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Run for cover–the 'right' species of under-vine cover crops do not influence yield in an Australian vineyard

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ABSTRACT

Reducing herbicide application in viticulture is of increasing importance due to tightening regulations in multiple export markets, and consumer demands. As a result, alternative vineyard floor management options are becoming increasingly sought after in the context of sustainable viticulture. This trial aimed to quantify the effects of different under-vine ground cover species on soil properties and vine productivity and to reassess whether bare soil under-vine compares with a covered under-vine zone.

In 2014, six under-vine treatments, including straw mulch, herbicide, medic (*Medicago* L.) mixes, and grasses (Wallaby grass [*Rytidosperma* sp. L], fescue [*Festuca* sp. L], and Cocksfoot [*Dactylis* sp. L]) were established in an irrigated vineyard field trial at Langhorne Creek, South Australia. In 2020, data on soil physical and chemical properties, moisture content, and leaf nutrient status were collected from the site. Yield data from 2016–2021 were also collected.

Cover crop species seeded in the under-vine space play a major role in defining vine productivity but do not necessarily have a negative impact. Analysed using a linear mixed model, yields over six seasons were no different between the herbicide control, straw mulch, and the medic treatments. That is, there was no yield penalty with specific cover crop treatments. However, significant negative effects on yield were observed relative to the herbicide control, with more aggressively growing perennial grass treatments. In Australia, seed blends including *Medicago* species provide viticulturists with new tools to enhance their vineyard's soil health and hence sustainability of their operations.

KEYWORDS: vineyard flood, viticulture, cover crop, under-vine, management pratice, weed management

INTRODUCTION

Vineyard floor management in many viticulture regions globally has included maintaining bare soil in the under-vine region. Maintenance of a bare under-vine zone is typically achieved with the use of herbicide (Tesic *et al.*, 2007; Tourte *et al.*, 2008), although cultivation of the soil is still carried out by some growers (Longbottom & Petrie, 2015). The rationale behind these practices is to remove competitive species from the root zone of the vine, thereby ensuring maximum water and nutrient availability in the soil (Smith *et al.,* 2008; Giese *et al.,* 2014; Steenworth *et al.,* 2016), reducing weed presence in the canopy [which can be an issue during harvest] (Guerra *et al.*, 2022; Wolf *et al.*, 2008), and reducing frost risk (Battany, 2016). However, with tightening restrictions on herbicide use in vineyards (Walsh & Kingwell, 2021; Wynn & Webb, 2022), and a shift towards ecological intensification of agroecosystems [*i.e.*, more plant cover, less disturbance] (Bommarco *et al.*, 2013; Bowles *et al.*, 2016), there is growing interest in under-vine cover cropping (*e.g.*, Vanden Heuvel & Centinari, 2021). Arguments, both for and against maintaining bare soil under vines exist; however, there is relatively little empirical evidence in support of either approach, particularly in viticultural regions where irrigation is necessary.

While the presence of competing under-vine species is typically seen as detrimental to grapevine vegetative growth and yields, this may not always be the case, with evidence from literature suggesting a multitude of site-specific factors affecting results (Nordblom *et al.*, 2021). For example, Hickey *et al.* (2016) found fescue grown under-vine reduced yields by 610 kg/ha on average over six years relative to a bare under-vine in a dry-farmed Virginia (USA) vineyard. Similarly, in an unirrigated vineyard in upper New York State, Karl *et al.* (2016a) observed a 50 % reduction in yield and pruning weight with a clover cover crop vs bare soil. In contrast, at a drip-irrigated vineyard, this one on silty loam near Sacramento, California, Ingels *et al.* (2005) found no significant impact on yields over three years with under-vine treatments including grasses, legumes, and tilling. The contradictory evidence present in the literature suggests a more nuanced system in action–one capable of returning contrasting results depending on a suite of local factors and their interactions, including soil type, site management, climate, vine genetics, and cover crop species (Vanden Heuvel & Centinari, 2021).

The presence of plants compared to bare soil in viticultural soils is known to contribute to a suite of ecosystem services facilitated by their inherent above- and below-ground characteristics (Danne *et al.*, 2010; Garcia *et al.*, 2018; Kesser *et al.*, 2023). The benefits of ecosystem service provisions include, for example, their ability to improve soil organic carbon stocks by up to 23 % after five years, evidenced in South Australia by Marks *et al.* (2022). Improvements in soil organic carbon in vineyards provide multiple other benefits, including improving aggregate stability, improved water infiltration and retention (García-Díaz *et al.*, 2018; Abad *et al.*, 2021), as well as facilitating the proliferation of a more diverse and functional soil microbial community (Capó-Bauçà *et al.*, 2019). Information about how under-vine cover crops interact with soil microbiota and influence their composition and functioning is also limited (Baumgartner *et al.*, 2005; Burns *et al.*, 2016; Holland *et al.*, 2016). Microbes (fungi and bacteria predominantly) play crucial roles in plant decomposition and therefore the cycling of organic carbon (Pingel *et al.,* 2019; Romdhane *et al.,* 2019) and mineralisation of essential plant nutrients, including in the nitrogen cycle (Ball *et al.*, 2020; Cheng & Baumgartner, 2004). Within this, they also enable colonisation by later-stage macro-fauna, such as earthworm populations (Andersen *et al.*, 2013).

Our study addresses several distinct knowledge gaps in under-vine management, notably, exploring the influence of *seven* under-vine treatments (five cover crops, a mulch, and a herbicide control) and the impact of under-vine management in a Mediterranean climate. The selection of cover crops aimed to explore several distinct ecosystem service provisions, *e.g.*, grasses (annual rye, cocksfoot, and fescue) grown in combination with leguminous cover crops (medic and clover) increase organic carbon without depleting soil nitrogen (Karl *et al.*, 2016b). Moreover, where most under-vine studies have been conducted in high rainfall, cool-climate regions, ours seeks to understand their effect on vineyards in hot, dry, Mediterranean-type climates.

Ultimately, this study aims to test the hypothesis that certain under-vine cover crops can have either neutral or beneficial impacts on soil properties and grapevine productivity. In testing this hypothesis, we aim to quantify several metrics including grapevine yield and fruit chemistry; soil nutrients and organic carbon concentrations; soil moisture, vine canopy density, and water status. These metrics are the focus of our study in a long-term trial in a commercial vineyard in the Langhorne Creek region of South Australia. The findings are expected to contribute to a broader understanding of how under-vine cover crops can be effectively used to enhance vineyard sustainability while maintaining or improving grape yield and quality.

MATERIALS AND METHODS

1. Trial layout

The trial site, in the Langhorne Creek wine region of South Australia; (–35.302” S, 138.952” E), was established in 2014 (Penfold e*t al.,* 2018) on Cabernet-Sauvignon vines planted in 1999. This initial project ran until 2018 before a new project at the same trial site began, and which ran from 2018 to 2021. Sampling throughout the previous trial was non-destructive and management of the vineyard was continuous across both periods. The trial was established in 2014 using a randomised complete block design (seven treatments, four blocks, nine vines per plot). The seven treatments (repeated four times; i.e. once per experimental block) presented here included a control (herbicide-managed bare soil using glyphosate and glufosinate at recommended application rates; “Herbicide”, hereafter), a mulch treatment (wheat straw applied at a rate of 50 tonne ha^{-1}; "Mulch", hereafter), and under-vine cover crop treatments including Kasbah Cocksfoot (*Dactylis glomerate* L.; "Cocksfoot", hereafter, perennial grass), Wallaby grass *(Rytidosperma geniculatum* L.; "Wallaby Grass", hereafter, perennial grass), a *Medicago* sp. mixture (*Medicago littoralis* L. with *Medicago truncatulata* L.; "Medic Mix", hereafter, annual legume), a medic and rye mix (*Lolium rigidum* L. with *Medicago polymorpha* L.; "Medic & Rye", hereafter, annual grass and legume, respectively), and a fescue and clover mix (*Festuca rubra* L. with *Trifolium fragiferum* L.; "Fescue & Clover", hereafter, perennial grass and annual legume). All treatments were allowed to complete their life cycle and were not sprayed with herbicide–annuals senesced after seed set, while perennials continued to grow over the summer months. All treatments received the same irrigation (3.5 ML ha^{-1}, with a dripper spacing of 2.5 m per row at 4 hr d^{-1}, at a rate of 4 L hr^{-1}) as the larger vineyard block within which the trial was sited. It is also important to note that the relative proportion of the species mix changed throughout the trial. Cover crop treatment plots were allowed to recruit 'volunteer' species. The purpose of allowing this to occur is to accurately quantify the effects of a management practice as opposed to highly specific interactions between a limited number of species that would provide little real-world application. See Table S1 and Figure S1 for species composition at the beginning of the 2020 vintage. Botanical analyses performed as described by Tothill *et al.* (1992).

2. Soil moisture profiles

Soil moisture was quantified using multi-depth capacitance probes (Dataflow Systems, Christchurch, New Zealand) at depths of 20 cm, 40 cm, 60 cm, and 80 cm (one probe per treatment, per block). Readings were collected fortnightly throughout the growing season when possible (due to a malfunction of the probe, data was lost at some timepoints). For each sensor (one at each depth), the maximum and minimum reading across the whole season was taken and used to define the max (100 %) and min (0 %) conductance. Given conductance and soil moisture are related linearly (Chanzy *et al.*, 1998), comparisons can be made among treatments at each depth. All data points were then normalised and expressed as relative field capacity [%] (*i.e.*, a percentage of this range) and compared using a linear mixed model (see below).

3. Soil physicochemistry

At the beginning of the 2019–2020 and 2020–2021 grape growing seasons, three 10 cm deep soil cores were taken per plot from the under-vine space, within one meter of a vine trunk, and homogenised. The soil from each plot was sieved to < 2 mm and air-dried before being sent to Eurofins APAL (Adelaide, Australia) for analysis. Total carbon (Total C) and total nitrogen (Total N) were measured by dry combustion. Using 1:5 soil:water extracts, soil pH and electrical conductivity (EC) were measured. Plant-available phosphorous (Colwell P) was measured colorimetrically after bicarbonate extraction. Exchangeable cations (calcium, magnesium, potassium, and sodium) were quantified by the ammonium acetate extraction method.

In August 2019 (winter), three samples of 5 kg of soil (0–15 cm) were taken from the under-vine area of each treatment plot and pooled. Samples were then sorted by hand, and earthworms were counted and weighed. This process was performed after five years of trial operation to explore the cumulative effects of the treatments.

4. Yield components

At the end of the 2019, 2020, and 2021 growing seasons, one day prior to commercial harvest occurring at the trial site, three vines per plot were hand-picked, and bunches were counted before the total yield of the vine was weighed. Cordon length was measured and used to normalise yield components on a cordon length basis (to account for variability in cordon length caused by *Eutypa*). In 2020, samples were taken for berry chemistry analysis. Berry weights were calculated as the average weight of 100 individual berries.

In earlier seasons (2016–2018), yield from each plot as a whole was obtained using a mechanical harvester equipped with a load cell and yield was calculated on a 'per plot' basis which could then be converted to the units provided here (tonnes per hectare). These differing methods for harvest collection were a result of the transition between funding schemes and project staffing over such an extended period.

5. Berry chemistry

The effects of cover crops on fruit quality were quantified by the Australian Wine Research Institute's commercial services (AWRI; Urrbrae, Australia). Samples of grapes (> 1 kg) from each treatment plot were sent for analysis: the spectroscopic methods of Somers and Evans (1977) were used to quantify anthocyanins, phenolics, and tannins. Titratable acidity (TA) and pH were quantified using the standard titration method. The total soluble solids (TSS) was measured using a refractometer. Ammonia, alpha-amino nitrogen (Dukes & Butzke, 1998), and thus yeast assimilable nitrogen (YAN) were quantified using a Thermo Fisher Gallery Discrete Analyser.

6. Leaf area index (LAI)

A ceptometer (Accupar: LP-80, Washington, USA) was used to estimate LAI in the 2019–2020 season. On the 28th of Jan, 2020, a clear, cloudless day, LAI data was collected within one hour of the daily solar zenith. Uninterrupted solar irradiance was measured at each plot before LAI readings were taken below the vine canopy, inline, and level with the cordon. Measurements were taken in triplicate and averaged for each plot.

7. Statistical analysis

Linear mixed models (LMM) were fitted to analyse the effects of treatments on variables ($p < 0.05$). Fixed effects in the model that comprised of the year (used here interchangeably with vintage), treatment, and their interaction, were aimed at quantifying their direct impacts on yield. The random variability between blocks was accounted for by treating the block as a random factor. The model's residual structure was specifically designed to capture correlations within each block-treatment combination across different years, thus addressing the structured variability unexplained by fixed and random factors. Year, treatment, and year:treatment terms were significant after the Wald test. Where normality assumptions were not met (as per Shapiro–Wilks non-parametric normality test), data was transformed using the inverse or log methods. Values of $p < 0.05$ were used to define the significance of Tukey's post-hoc multiple comparisons test. Results of these analyses are plotted as predicted means with 95 % confidence intervals, or alternatively in tables, treatment averages with letters defining significance can be found. Software used for LMM analysis was ASReml-R (Butler *et al.,* 2017) in R studio (RStudio-Team 2021, v1.4.1725) with R (version 4.1.0) in concert with the BiometryAssist package (Nielsen *et al.,* 2021).

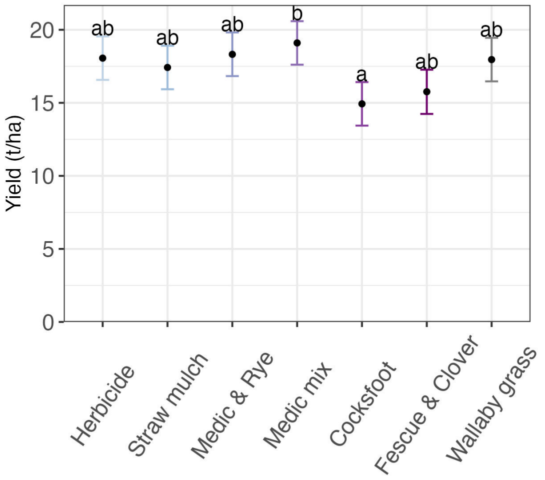

FIGURE 1. Predicted means of yield from the Langhorne Creek trial site from 2016–2021. Predicted means, labelled with the same letter, are not significantly different (p < 0.05) as determined via Tukey's multiple comparisons test. Values are predicted means (derived from the linear mixed model of annual yield data) ± 95 % confidence intervals (n = 4).

PCAs were carried out with the package ggfortifiy and plotted using the ggbiplot package. PCA was specifically used to assess the combined effects of various under-vine treatments on soil and vine parameters. The variables included in the PCA were total soil nitrogen (Total N), yeast assimilable nitrogen (YAN) in the fruit, yield, and leaf area index (LAI). These variables were selected because they represent critical aspects of soil health, vine growth, and fruit quality, which are influenced by the different under-vine management practices.

RESULTS

To determine treatment effects on yield across all seasons (Figure 1) a linear mixed model including year (of harvest) as a factor (2016 to 2021) was used. This analysis revealed that the medic treatments suffered no loss of yield when compared to the Herbicide treatment. Further, the Medic Mix treatment yielded significantly higher than the Cocksfoot treatment. There were no other significant differences among the treatments across this timespan.

Separating yield based on individual vintage (Figure 2) showed no statistical difference among treatments for the first four years of the trial (2016–2019). In 2020, yield was highest for the Herbicide treatment and lowest for the Fescue & Clover, but not significantly higher than for the Mulch, Medic & Rye, or Medic Mix treatments (Table 1). The yield of the Fescue & Clover treatment was significantly reduced, compared to the Herbicide treatment, which was 175 % greater. Data from the 2021 vintage showed similarities to that of the 2020 vintage, albeit with the Medic Mix presenting the overall highest mean yield at 26.54 t ha^{-1} and Kasbah presenting the lowest at 13.04 t ha^{-1}. Although

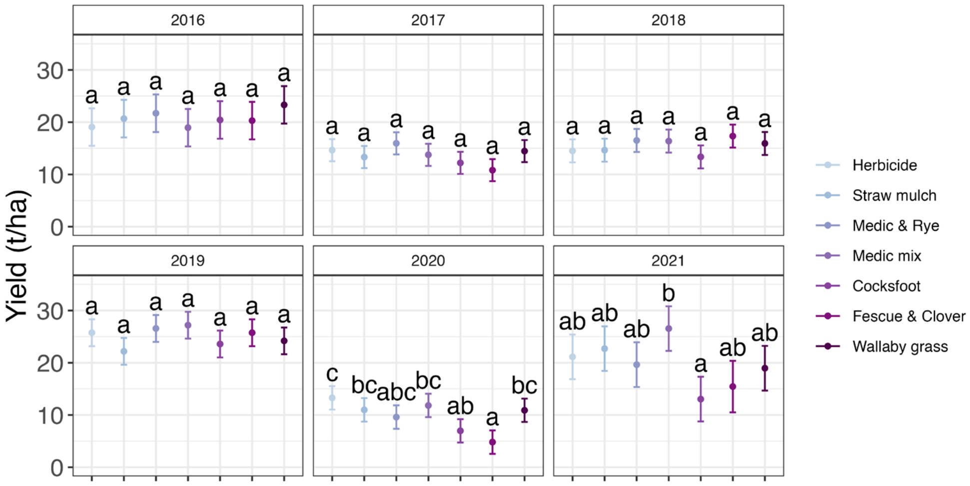

FIGURE 2. Annual predicted means of yield from the linear mixed model of yield data from the Langhorne Creek trial site from 2016–2021. Predicted means labelled with the same letter are not significantly different (p < 0.05) as determined via Tukey's multiple comparisons test. Values are predicted means ± 95 % confidence intervals (n = 4).

vintage variation was evident–especially between 2019 and 2020–treatment effects were evident (2020 and 2021) and presented a similar treatment pattern.

Data on berry size and bunch counts were collected for the 2019 and 2020 seasons (Table 1). In 2020, the highest-yielding treatments (namely Herbicide, Mulch, and Medic Mix) also had the highest bunch weights, which were 43.9, 39.9, and 39.5 g, respectively, compared to an average bunch weight of 23.6 g for the Fescue & Clover treatment. The Medic & Rye and Herbicide treatments both had more berries per bunch than the Fescue & Clover treatment, with 54 % and 51 % higher predicted means, respectively (Table 1). None of the treatments resulted in significant differences in individual berry weights, which on average ranged from 0.7 to 0.9 g across all treatments (Table 1).

Berry chemical profiling was also carried out from samples in 2020 (Table 2). TSS was not different amongst the treatments, while the Fescue & Clover treatment led to significantly higher anthocyanins than the Medic Mix and Medic & Rye treatments, by 21.4 %. Juice pH was significantly higher for the Fescue & Clover treatment than all other treatments except Medic & Rye, although all treatments ranged from 3.4

TABLE 1. Yield components (including bunch weight, berry weight, and berries per bunch) in the 2019, 2020, and 2021 vintages (n = 4). Data are the mean and standard deviation (in brackets). Significance-defined letters using Tukey's multiple comparisons test ($p < 0.05$).

	Herbicide	Mulch	Medic & Rye	Medic Mix	Cocksfoot	Fescue & Clover	Wallaby Grass
Vintage 2019							
Yield (t ha^{-1})	25.7 (1.1)	22.2 (0.9)	26.6 (1.4)	27.2 (1.2)	23.6 (1.4)	25.7 (1.3)	24.2 (2.6)
Bunch weight (g)	99.5 (4.7)	90.6 (7.2)	96.4 (7.2)	106 (7.3)	99.8 (4.0)	109.8 (5.8)	115.10 (14.5)
Berry weight (g)	1.06 (0.0)	1.07 (0.1)	1.11 (0.0)	1.11 (0.0)	1.04 (0.0)	1.05 (0.0)	1.06 (0.0)
Vintage 2020							
Yield (t ha^{-1})	13.3 (2.8) c	11.0 (0.4) bc	9.6 (0.5) abc	11.8 (2.0) bc	7.0 (3.2) ab	4.8 (2.8) a	10.9 (4.0) bc
Bunch weight (g)	43.9 (5.0) c	39.9 (2.6) bc	38.2 (4.9) bc	39.5 (3.5) bc	30.2 (7.0) ab	23.6 (5.3) a	38.4 (5.8) bc
Berry weight (g)	0.9 (0.0)	0.8 (0.1)	0.8 (0.2)	0.8 (0.1)	0.8 (0.1)	0.7 (0.1)	0.8 (0.1)
Vintage 2021							
Yield (t ha^{-1})	21.1 (1.6) ab	22.7 (2.2) ab	19.6 (1.3) ab	26.5 (4.3) b	13.0 (1.8) a	16.5 (2.7) ab	18.95 (2.1) ab
Bunch weight (g)	54.3 (5.5)	69.10 (23.1)	56.6 (7.7)	56.6 (7.7)	40.07 (3.4)	69.1 (23.1)	47.77 (4.4)
Berry weight (g)*	-	-	-	-	-	-	-

**Data not collected*

TABLE 2. Grape juice chemical properties in the 2020 vintage (n = 4). Data is mean and standard deviation (in brackets). Significance-defined letters using Tukey's multiple comparisons test ($p < 0.05$).

	Herbicide	Mulch	Medic & Rye	Medic Mix	Cocksfoot	Fescue & Clover	Wallaby Grass
Total soluble solids (°Brix)	26.0 (0.6)	24.5 (1.5)	25.4 (1.7)	24.8 (1.9)	25.4 (0.3)	26.0 (0.6)	25.0 (1.3)
Free Anthocyanins (mg g^{-1})	1.52 (0.1) ab	1.42 (0.2) ab	1.35 (0.1) a	1.35 (0.2) a	1.52 (0.1) ab	1.71 (0.1) b	1.42 (0.1) ab
pH	3.43 (0.0) a	3.45 (0.1) a	3.49 (0.1) ab	3.45 (0.1) a	3.44 (0.0) a	3.62 (0.1) b	3.46 (0.0) a
Titratable acidity pH 7.0 (g L^{-1})	7.27 (0.3) b	6.93 (0.5) b	7.53 (0.9) b	7.68 (1.2) b	6.33 (0.5) ab	5.28 (0.4) a	6.75 (0.4) ab
Total phenolics (absorbance)	147.6 (7.2) bc	140.5 (9.4) ab	136. 5 (5.0) ab	131.0 (11.3) a	152.1 (2.2) bc	162.3 (4.7) c	140.3 (6.3) ab
Total tannin (mg g^{-1})	6.01 (0.3) abc	5.72 (0.6) abc	5.58 (0.2) ab	5.24 (0.3) a	6.30 (0.1) bc	6.55 (0.5) c	5.74 (0.4) abc
Yeast assimilable nitrogen (mg L^{-1})	149.5 (33.7) a	158.0 (26.5) ab	209.2 (25.0) bc	219.8 (30.6) c	139.2 (21.4) a	156.0 (26.6) a	151.5 (9.1) a

to 3.6 pH. TA was lowest for the Fescue & Clover treatment, and similar to that of Cocksfoot and Wallaby Grass, whereas Herbicide, Mulch, Medic & Rye, and the Medic Mix were all significantly higher than Fescue & Clover.

The Fescue & Clover treatment led to the highest phenolic concentration, which was significantly higher than the Mulch, Medic & Rye, Medic Mix, and Wallaby Grass treatments. The Medic & Rye and Medic Mix treatments possessed significantly fewer total tannins, by 13.8 % and 20 %, respectively, than the Fescue & Clover treatments.

YAN was highest for the Medic Mix and Medic & Rye treatments (219 and 209 mg L^{-1}, respectively). These were significantly higher than the Herbicide, Cocksfoot, Fescue & Clover, and Wallaby Grass treatments, which ranged between 139.2 mg L^{-1} for Cocksfoot to 158.0 mg L^{-1} for Mulch.

LAI, a measure of canopy density, can be seen in Figure 3. Medic treatments had the highest LAIs, though not significantly different from the Herbicide, Mulch, and Wallaby Grass treatments. Cocksfoot and Fescue & Clover treatments had the lowest LAIs in 2020. Specifically, the Medic & Rye treatment was 51.5 % greater than the Cocksfoot treatment and 36.7 % greater than the Fescue & Clover treatment, while the Medic Mix was 49.6 % and 35.0 % greater than the Cocksfoot and Fescue & Clover treatments, respectively.

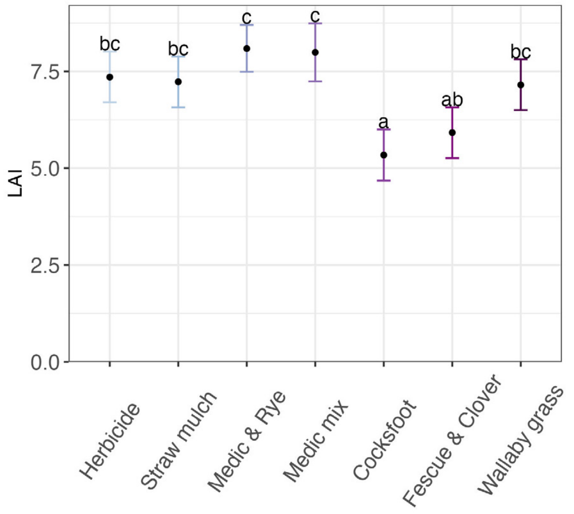

FIGURE 3. Leaf area index of vines on the 28th of Feb 2020. Letters indicate significant differences ($p < 0.05$) as determined via Tukey's multiple comparisons test. Values are predicted means ± 95 % confidence intervals ($n = 4$).

Soil physicochemical properties differed significantly among treatments. In 2019 (Table 3), few differences among treatments were significant. Notably, total nitrogen was highest for the Medic Mix, and lowest for the Herbicide and Mulch treatments. Similarly, soil from the Medic Mix treatment had the highest total carbon, though this was not significantly different from other treatments.

In 2020, the soil pH was significantly higher for the Herbicide than for all other treatments except Wallaby grass. Although plant-available (Colwell) P was low across all treatments (Oliver *et al.*, 2013), it was significantly higher for the Mulch treatment than the Medic Mix treatment. Sodium levels were highest for the Herbicide treatment (431 mg kg^{-1} kg) and lowest for the Mulch treatment (113 mg kg^{-1}), which were significantly different from each other, while all other treatments lay somewhere between and within an acceptable range (Oliver *et al.*, 2013) Total soil N was lowest for both the Herbicide and Mulch treatments, 0.23 % and 0.22 %, respectively. Higher were all other active cover-crop treatments, which ranged between 0.27 % for Medic & Rye and Wallaby Grass treatments to 0.29 % for Medic Mix, Cocksfoot, and Fescue & Clover treatments. Some of these differences were significant. Total soil carbon was highest for the Cocksfoot (2.2 %) and lowest for the Herbicide treatment (1.1 %), indicating a doubling by maintaining coverage with Cocksfoot for six years. The Medic & Rye and Medic Mix treatments had total carbon concentrations of 1.8 % and 1.7 %, respectively, though these levels were not significantly different from other treatments.

Soil moisture at depths from 20 cm to 80 cm, at 20 cm intervals, was measured using a capacitance probe across the 2020 vintage growing season (Figure 4). No significant differences were found among treatments when respective depths were separately compared. Despite a lack of

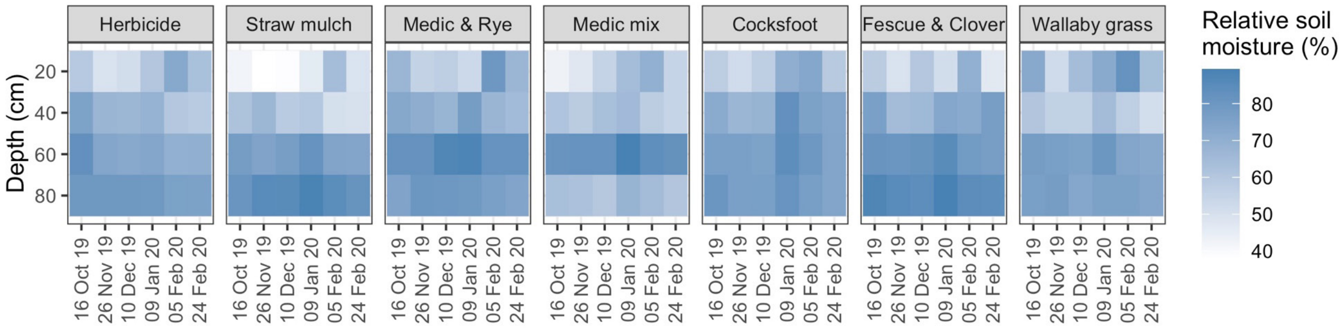

FIGURE 4. Soil moisture from 20 cm to 80 cm at 20 cm intervals, as a percentage of the maximum moisture detected throughout the 2019–2020 growing season at each depth, as measured using a capacitance probe. No significant differences between treatments at specific depths were detected. Sampling occurred throughout the season and phenological stages of development, including budburst (October), veraison (January), and harvest (late February).

TABLE 3. Soil physicochemical properties from Langhorne Creek in the 2019 vintage (n = 4). Data are mean and standard deviation (in brackets). Significance is defined by letters as tested by a linear mixed model ($p < 0.05$).

	Herbicide	Mulch	Medic & Rye	Medic Mix	Cocksfoot	Fescue & Clover	Wallaby Grass
pH $_{1:5\ water}$	7.76 (0.04) b	7.53 (0.1) ab	7.22 (0.04) a	7.20 (0.1) a	7.43 (0.1) ab	7.38 (0.1) ab	7.35 (0.04) a
pH $CaCl_2$	7.28 (0.1) b	7.09 (0.1) ab	6.90 (0.1) a	6.82 (0.1) a	7.10 (0.1) ab	7.00 (0.1) ab	7.07 (0.1) ab
Colwell Phosphorus	19.5 (4.03)	43.3 (8.51)	22.5 (2.86)	20.0 (4.17)	50.75 (13.81)	29.25 (7.19)	27.25 (6.26)
Calcium (mg/kg)	1412.5 (123.9) a	1470.0 (154.2) ab	1907.5 (216.9) abc	1770.0 (207.7) abc	2145.0 (168.8) bc	1720.0 (188.9) abc	2202.5 (240.6) c
Magnesium (mg/kg)	339.0 (35.3)	327.0 (12.5)	344.8 (25.9)	339.3 (36.4)	351.8 (24.5)	376.5 (29.3)	338.8 (24.1)
Potassium (mg/kg)	97.8 (9.3)	120.8 (26.6)	116.3 (13.9)	103.0 (9.8)	166.5 (30.9)	106.3 (21.6)	125.0 (20.2)
Sodium (mg/kg)	327.5 (136.7)	202.8 (45.7)	214.0 (48.2)	197.5 (25.7)	145.5 (18.6)	280.5 (47.7)	179.5 (34.2)
Salinity ($EC_{1:5}$ dS/m)	0.53 (0.2)	0.43 (0.1)	0.7 (0.2)	0.57 (0.2)	0.59 (0.1)	0.69 (0.2)	0.83 (0.2)
Iron (mg/kg)	8.40 (0.5)	11.60 (3.5)	11.03 (1.4)	11.93 (1.8)	10.33 (1.6)	9.90 (0.5)	8.25 (0.4)
Manganese (mg/kg)	8.30 (1.1)	7.68 (0.6)	10.13 (2.4)	12.2 (1.9)	10.50 (1.0)	11.50 (1.7)	9.95 (1.8)
Copper (mg/kg)	6.65 (0.7)	5.40 (1.1)	7.18 (0.7)	5.63 (0.7)	6.50 (0.5)	5.00 (0.7)	6.03 (0.1)
Zinc (mg/kg)	4.20 (0.6)	4.23 (0.6)	5.18 (0.9)	4.63 (0.9)	4.75 (0.5)	3.78 (0.8)	4.28 (0.4)
Total nitrogen (%)	0.17 (0.01) a	0.17 (0.01) a	0.25 (0.04) b	0.19 (0.01) ab	0.21 (0.02) ab	0.21 (0.01) ab	0.19 (0.1) ab
Total carbon (%)	1.52 (0.1) ab	1.35 (0.2) a	2.35 (0.1) b	1.58 (0.4) ab	2.10 (0.2) ab	1.95 (0.2) ab	1.68 (0.1) ab

TABLE 4. Soil physicochemical properties from Langhorne Creek in the 2020 vintage (n = 4). Data are mean and standard deviation (in brackets). Significance is defined by letters as tested by a mixed model ($p < 0.05$).

	Herbicide	Mulch	Medic & Rye	Medic Mix	Cocksfoot	Fescue & Clover	Wallaby Grass
pH $_{1:5\ water}$	8.15 (0.1) b	7.76 (0.2) a	7.67 (0.3) a	7.61 (0.2) a	7.56 (0.2) a	7.56 (0.1) a	7.79 (0.1) ab
pH $CaCl_2$	7.53 (0.3) b	7.14 (0.2) ab	7.01 (0.1) a	6.87 (0.1) a	6.99 (0.2) a	7.09 (0.1) a	7.22 (0.2) ab
Colwell Phosphorus	38.8 (21.7) ab	45.8 (27.2) b	25.2 (10.4) ab	15.0 (5.9) a	24.5 (5.7) ab	25.2 (3.9) ab	29.5 (7.9) ab
Calcium (mg/kg)	1465 (364)	1185 (145)	1500 (101)	1385 (103)	1595 (292)	1613 (100)	1455 (123)
Magnesium (mg/kg)	308.8 (46.3) ab	223.5 (32.9) a	285.5 (32.2) ab	316.2 (64.2) ab	372.2 (48.6) b	371.5 (22.9) b	278.8 (77.8) ab
Potassium (mg/kg)	145.8 (74.0) a	155.0 (94.4) a	70.8 (10.3) a	60.2 (5.5) a	85.2 (8.7) a	63.0 (17.6) a	117.0 (50.1) a
Sodium (mg/kg)	431.5 (260.2) c	112.9 (25.2) a	194.8 (45.6) abc	236.5 (50.6) abc	263.8 (90.6) abc	385.5 (79.2) bc	147.6 (40.3) ab
Salinity ($EC_{1:5}$ dS/m)	0.64 (0.60)	0.20 (0.06)	0.20 (0.04)	0.20 (0.06)	0.32 (0.13)	0.49 (0.10)	0.27 (0.21)
Iron (mg/kg)	6.45 (2.1)	8.92 (1.7)	13.75 (4.2)	12.75 (2.5)	19.25 (14.0)	12.22 (1.8)	9.05 (3.1)
Manganese (mg/kg)	8.52 (2.3) a	9.70 (1.1) abc	14.25 (1.5) cd	14.0 (2.8) bcd	15.50 (3.3) d	15.0 (3.6) d	8.90 (1.5) ab
Copper (mg/kg)	6.08 (1.3) ab	4.68 (1.1) a	7.82 (1.3) bc	7.45 (0.5) abc	9.05 (2.1) c	7.50 (0.4) abc	6.88 (1.4) abc
Zinc (mg/kg)	3.45 (0.9) ab	2.83 (0.7) a	5.63 (1.3) bc	5.23 (0.3) abc	7.60 (1.8) c	5.85 (0.8) bc	4.38 (1.3) ab
Total nitrogen (%)	0.23 (0.02) ab	0.22 (0.02) a	0.27 (0.03) bc	0.29 (0.04) c	0.29 (0.06) c	0.29 (0.01) c	0.27 (0.02) abc
Total carbon (%)	1.15 (0.1) a	1.28 (0.2) a	1.76 (0.3) ab	1.72 (0.3) ab	2.21 (0.8) b	1.74 (0.2) ab	1.45 (0.2) a

difference in soil moisture among treatments, there was a seasonal pattern. Specifically, water content was higher at the 80 cm depth at the beginning of the growing season in October 2019 in all treatments. This decreased through summer as available moisture reduced at this depth. Summer rains and irrigation led to the highest levels of surface moisture in late January 2020.

Earthworm presence in the under-vine area was significantly higher in the Wallaby Grass treatment than in the Herbicide treatment, with all other treatments intermediate (Figure 5). The Cocksfoot treatment also supported 10 times more earthworms than the Herbicide treatment, although these were not significantly different from one another.

Select data from the 2020 season, specifically total soil N, LAI, yield, and YAN, were used for principal components analysis. Two principal components explained 85.5 % of the variation (Figure 6). Loadings suggest the presence of medics as under-vine cover crops heavily influenced YAN in the fruit.

DISCUSSION

This study aimed to test the hypothesis that certain under-vine cover crops can have either a neutral or beneficial impact on soil properties and grapevine productivity. In testing this hypothesis, we quantified several metrics including grapevine yield and fruit chemistry; soil nutrients and organic carbon concentrations; soil moisture to a depth of one metre through the soil profile and vine canopy density and water status. These results are discussed in relation to the understanding of the drivers of variations in grapevine productivity, water and nutrient competition.

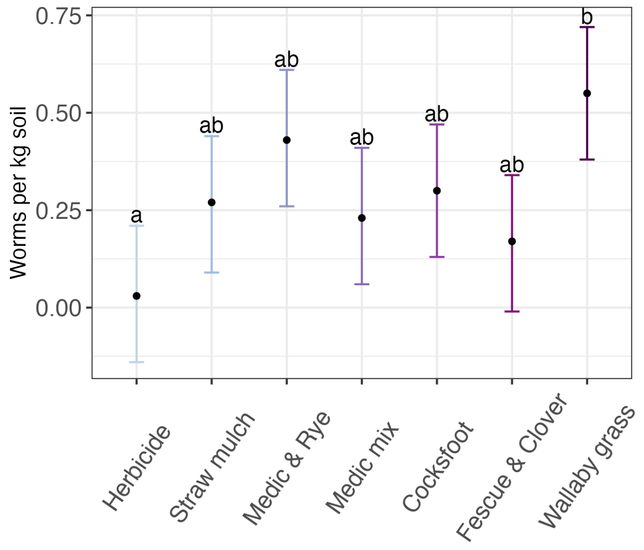

FIGURE 5. Number of earthworms per kg of soil. Letters indicate significant differences ($p < 0.05$) as determined via Tukey's multiple comparisons test. Values are predicted means ± 95 % confidence intervals (n = 4).

The results of a six-year field trial presented here, raise doubts about standard under-vine viticultural soil management practice. In terms of crop yield and quality, the Herbicide treatment provided no quantifiable advantage over the Medic Mix, Medic & Rye, Wallaby Grass, or Mulch treatments. Moreover, the inclusion of cover crops in some instances resulted in improvements in soil properties, broadly expected when compared to bare soil (Abad *et al.*, 2023). There is, however, a cautionary tale that the selection of cover crop–species or combination thereof–is important, as some cover crop species (in this case Fescue & Clover and Cocksfoot, both perennial grasses), resulted in yield reductions. Taken together, these data demonstrate that previous fears of resource competition by under-vine cover crops may be allayed when weighed against continued herbicide use.

The findings of this research have important implications for grape growers. Reducing or even eliminating under-vine herbicide application is no longer the domain of growers looking to sell their fruit as organic, or to improve sustainability at the cost of yields and profit. The detrimental effects of herbicides on soil ecology, water systems, and human health worldwide are attracting attention and may reduce market access via a possible ban or limitations on their use in the immediate future (Walsh & Kingwell, 2021; Wynn & Webb, 2022). Likewise, consumers are willing to pay more for a wine produced where cover crops were an integral part of their farming practices, indicating the value of expanding the use of cover crops in vineyards, not only the mid-row but under-vine as well (Kelley *et al.*, 2022).

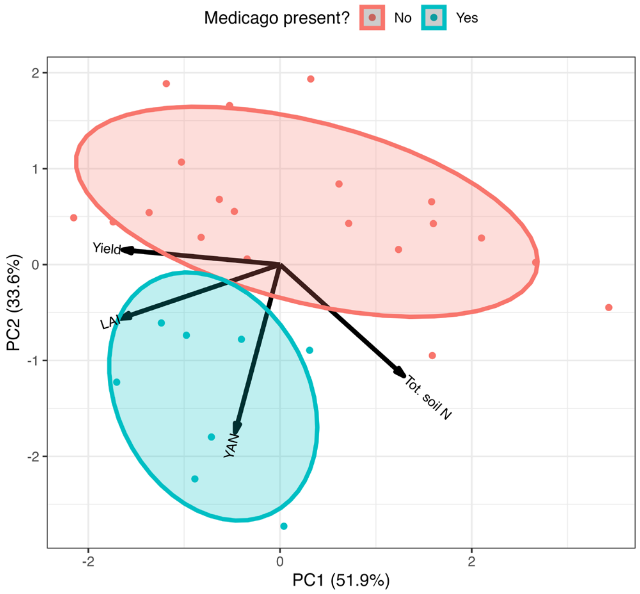

FIGURE 6. Principal coordinates analysis with samples grouped by the presence (blue ring) or absence (orange ring) of Medic (*Medicago* L.) during original seed sowing. Ellipses represent regions of 68 % confidence intervals. Arrows define the loading of the variables contributing to the principal components (Abbreviations are YAN = yeast assimilable nitrogen, Tot. soil N = Total soil nitrogen, LAI = Leaf area index).

1. Drivers of yield variation

For the most part, neither under-vine cover crops, nor Mulch, resulted in reduced yields relative to the Herbicide treatment, and indeed to the contrary, yield data from 2021 showed the Medic Mix to have the highest yield. The notable exceptions are that of the Fescue & Clover and Cocksfoot, which both lead to reduced yields relative to the Herbicide treatment. It is important to note here that for “Fescue & Clover”, the species composition shifted from a blend of Fescue and Clover to one dominated by Fescue (Table S1 and Figure S1). In a Southern France vineyard, the whole floor covered by tall fescue (*Festuca arundinacea* L.) reduced yields compared to when the mid-rows were kept bare (Celette *et al*., 2008). Conversely, creeping red fescue did not reduce vine yields in a high-rainfall vineyard, although pruning weights were lower than the under-vine herbicide treatment (DeVetter *et al.*, 2015). Where soil water is limited, cover crop selection is crucial, as demonstrated by a North Carolina study where climatic conditions provided ample moisture for both under-vine grass and grapevine (Giese *et al.*, 2014).

One advantage of the present study over previous work is the longevity of the trial. Yields were not immediately affected by the under-vine cover crop treatments; it took many years for these effects to propagate through the system and influence vine productivity. This may not be the case for vineyards less regularly watered. Together, these examples along with the present study, highlight the value of studies in different regions, and the correct selection of under-vine cover crops.

2. Water and nitrogen drive variations in yield

Given yields vary with ground cover species, we attempted to understand the drivers of this effect. Nitrogen levels in the grapevines at numerous trials, where under-vine perennial grasses were included, demonstrated that grapevine nitrogen is commonly reduced as a result of this competition (Giese *et al.*, 2014; Klodd *et al.*, 2016; Fleishman *et al.*, 2019); however, it is apparent that the degree and relevance of this reduction on grapevine yield depends on a hierarchy of limiting factors, soil moisture key among them. There have been relatively few studies that have looked at the impacts of under-vine vegetation in drier regions, and fewer again that have looked at management practices that have been in place for as long as those in this study, thus it is difficult to directly compare our findings. Nonetheless, some evidence exists from similar climates. An under-vine study in an Italian vineyard showed reduced chlorophyll concentration in vines grown above a grass mixture compared to a legume mix (Muscas *et al.*, 2017). During the second and third years of under-vine trials, yields, clusters per vine, and pruning weights were lowest in the grass mixture plots, compared to the natural cover and legume mixture. The authors concluded that this was likely due to a combined effect of water and nutrient competition with the grapevines (Muscas *et al.*, 2017). Our study supported these findings, as it was only the vigorous grass treatments of Cocksfoot and Fescue & Clover that had significantly lower yields. The Wallaby Grass, endemic to Australia and thus well-adapted to growing in conditions of low soil moisture and nutrients, did not appear to compete as aggressively as the Cocksfoot and Fescue treatments (based on its limited effect on LAI and yield relative to the herbicide treatment). Treatments of Medic Mix and Medic & Ryegrass performed similarly well, a result likely owing to nitrogen fixation. The *Medicago* sp. essentially supplies nitrogen in organic form to the soil, thus reducing competition by grass species in the mix. Although we may have expected similar results with the Fescue and (N-fixing) Clover, the species composition shift was dominated by Fescue (Figure S1), which effectively nullified organic nitrogen provision by the Clover. A similar study in the Barossa winegrowing region reported similar results, with combinations of legume and grass able to build soil carbon and nitrogen, while showing neutral or positive effects on grapevine yield versus the control (Ball *et al.*, 2020).

These results further demonstrate that with strategic selection of cover crop (or combination thereof) in the under-vine space, a grower can plausibly reduce herbicide application while yielding the same or more than with the traditional practice. This result is visible in the repeated measures analysis, and data from 2020, which, while being a low-yielding season in this region, showed significant differences among the treatments. In 2021, yields returned to their anticipated level, however, patterns of treatment effects were the same. This result shows that although vintage variation is a factor, treatment effects are still statistically significant. Moreover, it highlights a hierarchy of needs by both cover crops and grapevine–water and nitrogen chief among them–where dryer surface soils limit nitrogen mineralisation. More specifically, senesced above-ground biomass reduces evaporation, increasing surface moisture during dry periods, and enabling the breakdown of the high N organic material. In irrigated vineyards, this mechanism should apply (unless sprinkler irrigated), as drippers provide ample area for surface soil to dry and thus limit the mineralisation processes. The vineyard chosen for this study was highly irrigated, which may have led to a limit on the observable competitive effects pertaining to available water. However, over small scales, the hypothesised mechanism for moisture influencing N mineralisation should still apply.

Leguminous cover crops (with or without annual grasses) influence grapevine canopy and YANs. The observed yield reductions associated with the Cocksfoot and Fescue & Clover were likely due to persistent competition for resources in the root zone. This conclusion is further supported by the reduction in leaf area index (LAI) for these treatments (Keller, 2005). Additionally, and in conjunction with yield, it could be inferred that the treatments containing Medic contributed positively to the nitrogen requirements of the vines, without competing with vine roots due to senescence in late spring. Surprisingly, the disparity in fruit nitrogen was not evident in total soil N and, although lower in Herbicide and Mulch, remained non-significant between treatments containing Medic vs Cocksfoot and Fescue & Clover. Reduced total soil N in the Herbicide and Mulch treatments may be a result of leaching from high irrigation, or simply a reduced C content in the form of microbes and roots. The medic treatments possessed the greatest LAI values in the trial, which we attribute to the provision of N by the cover crop to the soil via associations with nitrogen-fixing bacteria (Sabagh *et al.*, 2020). Additionally, yeast assimilable nitrogen (YAN) was significantly higher (40–46 %) in juice from grapes grown under treatments of Medic than Herbicide. Results from the PCA analysis (Figure 6) demonstrate an observably strong correlation between YANs, LAI, and the presence of leguminous cover crops. Increased organic N supplied by leguminous cover crops encourages microbial activity, leading to N mineralisation (Griesser *et al.*, 2022). Thus, we would expect to observe greater plant-available N uptake by grapevines and thus higher LAI values (Segura-Borrego *et al.*, 2024) and YAN concentrations, consistent with our data. We hypothesise that increased organic N, provided by N-fixation in the Medic treatments, increases mineral N availability, and thus grapevines present significantly higher N concentrations in their fruit (and likely their other biomass).

Plant available P was lower in the treatments with under-vine cover crops than the mulch and herbicide treatment in the 0–10 cm soil layer. Storage of P in cover crops (above ground) may explain this reduction in soil P. Over time, as the cover crop ecosystem reaches a steady state, P fluxes should reduce as P is released into the soil with cycling of the cover crops and microbes (the same effect is likely with N). To overcome this initial reduction in soil P, fertiliser addition may be helpful when establishing cover crops, though grape vines have a low requirement for P (Keller, 2005) and the observed levels are not expected to have an impact on vine health.

3. Native cover crops increase earthworm frequency

Earthworms (number of earthworms per kg surface soi), crucial soil ecosystem ‘engineers’ (Meissner *et al.*, 2019), were significantly lower for the Herbicide treatment relative to the Wallaby Grass. The herbicide treatment had lower worm frequency than all other treatments, though this was not significant. Although there are relatively few studies specifically reporting on the effects of herbicide use in vineyards on worm populations, Zaller *et al.* (2018) did not find any significant effects of herbicides on earthworm populations in an Austrian vineyard, although the herbicide treatments did significantly reduce mycorrhizal colonisation of vine roots, compared to under-vine tillage by approximately 53 %. Alternatively, organic and biodynamic vineyard plots that did not use herbicides in Germany reported 45 % and 94 %, respectively, more earthworms than the herbicide treatment, although these were not statistically significant findings (Meissner *et al.*, 2019). The effects of herbicide may not be directly affecting worm presence–the reduction in food sources likely limits the dispersal of earthworms into areas devoid of a nutrient source. This result highlights the benefit of cover–whether it is living or mulch–in its ability to provide organic matter or retain soil moisture and thus provide habitat resources that encourage macro-faunal proliferation.

CONCLUSION

This study investigated the effects of seven different under-vine treatments on soil physiochemical properties, grapevine productivity, and fruit composition after six years in South Australia. The main findings of this research point to the promising potential of including Medic (*Medicago* L.) species in the under-vine area, as they did not reduce yields compared to the herbicide treatment, and they increased LAI and fruit YAN content relative to perennial grass under-vine treatments. The observed higher YAN and LAI are plausibly explained by the N fixed by the Medic species, which was valuable for grapevine development. Under-vine grass treatments, Cocksfoot and Fescue & Clover had the opposite effect. Specifically, they significantly reduced LAI and grapevine yield, which we suggest was a result of increased competition for soil water and nutrients, thus reducing availability to the grapevines at crucial timepoints in the growing season. A native perennial grass species (Wallaby Grass), on the other hand, did not impact yield, growth, or fruit composition, whilst benefitting earthworm populations compared to the Herbicide treatment. This shows that selecting species that have low water and nutrient requirements, or ones that are specifically adapted to the local environment, may prove beneficial additions to vineyards adopted under-vine or whole-floor cover.

The key implication of this research is that under-vine cover crop species selection is critical to success. Unfavourable cover crop selection, particularly in water-limiting environments can lead to large reductions in yield; however, this is very dependent on the context (interaction between vineyard age, soil type, climate, and production goals). Thus, there is a need for similar work in more regions, particularly those where water is limited. In addition to highlighting the potential of *Medicago* species as an important contribution to the under-vine area, this research also highlights the potential of including a native grass in the under-vine space of this system, with no impact on yield, fruit composition, or LAI, relative to the Herbicide treatment, and a positive effect of earthworm numbers observed.

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