Fertilize or Supplement: The Impact of Nitrogen on Vine Productivity and Wine Sensory Properties in Chardonnay

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Abstract: The impact of nitrogen (N) fertilization in the vineyard on vine productivity, fermentation, and wine sensory properties was compared to that of winery N addition on enological characters in Chardonnay between 2016 and 2018. Five treatments, including no vineyard or winery N addition (No N), addition of diammonium phosphate in the winery (+DAP), addition of organic N in the winery (+Org N), and addition of N to the vineyard soil (Soil N), or to foliage (Foliar N) were evaluated. The Foliar N treatment was evaluated in 2017 and 2018 only, while the other treatments were assessed in all years. Soil N increased leaf and petiole N status in all years and increased canopy growth and yield in years two and three. Foliar N had only a minor influence on leaf or petiole N status and did not alter vine growth or yield. Both Soil N and Foliar N elevated juice yeast assimilable nitrogen (YAN), although the extent of increase was greater for Soil N. Addition of DAP in the winery boosted juice YAN similar to the Soil N treatment, and addition of organic N was similar to the Foliar N musts. Fermentations proceeded more quickly in Soil N musts than in No N musts, with the Foliar N, +DAP, and +Org N treatments intermediate between Soil N and No N treatments. Wine sensory analysis revealed that Soil N wines were most distinct, with greater tropical fruit aromas. These findings show that while winery N additions provide similar fermentation kinetics to vineyard N fertilization in Chardonnay, they may not produce a wine with similar sensory characteristics.

Key words: nitrogen fertilization, nitrogen supplementation, *Vitis vinifera,* wine style, yeast assimilable nitrogen (YAN)

Nitrogen (N) is an essential nutrient required by grapevines and wine yeasts. In the vineyard, N availability influences vine N status, vine growth, and fruit composition, and is often the most important nutrient to manage

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(Bell and Robson 1999, Conradie 2001a, Linsenmeier et al. 2008, Schreiner et al. 2018). In the winery, must N, especially yeast assimilable nitrogen (YAN), plays a critical role in fermentation kinetics and the production of fermentation-derived aromas (Bell and Henschke 2005, Ugliano et al. 2008, Torrea et al. 2011). Since N has broad impacts in the vineyard and winery, studies have evaluated how vineyard N application or winery N supplementation influences must composition and wine style (Webster et al. 1993, Conradie 2001b, Ugliano et al. 2008, Torrea et al. 2011, Schreiner et al. 2014, 2018, Tahim and Mansfield 2019). However, no published research to date has compared vineyard N fertilization to winery N addition for their effects on must and wine composition in a systematic manner.

An important goal for N management in the vineyard and winery is to ensure that there is sufficient YAN present in the must for a successful fermentation. The minimum YAN required for successful fermentation is between 120 and 140 mg N/L (Bell and Henschke 2005), although others propose higher targets for must YAN (200 to 250 mg N/L) to reduce the risk of stuck or sluggish fermentation (Bisson and Butzke 2000, Mendes-Ferreira et al. 2004). While grape musts with low levels of YAN (<100 mg N/L) completed fermentation, the fermentation rate was reduced (Spayd et al. 1994, Ugliano et al. 2009, Stockert et al. 2013, Schreiner et al. 2018). Reducing N status in the vineyard and the resulting must improves berry and wine composition, particularly in red varieties (Bell and Robson 1999, Treeby et al. 2000, Schreiner et al. 2014, Yuan et al. 2018a, 2018b). Low N musts can produce lower concentrations of desirable esters and higher

alcohols in Shiraz and Chardonnay wines, as compared to low N musts supplemented with DAP in the winery (Ugliano et al. 2008, 2010, Torrea et al. 2011). As a result, it is unclear whether maintaining low N status throughout the wine production system or boosting must YAN levels in the vineyard, or in the winery, results in better wine quality (Webster et al. 1993, Ugliano et al. 2008, 2010, Torrea et al. 2011, Schreiner et al. 2018).

In the vineyard, N can be applied to the soil or to the foliage, and both application methods can influence fruit composition. N applied to the soil consistently results in a significant increase in vine N status and fruit N status across varieties and growing regions (Bell and Robson 1999, Conradie 2001a, 2001b, Linsenmeier et al. 2008, Schreiner et al. 2013, 2018). Since vine N status is a primary driver of vine growth, soil N application often results in larger canopies and higher yields (Bell and Robson 1999, Conradie 2001a, Linsenmeier et al. 2008, Schreiner et al. 2013, 2018). However, when N fertilization boosts vine N status to an excessive level, an overly vigorous canopy leads to excessive fruit shading, less color development in berries, and a greater chance of Botrytis infection (Keller et al. 1999, Hilbert et al. 2003). Foliar N application, on the other hand, can increase fruit YAN without altering canopy growth or yield. N applied to the canopy (most often as urea) between bloom and harvest diffuses into the leaves and is translocated to the fruit, or diffuses into the fruit directly, resulting in higher fruit N with limited impact on vine growth or yield (Lasa et al. 2012, Tozzini et al. 2013, Hannam et al. 2014). Foliar N use also carries less risk of nitrate leaching to groundwater as compared to soil N applications, since lower quantities of N are applied directly to the foliage (Lasa et al. 2012, Hannam et al. 2016).

Compared to vineyard N additions, winery N supplementation alters the concentration and composition of must YAN in a more specific and precise manner. When must YAN is low, diammonium phosphate (DAP) is added routinely to boost the level of ammonium-N (NH_4^+-N), and thus YAN, largely as a precaution to prevent slow fermentation and to reduce the production of undesirable sulfur compounds, such as H_2S (Jiranek et al. 1995). Although added DAP can increase fermentation rate, elevating ammonium concentration to an excessive level may increase production of H_2S and undesired acetic acid and ethyl acetate characters in wines, decreasing wine sensory quality (Ugliano et al. 2010, Torrea et al. 2011, Tahim and Mansfield 2019). Organic N supplements increase must YAN by increasing primary amino acids, rather than ammonium. Since amino acids are precursors of some fermentation-derived bouquets (e.g., branched-chained and acetate esters), adding amino-N may enhance wine aroma perception while promoting a successful fermentation (Miller et al. 2007, Torrea et al. 2011).

Both vineyard and winery N inputs are expected to affect wine aroma, because must N composition contributes to and regulates the formation of many volatile compounds during fermentation (Bell and Henschke 2005). While prior work has investigated how the concentrations of specific aroma compounds in wine, and the resulting aroma of those wines,

respond to either vineyard or winery N inputs (Garde-Cerdán and Ancín-Azpilicueta 2008, Ugliano et al. 2009, Siebert et al. 2018, Yuan et al. 2018b), a direct comparison of how vineyard N inputs compare to winery N inputs to alter the aroma perception of wines has not been reported. Moreover, how the concentration and composition of must YAN affect the mouthfeel of white wines remains unclear. N application in the vineyard alters phenolic composition in berries and wines in red varieties (Hilbert et al. 2003, Schreiner et al. 2014, Yuan et al. 2018b), but in white wines, it is unlikely that the change in phenolics would be large enough to alter mouthfeel perception. As with the wine aroma studies, research investigating changes to phenolic composition have not included a sensory component.

We investigated how boosting must N in the vineyard via fertilization, or in the winery through N supplementation of low N must, altered the sensory properties of Chardonnay wines, compared to maintaining low N status in both vineyard and winery. The specific goals of this study were to understand how fertilization of either soil or foliage with N influences vine N status, canopy growth, yield, and fruit composition, and to compare the effects of vineyard N fertilization to winery N supplementation on must composition, fermentation kinetics, and wine sensory properties. The knowledge gained through this study provides viticulturists and winemakers greater insight on how to manage N in the vineyard and winery to achieve a desired wine style.

Materials and Methods

The effects of vineyard N fertilization on vine productivity and wine sensory properties and a comparison of vineyard N use to winery N supplementation on must composition, fermentation kinetics, and sensory properties in Chardonnay were investigated. The overall experiment included five treatments, including two vineyard N additions, two winery N additions, and a control that received no N in either location (No N). N fertilizer was applied to the soil as urea-ammonium nitrate (Soil N) or to the foliage as urea (Foliar N) in the vineyard. Winery N additions included either an inorganic source of N (+DAP) or an organic N supplement (+Org N). Since fruit for the +DAP and +Org N treatments was obtained from the No N plots, only three treatments (No N, Soil N, and Foliar N) were evaluated in the vineyard. Fruit from the Soil N and Foliar N vines received no N additions in the winery. Each treatment was replicated four times using a random design in the vineyard and each treatment replicate was fermented separately in the winery. The four replicate finished wines for each treatment in each year were blended for sensory analysis. Data were collected over three years between 2016 and 2018, although the Foliar N treatment was assessed only in the last two years of this study.

Study vineyard. The commercial vineyard used in this study is located near Amity, OR (45°11′N; 123°20′W) and was planted in 2006 with Chardonnay (*Vitis vinifera* L., FPS clone 37) grafted onto Riparia Glorie (*Vitis riparia*). Vine rows are oriented north-south, with a spacing of 1 m × 1.75 m (vine × row, 5714 vines/ha). Vines were cane-pruned and trained

to a double Guyot system with vertical shoot-positioning. Canopy, weed, pest, and disease management were consistent with standard commercial practices used in the region. The soil in this vineyard is a mixture of Steiwer and Chehulpum soils (fine-loamy, mixed, superactive, mesic, shallow Ultic Haploxerolls). A grass cover crop in the alleys between vine rows was established at the time of planting and the remnant grass and volunteer weeds were mowed two to three times in each growing season. The vineyard was drip-irrigated and irrigation was applied between fruit set and harvest as per the cooperators' standard practice, based on visual assessments of shoot tips, weather conditions, the level of vine water stress determined by measuring leaf water potential, and past experience at the site.

Vineyard N applications. Within each of the four replicates in the vineyard, the No N and the Soil N treatments were assigned randomly to three entire rows of vines with a border row in between. A minimum of 128 vines occurred in the middle row of each replicate plot, where data were collected. In 2016 and 2017, vines in the Soil N treatment were fertilized three times each year: about one month before bloom, about one month after fruit set, and at veraison (Supplemental Table 1). In 2018, Soil N vines were fertilized twice (one month before bloom and one month after fruit set) to avoid boosting vine N status to an excessive level. At each application, urea ammonium nitrate solution (UAN-32, Oregon Vineyard Supply, McMinnville, OR) was diluted with water and applied at the rate of 17.8 kg/ha to the Soil N vines through the drip irrigation system. In total, 67.2 kg N/ha (~11.8 g N/vine) was applied to Soil N vines in 2016 and 2017 and 44.8 kg N/ha (~7.8 g N/vine) was applied in 2018.

The Foliar N treatment was applied to 25 continuous vines, at a random location within the middle row of the No N treatment in each replicate. Since N was applied only to the canopy and would not interfere with the growth of vines in adjacent rows, no buffer rows were used for the Foliar N treatment. Foliar N was applied three times each year in 2017 and 2018: about one month after fruit set, at two weeks before veraison (lag phase), and two weeks postveraison (Supplemental Table 1). At each application, 4.7 L urea (ACS certified, Fisher Scientific Inc., Fair Lawn, NJ) solution was applied to the canopy using a backpack sprayer, ensuring even coverage for east and west aspects of the canopy and the adaxial and abaxial sides of leaves. The concentration of urea was 0.85% in 2017, but was reduced to 0.72% in 2018, as some minor marginal leafburn occurred in the lower canopy in 2017. To avoid drift, foliar N sprays were applied in the morning (before 1000 hr, PDT) on days with little or no wind. In total, 25 kg/ha (~4.3 g N/vine) was applied in 2017 and 22 kg N/acre (~3.9 g N/vine) was applied in 2018. More N was applied in the Soil N treatment than in the Foliar N treatment to achieve a similar level of must YAN at harvest (D'Attilio 2014, Hannam et al. 2016, Moss 2016). The same vines in each plot were treated with soil N (entire rows via fertigation) or foliar N (25 vines in a single row) each year.

Vine N and leaf greenness. To assess the impact of vineyard N application on vine N status, leaf blades and petioles were sampled at 50% bloom and 50% veraison in all experimental years. For each plot, 15 opposite-cluster leaves were collected at bloom and 10 pairs of leaves, each including one opposite-cluster leaf and one recently-expanded leaf, were sampled at veraison. Leaves were collected from both sides of the canopy. Petioles and leaf blades were rinsed in distilled water, oven-dried at 65°C, ground to a fine powder, and the N concentration in each tissue was determined using a C-H-N analyzer as described (Schreiner et al. 2018). Leaf greenness associated with chlorophyll concentrations was assessed periodically from budbreak to harvest using a SPAD meter (model 502, Konica Minolta, Osaka, Japan) on opposite-cluster leaves throughout the season and on recently-expanded leaves late in the season. A single reading per leaf was taken from 30 leaves of each age class per plot on 30 separate shoots on a minimum of 15 vines.

Soil N analysis. Soil samples were collected using a soil auger (2.2 cm diam) from the weed-free vine row three times in 2017 and 2018: at about one month after fruit set, about two weeks before veraison, and about one month postharvest. Five cores were collected from each plot 15 to 20 cm offset from drip irrigation emitters to a depth of 45 cm and pooled. Available nitrate and ammonium were determined in air-dried, pulverized soils using standard methods for western Oregon soil (Miller et al. 2013). Briefly, soil was extracted with 2 M KCl, filtered with Whatman No. 42 paper, and nitrate and ammonium in the filtrate were determined with a Lachat flow injection auto-analyzer (Honeywell Analytics Inc., Lincolnshire, IL).

Vine vegetative growth, vine water potential, and photosynthesis. Shoot length was determined at bloom using a flexible measuring tape. Five vines were selected at random in each plot, and all shoots on one arm were measured. Leaf area was determined at veraison using a non-destructive method by comparing leaves to a series of concentric circles of known area (Schreiner et al. 2012). Four vines were chosen randomly per plot. The total shoot number per vine was recorded, and leaf area was measured on three random shoots. The total leaf area per vine was calculated by multiplying the average leaf area per shoot by the number of shoots per vine. The pruning mass of fruiting shoots in the dormant season was determined by collecting data from five sets of three continuous vines for each plot, with cane number and total cane mass recorded for each panel and converted to a per vine basis. All measures of growth were recorded each year.

Midday leaf water potential (LWP) was determined periodically between 1400 and 1700 hr on cloudless days between fruit set and harvest (see Tian and Schreiner 2021) using a pressure chamber (model 610, PMS instrument company, Albany, OR) on two leaves per plot from different vines. Leaf gas exchange was determined periodically on cloudless days between bloom and veraison using a portable photosynthesis system (model 6400 in 2016 and 2017 and model 6800 in 2018; LI-COR Biosciences, Lincoln, NE). Two leaves per plot on different vines were measured under ambient light (sun + sky), 400 ppm carbon dioxide, and chamber temperature control set at the ambient air

temperature at the start of a measurement period. Cluster solar exposure was measured on cloudless days near veraison each year using a ceptometer (AccuPAR model LP-80, Decagon Devices, Pullman, WA). Measurements were performed at 0900, 1100, 1300, 1500, and 1700 hr in 2016, and at 1000, 1200, 1400, and 1600 hr in 2017 and 2018. The level of cluster exposure was expressed as the percentage of radiation recorded in the fruit zone, compared to full sunlight.

Vine reproductive growth and yield parameters. To assess vine fruitfulness in each year, six vines were chosen randomly from each plot after shoot-thinning in the spring. The number of shoots and the number of inflorescences were recorded for all shoots on individual vines, and fruitfulness was expressed as the number of inflorescences per shoot.

Fruit was harvested in each year one day prior to commercial harvest, when the total soluble solids (TSS) of fruit was between 21.5 and 23.0 Brix. Clusters were removed from five sets of three continuous vines per plot, and clusters were counted and weighed. A subsample of five clusters was randomly chosen from each plot to determine the number of berries per cluster and average berry weight. The berries from the subsample were pressed using a stainless steel handcrank press. The concentration of nutrients other than YAN (phosphorus, potassium, calcium, copper, magnesium, sulfur, iron, manganese, boron, zinc, and sodium) in the juice was determined by inductively coupled plasma-optical emission spectrometry (Perkin Elmer Optima 3000DV) after microwave digestion in nitric acid (Jones and Case 1990).

Weather data. Weather data were collected between budbreak and harvest for each experimental year, including daily maximum, minimum, and average temperature; daily solar radiation; and daily precipitation from the closest Agrimet weather station, located in Aurora, OR (U.S. Department of Interior - Bureau of Reclamation, https://www.usbr.gov/pn/agrimet/webarcread.html). The weather station is ~37 km from the experimental site.

Winery N supplementation, juice chemistry, and fermentation. Fruit harvested from the No N plot in each field replicate was well mixed, split evenly into three groups, and two of the groups were assigned to either the +DAP or +Org N winery treatments. About 34 kg of fruit was used for each fermentation replicate, and all harvested fruit was stored overnight at 4°C in the winery. Fruit from individual replicates was destemmed the next day and pressed for five minutes at 0.15 mPa using a bladder press. Juice was placed in 19 L (5 gallon) glass carboys, 50 mg/L SO_2 (as potassium metabisulfite) was added, and the juices were allowed to settle for 24 hrs at 4°C. Twelve L juice for each replicate was racked into clean and sanitized 19 L glass carboys and subsamples of juice were collected from each carboy to determine basic juice chemistry parameters, including YAN. The concentration of juice YAN was calculated from the sum of free amino acid-N (FAN-N) as determined by the OPA (o-phthaldialdehyde) colorimetric assay (Dukes and Butzke 1998) and ammonium-N by enzymatic assay (Sigma ammonia assay kit; Sigma Chemical Co.). After determining juice YAN concentrations, DAP and organic N nutrition

(NutriFerm Arom Plus) were added to the juice according to the treatment. The concentration of ammonium-N and FAN-N provided by DAP and NutriFerm Arom Plus was determined previously by making additions of DAP or NutriFerm Arom Plus to white grape juice (Santa Cruz Organic) and measuring NH_4^+-N and FAN-N before and after additions. In each year, the level of juice YAN in the +DAP and +Org N treatments was boosted prior to fermentation to roughly match the +Soil N treatment. After winery N additions, the juice was well mixed and subsamples were collected from all treatments for post-addition analysis of YAN and other juice components. The TSS and pH of juice were determined using a refractometer and a pH meter, respectively. The level of titratable acids (TA) in juice was measured by titrating with 0.1 M NaOH to an end point of 8.2.

Juices were placed in a temperature-controlled room set at 15°C and inoculated with *Saccharomyces cerevisiae* D47 (Lallemand, Montreal, Canada) following manufacturer's instructions. The soluble solids in all musts were monitored daily using an Anton-Paar DMA 35N Density Meter. Once fermentation was completed, 50 mg/L SO_2 was added to the wines. After settling at 4°C for 48 hrs, wines were racked. Bentonite was added at 0.12 g/L to clarify wines and they were racked again after an additional 48 hrs. Preliminary sensory evaluation of the replicates by the investigators revealed an absence of winemaking faults or discernible differences among replicates within each treatment. Therefore, the four fermentation replicates for each treatment were combined and blended to make a representative wine sample for sensory evaluation. Individual samples from each replicate were taken prior to blending for chemical evaluation. Wines were stored in stainless steel tanks at 4°C until bottling. Before bottling, wine was filtered through a 1-μm nylon cartridge filter (G.W. Kent, MI, USA), followed by a 0.45-μm sterile PES cartridge filter (Merck-Millipore, MA, USA). Wines were bottled in 750-mL screwcapped bottles (Stelvin, Amcor, Zurich) and stored at 13°C until required for analysis.

Wine sensory analysis. Sensory analysis of Chardonnay wines, including Napping and Ultra-flash profiling (UFP), was conducted by wine experts after six months of bottle aging. Sensory analysis was approved by the Institutional Review Board at Oregon State University (#8781). To be included in the study, a panelist had to be over 21-years-old; a non-smoker; not currently pregnant; free of any taste deficits or oral disorders; free of oral lesions, cankers, sores, and piercings of the lip, tongue, or cheek; and have no allergies to wine. All panelists had worked in the wine industry, specifically with white wines, for a minimum of five years.

Panels were held at the Oregon State University Yamhill County Extension Office in McMinnville, OR. Panelists evaluated samples in custom-built tabletop booths (61 cm × 71 cm back, 61 cm × 65 cm sides, white corrugated plastic). The room contained both natural and artificial light, was kept at 20°C ± 2, and two air purifiers (Winix, Vernon Hills, IL) were used to maintain air quality in the space. A total of 17 wine experts (10 male, seven female) evaluated the 2016 wines in August 2017, and 20 wine experts (10 male, 10 female)

evaluated the 2017 wines in August 2018. The 2018 wines were evaluated in August 2019 by 22 wine experts (12 male, 10 female). Approximately 80% of the tasters each year were the same individuals.

Napping and UFP were conducted as described (Perrin and Pagès 2009, Reinbach et al. 2014). In each tasting event, panelists completed two Napping and UFP tests, one for aroma and one for mouthfeel. Half of the panelists started with aroma and the other half began with mouthfeel. For each test, individuals were presented with all treatments in duplicate; eight samples in total for 2016 wines, and 10 samples in total for 2017 and 2018 wines.

Panelists were instructed to group wines based on similarity in aroma or mouthfeel. Panelists were asked to only smell the wine for the aroma Napping and only taste wines for the mouthfeel Napping. To reduce the influence of perceived aroma on the evaluation of mouthfeel, panelists were required to wear nose clips during this portion of the test in 2018, as the prior results showed use of some aroma-related terms for mouthfeel (Sereni et al. 2016). During each evaluation, panelists were instructed to smell/taste the eight or 10 wines from left to right and mark the placement of wines on the provided paper (18 × 14 inches, Strathmore Drawing Paper Pad). Wines that were similar were to be placed closer together and wines that were very different placed farther apart. Once the wines were placed on the paper, they were instructed to enrich each wine/group with aroma descriptors (UFP). When the panelists finished with the test, the location of the wine glasses was marked by the instructors of the sensory tests.

Data analysis. Data were analyzed separately for each experimental year. Using the average value for each plot, leaf and petiole N concentrations, vegetative and reproductive growth parameters, must chemistry variables, and the number of days to complete fermentation were analyzed using N treatment as the main factor. A Student t-test was used when only the No N and Soil N treatments were compared (vine growth parameters in 2016), and analysis of variance (ANOVA) was performed when more than two treatments were assessed. Means were compared using Tukey's honest significant difference test at 95% confidence. Assumptions of normality and homogeneity of variance were examined using the Shapiro-Wilk test and Levene's test prior to ANOVA. Must FAN-N and must YAN were log-transformed before ANOVA to satisfy the homogeneity of variance assumption in 2017. Must ammonium-N in 2016 was analyzed by Kruskal-Wallis test and means were compared with Dunn's test at 95% confidence. Soil ammonium-N and nitrate-N were analyzed using a Mann-Whitney test when comparing the Soil N and No N treatments, or using a Kruskal-Wallis test when three treatments were evaluated. The mean and standard error of the mean are reported in figures and tables for simplicity. The treatment effect was considered significant when $p < 0.05$.

Raw data for must YAN concentrations and the number of days to complete fermentation were combined for all three years and analyzed by linear regression. Since the relationship between must YAN and fermentation time differed

significantly among years, this relationship was analyzed separately for each year. The assumption of normality was examined by the Kolmogorov-Smirnov and the Shapiro-Wilk tests. Statistical analyses mentioned above were conducted using R (version 3.5.3, R Core Team, 2019).

Sensory data were analyzed using XLSTAT ver 2019.3.1.61246 (Addinsoft, Paris, France). Napping data were obtained using a ruler (inches) and measuring from left (X) and bottom edges (Y) relative to the original paper's orientation in relation to each panelist. Multiple factor analysis (MFA) was conducted using the X and Y coordinates for each wine to analyze the effects of treatment. For UFP, the frequency of the terms used were placed into a matrix (treatment by term), and terms were condensed for redundancy. Words with similar meanings were grouped (e.g., sour, acidic, tart). Terms that were used in <15% of the total calculated frequencies were excluded from further analysis (Perrin and Pagès 2009). Correspondence analysis (CA) was used to evaluate the UFP terms. For both MFA and CA coordinates, hierarchial clustering (HC) and then k-means clustering were used on both aroma and mouthfeel data to determine how different wines grouped (Pelonnier-Magimel et al. 2020).

Results

Weather and vine phenology. Weather patterns and vine phenological development differed among years (Supplemental Table 2). Budbreak occurred 10 days earlier in 2016 than in 2017 and 2018. The cumulative growing degree days in March and April was 225°C in 2016, but between 130 and 150°C in the latter two years of the study. The time of bloom, veraison, and harvest were each advanced by 13 to 20 calendar days in 2016 as compared to the other two growing seasons, even though vines experienced warmer weather between bloom and veraison in 2017 and 2018. The major phenological stages occurred at similar times in 2017 and 2018, although bloom and veraison were delayed by about five days in 2017. The 2018 season was the driest among the three years.

Vine N status and leaf greenness. Soil N application improved vine N status in all years, while N applied to foliage had only a minor influence on vine N status (Table 1). Across three years, bloom leaf blade and petiole N was 10 to 30% higher in Soil N vines than in No N vines, even in the first year, when the Soil N vines had received only one N application (~3.9 g/vine) prior to sampling. The difference in leaf blade and petiole N between the Soil N and the No N vines became greater at veraison than at bloom. The concentration of veraison leaf blade and petiole N was 20 to 35% higher in the Soil N vines than in the control. Foliar N generally did not affect leaf blade and petiole N at bloom and veraison, except that veraison leaf blade N increased slightly in the Foliar N vines in 2018.

Leaf greenness increased with soil N application by midsummer, but was not affected by foliar N application in any year (Supplemental Table 3). The SPAD values of opposite-cluster leaves did not differ between the No N and Soil N vines from mid-May to the end of July, except in one case in 2018. SPAD values were higher in the Soil N vines

beginning in August of each year, and differences in SPAD of opposite-cluster leaves grew larger between the Soil N and No N vines as each growing season progressed. There was a similar pattern in the upper canopy leaves. On the other hand, Foliar N had no impact on leaf greenness. The SPAD values of opposite-cluster leaves and upper canopy leaves never differed between Foliar N vines and No N vines.

Vine vegetative growth. Soil N application stimulated canopy growth beginning in the second year, but foliar N application did not alter vegetative growth parameters (Figure 1). The responses of total shoot length at bloom, leaf area at veraison, and pruning mass at dormancy to soil N addition followed the same pattern. Those parameters of vegetative growth were 15 to 33% greater in Soil N vines than in No N vines in 2017 and 2018.

Soil N application had only a minor influence on single-leaf photosynthesis rates, increasing it on one of five days when gas exchange was measured (Supplemental Table 4). LWP did not differ between the No N and Soil N vines on any single measurement day (Supplemental Table 5). However, when data were combined for each season, the seasonal mean value of LWP was slightly lower in the Soil N vines than the No N vines in 2016 and 2018. LWP was measured in the Foliar N vines on three days in 2018 and did not differ from the No N vines on those days (data not shown). Soil N vines had less fruit exposure to sunlight than the No N vines at some times, most consistently in 2017, but the Foliar N vines did not differ from the No N controls (Supplemental Table 6).

Vine reproductive development. Soil N application increased fruit yield beginning in the second year, but fruit yield was unaffected by foliar N application (Figure 2). Fruit yield did not differ between the No N and the Soil N vines in 2016. However, yield increased 30% in the Soil N vines in 2017, due to greater cluster weights and more berries

per cluster. In 2018, although no yield components were affected by treatment, yield was 36% higher in the Soil N vines than in the No N vines. Fruitfulness, number of clusters per vine, and average berry weight did not vary among treatments in any year.

Soil inorganic N. Soil ammonium and nitrate levels were more responsive to soil N application in 2017 than in 2018, most likely because the Soil N vines received an additional dose of N (22.4 kg N/ha) in 2017 (Table 2). The concentration of NH_4^+-N and nitrate-N (NO_3^--N) in soil was relatively consistent in the No N vines over time, but both sources of N were elevated after harvest in the Soil N treatment in 2017. Nitrate was also much greater in the Soil N vines in August 2017. Foliar N applications did not affect soil inorganic N levels.

Must composition, nutrients, and alcoholic fermentation. Vineyard N and winery N additions on must maturity components (TSS, TA, and pH) were minor and not consistent across years (Table 3). The Soil N musts had a higher concentration of TSS than the No N and +DAP musts in 2016 and a higher pH than the No N, Foliar N, and +Org N musts in 2017. Must TA was not affected by vineyard or winery N treatments. Soil N fertilization altered the concentrations of several mineral nutrients besides N in the must over three years, but foliar N sprays did not affect must nutrients in 2017 and 2018 (Supplemental Table 7). The concentrations of Ca and Mg were greater in Soil N musts than in No N musts in 2016, but the opposite pattern occurred in 2018. The level of S in musts increased with soil N addition in 2016 and 2017, but not in 2018. Must P concentrations, however, were reduced in response to soil N application in the latter two years of this study.

Both vineyard and winery N additions effectively increased must YAN levels prior to fermentation, but the impacts on NH_4^+-N and FAN-N differed among treatments (Table 3). Must YAN was low in the No N treatment in all years. Soil N application in the vineyard elevated must YAN by 90 to 200% across three years, owing to increases in both NH_4^+-N and FAN-N. Foliar N application in the vineyard improved must YAN by 105% in 2017 and by 61% in 2018. The concentration of FAN-N in must was greater in the Foliar N treatment than in the control in 2017, but not in 2018, though must NH_4^+-N was unaffected by foliar N application in either year. The addition of DAP at the winery boosted must YAN to a similar level as in the Soil N musts in all three years, by increasing only NH_4^+-N in the must. As a result, NH_4^+-N accounted for more than 50% of the must YAN in the +DAP treatment, but only 7 to 30% of must YAN in other treatments. Organic N addition in the winery increased must YAN to match the Soil N treatment in 2016, but the concentration of must YAN in the +Org N treatment was similar to the Foliar N treatment in 2017 and 2018. Supplementing organic N in the winery had no influence on must NH_4^+-N, but increased must FAN-N by 83 to 160% compared to the control. The concentration of must FAN-N in the +Org N treatment was similar to the Soil N treatment in 2016 and 2018, but matched the Foliar N treatment in 2017.

Table 1 Effect of vineyard nitrogen (N) applications (Soil N and Foliar N) on leaf blade and petiole N concentrations in Chardonnay grapevines from 2016 to 2018. Vines that received no N supply (No N) served as the control. Values represent means (standard deviation) for each treatment at each phenological stage (n = 4). DW, dry weight.

	Bloom N (g N/kg DW)		Veraison N (g N/kg DW)	
	Leaf blade	Petiole	Leaf blade	Petiole
2016				
No N	25.4 b[a] (1.3)	6.2 b (0.4)	15.5 b (1.0)	2.9 b (0.2)
Soil N	28.0 a (1.6)	8.0 a (1.0)	20.0 a (0.8)	3.6 a (0.2)
2017				
No N	24.3 b (1.7)	5.7 b (0.9)	16.5 b (1.7)	3.3 b (0.4)
Soil N	27.3 a (1.0)	7.1 a (0.7)	22.5 a (1.2)	4.4 a (0.2)
Foliar N	-	-	17.6 b (1.0)	3.3 b (0.3)
2018				
No N	24.8 b (1.1)	6.3 b (0.4)	17.2 c (0.6)	3.6 b (0.3)
Soil N	27.3 a (0.9)	7.2 a (0.7)	21.9 a (0.5)	4.4 a (0.1)
Foliar N	23.8 b (1.1)	6.1 b (0.2)	18.3 b (0.4)	3.7 b (0.2)

[a]Data were analyzed separately for each experimental year. Means followed by a different letter between treatments within each year differ significantly based on t-test or Tukey's honest significant difference test at 95% confidence.

The level of must YAN influenced the length of time required for yeast to complete alcoholic fermentation (Table 3). Generally, fermentation proceeded more slowly in the No N treatment musts with 60 to 120 mg YAN/L than in the other, higher-YAN musts. While treatment was not significant in 2016, it took about three days longer for the No N musts to finish fermentation than for the Soil N, +DAP, and +Org N treatments. In 2017, fermentation finished 14 days sooner in the Soil N treatment than in the No N treatment, while the Foliar N, +DAP, and +Org N treatments were intermediate between these extremes. A similar pattern was observed in 2018 as in 2017. The number of days to complete fermentation correlated well with the level of must YAN in each year ($R^2 > 0.59$ each season), showing that must YAN was the primary driver of fermentation speed (Figure 3).

Sensory analysis - aroma. Vineyard N fertilization and winery N supplementation had varying influences on the aroma of Chardonnay wines (Figure 4, Supplemental Figure 1). MFA incorporating the spatial Napping data showed that the first two factors (F1 and F2) accounted for 49.2% of the total variance in 2016, 42.7% in 2017, and 36.8% in 2018 wines (Supplemental Figure 1). In each year, the wines clustered into three groups. Across all three years, the +Org N sensory replicates always clustered in the same group. In the other treatments, the sensory replicates showed some similarity, but were not always in the same cluster group.

Correspondence analysis that used the UFP data for aroma indicated that the first two factors explained 58.3% of the variance in 2016, 67.2% in 2017, and 45.8% in 2018 wines (Figure 4). Clustering showed three distinct groups in each year, although across years, the wines and terms associated with each group differed. However, some consistencies were found. The No N wines were characterized by peach and stone fruit aromas in 2016 and 2018, while they were

Figure 1 Effect of vineyard nitrogen (N) applications (Soil N and Foliar N) on vegetative growth of Chardonnay grapevines from 2016 to 2018. Vines that received no N supply (No N) served as the control. Data points represent means and standard error for each treatment in each year (n = 4). Data were analyzed separately for each experimental year. Letters near each symbol designate significant groups based on t-test or Tukey's honest significant difference test at 95% confidence. FW, fresh weight.

described by apple aroma and some negative descriptors, such as oxidized, closed, and tired, in 2017. The Soil N wines consistently grouped with tropical and fruity notes across all years of the study. Beside the clear association with tropical aromas, the Soil N wines also stood out as most different from all other treatments based on the spatial Napping in 2016 (Supplemental Figure 1). The aroma of the Foliar N wines was not consistent across wine replicates in a given year, nor across the two years that it was applied (Figure 4). However, one replicate in 2017 and 2018 was associated with citrus aromas, while the other replicate of the Foliar N treatment was linked with green apple (2017) or vegetal (2018) aromas. Similarly, both winery N treatments did not produce wines with consistent aromas across years based on UFP analysis. In 2016, the +DAP and +Org N replicates split into two groups, one characterized by more negatively associated descriptors and the other by fruity, pear, and citrus aromas.

In 2017, the +DAP wines were associated with tropical and fruity aromas, while the +Org N wines were split between two groups, but closest to orange or mild fruit aromas. In 2018, the +DAP wines were described as sweet/candied, fruity, or floral, and the +Org N wines were closest to peach/stone fruit aromas.

Sensory analysis - mouthfeel. MFA incorporating the spatial Napping data for mouthfeel accounted for a similar amount (44.7 to 45.5%) of the variance in the F1 and F2 axes in 2016 and 2017, but less so (36.0%) in 2018 (Supplemental Figure 2). In each year, wines clustered into three groups, but in 2016, only the Soil N wines were in the same group. The other treatments in 2016 had replicates in different groups. In 2017 and 2018, the wines from the vineyard fertilizer treatments (Soil N and Foliar N) were in the same group, while +DAP wines were always a unique group. However, the No N wines formed the third group in 2017, but the +Org N wines formed the third group in 2018.

The variance for the first two factors from the CA analysis each year explained more of the total variance (45.6 to 73.6%) in mouthfeel than MFA. Additionally for the 2018 wines, four clusters were found rather than three. The UFP data did not support the groupings identified by the MFA based on Napping (Supplemental Figure 3). Participants struggled to agree on terms for many of the wines, and replicates of the same treatment in a given year were often described using different terms. Additionally, some aroma terms were used for the 2016 and 2017 wines, but not the 2018 wines, due to the usage of a nose clip. Across the three years, the different N treatments did not associate consistently with specific descriptors. It should be noted that the No N wines from 2017 grouped clearly with terms linked to acidity, and the F1 axis for CA from that year is associated with high acid (positive direction) and low acid descriptors (negative direction). No other clear association with terms and the axes of the CA was seen.

Discussion

The purpose of this study was to understand whether wine composition of Chardonnay could be improved by applying N in the vineyard, by supplementing N in the winery, or by maintaining low N status in both vines and musts. Thus, we examined how vineyard N applications and winery N additions affected must YAN levels, fermentation kinetics, and wine sensory properties in comparison to maintaining low N in both vineyard and winery. Soil N application
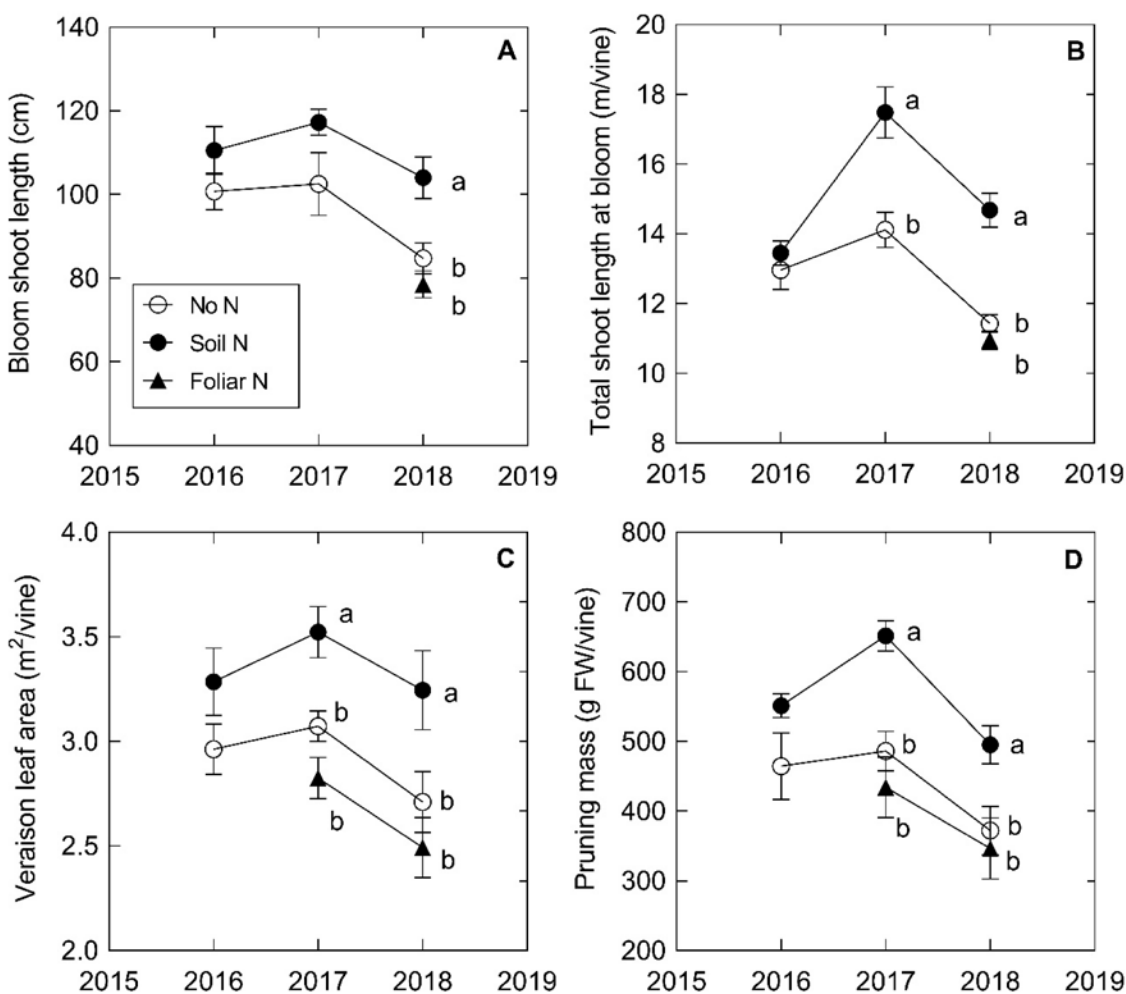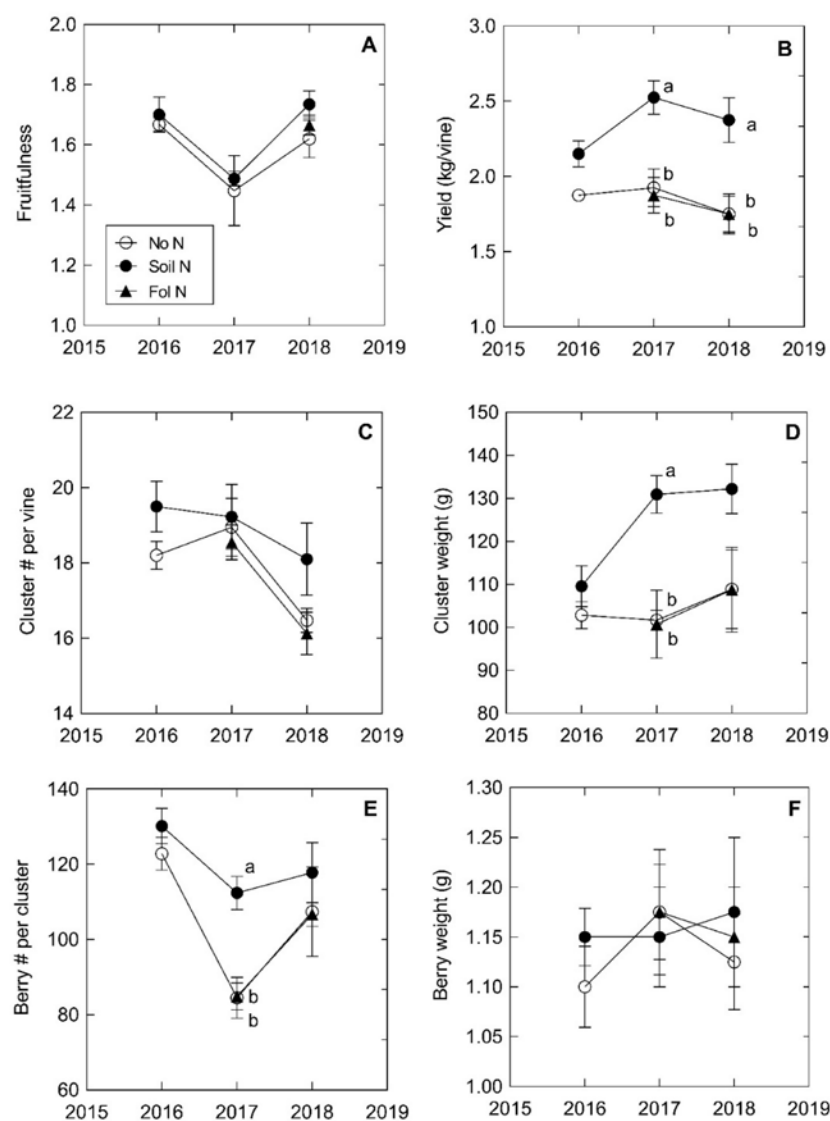

Figure 2 Effect of vineyard nitrogen (N) applications (Soil N and Foliar N) on reproductive parameters of Chardonnay grapevines from 2016 to 2018. Vines that received no N supply (No N) served as the control. Data points represent means and standard error for each treatment in each year (n = 4). Data were analyzed separately for each experimental year. Letters near each symbol designate significant groups based on t-test or Tukey's honest significant difference test at 95% confidence.

increased vine N status, improved vine productivity, and elevated fruit YAN concentrations, while foliar N did not affect vine N status or vine growth. Foliar N sprays also increased fruit YAN level, although to a smaller extent than soil N application in this vineyard. Winery N supplementation with DAP boosted must YAN to the same level as soil N fertilization in the vineyard, but only the soil N treatment consistently accelerated fermentation. As we expected, the N source affected the sensory properties of Chardonnay wines. Among all treatments, soil N application in the vineyard altered wine sensory characteristics to the greatest extent, with a consistent increase in tropical fruit aromas.

Soil and foliar N applications had varying influences on vine growth and fruit composition in the low N vineyard used in this study. The N concentrations in leaf blades at veraison in the No N vines were well below critical values proposed for Pinot noir grown in the region (Schreiner et al. 2018). Since Chardonnay vines are typically cropped at higher levels and often have larger canopies than Pinot noir, they should have a greater N demand and possibly a higher critical N level. Also, the level of YAN was as low as ~60 mg N/L in the No N musts in the last two years of the study, further confirming the low N status of the vineyard without N fertilization. That Soil N fertilization increased vine N status, vegetative growth, yield, and fruit YAN levels, but foliar N fertilization increased YAN without altering productivity agrees with prior studies (Bell and Robson 1999, Linsenmeier et al. 2008, Hannam et al. 2014, 2016, Moss 2016, Schreiner et al. 2018). However, that soil N increased fruit YAN more effectively than foliar N, despite also increasing yield, was not expected given past findings. More N must be applied to the soil to achieve a similar increase in fruit YAN. Foliar N sprays increased fruit YAN more than soil N applications when N was applied at the same rate (13.5 kg N/ha) in Merlot and Pinot gris at veraison (Hannam et al. 2016). Additionally, when N was applied at 30 to 40 kg N/ha to the foliage between bloom and veraison, it elevated fruit YAN to a similar or greater extent, as compared to N applied to the soil at 60 kg N/ha

near bloom in Sauvignon blanc and Petite Manseng grown in Virginia (D'Attilio 2014, Moss 2016). We suspect that the Foliar N treatment was less effective in this study, due to the low N status of the vines. Indeed, the N concentration of leaf petioles at bloom was ~6 g N/kg dry weight (DW) in No N vines, while it was between 8 and 9 g N/kg DW for unfertilized Sauvignon blanc and Petite Manseng vines studied in Virginia (D'Attilio 2014). Even though soil N application was more effective here in boosting must YAN levels than foliar N, promoting shoot growth could be a concern for vigorous sites. Thus, N application to the foliage offers a practical advantage over soil N application in vineyards with adequate canopy size, since it can improve must YAN to ensure a successful fermentation while avoiding excess vigor.

As expected, winery N supplementation boosted must YAN, but also altered must YAN composition, compared to vineyard N applications. The YAN concentration of the +DAP must was between 150 and 190 mg N/L, comparable to that of the Soil N must in all years (Table 3). However, the composition of must YAN differed between those two treatments, since DAP addition increased only NH_4^+-N while soil N fertilization boosted both NH_4^+-N and FAN-N. The concentration of must YAN in the +Org N treatment ranged from 110 to 160 mg N/L, matching the Soil N treatment in 2016 and the Foliar N treatment in the subsequent two seasons. Since the addition of organic N supplement (Nutriferm Aroma Plus) boosted only FAN-N, the proportion of FAN-N in must YAN was higher in this treatment than in the others. The time required to complete fermentation correlated negatively with must YAN concentration in each year (Figure 3). However, the average fermentation time remained unaffected by treatments in 2016. In the last two years, soil N application in the vineyard accelerated fermentation by 12 to 14 days, though the time required for fermentation did not improve as much with foliar N application or N supplementation in the winery (Table 3). Interestingly, even though YAN concentration was comparable in Soil N and +DAP musts, the time needed to finish fermentation was reduced by Soil N, but not

Table 2 Effect of vineyard nitrogen (N) applications (Soil N and Foliar N) on soil ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) concentrations in a Chardonnay vineyard in 2017 and 2018. Vines that received no N supply (No N) served as the control. Values represent means (standard deviation) for each treatment on each sampling date (n = 4).

	5 July 2017	14 Aug 2017	20 Oct 2017	11 July 2018	13 Aug 2018	21 Oct 2018
NH_4^+ -N[a]						
No N	4.5[b] (0.5)	4.5 (0.7)	3.5 b (0.3)	4.2 (0.4)	3.2 (0.5)	2.7 (0.5)
Soil N	6.0 (0.4)	7.3 (3.5)	5.8 a (2.1)	5.1 (2.3)	3.4 (0.7)	2.9 (0.4)
Foliar N	-	-	3.9 ab (0.4)	-	-	2.8 (0.2)
NO_3^- -N[a]						
No N	0.7 (0.4)	1.3 b (0.8)	1.1 b (0.5)	0.7 (0.3)	2 (1.3)	1.1 (0.8)
Soil N	2.3 (1.3)	12.6 a (6.8)	5.7 a (3.3)	3.0 (2.7)	4.5 (2.8)	2.2 (0.6)
Foliar N	-	-	1.7 ab (1.6)	-	-	1.9 (1.4)

[a]Concentrations of soil NH_4^+-N and NO_3^--N are expressed as mg N/kg dry soil.
[b]Data were analyzed separately at each sample date. Means followed by a different letter between treatments for a given variable differ significantly based on Mann-Whitney test or Dunn's test at 95% confidence.

by DAP addition, in 2017 and 2018. There are two possible explanations for this observation. First, soil N application may increase accumulation of other fruit nutrients that are essential for fermentation. Second, the shift in YAN composition (NH_4^+-N versus FAN-N) may lead to the difference in fermentation rate between the Soil N and +DAP treatments (Torrea et al. 2011).

While results from this study confirmed the importance of YAN concentration with regard to fermentation kinetics, sensory analysis of the wines also demonstrated the role that YAN concentration and composition plays in wine organoleptic properties. Among all treatments, soil N application in the vineyard had the most consistent effect on wine sensory attributes across years, by improving tropical and fruity aroma perception of Chardonnay wines. The increase in tropical notes was not associated with higher sugars in the Soil N treatment wines. Since the tropical aroma of white wines is associated with some grape-derived thiols, soil N fertilization may influence wine aroma by increasing the concentration of thiols in wine (Coetzee and du Toit 2012, Helwi et al. 2016). One explanation for our findings is that soil N application increased the accumulation of S-cysteine conjugates in the fruit, which are precursors of grape-derived thiols. Indeed, Choné et al. (2006) reported an increase of S-cysteine conjugates in Sauvignon blanc berries after soil N application at 60 kg N/ha in a low N vineyard. In this study, we did not examine the concentration of thiol precursors, but a higher S concentration occurred in the Soil N musts, indicating that

soil N fertilization increased accumulation of S-related compounds in musts. Higher N supply to soil boosted S concentrations in leaves and petioles of Pinot noir (Schreiner et al. 2018). Moreover, the increased NH_4^+-N in the Soil N musts may also play a role in promoting production of grape-derived thiols during fermentation (Garde-Cerdán and Ancín-Azpilicueta 2008). It is unlikely that the increased shading of fruit clusters that we observed in the Soil N vines contributed to greater tropical aromas in the resulting wines, since shading typically reduces fruity aromas in wine (Marais et al. 1999) and appears to have little influence on wine thiol concentrations (Martin et al. 2016).

The No N wines were characterized by peach and stone fruit aromas in 2016 and 2018, but not in 2017. Peach and stone fruit aromas are associated with branched-chain esters and terpenes in white wines (Siebert et al. 2018). It is possible that maintaining low N status in the vineyard and winery favored formation of those compounds in two of the three years. Indeed, previous work in Pinot noir found that decreasing vine N status led to the increase of branched-chain esters in finished wines, although vine N status had an inconsistent effect on terpenes (Yuan et al. 2018b). Another possibility for the lack of peach aroma in 2017 was that the no N wines exhibited some oxidized aromas, as noted by the sensory panelists for both replicates in 2017 and one replicate in 2018. Oxidation can alter the compounds thought to cause peach aromas, which may explain the lack of peach particularly in the 2017 wines (Espinase Nandorfy et al. 2021).

Table 3 Effect of vineyard nitrogen (N) applications (Soil N and Foliar N) and winery N additions (+DAP and +Org N) on must chemistry and fermentation kinetics in Chardonnay between 2016 and 2018. Musts that received no N inputs (no N) in the vineyard or winery served as the control. Values represent means (standard deviation) for each treatment in each year (n = 4). TSS, total soluble solids; YAN, yeast assimilable nitrogen; NH_4^+-N, ammonium-N; FAN-N, free amino acid-N.

	TSS (Brix)	pH	TA[a] (g/L)	Must NH_4^+-N (mg/L)	Must FAN-N (mg/L)	Must YAN (mg/L)	Days to complete fermentation
2016							
No N	22.1 b[b] (0.14)	3.14 (0.03)	7.0 (0.5)	23 b[c] (6.6)	79 b (9.0)	99 b (9.3)	18 (1.5)
Soil N	22.6 a (0.13)	3.21 (0.02)	7.8 (1.0)	40 ab (2.4)	149 a (10.0)	189 a (10.3)	14.7 (1.1)
+DAP	22.2 b (0.17)	3.19 (0.06)	7.0 (0.5)	104 a (6.9)	87 b (15.1)	191 a (21.6)	15.2 (2.8)
+Org N	22.4 ab (0.13)	3.19 (0.05)	7.1 (0.5)	26 b (2.7)	135 a (11.7)	162 a (14.2)	15.3 (1.5)
2017							
No N	21.4 (0.57)	3.31 b (0.08)	5.5 (0.5)	15 c[d] (4.0)	43 c[d] (7.7)	59 c[d] (4.2)	30.9 a (4.0)
Soil N	21.6 (0.54)	3.50 a (0.06)	5.8 (0.4)	31 b (1.1)	148 a (20.6)	179 a (21.4)	17.1 b (3.1)
Foliar N	22.7 (0.45)	3.37 b (0.04)	5.7 (0.4)	23 bc (3.4)	98 b (3.4)	121 b (3.7)	23.2 ab (2.8)
+DAP	21.8 (0.74)	3.41 ab (0.03)	6.1 (0.6)	121 a (9.7)	44 c (5.6)	166 a (10.1)	21.4 ab (2.5)
+Org N	21.7 (0.84)	3.29 b (0.04)	6.3 (0.3)	9 d (2.4)	113 b (7.5)	123 b (7.1)	24.0 ab (4.8)
2018							
No N	21.9 (0.86)	3.25 (0.03)	7.4 (0.9)	11 c (6.3)	53 b (13.0)	64 c (18.7)	30.2 a (7.1)
Soil N	22.2 (0.74)	3.26 (0.03)	7.8 (0.6)	40 b (7.3)	106 a (15.2)	147 a (11.3)	18.7 b (3.3)
Foliar N	22.6 (0.58)	3.32 (0.04)	6.6 (1.0)	20 bc (5.8)	83 ab (18.1)	103 bc (20.2)	25.0 ab (4.2)
+DAP	21.7 (0.79)	3.32 (0.05)	7.8 (1.2)	93 a (7.9)	56 b (12.8)	150 a (23.4)	21.1 ab (3.4)
+Org N	21.6 (0.95)	3.28 (0.02)	7.3 (0.7)	14 c (5.3)	97 a (11.56)	113 ab (17.8)	20.3 ab (2.8)

[a]Titratable acids (TA) are expressed as tartaric acid equivalents.
[b]Data were analyzed separately for each experimental year. Means followed by a different letter within each year differ significantly based on Tukey's honest significant difference test at 95% confidence.
[c]Data for must NH_4^+-N were analyzed using Kruskal-Wallis test in 2016 and Dunn's test was applied to separate means at 95% confidence.
[d]Data for must NH_4^+-N, must FAN-N, and must YAN were log-transformed prior to analysis in 2017.

While aromatic differences were noticed between treatments, it was expected that the differences between some treatments, such as +DAP and +Org N, would be greater than was observed. This was interesting as these forms of N, NH_4^+-N, or FAN-N have previously been shown to alter aroma composition in different ways (Styger et al. 2011, Torrea et al. 2011, Wang et al. 2016). While we thought that these wines would be very different simply due to the different types of N added in the winery, those differences were not large enough to produce aromatically unique wines. Clearly, the type of N added in the winery (DAP or Organic N) had less impact on wine aroma sensory characteristics than vineyard application of N.

Unlike aroma, the influence of N on wine mouthfeel parameters is not well understood, with previous research focusing on how N application affected formation of phenolics in grapes (Portu et al. 2015, Gutiérrez-Gamboa et al. 2017). The majority of descriptors for the different wine groups identified by CA in this study were linked to acidity and sugar. Interestingly, the wines associated with tart, sour, and acidity in each year did not correlate to either the juice or final wine with the highest TA or lowest pH (Table 3, wine data not shown). This suggests that N concentration and composition is likely influencing other components in wine that alter the acid balance. Additionally, the wines that were grouped together changed from year to year, suggesting that

Figure 3 Relationship between must yeast assimilable nitrogen (YAN) concentration prior to fermentation and number of days to complete fermentation in Chardonnay musts that received vineyard N applications (Soil N or Foliar N) or winery N additions (+DAP or +Org N) between 2016 and 2018. Musts that received no N input in the vineyard or winery (No N) served as the control. Data points represent raw data and the linear coefficient of determination (R^2) was based on all treatments in each year (n = 16 or 20).

Figure 4 Correspondence analysis of ultra-flash profiling (UFP) data for aroma of Chardonnay wines that received vineyard N applications (Soil N or Foliar N), winery N supplementations (+DAP or +Org N), or no N inputs (No N) between 2016 and 2018. Ellipses indicate groupings calculated using k-means clustering. Two samples of each wine were evaluated in each session.

N does not directly influence mouthfeel components, but may do so indirectly. We recognize that prior training and consensus for mouthfeel terms could have improved the consistency of results across years. The inconsistency could also be due to the difference in panelists between years. However, panelist performance was analyzed using all the raw data (not shown) and the 20% difference in panelists each year did not appear to account for these yearly inconsistencies. Additional studies investigating mouthfeel are warranted.

In addition to the influence of N on must composition and wine style, the environmental impact of vineyard N use is also important. N fertilizer applied at an excessive level can significantly increase nitrate in the soil, impose a greater risk of nitrate contamination in groundwater and waterways, and adversely influence the health of humans and wildlife (Schaller 1991, Barlow and Kröger 2014). The level of nitrate in soil in late October, prior to winter rains in this region, should indicate the potential for nitrate leaching to groundwater. The concentration of soil nitrate in the root zone (from 0 to 45 cm deep) at this time was <6 mg N/kg in 2017, even when N was applied at 67 kg N/ha/year. Such a low level of soil nitrate in the fall poses little risk of nitrate leaching (Fraser et al. 2013). This finding is consistent with other vineyard studies, where soil N applied at 50 to 60 kg N/ha had a small influence on the level of nitrate in the root zone (Conradie 2001a, Linsenmeier et al. 2008).

Overall, this study shows that viticulturists and winemakers may alter wine style of Chardonnay by how and where they manage N inputs in the vineyard or winery. A diversity of wine styles is important to attract new wine consumers, such as millennials, largely because they seek a sense of discovery in wine-drinking experiences (McMillan 2020). In this study, soil N application in the vineyard decreased fermentation times and consistently increased the tropical fruit aroma of wines, while foliar N applications and winery N additions did not have a consistent influence on fermentation and wine characteristics. The smaller impact of Foliar N and +Org N treatments on fermentation and wines could be attributed in part to the fact that those two treatments did not boost must YAN as high as the Soil N or +DAP treatments. Even though the +DAP treatment had the same level of must YAN as the Soil N treatment, it did not impact wines in the same manner as Soil N, highlighting the influence of N source on fermentation and wine sensory properties. It is important to note that we did not boost must YAN level (<200 mg N/L) as high as many previous studies (300 to 500 mg N/L; Bisson and Butzke 2000, Linsenmeier et al. 2008, Torrea et al. 2011). In fact, the must YAN levels obtained here are closer to the targets used by the industry for commercial production. Although sufficient YAN is critical for a successful fermentation, YAN at excessive levels can result in high residual N in wines after fermentation, increasing the risk of spoilage during aging (Bell and Henschke 2005), and may lead to elevated production of undesirable volatile sulfur compounds (Ugliano et al. 2009).

Conclusions

The source of N that contributes to YAN determines vine productivity and alters Chardonnay wine sensory characteristics. Winery N additions do not substitute for YAN obtained in the vineyard, as sensory differences were noted between vineyard and winery N-supplemented wines. In particular, soil N application consistently boosted tropical aromas, a trait that may or may not be desirable in a Chardonnay wine, depending on the desired wine style. However, using soil N to boost productivity and make a more tropical wine style may be economically beneficial for growers (yield) and winemakers (volume), since fruity wines are more attractive to younger wine consumers. In the vineyard, foliar N was not a replacement for soil N, since foliar N was not as effective as soil N in boosting juice YAN levels, even though vine N status was very low in this vineyard. Even so, foliar N is a good tool to increase YAN at vigorous sites, since it does not stimulate vine growth.

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