover water use

The standard method for managing vineyard floor vegetation in higher rainfall vineyards is mowing or slashing in the mid-row, and mowing or herbicide underneath the vine row. Cultivation is also sometimes used under the row in organic vineyards, but rarely in the mid-row unless for the establishment of a cover crop or new permanent ground cover.

To assess the impact of mid-row mowing in the current study, a preliminary estimate of the crop coefficient (Kc) was made for each interval between the soil water measurements at Site BA. This was calculated using the change in soil water content between dates, and rainfall and ETo data from SILO to the nearest full day. This could be improved using the trial site weather station, which recorded these parameters at 15 minute intervals, but this was not installed until the start of the 2020/21 season. Each Kc value was then assigned to one of eight classes, describing the condition or management of the vineyard floor vegetation from photos taken at each measurement date (Figure 47). Periods when the soil was at field capacity or above have been removed, but there are still some outliers relating to high rainfall events or possible differences between SILO and actual rainfall at the vineyard.

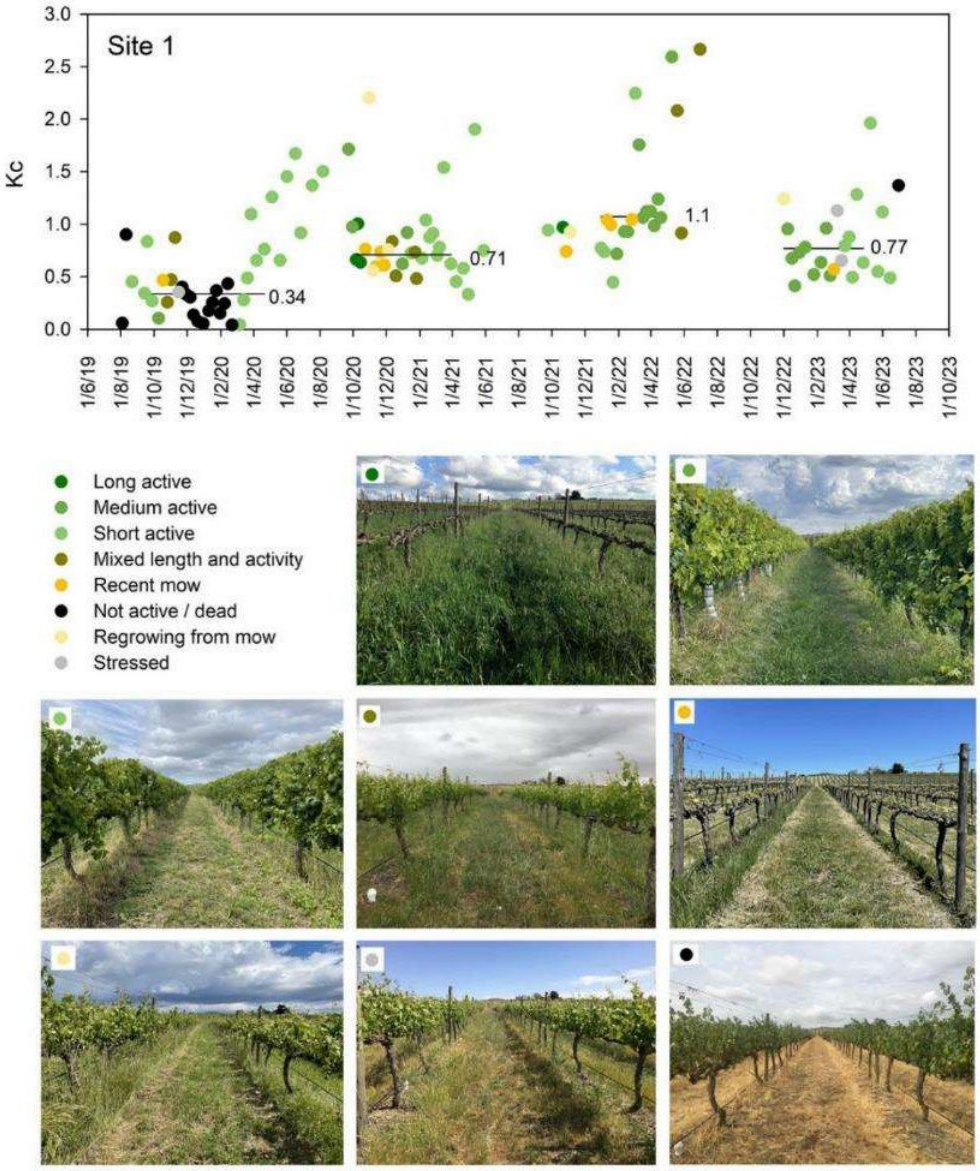


Figure 47. Comparison of vineyard floor vegetation condition to vineyard water use relative to ETo between each measurement interval. Kc values estimated from the change in soil water profile sum between measurement dates and rainfall and ETo data from SILO to the nearest day.

The only factor that appeared to slow vineyard water use was water stress during the 2019/20 season, and there was a trend in the Kc values to decrease as the soil water content dried beyond 40% of the remaining water (not shown). Mowing has been shown to temporarily reduce transpiration of cover crops (Centinari *et al.* 2013), but there was no apparent slowing of water use at the whole vineyard level. Given some overlap of the grapevine root system with the ground cover species, it is possible that mowing caused a shift in water availability in favour of the vines that would not be evident in the soil moisture readings. One additional observation from this comparison is that there seemed to be a difference in the Kc values in the 2021/22, with higher values in spring compared to the season before and after. Despite the noisy data, this suggests there could be other factors that can cause a small seasonal variation in the collective transpiration of the vineyard vegetation.

6.4 Modelling management impacts on vineyard water use

6.4.1 Model validation

With the uncertainty of the total soil water sums at Site RH and AN, the decision was made to undertake the initial validation of the water balance model using the data collected at Site BA (Figure 48). As the ability of the model to simulate seasonal soil water dynamics has already been demonstrated (Hofmann *et al.* 2014), the purpose of the validation was primarily to confirm the adaptation to the southern hemisphere. In particular, the radiation interception model, the offset of the season by six months to start from July rather than 1 January, and the modification to read standard SILO data files for the required climatic input variables. As a rain-fed vineyard with a VSP canopy and similar climatic conditions to where the model was developed, it was also the most suitable of the three sites to test the initial transition from the northern to southern hemisphere.

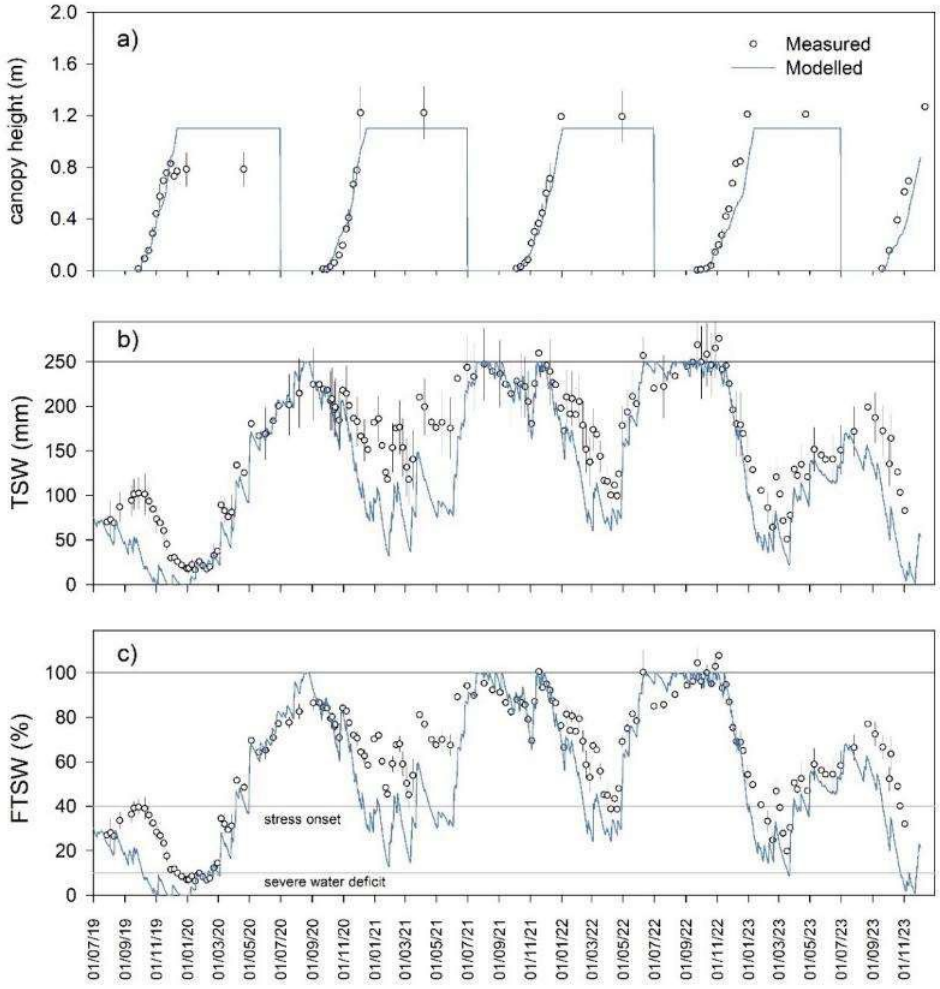


Figure 48. Comparison of modelled canopy height with measured shoot length (a), modelled total soil water with the average of the four replicate access tubes (b), and the same soil water data converted to FTSW with values of 0.4 and 0.1 indicating the onset of mild stress and severe stress thresholds (c). Error bars ± SE mean.

For canopy height, which is one of the parameters that defines the canopy architecture for the radiation interception calculations, the model showed a good correlation with shoot length and predicted the differences in budbreak that varied by about 20 days during the study period (Figure

48a). The trimming height (from the cordon) was set as a fixed value of 1.1 m based on measurements from the 2020/21 season onwards, but as not all shoots grew exactly vertically, the final average shoot lengths ended up slightly longer than the trimming height. At present, the model does not feed back water stress effects on shoot growth so the canopy did not reach the height used in the model for the 2019/20 season. However, with water stress progressively limiting transpiration in the model, the water use calculation becomes less sensitive to differences in canopy size.

For available soil water, the simulated values followed the measured values very closely for most of the study period (Figure 48b). At budbreak in the 2019/20 season, there was a difference at least partly due to rainfall at the vineyard that does not appear in the interpolated SILO data. The trial site weather station was yet to be installed, but rainfall events were recorded at the vineyard weather station located about 500 m from the trial site. A more accurate measured rainfall record could be therefore be combined from vineyard and trial site stations for later model runs. However, in demonstrating sufficient model performance with interpolated data, it opens the possibility for use of the model on a much wider scale, and also for retrospective analysis or future climatic conditions. Any cumulative difference between actual and interpolated rainfall are also reset each time a profile is fully recharged (or dried), and therefore errors do not propagate through longer term simulations.

To provide a standardised method of comparison across vineyards, and to link the soil water content to transpiration, the model converts the total water sum at the daily time step to an FTSW value. This provides the feedback to drive a linear reduction in transpiration as the water content decreases from an FTSW of 0.4 to zero. As discussed previously, an onset of stomatal closure at about this threshold has been demonstrated for a range of species, including some of the plants present on the vineyard floor at Site BA. With the original model validation, this was confirmed in the relationship between FTSW and the ratio of whole vine transpiration measured with sapflow gauges and expected transpiration in the absence of water stress. Based on a very preliminary comparison of the Kc values from section 6.3.5 and soil water (not shown), Kc values also declined at about and FTSW of 0.4, which corresponds to 100 mm remaining in the profile. Severe water stress, based here on the stem water potential threshold of -1.4 MPa, corresponded to an FTSW of approximately 0.1, or 25 mm of deficit available water remaining. These two thresholds are shown in Figure 48c, and have been used to summarise the first adaptation strategy simulations presented in section 6.4.3.

6.4.2 Water use components

Water use partitioning in the model is linked to the relative share of radiation intercepted by the grapevine canopy or ground cover, or evaporation to a depth of 15 cm for the proportion of the vineyard floor without ground cover. After validation of the model at Site BA, preliminary simulations were also run for the other two vineyards using interpolated SILO data from the corresponding 5 km grid, a total soil water of 300 mm and irrigation for the seasons the data were available. Some additional grower records are needed to finalise the irrigation amounts, as well as particle size analysis from the final soil coring to provide a more accurate estimate of total plant available water capacity of these soils. However, based on the validation at Site BA, the model can still be run for these sites to calculate vineyard water use and the relative share between the grapevine canopy and ground cover or bare ground in the undervine area.

For Site BA, the model calculations indicate that only 13% of the total water used during the period of the study was transpired through the grapevine canopy. The relative share approaches 30% when the grapevine canopy is at its maximum size, but with 3 m wide rows and green cover present on the vineyard floor for many months prior to the canopy reaching full size, total water use for the season is dominated by the ground cover species (Figure 49a). At Site RH, which has been modelled with bare soil under the vine row and a wider and denser canopy, the total grapevine share increases to 19%.

Transpiration from the vineyard floor used 52%, and the balance was evaporation from the bare soil (Figure 49b). At Site AN (Figure 49c), the grapevine water use represented 16%, the ground cover 55% and the balance evaporation from bare soil. Again for both of these sites, the model suggests a significant competitive advantage for the ground cover plants due the relatively late development of the grapevine canopy.

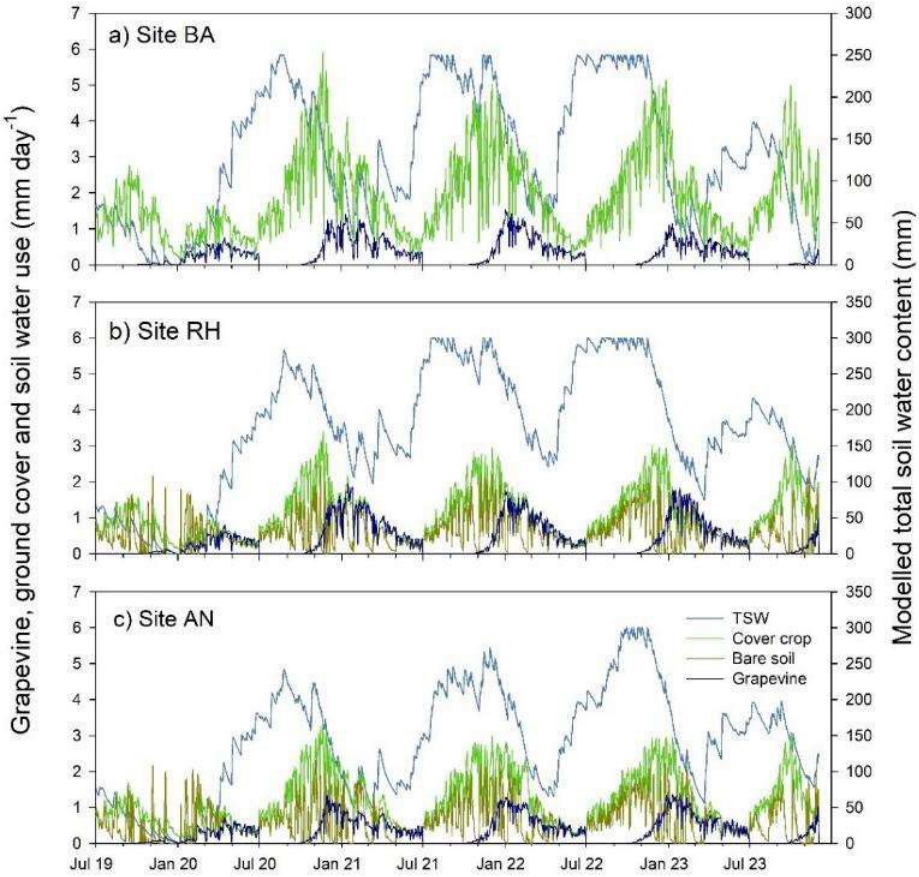


Figure 49. Modelled water use components of the three trial site vineyards, and comparison with modelled total soil water.

Under the cooler climate of the Orange region, grapevine canopies just reach their maximum extent as ET_O peaks through the last week of December and early January. Therefore, as ETo rises through spring from approximately 2 to 5 mm/day, most of the water use will be due to ground cover transpiration, or evaporation from any bare soil. In Spring 2019, the model simulations suggest that this was the fate of limited water that was still available in the profile. Leaving bare soil does not stop surface water losses, as suggested from the evaporation share at Sites RH and AN, and it may actually be higher in the event of frequent smaller rainfall events. However, water below approximately 15 cm will be conserved if not otherwise accessed by roots from neighbouring plants.

6.4.3 Modelling vineyard management and design adaptations

One key objective of the project was to use the model to test possible drought adaption strategies, and a number of simulations were run for Site BA. As a rain-fed vineyard, this allowed the simulations to be run with historical data without the need for irrigation records. Further simulations will be run for the study seasons at Site RH and AN once all of the irrigation records are

finalised. For Site BA, simulations were run for two time periods. The first from 1 July 1889 to the present, which covers the full SILO record to the nearest growing season (Appendix 4, Figure 3), and then the second period from 1 July 1970 to the present (Figure 50). The longer term simulation was just run using the current vineyard parameters, so comparing the differences between seasons, while the simulations from 1970 varied the percentage of ground cover, ground cover root depth and row spacing.

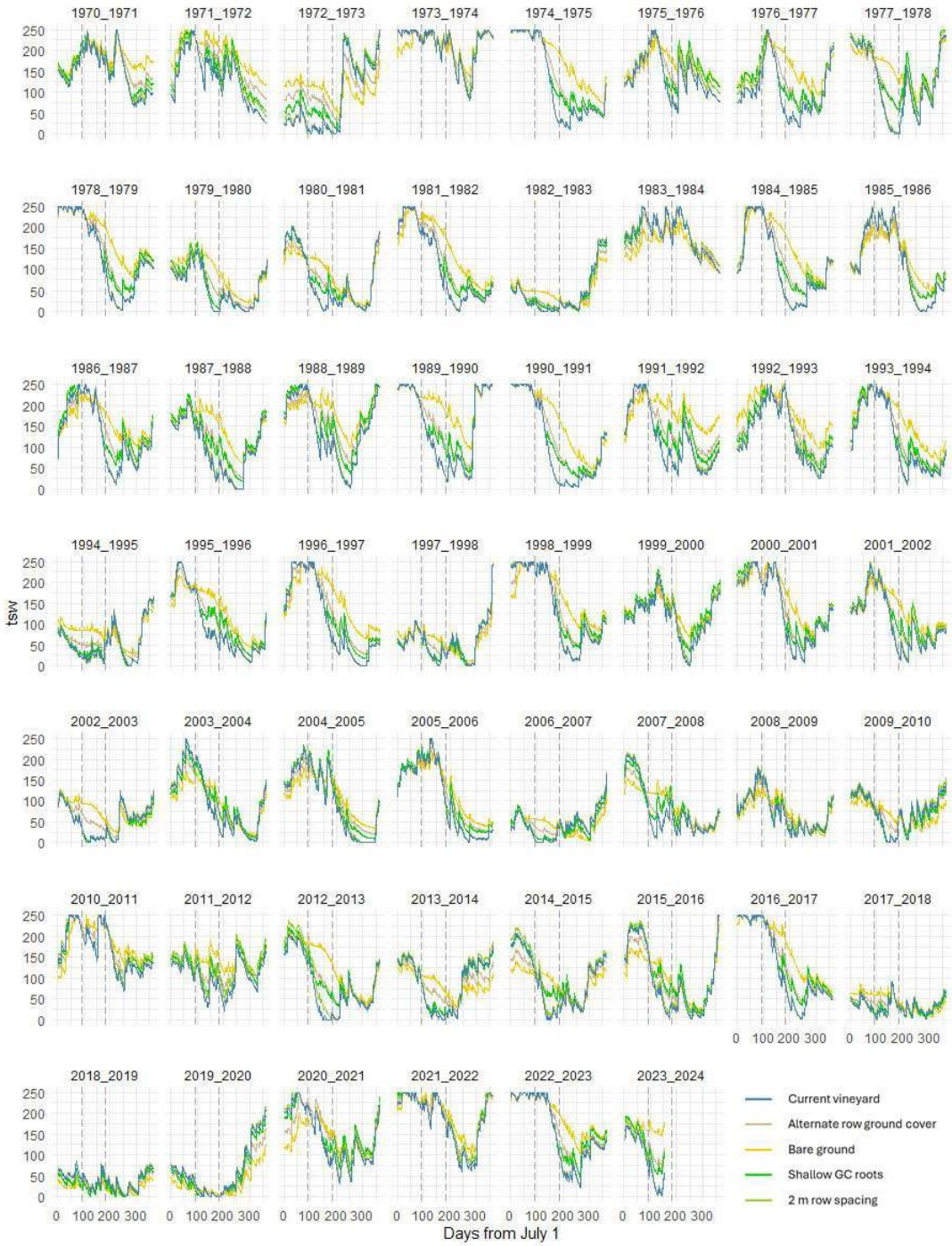


Figure 50. Simulated impact of floor management practice, ground cover root depth and row spacing for historical weather conditions for Site BA. Vertical lines show 1 October and 31 January.

Based on a preliminary assessment of the longer term simulation, the dry conditions experienced between 2017 and early 2020 were the most severe on record. There were individual seasons during the Federation drought from 1895 to 1902 that would have dried the soil of a hypothetical vineyard at this location to the same extent in some years, and also in the WW II drought from 1937 to 1945, the 1982 to 1983 drought, and the Millennium drought from 1997 to 2009. However, the recent drought appeared to be the first time the soil profiles had remained in significant deficit for three full years without some recharge.

The simulations run from the 1970/71 season showed that the number of days in water stress can be reduced by removing all ground cover plant competition (Figure 51a), which is well understood from practical experience in non-irrigated viticulture in dry climates. This was primarily due to slower water use during spring and early summer when the competition was at the highest. Simulating alternating mid-rows of ground cover and bare ground provided an intermediate response at the mild stress threshold, but almost reduced the number of days to the same as bare ground at the severe water stress threshold. In the period between flowering an version, where severe water stress would have the most impact on yield, an average of 19 % of the days would be drier than 0.1 FTSW under full cover crop, 5.5% for the alternate row cover crop and 4% for the bare ground. For a hypothetical shallow-rooted plant species that did not compete for all of the water in the grapevine rootzone, the effect was intermediate.

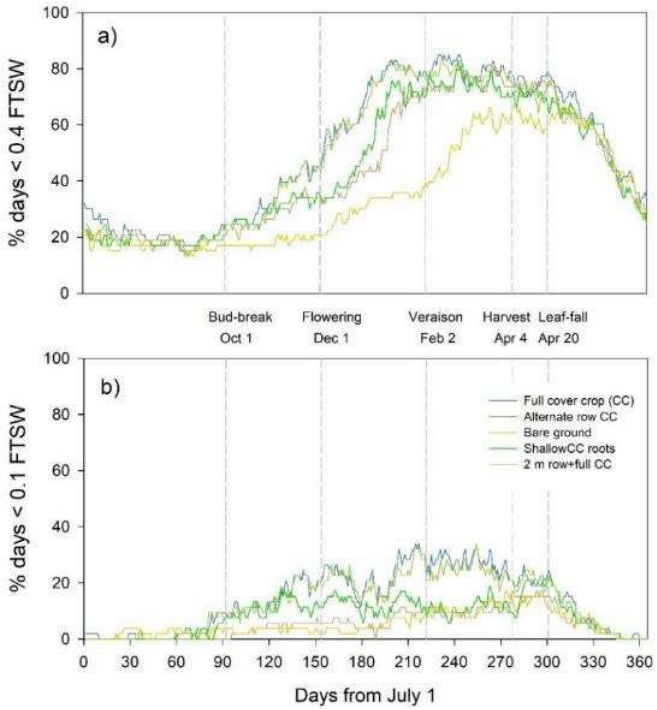


Figure 51. Based on 1970-2023 simulations, number of days passing mild stress threshold and severe stress threshold. Vertical lines indicated the average date for bud-break, flowering, veraison, harvest and leaf-fall recorded from spring 2019 to autumn 2023.

In the final simulation, the row spacing was reduced from 3 m to 2 m while keeping the full ground cover and canopy dimensions as the current vineyard. This had no impact on the number of days under the two water stress thresholds, but would have increased production per volume of water used due to the increased planting density.

6.5 Drought adaptation strategies for existing vineyards

6.5.1 Defining drought

Drought has varying definitions and varying impacts depending on the production system. The Bureau of Meteorology defines a rainfall deficiency as serious when rainfall is between ten and five percent of the lowest recorded over a three months period of time or longer, and severe if rainfall is within the lowest five percent of recorded rainfall[3]. In a recent survey undertaken across southern NSW to baseline drought from an agricultural perspective, it was found that a three year drought appeared to be beyond the capacity of many farmers to prepare for and manage (Brown 2022). Although a drought definition was not an objective of the present study, the impact on the region as a whole was severe, as surface and ground water sources used for irrigation were progressively depleted during the first two seasons, leaving little capacity to mitigate the impact of high temperatures and low rainfall in 2019 and early 2020. This was clearly evident in the impact on the vineyards in the study, and also the surrounding landscape (Figure 52).



Figure 52. Images from December 2019 and January 2020 highlighting the impact of a three year drought on both the vineyards and surrounding landscape.

In the model simulations for Site BA, none of the hypothetical practice changes at the existing row spacing would have been sufficient to slow water use beyond the first few months as the profile dried down from the last full recharge in 2016. If a similar drought was to occur that extended into a record fourth season, it is difficult to see how a vineyard could be sustained through this period without a source of more secure irrigation. Even if this was just a maintenance amount of water that ensures the viability of the vines and a more rapid production recovery once conditions improve. The scope for improving productivity in relation to water use in existing vineyard may be in the average seasons, adapting to any underlying drying trend, or during shorter term drought where management responses can be made while there is still sufficient water remaining to have an impact.

An additional point made by Brown (2022) was that the extremely wet conditions that followed the drought could be just as challenging, and this was also the case for some of the vineyards in the present study. Either higher vigour and disease pressure, or in the case of the extremely wet spring in 2022, soil that was too wet for vineyard machinery. The presence of well-established perennial grasses

[3] http://www.bom.gov.au/climate/glossary/drought.shtml

in the mid-row would have helped with the use of excess water and allowed earlier access to the vineyard. Therefore, in the space of a few seasons, the disadvantages and advantages of ground cover in a variable climate were demonstrated.

6.5.2 Soil moisture monitoring

At all three vineyards in the study, the broad seasonal trends in soil water content were driven by rainfall, with limited recharge leading into the 2019/20 season (as shown in Figure 52), followed by three wetter than average seasons. Where irrigation was used, it was supplementary and significantly less than the volume of water able to be stored in the profile at field capacity. Irrigation volumes were also significantly less than the amount of growing season rainfall in an average season, limiting the capacity to compensate for large rainfall deficits in a dry season. Making a distinction between the irrigated and rainfall-only supplied parts of the rootzone could therefore provide the possibility to quantify the available soil water, and to use this information to provide a more complete picture of water availability entering into a new season.

Soil moisture monitoring is already widely used in commercial vineyards, and probes are usually installed where they can record irrigation events. In regions or vineyards where irrigation is the main source of water, this is potentially sufficient to support irrigation management decisions. However, if a larger proportion of the soil water is derived from rainfall across the whole rootzone, then deep profile probes may be a useful complement for quantifying winter recharge and broader trends driven by rainfall. With profile probes that make readings at every depth increment it also possible to estimate total available soil water once field capacity and lower limit of extraction are established. This may be particularly useful to allow stored soil water to be compared on a volumetric basis with irrigation availability and long range rainfall forecasts for seasonal water budgeting.

To test this approach for future studies, three Sentek EnviroScan probes with sensors at 10 cm depth increments were purchased through separate funding, with the possibility to install in one of the existing access tubes at each trial site. These probes were chosen because they have the same sensor as used with the Diviner probe, but there are other options including 1.6 m versions of the EnviroPro probes installed under the drippers in the present study. One probe was installed at Site BA in December 2023 in the vine row tube closest to the average of the four replicates, with a screenshot of the individual sensors and profile sum shown in Figure 53.

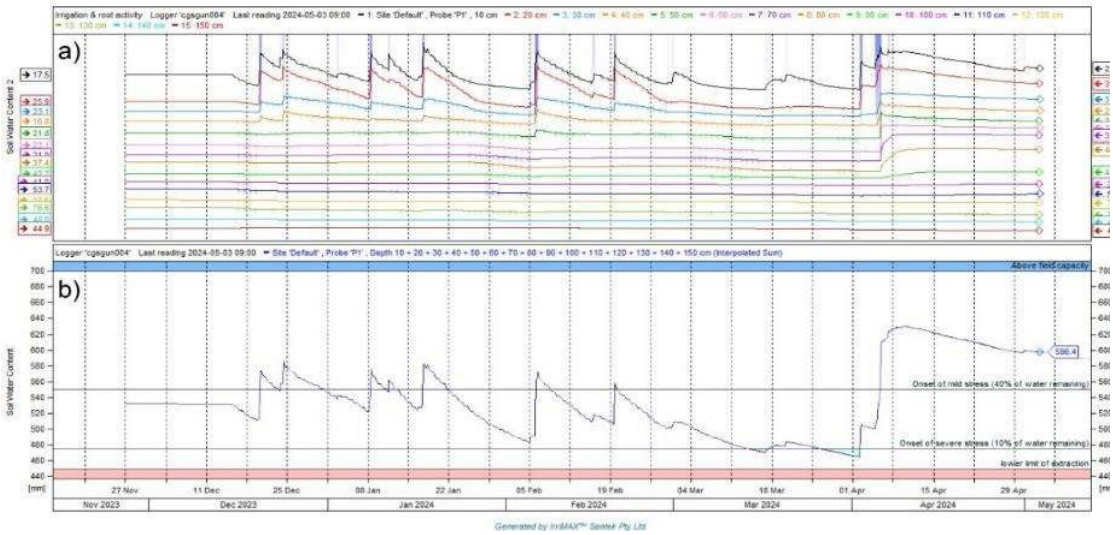


Figure 53. Screenshot from Sentek Irrimax software showing the output of a 1.5 m / 15 sensor EnviroScan

soil moisture probe installed at Site BA.

At the time of writing, the upper and lower limits for the new probe had not been established, but the approximate values shown for example are based on the previous manual readings for the tube. At the most recent reading there was approximately 150 mm or the equivalent of 1.5 ML/ha above the estimated lower limit of extraction. Within the individual depths, water extraction (set at 0.1 mm/day in the Irrimax software) can been seen to 1.3 m following rainfall events. From the soil coring results, this would be shared water extraction by the grapevines and ground cover species, but potentially increasing in favour of grapevine roots with depth.

Assumptions in suggesting this approach are the accuracy of the measurements without site specific soil calibrations and, regardless of the sensor type, whether technical issues around installation of deep probes on different soil types can be minimised. Although soil moisture probes are relatively mature technology, some work to address these questions still seems warranted, particularly in combination with the positioning of probes relative to grapevine rootzone in vineyards with permanent ground cover. Estimates of total water holding capacity could be checked from laboratory based soil analysis if direct *in situ* calibration is not feasible. Pre-dawn or stem water potential measurements provide a method to relate the output of soil moisture probes to the plant water status.

6.5.3 Managing ground cover water competition

The primary aim of the field survey, plot experiment and pot study was to gain a better understanding of the variation in water use traits of the plants that were present at the vineyard trial sites. These measurements mainly focussed on leaf water use traits, but combined with the information from the soil coring and root distribution study, provide an insight into how this water demand might translate to competition with grapevines below ground.

The results suggest that many common ground cover species, when the leaves are green and actively transpiring, will exceed the water use of grapevine leaves on a per unit leaf area basis. In the only species on the vineyard floor where transpiration rates were similar, the C4 grass *P. dilatatum*, photosynthesis rates were much higher than for the neighbouring grapevine leaves. This in turn would confer a competitive advantage for water if more leaf area can be produced as a result of the higher WUE. Below ground, the perennial grasses all appeared to be able to match the grapevine root system for depth, as could a number of the annual grasses and perennial dicots. Therefore, the higher transpiration demand of the leaves of most of these species could translate to water competition in much of the grapevine rootzone.

Selecting species for lower water competition provides one possible management approach to balancing water competition against the benefits of ground cover. However, the plot trial on the CSU campus demonstrated the practical difficulties of establishing new ground cover species, at least without significant effort to remove more competitive species, reduce the seed bank and allow the new plants to fully establish. Alternative strategies, for growers who would prefer to retain permanent ground cover, could be species that compete less during the critical spring period, or that do not compete in the whole rootzone. As one example from the present study with a shorter season, the native C4 perennial grass *B. macra* was one of the main ground cover components in the mid-row at Site AN, was most active through summer and early autumn. For a shallower root system, assuming species could be identified that did not eventually reach the full profile depth, the modelling suggests that this would reduce water risk by retaining some of the soil volume just for the grapevine root system. Another possibility would be short term sown cover crops that reduce the opportunity for the establishment of extensive competitive root systems.

Vineyards in the Orange wine region, as well as other Australian regions that derive more of their seasonal water requirement from rainfall, are potentially more exposed to the impact of seasonal rainfall variability than warmer inland regions. In the Riverina, as an example, there will be a large deficit between rainfall and ET_o through each growing season and irrigation provides growers with the ability to control the extent of deficit according to their production objectives. Where rainfall is closer to meeting seasonal demands, and there is not capacity in the irrigation systems to buffer seasonal extremes, the water situation from season to season may be much more variable. This has significant implications for ground cover management strategies where there is sufficient rainfall on average to maintain plant coverage across the full vineyard floor, but not always enough to afford the water competition. In wetter seasons the presence of permanent ground cover and the additional water competition is beneficial, but in drier seasons or locations this will compete for water to the detriment of grapevine growth and yield. This raises the question about the relative benefits of more a more stable permanent ground cover system, which requires fewer inputs, compared to a higher input system where ground cover management is varied according to seasonal water supply.

Slashing or mowing is the most commonly used tool for managing ground cover, and the most easily varied practice in relation to seasonal water availability. However, there was no obvious effect on whole vineyard water use when this was compared with a preliminary analysis of Kc values at the rain-fed vineyard. It is possible there was a temporary shift in water use in favour of the grapevines, as other studies show that mowing does cause a temporary reduction in ground cover transpiration, but it did not seem to slow the use of water by the vineyard as a whole. A next step, apart from refinements such as mulching or rolling which were not examined with the current study, is a more substantial disruption of ground cover transpiration. Traditionally this has been one of main the reasons why cultivation has been used in non-irrigated vineyards in dry climates, or more recently, in alternating mid-rows with perennial grasses or sown cover crops. If the equipment is available for cultivation and sowing cover crops, then the possibility is there to re-establish ground cover if wetter seasons are expected, or to sow shorter term cover crops that do not have sufficient time to establish a deep competitive root system.

The model simulations showed that bare ground reduced water risk to the greatest extent, although these were run to establish the boundaries of floor management based water use control, rather than advocate for this being undertaken in practice. Without ground cover, aside from all the soil related risks, the proportion of water lost to evaporation increases, and in some seasons the simulations show that the soil ended the season drier than in the presence of ground cover. However, with alternate row ground cover there was an intermediate reduction in the numbers of days reaching mild stress early in the season that lasted until about the onset of veraison (Figure 51). At the severe stress threshold, which is more critical for yield, there was a greater relative reduction in water risk with alternate row ground cover closely matching completely bare ground for days under 10% of remaining plant available water. How this is achieved in practice is a separate question, but it demonstrates that there is still significant room in the current systems to shift the water balance in favour of the grapevines when needed under dry conditions.

6.5.4 Use of long range rainfall forecasts

For seasonal irrigation planning, and any major intervention to modify ground cover water competition, long range rainfall forecasts are particularly valuable. The challenge is making a decision based on these forecasts and balancing the extent of a pre-emptive response against the possibility that the predicted conditions do not eventuate. During the course of the study, the long range forecasts from the Agriculture Victoria and GRDC-funded 'Using Seasonal Forecasts Project' were followed for Southern NSW. Although this project has since concluded, the summaries covered the full period of the current study with the output of 12 climate models compiled on a monthly basis with additional

updates on the driving climate patterns. The format was the same as the Victorian information that is still being produced[4]. These summaries included the rainfall and temperature predictions of the models for the six following months in two three-month periods. The rainfall predictions available at the end of July each year are summarised graphically below in relation to the measured soil water content at the rain-fed trial site (Figure 54). Model confidence is higher through the winter months, and for viticulture, this also corresponds to the time where growers might start planning according the expected water availability for the coming season.

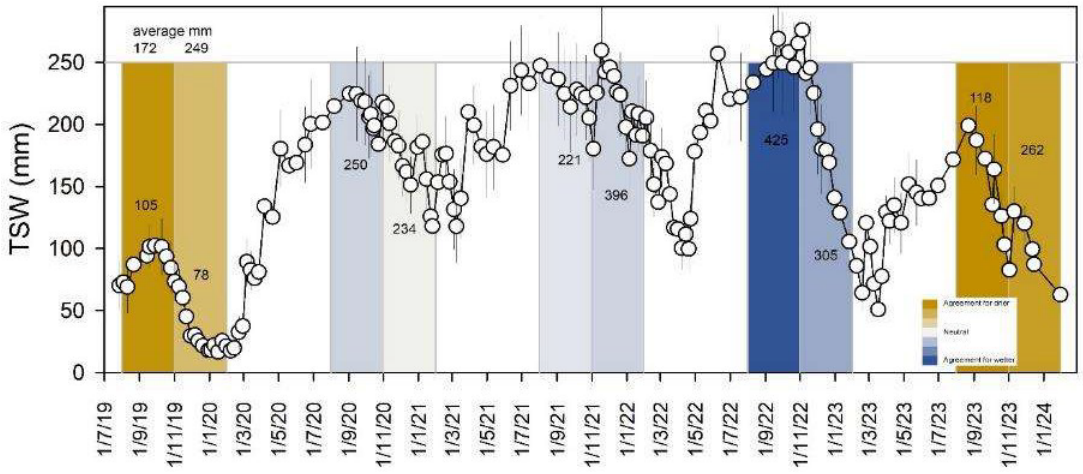


Figure 54. Measured available soil water at Site BA in comparison to the July long range forecasts for the periods 1 Aug - 31 Oct and 1 Nov – 31 Jan. Text values show total rainfall for each period measured at the Borenore BOM station. The colour range indicates consensus of the 12 climate models within the forecast for lower to neutral to higher than average rainfall.

The study period spanned three consecutive wetter than normal seasons with La Niña events, book-ended by weak and then stronger El Niño events. The El Niño–Southern Oscillation did not play a major role in the 2017-2019 drought (Devanand *et al.*, 2024), but the model predications from July were in strong agreement for drier conditions. For the three seasons with La Niña events, the profile was full or close to full at the time of the end of July forecasts, and the rainfall that eventuated was in broad agreement with the consensus of the 12 models. For the 2023 El Niño event, the models were in agreement for a dry start to the season, which did eventuate with rainfall below average between August and October, and similar to the same period 2019. The key difference with 2023/24 season was that it started with more water in the profile and returned to average rainfall through late spring and summer. This is consistent with previous rainfall correlations with the El Niño–Southern Oscillation where drought is often, but not always associated with El Niño events.

The project did not get to the point where particular responses to long range forecasts could be recommended, and this may vary considerably according to the situation at individual vineyards. It would also depend on the level of risk that is accepted by growers and what responses in the vineyard are feasible to implement. However, it does demonstrate that the combination of quantified soil water and long range rainfall forecasts provide valuable planning information leading into a new growing season. For example, if available irrigation leading into an expected dry season will be sufficient, if certain blocks need to be prioritised, or if attention is turned to conserving the available soil water by reducing ground cover competition. The model simulations for the first part of the 2023/24 season (Figure 50) suggest show a marked response in soil water in relation the percentage of ground cover,

[4] https://agriculture.vic.gov.au/support-and-resources/newsletters/the-break/the-fast-break-victoria#

conserving from 50 to 150 mm of water. For the same period in the 2019/20 season the same simulation showed limited effect as the vineyard was already in stress. As the 2023/24 season eventuated, this response would not have been needed, but without the summer rainfall, the profile would have dried into severe deficit by veraison. This might highlight a key question with pre-emptive responses to drought. By the time it is clear that that a response is needed it may be too late to have an impact, and when management responses can have an impact, it is not yet known if they will be really needed. Treating management responses as long term risk-reduction strategies could be more feasible than trying develop criteria specific for every situation and vineyard.

6.5.5 Use of supplementary irrigation

At the two vineyards in the study that normally used irrigation, the volumes available were less than the amount of winter rainfall that could be stored in the soil profile and also less than the amount of rain that would fall during an average growing season. Therefore it would not be possible to recharge a profile in the event of low winter rainfall, or compensate for large within season rainfall deficits. This raises a question as to how and when small amounts of irrigation can be best used, and what it achieves in terms of benefits for the vine.

From previous industry experience with poor growth following low winter rainfall, and research into the issue in warmer climates, winter irrigation has become a management consideration for drought affected vineyards. At Site BA, although there was no irrigation available leading into the 2019/20 season, the water that was available in the profile was centred around 60-70 cm where the total root length density was lower, and potentially where grapevine roots would have been more competitive. If irrigation was available, there possibly would have been no advantage in a winter irrigation that would have mainly benefited the ground cover plants. Instead, a strategy may be to wait until the ground cover plants have senesced, and just use irrigation at around flowering and fruit-set, or in advance of heat extremes during summer. In general, the leaves and canopy at Site BA remained in surprisingly good condition despite the severe water stress and significant reduced canopy growth compared to later seasons. This would suggest a degree of acclimation with progressive water deficits developing from bud-break, and it may be better to allow this to occur rather than using irrigation early in the season and then running out. During the 2019/20 season, the small amount of irrigation at Site RH did seem to make a difference to the condition of the canopy even though the volumes were small relative to the large soil water deficits. It also appeared there was some water deeper in the profile that would have been accessible to the grapevine root system, but without being able to establish an accurate lower limit, the starting soil water conditions are less clear than for Site BA.

The contrast between the deeper manual probes and the automated probes, although the latter were not installed until later in 2021, also raises questions about the impact of irrigation on grapevine nutrient uptake. At the start of the season, both the ground cover plants and grapevines extracted water from the whole rootzone, and then transitioned to a smaller volume of wetted soil once irrigation commenced. The automated probes show there is very rapid cycling from this volume of soil compared to slower changes in the rootzone water as a whole. At Site AN, the transition from profile stored water to irrigation occurred earlier in the season than at the other irrigated vineyard with Site RH. At Site RH, there was also more extensive grapevine root growth into the mid-row compared to Site AN with a clearer distinction between grapevine roots under the vine row and mainly grass roots under the mid-row. Potentially this offers an explanation for the differences in juice yeast assimilable nitrogen between the two sites as water uptake concentrated to a relatively smaller part of the rootzone, and the opportunity to target this transition with fertigation if needed.

6.6 Designing drought resilient vineyards

The combination of field based observations and modelling results suggests that there is scope to improve the drought resilience of future vineyards without increasing reliance on irrigation, at least outside further record-breaking drought. From the soil coring and root distribution study there were clear management related differences in grapevine root distribution, and by inference, the total amount of plant available soil water the grapevine roots can draw on. Although further work would be required to confirm the impact on the relative share of soil water, encouraging greater grapevine root exploration of the available soil volume may have a greater impact on soil water availability than other manageable factors. This potentially includes increasing soil organic matter, which is often proposed as a method for increasing soil water holding capacity, but evidence from the scientific literature suggests that any gains are likely to be small (Minasny and McBratney 2018).

In an earlier study undertaken in the Riverland, root system responses of mature grapevines were followed after the conversion from overhead sprinkler to drip irrigation (Soar and Loveys 2007). It was found that the conversion to drip led to increased root proliferation under the drip line, but did not cause a loss of roots from the soil under the mid-row. Despite the difference in production conditions, the root distribution response to drip irrigation and rainfall in the present study was very consistent with these earlier observations. The authors suggested that establishing a new vineyard under sprinkler irrigation and then converting to drip irrigation to generate a larger root system would produce a more drought tolerant vineyard than one established from the start under drip irrigation. Whether by timing a new planting with a wet season, or the use of a temporary (or permanent) mid-row drip line, a similar approach could be trialled for higher rainfall viticulture.

An additional consideration, given that ground cover is also likely to be present in higher rainfall vineyards, is the way the mid-row and undervine area is managed in the first seasons after planting. In the present study, the high root length density of the ground cover species appeared to exclude grapevine roots from the soil immediately under the mid-row. It is possible that by deferring the establishment of mid-row ground cover, it could allow more extensive and also shallower exploration of the mid-row soil volume by new grapevine roots. Unless timed with a wet season, this might require temporary irrigation in the mid-row or even a permanent sub-surface installation. Once an extensive structural root system is in place, there is potentially greater control provided over grapevine water supply and nutrition through floor management practices. If the top 30 to 40 cm of soil is dominated by ground cover roots, or drip irrigation aligns the root system with the vine row, then limiting ground cover water use will not necessarily have a proportional effect on water availability for the grapevines. If the grapevine roots occupy more of the soil volume they have access to more soil water storage capacity, then floor management practices will offer more control over water availability.

There may also be advantages for the uptake of some nutrients if grapevines roots can be encouraged into the shallower soil. The microbial profiling showed numerous taxa associated with nitrogen fixation and cycling amongst the roots of the ground cover plants and potentially the grapevines would benefit from having roots where these processes are taking place, rather than being positioned below and relying on nitrogen (or other nutrients cycled by deeper roots) that makes it past the denser root systems of the ground cover plants.

For new vineyard plantings there are also other possibilities for improving adaptation to drought and rainfall variability. Where there is the opportunity to select new locations for a vineyard, deeper soils with higher water holding capacity may allow more buffering from high rainfall seasons to low, and the use of slope and aspect can also reduce the amount of radiation reaching the vineyard and in turn reduce evapotranspiration. For both new or existing locations, there is also the capacity to vary row

spacing, orientation and canopy architecture to modify the relative partitioning of radiation between the grapevine canopy and vineyard floor. The water balance model used for the project was originally developed to evaluate future drought risk for vineyards in a region where wide variation in soil water holding capacity, topography and row orientation already exists. This means it could also be used for future studies to test hypothetical combinations of vineyard location and design as part of a more strategic drought risk assessment for future climate scenarios. For the present study, one example of reducing row spacing was tested using historical weather data. This did not result in a reduction in water use, but for no other change to the canopy architecture or percentage ground cover, it would potentially increase the vineyard on a per hectare basis. Potentially, increasing yield could also be viewed as a risk reduction strategy if it increases the return on the vineyard relative to water used.

7. Outcome/Conclusion

The project delivered on most of the planned outputs and performance targets, and with the contribution of the results from the earlier lead-in study, has provided significant new insight into the extent of management control over water use in rain-dependent production systems. The combined soil coring and molecular study in particular made a major advance in understanding of how irrigation and root competition modify the growth of the grapevine root system and access to water. With the microbial profiling, this added insight into the benefits of plant diversity in the vineyard, but also the complexity in attempting to deliberately manage these and link particular populations to grapevine root function. Due to the high labour requirements associated with soil coring and root sample processing we were not able to undertake further coring outside of the Orange region. However, more detail was obtained from the vineyards that were studied, and methodology is in place that could be confidently extended to other regions.

The water balance modelling was also very successful, with the rain-fed trial site similar to the vineyards for which it was originally developed, and providing an ideal transition point from the northern to southern hemisphere. Enough simulations were run to demonstrate the potential for testing the practical implications of field based research findings, including with historical weather that provides context with real droughts in the climate record. The modelling work did include irrigation inputs, but with more field based validation it would be possible to incorporate irrigation management scenarios into longer term simulations. One question is how to provide transpiration feedback to the vine when only part of the root system is dry, which can possibly be addressed with more in-depth analysis of the sap-flow data. Another is the criterion used to generate irrigation events for simulation under future climate conditions, or with historical climate data where irrigation data are not available.

The project did include a considerable instrumentation component, with a range of sensors for recording weather conditions, and parameters relating to plant and soil water dynamics. These have provided detail and resolution that will significantly strengthen the depth of interpretation and strength of the scientific outputs. However, from a practical industry perspective, the most value from this instrumentation may be in demonstrating that quite good predictions of vineyard water use can be made with regionally available weather data. From the ground based measurements, the most valuable parameter was total soil water holding capacity, which in turn could be obtained from soil moisture monitoring if feasible with the soil type, or calculated from soil physical properties in combination with water balance modelling. Other data collected in the study, including phenology and the berry and shoot growth responses to water deficit, could be used to further refine the model.

The broad context for the project was maintaining sustainable production in the context of a variable and changing climate, and the results suggest that there is still significant scope to adapt to these changes within the existing production system through the way grapevines and vineyard ground

cover are established and managed in the future. However, this in turn will require flexibility in management approaches to follow this variability. Grapevines do not appear to be rapid consumers of water, but traits such as low root length density, lower leaf transpiration rates and shorter growth cycles mean they can be out-competed for water by ground cover species. Particularly from diverse multispecies populations that develop with long term permanent ground cover, which often ends up being dominated by perennial grasses. A well designed system of vineyard floor management could use high rainfall seasons to obtain benefits from ground cover plants, but retain the flexibility to reduce competition in favour of grapevines in drier seasons.

8. Recommendations

This project set out to investigate the agronomic possibilities to improve drought resilience in rain dependent viticulture and found there is potentially sufficient scope within existing higher rainfall production systems to respond to shorter term drought or adapt to a gradual rise in water demand with climate change. Except for a drought more severe than experienced between 2017 and 2019, this may be possible to achieve without increasing reliance on irrigation. The work included a significant component on ground cover competition for water, including from the below ground perspective which has had limited previous attention, and the possibilities to select plant species according to water use traits. However, the combination of field based measurements and water balance modelling suggested that the way ground cover is introduced to the vineyard and then managed is more likely to improve drought resilience than ground cover species selection alone.

A challenge in making recommendations based on these findings is that the most effective control over ground cover water use requires a disruption of transpiration or root water transport. Traditionally this has been done with tillage, but herbicides or non-synthetic alternatives provide other options. An additional challenge is that despite the strong competition, the presence of other plants in the vineyard also brings many benefits. These include from many soil physical, chemical and biological parameters to practical aspects such improving trafficability in wet weather and providing competition that may be wanted in wet seasons or high vigour sites. An opportunity for future research would be to understand how to retain these benefits through floor management systems that could also be varied to adjust to short or longer term changes in water availability.

For future research there would also be an opportunity to look at the drought resilience gains that can be made in the establishment of new vineyards. This returns to the idea of built-in drought resilience raised in the original project application. The results of the present study suggest that just through encouraging greater root exploration there may be significant scope to improve water availability by increasing the volume of soil occupied by the grapevine root system. Potentially this would involve providing soil conditions that encourage root growth laterally into the mid-row as well as to depth under the vine row, which could be achieved with irrigation and a delayed introduction of ground cover in the season or two following planting. Even the question of root access to residual soil water, which has been examined in annual crops, could provide further insight into the capacity of grapevines to withstand extended drought.

Finally, with the water balancing modelling, scaling from a single point to a whole property would be possible with spatial information on total soil water holding capacity. There is also potential to run more combinations of above and below ground parameters, possibly incorporating other floor management options, to establish the full extent of water control for a given location. The use of historical climate data appeared quite sufficient for this purpose, but projecting these simulations forward in time would provide context for changes in water availability expected in coming decades.

9. Appendix 1: Communication

NSW DPI Spring Vine Field Health Days: 'Vineyard water use, cover crops and drought adaptation' Tumbarumba, Murrumbateman and Orange workshops in 2022 and 2023.

18th AWITC workshop presentation 26 July 2022: Understanding ground cover water competition in rain dependent and supplementary irrigated vineyards

ASVO Viticulture seminar 2023. 'Irrigation and ground cover management considerations for the establishment of new vineyards'.

Project workshops on the Orange CSU campus held with collaborating producers, advisory panels and industry and academic participants. 4 July 2022, 8 Aug 2023 and 5 Dec 2023.

10. Appendix 2: Intellectual Property

The southern hemisphere adaptation of the water balance model was undertaken as an in-kind contribution and the intellectual property remains solely with the Hochschule Geisenheim University. The model simulations and all other project findings will be in the public domain in the form or reports, presentations and scientific publications.

11. Appendix 3: References

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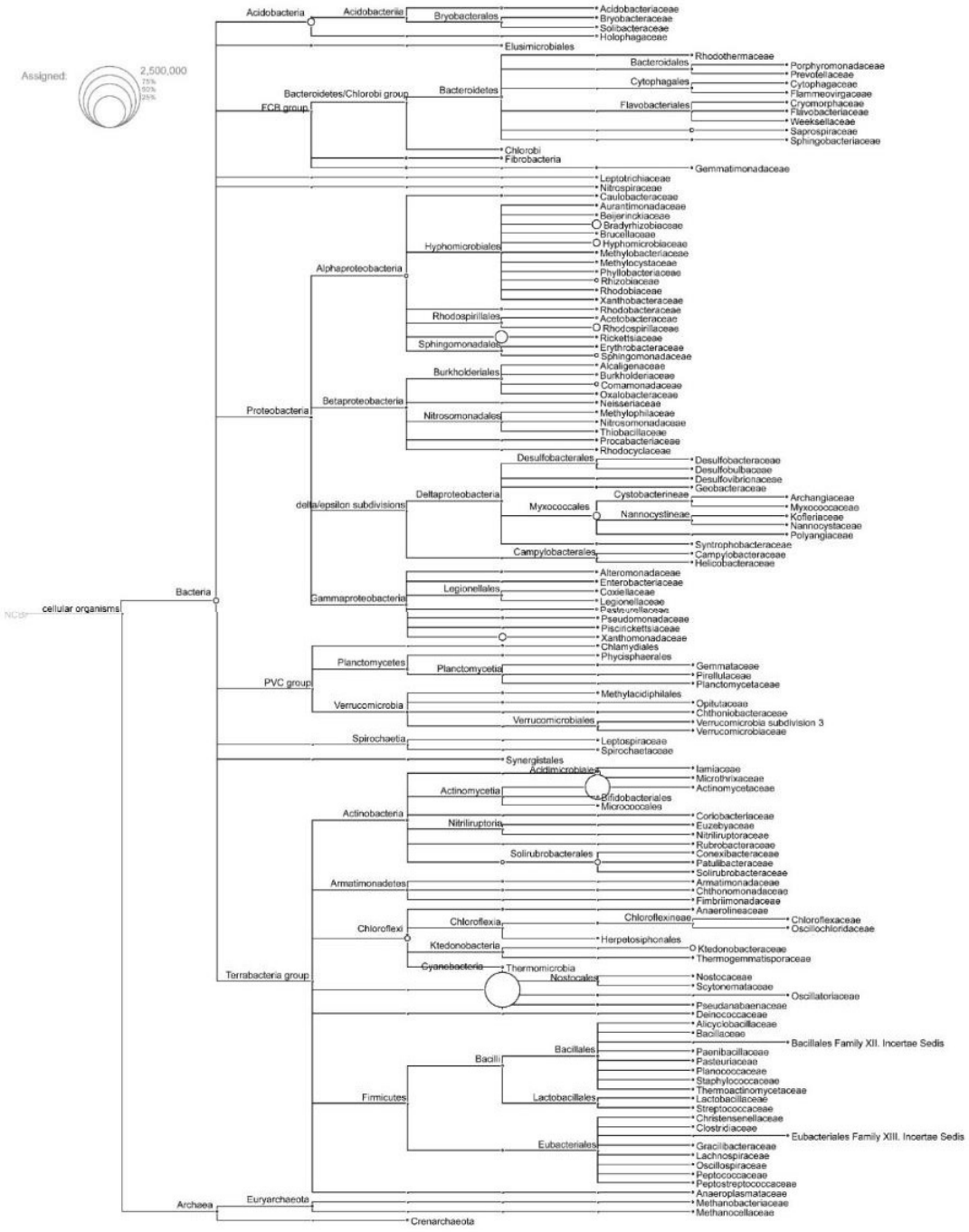
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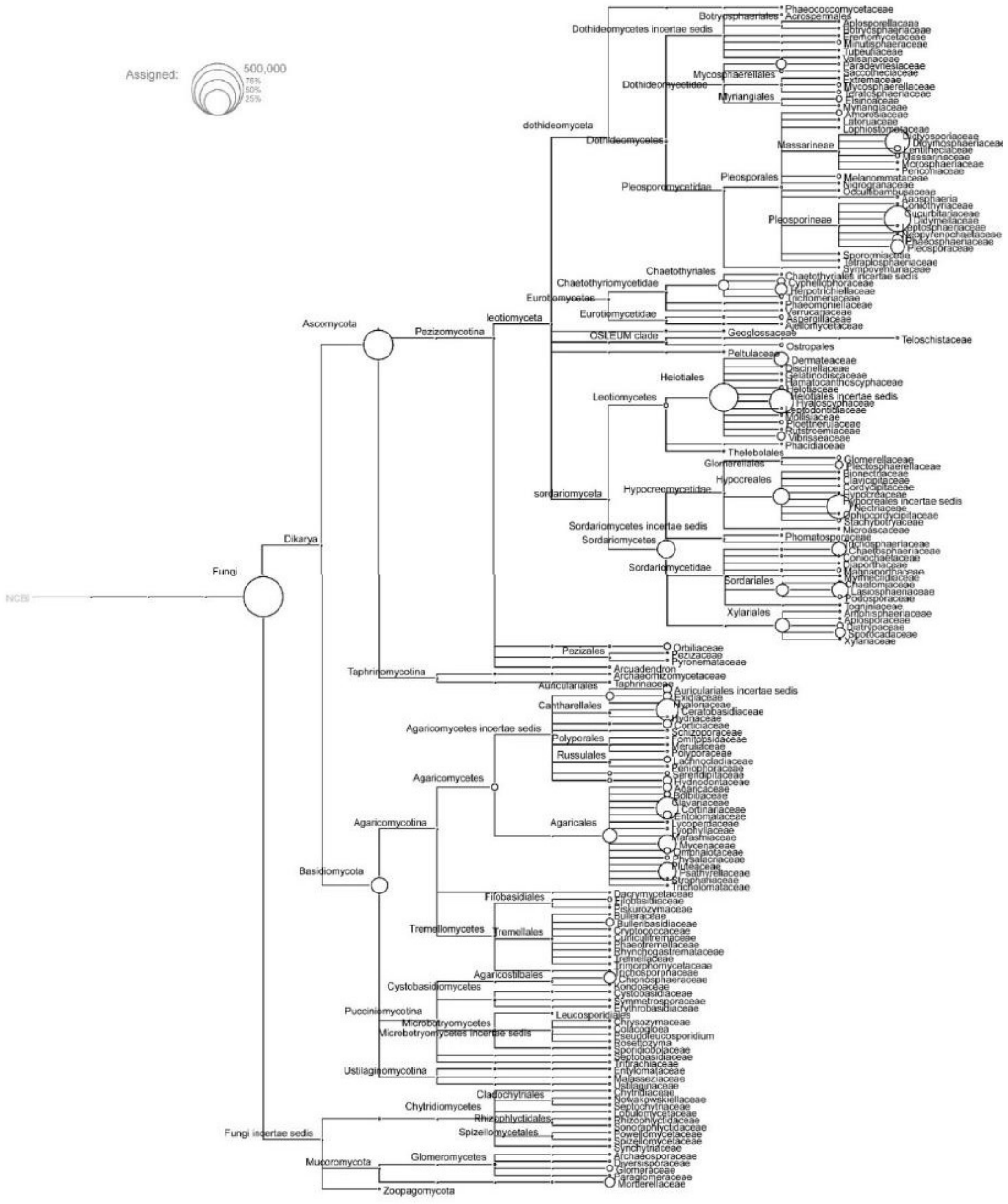
12: Appendix 4: Staff

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13. Appendix 5: Additional microbial profiling data

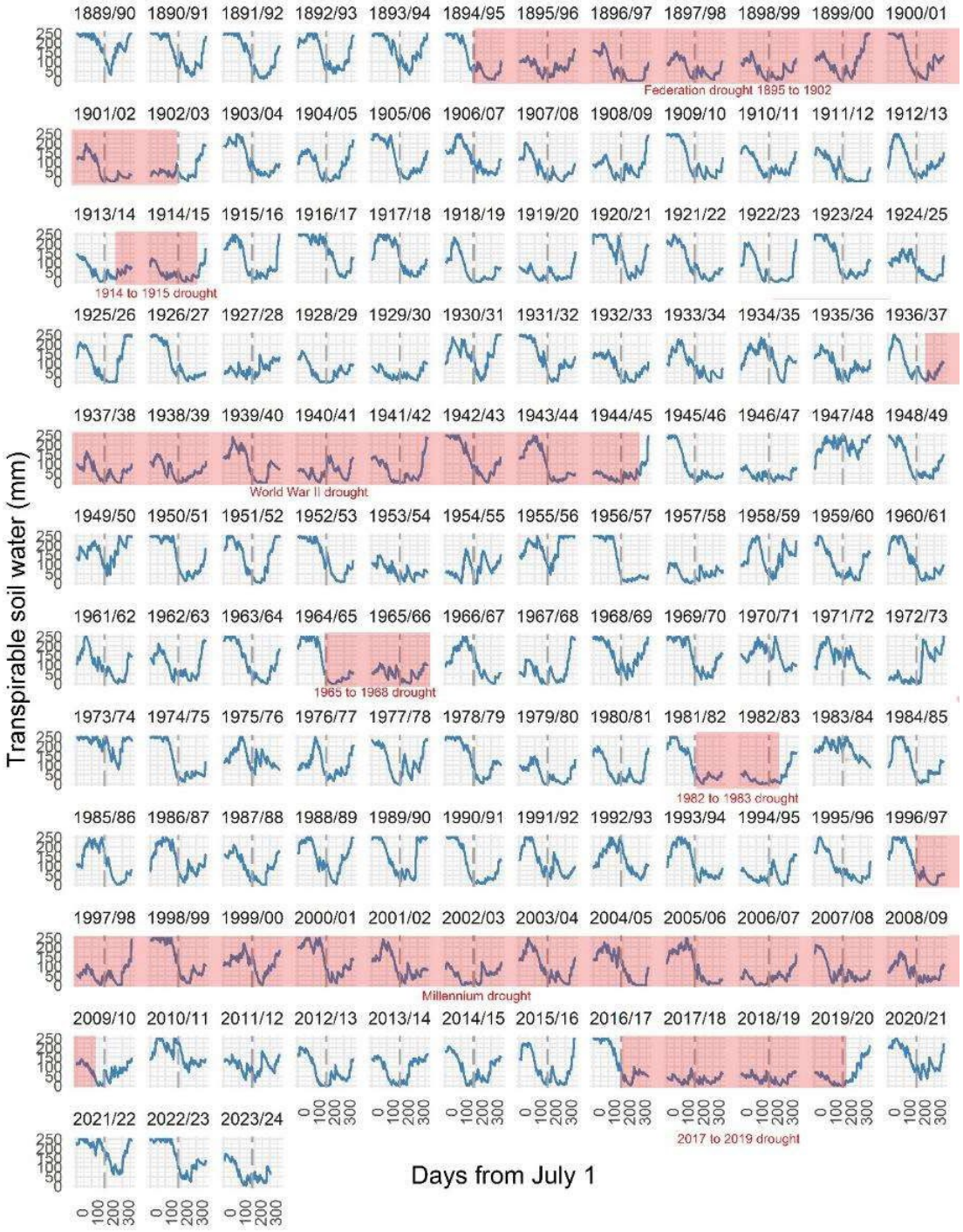


Appendix 5a. Phylogenetic tree to family level for the 16S rRNA bacterial diversity sequencing of roots showing the combined data and relative abundance from the three trial sites.



Appendix 5b. Phylogenetic tree to family level for the ITS fungal diversity sequencing of roots showing the combined data and relative abundance from the three trial sites.

14. Appendix 6: Additional water balance modelling data



Appendix 6. Simulations of soil water dynamics for Site BA, using interpolated weather data from SILO, had the vineyard been located at the same site since the 1889/90 season. The shaded area shows the approximate periods of historical droughts, noting that there was spatial and seasonal variation in the severity of these droughts across South Eastern Australia.