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Implications of Sustainable Vineyard Floor Management Practices on California Vineyard
Agroecosystems: The Effect of Cover Crops and Tillage on Whole Grapevine Physiology and Net
Ecosystem Carbon Balance

By

MARIA ZUMKELLER
THESIS

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Abstract

Vineyard floor management is comprised of several cultural practices used to accomplish various goals including weed control, water, nutrient, and fertilizer availability. The selection of floor management practices should thus balance the goals of the given vineyard, while taking into consideration factors such as vineyard age, climate, and soil physio-chemical properties (i.e., texture, pH, CEC, etc.). One such floor management practice is the adoption of winter cover crops. The use of this technique has increased across vineyards of Mediterranean climates over the last decade (Karlen et al., 2019). This is especially true among California vineyards, where adoption of climate smart agriculture practices is encouraged and, in some instances, incentivized (Lewis and Rudnick, 2019). Furthermore, reducing soil tillage has been identified as an integral strategy to meeting the State of California's greenhouse gas emissions (GHG) reduction goals (Steenwerth et al., 2016).

Cover crops may include a single or mixture of species, are seeded in the fall prior to winter rains, and allowed to grow in vineyard alleyways during the dormancy season. This complementarity in space and time is a key advantage to using cover crops to reduce soil erosion, improve water infiltration, and increase above and belowground biodiversity (Bowles et al., 2017a). Despite the well-documented benefits of cover crops on soil, concern exists regarding competition between the grapevine and cover crop for water and nutrients which may cause reductions in vegetative growth and yield (Ingels et al., 2005; Celette et al., 2009a). However, reports of the influence of cover crops and tillage on grapevine growth, yield, and juice characteristics in irrigated vineyards are inconsistent (Monteiro and Lopes, 2007a; Costello, 2010; Reeve et al., 2016a; Coniberti et al., 2018; Delpuech and Metay, 2018). Tillage, while an effective weed control method, exacerbates soil erosion and can result in soil compaction over time (Álvaro-Fuentes et al., 2008a; Lal, 2012b). Reduced tillage is defined as any tillage system that utilizes

fewer cultivation passes compared to what is considered conventional at a given site. While reduced and no-till management have been found to increase soil organic matter (SOM) and improve soil aggregation in soils of most semi-arid regions, the influence of various tillage management systems on grapevine performance is also uncertain (Myburgh, 2013a; Steenwerth et al., 2013a; Wolff et al., 2018).

Both cover cropping and reduced tillage have been identified as soil carbon sequestration (SCS) strategies, despite skepticism of the longevity and stability of reported increases in carbon (C). While cover crops and reduced tillage are widely regarded as sustainable floor management practices, the utility of these practices as climate change mitigation tools must be investigated at both the grapevine and vineyard scale. Thus, this thesis research sought to quantify the effect of different combinations of cover crop and tillage systems on grapevine performance as well as C storage potential of the whole vineyard, including C contributions and losses of both the grapevine and vineyard floor. This work was conducted in two commercial vineyards in Fresno and Napa Counties, over two years (2019-2021).

The first component of this work investigated the effects of cover crops and tillage on whole grapevine physiology and berry composition. At both sites, there were no treatment effects on leaf gas exchange which suggested negligible effect of cover crop and tillage on grapevine physiology in vineyards of these climates. In the mature Ruby Cabernet vineyard in Fresno, no changes to yield components or berry composition among cover crop and tillage were measured. In the young Merlot vineyard in Oakville, adoption of no-till management detrimentally affected grapevine water status, as grapevines under conventional tillage displayed higher water status over the two experimental seasons. Despite this effect, no changes to yield or water footprint were observed. Combined, these results provided evidence that both in mature and young vineyards cover cropping had negligible beneficial effects on grapevine physiology, mineral nutrition, or

production; and tillage may be of benefit to young vineyards in semi-arid regions to improve plant water status.

The second component of this work investigated the impact of cover crops and no-till management on net ecosystem carbon balance and the potential for vineyard systems to serve as carbon sinks. Results indicated that while vineyards of the investigated climates served as C sinks over the two experimental seasons, the influence of cover crops and tillage systems varied between the two sites. At the Fresno County vineyard, where soil texture is coarse and largely sandy, tillage and type of cover crop did not affect net ecosystem carbon balance (NECB). However, under the finer textured soil at the Napa County site, tillage reduced NECB via losses of soil organic carbon, and increased CO₂ efflux (soil respiration) compared to no-till management. Grapevines under conventional tillage also contributed higher C inputs through annual growth of shoots and leaves. Furthermore, the cover crops that produced the greatest amount of biomass increased NECB. A lack of statistical interaction between the cover crop and tillage factors may suggest that organic C inputs from the cover crop is a key determinant of C storage potential in addition to soil texture.

Combined, these results indicate that site characteristics such as vineyard age and soil texture may modulate the effect of cover crops and tillage systems observed on whole grapevine physiology and net ecosystem carbon balance. These findings reveal that the general considerations of cover cropping and reduced tillage as sustainable vineyard floor management practices may overlook important considerations to the grapevine itself as well as the vineyard as a whole.

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Chapter 1: Cover Crops and No-Tillage Show Negligible Effects on Grapevine Physiology in Mediterranean Vineyard Agroecosystems

Keywords: Cover crops, climate change, tillage, berry composition, grapevine physiology

Abstract

This study aimed to compare the effects of annual or perennial cover crops and tillage regimes on whole grapevine physiology and berry composition. We studied the interactive effects of tillage and cover crops on grapevine water status, leaf gas exchange, components of yield, berry composition and resulting water footprint in two contrasting production regions (Fresno County-hot climate and Napa County-warm climate) of California. The treatments included a perennial grass (PG), resident vegetation (RV), and an annual grass (AG) grown under conventional tillage (CT) and no-till (NT) settings. Neither cover crop nor tillage affected grapevine leaf gas exchange. However, at the Napa County vineyard, NT detrimentally affected grapevine water status compared to CT. Grapevine mineral nutrition, when assessed during anthesis revealed no effects of cover cropping in either year or at either location. Cover crop type did not affect yield components or berry composition, however CT increased titratable acidity (TA) at both sites. The water footprint of vineyards at either location was not affected by cover crops or tillage. Our results provided evidence that both in hot and warm climate vineyards cover cropping had negligible beneficial effects on grapevine physiology, mineral nutrition or productivity with no detrimental effects on vineyard water footprint; tillage was beneficial in younger vineyards to improve plant water status in semi-arid regions.

1. Introduction

In the last decade there has been a rise in adoption of sustainable soil management practices that reduce soil erosion and bolster soil organic matter to counter the impacts of climate change on agricultural soils (Lal, 2004a; Powlson et al., 2011a; Lal, 2012a; Lazcano et al., 2020a). Traditionally, vineyard rows were kept bare, with the use of herbicides and tillage. However, there is disagreement on the utility of this practice due to the detrimental effects of tillage on air quality and soil physical, chemical, and biological properties (Patiño-Zúñiga et al., 2009a; Ferreira et al., 2020a; Gatti et al., 2022a). Thus, the adoption of cover crops and reduced tillage is considered a sustainable alternative to traditional management of vineyard floors (Alsina et al., 2013b). Furthermore, environmental regulations and public perception serve as additional incentive to adopt climate-smart practices (Guerra and Steenwerth, 2012).

The benefits of cover crops on the properties of soils are well documented. They can increase soil organic matter (SOM), improve water infiltration and aggregate stability, reduce soil erosion and greenhouse gas emissions (GHG), and increase vineyard biodiversity (Ingels et al., 2005; Steenwerth and Belina, 2008b; Abad et al., 2021). Nevertheless, the adoption of cover crops in vineyards is limited by the concern of excessive competition between the cover crop and grapevine for water and nutrients (Smith et al., 2008; Steenwerth and Belina, 2008a; Celette et al., 2009a; Steenwerth et al., 2013; Pérez-Bermúdez et al., 2016). Many studies have sought to quantify the effects of cover cropping on the grapevine, yet the influence of cover crop adoption on grapevine physiology and production remains elusive.

There is agreement in literature that cover crops may reduce vegetative growth, with more pronounced yield losses in warmer regions. However some studies have found no effect (Monteiro and Lopes, 2007; Lopes et al., 2008a; Smith et al., 2008; Costello, 2010; Jordan et al., 2016; Steenwerth et al., 2016). Grapevine physiological responses to cover cropping were also

documented, and minimal effects on leaf gas exchange have been found. The presence of a cover crop is generally reported to detrimentally affect grapevine water status (Naor et al., 1997; Monteiro and Lopes, 2007; Hatch et al., 2011a; Pou et al., 2011; Steenwerth et al., 2016; Tomaz et al., 2021). Despite wide acceptance of this particular effect, results are inconsistent as some studies have shown that cover crops may improve early season water status, yet others have concluded that cover cropped vineyards do not display better water status compared to those with bare soil (Celette et al., 2005; Ingels et al., 2005; Monteiro and Lopes, 2007; Costello, 2010; Reeve et al., 2016a; Coniberti et al., 2018; Daane et al., 2018; Delpuech and Metay, 2018). Ultimately, previous works agree that changes in grapevine physiological response to cover crop adoption are largely driven by the climatic conditions and irrigation regime at a given site (Delpuech and Metay, 2018; Tomaz et al., 2021).

Likewise, it was assumed that the competition between the grapevine and cover crop for water and nutrients resulted in yield decreases, but this effect was also not consistent (Morlat and Jacquet, 2003; Ingels et al., 2005; Tesic et al., 2007; Lopes et al., 2008a; Smith et al., 2008; Costello, 2010; Pou et al., 2011; Steenwerth et al., 2013; Giese et al., 2015). Yield reductions and/or no effect on yield are the most common results from such work. However, cover crop species appears to be a more influential factor than merely the presence (or absence) of a cover crop. Yield increases have even been reported in some vineyards planted with annual species such as oats or legumes (Fourie and Freitag, 2010; Ovalle et al., 2010; Steenwerth et al., 2013, 2016; Messiga et al., 2016; Fourie et al., 2017). Consequently, any changes to berry composition as a result of cover crop adoption is closely associated with changes in yield, such as smaller berry size and purportedly greater content berry flavonoids (Lopes et al., 2008b, 2011; Lee and Steenwerth, 2013; Tomaz et al., 2021).

The adoption of reduced or no-till management preserves SOM, reduces soil erosion, improves soil structure, and is considered integral to reducing GHG emissions from the agriculture

sector (Álvaro-Fuentes et al., 2008b; Gaudin et al., 2010; Dobrei et al., 2015; Wolff et al., 2018a). The influence of tillage on soil properties, while not entirely understood, is more studied than the impact on crops themselves, particularly in permanent cropping systems such as vineyards. However, few reports have investigated the influence of tillage on grapevine physiology under the presence of a cover crop. While differences in leaf gas exchange have been reported between grapevines grown under conventional tillage compared to those under permanent cover crops, there is little evidence of tillage having a direct influence on grapevine stomatal conductance and net photosynthesis (Pou et al., 2011). Although no-till practices are often promoted for their positive influence on soil infiltration and conservation of soil water, few studies have found that this effect translated to ameliorated plant water status in grapevine (Pool et al., 1990; Patiño-Zúñiga et al., 2009b; Myburgh, 2013; Steenwerth et al., 2016; Van Huyssteen and Weber, 2017).

Previous works indicated that vegetative growth is greater under conventional tillage while yield reductions are typically associated with no-till management, despite research that indicated no effect on production (Myburgh, 2013; Steenwerth et al., 2013; Wolff et al., 2018a). Furthermore, while some studies have reported increased berry skin anthocyanin content under no-till management, overall there is limited impact of tillage on berry composition (Van Huyssteen and Weber, 1980; Lee and Steenwerth, 2013; Reeve et al., 2016a; Chrysargyris et al., 2018; Buesa et al., 2021).

Cover cropping and reduced tillage management are two practices that directly alter the growing environment of the grapevines. Thus, the selection of appropriate vineyard floor management practices is critical in order to maximize benefits to the soil while minimizing the impact on grapevine function and productivity. This selection involves decisions in space (cover crop in vineyard rows vs. under-vine), type (grasses vs. broadleaves; monoculture or species mixture), and time including perennial vs. annual species selection and timing of termination (Bowles et al., 2017b; Gatti et al., 2022b). Factors such as cultivar, vineyard age, macroclimate,

soil physiochemical characteristics, and the overall goals for the use of the selected cover crop and tillage system must also be considered. These elements have been shown to contribute to the effect of the practices on grapevine functioning and production (Ingels et al., 2005; Sweet and Schreiner, 2010; Steenwerth et al., 2013; Abad et al., 2021).

The objective of this work was to investigate the effects of cover cropping and tillage on whole grapevine physiology in two contrasting production regions in California, USA. Specifically, we studied the interactive effects of tillage and cover crops on grapevine water status, leaf gas exchange, components of yield, berry composition and resulting water footprint in two contrasting production regions of California during arid seasons.

2. Materials and methods

2.1. Site descriptions and experimental design

Field experiments were conducted at two sites for two consecutive growing seasons (2019-2020 and 2020-2021). The first site was located at a Winkler Index V vineyard in Fresno, CA (36.67151, -119.925823) in a Ruby Cabernet/Freedom (27% *V. vinifera* hybrid) vineyard. Grapevines were planted in 2012 with a spacing of 3.0 x 1.2 m (row x vine) with a row orientation of E-W. The grapevines were cane-pruned and trained to quadrilateral cordons 1.38 m with catch wires at 1.54 m and at 1.68 m above vineyard floor. The soil texture of the site was classified as a sandy loam and vines were drip-irrigated with two emitters per plant delivering 4.0 L/h each. The second site was located at a Winkler Index III vineyard in Oakville, CA (38.428000, -122.409000) in a Merlot (clone 181)/ 3309 C (*V. riparia* × *V. rupestris*) vineyard. Grapevines were planted in 2018 at a spacing of 3.0 by 2.0 m (row × vine) with a row orientation of E-W. The grapevines were spur-pruned and trained to quadrilateral cordons 1.38 m above vineyard floor with catch wires at 1.68 m. The soil texture of the site was classified as clay loam and grapevines were drip-irrigated with two emitters per plant delivering 2 L/h each.

At both experimental sites, the experiments were arranged as a split-plot 3×2 factorial arrangement of treatments (three cover crops and two tillage managements) with four (Oakville) and three replications (Fresno). Each treatment-replicate consisted of 15 grapevines. Three grapevines in the middle of each replicate were used for measurements and the distal plants on either end served as buffer plants. Treatments included tillage as the main plot [conventional tillage (CT) and no-till (NT)] and the sub-plot was randomly applied within the main plots as i) Perennial grass (*Poa bulbosa* hybrid cv. Oakville Blue); ii) Annual grass (Barley, *Hordeum vulgare*); iii) Resident vegetation (natural weed population). The cover crop seed was drilled in a 1.5 m wide strip according to seed manufacturer's recommended rate prior to receiving fall/winter rains in 2019 and 2020 at a rate of 605 kg/ha and 84 kg/ha for the perennial grass (PG) and annual grass (AG) treatments, respectively. Resident vegetation (RV) was allowed to grow within the 1.5 m strip and mowed at the vineyard manager's discretion. All other cultural practices were conducted according to University of California Cooperative Extension guidelines (Christensen, 2000).

2.2. Weather conditions

Weather data at both sites was obtained from California Irrigation Management Information System (CIMIS) stations nearest the experimental vineyard (station #77 in Napa County, CA and station #2 in Fresno County, CA). Growing degree day values were calculated using a base of 10 °C from 1 April through 30 September of each year.

2.3. Grapevine water status and gas exchange parameters (Ψ , A_{net} , g_s)

Plant water status was measured as stem water potential (Ψ_s) every 2 weeks (Oakville) and four times (Fresno) during each growing season within 1.5 h of solar noon and integrals calculated as previously reported (Yu et al., 2021). Two fully expanded leaves exposed to sun and without signs of disease and/or damage were selected per treatment-replicate. For Ψ_s , leaves were then covered 1.5 hours before measurements with a reflective foil-lined zip-top plastic bag to suppress

transpiration. The Ψ_s was measured with a pressure chamber (Model 610 Pressure Chamber Instrument, PMS Instrument Co., Corvallis, OR USA).

Leaf gas exchanges of stomatal conductance (g_s) and net carbon assimilation (A_{net}) were measured at solar noon on three fully expanded and sun-exposed leaves with a CIRAS-3 infra-red gas analyzer (PP Systems, Amesbury, MA, USA) equipped with a leaf chamber with a 4.5 cm² window. The reference CO₂ was set to 400 $\mu\text{mol/mol}$ CO₂ at a flow rate of 100 mL/min. The window of the chamber was oriented perpendicularly toward the sun to allow for saturating light conditions and the cuvette was left attached to the leaf for 40–60 s until a steady state was reached. Three grapevines were measured from each treatment-replicate.

2.4. Grapevine mineral nutrient status

Grapevine petiole samples were collected for nutrient analysis at bloom at both sites. Bloom (Oakville: 29 May 2020, 25 May 2021; Fresno: 10 May 2020, 15 May 2021) was defined as when >50% of flowers opened. Leaves with petioles were collected from the north side of the three middle data vines in each replicate and the blade removed. Petioles were delivered to a commercial laboratory for mineral analysis which was carried out by using coupled plasma-mass spectrometry. Nitrogen (N) was determined via automated combustion analysis (method B-2.20) while phosphorus (P), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), zinc (Zn), manganese (Mn), boron (B), iron (Fe), and copper (Cu) were analyzed via Nitric/Perchloric Acid Digestion (method B-4.20) as described by Gavlak et al. (1994).

2.5. Leaf area and yield components

At the Fresno site, leaf area index (LAI) was measured in late spring to characterize grapevine canopy growth and converted into leaf area by a smartphone program, VitiCanopy, via iOS system (Apple Inc., Cupertino, CA, USA) (De Bei et al., 2016). The gap fraction threshold was set to 0.75, extinction coefficient was set to 0.7, and sub-divisions were 25. A extendable mounting device was used to effectively position the device approximately 75 cm underneath the canopy. The

device was positioned with the maximum length of the screen being perpendicular to the cordon, and the cordon in line with the middle of the screen according to previous work (De Bei et al., 2016; Yu and Kurtural, 2020). In each experimental unit, three images were taken to capture half canopy of each vine and analyzed by the software. The relationship between leaf dry mass and area was determined on a subsample of leaves using a leaf area meter (Li-Cor 3300, Lincoln, NE USA).

Harvest commenced when the fruit reached approximately 25 °Brix in Oakville (August 25, 2020, and September 1, 2021) and 21 °Brix in Fresno (October 6, 2020, and September 7, 2021). At harvest at both sites, clusters from three data vines per treatment replicate were manually removed, counted, and weighed on a top-loading balance. Leaf area to fruit ratio was calculated by dividing leaf area with crop weight.

2.6. Berry composition

At harvest, fifty berries were randomly collected from the three middle grapevines within each replicate and immediately processed. Berries were weighed and gently pressed by hand to squeeze the juice. Total soluble solids (TSS) were determined using a temperature compensating digital refractometer (Atago PR-32, Bellevue, WA, USA). Must pH and titratable acidity (TA) were determined with an autotitrator (Metrohm 862 Compact Titrosampler, Herisau, Switzerland). TA was determined by titrating with 0.1 N sodium hydroxide to an end point of 8.3 pH and reported as g/L of tartaric acid.

Berry skin anthocyanin content was determined at harvest from 20 berries randomly collected from each treatment-replicate. Berries were gently peeled, skins were freeze-dried (Cold Trap 7385020, Labconco, Kansas City, MO, USA). Freeze-dried tissue was ground with a tissue lyser (MM400, Retsch, Germany). Fifty milligrams of the resultant powder was extracted in methanol: water: 7 M hydrochloric acid (70:29:1, V:V:V) to determine anthocyanin content. Extracts were filtered using a 0.45 µm filter (Thermo Fisher Scientific, San Jose, CA, USA) and

analyzed using an Agilent 1260 series reversed-phase high performance liquid chromatography (HPLC) system (Agilent 1260, Santa Clara, CA, USA) coupled to a diode array detector. Separation was performed on a reversed-phase C18 column LiChrospher 100, 250 mm × 4 mm with a 5 µm particle size and a 4 mm guard column of the same material at 25 °C with elution at 0.5 mL per minute. The mobile phase consisted of a constant 5% of acetic acid and the following gradient (v/v) of acetonitrile in water: 0 min 8%, at 25 min 12.2%, at 35 min 16.9%, at 70 min 35.7%, 65% between 70–75 min, and 8% between 80–90 min. The identification of compounds was conducted by determining the peak area of the absorbance at 520 nm for anthocyanins and made by comparison of the commercial standard retention times found in the literature. Commercial standard of oenin (Extrasynthese, Genay France) was used for the quantification of anthocyanins.

2.7. Water footprint assessment

Water footprint (WF) was calculated following the methods described in Zotou and Tshrintzis (2017) with slight modifications (Torres et al., 2021). Total WF was derived as the sum of the green, blue and grey WFs and expressed in m³ of water consumed per ton of fruit harvested. Cover crop WF was derived in the same manner and expressed in m³ of water consumed per kg of cover crop biomass. Green, blue, and grey components were given by following equations:

$$\text{Eq. 1. } \text{green WF} = \frac{\sum P_m}{Y}$$

where P_m is the monthly effective precipitation expressed in m³·ha⁻¹ after applying a conversion factor of 10 and Y is the yield of grapevines expressed in tonne·ha⁻¹.

$$\text{Eq. 2. } \text{blue WF} = \frac{\sum WU_m}{Y}$$

where WU_m is the total amount of irrigation water received by the grapevines monthly expressed in m³·ha⁻¹ and Y is the yield of grapevines expressed in tonne·ha⁻¹

$$\text{Eq. 3. } \text{grey WF} = \frac{\alpha AR}{(c_{max} - c_{nat}) Y}$$

where α is the percentage of fertilizer that leaches to the receiving aquatic system; AR is the amount of fertilizer applied to the grapevines expressed in $\text{kg} \cdot \text{ha}^{-1}$; c_{max} is the maximum acceptable concentration of fertilizer in the aquatic system ($\text{mg} \cdot \text{L}^{-1}$); and c_{nat} is the natural concentration of the pollutant in the aquatic system ($\text{mg} \cdot \text{L}^{-1}$). For grey component calculation, only nitrogen fertilization was considered given that N use in agriculture presents the largest environmental concern (UC Davis, 2016). The percentage of nitrogen entering the water system of the area was assumed 10% according to Mekonnen and Hoekstra (2011). The maximum acceptable concentration of nitrogen ($45 \text{ mg} \cdot \text{L}^{-1}$) was obtained from CDFFA (2020). According to Hoekstra et al. (2011), the natural concentration of pollutants was taken equal to zero, as proposed when data was missing.

2.8. Statistical analyses

Statistical analyses were conducted with R studio version 3.6.1 (RStudio: Integrated Development for R., Boston, MA, USA) for Mac OS. After normality assessment, data was submitted to a three-way analysis of variance (ANOVA) to assess the statistical differences between the different cover crop and tillage treatments and the respective interaction effects over two years. Means \pm standard errors (SE) were calculated and when the F value was significant ($P \leq 0.05$), a Tukey's 'Honest Significant Difference' (HSD) *post hoc* test was executed by using "agricolae" 1.2-8 R package. Plots were generated using GraphPad Prism v8.1.2 for Windows (Graph Pad Inc., San Diego, CA, USA).

3. Results

3.1. Weather conditions at experimental sites

Temperature minima, maxima, and their average were calculated daily (air and soil) and annually (air) from the CIMIS station data for the 2019-20 and 2020-21 seasons (Table 1). In comparison to the ten-year average (2011-2021), both sites experienced warmer and drier conditions over the course of the experiment. During both years in Fresno, total precipitation was lower than the ten-year average for the same period. Specifically, the 2019-20 season received 10.6 mm less

precipitation, while the 2020-21 season received 57.1 mm less. Average daily air temperature during the growing season was also 0.1 °C higher in 2020 and 0.4 °C higher in 2021 compared to the ten-year average. Average daily soil temperatures were 0.3 °C and 0.5 °C higher in 2020 and 2021, respectively. Despite one degree increases in average monthly temperature between the two years, mean daily air and soil temperatures were similar. The greatest number of growing degree days in Fresno were accumulated in 2021 (2488 GDD₁₀), compared to 2020 (2358 GDD₁₀).

In Oakville, drought conditions were more pronounced, as the 2019-20 season received 343.6 mm less precipitation than the ten-year average, and 299.5 mm less during the 2020-21 year (Table 1). However, there was a 43.8 mm increase in precipitation received in the second year of the experiment compared to the first year. Average daily temperature during the growing season was 0.4 °C higher in 2020 and 0.3 °C lower in 2021 compared to ten-year average values. Average daily soil temperatures were 0.5 °C and 0.4 °C higher in 2020 and 2021, respectively. As was observed at the Fresno vineyard, average daily air and soil temperatures were similar between the two years of the study. Contrarily, the greatest number of growing degree days in Oakville (1647 GDD₁₀) were accumulated in the 2020 growing season compared to 2021 (1519 GDD₁₀).

3.2. Leaf gas exchanges and plant water status

In Fresno, no treatment effects were observed on season-long integrals of g_s (Table 2). Likewise, season long integrals of A_{net} were also not affected by the treatments applied. However, in 2020 both g_s and A_{net} were significantly lower than 2021 in Fresno.

In Oakville, g_s was significantly lower among grapevines grown with AG compared to RV and PG in 2020 96 DAF and 110 DAF (Table 3). However, a similar effect was not evident throughout the rest of the experiment. Additionally, no differences were measured in A_{net} , although 2020 values were lower than 2021. A similar response was observed at the Fresno vineyard.

In Fresno, Ψ_s values ranged from -1.68 to -0.70 MPa in 2020 and -1.75 to -0.73 MPa in 2021. The cover crop and tillage treatments did not affect $\Psi_{s.integrals}$, and there was not an interaction

of cover crop and tillage, or an effect of year (data not shown). Conversely, at Oakville, Ψ_S integrals ranged from -1.49 to -0.64 MPa in 2020 and -1.46 to -0.77 in 2021. As presented in Figure 1, in 2020 Ψ_S integrals was affected by cover crops. Grapevines grown with AG displayed the most negative Ψ_S integrals, with PG the least negative. However, this effect was not observed in the second year of the study nor was there an interaction effect between cover crop and tillage system. Tillage was more effective in eliciting a Ψ_S integrals response more so than cover crop type. Grapevine water status was significantly lower in NT vines (-1.07 MPa) than CT vines (-1.01 MPa) over both years at the Oakville vineyard.

3.3. Grapevine mineral nutrient status

Grapevine mineral nutrition was assessed at bloom and there were year-to-year differences at both locations. At the Fresno vineyard, Nitrogen (%), Phosphorus (%), and Zinc (mg/kg) concentrations in petioles were greater in 2020 than in 2021 (**Table 4**). There was also an interaction of year and tillage where Calcium concentration was greater with tillage during 2020. In Fresno, Manganese, Magnesium, Iron, and Copper values were greater in 2021 than 2020. In Oakville, Nitrogen, Magnesium, and Iron petiole concentrations were greater in 2021 than 2020 (**Table 5**). Conversely, petiole concentrations of P, Zn, Mg, and Cu values were greater in 2020 compared to 2021. At Oakville we measured lower petiole Nitrogen concentrations with NT (1.18%) when compared to CT (1.25%). Again, an interaction of year and tillage within Calcium was measured in 2020 in Oakville as well.

3.4. Yield and yield components

In Fresno there were no differences in cluster number per vine, average cluster mass, yield per vine, or leaf area to fruit ratio (**Table 6**). On average, number of clusters per plant was higher in 2021 than 2020, although average cluster mass was lower in 2021 compared to 2020. Year-to-year differences were also observed at the Oakville site whereby cluster number per vine, average cluster mass and yield per vine was greater in 2021 compared to 2020, while leaf area to fruit ratio

was greater in 2020. There was no treatment effect of cover crop or tillage, nor an interaction measured at either site.

3.5. Grape berry composition

In Fresno juice pH, TA, TSS, total anthocyanin content, average berry mass, and average skin mass was not affected by cover crop or tillage system applied. The TSS and TA were higher in 2021, and TA was lower under NT (5.92 g/L) compared to CT (6.53 g/L) (Table 7).

At Oakville the TSS and average berry mass were higher in 2020 than in 2021. Total anthocyanin content and average skin mass were higher in 2021 than in 2020. There was a main effect of tillage on juice pH, as mean values were lower in CT (3.43) compared to NT (3.46) over the two seasons. Furthermore, there was also a year by tillage interaction among pH due to a greater difference between tillage treatments in 2021. Conversely, mean juice TA values were lower in NT (6.48 g/L) compared to CT (7.13 g/L) in only 2021, resulting in a year by tillage interaction and without the main effect of tillage.

3.6. Vineyard water footprint

The analysis of the effect of treatments applied on WF components indicated the same pattern regardless of the growing season at both sites. In Fresno, green water footprint was greater in 2020 than in 2021 (**Table 8**). However, the treatments applied did not affect the WF components at Fresno. At Oakville, year to year differences were observed in all WF components, with 2020 values greater than 2021 which was expected due to differences in yield between the two years (**Table 6**). The treatments applied did not affect the WF of the vineyard at Oakville in 2020 or 2021.

4. Discussion

4.1. Cover crop and tillage system did not affect leaf gas exchange

At both sites, there were no effects of cover crop or tillage on g_s or A_{net} . This may indicate that despite different climatic and site conditions, whole grapevine physiology was not affected by the presence of a cover crop or tillage. These results are corroborated with previous work that have

measured leaf gas exchange between grapevines grown with and without interrow cover crops and found negligible differences (Celette et al., 2009b; Sweet and Schreiner, 2010; Hatch et al., 2011b; Reeve et al., 2016b). Previous works that reported changes to leaf gas exchange of the grapevine have either contrasting applied water amounts (Torres et al., 2021), variation in leaf area to shoot ration (Martinez-Luscher and Kurtural, 2021) or soil spatial variability that affected grapevine water status (Brillante et al., 2018, Yu et al., 2021). Since neither factor had a significant effect on these parameters it is plausible that cover crops or tillage would not affect grapevine leaf gas exchange.

4.2. Tillage was more influential than cover crop on grapevine water status and mineral nutrition

Much of the literature regarding competition between cover crop and grapevine suggested that the presence of a cover crop increased the competition for nutrients, namely nitrogen (Calderón et al., 2001; Celette et al., 2009b; Reeve et al., 2016b). Although it was hypothesized that RV and AG would decrease grapevine mineral nutrition status, there were no effects of cover crop on grapevine mineral nutrition in the present study. In Fresno, the nutrient status of the grapevines at bloom only differed between years and was not affected by treatments, indicating little to no competition with the cover crop for nutrients in mature vineyards under arid conditions.

However, in Oakville tillage appeared to be minimally impactful on grapevine mineral nutrition, and CT vines demonstrated a higher nitrogen content at bloom compared to NT in both years. Higher N in both leaves and juice in response to tillage was previously reported (Rodriguez-Lovelle et al., 2000b; Guerra and Steenwerth, 2012). In one particular three-year study, NO₃-N petiole values of grapevines with interrow tillage were found to be up to 2 × greater than those of no-till grapevines, suggesting a possible temporal offset between soil N availability and plant uptake related to tillage (Steenwerth et al., 2013b; Reeve et al., 2016b). It is well demonstrated that soil tillage affects the decomposition and mineralization of N from plant residues and existing

pool which regulates the inorganic N pool available for uptake by the grapevines (Guerra and Steenwerth, 2012). Thus, while the type of cover crop did not influence nitrogen content in the present study, nor was there an interaction between cover crop and tillage.

It was also hypothesized that PG would improve grapevine water status due to spatial and temporal complementarity, whereby the shallow rooting depth would be less likely to compete with grapevines and peak water use of PG would occur during grapevine dormancy. In Fresno, no treatment effects were observed on grapevine water status nor among WF components. This result is particularly important as it indicated that despite different growth habits of the cover crop (AG is a tall stature grass and thus produces more biomass than the low stature PG), there was no competition with the grapevines for water, as also indicated by a lack of differences in Ψ s between treatments. In Oakville, PG did indeed improve grapevine water status compared to RV and AG in one instance during 2020, but this effect was not observed in the second year of the study, nor over the two seasons. Furthermore, there was no interaction effect between the PG cover crop and NT factor, which is particularly important as the greatest benefits to the soil from a permanent cover crop were observed under no-till environments (Rodriguez-Lovelle et al., 2000a; Morlat and Jacquet, 2003b; Volaire and Lelièvre, 2010).

While the type of cover crop again had little influence, CT improved grapevine water status (i.e., more positive Ψ S) compared to grapevines under NT. Although Steenwerth et al. (2016) previously reported reduced soil water content under no-till settings, no association was found with Ψ S. While contradictory to some reports that indicated tillage did not affect grapevine water status (Van Huyssteen and Weber, 1980; Steenwerth et al., 2013; Myburgh, 2013), this result provided further evidence of tillage in semi-arid regions to preserve water in the soil through early season cultivation. This was based on the notion that while evaporative losses of the upper tilled layer of soil immediately increase, overall losses are minimized as a barrier that restricts capillary water movement is created which preserves moisture in the deeper layers of the soil (Hillel, 1998;

Myburgh, 2013b). Furthermore, seasonal grapevine water status in irrigated viticulture was shown to be more influenced with subsoil conditions than the topsoil which dries quite early (Yu et al., 2021). As the presence of vegetation was shown to deplete water out of the upper portion of the soil more rapidly than bare soil (Monteiro and Lopes, 2007b; Celette et al., 2008; Novara et al., 2018), it is possible that the complete termination and incorporation of vegetation helped preserved moisture in the soil compared to NT, where vegetation was able to remain in competition with the grapevines. However, no conclusions can be made as to the mechanism of reduced water stress under CT vines as root structure was not examined in the present study (Hunter, 1998; Myburgh, 2013b). Ultimately, these differences in plant water status between tillage systems in Oakville did not affect WF components, as irrigation amounts remained unchanged.

4.3. Minimal effects observed on yield components and berry composition

In regard to grapevine yield and berry composition, most differences were observed between years at the Fresno vineyard. Although no yield components were affected by cover crop or tillage, minimal effects of tillage on berry composition were seen. The TA was significantly higher in CT compared to NT in both years, as has been previously reported when permanent grass was compared to conventionally tilled soil (Reeve et al., 2016b). This may suggest that tillage hastens the ripening process, however, no statistically significant effects were observed on TSS which would support such a claim (Kennedy, 2002). Other studies that investigated the influence of vineyard floor management in mature vineyards reported similar findings with reduced effect of soil management practices as grapevines aged. It was possible that mature grapevines may be more resilient to the of the adoption of cover crops due to their well-established root systems that can more effectively compete with the cover crop (Van Huyssteen and Weber, 1980; King and Berry, 2005; Steenwerth et al., 2013b; Fourie et al., 2017b). Combined, these results provided evidence that the use of the annual or perennial grass cover crops and/or no-till practices may be

implemented in mature irrigated vineyards in the San Joaquin Valley with little to no effect on production.

In the Oakville vineyard, TA was again significantly higher under CT compared to NT in 2021. However, this effect was only seen in the second year of the study resulting in a year by tillage interaction without a significant main effect. The juice pH was also reduced under CT grapevines, which has been a reported effect of cover crop adoption rather than tillage as a result of the release of potassium (K) when the cover crop decomposed (Wheeler et al., 2005; Guerra and Steenwerth, 2012; Chrysargyris et al., 2018b; Cataldo et al., 2020). While several studies have reported no reductions to yield in response to cover crops, others that assessed permanent cover crops observed decreased yield after 2 to 3 years (Morlat and Jacquet, 2003b; Tesic et al., 2007; Lopes et al., 2008a; Steenwerth and Belina, 2008b; Hatch et al., 2011b; Giese et al., 2015b). It is not clear whether competition for water or N was the primary cause as the two factors are interconnected (Celette et al., 2008, 2009b). The absence of effect on yield, as seen in this study, may be a result of the shorter length of experiment, and/or shallower rooting depth of the perennial grass used compared to deeper rooting and higher biomass producing grasses investigated in the aforementioned studies. Ultimately, the adoption of cover crops under both tillage systems in the present study did not affect production despite great differences in soil type, vineyard age, and climate between the two sites.

5. Conclusion

While the benefits of cover crops and reduced tillage on soil physical, chemical, and biological properties are well documented, the link between these management practices and grapevine physiology, components of yield and berry composition remain unclear. At both sites, there were no treatment effects on leaf gas exchange which suggested negligible effect of cover crop and tillage on grapevine physiology in vineyards of these climates. In the mature Ruby Cabernet vineyard in Fresno, no changes to yield components or berry composition among cover crop and

tillage were measured. In the young Merlot vineyard in Oakville, NT detrimentally affected grapevine water status. This indicated that the presence of vegetation in the early spring increased competition for water, but ultimately no changes to yield or water footprint were observed. Our results provided evidence that both in mature and young vineyards cover cropping had negligible beneficial effects on grapevine physiology, mineral nutrition or production; and tillage was beneficial in young vineyards to improve plant water status in semi-arid regions.

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Chapter 1: Tables and Figures

Table 1 Site conditions at two commercial vineyards in Fresno and Napa Co. from experimental years (2019-2021) compared to long-term mean values (2011-2021) ^{a, b, c}.

Fresno County (Five Points)								
Year	Air Temperature (°C)			Soil Temperature (°C)			Precipitation (mm)	Growing Degree Days
	Daily Max	Daily Min	Daily Average	Daily Max	Daily Min	Daily Average	Total	Total
2020								
Mean	25.6	9.1	17.3	25.7	10.7	22.6	199.0	2358
Annual max	35.8	18.0	27.3	--	--	--	--	--
Annual min	15.6	0.6	7.6	--	--	--	--	--
2021								
Mean	25.8	9.2	17.6	26.6	10.4	22.8	152.5	2488
Annual max	37.8	18.7	28.5	--	--	--	--	--
Annual min	12.5	3.1	7.7	--	--	--	--	--
2011-2021								
Mean	25.4	9.4	17.2	25.6	10.1	22.3	209.6	2259
Napa County (Oakville)								
2020								
Mean	24.4	7.0	14.9	22.5	10	16.5	234.2	1647
Annual max	31.8	12.3	21.1	--	--	--	--	--
Annual min	17.1	2.0	8.5	--	--	--	--	--
2021								
Mean	23.1	6.3	14.2	22.8	10.2	16.4	278.3	1519
Annual max	30.0	10.8	19.2	--	--	--	--	--
Annual min	12.5	2.6	7.6	--	--	--	--	--
2011-2021								
Mean	23.2	7.1	14.5	22.8	7.9	16.0	577.8	1504

a Annual maximum (max) and annual minimum (min) indicate the greatest or lowest value observed during the respective year. b Total precipitation occurred during the annual winter rainy season, calculated from October of the preceding year through September of the following year (e.g., 2020 values were calculated from October 1, 2019– September 30, 2020). c Abbreviations: --: not applicable.

Table 2 Stomatal conductance and net carbon assimilation values of Ruby Cabernet grapevines subjected to different cover crops and tillage systems, as collected at various points in the 2019-20 and 2020-21 growing seasons^{a, b}

Fresno County (Five Points)						
Treatment	Stomatal conductance (mmol m ⁻² s ⁻¹)			Net carbon assimilation rate (μmol CO ₂ m ⁻² s ⁻¹)		
	78 DAF	184 DAF	--	78 DAF	184 DAF	--
2020	78 DAF	184 DAF	--	78 DAF	184 DAF	--
No Till - AG	259.5 ± 23.4	266.7 ± 54.5	--	10.2 ± 1.6	13.4 ± 0.7	--
No Till - RV	308.7 ± 34.7	230.8 ± 21.6	--	12.2 ± 1.9	13.5 ± 3.7	--
No Till - PG	380.1 ± 60.1	252.5 ± 7.4	--	15.7 ± 0.5	14.5 ± 1.9	--
Till - AG	281.2 ± 30.1	260.2 ± 24.4	--	16.2 ± 1.7	12.9 ± 1.9	--
Till - RV	262.7 ± 24.4	301.8 ± 59.9	--	14.5 ± 0.9	11.3 ± 3.9	--
Till - PG	403.0 ± 64.2	189.8 ± 3.5	--	14.8 ± 0.2	9.97 ± 2.9	--
<i>Cover crop (CC)</i>	ns	ns	--	ns	ns	--
<i>Tillage (T)</i>	ns	ns	--	ns	ns	--
<i>CC x T</i>	ns	ns	--	ns	ns	--
2021	26 DAF	91 DAF	128 DAF	26 DAF	91 DAF	128 DAF
No Till - AG	178.4 ± 41.92	415.0 ± 68.0	349.8 ± 56.5	12.2 ± 3.6	15.8 ± 2.9	18.5 ± 2.7
No Till - RV	139.9 ± 22.21	352.1 ± 55.8	349.6 ± 30.2	6.33 ± 1.9	16.1 ± 0.9	20.8 ± 2.7
No Till - PG	149.5 ± 18.10	293.5 ± 68.2	382.0 ± 60.8	8.67 ± 1.5	18.1 ± 3.1	19.8 ± 1.8
Till - AG	133.2 ± 16.63	418.4 ± 51.2	318.0 ± 59.9	9.27 ± 1.2	18.5 ± 1.4	20.4 ± 3.0
Till - RV	137.7 ± 30.52	360.5 ± 70.7	413.9 ± 42.5	5.57 ± 1.6	17.2 ± 1.9	19.1 ± 2.1
Till - PG	137.9 ± 7.87	421.2 ± 89.4	407.0 ± 73.9	9.07 ± 1.7	19.7 ± 2.7	19.8 ± 1.9
<i>Cover crop (CC)</i>	ns	ns	ns	ns	ns	ns
<i>Tillage (T)</i>	ns	ns	ns	ns	ns	ns
<i>CC x T</i>	ns	ns	ns	ns	ns	ns
<i>Year</i>		ns			**	
<i>Year x CC</i>		ns			ns	
<i>Year x T</i>		ns			ns	
<i>Year x CC x T</i>		ns			ns	

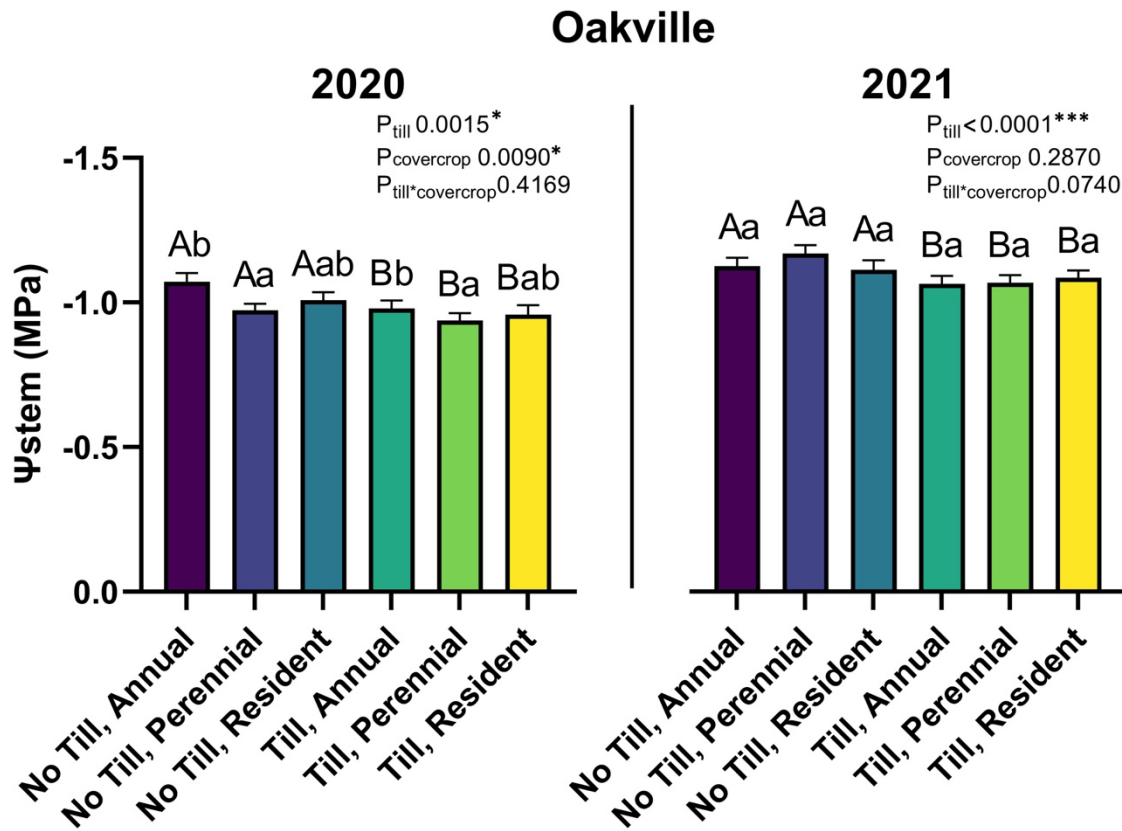
^a ANOVA to compare data (p value indicated); Letters within columns indicate significant mean separation according to Tukey's honest significance difference (HSD) test at p value < 0.05, where "**": p value < 0.05; "***": p value < 0.001, "****": p value < 0.0001. ^b Abbreviations: AG: Annual grass; RV: resident vegetation; PG: perennial grass; DAF: days after flowering; na: not applicable; ns: not significant; --: not applicable.

Table 3 Stomatal conductance and net carbon assimilation values of Merlot grapevines subjected to different cover crops and tillage systems, as collected at various points in the 2019-20 and 2020-21 growing seasons^{a, b}

Napa County (Oakville)									
Treatment	Stomatal Conductance (mmol m ⁻² s ⁻¹)				Net Carbon Assimilation Rate (μmol CO ₂ m ⁻² s ⁻¹)				
	61 DAF	96 DAF	110 DAF	145 DAF	61 DAF	96 DAF	110 DAF	145 DAF	
2020									
No Till - AG	136.6 ± 18.9	141.5 ± 6.3	147.7 ± 18.2	220.4 ± 22.2	10.0 ± 1.35	13.1 ± 0.96	19.2 ± 7.99	16.5 ± 1.6	
No Till - RV	161.3 ± 27.6	95.5 ± 26.5	13.7 ± 14.0	217.0 ± 29.1	7.7 ± 0.72	17.3 ± 0.94	14.4 ± 1.04	16.6 ± 1.7	
No Till - PG	174.0 ± 28.5	82.5 ± 17.1	17.0 ± 29.1	205.4 ± 17.3	11.7 ± 2.01	14.3 ± 1.85	17.1 ± 1.67	17.0 ± 0.9	
Till - AG	109.2 ± 13.0	24.3 ± 19.3	67.8 ± 11.7	159.0 ± 18.9	7.7 ± 0.24	11.4 ± 1.31	13.2 ± 0.95	14.8 ± 1.0	
Till - RV	143.0 ± 25.1	94.2 ± 32.5	29.2 ± 10.8	184.4 ± 10.8	6.3 ± 2.58	16.0 ± 2.32	16.0 ± 1.75	17.2 ± 0.3	
Till - PG	172.2 ± 26.3	67.2 ± 30.0	01.4 ± 24.5	237.0 ± 20.8	10.4 ± 2.44	12.9 ± 2.07	14.0 ± 2.14	19.0 ± 0.9	
<i>Cover crop (CC)</i>	ns	*	*	ns	ns	ns	ns	ns	
<i>Tillage (T)</i>	ns	ns	ns	ns	ns	ns	ns	ns	
<i>CC x T</i>	ns	ns	ns	ns	ns	ns	ns	ns	
2021									
No Till - AG	198.3 ± 26.4	191.7 ± 18.1	1179.0 ± 17.9	150.3 ± 15.9	9.0 ± 2.06	13.1 ± 2.01	13.6 ± 1.51	12.4 ± 1.2	
No Till - RV	180.9 ± 23.2	156.6 ± 25.1	1155.0 ± 24.4	173.6 ± 18.7	9.4 ± 1.42	11.9 ± 1.41	11.5 ± 1.15	13.2 ± 1.3	
No Till - PG	209.0 ± 28.9	146.5 ± 13.3	165.7 ± 16.9	177.1 ± 25.6	11.5 ± 2.08	13.2 ± 0.47	13.2 ± 1.63	12.2 ± 1.8	
Till - AG	193.5 ± 19.2	160.5 ± 32.8	194.2 ± 21.5	163.2 ± 27.1	9.5 ± 1.31	14.8 ± 1.70	11.9 ± 1.51	12.1 ± 2.0	
Till - RV	231.0 ± 24.6	168.7 ± 32.1	1177.4 ± 26.7	193.3 ± 23.3	10.8 ± 1.87	13.4 ± 1.29	10.9 ± 1.85	13.3 ± 1.6	
Till - PG	199.3 ± 26.2	171.4 ± 38.6	145.8 ± 18.3	181.2 ± 20.3	10.5 ± 1.73	12.5 ± 2.72	10.3 ± 1.59	13.9 ± 1.1	
<i>Cover crop (CC)</i>	ns	ns	ns	ns	ns	ns	ns	ns	
<i>Tillage (T)</i>	ns	ns	ns	ns	ns	ns	ns	ns	
<i>CC x T</i>	ns	ns	ns	ns	ns	ns	ns	ns	
<i>Year</i>			ns				***		
<i>Year x CC</i>			ns				ns		
<i>Year x T</i>			ns				ns		
<i>Year x CC x T</i>			ns				ns		

^a ANOVA to compare data (p value indicated); Letters within columns indicate significant mean separation according to Tukey's honest significance difference test at p value < 0.05, where "*": p value < 0.05; "***": p value < 0.001, "****": p value < 0.0001. ^b Abbreviations: AG: Annual grass; RV: resident vegetation; PG: perennial grass; DAF: days after flowering; na: not applicable; ns: not significant.

Figure 1. Mid-day stem water potential *integrals* of Merlot grapevines subjected to different cover crop and tillage systems at the Oakville vineyard (Napa County) over the course of the 2019-20 and 2020-21 growing season ^a



^a ANOVA to compare data (p value of the respective factor indicated); Letters above treatment bars indicate significant mean separation according to *Tukey's honest significance difference (HSD)* test at p value < 0.05, where "*": p value < 0.05; "***": p value < 0.001, "****": p value < 0.0001

Table 4 Petiole nutrition at bloom of Ruby Cabernet grapevines subjected to different cover crops and tillage systems collected in the 2019–20 and 2020-21 seasons ^{a, b}

Fresno County (Five Points)										
Treatment	N (%)	P (%)	K (%)	Zn (mg/kg)	Mn (mg/kg)	B (mg/kg)	Ca (%)	Mg (%)	Fe (mg/kg)	Cu (mg/kg)
2020										
No Till - AG	0.76 ± 0.06	0.13 ± 0.01	3.17 ± 0.24	30.3 ± 0.3	46.0 ± 14.6	31.3 ± 1.5	1.35 ± 0.07	0.88 ± 0.03	48.3 ± 6.6	3.00 ± 0.58
No Till - RV	0.69 ± 0.06	0.15 ± 0.02	3.51 ± 0.17	30.7 ± 3.2	48.0 ± 14.8	31.3 ± 0.1	1.31 ± 0.12	0.82 ± 0.08	46.7 ± 2.2	2.00 ± 0.03
No Till - PG	1.01 ± 0.35	0.17 ± 0.06	2.89 ± 0.47	33.0 ± 6.7	68.7 ± 19.4	32.0 ± 2.1	1.47 ± 0.04	0.86 ± 0.09	101.3 ± 54.9	2.33 ± 0.67
Till - AG	0.95 ± 0.24	0.17 ± 0.03	3.56 ± 0.43	34.3 ± 6.2	65.0 ± 19.4	34.0 ± 0.6	1.44 ± 0.07	0.84 ± 0.08	94.0 ± 52.0	2.33 ± 0.33
Till - RV	0.72 ± 0.02	0.19 ± 0.05	3.59 ± 0.10	27.7 ± 4.4	43.3 ± 14.1	30.7 ± 0.9	1.28 ± 0.05	0.79 ± 0.05	48.0 ± 5.3	2.33 ± 0.33
Till - PG	0.86 ± 0.23	0.18 ± 0.04	3.41 ± 0.19	32.0 ± 4.4	65.8 ± 18.2	32.3 ± 0.7	1.37 ± 0.19	0.86 ± 0.21	98.0 ± 50.5	2.67 ± 0.33
<i>Cover crop (CC)</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Tillage (T)</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>CC x T</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2021										
No Till - AG	0.65 ± 0.03	0.12 ± 0.01	3.16 ± 0.07	26.7 ± 4.3	68.7 ± 5.1	31.3 ± 0.4	1.79 ± 0.05	1.09 ± 0.05	155.1 ± 15.9	3.33 ± 0.44
No Till - RV	0.63 ± 0.03	0.11 ± 0.01	3.46 ± 0.17	26.7 ± 1.6	81.0 ± 15.2	31.7 ± 1.9	1.85 ± 0.05	1.14 ± 0.03	133.0 ± 24.6	3.33 ± 0.44
No Till - PG	0.72 ± 0.10	0.13 ± 0.04	3.06 ± 0.27	29.3 ± 5.2	64.3 ± 4.9	34.3 ± 3.1	1.58 ± 0.03	0.91 ± 0.10	143.0 ± 41.7	4.00 ± 0.76
Till - AG	0.66 ± 0.01	0.11 ± 0.01	3.49 ± 0.11	32.0 ± 5.9	84.7 ± 23.5	29.0 ± 1.3	1.72 ± 0.11	1.04 ± 0.07	192.1 ± 38.6	4.00 ± 0.76
Till - RV	0.64 ± 0.02	0.11 ± 0.02	3.37 ± 0.09	27.3 ± 1.8	83.3 ± 6.5	34.3 ± 5.1	1.70 ± 0.12	1.06 ± 0.07	138.0 ± 15.6	4.00 ± 1.32
Till - PG	0.64 ± 0.03	0.10 ± 0.01	3.81 ± 0.56	26.3 ± 1.9	84.3 ± 19.0	31.3 ± 1.6	1.88 ± 0.17	1.16 ± 0.75	134.0 ± 6.5	3.00 ± 0.44
<i>Cover crop (CC)</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Tillage (T)</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>CC x T</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Year</i>	***	***	ns	***	***	ns	ns	***	***	***
<i>Year x CC</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Year x T</i>	ns	ns	ns	ns	ns	ns	*	ns	ns	ns
<i>Year x CC x T</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

^a ANOVA to compare data (p value indicated); Letters within columns indicate significant mean separation according to Tukey's honest significance difference (HSD) test at p value < 0.05, where "**": p value < 0.05; "***": p value < 0.001, "****": p value < 0.0001. ^b Abbreviations: N: Nitrogen; P: Phosphorus; K: Potassium; Zn: Zinc; Mn: Manganese; B: Boron; Ca: Calcium; Mg: Magnesium; Fe: Iron; Cu: Copper; na: not applicable; ns: not significant.

Table 5 Petiole nutrition at bloom of Merlot grapevines subjected to different cover crops and tillage systems collected in the 2019–20 and 2020-21 seasons ^{a, b}

Napa County (Oakville)										
Treatment	N (%)	P (%)	K (%)	Zn (mg/kg)	Mn (mg/kg)	B (mg/kg)	Ca (%)	Mg (%)	Fe (mg/kg)	Cu (mg/kg)
2020										
No Till - AG	1.07 ± 0.09	0.43 ± 0.03	3.19 ± 0.18	98.8 ± 4.33	39.0 ± 4.64	43.5 ± 2.60	1.73 ± 0.14	0.63 ± 0.06	32.3 ± 3.60	10.00 ± 1.56
No Till - RV	0.88 ± 0.06	0.53 ± 0.06	3.12 ± 0.10	100.8 ± 3.57	37.3 ± 4.01	44.5 ± 1.37	1.99 ± 0.23	0.65 ± 0.05	32.0 ± 3.16	12.50 ± 1.20
No Till - PG	0.92 ± 0.09	0.51 ± 0.03	3.02 ± 0.36	101.0 ± 3.68	37.3 ± 3.07	48.0 ± 3.09	1.87 ± 0.20	0.79 ± 0.02	36.3 ± 2.18	13.00 ± 0.81
Till - AG	0.88 ± 0.04	0.51 ± 0.02	3.12 ± 0.20	99.5 ± 1.11	35.8 ± 0.55	46.3 ± 0.29	1.90 ± 0.08	0.68 ± 0.07	40.0 ± 1.16	12.50 ± 0.58
Till - RV	0.96 ± 0.09	0.46 ± 0.06	3.22 ± 0.17	96.3 ± 5.94	40.8 ± 4.63	45.0 ± 3.86	1.65 ± 0.14	0.60 ± 0.09	33.0 ± 5.27	10.25 ± 1.66
Till - PG	1.11 ± 0.12	0.46 ± 0.02	3.17 ± 0.19	91.8 ± 7.78	35.8 ± 4.86	47.0 ± 3.74	1.66 ± 0.10	0.63 ± 0.09	34.3 ± 3.31	11.00 ± 0.67
<i>Cover crop (CC)</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Tillage (T)</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>CC x T</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2021										
No Till - AG	1.39 ± 0.07	0.38 ± 0.02	3.04 ± 0.28	43.0 ± 2.45	63.0 ± 7.96	45.8 ± 1.91	1.74 ± 0.15	0.51 ± 0.05	46.3 ± 1.91	8.75 ± 0.29
No Till - RV	1.45 ± 0.07	0.39 ± 0.02	3.16 ± 0.29	40.3 ± 3.10	66.0 ± 5.06	45.8 ± 1.09	1.86 ± 0.14	0.51 ± 0.02	46.0 ± 0.82	8.75 ± 0.55
No Till - PG	1.39 ± 0.06	0.41 ± 0.02	3.09 ± 0.23	45.8 ± 1.52	65.0 ± 13.76	47.5 ± 1.67	1.80 ± 0.05	0.56 ± 0.01	51.3 ± 5.42	9.00 ± 0.82
Till - AG	1.54 ± 0.03	0.38 ± 0.02	3.17 ± 0.10	39.5 ± 2.33	70.0 ± 12.82	45.8 ± 1.28	1.95 ± 0.11	0.56 ± 0.04	44.8 ± 3.60	8.75 ± 0.33
Till - RV	1.50 ± 0.11	0.42 ± 0.04	2.99 ± 0.07	40.3 ± 4.01	77.5 ± 16.93	46.8 ± 1.44	1.92 ± 0.15	0.54 ± 0.01	42.8 ± 4.25	8.75 ± 0.55
Till - PG	1.51 ± 0.04	0.42 ± 0.03	3.44 ± 0.18	43.0 ± 1.33	79.0 ± 13.56	48.8 ± 1.36	1.98 ± 0.05	0.52 ± 0.02	52.5 ± 3.67	9.25 ± 0.55
<i>Cover crop (CC)</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Tillage (T)</i>	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>CC x T</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Year</i>	**	**	ns	***	***	ns	ns	***	***	***
<i>Year x CC</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Year x T</i>	ns	ns	ns	ns	ns	ns	*	ns	ns	ns
<i>Year x CC x T</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	*

^a ANOVA to compare data (p value indicated); Letters within columns indicate significant mean separation according to Tukey's honest significance difference (HSD) test at p value < 0.05, where "**": p value < 0.05; "***": p value < 0.001, "****": p value < 0.0001. ^b Abbreviations: N: Nitrogen; P: Phosphorus; K: Potassium; Zn: Zinc; Mn: Manganese; B: Boron; Ca: Calcium; Mg: Magnesium; Fe: Iron; Cu: Copper; na: not applicable; ns: not significant.

Table 6. Yield components of Ruby Cabernet (Fresno County) and Merlot grapevines (Napa County) subjected to different cover crops and tillage systems, collected in the 2019–20 and 2020–21 seasons ^{a, b}

Fresno County (Five Points)					Napa County (Oakville)				
Treatment	Cluster No.	Average Cluster Mass (g)	Yield (kg/vine)	Leaf Area: Fruit (m ² /kg)	Treatment	Cluster No.	Average Cluster Mass (g)	Yield (kg/vine)	Leaf Area: Fruit (m ² /kg)
2020					2020				
No Till - AG	139 ± 9.92	128.6 ± 7.9	5.92 ± 0.98	1.94 ± 0.11	No Till - AG	18 ± 1.29	64.5 ± 6.5	0.40 ± 0.17	1.74 ± 0.41
No Till - RV	122 ± 20.17	128.4 ± 16.9	5.11 ± 0.77	1.42 ± 0.68	No Till - RV	19 ± 2.67	74.2 ± 12.4	0.47 ± 0.28	1.38 ± 0.23
No Till - PG	115 ± 20.13	145.6 ± 16.8	5.44 ± 0.95	1.55 ± 0.66	No Till - PG	17 ± 1.57	73.1 ± 7.1	0.41 ± 0.08	1.54 ± 0.10
Till - AG	139 ± 25.89	112.8 ± 18.7	5.32 ± 0.62	1.72 ± 0.25	Till - AG	17 ± 1.57	82.6 ± 15.4	0.47 ± 0.32	1.37 ± 0.26
Till - RV	146 ± 26.35	121.9 ± 9.9	5.86 ± 1.02	1.58 ± 0.25	Till - RV	17 ± 1.06	80.0 ± 10.9	0.46 ± 0.26	1.54 ± 0.32
Till - PG	129 ± 36.47	157.1 ± 11.6	6.58 ± 0.98	1.60 ± 0.45	Till - PG	18 ± 0.50	78.9 ± 7.8	0.47 ± 0.24	1.41 ± 0.28
<i>Cover crop (CC)</i>	ns	ns	ns	ns	<i>Cover crop (CC)</i>	ns	ns	ns	ns
<i>Tillage (T)</i>	ns	ns	ns	ns	<i>Tillage (T)</i>	ns	ns	ns	ns
<i>CC x T</i>	ns	ns	ns	ns	<i>CC x T</i>	ns	ns	ns	ns
2021					2021				
No Till - AG	111 ± 15.59	160.2 ± 14.2	5.85 ± 0.67	1.97 ± 0.12	No Till - AG	34 ± 4.71	100.6 ± 19.0	1.16 ± 1.05	0.65 ± 0.12
No Till - RV	106 ± 23.11	134.4 ± 25.4	4.53 ± 0.75	1.64 ± 0.94	No Till - RV	32 ± 5.52	79.8 ± 5.3	0.83 ± 0.24	0.70 ± 0.13
No Till - PG	100 ± 9.33	147.7 ± 33.2	4.83 ± 0.46	1.59 ± 0.58	No Till - PG	37 ± 0.50	81.0 ± 2.7	0.88 ± 0.06	0.72 ± 0.45
Till - AG	124 ± 28.06	168.9 ± 21.3	6.71 ± 0.81	1.40 ± 0.26	Till - AG	32 ± 1.22	105.1 ± 26.6	1.09 ± 0.74	0.60 ± 0.09
Till - RV	137 ± 16.52	131.8 ± 28.5	5.91 ± 0.22	1.52 ± 0.40	Till - RV	41 ± 7.80	104.0 ± 17.1	1.52 ± 0.78	0.57 ± 0.10
Till - PG	116 ± 26.39	172.8 ± 45.0	6.66 ± 0.10	1.63 ± 0.48	Till - PG	40 ± 7.17	82.9 ± 11.3	1.11 ± 0.85	0.76 ± 0.32
<i>Cover crop (CC)</i>	ns	ns	ns	ns	<i>Cover crop (CC)</i>	ns	ns	ns	ns
<i>Tillage (T)</i>	ns	ns	ns	ns	<i>Tillage (T)</i>	ns	ns	ns	ns
<i>CC x T</i>	ns	ns	ns	ns	<i>CC x T</i>	ns	ns	ns	ns
<i>Year</i>	*	*	ns	ns	<i>Year</i>	***	**	***	***
<i>Year x CC</i>	ns	ns	ns	ns	<i>Year x CC</i>	ns	ns	ns	ns
<i>Year x T</i>	*	***	ns	ns	<i>Year x T</i>	*	***	ns	ns
<i>Year x T x CC</i>	ns	ns	ns	ns	<i>Year x T x CC</i>	ns	ns	ns	ns

^a ANOVA to compare data (p value indicated); Letters within columns indicate significant mean separation according to Tukey's honest significance difference (HSD) test at p value < 0.05, where "**": p value < 0.05; "***": p value < 0.001, "****": p value < 0.0001. ^b Abbreviations: ns: not significant; AG: Annual grass; RV: resident vegetation; PG: perennial grass.

Table 7. Berry composition of Ruby Cabernet (Fresno County) and Merlot (Napa County) grapevines subjected to different cover crops and tillage collected in the 2019–20 and 2020-21 seasons ^{a, b}

Fresno County (Five Points)						
Treatment	Juice pH	TA (g/L)	TSS (°Brix)	Total Anthocyanins (mg/g Berry FM)	Average Berry Mass (g/berry)	Average Skin Mass (g)
2020						
No Till - AG	3.89 ± 0.04	5.37 ± 0.38	21.8 ± 1.5	1.45 ± 0.23	1.83 ± 0.09	1.31 ± 0.07
No Till - RV	3.84 ± 0.04	5.73 ± 0.23	17.2 ± 4.3	1.44 ± 0.24	1.78 ± 0.05	1.41 ± 0.12
No Till - PG	3.88 ± 0.05	5.68 ± 0.71	19.5 ± 2.6	1.48 ± 0.27	1.54 ± 0.08	1.24 ± 0.22
Till - AG	3.80 ± 0.06	6.30 ± 0.50	20.4 ± 1.3	1.20 ± 0.21	1.72 ± 0.22	1.32 ± 0.21
Till - RV	3.89 ± 0.02	5.60 ± 0.27	20.4 ± 1.2	1.55 ± 0.04	1.80 ± 0.02	1.42 ± 0.17
Till - PG	3.88 ± 0.02	5.77 ± 0.56	20.8 ± 0.8	1.40 ± 0.03	1.77 ± 0.12	1.51 ± 0.34
<i>Cover crop (CC)</i>	ns	ns	ns	ns	ns	ns
<i>Tillage (T)</i>	ns	ns	ns	ns	ns	ns
<i>CC x T</i>	ns	ns	ns	ns	ns	ns
2021						
No Till - AG	3.86 ± 0.03	6.18 ± 0.24 b	21.1 ± 1.4	1.85 ± 0.44	1.74 ± 0.11	1.37 ± 0.29
No Till - RV	3.87 ± 0.03	5.92 ± 0.61 b	22.2 ± 1.3	1.41 ± 0.39	1.67 ± 0.08	1.25 ± 0.20
No Till - PG	3.82 ± 0.04	6.13 ± 0.78 b	19.6 ± 2.4	1.60 ± 0.30	1.68 ± 0.11	1.34 ± 0.12
Till - AG	3.77 ± 0.06	6.98 ± 0.86 a	20.8 ± 1.1	1.27 ± 0.10	1.75 ± 0.14	1.28 ± 0.23
Till - RV	3.82 ± 0.09	6.95 ± 0.95 a	21.9 ± 0.9	1.80 ± 0.35	1.78 ± 0.07	1.45 ± 0.23
Till - PG	3.88 ± 0.09	6.35 ± 1.33 a	22.1 ± 1.2	2.04 ± 0.42	1.68 ± 0.04	1.62 ± 0.20
<i>Cover crop (CC)</i>	ns	ns	ns	ns	ns	ns
<i>Tillage (T)</i>	ns	*	ns	ns	ns	ns
<i>CC x T</i>	ns	ns	ns	ns	ns	ns
<i>Year</i>	ns	***	**	ns	ns	ns
<i>Year x CC</i>	ns	ns	ns	ns	ns	ns
<i>Year x T</i>	ns	ns	ns	ns	ns	ns
<i>Year x T x CC</i>	ns	ns	ns	ns	ns	ns
Napa County (Oakville)						
2020						
No Till - AG	3.33 ± 0.02	7.70 ± 0.43	25.9 ± 1.0	1.16 ± 0.08	0.94 ± 0.06	0.78 ± 0.08
No Till - RV	3.32 ± 0.01	7.65 ± 0.29	25.2 ± 0.6	1.50 ± 0.44	0.94 ± 0.05	0.61 ± 0.07
No Till - PG	3.30 ± 0.01	7.85 ± 0.05	24.8 ± 0.5	1.37 ± 0.48	1.02 ± 0.08	0.63 ± 0.11
Till - AG	3.31 ± 0.02	7.50 ± 0.13	25.4 ± 0.2	1.49 ± 0.30	1.00 ± 0.10	0.61 ± 0.09
Till - RV	3.33 ± 0.02	7.50 ± 0.24	24.7 ± 1.6	1.42 ± 0.12	0.96 ± 0.06	0.59 ± 0.06
Till - PG	3.32 ± 0.02	7.68 ± 0.24	25.7 ± 0.5	1.36 ± 0.11	0.94 ± 0.07	0.57 ± 0.09
<i>Cover crop (CC)</i>	ns	ns	ns	ns	ns	ns
<i>Tillage (T)</i>	ns	ns	ns	ns	ns	ns
<i>CC x T</i>	ns	ns	ns	ns	ns	ns
2021						
No Till - AG	3.58 ± 0.05 a	6.58 ± 0.43	24.1 ± 0.5	2.40 ± 0.44	0.91 ± 0.13	1.14 ± 0.13
No Till - RV	3.65 ± 0.10 a	6.73 ± 0.11	24.7 ± 0.5	1.20 ± 0.32	0.99 ± 0.05	1.26 ± 0.26
No Till - PG	3.59 ± 0.03 a	6.30 ± 0.30	25.6 ± 0.9	2.93 ± 0.89	0.83 ± 0.04	1.37 ± 0.08
Till - AG	3.54 ± 0.01 b	7.00 ± 0.32	24.9 ± 0.6	1.91 ± 0.39	0.96 ± 0.03	1.40 ± 0.29
Till - RV	3.54 ± 0.03 b	7.30 ± 0.33	24.4 ± 0.5	2.28 ± 0.41	1.04 ± 0.05	1.18 ± 0.21
Till - PG	3.54 ± 0.01 b	7.08 ± 0.23	24.1 ± 0.4	2.05 ± 0.19	0.89 ± 0.04	1.05 ± 0.15
<i>Cover crop (CC)</i>	ns	ns	ns	ns	ns	ns
<i>Tillage (T)</i>	*	ns	ns	ns	ns	ns
<i>CC x T</i>	ns	ns	ns	ns	ns	ns
<i>Year</i>	***	***	*	***	ns	***
<i>Year x CC</i>	ns	ns	ns	ns	ns	ns
<i>Year x T</i>	*	***	ns	ns	ns	ns
<i>Year x T x CC</i>	ns	ns	ns	ns	ns	ns

a ANOVA to compare data (p value indicated); Letters within columns indicate significant mean separation according to Tukey's honest significance difference (HSD) test at p value < 0.05, where "*": p value < 0.05; "***": p value < 0.001, "****": p value < 0.0001. b Abbreviations: TA: titratable acidity; TSS: total soluble solids; ns: not significant; AG: Annual grass; RV: resident vegetation; PG: perennial grass.

Table 8. Water footprint (m³/ton) of the Ruby Cabernet (Fresno County) and Merlot (Napa County) vineyard subjected to different cover crops and tillage collected in the 2019–20 and 2020–21 seasons ^{a, b}

Fresno County (Five Points)					Napa County (Oakville)				
Treatment	Green Water Footprint (m ³ /ton)	Blue Water Footprint (m ³ /ton)	Grey Water Footprint (m ³ /ton)	Total Water Footprint (m ³ /ton)	Treatment	Green Water Footprint (m ³ /ton)	Blue Water Footprint (m ³ /ton)	Grey Water Footprint (m ³ /ton)	Total Water Footprint (m ³ /ton)
2020					2020				
No Till - AG	170 ± 9.3	248 ± 13.6	50 ± 2.7	468 ± 25.7	No Till - AG	2803 ± 369.3	1170 ± 154.1	116 ± 15.2	4088 ± 538.7
No Till - RV	197 ± 10.2	288 ± 14.9	58 ± 3.0	542 ± 28.0	No Till - RV	2344 ± 376.1	978 ± 157.0	97 ± 15.5	3419 ± 548.6
No Till - PG	180 ± 9.3	263 ± 13.5	53 ± 2.7	496 ± 25.5	No Till - PG	2347 ± 173.5	979 ± 72.4	97 ± 7.2	3423 ± 253.0
Till - AG	211 ± 63.2	308 ± 92.4	62 ± 18.5	500 ± 174.1	Till - AG	2316 ± 274.0	966 ± 114.3	95 ± 11.3	3378 ± 399.6
Till - RV	173 ± 18.4	254 ± 26.8	51 ± 5.4	478 ± 50.6	Till - RV	2366 ± 337.9	987 ± 141.0	98 ± 13.9	3451 ± 492.9
Till - PG	159 ± 29.4	233 ± 43.0	47 ± 8.6	439 ± 81.0	Till - PG	2281 ± 319.5	952 ± 133.3	94 ± 13.2	3328 ± 466.0
<i>Cover crop (CC)</i>	ns	ns	ns	ns	<i>Cover crop (CC)</i>	ns	ns	ns	ns
<i>Tillage (T)</i>	ns	ns	ns	ns	<i>Tillage (T)</i>	ns	ns	ns	ns
<i>CC x T</i>	ns	ns	ns	ns	<i>CC x T</i>	ns	ns	ns	ns
2021					2021				
No Till - AG	137 ± 13.9	276 ± 28.1	52 ± 5.3	466 ± 47.3	No Till - AG	1213 ± 332.9	359 ± 98.6	87 ± 23.8	1659 ± 455.3
No Till - RV	187 ± 32.6	377 ± 65.6	72 ± 12.5	635 ± 110.6	No Till - RV	1481 ± 97.4	439 ± 28.9	106 ± 7.0	2026 ± 133.3
No Till - PG	177 ± 38.2	357 ± 77.1	68 ± 14.6	603 ± 129.9	No Till - PG	1417 ± 142.5	420 ± 42.2	101 ± 10.2	1938 ± 194.9
Till - AG	121 ± 9.8	244 ± 19.8	46 ± 3.8	412 ± 33.4	Till - AG	1231 ± 225.8	365 ± 66.9	88 ± 16.2	1684 ± 308.8
Till - RV	133 ± 28.1	277 ± 56.7	51 ± 10.8	452 ± 95.5	Till - RV	932 ± 106.4	276 ± 31.5	67 ± 7.6	1275 ± 145.6
Till - PG	138 ± 56.6	278 ± 114.1	53 ± 21.7	469 ± 192.4	Till - PG	1208 ± 229.1	358 ± 67.9	87 ± 16.4	1653 ± 313.4
<i>Cover crop (CC)</i>	ns	ns	ns	ns	<i>Cover crop (CC)</i>	ns	ns	ns	ns
<i>Tillage (T)</i>	ns	ns	ns	ns	<i>Tillage (T)</i>	ns	ns	ns	ns
<i>CC x T</i>	ns	ns	ns	ns	<i>CC x T</i>	ns	ns	ns	ns
<i>Year</i>	*	ns	ns	ns	<i>Year</i>	**	**	**	**
<i>Year x CC</i>	ns	ns	ns	ns	<i>Year x CC</i>	ns	ns	ns	Ns
<i>Year x T</i>	ns	ns	ns	ns	<i>Year x T</i>	ns	ns	ns	ns
<i>CC x T x Year</i>	ns	ns	ns	ns	<i>CC x T x Year</i>	ns	ns	ns	ns

^a ANOVA to compare data (p value indicated); Letters within columns indicate significant mean separation according to Tukey's honest significance difference (HSD) test at p value < 0.05, where "*": p value < 0.05; "**": p value < 0.001, "***": p value < 0.0001. ^b Abbreviations: ns: not significant; AG: Annual grass; RV: resident vegetation; PG: perennial grass.

Chapter 2: Cover Crop and Tillage Effects on Vineyard Net Ecosystem Carbon Balance in California Vineyard Agroecosystems

Keywords: Climate change, carbon sequestration, cover crop, tillage, carbon storage

Abstract

Considering global wine grape production covers approximately 7.4 M ha, the potential for carbon (C) storage in vineyard systems is of great interest to offset greenhouse gas emissions and mitigate the effects of climate change. Sustainable soil management practices such as cover crop adoption and reduced tillage may contribute to soil organic carbon (SOC) sequestration. However, site specific factors such as soil texture, other soil physicochemical properties, and climate largely influence the range and rate to which SOC may be stored. To measure the potential for C storage in vineyard systems under varying sustainable soil management practices, we calculated net ecosystem carbon balance (NECB) of three cover crops (Perennial grass (*Poa bulbosa* hybrid cv. Oakville Blue); Annual grass (Barley, *Hordeum vulgare*); Resident vegetation (natural weed population) under conventional tillage (CT) and no-till (NT) management. Results provided evidence that vineyards under the current management practices serve as C sinks. In sandy soils, the type of cover crop and tillage system may be of little influence on NECB, while in finer textured soils tillage reduced NECB and higher biomass producing cover crops enhanced the overall carbon storage potential of the vineyard agroecosystem. Our results revealed that soil texture and site characteristics were key determinants of the C storage potential of vineyards in Mediterranean climates such as those found in coastal and inland California production regions.

1. Introduction

As temperatures rise and rain events become more unpredictable, soils are under threat of loss of soil organic matter (SOM), soil nutrient imbalances, loss of soil biodiversity, contamination, and compaction (Panagos et al., 2020). Moreover, it is estimated that almost 36 billion tons of soil are lost annually due to water and wind erosion (Alsina et al., 2014; Borrelli et al., 2017; Wolff et al., 2018). Soil erosion is further accelerated by mechanical tillage, and an over reliance on soil cultivation for weed control and aeration over the past half century has resulted in a significant loss of SOM across agricultural soils (Alsina et al., 2014; Mitchell et al., 2017). Thus, over the last decade there has been a substantial increase in attention towards rebuilding SOM and using soils as a tool to mitigate the effects of climate change (Lal, 2004; Powlson et al., 2011; Lazcano et al., 2020; Nilahyane et al., 2020).

Traditionally, the inter-rows (also known as ‘alleyways’ or ‘tractor rows’) of vineyards were kept barren with the use of herbicides and tillage. However, it has been shown that both practices may have detrimental effects on soil quality as well as the surrounding ecosystem (Patiño-Zúñiga et al., 2009b; Ferreira et al., 2020b; Gatti et al., 2022c). Thus, the adoption of cover crops and reduction of soil tillage have been proposed as sustainable alternatives to traditional management of the vineyard floor (Alsina et al., 2013). Research suggests that cover crops may not only reduce soil erosion and runoff, but also improve water infiltration in most soils of temperate regions by increasing SOM (Álvaro-Fuentes et al., 2008; Steenwerth and Belina, 2008a; Belmonte et al., 2018; Cataldo et al., 2020). SOM is further preserved under reduced or no-till settings whereby soil aggregates and accompanying SOM remain undisturbed (Šimon et al., 2009; Peregrina et al., 2010; Seddaiu et al., 2013). The preservation of SOM in turn bolsters SOC (soil organic carbon), which has been identified as a key target in mitigating climate change via carbon sequestration. In fact, the Intergovernmental Panel on Climate Change (IPCC) has estimated that by 2030, global SOC sequestration has the potential to mitigate up to about 5.3 Gt CO₂ per year (Porter et al., 2017). Vineyard systems represent large potential for agricultural soil carbon sequestration (SCS), as grapevines long-life cycle and deep rooting systems allow them to potentially sequester higher amounts of C compared to annual crops (Peregrina et al., 2014; Nistor et al., 2018). However, there are limitations to SCS, including

a lack of standardized methods of SOC determination as well as uncertainty regarding the stability of different soil carbon pools (Powlson et al., 2011). Furthermore, the effectiveness and rate of long-term SOC sequestration in agricultural soils is largely influenced by site specific conditions including climate, soil texture, other soil physiochemical properties, and management practices (Carlisle et al., 2010; Powlson et al., 2011).

Previous literature has indeed identified vineyards as C sinks; however, management practices largely influenced the storage potential of the systems (Brunori et al., 2016; Payen et al., 2021). Subsequently, management practices that increase SOM have been encouraged over the last decade as a SCS strategy; yet their impact on SOC storage rates remains unclear (Novara et al., 2019). While some authors have recorded an increase in a soil organic carbon (SOC) sequestration rate due to cover crop adoption (Steenwerth and Belina, 2008a; Peregrina et al., 2014), others have reported the opposite (Celette, 2007; Novara et al., 2019; Jian et al., 2020).

One source of contradictions in previous literature may be the presence or absence of measurement of CO₂ efflux (soil respiration). Soil CO₂ efflux results from the combination of biological and physical processes, both of which are sensitive to edaphic factors and highly variable in space and time. In soils of hot climates, CO₂ efflux may be great enough to potentially offset short term storage of C from cover crop adoption (Yu et al., 2019). Furthermore, some studies have shown that cover crops enhance microbial activity, thus increasing CO₂ efflux (Steenwerth et al., 2010; Maier et al., 2011; Freidenreich et al., 2021). In fact, CO₂ emissions may be further increased under some types of cover crops due to the lower C:N ratios of certain plant material, such as legumes (Alluvione et al., 2010; Freidenreich et al., 2021). Since recent work has shown that SOC increases as tillage frequency is reduced, some studies have combined various cover crops with reduced or no-till management in California vineyards (Conant et al., 2007; Steenwerth and Belina, 2008a; Wolff et al., 2018b). Furthermore, there is a lack of information regarding the potential of C storage in vineyard systems under combinations of soil management practices, considering C losses at both the grapevine and vineyard scale.

Thus, a NCEB is needed to elucidate C inputs and outputs in a commercial production setting. One method of estimating net C storage within a system is through the net ecosystem production (NEP) methodology (Randerson et al., 2002). NEP reflects the balance of ecosystem primary production minus ecosystem respiration to determine whether the net gain or loss of C. NEP is effectively parallel to net primary production (NPP), as NPP quantifies the C input by plants only and NEP includes C input by plants in addition to C release by soils. By combining NPP of the cover crop and grapevine with soil CO₂ efflux and C losses at harvest, NECB is revealed (Cates and Jackson, 2019).

This two-year study (2019-2021) was conducted to i) quantify carbon inputs and losses at the vineyard scale to determine NECB; ii) compare these values among different vineyard floor management systems in two California vineyards in different regions over two arid seasons.

2. Material and methods

2.1. Experimental design

Field experiments were conducted at two sites for two consecutive growing seasons (2019-2020 and 2020-2021). The first site was located in Fresno, CA (36.671514, -119.925823) in a Ruby Cabernet/Freedom (27% *V. vinifera* hybrid) vineyard. Grapevines were planted in 2012 with a spacing of 3.0 × 1.2 m (row × vine) with a row orientation of E-W. The grapevines were head-trained and cane pruned. The vineyard is under a sprawling trellis system with catch wires at 1.54 m and at 1.68 m above vineyard floor. The soil texture of the site is classified as a sandy loam and vines were drip-irrigated with two emitters per plant delivering 4.0 L/h each. The second site in Oakville, CA (38.428° N, 122.409° W) in a Merlot (clone 181)/3309 C (*V. riparia* × *V. rupestris*) vineyard. Grapevines were planted in 2018 with a spacing of 3.0 × 2.0 m (row × vine) with a row orientation of E-W. The grapevines were spur-pruned and trained to quadrilateral cordons 1.38 m above vineyard floor with catch wires at 1.68 m. The soil texture of the site is classified as clay loam and vines were drip-irrigated with two emitters per plant delivering 2 L/h each.

At both sites, experiments were arranged in a split-plot 3 × 2 factorial design (three different cover crops subjected to two tillage managements) with four (Oakville) and three replications (Fresno). At both vineyards, each treatment replicate consisted of 15 grapevines. Three vines in the middle of each replicate

were used for measurements, while the distal vines on either end were treated as border plants. Cover crop treatments included a i) Perennial grass (*Poa bulbosa* hybrid cv. Oakville Blue); ii) Annual grass (Barley, *Hordeum vulgare*); iii) Resident vegetation (natural weed population). Tillage management consisted of no-till (NT) in which inter-rows were disked 2-3 cm only once in the fall prior to seeding and conventional tillage (CT) which was disked to a depth of 10 cm once in the fall and twice in the spring to incorporate the cover crop residue. The cover crop seed was drilled to a 1.5 m wide strip according to seed manufacturer's recommended practices prior to receiving fall/winter rains in 2019 and 2020 at a rate of 605 kg/ha and 84 kg/ha for the perennial grass (PG) and annual grass (AG) treatments, respectively. Resident vegetation (RV) was allowed to grow within a 1.5 m strip and mowed according to vineyard manager's discretion. Berms were 1.0 m wide and kept free of vegetation using a glyphosate herbicide application in the spring.

2.2. Site conditions

Annual (January 1 to December 31) mean daily air and soil temperatures, as well as maximum and minimum air temperatures from the two sites were obtained from California Irrigation Management Information System (CIMIS) stations nearest the experimental vineyard (station #77 in Napa County, CA and station #2 in Fresno County, CA).

Soil texture was assessed using hydrometer method (S – 14.10) from the North American Proficiency Testing (NAPT) program. Soil organic carbon (SOC) content was measured in the inter-rows of each experimental unit under dry conditions. In July 2020, bulk density was assessed at the centers of inter-rows (122 cm from the vine rows) as well as the edges of the berms (61 cm), using brass rings of 10 cm internal diameter and 7 cm length. No differences in bulk densities were found in CT versus NT inter-rows, thus all soil samples were taken at the same depths. Three soil cores were randomly collected per experimental unit to a depth of 30 cm and partitioned into 0-15 cm and 15-30 cm sub-samples. Sub-samples were homogenized and kept in a cool environment until analysis. At analysis, samples were dried, sieved to <2 mm, ball-milled, and analyzed for SOC by combustion. Soil pH was determined via saturated paste method as described by Gavlak, 1994. Saturated paste percentage was also reported as a reflectance of soil moisture status (Table 1).

2.3. Soil respiration (R_s)

To calculate the losses of soil C through soil respiration, soil CO₂ efflux was measured in situ using a CIRAS-3 (PP Systems, Amesbury, MA) portable gas exchange system coupled with a closed system soil respiration chamber (SRC-2). The SRC-2 chamber consisted of a soil surface area of 78 cm² and a system volume of 1171 ml. Upon measurement, the chamber was allowed to stabilize for one minute before the gas accumulated in the chamber headspace was continuously sampled in the closed circuit. Efflux rate of CO₂ (μmol CO₂ m⁻² s⁻¹) was calculated based on linear fit by the CIRAS-3 analyzer as shown in Eq. 1

$$\text{Eq. 1. } R = \frac{C_n - C_o}{T_n} \times \frac{V}{A}$$

where R is the respiration rate (CO₂ flux, or moles of CO₂ unit area⁻¹ unit time⁻¹), C_o is the CO₂ concentration at T=0 and C_n is the concentration at a time T_n later. A is the area of soil exposed and V the total system volume. Soil surface temperature, relative humidity, and surface evaporation were also simultaneously recorded. The air within the chamber was continuously and automatically mixed during the measurement period to ensure representative samples.

To minimize leakage, the chamber was fit onto a 10 cm diameter polyvinyl chloride (PVC ring) placed 5 cm deep in the vineyard inter-row of each experimental unit. The PVC rings remained in the soil throughout the experiment except for removal for mowing and tillage whereby the ring was replaced at least 24 hr prior to sampling. Sampling areas were selected with careful consideration for the least plant material to avoid CO₂ contributions from aboveground plant parts. Additionally, as autotrophic and heterotrophic respiration could not be separated in this study, the measured CO₂ efflux included emissions from all soil processes. Soil respiration measurements took place no more than 2 hours after solar noon and was measured at six timepoints per experimental unit in each season in Oakville (2020-01-24, 2020-04-13, 2020-04-21, 2020-04-23, 2020-05-22, 2020-06-19, 2021-01-29, 2021-04-14, 2021-04-16, 2021-05-14, 2021-06-15, and 2021-07-0), and five timepoints in Fresno (2020-03-02, 2020-03-25, 2020-04-16, 2020-04-17, 2020-6-12, 2021-02-14, 2021-03-24, 2021-04-28, 2021-04-29, and 2021-07-01). In addition, R_s was measured under the vines on bare soil at each measurement. Measurement timepoints were selected to

represent conditions throughout the season, including the day before and after a tillage event at both sites. Mean R_s values for the season were calculated for each treatment and the bare soil control.

2.4. Estimates of net primary productivity (NPP)

2.4.1 Grapevine NPP

$NPP_{\text{grapevine}}$ was estimated as the summation of annual production (harvest yield, leaf biomass, and cane production) and permanent organs (trunk and root biomass). Harvest commenced when the fruit reached approximately 25 °Brix in Oakville (August 25, 2020, and September 1, 2021) and 21 °Brix in Fresno (October 6, 2020, and September 7, 2021). At both sites, clusters from three data vines per experimental unit were manually removed, counted, and weighed on a top-loading balance. Sub-samples were collected from clusters within each experimental unit at harvest and carbon content (% mass) was determined via combustion (Western Region Method S-9.30)(Gavlak et al., 1994).

At the Fresno vineyard, leaf area index (LAI) was measured in late spring to characterize grapevine canopy growth by a smartphone program, [VitiCanopy, via iOS system \(Apple Inc., Cupertino, CA, USA\)](#) (De Bei et al., 2016) and converted into leaf area. The gap fraction threshold was set to 0.75, extinction coefficient was set to 0.7, and sub-divisions were 25. An extendable mounting device was used to effectively position the device approximately 75 cm underneath the canopy. The device was positioned with the maximum length of the screen being perpendicular to the cordon, and the cordon in line with the middle of the screen according to previous work (De Bei et al., 2016; Yu and Kurtural, 2020). In each experimental unit, three images were taken to capture half canopy of each vine and analyzed by the software. Subsamples of 100 leaves were collected per experimental unit and leaf area (cm²) was determined by leaf area meter (Li-Cor 3300, Lincoln, NE USA), dried at 80 °C, and dry weight (g) was recorded, and values extrapolated to determine LAI. At the Oakville vineyard, two vines per treatment were completely defoliated and biomass was measured. Carbon content (% mass) of leaves was estimated as 56% of dry weight (Zhang et al., 2021).

Cane production (pruning wood weights) was measured at dormancy among the three data vines per experiment unit. The carbon content (%) of pruning wood was estimated based on previous literature

(Morandé et al., 2017). Annual biomass accumulation in permanent organs (trunk, cordon, and roots) was also literature driven and included one study based at the same experimental site (Oakville, CA) and another of a vineyard of similar age and productivity in the San Joaquin Valley (Williams et al., 2011; Martínez-Lüscher and Kurtural, 2021).

2.4.2 Cover crop NPP

Cover crop NPP was estimated by collecting above and below-ground biomass at crop physiological maturity as described in Steenwerth and Belina, 2008b, or just before termination. A one 1 m² quadrat was randomly placed in the inter-row of each experimental unit and all aboveground biomass was collected. Below ground biomass (roots) was also collected to a depth of 15 cm. Cover crop fresh biomass was determined and then dried at 60 °C for 48 hours to obtain the dry biomass. The carbon contribution (%) of the cover crop was estimated as 50% C of the dry biomass (Cates and Jackson, 2019).

2.5. Determination of net ecosystem carbon balance (NECB)

NECB (Mg C ha⁻¹ yr⁻¹) was calculated as follows:

$$\text{Eq. 2. } \text{NECB} = \text{grapevine NPP} + \text{SOC} + \text{cover crop NPP} - R_s \text{ inter-row} - \text{harvest} - R_s \text{ under vine}$$

Where grapevine NPP is the summation of annual and perennial growth (+), SOC is the soil organic carbon sequestered to a depth of 30 cm adjusted for spatial coverage of the inter-row (+), cover crop NPP is the sum of above and belowground cover crop biomass to 10 cm (+), R_s inter-row is the soil respiration of the portion of the vineyard where the cover crop is grown (-), harvest is the amount of C removed through yield (-), and R_s under vine is the soil respiration of the portion of the soil left bare (-). Inter-row coverage was estimated as 48% of one hectare and bare soil 52% of one hectare. A positive NECB signifies the system is net sink of C and a negative NECB signifies a net source of C to the atmosphere.

2.6. Statistical Analyses

Statistical analyses were conducted with R studio version 3.6.1 (RStudio: Integrated Development for R., Boston, MA, USA) for Mac OS. After normality assessment, data were submitted to a two-way analysis of variance (ANOVA) to assess the statistical differences between the different cover crop and tillage treatments and the respective interaction effects. Means \pm standard errors (SE) were calculated and when

the F value was significant ($P \leq 0.05$), a Tukey's 'Honest Significant Difference' (HSD) *post hoc* test was executed by using "agricolae" 1.2-8 R package. Plots were generated using GraphPad Prism v8.1.2 for Windows (Graph Pad Inc., San Diego, CA, USA).

3. Results

3.1 Site conditions

The site conditions in both Fresno County and Napa County vineyards were shown in **Table 1**. At the Fresno County vineyard, during the period of growth of the cover crop, beginning in October of the preceding year, approximately 199.0 mm of rain fell in year 1 of the study, while 152.5 mm of rain fell during the same period in year 2. In year 1 of the study, March received the greatest amount of precipitation (67 mm) while in year 2, January received the highest amount (87 mm) followed by December and October. Thus, compared to year 1, year 2 received more fall-to-winter precipitation.

The Napa County (Oakville) vineyard received a greater amount of rainfall compared to that of Fresno County, as 234.2 mm of rain fell in year 1 and 278.3 mm of rain fell in year 2. December and January received the greatest amount of precipitation in both year 1 and 2. At both sites, air temperatures were consistent between years, however the mean daily maximum temperature was 0.2 °C higher in 2021 than 2020 in Fresno County while it was 1.3°C lower in 2021 compared to 2020 in Oakville County. Daily maximum soil temperature was 0.9 °C and 0.3 °C higher in 2021 in both Fresno County and Napa County, respectively. Daily average soil temperatures were also slightly higher, by a degree of 0.1-0.2 °C at both sites in 2021. Furthermore, average soil temperatures during both years were 0.3-0.5°C higher in Fresno County and 0.4-0.5°C higher in Oakville County compared to the long-term average for the region over the past ten years (2011-2021).

3.2 Soil properties

The soil texture at the Fresno County vineyard is classified as a sandy loam with approximately 66% sand, 22% silt, and 12% clay, and a bulk density value 1.4 g cm⁻³. **Table 2** shows SOC (% mass C) was not affected by type of cover crop nor tillage system over the course of the experiment at either depth in Fresno site. Although SOC was not significantly different between years, greater SOC was observed in the 15-30

cm portion of the soil compared to the upper 0-15 cm portion. The soil texture at the Napa County (Oakville) vineyard was classified as a loam with approximately 33% sand, 42% silt, and 25% clay, and a bulk density value of 1.3 g cm⁻³. As was observed at the Fresno vineyard, SOC was greater in the lower 15-30 cm portion of the soil (**Table 2**). While type of cover crop again had no influence on SOC at either depth, tillage reduced SOC at both depths.

3.3 Soil respiration

Across the five readings of soil respiration (R_s) measured in the inter-rows at the Fresno County vineyard (**Table 3**), type of cover crop affected soil respiration rates early in the season, however trends in increased CO₂ production were not consistent between the cover crops. However, when readings were averaged to estimate seasonal mean R_s , no overall differences were observed over the course of the experiment. At the Napa County (Oakville) site, the perennial grass cover crop also increased R_s in early spring. Tillage also increased R_s in the first measurement. When readings were averaged to yield seasonal R_s , tillage displayed an overall effect and increased R_s . No interactions were observed between type of cover crop and tillage system at either site. Under vine R_s was higher in 2021 than in 2020 at both sites as well.

3.4 Estimates of grapevine and cover crop net primary productivity

At the Fresno County vineyard, there was an effect of cover crop on pruning wood and leaf C contributions (**Table 4**), whereby the PG reduced C input through production of annual growth (canes and leaves) compared to the AG and RV. However, this affect did not influence yield or C input from fruit. Ultimately, no differences in grapevine net NPP were observed between different cover crops nor tillage system. Contrary to the Fresno site, at the Napa County (Oakville) vineyard annual growth (harvest, pruning wood, leaves) was affected by tillage system rather than cover crop. Grapevines under tillage resulted in higher C contributions from pruning wood and leaves. This contributed to differences in grapevine NPP, whereby tillage increased NPP, RV resulted in highest NPP compared to AG and then PG. A significant interaction of the two factors was also found. Year-to-year differences were also observed at the Napa County site whereby C contributions from harvest and pruning wood were higher in 2021 compared to 2020, thus the same trend observed among grapevine NPP was as well.

3.5 Estimates of net carbon balance at the grapevine and vineyard scale

When harvest and R_s under vine were subtracted from grapevine NPP to generate grapevine net carbon balance (NCB), a year-to-year difference was observed at the Fresno County vineyard as values in 2020 were greater than that of 2021 (**Table 4**). At the Napa County vineyard, there was an effect of cover crop, tillage, and an interaction of the two factors on grapevine NCB. The same pattern as grapevine NPP was observed whereby NCB values were greatest under RV followed by the AG and then PG. Tillage resulted in greater NCB. When SOC adjusted for spatial coverage of the inter-row (+), R_s of the inter-row (-), and cover crop NPP (+) were added to NCB to yield NECB for the six different cover crop and tillage systems, no treatment effects were observed at the Fresno County site. At the Napa County site, however, some previous statistical trends from grapevine NCB reversed when the remaining components of the vineyard ecosystem were added. Overall, PG and tillage factors reduced NECB, however there was no interaction between the two factors (**Figure 1**). Greater NECB was also observed in 2020 compared to 2021.

4. Discussion

4.1 Soil texture plays a key role in the effect of cover crop and tillage system on soil organic carbon

After two years of the adoption of treatments, there were no statistical differences between type of cover crop on SOC at either experimental vineyard. While previous studies have reported an increase in SOM under a perennial cover crop, the species used in these experiments were native to the experimental region, contrary to the species used in the present study (Morlat and Jacquet, 2003). As expected, greater SOC content was found in the deeper portion of the soil (15-30 cm) compared to the upper 15 cm (Steenwerth and Belina, 2008b; Alsina et al., 2014; Yu et al., 2019). Previous literature has shown this effect to be quite consistent among most soil textures of temperate regions (Krull et al., 2001; Tautges et al., 2019). Likewise, SOC values at both sites were consistent with other studies under similar soil texture and climates (Steenwerth et al., 2010; Wolff et al., 2018; Yu et al., 2019). Tillage reduced SOC at only the Napa County vineyard. The lack of this effect at the Fresno County vineyard can likely be attributed to the greater SOM and finer soil texture at the Napa County vineyard. There has been recent work in Mediterranean climates that is generally agreement with these results, reporting increased SOC accumulation under minimum and

complete lack of tillage (Álvaro-Fuentes et al., 2008b; López-Bellido et al., 2010; Wolff et al., 2018b). However, it should be noted that broad conclusions regarding increased SOC under NT management are nuanced, as other studies have reported to effect of reduced tillage on SOC content (Janzen, 2006; Baker et al., 2007; Blanco-Canqui and Lal, 2008; Derpsch et al., 2014). All in all, results of the present study indicate a clear reduction of SOC under conventional tillage under the characteristics of the experimental site.

4.2 R_s and cover crop biomass are main drivers of NECB

At the Fresno County vineyard, while the soils under the RV and PG cover crop displayed increased R_s in early season measurements, ultimately no seasonal differences were observed. This may be due in part to the high sand content of the soil, and greater moisture content of the soil during the spring measurements (Nilahyane et al., 2020). While soil respiration indeed increased after tillage events during the season due to oxidation of organic matter from exposed soil aggregates, overall, there was no effect of tillage on average soil respiration over the two seasons. This result is in line with previous research that has found soil respiration to be reduced in sandy soils compared to finer textured soils (Bouma and Bryla, 2000). Although PG also decreased NPP via a reduction in annual production (canes and leaves), ultimately no differences in grapevine NCB or NECB were observed among the different cover crop and tillage systems. This is likely due to the compensation of C input through biomass from the other cover crop systems.

At the Napa County vineyard, R_s interrow was also increased under the PG cover crop compared to RV and AG in early season measurements, despite no significant differences of cover crop type over season long averages. Tillage, however increased R_s over seasonal averages, which may be due in part to greater clay content and thus SOM in the Napa County soil compared to that of Fresno County. Annual production was also reduced under NT, thus NPP and NCB were also significantly lower than under CT. However, when the remaining components of the vineyard agroecosystem were added, and despite a reduction of C inputs at the grapevine scale under NT, NECB was enhanced compared to CT. This indicates that R_s and SOC may play a greater role in determining NECB compared to the other factors, as losses of C through inter-row R_s and SOC under tillage were large enough to negate the previous increase in NPP.

High variability in biomass also led to a significant effect of cover crop type on NECB at the Napa County vineyard, as the AG and RV had contrasting growth patterns compared to the low stature PG. Changes in NECB as affected by cover crop paralleled cover crop NPP (biomass) and was greatest under RV, followed by AG, and PG. There was no significant interaction of the cover crop and tillage factors on NECB. [This loss of interaction between the two factors may again indicate that inter-row \$R_s\$ plays a significant role in NECB determination and thus, the carbon storage potential of vineyards.](#) On the other hand, interactions between both soil managements were observed on grapevine NPP and NCB.

5. Conclusion

Together, these findings indicate the potential for these vineyards to serve as a C sink, in line with previous research works. [Under sandy soils, tillage and type of cover crop had little to no effect on net ecosystem carbon balance \(NECB\). However, under the finer textured soil, tillage reduced NECB largely through a reduction in soil organic carbon and increase in soil respiration, or CO₂ efflux. The type of cover crop also impacted NECB, as cover crops that produced greater biomass increased NECB. Ultimately, soil texture and vineyard site characteristics were key determinants of the C storage potential of vineyards in Mediterranean climates like that of Fresno and Napa Counties.](#) However, further research is required to explore the impact of fossil fuel consumption on the net carbon balance at a landscape level.

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Chapter 2: Tables and Figures

Table 1 Site conditions at two commercial vineyards in Fresno and Napa Co. from experimental years (2019-2021) compared to long-term mean values (2011-2021) ^{a, b, c}.

Fresno County (Five Points)								
Year(s)	Air Temperature (°C)			Soil Temperature (°C)			Precipitation (mm)	Growing Degree Days
	Daily Max	Daily Min	Daily Average	Daily Max	Daily Min	Daily Average	Total	Total
2020								
Mean	25.6	9.1	17.3	25.7	10.7	22.6	199	2358
Annual max	35.8	18	27.3	--	--	--	--	--
Annual min	15.6	0.6	7.6	--	--	--	--	--
2021								
Mean	25.8	9.2	17.6	26.6	10.4	22.8	152.5	2488
Annual max	37.8	18.7	28.5	--	--	--	--	--
Annual min	12.5	3.1	7.7	--	--	--	--	--
2011-2021								
Mean	25.4	9.4	17.2	25.6	10.1	22.3	209.6	2259
Napa County (Oakville)								
2020								
Mean	24.4	7	14.9	22.5	10	16.5	234.2	1647
Annual max	31.8	12.3	21.1	--	--	--	--	--
Annual min	17.1	2	8.5	--	--	--	--	--
2021								
Mean	23.1	6.3	14.2	22.8	10.2	16.4	278.3	1519
Annual max	30	10.8	19.2	--	--	--	--	--
Annual min	12.5	2.6	7.6	--	--	--	--	--
2011-2021								
Mean	23.2	7.1	14.5	22.8	7.9	16	577.8	1504

a Annual maximum (max) and annual minimum (min) indicate the greatest or lowest value observed during the respective year. b Total precipitation occurred during the annual winter rainy season, calculated from October of the preceding year through September of the following year (e.g., 2020 values were calculated from October 1, 2019– September 30, 2020). c Abbreviations: --: not applicable.

Table 2. Soil organic carbon (% by mass) and total C (Mg ha⁻¹) determined at two depths (0-15 cm and 15-30 cm) and mean values (0-30 cm) in Fresno County and Napa County, respectively. Bulk density was determined in year 2 of the study.

Factors and treatment	Fresno County						Napa County					
	Average C (% mass)	<i>p</i> -value	Bulk Density (g cm ⁻³)	<i>p</i> -value	C (t ha ⁻¹)	<i>p</i> -value	Average C (% mass)	<i>p</i> -value	Bulk Density (g cm ⁻³)	<i>p</i> -value	C (t ha ⁻¹)	<i>p</i> -value
Tillage System (T)												
No Till	0.70	ns	1.4	ns	14.68	ns	1.44 a	**	1.3	ns	28.15 a	**
Till	0.69	ns	1.4	ns	14.39	ns	1.37 b		1.3	ns	26.62 b	
Cover Crop (CC)												
Annual grass	0.88	ns	1.4	ns	18.56	ns	1.47	ns	1.3	ns	28.67	ns
Resident vegetation	0.90	ns	1.4	ns	18.93	ns	1.45	ns	1.3	ns	28.33	ns
Perennial grass	0.86	ns	1.4	ns	17.99	ns	1.41	ns	1.3	ns	27.55	ns
Depth (D)												
0-15 cm	0.88 a	***	1.4	ns	18.50 a	***	1.45 a	**	1.3	ns	28.18 a	**
15-30 cm	0.50 b		1.4	ns	10.57 b		1.36 b		1.3	ns	26.59 b	
Year (Y)												
2020	0.64	ns	1.4	ns	13.42	ns	1.30	ns	1.3	ns	25.37	ns
2021	0.69	ns	1.4	ns	14.53	ns	1.40	ns	1.3	ns	27.29	ns
<i>CC x T</i>		ns		ns		ns		ns		ns		ns
<i>CC x T x D</i>		ns		ns		ns		ns		ns		ns
<i>CC x T x D x Y</i>		ns		ns		ns		ns		ns		ns

^a ANOVA to compare data (p value indicated); Letters within columns indicate significant mean separation according to Tukey's honest significance difference (HSD) test (at p = 0.05), where "**": p value < 0.05; "***": p value < 0.001, "****": p value < 0.0001. b Abbreviations: ns: not significant.

Table 3. Average soil respiration (R_s) (ton C ha⁻¹ yr⁻¹) analyzed over the two-year study across five (Fresno) and six (Napa) timepoints as measured in each cover crop and tillage treatment combination and under vine (bare soil).

Fresno County								
	Tillage System			Cover Crop				
	No Till	Till	<i>p</i> -value	Annual grass	Resident vegetation	Perennial grass	Under vine	<i>p</i> -value
2020								
3-Feb	3.45	2.12	ns	2.15	3.43	2.78	1.03	ns
25-Mar	4.27	5.13	ns	4.26	5.43	4.41	1.23	ns
16-Apr	4.34	3.60	ns	2.85	3.99	5.07	2.04	ns
17-Apr	3.80	4.63	ns	4.14	4.20	4.32	2.42	ns
12-Jun	2.10	2.11	ns	2.12	2.15	2.04	2.46	ns
Season-long Average R_s (ton C ha yr)	3.59	3.52	ns	3.10	3.84	3.72	1.84	ns
2021								
14-Feb	2.41	3.24	ns	3.42 a	2.20 b	2.86 b	1.92 c	*
24-Mar	3.35	5.35	ns	4.02 b	4.02 b	5.01 a	1.38 c	*
28-Apr	4.99	4.32	ns	5.10	4.12	4.74	1.85	ns
29-Apr	4.29	7.52	ns	6.48	5.39	5.84	3.34	ns
1-Jul	3.66	4.32	ns	4.79	3.76	3.42	6.30	ns
Season-long Average R_s (ton C ha yr)	3.74	4.95	ns	4.76	3.90	4.37	2.96	ns
<i>CC x T</i>	--	--	ns	--	--	--	--	ns
<i>Year (Y)</i>	--	--	ns	--	--	--	--	ns
<i>CC x T x Y</i>	--	--	ns	--	--	--	--	ns
Napa County								
	Tillage System			Cover Crop				
	No Till	Till	<i>p</i> -value	Annual grass	Resident vegetation	Perennial grass	Under vine	<i>p</i> -value
2020								
24-Jan	7.14 b	8.52 a	*	10.02 a	6.19 b	7.29 b	5.29 c	*
13-Apr	6.70 b	9.61 a	*	8.30	7.02	9.14	8.13	ns
21-Apr	6.78	6.90	ns	8.21	6.75	5.56	2.82	ns
23-Apr	5.52	5.43	ns	5.09	5.15	6.19	2.05	ns
22-May	0.91	1.60	ns	0.67	1.02	2.08	1.36	ns
19-Jun	5.30	4.27	ns	4.93	4.56	4.86	2.64	ns
Seasonal Average R_s (ton C ha yr)	5.39 b	6.06 a	**	6.20	5.12	5.85	3.71	ns
2021								
29-Jan	14.78 b	15.87 a	*	15.60 b	10.06 c	20.32 a	12.48 b	*
14-Apr	7.14	5.42	ns	5.89	6.19	6.76	5.29	ns
16-Apr	5.72	8.50	ns	8.74	7.14	5.45	2.40	ns
14-May	6.78	6.90	ns	8.21	6.75	5.56	2.82	ns
15-Jun	7.26	5.43	ns	5.09	7.76	6.19	2.05	ns
9-Jul	0.91	1.60	ns	0.67	1.02	2.08	1.36	ns
Seasonal Average R_s (ton C ha yr)	7.10 b	7.29 a	*	7.36	6.49	7.73	4.40	ns
<i>CC x T</i>	--	--	ns	--	--	--	--	ns
<i>Year (Y)</i>	--	--	ns	--	--	--	--	ns
<i>CC x T x Y</i>	--	--	ns	--	--	--	--	ns

^a ANOVA to compare data (p value indicated); Letters within columns indicate significant mean separation according to Tukey's honest significance difference (HSD) test (at p = 0.05), where "": p value < 0.05; "**": p value < 0.001, "***": p value < 0.0001. b Abbreviations: ns: not significant; --: not applicable.

Table 4. Components of vineyard net primary production (NPP as t/ha of dry matter), whole vine (NCB) and vineyard net ecosystem carbon balance (NECB) calculated over the two year study for six different cover crop and tillage systems. ^{a,b}

Treatment	Harvest (Mg C ha-1)	Pruning Wood (Mg C ha-1)	Leaves (Mg C ha-1)	Permanent Organs (Mg C ha-1)	Grapevine NPP (Mg C ha-1 yr-1)	Rs Under Vine (Mg C ha-1 yr-1)	Grapevine NCB (Mg C yr-1)	Cover Crop NPP (Mg C ha-1)	Rs Interrow (Mg C ha-1 yr-1)	SOC (Mg C ha-1)	NECB (Mg C ha-1 yr-1)
Fresno County											
2020											
No Till - Annual grass	1.39	1.76	3.02	11.5	17.7	1.84	15.35	1.80	2.81	17.71	23.36
No Till - Perennial grass	1.41	1.30	2.97	11.5	17.2	1.84	14.83	1.70	4.02	19.25	22.96
No Till - Resident vegetatiior	1.32	1.73	3.53	11.5	18.1	1.84	15.82	2.30	3.94	17.64	23.5
Till - Annual grass	1.25	1.75	3.22	11.5	17.7	1.84	15.54	3.93	3.39	19.81	25.31
Till - Perennial grass	1.48	1.56	3.27	11.5	17.8	1.84	15.39	1.83	3.42	18.97	23.74
Till - Resident vegetation	1.65	2.17	2.93	11.5	18.3	1.84	15.66	1.17	3.74	17.64	22.89
2021											
No Till - Annual grass	1.24	1.61	2.98	11.5	17.3	2.96	14.56	2.00	3.71	17.71	22.24
No Till - Perennial grass	1.63	1.25	2.92	11.5	17.3	2.96	14.15	2.15	3.69	19.32	22.69
No Till - Resident vegetatiior	1.80	1.53	3.48	11.5	18.3	2.96	14.99	1.83	3.81	17.71	22.54
Till - Annual grass	1.11	1.75	3.18	11.5	17.6	2.96	14.91	4.08	5.81	19.88	23.63
Till - Perennial grass	1.21	1.50	3.09	11.5	17.3	2.96	14.58	2.07	5.06	18.97	22.25
Till - Resident vegetation	1.34	2.01	2.88	11.5	17.8	2.96	14.88	1.33	3.98	17.36	21.94
<i>Cover crop (CC)</i>	ns	*	*		ns		ns	**	ns	ns	ns
<i>Tillage (T)</i>	ns	ns	ns		ns		ns	ns	ns	ns	ns
<i>CC x T</i>	ns	ns	ns		ns		ns	ns	ns	ns	ns
<i>Year</i>	ns	ns	ns	--	ns	--	*	*	ns	ns	ns
<i>Year x CC</i>	ns	ns	ns		ns		ns	ns	ns	ns	ns
<i>Year x T</i>	ns	ns	ns		ns		ns	ns	ns	ns	ns
<i>Year x CC x T</i>	ns	ns	ns		ns		ns	ns	ns	ns	ns
Napa County (Oakville)											
2020											
No Till - Annual grass	0.15	0.37	0.57	6.7	7.8	3.71	5.71	2.92	5.56	29.25	18.49
No Till - Perennial grass	0.17	0.21	0.49	6.7	7.6	3.71	5.47	0.96	5.89	28.28	16.68
No Till - Resident vegetatiior	0.14	0.43	0.44	6.7	7.7	3.71	5.63	2.59	4.73	28.76	18.41
Till - Annual grass	0.16	0.51	0.55	6.7	7.9	3.71	5.83	2.96	6.85	29.25	18.00
Till - Perennial grass	0.16	0.32	0.64	6.7	7.8	3.71	5.72	1.19	5.82	27.30	16.61
Till - Resident vegetation	0.16	0.77	0.74	6.7	8.4	3.71	6.29	3.20	5.50	27.30	18.29
2021											
No Till - Annual grass	0.44	0.56	0.61	7.0	8.6	4.40	5.88	3.20	7.25	28.76	17.74
No Till - Perennial grass	0.33	0.39	0.53	7.0	8.3	4.40	5.63	1.62	7.70	27.79	16.05
No Till - Resident vegetatiior	0.26	0.62	0.47	7.0	8.3	4.40	5.80	3.60	6.68	30.23	18.83
Till - Annual grass	0.37	0.68	0.58	7.0	8.6	4.40	5.97	3.32	7.48	27.30	17.08
Till - Perennial grass	0.38	0.48	0.67	7.0	8.5	4.40	5.86	1.47	7.75	26.81	15.72
Till - Resident vegetation	0.46	0.96	0.77	7.0	9.2	4.40	6.45	3.46	5.64	26.81	18.27
<i>Cover crop (CC)</i>	ns	ns	ns		*		**	**	ns	ns	***
<i>Tillage (T)</i>	ns	**	*		***		***	ns	**	**	*
<i>CC x T</i>	ns	ns	ns		*		*	ns	ns	ns	ns
<i>Year</i>	***	**	ns	--	***	--	ns	ns	ns	ns	*
<i>Year x CC</i>	ns	ns	ns		ns		ns	ns	ns	ns	ns
<i>Year x T</i>	ns	ns	ns		ns		ns	ns	ns	ns	ns
<i>Year x CC x T</i>	ns	ns	ns		ns		ns	ns	ns	ns	ns

^a ANOVA to compare data (p value indicated); Letters within columns indicate significant mean separation according to Tukey's honest significance difference (HSD) test (at p = 0.05), where "**": p value < 0.05; "***": p value < 0.001, "****": p value < 0.0001. ^b Abbreviations: ns: not significant; --: not applicable.

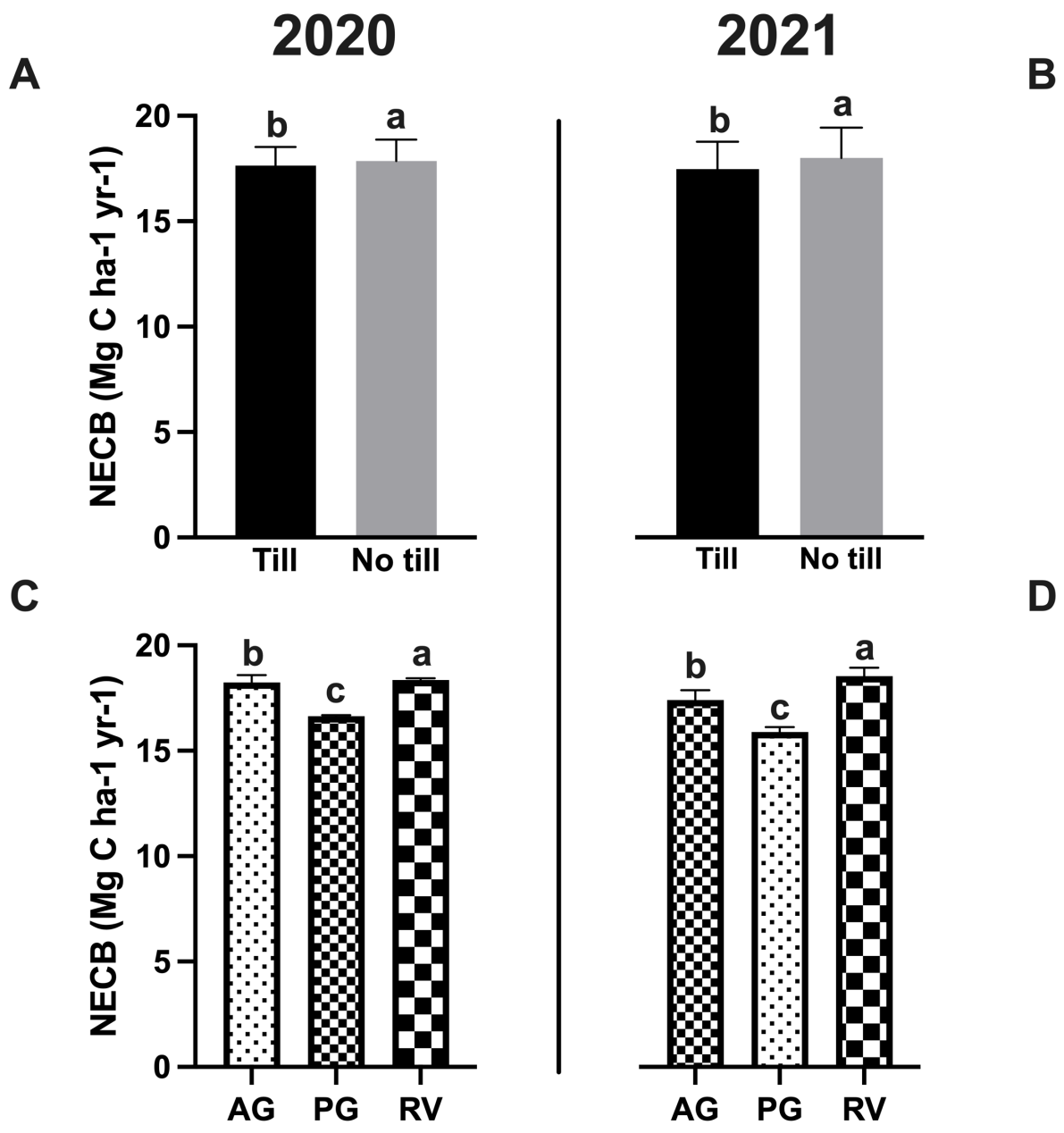


Figure 1. (A, B) Net ecosystem carbon balance (NECB) as affected by tillage system at the Oakville site. (C, D) NECB as affected by cover crop treatment. Values represent means \pm SE. Different letters indicate significant differences ($p \leq 0.05$) between respective treatments according to two-way ANOVA followed by Tukey's honest significance (HSD) test.

Summary:

Intensive agricultural practices over the last century have resulted in severe soil degradation and losses of soil fertility and biodiversity (Lal, 2012b). In response, alternative farming practices, and larger movements, have developed to bring attention to conservation of soils, bolster SOC stocks, and harness soil ecosystem services. The use of cover crops and reduction in soil tillage are two common sustainable soil management practices often utilized to increase SOM and thus enhance soil infiltration, water holding capacity, reduce soil erosion. These practices have also been targeted as soil carbon sequestration strategies to mitigate the effects of climate change. However, the ambitious goals these practices seek to accomplish in regard to long-term carbon storage in agricultural settings may fail to consider critical components of a functioning agroecosystem, such as effects on the target crop itself (including yield) and trickle-down effects on soil carbon dynamics. Thus, the present trial was conducted to investigate the impact of various cover crops under different tillage regimes on grapevine physiology, production, and the net carbon storage potential of the agroecosystem in two commercial production regions.

Cover cropping and reduced soil tillage directly alter the growing environment of grapevines. Thus, the selection of appropriate vineyard floor management practices may be critical in order to maximize benefits to the soil while minimizing the impact on grapevine functioning and production. This selection involves decisions in space (cover crop in vineyard rows vs. under-vine), type (grasses vs. broadleaves; monoculture or species mixture), and time (perennial vs. annual species; and timing and termination (*i.e.* tillage)) (Bowles et al., 2017b; Gatti et al., 2022b). Factors such as cultivar, vineyard age, macroclimate, soil physiochemical characteristics, and the overall motivation for the use of the selected cover crop and tillage system must also be considered as these elements have been shown to contribute to the effect of the practices on grapevine

functioning and production (Ingels et al., 2005; Sweet and Schreiner, 2010; Steenwerth et al., 2013; Abad et al., 2021).

While the benefits of cover crops and reduced tillage on soil physical properties are well studied, the influence of these practices on grapevine physiology and production is less clear (Ingels et al., 2005; Steenwerth and Belina, 2008b; Abad et al., 2021). Although there is agreement in literature that cover crops may reduce vegetative growth, with more pronounced yield losses in warmer regions, some studies have contrastingly reported no effect (Monteiro and Lopes, 2007; Lopes et al., 2008a; Smith et al., 2008; Costello, 2010; Jordan et al., 2016; Steenwerth et al., 2016). Grapevine physiological responses to cover cropping have also been documented, and the presence of a cover crop is generally reported to detrimentally affect grapevine water status (Naor et al., 1997; Monteiro and Lopes, 2007; Hatch et al., 2011a; Pou et al., 2011; Steenwerth et al., 2016; Tomaz et al., 2021). Furthermore, while yield reductions and/or no effect on yield are the most common results from such work, cover crop species appears to be a more influential factor than merely the presence (or absence) of a cover crop. In fact, yield increases have even been reported in some vineyards planted with annual species such as oats or legumes (Fourie and Freitag, 2010; Ovalle et al., 2010; Steenwerth et al., 2013, 2016; Messiga et al., 2016; Fourie et al., 2017).

Results from the present trial indicated that whole grapevine physiology was not affected by the presence of a cover crop or tillage system at either experimental vineyard. Overall, little to no effect of cover crop type or tillage system on grapevine physiology or yield were observed at the Fresno County vineyard. However, minimal effects were reported at the Napa County (Oakville) site. While it was hypothesized that the perennial grass cover crop would preserve plant available water early in the season due to spatio-temporal compatibility in vineyard systems, this effect was not consistently seen. However, grapevines under conventional tillage displayed improved grapevine water status (*i.e.*, more positive Ψ_s) compared to grapevines under no-till management. While this finding is contradictory to some reports that indicated tillage did not affect

grapevine water status, this result provided further evidence of tillage in semi-arid regions to preserve water in the soil through early season cultivation. This finding may also indicate that the presence of vegetation in the early spring under no-till increased competition for water. However, differences observed among plant water status throughout the two experimental seasons did not ultimately affect water yield or yield components. Combined, these results provide evidence that both in mature and young vineyards cover cropping imparts negligible beneficial effects on grapevine physiology, mineral nutrition or production; while tillage may be beneficial in young vineyards to improve plant water status in semi-arid regions.

While the open alleyways and permanent organs of vineyards present a considerable opportunity for carbon sequestration in agricultural settings, the ability of cover crops and reduced tillage to increase SOC stocks long-term may be overestimated (Novara et al., 2018). While some authors have recorded an increase in a soil organic carbon (SOC) sequestration rate due to cover crop adoption (Steenwerth and Belina, 2008a; Peregrina et al., 2014), others have recorded the contrary (Celette, 2007; Novara et al., 2019; Jian et al., 2020). The second component of the present trial investigated the impact of cover crops and no-till management on net ecosystem carbon balance and the potential for vineyard systems to serve as carbon sinks.

Net ecosystem carbon balance was chosen as the most appropriate method for evaluation of C storage potential as this technique incorporates C influxes and outfluxes of the vineyard, including both the grapevine and vineyard floor. One key component of C balances in vineyards is losses of CO₂ via soil respiration. Soil CO₂ efflux results from the combination of biological and physical processes, both of which are sensitive to edaphic factors and highly variable in space and time. In soils of some climates, CO₂ efflux may be great enough to potentially offset short term storage of C from cover crop adoption (Yu et al., 2019). Furthermore, some studies have shown that cover crops enhance microbial activity, thus increasing CO₂ efflux (Steenwerth et al., 2010; Maier et al., 2011; Freidenreich et al., 2021). In fact, CO₂ emissions may be further increased under some types

of cover crops due to the lower C:N ratios of certain plant material, such as legumes (Alluvione et al., 2010; Freidenreich et al., 2021). While recent work has shown that SOC increases as tillage frequency is reduced, few studies have combined various cover crops with reduced or no-till management in California vineyards (Conant et al., 2007; Steenwerth and Belina, 2008a; Wolff et al., 2018b). Furthermore, there is a lack of information regarding the potential of C storage in vineyard systems under combinations of soil management practices, considering C losses at both the grapevine and vineyard scale. This two-year study (2019-2021) was conducted with the objectives to i) quantify carbon inputs and losses at the vineyard scale to determine net ecosystem carbon balance (NECB); ii) compare these values among different vineyard floor management systems in two California vineyards over two arid seasons.

Findings suggested that while vineyards of the investigated climates are indeed C sinks, in line with previous vineyard C budget research. However, further research is required to explore the impact of fossil fuel consumption on the net carbon balance at a landscape level. Under sandy soils like that at the Fresno County site, tillage and type of cover crop had little to no effect on net ecosystem carbon balance (NECB). However, under the finer textured soil at the Napa County site, tillage reduced NECB. The primary component of the vineyard that resulted in a reduction of NECB was identified as losses of soil organic carbon and increased CO₂ efflux under conventional tillage compared to no-till management. Furthermore, grapevines under conventional tillage also contributed higher C input through annual production (leaves and canes) compared to vines under no-till. The type of cover crop also impacted NECB, as the cover crops that produced greater biomass (Resident vegetation and annual grass) increased NECB. In conclusion, site characteristics (soil texture) and cover crop biomass were key determinants of the C storage potential of vineyards in Mediterranean climates like that of Fresno and Napa Counties. Taken together, results from both components of this work indicate that vineyard age and soil texture modulate the effect of cover crops and tillage systems observed on whole grapevine physiology

and net ecosystem carbon balance. These findings reveal that the general considerations of cover cropping, and reduced tillage as sustainable vineyard floor management practices may overlook