# Optimizing nitrogen fertilization practices under intensive vineyard cover cropping floor management systems

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## ABSTRACT

Under-trellis cover crops have become more prevalent in East Coast grape growing regions through either intentional planting or adoption of native vegetation, to minimize the potential for erosion and to help regulate grapevine size and vigor. These companion crops, however, have sometimes resulted in increased competition for soil nitrogen, leading to decreased vine nitrogen status and berry yeast assimilable nitrogen (YAN). The aim of this study was to determine the effects of different nitrogen fertilization methods applied at varying doses and different times, on vine and berry nitrogen parameters of cover cropped grapevines. The research described herein involved Sauvignon blanc, Merlot, and Petit Manseng grapevines (*Vitis vinifera* L.) subjected to different sets of nitrogen treatments, and was primarily conducted over two years. There were very few differences in pruning weights, canopy architecture, components of yield, and primary fruit chemistry amongst nitrogen treatments. Sauvignon blanc petiole nitrogen concentration, season-long chlorophyll content index (CCI) values, and berry YAN were most affected by the highest rate of soil nitrogen treatment (60 kg N/ha total split between two calcium nitrate applications at bloom and six weeks post bloom) and foliar fertilization (40 kg N/ha split over seven to nine urea applications); however, the foliar fertilization was most effective at increasing the concentration of certain individual amino acids. Petit Manseng berry YAN at harvest was increased in response to post-véraison foliar applications (10 kg N/ha split between two urea applications), corresponding to an increased concentration of nine amino acids. Merlot berry YAN, petiole nitrogen concentration, and season-long CCI values were most affected by a high rate of soil nitrogen treatment (60 kg N/ha total split between two calcium nitrate applications at bloom and six weeks post bloom) and establishing clover as the under-trellis cover crop. This study identified nitrogen treatments that improved berry nitrogen concentration and content in cover cropped sites.

## Dedication

To my better half, James— the best data recorder and editor a girl could ask for.

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# Table of Contents

# List of Figures

# List of Tables

# List of Abbreviations

Ala - Alanine
AREC - Alson H. Smith Jr. AREC (Petit Manseng)
Arg - Arginine
Asn - Asparagine
Asp - Aspartic acid
CCI - Chlorophyll Content Index
CEFA - Cluster Exposure Flux Availability
CEL - Cluster Exposure Layer
CON - Chateau O'Brien at Northpoint (Merlot)
Cys - Cysteine
EPQA - Enhanced Point Quadrat Analysis
GDD - Growing Degree Days
Gln - Glutamine
Glu - Glutamic Acid
Gly - Glycine
GMV - Glen Manor Vineyards (Sauvignon blanc)
His - Histidine
Ile - Isoleucine
LEFA - Leaf Exposure Flux Availability
LEL - Leaf Exposure Layer
Leu - Leucine
Lys - Lysine
Met - Methionine
OLN - Occlusion Layer Number
Phe - Phenylalanine
PPFD – Photosynthetic Photon Flux Density
Pro - Proline
Ser – Serine
TA – Titratable Ccidity
Thr – Threonine
TSS – Total Soluble Solids
Tyr - Tyrosine
UPLC - Ultra Performance Liquid Chromatography
Val - Valine
YAN - Yeast Assimilable Nitrogen

## Introduction

In recent years Virginia vineyards have employed intensive cover cropping as a vineyard management strategy to suppress excess vine vigor. Additional benefits associated with cover cropping include: reducing soil nutrient leaching, improving soil organic matter and soil structure, suppressing weed populations, improving conditions for tractors in wet periods, moderating soil temperatures and water status, reducing erosion, enhancing biodiversity, and improving water filtration (Baumgartner, Smith, and Bettiga 2005; Celette, Findeling, and Gary 2009; Fourie, Louw, and Agenbag 2007; Gulick et al. 1994). In many studies cover crops have been shown to compete for soil nitrogen, leading to diminished berry nitrogen, and specifically, reduced yeast assimilable nitrogen (YAN) in berries (Larchevêque, Casanova, and Dupuch 1998; Maigre 1997, 2002; Maigre and Aerny 2000; Spring and Lorenzini 2006; Xi et al. 2010). There is an urgent need to address this concern, because reduced berry YAN contributes to stuck or slow fermentations and reduced wine quality (Bell and Henschke 2005). Standard nitrogen application rates, which have previously been successful for vines grown in the presence of an herbicide strip, may be insufficient to maintain or increase vine nitrogen and berry nitrogen status, due to the cover crop successfully competing for the fertilizer addition. Other methods—such as utilizing leguminous cover crops, applying compost, spraying foliar nitrogen applications, or a combination thereof—have potential to be successful alternatives, and were thus explored in this study (Guerra and Steenwerth 2012; Jreij et al. 2009; Schreiber et al. 2002; Spring 2001; Spring and Lorenzini 2006).

# Review of Literature

## Nitrogen Cycling

### *Nitrogen partitioning overview*

A fundamental role of nitrogen in grapevines is to produce amino acids that can later be assembled into proteins vital to new tissue growth, vine metabolism, and energy generation (Keller 2010). It is well documented that annual vine growth is sustained by carbohydrates and nutrients stored in woody tissues, as well as those obtained from the soil on a seasonal basis. For this reason, seasonal nitrogen uptake at critical demand periods has often been studied in conjunction with annual carbohydrate cycling in grapevines.

The most general summary of nitrogen and carbohydrate partitioning suggests that early season root and shoot growth are supported by reserve nitrogen and carbohydrates throughout active shoot growth. By the beginning of bloom, reserves are often depleted and future growth is reliant on nitrogen uptake and carbon assimilation until at least véraison. For this reason, the critical demand period for new nitrogen is thought to be toward the end of active shoot growth through early berry development (Christensen, Kasimatis, and Jensen 1978; Conradie 1986; Wermelinger, Baumgärtner, and Gutierrez 1991). Uptake during this critical demand period is dependent on soil nitrogen availability and is closely linked to climatic conditions that influence nitrogen mineralization rates. Soil microbes mineralize more nitrogen in moist soil, consequently resulting in a limited nitrogen pool in dryer soils (Keller 2010). After harvest, nitrogen uptake and translocation of nutrients from leaf tissue prior to senescence are responsible for renewing the nitrogen and carbohydrate reserves in woody tissues (Conradie 1986).

### *Early-season nitrogen dynamics*

Initial spring growth from budbreak throughout rapid shoot growth is heavily dependent on reserve nitrogen and total nonstructural carbohydrates being remobilized. In a study conducted on three-year-old Pinot noir vines, roots stored 90% of total starch, 75% of total nitrogen at the beginning of the season, but exhibited a 70% starch loss by bloom (Zapata et al. 2004). Destructive analyses were conducted throughout the season to measure starch content, and to perform a quantitative nitrogen analysis through the use of a $^{15}$N tracer. During the dormant analysis, nitrogen represented only 1.6% root dry weight, while starch, represented 33% dry weight. No growth or yield data was provided, but the authors of this study suggest that their findings illustrate that carbohydrates (and starch specifically) are the primary contributor to initial spring growth (Zapata et al. 2004). In a similar study conducted on three-year-old Concord vines, roots stored 84% of total vine starch and 75% of total vine nitrogen, with trunks and shoots contributing the remaining storage units (Bates, Dunst, and Joy 2002). In accordance with Zapata et al. 2004, the Concord vines exhibited a 78% loss of the reserve starch, which was used for pre-bloom root and shoot growth. Fine root nitrogen uptake accounted for at least 84% of spring growth nitrogen, indicating that stored nitrogen is less important than stored starch for initial spring growth. This data conflicts with a separate nitrogen fertilization experiment which reported that 8 weeks of mid-summer fertigation with ammonium nitrate did not have an impact on total vine nitrogen in one-year-old Concord vines, but total nonstructural carbohydrates decreased slightly as determined by a destructive analysis (Xia and Cheng 2004). In a separate publication, data is presented that demonstrates total leaf area, fruit yield, and total dry weight increased in accordance with the rate of nitrogen applied the previous year (0, 5, 10, 15, or 20 mM N): those that received foliar nitrogen combined with fertigation had greater yield data than the fertigation treatments alone—despite the decrease in total nonstructural carbohydrates—suggesting that reserve nitrogen is actually more important than vine total nonstructural carbohydrates in terms of contributions to growth and development (Cheng, Xia, and Bates 2004). The differences in these studies may be attributed to the differences in vine age or environmental conditions such as precipitation and temperature patterns. The fact remains that replenishing storage nitrogen is important for maintaining overall vine nitrogen status for future years, and the fertilization methods proposed in this Virginia study take this into account.

### *Mid-season nitrogen dynamics*

Rapid absorption of nitrogen into the roots occurs during active shoot growth (Hanson and Howell 1995). For Chenin blanc grapevines grown in sand culture, fertilization during different phenological periods found that flowering through véraison was considered a critical period, as nitrogen uptake is highest at this point, while storage nitrogen is depleted. Vines rely on this stage to absorb enough nitrogen to supply clusters during maturation and growth (Conradie 1986). One study reports that 46% of the nitrogen supplied at the end of rapid shoot growth will end up in mature berries (Conradie 1991). Moreover, bloom commences this important uptake period because it coincides with a fully developed fine root system.  Beginning at bloom through early berry development, nitrogen uptake and carbon assimilation support berry growth (Zapata et al. 2001). Reserve nitrogen and carbohydrate accumulation also begin at bloom and continue through harvest, with the majority of accumulation occurring at the end of active shoot growth onward (Bates, Dunst, and Joy 2002; Pradubsuk and Davenport 2010; Zapata et al. 2001). Given the significance of the bloom-harvest period on N absorption and accumulation, it is unlikely that nitrogen applications prior to this time will contribute meaningfully to either current season nitrogen or nitrogen reserves, and therefore was not considered in this study.

### *Pre-harvest through senescence nitrogen dynamics*

Similar fertilization studies conducted in South Africa demonstrate that up to 37% of annual nitrogen absorption occurs between harvest and leaf senescence, thereby providing 60% of the stored nitrogen for the following season (Conradie 1986; Conradie 1992). It appears that roots are still able to absorb nitrogen after harvest; therefore, fertilization at this time replenishes storage nitrogen considerably (Treeby and Wheatley 2006). Post-harvest, nitrogen is remobilized from leaves to storage units in the trunk and roots; however, this remobilization can be impaired if temperature drops rapidly or the canopy defoliates (Cheng, Xia, and Bates 2004). For the purposes of my study, I aimed to assess whether post-harvest nitrogen applications improve berry YAN levels in future years, and specifically whether this strategy can be considered a feasible nitrogen management practice in cover cropped vineyards.

## Cover Crop Use

### *Benefits of cover crops*

Growing grapevines in conjunction with alleyway cover crops or under-trellis cover crops is now a long-standing viticulture practice. There are many perceived benefits of cover cropping systems, which include: reducing excess vine vigor, improving soil organic matter, soil structure and water holding capacity, reducing nitrate leaching, suppressing weed populations, improving traffic conditions in wet periods, moderating soil temperatures and water status, reducing erosion, enhancing biodiversity, and improving water infiltration (Baumgartner, Smith, and Bettiga 2005; Celette, Findeling, and Gary 2009; Fourie, Louw, and Agenbag 2007; Gulick et al. 1994). Most of these effects also apply to other agricultural crops, but specific effects of cover crops on grapevines include achieving higher total soil microbial biomass, an effect that has potential for increased arbuscular mycorrhizal fungal interactions, pending grape root overlap with cover crop roots (Ingels et al. 2005). Increased arbuscular mycorrhizal fungi interactions have potential to improve vine nutrition by increasing phosphorous uptake, providing greater protection from soil borne pathogens, and improving water acquisition (Cheng and Baumgartner 2004; Schreiner 2005).

### *Effects of cover crops on vine yield and berry YAN*

Many studies have illustrated that vines grown with cover crops exhibit reduced vine vigor and significant decreases in yield (Celette, Findeling, and Gary 2009; Hatch, Hickey, and Wolf 2011; Larchevêque, Casanova, and Dupuch 1998; Lavezzi et al. 2005;

Tan and Crabtree 1990; Tesic, Keller, and Hutton 2007; Wolpert et al. 1993). Chardonnay vines grown with sward competition exhibited reduced vine vigor, more open canopies, lower yields, and overall increased fruit maturity (Tesic, Keller, and Hutton 2007). While decreased vigor and yield is considered beneficial in some scenarios, the Chardonnay also exhibited reduced petiole nutrient status, an outcome that may be detrimental to overall vine health. The reduced nutrient status was attributed to the observed decrease in water supply and subsequent reduced nutrient uptake (Tesic, Keller, and Hutton 2007). This data is in accordance with Hatch et al. 2011, who suggested that under-trellis cover crops are a powerful tool for restricting vine growth by limiting water supply, especially in regions prone to excess vigor. These studies justify experimentation with alternate forms of nutrient additions less dependent on water uptake to offset nutrient deficiencies, such as foliar applications. Studies that examine the effects of cover crops on grape yield while providing irrigation to experiment vines often indicate little or no reduction in vigor (Ingels et al. 2005). The lack of water stress and ability to uptake minerals likely enables vigor, yield, and nutrient content to remain unaffected in most cases, unless cover crops are excessively competitive in nature. Furthermore, studies that examine the effects of alleyway cover crops on vine vigor and yield are often unaffected if an herbicide strip is maintained under the trellis; however, nutrient deficiencies in leaf tissue and berry YAN are often observed and have potential to reduce wine quality (Baumgartner, Steenwerth, and Veilleux 2008; Sweet and Schreiner 2010).

Celette et al. 2009 found that for Mediterranean Aranel/Fercal vines grown with a permanent tall fescue or a nonpermanent barley intercrop, nitrogen uptake and growth were reduced. This effect was greatest for the permanent tall fescue intercrop, leading to reduced nitrogen reserves. The authors attributed the decrease in nitrogen to the cover crops directly removing nitrogen and water from the mineralization zone, causing drought and reduced nitrate reductase activity. This effect was exacerbated in the fescue treatment because the crop's early development, prior to vine rapid shoot growth, enabled rapid removal of soil water at the same time that the vines were utilizing perennial nitrogen rather than seeking absorption of early season soil nitrogen. In contrast, vines grown with barley were able to take advantage of newly formed inorganic nitrogen present in the soil, due to favorable spring conditions of warm temperatures and rainfall, prior to barley emergence. The barley was also less competitive because it was destroyed chemically by the time of bloom and therefore did not continue to compete for nitrogen after that point (Celette et al. 2009). Reductions in growth are therefore attributed to in-season nitrogen removal as well as reduced nitrogen accumulation for initial spring growth in the following season. It should be noted that the degree of competition for water and nitrogen is most likely dependent on the actual cover crop biomass produced, which is affected by climatic and site-specific parameters.

In addition to the devigorating effects caused by reduced water and nitrogen uptake, significant decreases in berry YAN levels in competitive cover cropped systems are well documented (Larchevêque, Casanova, and Dupuch 1998; Maigre 1997, 2002; Maigre and Aerny 2000; Spring and Lorenzini 2006; Xi et al. 2010). While the overall decrease in YAN is primarily explained by increased competition for soil nitrogen, and reduced uptake due to water availability, it may also be due to the increase in proline relative to other amino acids. Environmental stressors, including water and heat stress, induce proline accumulation which has been shown to protect proteins and cell membranes against osmotic damage (Schaller 2005). If the competing cover crop limits water to the point of inducing stress, then increased berry proline concentrations are certainly plausible. This contrasts with the results of a cool climate experiment with adequate soil moisture, where the presence of cover crops improved the relative composition of amino acids in berries such that the content of assimilable amino acids, as a percentage of total amino acids, was higher in cover cropped vines compared to the control; however, total berry nitrogen concentration still decreased (Gouthu et al. 2012). Insufficient YAN can be detrimental to wine production and will be discussed in section III.

### *Leguminous cover crops*

The use of leguminous cover crops to overcome nitrogen deficiencies has been explored in vineyards. Compared to grasses, legumes have a much lower C:N ratio which enables them to decompose rapidly after incorporation, thereby providing nitrogen to the vines (Fourie, Louw, and Agenbag 2007). In order for the cover crops to be successful, tilling in of the legumes must be synchronized with the critical demand period between active shoot growth and véraison, and the relative biomass of the cover crop must be large enough to contribute meaningfully to the nitrogen soil pool. This synchronization will depend on the decomposition rate for the cover crop being used—a value that is rarely documented in the literature for cover crops conducive to Virginia growing conditions. Hairy vetch (*Vicia villosa* subsp. *villosa* L.) and yellow sweet clover (*Melilotus officinalis* (L.) Lam.) were analyzed in a Pacific Northwest vineyard for their potential contribution of nitrogen to four year-old Concord vines during the critical demand period (Bair, Davenport, and Stevens 2008). Incorporation of either cover crop at vine bloom appeared to significantly increase soil nitrate levels from bloom through véraison compared to no nitrogen control treatments. The relative increase in soil nitrogen was largely dependent on achieving adequate biomass, indicating an urgent need to improve the methods of cover crop establishment if leguminous cover crops are to be adopted as a commonplace grape growing practice in Virginia. In some cases, even substantial biomass may not be fully sufficient to provide comparable nitrogen inputs as conventional practices, but a study conducted in Egypt reported that when combined with compost, leguminous cover crops can provide similar levels of nitrogen as synthetic fertilizers after three years of establishment (Rizk 2012). My study aims to determine if this is a viable option for Virginia growers.

Simply establishing a strong legume stand may suffice for improving available soil nitrogen in vineyards, if the established legume exhibits substantial rhizodeposition of nitrogen (Fustec et al. 2010). Rhizodeposition occurs via decomposition and decay of nodules and roots cells, or through direct exudation of soluble nitrogen compounds (namely ammonium and amino acids). Nitrogen derived from rhizodeposition can account for 7-57% of the mature leguminous plant's total nitrogen content, and is readily available to surrounding plants (Fustec et al. 2010). If we consider that some leguminous species such as white clover can fix upwards of 296 kg N/ha in a pure stand, then the vital role rhizodeposition may play a role in using leguminous crops as a primary nitrogen source becomes evident (Ledgard and Steele 1992). Nitrogen fixation has been reported to increase when legumes are grown in association with non-legumes species, with maximum reported nitrogen fixation values of 682 kg N/ha (Ledgard and Steele 1992). Such findings suggest that establishing legume-grass cover crop stands in vineyards should be considered as a practice for commercial vineyards.

A Merlot study comparing leguminous cover crops in conjunction with different methods of incorporation found that vines grown with a green manure mix (bell bean, field pea, common vetch, barley) disked in the spring had significantly higher petiole nitrogen concentration compared to vines grown with an annual clover mix (subterranean clover, bur medic, crimson clover, rose clover) mowed in the spring (Ingels et al. 2005). This may be explained by the green manure mix reaching a larger biomass and keeping non-leguminous weed populations lower. The increase in higher petiole nitrogen concentration was likely due to both the increased biomass available, as well as incorporation of the mix with disking. While not mentioned in the publication as a potential cause for biomass disparity, the green manure mix was seeded at a much higher rate than the annual clover mix (90 ka/ha, and 28 kg/ha respectively), which may have affected the cover crop establishment. However, the rate may not be the cause, as suggested by a separate study where low seeding rates of clover mix (4.2 kg/ha) in an Oregon vineyard provided up to 86 kg N/ha to Pinot noir vines when percent cover crop establishment reached 80% as estimated by 0.25 meter quadrant plots (Sweet and Schreiner 2010). In the same study, the clover mix produced increased vine nitrogen content and berry YAN compared to the clean cultivated vines in the second year of data collection. This data provides justification for further experimentation with clover mixes as a method to improve berry YAN.

$^{15}$N-enriched fertilizer studies have been used to assess the percent nitrogen that cover crops actually contribute to grape-leaf nitrogen concentration. A large range of values (0.28-25%) have been reported, which can be attributed to many potential confounding variables that include: whether the study is conducted on young vines with low nitrogen reserves or old vines that tend to have larger nitrogen reserves; whether the cover crop is planted in the inter-row or under the trellis; whether fine root growth is disturbed by tilling; the soil type present; and the cultivar and rootstock in the experiment (Keller, Kummer, and Vasconcelos 2001; Ovalle et al. 2010; Patrick et al. 2004). Rainfall also likely plays an important factor as reported by Patrick et al. 2004, where the 0.28% contribution of legume nitrogen to vine nitrogen concentration occurred during a season that received 45 mm of rain, which would undoubtedly reduce plant decomposition, and nitrogen mineralization and uptake.

Legumes can also increase nitrogen availability through nitrogen fixation – strawberry clover, for example, is capable of providing up to 330kg/ha of nitrogen annually (Ingels 1998). The amount of nitrogen provided through fixation is dependent on many variables, including: legume variety, inoculation effectiveness, soil moisture, temperature, and phosphorous availability (Chaudhary et al. 2008; Madge 2005). Leguminous cover crops were found to be more effective in a California vineyard at improving soil water content than native grasses, thereby further improving the potential for increased soil nitrogen levels via mineralization processes (King and Berry 2005). Because nitrogen fixation is an extremely energy intensive process, legumes will preferentially use soil nitrogen if it is available. For this reason, nitrogen applications made to leguminous cover crops may halt nitrogen fixation (Salvagiotti et al. 2008). The large range of variables that affect nitrogen fixation rates provide ample justification for incorporating leguminous cover crops to ensure significant nitrogen contributions to the system.

### *Effect of cover crops on berry chemistry and wine quality*

The effect of cover crops on primary grape chemistry generally depends on the level of competition between the cover crop and the vine. Systems in which no herbicide strip is employed and irrigation is unavailable exhibit the most aggressive competition for limited nutrients and water; competition of systems in which an herbicide strip is present will heavily depend on the width of the herbicide strip.

Many studies illustrate that cover cropped systems can increase total soluble solids (TSS) and decrease titratable acidity (TA) of harvested berries (Larchevêque, Casanova, and Dupuch 1998; Wheeler, Black, and Pickering 2005). The increase in TSS can likely be attributed to a concentrating effect induced by the reduction in yield. Other studies indicate cover crops have no affect on TSS, TA, or pH (Ingels et al. 2005; Sweet and Schreiner 2010). It is important to note that in these studies, less aggressive, inter-row cropping systems were used, and yield and pruning weights were also unaffected. There is also data that indicates nominal differences in primary chemistry attributes, but TSS still increased and TA decreased for complete vineyard floor cropping systems (Tesic, Keller, and Hutton 2007).

In aggressive systems where vine vigor is decreased, canopy porosity has been found to increase, enabling greater sun exposure and increased cluster surface temperatures (Maigre 2001). Price et al. (1995) report that greater exposure to direct sunlight increased total polyphenols, anthocyananins, and flavonols. Yet, other reports show no significant difference in anthocyanin or total phenolics for cover cropped vines (Hostetler et al. 2007; Sweet and Schreiner 2010).

Reports on the effect of cover crops on wine quality vary. Off-aromas and loss of aromas are expected results of fermentations on musts deficient in YAN due to cover cropping. Schultz and Lohnertz (2003) report that sward-vine competition resulted in nitrogen deficiencies which contributed to off-flavor development in white German wines. On a similar note, a Swiss study conducted on Pinot noir vines found that wines produced from cover cropped treatments contained more aggressive tannins and reduced aroma potency (Maigre 2002). The increased water and nitrogen stress associated with cover crops has also been associated with the presence of ortho-aminoacetophenone (O-AAP) in wines, the compound responsible for atypical aging (Bell and Henschke 2005).

More recent literature suggests that, despite decreases in total berry nitrogen, the total free amino acid concentration of wine and grape increases in cover cropped vines. Xi et al. (2011) found that five-year-old Cabernet Sauvignon vines produced wines with a greater number of aroma compounds when grown with cover crops (such as alfalfa, tall fescue, and white clover), compared to wines produced from vines grown under clean tillage. In the same study, wines produced from grapevines grown with cover crops rated higher for aroma (rating more abundant in floral aroma and sweet and ripened fruit notes), taste analysis, and harmony in a sensory study, than did wines produced from grapevines grown in the presence of an herbicide strip.

## Effects of nitrogen on berry development and fermentation

### *Importance of nitrogen balance*

Nitrogen is an integral component of vine and berry development. Without nitrogen, vines would be unable to produce the amino acids required for tissue growth or have the energy necessary for metabolic functions (Keller 2010). Nitrogen is also a fundamental driver of fermentation success by affecting yeast growth, fermentation kinetics, and flavor metabolism. While there are many forms of nitrogen present in grape berries, wine yeasts preferentially metabolize ammonia and primary amino acids (collectively referred to as the yeast assimilable nitrogen or YAN) during fermentation. Ultimately, it is the amount of YAN that regulates fermentation processes and wine production (Bell and Henschke 2005).

### *Effect of YAN on fermentation success and aroma development*

Extremely low or extremely high nitrogen YAN levels contribute to unbalanced musts and the risk of consequences detrimental to wine quality. Nitrogen requirements are dependent on the specific yeast strain used, but the culmination of many experiments has suggested that 140 mg N/L is considered a minimum to complete fermentation. The relative vigor of fermentations (as defined by sugar consumption per day) is influenced by YAN, but is also heavily dependent on fermentation conditions and yeast strain (Bell and Henschke 2005). Data from Ugliano et al. (2009) suggests that musts with YAN levels above 400 mg N/L are able to finish fermentation up to one week faster than musts containing 100 mg N/L. Although temperature, pH, sugar, clarification, aeration, and vitamins all affect production of volatile compounds in wine, there are many studies to suggest that there are significant relationships between aroma compounds and must nitrogen levels. Most notably, low YAN is associated with an increased production of volatile sulfur compounds, which include hydrogen sulfide, mercaptans and disulfides—all of which contribute to rotten egg, cabbage, and onion aromas (Bell and Henschke 2005). There is a direct relationship between YAN and higher alcohol production, a group of compounds, which impart solvent and fusel-like aromas to wines. When YAN is less than 200-300 mg N/L, there is a direct relationship between higher alcohol production and YAN, which becomes inverse at high nitrogen conditions (Vilanova et al. 2012; Vilanova et al. 2007). Studies conducted on both model solutions and wine solutions determined that 350-450 mg N/L is an ideal range for optimal fatty acid ester and ethyl ester formation, which impart floral and fruit aromas to wines (Hernández-Orte et al. 2005; Torrea et al. 2011; Vilanova et al. 2012; Vilanova et al. 2007). However, the higher end of the range also correlates with drastic increases in ethyl acetate, acetic acid and volatile acidity; aromas that will surely mask any fruit or floral notes obtained by higher ester concentrations (Torrea et al. 2011). Bell and Henschke (2005) review further consequences of high nitrogen in must, which include: increased presence of haze-causing proteins, higher instances of ethyl carbamate and biogenic amine formation, increased risk of *Brettanomyces* spoilage, and higher heat outputs due to increases in fermentation vigor.

### *Effect of amino acid composition on fermentation success and aroma development*

Many studies focus on how the amino acid composition of juice correlates with ester development in the final wine, due to the integral part amino acids play in ester development (Bell and Henschke 2005). Such studies have yielded conflicting results, which can likely be explained by whether the substrate of study was a natural must or model solution, differences in the amount of juice used, timing of any additions made (prior to fermentation or after a specific amount of sugar has been consumed), and specific yeast strain used (Beltran et al. 2005; Garde-Cerdán and Ancín-Azpilicueta 2008; Hernández-Orte, Cacho, and Ferreira 2002; Hernández-Orte et al. 2005; Miller et al. 2007). Miller et al. (2007) found that in Chardonnay juice, yeast preferentially utilize ammonium over amino acids, and postulated that under high ammonium availability, ester formation occurs via the biosynthetic pathway rather than from amino acid degradation. For example, under additions of the amino acid leucine, the corresponding ester (isoamyl acetate) did not increase. In cases were DAP was added, utilization of the leucine in the must decreased, but the production of isoamyl acetate increased. This contrasts with results previously obtained on both model solutions and Mazuelo (also known as Carignan) musts, where relative ester formation increased when specific amino acids were present at higher concentrations (Garde-Cerdán and Ancín-Azpilicueta 2008; Hernández-Orte, Cacho, and Ferreira 2002). The effect of nitrogen source on ester development has been found to be dependent on yeast strain in Airen juice where supplementation with amino acids resulted in statistically similar aroma profiles to those supplemented with ammonia with one yeast strain, while fermentation with amino acid supplementation under a different yeast strain increased fruit and floral notes. Moreover, when musts had sufficient assimilable primary amino acids, further additions did not increase the concentrations of many esters (Hernández-Orte et al. 2005). This might be explained by an observation made in supplemented Chardonnay grape juice with both inorganic and amino acid additions: statistical differences were observed between residual amino acids, suggesting that total concentration of nitrogen, rather than concentration of individual amino acids, is the primary determinant of amino acid uptake (Torrea et al. 2011). This study did, however, demonstrate that musts containing a mixture of inorganic nitrogen and high amino acid additions produced wines with a more intense fruity profile, favored by the production of ethyl and acetate esters.

### *Must nitrogen additions*

Diammonium phosphate (DAP) additions have been widely used to correct nitrogen deficiencies in grape must, ensure successful fermentations, decrease fermentation time, and prevent the formation of volatile sulfur compounds. While there is certainly plenty of evidence indicating DAP additions decrease fermentation time, it should be noted that musts naturally high in YAN are considered superior to those additions, in that balanced mixtures of amino acids exhibit enhanced yeast growth. This is because yeast directly incorporate the amino acids into protein, rather than expensing energy to synthesize new amino acids (Bell and Henschke 2005). Many studies that have analyzed the relationships between YAN and aroma compounds have utilized model solutions or grape juice with a relatively high YAN starting point, which has contributed to some of the discrepancies in reports. For this reason, further analyses on low YAN musts should be considered to delineate these specific relationships (Torrea et al. 2011).

Recent literature suggests that DAP additions may not necessarily reduce the presence of volatile sulfur compounds, despite their widespread use for this purpose. For Shiraz grape juice supplemented to 250 mg N/L and 400 mg N/ L with two different yeast strains, supplementation increased final levels of sulfides, disulfides, mercaptans, and mercaptoesters in the finished wines (Ugliano et al. 2009). In a separate study, actual production of hydrogen sulfide increased with increased YAN during fermentation; however, residual hydrogen sulfide concentrations were lowest in the low and moderate YAN Chardonnay musts. This reduction was attributed to a decrease in fermentation vigor, which can result in less carbon dioxide production and a reduced ability to purge the volatile compounds from the media (Ugliano, Kolouchova, and Henschke 2011). These studies raise questions as to whether DAP additions are warranted as a way of reducing volatile sulfur compounds in wine, and suggest further experimentation analyzing this relationship with musts naturally high in YAN, such as those obtained from nitrogen fertilizers in optimally managed vineyards.

Other studies document clear relationships between DAP additions and the production of ethyl esters, acetate esters, and fatty acid esters (Vilanova et al. 2012; Vilanova et al. 2007). A study conducted on Albariño grape juice demonstrates that moderate DAP additions (up to 350 mg N/L) were associated with an increase in the concentration of glycoside precursors of varietal volatile compounds, ethyl esters, volatile fatty acids, and higher alcohols. The moderate nitrogen supplementation (to 350mg N/L) also produced the most favorable intensity of ethyl esters, terpenes, and C13-norisoprenoids—contributing to fruity and floral aromas—while high supplementation (450 mg N/L) reduced the concentration of ethyl esters, terpenes, and C13-norisoprenoids. The study suggests that very high nitrogen availability inhibits release of free volatile compounds (Vilanova et al. 2012). These results are in close agreement with other reports indicating fatty acid ester and ethyl ester production are highest when YAN ranges from 350-450 mg N/L (Torrea et al. 2011; Vilanova et al. 2007). However, these relationships are not concrete, as the results of separate studies have shown many discrepancies. Such discrepancies may be attributed to differences in starting YAN concentrations, yeast strain used, and fermentation conditions. For example, Ugliano et al. (2010) found that DAP additions up to 250 mg N/L resulted in higher reduced attributes, while additions up to 400 mg N/L resulted in higher red fruit and dark fruit attributes and decreased yeast, cheese, vegetal, and earth attributes. This contrasts with the reports of Torrea et al. (2011) where high concentrations of the esters obtained by increased YAN levels did not result in wines rated highest for fruity and floral attributes. Rather, nitrogen supplementation to 480 mg N/L produced musts dominated by nail polish aromas as a result of increased ethyl acetate and acetic acid concentrations, an outcome that was in agreement with high nitrogen supplementation experiments (Vilanova et al. 2007).

### *Factors affecting YAN concentration and amino acid composition in grapes*

Individual grape varieties have characteristic amino acid compositions, which appear to be genetically determined, although moderated by environment and cultural factors (Huang and Ough 1991; Stines et al. 2000; Stewart 2013). For all *V. vinifera* varieties, proline and arginine concentrations are the highest, but some varieties accumulate much greater concentrations of proline (e.g., Cabernet Sauvignon and Merlot) while others accumulate greater concentrations of arginine (e.g., Tempranillo). There are also varieties that show an accumulation of both (e.g., Sauvignon blanc). Proline accumulators are more likely to have insufficient assimilable nitrogen for fermentation due to the developmental regulation of proline and arginine concentrations: proline begins to accumulate late in the ripening period (four weeks post-véraison) while arginine increases pre-véraison and continues to the end of berry maturation, *unless* proline accumulation is high, in which case arginine will no longer accumulate. If arginine accumulation plateaus without having reached a sufficient concentration for fermentation, the continued increase of proline will not contribute to YAN (Stines et al. 2000). The timing of this developmental regulation may be an important consideration for nitrogen fertilization practices seeking to increase YAN levels in proline accumulating varieties.

Rootstock selection also appears to affect assimilable amino acid concentrations. Own-rooted Chardonnay vines and vines grafted to Schwarzmann and K51-40 had significantly greater YAN than vines grafted to 140 Ruggeri and 101-14 in an Australian study. Moreover, vines grafted to K51-40 had a greater concentration of six amino acids than own-rooted vines (Treeby et al. 1998). Similarly, Cabernet Sauvignon vines grafted to 110R exhibited greater YAN levels and higher concentrations of seven amino acids compared to a less vigorous rootstock, 420A. For both rootstocks, juices were higher than berry extracts in ammonia, but lower in total free amino acid concentration (Lee and Steenwerth 2011).

Many studies indicate the role sampling protocol plays in accurately determining final YAN concentrations. Wineries using juice for sampling do not account for skin-derived YAN and may add too much artificial nitrogen during fermentation. Exhaustive extraction is more representative for reds and should be done prior to any nitrogen supplementation to the must to avoid the negative consequences of high nitrogen (Lee 2010).

On a molecular level, environmental factors such as drought stress have been found to increase the expression of many transcripts associated with glutamate and proline biosynthesis (Deluc et al. 2009). On a whole-plant level, factors affecting vine nitrogen uptake—rate of soil nitrogen mineralization, soil type, water availability, vine water potential, and their interactions—will also have a direct affect on final YAN concentrations. Vineyard nitrogen applications also have a significant effect on berry nitrogen status and will be considered in greater detail in the next section.

## Vineyard Nitrogen Additions

### *Effects of soil nitrogen applications on berry YAN*

Earlier in this review, the flowering through véraison and post-harvest periods were discussed as the two critical demand periods for vine nitrogen uptake, and consequently the most efficacious time periods to supply nitrogen. However, these periods have been determined by conducting leaf tissue analyses, rather than considering berry YAN or berry yield. The effect of fertilization practices on YAN has yielded varying results depending on the method, timing, and rate of fertilizer application, but some generalities persist. The first generality is that fertilization applications made too early (i.e., around budbreak) contribute to excessive vegetative growth and decreased berry yield (Linsenmeier, Loos, and Lohnertz 2008; Neilsen et al. 2010). The second generality is that nitrogen applications made after fruit set, and even after véraison, are most effective at increasing berry YAN (Linsenmeier, Loos, and Lohnertz 2008). The third generality is that high fertilization rates may be required to obtain adequate berry YAN in nitrogen deficient sites, but high fertilization rates heavily favor shoot growth over fruit set (Goldspink 1991; Linsenmeier, Loos, and Lohnertz 2008; Spayd et al. 2000).

While budbreak nitrogen applications were found to be most effective at incorporating nitrogen into vegetative growth in a Canadian study, bloom fertilization was superior to budbreak applications for Merlot vines where the goal was to improve berry yield and reduce canopy density (Neilsen et al. 2010). Rates of 80 kg N/ha were required for the berries to obtain adequate YAN levels; yet, the effect of fertilization timing on YAN concentration was not consistent for Cabernet Sauvignon vines, as there were no differences between budbreak and bloom nitrogen applications applied at the same rate. Holzapfel and Treeby (2007) observed that Shiraz vines grafted to different rootstocks responded differently to nitrogen application timing, as well as fertilization rate. Specifically, greater nitrogen inputs were required to obtain minimal YAN levels for Teleki 5C compared to Ramsey and Schwarzmann rootstocks. While these disparities may be due to a difference in site history, this phenomenon justifies experimentation with multiple varieties for the current Virginia study.

A hydroponic study conducted on Cabernet Sauvignon vines suggests that post-véraison nitrogen applications are most effective at stimulating berry growth and achieving adequate YAN (Rodriguez-Lovelle and Gaudillere 2002). The authors suggest this is due to the increased fruit-sink strength after véraison, but they caution growers who apply nitrogen solely during this time, warning that as roots become a weaker sink, their growth will be sacrificed. Failure of root systems to fully develop reduces the overall nitrogen uptake capacity of vines in future years, resulting in vines that are extremely dependent on nitrogen applications to improve both vine nitrogen status as well as berry YAN.

Limitations of soil based nitrogen applications include favoring excessive vegetative growth at the expense of fruit maturation, delayed harvest, increased susceptibility to pathogens, and increased leaching in vineyards. For drip-irrigated Riesling vines in Washington State, a minimum of 112 kg N/ha was required for YAN levels to exceed the recommended minimal range for fermentation success. However, such a high rate resulted in excessive vine growth, fruit shading, delayed ripening, and ultimately reduced berry quality (Spayd et al. 2000). Shading is particularity problematic given its effect on bud fruitfulness and yields the following year. Delayed ripening is also an important consideration because there are many risks associated with a later harvest, such as: loss of crop to frost, poor berry quality due to higher rainfall, and increased instances of disease. Many other fertilization studies have noted the increased sensitivity of berries to *Botrytis cinerea* in response to higher nitrogen applications (Eynard 2000; Martinson 2012). Other berry quality parameters such as Brix, TA, pH, and anthocyananins have varied considerably in fertilization studies, and appear to be more heavily dependent on the combination of all cultural practices at the specific vineyard site (Bell and Henschke 2005).

### *Effects of foliar nutrient applications on berry YAN*

Foliar nitrogen applications have shown great promise in improving amino acid compositions and wine aroma profiles. Twenty-year-old Sauvignon blanc vines underwent treatment of various foliar nitrogen application rates with the specific goal of increasing berry YAN and improving aromatic expression in wine. Two foliar applications made at 10 kg N/ha prior to véraison increased YAN by 60% without affecting vine vigor or susceptibility to *Botrytis cinerea* (Lacroux et al. 2008). When the same application was applied with an addition of 5 kg S/ha YAN levels further increased, as did the concentration of wine volatile thiols, the compounds responsible for contributing to varietal aromas in Sauvignon blanc wines. Compared to the no nitrogen control, soil applications did not contribute to significant differences in YAN levels or volatile thiol concentration. This work justifies further experimentation with using foliar applications to improve berry YAN and aromatic expression in other prominent grape varieties grown in the state of Virginia.

Other studies have also found a synergism between coupled sulfur and nitrogen applications in crops such as wheat, contributing to increased nitrogen use efficiency (28%), and the subsequent concentration of cysteine (27%) and methionine (14%) (Habtegebrial and Singh 2009). In an Argentine wheat study, this observation was attributed to sulfur additions improving nitrogen use efficiency by increasing nitrogen recovery from the soil rather than an improved internal efficiency (Salvagiotti et al. 2009). Given the success of both foliar and soil sulfur supplements in routine nitrogen applications, it would be interesting to test both conditions in one controlled experiment.

In a New York study, foliar nitrogen applications at véraison to Riesling vines increased the concentrations of all amino acids in juice, while soil nitrogen applications or the combination of soil and foliar applications increased fewer amino acid concentrations (Cheng 2010). This suggests that the method, and perhaps timing, of nitrogen applications does in fact alter final amino acid composition. The potential for increased concentrations of specific amino acids, which may result in increased fruity aromas, justifies experimentation with vineyard practices that have potential to alter grape amino acid composition. In the same token, we should be interested in evaluating practices that may decrease the concentration of specific amino acids. A study conducted on Monastrell grapes found that the application of six different fungicides all decreased the concentration of amino acids and ammonium at varying levels (Oliva et al., 2011).

In a study analyzing nitrogen accumulation and cycling, $^{15}N$ labeled ammonium nitrate was applied as a broadcast application to Seyval blanc vines at budbreak or at bloom. Recovery of the nitrogen fertilizer by the vines, determined by the percent of labeled N of total N present in the vine, was 7.1% and 10.6%, respectively. Recovery of the nitrogen fertilizer by the inter-row sod was 13.0-20.2% of the total nitrogen present in the plant (Hanson and Howell 1995). This study demonstrates the significant competition that can exist between cover crops and their surrounding vines, and justifies experimentation with foliar-based nitrogen sources that may increase the efficiency of vine utilization of applied nitrogen sources.

Lasa et al. (2012) assessed the effects of foliar N applications of 10 kg N/ha and 50 kg N/ha applied to Merlot and Sauvignon blanc vines at three time periods – 3 weeks pre-véraison, véraison, and 3 weeks post-véraison—on grape juice quality; concluding that the low rate (10 kg N/ha) urea application made post-véraison were more effective at increasing berry YAN in both varieties. The urea applications in this study contained a 1% $^{15}N$ tracer, which enabled the determination of how much nitrogen was transferred to the berries after incorporation by the leaf tissue. The group determined that 17-80% of nitrogen applied to Merlot and Sauvignon blanc leaves was absorbed by the berries, with maximum translocation occurring as a result of véraison and post-véraison urea applications. These results were higher than those previously reported, where only 30% of foliar nitrogen absorbed by grapevine leaves ended up in the berries (Schreiber et al. 2002). The disparity between these studies justifies investigating the ideal timing of foliar applications for individual cultivars.

### *Factors affecting efficacy of foliar nutrient applications*

High penetration rate is an important prerequisite for effective foliar applications. (Mengel 2002). Urea has long been selected as a foliar nitrogen application source due to its ability to readily penetrate the epicuticular wax and the cutin layer of leaves. The primary limitation of urea is its potential to induce phytotoxicity (Mengel 2002).

There are disparities regarding whether new or old leaves have better nitrogen uptake. One theory argues that because nitrogen is transported under deficiency conditions from old leaves to new leaves, old leaves must have a better uptake capacity—and that old leaves are damaged in such a way that readily provides pathways for applied solutions (Mengel 2002). A second theory supports improved uptake for apical leaves, specifically in apples, where absorption has been found to be 32% greater than in basal leaves (Toselli, Thalheimer, and Tagliavini 2004). This theory seems more likely, as the greater absorption by young leaves may be explained by their thinner cuticles and reduced barrier (Boynton, 1954).

Other factors affecting absorption of nutrient solutions include contact angle, surface wetting, temperature, humidity, vapor pressure, and loss of the spray to the atmosphere (Boynton 1954). Losses may occur through volatilization, large spray volumes that result in the liquid dripping off the leaves, and poor sprayer set-up resulting in drift (Boynton 1954). Toselli, Thalheimer, and Tagliavini (2004) postulate that the spray carrier water volume in urea applications is only significant in the first 48 hours after application, where larger volumes result in longer wetting periods that favor absorption. In the following 72 hours, total absorption will reach an equilibrium across treatments receiving the same concentration at varying application volumes because the repeated drying and wetting cycles that occur increase cuticle pore size and cuticle penetration. Moreover, similar studies on apples show that highest uptake of urea in the first 48 hours occurs when applied in low concentrations (Toselli, Thalheimer, and Tagliavini 2004). This suggests that multiple applications throughout the season may be superior to single applications—a strategy that will be tested in my Virginia experiment.

### *Nitrogen applications in cover cropped systems*

While many studies discuss nitrogen limitations in cover cropped systems, to my knowledge, few studies have attempted to determine the most efficient method for improving berry YAN in cover-cropped vines. Nonetheless, there are studies that assess the effects of soil and foliar applications on berry YAN levels, and in some cases, these studies were conducted on vines experiencing similar stresses brought about by cover crops (e.g., water stress).

In a permanently grassed twelve-year-old Riesling vineyard, 30% of nitrogen from foliar applications was partitioned to clusters, which was significantly greater than the 2% partitioned to berries in response to soil nitrogen applications made at the same time. In this study, foliar fertilization was combined with soil applications (0 or 60 kg N/ha). When only foliar applications were made, berry YAN increased 30%, but when foliar application were combined with soil applications of 60 kg N/ha, YAN levels increased 100%. However, YAN levels in this study were still far below the 140mg N/L minimum requirement, which warrants exploring additional nitrogen management practices, such as leguminous cover crops to achieve adequate berry YAN (Schreiber et al. 2002).

For Sauvignon blanc vines experiencing mild water deficit and previous nitrogen deficiency, soil nitrogen applications at budbreak (30 or 60 kg N/ha) were combined with foliar nitrogen applications at véraison (2.5 or 5 kg N/ha). The foliar supplementation of 5 kg N/ha increased YAN levels across all treatments, but increased YAN by 143% under the highest fertilization treatment (60 kg N to soil + 5 kg N foliar). The authors of this study concluded that foliar supplementation was an appropriate solution for water-stressed vines late in the season when nitrogen uptake is low (Jreij et al. 2009). The lack of literature on fertilization management in cover cropped systems, and specifically regarding adaption of a solely foliar-based program to compensate for nitrogen competition, justifies experimentation with optimizing fertilization practices in cover cropped Virginia vineyards.

## Materials and Methods

### Research objectives

While many studies discuss nitrogen limitations in cover cropped systems, few studies have attempted to determine the most efficient method for improving vine nitrogen and berry YAN in these cases. The primary objective of this study was to determine the most efficient method and optimal time for annual nitrogen applications, such that cover crop growth is maintained for known benefits, while adequate vine nitrogen and final berry YAN are achieved. This research explores differences in nitrogen contributions from both foliar nitrogen applications and sustainable alternatives, such as composting and utilizing leguminous cover crops.

An additional objective of this study is to determine if different fertilization methods in cover cropped vineyards affect the amino acid composition of mature berries, and to consider how that may affect the sensory component of the produced wines.

### Site details

Research was conducted at three experimental sites in this study. The first site was located at Glen Manor Vineyards (GMV) near Front Royal, VA. The Sauvignon blanc vines were planted in 1995 and trellised to an open Lyre system. Soil is a Myersville-Catoctin silt loam that is considered deep and well drained. Located approximately 335 meters above sea level, the vineyard rows run north/south on a 15% slope with an inter row spacing of 3.7 meters and an inter-planting spacing of 2.4 m. The vineyard floor has consisted of natural vegetation, predominately tall fescue (*Festuca arundinacea)* in the inter-row and creeping red fescue (*Festuca rubra)* in the intra-row, for the past six years, maintained by mowing. This site was selected because it exhibits low nitrogen status in the vines and in the must.

The second site was located at Chateau O'Brien at Northpoint (CON) in Markham, VA. The Merlot vines were planted in 2004 and trellised to a VSP system. Soil is a Tankerville loam. Located approximately 200 meters above sea level, the vineyard rows run north/south, on a 5 % slope, with an inter-row spacing of 2.1 m and an inter-planting spacing of 1.2 m. The vineyard floor has permanent row middle fescue, with intra-row consisting of native vegetation, maintained with a hand-held line trimmer. This site was selected due to chronically low nitrogen levels in the vines and in the must and chronically inferior vine capacity. It is also the platform for exploring sustainable alternatives to conventional fertilizer practices.

The third site was located at the Virginia Tech Alson H. Smith Jr. Agricultural Research and Extension Center (AREC) in Winchester, VA. The Petit Manseng vines were planted in 2007 and trellised to a VSP system. Soil was a Frederick-Poplimento sandy loam. Located approximately 300 meters above sea level, the rows run northeast/southwest on a 2% slope with an inter-row spacing of 3 meters and an inter-planting spacing of 1.5 meters. Inter-rows consist of a mixed sward of tall fescue (*Festuca arundinacea*) and orchard grass (*Dactylis glomerata*), with under-trellis cover crop consisting of creeping red fescue infested with native weed vegetation (primarily *Trifolium arvense* L. and *Medicago lupulina* L.), maintained by mowing. This site was selected to evaluate the relative difference in nitrogen status for cover cropped panels compared to herbicide panels, and to extend my research on foliar nitrogen applications.

**Explanation of treatments**

Nitrogen fertilizer treatment was the sole nutrient factor evaluated in this study, at three different vineyard locations.

The experiment was first implemented in 2010 at GMV where four treatments were evaluated in a randomized complete block experimental design. Experimental units included three-vine panels. The following treatments were applied to cover cropped vines: a control treatment that received no nitrogen (Control-GMV), a low soil rate of 30kg N/ha calcium nitrate at bloom (Low soil-GMV), a high soil rate of 30 kg N /ha of calcium nitrate at bloom and an additional 30 kg N /ha calcium nitrate six weeks after bloom (High soil-GMV), and a foliar nitrogen treatment of 40 kg N/ha of urea spread over multiple applications, beginning at bloom and ending at véraison (Foliar-GMV). Between 2011 and 2013, the number of applications increased, from 5 to 7 to 9 respectively, to reduce instances of phytotoxicity and enable more efficient N absorption. Due to a miscalculation of plot area, nitrogen treatments were under-applied by 33.3% in 2011; however, this error was corrected for in 2012 and 2013.

In January 2012, a second research plot was implemented at CON where eight treatments were evaluated in a randomized complete block experimental design. Experimental units included six-vine panels. The following treatments were applied to cover cropped vines: a control treatment that received no nitrogen (Control-CON), a low rate compost treatment of 33.5 kg N/ha in the first year (Low compost), a high compost treatment of 67 kg N/ha in the first year (High compost), a combination of the low rate compost treatment with clover as the planted cover crop (Low compost+ clover), a combination of the high rate compost treatment with clover as the planted cover crop

(High compost + clover), a low soil rate of 15 kg N/ha of calcium nitrate at bloom and an additional 15 kg N/ha of calcium nitrate six weeks after bloom (Low soil-CON), a high soil rate of 30 kg N/ha of calcium nitrate at bloom and an additional 30 kg N/ha of calcium nitrate six weeks after bloom (High soil-CON), and a low soil rate of 30 kg N/ha of calcium nitrate post-harvest (Post-harvest). In 2012, Merlot vines exhibited phosphorous deficiency, which was corrected for with 112 kg/ha triple super phosphate ($P_2O_5$) application made to all panels. $P_2O_5$ was selected because it contains no nitrogen that could confound treatment effects. The compost used in this study was sourced from Cow Pie Compost (Midland, VA) and comprised primarily cow manure and wood chips. A compost sample was sent to A&L Great Lakes Laboratories, Inc. (Fort Wayne, IN) for a full analysis to enable the calculation of 33.5 kg N/ha and 67 kg N/ha compost treatments in this study. In 2012, clover plots were roto-tilled to 45.7 cm soil depth on each side of the panels. Red clover (*Trifolium pratense)* was then sown at a rate of 22.4 kg/ha and raked in. Due to poor germination in 2012, the sowing method was modified in 2013. Clover panels received two applications of 1% TouchDown (glyphosate) in early spring. At the time of sowing, panels received double the suggested forage rate of red clover (*Trifolium pratense;* 35.9 kg/ha) and of crimson clover (*Trifolium incarnatum;* 67.3 kg/ha) and the seed was raked in to a depth of 0.64cm. Clover was mowed to 7.6cm and raked to evenly distribute the residue under the trellis two weeks post-bloom in 2013.

In the summer of 2012 a third research plot was implemented at AREC where four treatments were evaluated in a randomized design. Blocking was not an option due to the limited number of available panels that have been continuously maintained with an herbicide strip. Experimental units included five-vine panels and were replicated five times. All units received a baseline of 10 kg N/ha as calcium nitrate at bloom in 2012 and in 2013, as part of annual vineyard management routine. The treatments included: a cover crop control treatment with no additional nitrogen (CC-control), an herbicide control treatment whereby the panels were maintained with an herbicide strip and received no additional nitrogen (Herbicide-control), a pre-véraison foliar treatment to cover cropped vines of 5kg N/ha as foliar urea 2 weeks pre-véraison followed by an additional 5kg N/ha as foliar urea 1 week pre-véraison (Pre-véraison foliar), a post-véraison foliar treatment to cover cropped vines of 5kg N/ha as foliar urea 1 week post-véraison followed by an additional 5kg N/ha as foliar urea 2 weeks post-véraison (Post-véraison foliar).

### Temperature and rainfall

Weather data was logged daily using an ET106 weather station (Campbell Scientific, Inc., Logan, UT) on site at AREC in 2012 and 2013. Within weather data was not available for GMV or CON.

### Plant tissue analysis

GMV: Leaf petioles were collected at bloom and véraison and analyzed for nutrient levels in 2011, 2012, and 2013. Thirty petioles per panel (15 per side) were collected. Leaf blades were collected at véraison in 2011 and 2013. Ten blades per panel (5 per side) were collected. Petioles and blades were collected opposite an inflorescence at bloom and from the first fully expanded leaf at véraison, and triple rinsed in tap water prior to bagging. Samples were oven dried (60˚C) and sent to Pennsylvania State University's Agricultural Analytical Service's Laboratory (University Park, PA) for analysis of essential mineral nutrients.

CON: Baseline leaf petiole samples were collected at the time of experiment implementation (bloom) in 2012, and analyzed for nutrient levels. 80 petioles were collected for each block sample, whereby 10 petioles were collected from each experimental unit. Leaf petioles were collected at véraison in 2012 and 2013, and at bloom in 2013 and analyzed for nutrient levels. Due to a limited number of canopy leaves at véraison in 2012, only 20 petioles were taken from each experimental unit. Petioles from individual experimental units were consolidated into 50 petiole samples by taking the collected petioles from 2.5 experimental units to produce two replicates from each experimental treatment. Thirty petioles per panel (15 per side) were collected at both sampling times in 2013. Petioles and blades were collected opposite an inflorescence at bloom and from the first fully expanded leaf at véraison and triple rinsed in tap water prior to bagging. Samples were oven dried (60˚C) and sent to Pennsylvania State University's Agricultural Analytical Service's Laboratory (University Park, PA) for analysis of essential mineral nutrients.

AREC: Baseline leaf petiole samples were collected at the time of experiment implementation (post-bloom) in 2012 and analyzed for nutrient levels. 100 total petioles were collected from all cover cropped experimental units and 100 total petioles were collected from experimental units with a maintained herbicide strip. Leaf petioles were collected at bloom and véraison in 2013. Sixty petioles (30 petioles per side) were collected opposite an inflorescence at bloom and from the first fully expanded leaf at véraison, and triple rinsed in tap water prior to bagging. Samples were oven dried (60˚C) and sent to Pennsylvania State University's Agricultural Analytical Service's Laboratory (University Park, PA) for analysis of essential mineral nutrients.

### Soil nutrient analysis

Soil samples were collected in 2011, 2012, and 2013 at GMV, and 2012 and 2013 at CON to benchmark soil organic matter (OM), cation exchange capacity (CEC), and plant available nutrient levels. Two subsamples were collected from each block. Subsamples were a composite of four 25cm deep soil cores with the top 5cm of soil discarded, collected with a handheld augur. Samples were only collected in 2013 at AREC. Due to the lack of blocking, two 25cm cores with the top 5cm discarded were collected from each experimental unit. Samples submitted consisted of a composite of four samples in relative proximity to each other in the experimental design. Samples were sent to Virginia Tech's Soil Testing Lab (Blacksburg, VA) for analysis.

### Chlorophyll content index

A handheld chlorophyll meter (CCM-200 plus, Apogee Instruments, Logan, UT) was used to record the chlorophyll content index (CCI) by measuring the ratio of radiation transmitted through a 0.71 $cm^2$ segment of the leaf at 940nm and 660nm. In 2012, one CCI reading was taken from two leaves per vine, roughly located at the 7th node, at véraison. In 2013, individual leaves were tagged at bloom on representative vines from each experimental unit at all sites. At GMV, eight leaves located between the 5-8th node positions were tagged per vine and two CCI measurements were recorded per leaf post- bloom, véraison, post-véraison and harvest. At CON, five leaves located between the 5th-8th node position were tagged per vine and two CCI measurements were recorded per leaf post-bloom, véraison, and post-véraison. Due to suspected raccoon feeding, harvest at CON occurred very early in the season and a pre-harvest reading was not attained. At AREC, five leaves located between the 5th and 8th node position were tagged per vine and two CCI measurements per leaf were recorded post-bloom, véraison, post-harvest, and harvest.

### Canopy architecture and fruit exposure

The Point Quadrat Analysis (PQA), developed by Smart and Robinson (1991), was used to provide an assessment of canopy density. To conduct the PQA for this study, a measuring tape was tied to the end posts of each treatment panel at the center of the fruiting zone. Canopy insertions were made at 30cm intervals with a thin metal probe directly below the measuring tape, resulting in approximately 24 insertions per treatment replicate. For each insertion, data was recorded as the probe came in contact with leaves, clusters, or “gaps.” In this study, an Enhanced Point Quadrat Analysis (EPQA) was performed (Meyers and Vanden Heuvel, 2008) to include the characterization of fruit exposure to sunlight. Photosynthetic photon flux density (PPFD) was recorded at solar noon on cloudless days during véraison with an AccuPAR ceptometer (AccuPAR80, Decagon Devices, Inc., Pullman, WA). Before and after taking the canopy measurements for each panel, an unobstructed ambient PPFD reading was taken. The ceptometer was placed within the fruiting zone, such that it lay parallel to the cordon, and the PPFD was recorded on a per vine basis. A vertical, east, and west measurement was recorded and averaged. Data were analyzed with EPQA software (Meyers and Vanden-Heuvel 2008).

### Primary chemistry and yeast assimilable nitrogen (YAN) of mature fruit

In 2011, berries were collected from GMV at different sampling dates. Fifty berries were collected from the west side of the canopy approximately 10 days earlier than the 50 berries collected from the east side of the canopy. Both sample sets were analyzed for primary fruit chemistry within 24 hours of collection. At all sites in 2012, 60 berries were collected per experimental unit at harvest and then frozen for future analysis. Prior to analysis, samples were thawed in a warm water bath. At all sites in 2013, 100 berries were collected per experimental unit at harvest and analyzed for primary fruit chemistry within 48 hours. In all years, juice samples were obtained by hand-pressing fresh (non-frozen) berry samples such that full maceration was achieved and seed breakage avoided. Fifteen milliliters of the juice was poured into a test tube and centrifuged for 5 minutes at 4,500 rpm. One milliliter was allocated to a 96-well plate and sent to the Virginia Tech Enology Service Lab for a yeast assimilable nitrogen (YAN) analysis. In 2012 and 2013, 2mL were allocated for amino acid analysis (see: "UPLC analysis of mature fruit"). Juice pH was measured directly in the test tube with an Orion 3 Star pH meter (Thermo Fisher Scientific, Beverly, MA). Soluble solids (˚Brix) were measured with a digital refractometer (Pocket PAL-1, ATAGO USA, Inc., Bellevue, WA). Titratable acidity (TA) was measured using an automatic titrator (848 Titrino Plus, Metrohm, Herisau, Switzerland) with 0.1 N NaOH to an endpoint detection of pH 8.2 in 2012 and 2013.

### UPLC analysis of mature fruit

AccQ•Fluor reagent Kit (Waters Corporation, Milford, MA, USA) was used for the UPLC analysis and the derivation was conducted according to the manufacturer's protocol. The standard 2.5 mM amino acid solution containing hydrochloric acid and the following amino acids: His, Ser, Arg, Gly, Asp, Glu, Thr, Ala, Pro, Cys Lys, Tyr, Met, Val, Ile, Leu, and Phe was obtained from Waters Corporation. Standards for 2.5mM Gln and Asp were prepared and added to the standard solution prior to analysis. In 2012 and

2013, 20 μL of the standard were mixed with 80 μL of AccQ•Tag Ultra borate buffer (pH 8.8), and 20 μL of the AccQ•Tag Ultra reagent (containing 6-acetamido-4-hydroxy-2-methyl quinolone) previously diluted in 1.0mL of AccQ•Tag Ultra reagent diluent. The mixture was vortexed and the reaction was allowed to proceed in a heating block at 55°C for 10 minutes. Juice samples were prepared as described above and then diluted 1:8 with water. In 2012, juice samples were prepared in the same way, with the standard mix being substituted by diluted juice. In 2013, sample reactions were quartered, such that 5 μL of wine were mixed with 20 μL of the AccQ•Tag Ultra borate buffer and 5 μL of the diluted AccQ•Tag Ultra reagent.

In 2012 and 2013, the UPLC analysis was performed on a Waters AccQ Tag TM Ultra C18 column (2.1 mm x 100 mm, 5 microm). Water/acetonitrile (95:5) was used as the weak needle wash solvent, and water/acetonitrile (5:95) were used as the strong needle was solvent. The derivatives were eluted with the specified dilutions of Waters AccQ•Tag Ultra Eluent A and Waters AccQ•Tag Eluent B at a flow rate of 0.7mL per minute (Aubin et al., 2007). The column heater was set to 55°C. Wavelength detection occurred at 260nm. The system was controlled and data were collected using Empower 2 Software (Waters Corporation, Milford, MA, USA).

**Components of yield**

GMV: Crop yield data were collected at harvest in 2011, 2012, and 2013. Fruit mass and number of clusters were recorded on a per-vine basis, and used to calculate the average cluster weight. Individual berry weights were determined in 2011 by weighing the berry samples used for primary fruit chemistry analysis and dividing by the sample size.

CON: Crop yield data were collected at harvest in 2012 and 2013. Yields in both years were extremely small due to the culmination of poor fruit set, frost damage, disease, and raccoon and bird herbivory. In 2012, fruit mass and number of clusters were recorded on a per-panel basis. The number of healthy vines producing fruit was recorded, and used to estimate the average yield per vine. Average cluster weights were estimated by dividing the total yield by the total number of clusters. In 2013, raccoon damage was too severe in experimental units to record precise yield data. Projected yield was calculated by taking the average cluster weight—as determined by sampling and weighing 100 representative, undamaged clusters in triplicate—and multiplying it by the total number of clusters on the vine, inclusive of completely bare rachises, at harvest. Individual berry weights were determined by weighing the berry samples used for primary fruit chemistry analysis and dividing by the sample size.

AREC: Crop yield data were collected at harvest in 2012, and 2013. Fruit mass and number of clusters were recorded on a per-vine basis, and used to calculate the average cluster weight. Individual berry weights were determined by weighing the berry samples used for primary fruit chemistry analysis and dividing by the sample size.

### Pruning weights

Vine pruning weights were recorded by vine each winter at GMV and AREC. Vine pruning weights were recorded by panel at CON due to the small vine size at the site.

## Data analysis

GMV: Data were analyzed using JMP version 11 (SAS; Cary, NC). Two-way analysis of variance (ANOVA) was computed for the following response variables: petiole nutrients, primary fruit chemistry and YAN, components of yield, pruning weights, EPQA parameters, and individual amino acid concentrations. Model effects tested were treatment and block. Their interaction could not be tested due to insufficient degrees of freedom. Differences of least square means were analyzed using Tukey's HSD test. All tests evaluated the responses for significance at the 95% confidence level.

CON: Data were analyzed using JMP version 11 (SAS; Cary, NC). Two-way analysis of variance (ANOVA) was computed for the following data obtained: plant nutrients, primary fruit chemistry and YAN, components of yield, pruning weights, EPQA parameters, and individual amino acid concentrations. Model effects tested were treatment and block. Their interaction could not be tested due to insufficient degrees of freedom. Differences of least square means were analyzed using Tukey's HSD test. All tests evaluated the responses for significance at the 95% confidence level.

AREC: Data were analyzed using JMP version 11 (SAS; Cary, NC). One-way analysis of variance (ANOVA) was computed for the completely randomized design where the following response varaibles were analyzed: plant nutrients, primary fruit chemistry and YAN, components of yield, pruning weights, EPQA parameters, and individual amino acid concentrations. Differences of least square means were analyzed using Tukey's HSD test. All tests evaluated the responses for significance at the 95% confidence level.

All sites: A univariate repeated measures ANOVA was conducted on averaged

2013 CCI readings (two readings per leaf at each time point) from all sites to test the effect of treatment over time. In all cases the sphericity test chi-square was not significant, and therefore the unadjusted univariate F tests were used. Time was the only within subject variable modeled, and treatment was the only between subject variable modeled. The univarate approach was selected due to occasional missing CCI values (i.e., if the tag blew away or couldn't be located), for which the multivariate analysis would exclude all non-missing data values for those individuals.

## Results

### Temperature and rainfall

The 2012 and 2013 seasons had comparable mean July temperatures of 24.91C$^o$ and 23.65C$^o$ respectively (Table 1) that approximated the historical average of 23.67C$^o$ (Wolf et al. 2008). The accumulated growing degree days (GDD) at the end of October in 2012 were 119 GDD higher than in 2013, indicating 2012 was a warmer season (Table 1). The cumulative rainfall during the active growing season was 165.35mm greater in 2012 than in 2013 (Table 1).

**Table 1. Temperature and rainfall data for AREC (Winchester, VA) experimental vineyard site 2011-2013.**

|  |  |  |  |  | Heat summation (10 C$^o$ base) | Precipitation (mm) |  |
|---|---|---|---|---|---|---|---|
| Year | GMV[a] harvest date | CON[b] harvest date | AREC harvest date | Mean July temperature (C$^o$) | Apr-Oct inclusive | Apr-Oct inclusive | Jan-Dec inclusive |
| 2011 | 3-Sept | n/a | n/a | 25.37 | 2019 | 645.65 | 902.96 |
| 2012 | 21-Aug | 1-Oct | 14-Sept | 24.91 | 1941 | 785.15 | 972.85 |
| 2013 | 9-Sept | 25-Sept | 21-Oct | 23.65 | 1820 | 619.80 | 932.98 |

[ab]No official weather station serves GMV or CON therefore weather data from AREC (47.5km from GMV; 52km from CON) is listed for these sites.

**Plant tissue analysis**

GMV: Nitrogen concentration in petioles was increased by the high calcium nitrate rate (High soil-GMV) at véraison in 2011 and 2012, and by foliar fertilization (Foliar-GMV) at véraison in 2011 (Table 2). Nitrogen concentration in blades was increased by the high calcium nitrate rate (High soil-GMV) and foliar fertilization (Foliar-GMV) at véraison 2013 (Table 2). Phosphorous concentration in petioles was reduced by all fertilization treatments at véraison in 2012 and 2013 and at bloom in 2013 (Table 2). Potassium concentration was reduced in petioles at véraison in 2012 and 2013, bloom 2013, and in blades at véraison in 2013 by the high calcium nitrate rate (High soil-GMV) (Table 2). Sulfur concentration was reduced by the high calcium nitrate rate (High soil-GMV) at véraison in 2012 and 2013, and in bloom 2013 (Table 2).

CON: Both clover treatments (Clover + low compost and clover+high compost) increased petiole nitrogen concentration and decreased calcium concentration at bloom 2013 (Table 4). Phosphorous concentration in petioles was decreased by both clover treatments (Clover + low compost and Clover + high compost) at bloom 2012 and véraison 2013 (Table 4). Potassium concentration in petioles was unaffected by all treatments. Magnesium concentration in petioles was decreased by the two compost treatments (Low compost and High compost) and increased by the high calcium nitrate rate (High soil-CON) treatment at véraison 2013.

AREC: Nitrogen and phosphorous concentrations in petioles from vines grown in the presence of an herbicide strip were consistently the greatest (Herbicide control) (Table 6). Magnesium concentration in petioles from vines grown in the presence of an herbicide strip was consistently the lowest (Herbicide control) (Table 7).

**Table 2. GMV: Treatment effect on percent macronutrient composition of Sauvignon blanc petioles and blades at bloom and véraison in 2011-2013.**

| | **Treatment[ab]** | **N %** | | **P %** | | **K %** | | **Ca %** | | **Mg %** | | **S%** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tissue[c] | P | B | P | B | P | B | P | B | P | B | P | B |
| **Bloom 2011** | | 0.88 | 2.87 | 0.50 | 0.22 | 4.87 | 1.18 | 1.71 | 1.85 | 0.67 | 0.33 | 0.26 | 0.51 |
| **Véraison 2011** | Significance[d] | **0.0112** | ns | ns | ns | ns | ns | ns | ns | **0.0173** | ns | ns | ns |
| | Control | 0.43b | 2.50 | 0.21 | 0.17 | 4.58 | 1.45 | 1.13 | 1.48 | 0.53b | 0.31 | 0.15 | 0.27 |
| | Low soil | 0.47ab | 2.53 | 0.20 | 0.18 | 4.62 | 1.43 | 1.14 | 1.60 | 0.55ab | 0.31 | 0.15 | 0.27 |
| | High soil | 0.48a | 2.59 | 0.18 | 0.18 | 4.27 | 1.38 | 1.12 | 1.50 | 0.59a | 0.32 | 0.14 | 0.26 |
| | Foliar | 0.48a | 2.54 | 0.19 | 0.18 | 4.21 | 1.33 | 1.12 | 1.57 | 0.59a | 0.31 | 0.15 | 0.27 |
| **Bloom 2012** | Significance | ns | ns | ns | ns | ns | ns | ns | ns | ns | **0.0143** | ns | ns |
| | Control | 0.89 | 2.56 | 0.46 | 0.23 | 3.47 | 1.24 | 1.51 | 1.88 | 0.51 | 0.32ab | 0.24 | 0.29 |
| | Low soil | 0.85 | 2.62 | 0.48 | 0.22 | 3.32 | 1.17 | 1.63 | 1.91 | 0.49 | 0.33a | 0.26 | 0.28 |
| | High soil | 0.86 | 2.52 | 0.44 | 0.22 | 3.42 | 1.22 | 1.50 | 1.73 | 0.51 | 0.31b | 0.25 | 0.29 |
| | Foliar | 0.89 | 2.63 | 0.41 | 0.22 | 3.35 | 1.22 | 1.51 | 1.80 | 0.51 | 0.32ab | 0.27 | 0.29 |
| **Véraison 2012[e]** | Significance | **0.0138** | | ns | | **0.0475** | | ns | | ns | | **0.0110** | |
| | Control | 0.81b | | 0.37 | | 4.40a | | 1.01 | | 0.60 | | 0.13a | |
| | Low soil | 0.91ab | | 0.33 | | 3.96ab | | 1.00 | | 0.63 | | 0.12ab | |
| | High soil | 0.94a | | 0.31 | | 3.85b | | 1.03 | | 0.69 | | 0.10b | |
| | Foliar | 0.84ab | | 0.31 | | 4.11ab | | 0.98 | | 0.60 | | 0.12ab | |
| **Bloom 2013[d]** | Significance | ns | | **<.0001** | | **<.0001** | | ns | | **0.0045** | | **0.0090** | |
| | Control | 0.82 | | 0.62a | | 3.14a | | 1.26 | | 0.43b | | 0.22a | |
| | Low soil | 0.80 | | 0.39c | | 2.06bc | | 1.25 | | 0.45ab | | 0.20ab | |
| | High soil | 0.86 | | 0.26d | | 1.75c | | 1.17 | | 0.52a | | 0.18b | |
| | Foliar | 0.81 | | 0.51b | | 2.56b | | 1.22 | | 0.43b | | 0.20ab | |
| **Véraison 2013** | Significance | ns | **0.0005** | **<.0001** | ns | **0.0194** | **0.0021** | ns | ns | **<.0001** | ns | **<.0001** | ns |
| | Control | 0.52 | 2.52b | 0.49a | 0.22 | 4.26a | 1.66a | 1.19 | 1.61 | 0.51c | 0.30 | 0.21a | 0.33 |
| | Low soil | 0.53 | 2.58b | 0.18b | 0.21 | 3.83ab | 1.59ab | 1.22 | 1.73 | 0.60b | 0.30 | 0.18b | 0.32 |
| | High soil | 0.55 | 2.72a | 0.15b | 0.21 | 3.42b | 1.50b | 1.20 | 1.74 | 0.73a | .032 | 0.16c | 0.30 |
| | Foliar | 0.52 | 2.72a | 0.24b | 0.21 | 3.70ab | 1.59ab | 1.18 | 1.51 | 0.57bc | .028 | 0.18b | 0.29 |

[a] Control= no nitrogen; Low soil= 30kg N/ha of calcium nitrate at bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Foliar= 40 kg N/ha of urea applied evenly in roughly 7 applications between bloom and véraison.

[b] Separation of means using Tukey's HSD (α=0.05).

[c] P= petiole; B= blade.

[d] Significance of treatment effects on response variables using two-way ANOVA (p>F; ns= not significant).

[e] Blade samples were not taken at véraison in 2012 or at bloom in 2013.

## Table 3. GMV: Treatment effect on micronutrient composition of Sauvingon blanc petioles and blades at bloom and véraison in 2011-2013.

| | Treatment[ab] | Mn ppm | | Fe ppm | | Cu ppm | | B ppm | | Al ppm | | Zn ppm | | Na ppm | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tissue[c] | P | B | P | B | P | B | P | B | P | B | P | B | P | B |
| **Bloom 2011** | Vineyard Average | 376 | 659 | 16 | 57 | 12 | 17 | 44 | 64 | 7 | 8 | 105 | 85 | 308 | 126 |
| **Véraison 2011** | Significance[d] | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | **<.0001** | **0.0406** |
| | Control | 303 | 166 | 10 | 51 | 4 | 13 | 33 | 33 | 4 | 10 | 46 | 26 | 113b | 86 a |
| | Low soil | 328 | 199 | 8 | 53 | 4 | 10 | 33 | 33 | 4 | 10 | 45 | 27 | 130b | 112 a |
| | High soil | 284 | 176 | 8 | 52 | 4 | 9 | 33 | 33 | 3 | 10 | 48 | 28 | 122b | 86 a |
| | Foliar | 309 | 171 | 9 | 52 | 4 | 9 | 32 | 30 | 4 | 11 | 46 | 24 | 210a | 130a |
| **Bloom 2012** | Significance | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | Control | 378 | 544 | 11 | 44 | 9 | 13 | 42 | 47 | 5 | 2 | 125 | 96 | 322 | 207 |
| | Low soil | 422 | 585 | 12 | 42 | 9 | 13 | 41 | 49 | 12 | 2 | 135 | 99 | 350 | 224 |
| | High soil | 380 | 551 | 12 | 58 | 9 | 13 | 40 | 48 | 7 | 2 | 125 | 102 | 323 | 178 |
| | Foliar | 395 | 560 | 14 | 48 | 10 | 13 | 41 | 44 | 5 | 4 | 142 | 101 | 340 | 201 |
| **Veraison 2012[e]** | Significance | ns | | ns | | ns | | ns | | ns | | ns | | **<.0001** | |
| | Control | 414 | | 11 | | 5 | | 38 | | 2 | | 60 | | 60b | |
| | Low soil | 428 | | 12 | | 5 | | 35 | | 2 | | 60 | | 54b | |
| | High soil | 396 | | 13 | | 5 | | 36 | | 2 | | 58 | | 52b | |
| | Foliar | 416 | | 13 | | 6 | | 36 | | 2 | | 60 | | 113a | |
| **Bloom 2013** | Significance | ns | | ns | | ns | | ns | | ns | | **0.0076** | | Ns | |
| | Control | 536 | | 11 | | 6 | | 42 | | 8 | | 88a | | 322 | |
| | Low soil | 490 | | 9 | | 6 | | 38 | | 6 | | 86ab | | 325 | |
| | High soil | 394 | | 10 | | 5 | | 38 | | 6 | | 73b | | 311 | |
| | Foliar | 354 | | 11 | | 6 | | 38 | | 5 | | 80a | | 327 | |
| **Véraison 2013** | Significance | ns | ns | ns | **0.0149** | ns | ns | **0.0005** | **0.0121** | ns | **0.0050** | ns | ns | **<.0001** | **<.0001** |
| | Control | 515 | 368 | 6 | 57b | 6 | 10 | 38a | 34a | 8 | 10 | 66 | 67 | 185b | 38b |
| | Low soil | 507 | 417 | 6 | 64ab | 6 | 10 | 36b | 30ab | 6 | 11 | 69 | 75 | 188b | 45 |
| | High soil | 435 | 378 | 7 | 63ab | 5 | 10 | 34b | 30ab | 7 | 12 | 66 | 76 | 193b | 42b |
| | Foliar | 496 | 376 | 7 | 65a | 6 | 10 | 35b | 29b | 6 | 11 | 68 | 73 | 519a | 123a |

[a.] Control= no nitrogen; Low soil= 30kg N/ha of calcium nitrate at bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Foliar= 40 kg N/ha of urea applied evenly in roughly 7 applications between bloom and véraison.

[b.] Separation of means using Tukey's HSD (α=0.05).

[c.] P= petiole; B= blade.

[d.] Significance of treatment effects on response variables using two-way ANOVA (p>F; ns= not significant).

[e.] Blade samples were not taken at véraison in 2012 or at bloom in 2013.

## Table 4. CON: 2012 and 2013 Nitrogen treatment effect on percent macronutrient composition of Merlot petioles at bloom and véraison in 2012-2013.

| | **Treatment[ab]** | **N %** | **P %** | **K %** | **Ca %** | **Mg %** | **S %** |
|---|---|---|---|---|---|---|---|
| **Bloom 2012** | Vineyard Average | 1.06 | 0.16 | 4.74 | 1.48 | 0.44 | 0.48 |
| **Véraison 2012** | Significance [c] | ns | ns | ns | ns | ns | ns |
| | Control | 0.74 | 0.16 | 5.20 | 1.22 | 0.30 | 0.28 |
| | Low compost | 0.75 | 0.16 | 5.40 | 1.14 | 0.28 | 0.28 |
| | High compost | 0.75 | 0.14 | 5.72 | 1.16 | 0.26 | 0.32 |
| | Clover + low compost | 0.74 | 0.14 | 6.22 | 1.19 | 0.29 | 0.34 |
| | Clover + high compost | 0.72 | 0.14 | 5.97 | 1.19 | 0.29 | 0.32 |
| | Low soil | 0.77 | 0.15 | 5.42 | 1.21 | 0.33 | 0.31 |
| | High soil | 0.82 | 0.12 | 6.25 | 1.19 | 0.33 | 0.30 |
| | Post harvest | 0.77 | 0.14 | 5.90 | 1.18 | 0.29 | 0.32 |
| **Bloom 2013** | Significance | **<.0001** | **0.0001** | ns | **0.0209** | ns | **0.0044** |
| | Control | 0.86c | 0.33a | 5.17 | 1.25ab | 0.29 | 0.35abc |
| | Low compost | 0.96bc | 0.41a | 4.45 | 1.13ab | 0.29 | 0.35bc |
| | High compost | 0.88c | 0.38a | 4.46 | 1.17ab | 0.31 | 0.31c |
| | Clover + low compost | 1.16ab | 0.14b | 4.20 | 1.10ab | 0.37 | 0.39ab |
| | Clover + high compost | 1.23a | 0.14b | 4.59 | 1.08b | 0.34 | 0.42a |
| | Low soil | 0.97bc | 0.37a | 4.96 | 1.23ab | 0.34 | 0.37abc |
| | High soil | 0.92bc | 0.28ab | 4.69 | 1.25ab | 0.37 | 0.37abc |
| | Post-harvest | 0.93bc | 0.30ab | 4.25 | 1.34a | 0.42 | 0.36abc |
| **Véraison 2013** | Significance | ns | **<.0001** | ns | ns | **0.0085** | ns |
| | Control | 0.63 | 0.40abc | 4.99 | 1.26 | 0.31ab | 0.26 |
| | Low compost | 0.63 | 0.50a | 5.34 | 1.21 | 0.28b | 0.27 |
| | High compost | 0.62 | 0.47ab | 5.34 | 1.17 | 0.28b | 0.27 |
| | Clover + low compost | 0.60 | 0.19d | 5.48 | 1.15 | 0.36ab | 0.27 |
| | Clover + high compost | 0.62 | 0.21d | 5.36 | 1.17 | 0.37ab | 0.27 |
| | Low soil | 0.63 | 0.28cd | 5.36 | 1.24 | 0.35ab | 0.25 |
| | High soil | 0.67 | 0.18d | 5.49 | 1.19 | 0.40a | 0.26 |
| | Post-harvest | 0.61 | 0.32bcd | 5.51 | 1.19 | 0.32ab | 0.28 |

[a] Control= no nitrogen; Low compost= 33.5 kg N/ha applied as compost at bloom in 2012; High compost= 67 kg N/ha applied as compost at bloom in 2012; Clover + low compost= 33.5 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Clover + high compost= 67 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Low soil= 15kg N/ha of calcium nitrate at bloom + 15kg N/ha of calcium nitrate 6 weeks post-bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Post-harvest= 30kg N/ha of calcium nitrate applied post-harvest.

[b] Separation of means using Tukey's HSD (α=0.05).

[c] Significance of treatment effects on response variables using two-way ANOVA (p>F; ns= not significant).

## Table 5. CON: Treatment effect on micronutrient composition of Merlot petioles at bloom and véraison in 2012-2013.

| | Treatment[ab] | Mn ppm | Fe ppm | Cu ppm | B ppm | Al ppm | Zn ppm | Na ppm |
|---|---|---|---|---|---|---|---|---|
| **Bloom 2012** | Vineyard Average | 416 | 28 | 96 | 46 | 2 | 167 | 398 |
| **Véraison 2012** | Significance[c] | ns | ns | ns | ns | ns | ns | ns |
| | Control | 285 | 18 | 75 | 42 | 2 | 103 | 151 |
| | Low compost | 323 | 17 | 99 | 41 | 3 | 119 | 158 |
| | High compost | 302 | 17 | 99 | 41 | 2 | 121 | 160 |
| | Clover + low compost | 363 | 13 | 83 | 40 | 2 | 118 | 135 |
| | Clove + high compost | 371 | 16 | 94 | 43 | 3 | 103 | 146 |
| | Low soil | 377 | 18 | 94 | 43 | 2 | 104 | 150 |
| | High soil | 323 | 16 | 101 | 41 | 2 | 97 | 152 |
| | Post-harvest | 322 | 18 | 106 | 42 | 3 | 114 | 163 |
| **Bloom 2013** | Significance | **0.0043** | ns | ns | ns | ns | ns | ns |
| | Control | 303b | 13 | 13 | 36 | 3 | 130 | 323 |
| | Low compost | 339ab | 13 | 13 | 38 | 2 | 132 | 313 |
| | High compost | 305b | 11 | 11 | 35 | 2 | 122 | 248 |
| | Clover + low compost | 406a | 14 | 11 | 38 | 2 | 111 | 279 |
| | Clove + high compost | 377ab | 13 | 14 | 38 | 2 | 125 | 265 |
| | Low soil | 343ab | 13 | 13 | 39 | 2 | 122 | 319 |
| | High soil | 350ab | 14 | 12 | 37 | 2 | 115 | 311 |
| | Post-harvest | 407a | 13 | 12 | 36 | 2 | 114 | 263 |
| **Véraison 2013** | Significance | **0.0138** | ns | ns | ns | ns | ns | ns |
| | Control | 209b | 11 | 174 | 41 | 4 | 103 | 211 |
| | Low compost | 228ab | 11 | 162 | 43 | 3 | 106 | 212 |
| | High compost | 197b | 9 | 140 | 41 | 3 | 100 | 203 |
| | Clover + low compost | 297a | 11 | 148 | 41 | 3 | 98 | 231 |
| | Clove + high compost | 275ab | 10 | 153 | 40 | 3 | 112 | 226 |
| | Low soil | 228ab | 13 | 177 | 41 | 4 | 104 | 221 |
| | High soil | 242ab | 10 | 138 | 39 | 3 | 103 | 193 |
| | Post-harvest | 252ab | 10 | 134 | 43 | 3 | 103 | 199 |

[a] Control= no nitrogen; Low compost= 33.5 kg N/ha applied as compost at bloom in 2012; High compost= 67 kg N/ha applied as compost at bloom in 2012; Clover + low compost= 33.5 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Clover + high compost= 67 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Low soil= 15kg N/ha of calcium nitrate at bloom + 15kg N/ha of calcium nitrate 6 weeks post-bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Post-harvest= 30kg N/ha of calcium nitrate applied post-harvest.

[b] Separation of means using Tukey's HSD (α=0.05).

[c] Significance of treatment effects on response variables using two-way ANOVA (p>F; ns= not significant).

## Table 6. AREC: Treatment effect on percent macronutrient composition of Petit Manseng petioles at véraison in 2012-2013 and bloom in 2013.

| | **Treatment**[ab] | **N %** | **P %** | **K %** | **Ca %** | **Mg %** | **S %** |
|---|---|---|---|---|---|---|---|
| **July 2012** | Cover crop panels | 0.40 | 0.08 | 3.00 | 2.08 | 0.32 | 0.14 |
| | Herbicide panels | 0.48 | 0.18 | 3.15 | 2.05 | 0.36 | 0.18 |
| **Bloom 2013** | Significance[c] | ns | **0.0069** | ns | ns | **0.0499** | ns |
| | CC control | 0.80 | 0.34 ab | 2.01 | 2.01 | 0.22 ab | 0.24 |
| | Herbicide control | 0.92 | 0.46 a | 1.69 | 2.00 | 0.26 a | 0.25 |
| | Pre-véraison foliar | 0.84 | 0.32 ab | 1.66 | 2.00 | 0.23 ab | 0.23 |
| | Post-véraison foliar | 0.79 | 0.25 b | 1.69 | 1.96 | 0.21 b | 0.23 |
| **Véraison 2013** | Significance | **0.0072** | ns | ns | ns | ns | ns |
| | CC control | 0.61 b | 0.20 | 4.71 | 2.62 | 0.33 | 0.24 |
| | Herbicide control | 0.81 a | 0.27 | 4.89 | 2.69 | 0.38 | 0.22 |
| | Pre-véraison Foliar | 0.68 ab | 0.19 | 4.93 | 2.52 | 0.35 | 0.23 |
| | Post-véraison foliar | 0.63 b | 0.16 | 4.94 | 2.45 | 0.33 | 0.24 |

[a] CC control= cover cropped panels received no additional nitrogen; Herbicide control= herbicide panels received no additional nitrogen; Pre-véraison foliar= 5 kg N/ha foliar urea applied 2 weeks pre-véraison + 5 kg N/ha foliar urea applied 1 weeks pre-véraison to cover cropped vines; Post-véraison foliar= 5 kg N/ha foliar urea applied 1 week post-véraison + 5 kg N/ha foliar urea applied 2 weeks post-véraison to cover cropped vines. All treatments received 10kg N/ha as calcium nitrate at bloom in 2012 and 2013 as part of general vineyard maintenance.

[b] Separation of means using Tukey's HSD (α=0.05).

[c] Significance of treatment effects on response variables using one-way ANOVA (p>F; ns= not significant).

**Table 7. AREC: Treatment effect on micronutrient composition of Petit Manseng petioles at véraison in 2012-2013 and bloom in 2013.**

| | **Treatment[ab]** | **Mn ppm** | **Fe ppm** | **Cu ppm** | **B ppm** | **Al ppm** | **Zn ppm** | **Na ppm** |
|---|---|---|---|---|---|---|---|---|
| **July 2012** | Cover crop panels | 229 | 12 | 3 | 25 | 2 | 73 | 311 |
| | Herbicide panels | 169 | 9 | 4 | 25 | 2 | 82 | 248 |
| **Bloom 2013** | Significance[c] | **0.0209** | ns | ns | ns | ns | ns | ns |
| | CC control | 220 ab | 16 | 9 | 40 | 9 | 74 | 189 |
| | Herbicide control | 159 b | 18 | 10 | 40 | 9 | 76 | 158 |
| | Pre-véraison foliar | 241 a | 18 | 10 | 40 | 10 | 80 | 178 |
| | Post-véraison foliar | 216 ab | 16 | 8 | 39 | 9 | 76 | 183 |
| **Véraison 2013** | Significance | **0.0049** | ns | ns | ns | ns | ns | ns |
| | CC control | 295a | 7 | 6 | 37 | 3 | 70 | 290 |
| | Herbicide control | 166b | 7 | 6 | 37 | 3 | 71 | 275 |
| | Pre-véraison Foliar | 292a | 7 | 6 | 36 | 3 | 66 | 326 |
| | Post-véraison foliar | 258ab | 6 | 6 | 37 | 3 | 72 | 333 |

[a] CC control= cover cropped panels received no additional nitrogen; Herbicide control= herbicide panels received no additional nitrogen; Pre-véraison foliar= 5 kg N/ha foliar urea applied 2 weeks pre-véraison + 5 kg N/ha foliar urea applied 1 weeks pre-véraison to cover cropped vines; Post-véraison foliar= 5 kg N/ha foliar urea applied 1 week post-véraison + 5 kg N/ha foliar urea applied 2 weeks post-véraison to cover cropped vines. All treatments received 10kg N/ha as calcium nitrate at bloom in 2012 and 2013 as part of general vineyard maintenance.

[b] Separation of means using Tukey's HSD (α=0.05).

[c] Significance of treatment effects on response variables using one-way ANOVA (p>F; ns= not significant).

## Soil Nutrient Analysis

AREC and GMV soils were found to have low soil organic matter and pH ranges suitable for growing *V. vinifera* (Table 8). In both years the pH at CON was below the recommended pH of 6.5; however the increase from 5.55 to 5.97 is attributed to a lime application made in 2012 (Table 8). All sites were highly deficient in phosphorous and moderately deficient in calcium (Table 8).

**Table 8. Averaged[a] soil nutrient analyses for AREC in 2013, and for GMV and CON in 2012 and 2013.**

| | | Vineyard Site | | | | |
|---|---|---|---|---|---|---|
| | | GMV | | CON | | AREC |
| **Nutrient** | **Target Value [b]** | **2012** | **2013** | **2012** | **2013** | **2013** |
| pH | 6.5[c] | 6.34 | 6.25 | 5.55 | 5.97 | 6.73 |
| % OM | 3-5 | 2.3 | 2.0 | 3.43 | 3.38 | 1.34 |
| P (lbs/A) | 40-100 | 2 | 2 | 2 | 4 | 3 |
| K (lbs/A) | 150-200 | 70 | 58 | 111 | 109 | 68 |
| Ca (lbs/A) | 1,000-4,000 | 559 | 540 | 557 | 639 | 723 |
| Mg (lbs/A) | 200-500 | 85 | 90 | 83 | 123 | 85 |
| Zn (ppm) | 2 | 1.3 | 0.8 | 1.1 | 1.2 | 1.7 |
| Mn (ppm) | 20 | 7.9 | 6.1 | 5.4 | 5.3 | 9.8 |
| Cu (ppm) | 0.5 | 0.8 | 0.6 | 3.0 | 3.3 | 0.6 |
| Fe (ppm) | 20 | 6.8 | 7.7 | 15.9 | 13.3 | 6.9 |
| B (ppm) | 0.3-2.0 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |

[a] Soil samples collected for each block were averaged to provide a baseline description of soil nutrients at each site.
[b] Target value recommendations obtained from Wolf et al. (2008).
[c] Recommendation for *V. vinifera*.

**Chlorophyll content index**

GMV: No treatment significantly affected CCI values at véraison in 2012 (Table 9). Sauvignon blanc CCI values increased continuously between the post-bloom sampling period and harvest in response to all treatments in 2013, with the high rate of calcium nitrate being most effective at increasing average season-long CCI values by 26.0% (Figure 1, Table 12).

CON**:** No treatment significantly affected CCI values at véraison in 2012 (Table 10). Merlot leaf CCI values varied by treatment between the post-bloom and véraison sampling periods (Figure 2). Merlot leaf CCI values increased in response to the high calcium nitrate rate and clover combined with a low compost rate between the post-bloom and véraison sampling periods (Figure 2). In contrast, leaves from the post-harvest, clover + high compost, and both compost treatments exhibited a decrease in CCI values between the post-bloom and véraison sampling periods; while leaves from the low calcium nitrate rate and control treatments remained stagnant (Figure 2). All treatments exhibited a decline in CCI values at harvest. Both clover treatments, the high rate of calcium nitrate, and the post harvest calcium nitrate treatments increased average season-long CCI values in 2013, but the high rate of calcium nitrate was most effective by increasing CCI values by 57.6% (Figure 2, Table 14).

AREC: Vines grown in the presence of an herbicide strip exhibited the highest CCI values at véraison in 2012 (Table 11) and average season-long CCI values in 2013 (Figure 3, Table 16). Sauvignon blanc leaf CCI values increased between the post-bloom and véraison sampling periods and decreased between the pre-harvest and harvest sampling periods in response to all treatments (Figure 3). Leaves from vines grown in the presence of an herbicide strip, and from the post-véraison foliar, and cover crop control treatments all showed slight increases in CCI values between véraison and harvest; in contrast, the pre-véraison foliar treatment resulted in decreased CCI values between these sampling periods.

**Table 9. GMV: Treatment effect on Sauvignon blanc leaf chlorophyll content index at véraison in 2012.**

| Treatment[a] | CCI at véraison |
|---|---|
| Significance[b] | ns |
| Control | 14.92 |
| Low soil | 14.68 |
| High S\soil | 15.99 |
| Foliar | 16.32 |

[a] Control= no nitrogen; Low soil= 30kg N/ha of calcium nitrate at bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Foliar= 40 kg N/ha of urea applied evenly in roughly 7 applications between bloom and véraison.

[b] Significance of treatment effects on response variables using two-way ANOVA (p>F; ns= not significant).

**Table 10. CON: Treatment effect on Merlot leaf chlorophyll content index at véraison in 2012.**

| Treatment[a] | CCI at véraison |
|---|---|
| Significance[b] | ns |
| Control | 19.14 |
| Low compost | 18.98 |
| High compost | 19.34 |
| Clover + low compost | 20.58 |
| Clover + high compost | 21.48 |
| Low soil | 19.75 |
| High soil | 21.41 |
| Post-harvest | 20.02 |

[a] Control= no nitrogen; Low compost= 33.5 kg N/ha applied as compost at bloom in 2012; High compost= 67 kg N/ha applied as compost at bloom in 2012; Clover + low compost= 33.5 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Clover + high compost= 67 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Low soil= 15kg N/ha of calcium nitrate at bloom + 15kg N/ha of calcium nitrate 6 weeks post-bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Post-harvest= 30kg N/ha of calcium nitrate applied post-harvest.

[b] Separation of means using Tukey's HSD (α=0.05).

**Table 11. AREC: Treatment effect on Petit Manseng leaf chlorophyll content index at véraison in 2012.**

| Treatment[ab] | CCI at véraison |
|---|---|
| Significance[c] | **0.0012** |
| CC control | 14.72 b |
| Herbicide control | 18.39 a |
| Pre-véraison foliar | 17.13 ab |
| Post- véraison foliar | 16.82 ab |

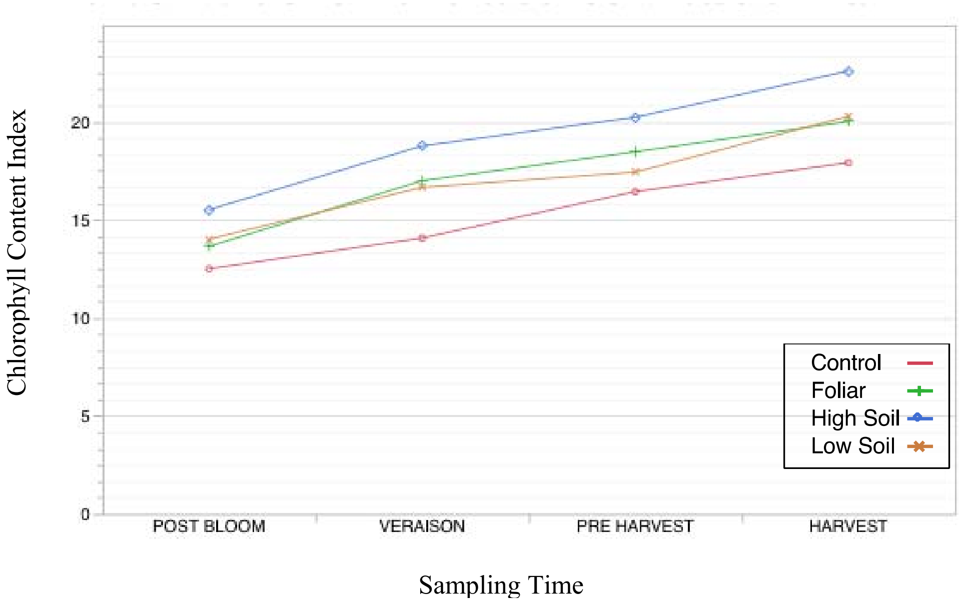
[a] CC Control= cover cropped panels received no additional nitrogen; Herbicide control= herbicide panels received no additional nitrogen; Pre-véraison foliar= 5 kg N/ha foliar urea applied 2 weeks pre-véraison + 5 kg N/ha foliar urea applied 1 weeks pre-véraison to cover cropped vines; Post-véraison foliar= 5 kg N/ha foliar urea applied 1 week post-véraison + 5 kg N/ha foliar urea applied 2 weeks post-véraison to cover cropped vines. All treatments received 10kg N/ha as calcium nitrate at bloom in 2012 and 2013 as part of general vineyard maintenance.

[b] Separation of means using Tukey's HSD (α=0.05).

[c] Significance of treatment effects on response variables using one-way ANOVA (p>F; ns= not significant)

## Figure 1. GMV: 2013 treatment effects on Sauvignon blanc leaf chlorophyll content index values over time.

Graph represents the average of CCI readings across n=48 leaves per treatment. Treatments include: Control= no nitrogen; Low soil= 30kg N/ha of calcium nitrate at bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Foliar= 40 kg N/ha of urea applied evenly in roughly 7 applications between bloom and véraison.



## Table 12. GMV: Season long treatment effect on Sauvignon blanc leaf chlorophyll content index in 2013.

| **Treatment**[ab] | **Average CCI**[c] |
|---|---|
| Significance[d] | **<.0001** |
| Control | 15.24 c |
| Low soil | 17.15 b |
| High soil | 19.20 a |
| Foliar | 17.29 b |

[a] Control= no nitrogen; Low soil= 30kg N/ha of calcium nitrate at bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Foliar= 40 kg N/ha of urea applied evenly in roughly 7 applications between bloom and véraison.

[s]eparation of means using Tukey's HSD (α=0.05).

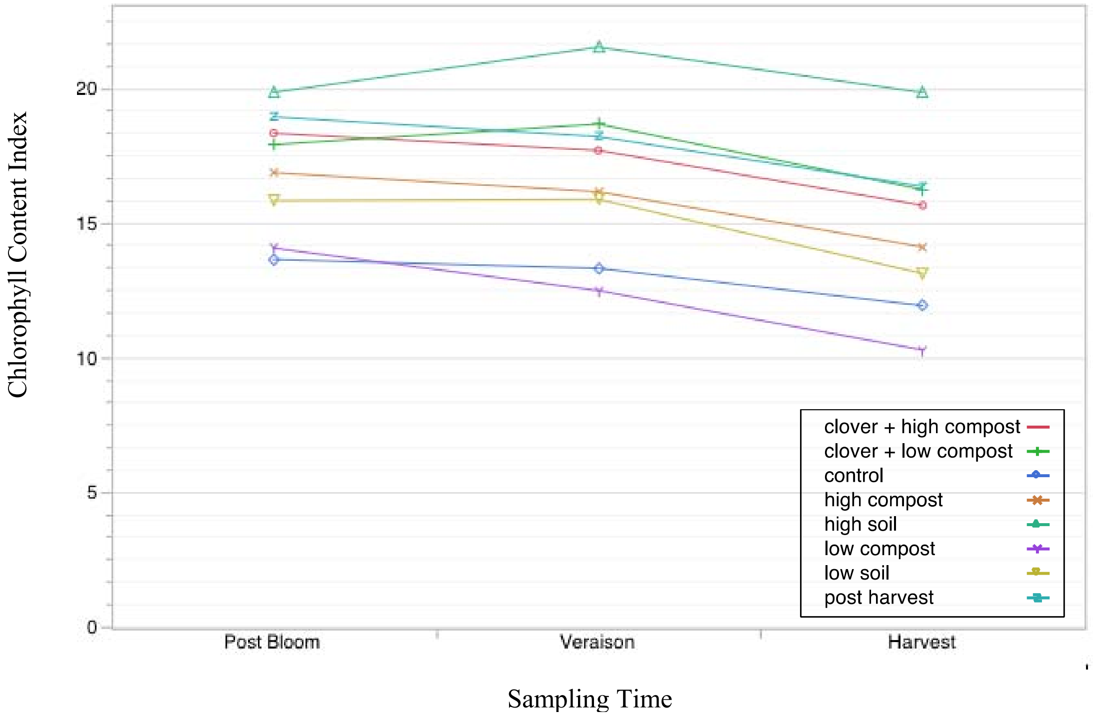
[c] Average CCI includes all values from post-bloom, véraison, pre-harvest, and harvest readings.

[d] Significance of treatment effects on response variables using a repeated measures univarate ANOVA (p>F; ns= not significant).

## Table 13. GMV: Repeated measures univariate ANOVA Fixed Effect Tests for Sauvignon blanc chlorophyll content index measurements.

| **Source** | **F Ratio** | **Prob > F** |
|---|---|---|
| Treatment | 19.0845 | <.0001 |
| Time | 175.0368 | <.0001 |
| Treatment x Time | 1.3486 | 0.2087 |

**Figure 2. CON: 2013 treatment effects on Merlot leaf chlorophyll content index values over time.**
Graph represents the average of CCI readings across n=40 leaves per treatment. Treatments include: Control= no nitrogen; Low compost= 33.5 kg N/ha applied as compost at bloom in 2012; High compost= 67 kg N/ha applied as compost at bloom in 2012; Clover + low compost= 33.5 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Clover + high compost= 67 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Low soil= 15kg N/ha of calcium nitrate at bloom + 15kg N/ha of calcium nitrate 6 weeks post-bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Post-harvest= 30kg N/ha of calcium nitrate applied post-harvest.



## Table 14. CON: Season long treatment effect on Merlot leaf chlorophyll content index in 2013.

| **Treatment**[ab] | **Average CCI**[c] |
|---|---|
| Significance[d] | **<.0001** |
| Control | 12.94 cd |
| Low compost | 12.25 d |
| High compost | 15.69bc |
| Clover + low compost | 17.44 ab |
| Clover + high compost | 17.21 ab |
| Low soil | 14.92 bcd |
| High soil | 20.39 a |
| Post-harvest | 17.81ab |

[a] Control= no nitrogen; Low compost= 33.5 kg N/ha applied as compost at bloom in 2012; High compost= 67 kg N/ha applied as compost at bloom in 2012; Clover + low compost= 33.5 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Clover + high compost= 67 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Low soil= 15kg N/ha of calcium nitrate at bloom + 15kg N/ha of calcium nitrate 6 weeks post-bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Post-harvest= 30kg N/ha of calcium nitrate applied post-harvest.

[b] Separation of means using Tukey's HSD (α=0.05).

[c] Average CCI includes all values from post-bloom, véraison, and harvest readings.

[d] Significance of treatment effects on response variables using a repeated measures univarate ANOVA (p>F; ns= not significant).

## Table 15. CON: Repeated measures univariate ANOVA Fixed Effect Tests for Merlot chlorophyll content index measurements.

| **Source** | **F Ratio** | **Prob > F** |
|---|---|---|
| Treatment | 13.3636 | <.0001 |
| Time | 58.6467 | <.0001 |
| Treatment x Time | 1.7245 | 0.0488 |

**Figure 3. AREC: 2013 treatment effects on Petit Manseng leaf chlorophyll content index values over time.** Graph represents the average of CCI readings across n=25 leaves per treatment. Treatments include: CC control= cover cropped panels received no additional nitrogen; Herbicide control= herbicide panels received no additional nitrogen; Pre-véraison foliar= 5 kg N/ha foliar urea applied 2 weeks pre-véraison + 5 kg N/ha foliar urea applied 1 weeks pre-véraison to cover cropped vines; Post-véraison foliar= 5 kg N/ha foliar urea applied 1 week post-véraison + 5 kg N/ha foliar urea applied 2 weeks post-véraison to cover cropped vines. All treatments received 10kg N/ha as calcium nitrate at bloom in 2012 and 2013 as part of general vineyard maintenance.

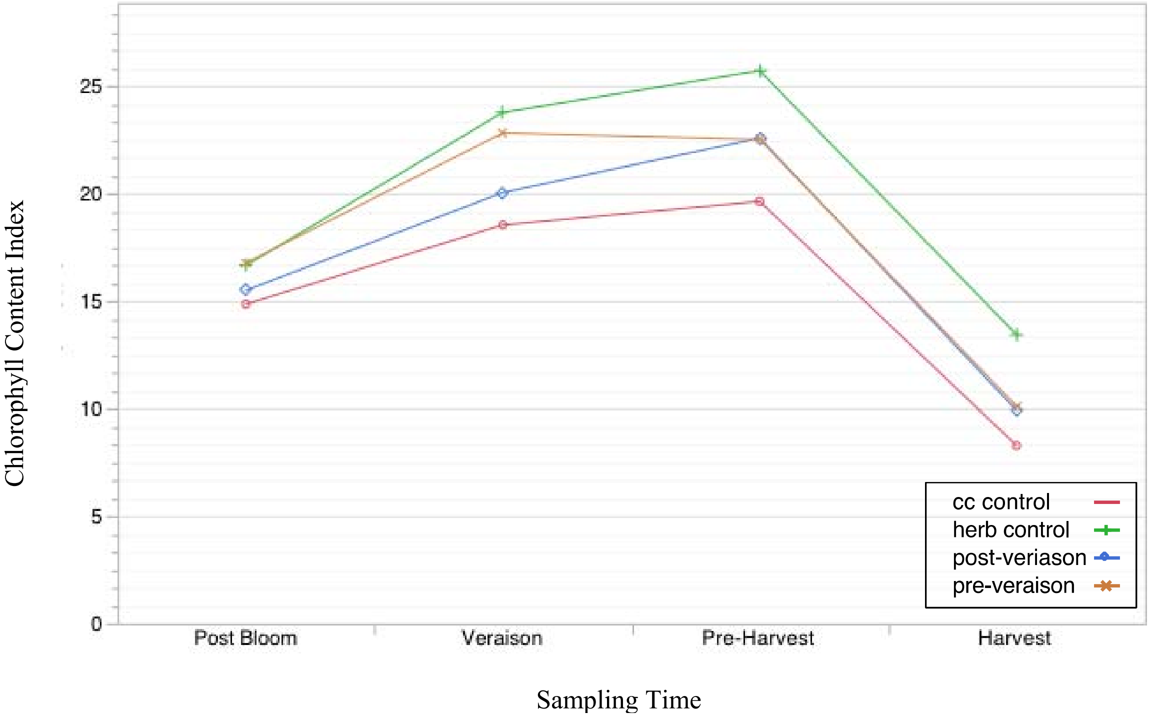


**Table 16. AREC: Season long treatment effect on Petit Manseng leaf chlorophyll content index in 2013.**

| Treatment[ab] | Average CCI[c] |
|---|---|
| Significance[d] | **0.0003** |
| CC control | 15.33b |
| Herbicide control | 19.68a |
| Pre-véraison foliar | 17.74ab |
| Post-véraison foliar | 17.01b |

[a.] CC control= cover cropped panels received no additional nitrogen; Herbicide control= herbicide panels received no additional nitrogen; Pre-véraison foliar= 5 kg N/ha foliar urea applied 2 weeks pre-véraison + 5 kg N/ha foliar urea applied 1 weeks pre-véraison to cover cropped vines; Post-véraison foliar= 5 kg N/ha foliar urea applied 1 week post-véraison + 5 kg N/ha foliar urea applied 2 weeks post-véraison to cover cropped vines. All treatments received 10kg N/ha as calcium nitrate at bloom in 2012 and 2013 as part of general vineyard maintenance.

[b.] Separation of means using Tukey's HSD (α=0.05).

[c.] Average CCI includes all values from post-bloom, véraison, pre-harvest, and harvest readings.

[d.] Significance of treatment effects on response variables using a repeated measures univarate ANOVA (p>F; ns= not significant).

**Table 17. AREC: Repeated measures univariate ANOVA Fixed Effect Tests for Petit Manseng chlorophyll content index measurements.**

| Source | F Ratio | Prob > F |
|---|---|---|
| Treatment | 97.18 | 0.0003 |
| Time | 271.2 | <.0001 |
| Treatment x Time | 271.1 | 0.0020 |

## Linear regression analysis

To assess the value of CCI readings as an indicator of chlorophyll content and subsequent leaf nitrogen values, a linear regression analysis was performed on petiole and blade nitrogen values (independent variables) and CCI readings (dependent variables). There was significant evidence that CCI values were linearly associated with Sauvignon blanc blade nitrogen at GMV; however, the coefficient of determination was low ($R^2$=0.223) (Figure 4). AREC was the only site for which there was significant evidence that petiole nitrogen and CCI values were linearly associated, and the coefficient of determination was slightly stronger ($R^2$=0.337) than for the Sauvignon blanc blades and CCI values (Figure 5).

**Figure 4. GMV: Regression analysis of average 2013 Sauvignon blanc véraison CCI readings and %N in blades.**

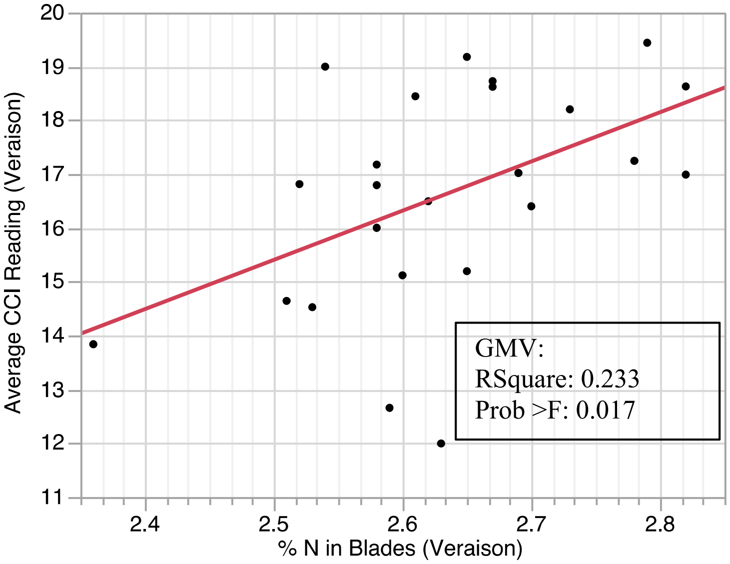
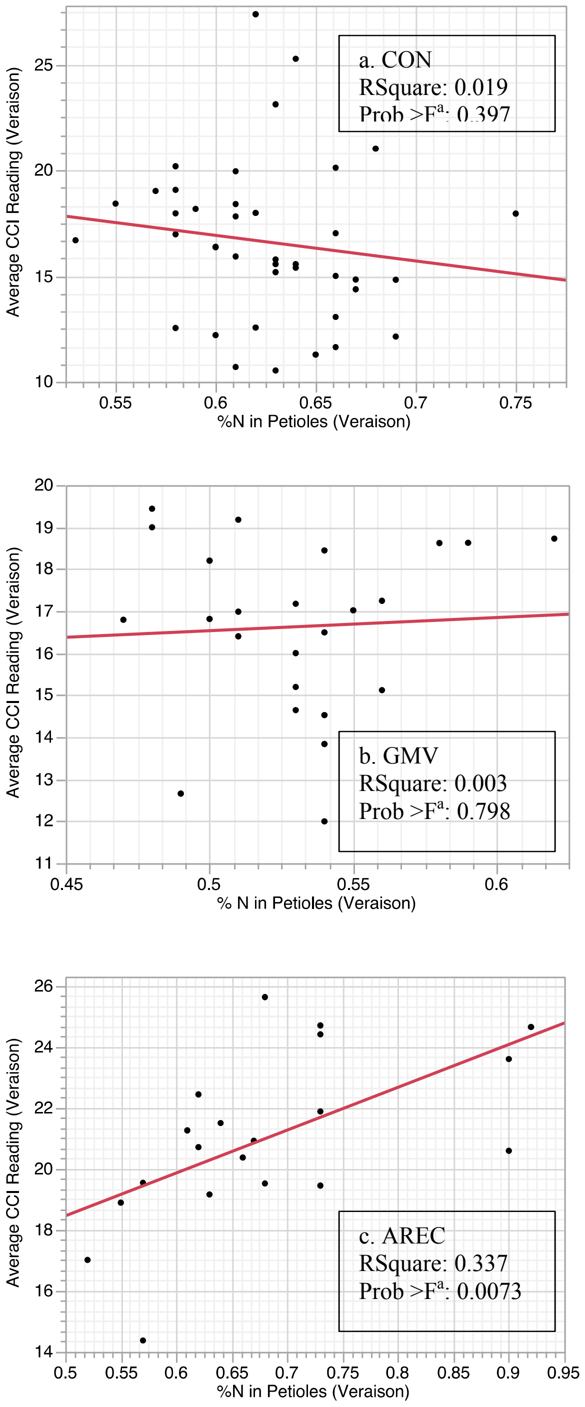


**Figure 5. Regression analysis of average 2013 véraison CCI readings from all sites and %N in petioles.**



## Canopy architecture and fruit exposure

The canopy density did not change as a function of nitrogen treatment for any site in this study as indicated by the EQPA parameters of interest (Tables 18, 19, and 20). Fruit zone light interception (fruit zone PPFD) was decreased by the two compost treatments (Low compost and High compost), the clover combined with a low compost rate (Clover + low compost), and both calcium nitrate rates (Low soil-CON and High soil-CON) at CON in 2013 (Table 19).

**Table 18. GMV: Treatment effects on Sauvignon blanc EPQA parameters of interest in 2012 and 2013.**

| | **Occlusion layer number (OLN)** | | **Cluster exposure layer (CEL)** | | **Leaf exposure layer (LEL)** | | **Cluster exposure flux availability (CEFA)** | | **Leaf exposure flux availability (LEFA)** | | **PPFD (µmol sec -1 meter -2)** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment**[a] | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 |
| Significance[b] | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Control | 1.01 | 1.24 | 0.20 | 0.19 | 0.06 | 0.14 | 0.54 | 0.53 | 0.60 | 0.53 | 6.06 | 3.57 |
| Low soil | 1.10 | 1.44 | 0.13 | 0.28 | 0.05 | 0.12 | 0.63 | 0.42 | 0.53 | 0.56 | 5.06 | 4.77 |
| High soil | 0.93 | 1.39 | 0.07 | 0.25 | 0.19 | 0.18 | 0.67 | 0.44 | 0.54 | 0.53 | 7.14 | 4.16 |
| Foliar | 0.91 | 1.17 | 0.12 | 0.14 | 0.09 | 0.10 | 0.67 | 0.51 | 0.60 | 0.61 | 7.22 | 3.87 |

[a] Control= no nitrogen; Low soil= 30kg N/ha of calcium nitrate at bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Foliar= 40 kg N/ha of urea applied evenly in roughly 7 applications between bloom and véraison.

[b] Significance of treatment effects on response variables using two-way ANOVA (p>F; ns= not significant).

**Table 19. CON: Treatment effects on Merlot EPQA parameters of interest in 2012 and 2013.**

| | **Occlusion layer number (OLN)** | | **Cluster exposure layer (CEL)** | | **Leaf exposure layer (LEL)** | | **Cluster exposure flux availability (CEFA)** | | **Leaf exposure flux availability (LEFA)** | | **PPFD (µmol sec -1 meter -2)** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment**[ab] | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 |
| Significance[c] | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | **0.0022** |
| Control | 0.95 | 2.03 | 0.02 | 0.38 | 0.06 | 0.22 | 0.76 | 0.48 | 0.68 | 0.54 | 10.93 | 22.87 a |
| Low compost | 0.80 | 2.02 | 0.00 | 0.57 | 0.08 | 0.18 | 0.75 | 0.35 | 0.67 | 0.50 | 14.96 | 12.23 ab |
| High compost | 0.98 | 2.03 | 0.16 | 0.64 | 0.08 | 0.21 | 0.65 | 0.30 | 0.66 | 0.47 | 13.15 | 8.45 b |
| Clover + low compost | 1.20 | 2.22 | 0.09 | 0.47 | 0.07 | 0.18 | 0.70 | 0.37 | 0.61 | 0.49 | 9.98 | 7.87 b |
| Clover + high compost | 0.97 | 2.28 | 0.05 | 0.53 | 0.04 | 0.21 | 0.76 | 0.30 | 0.68 | 0.48 | 7.70 | 7.69 b |
| Low soil | 0.91 | 2.12 | 0.04 | 0.61 | 0.05 | 0.18 | 0.71 | 0.31 | 0.70 | 0.53 | 8.38 | 14.70 ab |
| High soil | 1.03 | 2.10 | 0.09 | 0.32 | 0.13 | 0.19 | 0.71 | 0.41 | 0.62 | 0.46 | 11.48 | 7.13 b |
| Post-harvest | 0.97 | 2.32 | 0.04 | 0.66 | 0.11 | 0.27 | 0.70 | 0.29 | 0.63 | 0.44 | 13.84 | 9.14 b |

[a] Control= no nitrogen; Low compost= 33.5 kg N/ha applied as compost at bloom in 2012; High compost= 67 kg N/ha applied as compost at bloom in 2012; Clover + low compost= 33.5 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Clover + high compost= 67 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Low soil= 15kg N/ha of calcium nitrate at bloom + 15kg N/ha of calcium nitrate 6 weeks post-bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Post-harvest= 30kg N/ha of calcium nitrate applied post-harvest.

[b] Separation of means using Tukey's HSD (α=0.05).

[c] Significance of treatment effects on response variables using two -way ANOVA (p>F; ns= not significant).

**Table 20. AREC: Treatment effects on Petit Manseng EPQA parameters of interest in 2012 and 2013.**

| | **Occlusion layer number (OLN)** | | **Cluster exposure layer (CEL)** | | **Leaf exposure layer (LEL)** | | **Cluster exposure flux availability (CEFA)** | | **Leaf exposure flux availability (LEFA)** | | **PPFD (µmol sec -1 meter -2)** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment**[ab] | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 |
| Significance[c] | ns | ns | ns | ns | ns | ns | ns | ns | **0.0480** | ns | ns | ns |
| CC control | 2.11 | 3.01 | 0.45 | 0.65 | 0.10 | 0.28 | 0.30 | 0.21 | 0.53a | 0.38 | 6.06 | 3.57 |
| Herb control | 2.46 | 3.03 | 0.51 | 0.66 | 0.15 | 0.31 | 0.29 | 0.19 | 0.47a | 0.38 | 5.06 | 4.77 |
| Pre-véraison foliar | 2.10 | 2.89 | 0.52 | 0.56 | 0.20 | 0.22 | 0.30 | 0.26 | 0.45a | 0.41 | 7.14 | 4.16 |
| Post-véraison foliar | 2.33 | 2.63 | 0.50 | 0.57 | 0.14 | 0.29 | 0.29 | 0.25 | 0.46a | 0.39 | 7.22 | 3.87 |

[a] CC control= cover cropped panels received no additional nitrogen; Herbicide control= herbicide panels received no additional nitrogen; Pre-véraison foliar= 5 kg N/ha foliar urea applied 2 weeks pre-véraison + 5 kg N/ha foliar urea applied 1 weeks pre-véraison to cover cropped vines; Post-véraison foliar= 5 kg N/ha foliar urea applied 1 week post-véraison + 5 kg N/ha foliar urea applied 2 weeks post-véraison to cover cropped vines. All treatments received 10kg N/ha as calcium nitrate at bloom in 2012 and 2013 as part of general vineyard maintenance.

[b] Separation of means using Tukey's HSD (α=0.05).

[c] Significance of treatment effects on response variables using one-way ANOVA (p>F; ns= not significant).

### Primary chemistry and yeast assimilable nitrogen (YAN) of mature fruit

GMV: Treatments had no effect on primary juice chemistry in 2011, 2012, or 2013 (Table 21). Foliar fertilization (Foliar-GMV) consistently increased berry YAN at harvest in all three years by 29.4%, 45.2%, and 88.8% respectively (Figure 6). The high calcium nitrate rate (High soil-GMV) increased berry YAN at harvest in 2011 and 2013 (Figure 6). The low calcium nitrate rate (Low soil-GMV) only affected berry YAN at harvest in 2013 (Figure 6).

CON: Treatments had no effect on primary juice chemistry in 2011, 2012, or 2013 (Table 22). The high calcium nitrate rate (High soil-CON) increased berry YAN at harvest by 66.2% in 2013 (Figure 7).

AREC: Treatments had no effect on juice pH or TA (g/L) in 2012 or 2013 (Table 23). Vines grown in the presence of an herbicide strip (Herbicide control) produced berries with decreased °Brix at harvest in 2013 (Table 23). The post-véraison foliar application (Post-véraison foliar) increased berry YAN at harvest by 69.8% in 2013 (Figure 8).

**Table 21. GMV: Treatment effect on Sauvignon blanc primary fruit chemistry and yeast assimilable nitrogen (YAN) in 2011-2013.**

| | **pH** | | | **TA (g/L)** | | | **°Brix** | | | **YAN (mg N/L)** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment**[ab] | 2011 | 2012 | 2013 | 2011 | 2012 | 2013 | 2011 | 2012 | 2013 | 2011 | 2012 | 2013 |
| **Significance**[c] | ns | ns | ns | ns | ns | ns | ns | ns | ns | **0.0097** | **0.0061** | **0.0005** |
| Control | 3.42 | 3.28 | 3.30 | 6.42 | 4.56 | 8.76 | 21.9 | 21.4 | 22.7 | 119 b | 157 b | 125 b |
| Low soil | 3.43 | 3.28 | 3.31 | 6.05 | 4.80 | 9.35 | 21.5 | 21.5 | 22.3 | 138 ab | 175 b | 189 a |
| High soil | 3.43 | 3.27 | 3.31 | 6.13 | 5.57 | 8.54 | 21.8 | 21.4 | 22.3 | 154 a | 174 b | 194 a |
| Foliar | 3.44 | 3.31 | 3.31 | 6.14 | 4.75 | 9.06 | 22.2 | 21.4 | 22.3 | 154 a | 228 a | 236 a |

[a.] Control= no nitrogen; Low soil= 30kg N/ha of calcium nitrate at bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Foliar= 40 kg N/ha of urea applied evenly in roughly 7 applications between bloom and véraison.

[b.] Separation of means using Tukey's HSD (α=0.05).

[c.] Significance of treatment effects on response variables using two-way ANOVA (p>F; ns= not significant).

**Figure 6. GMV: Treatment effect on Sauvignon blanc yeast assimilable nitrogen (YAN) in 2011-2013.** Chart represents the average YAN values across n=6 samples from each treatment. Error bars represent standard error.

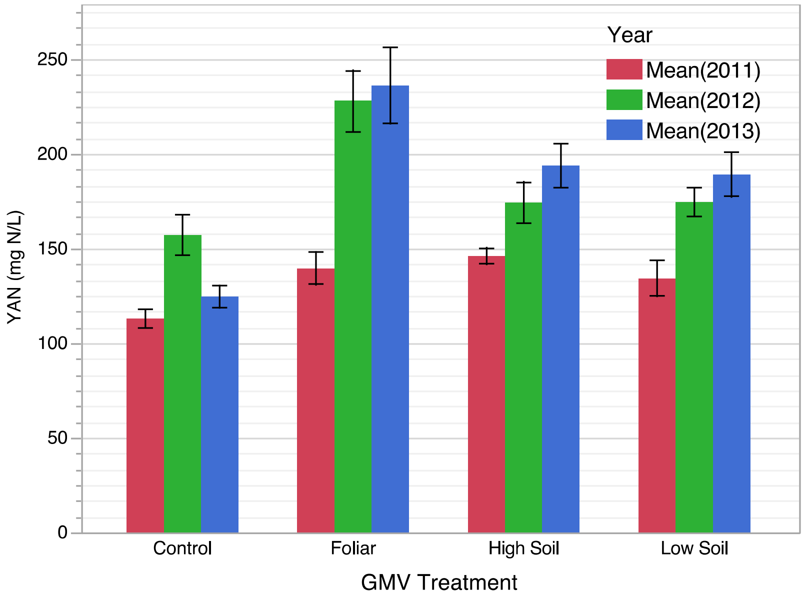


**Table 22. CON: Treatment effect on Merlot primary fruit chemistry and yeast assimilable nitrogen (YAN) in 2011-2013.**

| **Treatment**[ab] | **pH 2012** | **pH 2013** | **TA (g/L) 2012** | **TA (g/L) 2013** | **°Brix 2012** | **°Brix 2013** | **YAN (mg N/L) 2012** | **YAN (mg N/L) 2013** |
|---|---|---|---|---|---|---|---|---|
| **Significance**[c] | ns | ns | ns | ns | ns | ns | ns | **0.0090** |
| Control | 4.02 | 5.34 | 1.29 | 2.19 | 18.7 | 20.7 | 73 | 65 b |
| Low compost | 4.11 | 4.82 | 1.42 | 2.90 | 20.0 | 20.2 | 58 | 66 b |
| High compost | 4.16 | 4.35 | 1.40 | 3.49 | 20.0 | 20.3 | 63 | 73 ab |
| Clover + low compost | 4.15 | 4.44 | 1.60 | 3.40 | 20.2 | 20.8 | 75 | 94 ab |
| Clover + high compost | 4.07 | 4.75 | 1.72 | 3.02 | 20.4 | 20.4 | 69 | 94 ab |
| Low soil | 4.01 | 4.76 | 1.53 | 2.92 | 19.9 | 20.4 | 78 | 75 ab |
| High soil | 4.14 | 4.19 | 1.58 | 3.85 | 20.0 | 20.1 | 88 | 108 a |
| Post-harvest | 4.16 | 4.04 | 1.60 | 4.02 | 20.2 | 20.5 | 67 | 100 ab |

[a.] Control= no nitrogen; Low compost= 33.5 kg N/ha applied as compost at bloom in 2012; High compost= 67 kg N/ha applied as compost at bloom in 2012; Clover + low compost= 33.5 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Clover + high compost= 67 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Low soil= 15kg N/ha of calcium nitrate at bloom + 15kg N/ha of calcium nitrate 6 weeks post-bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Post-harvest= 30kg N/ha of calcium nitrate applied post-harvest.

[b.] Separation of means using Tukey's HSD (α=0.05).

[c.] Significance of treatment effects on response variables using two-way ANOVA (p>F; ns= not significant).

**Figure 7. CON: Treatment effect on Merlot yeast assimilable nitrogen (YAN) in 2012-2013.** Chart represents the average YAN values across n=5 samples from each treatment. Error bars represent standard error.

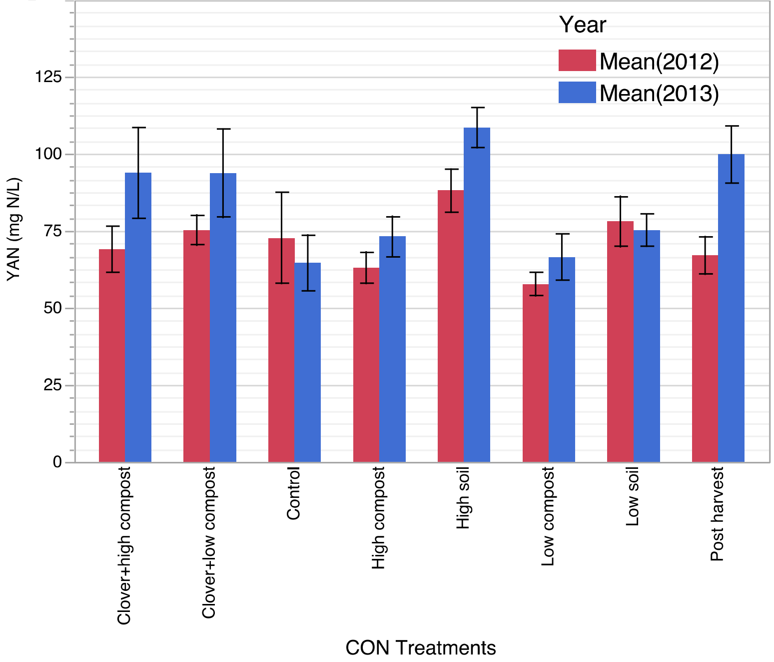


**Table 23. AREC: Treatment effect on Petit Manseng primary fruit chemistry and yeast assimilable nitrogen (YAN) in 2011-2013.**

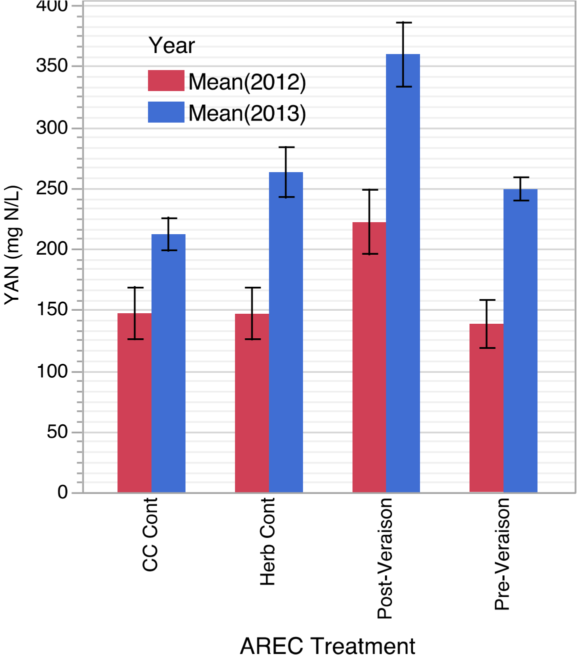
| Treatment[ab] | pH 2012 | pH 2013 | TA (g/L) 2012 | TA (g/L) 2013 | °Brix 2012 | °Brix 2013 | YAN (mg N/L) 2012 | YAN (mg N/L) 2013 |
|---|---|---|---|---|---|---|---|---|
| **Significance[c]** | ns | ns | ns | ns | ns | **0.0427** | ns | **0.0003** |
| CC control | 3.38 | 3.29 | 5.24 | 11.04 | 26.9 | 28.3 a | 147 | 212 b |
| Herbicide control | 3.35 | 3.27 | 5.43 | 11.58 | 27.0 | 27.3 b | 147 | 263 b |
| Pre-véraison foliar | 3.33 | 3.27 | 5.16 | 11.66 | 26.5 | 28.0 ab | 138 | 249 b |
| Post-véraison foliar | 3.37 | 3.27 | 5.02 | 12.08 | 26.6 | 28.0 ab | 222 | 360 a |

[a] CC control= cover cropped panels received no additional nitrogen; Herbicide control= herbicide panels received no additional nitrogen; Pre-véraison foliar= 5 kg N/ha foliar urea applied 2 weeks pre-véraison + 5 kg N/ha foliar urea applied 1 weeks pre-véraison to cover cropped vines; Post-véraison foliar= 5 kg N/ha foliar urea applied 1 week post-véraison + 5 kg N/ha foliar urea applied 2 weeks post-véraison to cover cropped vines. All treatments received 10kg N/ha as calcium nitrate at bloom in 2012 and 2013 as part of general vineyard maintenance.

[b] Separation of means using Tukey's HSD (α=0.05).

[c] Significance of treatment effects on response variables using one-way ANOVA (p>F; ns= not significant).

**Figure 8. AREC: Treatment effect on Petit Manseng yeast assimilable nitrogen (YAN) in 2012-2013.** Chart represents the average YAN values across n=5 samples from each treatment. Error bars represent standard error.



## UPLC analysis of mature fruit

GMV: Among free amino acids, Gln (47.76-68.70 mg/L), Arg (79.29-125.41 mg/L), Thr (68.93-96.69 mg/L), Ala (93.36-125.79 mg/L) and Pro (210.18-284.67 mg/L) were the most abundant in 2012 Sauvignon blanc juice (Table 24). The high rate of calcium nitrate (High soil-GMV) increased the concentrations of Ser and Gln in 2012 (Table 24). In 2013, Gln (46.30-87.06 mg/L), Arg (103.04-269.44 mg/L), Glu (108.55-139.75 mg/L), Ala (86.65-147.35 mg/L), and Pro (158.14-222.31) were among the most abundant free amino acids in Sauvignon blanc juice (Table 25). In 2013, the low rate of calcium nitrate (Low soil-GMV) increased the concentration of Arg; the high rate of calcium nitrate (High soil-GMV) increased the concentration of five amino acids (Gln, Arg, Thr, Ala, and Ile); and season long foliar nitrogen applications (Foliar-GMV) increased the concentration of eleven amino acids (Gln, Arg, Thr, Ala, Lys, Tyr, Met, Val, Ile, Leu, and Phe) (Table 25). Berries treated with season-long foliar nitrogen (Foliar-GMV) exhibited a 65.6% increase in total free amino acids (1164.77 mg/L) in 2013 (Table 25).

CON: Among free amino acids, Ser (24.52-37.36 mg/L), Gln (28.81-41.11 mg/L), Arg (53.48-95.13 mg/L), Ala (22.84-38.91 mg/L), and Pro (740.09-1078.24 mg/L) were the most abundant in 2012 Merlot juice (Table 26). In 2013, His (50.03-65.52 mg/L), Arg (62.18-130.86 mg/L), Glu (44.19-70.43 mg/L), Ala (55.75-80.67 mg/L), and Pro (573.30-932.59 mg/L) were among the most abundant free amino acids in Merlot juice (Table 27). Treatments had no effect on total free amino acids or individual amino acid concentrations in 2012 or 2013 (Table 26 and Table 27).

AREC: Among free amino acids, Ser (60.94-81.47 mg/L), Gln (42.92-75.32 mg/L), Arg (230.08-313.85 mg/L), Thr (68.13-90.10 mg/L), and Pro (1841.89-2165.85 mg/L) were the most abundant in 2012 Petit Manseng juice (Table 28). Treatments had no effect on total free amino acids or individual amino acid concentrations in 2012. In 2013, Ser (80.38-145.72 mg/L), Gln (67.72-135.76 mg/L), Arg (284.74-612.26 mg/L), Thr (90.00-158.66 mg/L), and Pro (2309.71-2591.74 mg/L) were among the most abundant amino acids in Petit Manseng juice (Table 29). In 2013, the post-véraison foliar treatment (Post-véraison foliar) increased the concentration of total free amino acids by 30.3%, which corresponded to an increase in 9 individual amino acids (Ser, Gln, Arg, Gly, Glu, Thr, Ala, Lys, and Met) (Table 29).

## Table 24. GMV: Treatment effects on Sauvignon blanc juice amino acid concentration (mg/L) at harvest in 2012.

| | | Free amino acid concentration (mg/L)[e] | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Amino acids[c]** | **His** | **Asn** | **Ser** | **Gln** | **Arg** | **Gly** | **Asp** | **Glu** | **Thr** | **Ala** | **Pro** | **Lys** | **Tyr** | **Met** | **Val** | **Ile** | **Leu** | **Phe** | **Total:** | **# Increased by treatment** |
| **Treatment[a,b]** | **Significance[d]** | ns | ns | **0.0345** | **0.0312** | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | **--** |
| | **Control** | 18.04 | 5.26 | 47.76b | 79.29b | 243.25 | 2.91 | 41.46 | 50.97 | 68.93 | 93.36 | 210.18 | 3.11 | 4.08 | 0.91 | 19.73 | 8.34 | 9.96 | 7.95 | 915.48 | **--** |
| | **Low soil** | 17.95 | 14.02 | 59.90ab | 101.51ab | 317.19 | 3.47 | 48.6 | 62.21 | 87.39 | 118.73 | 268.39 | 3.86 | 5.5 | 1.26 | 25.01 | 10.28 | 12.82 | 9.99 | 1168.07 | **0** |
| | **High soil** | 18.81 | 7.27 | 68.70a | 125.41a | 364.32 | 3.92 | 50.88 | 69.82 | 96.69 | 125.79 | 284.67 | 4.01 | 5.8 | 1.31 | 26.13 | 10.89 | 13.53 | 10.12 | 1288.1 | **2** |
| | **Foliar** | 20.55 | 5.65 | 57.00ab | 110.62ab | 358.88 | 3.75 | 45.71 | 57.76 | 87.5 | 118.94 | 255.41 | 3.28 | 5.28 | 1.56 | 24.45 | 10.14 | 12.6 | 10 | 1189.08 | **0** |

[a] Control= no nitrogen; Low soil= 30kg N/ha of calcium nitrate at bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Foliar= 40 kg N/ha of urea applied evenly in roughly 7 applications between bloom and véraison.

[b] Separation of means using Tukey's HSD (α=0.05).

[c] Cysteine was not identified in 2012 samples.

[d] Significance of treatment effects on response variables using two-way ANOVA (p>F; ns= not significant).

[e] Table represents the average amino acid concentrations from n=6 biological repetitions.

## Table 25. GMV: Treatment effects on Sauvignon blanc juice amino acid concentration (mg/L) at harvest in 2013.

| | | Free amino acid concentration (mg/L)[e] | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Amino acids[c]** | **His** | **Ser** | **Gln** | **Arg** | **Gly** | **Asp** | **Glu** | **Thr** | **Ala** | **Pro** | **Lys** | **Tyr** | **Met** | **Val** | **Ile** | **Leu** | **Phe** | **Total:** | **# Increased by treatment** |
| **Treatment[a,b]** | **Significance[d]** | ns | **0.0046** | **0.0064** | **0.0006** | ns | **0.0318** | ns | **0.001** | **0.0043** | ns | **0.0227** | **0.0369** | **0.0169** | **0.023** | **0.0056** | **0.0147** | **0.0237** | **0.0063** | **--** |
| | **Control** | 46.45 | 32.63b | 46.30b | 103.04b | 3.39 | 25.03ab | 108.55 | 39.11b | 86.65b | 158.14 | 2.75b | 4.27b | 2.43b | 18.98b | 9.81b | 9.47b | 8.45b | 703.47b | **--** |
| | **Low soil** | 46.05 | 41.89ab | 69.94ab | 194.70a | 4.02 | 22.97b | 119.71 | 54.85ab | 117.95ab | 195.53 | 3.29ab | 5.90ab | 2.911ab | 23.01ab | 12.44ab | 12.34ab | 9.81ab | 937.33ab | **1** |
| | **High soil** | 48.78 | 44.29ab | 77.78a | 200.19a | 4.33 | 26.83ab | 134.4 | 60.26a | 134.94a | 202.66 | 3.43ab | 5.30ab | 2.64b | 24.26ab | 13.12a | 13.24ab | 10.10ab | 1006.64ab | **5** |
| | **Foliar** | 56.03 | 53.33a | 87.06a | 269.44a | 4.82 | 29.95a | 139.75 | 73.03a | 147.35a | 222.31 | 4.12a | 6.12a | 4.29a | 26.82a | 14.11a | 14.31a | 11.93a | 1164.77a | **11** |

[a] Control= no nitrogen; Low soil= 30kg N/ha of calcium nitrate at bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Foliar= 40 kg N/ha of urea applied evenly in roughly 7 applications between bloom and véraison.

[b] Separation of means using Tukey's HSD (α=0.05).

[c] Cysteine and asparagine was not identified in 2013 samples.

[d] Significance of treatment effects on response variables using two-way ANOVA (p>F; ns= not significant).

[e] Table represents the average amino acid concentrations from n=6 biological repetitions.

## Table 26. CON: Treatment effects on Merlot juice amino acid concentration (mg/L) at harvest in 2012.

| | | Free amino acid concentration (mg/L)[e] | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Amino acids[c]** | **His** | **Asn** | **Ser** | **Gln** | **Arg** | **Gly** | **Asp** | **Glu** | **Thr** | **Ala** | **Pro** | **Lys** | **Tyr** | **Met** | **Val** | **Ile** | **Leu** | **Phe** | **Total:** | **# Increased by treatment** |
| | **Significance[d]** | ns | ns | ns | ns | **0.0157** | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | |
| **Treatment[ab]** | **Control** | 12.71 | 10.51 | 28.99 | 32.75 | 62.56ab | 2.14 | 13.75 | 13.41 | 15.36 | 27.18 | 783.48 | 1.58 | 1.05 | 0.55 | 21.21 | 13.05 | 13.68 | 2.81 | 1056.79 | -- |
| | **Low compost** | 14.77 | 11.74 | 33.28 | 38.11 | 56.74ab | 5.22 | 14.49 | 13.2 | 16.75 | 22.84 | 878.75 | 2.42 | 2.23 | 1.14 | 19.04 | 16 | 15.87 | 2.92 | 1165.5 | 0 |
| | **High compost** | 12.01 | 11.38 | 28.7 | 31.76 | 64.11ab | 2.24 | 13.76 | 13.07 | 15.21 | 28.29 | 740.13 | 1.85 | 1.4 | 1.17 | 20.99 | 12.35 | 13 | 2.6 | 1014.05 | 0 |
| | **Clover + low compost** | 15.44 | 13.47 | 30.55 | 34.61 | 75.74ab | 2.19 | 11.97 | 12.78 | 17.31 | 24.6 | 949.46 | 1.56 | 0.73 | 0.74 | 24.36 | 14.85 | 16.14 | 3.07 | 1249.58 | 0 |
| | **Clover + high compost** | 10.43 | 11.61 | 24.52 | 28.81 | 53.48b | 1.89 | 12.3 | 12.11 | 12.57 | 26.13 | 740.09 | 1.51 | 1.32 | 0.67 | 19.84 | 12.48 | 12.39 | 2.11 | 984.27 | 0 |
| | **Low soil** | 13.16 | 12.17 | 37.36 | 41.11 | 88.23ab | 2.67 | 15.26 | 15.98 | 21.74 | 36.12 | 1004.04 | 1.97 | 1.36 | 1.23 | 27.58 | 17.15 | 18.47 | 3.7 | 1359.31 | 0 |
| | **High soil** | 16.5 | 15.29 | 33.96 | 40.27 | 95.13a | 2.54 | 16.4 | 16.65 | 21.1 | 38.91 | 1078.24 | 1.62 | 1.28 | 0.74 | 26.36 | 15.47 | 18.25 | 3.9 | 1442.66 | 0 |
| | **Post-harvest** | 10.62 | 12.62 | 29.02 | 32.88 | 61.59ab | 2.2 | 14.33 | 13.1 | 14.88 | 31.4 | 810.86 | 1.79 | 1.16 | 0.74 | 23.37 | 14.1 | 14.71 | 3.03 | 1092.42 | 0 |

[a] Control= no nitrogen; Low compost= 33.5 kg N/ha applied as compost at bloom in 2012; High compost= 67 kg N/ha applied as compost at bloom in 2012; Clover + low compost= 33.5 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Clover + high compost= 67 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Low soil= 15kg N/ha of calcium nitrate at bloom + 15kg N/ha of calcium nitrate 6 weeks post-bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Post-harvest= 30kg N/ha of calcium nitrate applied post-harvest.

[b] Separation of means using Tukey's HSD (α=0.05).

[c] Cysteine was not identified in 2012 samples.

[d] Significance of treatment effects on response variables using two-way ANOVA (p>F; ns= not significant).

[e] Table represents the average amino acid concentrations from n=6 biological repetitions.

## Table 27. CON: Treatment effects on Merlot juice amino acid concentration (mg/L) at harvest in 2013.

| | | Free amino acid concentration (mg/L)[e] | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Amino acids[c]** | **His** | **Ser** | **Gln** | **Arg** | **Gly** | **Asp** | **Glu** | **Thr** | **Ala** | **Pro** | **Lys** | **Tyr** | **Met** | **Val** | **Ile** | **Leu** | **Phe** | **Total:** | **# Increased by treatment** |
| | **Significance[d]** | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | -- |
| **Treatment[ab]** | **Control** | 50.75 | 54.53 | 40.64 | 65.77 | 4.81 | 24.03 | 55.87 | 37.96 | 67.02 | 788.23 | 3.93 | 3.74 | 4.21 | 24.29 | 12.72 | 13.78 | 11.12 | 1265.38 | -- |
| | **Low compost** | 53.95 | 48.21 | 40.45 | 62.18 | 3.74 | 16.65 | 55.58 | 27.89 | 57.53 | 573.3 | 3.73 | 3.3 | 4.37 | 22.36 | 13.88 | 14.03 | 10.53 | 1011.69 | 0 |
| | **High compost** | 50.83 | 40.76 | 33.38 | 66.99 | 3.36 | 11.76 | 44.19 | 24.79 | 54.9 | 587.2 | 2.44 | 3.71 | 3.46 | 19.01 | 11.38 | 13.12 | 10.86 | 982.14 | 0 |
| | **Clover + low compost** | 59.66 | 55.9 | 50.2 | 97.51 | 3.66 | 17.33 | 64.55 | 25.81 | 72.52 | 887.39 | 4.19 | 4.08 | 4.03 | 26.23 | 12.75 | 16.73 | 13.22 | 1415.77 | 0 |
| | **Clover + high compost** | 65.52 | 51.97 | 46.9 | 108.85 | 4.14 | 25.86 | 56.18 | 21.47 | 67.76 | 699.15 | 3.41 | 5.74 | 4.92 | 22.12 | 13.67 | 14.43 | 13.3 | 1225.41 | 0 |
| | **Low soil** | 51.22 | 44.04 | 33.03 | 65.83 | 3.25 | 13.37 | 50.03 | 27.89 | 55.73 | 709.57 | 2.63 | 3.55 | 3.47 | 20.8 | 12.7 | 14.04 | 9.73 | 1120.89 | 0 |
| | **High soil** | 50.03 | 52.22 | 48.97 | 130.86 | 4.13 | 17.52 | 57.83 | 37.09 | 72.16 | 882.91 | 3.66 | 4.78 | 4.53 | 24.36 | 13.44 | 16.17 | 13.91 | 1434.58 | 0 |
| | **Post-harvest** | 59.37 | 58.32 | 48.2 | 130.25 | 4.55 | 17.04 | 70.43 | 39.77 | 80.67 | 932.59 | 3.96 | 4.05 | 4.37 | 26.21 | 16.49 | 12.7 | 16.08 | 1525.05 | 0 |

[a] Control= no nitrogen; Low compost= 33.5 kg N/ha applied as compost at bloom in 2012; High compost= 67 kg N/ha applied as compost at bloom in 2012; Clover + low compost= 33.5 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Clover + high compost= 67 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Low soil= 15kg N/ha of calcium nitrate at bloom + 15kg N/ha of calcium nitrate 6 weeks post-bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Post-harvest= 30kg N/ha of calcium nitrate applied post-harvest.

[b] Separation of means using Tukey's HSD (α=0.05).

[c] Cysteine and asparagine was not identified in 2013 samples.

[d] Significance of treatment effects on response variables using two-way ANOVA (p>F; ns= not significant).

[e] Table represents the average amino acid concentrations from n=6 biological repetitions.

## Table 28. AREC: Treatment effects on Petit Manseng juice amino acid concentration (mg/L) at harvest in 2012.

| | | Free amino acid concentration (mg/L)[e] | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amino acids[c] | His | Asn | Ser | Gln | Arg | Gly | Asp | Glu | Thr | Ala | Pro | Lys | Tyr | Met | Val | Ile | Leu | Phe | Total: | # Increased by treatment |
| Treatment[ab] | **Significance[d]** | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | -- |
| | **Control** | 37.22 | 5.70 | 65.64 | 47.64 | 236.53 | 4.36 | 14.12 | 30.96 | 68.13 | 46.62 | 1977.01 | 2.11 | 19.08 | 2.53 | 43.7 | 22.59 | 41.47 | 18.27 | 2678.69 | -- |
| | **Herbicide Control** | 20.66 | 29.64 | 61.42 | 49.34 | 230.08 | 4.75 | 17.49 | 28.37 | 68.46 | 34.10 | 1841.89 | 2.39 | 13.87 | 4.17 | 38.27 | 21.51 | 38.57 | 18.90 | 2523.9 | 0 |
| | **Pre-Véraison Foliar** | 29.34 | 5.73 | 60.94 | 42.92 | 233.03 | 4.62 | 13.83 | 27.71 | 69.7 | 38.88 | 1933.35 | 1.96 | 19.12 | 2.30 | 44.95 | 24.48 | 44.04 | 19.97 | 2616.85 | 0 |
| | **Post-Véraison Foliar** | 32.53 | 5.82 | 81.47 | 75.32 | 313.85 | 5.71 | 15.5 | 34.86 | 90.1 | 59.87 | 2165.85 | 2.17 | 22.75 | 4.01 | 52.17 | 30.08 | 40.72 | 17.09 | 3049.87 | 0 |

[a] CC control= cover cropped panels received no additional nitrogen; Herbicide control= herbicide panels received no additional nitrogen; Pre-véraison foliar= 5 kg N/ha foliar urea applied 2 weeks pre-véraison + 5 kg N/ha foliar urea applied 1 weeks pre-véraison to cover cropped vines; Post-véraison foliar= 5 kg N/ha foliar urea applied 1 week post-véraison + 5 kg N/ha foliar urea applied 2 weeks post-véraison to cover cropped vines. All treatments received 10kg N/ha as calcium nitrate at bloom in 2012 and 2013 as part of general vineyard maintenance.

[b] Separation of means using Tukey's HSD (α=0.05).

[c] Cysteine was not identified in 2012 samples.

[d] Significance of treatment effects on response variables using two-way ANOVA (p>F; ns= not significant).

[e] Table represents the average amino acid concentrations from n=6 biological repetitions.

## Table 29. AREC: Treatment effects on Petit Manseng juice amino acid concentration (mg/L) at harvest in 2013.

| | | Free amino acid concentration (mg/L)[e] | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amino acids[c] | His | Ser | Gln | Arg | Gly | Asp | Glu | Thr | Ala | Pro | Lys | Tyr | Met | Val | Ile | Leu | Phe | Total: | # Increased by treatment |
| **Treatment[ab]** | **Significance[d]** | ns | **0.0101** | **0.0042** | **0.0131** | **0.0076** | **0.0265** | **0.0069** | **0.0268** | **0.0003** | ns | **0.0096** | ns | **0.0061** | ns | ns | ns | ns | ns | -- |
| | **Control** | 65.12 | 80.38b | 67.72b | 284.74b | 6.17b | 28.63 | 36.35b | 90.00b | 37.34b | 2309.71 | 3.44b | 24.57 | 5.11b | 43.99 | 26.46 | 40.87 | 21.35 | 3171.94 | -- |
| | **Herbicide Control** | 68.52 | 105.93ab | 90.48ab | 371.32ab | 7.34ab | 43.05 | 50.37ab | 115.40ab | 57.44b | 2520.2 | 4.43ab | 26.25 | 6.17b | 51.41 | 27.67 | 43.40 | 24.97 | 3616.37 | 0 |
| | **Pre-Véraison Foliar** | 63.91 | 100.07ab | 80.06b | 357.62ab | 7.82ab | 30.70 | 40.78b | 110.65ab | 48.67b | 2284.17 | 4.83b | 26.99 | 5.57b | 48.56 | 28.77 | 41.00 | 23.58 | 3299.8 | 0 |
| | **Post-Véraison Foliar** | 78.45 | 145.72a | 135.76a | 612.26a | 10.14a | 40.68 | 58.74a | 158.66a | 86.35a | 2591.74 | 3.64a | 36.55 | 9.36a | 58.94 | 25.99 | 48.4 | 30.11 | 4135.46 | **9** |

[a] CC control= cover cropped panels received no additional nitrogen; Herbicide control= herbicide panels received no additional nitrogen; Pre-véraison foliar= 5 kg N/ha foliar urea applied 2 weeks pre-véraison + 5 kg N/ha foliar urea applied 1 weeks pre-véraison to cover cropped vines; Post-véraison foliar= 5 kg N/ha foliar urea applied 1 week post-véraison + 5 kg N/ha foliar urea applied 2 weeks post-véraison to cover cropped vines. All treatments received 10kg N/ha as calcium nitrate at bloom in 2012 and 2013 as part of general vineyard maintenance.

[b] Separation of means using Tukey's HSD (α=0.05).

[c] Cysteine and asparagine was not identified in 2013 samples.

[d] Significance of treatment effects on response variables using two-way ANOVA (p>F; ns= not significant).

[e] Table represents the average amino acid concentrations from n=6 biological repetitions

## Components of yield

GMV: Treatments had no effect on components of yield in 2011, 2012, or 2013 (Table 30).

CON: Treatments had no effect on components of yield in 2012 or 2013 (Table 31).

AREC: Treatment had no effect on number of clusters per vine in 2012 or 2013 (Table 32). Vines grown in the presence of an herbicide strip (Herbicide control) exhibited increased cluster weights, corresponding to a 50.0% increase in total vine yield in 2012 (Table 32). Berries from herbicide plots (Herbicide control) had greater berry weights than berries treated with a post-véraison foliar (Post-véraison foliar) application, but they did not have significantly greater berry weights than the cover crop control (CC control) (Table 32).

## Table 30. GMV: Treatment effects on Sauvignon blanc components of yield in 2011-2013.

| | 2011 Harvest | | | | 2012 Harvest | | | | 2013 Harvest | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment**[a] | Individual berry weight (g) | Clusters per vine | Vine Yield (kg) | Cluster weight (g) | Individual berry weight (g) | Clusters per vine | Vine Yield (kg) | Cluster weight (g) | Individual berry weight (g) | Clusters per vine | Projected vine yield (kg) | Projected cluster weight (g) |
| Significance[b] | ns | ns | ns | ns | n/a | ns | ns | ns | n/a | ns | ns | ns |
| Control | 2.0 | 21 | 6.1 | 291.0 | . | 41 | 5.2 | 128.5 | . | 50 | 9.1 | 179.7 |
| Low soil | 2.1 | 22 | 6.5 | 296.7 | . | 42 | 5.4 | 127.0 | . | 50 | 9.8 | 196.0 |
| High soil | 2.0 | 23 | 6.5 | 284.8 | . | 41 | 4.9 | 119.8 | . | 55 | 10.6 | 193.8 |
| Foliar | 2.0 | 22 | 6.0 | 277.2 | . | 38 | 4.5 | 122.3 | . | 50 | 9.3 | 184.3 |

[a] Control= no nitrogen; Low soil= 30kg N/ha of calcium nitrate at bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Foliar= 40 kg N/ha of urea applied evenly in roughly 7 applications between bloom and véraison.

[b] Significance of treatment effects on response variables using two-way ANOVA (p>F; ns= not significant).

## Table 31. CON: Treatment effects on Merlot components of yield in 2012 and 2013.

| | 2012 Harvest | | | | 2013 Harvest | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment**[a] | Individual berry weight (g) | Clusters per vine | Vine Yield (kg) | Cluster weight (g) | Individual berry weight (g) | Clusters per vine | Projected vine yield (kg) | Projected cluster weight (g) |
| Significance[b] | ns | ns | ns | ns | ns | ns | ns | ns |
| Control | 0.99 | 64 | 2.7 | 41.9 | 1.18 | 60 | 0.3 | 27.9 |
| Low compost | 0.99 | 60 | 3.1 | 48.8 | 1.20 | 70 | 0.3 | 24.1 |
| High compost | 1.01 | 63 | 3.3 | 51.4 | 1.22 | 76 | 0.4 | 25.3 |
| Clover+ low compost | 0.95 | 70 | 3.4 | 48.1 | 1.18 | 88 | 0.4 | 22.5 |
| Clover+ high compost | 0.99 | 75 | 3.4 | 45.5 | 1.20 | 97 | 0.4 | 21.4 |
| Low soil | 0.93 | 62 | 2.7 | 41.4 | 1.21 | 73 | 0.3 | 21.1 |
| High soil | 0.99 | 68 | 2.6 | 38.1 | 1.23 | 79 | 0.4 | 24.0 |
| Post-harvest | 1.07 | 72 | 3.7 | 51.9 | 1.23 | 76 | 0.4 | 25.3 |

[a] Control= no nitrogen; Low compost= 33.5 kg N/ha applied as compost at bloom in 2012; High compost= 67 kg N/ha applied as compost at bloom in 2012; Clover + low compost= 33.5 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Clover + high compost= 67 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Low soil= 15kg N/ha of calcium nitrate at bloom + 15kg N/ha of calcium nitrate 6 weeks post-bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Post-harvest= 30kg N/ha of calcium nitrate applied post-harvest.

[b] Significance of treatment effects on response variables using two-way ANOVA (p>F; ns= not significant).

**Table 32. AREC: Treatment effects on Petit Manseng components of yield in 2012 and 2013.**

| | 2012 Harvest | | | | 2013 Harvest | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment**[ab] | Individual berry weight (g) | Clusters per vine | Vine Yield (kg) | Cluster weight (g) | Individual berry weight (g) | Clusters per vine | Vine Yield (kg) | Cluster weight (g) |
| **Significance**[c] | ns | ns | **0.0004** | **<0.0001** | **0.0423** | ns | ns | ns |
| CC control | 1.13 | 37 | 2.8 b | 74.0 b | 1.08 ab | 39 | 3.7 | 94.7 |
| Herbicide control | 1.18 | 40 | 4.20 a | 104.8 a | 1.11 a | 41 | 4.1 | 101.6 |
| Pre-véraison Foliar | 1.16 | 39 | 3.1 b | 79.6 b | 1.10 ab | 38 | 3.9 | 101.8 |
| Post-véraison foliar | 1.15 | 39 | 3.3 ab | 84.1 b | 1.06 b | 40 | 3.9 | 96.2 |

[a] CC control= cover cropped panels received no additional nitrogen; Herbicide control= herbicide panels received no additional nitrogen; Pre-véraison foliar= 5 kg N/ha foliar urea applied 2 weeks pre-véraison + 5 kg N/ha foliar urea applied 1 weeks pre-véraison to cover cropped vines; Post-véraison foliar= 5 kg N/ha foliar urea applied 1 week post-véraison + 5 kg N/ha foliar urea applied 2 weeks post-véraison to cover cropped vines. All treatments received 10kg N/ha as calcium nitrate at bloom in 2012 and 2013 as part of general vineyard maintenance.

[b] Separation of means using Tukey's HSD (α=0.05).

[c] Significance of treatment effects on response variables using one-way ANOVA (p>F; ns= not significant).

## Pruning weights

GMV: Treatment had no effect on pruning weights in 2011 or 2012.

CON: The clover combined with a high compost rate (Clover + high compost) increased pruning weights by 63.6% in 2012 (Table 28). All other treatments had no effect.

AREC: Vines grown in the presence of an herbicide strip (Herbicide control) exhibited a 66.6% increase in pruning weights compared to vines grown with companion cover crops (CC control) (Table 29). All other treatments had no effect.

### Table 33. GMV: Treatment effects on Sauvignon blanc pruning weights in 2011 and 2012.

| Treatment[a] | 2011 Pruning Weight (kg/m canopy)[b] | 2012 Pruning Weight (kg/m canopy) |
|---|---|---|
| **Significance[c]** | ns | ns |
| Control | 0.31 | 0.29 |
| Low Soil | 0.35 | 0.31 |
| High Soil | 0.32 | 0.29 |
| Foliar | 0.28 | 0.24 |

[a] Control= no nitrogen; Low soil= 30kg N/ha of calcium nitrate at bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Foliar= 40 kg N/ha of urea applied evenly in roughly 7 applications between bloom and véraison.

[b] Pre-treatment (December 2010) pruning weight average: 0.32 kg/m canopy.

[c] Significance of treatment effects on response variables using two-way ANOVA (p>F; ns= not significant).

### Table 34. CON: Treatment effects on Merlot pruning weights in 2012.

| Treatment[ab] | 2012 Pruning Weight (kg/m canopy)[c] |
|---|---|
| **Significance[d]** | **0.0016** |
| Control | 0.11 b |
| Low compost | 0.12 b |
| High compost | 0.11 b |
| Clover+ low compost | 0.15 ab |
| Clover+ high compost | 0.18 a |
| Low soil | 0.12 b |
| High soil | 0.14 ab |
| Post-harvest | 0.13 ab |

[a] Control= no nitrogen; Low compost= 33.5 kg N/ha applied as compost at bloom in 2012; High compost= 67 kg N/ha applied as compost at bloom in 2012; Clover + low compost= 33.5 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Clover + high compost= 67 kg N/ha applied as compost at bloom in 2012 + early season establishment of red clover in 2012, and red clover+crimson clover in 2013; Low soil= 15kg N/ha of calcium nitrate at bloom + 15kg N/ha of calcium nitrate 6 weeks post-bloom; High soil= 30kg N/ha of calcium nitrate at bloom + 30kg N/ha of calcium nitrate 6 weeks post-bloom; Post-harvest= 30kg N/ha of calcium nitrate applied post-harvest.

[b] Separation of means using Tukey's HSD ($\alpha$=0.05).

[c] Pre-treatment (December 2010) pruning weight average: 0.185 kg/m canopy.

[d] Significance of treatment effects on response variables using two-way ANOVA (p>F; ns= not significant).

### Table 35. AREC: Treatment effects on Petit Manseng pruning weights in 2012.

| Treatment[ab] | 2012 Pruning Weight (kg/meter canopy)[c] |
|---|---|
| **Significance[d]** | **<0.0001** |
| CC control | 0.35 b |
| Herbicide control | 0.58 a |
| Pre-véraison foliar | 0.43 b |
| Post-véraison foliar | 0.42 b |

[a] CC control= cover cropped panels received no additional nitrogen; Herbicide control= herbicide panels received no additional nitrogen; Pre-véraison foliar= 5 kg N/ha foliar urea applied 2 weeks pre-véraison + 5 kg N/ha foliar urea applied 1 weeks pre-véraison to cover cropped vines; Post-véraison foliar= 5 kg N/ha foliar urea applied 1 week post-véraison + 5 kg N/ha foliar urea applied 2 weeks post-véraison to cover cropped vines. All treatments received 10kg N/ha as calcium nitrate at bloom in 2012 and 2013 as part of general vineyard maintenance.

[b] Separation of means using Tukey's HSD ($\alpha$=0.05).

[c] No pre-treatment pruning weights are available due to late implementation of experiment.

[d] Significance of treatment effects on response variables using one-way ANOVA (p>F; ns= not significant).

## Discussion

Establishing cover crops as a floor management system in vineyards is an effective method to battle excess vine vigor, reduce erosion, reduce soil nitrate leaching, and improve soil structure (Baumgartner, Smith, and Bettiga 2005; Celettte, Findeling, and Gary 2009; Fourie, Louw, and Agenbag 2007; Gulick et al. 1994). In grape growing regions such as Virginia, where many sites are characterized by steep terrain and the growing season is conducive to excess vegetative vine growth, cover crops have become an increasingly common component of routine vineyard management. The consequences of allowing cover crops to become intensively established in areas that do not perform annual hilling-up are evident by intense competition for soil nitrogen, leading to diminished vine and berry nitrogen concentrations.

The objectives of this study were two-fold. First, I sought to determine the most efficient method and optimal time for annual nitrogen applications, such that cover crop growth could be maintained for its intended benefits, while adequate vine nitrogen and berry YAN were achieved. Second, I sought to determine if different fertilization methods affected the amino acid concentration of mature berries and, if so, to consider how that may affect the sensory component of the resultant wines. A wide range of treatments were evaluated in this study: some were common practices currently employed on many vineyards (e.g., soil nitrogen treatments); others have yet to gain common usage in commercial vineyards (e.g., compost and leguminous cover crops); still others are not typically considered sufficient for perennial nitrogen requirements (e.g., sole reliance on foliar fertilization). This study sought to develop a fertilization management approach that could balance the competition for nitrogen uptake between established cover crops and grapevines.

As demonstrated with Sauvignon blanc at GMV, both the high calcium nitrate rate (High soil-GMV) and repeated foliar urea (Foliar-GMV) applications were effective at increasing petiole nitrogen concentration at véraison 2011 and 2012, and in blades at véraison 2013. Petioles of vines subject to the high calcium nitrate rate (High soil-GMV) exhibited decreased sulfur concentration at véraison 2012 and 2013, and bloom 2013. The decrease in sulfur in vines treated with soil nitrogen compared to vines treated with foliar nitrogen may be due to the implicit role that sulfur plays in improving nitrogen use efficiency by increasing nitrogen recovery from the soil (Salvagiotti et al. 2009). Treatments were applied over three years at GMV, so I expected to observe more pronounced trends in comparison with the other two sites; however, there were no treatment effects on tissue nitrogen at either bloom 2012 or 2013. The lack of differences at bloom may be have been due to oscillations in stability periods of total nitrogen concentration in petioles. A study conducted on Tempranillo found stability periods in petiole nitrogen included the complete flowering stage, and two weeks after bloom throughout harvest (Romero, García-Escudero, Martín 2013). Despite targeting the same percent bloom for sampling periods each year, environmental conditions are strong determinants of the bloom period duration, and it is difficult to pinpoint exact phenological stages yearly. In the event that my samples were taken between these two stability periods, they may not provide an accurate representation of the treatment effects. For this reason, Romero et al. (2013) suggests that leaf blades are preferable for total nitrogen diagnosis at flowering and at véraison, as the nitrogen concentration in these tissues have longer stability periods. My data indicates that both the high calcium nitrate rate (High soil-GMV) and the repeated foliar applications (Foliar-GMV) equally increased nitrogen concentration in blades relative to the control, at véraison 2013. Differences in the nitrogen stability periods between blades and petioles may also account for significant treatment effects being observed in one tissue but not the other at véraison 2011 and 2013.

At CON both clover treatments (Low compost + clover and High compost + clover) increased petiole nitrogen at bloom, and decreased phosphorous at bloom and véraison. Although I did not have quantitative clover establishment data, as I would have determined by clover density relative to other species in a given area, my field notes indicate that the red and crimson clovers were fully established and robust prior to the first sampling period (Figure 9).

Figure 9. CON: May 2013 establishment of crimson clover and red clover.

The increased petiole nitrogen may be due to rhizodeposition of nitrogen from the legumes in those plots (Fustec et al. 2010). The decrease in phosphorous may be attributed to the essential role phosphorous plays in the symbiosis of *Rhizobium* bacteria and host plants. *Rhizobium* bacteria require phosphorous, in the form of ATP, in order to fix atmospheric nitrogen into ammonium. It is likely that the clover plants were aggressively competing with the grapevines to obtain enough phosphorous to support the energy demands of their nodules, and subsequently those vines exhibited a decrease in phosphorous (International Rice Research 1990).

Foliar applications (Post-véraison foliar and Pre-véraison foliar) did not affect the macronutrient concentration or micronutrient content in Petit Manseng vines. Vines grown in the presence of an herbicide strip (Herbicide control) exhibited increased nitrogen content in petioles, relative to vines grown with companion cover crops (CC control). This is in agreement with other studies that have observed reduced petiole nutrient status in Chardonnay vines grown in the presence of under-trellis cover crops, which they attribute to a decrease in water supply and subsequent nutrient uptake (Tesic, Keller, and Hutton 2007).

A handheld chlorophyll meter was used to assess chlorophyll content because it was previously demonstrated that CCI values are significantly associated with leaf chlorophyll content and leaf nitrogen concentration in grapevines (Porro et al. 2001). In a study on Chardonnay vines, the coefficients of determination for CCI readings and leaf nitrogen content ranged from 0.95 at fruit set to 0.64 at harvest (Porro et al. 2001). The coefficients of determination for CCI readings and petiole or blade nitrogen concentration were much weaker in my experiment than values previously reported, but they were also computed on a season-long basis rather than at individual sampling periods. Of the three varieties in this study, a linear relationship was only significant between CCI readings and nitrogen concentration in Petit Manseng petioles and Sauvignon blanc blades. The lack of a strong correlation between Merlot and Sauvignon blanc petiole nitrogen concentration and CCI values and variations in leaf greenness, may be a result of vine age, rootstock, stage of growth, sun exposure, air temperature, or other nutrient deficiencies (Porro et al. 2001). Due to the fact that a calibration standard – plotting measured chlorophyll content against CCI readings – was not performed for each variety in this study, CCI values were not adjusted and cannot be compared across varieties. My decision to not perform a calibration standard was justified by my primary interest being the relative CCI values within each variety, as the treatments in this study were not applied to all subjects.

Despite the poor correlations found in the regression analysis, the effect of nitrogen treatment on CCI values for all varieties closely resembled the outcomes of the petiole nutrient analysis. The high calcium nitrate rate (High soil-GMV) was most effective at increasing leaf chlorophyll content in Sauvignon blanc vines. Similarly a high calcium nitrate rate (High soil-CON) was the most effective at increasing leaf chlorophyll content in Merlot vines. The clover treatments (Low compost + clover, High compost + clover) and post-harvest calcium nitrate treatment (Post-harvest) also increased Merlot CCI values, but not as dramatically. The substantial effect of the 2012 post-harvest calcium nitrate (Post-harvest) treatment on 2013 leaf chlorophyll content was unexpected, given its low rate compared to other treatments. One explanation for the increased CCI values may be attributed to efficient nitrogen uptake and translocation by Merlot after cover crop growth and competition had been suppressed late in the 2012 season. Neither of the foliar applications made to Petit Manseng vines had an effect on CCI values. This was not unexpected, given that the pre-véraison foliar treatment was only implemented for three of the four sampling periods and that the post-véraison foliar treatment was only implemented for half of the sampling periods. Petit Manseng vines grown in the presence of an herbicide strip had 28.4% higher CCI values than vines grown with an under-trellis cover crop present. This supports previous findings of under-trellis cover crops reducing vine nutrient status (Tesic, Keller, and Hutton 2007).

Canopy density did not change as a function of nitrogen treatment for any variety in this study as indicated by the EPQA parameters of interest. Moreover, vines grown in the presence of an herbicide strip (Herbicide control) did not have less dense canopies when compared to vines grown with companion cover crops. This contradicts previous reports of under-trellis cover crops significantly reducing canopy congestion in Cabernet Sauvignon vines (Hatch, Hickey, Wolf 2011). Fruit zone light interception was decreased by the two compost treatments (Low compost and High compost), the clover combined with a low compost rate (Clover + low compost), and both calcium nitrate rates in Merlot (Low soil-CON and High-soil CON); however, given the lack of treatment effect on other growth parameters, it is more likely that inconsistent leaf removal was responsible for differences in PPFD readings. The lack of treatments effects on EPQA parameters may be explained by adequate rainfall being received in both years of the experiment. Ingels et al. (2005) found that when Merlot vines in intensively cover cropped systems were supplemented with adequate irrigation, there were no cover crop treatment effects on shoot growth.

While my primary goal was to assess which nitrogen fertilization methods are most efficient at increasing vine and berry nitrogen, I also sought to determine whether any of the fertilization methods had unwanted consequences on berry maturation.

Nitrogen treatments had no effect on berry ripening and maturation in Merlot, Sauvignon blanc, or Petit Manseng berries, as determined by soluble solids, titratable acidity, and pH. One exception was with Petit Manseng vines grown in the presence of an herbicide strip (Herbicide control) which demonstrated decreased °Brix at harvest 2013. This is in agreement with previous reports of under-trellis cover crops increasing total soluble solids in Cabernet Sauvignon (Wheeler, Black, and Pickering 2005).

The recommended concentration for YAN is a function of the pre-fermentation sugar concentration of juice, but 140 mg N/L must is generally considered the absolute minimum concentration required to successfully complete fermentation (Bell et al. 2005). Although diammonium phosphate (DAP) and autolyzed yeast can be added to musts to increase YAN levels, there are some documented  drawbacks to their use. First, there are legal restrains on the amount of DAP that can be added to a must. In the United States it corresponds to an increase of 200 mg N/L, and in the European Union it corresponds to an increase of only 68 mg N/L (TTB, 21 CFR 184.1141). In situations where starting YAN is extremely low, DAP additions may not provide adequate nitrogen for yeast metabolism. Nonetheless, DAP additions are considered inferior to musts containing a naturally abundant mixture of amino acids because yeast can directly incorporate the amino acids into protein, rather than synthesizing new amino acids and wasting energy (Bell and Henschke 2005). The use of autolyzed yeast are proposed as a retort to this concern because they provide the must with a source of amino nitrogen and are marketed as promoting more complex aromas in wine. Yet, evidence suggests that musts higher in naturally occurring amino acids produce superior wine. Previous authors report that berry tissue amino acid composition is genetically fixed for each variety, while the amino acid composition of other tissues (e.g., roots) is approximately the same across varieties (Oungoulian 2012). Consequently, signature amino acid profiles are considered to play an integral role in the formation of varietal character (Oungoulian 2012). By this logic, naturally enhancing a variety's pre-determined amino acid profile is more likely to improve the formation of varietal aromas.

Although not significant in 2012, the post-véraison foliar treatment (Post-véraison foliar) increased Petit Manseng berry YAN at harvest by 51.0% and 69.8% in 2012 and 2013, respectively. In 2012 the post-véraison foliar treatment (Post-véraison foliar) did not increase the concentration of any free amino acid; however, it increased the concentration of nine amino acids in 2013. Threonine has been demonstrated to have the greatest influence on wine aroma composition by way of its concentration being most correlated with aromas related to esters and fatty acid synthesis (Hernández-Orte, Cacho, and Ferreira 2002). In this study, the concentration of threonine was increased by the post-véraison foliar treatment. The pre-véraison foliar treatment (Pre-véraison foliar) did not have an affect on berry YAN or amino acid composition. This is possibly due to the pre-véraison applied nitrogen being diverted to vegetative growth, instead of to the berries. My findings are in accordance with previous reports of post-véraison nitrogen applications increasing berry YAN due to increased fruit-sink strength at this time (Rodriguez-Lovelle and Gaudillere 2002).

The high calcium nitrate rate (High soil-CON) was the only treatment that increased Merlot berry YAN at harvest, and was limited to 2013. Moreover, no treatment affected the free amino acid composition of Merlot berries at harvest in either year. Given the low berry YAN values each year (58-108 mg N/L) compared to other varieties in this study (119-306 mg N/L) UPLC reaction volumes could have been reduced for the Merlot samples to enable more accurate quantifications.

Foliar fertilization (Foliar-GMV) consistently increased Sauvignon blanc berry YAN in all three years of this study and the high calcium nitrate rate (High soil-GMV) increased berry YAN in 2011 and 2013. Although the high calcium nitrate rate (High soil-GMV) did not significantly increase berry YAN in 2012, it increased the individual concentration of Ser and Gln. This result has limited practical importance due to the fact that other studies have demonstrated glutamine does not have an influential role in aroma development (Hernández-Orte, Cacho, and Ferreira 2002). The foliar treatment did not have an affect on individual free amino acid concentrations in 2012 despite its affect on berry YAN. In 2013, the low calcium nitrate rate (Low soil-GMV), high calcium nitrate rate (High soil-GMV) and foliar fertilization (Foliar-GMV) all significantly increased YAN by one, five, and eleven amino acids, respectively. My results are in close agreement with previous reports of foliar nitrogen fertilization increasing the concentration of all measured amino acids (except lysine) in Riesling berries, with soil-applied ammonium nitrate only increasing the concentration of five amino acids (asparagine, glutamic acid, arginine, threonine, and alanine) (Cheng and Lakso 2010). Of the amino acids identified as having an influential role in aroma development (Hernández-Orte, Cacho, and Ferreira 2002), threonine and alanine were both increased by the high calcium nitrate rate (High soil-GMV) and foliar fertilization (Foliar-GMV).

Nitrogen treatments had no effect on components of yield in Merlot, Sauvignon blanc, or Petit Manseng vines; however, vines grown in the presence of an herbicide strip (Herbicide control) exhibited increased cluster weights, corresponding to a 50% increase in total vine yield in 2012. Similarly, nitrogen treatments had limited effects on pruning weights, with the exception of vines grown in the presence of an herbicide strip (Herbicide control) exhibiting a 66.6% increase compared to the cover cropped control vines (CC control). These findings were in agreement with previous reports of under-trellis cover crops decreasing vine yields and pruning weights (Hatch, Hickey, Wolf 2011; Tesic, Keller, and Hutton 2007). In 2012, the clover combined with a high compost rate increased pruning rates by 63.6% compared to the control. Given the poor establishment of the clover in 2012, I attribute this increase to the combined effects of the high compost rate with disking the plots and subsequently temporarily removing the cover crop competition. The clover combined with the low compost rate also increased pruning weights by 36.4%, although this difference was not significant.

## Conclusion

Results from this study varied, possibly due to the different varieties being tested, the different sites under consideration, the varying degrees of vineyard floor establishment, and other variations in routine vineyard management practices (e.g., spray schedule, leaf pulling, pruning, fertilization of other nutrients). Nonetheless, this study helped identify nitrogen treatments that effectively increase berry YAN and the concentration of specific amino acids, without interfering with berry ripening or inhibiting the primary purpose of utilizing cover crops to curtail vine vigor. Although no treatment in this study wholly increased all parameters of vine nitrogen status (as determined by nutrient analyses of petioles and blades, and CCI readings), it did provide a baseline of how growers might integrate fertilization approaches. Given the combined success of foliar nitrogen applications to Petit Manseng and Sauvignon blanc increasing berry YAN and individual amino acids, with the positive effects of high rates of soil-applied calcium nitrate on improving chlorophyll content and petiole nitrogen in Merlot and Sauvignon blanc, I believe an integrated approach may be an effective fertilization practice to help alleviate some of the competition for nitrogen uptake between established cover crops and grapevines. Coupling a high rate of soil-applied calcium nitrate with a post-véraison foliar application of urea may facilitate adequate vine nitrogen status while significantly increasing berry YAN and individual amino acids, thereby boosting the potential to improve the overall aroma profile of resultant wines. The results from this study suggest establishing clover as the under-trellis cover crop may aid in improving vine nitrogen status; as such, future work could focus on improving establishment of clover in vineyards and potentially combining leguminous cover crops with post-véraison foliar nitrogen applications. In terms of how increasing the concentration of individual amino acids in berries may affect sensory components of wine, future work should include metabolomics profiling and be conducted on multiple varieties to account for the genetic differences in amino acid accumulation and the formation of specific metabolites. In those studies, it would be valuable to correlate berry amino acid concentrations and specific metabolites with sensory analyses.

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