VINEYARD FLOOR MANAGEMENT IN THE FINGER LAKES REGION: PHYSIOLOGICAL AND MICROBIAL PERSPECTIVES

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ABSTRACT

VINEYARD FLOOR MANAGEMENT IN THE FINGER LAKES REGION: PHYSIOLOGICAL AND MICROBIAL PERSPECTIVES

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Excessive vine vegetative growth in wet, cool climates increases management costs and compromises grape quality. However, the standard practice of bare soil under vines exacerbates the vigor problem. Previous studies found that using under-vine cover crops reduced vine vigor in young vineyards but had little to no impact on mature vines. Wine sensory properties were impacted by under-vine cover crops although the cause was not clear. A study conducted in a mature Cabernet franc (*Vitis vinifera* L.) vineyard in the Finger Lakes region showed that chicory was the most effective cover crop to consistently reduce pruning weight and canopy leaf layer number without reducing yield compared to glyphosate maintained bare soil, whereas other under-vine cover crop treatments were not as consistent. In a three year study conducted in a mature Riesling (*Vitis vinifera* L.) vineyard, under-vine natural vegetation reduced vine canopy leaf layers and occlusion layers in one of the years compared to glyphosate maintained bare soil but there were no detectable sensory differences among wines from different under-vine floor treatments in any year. Profiling of soil microbiome using high-throughput sequencing showed that microbial community of natural vegetation diverged from the cultivation and glyphosate maintained treatments. However, no corresponding change in fungal community structure was observed on grapes or in simulated spontaneous fermentations. Undiscernible wine sensory properties also confirmed the lack of treatment effects in wines. Although under-vine cover crops

impact on vine vegetative growth varied, no reduction in yield suggested that under-vine cover crops could serve as beneficial alternatives to bare soil for sustainable vineyard management. Further studies on how under-vine cover crops impact wine sensory properties are required to evaluate their practical adoptability.

BIOGRAPHICAL SKETCH

Ming-Yi Chou was born in Taipei, Taiwan. He graduated from High School of National Taiwan Normal University in 2006 and received his Bachelor of Science in Horticulture from National Taiwan University in 2010. Starting in the junior year of his undergraduate study, he was dedicated to table grape research under Dr. Kou-Tan Li's guidance in Miaoli, Taiwan, where he fell in love with viticulture and decided to pursue doctoral study in the field.

After the one year of mandatory military service, he pursued a Master of Science in Wine Business at Ecole Supérieure de Commerce de Dijon (Burgundy School of Business) in Burgundy, France, where he honed his wine appreciation skills. In 2013, he started working at L'Atelier De Joël Robuchon in Taipei while finishing his master dissertation, until he was offered admission to Cornell University for his Ph.D. study in Dr. Justine Vanden Heuvel's lab. During his time at Cornell University, he was invited to co-establish the CUVEE wine blind tasting and educational society. Academically, he was also the recipient of the American Society of Enology and Viticulture Eastern Section Scholarship and Professor Robert M. Smock Scholarship.

Following the completion of his Ph.D., he plans to work in fine wine (grape) production in the U.S. and aims to become a professional vineyard and winery consultant.

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CHAPTER 1

Literature review

Introduction

Excessive vegetative growth of the grapevine, *Vitis vinifera* L.*,* is a primary viticultural challenge in Finger Lakes. If incorrectly managed, excessive vegetative growth can incur high vineyard management costs and compromised fruit quality. A high source-sink ratio promotes growth in woody plants (Kozlowski, 1992). In cool climate, the factors limiting source supply are minimized due to ideal growing season temperatures, combined with an abundance of precipitation and fertile soil with 3-5% organic matter content (Wolf, 2008). The high supply of source and the indeterminate shoot growth habit of grapevines (as a strong sink) result in excessive vegetative growth. Furthermore, many vineyard management practices inadvertently promote further vine vegetative growth, such as inappropriate planting densities, rootstocks, trellis systems, and floor management. Among these practices, floor management is readily adjustable, especially in a well-established and mature vineyard. In the Finger Lakes, maintaining a bare soil strip directly under the vine with a mixed vegetation inter-row is the most common vineyard floor management practice (Wolf 2008). However, this floor management scheme further promotes vine vigor, as maintaining a bare soil strip denies resource competition from under-vine vegetation (Wheeler et al., 2005). As a result, the winter pruning weight of mature *Vitis vinifera* vines, vertically trained and maintained for wine grape production, often exceeds one kilogram per vine in vineyards with 1.8m in-row spacing in many cool climate regions, even when hedging is commonly performed throughout the growing season.

Excessive vegetative growth can result in high canopy density and fruit shading. It has been found that high canopy density favors disease development and increases the difficulty of disease management due to reduced sunlight exposure, airflow, and pesticide penetration in the fruiting zone (Austin et al., 2011). In addition, berry shading has been found to negatively impact grape and must composition influencing soluble solids, pH, tartrate/malic acid ratio, polyphenol and anthocyanin concentration (Smart, 1985), and accumulation of highly organoleptic compounds, such as methoxypyrazines (Scheiner et al., 2012) and rotundone (Geffroy et al., 2014). Wine sensory properties can also be impacted by cluster shading. Traminette wines made from exposed grapes were described as more fruity, floral, and spicy than the wines made from shaded grapes (Skinkis et al., 2010). Wines made from shaded Shiraz grapes had less fruit flavors on the palate compared with wines made from grapes that received higher sunlight exposure (Ristic et al., 2007). Managing vines with an adequate vegetative and reproductive balance is crucial for sustainable and high quality grape and wine production (Howell, 2001, Kliewer and Dokoozlian, 2005), and excessive vine vigor is an issue that needs to be addressed in overly vigorous vineyards such as vineyards in Finger Lakes.

A bare soil strip is often achieved by applying herbicide or with soil cultivation. Glyphosate, the most widely used herbicide in the vineyards of the Northeastern U.S. (Wolf, 2008, Yeh et al., 2014), selectively disrupts plant enolpyruvylshikimate phosphate synthase when in contact with plant tissue. It is considered to be an herbicide with relatively low environmental risk (Duke and Powles, 2008) due to its high microbial degradability, since glyphosate per se is a carbon and nitrogen metabolic substrate for microorganisms (especially fungi) when it adsorbs to soil (Sprankle et al., 1975). However, it has become a great concern that intensive application of glyphosate accelerates the evolution of glyphosate resistant weeds (Yamada et al., 2009). There

are 293 plant species that have been identified as glyphosate resistant worldwide (Heap, 2018). Compared to herbicide use, soil cultivation is more time and energy demanding, but is favored by organic growers as it enables weed management without the application of synthetic herbicides. However, soil cultivation physically breaks down soil aggregates and promote losses in the mineral nutrient pool (Paustian et al., 1997, Elliott, 1986). Aside from promoting vine vigor, maintaining bare soil in the vineyard was also found to enhance soil erosion and runoff (Battany and Grismer, 2000, Napoli et al., 2017), resulting in pesticide and nutrient leaching into the waterway (Karl et al., 2016b), and potential reduction of long-term soil health (Blanco-Canqui et al., 2011, Peregrina et al., 2010).

A recent study revealed that grapevine aerial organs shared high amount of bacterial OTUs with soil, and theorized that microbes present on grape berries may have originated from the soil (Zarraonaindia et al., 2015). Hence, vineyard floor management practices should not be examined solely for their impact on soil health and vine physiological parameters. One study on under-vine cover crops found that although vine growth and grape harvest parameters were not impacted by under-vine cover crops, wine sensory properties were (Jordan et al., 2016). This raises suspicions about the effects of under-vine cover crops on the wine sensory properties through alteration of the vineyard microbiome. Thus, further study is warranted on the use of aggressive under-vine cover crops to mitigate vine vigor in cool climate mature vineyards and their effect on wine sensory properties through vine physiological and vineyard microbial routes.

Grapevine and wine sensory properties respond to under-vine floor management practices

Cover crop establishment is not always recommended, as resource competition can substantially reduce vine growth and yield in hot and arid regions (Medrano et al., 2015, Tesic et al., 2007). The efficacy of cover crops in different climatic regions varies; complete vineyard floor vegetation compared with maintaining bare soil was shown to drastically reduce Chardonnay vine yields in an arid climate, but only moderately reduce yield in a climate with higher rainfall (Tesic et al., 2007). That study showed reduced shoot growth and canopy density correlated with a decrease in petiole nutrient and soil water content, but not soil nutrient content. This indicates that reduced soil water content suppressed soil nutrient mineralization and led to decreased vine vegetative growth and yield capacity (Tesic et al., 2007). Another study showed that cover crops competed for soil nitrogen with grapevines, after cover crop establishment (Pérez-Álvarez et al., 2015). This resulted in reduced vine petiole nitrogen content in the third year, and reduced berry YAN in the fourth year. These sequential results indicated direct nutrient competition between cover crops and grapevines. However, mitigation of excessive vine vigor due to resource competition from under-vine ground vegetation could be beneficial to growers in wet and cool climate regions.

In cool climates, it was found that chicory growing under-vine did not impact vine yield, but reduced young vine vegetative growth, including shoot growth rate, leaf size, leaf layers, and pruning weight in a one year study (Wheeler et al., 2005). Consequently, berry ripeness improved, including increased soluble solids and reduced TA. In the same study, sensory evaluation of the wines made from vines with under-vine chicory crops had higher rated attributes for appearance, aroma, palate, and higher overall scores than wines from vines with soil cultivation treatment. Another study conducted in the humid climate of southern Uruguay

showed that under-vine tall fescue reduced pruning weight, yield, canopy density, and berry size of young Tannat vines, while simultaneously increasing sunlight penetration in the canopy and berry soluble solids compared to vines with bare soil maintenance with herbicide treatment (Coniberti et al., 2018). Wines made from Tannat vines with under-vine cover crop treatment had a greater anthocyanin concentration than those from vines with herbicide treatment. In the cool climate Finger Lakes, it was also shown that under-vine native vegetation and white clover can reduce pruning weight, canopy leaf layers, and yield in young Cabernet Franc vines while maintaining the same Ravaz index and juice soluble solids, pH, and TA (Karl et al., 2016a). However, wines from this study and their aromatic properties were indistinguishable to a sensory panel. In a study performed in North Carolina, where the climate is warm and humid, under-trellis KY-31 tall fescue, Aurora Gold fescue, perennial ryegrass, orchardgrass, and Elite II tall fescue cover crops were found to effectively reduce pruning weights and the percentage of shaded clusters without impacting yield in a young (6 years old at onset of the experiment) Cabernet Sauvignon vineyard; these effects were likely due to nutrient competition as vine water potential was minimally impacted (Giese et al., 2014).

Another study completed in warm and humid Virginia showed that an under-trellis cover crop mix including creeping red fescue, tall fescue, and orchard grass limited lateral shoot leaf growth, reduced pruning weight by 47%, and enhanced canopy light environment by reducing canopy density mainly by increasing water competition in a three year old Cabernet Sauvignon vineyard (Hatch et al., 2011). Similar results were observed in the continued study of the same vineyard, where under-vine perennial creeping red fescue reduced pruning weight by an average of 26% and improved cluster light exposure by an average of 35% compared with bare soil maintained with herbicide over seven years of assessment (Hickey et al., 2016). However, it was

also concluded that nutrient competition, especially for nitrogen, contributed more to the reduced vegetative growth compared to water competition as a function of under-vine cover crops. The results from these studies indicate increased water competition resilience in mature grapevines.

In a mature Cabernet Franc vineyard (9 years old when the experiment was started) in the Finger Lakes, under-vine buckwheat and rosette forming turnips had no impact on yield, pruning weight, or Ravaz index. It was hypothesized that vines shed fine roots in areas of high competition, but maintained fine roots longer in the low competition areas to better explore soil for water and nutrients (Centinari et al., 2016). In the same study, under-vine annual ryegrass reduced pruning weight by 34% in the third year, showing the ability of under-vine cover crops to mitigate mature vine vigor. In the same region, residential vegetation, annual ryegrasss, and buckwheat were found to have no impact on 16 years old Riesling vine growth, as canopy structure, pruning weight, yield, and berry harvest parameters were unaffected in all three years of the study (Jordan et al., 2016). However, wines made from the different treatments had sensory differences in the second year of the study, and the wine from vines with herbicide treatment differed from the others in the third year. These results indicated better resource competition resilience in mature vines than that of young vines. It has been proposed that the increased resilience of mature vines faced with variations in morphology, water, and nutrient status in mature vines are likely due to larger root systems and permanent structures with higher nutrient reserves (Zufferey and Maigre, 2007, Grigg et al., 2018, Holzapfel et al., 2010). The wine sensory results from Jordan et al. (2016) also indicated that under-vine cover crops affect wine sensory properties through non-physiological routes.

Floor management practices impact soil conditions

Bare soil in the vineyard intensifies soil erosion and runoff. There is a significant negative correlation between soil erosion and runoff and vineyard ground cover; soil loss was reduced with increased ground coverage (Battany and Grismer, 2000). An eight year study in a Mediterranean vineyard found an overall 68.5% reduction in eroded soil and significantly lower levels of nitrogen and phosphorous loss in a vineyard with floor vegetation compared to a vineyard with soil cultivation (Napoli et al., 2017). Compared to herbicide treatment, cultivation maintained greater weed biomass which contributed to labile carbon in the soil, and had higher nitrogen retention (Steenwerth and Belina, 2010). However, soil cultivation mechanically breaks down soil aggregates, which reduced macroaggregate stabilization and resulted in more microaggregates, in which the carbon and nitrogen were less labile (Elliott, 1986). The destabilization of aggregates increases organic content availability for utilization by microorganisms and results in lower soil organic matter in the long term (Snyder and Vázquez, 2005, Six et al., 2002). Compared to cover crops, many studies have shown that cultivation and tillage decrease organic matter content, microbial activity, and soil aggregation, while increasing subsoil bulk density and soil erosion (Zehetner et al 2015, Steenwerth and Belina 2008b, Six et al 1999). A study showed that 15 years of perennial cover crop rotation reduced soil density by 4%, while increasing top soil aggregate size by 80% and organic matter content up to 30% compared to cultivated bare soil (Blanco-Canqui et al., 2011). In a California vineyard, cover crop Trios 102 and Merceds Rye enhanced soil carbon mineralization, microbial respiration, and microbial biomass compared with cultivated soil (Steenwerth and Belina, 2008b). It was also found in cool climates that maintaining under-vine native vegetation or white clover enhanced soil microbial respiration rate and reduced dissolvable organic carbon in soil leachate in all four years of the

study. Additionally, soil organic matter content increased in the fourth year compared with cultivated soil (Karl et al., 2016a).

Soil nitrogen content was reduced in a Spanish vineyard with cover crops in comparison with tilled bare soil (Pérez-Álvarez et al., 2015). Another study also showed that non-permanent, inter-row cover crops reduced surface soil nitrogen in a Mediterranean vineyard (Celette et al., 2009). Aside from soil nutrient content, many studies found that vineyard cover crops increased vineyard water use and reduced soil water content. Vineyard permanent residential vegetation and sowed cover crop mix increased water use before bloom in a Mediterranean climate vineyard (Monteiro and Lopes, 2007). Increased floor vegetation coverage led to reduced soil volumetric water content in a hot and arid climatic condition (Tesic et al., 2007). It was also demonstrated that permanent vineyard cover crops dried out the top soil zone and causing the vine to extend its root system to explore water in the deeper soil zone (Celette et al., 2008). In wet climate vineyards, soil volumetric water content was found to be reduced by under-vine chicory, white clover, and native weeds in comparison with bare soil maintained by herbicide or soil cultivation (Wheeler et al., 2005, Karl et al., 2016a). Reduced soil water could also relate to reduced soil nutrient mineralization which leads to reduced vine growth. One study observed that cover crops reduced vine yield, pruning weight, and petiole nutrients including nitrogen and magnesium (Tesic et al., 2007). In that study, reduced soil moisture, combined with an unchanged soil nutrient concentration indicates that the reduced vine nutrient uptake was due to lower soil nutrient mineralization under reduced soil moisture. However, a study done in a California vineyard showed that microbial biomass, nitrogen, and soil nitrogen mineralization were increased using a cover crop of Trios 102 and Merceds Rye without impacting soil moisture, despite the fact that total nitrogen in the dry soil was lower compared to cultivated soil

(Steenwerth and Belina, 2008a). These previous findings indicate that cover crops could potentially increase the easily mineralizable nitrogen pool in the soil, which is beneficial to grapevine nitrogen uptake. The findings also suggested that cover crop effects on soil water and nutrient status are likely weather dependent.

Vineyard management practices and vineyard microbiome

The impact of the vineyard microbiome and its potential to generate wines with a regional typicity is referred to as microbial terroir (Gilbert et al., 2014). The potential of endophytes to regulate plant metabolism and produce volatile compounds that could impact grape and wine aromatic profiles has been suggested (Abrahão et al., 2013, Yang et al., 2016). For example, the endophytic pathogen *Botrytis cinerea* was found to impact the synthesis of a wide variety of berry secondary metabolites, including many aroma precursors such as fatty acids, amino acids, lipids and polyols, and aromatic compounds such as benzoic acid (Agudelo-Romero et al., 2015). There have been many endophytic bacteria and fungi identified in grapes (Compant et al., 2011, González and Tello, 2011), including yeast genera that can negatively impact wine fermentation and wine organoleptic properties. *Acremonium*, *Aspergillus,* and *Penicillium* were a few of the pernicious genera identified.

Recent studies have shown that the climatic conditions, vintage, and grape varieties were crucial factors that shaped grape must microbiome and microbial biogeography (Bokulich et al., 2014). One study showed that use of selected regional *Saccharomyces* yeast genotypes in wine fermentation affected the resulting wine chemical composition (Knight et al., 2015). A study showed that vineyard sites had differentiated grape microbiomes, which correlated with the wine

metabolome, and suggested that selected microbiota in grapes could be used as wine metabolite abundance predictors (Bokulich et al., 2016). These studies indicated the importance of the grape and vineyard microbiome and their possible contribution to wine phenotypes, but the mechanism of how microbiome variance contributes to wine chemistry has yet investigated. The geographical pattern of grape associated microbiomes could also be linked to the soil microbiome. The grapevine aerial organs shared a considerate amount of bacterial OTUs with soil which indicated the possibility of grape microbiome's soil origination (Zarraonaindia et al., 2015). The results of that study further emphasized the importance of investigating vineyard soil microbiome management and its link to the grape and wine fermentation microbiomes.

Glyphosate is readily biodegradable when bonding to soil (Sprankle et al., 1975), and thus is considered to have a low environmental risk. A long term study on repeated glyphosate application found that the culturable bacterial population was not impacted, but fungal population increased (Araújo et al., 2003). However, repeated application of glyphosate for four years was found to reduce organic carbon content by 46%, nitrogen by 15%, and acid phosphate activity by 64% in Haplorthod soil (Pe´rie´ and Munson, 2000) in a cool climate. These soil properties could be unsupportive to microbial population, and hence negatively impact soil microbial health. It was concluded that glyphosate application had few direct impacts on soil microbial activity, biomass, and structure, but had indirect impacts on the microbial population such as reducing soil vegetation and organic matters were more prominent (Rose et al., 2016). Compared with glyphosate application, soil cultivation seemed to have a more consistent negative impact on soil microbiota according to previous studies. Cultivated soil was found to have lower carbon, nitrogen, and microbial biomass compared with vegetated soil in a study of 42 coastal land sites in California (Steenwerth et al., 2002). In a California vineyard, higher

microbial biomass was found in soil with cover crop treatments, including native grass and clover, in comparison with tilled soil (Ingels et al., 2005). Compared to bare soil maintained with manual cultivation and glyphosate application, microbial activity as measured by microbial respiration was higher in an under-vine native vegetation treatment in three out of four years, and in a white clover treatment in two out of four years (Karl et al., 2016b).

Aside from floor management, other vineyard management practices were also studied. Grape epiphytic bacterial cell density was negatively correlated with the copper from phytosanitary spray (Martins et al., 2012). Similarly, epiphytic yeasts and yeast-like fungus showed the same response to copper originating from pesticide use (Martins et al., 2014). In these studies, culturable bacterial and fungal community structure in organic vineyards were found to be different from that of a conventional vineyard (Martins et al., 2014, Martins et al., 2012). Fungal phospholipid fatty acids markers are negatively associated with soil copper, which indicated the impact of copper pesticides on vineyard soil microbiome properties (Zehetner et al., 2015). In a study of different vineyard farming systems, dominant non-*Saccharomyces* yeasts in the must differed if the vineyard management practices were conventional, biodynamic, or integrated pest management, but diverged non-*Saccharomyces* yeasts did not affect the growth of *Saccharomyces* yeast during spontaneous fermentation (Bagheri et al., 2015). However, using a culture dependent method (Martins et al., 2012, Martins et al., 2014, Bagheri et al., 2015) without proper field replications (Bagheri et al., 2015) greatly limited the scope of microbial populations studied and possibilities of conducting statistical analysis. A more recent study adopted next generation sequencing and found that soil, and grape associated fungal community of conventionally managed vineyards differed from that of biodynamically managed vineyards (Morrison-Whittle et al., 2017). In that study, vineyard management approaches were studied

while it was unclear which management practices impacted aspects of the fungal community, and to what degree.

Conclusion

Excessive vine vigor in cool climate regions is a major challenge for growers. Using under-vine cover crops to mitigate vigor in young vines is effective, but the efficacy on mature vine is unclear. Also, the use of under-vine floor management practices was found to impact wine sensory properties without influencing vine physiological parameters. This indicates that under-vine floor management may affect wine sensory properties through alteration of the vineyard microbiome.

This study evaluated the effects of aggressive under-vine cover crops in mitigation of high vine vigor in a mature vineyard of the Finger Lakes region. In addition, under-vine floor management effects on wine sensory properties through vine physiological and microbial routes were examined. The objective of this study is to assess how under-vine floor management practices impact vine growth, yield, and wine sensory properties for practical use in cool climate mature vineyards in order to achieve sustainable vineyard management.

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CHAPTER TWO

Under-vine cover crops mitigated vine vigor in a mature and vigorous Cabernet franc vineyard

Introduction

Maintaining bare soil under-vine by applying herbicide is the most common vineyard under-vine floor management practice around the world. While vineyard ground cover provides water and nutrient competition (Wheeler et al., 2005, Lopes et al., 2008, Celette et al., 2009), bare soil maintained under-vine enables higher water and nutrient availability and promotes vine vegetative growth. In the Finger Lakes region of New York State, frequent growing season precipitation and high soil organic matter combine to result in excessive vine vigor, which is one of the main viticultural challenges.

Excessive vine vegetative growth can lead to high canopy management costs (Smart and Robinson, 1991), reduced fruit sunlight exposure and increased disease incidence (Austin et al., 2011, Valdés-Gómez et al., 2008), which compromise fruit quality (Smart, 1985). Bare soil maintained with either herbicide or soil tillage risks degradation of soil health such as soil erosion, breakdown of soil aggregates, depletion of organic matter and deterioration of the microbial environment (Blanco-Canqui et al., 2011, Napoli et al., 2017, Peregrina et al., 2010). Moreover, vineyard soil without groundcover results in pesticide and nutrient leaching which contaminates groundwater (Karl et al., 2016b).

Many studies have investigated the effect of under-vine cover crops in young vineyards and found some cover crop species were able to reduce vine vegetative growth such as pruning weight, leaf layers and shoot length (Hatch et al., 2011, Giese et al., 2014, Karl et al., 2016a), but

studies completed in mature vineyards are lacking. One study done in a mature vineyard in cool climate Finger Lakes found that annual ryegrass inconsistently reduced vine pruning weight with yield being affected but the grape composition was not examined (Centinari et al., 2016). Another study done in the same region showed that vine growth, yield, grape composition were not impacted by annual ryegrass, buckwheat and resident vegetation growing under-vine in three years on one site (Jordan et al., 2016) but chicory reduced shoot growth, pruning weight and yield in the second year of establishment on the other site (Jordan, 2014). The inconsistent results implied the uncertainty of using under-vine cover crops in mature vineyards. Thus, a study on aggressive under-vine cover crops and their effects on vine growth, yield and berry composition was needed.

This study aimed to employ aggressive under-vine cover crops, including chicory, fescue, tillage radish, alfalfa and natural vegetation, to determine if vigorous resource competition coming from the under-vine cover crops would consistently reduce vine growth, and also to evaluate how yield and berry composition would be impacted by under-vine cover crops in a mature vineyard in the cool climate region of the Finger Lakes. It was hypothesized that under-vine cover crops would reduce vine vegetative growth through reduce vine water potential and nutritional status. The objective of this study was to investigate under-vine cover crops in the vineyard to mitigate vine vigor and improve soil health to facilitate sustainable vineyard operation.

Material and methods

Experimental setup

This study was conducted from 2014 to 2016 at a commercial vineyard in Ovid, NY

(42.66˚N, -76.71˚W). The soil type was Howard gravely loam with less than 5% slope (Soil Survey Staff 1975).

The climate in 2016 was the warmest during the three years of the experiment with total 1648 GDD followed by 2015 (1586 GDD) and 2014 (1431 GDD) based on 10˚C (Table 2.1). Although the sum of the precipitation was higher in 2016, the early growing season from late May to early August was the driest compared to 2014 and 2015. The early season precipitation, June and July, of 2014 and 2015 was 127% and 93% more than that of 2016. In 2016, many sites in the Finger Lakes were listed at level three drought according to U.S. Drought Monitor (http://droughtmonitor.unl.edu/) but the experimental site had ample precipitation through much of the season.

The Cabernet franc cl.UC Davis 1 grafted onto Courderc 3309 rootstock vines were planted in 1999 in a North-South row orientation and trained on Scott-Henry trellis. The in-row vine spacing was 2.13m and inter-row spacing was 2.74m. According to the standard practices of the region (Wolf 2008), the vines were late winter cane-pruned around February each year to a consistent bud number, on average 16.4 buds per linear meter and 40 buds per vine, not including one extra cane that served the dual function of kicker and winter damage back-up cane, which was removed by bloom.

An experimental plot was set up for five cover crop treatments to compare with glyphosate maintained bare soil as the control. The randomized complete block design (RCBD) was applied across four adjacent vineyard rows of the experimental site for four replications, with treatments and control randomly assigned within each replication. Each experimental unit was comprised of four consecutive panels with three vines per panel (12 vines per experimental unit). The middle two panels (six vines) were used for data collection for a total of 48 panels

(144 vines) in the experiment. Cover crops were seeded, and the herbicide was applied to approximately 1.2m wide under-vine strip along the row. A permanent between-row cover crop, a mix of fescue, white clover and weeds, was maintained separately and mowed periodically.

Table 2.2. Growing Degree Days (GDD) base on 10˚C and precipitation of the experimental site during the growing season from 2014 to 2016.

Month	GDD (˚C)			Precipitation (cm)		
	2014[a]	2015[b]	2016	2014[c]	2015	2016
April	28.3	29.9	24.4	7.1	6.6	3.8
May	165.2	210.6	125.2	4.6	3.4	5.8
June	294.0	293.7	291.2	5.5	3.8	1.8
July	335.7	360.3	406.7	7.0	6.8	3.7
August	289.2	328.2	423.8	8.1	3.3	11.0
September	225.0	297.3	271.7	1.3	7.9	7.6
October	94.1	65.5	104.6	2.2	5.2	10.9
Sum	1431.4	1585.6	1647.5	35.7	37.1	44.6

[a]Data obtained from Romulus, NY station.
[b]GDD and precipitation data of 2015 and 2016 were obtained from Ovid, NY station.
[c]Data obtained from Varick, NY station.

Under-vine cover crop establishment

The five under-vine cover crops treatments were natural vegetation (NV), alfalfa (ALF), fescue (FES), tillage radish (TR) and chicory (CHI). Seeding rates varied by treatment (Table 2.1). The control was maintained by applying Roundup (Roundup® PRO concentrate, Monsanto, St. Louis MO). The under-vine cover crops and herbicide stripes were established on an annual basis.

For the NV treatment, the weeds were allowed to grow freely whereas the other cover crops treatments were seeded. Seeds of ALF, FES, CHI and TR treatments were hand broadcasted on 26 May to 2 June 2014, 13 to 15 May 2015 and 25 to 26 May 2016. The seeding rates were the same for FES across all three years but increased in 2015 and 2016 for the other

cover crops due to poor establishment in 2014. The control was established with Roundup application, in which Glyphosate was the active ingredient, with 2.9 kg a.i./ha application rate of 2% solution on 24 June and 16 July 2014, 16 June 2015 and 15 June 2016. The cover crop treatments were trimmed using a string trimmer on 8 to 9 August 2015 as the vegetation was reaching the fruiting zone.

Ground coverage assessment and weed identification

In each experimental unit, two 400cm^2 square-shaped grids were randomly chosen using a square wooden frame with 0.2m inner length of each side at veraison in 2015 and 2016. A digital photo was taken at 1.5m vertically above each chosen grid with measuring tape placed horizontally on the ground to be used as photo scaling reference. The above ground tissue of cover crop and weeds were separately harvested from each of the chosen grid, contained in separate paper bags, dried in oven at 60˚C overnight and weighed. The chosen grid within each digital photo was analyzed with ImageJ Version 1.50b (open resource via http://imagej.nih.gov/) to define the proportion of ground coverage with image processing steps similar to Ricotta et al. (2014). The percentage of cover crop coverage was determined by dividing cover crop biomass by total biomass for each of the experimental unit. The weeds in the NV treatment were identified visually using the same digital photos for percent ground cover measurement.

Table 2.1. Scientific name, common name and seeding rate of under-vine cover crop treatments used in the experiment. The seeds were purchased from Ernst Seeds, PA, USA.

Abbreviation	Scientific name	Variety/Common name	Seeding rates (kg/ha)	
			2014	2015&16
CHI	*Cichorium intybus*	Blue Chicory	7.01	8.76
ALF	*Medicago sativa*	Alfalfa, Vernal	28	35
TR	*Raphanus sativus*	Tillage Radish, Ground hog	14	17.4
FES	*Festuca arundinacea*	Tall Fescue, Kentucky 31	196	196

Shoot growth measurement

Four shoots per data vine were randomly marked in the beginning of each growing season to represent the primary shoot growth dynamic for each vine throughout the growing season. Shoot diameters were measured (mm) by using calipers at the middle of internode one above the first fully developed bud where two measurements were taken per shoot. The average of the two numbers was used to represent each shoot. Shoot length was measured (cm) with measuring tape from primary shoot base to shoot tip. Lateral shoot from the primary shoot marked for primary shoot measurement were measured using the same methods described for the primary shoot beginning after the first hedging and continuing until the second hedging or shoot thinning which imposed missing tagged shoots.

Canopy architecture - EPQA

Point quadrat analysis (PQA) (Smart and Robinson, 1991) and enhanced point quadrat analysis (EPQA) (Meyers and Vanden Heuvel, 2008) were conducted to characterize canopy light environment at veraison, 25 August 2015 and 19 August 2016. To measure basic PQA, a thin wooden stick horizontally inserted through the fruiting zone in perpendicular to the row at 20cm interval on a per panel basis while recording any leaf and cluster contact with the stick end. Light environment of the canopy at fruiting zone was also measured on the same day of measuring PQA using a ceptometer (Decagon, model AccuPAR LP-80, Pullman, WA), which recorded the photon flux, with an ambient flux sensor attached. Two measurements were taken per data vine on the fruiting zone during the solar noon with an hour deviation. The ambient flux sensor was pointed vertically toward the sky above canopy without any shade throughout the ceptometer measurement. The proportion light interception was calculated dividing fruiting zone

photon flux by ambient photon flux. Light interception and PQA data were uploaded into Canopy Exposure Mapping Tools, version 1.7 (available via Jim Meyers, jmm533@cornell.edu) to calculate leaf layer number, occlusion layer number, interior leaf percentage, interior cluster percentage, cluster exposure layer and cluster exposure flux availability.

Vine water and nutrient status measurements

Vine midday stem and predawn leaf water potential were measured according to Fulton et al. (2001) with a pressure chamber (Soil Moisture Equipment Corporation, model 3005F01, Santa Barbara, CA). Midday stem water potential measurements were performed at solar noon with one hour deviation on a biweekly basis and predawn water potential was measured during late growing season during fruit ripening 11 September 2015and 13 September 2016 at 4AM EST with one hour deviation. For midday stem water potential measurement, fully expanded healthy young leaves were bagged with a 500ml alumina foil covered Ziploc bag for 15mins before measurement. Each leaf was cut off with a sharp blade, transferred immediately into the pressure chamber, and pressurized at about 1bar/sec to the point when xylem sap moisturized the cut surface of the petiole.

One hundred petiole samples per experimental unit, from young fully expanded leaves, were collected at roughly full bloom on 20 June 2015 and 24 June 2016, and veraison on 4 September 2014, 24 August 2015 and 26 August 2016. The petioles were washed with mild soap, rinsed with deionized water and sent to Cornell Nutrient Analysis Laboratory (CNAL) for total Carbon, Nitrogen using combustion method, and macro- and micronutrients (Al, B, Ca, Cu, Fe, K, Mg, Mo, Mn, Na, P, Zn) using dry ash extraction method according to Campbell et al (1998).

Yield components and juice composition measurements

The yield data was collected at commercial harvest as determined by the grower on 25 October 2014, 17 October 2015 and 22 October 2016. For each year, the harvests were done manually on a per vine basis. The clusters from each vine were clipped, counted and pooled in a plastic lug to determine the yield by weighing using a hanging scale (Salter Brecknell, model SA3N340, accuracy ±0.1kg, Fairmont, MN). The total yield per vine was then divided by the number of clusters to determine the average weight per cluster. An extra 100 berries per experimental unit was collected at harvest, stored in Ziploc bag at -20˚C until weighed (Santorius ELT103, accuracy ±0.001g, Goettingen, Germany) to determine the average berry weight.

Pruning weight was collected on a per vine basis from upward shoots in 2014 and from both upwards and downwards shoots in 2015 and 2016 in the early winter for downward shoots and late winter for the upwards shoots of each year (as determined by the cooperating grower's standard practice). Only upward shoot pruning weight was collected in 2014 because the downward shoots were pruned in an untraceable manner by the vineyard worker prior to data collection. In 2016, the downward shoots were pruned by the vineyard worker prior to the data collection. However, the shoots remained directly under the vine so the data was collected from the shoots reconstructed by identifying the size, shape and color of the cut surfaces. Pruning weight was used as an indicator for vine vegetative growth and to determine the Ravaz index by dividing yield by pruning weight.

Twenty clusters were collected randomly from each experimental unit at harvest and stored in a -20˚C freezer before juice composition analysis. The clusters were then thawed at room temperature, whole cluster pressed and the juice was filtered with cheesecloth. The juice soluble solids, titratable acid (TA), pH and yeast assimilable nitrogen (YAN) were analyzed

using temperature compensating digital refractometer, titration 50 mL aliquot of juice against 0.10 M NaOH to pH 8.2, a benchtop pH meter (VWR Symphony pH Meter, model SB80RI, Radnor, PA), and a Chemwell 2910 multianalyzer to measure ammonia and spectrophotometry to measure primary amino nitrogen (Nisbet et al., 2013). YAN was quantified in 2015 and 2016 only. Cluster compactness was measured in the third year of study in 2016 where 10 clusters from each of the experimental unit was collected at harvest, berries from each cluster were counted and removed to measure the naked rachises length. The compactness was presented as number of berries per cm of rachis.

Analysis of soil properties

Under-vine soil samples were collected at the end of the growing season in November 2015 and 2016. Six soil cores to depth 20 cm were taken from each experimental unit, combined, and analyzed for wet aggregate stability, organic matter content and microbial respiration rate.

Soil properties were measured according to Gugino et al. (2009) and Karl et al. (2016a). Briefly, aggregate stability was measured with dried soil that was sieved to select particle size between 0.25-2mm. Water droplets in a 0.042mm/s rate generated from a rain simulator were applied to the soil placed on 0.25mm sieve for 5min. Soil particles retained on and passed through the sieve were collected, dried and weighed to determine the proportion of stable soil aggregates. Soil organic matter content was measures by dry combustion at 550˚C for two hours. For cumulative microbial respiration measurement, 50g soil from each experimental unit with particle size smaller than 2mm in diameter was placed in a 250ml airtight and sterilized glass jar along with 20ml of 0.5M NaOH contained in a plastic tube. The jars were placed in darkness at 30 ˚C for two weeks. Electrical conductivity of NaOH in the plastic tube in each jar was

measured and compared with a control solution to calculate the CO2 generated during the two weeks of incubation. Carbon mineralizability was calculated dividing CO2 generation rate by organic carbon content in the soil.

Additional tests of Morgan-extractable phosphorus, potassium and micronutrients were conducted in 2015. Briefly, soil nutrients were extracted using Morgan's solution and quantified with Inductively Coupled Argon Plasma Spectrophotometry. In November 2016, four intact soil cores per experimental unit were collected for soil bulk density measurement. The soil samples were stratified into 0-5, 5-10, and 10-15cm by hand, dried in oven at 60˚C for 24hrs, weighed and divided the weight by volume.

Statistical methods

The data were checked for normality assumption and analyzed with mixed-model ANOVA, where under-vine floor treatments were classified as a fixed effect and blocks as random effects, using JMP Pro version 12.0.1. The Dunnett's test was adopted for post-hoc comparison of treatment means compared to the mean of the GLY control at $\alpha=0.05$.

Results

Cover crop establishment

Cover crops were not well established in the first year of the experiment, likely due to residual herbicide remaining in the treatment plots from the previous seasons. In the second and third years of the experiment, the area under the vines was well covered with cover crops and weeds (Fig. 2.1.). The glyphosate control remained relatively bare while the coverage was 30% in 2015 and less than 10% in 2016 at veraison (Fig 2.1) whereas more than 70% of the

proportion of ground coverage(cover crops and weeds combined) was achieved with cover crops.

Natural vegetation had more than 70% ground coverage for 2015 and 2016. The weed species identified are listed in Table 2.3. Among the cover crops, TR was the most difficult to establish and resulted in the lowest coverage at about 27% and 38% in 2015 and 2016 respectively. Unlike TR, ALF had poor establishment in 2015 at 24% coverage but grew well in 2016 and reached 67% ground coverage. Cover crops of FES and CHI treatments were relatively well established for both years where CHI had 50% coverage in both years and FES had 53% and 62% in 2015 and 2016, respectively.

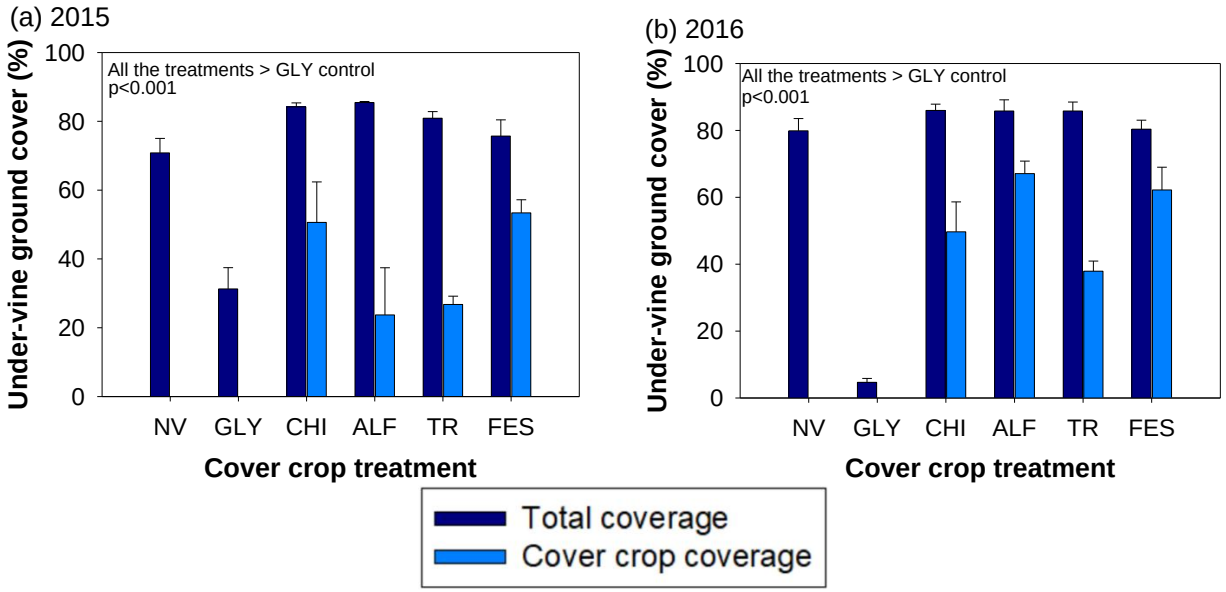


Figure 2.1. Proportion of under-vine soil covered with weeds and cover crops in a Cabernet franc vineyard at Veraison in (a) 2015 and (b) 2016. NV = Natural Vegetation, GLY = Glyphosate, CHI = Chicory, ALF = Alfalfa, FES = Fescue and TR = Tillage Radish. The bars indicate standard errors. The significant differences between each of the treatment and control were found using mixed model ANOVA following with Dunnett's test at α=0.05.

Table 2.3. Weed species identified in under-vine natural vegetation treatment at veraison in 2015 and 2016.

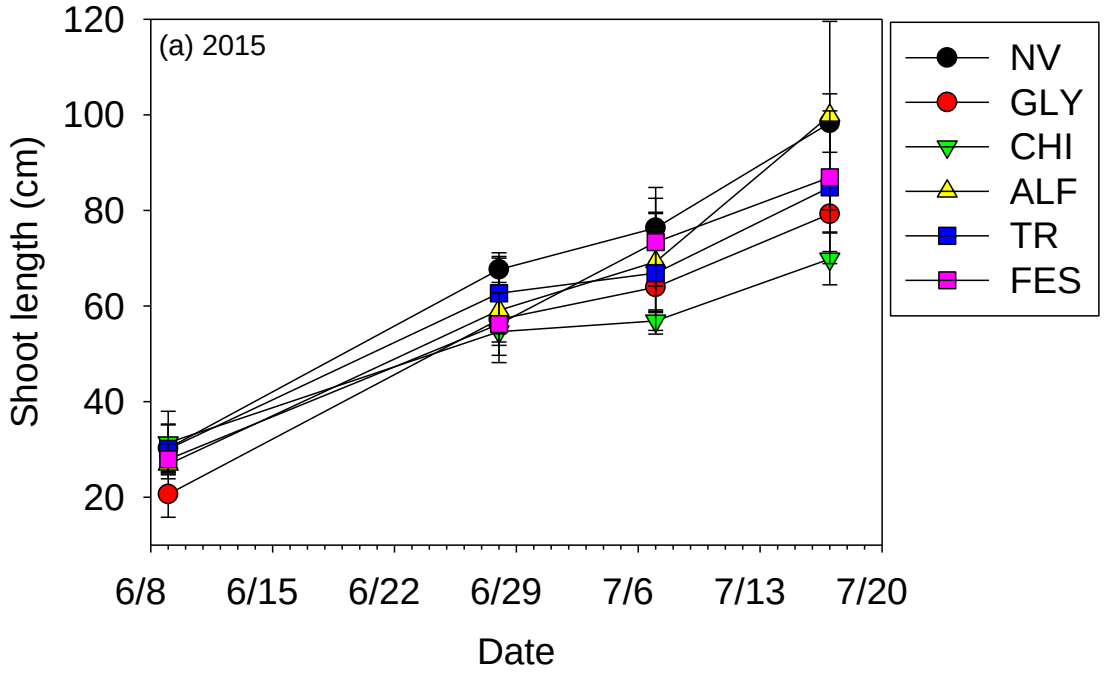
2015		2016	
Common name	Scientific name	Common name	Scientific name
Blackseed plantain	*Plantago lanceolata* L.	Blackseed plantain	*Plantago lanceolata* L.
Common blue violet	*Viola sororia* Willd.	Common burdock	*Arctium minus* Bernh.
Common mallow	*Malva neglecta* Wallr.	Common milk weed	*Asclepias syriaca* L.
Dallisgrass	*Paspalum dimidiatum* L.	Dallisgrass	*Paspalum dimidiatum* L.
Dandelion	*Taraxacum officinale* F.H. Wigg.	Dandelion	*Taraxacum officinale* F.H. Wigg.
Horsenettle	*Solanum carolinense* L.	Eastern black nightshade	*Solanum ptychanthum* Dunal.
Horseweed	*Erigeron canadensis* (L.) Cronquist	Horsenettle	*Solanum carolinense* L.
Johnsongrass	*Sorghum halepense* (L.) Pers.	Lesser-seeded bittercress	*Cardamine oligosperma* Nutt.
Large crabgrass	*Digitaria sanguinalis* (L.) Scop.	Oxeye daisy	*Leucanthemum vulgare* Lam.
Red clover	*Trifolium pratense* L.	Powell amaranth	*Amaranthus powellii* S.Wats.
Roughstalk bluegrass	*Poa trivialis* L.	Red clover	*Trifolium pratense* L.
Smartweed	*Persicaria lapathifolia* (L.) Delabre.	Roughstalk bluegrass	*Poa trivialis* L.
Smooth pig weed	*Amaranthus hybridus* L.	Smartweed	*Persicaria lapathifolia* (L.) Delabre.
Sow thistles	*Sonchus oleraceus* L.	Smooth pig weed	*Amaranthus hybridus* L.
Tumble mustard	*Sisymbrium altissimum* L.	Sow thistles	*Sonchus oleraceus* L.
Tall fescue	*Festuca arundinacea* Schreb.	Tall fescue	*Festuca arundinacea* Schreb.
Velvetleaf	*Abutilon theophrasti* Medik.	Velvetleaf	*Abutilon theophrasti* Medik.
White clover	*Trifolium repens* L.	White clover	*Trifolium repens* L.
Wild buckwheat	*Fallopia convolvulus* (L.) Á.Löve	Wild buckwheat	*Fallopia convolvulus* (L.) Á.Löve
Yellow foxtail	*Setaria pumila* (Poir.) Roem. & Schult.	Yellow foxtail	*Setaria pumila* (Poir.) Roem. & Schul
Yellow woodsorrel	*Oxalis stricta* L.	Yellow woodsorrel	*Oxalis stricta* L.

Shoot growth

Shoot length was not statistically different whether cover crops were utilized or not throughout the first half of the 2015 growing season (Fig. 2.2a). In 2016 early season, the primary shoot length was longer in vines of FES and TR treatments than control vines (Fig. 2.2b). Primary shoot length of FES was about 16% and 30% longer than that of control on 6 and 20 June respectively. Primary shoot length of TR was about 25% longer than that of control on both 6 and 20 June. Primary shoot length was reduced by CHI in the middle of the growing season by 29% and 39% compared to GLY on 12 and 25 July, respectively. There were no primary shoot diameter differences found between any ground cover management in 2015 and 2016 (Fig. 2.3).

Lateral shoot length did not differ between any of the treatments and control in both years (Fig. 2.4). The proportion of primary shoots with laterals was not different between any of

the treatments and control in 2015 (Fig. 2.5a) but in 2016 fewer primary shoots in CHI had laterals compared to the GLY control on 12 September (Fig. 2.5b).



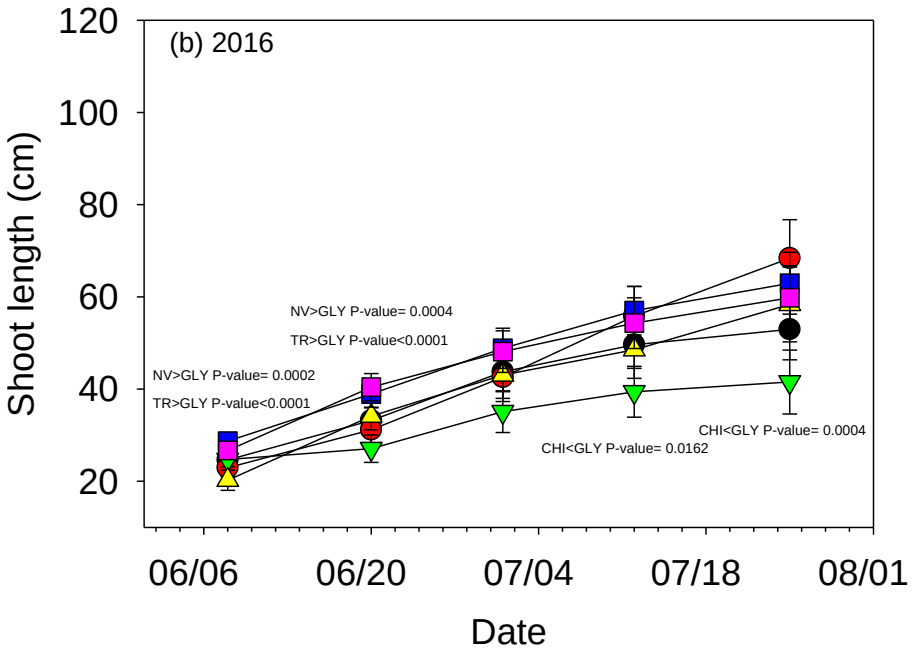


Figure 2.2. Primary shoots length of Cabernet franc vine growing with different under-vine cover crop treatments throughout the early to mid-growing season in (a) 2015 and (b) 2016. NV = Natural Vegetation, GLY = Glyphosate, CHI = Chicory, ALF = Alfalfa, FES = Fescue and TR = Tillage Radish. The significant differences between each of the treatment and control were tested using mixed model ANOVA following with Dunnett's test at α=0.05.

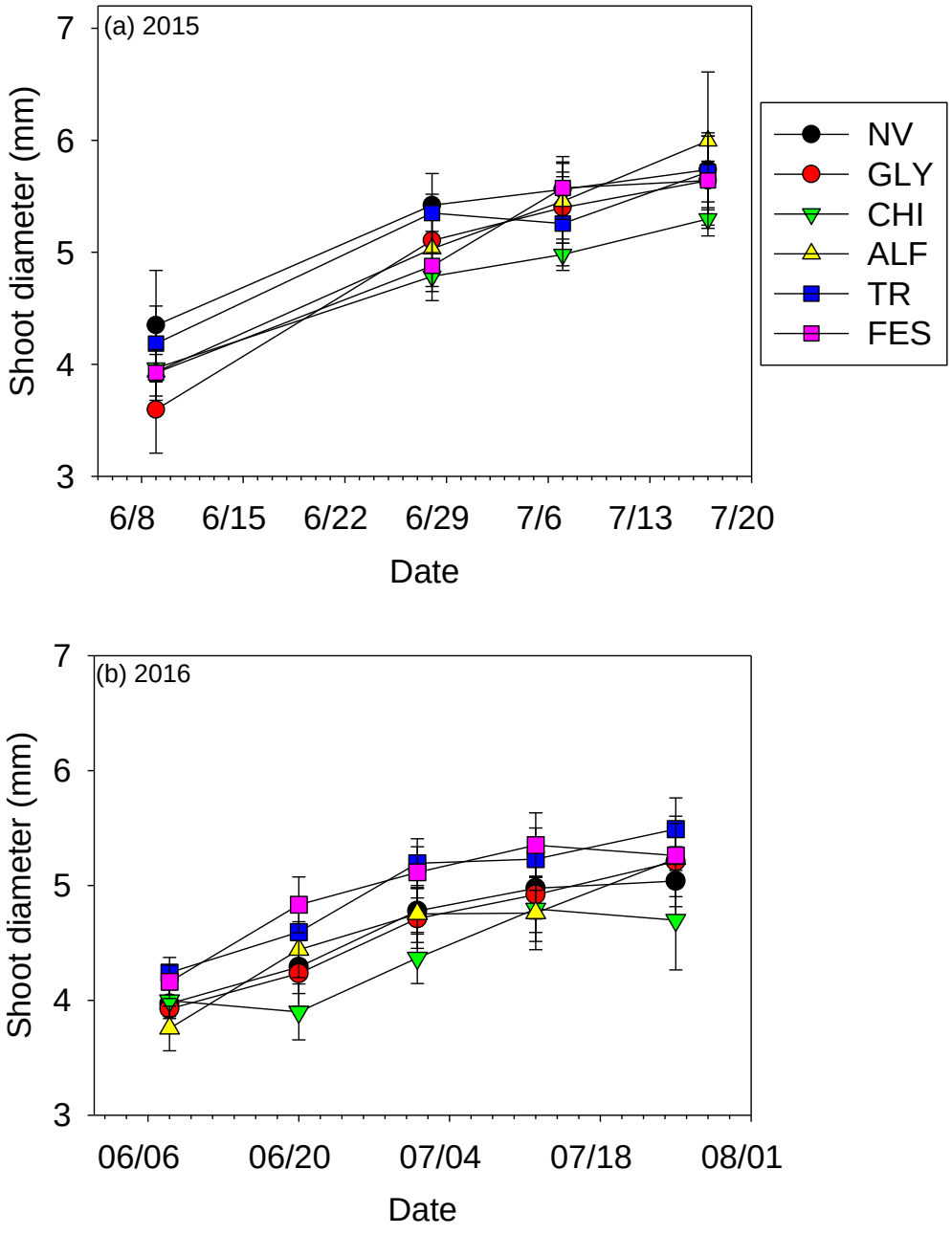


Figure 2.3. Primary shoots diameter of Cabernet franc vine growing with different under-vine cover crop treatments throughout the early to mid-growing season in (a)2015 and (b) 2016. NV = Natural Vegetation, GLY = Glyphosate, CHI = Chicory, ALF = Alfalfa, FES = Fescue and TR = Tillage Radish. The significant differences between each of the treatment and control were tested using mixed model ANOVA following with Dunnett's test at α=0.05.

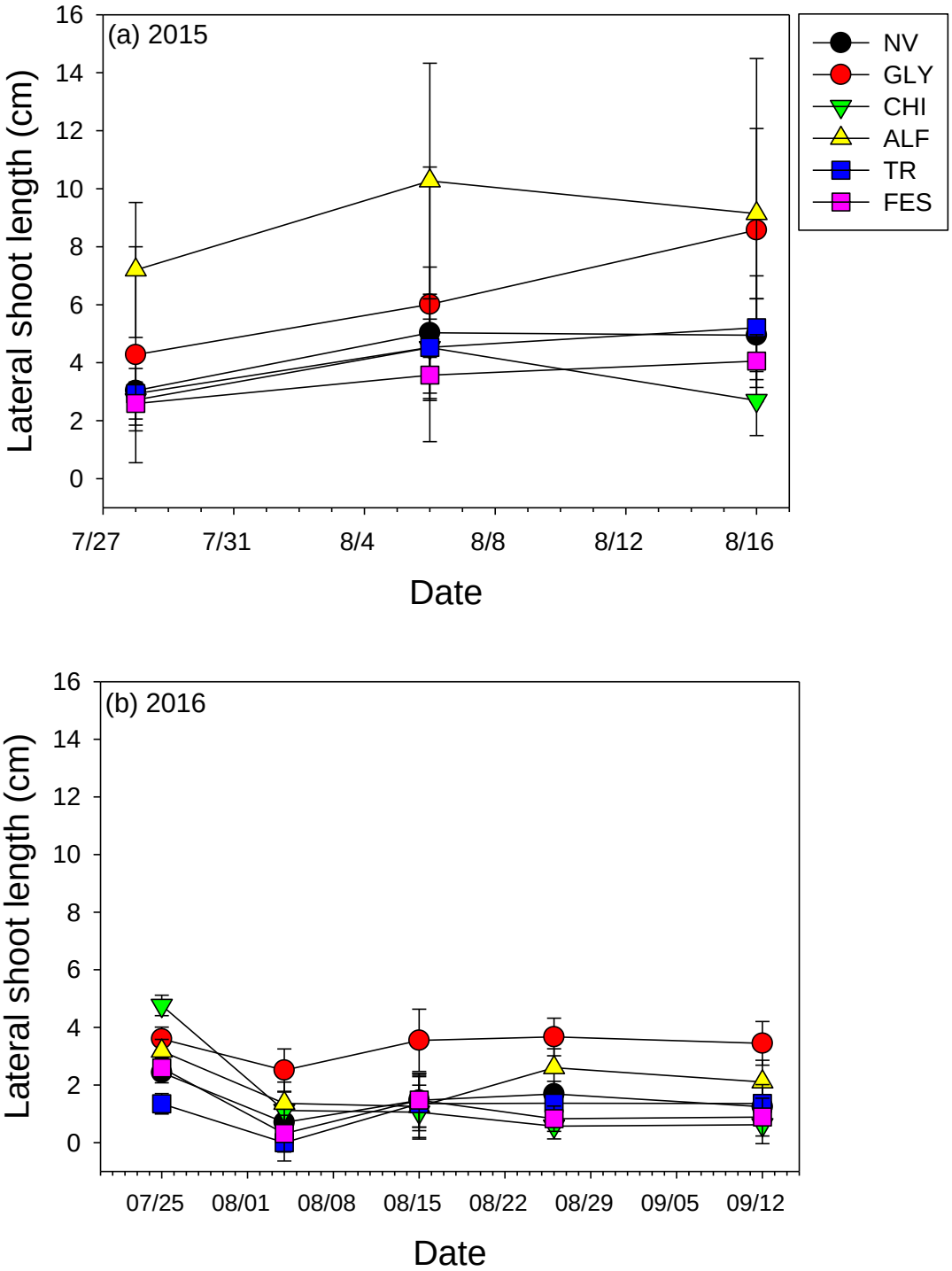


Figure 2.4. Lateral shoot growth of Cabernet franc vines with different cover crops growing under-vine throughout the mid to late growing season in (a)2015 and (b)2016. NV = Natural Vegetation, GLY = Glyphosate, CHI = Chicory, ALF = Alfalfa, FES = Fescue and TR = Tillage Radish. The significant differences between each of the treatment and control were tested using mixed model ANOVA following with Dunnett's test at α=0.05.

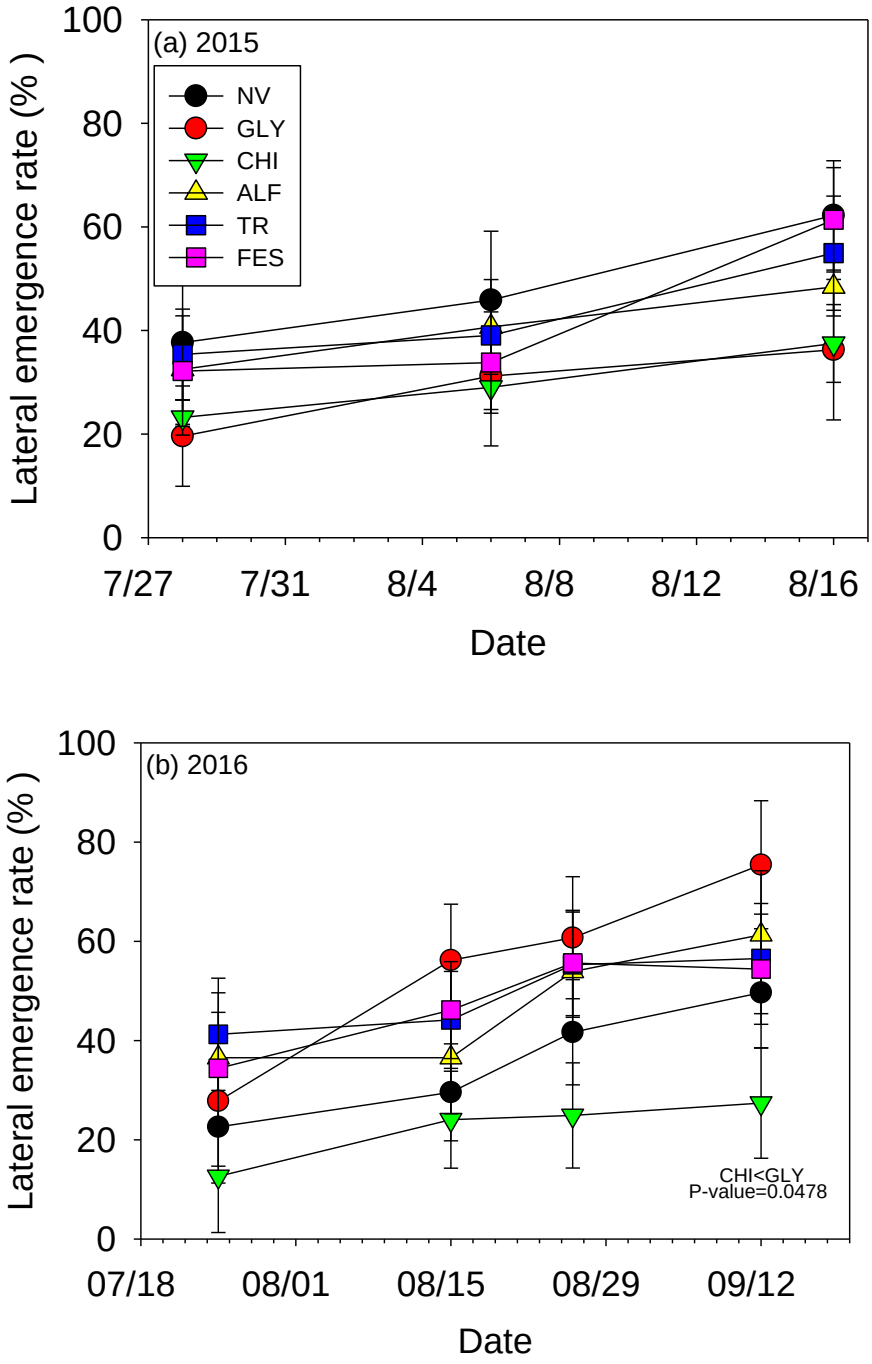


Figure 2.5. Proportion of primary shoot with laterals of Cabernet franc vines with different cover crops growing under-vine throughout the mid to late growing season in (a)2015 and (b)2016. NV = Natural Vegetation, GLY = Glyphosate, CHI = Chicory, ALF = Alfalfa, FES = Fescue and TR = Tillage Radish. The significant differences between each of the treatment and control were tested using mixed model ANOVA following with Dunnett's test at α=0.05. The significance symbol * indicates P-value <0.05.

EPQA

Grapevine canopy structure was impacted in the second and third year of the experiment (Table 2.4) (EPQA data was not collected in 2014). In 2015, planting cover crops reduced the leaf layer number in the fruiting zone, and NV, TR and FES resulted in reduced occlusion layer numbers compared to the GLY control. The proportion of interior leaves was reduced to 8.9% in FES compared to 20.3% in GLY and the proportion of interior clusters was reduced to 15.5% and 17% in NV and ALF, respectively, compared to 43.2% in the GLY control. Although the canopy structure was impacted by the under-vine cover crops in 2015, the light environment parameters including cluster exposure layer and cluster exposure flux availability were not significantly impacted. In 2016, the leaf layer number was impacted in the fruiting zone where CHI reduced 35% and FES reduced 28% of the leaf layer compared to GLY. Mixed model ANOVA also showed that the cluster exposure layer and cluster exposure flux availability were significantly impacted by the different under-vine cover crops but no pairwise differences between any of the treatments and control were found using the Dunnett's test.

Table 2.4. Canopy architecture approximation using EPQA analysis of Cabernet franc vines with different under-vine cover crops in 2015 and 2016 at veraison.

2015 veraison			
Treatment[a]	Leaf Layer Number	Occlusion Layer Number	% Interior Leaves
GLY	1.75±0.24[c]	2.33±0.15	20.3±4.39
NV	1.18±0.23 *[d]	1.84±0.15 *	15.0±4.20
CHI	1.22±0.23 *	2.11±0.15	12.4±4.20
TR	1.08±0.23 **	1.82±0.15 **	14.7±4.20
ALF	1.21±0.23 *	1.95±0.15	10.2±4.20
FES	1.20±0.23 *	1.80±0.15 **	8.93±4.20 *
P-value[b]	0.022	0.009	0.146
Treatment	% Interior Clusters	Cluster Exposure Layer	Cluster Exposure Flux Availability
GLY	43.2±8.01	0.44±0.08	0.42±0.07
NV	15.5±7.20 *	0.19±0.08	0.54±0.06
CHI	28.3±7.20	0.30±0.08	0.49±0.06
TR	20.9±7.20	0.24±0.08	0.57±0.06
ALF	17.0±7.20 *	0.20±0.08	0.51±0.06
FES	26.1±7.20	0.26±0.08	0.56±0.06
P-value	0.091	0.225	0.416
2016 veraison			
Treatment	Leaf Layer Number	Occlusion Layer Number	Interior Leaves (%)
GLY	1.34±0.07	2.26±0.07	20.2±2.3
NV	1.27±0.06	2.29±0.06	19.6±2.0
CHI	0.87±0.06 ***	1.93±0.06	13.5±2.0
TR	1.16±0.06	2.31±0.06	17.2±2.0
ALF	1.25±0.08	2.31±0.08	14.2±2.6
FES	0.96±0.07 *	2.01±0.07	20.4±2.3
P-value	0.001	0.055	0.316
Treatment	% Interior Clusters	Cluster Exposure Layer	Cluster Exposure Flux Availability
GLY	22.41±4.3	0.24±0.05	0.56±0.06
NV	26.87±3.7	0.28±0.04	0.58±0.05
CHI	9.48±3.7	0.11±0.04	0.58±0.05
TR	27.41±3.7	0.29±0.04	0.58±0.05
ALF	33.32±4.3	0.33±0.05	0.49±0.06
FES	17.56±4.3	0.18±0.05	0.65±0.06
P-value	0.003	0.013	0.595

[a]Treatment: GLY = Glyphosate, NV = Natural vegetation, CHI = Chicory, TR = Tillage Radish, ALF = Alfalfa, FES = Fescue.
[b]P-value: The P-value was derived from mixed model ANOVA following at α=0.05.
[c]Pooled standard error
[d]Significance designation of Dunnett's test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Yield components and berry composition

Yield per vine was not impacted by the under-vine cover crops in 2014 and 2015, but in 2016 NV and TR increased the yield by 100% and 77% respectively compared to GLY (Table 2.5). Since the cluster weight was not impacted by any of the under-vine cover crops, the yield increment in NV and TR was mainly due to the increased number of clusters per vine. Only upward shoot pruning weight was collected in 2014 which indicated no differences ($p=0.4673$, data not shown). Compared to GLY, CHI reduced pruning weight by 65% in 2015 and 59% in 2016. Pruning weight was reduced about 54% by TR and FES compared to the control in 2015. However, the Ravaz index (yield/pruning weight) was only impacted by CHI (increase of 129%) in 2015.

Cluster number per vine was increased by NV, TR, FES in 2016 by 95%, 66% and 73% respectively. Berry size was increased by using under-vine cover crops in 2016. Number of berries per cluster was reduced by CHI and FES in 2016 by 31% and 25% respectively. In 2016, the third year of the experiment, berries from all the cover crop treatments increased the berry size by about 10 to 17%. Cluster compactness was only measured in 2016. The cluster compactness, presented as number of berry per cm rachis, was impacted by the under-vine floor treatments as revealed by ANOVA but the pairwise comparison showed no differences between any of the treatments and the GLY control. The modification of the cluster compactness was possibly because that the rachis length was reduced 18.6% by CHI compared to the control (Table 2.6). Berry soluble solids and TA were not impacted by any of the under-vine treatments in all three years (Table 2.7). Juice pH was reduced 5% by FES in 2014 and YAN was reduced 40% by CHI in 2015 compare to those of GLY control which were pH 3.42 and 112mg/L, respectively.

Table 2.5. Yield components of Cabernet franc vines growing with different under-vine cover crops.

Treatment[a]	Yield (kg/vine)			Pruning Weight (kg/vine)		
	2014	2015	2016	2014	2015	2016
GLY	4.61±0.85[c]	3.90±0.57	2.89±0.73	-	1.15±0.18	0.44±0.09
NV	4.25±0.85	4.11±0.47	5.80±0.63***[d]	-	0.81±0.18	0.36±0.08
TR	6.51±0.85	4.74±0.47	5.12±0.63**	-	0.53±0.18*	0.34±0.08
CHI	6.11±0.85	5.22±0.46	3.03±0.63	-	0.40±0.18**	0.18±0.08
FES	5.57±0.85	4.28±0.51	4.18±0.63	-	0.58±0.18*	0.36±0.08
ALF	5.68±0.85	4.61±0.49	2.88±0.73	-	0.86±0.19	0.35±0.09
P-value[b]	0.1123	0.4563	0.0008		0.008	0.1231
Treatment	Ravaz-Index (yield/pruning weight)			Number of Cluster (cluster/vine)		
	2014	2015	2016	2014	2015	2016
GLY	-	7.5±4.2	10.8±5.2	37.9±5.5	31.3±5.1	32.3±7.1
NV	-	6.1±3.6	20.7±4.5	33.5±5.5	38.3±4.1	62.9±6.0***
TR	-	10.5±3.6	18.0±4.5	47.7±5.5	47.1±4.7	53.7±6.1*
CHI	-	17.3±3.6*	18.2±4.5	45.7±5.5	41.8±4.2	38.5±6.2
FES	-	8.2±3.8	13.2±4.5	45.2±5.5	33.0±4.7	55.9±6.3**
ALF	-	13.6±3.7	14.1±5.2	46.8±5.5	38.9±4.2	30.5±7.2
P-value		0.001	0.3761	0.1119	0.236	<0.0001
Treatment	Number of berry (kg/vine)			Cluster weight (g/cluster)		
	2014	2015	2016	2014	2015	2016
GLY	70.7±5.9	84.3±7.7	77.9±7.2	122.0±10.9	122.9±10.7	97.0±10.0
NV	73.6±5.9	72.6±6.7	63.8±6.2	130.7±10.9	128.3±8.7	94.0±8.5
TR	75.6±5.9	67.8±6.9	68.9±6.2	141.1±10.9	115.1±9.8	95.9±8.5
CHI	78.0±5.9	87.6±6.7	53.8±6.3**	133.9±10.9	131.9±8.9	79.8±8.7
FES	68.0±5.9	90.3±7.1	58.4±6.4*	125.2±10.9	143.9±9.9	81.3±8.8
ALF	64.0±5.9	74.8±6.7	73.7±7.3	118.5±10.9	119.9±9.4	101.9±10.1
P-value	0.5941	<0.0001	0.0052	0.6788	0.2478	0.0991
Treatment	Berry weight (g/berry)					
	2014	2015	2016			
GLY	1.60±0.05	1.52±0.03	1.26±0.03			
NV	1.75±0.05	1.68±0.03*	1.47±0.02****			
TR	1.95±0.05*	1.63±0.03	1.39±0.02***			
CHI	1.63±0.05	1.53±0.03	1.47±0.02****			
FES	1.75±0.05	1.57±0.03	1.40±0.02****			
ALF	1.83±0.05	1.57±0.03	1.39±0.02***			
P-value	0.0117	<0.0001	<0.0001			

[a]Treatment: GLY = Glyphosate, NV = Natural vegetation, CHI = Chicory, TR = Tillage Radish, ALF = Alfalfa, FES = Fescue.

[b]P-value: The P-value was derived from mixed model ANOVA following at $\alpha=0.05$.

[c]Pooled standard error

[d]Significance designation of Dunnett's test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Table 2.6. Cluster compactness measurement of mature Cabernet franc vines with different cover crops growing under-vine

Treatment[a]	Cluster compactness (berry number/cm)	Rachis length (cm)	Berry number per cluster
GLY	7.63 ± 0.85[c]	11.8 ± 1.32	89.3 ± 9.21
NV	8.05 ± 0.74	11.2 ± 1.14	91.4 ± 7.97
TR	7.99 ± 0.74	10.7 ± 1.14	84.9 ± 7.97
CHI	8.52 ± 0.74	9.61 ± 1.14 *[d]	76.7 ± 7.97
FES	6.48 ± 0.74	12.2 ± 1.14	78.0 ± 7.97
ALF	8.39 ± 0.85	10.1 ± 1.32	83.3 ± 9.21
P-value[b]	0.0024	<0.0001	0.3539

[a]Treatment: GLY = Glyphosate, NV = Natural vegetation, CHI = Chicory, TR = Tillage Radish, ALF = Alfalfa, FES = Fescue.
[b]P-value: The P-value was derived from mixed model ANOVA at $\alpha=0.05$.
[c]Pooled standard error
[d]Significance designation of Dunnett's test: *$p<0.05$, **$p<0.01$, ***$p<0.001$

Table 2.7. Berry composition of Cabernet franc vines growing with different under-vine cover crops.

Treatment[a]	Soluble Solids (Brix)			pH		
	2014	2015	2016	2014	2015	2016
GLY	22.2±0.4[c]	18.8±0.8	16.9±1.3	3.42±0.03	3.57±0.05	3.70±0.08
NV	21.8±0.4	20.8±0.7	15.8±1.1	3.44±0.03	3.68±0.04	3.63±0.07
TR	20.9±0.4	20.5±0.7	19.3±1.1	3.37±0.03	3.59±0.04	3.49±0.07
CHI	21.3±0.4	20.0±0.7	18.1±1.1	3.41±0.03	3.51±0.04	3.57±0.07
FES	21.3±0.4	20.3±0.7	17.3±1.1	3.25±0.03*[d]	3.59±0.04	3.56±0.07
ALF	21.1±0.4	18.9±0.7	17.2±1.3	3.41±0.03	3.59±0.04	3.62±0.08
P-value[b]	0.5138	0.2043	0.4563	0.0123	0.1975	0.4513
Treatment	Titratable Acid (g/L)			YAN (mg/L)		
	2014	2015	2016	2014	2015	2016
GLY	5.84±0.23	5.86±0.33	3.40±0.32	-	112.0±11.2	71.7±26.9
NV	5.59±0.23	4.78±0.29	3.59±0.28	-	85.9±9.7	72.8±23.3
TR	5.87±0.23	4.96±0.29	4.05±0.28	-	73.7±9.7	76.9±23.3
CHI	5.36±0.23	5.00±0.29	3.26±0.28	-	67.5±9.7*	51.0±23.3
FES	5.81±0.23	5.09±0.29	3.82±0.28	-	70.4±9.7	57.0±23.3
ALF	5.89±0.23	5.47±0.29	4.41±0.32	-	106.0±9.7	100±26.9
P-value	0.1915	0.1585	0.1075	-	0.0389	0.7576

[a]Treatment: GLY = Glyphosate, NV = Natural vegetation, CHI = Chicory, TR = Tillage Radish, ALF = Alfalfa, FES = Fescue.

[b]P-value: The P-value was derived from mixed model ANOVA following at $\alpha=0.05$.

[c]Pooled standard error

[d]Significance designation of Dunnett's test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Nutrient and water status

Petiole nutrient differences were found at bloom in 2015 and veraison in 2015 and 2016. In 2015 bloom, CHI reduced B by 10.7mg/kg (23%), ALF increased Cu by 2.7mg/kg (24%), ALF reduced Na by 0.05 g/kg (28%), all the treatments except NV increased P by up to 1.21 g/kg (48%), and NV, CHI and FES reduced Zn by up to 10 mg/kg (20%) compared to the control (Table 2.8a). By veraison in 2015, TR and CHI increased petiole Mg content by up to 2.64 g/mg (48%) and TR increased petiole P content by 1.39 g/kg (45%) (Table 2.8b). In 2016 at veraison, the petiole C content was reduced 1.4% by NV and increased 0.9 and 1% by CHI and FES respectively compared to GLY (Table 2.8c). At the same time, NV increased the petiole Fe and Na content by 16.3 mg/kg (75%) and 0.94 g/kg (300%) respectively compared to GLY.

There were no vine midday stem water potential (SWP) differences found between any of the treatments and the GLY control in 2015 (Fig. 2.6a). In 2016 vines from FES treatment had lower mean SWP (-8.1bar) on 20 June, vines from FES treatment had higher mean SWP (-6.6 bar) on 3 August, vines from NV treatment had lower mean SWP (-9.9 bar) on 27 August and vines from NV, ALF and FES treatment had lower mean SWP, ranging from -12 to -13 bars, on late growing season 13 September which is when the lowest SWP in the growing season was observed (Fig. 2.6b).

Differences in late season predawn leaf water potential between treatment and control were found in both 2015 and 2016. Vines in TR treatments constantly had lower predawn leaf water potential values, 42% in 2015 and 55% in 2016 compared to vines in GLY (Fig. 2.6c). In 2016, FES reduced the predawn leaf water potential by 60% compared to the GLY control (Fig. 2.6d).

Table 2.8. Vine petiole nutrient analysis of Cabernet franc vines growing with different under-vine cover crops in 2015 at (a) bloom and (b) veraison and 2016 at (c) veraison.

(a) 2015 Bloom

Treatment[a]	**N** %	**C** %	**Al** mg/kg	**B** mg/kg	**Ca** g/kg	**Cu** mg/kg	**Fe** mg/kg
GLY	0.91 ± 0.05[c]	38.7 ± 0.2	10.1 ± 2.5	46.2 ± 2.3	13.0 ± 0.66	11.4 ± 0.4	20.4 ± 2.8
NV	0.84 ± 0.05	38.6 ± 0.2	15.2 ± 2.5	42.4 ± 2.3	13.4 ± 0.66	11.7 ± 0.4	20.0 ± 2.8
TR	0.80 ± 0.05	38.4 ± 0.2	10.5 ± 2.5	41.6 ± 2.3	13.3 ± 0.66	12.8 ± 0.4	20.0 ± 2.8
CHI	0.75 ± 0.05	38.3 ± 0.2	13.7 ± 2.5	35.5 ± 2.3 *[d]	14.0 ± 0.66	12.4 ± 0.4	24.0 ± 2.8
FES	0.85 ± 0.05	38.6 ± 0.2	10.0 ± 2.5	42.7 ± 2.3	13.3 ± 0.66	12.7 ± 0.4	17.7 ± 2.8
ALF	0.89 ± 0.05	38.0 ± 0.2	9.30 ± 2.5	44.9 ± 2.3	14.9 ± 0.66	14.1 ± 0.4 **	16.6 ± 2.8
P-value[b]	0.4454	0.3567	0.4669	0.0902	0.2674	0.0062	0.5664

Treatment	**K** g/kg	**Mg** g/kg	**Mn** mg/kg	**Na** g/kg	**P** g/kg	**Zn** mg/kg
GLY	14.3 ± 0.9	3.99 ± 0.16	69.4 ± 10	0.18 ± 0.01	2.54 ± 0.20	57.8 ± 2.4
NV	14.5 ± 0.9	3.62 ± 0.16	51.3 ± 10	0.19 ± 0.01	2.81 ± 0.20	46.8 ± 2.4 *
TR	15.0 ± 0.9	3.88 ± 0.16	50.2 ± 10	0.14 ± 0.01	3.50 ± 0.20 *	50.4 ± 2.4
CHI	14.3 ± 0.9	4.25 ± 0.16	66.1 ± 10	0.17 ± 0.01	3.46 ± 0.20 *	47.8 ± 2.4 *
FES	15.0 ± 0.9	3.50 ± 0.16	45.0 ± 10	0.13 ± 0.01 *	3.32 ± 0.20 *	46.1 ± 2.4 **
ALF	15.3 ± 0.9	4.52 ± 0.16	72.2 ± 10	0.15 ± 0.01	3.75 ± 0.20 **	54.2 ± 2.4
P-value	0.9574	0.0025	0.3467	0.0025	0.0032	0.0108

(b) 2015 Veraison

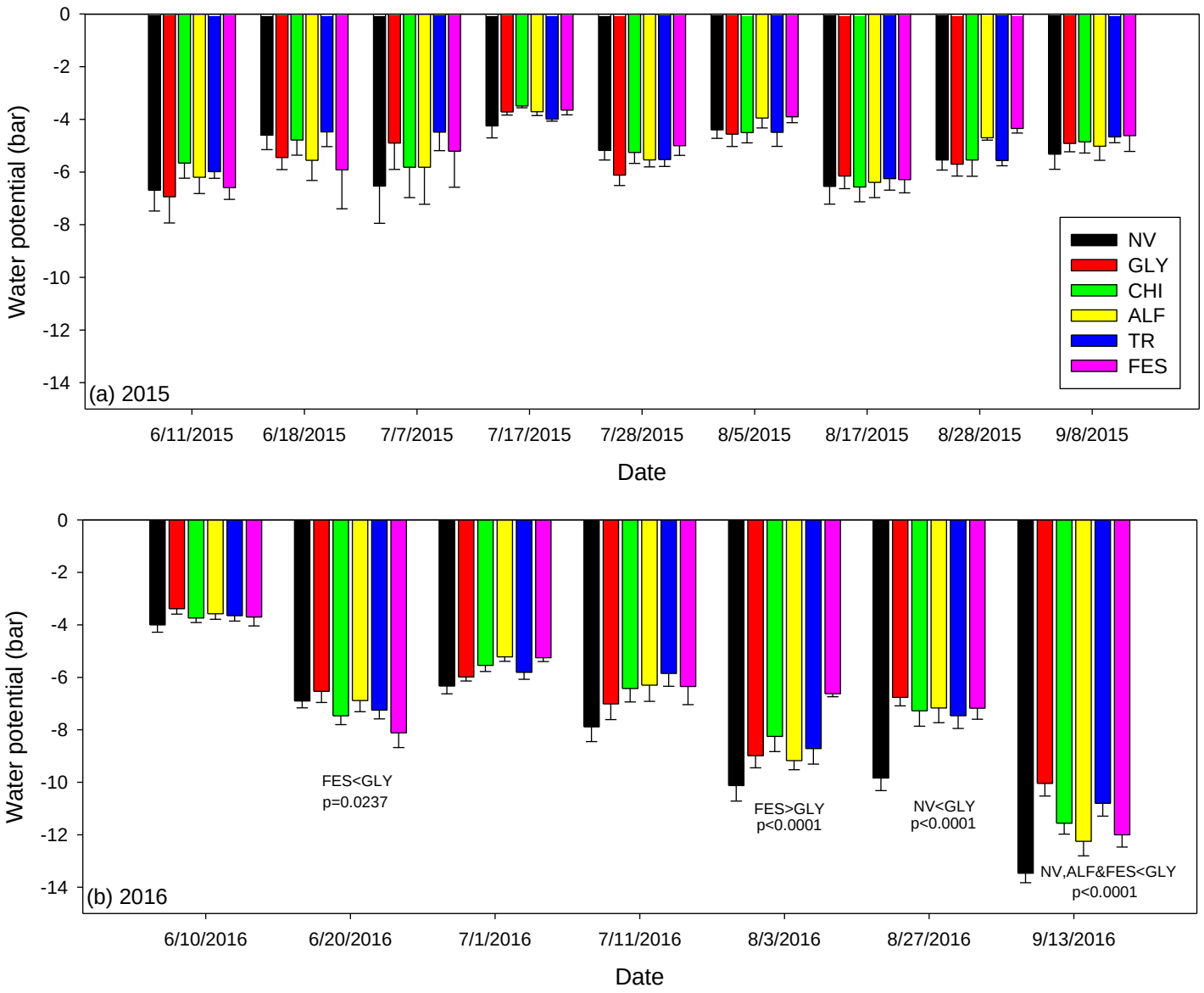
Treatment	N %	C %	Al mg/kg	B mg/kg	Ca g/kg	Cu mg/kg	Fe mg/kg
GLY	0.81 ± 0.08	44.8 ± 4.4	3.76 ± 0.61	36.2 ± 2.6	16.3 ± 1.1	6.59 ± 0.43	13.1 ± 0.90
NV	0.80 ± 0.08	43.2 ± 4.4	3.93 ± 0.61	38.1 ± 2.6	16.2 ± 1.1	6.71 ± 0.43	12.2 ± 0.90
TR	0.71 ± 0.08	41.3 ± 4.4	4.25 ± 0.61	36.6 ± 2.6	17.6 ± 1.1	6.45 ± 0.43	14.0 ± 0.90
CHI	0.67 ± 0.08	41.1 ± 4.4	5.59 ± 0.61	33.7 ± 2.6	17.2 ± 1.1	5.96 ± 0.43	15.3 ± 0.90
FES	0.67 ± 0.08	43.2 ± 4.4	4.08 ± 0.61	37.4 ± 2.6	16.1 ± 1.1	6.49 ± 0.43	14.8 ± 1.02
ALF	0.75 ± 0.08	42.1 ± 4.4	3.42 ± 0.61	36.3 ± 2.6	17.6 ± 1.1	6.36 ± 0.43	13.5 ± 0.90
P-value	0.8396	0.9868	0.2409	0.9229	0.7288	0.381	0.3138

Treatment	K g/kg	Mg g/kg	Mn mg/kg	Na g/kg	P g/kg	Zn mg/kg
GLY	28.9 ± 1.5	5.48 ± 0.64	75.7 ± 12.3	0.47 ± 0.04	3.11 ± 0.32	67.3 ± 2.8
NV	26.5 ± 1.5	6.79 ± 0.64	85.9 ± 12.3	0.49 ± 0.04	4.11 ± 0.32	67.2 ± 2.8
TR	24.4 ± 1.5	8.09 ± 0.64	87.9 ± 12.3	0.50 ± 0.04	4.50 ± 0.32	67.2 ± 2.8
CHI	22.6 ± 1.5	8.12 ± 0.64	87.7 ± 12.3	0.53 ± 0.04	4.29 ± 0.32	61.0 ± 2.8
FES	24.9 ± 1.5	6.76 ± 0.74	72.0 ± 14.2	0.57 ± 0.05	3.62 ± 0.37	66.9 ± 3.3
ALF	23.7 ± 1.8	7.11 ± 0.64	95.4 ± 12.3	0.48 ± 0.04	3.97 ± 0.32	65.6 ± 2.8
P-value	0.1772	0.0872	0.822	0.6908	0.1425	0.6438

(c) 2016 Veraison							
Treatment	**N** %	**C** %	**Al** mg/kg	**B** mg/kg	**Ca** g/kg	**Cu** mg/kg	**Fe** mg/kg
GLY	0.57 ± 0.03	38.4 ± 0.3	32.6 ± 4.6	37.3 ± 1.8	28.3 ± 1.1	33.0 ± 6.8	22.5 ± 4.2
NV	0.61 ± 0.03	37.0 ± 0.3 **	32.4 ± 4.6	38.0 ± 1.8	28.8 ± 1.1	22.7 ± 6.8	39.3 ± 4.2 *
TR	0.54 ± 0.03	39.0 ± 0.3	15.9 ± 4.6	40.5 ± 1.8	26.0 ± 1.1	14.6 ± 6.8	22.8 ± 4.2
CHI	0.52 ± 0.03	39.3 ± 0.3 *	17.6 ± 4.6	37.7 ± 1.8	25.1 ± 1.1	24.7 ± 6.8	28.1 ± 4.2
FES	0.51 ± 0.03	39.4 ± 0.3 *	15.7 ± 4.6	39.5 ± 1.8	25.8 ± 1.1	9.31 ± 6.8	18.9 ± 4.2
ALF	0.51 ± 0.03	39.3 ± 0.3	17.4 ± 4.6	37.7 ± 1.8	25.2 ± 1.1	11.5 ± 6.8	26.6 ± 4.2
P-value	0.1571	<0.0001	0.048	0.8298	0.138	0.2168	0.0391

Treatment	**K** g/kg	**Mg** g/kg	**Mn** mg/kg	**Na** g/kg	**P** g/kg	**Zn** mg/kg
GLY	18.2 ± 2.5	16.2 ± 1.3	161 ± 25	0.31 ± 0.13	1.20 ± 0.25	80.1 ± 4.4
NV	18.7 ± 2.5	18.2 ± 1.3	174 ± 25	1.25 ± 0.13 **	1.59 ± 0.25	77.0 ± 4.4
TR	10.5 ± 2.5	19.6 ± 1.3	166 ± 25	0.25 ± 0.13	2.18 ± 0.25	83.6 ± 4.4
CHI	11.6 ± 2.5	18.4 ± 1.3	172 ± 25	0.32 ± 0.13	1.90 ± 0.25	90.2 ± 4.4
FES	15.2 ± 2.5	16.3 ± 1.3	113 ± 25	0.29 ± 0.13	1.97 ± 0.25	81.5 ± 4.4
ALF	12.7 ± 2.5	17.8 ± 1.3	173 ± 25	0.28 ± 0.13	1.49 ± 0.25	80.0 ± 4.4
P-value	0.1634	0.5208	0.6007	0.0005	0.109	0.4551

[a]Treatment: GLY = Glyphosate, NV = Natural vegetation, CHI = Chicory, TR = Tillage Radish, ALF = Alfalfa, FES = Fescue.
[b]P-value: The P-value was derived from mixed model ANOVA following with Dunnett's test at α=0.05.
[c]Pooled standard error
[c]Significance designation of Dunnett's test: *p<0.05, **p<0.01, ***p<0.001


Water potential (bar)
0
-2
-4
-6
-8
-10
-12
-14
(a) 2015
NV
GLY
CHI
ALF
TR
FES
6/11/2015
6/18/2015
7/7/2015
7/17/2015
7/28/2015
8/5/2015
8/17/2015
8/28/2015
9/8/2015
Date
Water potential (bar)
(b) 2016
FES<GLY
p=0.0237
FES>GLY
p<0.0001
NV<GLY
p<0.0001
NV,ALF&FES<GLY
p<0.0001
6/10/2016
6/20/2016
7/1/2016
7/11/2016
8/3/2016
8/27/2016
9/13/2016
Date

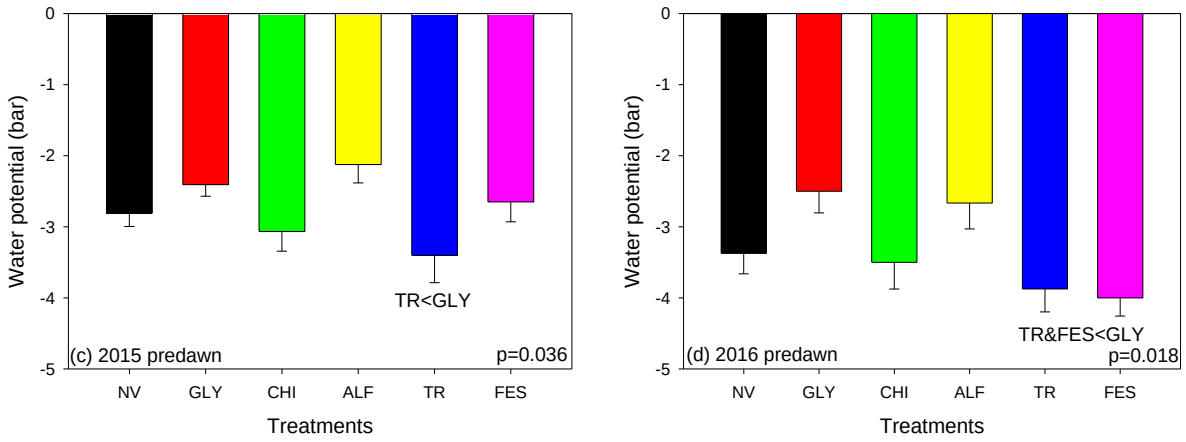


Figure 2.6. Midday stem water potential throughout the first half of growing seasons in (a) 2015 and (b) 2016, and late season predawn water potential of Cabernet franc vines growing with different under-vine cover crops in (c) 2015 and (d) 2016. There were no significant differences among the treatments in midday stem water potential for both 2015 and 2016 and late season predawn water potential in 2016 using mixed model ANOVA following with Dunnett's test at 5% significance level. NV = Natural Vegetation, GLY = Glyphosate, CHI = Chicory, ALF = Alfalfa, FES = Fescue and TR = Tillage Radish.

Soil property parameters

Soil property parameters were generally increased by cover crops except for TR compared to the GLY control (Table 2.9). Stable wet soil aggregates were improved to 20.3% in NV and 20.5% in ALF compared to 11.1% in GLY in 2015. Organic matter content was raised to 3.36% in CHI compared to 2.97% in GLY in 2015, and increased to 3.33 and 3.36% in NV and FES respectively compared to 3.02% in GLY in 2016. Microbial respiration rate was increased 64% by NV in 2015 and 75% by FES in 2016. Soil carbon mineralizability lined-up with the microbial respiration rate where NV in 2015 and FES in 2016 was 54% and 68% higher, respectively, compared to GLY in each year.

In 2015, soil morgan-extractable nutrients were analyzed (Table 2.10). There were no differences found between any of the treatments and control soil regarding P, K, Fe and Zn content. Soil Mg content was increased 21%, 18% and 17% by NV, CHI and FES respectively; while Mn content was increased 35% and 38% by CHI and FES respectively. Soil nutrient data was not collected in 2016. There were no soil bulk density differences found between any of the treatments and control in any of the soil depth from 0-15cm (Table 2.11).

Table 2.9. Property parameters of the under-vine soil treated with different cover crops.

Treatment[a]	Aggregate Stability (%)		Organic Matter (%)	
	2015	2016	2015	2016
GLY	11.1±2.1[c]	17.1±2.0	2.97±0.10	3.02±0.07
NV	20.3±2.1*[d]	21.5±2.0	3.13±0.10	3.33±0.07
TR	12.1±2.1	17.5±2.0	3.19±0.10	3.28±0.07
CHI	15.5±2.1	22.5±2.0	3.36±0.10	3.24±0.07
FES	12.1±2.1	20.3±2.0	3.21±0.10	3.36±0.07
ALF	20.5±2.1*	23.6±2.0	3.27±0.10	3.15±0.07
P-value[b]	0.028	0.196	0.065	0.057
Treatment	Microbial respiration (mg CO2 g/14days)		C Mineralizability (mg CO2/g OC)	
	2015	2016	2015	2016
GLY	1.17±0.18	0.89±0.11	39.5±4.78	29.34±3.08
NV	1.92±0.18	1.33±0.11	60.8±4.78*	39.84±3.08
TR	1.55±0.18	1.16±0.11	48.2±4.78	35.5±3.08
CHI	1.58±0.18	1.09±0.11	46.6±4.78	33.8±3.08
FES	1.33±0.18	1.56±0.11**	41.4±4.78	46.2±3.08*
ALF	1.56±0.18	1.29±0.11	47.8±4.78	41.4±3.08
P-value	0.072	0.025	0.046	0.035

[a]Treatment: GLY = Glyphosate, NV = Natural vegetation, CHI = Chicory, TR = Tillage Radish, ALF = Alfalfa, FES = Fescue.
[b]P-value: The P-value was derived from mixed model ANOVA at $\alpha=0.05$.
[c]Pooled standard error
[d]Significance designation of Dunnett's test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Table 2.10. Soil Morgan-extractable nutrients analysis of under-vine soil treated with different cover crops in 2015 after harvest

Treatment[a]	P	K	Mg	Fe	Mn	Zn
	(mg/kg soil)					
GLY	20.0 ± 1.86[c]	344 ± 34.5	128 ± 5.33	0.59 ± 0.06	8.33 ± 0.57	1.75 ± 0.16
NV	21.5 ± 1.86	402 ± 34.5	155 ± 5.33 **[d]	0.48 ± 0.06	9.82 ± 0.57	1.54 ± 0.16
TR	20.2 ± 1.86	383 ± 34.5	142 ± 5.33	0.53 ± 0.06	10.4 ± 0.57	1.68 ± 0.16
CHI	21.2 ± 1.86	459 ± 34.5	151 ± 5.33 **	0.62 ± 0.06	11.2 ± 0.57 *	1.97 ± 0.16
FES	22.0 ± 1.86	404 ± 34.5	150 ± 5.33 *	0.53 ± 0.06	11.5 ± 0.57 **	1.63 ± 0.16
ALF	19.4 ± 1.86	384 ± 34.5	138 ± 5.33	0.71 ± 0.06	10.4 ± 0.57	1.90 ± 0.16
P-value[b]	0.914	0.350	0.005	0.216	0.022	0.479

[a]Treatment: GLY = Glyphosate, NV = Natural vegetation, CHI = Chicory, TR = Tillage Radish, ALF = Alfalfa, FES = Fescue.
[b]P-value: The P-value was derived from mixed model ANOVA following with Dunnett's test at α=0.05.
[c]Pooled standard error
[d]Significance designation of Dunnett's test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Table 2.11. Bulk density of under-vine soil treated with different under-vine cover crops at different depths in third year of the experiment (2016)

Treatment[a]	0-5cm	5-10cm	10-15cm
	(g/cm^3)		
GLY	1.11 ± 0.05[c]	1.23 ± 0.03	1.30 ± 0.03
NV	1.06 ± 0.05	1.21 ± 0.03	1.29 ± 0.04
TR	1.07 ± 0.05	1.20 ± 0.03	1.30 ± 0.04
CHI	1.12 ± 0.05	1.18 ± 0.03	1.25 ± 0.03
FES	1.17 ± 0.05	1.19 ± 0.03	1.23 ± 0.03
ALF	1.12 ± 0.05	1.17 ± 0.03	1.31 ± 0.03
P-value[b]	0.238	0.706	0.448

[a]Treatment: GLY = Glyphosate, NV = Natural vegetation, CHI = Chicory, TR = Tillage Radish, ALF = Alfalfa, FES = Fescue.
[b]P-value: The P-value was derived from mixed model ANOVA following with Dunnett's test at α=0.05.
[c]Pooled standard error

Discussion

This study revealed that under-vine cover crops can consistently reduce vine vegetative growth in a vigorous, mature, cool climate vineyard without reducing yield, which is a major economic concern to growers. Previous studies have demonstrated that alleyway cover crops altered vine balance by maintaining the same level of yield with reduced pruning weight in warm and hot climate mature vineyards in Spain (Pérez-Álvarez et al., 2015) and Portugal (Lopes et al., 2008). However, under-vine cover crop studies done in cool climates demonstrated little to no impact on vegetative growth and Ravaz index (Jordan, 2014, Centinari et al., 2016). Chicory growing under-vine was found to reduce vine canopy density and pruning weight but did not impact on yield in New Zealand (Wheeler et al., 2005). However, the study was conducted in a young vineyard and presented only a single year of results.

In this study, chicory growing under-vine most effectively and consistently reduced the vine vegetative growth including pruning weight and canopy structure, while other cover crops showed inconsistent effects on the same parameters. The inconsistency of the cover crop effect on vine growth may due to different growing habits of the cover crops such as rooting pattern, depth and density that may trigger different water and nutrient dynamics in the soil (Perkons et al., 2014, Sainju et al., 1998, Karl et al., 2016a). The timing of the competition was likely different between weeds and cover crop due to different timing of establishment and growth. Also, the interaction of the cover crop with year to year weather variation especially the uneven precipitation resulting in inconsistent ground coverage by the cover crops might have a significant impact. As for the experimental site, drastically different weather patterns were found during the experiment which may have confounded results.

The reduction of vegetative growth in the CHI treatment led to the high crop load as

shown by Ravaz index which exceeded the recommended range of 5 to 10 for quality table and wine grapes production under various of trellis systems (Kliewer and Dokoozlian, 2005). However, the grape ripeness level was not compromised as the berry soluble solids and TA of CHI treatment did not statistically differ from that of GLY control. Although wines were not made in this study, the grape harvest parameters indicated that the reduced vegetative tissue including leaf layers and shoots were unnecessary to ripen the fruit to commercially acceptable standards for the region. Further study on wine sensory properties and other chemical compounds such as secondary metabolites are required.

Resource competition was considered to be the reason for reduced vine growth in the previous cover crop studies (Wheeler et al., 2005, Monteiro and Lopes, 2007, Tan and Crabtree, 1990). In our study, however, there were no clear associations of reduction of water potential and nutrient content with the vine growth in the cover crop treatments except for TR and FES. Predawn soil water potential, which is often used as soil water indicator (Winkel and Rambal, 1993), was reduced in 2015 by TR. Pruning weight and leaf layer number were also reduced in the TR in 2015. In 2016, the reduction of midday stem water potential early and late growing season, and late season predawn water potential in FES treatment may also explain the reduced leaf layer number. Since the seeds of fescue were broadcast in late May, they likely grew vigorously starting June to provide aggressive water competition. The water competition of FES might have a critical impact on vine vegetative growth especially in the early growing season as it was found that vines are sensitive to water deficit before veraison and can lead to inhibition of vegetative and reproductive growth (Matthews and Anderson, 1989, Hardie and Considine, 1976).

The mechanism behind the reduction of vegetative growth in CHI treatment remained

unclear as the treatment did not significantly impact vine water and nutrient status. For nutrient status, petiole analysis showed that CHI led to minor fluctuations in some of the nutrients but these nutrients were all in the optimal range (Wolf, 2008). Nitrogen competition was suggested as the reason for reduced vine vegetative growth in many cover crop studies in various climates (Celette et al., 2009, Pérez-Álvarez et al., 2015, Wheeler et al., 2005). However, there were no statistical differences between any of the treatments and control vines in the petiole N content in this study. Although the nitrogen content of vines from both treatments and control were at the borderline or lower than the recommended value (1.2% at bloom and 0.8% at latter stage) according to Wolf (2008), there was no visual nutrient deficiency symptom observed. This phenomenon was also observed in previous studies done in the region (Centinari et al., 2016, Jordan et al., 2016, Karl et al., 2016a). Previous studies showed that using barley as a cover crop reduced top soil nitrate availability since the first year of the experiment and yet the effect of reduction of nitrogen only showed up in plant tissue in the third year and grape must in the fourth (Pérez-Álvarez et al., 2015). This finding suggested that the nutrient competition started at the soil first, and then the reduction in nutrient showed up in vegetative tissues and grapes. With only petiole N and fruit YAN measured in this study, it was unknown if the N competition actually happened at the soil level in this study. Although the same petiole nutrient status were found in vigorous vines in GLY control and smaller vines in CHI treatment, the equality of the nutrient status may due to the dilution effect (Jarrell and Beverly, 1981) where the nutrient uptake in a large vine with high nutrient availability equals to that in a small vine with low nutrient availability.

Although the range of predawn water potential found in our study falls between -2 to -4 bars, which is classified as mild water stress range (Ojeda et al., 2002), no visual symptom of

water stress in any of the growing seasons was observed. Although CHI reduced vine vigor, it did not impact on the vine water status in any of the years. This was not the first study to report that reduced vine vigor as a result of cover cropping had no association with vine water status. One study found that interrow tall fescue growth did not impact vine midday stomata conductance and predawn leaf water potential but reduced pruning weight by approximately five t/ha (Celette et al., 2005a) in a young Sauvignon Blanc vineyard in a Mediterranean climate. Another study done in the cool climate Finger Lakes region of New York also showed that under-vine white clover reduced pruning weight of young Cabernet franc vines without impacting midday stem and predawn leaf water potentials (Karl et al., 2016a). Aside from water and nutrient competition, cover crops may also have allelopathic effects on vine growth as suspected by previous studies (Wolpert et al., 1993, Celette et al., 2005b). In fact, tall fescue has been demonstrated to allelopathically suppress the below- and aboveground growth of young pecan trees (Smith et al., 2001).

Berry composition was not impacted by most of the cover crops which was similar to previous studies (Monteiro and Lopes, 2007, Tesic et al., 2007). As an exception, CHI reduced berry YAN compared to GLY in 2015. A reduction in grape must YAN as a result of cover crops has often been coupled with an association with reduced petiolar nitrogen content (Sweet and Schreiner, 2010, Pérez-Álvarez et al., 2015, Karl et al., 2016a). Previous studies showed that winegrapes in Finger Lakes generally had low YAN (Karl et al., 2016a, Nisbet et al., 2014). The YAN of grapes from all of the treatments and control in this study also had lower than the recommended content for healthy fermentation (Boulton et al., 2013, Bell and Henschke, 2005) so nitrogen adjustment may be required regardless of under-vine cover crop treatment.

Under-vine soil physical, chemical and microbial health were improved by under-vine

cover crops except TR. The lack of improved soil properties with TR may because of its growing habit. Tillage radish is known to grow actively in the fall, producing a high amount of biomass with a low C/N ratio (Weil et al., 2009) but does not favor the growth of plant beneficial fungi and other soil microorganisms due to its *Brassicaceae* biofumigation effect (White and Weil, 2010, Sarwar et al., 1998). Thus, great amounts of biomass were still produced but did not built soil OC. Although a previous study showed that using barley and clover as cover crops did not impact vineyard soil P, K and Mg content in Spain (Pérez-Álvarez et al., 2015), Mg was increased in the soil by NV, CHI and FES treatments in 2015 in this study. Cover crops enriched vineyard soil organic matter, increased soil aggregation, improved microbial respiration rate, and resulted in higher nutrient mineralization in the previous studies (Steenwerth and Belina, 2008, Peregrina et al., 2010, Ruiz-Colmenero et al., 2013). In this study, organic matter in the soil was not significantly impacted while microbial respiration rate and mineralizability were improved by FES. The decoupling of soil organic matter content and microbial activity may indicate that FES effectively built the labile carbon in the soil and created microbial friendlily soil environment but the contribution to stable soil organic carbon was dismissible in the short term. Microbial activity is sensitive to the short-term enrichment of labile carbon, such as cover crop residues in this study, that can be readily used as metabolism substrates (Sparling 1997). However, the stabilized soil organic carbon pool takes a long time to accumulate (Smith 2004, Wander et al. 1994).

Conclusion

This experiment showed that the under-vine cover crops can be used to reduce the need for herbicide use, mitigate vine vigor and improve soil health in a mature vineyard in a cool climate region. A previous analysis conducted in the same region found growing under-vine cover crops had a lower cost than maintaining bare soil by applying herbicide (Karl et al., 2016a). However, that study did not recommend using cover crops due to the fact the yield reduction in cover crops treatment could lead to loss of total revenue up to $4,000/ha in a young vineyard. In the Northeastern U.S., yield is key to economic viability for grape growers due to the low profit margin (Yeh et al., 2014). In contrast, this study found that under-vine cover crops could reduce vine vegetative growth but maintain the vine yield. This may because this experiment was conducted in a mature vineyard, which likely had a more extensive root system and carbohydrate reserves than young vines (Holzapfel et al., 2010) potentially making it more resilient to resource competition. However, the practical adoption of the cover crops will require further investigation of their impact on wine quality and careful assessment on their adaptation to the specific sites, grape cultivars and the resulting financial outcomes.

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CHAPTER THREE

Under-vine soil management practices impact vine growth but not wine sensory properties in mature Riesling grapevines

Introduction

The vineyard floor directly under vines is most commonly managed as bare soil by applying herbicide or adopting soil cultivation around the world. However, bare soil is susceptible to soil erosion and run-off (Battany and Grismer, 2000) and can result in groundwater pollution due to pesticides and nutrients leaching into the waterway (Karl et al., 2016a). In addition, constant herbicide application promotes herbicide-resistant weeds (Heap, 2014). Soil cultivation, or tillage, physically breaks down the soil aggregates, increases dissolvable carbon in the soil leachate and risks reduction of the long-term mineral nutrient pool (Paustian et al., 1997, Elliott, 1986, Karl et al., 2016a)

Under the cool and humid conditions in the Finger Lakes, excessive vegetative growth of grapevines is the result of high precipitation during the growing season and fertile soil. In wet years, pruning weight often exceeds one kilogram per vine for vines on1.8m in-row spacing (Jordan et al., 2016, Karl et al., 2016b). Since under-vine vegetation can provide water and nutrient competition to the vines, reducing competition by reducing or eradicating the vegetation enables higher resource availability and further promotes vine vigor (Wheeler et al., 2005, Giese et al., 2014, Karl et al., 2016b). High vigor vines give rise to increased canopy density and shaded fruit, reducing quality attributes including soluble solid, pH, tartrate/malic acid ratio, polyphenol, coloration (Smart, 1985), and wine sensory properties (Morrison and Noble, 1990,

Cortell et al., 2008). Many aromatic compounds including 1,1,6-trimethyl1,2-dihydronaphthalene (TDN) and b-damascenone are increased with reduced canopy density and increased cluster light exposure in Riesling (Meyers et al., 2013), although whether the increase in TDN is positive from a consumer response is debatable (Sacks et al., 2012, Ross et al., 2015). Thus, in cool climate viticultural regions, allowing vegetation to grow under vines as a substitution for bare soil may offer an opportunity for reducing herbicide pollution, reducing erosion, lowering nutrient leaching, and mitigating vine vigor through water and nutrient competition which could be beneficial to commercial growers.

In a young vineyard, chicory growing under vines effectively reduced the Cabernet Sauvignon vine petiole nitrogen content, shoot growth, leaf size, pruning weight and resulted in riper grapes and better rated wines in the cool climate of New Zealand (Wheeler et al., 2005). In the Finger Lakes region of New York state, under-vine native vegetation reduced vine pruning weight, canopy density and berry pH, titratable acidity and yeast assimilable nitrogen content of young Cabernet franc vines but had no noticeable impact on wine sensory properties (Karl et al., 2016b). However, young grapevines with smaller root systems may be more effected by the cover crop induced resource competition in the top soil horizons.

Studies of under-vine floor management affects in mature vineyards are limited and suggest inconsistent results with respect to vine vegetative growth, reproductive growth and wine sensory properties. A previous study conducted in a mature Cabernet franc vineyard demonstrated that under-vine annual ryegrass reduced pruning weight and canopy density in the third year of the experiment without impacting on berry brix, pH and titratable acidity (Centinari et al., 2016) but wine sensory properties were not examined. Another study in a mature Riesling vineyard found that under-vine annual ryegrass, buckwheat and resident vegetation had no

impact on vine petiole nutrient content at veraison, midday stem water potential, predawn leaf water potential, vegetative growth, yield, and berry composition (Jordan et al., 2016). In that same study, aroma characteristics of wines made from vines with under-vine cover crops and herbicide strip diverged from each other but the reasons remained unknown. This indicated that further research would be required to understand the mechanism and the effects of under-vine floor management practices on mature vine growth and wine sensory properties, as well as the potential use in mature vineyards.

To investigate the under-vine floor management practices impact on wine sensory properties, vine physiological and vineyard microbial factors were examined in this and next Chapter respectively. This study aimed to examine the vine physiological factors including shoot growth, canopy structure, water status and nutrient status of mature and vigorous Riesling vine in the cool climate region of the Finger Lakes, NY. In this presented study, it was hypothesized that allowing weeds to grow under vines would improve soil health, engage resource competition, mitigate vine vigor and reduce canopy density, thereby affecting berry composition and wine sensory properties.

Material and methods

Experimental setup

This experimental setup was used from 2014-2016 for both the vine physiological study and the vineyard microbiome study presented in Chapter 3 and Chapter 4, respectively. The research plot was located in Ovid, NY (42.66˚N, -76.71˚W) with soil type of Howard gravely loam according to Soil Survey Staff (1975). Riesling cl. 239 vines grafted onto Couderc 3309 rootstock were planted in 1999 in a South-North row orientation and trained to a Scott-Henry trellis system. The in-row vine spacing was 2.13m and inter-row spacing was 2.74m. The vineyard was managed according to standard practices (Wolf, 2008). The vines were cane-pruned in February each year to a consistent bud number, on average 16.4 buds per linear meter (40 buds per vine), not including one extra cane that served the dual function of kicker and winter damage back-up cane, which was removed before bloom. The vines were hedged periodically throughout the growing season when shoots grew well above the top catch wires.

The experiment investigated two under-vine floor treatments in addition to a glyphosate maintained bare soil as the control. A randomized complete block design was applied to enable four replicates for each of the three treatments. Treatments were randomly assigned to the experimental units, which are one meter wide under-vine soil strips, within each block. Each experimental unit was across three rows with nine consecutive vines in a row (27 vines per experimental unit, 81 vines per rep, 324 vines in the experiment). The vine growth, yield and soil samples were measured and collected from the middle three vines and the 1m × 6m under-vine soil strip, in the middle row from each of the experimental unit where the other vines were served as guards for physical and spatial buffering. A permanent interrow cover crop, a mix of fescue, white clover and weeds, was maintained separately and mowed periodically.

Under-vine floor management practices

The under-vine floor management treatments included natural vegetation (NV), soil cultivation (CULT) and Glyphosate (GLY) application. The GLY treatment (control) was maintained by applying Roundup (Roundup® PRO concentrate, Monsanto, St. Louis MO). For the NV treatment, the weeds were allowed to grow freely. Soil was cultivated, when the weeds reached 50% ground coverage by visual assessment, in June 2014, June and July 2015, and June and July 2016 for CULT treatment. The GLY control was established with Roundup application, in which Glyphosate was the active ingredient, with 2.9 kg a.i./ha application rate of 2% solution on 24 June and 16 July 2014, 16 June 2015 and 15 June 2016. The groundcover in NV was trimmed only in 2015 using a string trimmer on 8 - 9 August as the vegetation was reaching the fruiting zone.

Ground coverage assessment and weed identification

On September 2015 and August 2016 at late veraison, two 400cm^2 square-shaped grids were randomly chosen in each experimental unit. For each chosen grid, a digital photo was taken at 1.5m vertically above with measuring tape placed horizontally on the ground as photo scaling reference. The chosen grid within each digital photo was analyzed with ImageJ Version 1.50b (open resource via http://imagej.nih.gov/) to define ground percentage cover with image processing steps similar to Ricotta et al. (2014). The digital photos taken from the NV treatment were also used for visual weed species identification.

Shoot growth measurement

Length and diameter of primary and lateral shoots were measured. Four shoots per data

vine were randomly marked in the beginning of each growing season to represent the primary shoot growth dynamic for that vine throughout the growing season. Shoot diameter was measured (mm) by using calipers at the middle of internode one above the first fully developed bud where two measurements were taken per shoot. The two numbers were averaged to represent the diameter of each shoot. Shoot length was measured (cm) with a measuring tape from primary shoot base to shoot tip. Length and diameter of the basal lateral shoot were recorded using the same methods described for the primary shoot. Lateral shoots from the same primary shoots were measured starting from the first hedging and continuing until the second hedging or shoot thinning which led to an inability to track tagged lateral shoots.

Canopy architecture - EPQA

Point quadrat analysis (PQA) (Smart and Robinson, 1991) and enhanced point quadrat analysis (EPQA) (Meyers and Vanden Heuvel, 2008) were conducted to characterize canopy light environment at veraison, 25 August 2015 and 19 August 2016. Basic PQA was conducted by inserting a thin wooden stick horizontally through the fruiting zone in perpendicular to the row at 20cm interval on a per panel basis. Any leaf and cluster contact with the stick end was recorded. On the same day of measuring PQA, light environment of the canopy at fruiting zone was also measured using a ceptometer (Decagon, model AccuPAR LP-80, Pullman, WA), which recorded the photon flux, with an ambient flux sensor attached. Two measurements were taken per data vine on each side of the fruiting zone within one hour of solar noon. The ambient flux sensor was pointed vertically toward the sky above canopy without any shade throughout the ceptometer measurement. The light interception was calculated dividing fruiting zone photon flux by ambient photon flux and presented in percentage. Light interception and PQA data were

pooled into Canopy Exposure Mapping Tools, version 1.7 (available via Jim Meyers, jmm533@cornell.edu) to calculate leaf layer number, occlusion layer number, interior leaf percentage, interior cluster percentage, cluster exposure layer and cluster exposure flux availability.

Vine water and nutrient status measurements

Vine water status including midday stem and predawn leaf water potential were measured according to Fulton et al. (2001) with a pressure chamber (Soil Moisture Equipment Corporation, model 3005F01, Santa Barbara, CA). Midday stem water potential was measured roughly every two weeks at solar noon with one hour deviation and predawn water potential was measured during the late growing season during fruit ripening on 11 September 2015and 13 September 2016 at 4AM EST with one hour deviation. For midday stem water potential measurements, fully expanded healthy young leaves were bagged with a 500ml alumina foil covered Ziploc bag for 15mins before measurement. Each leaf was cut from the shoot with a sharp blade, transferred immediately into the pressure chamber, and pressurized at about 1bar/sec to the point when xylem sap moisturized the cut surface of the petiole.

For each experimental unit, 100 petiole samples were collected from young fully expanded leaves at roughly full bloom on 20 June 2015 and 24 June 2016, and veraison on 4 September 2014, 24 August 2015 and 26 August 2016. The samples were washed with mild soap, rinsed with deionized water and sent to Cornell Nutrient Analysis Laboratory (CNAL) for total carbon and nitrogen using a combustion method (Campbell and Plank, 1998). Macro- and micronutrients (Al, B, Ca, Cu, Fe, K, Mg, Mo, Mn, Na, P, Zn) were measured using dry ash extraction method.

Yield components and juice composition measurements

Yield data were collected on a per vine basis at commercial harvest as determined by the grower on 30 October 2014, 11 October 2015 and 15 October 2016. The clusters were harvested from each vine and counted before being pooled in a plastic lug to measure the total yield per vine by weighing with the hanging scale (Salter Brecknell, model SA3N340, accuracy ±0.1kg, Fairmont, MN). The average weight per cluster was calculated by dividing the total weight of the fruit per vine by the number of clusters. The berry weight was determined by averaging the weight of 100 berries that were collected on a per experimental unit basis at harvest. The berries were stored in Ziploc bag at -20˚C after harvest until weighed (Santorius ELT103, accuracy ±0.001g, Goettingen, Germany).

Winter pruning weight was collected on a per vine basis in December for downward shoots and February for the upwards shoots of each year (as determined by the cooperating grower's standard practice). In 2016, although the downward shoots were pruned by the vineyard crew prior to data collection, the pruned shoots remained directly under the vine. As a result, the data were collected from the shoots that were reconstructed by identifying the size, shape and color of the cut surfaces. Pruning weight was used as an indicator for vine vegetative growth. To determine Ravaz index, yield was divided by pruning weight. Cluster compactness parameters were measured in the third year of the experiment where 10 clusters were randomly sampled from each of the experimental unit. Berries of each cluster were detached from the rachis and counted. The length of the rachises (cm) were recorded.

Twenty clusters were randomly collected from each experimental unit at harvest for juice composition analysis. The clusters were stored at -20˚C freezer until analysis. They were thawed in a 4˚C cooler, whole cluster pressed and the juice was filtered through cheesecloth

before measuring soluble solids, titratable acid (TA), pH, and yeast assimilable nitrogen (YAN) using a temperature compensating digital refractometer, titration 50 mL aliquot of juice against 0.10 M NaOH to pH 8.2, a benchtop pH meter (VWR Symphony pH Meter, model SB80RI, Radnor, PA), and a Chemwell 2910 multianalyzer to measure ammonia and spectrophotometry to measure primary amino nitrogen (Nisbet et al., 2013). YAN was only quantified in 2014 and 2015.

Soil property parameters

Under-vine soil samples were collected at the end of growing season in November 2015 and 2016 for soil health analysis according to Gugino et al. (2009). Six soil cores from the top 20 cm soil were taken from each experimental unit, combined, and sent to CNAL for soil health assessment including wet aggregate stability, organic matter content and microbial respiration rate for both 2015 and 2016. Extra tests of organic matter, Morgan-extractable Phosphorus, Potassium, Iron, Manganese and Zinc were conducted in 2015 only. In November 2016, four intact soil cores were taken per experimental unit from the top 15cm soil for soil bulk density measurement. The soil samples were manually stratified in an interval of 5cm, dried in oven at 60˚C overnight, weighed, and divided the weight by volume for bulk density calculation.

Winemaking and Sensory analysis

In each year, grapes from each treatment were obtained at commercial harvest as described above and fermented in duplicate at the Vinification and Brewing Laboratory at Geneva, NY using standard white winemaking procedures. Briefly, within 24hr after harvest the clusters were destemmed, crushed, pressed, 50ppm SO2 added to the juice, settled overnight at

4°C, racked from the sediment and contained in 114L stainless steel jacketed fermenters. Commercial yeast *Sacharomyces cerevisiae* strain EC-1118 at 0.25 g/L, fermentation aid Go-Ferm Protect® and Fermaid® K (Lallemand, Petaluma, CA) as per manufacturer's directions were added to conduct the fermentation. The wines were fermented at 15°C until dryness as tested with Clinitest tablet (Bayer, West Haven, CT) and did not undergo malolactic fermentation nor acid adjustment. Wines were brought up to 40 ppm SO2, cold stabilized at 2°C for three months, tasted for faults before being bottled in 750ml green glass bottles and stored at 15°C.

Wines derived from the process described above were subjected to sensory evaluation to determine whether they were aromatically similar or different. The sensory studies were conducted on 13 September 2016, 21 April 2017 and 18 May 2017 for the wines of 2014, 2015 and 2016 vintages, respectively, at Cornell University, Ithaca, NY. The sensory evaluation process was according to Lawless and Heymann (2010) and Jordan et al. (2016). For each year, the panelists were comprised of 100 panelists who self-reported to drink white wine at least once a month. The panelists were seated in a room with white fluorescence light at a wooden table separated by white cardboard partitions to isolate each of them. The setting of each spot included 50ml of each wines, all the field treatments and control in duplicate, contained in ISO tasting glasses with plastic lids on top, a sorting sheet that included a short survey about drinking frequency, age and gender, and a pencil to fill out the sheet. The wines were presented simultaneously with randomly generated three-digit codes on each of the glasses. The panelists were asked to smell all the wine, group the wines based on the overall aroma similarity, and to complete the survey without time limitation. Similarity scores were assigned to the results derived from each of the sensory panelist. Score of one was given to the wines that were grouped, while zero was given to the wines that were not in the same group. The cumulative

scoring results of each year were transferred into a similarity square matrix and analyzed using single dimensional scaling in R version 3.2.4.

Climate data

Climate data from Romulus, NY and Varick, NY for 2014 and Ovid, NY stations for 2015 and 2016 were obtained from the Cornell University Network for Environment and Weather Applications (NEWA). The distances between each of the weather stations and the experimental site were 17.8km, 5km and 4.1km for Varick, Romulus, and Ovid respectively. The weather stations were all northwest of the experimental site. The Romulus and Varick data were used for Growing Degree Days (GDD) and precipitation respectively due to the weather station at Ovid malfunctioning in 2014. The growing degree days were calculated using 10˚C as baseline for the growing seasons.

Table 3.1. Growing Degree Days (GDD) base on 10˚C and precipitation of the experimental site during the growing season from 2014 to 2016.

Month	GDD (˚C)			Precipitation (cm)		
	2014[a]	2015[b]	2016	2014[c]	2015	2016
April	28.3	29.9	24.4	7.1	6.6	3.8
May	165.2	210.6	125.2	4.6	3.4	5.8
June	294.0	293.7	291.2	5.5	3.8	1.8
July	335.7	360.3	406.7	7.0	6.8	3.7
August	289.2	328.2	423.8	8.1	3.3	11.0
September	225.0	297.3	271.7	1.3	7.9	7.6
October	94.1	65.5	104.6	2.2	5.2	10.9
Sum	1431.4	1585.6	1647.5	35.7	37.1	44.6

[a]Data obtained from Romulus, NY station.
[b]GDD and precipitation data of 2015 and 2016 were obtained from Ovid, NY station.
[c]Data obtained from Varick, NY station.

Year 2016 had highest overall precipitation among the three experimental years (Table 3.1). However, due to the high precipitation in August and October, the growing season of 2016 from late May to early August was the driest among the three years. Precipitation in 2015 was more equally distributed during the growing season whereas 2014 had wetter early growing season and drier late growing season from September to October. The temperature in 2016 was the warmest with 16478GDD followed by 2015 with 15856GDD and 2014 with 1431GDD.

Statistical methods

Normality assumptions were checked for all the data. The data were analyzed with mixed-model ANOVA, where under-vine floor treatment was classified as a fixed effect and blocks as a random effect, using JMP Pro version 12.0.1. The post-hoc analysis for pair-wised comparison was performed using Dunnett's test comparing treatment means with mean of GLY control at $\alpha=0.05$ significance level.

Results

Under-vine floor coverage

The ground coverage in the NV treatment was significantly higher than that of GLY control, while CULT was the same as GLY in both 2015 and 2016 (Fig. 3.1). NV ground coverage was four and 42 times more than the vegetation coverage of GLY in 2015 and 2106, respectively. There was no difference in the CULT and GLY in proportion ground coverage in 2015 and 2016. The weeds identified in the NV treatment are listed in Table. 3.2.

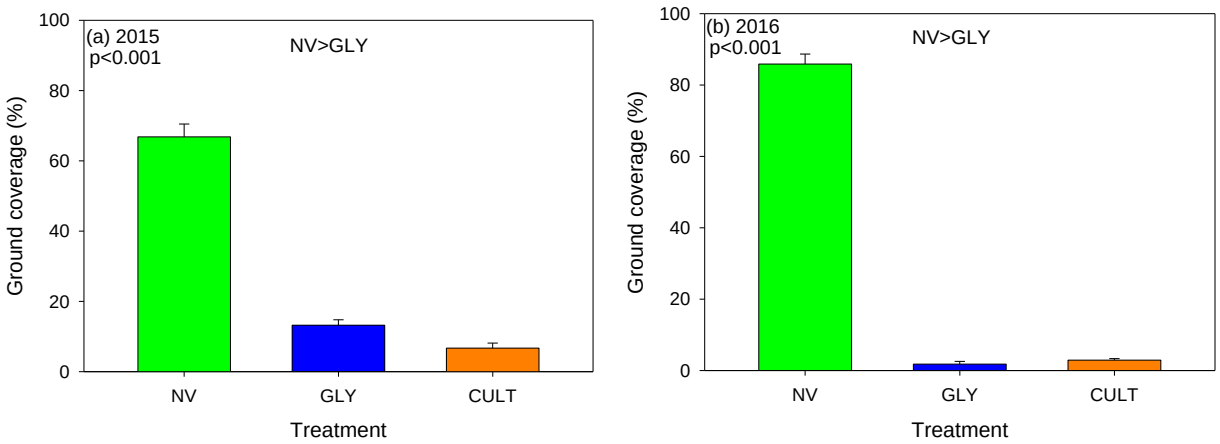


Figure 3.1. Proportion of Riesling vineyard under-vine soil covered with weeds at veraison in (a) 2015 and (b) 2016. NV = Natural Vegetation, GLY = Glyphosate, CULT = Soil Cultivation. The bars indicate standard errors. The significant differences between treatment and control were found using mixed model ANOVA following with Dunnett's test at 5% significance level. The significance symbol *** indicates P-value <0.001.

Table 3.2 Weed species identified in under-vine natural vegetaion treatment at veraison in 2015 and 2016

2015		2016	
Common name	Scientific name	Common name	Scientific name
Birdseye pearlwort	*Sagina procumbens* L.	Birdseye pearlwort	*Sagina procumbens* L.
Blackseed plantain	*Plantago lanceolata* L.	Blackseed plantain	*Plantago lanceolata* L.
Clammy ground cherry	*Physalis heterophylla* Nees.	Clammy ground cherry	*Physalis heterophylla* Nees.
Common blue violet	*Viola sororia* Willd.	Common blue violet	*Viola sororia* Willd.
Common burdock	*Arctium minus* Bernh.	Common burdock	*Arctium minus* Bernh.
Common lambsquarters	*Chenopodium album* L.	Common milk weed	*Asclepias syriaca* L.
Common mallow	*Malva neglecta* Wallr.	Dallisgrass	*Paspalum dimidiatum* L.
Common milk weed	*Asclepias syriaca* L.	Dandelion	*Taraxacum officinale* F.H. Wigg.
Dallisgrass	*Paspalum dimidiatum* L.	Eastern black nightshade	*Solanum ptychanthum* Dunal.
Dandelion	*Taraxacum officinale* F.H. Wigg.	Horsenettle	*Solanum carolinense* L.
Eastern black nightshade	*Solanum ptychanthum* Dunal.	Johnsongrass	*Sorghum halepense* (L.) Pers.
Horsenettle	*Solanum carolinense* L.	Lesser-seeded bittercress	*Cardamine oligosperma* Nutt.
Horseweed	*Erigeron canadensis* (L.) Cronquist	Oxeye daisy	*Leucanthemum vulgare* Lam.
Johnsongrass	*Sorghum halepense* (L.) Pers.	Powell amaranth	*Amaranthus powellii* S.Wats.
Large crabgrass	*Digitaria sanguinalis* (L.) Scop.	Red clover	*Trifolium pratense* L.
Lesser-seeded bittercress	*Cardamine oligosperma* Nutt.	Roughstalk bluegrass	*Poa trivialis* L.
Oxeye daisy	*Leucanthemum vulgare* Lam.	Smartweed	*Persicaria lapathifolia* (L.) Delabre.
Powell amaranth	*Amaranthus powellii* S.Wats.	Smooth pig weed	*Amaranthus hybridus* L.
Red clover	*Trifolium pratense* L.	Sow thistles	*Sonchus oleraceus* L.
Rough Cinquefoil	*Potentilla norvegica* L.	Speedwell	*Veronica officinalis* L.
Roughstalk bluegrass	*Poa trivialis* L.	Staghorn sumac	*Rhus typhina* L.
Smartweed	*Persicaria lapathifolia* (L.) Delabre.	Tall fescue	*Festuca arundinacea* Schreb.
Smooth pig weed	*Amaranthus hybridus* L.	Tree of heaven	*Ailanthus altissima* (Mill.) Swingle
Sow thistles	*Sonchus oleraceus* L.	Tumble mustard	*Sisymbrium altissimum* L.
Speedwell	*Veronica officinalis* L.	Velvetleaf	*Abutilon theophrasti* Medik.
Staghorn sumac	*Rhus typhina* L.	White clover	*Trifolium repens* L.
Tree of heaven	*Ailanthus altissima* (Mill.) Swingle	Wild buckwheat	*Fallopia convolvulus* (L.) Á.Löve
Tumble mustard	*Sisymbrium altissimum* L.	Yellow foxtail	*Setaria pumila* (Poir.) Roem. & Schult.
Velvetleaf	*Abutilon theophrasti* Medik.	Yellow woodsorrel	*Oxalis stricta* L.
White clover	*Trifolium repens* L.		
Wild buckwheat	*Fallopia convolvulus* (L.) Á.Löve		
Yellow foxtail	*Setaria pumila* (Poir.) Roem. & Schult.		
Yellow woodsorrel	*Oxalis stricta* L.		

Vegetative growth

There were no primary and lateral shoot length differences between either of the two treatments (NV, CULT) compared to (GLY) control except the primary shoot length in mid July 2016 where the primary shoot of NV was 33% longer than the control (Fig. 3.2). The proportion of primary shoots that had lateral shoots was the same between either of the treatments and control.

The fruiting zone canopy structure and light environment were impacted by NV in 2015 but not in 2016 (Table 3.3). In 2015, NV reduced the leaf layer number by 36%, occlusion layer

number by 41% and percent interior cluster by 49% compare to that of GLY control. The change of the canopy structure resulted in change of the canopy light environment where the cluster exposure layer and cluster exposure flux availability was reduced 50% and increased 76%, respectively, by NV compared to GLY in 2015. There were no canopy structure and light environment differences found between CULT treatment and GLY in both 2015 and 2016.

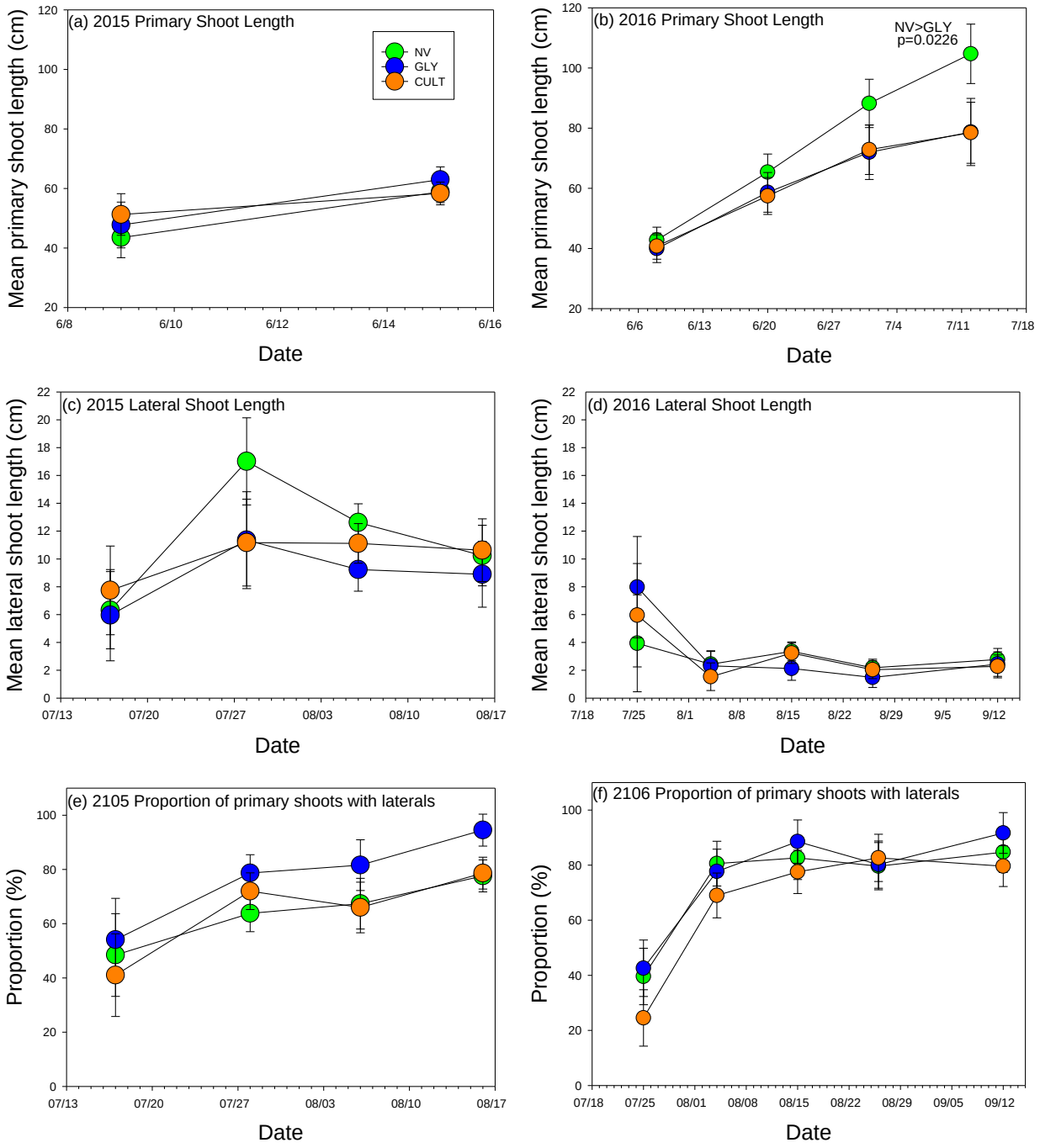


Figure 3.2. Shoot growth of Riesling vine growing with different under-vine cover crop treatments. Primary shoot length before hedging in (a) 2015 and (b) 2016. Lateral shoot in (c) 2015 and (d) 2016 and proportion of primary shoot with laterals in (e) 2015 and (f) 2016 after hedging. NV = Natural Vegetation, GLY = Glyphosate, CULT = Soil Cultivation. Significant difference between treatment and control was found using mixed model ANOVA following with Dunnett's test at 5% significance level.

Table 3.3. Canopy architecture approximation using EPQA analysis of Riesling vines with different under-vine floor management practices in 2015 and 2016 at veraison

2015 veraison			
Treatment[a]	Leaf Layer Number	Occlusion Layer Number	% Interior Leaf
GLY	2.08 ± 0.15[c]	2.99 ± 0.28	32.3 ± 4.5
NV	1.33 ± 0.15 *[d]	1.76 ± 0.28 *	23.5 ± 4.5
CULT	2.08 ± 0.17	2.70 ± 0.33	31.3 ± 5.2
P-value[b]	0.0281	0.042	0.5067
Treatment	% interior cluster	Cluster Exposure Layer	Cluster Exposure Flux Availability
GLY	52.3 ± 5.5	0.56 ± 0.06	0.33 ± 0.03
NV	26.6 ± 5.5 *	0.28 ± 0.06 *	0.58 ± 0.03 **
CULT	48.8 ± 6.4	0.53 ± 0.06	0.48 ± 0.04
P-value	0.0452	0.0303	0.0138

2016 veraison			
Treatment	Leaf Layer Number	Occlusion Layer Number	% Interior Leaf
GLY	0.89 ± 0.10	2.16 ± 0.16	9.65 ± 4.42
NV	0.72 ± 0.10	1.90 ± 0.16	11.6 ± 4.42
CULT	0.74 ± 0.12	1.76 ± 0.19	6.30 ± 5.11
P-value	0.1056	0.2957	0.7613
Treatment	% interior cluster	Cluster Exposure Layer	Cluster Exposure Flux Availability
GLY	21.3 ± 3.7	0.25 ± 0.05	0.51 ± 0.04
NV	15.4 ± 3.7	0.15 ± 0.05	0.57 ± 0.04
CULT	14.3 ± 4.3	0.14 ± 0.06	0.57 ± 0.05
P-value	0.4083	0.2091	0.6516

[a]Treatment: GLY = Glyphosate, NV = Natural vegetation, CULT = Soil Cultivation.
[b]P-value: The P-value was derived from mixed model ANOVA at α=0.05.
[c]Pooled standard error
[d]Significance designation of Dunnett's test: *p<0.05, **p<0.01, ***p<0.001

Yield components, cluster compactness and berry composition

Yield components including yield per vine, Ravaz index, cluster weight, number of clusters per vine, and number of berries per cluster were not affected by the under-vine floor treatments. (Table 3.4). Berry weight was impacted by the under-vine floor management practices in 2014 and 2016 as revealed by mixed model ANOVA with no pairwise differences. In

2015, the vine pruning weight was considerably but not significantly reduced by NV compared to vines from GLY control. No significant difference was found in terms of cropload as Ravaz index showed no differences between either of the treatments and GLY.

Berry composition including pH, Brix, TA and YAN were not impacted by the under-vine floor treatments in all three years of the experiment (Table 3.5). Cluster compactness, measured in the third year of the experiment, suggested that rachis length, berry number per rachis and berry number per unit rachis were all similar between either of the treatment and control (Table 3.6).

Table 3.4. Yield components of the Riesling vines growing with different under-vine floor management practices

Treatment[a]	Yield kg/vine			Pruning Weight kg/vine		
	2014	2015	2016	2014	2015	2016
GLY	7.10 ± 1.08	4.78 ± 0.92	5.69 ± 0.67	1.05 ± 0.13	1.13 ± 0.13	0.91 ± 0.12
NV	5.25 ± 1.05	4.49 ± 0.83	5.11 ± 0.67	0.89 ± 0.12	0.71 ± 0.12 *[c]	0.69 ± 0.10
CULT	5.29 ± 1.05	5.30 ± 0.86	6.79 ± 0.67	0.95 ± 0.12	0.83 ± 0.12	0.94 ± 0.10
P-value[b]	0.3998	0.7713	0.2741	0.7228	0.0712	0.2405

Treatment	Ravaz-Index yield/pruning weight			Number of Cluster cluster/vine		
	2014	2015	2016	2014	2015	2016
GLY	18.41 ± 2.89	4.54 ± 1.53	6.11 ± 1.37	55.2 ± 8.5	54.6 ± 7.3	65.7 ± 6.0
NV	18.19 ± 2.66	8.48 ± 1.33	7.53 ± 1.12	45.3 ± 8.3	51.6 ± 6.5	57.0 ± 5.5
CULT	13.99 ± 2.66	5.93 ± 1.31	7.55 ± 1.12	42.2 ± 8.3	58.7 ± 6.9	71.0 ± 5.9
P-value	0.3779	0.1772	0.686	0.4489	0.8002	0.1572

Treatment	Number of berry kg/cluster			Cluster weight g/cluster		
	2014	2015	2016	2014	2015	2016
GLY	87.0 ± 5.8	52.3 ± 5.2	76.8 ± 7.3	130.7 ± 9.0	81.7 ± 9.0	90.5 ± 7.4
NV	81.4 ± 6.5	52.7 ± 4.7	82.4 ± 6.5	120.4 ± 8.5	85.1 ± 8.3	97.7 ± 6.9
CULT	79.4 ± 5.8	54.2 ± 4.9	90.4 ± 7.3	124.9 ± 8.5	89.2 ± 8.6	95.3 ± 8.0
P-value	0.7165	0.9507	0.4378	0.7433	0.7646	0.8369

Treatment	Berry weight g/berry		
	2014	2015	2016
GLY	1.49 ± 0.04	1.58 ± 0.07	1.12 ± 0.05
NV	1.49 ± 0.04	1.64 ± 0.07	1.19 ± 0.05
CULT	1.57 ± 0.04	1.64 ± 0.07	1.07 ± 0.05
P-value	0.0466	0.475	0.0137

[a]Treatment: GLY = Glyphosate, NV = Natural vegetation, CULT = Soil Cultivation.
[b]P-value: The P-value was derived from mixed model ANOVA following with Dunnett's test at α=0.05.
[c]Significance designation of Dunnett's test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Table 3.5. Berry composition of Riesling vines growing with different under-vine floor management practices

Treatment[a]	Soluble solids Brix			pH		
	2014	2015	2016	2014	2015	2016
GLY	16.9 ± 1.2[c]	18.6 ± 0.4	20.8 ± 0.5	3.17 ± 0.03	3.26 ± 0.02	3.33 ± 0.06
NV	17.9 ± 1.2	18.3 ± 0.4	19.4 ± 0.5	3.10 ± 0.03	3.27 ± 0.02	3.29 ± 0.06
CULT	18.9 ± 1.2	19.1 ± 0.4	20.3 ± 0.5	3.15 ± 0.03	3.30 ± 0.02	3.21 ± 0.06
P-value[b]	0.5773	0.3582	0.2651	0.3359	0.3655	0.4166

Treatment	TA g/L			YAN mg/L		
	2014	2015	2016	2014	2015	2016[d]
GLY	8.13 ± 0.59	6.97 ± 0.33	7.07 ± 2.91	56.7 ± 9.1	107.0 ± 17.6	-
NV	9.63 ± 0.59	7.43 ± 0.33	10.80 ± 2.91	31.2 ± 9.1	93.5 ± 17.6	-
CULT	8.13 ± 0.59	7.30 ± 0.33	8.54 ± 2.91	66.8 ± 9.1	123.1 ± 17.6	-
P-value	0.2117	0.6699	0.4526	0.1015	0.4989	-

[a]Treatment: GLY = Glyphosate, NV = Natural vegetation, CULT = Soil Cultivation.
[b]P-value: The P-value was derived from mixed model ANOVA at α=0.05.
[c]Pooled standard error
[d]2016 YAN data was not collected

Table 3.6. Cluster compactness measurement of Riesling vines growing with different under-vine floor management practices in the third year of experiment in 2016

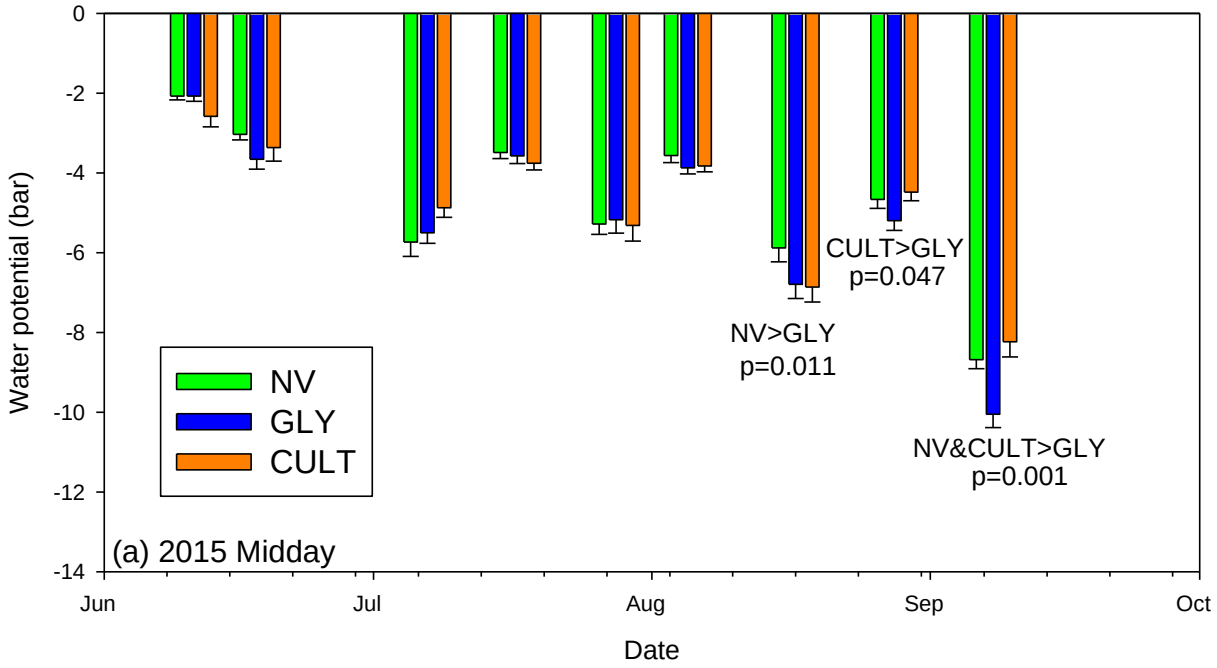
Treatment[a]	Cluster compactness (berry/rachis length)	Rachis length (cm)	Berry number per rachis
GLY	10.8 ± 0.5[c]	9.23 ± 0.54	94.7 ± 5.8
NV	9.42 ± 0.5	8.92 ± 0.54	82.4 ± 5.9
CULT	9.96 ± 0.4	9.07 ± 0.52	91.0 ± 5.4
P-value[b]	0.1430	0.8322	0.0529

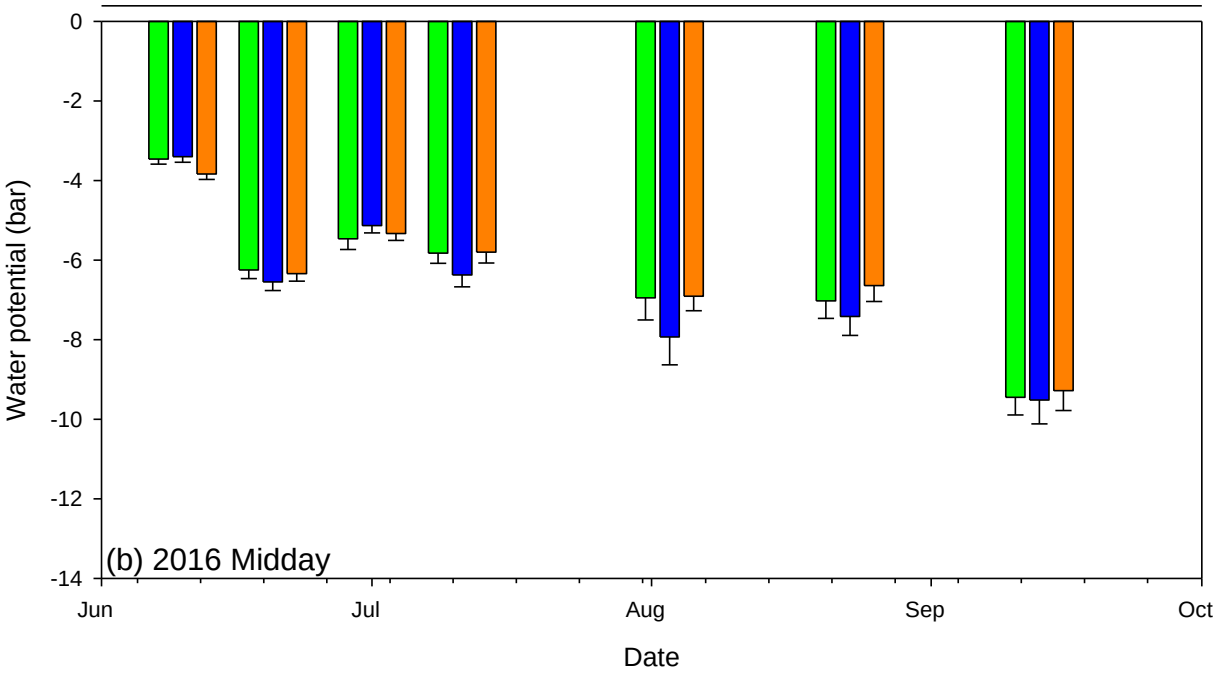
[a]Treatment: GLY = Glyphosate, NV = Natural vegetation, CULT = Soil Cultivation.
[b]P-value: The P-value was derived from mixed model ANOVA at α=0.05.
[c]Pooled standard error

Vine water and nutrient status

Midday stem water potentials of the treatment vines were higher than that of control vines in late season from early August to early September in 2015 (Fig. 3.3a). However, in late season 2015, NV led to lower predawn water potential (-2.13bar) than the GLY control (-1.74bar) (Fig. 3.3c). There were no predawn and midday stem water potential differences between either of the treatments and control in 2016.

Comparing to GLY, petiole N was reduced 12% and 11% by NV and CULT respectively, B was reduced 8% and 12% by NV and CULT respectively, Mn was reduced 20% by NV, and P was reduced 10% by CULT in 2015 by bloom (Table 3.7). NV increased Al by 81% by veraison 2015 and reduced Mn by 24% by veraison 2016 compared to GLY control. The rest of the nutrients in the petiole were the same between either of the under-vine floor treatments and GLY control.


0
-2
-4
-6
-8
-10
-12
-14
Water potential (bar)
NV
GLY
CULT
NV>GLY
p=0.011
CULT>GLY
p=0.047
NV&CULT>GLY
p=0.001
(a) 2015 Midday
Jun
Jul
Aug
Sep
Oct
Date


0
-2
-4
-6
-8
-10
-12
-14
Water potential (bar)
(b) 2016 Midday
Jun
Jul
Aug
Sep
Oct
Date

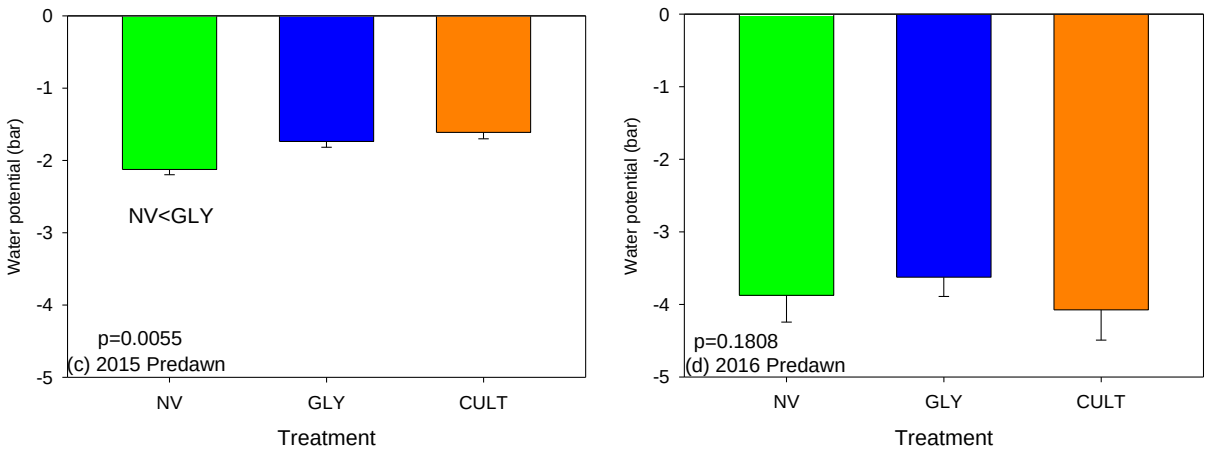


Figure 3.3. Midday stem water potential throughout the growing seasons in (a) 2015 and (b) 2016 and late season predawn water potential in (c) 2015 and (d) 2016 of Riesling vines growing with different under-vine floor management practices. The significant differences were tested using mixed model ANOVA followed with Dunnett's test at 5% significance level. NV = Natural Vegetation, CULT = Soil Cultivation, GLY = Glyphosate.

Table 3.7. Petiole nutrient analysis of Riesling vines growing with different under-vine floor management practices

2015 bloom

Treatment[a]	N	C	Al	B	Ca	Cu	Fe
	%	%	mg/kg	mg/kg	g/kg	mg/kg	mg/kg
NV	0.88 ± 0.03 *[c]	37.7 ± 0.2	11.9 ± 1.4	47.6 ± 1.3 *	15.0 ± 0.5	13.6 ± 0.5	21.8 ± 1.4
GLY	1.00 ± 0.03[d]	37.9 ± 0.2	11.3 ± 1.4	51.8 ± 1.3	15.1 ± 0.5	13.5 ± 0.5	18.8 ± 1.4
CULT	0.89 ± 0.03 *	38.0 ± 0.2	10.6 ± 1.4	45.8 ± 1.3 *	15.5 ± 0.5	12.5 ± 0.5	18.9 ± 1.4
P-value[b]	0.0215	0.565	0.8537	0.0165	0.7801	0.3217	0.2881

Treatment	K	Mg	Mn	Na	P	Zn
	g/kg	g/kg	mg/kg	g/kg	g/kg	mg/kg
NV	21.0 ± 0.9	2.80 ± 0.129	29.4 ± 2.2 **	0.23 ± 0.01	5.40 ± 0.17	37.3 ± 2.6
GLY	22.3 ± 0.9	3.05 ± 0.129	36.8 ± 2.2	0.22 ± 0.01	5.30 ± 0.17	41.9 ± 2.6
CULT	20.0 ± 0.9	2.78 ± 0.129	35.3 ± 2.2	0.23 ± 0.01	4.75 ± 0.17 *	33.9 ± 2.6
P-value	0.1899	0.0947	0.008	0.4932	0.0135	0.1628

2015 veraison

Treatment[a]	N	C	Al	B	Ca	Cu	Fe
	%	%	mg/kg	mg/kg	g/kg	mg/kg	mg/kg
NV	0.56 ± 0.03	40.0 ± 2.1	7.75 ± 0.61 *[d]	40.8 ± 1.4	25.4 ± 1.3	5.25 ± 0.15	11.0 ± 0.6
GLY	0.59 ± 0.03	40.8 ± 2.1	4.29 ± 0.61	38.6 ± 1.4	23.5 ± 1.3	5.13 ± 0.15	9.8 ± 0.6
CULT	0.55 ± 0.03	36.7 ± 2.1	5.16 ± 0.61	38.5 ± 1.4	24.0 ± 1.3	5.01 ± 0.15	10.5 ± 0.6
P-value[b]	0.4424	0.4451	0.0352	0.6006	0.5526	0.5633	0.4385

Treatment	K	Mg	Mn	Na	P	Zn
	g/kg	g/kg	mg/kg	g/kg	g/kg	mg/kg
NV	29.7 ± 0.8	3.50 ± 0.25	40.7 ± 4.8	0.54 ± 0.05	5.40 ± 0.25	45.7 ± 2.5
GLY	29.6 ± 0.8	3.70 ± 0.25	49.8 ± 4.8	0.43 ± 0.05	5.50 ± 0.25	43.3 ± 2.5
CULT	28.7 ± 0.8	3.60 ± 0.25	56.5 ± 4.8	0.45 ± 0.05	5.00 ± 0.25	46.2 ± 2.5
P-value	0.7107	0.886	0.0698	0.3171	0.3674	0.6818

2016 veraison

Treatment	N	C	Al	B	Ca	Cu	Fe
	%	%	mg/kg	mg/kg	g/kg	mg/kg	mg/kg
NV	0.49 ± 0.02	37.1 ± 0.6	43.9 ± 5.0	45.1 ± 1.4	40.4 ± 1.8	43.5 ± 9.5	33.0 ± 3.3
GLY	0.53 ± 0.02	37.1 ± 0.6	37.2 ± 5.0	45.9 ± 1.4	40.6 ± 1.8	24.8 ± 9.5	29.0 ± 3.3
CULT	0.48 ± 0.02	36.6 ± 0.6	38.8 ± 5.7	44.8 ± 1.6	38.6 ± 2.1	28.0 ± 10.9	27.7 ± 3.8
P-value	0.2106	0.7608	0.7612	0.8636	0.7872	0.5828	0.4314

[a]Treatment: GLY = Glyphosate, NV = Natural vegetation, CULT = Soil Cultivation.
[b]P-value: The P-value was derived from mixed model ANOVA at α=0.05.
[c]Significance designation of Dunnett's test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001
[d]Pooled standard error

Soil property parameters

Soil property parameters were generally improved by NV, where soil aggregate stability was increased by 50% and organic matter content was increased by 18% in 2016. Microbial respiration rate was increased by 70% and 27% in 2015 and 2016 respectively, and carbon mineralizability was increased 54% in 2015, when compared to the GLY control (Table 3.8). None of the soil health parameters differed between the CULT treatment and GLY control. For soil nutrients, NV and CULT treatments increased Mg by 10% and 12% respectively and NV increased Mn by 36% compared to GLY in 2015 (Table 3.9). Other nutrients including P, K, Fe and Zn were unaffected by the under-vine floor treatments. Soil bulk density in the top 5 cm was reduced 16% by NV compared to GLY but the bulk density of soil from 5-15cm was the same between either of the treatment and control in the third year of experiment in 2016 (Table 3.10).

Table 3.8. Health parameters of the under-vine soil treated with different floor management practices in a Riesling vineyard in the second and third year of the experiment (2015 and 2016)

Treatment[a]	Aggregate stability (%)		Organic matter (%)	
	2015	2016	2015	2016
NV	12.3 ± 1.24[c]	27.0 ± 2.38 *[d]	3.07 ± 0.10	3.26 ± 0.07 **
GLY	9.87 ± 1.24	18.0 ± 2.38	2.80 ± 0.10	2.77 ± 0.07
CULT	10.4 ± 1.24	15.6 ± 2.38	3.10 ± 0.10	2.74 ± 0.07
P-value[b]	0.4842	0.0251	0.1095	0.0069

Treatment	Microbial respiration (mg CO_2 g/14days)		C Mineralizability (mg CO_2/g OC)	
	2015	2016	2015	2016
NV	1.82 ± 0.15 *	1.08 ± 0.04***	59.0 ± 4.00 *	33.2 ± 1.36
GLY	1.07 ± 0.15	0.85 ± 0.04	38.3 ± 4.00	30.9 ± 1.36
CULT	1.21 ± 0.15	0.88 ± 0.04	39.0 ± 4.00	32.2 ± 1.36
P-value	0.0271	<0.0001	0.0211	0.4988

[a]Treatment: GLY = Glyphosate, NV = Natural vegetation, CULT = Soil Cultivation.
[b]P-value: The P-value is derived from mixed model ANOVA at α=0.05.
[c]Pooled standard error
[d]Significance designation of Dunnett's test: *p<0.05, **p<0.01, ***p<0.001

Table 3.9. Nutrient analysis of under-vine soil treated with different floor management practices in 2015 after harvest

Treatment[a]	P	K	Mg
	ppm		
GLY	22.6 ± 1.44[c]	349 ± 36.1	112 ± 8.46
NV	22.4 ± 1.44	356 ± 36.1	123 ± 8.46 *[d]
CULT	22.0 ± 1.44	346 ± 36.1	125 ± 8.46 *
P-value[b]	0.9691	0.8658	0.0186

Treatment	Fe	Mn	Zn
	ppm		
GLY	0.48 ± 0.09	8.12 ± 0.60	1.25 ± 0.10
NV	0.51 ± 0.09	11.0 ± 0.60 **	1.61 ± 0.10
CULT	0.50 ± 0.09	9.60 ± 0.60	1.52 ± 0.10
P-value	0.9141	0.0102	0.0718

[a]Treatment: GLY = Glyphosate, NV = Natural vegetation, CULT = Soil Cultivation.
[b]P-value: The P-value is derived from mixed model ANOVA at α=0.05.
[c]Pooled standard error
[d]Significance designation of Dunnett's test: *p<0.05, **p<0.01

Table 3.10. Bulk density of under-vine soil treated with different under-vine cover crops at different depths in third year of the experiment (2016)

Treatment[a]	0-5cm	5-10cm	10-15cm
	(g/cm^3)		
GLY	1.20 ± 0.06[c]	1.23 ± 0.04	1.36 ± 0.03
NV	1.01 ± 0.06 **[d]	1.17 ± 0.04	1.27 ± 0.03
CULT	1.12 ± 0.06	1.27 ± 0.04	1.28 ± 0.04
P-value[b]	0.0159	0.0564	0.0803

[a]Treatment: GLY = Glyphosate, NV = Natural vegetation, CULT = Soil Cultivation.
[b]P-value: The P-value is derived from mixed model ANOVA at α=0.05.
[c]Pooled standard error
[d]Significance designation of Dunnett's test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Winemaking and one-dimensional sorting on the wine aroma

Although statistical analysis was not performed, the mean values of pseudo-replicated wine composition parameters including the ethanol, TA and pH were very similar between either of the treatments and control (Table 3.11).

Based on the one-dimensional sorting of the sensory study data derived from 100

panelists each year, there were no grouping patterns of the replications or treatments across all three years (Fig. 3.4). The wine replications randomly spread along the sorting scale for each year which indicated that panelists were unable to differentiate the under-vine floor treatments from the control based on the aromatic properties of the wines in all three years of the study.

Table 3.11. Riesling juice soluble solid after press and wine ethanol, titratable acid and pH at bottling from grapes of different under-vine floor treatments in 2014, 2015 and 2016.

Treatment[a]	Rep[b]	Soluble solid (Brix) after press			Ethanol content (%) at bottling		
		2014	2015	2016	2014	2015	2016
GLY	1	19.6	20	22	-	11.7	12.8
GLY	2	19.6	20	22	-	11.6	12.8
NV	1	19.2	18.8	20.9	-	11.6	12.5
NV	2	19.2	18.8	20.9	-	11.6	12.5
CULT	1	19.3	19.8	20.9	-	11.5	12.6
CULT	2	19.3	19.8	20.9	-	11.5	12.7
Treatment	Rep	pH at bottling			Titratable acid (g/L) at bottling		
		2014	2015	2016	2014	2015	2016
GLY	1	3.19	3.22	2.97	8.55	7.94	8.9
GLY	2	3.33	3.17	2.98	8.21	8.06	8.61
NV	1	3.33	3.13	2.89	8.35	7.83	8.69
NV	2	3.3	3.17	2.91	8.08	7.93	9.05
CULT	1	3.21	3.18	2.91	8.61	7.7	8.94
CULT	2	3.23	3.14	2.9	8.71	8.23	9.09

[a]Treatment: GLY = Glyphosate, NV = Natural vegetation, CULT = Soil Cultivation.
[b]Rep: Fermentation replications of each treatment

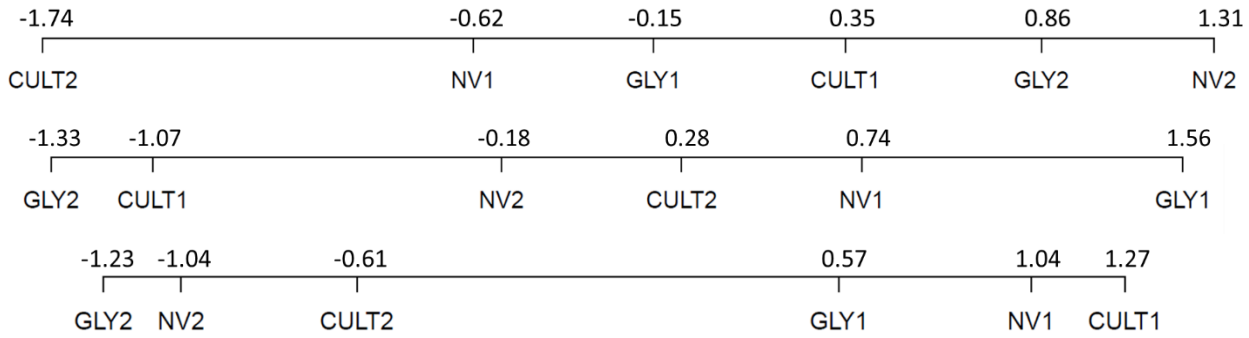


Figure 3.4. One dimensional scaling of wine sensory property similarity rating of Riesling wines made from grapes derived from vines treated with under vine Glyphosate application (GLY), soil cultivation (CULT) and natural vegetation (NV) in 2014, 2015 and 2016. n=100 for all three years.

Discussion

The major finding of this study was that allowing under-vine floor weeds to grow had little impact on mature vine growth and no discernable impact on wine sensory properties. Shoot growth, vine pruning weight, yield per vine, berry soluble solids, pH and TA were all unaffected by the under-vine soil treatments over the three year duration of the experiment. The only parameter impacted by treatments was canopy structure where leaf layers and occlusion layers were reduced and cluster light environment was improved in 2015, the year when the precipitation was reasonably equally distributed throughout the growing season. Previous studies have demonstrated that under-vine cover crops reduced vine canopy density and improved cluster light exposure (Wheeler et al., 2005, Hatch et al., 2011, Karl et al., 2016b, Hickey et al., 2016). In these studies, reduction in canopy density was always coupled to reduced shoot growth and pruning weight attributable to resource competition from under-vine cover crops. However, in this study pruning weight and shoot growth were not significantly reduced, though pruning weight was lower in NV than in GLY in all years of the study. Compared to previous studies (Wheeler et al., 2005, Hatch et al., 2011, Karl et al., 2016b, Hickey et al., 2016), direct water and nutrient competition between vine and floor vegetation were less prominent in this study. The midday stem water potential was the same in the early season and higher in the late season in NV compared to GLY in 2015. The resilience of grapevine to withstand competition from weeds observed in this study can likely be explained by an extensive root system and high carbohydrate reserve in mature vines (Holzapfel et al., 2010).

The reduction of fruiting zone leaf layer number and occlusion layer number in NV in 2015 may relate to the reduced vine nutrient status at bloom in 2015 where the N, B and Mn content were lower in the petiole compared to GLY control. This result may be due to reduced

soil nutrient content resulting in lower vine available nutrient (Pérez-Álvarez et al., 2015) or to lower soil nutrient mineralization while the soil overall nutrient was not reduced (Tesic et al., 2007). In this study, none of the soil nutrients were reduced in the NV treatment. Although soil N and B were not measured, Mn, which was reduced in the petiole, was higher in soil in the NV treatment than the control. Thus, the latter theory (lower soil nutrient mineralization) is more likely to explain the reduction of vine petiole nutrients.

Soil nutrient mineralization is mainly derived from biological process of soil microorganisms which can be impacted by substrate, temperature and moisture (Bardgett and Chan, 1999, Goncalves and Carlyle, 1994). Although soil temperature was not examined in this study, soil substrate content, microbial activity and moisture were indirectly quantified. In 2015 the soil nutrient concentration was not reduced by NV. Soil organic matter content was unaffected by the NV treatment but the organic carbon was more labile as higher carbon mineralizability was noted. Given the same moisture level, microbial population was more actively mineralizing nutrients in NV than in GLY as shown by the higher microbial respiration rate. Thus, it is more possible that reduced vine nutrient status was due to less favorable soil moisture conditions for nutrient mineralization in the NV treatment. Indeed, the soil water was found to be lower in the NV treatment than in the control in 2015 through an indirect measurement using predawn leaf water potential, which is often used as soil water indicator (Winkel and Rambal, 1993). As a result, the reduced vine canopy density found in NV treatment in 2015 was likely due to lower nutrient availability caused by drier soil with lower nutrient mobility and mineralization. The same phenomenon was also observed by Tesic et al. (2007), where the vine canopy density was reduced by complete floor coverage. They reported that floor vegetation reduced the soil volumetric water and consequently reduced the vine N and Mg

uptake due to lower nutrient mineralization rate in the soil. Groundcover may have redistributed the vine root to the deeper soil (Centinari et al., 2016) where the N mineralization rate was lower and that resulted in lower vine N status and reduced vegetative growth (Celette et al., 2009).

Although predawn leaf water potential was lower in the NV treatment compared to GLY in 2015, the surface soil may have been moister in the NV treatment during the late growing season, which was when many of the weeds stopped growing actively and served merely as living mulch preventing surface soil water evaporation. The plant residues left from soil cultivation had the same function for protecting surface soil moisture. The ability of vegetative mulch to protect soil water from evaporation loss was previously discussed (Frye et al., 1988). The evaporation loss prevention was demonstrated in cool climate vineyard where the growing season volumetric water top 20cm surface soil was higher in the manually cultivated soil compared to that of herbicide maintained bare soil in all three years of the study (Karl et al., 2016a). This likely explained why the midday stem water potential of vines in NV and CULT treatments were higher than the control in late season 2015. In additional to the soil surface water conservation effect of NV and CULT treatments, differentiated root distribution among the vines with different under-vine floor management practices may had an impact on the vine water status. Soil cultivation was observed to reduce the grapevine root distribution in the top soil horizon (Van Huyssteen and Weber, 1980). A study done in a humid climate demonstrated that mature grapevine growing with bare soil maintained with herbicide under vines had 49% more total absorptive root length distributed to the top 20cm soil than the grapevine growing with permanent under-vine grass (Klodd et al., 2016). Thus, if the surface soil dried out during the day it may have had more impact on the vines in GLY control than in other treatments if there was more water absorptive root distributed. This further explained the observation that late season

midday stem water potential of NV and CULT was higher compared to that of GLY control in late season 2015.

By examining the grapevine biomass distribution, it was suggested that early season growth is dependent on carbohydrate and nutrient reserves stored in permanent structures of the vine (Keller and Koblet, 1995). Although the nutrients including N, B and Mn were reduced in the vines in NV treatment in 2015, more vigorous primary shoot growth in the early 2016 did not suggest reduced nutrient reserves. This observation may be explained by post-harvest reserve nutrient replenishment. Twenty three years old mature grapevines in Oregon not only had great resilience of nutrient reserve but also relied heavily on the post-harvest nutrient acquirement for reserve nutrient replenishment (Schreiner et al., 2006). Since the soil organic matter in the NV treatment was more labile, the decomposition of the cumulative weeds residues plus the mineralization of nutrients in the late season and post-harvest period could have replenished and even enriched the nutrient reserve in the vines, and consequently led to more vigorous early season primary shoot growth. However, longer early season primary shoot length in the NV treatment did not result in higher winter pruning weight likely due to periodically hedging of all treatments and the compensation growth of the lateral shoots, mainly from concurrent season nutrient uptake.

Many studies have shown the link between reduced canopy density along with increased light environment by under-vine cover crops and enhanced berry ripeness including increased soluble solids and reduced TA (Hickey et al., 2016, Coniberti et al., 2018). Although with only one year of results, another study not only showed that chicory growing under-vine reduced vine canopy density which led to increased soluble solids and reduced TA in grapes but also observed higher rated wine sensory properties including appearance, aroma, palate and overall evaluation

of wines from the under-vine chicory compared to cultivated bare soil (Wheeler et al., 2005). However, in our study, grape soluble solids, pH and TA were not impacted by the under-vine floor treatment and the sensory study showed parallel results, even though the canopy structure and light environment were improved by NV treatment in 2015. In a New York study, mature Riesling vine growth including canopy density, pruning weight and yield were not impacted by the under-vine cover crop treatments but the wines were sensorially different from each other (Jordan et al., 2016). That study suggested that under-vine floor management practices could have altered the wine sensory properties through mechanisms other than vine balance, canopy light environment, and water and nutrient status. Under-vine cover crop effects on wine sensory properties through altering the grape associated microbial communities was suspected. Manipulated grapevine endophytic fungal community showed impact on grape secondary metabolites including total flavonoids, total phenols and trans-resveratrol (Yang et al., 2016). Thus, microbial factors of under-vine floor management practices impact on vineyard soil, grapes, spontaneously fermentation and the resulting wine sensory properties were examined and discussed in the next Chapter.

Letting weeds grow under-vine resulted in lower management costs compared to maintaining cover crops and bare soil using cultivation or herbicide but reduced overall revenue because of yield reduction derived from resource competition was demonstrated in a previous study done in a young Cabernet franc vineyard (Karl et al., 2016b). However, in this study, the NV treatment did not reduce vine yield and Ravaz index even under dramatic different climatic conditions throughout the experiment in a mature vineyard. Thus, letting weeds to grow under-vine, which is associated with lower management cost, in cool climate mature vineyards could be a beneficial management practice to the growers.

This study hypothesized that natural vegetation growing under-vine would reduce vine vegetative growth including shoot growth, pruning weight and canopy density through resource competition and consequent increases in canopy light environment and alteration of grape composition and wine sensory properties. The experimental results rejected the hypothesis. Vine physiological factors of under-vine floor management effects were examined in this chapter, microbial factors were also examined in Chapter 4 to better understand their impacts on wine sensory properties and adaptability for sustainable viticulture.

Conclusion

This study revealed the potential of using under-vine natural vegetation as an alternative to bare soil in a mature vineyard in cool climate environmental conditions. Under-vine NV treatment had no impact on vine vegetative growth, yield, fruit harvest parameters and wine sensory properties in all three years, and generally improved soil health parameters. These findings suggested that letting weeds to grow under-vine could provide a beneficial alternative to the traditional herbicide and/or cultivation maintained bare soil with lower management cost and improved soil health.

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CHAPTER FOUR

Fungal communities of grapes and spontaneous fermentations are resilient to vineyard management techniques that shift soil microbiome composition

Introduction

Vineyard management practices impact fruit and wine composition through many routes (Jackson and Lombard, 1993). Widely known effects include the modification of the leaf area to fruit ratio (Kliewer and Dokoozlian, 2005), alteration of the fruit microclimate (Smart and Robinson, 1991), and changes in nutrient and/or water uptake. However, the role of vineyard microbiology has been largely overlooked until recently, with researchers suggesting microbial composition as a possible driver of wine sensory properties (Gilbert et al., 2014).

Aside from intentional inoculation, the major sources of yeasts in wine fermentations are derived from the vineyard and winery (Sabate et al., 2002, Mortimer and Polsinelli, 1999). The impact of the winery environment on yeast dynamics during fermentation has been extensively studied (Sabate et al., 2002, Ciani et al., 2004, Bokulich et al., 2013, Perez-Martin et al., 2014), but vineyard factors have received less attention. Recent studies have found that the microbial communities present in wine fermentations are structured to reflect regional and vineyard site patterns (Bokulich et al., 2016), which suggests the importance of vineyard factors.

Grape microbiomes can be shaped by climate, region, site, and grape cultivar (Bokulich et al., 2014, Corneo et al., 2013, Setati et al., 2012, Burns et al., 2015), and also appear to be associated with the composition of the microbiome involved in wine fermentation, and with wine metabolite profiles and abundances (Bokulich et al., 2016). Regionally-differentiated yeast

genotypes collected from vineyards, forests, and spontaneous fermentations are confirmed to have different impacts on wine chemical composition (Knight et al., 2015). These studies suggest the importance of "microbial terroir" by indicating the significance of specific vineyard properties on wine characteristics as a function of microbiome composition. The term "microbial terroir" refers to a microbially-triggered grape and wine fermentation response that results in region-specific wine characteristics (Gilbert et al., 2014, Belda et al., 2017).

Management practices in the vineyard and winery, such as the use of fungal sprays and sulfiting fermentations, play important roles in the microbial dynamics of grape and wine fermentations that potentially contribute to terroir (Grangeteau et al., 2017). Yeast populations in vineyard soil (Zehetner et al., 2015), grapes, and wine fermentations (Martins et al., 2014, Bagheri et al., 2015, Patrignani et al., 2016) have been studied in the context of specific vineyard management techniques, such as organic or conventional management. A study that was conducted in a hot and arid climate in Spain suggested that soil tillage is related to high diversity in grape-associated yeast (Cordero-Bueso et al., 2011b). However, they were unable to statistically test this concept as they relied on culture-dependent techniques to characterize yeast diversity. Another study conducted in California (USA) used next generation sequencing methods to demonstrate that vineyard floor management impacted the community structure of soil bacteria(Burns et al., 2016). Fungi were not included in their study, nor was the association of soil microbial composition with grape or wine fermentation microbiomes.

The concept of soil as a source of microorganisms inhabiting grape surfaces and wine fermentations is easily understood, but challenging to examine systematically. A study conducted in Long Island, NY (USA) found that bacterial communities associated with grape leaves, flowers, and fruit shared a greater proportion of taxa found in soil compared with each other,

which they suggested as evidence of soil serving as a bacterial reservoir in vineyards (Zarraonaindia et al., 2015). There are several known microbial dispersal mechanisms that transport fungi and bacteria from the ground to crops, including rain (Madden, 1997) and wind (Bock et al., 2012). These routes of microbial dispersal are likely to hold in vineyards as well, although there are many other possible routes to be explored. Thus, it is possible that vineyard soil management practices could alter the microbiome in the vineyard - not only at the soil level, but also with aerial parts such as grapes. In one of our previous studies conducted in New York (Jordan et al., 2016), we showed that under-vine soil treatments had no impact on vine growth and yield components, but that wine sensory properties differed. We suspected that microorganisms in the vineyard, as a function of floor management practices, might have triggered changes in grape secondary metabolite production that altered wine sensory properties.

To understand the management impacts on microbial terroir, a three-year single-factor study was conducted within an experimental design that corresponds to our previous study (Jordan et al., 2016) in a commercial vineyard in the Finger Lakes Region of New York. Under-vine soil management was chosen as our vineyard management factor, as we expected that it would directly manipulate the vineyard microbial pool in soil. The objective was to examine how under-vine soil management practices, including herbicide application with Glyphosate (GLY), soil cultivation (CULT) using hand weeding, and under-vine natural vegetation (NV) with no cultivation/herbicide, impacted the microbiomes of soil, grapes, and simulated spontaneous fermentations. The goal of the study is to understand the role of specific vineyard soil management practices on microbial terroir. We hypothesized that specific types of under-vine soil management would alter the composition of the soil microbiome, and this impact would be reflected in the community found on grapes and in simulated spontaneous wine fermentations,

which would impact wine sensory properties.

Material and methods

Vineyard design

The experiment was conducted in a commercial vineyard on Howard gravelly loam soil located in Ovid, NY, USA for three consecutive years from 2014 to 2016. The vines, *V. vinifera* cultivar Riesling grafted onto 3309C rootstock, were planted in 2001 with 2.13m × 2.74m intra- and inter-row spacing. The trellis system was cane pruned Scott-Henry system with 10 buds per cane on each of four canes. A complete randomized block design was applied to enable four replicates for each treatment, and the treatments were randomly assigned to the experimental units, which are one meter wide under-vine soil strips, within each block. Each experimental unit was across three rows with nine consecutive vines in a row. The grape and soil samples were collected from the middle three vines and the accordance under-vine 1m × 5.8m soil strip, in the middle row from each of the experimental unit where the other vines were served as guards for physical and spatial buffering. The vineyard canopy, pest-control and amendments were managed following standard commercial practice in the Finger Lakes region (Wolf, 2008) by the professional vineyard crew.

Under-vine soil treatments

The experimental units were subjected to three different under-vine soil treatments in a one meter wide strip under vines including spot application of herbicide, in which the active ingredient was glyphosate, cultivation maintained bare soil, and natural vegetation, where weeds grew freely with periodic mowing to keep them out of the fruiting zone. Herbicide and

cultivation bare soil strips were established following the commercial standard. In brief, 2% Roundup (Monsanto, MO, USA) was sprayed with electronic pumped spraying nozzle in rate about 3kg a.i./ha. Cultivation was done by combining mechanical, rototiller to roughly 20cm depth, and manual tillage, cultivation with hoes. Herbicide was applied on June 23rd, July 9th, July 18th in 2014, June 16th in 2015 and June 15th in 2016. Soil cultivation was applied on June 27th, July 3rd, and July 18th in 2014, June 3rd, July 23rd to July 27th in 2015 and May 25th and June 24th in 2016. A permanent between-row cover crop was maintained separately and was a mix of fescue, white clover and weeds.

Sample collection

At bloom (2015 and 2016) and harvest (2014, 2015 and 2016), ten soil cores per experimental unit were collected using a core (6 cm diameter × 10 cm deep) attached to the slide hammer auger (AMS Inc, American Falls, ID, USA) in a grid pattern. Grape cluster samples were taken at commercial harvest with individual sterilized blazers for each of the experimental unit. Ten clusters from each experimental unit were randomly picked. The soil and grape samples from each experimental unit were contained in separate zip bags immediately after sampling, transported at 0˚C and stored in -20˚C until further analysis. Sub-samples of five berries per cluster, comprised of two from the top, two from the middle and one from the bottom of the cluster were detached in the original field sampling bag while frozen and allocated into a new zip bag to make 50 berries per experimental unit for grape microbial community DNA extraction.

Simulated spontaneous fermentation

Simulated spontaneous fermentations were duplicated for each field treatment (n=2) with a fermentation control (i.e., no inoculation). Riesling juice obtained from the Cornell University

property at Lansing, NY, pasteurized for more than 30 minutes with exit temperature 75˚C served as base juice for both rinsing and fermentation. The base juice was divided into eight sanitized 22.7L glass carboys with 16kg of juice in each. Eight sound clusters from each experimental unit were randomly harvested on Oct. 14th 2014, which was the commercial harvest date, and kept in 4˚C overnight. The following day the clusters from the same treatment, a total of 24 clusters per treatment, were combined in a carefully sanitized plastic rinsing bucket. The clusters from each treatment were then soaked in 4L of base juice and shaken for three hours to dislodge the surface fungi including yeasts (Renouf et al., 2005). The drench from each rinsing bucket was divided into two 1.8L inoculum batches, avoiding any solids. The inocula were then introduced into the carboys that contained pasteurized juice to start the fermentation. One un-inoculated fermentation control was lost due to a carboy flaw. The fermentation was conducted in an isolated 16˚C dark room and was terminated when the fermentation reached dryness. The fermentation was monitored and sampled on average once every three days with sterilized pipets. For monitoring during the fermentation 30ml of fermenting wine was drawn. Out of the 30ml sample, 15ml of well homogenized fermenting wine were kept in sterilized falcon tube in -20˚C freezer until process for DNA extraction and the other 15ml was tested with portable density meter DMA35 (Anton Paar, VA, USA) and, when close to dry as evidenced by a Clinitest (Bayer Corporation, IN, USA).

Sample DNA extraction, amplification and Sequencing

DNA extraction of soil samples followed the protocol for the PowerSoil DNA isolation kit (MO BIO Laboratories, CA, USA). For grape samples, the grapes were thawed and crushed in the zip bag before following the procedures, which were also used for fermenting wine

samples. Grape must and fermenting wine samples were vortexed and homogenized, and transferred into two 2ml Eppendorf tubes and centrifuged at 11600×g for 20 minutes. The pellets from the same sample were combined and washed two times with chilled PBS. The pellets were then used for DNA extraction following the protocol from the MoBio PowerPlant DNA isolation kit. The bacterial 16S rRNA gene V3/V4 regions and fungal ITS barcoded region were amplified with the universal bacterial primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') and fungal primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and 5.8A2R (5′-CTGCGTTCTTCATCGAT-3′), in which the Illumina adaptors at the 5' end of the primer sequences (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3' for the forward primer and 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3' for the reverse primer) were attached(Bell et al., 2016, Yergeau et al., 2015). The reaction was conducted in 20µl containing 9µl H_2O, 8µl 5prime HotMaster mix (5 PRIME Inc., MD, USA), 1 µl of each primer (forward and reverse) and 1 µl of 1:10 diluted DNA template in thermocycler (Bio-Rad, CA, USA) following the condition of 3 min at 95˚C and then 25 (bacteria) and 30 (fungi) cycles of 30 s at 95˚C, 35 s at 50˚C and 60s at 72˚C before entering the final step of 10 min at 72˚C. The amplicons were transferred into 96-well plates and cleaned with MagBio HighPrep PCR beads (MagBio Genomics, MD, USA). We then attached unique two-barcode indexes to cleaned amplicons by running PCR with 2.5 µl each of forward and reverse primers (10 µM) carrying designated barcodes, 12.5 µL of Q5 High Fidelity 2X Master Mix (New England Biolabs Inc., MA, USA), 5 µL of template, and 2,5 µl of water, with the following temperature protocol: 8 cycles of 15 s at 98˚C, 30 s at 55˚C and 20s at 72˚C after 1 min at 98˚C and before 3 min at 72˚C. Sample DNA was normalized with the SequalPrep Normalization Kit (ThermoFisher, Waltham,

MA), pooled using equal liquid volumes, and the pool purified with a PureLink QuickGel Extraction Kit (ThermoFisher). Each pool was sent to the Cornell Institute of Biotechnology (Ithaca, NY) for paired-end sequencing, using the 600-cycle MiSeq Reagent Kit v.3 for our 16S pool, and the 500-cycle MiSeq Reagent Kit v.2 for our ITS pool on the Illumina MiSeq platform (Illumina Inc., CA, USA). The sequencing process generated 4,060,310 ITS and 552,871 16S rRNA gene reads after downstream processing as described below. All the MiSeq data were uploaded to the NCBI Sequence Read Archive and are public accessible under the project number of SRP132177.

Bioinformatic and statistical analysis

The raw sequences were processed and aligned following the protocol described in the Brazilian Microbiome Project (Pylro et al., 2014) with some modifications (Howard et al., 2017). Briefly, paired-end sequence merging, primer trimming, and singleton sequence removal were performed in Mother v 1.36.1. Operational Taxonomic Units (OTU) were produced at 97% sequence similarity. Taxonomic classification of OTUs was performed in Mother using the GreenGenes v.13.8 database for 16S sequences and UNITE v. 7 database for ITS sequences. From this step, all the downstream data analysis was conducted in R version 3.3.3 with packages Vegan and Phyloseq. The microbial diversity was determined using Shannon Diversity Index. The β-diversity of the assemblage dissimilarity between samples were calculated with the Bray-Curtis distances for fungal community and weighted UniFrac distances for the bacterial community. The fungal β-diversity based on the Bray-Curtis distance metric was also tested on variables of vintage and under-vine soil treatments with Permutational Multivariate Analysis of Variance (PERMANOVA) and paired REMANOVA at $\alpha=0.05$. When three-year overall analysis

was done, the year was positioned as a fixed effect with samples within each block in constrained permutation to account for the repeated measures. The overall PERMANOVA was not performed for soil 16S data due to incomparable sequence reads between 2014 and the rest of the years. Paired-PERMANOVA was performed by subsetting the treatments and Bonferroni correction was applied to the P-values. The relative abundance of selected fungal genera in the samples were compared using one-way analysis of variance (ANOVA) test followed by Student's t test if there was only one group of comparison and Tukey HSD if there were more than one group of comparisons performing in JMP Pro 12.0.1 (SAS Institute, NC, USA), with log transformations when needed under violations of normality. For the comparison of the relative abundance of *Saccharomyces* and *Hanseniaspora*, each DAI was calculated by pooling samples across different treatments (n=7) to enable meaningful statistical analysis. Since the interaction of DAI and fungal genus was significant, the comparisons were made for each DAI individually.

Sensory evaluation

Wines derived from simulated spontaneous fermentations were subjected to sensory evaluation regarding the overall aroma similarity. The panelists were comprised of 97 male and female aged from 21 to 79 who self-reported drank white wine at least once a month. The panelists were seated in a room with white fluorescence light at a wooden table separated by white cardboard partitions to isolate each of them. The setting of each spot included the wines, all the field treatments and fermentation control in duplicate, contained in ISO tasting glasses with plastic lids on top, a sorting sheet that included a short survey about drinking frequency, age and gender, and a pencil to fill out the sheet. The wines were presented simultaneously with randomly generated three-digit codes on each of the glasses. The panelists were asked to smell

all the wine and group the wines based on the overall aroma similarity, and to complete the survey without time limitations (Lawless and Heymann, 2010).

Results

Fungal communities cluster distinctly between soil, grapes and inoculum, and wine fermentations

Fungal community profiles showed distinct clustering of samples derived from grapes and inoculum, wine fermentation, and soil collected under grapevines. The Bray-Curtis distance metric was used to determine multivariate sample distances, which were visualized through an ordination of a principal coordinates analysis (PCoA). Axes 1 and 2 explained 66% of the variance in the data. The inoculum for the fermentations clustered with the grapes, as they were the drenches of the grapes washed by base juice. Similarly, the soil samples clustered together distinctly, and separately from other sample sources according to both PCoA dimensions. However, the wine samples showed fungal communities that varied widely in taxonomic profiles, suggesting variability throughout the fermentation (Fig. 4.1). Shannon diversity indices for OTUs did not differ among treatments across grapes, inoculum, soil, or wine fermentations across years (Supplementary Fig. S1), thus PCoA analysis was not likely influenced substantially by differences in OTU diversity across treatments.

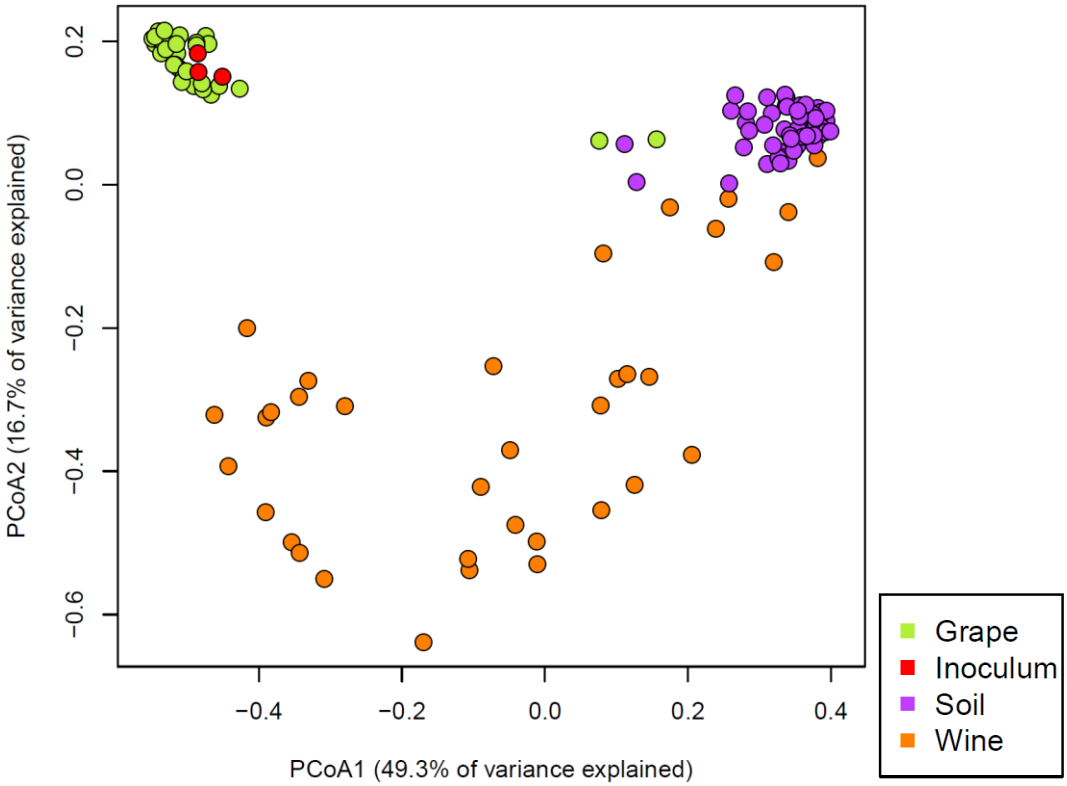


Figure 4.1. Principal coordinates analysis (PCoA) of fungal communities (ITS region) of soil, grape, inoculum (derived from grape must) and wine fermentations from all harvest years and management treatments. The ordination is based on the Bray-Curtis distance metric, with samples clustering by collection type (grape/inoculum, soil, and wine fermentations).

Under-vine soil management impacted soil fungal community structure

To evaluate the impact of under-vine soil management on microbial community composition, we first profiled the soil microbiome. The three-year average under-vine soil vegetation coverage rate for NV was more than 70%, while coverage rates for CULT and GLY were less than 20% at veraison. PCoA plots with samples from each of the three years of the study (generated using the Bray-Curtis distance metric) showed that NV soil fungal communities differed from those of GLY and CULT treatments (Fig. 4.2a). Over the three years of the experiment, sample clustering was based primarily on vintage, with each vintage clustered, and then by treatment, where NV separated from GLY and CULT. However, no clustering pattern was detected among the CULT and GLY samples. Notably, the dissimilarities between NV and

the other two soil treatments grew with time since groundcover establishment, suggesting possible intensification of the NV treatment effect over time. In 2015 and 2016, the soil samples were taken at two different vine phenological stages - bloom and harvest, which showed separation by PCoA ordination.

These observations were confirmed by statistical analysis. According to the three-year overall Permutational Multivariate Analysis of Variance (PERMANOVA), vintage and treatment effects were both significant ($P<0.001$), while vintage ($R^2=0.159$) explained more variation than treatment ($R^2=0.114$). The treatment effect was significant across all three years ($p=0.032$ in 2014, $p=0.001$ in 2015 and $p=0.001$ in 2016) when each year was analyzed individually. The phenological stage effect was significant in both year 2015 ($p=0.008$) and 2016 ($p=0.048$), when samples were not taken at vine full bloom in 2014 (Table 4.1a).

Unclassified fungal genera in soil samples ranged from around 10% to more than 25% relative abundance. However, analyses excluding the unidentified genera did not change the differentiation of NV samples from CULT and GLY samples on the ordination. The top five fungal genera found in the soil (excluding unclassified) were *Verticillium*, *Nectria*, *Mortierella*, *Gibberella* and *Fusarium*, based on average relative abundances across all soil samples (Fig. 4.4a). Fungal genera relative abundance differences were found in *Gibberella*, *Neopestalotiopsis*, *Verticillium* and an unclassified genus under *Amphisphaeriaceae* family, where soil of NV treatment had less *Gibberella* ($P<0.005$ in 2015), *Neopestalotiopsis* ($P<0.05$ in 2015 and 2016), unclassified *Amphisphaeriaceae* ($P<0.05$ in 2016) and more Verticillium ($P<0.05$ in 2015). Soil of CULT treatment had less *Neopestalotiopsis* ($P<0.05$ in 2015 and 2016) compared to soil of GLY (Fig. 4.4b). Among these genera, *Neopestalotiopsis* and *Verticillium* are found in the top five most important variables along with *Monographella*, *Paraphaeosphaeria* and unclassified

genera under *Nectriaceae* in the Random Forest model for soil treatment prediction

(Supplementary Fig. S2).

Table 4.1. Comparison of bacterial and fungal community structure dissimilarity in soil and grapes using (a) permutational multivariate analysis of variance (PERMANOVA) and (b) paired-PERMANOVA. The significance symbol *, ** and *** indicates P-value <0.05, <0.01 and <0.001 respectively.

(a) PERMANOVA

Factors	Overall		2014		2015		2016	
	R^2	P-value	R^2	P-value	R^2	P-value	R^2	P-value
Soil 16S								
Treatment	-	-	0.243	0.042*	0.097	0.181	0.104	0.013*
Stage	-	-	-	-	0.061	0.032*	0.094	<0.001**
Treatment*Stage	-	-	-	-	0.083	0.757	0.085	0.176
Soil ITS								
Treatment	0.114	<0.001**	0.246	0.032*	0.213	<0.001**	0.243	<0.001**
Stage	0.012	0.443	-	-	0.074	0.008**	0.054	0.048*
Year	0.159	<0.001**	-	-	-	-	-	-
Treatment*Stage	-	-	-	-	0.094	0.066	0.058	0.653
Treatment*Year	0.082	<0.001**	-	-	-	-	-	-
Grape ITS								
Treatment	0.026	0.658	0.138	0.492	0.211	0.472	0.169	0.278
Year	0.498	<0.001**	-	-	-	-	-	-
Treatment*Year	0.051	0.771	-	-	-	-	-	-

(b) Paired-PERMANOVA

Pairs	overall		2014		2015		2016	
	R^2	p value	R^2	p value	R^2	p value	R^2	p value
Soil 16S								
CULT vs GLY	-	-	0.135	0.621	0.070	0.481	0.064	0.455
CULT vs NV	-	-	0.186	0.033*	0.072	0.209	0.088	0.064
GLY vs NV	-	-	0.190	0.026*	0.083	0.086	0.088	0.036*
Soil ITS								
CULT vs GLY	0.061	0.005**	0.157	0.238	0.110	0.027*	0.103	0.022*
CULT vs NV	0.101	<0.001**	0.225	0.095	0.179	0.001**	0.251	0.002**
GLY vs NV	0.097	<0.001**	0.208	0.105	0.205	<0.001**	0.197	<0.001**
Grape ITS								
CULT vs GLY	0.022	0.860	0.186	0.103	0.089	0.809	0.135	0.608
CULT vs NV	0.021	0.894	0.113	0.912	0.172	0.421	0.155	0.514
GLY vs NV	0.017	0.834	0.051	0.581	0.205	0.206	0.105	0.835

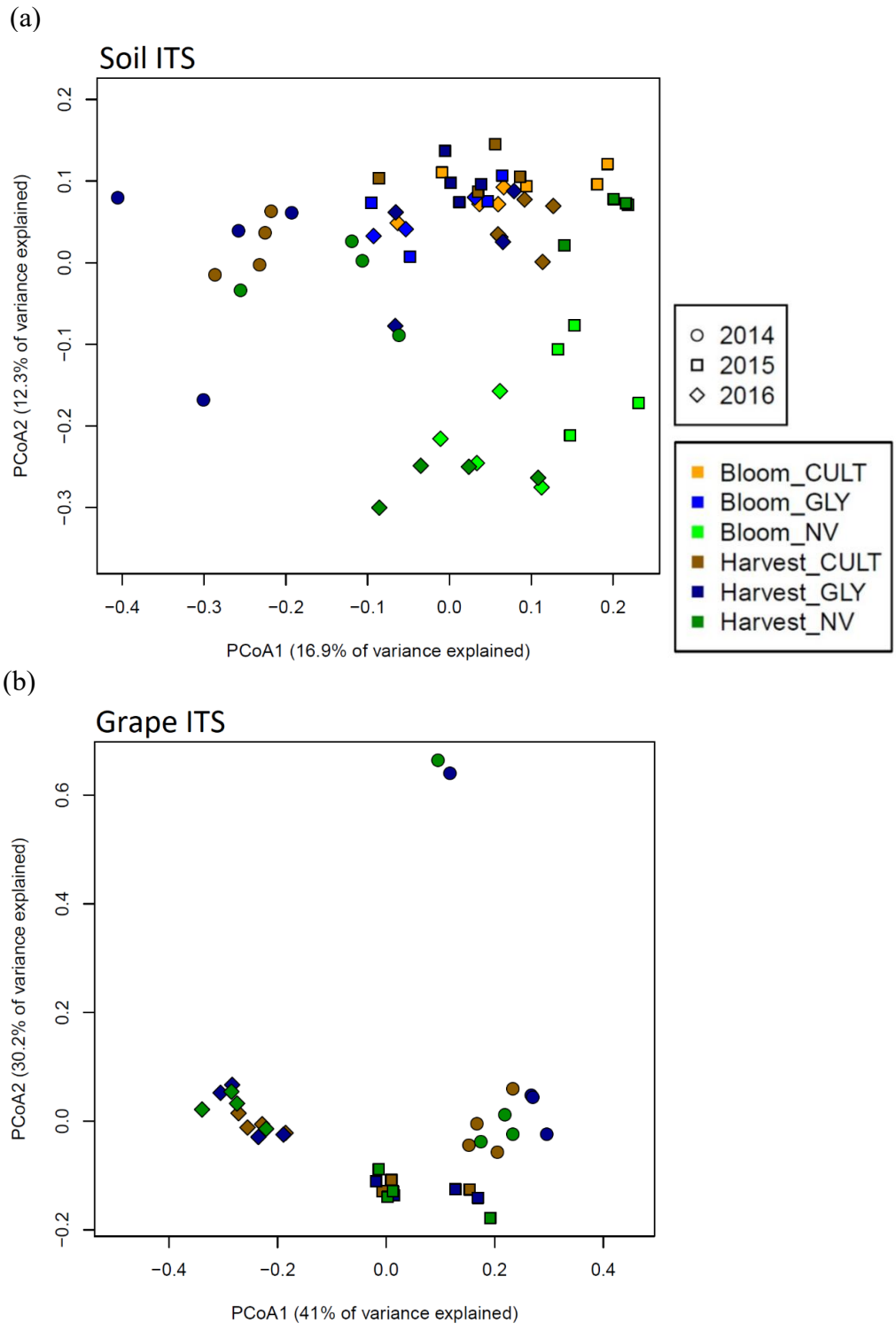


Figure 4.2. Principal coordinates analysis (PCoA) ordinations of fungal communities (ITS region) derived from (a) soil at grapevine bloom and harvest; and (b) grape at harvest. The three under-vine management treatments include Cultivation (CULT), Glyphosate (GLY) and Natural Vegetation (NV). The PCoA is based on the Bray-Curtis distance metric for three experimental years.

Under-vine soil bacterial community structure was not clearly impacted by floor management practice

The sequencing reads generated from the 2014 samples contained unexpectedly high amounts of short reads, whereas the sample sequences were comparatively low. Thus, the 2015 and 2016 soil bacterial samples were analyzed separately from the 2014 samples to generate the following results. Although the samples did not seem to cluster based on treatments on PCoA plots using UniFrac distance metrics (Fig.4.3), the treatment effect was significant in year 2014 ($p=0.042$) and 2016 ($p=0.013$) according to PERMANOVA (Table 4.1a). In fact, paired-PERMANOVA further revealed that the bacterial community structure among the treatments was different in 2014, where NV differed from GLY ($p=0.026$) and CULT ($p=0.033$), and 2016, where NV differed from GLY ($P=0.036$) (Table 4.1b). Grape- and wine-associated bacterial community structure was not further examined due to low yield of bacterial DNA resulting in low PCR amplification.

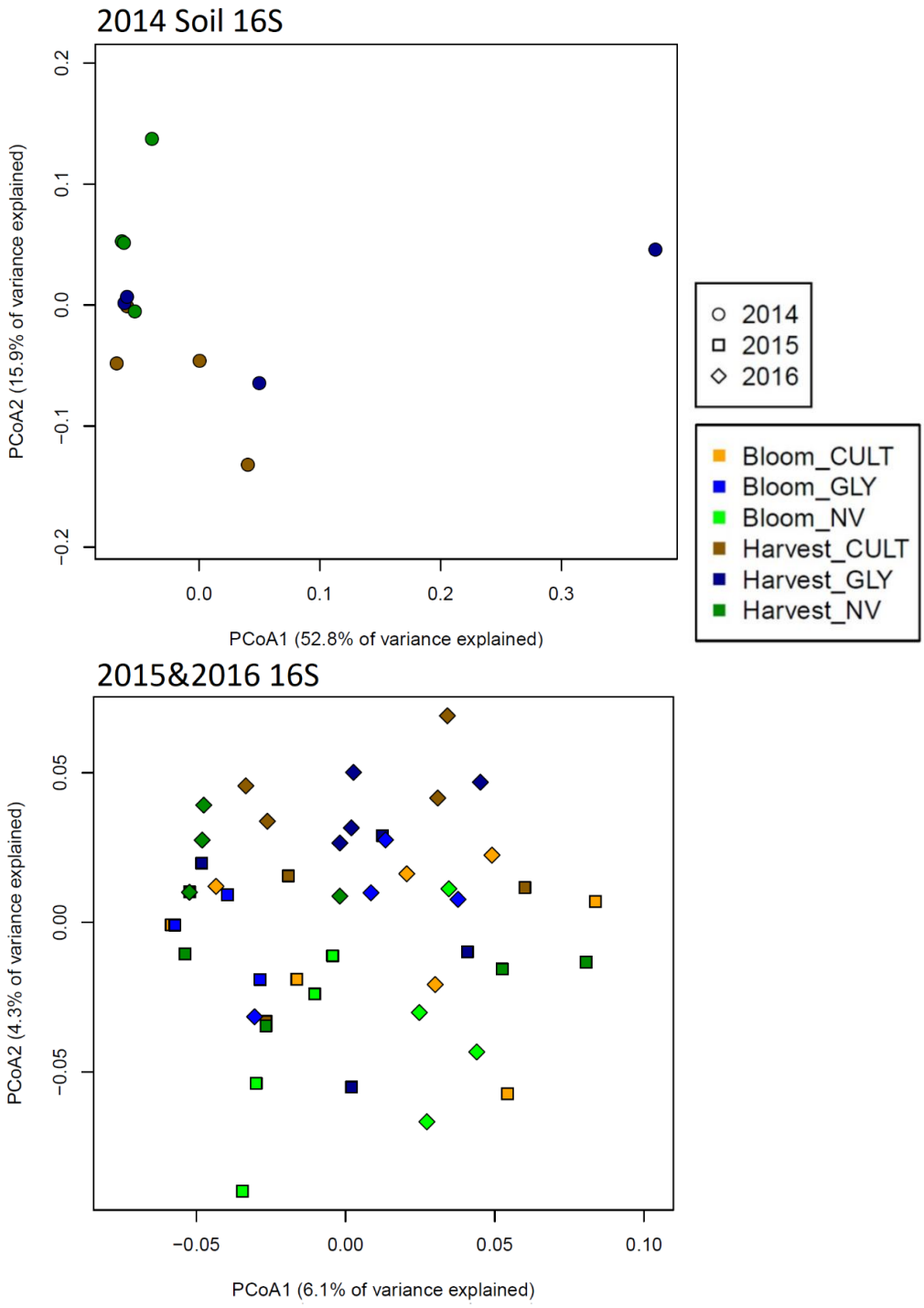
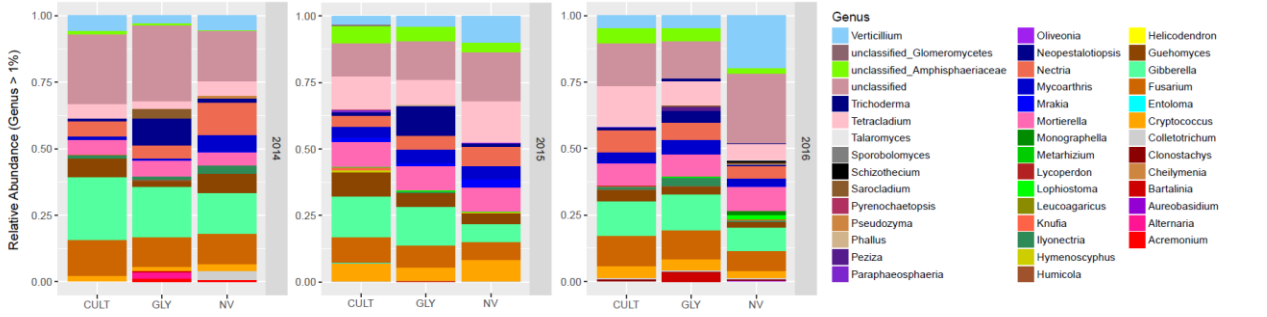


Figure 4.3. Principal coordinates analysis (PCoA) ordinations of soil sample bacterial microbiota derived from Cultivation (CULT), Glyphosate (GLY) and Natural vegetation (NV) field treatments at bloom(B) and harvest(H) based on weighted UniFrac distance metric for 2014, 2015 and 2016 experimental years, where year 2014 was analyzed apart from 2015 and 2016 due to the amount of sequences difference in the samples.

(a)



(b)

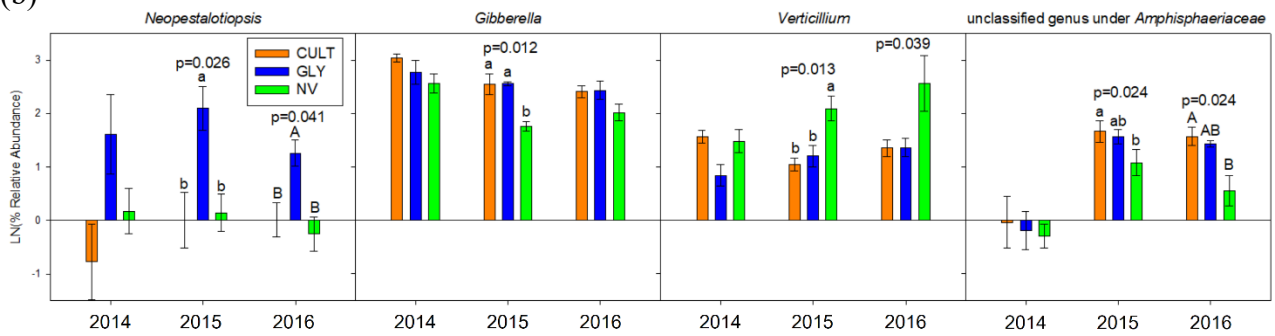


Figure 4.4. Soil fungal relative abundance at genus level. (a) full fungi profile (>1%) in the soil from Cultivation (CULT), Glyphosate (GLY) and Natural vegetation (NV) field treatments (n=4) and (b) Selective fungi that were different in relative abundance (n=4). The statistical differences were tested by using one-way analysis of variance (ANOVA) followed with Tukey HSD test comparing log mean relative abundance at α=0.05.

Under-vine soil management did not impact fungal communities on grapes

Grape samples were collected at commercial harvest in each year. Over 71% of the variance in grape fungal community structure was explained by the first two PCoA axes, but the grape samples were not structured as a function of under-vine soil treatments (Fig. 4.2b). PERMANOVA and paired PERMANOVA were used to confirm that no community composition differences were found among treatments. The three-year overall PERMANOVA showed that the year-to-year differences were the only significant effects (Table 4.1a).

Unclassified genera accounted for 5 to more than 30% of the relative abundance in grape samples. The top five fungal genera with the highest average relative abundance of the three

years in the grape samples were *Sporobolomyces*, *Aureobasidium*, *Rhodosporidium*, *Penicillium,* and *Entyloma*. The fungal genera that differed in relative abundance in soil were not found to differ in relative abundance in grapes. Differences in relative abundance in grape-associated fungal genera were found in *Penicillium*, *Sporobolomyces* and unidentified genera across the years. The fungal genus *Penicillium* was only found in the 2014 grape samples, which was 16.6% in relative abundance, and *Sporobolomyces* was highest in relative abundance in 2015 ($p<0.05$) and lowest in 2016 in grape samples ($p<0.01$), and the unidentified genera relative abundance in 2016 was higher than that in 2014 and 2015 ($p<0.0001$) (Supplementary Fig. S3). The differences in these fungal genera may account for the separation of the grape samples by vintage on the PCoA plot. The grape-specific (not found in soil nor wine) fungal genera detected included *Coprinellus*, *Ischnoderma*, *Mycosphaerella*, *Occultifur*, *Pestalotiopsis,* and *Tilletiopsis*. Many yeast genera commonly found in abundance in grapes, such as *Candida*, *Pichia*, *Debaryomyces*, *Lipomyces*, *Kluyveromyces*, and *Issatchenkia,* were not found or not abundant (<1% in relative abundance) in this study.

Simulated spontaneous fermentation

To simulate spontaneous fermentations, we used microbiomes present on grapes to inoculate the pasteurized (microbially-inactive) base Riesling juice. While the base juice was consistent across treatments, the initial grape microbiome was the only factor that differed in the fermentation reaction. Previous studies have demonstrated that under-vine soil management can alter grape chemistry and/or wine sensory properties(Jordan et al., 2016, Karl et al., 2016a).

The fermentations started when soluble solids content (˚Brix) dropped at the 9th day after inoculation (DAI) and reached the end of fermentation close to the 48th DAI. Soluble solid

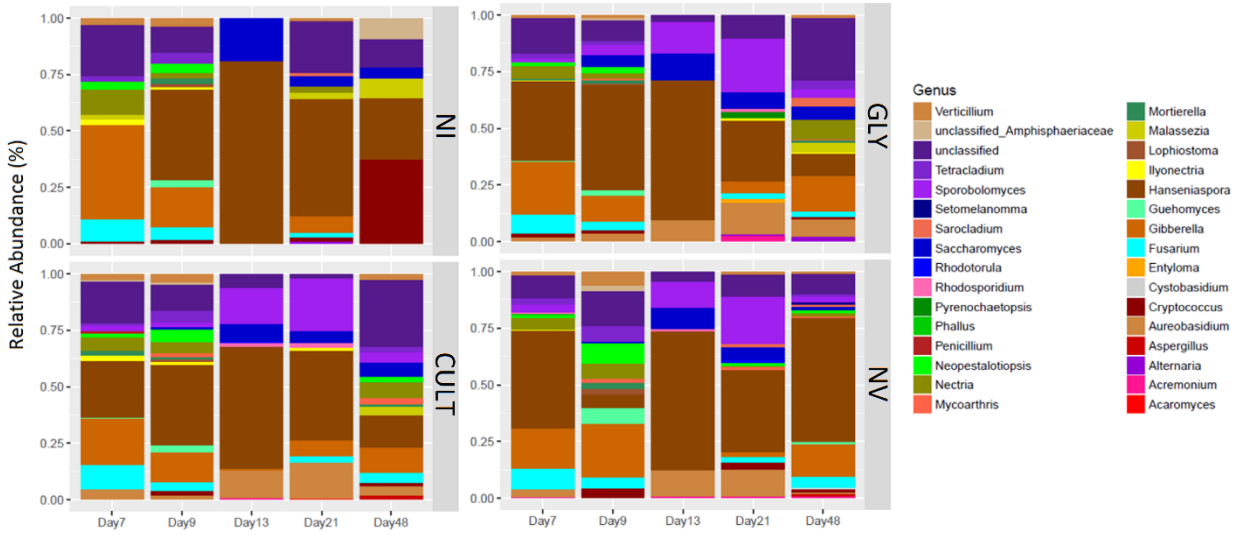
consumption during fermentation did not differ among treatments, except for the uninoculated control, which was delayed two days before the soluble solid content started dropping, and did not reach dryness in two months (Supplementary Fig. S4). The samples selected for analysis were 7th DAI (before the fermentation started), 9th DAI (right as the fermentation started), 13rd DAI (the peak of fermentation), 21st DAI (post-peak of fermentation), and 48th DAI (the end of fermentation where all the samples reached dryness except the uninoculated fermentation control).

The fungal genus *Hanseniaspora* had the highest relative abundance of all fungal genera over the course of fermentation. For the treatments GLY and CULT, the abundance of *Hanseniaspora* was reduced greatly by the end of the fermentation, but remained high in NV (Fig. 4.5a). The genus *Saccharromyces* is usually considered as the major wine fermenting yeast, however, its relative abundance was notably low (peaked at about 10% relative abundance) when compared to *Hanseniaspora* (peaked at 60% relative abundance) (Fig. 4.5b). We acknowledge that this could also be related to primer-specific biases. Although *Hanseniaspora* was present at a high relative abundance, we do not know what role it played in the fermentation. *Hanseniaspora*, *Saccharromyces*, *Sporobolomyces* and *Aureobasidium*, which are all yeast or yeast-like fungi, followed a similar succession pattern, with low relative abundance early in the fermentation, a peak midway, and a decline near the end of the fermentation (Fig. 4.5a).

There was no sample separation by treatment, but by stage of fermentation as shown on the non-metric multidimensional sorting table (Fig. 4.6). Samples of DAI 7th and 9th were similar, DAI 13th separated from all other stages, 21st DAI and 48th DAI overlapped mainly due to the uninoculated fermentation control sample as it remained unfinished in DAI 48th (Fig. 4.6). To compare the fungal relative abundance of each DAI, the sample separation of DAI 13th on the

PCoA plot may be due to the high abundance of a few genera, including *Hanseniaspora*, *Sporobolomyces*, *Saccharomyces,* and *Aureobasidium*, and their effect of displacing more rare genera toward the peak of fermentation. The PERMANOVA also confirmed that DAI had a significant effect ($P<0.001$) on fungal community composition in the fermentation (data not shown).

(a)



(b)

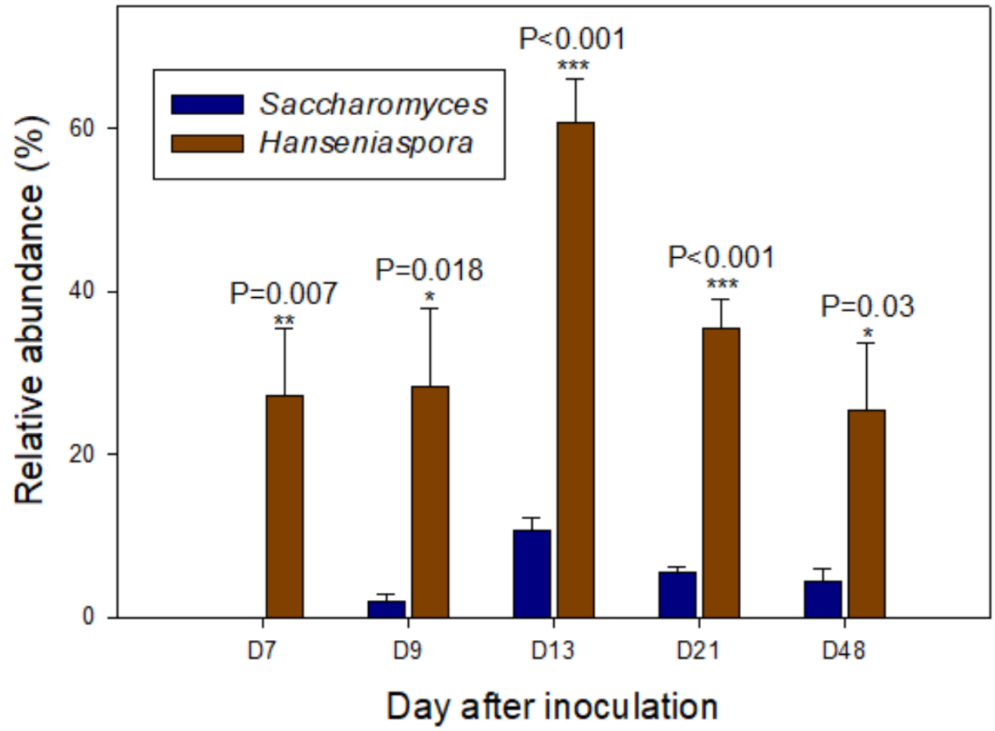


Figure 4.5. Simulated spontaneous fermentation fungal relative abundance at genus level. (a) Fungal relative abundance of wines fermented with treatments (GLY: glyphosate, CULT: soil cultivation, NV: natural vegetation) grape microbiome and non-inoculated (NI) fermentation control (n=2 for treatments and n=1 for NI). (b) *Saccharomyces* and *Hanseniaspora* overall mean relative abundance (n=7) throughout simulated spontaneous fermentation. The error bars indicate standard error. Only the fungi genera with more than 1% mean relative abundance of all the replications within each treatment were presented. The statistical differences were tested by using one-way analysis of variance (ANOVA) followed with Student's t test comparing log mean relative abundance at α=0.05.

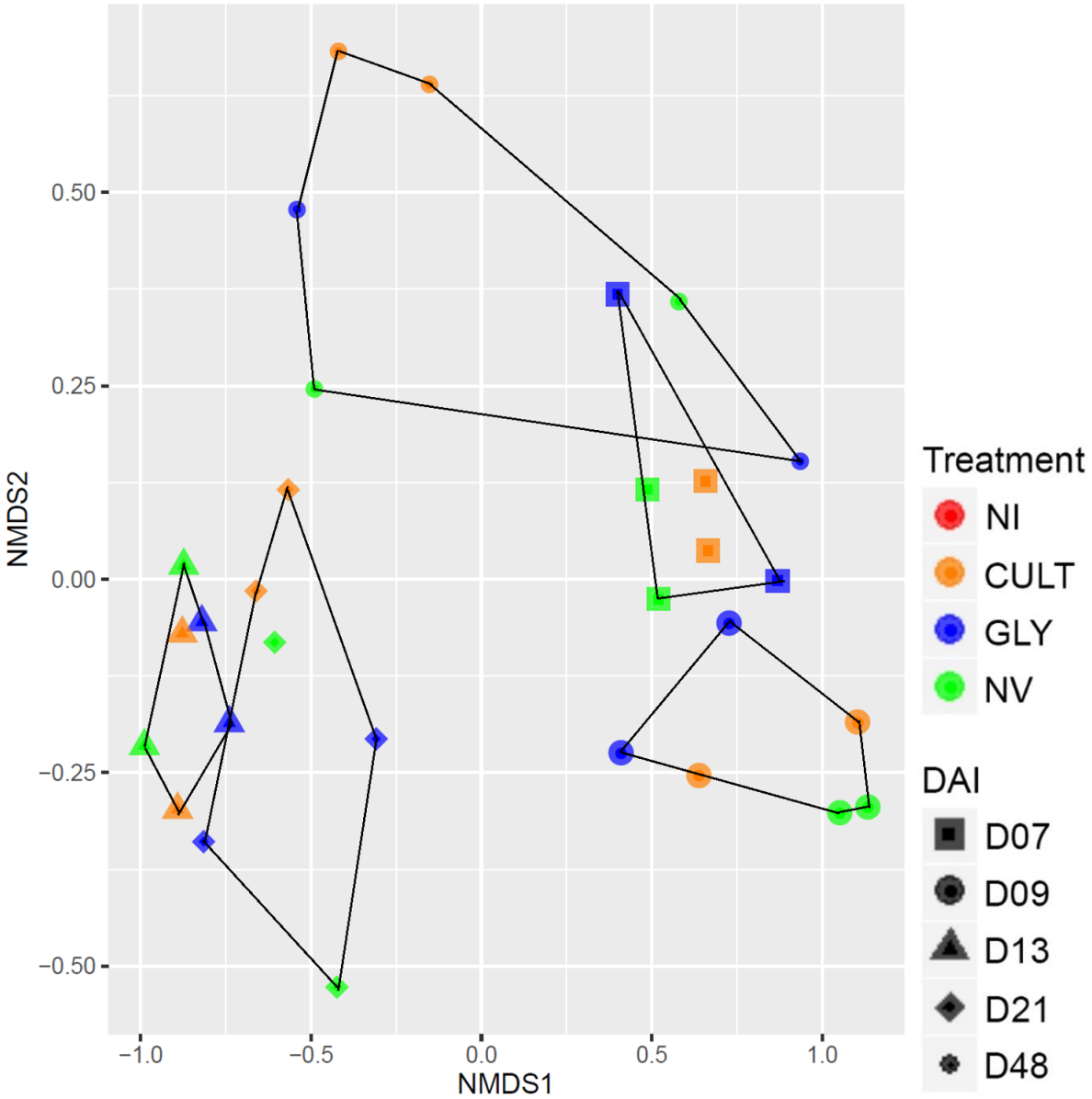


Figure 4.6. Fungal assemblage throughout simulated spontaneous fermentation using microbial wash from grapes of under-vine soil treatments including Soil cultivation (CULT), Glyphosate (GLY) and Natural vegetation (NV) with a non-inoculated fermentation control (NI) plotting on a non-metric multidimensional sorting table. The ellipses were drawn to show the sample clustering in a DAI basis.

Sensory analysis of resulting wines from simulated spontaneous fermentations

The wines (2014 only) were assessed by overall aroma similarity by 97 panelists who self-claimed to drink white wine at least once a month. Metric based single-dimensional scaling revealed that the distance of fermentation replications of GLY and NI samples was closer than that of NV and CULT. However, there was no identifiable clustering among the treatment duplicates (Fig. 4.7). This sensory result matches our findings from the grape and fermentation microbiome structure, in that the under-vine soil management effect was not found in grapes or in simulated spontaneous fermentations.

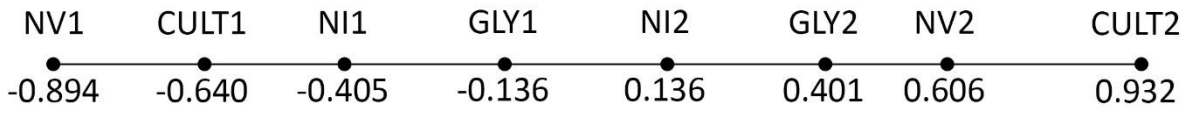


Figure 4.7. Metric-based one dimensional scaling of the overall aroma similarity of simulated spontaneously fermented Riesling (n=97). The base juice was inoculated with inoculums made of drench derived from grapes from different under-vine soil treatments including Cultivation (CULT), Glyphosate(GLY) and Natural vegetation (NV) washed by base juice. The NI indicates non-inoculated fermentation control and the numbers followed the treatments indicate fermentation replicates.

Supplementary data

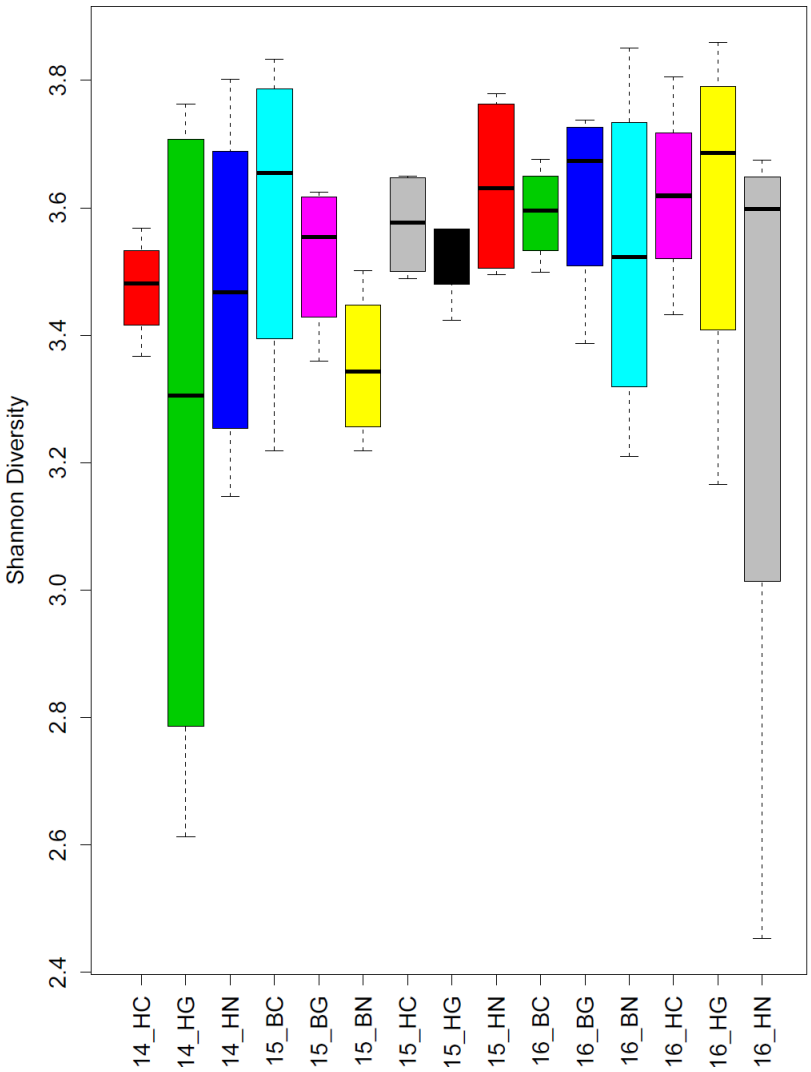
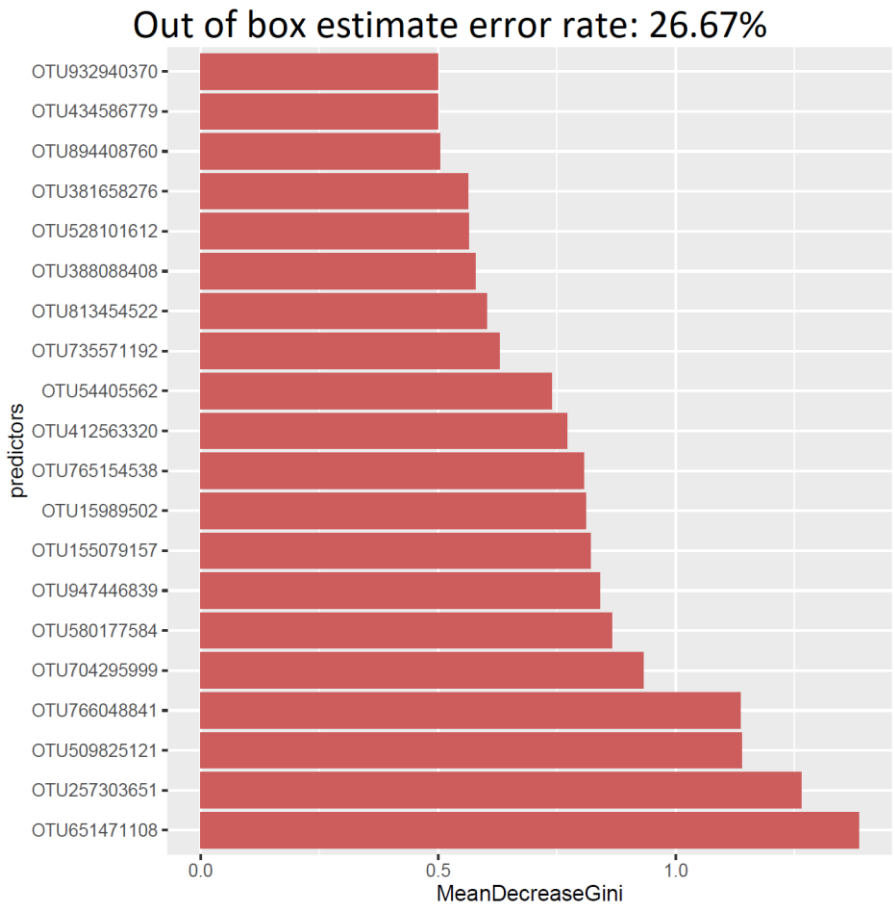


Figure S1. Fungal diversity in the under-vine soil from three experimental years (2014-2016) at two different vine developmental stages, bloom and harvest, analyzed using Shannon Diversity Index. In the x-axis, the numbers in the labels indicate vintage, H/B indicates the sampling stage at harvest/bloom and the C/G/G indicates the soil treatments CULT/GLY/NV.



	Family	Genus	Species
OTU155079157	*Chaetomiaceae*	*unclassified*	*unclassified*
OTU15989502	*Nectriaceae*	*Gibberella*	*unclassified*
OTU257303651	*Amphisphaeriaceae*	*Neopestalotiopsis*	*Neopestalotiopsis foedans*
OTU381658276	*unclassified*	*unclassified*	*unclassified*
OTU388088408	*Incertae sedis*	*Cryptococcus*	*Cryptococcus huempii*
OTU412563320	*Incertae sedis*	*Sarocladium*	*Sarocladium strictum*
OTU434586779	*Hypocreaceae*	*Trichoderma*	*unclassified*
OTU509825121	*Plectosphaerellaceae*	*Verticillium*	*Verticillium_dahliae*
OTU528101612	*Mortierellaceae*	*Mortierella*	*Mortierella acrotona*
OTU54405562	*Incertae sedis*	*Tetracladium*	*Tetracladium spp.*
OTU580177584	*Amphisphaeriaceae*	*Bartalinia*	*Bartalinia_robillardoides*
OTU651471108	*Incertae sedis*	*Monographella*	*Monographella cucumerina*
OTU704295999	*Nectriaceae*	*unclassified*	*unclassified*
OTU735571192	*Nectriaceae*	*Gibberella*	*Gibberella intricans*
OTU765154538	*Chaetomiaceae*	*unclassified*	*unclassified*
OTU766048841	*Montagnulaceae*	*Paraphaeosphaeria*	*Paraphaeosphaeria spp.*
OTU813454522	*Nectriaceae*	*Fusarium*	*unclassified*
OTU894408760	*unclassified*	*unclassified*	*unclassified*
OTU932940370	*Glomerellaceae*	*Colletotrichum*	*Colletotrichum anthrisci*
OTU947446839	*Incertae sedis*	*Acremonium*	*Acremonium dichromosporum*

Figure S2. Under-vine soil treatment prediction derived from Random Forest model using soil fungal OTUs as variables. List of top 20 most important predictors and their correspondent fungal taxonomy according to their mean decrease in Gini coefficients.

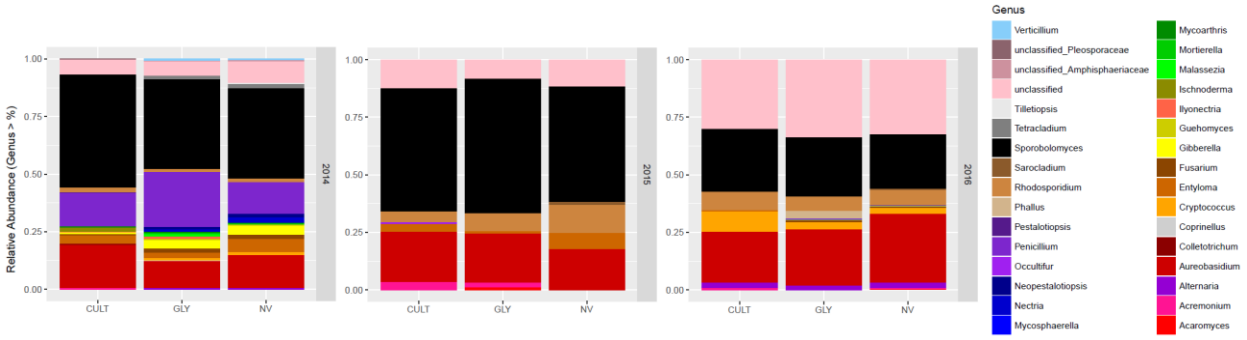


Figure S3. Fungal relative abundance of grape samples from Cultivation (CULT), Glyphosate (GLY) and Natural vegetation (NV) field treatments at genus level for three consecutive experimental years. Only the fungi genera with more than 1% mean relative abundance of all the replications within each treatment were presented.

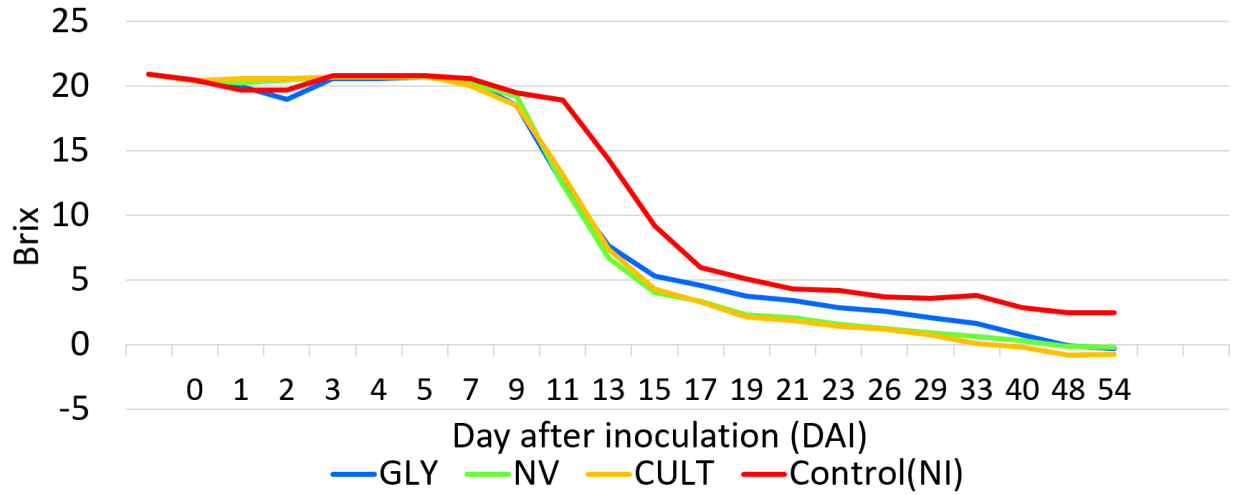


Figure S4. Simulated spontaneous fermentation soluble solid consumption curve. The fermentation was conducted using the grape wash drench where the grapes were harvested from the under-vine soil treatment blocks including Cultivation (CULT), Glyphosate (GLY) and Natural vegetation (NV). The uninoculated fermentation control (NI) was the base juice that was the same juice used for preparing inoculum and fermentation per se.

Discussion

The link between soil microbiome composition and regional wine characteristics has been recently studied (Bokulich et al., 2016, Knight et al., 2015), leading to greater interest in the role of microbial terroir (Gilbert et al., 2014). Our multiple-year experiment examined how different management practices could alter grape and fermentation microbial and wine sensory properties, through the influence of the microbiome. While a previous study suggested that soil management in the vineyard impacted soil microbial communities (Burns et al., 2016) and that grapevine aerial organ-associated microbiomes originated from soil (Zarraonaindia et al., 2015), we hypothesized that implementing different under-vine soil management practices would not only alter soil microbial composition, but that the grape-associated and wine fermentation microbiomes would reflect these changes.

In our study, changes in the fungal community of the soil, due to adopting different under-vine soil management practices, did not extend to the grapes or simulated spontaneous fermentations. As revealed by sensory evaluation, the simulated spontaneously fermented wines, where the inocula were gathered from the grapes grown under the varying management treatments, did not possess consistent detectable different sensory properties.

While this study showed a link between under-vine management practices and soil fungal composition, it did not reveal corresponding changes in grape and fermentation properties. Previous studies have shown that vineyard management alters grape and wine microbiome composition where systematic vineyard management practices or direct microbial management approaches were applied (Cordero-Bueso et al., 2011a, Martins et al., 2014, Bagheri et al., 2015, Grangeteau et al., 2017). In one study, for example, yeast dynamics during the spontaneous fermentation using grapes obtained from conventionally and non-conventionally

managed vineyards differed (Bagheri et al., 2015). Another study revealed that management practices applied directly onto grapes, such as pesticides, impacted grape-associated yeast diversity, which negatively correlated with the copper residuals found on the grapes (Martins et al., 2014). Unlike these studies, our study did not directly manage the microbes on the grapes, but applied indirect changes to microbial community structure in soils.

In our study, the under-vine soil effects on grape and simulated-spontaneous fermentation fungal community structure could also be masked by factors such as climate, geological properties (e.g. soil type), management practices associated with cool climate viticulture (e.g. trellis system, fungal spray use and frequency), vineyard management history, and inter-row vineyard floor management. Among these factors, many are specific to the region, such as large vine size with tall trellis systems, frequent pesticide applications, and hilling soil up over the graft union in winter and down off of the graft union in the spring. In a broader sense, climatic conditions play a significant role in microbiome structure, which is shown in our study, with year-to-year climate differences being the most significant factor explaining variance in the soil and grape fungal assemblages, which is consistent with a previous study (Bokulich et al., 2014).

With weather variability increasing as a function of climate change, there is renewed interest in improving resilience of vines to environmental stress. Cover crops are known to improve soil health by retaining soil moisture, enhancing drainage, raising soil organic matter content, maintaining soil physical structure, supporting soil microbial properties and processes (Doran and Zeiss, 2000, Steenwerth and Belina, 2008b, Peregrina et al., 2010, Ruiz-Colmenero et al., 2013, Karl et al., 2016b). Also, cover crops provide a prolific root zone (rhizosphere) that enriches for a diversity of microorganisms that perform many functions, such as mediating soil

nutrient cycling, impacting plant growth and development, and influencing pathogen interactions (Barrios, 2007, Steenwerth and Belina, 2008a, Steenwerth and Belina, 2008b, Doran and Zeiss, 2000, Gianinazzi et al., 2010). Although no evidence of higher microbial diversity was supported by under-vine cover crops in this study, possible grapevine pathogenic genera *Neopestalotiopsis* was reduced in vegetation covered soil.

Hight relative abundance of *Hanseniaspora* observed in this study may originated from grapes or winery environment. *Hanseniaspora* is known to be present in high abundance on grape, must (Zott et al., 2010), and in the early stages of controlled or uncontrolled fermentation (Fleet, 2003, Torija et al., 2001, Grangeteau et al., 2015, Bokulich et al., 2015). Previous studies have also shown that *Hanseniaspora*, even though occurring at very low relative abundance at the end of fermentations, persists throughout the fermentation without addition of SO_2 to tolerate alcohol levels up to 13% (Grangeteau et al., 2017). There is also a study demonstrating that *Hanseniaspora* has the ability to secrete toxins that suppress the activity of *Saccharomyces* during fermentation (Radler et al., 1990), which may also be the case in this study as low *Saccharomyces* relative abundance was observed. Although the actual role of *Hanseniaspora* was unknown in our study, its significance in abundance suggests that *Hanseniaspora* may have the potential to ferment wine to dryness with the help of *Saccharomyces*.

This study aimed to evaluate the role of management practices - specifically vineyard soil management - on microbial terroir. Our study is the first to examine the impact of management practices on soil tracked through to grapes and spontaneous wine fermentations. We found that bare soil maintained by soil cultivation and herbicide led to soil fungal communities that diverged from the non-cultivation natural vegetation treatment. The results indicate that vineyard microbial terroir could be susceptible to changes under different soil management

practices; however, the spatial gap between soil and the fruiting zone, and the frequent pesticide applications, could impact the level of soil management effects. It also suggests that future studies on the movement of microorganisms from soil to grape would be key to understanding the role of vineyard soil management in microbial terroir.

Despite the previous findings on vineyard management effects on vineyard microbiomes, our findings reveal that altering soil microbial composition in the vineyard through under-vine management practices does not result in corresponding changes to the grape and wine microbiome or the wine sensory properties. The concept that soil microbial composition could be driving microbial terroir should be examined in light of vineyard management practices that alter soil biotic components. Regional management practices that modify soil conditions could have a significant role in shaping microbial terroir in wine growing regions.

Conclusion

This study showed that letting weeds to grow under-vine shifted soil bacterial and fungal community structure. However, this fungal community shift in soil did not extend to the grapes or the simulated spontaneous fermentations. Sensory analysis of wine inoculated with the microbiome from grape washes showed no distinguishable patterns across treatments. Strong microbial association between soil and grapes suggested by previous studies was disapproved. While previous studies emphasized on geographical impact on vineyard microbiome, the results of this study suggested that regional vineyard management practices such as trellis system and pesticide application frequency may have greater impact on grape associated and wine fermentation microbial communities.

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Chapter 5

Conclusion and Future Perspectives

Vine vigor mitigation effects of under-vine cover crops were prominent in young vineyards where vines were susceptible to nutrient or water competition (Wheeler et al., 2005, Hickey et al., 2016, Karl et al., 2016, Coniberti et al., 2018), but mature vines were less impacted by under-vine vegetation (Centinari et al., 2016, Jordan et al., 2016). Resilience to resource competition in mature vines was confirmed by the results in Chapter 3 where under-vine natural vegetation had little impact on mature Riesling vines, but the study in Chapter 2 showed that excessive vigor of mature vines could still be reduced using aggressive under-vine cover crops such as chicory.

In the studies reported here, the yield was not reduced in any of the under-vine floor treatments across two grape cultivars and three years. Yield is crucial to financial sustainability especially to Finger Lakes growers due to low profit margins (Yeh et al., 2014). The ability to maintain stable yield with under-vine cover crops suggested the practical adoptability and advantage of using under-vine cover crops in mature vineyards rather than young ones. However, long-term effects of under-vine cover crops were not studied. Long-term evaluation of how under-vine cover crops impact vine growth, and grape and wine composition are required for commercial adoption.

In the first two chapters, grapes were only examined on their harvest parameters including soluble solids, pH, TA and YAN. Although the results suggested that under-vine cover crops did not compromise harvest parameters, other grape quality associated compounds such as

aroma precursors, colors, and tannins were not examined. Although wine sensory properties were evaluated and suggested no detectable differences among the wines, the untrained and skewed selection of panelists was not necessary the best representation of the regular wine consumers. A comprehensive chemical analysis of grape and wine composition could provide a thorough evaluation of the impact of under-vine floor management practices on fruit and wine, and could advance the understanding of their effects by tracing the influences backwards from wine to grape, grape to vine, and vine to soil. Using a trained sensory panel may also provide a more robust qualitative and quantitative sensory assessment of the wines.

A previous study conducted in the Finger Lakes region showed a surprising result where the under-vine floor management effect on wine sensory properties was decoupled from vine shoot growth, pruning weight, Ravaz index, canopy structure, midday stem water potential and petiole nutrient status when these parameters were not affected but wine sensory properties differed (Jordan et al., 2016). In the presented studies, under-vine cover crop impact on wine sensory properties were studied in a mature Riesling vineyard (Chapter 3 and 4). Sensory analysis on the wines made from inoculated winemaking processes revealed panelists did not consistently detect differences in aroma among wines while under-vine floor treatments had little impact on vine physiological parameters over the three years. Multi-dimensional sorting of the simulated spontaneous fermentations showed that there were no detectable differences among the wines from different under-vine floor management regimes and indicated no detectable effect of soil microbial community structure changes. Fungal community profiling of grapes from each of the under-vine floor management regimes confirmed that there were no fungal community structure differences among them, even though soil fungal communities differed. This result rejected the hypothesis derived from the study of Jordan et al. 2016, that the under-vine floor

management impacted wine sensory properties through the change of grape associated microbiome. However, this result revealed the importance of investigating vineyard microbiomes in a range of climatic and managerial environments in future studies. To answer the question of how under-vine cover crop impacted wine sensory properties without changing vine growth parameters, under-vine cover crops impact on microclimate such as humidity and temperature, and the subsequent impact on disease and pest incidence and severity may be interests of future studies.

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