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Research Article

# 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 as compared to winery N addition on enological characters was evaluated in Chardonnay between 2016 and 2018. Five treatments, including no

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vineyard or winery N addition (No N), addition of diammonium phosphate in the winery (+DAP), addition of organic N in the winery (+Org N), addition of N in the vineyard to the soil (Soil N), or to the foliage (Foliar N) were evaluated. The Foliar N treatment was evaluated in 2017 and 2018, 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 year 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 the level of 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 the Soil N musts than No N, with the Foliar N, +DAP, and +Org N treatments intermediate between Soil N and No N treatments. Wine sensory analysis revealed that the 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, they may not produce a wine with similar sensory characteristics as obtained using vineyard N fertilization in Chardonnay.

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

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# Introduction

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 it is often the most important nutrient to manage (Bell and Robson 1999, Conradie 2001a, Linsenmeier et al. 2008, Schreiner et al. 2018). In the winery, N in the must, 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, a number of 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, Schreiner et al. 2018, Tahim et al. 2019). However, no published research to date has compared vineyard N fertilization to winery N addition on must and wine composition in a systematic manner.

An important goal for N management in the vineyard and winery is to ensure there is sufficient YAN present in the must for a successful fermentation. The minimum YAN required for successful fermentation is between 120 to 140 mg N/L (Bell and Henschke 2005), although others proposed higher targets for must YAN (200 to 250 mg N/L) in order to reduce the risk of stuck or sluggish fermentation (Bisson and Butzke 2000, Mendes-Ferreira et al. 2004). Many studies have shown that grape musts with low levels of YAN (< 100 mg N/L) completed fermentation, although the fermentation rate was reduced (Spayd et al. 1994, Ugliano et al. 2009, Stockert et al. 2013, Schreiner et al. 2018). Some studies have shown that reducing N status in the vineyard and the resulting must improves berry and wine composition, particularly in red

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varieties (Bell and Robson 1999, Treeby et al. 2000, Schreiner et al. 2014, Yuan et al. 2018a, 2018b). Low N musts have been found to 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, Ugliano et al. 2010, Torrea et al. 2011). As a result, it is unclear if 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, Ugliano et al. 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. Nitrogen applied to the soil consistently results in a significant increase in vine N status and fruit N status across various varieties and growing regions (Bell and Robson 1999, Conradie 2001a, Conradie 2001b, Linsenmeier 2008, Schreiner et al. 2013, Schreiner et al. 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 2008, Schreiner et al. 2013, Schreiner et al. 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. A number of studies showed that 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

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applications since lower quantities of N are applied directly to the foliage (Lasa et al. 2012, Hannam et al. 2016).

As 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 routinely added to boost the level of $NH_4^+$-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 the addition of DAP can increase fermentation rate, elevating ammonium concentration to an excessive level may increase the 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 et al. 2019). Organic N supplements increase must YAN through increasing primary amino acids, rather than ammonium. Since amino acids are precursors of some fermentation-derived bouquets (e.g. branched-chained and acetate esters), the addition of amino-N might 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 impact mouthfeel of

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123 white wines remains unclear. Nitrogen application in the vineyard alters phenolic composition in
124 berries and wines in red varieties (Hilbert et al. 2003, Schreiner et al. 2014, Yuan et al. 2018b),
125 but for white wines it is unlikely that the change in phenolics would be large enough to alter
126 mouthfeel perception. As with the wine aroma studies, research investigating changes to
127 phenolic composition have not included a sensory component.

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

137 ## Materials and Methods

138 The effects of vineyard N fertilization on vine productivity and wine sensory properties
139 and a comparison of vineyard N use to winery N supplementation on must composition,
140 fermentation kinetics, and sensory properties in Chardonnay were investigated. The overall
141 experiment included five treatments, including two vineyard N additions, two winery N additions
142 and a control that did not receive N in either location (No N). Nitrogen fertilizer was applied to
143 the soil as urea-ammonium nitrate (Soil N) or to the foliage as urea (Foliar N) in the vineyard.
144 Winery N additions included either an inorganic source of N (+DAP) or an organic N

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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, 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 (45.1157° N, 123.2073° 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 management, as well as weed, pest, and disease management, was consistent with standard practices used in the region in commercial vineyards. 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 the 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 is drip-irrigated and irrigation was applied between fruit set and harvest as per the cooperators' standard practice, based on visual assessments of shoots 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 each randomly assigned to three entire rows of vines with a

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167 border row in between these two treatments. A minimum of 128 vines occurred in the middle
168 row of each replicate plot where data were collected. In 2016 and 2017, vines in the Soil N
169 treatment were fertilized three times each year; ~ one month before bloom, ~ one month after
170 fruit set, and at veraison (Supplemental Table 1). In 2018, Soil N vines were fertilized twice (one
171 month before bloom and one month after fruit set), to avoid boosting vine N status to an
172 excessive level. At each application, urea ammonium nitrate solution (UAN-32, Oregon
173 Vineyard Supply, McMinnville, OR) was diluted with water and then applied at the rate of 17.8
174 kg/ha to the Soil N vines through the drip irrigation system. In total, 67.2 kg N/ha (~ 11.8 g
175 N/vine) was applied to Soil N vines in 2016 and 2017 and 44.8 kg N/ha (~ 7.8 g N/vine) was
176 applied in 2018.

177 The Foliar N treatment was applied to 25 continuous vines, at a random location within
178 the middle row of the No N treatment in each replicate. Since N was applied only to the canopy
179 and would not interfere with the growth of vines in adjacent rows, no buffer rows were used for
180 the Foliar N treatment. Foliar N was applied three times each year in 2017 and 2018; ~ one
181 month after fruit set, at two weeks before veraison (lag phase), and two weeks post-veraison
182 (Supplemental Table 1). At each application, 4.7 L of urea (ACS certified, Fisher Scientific Inc.,
183 Fair Lawn, NJ) solution was applied to the canopy using a backpack sprayer, ensuring even
184 coverage for east and west aspects of the canopy and the adaxial and abaxial sides of leaves. The
185 concentration of urea was 0.85% in 2017, but reduced to 0.72% in 2018, as some minor marginal
186 leaf-burn occurred in the lower canopy in 2017. To avoid drift, foliar N sprays were applied in
187 the morning (before 10 AM, PDT) on days with little or no wind. In total, 25 kg/ha (~ 4.3 g
188 N/vine) was applied in 2017 and 22 kg N/acre (~ 3.9 g N/vine) was applied in 2018. More N was

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189 applied in the Soil N treatment compared to the Foliar N treatment, in an effort to achieve a
190 similar level of must YAN at harvest (D'Attilio 2014, Hannam et al. 2016, Moss 2016). The
191 same vines in each plot were treated with soil N (entire rows via fertigation) or foliar N (25 vines
192 in a single row) each year.

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

205 **Soil nitrogen analysis.** Soil samples were collected using a soil auger (2.2 cm in
206 diameter) from the weed-free, vine row three times in 2017 and 2018, at ~ one month after fruit
207 set, ~ two weeks before veraison, and ~ one month post harvest. Five cores were collected from
208 each plot 15 to 20 cm offset from drip irrigation emitters to a depth of 45 cm and pooled.
209 Available nitrate and ammonium was determined in air-dried, pulverized soils using standard
210 methods for western Oregon soil (Miller et al. 2013). Briefly, soil was extracted with 2 M KCl,

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filtered on 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, and 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. 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 between 1400 and 1700 hr on cloudless days periodically between fruit set to harvest (see Tian and Schreiner 2021), using a pressure chamber (model 610, PMS instrument company, Albany, OR) on two leaves per plot retrieved 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 levels, 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

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ceptometer (AccuPAR model LP-80, Decagon Devices, Pullman, WA). Measurements were performed at 0900 hr, 1100 hr, 1300 hr, 1500 hr, and 1700 hr in 2016, and at 1000 hr, 1200 hr, 1400 hr, and 1600 hr in 2017 and 2018. The level of cluster exposure was expressed as the percentage of radiation recorded in the fruit zone as 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 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 (P, K, Ca, Cu ,Mg, S, Fe, Mn, B, Zn, and Na) in the juice was determined by inductively coupled plasma-optical emission spectrometry (ICP-OES; Perkin Elmer Optima 3000DV) after microwave digestion in $HNO_3$ (Jones and Case 1990).

**Weather data.** Weather data between bud break and harvest for each experimental year, including daily maximum temperature, daily minimum temperature, daily average temperature, daily solar radiation, and daily precipitation were obtained 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 approximately 37 km

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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 and pressed for 5 minutes at 0.15 mPa using a bladder press the next day. 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 hr at 4 °C. Twelve liters of 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, Enartis, CA) were added to the juice according to the treatment. The concentration of ammonium-N and FAN-N provided by DAP and NutriFerm Arom Plus had previously been determined by making additions of DAP or NutriFerm Arom Plus to white grape juice (Santa Cruz Organic) and measuring ammonia-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 with 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 total soluble solid (TSS) and pH of juice were determined

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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 the 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 hr, wines were racked and bentonite was added at 0.12 g/L to clarify wines and racked again after an additional 48 hr. Preliminary sensory evaluation of the replicates by the investigators revealed an absence of winemaking faults or discernible differences between the 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 1µm 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 screw-capped (Stelvin™, Amcor, Zurich) bottles 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 6 months of bottle aging Sensory analysis was approved by the Institutional Review Board at Oregon State University (#8781). To be included in the study panelists had to be over 21 years of age, 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

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in the wine industry, specifically with white wines for a minimum of 5 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 x 71 cm center, 61 cm x 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, USA) were used to maintain air quality in the space. A total of 17 wine experts (10 male, 7 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 Ultra-flash profiling (UFP) were conducted as described by Perrin and Pagès (2009), and 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.

Napping® test was used to let participants group wines based on the level of similarity in wine 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 the usage of some aroma related terms for mouthfeel (Sereni et al. 2016). During each evaluation, panelists were instructed to smell/taste the eight or ten wines from left to right and mark the placement of wines on the provided paper (18 x 14

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inches, Strathmore Drawing Paper Pad). Wines that were similar were to be placed closer together and wines that are 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 HSD 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 the *P* value was lower than 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

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between must YAN and fermentation time differed significantly between 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 of the original paper 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 ultra-flash profiling 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). The terms that were used less than 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 co-ordinates, 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 between years (Supplemental Table 2). Bud break occurred 10 days earlier in 2016 as compared to 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

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365 veraison in 2017 and 2018. The major phenological stages occurred at similar times in 2017 and
366 2018, although bloom and veraison were delayed by ~ 5 days in 2017. The 2018 season was the
367 driest among the three years.

368 **Vine N status and leaf greenness.** Soil N application improved vine N status in all
369 years, while N applied to foliage had only a minor influence on vine N status (Table 1). Across
370 three years, bloom leaf blade and petiole N was 10 to 30% higher in Soil N vines than the No N
371 vines, even in the first year when the Soil N vines had received only one N application (~ 3.9
372 g/vine) prior to sampling. The difference in leaf blade and petiole N between the Soil N and the
373 No N vines became greater at veraison as compared to bloom. The concentration of veraison leaf
374 blade and petiole N was 20 to 35% higher in the Soil N vines than the control. Foliar N generally
375 had no effect on leaf blade and petiole N at bloom and veraison, except that veraison leaf blade
376 N increased slightly in the Foliar N vines in 2018.

377 Leaf greenness increased with soil N application by mid-summer, but was not affected by
378 foliar N application in any year (Supplemental Table 3). The SPAD values of opposite cluster
379 leaves did not differ between the No N and Soil N vines from mid-May to the end of July, except
380 in one case in 2018. Values of SPAD were higher in the Soil N vines beginning in August of
381 each year, and differences in SPAD of opposite cluster leaves grew larger between the Soil N
382 and No N vines as each growing season progressed. A similar pattern was observed in the upper
383 canopy leaves. On the other hand, Foliar N had no impact on leaf greenness. The SPAD values
384 of opposite cluster leaves and upper canopy leaves never differed between the Foliar N vines and
385 the No N vines.

386 **Vine vegetative growth.** Soil N application stimulated canopy growth beginning in the

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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. As compared to the No N vines, those parameters of vegetative growth were 15 to 33% greater in the Soil N vines in 2017 and 2018.

Soil N application had only a minor influence on rates of single-leaf photosynthesis, increasing it on one of five days when gas exchange was measured (Supplemental Table 4). Leaf water potential 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 leaf water potential was slightly lower in the Soil N vines than the No N vines in 2016 and 2018. Leaf water potential was measured in the Foliar N vines on three days in 2018, and it did not differ from the No N vines on those days (data not shown). Soil N vines had lower 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 a higher number of berries per cluster. In 2018, although none of the yield components were affected by treatment, yield was 36% higher in the Soil N vines than the No N vines. Fruitfulness, number of clusters per vine, and the average berry weight did not vary between treatments in any year.

**Soil inorganic N.** Soil ammonium and nitrate levels were more responsive to soil N application in 2017 than 2018, most likely because the Soil N vines received an additional dose

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of N (22.4 kg N/ha) during the 2017 season (Table 2). The concentration of $NH_4^+$-N and $NO_3^-$-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 of 2017. Foliar N applications did not have an impact on 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 greater 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 a few other mineral nutrients besides N in the must over three years, but foliar N sprays had no impact on must nutrients in 2017 and 2018 (Supplemental Table 7). The concentration of calcium and magnesium was greater in the Soil N musts than the No N musts in 2016, but the opposite pattern was observed in 2018. The level of sulfur in musts increased with soil N addition in 2016 and 2017, but not in 2018. Must phosphorus 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 between 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 an increase 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 higher in the Foliar N treatment than the control in 2017 but not in 2018, though must $NH_4^+$-N was unaffected by foliar N application in

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either year. The addition of DAP at the winery boosted must YAN to the 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, although it made up only 7 to 30% of must YAN in other treatments. The 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 it increased must FAN-N by 83 to 160% as 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 with Foliar N treatment in 2017.

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 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 the Soil N, +DAP, and +Org N treatments. In 2017, fermentation finished 14 days sooner in the Soil N treatment than 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 was well correlated 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). Multiple factor analysis incorporating the spatial Napping® data showed that the first two factors

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(F1 and F2) had 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 were clustered into three groups. Across all three years the +Org N sensory replicates were always in the same group. For the other treatments the sensory replicates showed some similarity, but were not always in the same cluster group.

Correspondence analysis that utilized 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 in each group and the 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 related to apple aroma and some negative descriptors, such as oxidized, closed, and tired, in 2017. The Soil N wines were consistently grouped with tropical and fruity notes across all years of the study. Besides 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 the UFP analysis. In 2016, the +DAP and +Org N replicates were 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

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+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.** Multi factor analysis 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 were 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 a 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 greater amounts of the total variance (45.6 to 73.6%) than MFA for mouthfeel. 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 this was not an issue for the 2018 wines due to the usage of a nose clip. Across the three years, the different N treatments did not result in consistent associations with specific descriptors. It should be noted that the No N wines from 2017 were clearly grouped with terms linked to the 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

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497 terms and the axes of the CA could be seen.

498 ## Discussion

499 The purpose of this study was to understand whether wine compostion of Chardonnay
500 could be improved by applying N in the vineyard, by supplementing N in the winery, or by
501 maintaining low N status in both vines and musts. Thus, we examined how vineyard N
502 applications and winery N additions affected must YAN levels, fermentation kinetics, and wine
503 sensory properties in comparison to maintaining low N in both the vineyard and winery. Our
504 results show that soil N application increases vine N status, improves vine productivity, and
505 elevates fruit YAN concentrations, while foliar N did not affect vine N status or vine growth.
506 Foliar N sprays also increased fruit YAN level, although to a smaller extent as compared to soil
507 N application in this vineyard. Winery N supplementation with DAP boosted must YAN to the
508 same level as soil N fertilization in the vineyard, but only the soil N treatment consistently
509 accelerated fermentation. As we expected, the sources of N affected the sensory properties of the
510 Chardonnay wines. Among all treatments, soil N application in the vineyard altered wine
511 sensory characteristics to the greatest extent with a consistent increase of tropical fruit aromas in
512 the Soil N wines.

513 Soil and foliar N applications had varying influences on vine growth and fruit
514 composition in the low N vineyard used in this study. The N concentrations in leaf blades at
515 veraison in the No N vines were well below critical values proposed for Pinot noir grown in the
516 region (Schreiner et al. 2018). Since Chardonnay vines are typically cropped at higher levels and
517 often have larger canopies than Pinot noir, they should have a greater N demand and possibly a
518 higher critical N level. Also, the level of YAN was as low as about 60 mg N/L in the No N musts

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519 in the last two years of the study, further confirming the low N status of the vineyard without N
520 fertilization. That Soil N fertilization increased vine N status, vegetative growth, and yield along
521 with fruit YAN levels, but foliar N fertilization increased YAN without altering productivity
522 agrees with a number of prior studies (Bell and Robson 1999, Linsenmeier et al. 2008, Hannam
523 et al. 2014, 2016, Moss 2016, Schreiner et al. 2018). However, soil N having increased fruit
524 YAN more effectively than foliar N despite also increasing yield was not expected given past
525 findings. More N needs to be applied to the soil to achieve a similar increase in fruit YAN. Foliar
526 N sprays increased fruit YAN more than soil N applications when N was applied at the same rate
527 (13.5 kg N/ha) in Merlot and Pinot gris at veraison (Hannam et al. 2016). Additionally, when N
528 was applied at 30 to 40 kg N/ha to the foliage between bloom and veraison, it elevated fruit YAN
529 to the similar or greater extent, as compared to N applied to the soil at 60 kg N/ha near bloom in
530 Sauvignon Blanc and Petite Manseng grown in Virginia (D'Attilio 2014, Moss 2016). We
531 suspect that the Foliar N treatment was less effective in this study, due to the low N status of the
532 vines. Indeed, the N concentration of leaf petioles at bloom was around 6 g N/kg DW here for
533 the No N vines, while it was between 8 and 9 g N/kg DW for unfertilized Sauvignon Blanc and
534 Petite Manseng vines studied in Virginia (D'Attilio 2014). Even though soil N application was
535 more effective here in boosting must YAN levels than foliar N, promoting shoot growth could be
536 a concern for vigorous sites. In this regard, N application to the foliage conveys a practical
537 advantage over soil N application in vineyards with adequate canopy size, since it can improve
538 must YAN to ensure a successful fermentation while avoiding excess vigor.

539 As expected, winery N supplementation boosted must YAN but also altered must YAN
540 composition as compared to vineyard N applications. The YAN concentration of the +DAP must

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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 with the Soil N treatment in 2016 and with the Foliar N treatment in the subsequent two seasons. Since the addition of organic N supplement (Nutriferm Aroma Plus) boosted solely FAN-N, the proportion of FAN-N in must YAN was higher in this treatment than others. The time required to complete fermentation was negatively correlated 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 the Soil N and +DAP musts, the time needed to finish fermentation was reduced by Soil N but not by DAP addition in 2017 and 2018. There are two possible explanations for this observation. First, soil N application might increase the accumulation of other nutrients in the fruit that are essential for fermentation. Second, the shift of YAN composition ($NH_4^+$-N vs FAN-N) might 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 regards to fermentation kinetics, sensory analysis of the wines also demonstrated the role that YAN concentration and composition plays to affect wine organoleptic properties. Among all treatments, soil N application in the vineyard had the most consistent effect on wine sensory

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563 attributes across years, by improving tropical and fruity aroma perception of Chardonnay wines.
564 The increase in tropical notes was not associated with higher sugars in the Soil N treatment
565 wines. Since the tropical aroma of white wines is associated with some grape-derived thiols, soil
566 N fertilization may influence wine aroma by increasing the concentration of thiols in wine
567 (Coetzee and du Toit 2012, Helwi et al. 2016). One explanation for our findings is that soil N
568 application increased the accumulation of S-cysteine conjugates in the fruit, which are the
569 precursors of grape-derived thiols. Indeed, Choné et al. (2006) reported an increase of S-cysteine
570 conjugates in Sauvignon Blanc berries after soil N application at 60 kg N/ha in a low N vineyard.
571 In this study, we did not examine the concentration of thiol precursors, but a higher sulfur
572 concentration occurred in the Soil N musts, indicating that soil N fertilization increased the
573 accumulation of sulfur related compounds in musts. Higher N supply to soil was shown to boost
574 S concentrations in leaves and petioles of Pinot noir previously (Schreiner et al. 2018).
575 Moreover, the increase of ammonium-N in the Soil N musts may also play a role in promoting
576 the production of grape-derived thiols during fermentation (Garde-Cerdán and Ancín-Azpilicueta
577 2008). It is unlikely that the greater shading of fruit clusters that we observed in the Soil N vines
578 contributed to greater tropical aromas in the resulting wines, since shading typically reduces
579 fruity aromas in wine (Marais et al. 1999) and appears to have little influence on thiol
580 concentrations in wine (Martin et al. 2016).

581 The No N wines were characterized by peach and stone fruit aromas in 2016 and 2018,
582 but not in 2017. Peach and stone fruit aromas are associated with branched-chain esters and
583 terpenes in white wines (Siebert et al. 2018). It is possible that maintaining a low N status in the
584 vineyard and winery favored the formation of those compounds in two of the three years. Indeed,

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585 previous work in Pinot noir found the decrease of vine N status led to the increase of branched-
586 chain ester in finished wines, although vine N status had an inconsistent effect on terpenes (Yuan
587 et al. 2018b). Another possibility for the lack of peach aroma in the 2017 was that the no N
588 wines exhibited some oxidized aromas, as noted by the sensory panelists for both replicates in
589 2017 and one replicate in 2018. Oxidation is known to alter the compounds thought to cause
590 peach aromas, which may explain the lack of peach particularly in the 2017 wines (Espinase
591 Nandorfy et al. 2021).

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

601 Unlike aroma, the influence of nitrogen on wine mouthfeel parameters is not well
602 understood with previous research focusing on how nitrogen application impacted the formation
603 of phenolics in grapes (Portu et al. 2015, Gutiérrez-Gamboa et al. 2017). The majority of
604 descriptors for the different wine groups identified by CA in this study were linked to acidity and
605 sugar. Interestingly, wines that were associated with tart, sour and acidity for each year did not
606 correlate to either the juice or final wine with the highest TA or lowest pH (Table 3, wine data

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not shown). This suggests that nitrogen 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 nitrogen is not directly influencing mouthfeel components but may be indirectly doing so. We recognize that prior training and consensus for mouthfeel terms could have improved the consistency of results across years. It is also possible that 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 warrants further investigation.

In addition to the influence of N on must composition and wine style, the environmental impact of vineyard N use is also important. Nitrogen 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 depth) at this time was below 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, results of this study show that viticulturists and wine makers may alter wine style of Chardonnay by how and where they manage N inputs in the vineyard and winery. A

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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 improved 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 and +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 in 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

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651 that may or may not be desirable in a Chardonnay wine depending on the desired wine style.
652 However, using soil N to boost productivity and make a more tropical style of wine may be
653 economically beneficial for growers (yield) and winemakers (volume), since fruity wines are
654 more attractive to younger wine consumers. In the vineyard, foliar N was not a replacement for
655 soil N, since foliar N was not as effective as soil N in boosting juice YAN levels, though vine N
656 status was very low in this vineyard. Even so, foliar N is a good tool to increase YAN at
657 vigorous sites, since it does not stimulate vine growth.

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811 **Table 1.** Effect of vineyard N applications (Soil N and Foliar N) on leaf blade and petiole N
812 concentrations in Chardonnay grapevines from 2016 to 2018. Vines that received no N supply (No
813 N) served as the control. Values represent means (standard deviation) for each treatment at each
814 phenological stage (n = 4).
815

| | 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 HSD at 95% confidence. DW, dry weight.
816

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817 **Table 2.** Effect of vineyard N applications (Soil N and Foliar N) on soil ammonium-N and
818 nitrate-N concentrations in a Chardonnay vineyard in 2017 and 2018. Vines that received no N
819 supply (No N) served as the control. Values represent means (standard deviation) for each
820 treatment on each sampling date (n = 4).
821

| | 5-Jul-17 | 14-Aug-17 | 20-Oct-17 | 11-Jul-18 | 13-Aug-18 | 21-Oct-18 |
|---|---|---|---|---|---|---|
| **$NH_4^+$ - 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 $NH4^+$-N and $NO3^-$-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.
822

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823 **Table 3.** Effect of vineyard N applications (Soil N and Foliar N) and winery N additions (+DAP and
824 +Org N) on must chemistry and fermentation kinetics in Chardonnay between 2016 and 2018. Musts that
825 received no N inputs (no N) in the vineyard or winery served as the control. Values represent means
826 (standard deviation) for each treatment in each year (n = 4).

| | TTS [a] (°Brix) | pH | TA [b] (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 [c] (0.14) | 3.14 (0.03) | 7.0 (0.5) | 23 b [d] (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 [e] (4.0) | 43 c [e] (7.7) | 59 c [e] (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] TTS, total soluble solids.
[b] Titratable acids (TA) are expressed as tartaric acid equivalents.
[c] Data were analyzed separately for each experimental year. Means followed by a different letter within each year differ significantly based on Tukey's HSD at 95% confidence.
[d] Data for must NH4-N were analyzed using Kruskal-Wallis test in 2016 and Dunn's test was applied to separate means at 95% confidence.
[e] Data for must NH4-N, must FAN-N, and must YAN were log-transformed prior to analysis in 2017.

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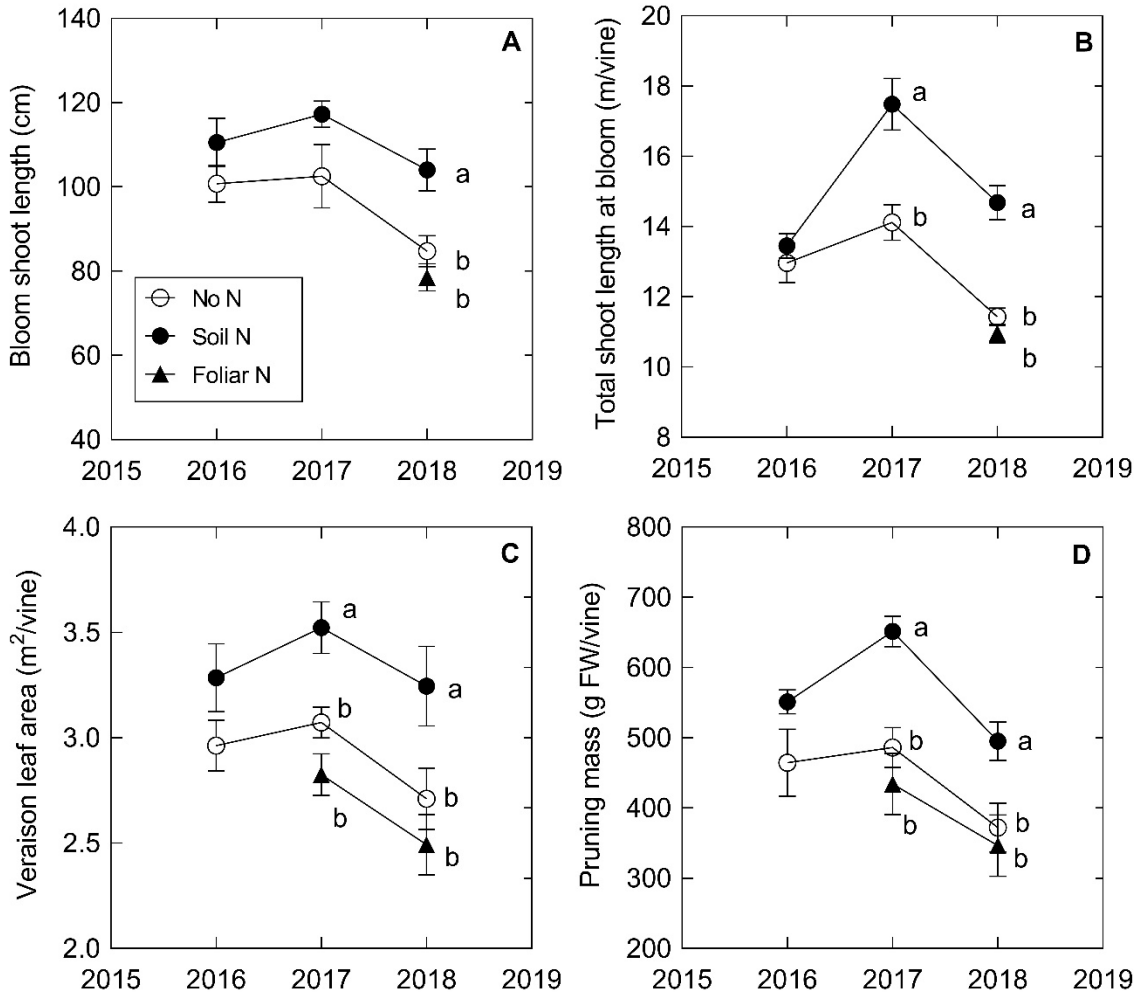


829 **Figure 1.** Effect of vineyard N applications (Soil N and Foliar N) on vegetative growth of
830 Chardonnay grapevines from 2016 to 2018. Vines that received no N supply (No N) served as
831 the control. Data points represent means and standard error for each treatment in each year (n =
832 4). Data were analyzed separately for each experimental year. Letters near each symbol
833 designate significant groups based on t-test or Tukey's HSD test at 95% confidence.
834

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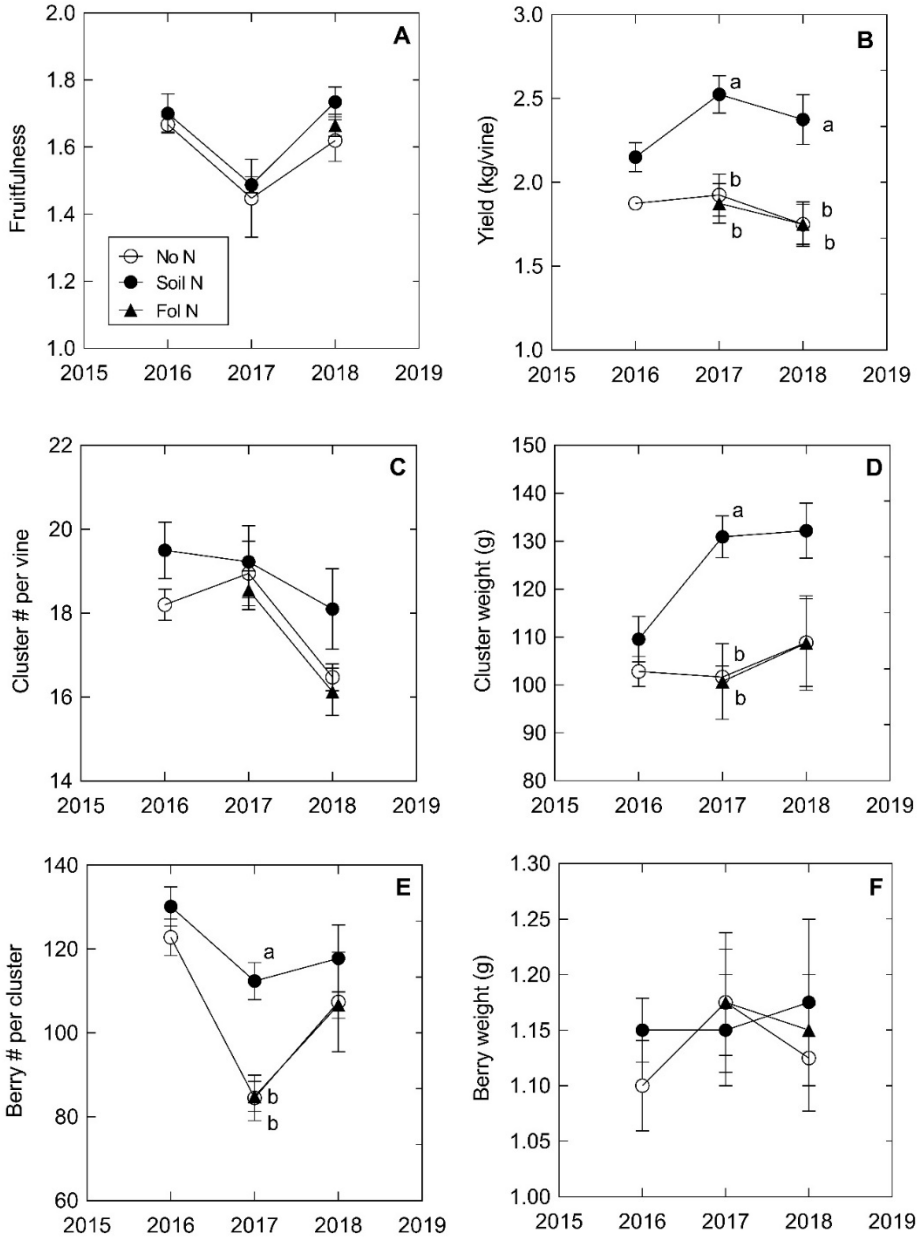


**Figure 2.** Effect of vineyard N applications (Soil N and Foliar N) on reproductive parameters of Chardonnay grapevines from 2016 to 2018. Vines that received no N supply (No N) served as the control. Data points represent means and standard error for each treatment in each year (n = 4). Data were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

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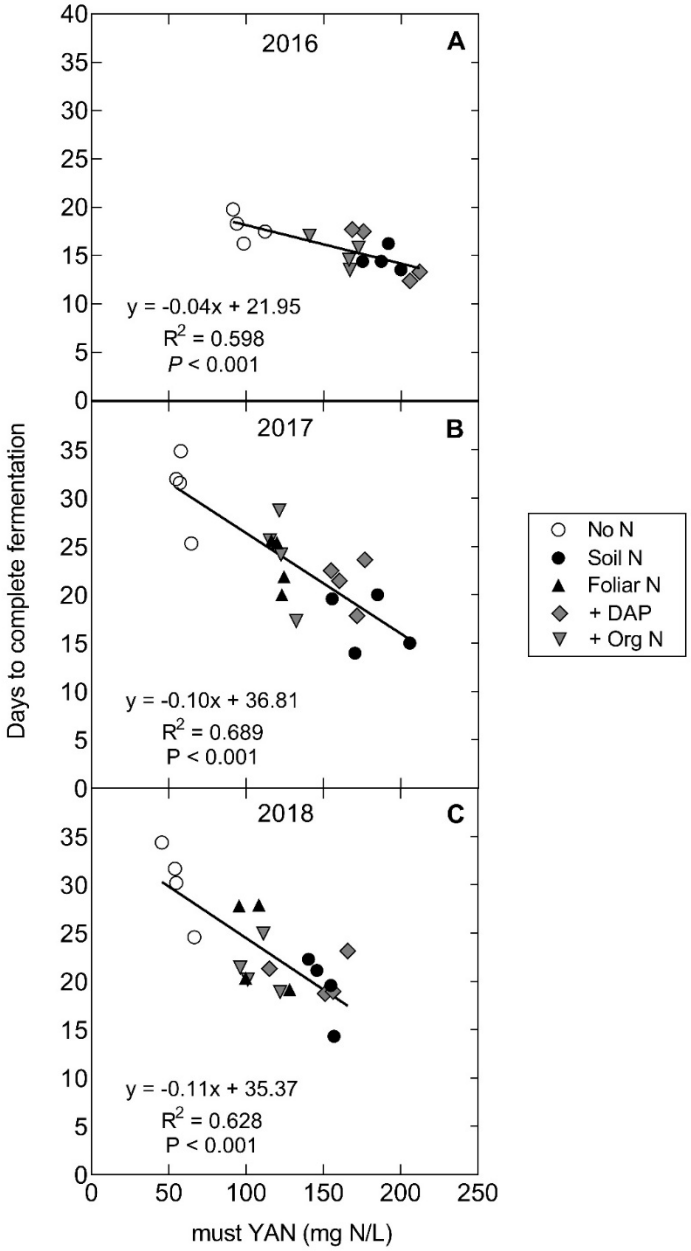


**Figure 3.** Relationship between must YAN concentration prior to fermentation and number of days to complete fermentation in Chardonnay musts that received vineyard N applications (Soil N or Foliar N) or winery N additions (+DAP or +Org N) between 2016 and 2018. Musts that received no N input in the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

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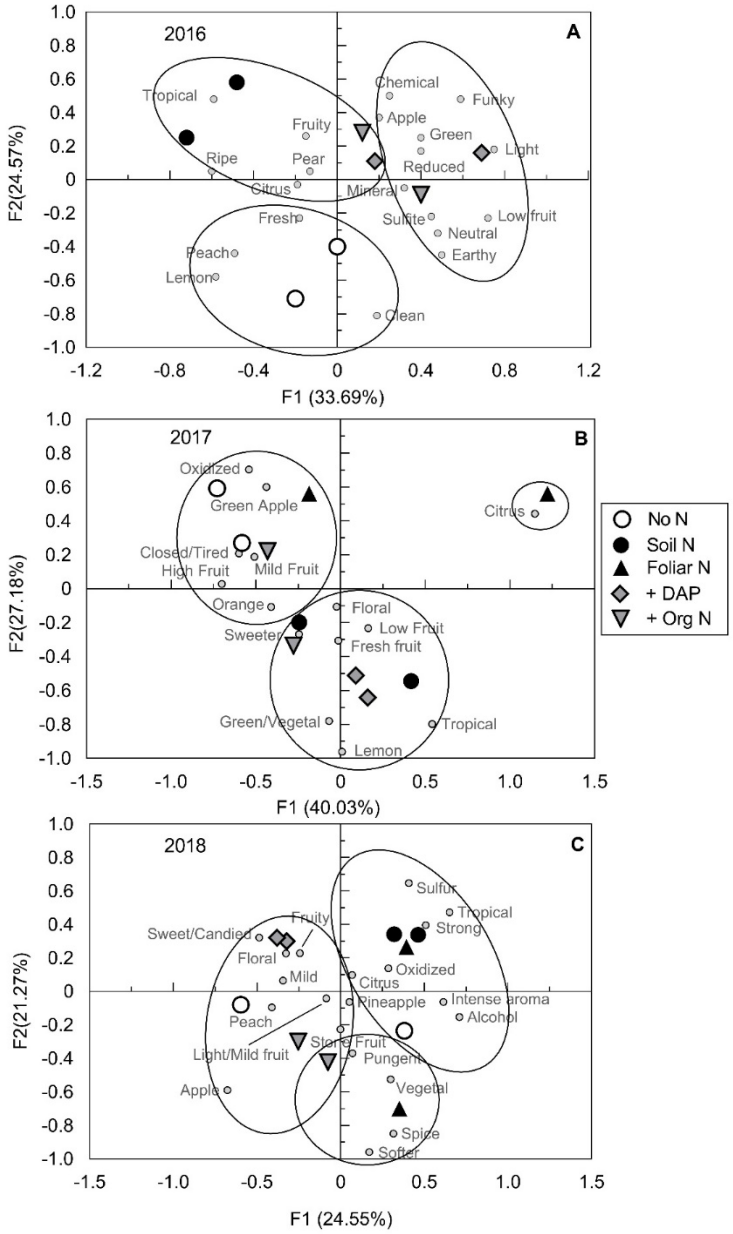


850 **Figure 4**. Correspondence analysis of ultra-flash profiling (UFP) data for aroma of Chardonnay
851 wines that received vineyard N applications (Soil N or Foliar N), winery N supplementations
852 (+DAP or +Org N), or no N inputs (No N) between 2016 and 2018. Ellipses indicate groupings
853 calculated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

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855 **Supplemental Table 1.** Dates when soil N (UAN) or foliar N (urea) fertilizers were applied to Chardonnay grapevines in 2016 to
856 2018.

| | Date of application | | | Number of days after bloom | | |
|---|---|---|---|---|---|---|
| | 1st application | 2nd application | 3rd application | 1st application | 2nd application | 3rd application |
| **2016** | | | | | | |
| Soil N[a] | 13 May | 30 Jun | 29 Jul | -19 | 29 | 58 |
| **2017** | | | | | | |
| Soil N | 30 May | 19 Jul | 25 Aug | -23 | 27 | 64 |
| Foliar N[b] | 18 Jul | 16 Aug | 8 Sep | 26 | 55 | 78 |
| **2018** | | | | | | |
| Soil N | 31 May | 20 Jul | - | -17 | 33 | |
| Foliar N | 18 Jul | 10 Aug | 6 Sep | 31 | 54 | 81 |

[a] UAN was applied at a rate of 17.8 kg N/hectare (20 pounds N/acre).

[b] Urea was applied at a rate of 8.2 kg N/hectare (7.3 pounds N/acre) in 2017 and 7.4 kg N/hectare (6.6 pounds N/acre) in 2018.

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858 **Supplemental Table 2.** Vine phenology and meteorological variables for Chardonnay grapevines from 2016 to 2018.

| Year/Growth Stage | GDD > 10 °C [a] | Mean daily temp (°C) | Precipitation (mm) | Total solar radiation (MJ/m2) |
|---|---|---|---|---|
| **2016** | | | | |
| Bud break to bloom — 6 Apr to 1 Jun | 336 | 15.0 | 81 | 1056 |
| Bloom to veraison — 2 Jun to 12 Aug | 672 | 19.0 | 41 | 1742 |
| Veraison to harvest — 13 Aug to 15 Sep | 354 | 20.0 | 3 | 748 |
| Season total | 1331 | | 126 | |
| **2017** | | | | |
| Bud break to bloom — 20 Apr to 22 Jun | 383 | 14.9 | 102 | 1209 |
| Bloom to veraison — 23 Jun to 26 Aug | 761 | 21.4 | 3 | 1638 |
| Veraison to harvest — 27 Aug to 28 Sep | 323 | 19.1 | 42 | 541 |
| Season total | 1466 | | 147 | |
| **2018** | | | | |
| Bud break to bloom — 21 Apr to 17 Jun | 365 | 15.3 | 43 | 1215 |
| Bloom to veraison — 18 Jun to 22 Aug | 762 | 21.3 | 0 | 1715 |
| Veraison to harvest — 23 Aug to 26 Sep | 217 | 16.7 | 15 | 609 |
| Season total | 1344 | | 58 | |

859 a GDD, growing degree days. To calculate GDD, the daily maximum and minimum temperatures were obtained from the Agrimet
860 weather station at Aurora, OR. On occasions where the daily minimum temperature was below 10°C, it was adjusted to 10°C prior to
861 calculation.

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862 **Supplemental Table 3.** Effect of vineyard N applications (Soil N and Foliar N) on leaf SPAD
863 values of Chardonnay grapevines from 2016 to 2018. Vines that received no N supply (No N)
864 served as the control. Values are means (standard deviation) for each treatment on each
865 measurement day (n = 4).

| Date | SPAD of opposite cluster leaves [a,c] | | | SPAD of upper canopy leaves [b,c] | | |
|---|---|---|---|---|---|---|
| | No N | Soil N | Foliar N | No N | Soil N | Foliar N |
| **2016** | | | | | | |
| 13-May | 35.1 (0.7) | 35.2 (0.9) | - | - | - | - |
| 30-Jun | 35.3 (1.7) | 36.9 (1.9) | - | - | - | - |
| 18-Jul | 34.5 (2.5) | 36.9 (1.9) | - | - | - | - |
| 5-Aug | 30.6 b (1.2) | 35.1 a (1.1) | - | - | - | - |
| 12-Aug | 30.5 b (3.6) | 35.8 a (2.8) | - | 32.5 b (1.2) | 37.6 a (1.8) | - |
| **2017** | | | | | | |
| 25-May | 26.8 (2.2) | 27.8 (1.3) | 26.8 (2.2) | - | - | - |
| 12-Jun | 31.8 (3.7) | 37.9 (2.0) | 31.9 (3.7) | - | - | - |
| 22-Jun | 33.8 (2.8) | 37.9 (2.2) | 34.8 (2.8) | - | - | - |
| 19-Jul | 34.5 b (1.7) | 39.4 a (1.8) | 34.5 b (1.7) | 31.5 (2.2) | 35.2 (2.0) | - |
| 4-Aug | 33.1 b (1.7) | 39.0 a (1.6) | 35.3 ab (2.5) | 31.7 b (1.0) | 36.0 a (1.4) | 32.0 b (1.4) |
| 28-Aug | 33.0 b (4.2) | 42.3 a (2.9) | 34.4 b (3.9) | 32.6 b (1.9) | 40.6 a (1.9) | 33.5 b (1.7) |
| 26-Sep | 29.8 b (1.5) | 40.2 a (1.3) | 31.9 b (1.4) | 30.4 b (3.2) | 39.1 a (3.0) | 30.9 b (2.7) |
| **2018** | | | | | | |
| 16-May | 28.3 b (0.3) | 30.9 a (0.6) | 28.0 b (0.9) | - | - | - |
| 31-May | 29.9 (1.2) | 32.1 (2.0) | 28.8 (2.3) | - | - | - |

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| | | | | | | |
|---|---|---|---|---|---|---|
| 18-Jun | 34.5 (2.8) | 37.8 (2.9) | 32.9 (2.6) | - | - | - |
| 24-Jul | 33.3 (2.7) | 37.2 (2.1) | 33.2 (2.4) | - | - | - |
| 10-Aug | 32.5 b (1.7) | 39.6 a (1.2) | 32.4 b (1.2) | 33.1 b (0.9) | 40.2 a (0.8) | 33.8 b (1.3) |
| 23-Aug | 31.2 b (2.8) | 39.1 a (2.8) | 31.0 b (2.1) | 33.1 b (2.7) | 40.0 a (2.0) | 33.9 b (2.2) |
| 25-Sep | 24.0 b (2.8) | 35.8 a (1.8) | 25.0 b (2.3) | 28.3 b (1.7) | 36.8 a (0.8) | 28.8 b (1.0) |

[a] Leaves that are opposite the clusters.

[b] Leaves that are most recently fully expanded on the main shoot, or located at least two nodes below the hedging point of the shoot.

[c] Data were analyzed separately for each sampling date and leaf type (n = 4). Means followed by a different letter in a row for each leaf type differ significantly based on Tukey's HSD test at 95% confidence.

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868 **Supplemental Table 4.** Effect of vineyard N applications (Soil N and Foliar N) on leaf
869 photosynthetic rates in Chardonnay grapevines between 2016 and 2018. Vines that received no N
870 supply (No N) served as the control. Values are means (standard deviation) for each treatment on
871 each measurement day (n = 4).
872

| | Leaf photosynthesis [a] (μmol CO2 fixed/m2 s) | | |
|---|---|---|---|
| Time/Date | No N | Soil N | Foliar N |
| 25 Jul 2016, 1500 hr | 16.7 (2.0) | 16.2 (3.8) | - |
| 21 Jun 2017, 1500 hr | 20.2 (2.4) | 22.3 (1.7) | - |
| 28 Jul 2017, 1500 hr | 10.6 (4.0) | 10.6 (5.0) | - |
| 31 Jul 2018, 1500 hr | 13.9 (1.1) | 14.5 (3.2) | 14.8 (1.4) |
| 6 Sep 2018, 1200 hr | 15.3 b[b] (1.7) | 20.3 a (1.7) | 15.4 b (2.3) |

[a] Measurements were conducted on sunny cloudless days when the ambient level of PAR (photosynthetically active radiation) was above 1700 μmol/m2/s. Data were analyzed separately for individual measurement days.
[b] Means followed by a different letter in a row differ significantly based on Tukey's HSD at 95% confidence.

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875 **Supplemental Table 5.** Effect of soil N applications on leaf water potential on individual
876 measurement days in Chardonnay grapevines between 2016 and 2018 (n=4). Vines that received
877 no N supply (No N) served as the control. Seasonal average is the mean of leaf water potential
878 for each treatment after pooling data from all measurement days for each year.
879

| | Leaf water potential (MPa) | |
|---|---|---|
| Date | No N | Soil N |
| **2016** | | |
| 20-Jul | -0.98 (0.08) | -0.99 (0.03) |
| 22-Aug | -1.28 (0.10) | -1.33 (0.10) |
| 12-Sep | -1.28 (0.24) | -1.53 (0.17) |
| 28-Sep | -1.02 (0.18) | -1.26 (0.10) |
| Seasonal average | -1.14 aa | -1.28 b |
| **2017** | | |
| 12-Jul | -0.80 (0.05) | -0.86 (0.12) |
| 26-Jul | -1.10 (0.10) | -1.17 (0.04) |
| 28-Jul | -0.99 (0.07) | -1.04 (0.15) |
| 9-Aug | -1.31 (0.24) | -1.39 (0.16) |
| 28-Aug | -1.45 (0.12) | -1.47 (0.11) |
| 12-Sep | -1.48 (0.21) | -1.56 (0.21) |
| 14-Sep | -1.06 (0.14) | -1.16 (0.10) |
| Seasonal average | -1.17 | -1.23 |
| **2018** | | |
| 31-Jul | -1.26 (0.14) | -1.26 (0.13) |
| 6-Aug | -1.14 (0.07) | -1.32 (0.17) |
| 22-Aug | -1.36 (0.09) | -1.49 (0.11) |
| 5-Sep | -1.36 (0.12) | -1.39 (0.13) |
| Seasonal average | -1.19 a | -1.37 b |

a Data were analyzed for each experimental year after pooling data from all measurement days. Means followed by a different letter in a row differ significantly based on *t*-test at 95% confidence interval. Leaf water potential did not differ between the No N and + Soil N treatments on any individual measurement days. Irrigation was not initiated until the beginning of July in all years. Standard deviation is shown in the parentheses.

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882 **Supplemental Table 6.** Effect of vineyard N applications (Soil N and Foliar N) on cluster zone
883 solar exposure in Chardonnay grapevines near veraison from 2016 to 2018. Vines that received
884 no N supply (No N) served as the control. Values represent means (standard deviation) for each
885 treatment on each measurement day (n = 4).

| Date/ Time | Cluster exposure (% PAR in cluster zone)a | | |
|---|---|---|---|
| | No N | Soil N | Foliar N |
| **July 27, 2016** | | | |
| 930 hr | 45[b] (3.1) | 51 (5.1) | - |
| 1120 hr | 33 (8.3) | 38 (10.8) | - |
| 1320 hr | 2 (1.0) | 2 (0.4) | - |
| 1520 hr | 24 (3.1) | 33 (6.1) | - |
| 1720 hr | 44 a (11.2) | 26 b (6.9) | - |
| **September 11, 2017** | | | |
| 1000 hr | 60 ab (3.8) | 50 b (5.1) | 66 a (7.2) |
| 1200 hr | 31 a (6.8) | 13 b (3.8) | 22 ab (9.5) |
| 1400 hr | 32 a (6.1) | 22 b (2.7) | 38 a (4.8) |
| 1600 hr | 56 (9.6) | 42 (4.7) | 50 (9.5) |
| **September 5, 2018** | | | |
| 1000 hr | 56 (6.1) | 46 (3.4) | 64 (6.1) |
| 1200 hr | 30 (17.3) | 20 (11.9) | 31 (13.7) |
| 1400 hr | 26 ab (8.0) | 22 b (9.5) | 31 a (12.5) |
| 1600 hr | 57 (6.1) | 44 (7.3) | 57 (7.9) |

a PAR, photosynthetically active radiation (400 to 700 nm). Basal leaves on the east aspect of the canopy were removed to improve cluster exposure prior to measurement, but leaves on the west aspect of the canopy were not removed. Measurements were performed in the east aspect of the canopy before 1300 hr and in the west aspect of the canopy afterwards.

b data were analyzed separately at each time point on each measurement day. Means followed by a different letter within each row differ significantly based on t-test or Tukey's HSD test at 95% confidence.

886

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887 **Supplemental Table 7.** Effect of vineyard N applications (Soil N and Foliar N) on must nutrient concentrations in Chardonnay
888 grapevines from 2016 to 2018. Vines that received no N supply (No N) served as the control. Values represent means (standard
889 deviation) for each treatment (n = 4).

| | B [a] | Ca | Cu | Fe | K | Mg | Mn | Na | P | S | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **2016** | | | | | | | | | | | |
| No N | 2.9 (0.2) | 79 b [b] (9) | 1.0 (0.3) | 3.1 (1.3) | 751 (72) | 74 b (5) | 0.6 (0.2) | 2.4 (0.7) | 105 (14) | 48 b (5) | 0.5 (0.10) |
| Soil N | 3.1 (0.2) | 103 a (11) | 1.1 (0.3) | 3.0 (0.6) | 836 (109) | 97 a (4) | 0.7 (0.2) | 3.2 (0.8) | 103 (7) | 74 a (5) | 0.5 (0.05) |
| **2017** | | | | | | | | | | | |
| No N | 1.5 (0.1) | 71 (3) | - | 0.8 (0.6) | 492 (27) | 67 (3) | 0.5 (0.2) | - | 94 a (12) | 45 b (4) | 0.3 (0.08) |
| Soil N | 1.3 (0.1) | 61 (6) | - | 0.4 (0.5) | 541 (95) | 62 (8) | 0.4 (0.3) | - | 57 b (10) | 57 a (3) | 0.2 (0.08) |
| Foliar N | 1.5 (0.1) | 64 (5) | - | 2.5 (0.8) | 516 (85) | 64 (5) | 0.6 (0.3) | - | 87 a (13) | 48 b (2) | 0.2 (0.04) |
| **2018** | | | | | | | | | | | |
| No N | 2.9 (0.6) | 115 a (7) | 0.5 (0.2) | 1.2 (0.3) | 959 (175) | 74 a (3) | 2.7 (0.2) | 1.4 b (1.4) | 98 a (10) | 51 (7) | 0.6 (0.11) |
| Soil N | 2.5 (0.5) | 88 b (9) | 0.4 (0.1) | 0.7 (0.2) | 790 (238) | 64 b (4) | 2.5 (0.1) | 1.0 b (0.9) | 70 b (13) | 55 (5) | 0.5 (0.19) |
| Foliar N | 3.3 (0.3) | 94 ab (4) | 0.4 (0.1) | 0.9 (0.3) | 785 (132) | 71 ab (3) | 3.0 (0.1) | 4.0 a (0.7) | 102 a (8) | 52 (5) | 0.6 (0.34) |

[a] Concentrations of all nutrients are expressed as mg/L.

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

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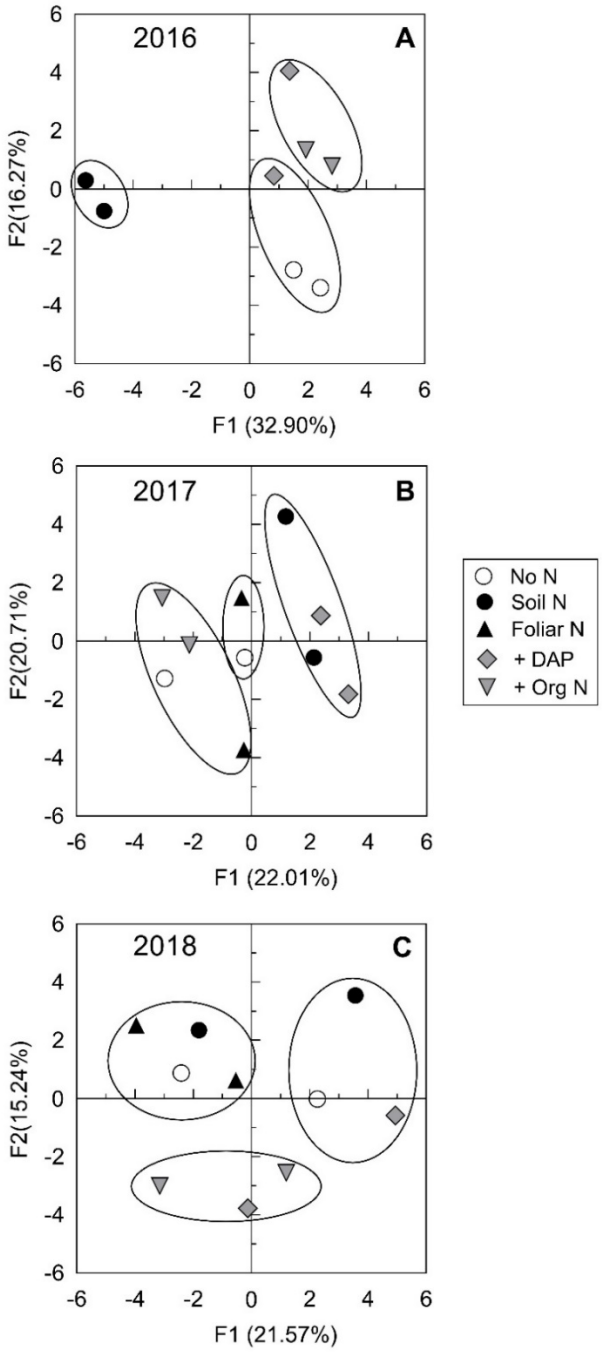


**Supplemental Figure 1.** Multiple factor analysis of Napping data for aroma of Chardonnay wines that received vineyard N applications (Soil N or Foliar N), winery N supplementations (+ DAP or + Org N), or no N inputs (No N) between 2016 and 2018. Ellipses indicate groupings calculated using k-means clustering. Two samples of each wine were evaluated in each session.

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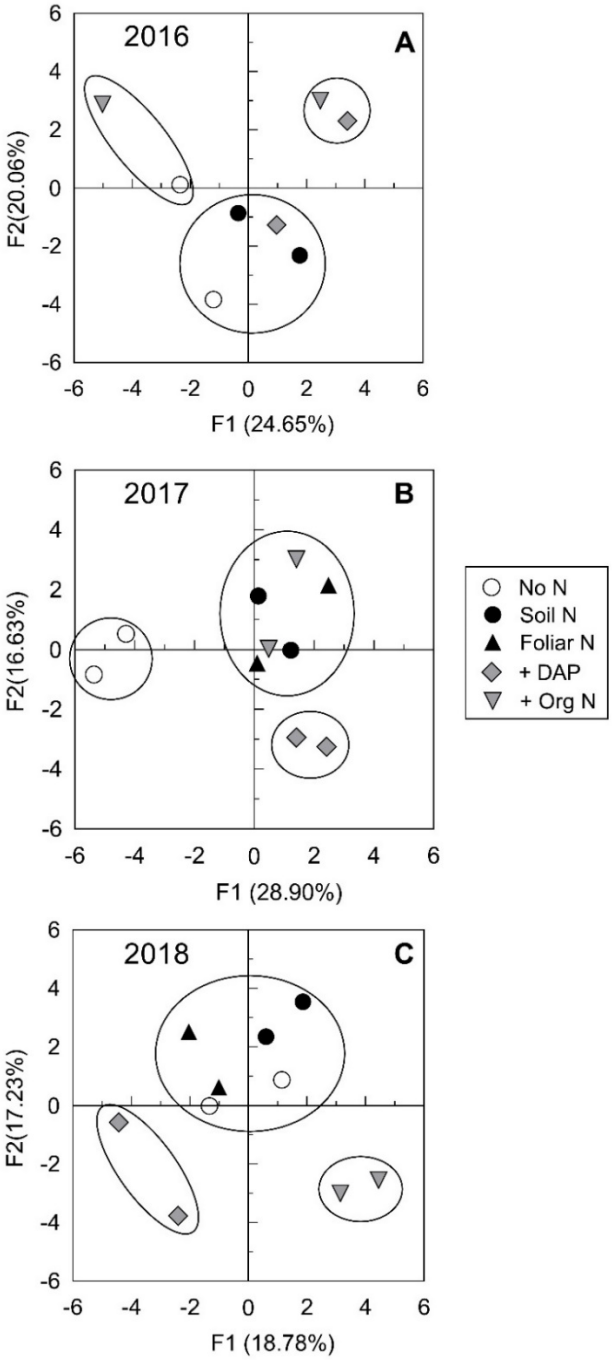


**Supplemental Figure 2**. Multiple factor analysis of Napping data for mouthfeel of Chardonnay wines that received vineyard N applications (Soil N or Foliar N), winery N supplementations (+ DAP or + Org N), or no N inputs (No N) between 2016 and 2018. Ellipses indicate groupings calculated using k-means clustering. Two samples of each wine were evaluated in each session.

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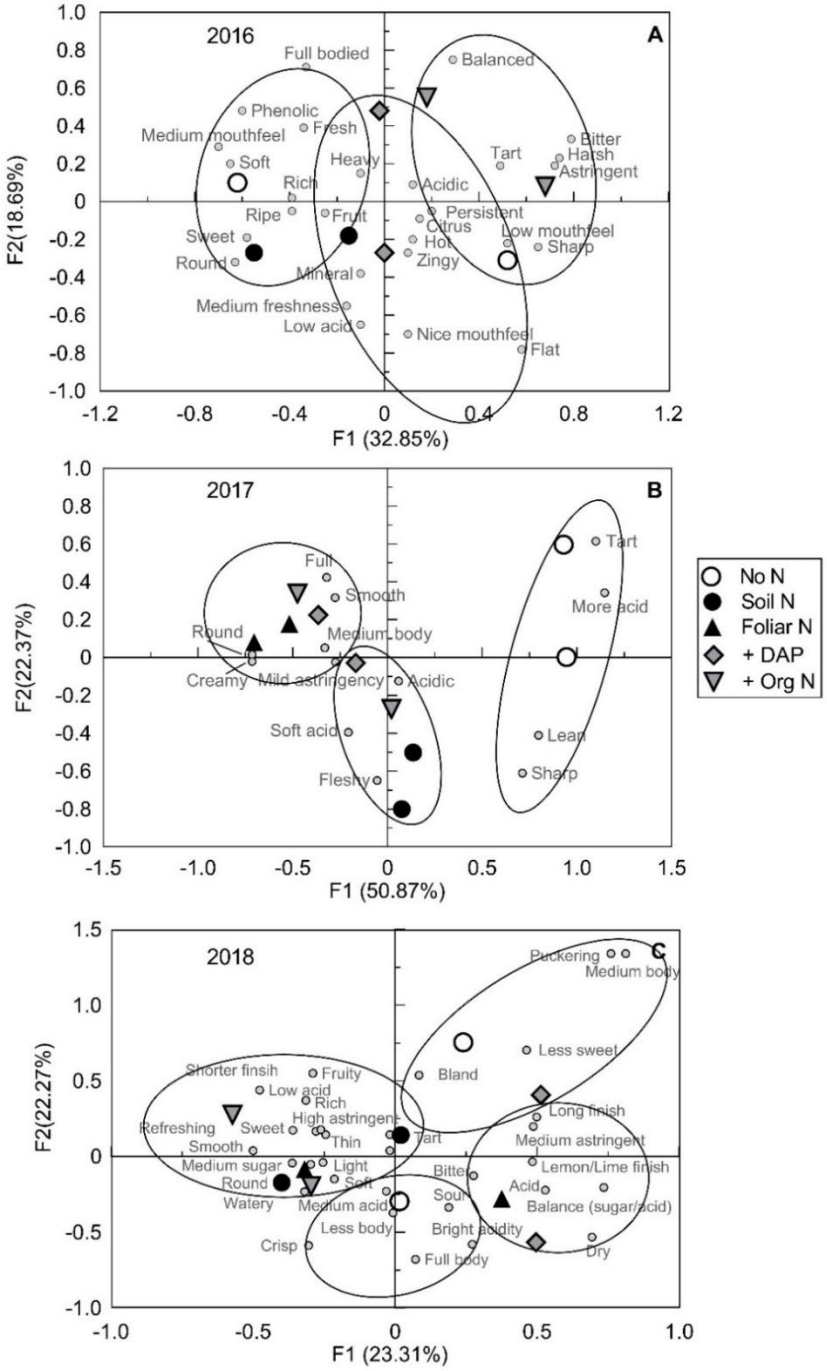


**Supplemental Figure 3**. Correspondence analysis of UFP data for mouthfeel of Chardonnay wines that received vineyard N applications (Soil N or Foliar N), winery N supplementations (+ DAP or + Org N), or no N inputs (No N) between 2016 and 2018. Ellipses indicate groupings calculated using k-means clustering. Two samples of each wine were evaluated in each session.