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Research Article

Nitrogen Requirements of Pinot noir Based on Growth Parameters, Must Composition, and Fermentation Behavior

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Abstract: A study to reassess the nitrogen (N) requirements for Pinot noir was carried out using a pot-in-pot vineyard where N inputs were carefully controlled. Pinot noir grafted on 101-14 rootstock was exposed to five levels of N supply beginning in their fourth growing season, and vine productivity, berry chemistry and must fermentation dynamics were studied over three years. N supply altered the N status of vines in accordance with expectations. Varying N had a greater impact on vegetative growth parameters than upon reproductive responses. For example, at veraison, leaf area of vines exposed to the three lowest rates of N was reduced in all years, but yield was only reduced at the lowest N rate in the first year, and the two lowest N rates in subsequent years. Fruitfulness and fruit set were slightly reduced by low N, while flower number of inflorescences was unaffected by N. Effects on berry maturity indices at harvest were generally small, but effects on must yeast-assimilable nitrogen (YAN) concentrations were large.

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YAN was reduced from about 200 mg N/L in the Control to as low as 25 mg N/L in the lowest N rate after three years. Treatments with lower YAN required more time to complete alcoholic fermentation, particularly those with YAN below 100 mg N/L. However, all musts fermented to dryness. Reducing vegetative growth of Pinot Noir can be achieved prior to reducing yield by reducing N when vines are cropped at typical levels for premium wine production in the region. YAN levels as low as 100 mg N/L might be a better production target for wineries to achieve minimum fermentation requirements of Pinot noir.

Key words: leaf nitrogen, pruning weight, *Vitis vinifera*, YAN, yield

Introduction

Nitrogen (N) is known to influence the productivity and fruit composition of winegrapes and is often the most important nutrient to manage in vineyards since it has a large impact on vine productivity (Roubelakis-Angelakis and Kliewer 1992, Bell and Henschke 2005). It is well established that excessive N supply results in increased vegetative growth (vigor) often at the expense of reproductive growth and/or fruit ripening (Wheeler and Pickering 2003, Delgado et al. 2004). High N supply resulting in increased vegetative growth, can also result in undesirable effects on berries due to increased shading of clusters decreasing color development (Keller et al. 1999, Hilbert et al. 2003) and increasing the incidence of *Botrytis* infection (Conradie and Saayman 1989). Too little N can reduce yield and quality of fruit by reducing fruit set, berry growth, and fruit ripening by reducing vegetative growth too severely (Kliewer et al. 1991, Bell and Robson 1999). Low N concentrations in berries leading to low YAN (yeast assimilable nitrogen) levels can reduce fermentation rates and presumably wine quality in many grape-

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51 growing regions (Bell and Henschke 2005). In commercial production it is generally accepted
52 that grape must YAN values of 140 mg N/L are required to obtain alcoholic fermentations that
53 complete to dryness and reduce the risk of yeast produced hydrogen sulfide (Jiranek et al. 1995,
54 Bell and Henschke 2005, Martinez-Moreno et al. 2012). While vine N status is routinely
55 measured by commercial vineyards using leaf blades or petioles collected at bloom or veraison
56 (see Schreiner and Scagel 2017 for a brief history on leaf and petiole testing), specific tissue N
57 status benchmarks required to meet specific production or fruit composition goals are not well
58 defined (Schreiner et al. 2013, 2014).

59 Recent work using a pot-in-pot system to investigate N requirements for own-rooted
60 Pinot Noir showed that lowering N status reduced amino acid contribution to YAN the most but
61 increased some berry secondary metabolites including anthocyanins, phenolic acids and
62 condensed tannins (Schreiner et al. 2013, 2014). An increase in condensed tannins and phenolic
63 acids was shown to occur independent of changes in berry size, while anthocyanins and other
64 phenols were related to N-induced changes in berry size. Others have also reported increases in
65 polyphenolics or tannins in red wine cultivars as N status declines (Keller et al. 1999, Hilbert et
66 al. 2003, Delgado et al 2004, Pérez-Álvarez et al. 2013), indicating that a balance of N status
67 needs to be achieved not only in terms of vegetative and reproductive growth, but also in terms
68 of primary (mainly amino-N) and secondary metabolites that are known to alter red wine quality.
69 In general, moderate to low levels of N appear to be beneficial for improving fruit quality in red
70 cultivars (Bell and Robson 1999, Treeby et al. 2000, Pérez-Álvarez et al. 2013).

71 Critical leaf blade values of 25 g N/kg DW (dry weight) at bloom and 18 g N/kg DW at
72 veraison were proposed for own-rooted Pinot Noir in order to maintain yields typical for

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commercial producers in the Willamette Valley of western Oregon and achieve must YAN values of 140 mg N/L (Schreiner et al. 2013). These N status targets were in general agreement or slightly higher than critical leaf blade N values from other studies (Conradie 2001, Robinson 2005, Linsenmeier et al. 2008). However, since tissue N concentrations that equate to yield or must YAN targets can vary among grape cultivars and rootstocks (Christensen et al. 1994, Conradie 2001), tissue test guidelines need to be defined for both cultivars and rootstocks used within a given production region.

The goal of this study was to evaluate how N supply affects both vegetative and reproductive parameters in grafted Pinot Noir grapevines and how fruit chemistry and fermentation behavior are altered by N status. Based on these responses, a second goal was to test if the proposed leaf blade and petiole N standards obtained previously (Schreiner et al. 2013) would apply also to grafted Pinot Noir vines. Pinot Noir was grown in a pot-in-pot vineyard where varying levels of N were precisely controlled so that vine responses to N could be carefully examined. Fruit was thinned on all vines by retaining a single cluster per shoot to achieve yields that are similar to current industry standards for production of premium Pinot Noir wines in western Oregon.

Materials and Methods

Pot-in-pot vineyard system and experimental design. The data used for this study was obtained over three consecutive growing seasons (2012-2014) from a microplot (pot-in-pot) vineyard (Schreiner et al. 2013) with Pinot Noir grapevines that received five different levels of N at the beginning of their fourth growing season as described by Schreiner and Scagel (2017).

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Briefly, grafted 'Pinot Noir' grapevines (certified *Vitis vinifera*, L. Pommard clone, FPS 91 on 101-14 rootstock, Duarte Nursery Inc., Hughson, CA) were grown in (60 L) pot-in-pot (Grip Lip 6900T, Nursery Supplies Inc., McMinnville, OR) microplots, installed at the Oregon State University, Lewis Brown Research Farm, Corvallis, OR, USA (44.553°N, 123.216°W). Pots were filled with 50 L of a mix including 3:1 coarse sand (Pre-stress sand mix, Knife River Inc., Corvallis, OR): Jory soil series (fine, mixed, active, mesic Xeric Palehumult collected from the Oregon State University, Woodhall Research Vineyard). Dormant 1-year-old vines were planted in the microplots in May of 2009, spaced at 1.0 m x 3.2 m, and trained on a single Guyot system using vertical shoot positioning. Vines were pruned to two buds in February of 2010 and two shoots were grown to the top wire until mid-July. At this time, the largest shoot was cut at 0.5 m from ground level and retained as the trunk and the smaller shoot was pruned off. Two laterals that developed just below the new trunk in 2010 were allowed to grow to the top wire in 2010. One of these laterals served as the fruiting cane for 2011, and the other was pruned to two buds to serve as the renewal spur. From 2011 to 2014, vines were pruned to 12 bud canes plus a two-bud renewal spur, and later thinned to 10 shoots per cane and a single renewal spur after threat of frost had passed each spring. Main shoots were trimmed (hedged) about two weeks after fruit set at a height of 2.2 m from ground level. All vines received complete nutrient solution (half-strength Hoagland's solution; Hoagland and Arnon, 1950) for the first three years after planting (2009 to 2011) delivered via fertigation three times per week from budbreak to veraison and approximately two times per week from veraison to harvest. In 2011, clusters were thinned to obtain five clusters per vine (a small crop) about 3 weeks after fruit set by retaining a single cluster on alternate shoots. Vines were irrigated during the growing season as needed based on

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volumetric soil water content (θ_v) and vine water status, using a similar approach as described by Schreiner et al. (2013). The θ_v was measured by time domain reflectometry (TDR; Soil Moisture Equipment Corp., Santa Barbara, CA) using 45 cm steel waveguides (rods) installed vertically in the pots halfway between the vine trunk and the pot edge. One set of waveguides was installed in each plot replicate in the center vine in each plot.

Treatments with varying N levels were applied to vines from 2012 to 2014. The concentration of N was supplied at 4 lower rates (75%, 50%, 30%, and 15% of Control rate) along with the Control (100%) where the total concentration of N in the Control during fertigation was 7.5 mM (equivalent to ½ strength Hoagland's solution) (Hoagland and Arnon 1950). All other nutrients (P, K, Ca, Mg, S, Fe, Mn, B, Zn, Cu, Mo) were held constant, with macronutrients except K supplied at the ½ strength Hoagland's rate and all micronutrients at full strength Hoagland's rate. Potassium was supplied at a higher rate of 4.5 mM total K (instead of 3 mM) based on the low K status of vines from the previous trial using similar microplots with 100% sand as a growing medium (Schreiner et al. 2013). Treatments are Control (100%N), 75%N, 50%N, 30%N, and 15%N. Each treatment was replicated four times in a randomized complete block design, and each replicate plot comprised five continuous vines. Border rows of Pinot Noir were also planted on both sides of the microplot vineyard and managed to obtain similar canopy size as the experimental vines. Vines were fertigated with varying N rates about three times per week from budbreak to veraison and approximately two times per week from veraison to harvest by supplying 4 L of the respective nutrient solution per fertigation event per microplot (see Table S-1 for actual dates and total N applied to the Control vines). To ensure that fertilizer salts did not accumulate during the summer, vines were irrigated for a 2 hour period (16

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L) on a single day at the beginning of July, August, and September in each year. The potential accumulation of salts was monitored by measuring the soil electrical conductivity (EC) using a soil probe at multiple depths (Model number 2265FSTP, Spectrum Technologies Inc. Plainfield IL). The EC values were always below 1.2 mS/cm.

Clusters were thinned approximately two weeks after fruit set in 2012 to 2014 to one cluster per shoot including the renewal shoot (spur) by retaining either the basal cluster (in most cases), or the second cluster. The second cluster was only retained if the basal cluster was unusually small, which occurred on the renewal spur shoots. Fungicides were used to manage powdery mildew (*Erysiphe necator* (Schw. Burr.)) and bunch rot (*Botrytis cinerea* L.) as per standard practices in the region. Differences in fruit cluster solar exposure and vine water status resulting from differences in canopy size among different N treatments were minimized by applying variable levels of leaf removal and irrigation as previously described (Schreiner et al. 2013). Minimizing the differences in cluster solar exposure as a result of canopy size changes in response to varying N was applied here to understand how N supply directly influenced berry composition without the interference of fruit shading, and also because commercial producers apply this practice irrespective of vine N status and canopy leaf density. Basal leaves (3-4 nodes) were first removed about two weeks after fruit set in the cluster zone on the east side of the canopy in all treatments to visually match cluster zone solar exposure. Immediately thereafter, cluster exposure was measured using a ceptometer (AccuPAR Model LP-80, Decagon, Pullman WA) at 0900, 1100, 1300, 1500 and 1700 hr by inserting the instrument in the cluster zone of the three individual (middle) vines per plot. The percent of photosynthetically active radiation in the cluster zone was calculated based on readings taken in full sunlight adjacent to each plot. Based

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on these results, more leaves were removed in any replicates with too much shading until treatment differences were no longer significant. In 2012 solar exposure was matched across all N levels by applying greater leaf removal in the higher N treatments. In 2013 and 2014, solar exposure in the higher N levels (100%N and 75%N) was matched to cluster solar exposure to the 50%N treatment, as canopy growth in the two lowest N levels (30%N and 15%N) was too insufficient to allow for the same cluster exposure as the higher N treatments. Therefore, solar exposure of fruit clusters was greater in the two lowest N level vines in 2013 and 2014.

Rates of irrigation were differentially applied as needed to each treatment to minimize any daily differences in soil and vine water status due to N treatment. Less water was supplied to the lowest N treatments (especially 30%N and 15%N vines) compared to the Controls in order to achieve the same θ_v. In addition, irrigation was adjusted seasonally based on vine phenology using θ_v so that all vines were not limited by water prior to fruit set, but experienced moderate water stress between fruit set and veraison, and slight water stress after veraison as described previously (Schreiner et al. 2013). New targets for θ_v were determined for this sand:soil mixture based on relationships between θ_v and midday leaf water potential (Ψ_{Leaf}) (pressure chamber, PMS Instrument Company, Albany OR) and stomatal conductance (g_s) (Licor 6400 photosynthesis system or LiCor 1600 steady state leaf porometer, LiCor Inc, Lincoln, NE) and are explained in greater detail in Schreiner and Scagel (2017). Before fruit set, irrigation was applied to maintain θ_v above 17% to ensure no water limitation. Between fruit set and veraison, irrigation was applied when θ_v was between 10 and 13% to help control canopy growth and expose vines to moderate water stress. After veraison, irrigation was applied to maintain θ_v

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between 14 and 15% to ensure only mild water stress. Irrigation was applied after 2100 hr (PST) and rates were adjusted daily as needed based on mean θ_v values per treatment.

Vine nutrient status. Vine leaf blades and petioles were collected to determine nutrient status at 50% bloom and at 50% veraison each year based on the average % of clusters at this stage of development assessed visually for all clusters per vine for each vine within each replicate. Ten leaves per plot were sampled and combined from count shoots at both bloom and veraison between 0900 to 1100 hr. Leaves opposite clusters were collected at bloom, and paired leaf samples comprising a leaf opposite a cluster and a recently expanded leaf were collected at veraison. Leaf blades and petioles were separated, rinsed in distilled water, dried at 65ºC for 48 h (Shel Lab FX 28-2, Sheldon Manufacturing Inc., Cornelius, OR), and ground to pass through a 425-µm-sieve. Nitrogen was determined via combustion analysis (Leco, Inc., St Louis, MO). Other nutrient (P, K, Ca, Mg, S, Fe, Mn, B, Zn Cu) concentrations were measured by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry; Perkin Elmer Optima 3000DV, Wellesley, MA) after microwave digestion in HNO_3 (Jones and Case 1990). Reference standard apple (*Malus domestica* L.) leaves (no. 151, National Institute of Standards and Technology) were included in each set of samples to ensure instrument and digestion procedures were accurate. Leaf blade and petiole concentrations are expressed on tissue dry weight (DW) basis.

Vine vegetative growth and photosynthesis. Shoot length and leaf area per vine was measured at bloom, and leaf area was measured at veraison in each year by first obtaining the primary shoot length and the length of all lateral shoots for all shoots on the middle three vines per plot. The area of leaves on main shoots and lateral shoots was then determined on 20 random shoots per treatment (100 total, ensuring that both larger and smaller shoots were included from

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each treatment) by comparing leaves to a series of concentric circles with known area as described in Schreiner et al. (2012). Leaf area per vine was calculated from the relationships between leaf area and shoot length for main shoots and for lateral shoots and summed for all shoots per vine. Dormant season pruning mass (fresh weight of 1-year-old canes) from the three middle vines per replicate was determined in the winter by weighing the count shoots from the previous season. Leaf gas exchange was measured using a portable infra-red gas analyzer system (LiCor 6400, LiCor Inc., Lincoln, NE) on a single leaf per plot. Fully exposed leaves (PAR>1800 μmol/m^2 s) on a main shoot in the lower or middle canopy were measured at bloom and veraison, respectively. Measurements of gas exchange were made at various times during the day, but data collected within one hour of solar noon (1300 hr) are shown here.

Vine reproductive growth and yield parameters. Flowers and fruit set were determined by placing fine mesh fabric bags on two random clusters per plot prior to the onset of flowering. The bags were carefully removed after fruit set ensuring that all flower caps were collected by inserting a small tray under each bag and the total number flower caps were counted. Each cluster used for this purpose was tagged to later sample just prior to commercial maturity and count the final number of berries to calculate fruit set. The date of fruit harvest in each year was based on a random sampling berries from all plots (3 berries per plot) when berries reached about 22-24 °Brix. However, in 2013, high rainfall just prior to fruit maturity decreased berry soluble solids below 20 °Brix, and fruit was eventually harvested at about 21 °Brix. All plots were harvested on the same day each year. Fruit clusters were removed from the three middle vines per plot, counted, and weighed to determine yield and average cluster

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weights. A subsample of five randomly selected clusters from each plot were transported back to lab to determine the number of berries per cluster and average berry weight.

Must chemistry and fermentation. The five cluster subsample from each plot was juiced using a stainless steel hand-crank press to obtain a yield equivalent to 625 mL must per kg fresh weight of clusters using at least two pressings that were combined for analysis. Fruit maturity indices (soluble solids, pH, and titratable acidity) were determined as previously described (Schreiner et al. 2013). Yeast assimilable nitrogen (YAN) concentration in must was determined by summing free amino acid-N (FAA-N) obtained by the OPA (*o*-phthaldialdehyde) colorimetric assay (Dukes and Butzke 1998) and ammonium-N by an enzymatic assay (Sigma ammonia assay kit; Sigma Chemical Co., St. Louis, MO). Must YAN concentrations are expressed as mg N/L. All must chemical parameters were analyzed in duplicate. The remaining fruit per plot was combined, stored overnight at 4°C, and destemmed the next day. Field replicates were processed separately, and 3 kg of each replicate was placed in 4L micro-fermenters as described by Sampaio et al. (2007). Fermenters were placed in a temperature controlled room set at 27°C, warmed to room temperature, and inoculated with *S. cerevisiae* RC212 (Lallemand, Montreal, Canada) at approximately 10^6 cfu/mL after rehydration according to the manufacturer's specifications. Fermentations were conducted with a submerged cap (Sampaio et al. 2007), and soluble solids monitored daily using an Anton-Paar DMA 35N Density Meter (Graz, Austria). After all fermentations reached dryness (< 0.5 g/L reducing sugar as measured by Clinitest®, Bayer, Leverkusen), they were pressed using a modified basket press with an applied constant pressure of 0.1 MPa for five min. An addition of 50 mg/L SO_2 (as

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potassium metabisulfite) was made to the wines before they were cold settled at 4°C for five days. Wines were then racked and an addition of SO_2 was made to achieve 25-30 mg/L free SO_2 prior to being bottled in 375 mL screw-capped (Stelvin™, Amcor, Zurich) wine bottles and stored at 13°C.

Statistical analysis. All statistics were conducted using Statistica software (version 12.7, Statsoft, Tulsa, OK). MANOVA was first conducted for groups of related variables to examine the interactive effect of year and treatment accounting for the contribution and experiment wide error associated with closely related variables. Variables were grouped according to vegetative and reproductive vine parameters, and must composition parameters using the average value for each replicate plot, since many variables had multiple subsampling observations per plot. After showing that there was a significant year by N treatment interaction (P<0.001) by MANOVA for each group of related variables, factorial ANOVA was used to examine how specific vine and fruit variables were altered by N supply and year accounting for block effects in the model. Variance assumptions were tested using Cochran's test and residuals were examined to ensure normality. Must free amino acid-N (FAA-N), must YAN, and the number of days to complete fermentation were log-transformed prior to ANOVA to satisfy variance assumptions. Means were compared using Tukey's post-hoc test at 95% confidence. For simplicity, the means and standard error of the mean are reported in all Tables and Figures. In addition, the data from the season prior to manipulating N (2011) is shown as a reference in figures, although this data was not included in analysis. The concentration of leaf blade N at veraison that corresponded to a 30% decrease in numerous vine and must response variables was calculated by first converting

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the quantities for each response variable relative to the Control treatment (100%N) mean value within each year. This allowed a comparison of different variables on the same scale. The point where a 30% decrease for each response variable had occurred was then computed from the regression line of the relative response values as a function of leaf N concentration at verasion.

Results

Weather differed between the three years of this study which led to differences in vine development (Table S-1). Air temperatures were cooler in 2012 leading to later development dates for bloom and veraison (about 2 weeks later than 2013 and 2014), and a later harvest (2-3 weeks later than 2013 and 2014). Overall 2014 was the warmest year, advancing budbreak and fruit harvest by about 10 days earlier than 2013 which was also a relatively warm year. High rainfall shortly before harvest in 2013 (including one day with 56 mm) thoroughly wetted fruit clusters and decreased soluble solids, thus delaying harvest that year. It should be noted that the fruit soluble solids never fully recovered and fruit was harvested at lower °Brix in 2013, ahead of another storm event.

Solar exposure of fruit clusters was not affected by N rates in 2012 (data not shown) but was greater in both the 30%N and 15%N treatments in 2013 and 2014 (Table S-2). Greater solar exposure was most evident in the lowest N vines (15%N) that had higher exposure than the Controls at 1300, 1500 and 1700 hr, while the 30%N vines had more solar exposure at 1500 and 1700 hr only. The 50%N and 75%N vines did not differ from the 100%N vines at any time.

Soil water content was altered by N supply on a few individual days each year as the irrigation adjustments to compensate for varying canopy size and water use among N treatments

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were not perfect (data not shown). However, these differences were generally small and were corrected the next day by adjusting irrigation rates. The season long average θ_v was not affected by N supply in 2012 or 2013, but θ_v was greater in the 30%N and 15%N treatment soils in 2014 as compared to the three higher N rates (Table S-3). The difference in the season long θ_v was about 1.0 to 1.6% higher in the two lowest N treatments than the higher N treatments in 2014.

As expected, year and N supply altered the concentration of N in leaf blades and petioles sampled at bloom and veraison in accordance with rates of N (Table 1). Leaf blade N concentration at bloom was affected by year and N supply without an interaction between them, as 2012 was lower than both 2013 and 2014, and leaf blade N was progressively reduced as N declined. Bloom petiole N concentrations showed a less marked decline as N rate decreased. A year by N supply interaction was observed for bloom petiole N concentrations because only the 30%N and 15%N vines had lower petiole N in 2012 and 2013 compared to the Control, while all lower N rates were lower than the Control by 2014. At veraison, leaf blade N concentrations were altered by the interaction between year and N rate, since veraison leaf blade N was lower in the 50%N, 30%N, and 15%N vines in 2013 and 2014, but only the 2 lowest N rates differed in 2012. The main effect of N on both leaf blade and petiole N concentrations at veraison was similar, as reductions in N supply resulted in lower N in both tissues. Petiole N at veraison was not affected by the interaction between year and N supply. Comparing the main effects of N supply at bloom and at veraison indicates that a greater separation among the N rates occurred at veraison as compared to bloom in both tissues. For example, the five N treatments resulted in four distinct leaf blade N concentration levels at bloom but separated further into five groups at

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veraison, while only three N levels were distinguished at bloom for petiole N but five groups differed at veraison.

Nitrogen influenced leaf blade and petiole concentrations of other plant mineral nutrients, most prominently P and S (data not shown). Focusing on the veraison data for brevity, low N supply increased P status and reduced S status of vines. Leaf blade and petiole P concentrations were higher in the 15%N (leaf blade P = 2.0 g/kg DW) vines than in the Control vines (leaf blade P = 1.5 g/kg DW) in 2012. In 2013 and 2014, the 15%N and 30%N vines had even higher P concentrations in both leaf blades and petioles (leaf blade ranging from 3.4-5.2 g P/kg DW) at veraison compared to the Control (leaf blade ranging from 1.7-1.8 g P/kg DW). Lower concentrations of S occurred in both leaf blades and petioles at veraison in all years in the 15%N and 30%N vines (leaf blade S = 1.4 g/kg DW) than in the Controls (leaf blade S = 1.9 g/kg DW), even though SO_4-S was supplied to all N treatments at the same concentration. The only other nutrient showing a consistent effect over time was B, where veraison leaf blade B concentrations were greater in the 15%N vines (ranging from 57-71 mg B/kg DW) than the Control (ranging from 40-50 mg B/kg DW) in all years, although petiole B concentrations did not differ in any year.

Vine vegetative growth parameters were highly responsive to N supply (Fig. 1) and an interaction with year was significant for all growth measures since effects became larger over time. Bloom shoot lengths showed no treatment differences in 2012, but by 2013 shoot length was lower in the three lowest N treatments compared to the Control, and by 2014 shoot length in the two lowest N treatments had dropped even further. The 75%N vines did not differ from the Controls in any year. Bloom leaf area responded similarly to bloom shoot length. By 2014 shoot

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length and leaf area at bloom in both the 30%N and 15%N vines was about 50% lower than the Controls, and the 50%N vines were about 25% lower than the Controls. At veraison, leaf area was already reduced in the three lowest N treatments in 2012 and by 2013 and 2014 leaf area was also lower in the 75%N vines than in Controls. The dormant season pruning weights showed a sharper decline over time in the low N treatments compared to leaf area, however, only vines in the two lowest N rates differed from the Controls in 2012 while vines in the three lowest N rates differed in 2013 and 2014.

Leaf photosynthesis at midday was affected by N rate, but not by year at both bloom and veraison (Fig. 1). The rate of photosynthetic carbon fixation at bloom was reduced by about 13% in the 30%N and 15%N vines, while at veraison photosynthetic rate in the 15%N vines was reduced even further than the 30%N vines and was about 30% lower than the Controls. Measurements taken later in the day (1500 - 1600 hr) across all years showed less impact of N supply on photosynthesis (data not shown). For example, late day photosynthesis rates at veraison did not differ among the different N treatments, and only the 30%N vines had a lower rate of photosynthesis at bloom in one year (2013) than the other treatments (including the 15%N vines). So, N status had a greater impact on single leaf photosynthesis at midday with little impact late in the day.

Vine reproductive parameters were influenced less by N supply than were vegetative parameters. Fruitfulness was reduced only in the lowest N treatment across all years, with 15%N vines having fewer clusters per shoot than the four higher N rates (Fig. 2). The number of individual flowers produced per inflorescence was not changed by N but was substantially higher in 2014 than in the prior two years (increased by about 40%). It should be noted that flower

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numbers per inflorescence were again lower in 2015 in all N treatments similar to 2012 and 2013 (data not shown), indicating that 2014 was an unusual year. The number of flowers that set fruit was only reduced by N in 2014 with lower fruit set in the 30%N vines and a further reduction in the 15%N vines. However, the final berry number per cluster at harvest was lower in both 2013 and 2014 in these two lowest N treatments, even though fruit set was only reduced in 2014. Yield was reduced in the 15%N vines in all years, while it was reduced in the 30%N vines in 2013 and 2014. Yield was not reduced in the 50%N or 75%N vines compared to the Control in any year. The average berry weight was reduced in all three of the lowest N treatments similarly across years, with the 15%N treatment showing a decrease of about 8% compared to the Control. All fruit was visually disease-free (no incidence of mildew or grey mold) at harvest in all years, except for 4 clusters in 2013 that had a few berries with grey mold (*Botrytis*).

Effects of N on must maturity indices (sugars, acids, pH) were minor and inconsistent across years (Fig. 3). Soluble solids differed in one treatment each in 2013 and 2014. The 50%N vines had higher soluble solids than all other treatments except the 75%N vines in 2013, and the 15%N vines had lower soluble solids than the three highest N levels (50-100%N) in 2014. No other treatments differed from the Control vines in other years, but 2013 (high rainfall prior to harvest) had lower soluble solids than 2012 and 2014. Must pH was altered by N supply only in 2014, with the two lowest N rates having lower pH that year. Must pH was also higher across all treatments in 2014 than in the previous years. Titratable acidity was about 12% lower in the 50%N vines compared to the Control vines across all years, but other N treatments did not differ from either of those treatments. Year also affected acids, as the 2014 musts had lower titratable acids than the prior two years, consistent with the higher pH in 2014.

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As expected, must N concentrations were strongly influenced by N supply. Must ammonium-N was reduced across all years as N rate declined. Both FAA-N and YAN showed nearly identical trends in response to N over time, as most of YAN was contributed by amino-N. Must FAA-N and YAN values were fairly consistent from 2012 to 2014 in the Control and 75%N vines, with values for YAN ranging from 190-230 mg N/L in the Controls and values from 145-175 in the 75%N vines. YAN was lower in the 75%N vines than the Control vines in 2012 and 2014. The three lowest N treatments had lower YAN than both the Control and 75%N vines in all years, and the two lowest N rates showed significant declines over time that were not significant for the 50%N vines. The 50%N vines had YAN concentrations of about 100 mg N/L across years, while the 30%N vines declined from about 80 mg N/L in 2012 to about 40 mg N/L by 2014, and the 15%N vines declined from about 70 mg N/L in 2012 to about 25 mg N/L in 2014.

The relative impact of N status on different vine response variables was compared by identifying the leaf blade N concentration at veraison that equated to a 30% reduction for each response parameter using regression (Table 2). This analysis (ranked in order of most to least affected by N status) revealed that vine N status has the greatest impact on must YAN levels. Must YAN was reduced by 30% when leaf blade N was between 19-22 g N/kg DW. Pruning weights were reduced by 30% at fairly similar leaf blade N status in 2013 and 2014 (20-21 g N/kg DW), but the point where pruning mass values were reduced by 30% in 2012 were considerably lower (17 g N/kg DW) since N effects on growth were cumulative over time. While a similar difference was also true for YAN in 2012, the difference in the N status in that year was not as great as it was for pruning mass indicating that N status influences YAN more quickly

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than growth. Leaf area at veraison was the next most sensitive variable to N status, showing a 30% reduction when leaf blade N was 16-20 g N/kg DW. Yield and bloom shoot length were less sensitive to N status, showing a 30% decline at leaf blade N concentrations averaged across all years of 16 g N/kg DW. Both yield and shoot length at bloom had much lower values in 2012, owing to cumulative impact of low N supply. Leaf photosynthesis was even less responsive with 30% lower rates occurring at a leaf blade N status of about 14 g N/kg DW.

Nitrogen supply and the resulting change in must YAN concentrations influenced alcoholic fermentation (Fig. 4). The musts from higher N treatments (100%N and 75%N) completed alcoholic fermentation within 4-5 days in all years and did not differ, while the 50%N musts took longer and completed ferment in about 7 d. Fermentations of the two lowest N treatments (30%N, 15%N) took between 8-10 days to complete in 2012 and 2013, and even longer (17 d for 15%N musts) in 2014 (Fig. 4A). However, musts reached dryness (fermentations completed) in all years, even when must YAN was as low as 25 mg N/L (15%N musts in 2014). Data from all years from the individual replicates showed that the time needed to complete fermentation increased dramatically when must YAN was below about 100 mg N/L (Fig 4B). The fitted curve (exponential decay) accounted for nearly 90% of the variation in ferment time as a function of must YAN level.

Discussion

The key finding from this study was that N supply altered vine production variables and must chemistry to different degrees. Vegetative growth parameters of Pinot Noir grafted onto 101-14 rootstock were constrained more than reproductive growth parameters as N status

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417 decreased. This is particularly relevant for yield and pruning mass of vines, two common
418 parameters used by viticulturists to understand vine vegetative and reproductive balance
419 (Jackson and Lombard 1993, Kliewer and Dokoozlian 2005). While the pruning mass and the
420 yield of vines were both reduced by low N with a greater impact over time, pruning mass
421 decreased to a greater degree than did yield. Impacts on yield had occurred at a lower vine N
422 status. A greater impact of N supply on vegetative pruning mass than on yield has been found in
423 previous N trials with grapevines (Kliewer at al. 1991, Bell and Robson 1999, Schreiner et al.
424 2013), but others have reported similar gains, or even inconsistent gains, in both parameters as N
425 status increased (Conradie and Saayman 1989, Conradie 2001, Pérez-Álvarez et al. 2013). The
426 inconsistent responses to N in vegetative versus reproductive growth across different studies may
427 reflect different N thresholds for canopy growth versus fruit development among different
428 cultivars of grapevines or may reflect the impact of other environmental factors that differed
429 across studies. The consistent finding of a greater impact on pruning mass than on yield from this
430 trial and a prior trial (Schreiner et al. 2013) with Pinot Noir utilizing microplots and fertigation to
431 carefully control vine N status, has important implications in managing Pinot Noir grapevines in
432 western Oregon, where canopies grow vigorously and yield is restricted to relatively low levels.
433 Both studies show that N reduction (or manipulation) can be used as tool to reduce vine
434 vegetative growth prior to impacting yield when producing low to moderate yield targets that are
435 typical for premium Pinot Noir. Based on these findings, viticulturists should be able to reduce
436 canopy size by using means to reduce vine N status within a given vineyard while maintaining
437 the same target yields. Since lowering N status generally improves fruit composition for red
438 cultivars (Jackson and Lombard 1993, Keller at al. 1999, Treeby et al. 2000, Hilbert et al. 2003,

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Schreiner et al. 2014), fruit and wine quality may also increase with lower N status. Our data suggest that viticulturists in the region have some room to adjust vine N status to reduce canopy size and possibly improve fruit composition before suffering a yield loss. A reduction in N status could be brought about by reducing N inputs directly, or by other means such as increasing competitive vineyard floor vegetation or using low N rootstocks (Reeve et al. 2016). The caveat is that must YAN levels appear to be slightly more sensitive to low N status, so lower YAN levels will accompany lower vegetative growth.

It has been suggested that for a 21 °Brix grape must, a minimum 200 mg N/L YAN is required to complete fermentation with an additional 25 mg N/L for every one degree increase in °Brix (Bisson and Butzke 2000). Mendes-Ferreira et al. (2004) also reported that a minimum YAN of 267 mg N/L was required for *S. cerevisiae* PYCC 4072 to complete alcoholic fermentation in a defined grape must-like media. In contrast, the data obtained here with low N fruit indicates that YAN levels may not need to be as high as is often suggested for completion of alcoholic fermentation. In this study, fermentation rate was not significantly reduced until must YAN levels dropped below about 100 mg N/L. Indeed, even the lowest N vines in 2014 with must YAN levels of 25 mg N/L completed ferments under experimental conditions, which was somewhat surprising given critical ranges reported in the literature. Stockert et al. (2013) also noted that Merlot musts completed fermentation with YAN values as low as about 60 mg N/L, although their fermentation rates for the higher N musts were slower than found here. Wang et al. (2003) and Ugliano et al. (2009) also reported that musts containing ≤ 100 mg N/L were fermented to dryness while others have suggested 140 mg N/L as the minimum YAN

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460 concentration required to ensure complete fermentation (Jiranek et al. 1995, Martinez-Moreno et
461 al. 2012). Indeed, a minimum YAN of 140 mg N/L is commonly used in the industry.
462 Determining minimum YAN requirements for fermentations is complicated by the fact
463 that there are large differences in N utilization between *S. cerevisiae* yeast strains (Jiranek et al.
464 1995, Mendes-Ferreira et al. 2004). The yeast strain used in our trial with Pinot Noir, *S.*
465 *cerevisiae* RC212, is considered (by the supplier) to be a moderate to high YAN requiring yeast.
466 However, this yeast strain was able to complete fermentation in a very low YAN must in our
467 study and suggests that lower YANs could still be considered even when yeast strains with
468 higher nutrient requirements are used. The method of wine fermentation employed here (small
469 lots with a submerged cap) might be an important factor as to why these musts completed
470 fermentation at such low YANs, but we could not find published work comparing small lab-scale
471 fermentations to larger scale ferments in relation to YAN requirements. Although, Casalta et al.
472 (2010) reported that 1 L ferments did not appreciably differ in fermentation kinetics compared to
473 100 L ferments for Grenache blanc and Sauvignon blanc as long as grape solids were included
474 with clarified musts. Greater skin contact due to the submerged cap fermentations may have
475 resulted in increased extraction of YAN from the skins, as Lee and Schreiner (2010) noted that
476 must YAN values were approximately 50% of the whole berry YAN with the majority of the
477 remaining YAN being located in the skins. Stines et al. (2000) also showed that skins contributed
478 up to 29% of total berry YAN. However, to our knowledge, there are no studies published on
479 the impact of cap management techniques (submerged vs. punch-down vs. pump-over) on YAN
480 extraction and availability to yeast during fermentation, so it is unknown if the use of submerged
481 cap fermentation impacted YAN availability from skins.

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Our findings suggest that 140 mg N/L may not be the critical level of YAN needed for red wines, and it is likely that Pinot Noir musts can complete fermentation with significantly less YAN. Whether this is the case for other red wines needs to be determined. Differences in amino acid composition between red winegrape varieties may impact YAN requirements. Pinot Noir is known to be a high arginine containing grape variety while others, such as Cabernet sauvignon, contain lower arginine and higher concentrations of proline (Spayd and Anderson-Bagge 1996). While proline cannot be utilized by *S. cerevisiae* under anaerobic conditions, arginine is a major source of primary amino acid N for the yeast during fermentation. Stockert et al. (2013) noted that amino acid composition in conjunction with overall YAN levels may better explain observed differences in fermentation rates. The authors also suggested that factors other than YAN in lower YAN musts may have influenced fermentation rates. Therefore, we suggest that minimum YAN requirements might be closer to 100 mg N/L for Pinot Noir and possibly other red cultivars on the basis of completing fermentation.

Aside from fermentation rate, YAN is also known to impact production of hydrogen sulfide (H_2S) with low YAN often cited as being a primary driver of yeast produced H_2S (Jiranek et al. 1995). However, others have noted that high YAN can also result in high H_2S production and that additional factors such as vitamin content and yeast strain may have a greater influence on H_2S production than YAN alone (Wang et al. 2003, Ugliano et al. 2009). While the present study does not report on wine H_2S concentration, additional analysis of the wines produced from this study has shown that all of the volatile S compounds known to impart unpleasant aromas in wines were either at lower concentrations in the low N wines or did not differ from the Control (100%N) wines (Yuan F, Schreiner RP, Osborne J and Qian MC, unpublished data, 2017). YAN

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504 can also impact desirable yeast-derived volatile compounds such as esters and higher alcohols
505 and this must be considered when discussing target YAN concentrations and their relationship
506 with wine quality (Ugliano et al. 2010). Volatile aroma analysis of wines produced from this trial
507 (Yuan F, Schreiner RP, Osborne J and Qian MC, unpublished data, 2017) will provide a more
508 comprehensive understanding of YAN targets that take into consideration fermentation rate as
509 well as other wine quality parameters.

510 Data from the 50%N treatment vines in this study are most appropriate for developing
511 tissue N guidelines for managing production of grafted Pinot Noir vines. The 50%N vines had
512 similar yields as the Control vines but with smaller, more manageable canopies (an important
513 goal for the region). Furthermore, 50%N musts with YAN values close to 100 mg N/L fermented
514 to dryness at only slightly longer time frame than the high N musts. Leaf blade N status of the
515 50%N vines ranged from 22.4 to 23.5 g N/kg DW at bloom and 18.8 to 19.3 g N/kg DW at
516 veraison across the 3 years of this study. Therefore, it appears that a good estimate for critical
517 values for leaf blade N are 23-24 g N/kg DW at bloom and 19 g N/kg DW at veraison. These leaf
518 blade N values agree with the previous values of 25 g N/kg DW at bloom and 18 g N/kg DW at
519 veraison determined from own-rooted Pinot Noir vines (Schreiner et al. 2013). Petiole N values
520 that correspond to these leaf blade values in the 50%N vines are 7.0 g N/kg DW at bloom and
521 4.4 g N/kg DW at veraison. Based on the responses of the two lowest N rates employed here, it
522 appears that a single year value of 15 g N/kg DW in leaf blades at veraison or two consecutive
523 years with veraison leaf blade N below 17.5 g N/kg DW will result in lower yield. If producers
524 want to obtain YAN levels of at least 140 mg N/L in must, then bloom leaf blade N based on the
525 75%N vines in 2013 and 2014 years where this YAN level had occurred would be: 25-26 g N/kg

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526 DW at bloom and 21-22 g N/kg DW in blades at veraison. Petiole values that equate to 140 YAN
527 found here in those same vines were 7.6-7.7 mg N/kg DW at bloom and 4.7-5.0 mg N/kg DW at
528 veraison. These guidelines for N status derived from this study agree with some previous studies
529 (Conradie 2001, Linsenmeier et al. 2008, Schreiner et al. 2013) or are slightly higher than others
530 (Robinson 2005) for winegrapes. The aforementioned leaf blade guidelines are more reliable
531 than the petiole values as recently shown using regression analysis of the raw plot data from this
532 vineyard, owing to wider year to year variation in petioles (Schreiner and Scagel 2017).
533 The aforementioned N guidelines may not apply to all grafted Pinot Noir vineyards in the
534 region. All data from this trial was based on vines carrying 1 cluster per shoot, which produced
535 fruit yields of 5600 to 7840 kg/ha (equivalent to 2.5 to 3.5 U.S. tons per acre). A slightly higher
536 N status may be required to achieve similar goals at higher crop loads. Indeed, our data support
537 that higher N status may be needed to produce higher yields, since the level of leaf blade N at
538 veraison that equated to a 30% yield loss in 2014 (the highest yield year) was 17.9 g N/kg DW
539 compared to 16.7 g N/kg DW in 2013. It seems likely and logical that a higher N status will be
540 needed to maintain higher yields over time. How much higher N status guidelines need to be
541 adjusted to accommodate higher yields is not known. The yield from this trial for the 50%N and
542 higher N level vines was above typical yields (2.0 U.S. tons per acre) traditionally carried in
543 western Oregon premium Pinot Noir vineyards.
544 Other nutrients besides N were above reported critical concentrations across all
545 treatments in any year (Conradie 2001, Robinson 2005, Schreiner et al. 2013), with the possible
546 exception of S. Tissue guidelines for S are not known, owing to a lack of S data in grapevines.
547 However, we have recorded S values in leaf blades in commercial Pinot Noir vineyards at

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548 veraison as low as 1.0 g S/kg DW without any apparent effects on vine health or presence of leaf
549 deficiency symptoms (Schreiner RP, unpublished data, 2017), and our bloom values for S were
550 above 2.2 g S/kg DW in leaf blades across all treatments. Critical leaf blade values for S near
551 harvest in other crops appears to be about 1.0 g S/kg DW, with higher values earlier in the season
552 near 2.0 g S/kg DW (Yoshida and Chaudhry 1979, Withers et al. 1995). We therefore suspect
553 that S status of our vines was in a healthy range.

554 The high yield in 2014 (equating to 7840 kg/ha or 3.5 U.S. tons per acre) was due to the
555 higher flower numbers per cluster that year. Nitrogen supply had no influence on flower numbers
556 here. The greater number of flowers in 2014 may be related to lower air temperatures near the
557 time of budbreak, as observed for Cabernet Sauvignon and Merlot where a temperature at
558 budbreak of 12°C resulted in more flowers per cluster than when budbreak temperatures were
559 near 20°C (see May 2004). The average daily air temperature in 2014 on the day of budbreak and
560 the following 5 days was 9.7°C, but the corresponding temperatures in 2012 and 2013 were
561 12.5°C and 12.1°C. These differences were not as large as those reported previously, but vines
562 here were grown under field conditions where solar heating may have also influenced bud
563 temperature (May 2004). Since N supply had no influence on flower number per inflorescence
564 here over a three year period, our data do not support recent findings that N status in the previous
565 growing season influences flower number in the subsequent year (Guilpart et al. 2014), nor
566 findings from a vineyard floor trial where lower flower number was related to lower N status of
567 vines (Reeve et al. 2016). Differences among these studies and the present findings may be due
568 to other factors including vine age and weather conditions that may interact with N status in
569 influencing flower numbers per inflorescence.

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In a prior N trial with own-rooted Pinot noir (Schreiner et al. 2013), we suggested that leaf N status guidelines should not be based on single leaf rates of photosynthesis because this measure appeared to be related to crop level and source:sink responses to low N as opposed to direct N limitation on the photosynthetic machinery (Chen and Cheng 2003, Prieto et al. 2012). That interpretation was based on the fact that leaf blade N concentrations in the lowest N vines in that study were the same in two years (14-15 g N/kg DW), but photosynthesis at veraison was reduced in only the second year when there was a simultaneous reduction in fruit yield. Photosynthesis was more consistently reduced by low N status here in grafted vines where veraison photosynthesis was reduced in the two lowest N rates as a main effect across all years. However, leaf blade N values in those vines showing lower rates of photosynthesis (15-17 g N/kg DW) were higher than the prior values. Yield was also more consistently reduced here than in the previous trial with own-rooted Pinot Noir (Schreiner et al. 2013). In addition, the point where photosynthesis was reduced by 30% occurred at a much lower N concentration in leaf blades compared to the N status where yield was reduced by 30% (Table 2). Results from this trial with grafted vines support our original interpretation that single leaf photosynthesis measures are not a good response variable upon which to base leaf N guidelines.

It was interesting that rates of photosynthesis in low N vines (15%N and 30%N) no longer differed from the Control vines when leaves were measured late in the day at veraison. The reason for this is not clear, although it is possible that vines with more N became sink-limited late in the day. Higher N vines had greater rates of leaf photosynthesis at midday and greater overall leaf area that may have saturated the supply of fixed carbon needed by developing fruit clusters, while the low N vines did not saturate the carbon supply needed by clusters by late

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592 afternoon since they had lower rates of gas exchange earlier in the day and fewer leaves.
593 Alternatively, the higher N vines may have had similar rates of gas exchange per leaf late in the
594 day as the low N vines because all vines were beginning to experience similar levels of water
595 limitation. Irrigation inputs were managed in this trial to obtain similar soil moisture levels late
596 in the day, so this latter explanation may be more likely to explain why high N and low N vines
597 had similar photosynthesis rates late in the day. Regardless of the underlying reason for these
598 differential effects of low N status on leaf level photosynthesis at different times of the day, the
599 higher N vines clearly fixed more carbon per vine to support the higher fruit yields.
600 If low N Pinot Noir fruit results in desirable phenolic and aromatic composition and
601 improves overall wine quality, or if low N fruit has a similar quality as high N fruit, then
602 reducing N status and vine canopy size would be strongly encouraged to reduce inputs and the
603 environmental footprint of vineyards. Often high N status vineyards are managed to deal with
604 excessive canopy growth by adopting expensive canopy management practices such as repeated
605 hedging of canopies. Reducing vine N status would help avoid these extra management costs.
606 Furthermore, repeated hedging of high N vines was found to result in poor color development in
607 Pinot Noir (Keller et al. 1999). Reducing N fertilizer use to achieve lower vine N status will
608 reduce the potential of nutrient leaching to groundwater. Growing smaller canopies in vigorous
609 vineyards by adopting practices to reduce vine N status will also result in lower water
610 requirements per unit land area. These production changes can provide significant environmental
611 benefits, but they are difficult to value economically. Reducing vineyard water use and the
612 potential for nutrient leaching to groundwater or watersheds should improve both the quantity of
613 water available for other uses, including late summer stream flows to protect aquatic habitat

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(Kaufmann and Hughes 2006), and the quality of that water (Carpenter et al. 1998). These goals are important components of vineyard sustainability programs that vineyards and wineries can utilize to gain market share of their products (Schäufele and Hamm 2017).

Nitrogen management will be more challenging to fine tune in non-irrigated vineyards, but some practices including the use of competitive cover crops can also reduce vine N status and canopy size with less impact on yield (Reeve et al. 2016). Ongoing research is addressing whether or not N fertilization in low N vineyards to boost native fruit YAN will produce better wines, or if maintaining low vine N status combined or not with winery YAN additions will produce better wines.

Conclusions

Lowering vine N status reduces vegetative growth more than reproductive growth for grafted Pinot Noir, indicating that growers can reduce N supply to limit vigor before suffering a yield loss at the current yield targets for premium wine production. However, must YAN appears to be more sensitive to N status than vegetative growth, so lower YAN values can be expected when N limitation is being used to reduce vigor. Further research needs to address whether the benefits of reducing N status in red wine cultivars in terms of fruit composition outweigh the negative consequences of lower must YAN. Results based on fermentation rates suggest that about 100 mg N/L might be a better target for minimum YAN requirements of Pinot Noir than 140 mg N/L suggested by others. The results from this study indicate that leaf blade N concentrations of 23-24 g N/kg DW at bloom and of 19 g N/kg DW at veraison allow for grafted Pinot Noir vines to maintain yield, reduce vigor and obtain YAN values of about 100 mg N/L.

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Corresponding petiole N concentrations are 7.0 g N/kg DW at bloom and 4.4 g N/kg DW at veraison. If producers wish to target YAN values of 140 mg N/L, then leaf blade N values should be 25-26 g N/kg DW at bloom and 21-22 g N/kg DW at veraison at current yield targets.

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Table 1 Leaf blade and petiole N concentrations at bloom and veraison in Pinot noir grapevines grown in microplots at varying rates of N from 2012 to 2014. Data are means and standard errors of the mean for each factor (n = 20 for year, n = 12 for N supply, n = 4 for Y · N Supply interaction).

		Bloom Nitrogen (g N/kg DW)		Veraison Nitrogen (g N/kg DW)	
Effect	Level	Leaf blade	Petiole	Leaf blade	Petiole
Year	2012	22.3 (0.5) b[1]	6.0 (0.2) b	17.9 (0.5) b	3.7 (0.1) c
	2013	23.3 (0.6) a	7.4 (0.2) a	19.1 (0.6) a	4.6 (0.1) a
	2014	23.6 (0.7) a	7.5 (0.2) a	19.6 (0.7) a	4.2 (0.1) b
	ANOVA sig. level (*p*)	<0.001	<0.001	<0.001	<0.001
N Supply[2]	100%	26.6 (0.4) a	8.2 (0.3) a	22.2 (0.4) a	4.8 (0.1) a
	75%	24.9 (0.3) b	7.2 (0.2) b	21.0 (0.4) b	4.6 (0.1) b
	50%	23.0 (0.3) c	6.9 (0.2) b	19.0 (0.3) c	4.2 (0.1) c
	30%	20.9 (0.3) d	6.4 (0.2) c	16.9 (0.2) d	3.8 (0.1) d
	15%	20.1 (0.3) d	6.1 (0.2) c	15.3 (0.3) e	3.6 (0.1) e
	ANOVA sig. level (*p*)	<0.001	<0.001	<0.001	<0.001
Y · N Supply	2012 - 100%	25.2 (0.2)	6.9 (0.3) cde	20.6 (0.3) bcd	4.3 (0.2)
	2012 - 75%	24.1 (0.4)	6.2 (0.2) def	19.7 (0.3) cd	4.1 (0.1)
	2012 - 50%	22.4 (0.5)	6.1 (0.1) efg	18.8 (0.4) de	3.9 (0.1)
	2012 - 30%	20.4 (0.5)	5.4 (0.1) fg	16.5 (0.4) f	3.4 (0.1)
	2012 - 15%	19.7 (0.5)	5.2 (0.1) g	14.2 (0.2) g	3.1 (0.1)
	2013 - 100%	26.4 (0.4)	8.5 (0.2) ab	22.2 (0.2) ab	5.1 (0.1)
	2013 - 75%	25.5 (0.6)	7.7 (0.2) bc	21.3 (0.4) bc	5.0 (0.1)
	2013 - 50%	23.5 (0.6)	7.5 (0.2) bc	18.9 (0.5) de	4.6 (0.1)
	2013 - 30%	21.2 (0.5)	6.8 (0.2) cde	17.2 (0.3) ef	4.2 (0.1)
	2013 -15%	20.2 (0.3)	6.5 (0.1) de	15.7 (0.4) fg	4.1 (0.1)
	2014 - 100%	28.1 (0.5)	9.3 (0.3) a	23.8 (0.4) a	5.0 (0.1)
	2014 - 75%	25.1 (0.3)	7.6 (0.2) bc	21.9 (0.5) ab	4.7 (0.1)
	2014 - 50%	23.3 (0.5)	7.1 (0.1) cd	19.3 (0.6) d	4.1 (0.1)
	2014 - 30%	21.0 (0.6)	7.0 (0.1) cde	17.0 (0.4) ef	3.7 (0.1)
	2014 - 15%	20.4 (0.6)	6.6 (0.1) de	16.2 (0.5) f	3.6 (0.1)
	ANOVA sig. level (*p*)	0.304	0.031	0.028	0.136

[1]Means followed by the same letter within an effect do not differ based on Tukey's HSD at 95% confidence.
[2]Nitrogen supply expressed as % of Control level of N supplied during fertigation events, 100% is equivalent to 7.5 mM N.

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Table 2 Veraison leaf blade N concentrations where key vine performance variables were reduced by 30% for Pinot noir grapevines grown in microplots at varying rates of N from 2012 to 2014. Data were derived from regressions of each variable relative to the Control (100% N) treatment against leaf blade N concentrations at veraison.

	Veraison Leaf Blade N Concentration (g N/kg DW)[1] where vine responses were reduced by 30% of Control vines.			
Variable	2012	2013	2014	All years
Must YAN (mg/L)	19.3	20.2	21.8	20.7
Pruning Mass (g FW)	16.9	19.8	21.3	19.7
Veraison Leaf Area (m^2)	16.4	18.4	20.0	18.4
Yield (kg)	13.5	16.7	17.9	16.1
Bloom Shoot Length (cm)	10.4	17.3	19.1	16.0
Veraison Photosynthesis ($\mu mol/m^2 \cdot s^1$)	14.4	12.8	14.5	14.0

[1] from regressions for each year independently (n = 20) or all years combined (n = 60).

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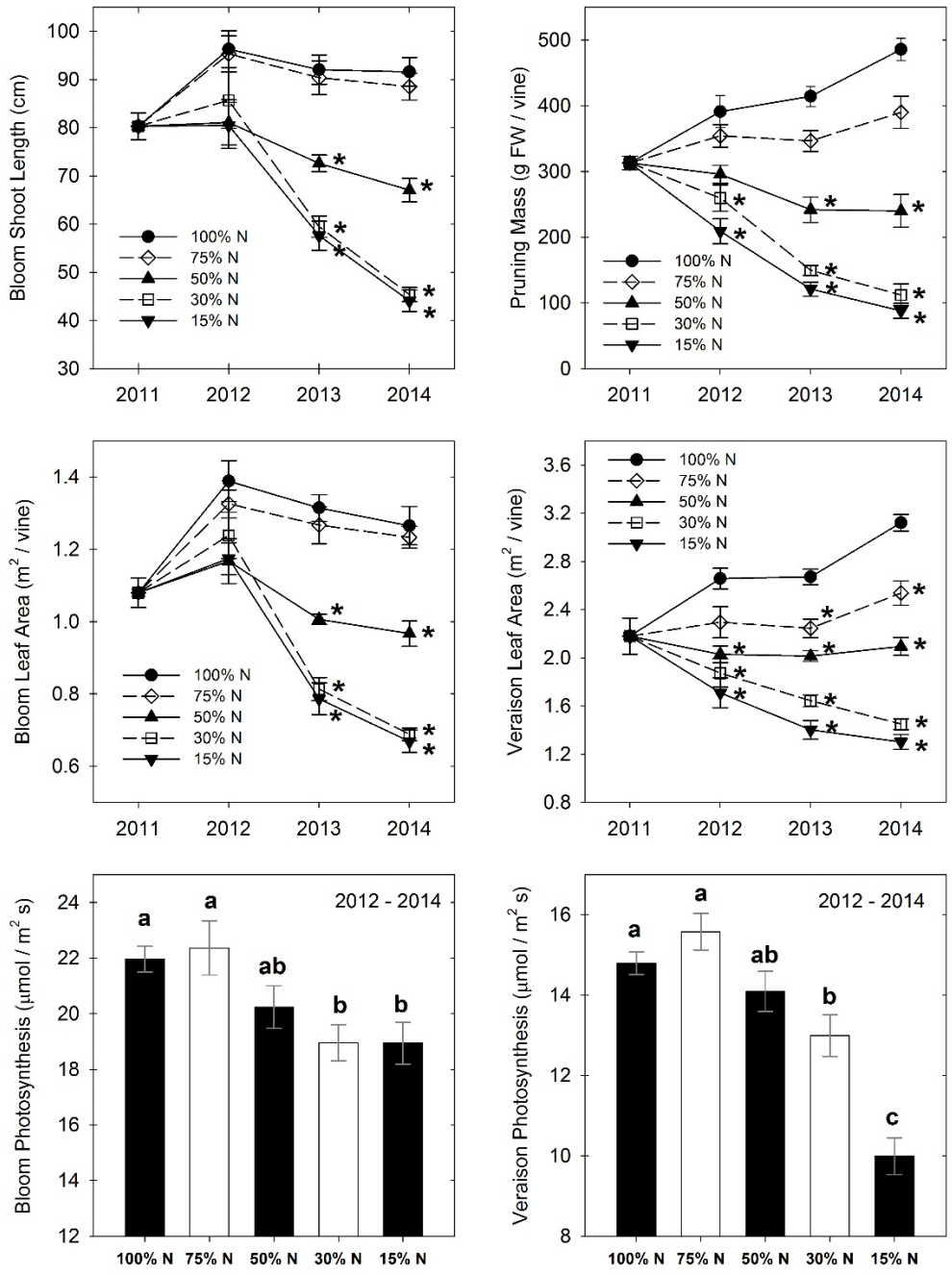

Figure 1 Effect of year and N supply on vegetative parameters of Pinot noir grown in microplots from 2012-2014. Interactive effect of year and N supply (scatter charts) on bloom shoot length, bloom leaf area, pruning mass, and veraison leaf area (n=4), and main effect of N supply (bar charts) on midday bloom and verasion single leaf photosynthesis (n=12). A * to the right of a symbol in interactive plots indicates those N treatments that differ from the Control (100%N) in each year based on Tukey's HSD at 95% confidence. Data from 2011 before N was manipulated are shown as a reference in interactive plots. Letters above means in bar charts indicate treatment differences based on Tukey's HSD at 95% confidence. Data are means and standard error of the mean for each plot.

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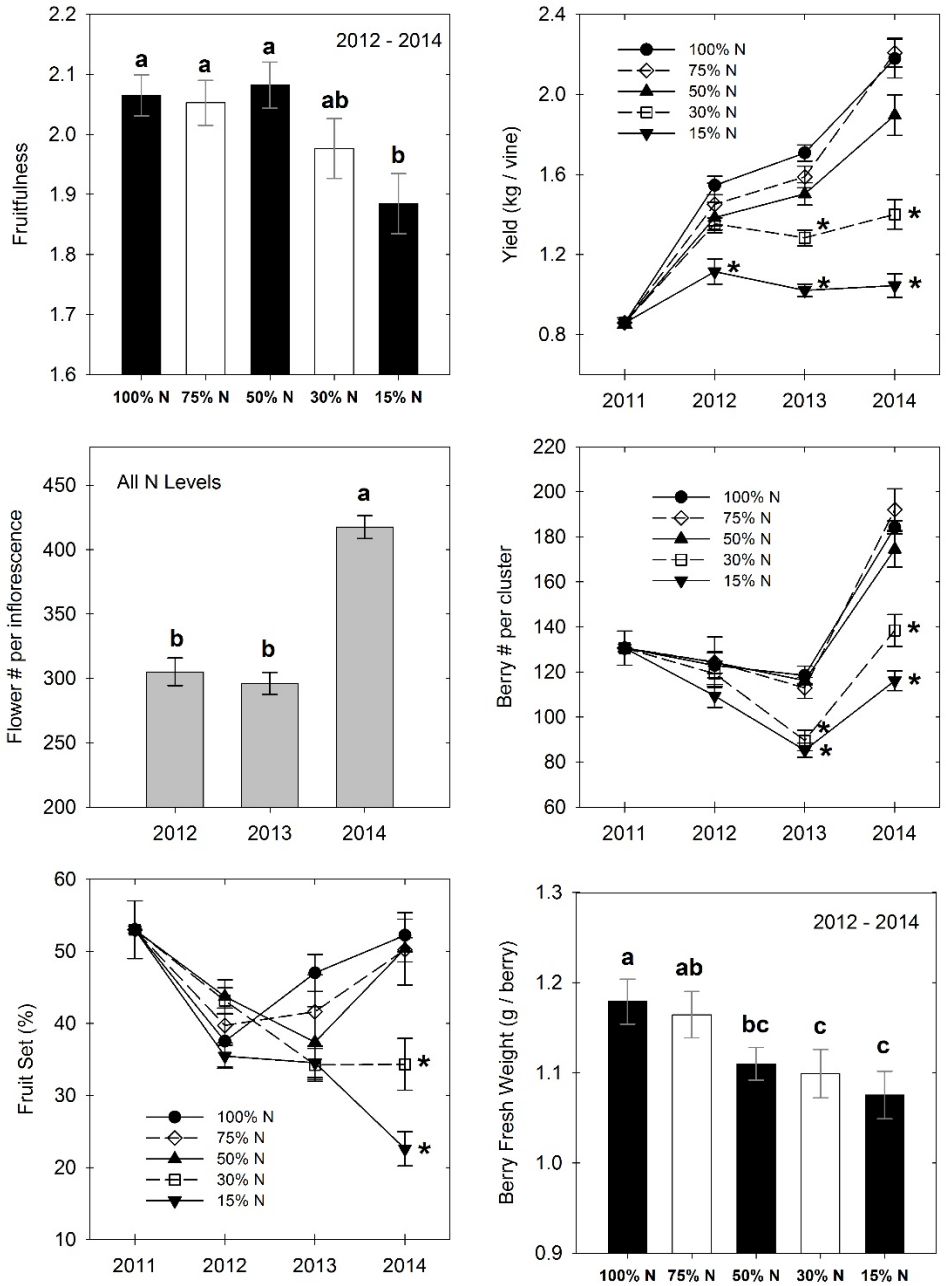

Figure 2 Effect of year and N supply on reproductive parameters of Pinot noir grown in microplots from 2012-2014. Interactive effect of year and N supply (scatter charts) on yield (n=4), fruit set (n=4) and berry number per cluster (n=4), and main effect of N supply (bar charts) on fruitfulness (inflorescences per shoot) and berry fresh mass (n=12), or main effect of year on flower number per inflorescence (n=20). A * to the right of a symbol in interactive plots indicates those N treatments that differ from the Control (100%N) in each year based on Tukey's HSD at 95% confidence. Data from 2011 before N was manipulated are shown as a reference in interactive plots. Letters above means in bar charts indicate treatment differences based on Tukey's HSD at 95% confidence. Data are means and standard error of the mean for each plot.

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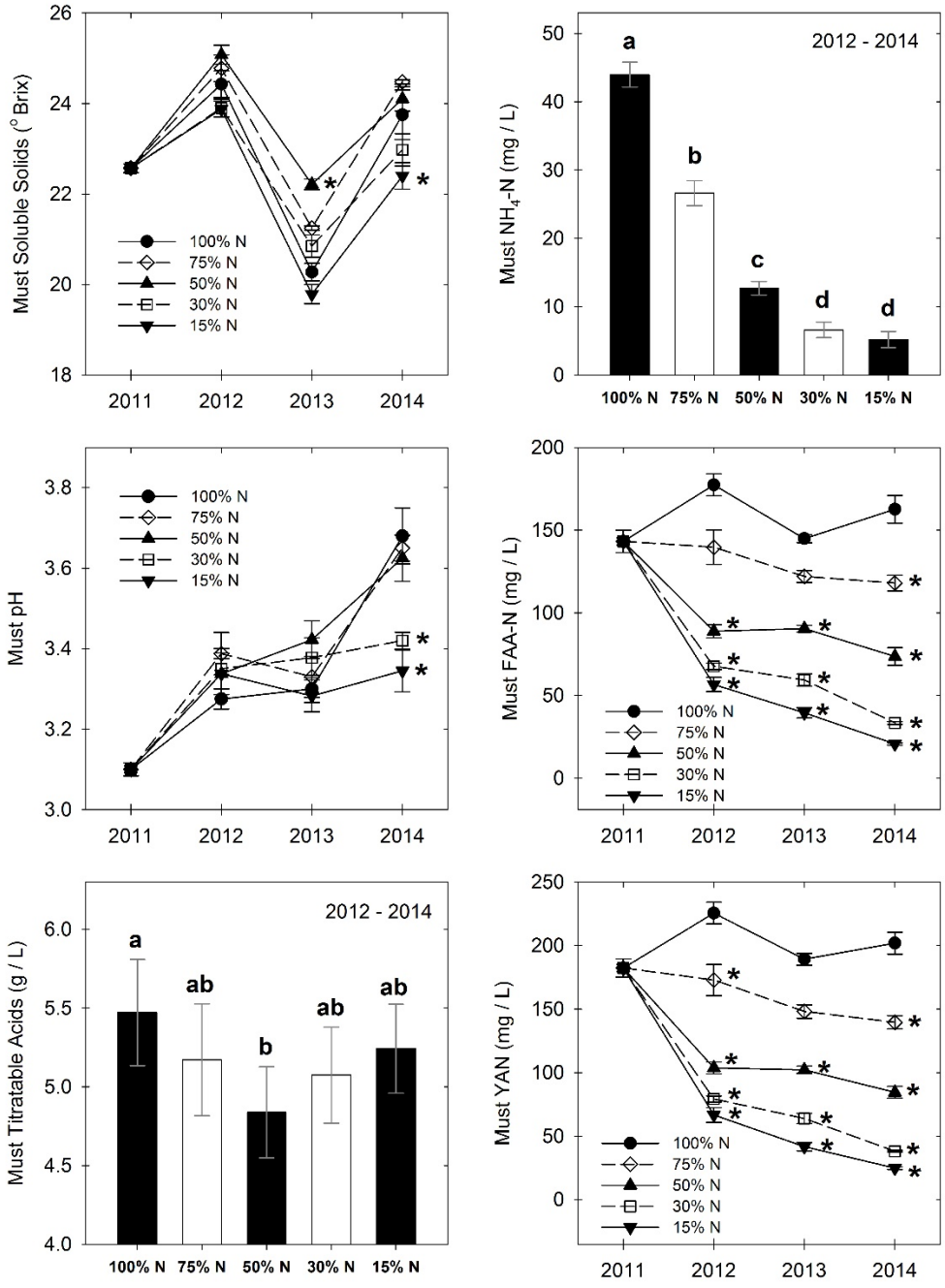

Figure 3 Effect of year and N supply on must parameters of Pinot noir grown in microplots from 2012-2014. Interactive effect of year and N supply (scatter charts) on soluble solids, pH, free amino acid-N, and YAN (n=4), and main effect of N supply (bar charts) on ammonium-N and titratable acidity (n=12). A * to the right of a symbol in interactive plots indicates those N treatments that differ from the Control (100%N) in each year based on Tukey's HSD at 95% confidence. Data from 2011 before N was manipulated are shown as a reference in interactive plots. Letters above means in bar charts indicate treatment differences based on Tukey's HSD at 95% confidence. Data are means and standard error of the mean for each plot.

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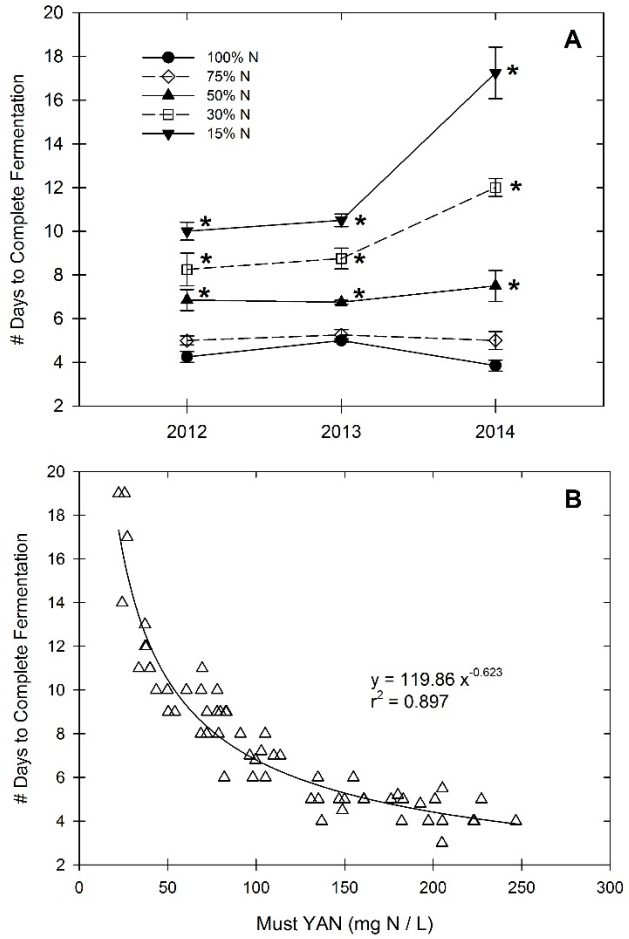

Figure 4 Effect of year and N supply on fermentation time (A) and the relationship between must YAN levels and fermentation time (B) in individual wine replicates. Data in (A) are means and standard error of the mean (n=4), and data in (B) are raw data points from all years and treatments. A * to the right of a symbol in (A) indicates those N treatments that differ from the Control (100%N) in each year based on Tukey's HSD at 95% confidence.

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Supplemental Table 1 Vine phenology, weather, and nitrogen inputs for Pinot noir grapevines grown in microplots at varying rates of N supply from 2012 to 2014.

Year/Growth stage	GDD>10°C	Mean daily temp. (°C)	Rainfall (mm)	Mean daily RH (%)	Solar radiation (MJ/m^2)	Nitrogen applied (kg/ha)[1]
2012						
Budbreak – bloom 24 Apr – 26 June	312	13.5	141	75	1356	18.5
Bloom – veraison 27 June – 30 Aug	613	19.2	15	69	1563	24.8
Veraison – harvest 31 Aug – 8 Oct	312	16.8	8	59	743	9.3
Season Total 24 Apr – 8 Oct	1237		164		3662	52.6
2013						
Budbreak – bloom 26 Apr – 10 June	273	14.7	59	71	1055	16.2
Bloom – veraison 11 June – 12 Aug	597	19.2	37	66	1607	24.8
Veraison – harvest 13 Aug – 26 Sep	417	19.1	90	73	758	7.4
Season Total 26 Apr – 26 Sep	1287		186		3420	48.4
2014						
Budbreak – bloom 16 Apr – 9 June	289	13.7	124	74	1195	17.2
Bloom – veraison 10 June – 12 Aug	641	19.8	28	66	1566	26.2
Veraison – harvest 13 Aug – 16 Sep	376	20.4	4	58	760	7.4
Season Total 16 Apr – 16 Sep	1306		156		3521	50.8

[1]N applied equates to the 100% N control treatment.

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Supplemental Table 2 Cluster zone solar exposure between veraison and harvest in Pinot noir grapevines grown in microplots at varying rates of N supply in 2013 and 2014. Data are means and standard errors of the mean at each timepoint (n = 4).

		% of PAR[1] in cluster zone	
Time of Day (PST)	N Supply[2]	Sep 10, 2013	Aug 28, 2014
9:00 AM	100%	83.8 (2.7)	79.9 (1.5)
	75%	83.9 (2.9)	81.7 (1.3)
	50%	81.2 (3.3)	79.3 (2.7)
	30%	80.6 (3.9)	74.3 (4.4)
	15%	85.3 (2.6)	79.2 (2.5)
	ANOVA sig. level (*p*)	0.780	0.449
11:00 AM	100%	65.0 (3.1)	64.9 (6.4)
	75%	72.1 (4.7)	62.7 (4.1)
	50%	67.0 (7.2)	57.7 (5.3)
	30%	72.8 (5.6)	52.3 (4.9)
	15%	78.3 (4.1)	55.4 (4.3)
	ANOVA sig. level (*p*)	0.456	0.435
1:00 PM	100%	13.7 (3.4)	14.1 (1.6) bc[3]
	75%	16.8 (3.7)	11.8 (1.1) b
	50%	15.4 (3.3)	15.5 (2.0) bc
	30%	18.7 (1.7)	21.6 (3.2) ab
	15%	26.7 (2.4)	26.8 (2.3) a
	ANOVA sig. level (*p*)	0.058	0.001
3:00 PM	100%	12.8 (2.2) b	37.5 (2.1) b
	75%	15.5 (1.5) b	33.3 (2.5) b
	50%	18.3 (3.5) b	41.7 (2.1) b
	30%	42.1 (3.7) a	58.6 (1.1) a
	15%	40.3 (1.8) a	58.0 (3.3) a
	ANOVA sig. level (*p*)	<0.001	<0.001
5:00 PM	100%	70.0 (5.0) b	51.6 (3.2) b
	75%	76.1 (3.6) ab	54.9 (2.2) b
	50%	77.0 (1.7) ab	60.9 (3.4) ab
	30%	84.0 (2.5) a	67.8 (2.3) a
	15%	83.6 (1.9) ab	71.3 (3.3) a
	ANOVA sig. level (*p*)	0.037	0.001

[1]PAR photosynthetically active radiation (400-700 nm)
[2]N supply expressed as % of Control level of N supplied during fertigation events (Control = 7.5 mM total N).
[3]Means followed by the same letter in a column within each time do not differ based on Tukey's HSD at 95% confidence.

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Supplemental Table 3 Soil water content, θ_v, averaged over the growing season within each N supply treatment for Pinot noir grapevines grown in microplots from 2012 to 2014. Data are means and standard errors of the mean for each year.

	Soil water content (% volumetric)		
N Supply	2012	2013	2014
100%	18.2 (0.2)	18.9 (0.2)	19.0 (0.3) b[1]
75%	18.1 (0.2)	18.8 (0.2)	18.6 (0.3) b
50%	18.3 (0.2)	18.9 (0.3)	18.7 (0.3) b
30%	18.6 (0.2)	19.3 (0.3)	20.2 (0.3) a
15%	18.6 (0.2)	19.4 (0.2)	20.0 (0.3) a
ANOVA sig. level	0.467	0.217	0.005
n	316	284	252

[1]Means followed by the same letter do not differ based on Tukey's HSD at 95% confidence.