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Research Article

The Effect of Postharvest Defoliation on Carbon and Nitrogen Resources of High Yielding Sauvignon blanc Grapevines

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Abstract: We quantified the importance of post-harvest carbohydrate assimilation and nitrogen availability to replenish vine reserves, additional to maintaining optimal growth, productivity and fruit quality of high yielding vigorous Sauvignon blanc grapevines. To create different carbohydrate (CHO) and nitrogen (N) reserve concentrations, our factorial-design trial consisted of a post-harvest defoliation treatment overlaid with a pruning treatment for which 48 and 72 nodes were retained on, respectively, four- and six-cane vertical shoot positioned (VSP) vines. For defoliation (Defol), immediately after fruit harvest, all the leaves of the vines were removed, compared to foliated vines (Fol) that went through normal senescence. From just after ecto-dormancy in 2008, samples of root and trunk tissue were taken throughout the years for CHO and N analyses, and results compared with annual yield data. In the seasons following the treatments, both the defoliation and node number treatments reduced vine growth and yield. Additionally, differences in CHO and N of the permanent structure were found. Depleted winter reserves

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in trunk and root were replenished during the next growth cycle, suggesting that grapevine N and CHO partitioning favor survival of the permanent structure over increasing vine size and yield. However, after two consecutive years of defoliation, the cumulative effects of smaller, less fruitful canes from year 1 and reduced carbohydrates from the subsequent year, did reduce both yield and vegetative growth in the third growing season. Therefore, even the short-lived post-harvest canopy in cool climates contributes to the vine CHO economy. Defoliation or excessive crop loads affected carbohydrate reserves in vines but only after a few consecutive years of low recharge was this manifested in lower yields and poorer vegetative growth.

Key words: carbohydrate, nitrogen, post-harvest defoliation, crop load, reserves

Introduction

Carbohydrates (CHO) are the direct products of photosynthesis and are therefore the primary energy storage compounds found in plants, from which most organic compounds are synthesized (Kozlowski and Pallardy 1997). The CHO accumulation in vine reserve organs depends on the photosynthesis rate and the CHO partitioning between shoot, root, and fruit growth and storage (Howell 2001). In grapevines the greatest proportion of total seasonally assimilated carbon is incorporated into structural cellulose compounds in roots, stems and shoots (Winkler and Williams 1938) and these complex structural CHOs cannot be remobilized, as plants lack the enzymes to degrade cellulose (Kozlowski and Pallardy 1997). Resumption of vegetative and reproductive growth in the new season depends on carbon stored as non-structural CHO reserves, mainly in the form of starch (Stoev et al. 1966). Other storage forms of CHOs are soluble sugars, mainly sucrose, glucose and fructose (Jones et al. 1999, Sepœlveda and Kliewer 1986). These non-structural CHO reserves support the production of new roots, shoots, leaves and clusters early in the new season (Greven et al. 2005). The storage of non-structural CHO is generally highest in the root tissue of grapevines (Bates et al. 2002, Uys and Orffer 1983) and root-derived CHO was found to be the principal reserve source for the annual re-establishment of growth in grapevines (Bates et al. 2002, Loescher et al. 1990, Zapata et al. 2004a). Reserve CHOs

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accumulate to their highest concentrations in all plant organs by leaf fall in autumn (Bennett et al. 2005, Williams 1996, Winkler and Williams 1945) and are mostly retained during ecto-dormancy (abbreviated to 'dormancy' throughout this work), apart from small respiratory losses. During leaf senescence in autumn, hydrolytic enzymes break down leaf proteins, carbohydrates and nucleic acids, which are transported in the phloem back into the permanent plant structure, where they are stored during dormancy and are re-mobilized in spring for early growth. Many minerals are also transported out of senescing leaves back into the vine's permanent structure. It has been shown that 78% of reserve starch in the vine present at budburst is used for shoot and root growth by the time of bloom (Bates et al. 2002).

In autumn, frosts can cause virtually instantaneous leaf death, premature leaf abscission, and loss of post-harvest photosynthate production as well as loss of an important pool of organic and inorganic nutrients. With almost 2000 wind machines in Marlborough to combat potential spring frosts, the question is raised whether it would be desirable or economic to use these machines to prevent autumn frost damage. Management practices after harvest such as pre-leaf fall pruning may also alter the capacity of the vine to "recycle" nutrients and replenish storage reserves.

The period immediately following harvest is important for root growth and nutrient uptake in grapevines (Conradie 1986, Mullins et al. 1992). Sufficient late-season nitrogen uptake and reserve accumulation is essential, since early nitrogen demand in spring cannot be met by root uptake (Conradie 1986, Löhnertz et al. 1989, Peacock et al. 1989). Imbalance in source-sink relationships in late season may limit potential assimilate supply to the roots in autumn. Autumn-stored assimilates are preferentially used for early shoot growth the following spring (Yang and Hori 1979).

Mobilization of CHO reserves in spring supplies energy and carbon skeletons for new shoot growth and flower development until photosynthesis becomes the primary source of carbon. Therefore, the post-harvest period may be important in determining vine vigor and productivity in the following season. It also allows the remobilization of nitrogen from the senescing leaves to the trunk and roots.

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The capacity for reserve replenishment increases after mid berry ripening (Candolfi-Vasconcelos et al. 1994a). Loss of photosynthetically active leaf area or excessive crop loads may deplete storage reserves (Candolfi-Vasconcelos et al. 1994b). High crop loads may reduce the amount of accumulated vine reserves before harvest, and the delayed fruit maturation may shorten the post-harvest period (Greven et al. 2015). These effects reduce the vine's capacity to accumulate carbohydrates for the following season. Some studies, however, found no effect of crop load (Bravdo et al. 1985) or harvest date (Wample and Bary 1992) on cane reserve carbohydrate concentration, despite reasonably high crop loads. The lack of effect on CHO reserves could not be ascribed to sink limitation, since both studies reported moderate to high crop loads, but it could be explained by the ability of the vine to maintain equilibrium by adjusting physiological processes (Poni et al. 2006, Smith and Holzapfel 2009).

Photosynthesis declines after harvest (Scholefield et al. 1978) along with leaf nitrogen concentration (Williams and Smith 1985), but remains important for reserve replenishment (Loescher et al. 1990). Scholefield et al. (1978) showed that leaf removal at harvest could lead to yield reduction of more than 50% in the following year in Sultana grapes. Fruit set depends strongly on the supply of carbohydrates to the inflorescences, which, in turn, is determined by the carbon balance between vine reserve status, current photosynthesis, and demand by competing sinks (Zapata et al. 2004). Holzapfel et al. (2006) showed that conditions during the post-harvest period could affect at least three stages of reproductive development: initiation, differentiation, and fruit set. However, studies by Trought et al. (2011) on pruning time in Sauvignon blanc showed no influence of pruning only 10 days after harvest, on yield or carbohydrate reserves in the following season.

Most factors that reduce storage CHO may concomitantly reduce nitrogen (N) reserves in vines (Loescher et al. 1990). Nitrogen is the mineral nutrient for which vines have the highest demand and the nutrient that most often limits growth (Keller 2010). Cheng et al. (2004) showed that differences in vegetative growth and yield were mainly determined by reserve N and not CHO. Nitrogen together with carbon is incorporated in many physiologically important plant compounds such as amino acids, proteins

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and enzymes (Kozlowski and Pallardy 1997). Leaf area development during spring growth was found to be directly correlated to N mobilization from wood (Weyand and Schultz 2006). Many studies have shown that CHO reserves are used in the development of new grapevine shoots and inflorescences in the following spring until shoots develop eight leaves and start exporting CHO (Scholefield et al. 1978, Yang and Hori 1979). Investigations into the effect of early pruning and hence leaf removal from vines has been undertaken in Australia on Shiraz vines in Wagga Wagga (Field et al. 2009) and in Semillon vines in Riverina (Holzapfel et al. 2006). Both these areas are warm climate grape growing regions where leaves stay on the vines for many weeks after harvest replenishing vine reserves. This longer period from harvest until leaf senescence (Field et al. 2009, Holzapfel et al. 2006) may be the main reason vineyards in warmer regions can support higher crop loads than those in cooler regions. In highly productive vineyards, it is important to sustain yields through good management and to optimize vine vigor and productivity for the subsequent season by manipulating the length and effectiveness of the post-harvest period, for instance by frost protection, irrigation, and nutrition management. However, in cool climate regions such as in New Zealand, where autumn temperatures are often limiting, it may be argued that post-harvest CHO accumulation is insufficient to warrant the expense of cultural practices aimed at maintaining an active canopy.

The present work investigates the role of the post-harvest period of high yielding Sauvignon blanc vines in the Marlborough, New Zealand, region on vine carbon and nitrogen status as vines approach onset of winter dormancy. For this purpose, besides the post-harvest leaf removal, an additional treatment was applied: increasing the number of canes laid down at pruning time from the standard four canes for Marlborough Sauvignon blanc, to six canes. It was hypothized that the additional fruit produced from these nodes would increase the drain on vine reserves and therefore emphasize the importance of these reserves.

The objectives of this work were: A) to quantify photosynthetic net carbon gain during the period after harvest until leaf fall; B) to investigate whether it is possible to maintain high crop yields without the

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contribution of post-harvest vine photosynthesis; and C) to evaluate whether post-harvest vine management practices such as frost protection are cost-effective in the long term.

Materials and Methods

This experiment was conducted in a high vigor Sauvignon blanc vineyard (clone UCD1MS on Schwarzmann rootstock, Vitis riparia x Vitis rupestris) located at Rowley in Blenheim, Marlborough (lat. 41°29'N; long. 173°57'E; 7 m asl). Gladstones (1992) describes Marlborough as a typical cool climate winegrowing region (Figure 1). Marlborough has a Heliothermal index (Tonietto & Carbonneau 2004), value of 1613, within the 1500-1800 class interval, suitable for cool climate viticulture.

Vines were planted in 2003 on a deep well drained silt-loam soil. The trickle-irrigated vineyard was managed to best industry practice following New Zealand Sustainable Winegrowing practice (http://www.nzwine.com/swnz/). Vine rows at the trial site were oriented NNW-SSE with 2.8 x 1.8 m row-by-vine spacing. The lowest fruiting wire was 90 cm from the ground, the top fruiting wire 110 cm. Vines were cane pruned to four 12-node canes (Marlborough Sauvignon blanc standard crop load, 48 nodes) or six (very high crop load, 72 nodes) 12-node canes (48N and 72N respectively). An additional fruiting wire was placed on the other side of the post, parallel to the top fruiting wire at 110 cm, to accommodate the two additional canes of the 72N treatment. All shoots were trained on vertical shoot positioned (VSP) vines upwards and positioned between three pairs of movable wires, as is typical in the region. A factorial design of node number x harvest defoliation was used. All the leaves from half the vines were removed immediately after harvest on 16 April 2009, 21 April 2010 and 19 April 2011. The experimental unit was a group of four similar, adjacent vines between two posts, and each treatment was replicated six times.

Gas-exchange. Stomatal conductance (g_s), photosynthesis (A), transpiration (E), water use efficiency (A/g_s) and sub-stomatal CO_2 concentration (C_i) were measured on well-exposed primary leaves arising from the tenth node from the shoot base of two representative shoots on each plot at two-week intervals from about three weeks after flowering until leaf fall, using a portable infra-red gas analyzer

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(Ciras-2, PP SYSTEMS, Hitchin, Herts SG5 1RT UK). In order to ensure measurements were fully comparable, gas exchange measurements were performed only under fully saturated light conditions and therefore the intervals were ± 1 day between the fortnightly measurements.

Chlorophyll content. Leaf greenness was measured non-destructively with a SPAD-502 chlorophyll meter (Minolta) on the same dates on the same leaves sampled for gas exchange. Six readings were taken per data leaf and then averaged. Chlorophyll content was calculated using the method described by Candolfi-Vasconcelos et al. (1994b).

Yield components and fruit composition. The fruit were harvested on 15 April 2009, 20 April 2010 and 19 April 2011. At harvest, the cluster number and yield per vine were recorded from which cluster weight, clusters per shoot and fruitfulness (fruit weight per shoot) were calculated. A sample of eight clusters per replicate, was collected randomly from both sides of the canopy, from lower and upper canes and different positions within the shoot. After stripping all berries from the eight clusters, a sub-sample of 100 berries per replicate was used to estimate berry weights and berries per cluster. The sample was crushed for determination of total soluble solids content (TSC), pH and titratable acidity.

Canopy development and vine vigor. Leaf area was measured at defoliation time after harvest on the vines used for the defoliation treatment. All leaves from these vines were removed and weighed. From each bay, the leaf area of a random sub-sample of 100 leaves was measured using a Li-Cor leaf area meter (LI-3100, Li-Cor Inc., USA). The total leaf area per bay was estimated from the total weight of the leaves of the four vines in the bay and the weight-area relationship from the 100-leaf sample. For comparison, Point Quadrat measurements of 48 points for one vine per plot were taken around véraison. The leaf area was used to calculate the leaf/fruit ratio. Over winter, the dormant canopy was assessed and then all vines were pruned back to their treatment node number. The canopy assessment included a count of all blind nodes (nodes that failed to break bud) and all shoots per vine. Canes were weighed to calculate total vine pruning weight, mean cane weight, clusters per shoot and the Ravaz index. The Ravaz index represents the ratio of reproductive to vegetative growth and balanced vines should remain between

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5-7 (Ravaz, 1903). Because of its practicality this measurement is readily used in Marlborough Sauvignon blanc. Total vine bud burst was calculated by dividing shoots per vine by nodes per vine at the start of the season.

Carbohydrate and nitrogen concentration (CHO and N respectively) of permanent structure. Wood samples from trunk and roots were collected, starting just after the dormant period 2008, at the five-leaf stage. From there onwards, samples were collected at bloom, lag phase, véraison, mid-ripening, harvest, leaf fall, dormancy and five-leaf stage again through to véraison in 2012. Trunk wood samples were taken from the midsection of the trunk of one vine in each plot to provide an estimate of the CHO status of grapevine trunks. For this, the old bark was peeled off and using a chisel, a small piece of wood and bark of approximately 2 cm in length, 1 cm in width and 3 mm in depth was collected (Candolfi-Vasconcelos and Koblet 1990). Root samples were taken from a mixture of old and younger roots varying from 1 to 5 mm in diameter at a depth of about 150 mm. The samples (0.8 to 1.2 cm^3 in volume) were freeze dried and stored at -20°C, then ground to a powder using a ring grinder (Rocklabs Ltd, Auckland, New Zealand). The carbohydrate analysis was undertaken on a 50-mg subsample of ground wood. Carbohydrates were ethanol extracted, analyzed using the method described by Smith et al. (1992) and reported as total soluble carbohydrates (TSC) and starch. Total nitrogen (N) was determined using a thermal combustion analyzer (VarioMAX, Elementar Analysensysteme GmbH, Germany). Because of large changes in the CHO found between mid-ripening and harvest, an additional sample was collected pre-harvest in the 2010 and 2011 seasons.

Data were submitted to analysis of variance using the Genstat 10.2 statistical package (Lawes Agricultural Trust). Mean separations were determined by least significant differences (LSD) at the 5% level of significance.

Results and Discussion

Photosynthesis and gas exchange. Overall photosynthetic rates of between 12 and 20 μmol $CHO/m^2/s$, were typical for those reported for normal grape leaf photosynthesis for the times of year. In

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2009, leaf photosynthesis was slightly lower early in the season on 72N vines but rates were similar during the ripening period. This was likely due to the initial 3-10% lower chlorophyll content in 72N vines (Figure 2D). Other key gas exchange parameters (stomatal conductance and transpiration) followed the same patterns for both treatments. No defoliation treatment had been applied at this stage of the study.

In 2010, with the exception of a short period in mid-February when 72N vines had a slightly higher photosynthetic rate, there were no gas exchange differences in response to the number of retained node number (Figure 3). The 48N vines had a 6-8% lower chlorophyll content throughout the measurement period during 2010, this was counter to what was found during 2009 and 2011 (Figures 2 and 4). The 2009 post-harvest defoliation did not affect gas exchange performance in 2010 (Figure 3) nor did eliminating post-harvest photosynthesis by defoliation straight after harvest affect gas-exchange performance during 2011. However, defoliated vines showed slightly lower leaf chlorophyll content following two consecutive seasons of defoliation (Figure 4).

Many studies have shown that photosynthesis adjusts dynamically to changes in sink demand (Candolfi-Vasconcelos et al. 1994b, Kliewer and Antcliff 1970, Petrie et al. 2000). However, none was found in the present study (Figures 2, 3 and 4). Nor were any treatment differences found in canopy density measured by Point Quadrat in 2010 or 2011 (data not shown). Because neither the increase in node number (Table 1) nor post-harvest defoliation (Table 2) significantly changed leaf area or fruit yield, the absence of significant differences in gas-exchange during the 2010 and 2011 seasons is not contradictory to the literature.

In interpreting the gas exchange results, it should be kept in mind that despite the higher number of nodes retained, the 72N treatment increased yields only in the first season (Table 1), in agreement with earlier work with Sauvignon blanc in Marlborough (Greven et al. 2014).

Vegetative growth and yield. By increasing the number of nodes from 48 to 72, highly significant increases for shoot number and therefore yield per vine were found in 2009 (Table 1). The number of clusters per shoot was not different, which is consistent with inflorescence primordia initiation

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234 occurring during the previous season, when all vines had the same retained node number. Berries per
235 cluster and berry weight were not affected by increasing the number of nodes, although berry weight
236 showed a trend to be slightly smaller (P = 0.07). Fruit yield on these vines, while significantly greater,
237 was increased by 21%, despite the node number set by pruning to be 41% higher for the 72N treatment.
238 This difference between potential and actual yield increase was largely accounted for by the significantly
239 lower budburst on 72N vines (and therefore a 84% higher number of blind buds) as well as a 27% lower
240 mean cane weights (Table 1). No difference was found in leaf area index, which combined with the 21%
241 higher yield for 72N resulted in a 13% lower leaf/fruit ratio.

242 When the 48N and 72N treatments were again applied in the 2010 season, the number of shoots
243 per vine remained significantly different. However, with the shoots arising from the 72N treatment now
244 originating from higher yielding vines of the previous season, the number of blind buds was 75% higher
245 in the 72N treatment than in the 48N treatment, and clusters/shoot, cluster weight and berry weight were
246 all reduced (Table 1). All these yield components had not been different in the previous year when both
247 treatments were applied for the first time. The lower mean cane weight, due to the higher shoot number in
248 72N vines resulted in lower fruitfulness per shoot so that in 2010, the yields of 48N and 72N vines were
249 not different. This is in contrast to a 21% difference in yield the previous year. Similarly, no significant
250 differences in yield were found in 2011. The higher number of shoots on the 72N vines resulted in lower
251 berry and cluster weight, decreasing fruitfulness . The lower cluster weight was likely due to three
252 consecutive years of lower reserves. These results mirror the outcome from a long-term study done in
253 Marlborough with vines pruned to 24, 36, 48, 60 or 72 nodes, where strong response mechanisms that
254 changed yield components were found according to the number of nodes left at pruning (Greven et al.
255 2014).

256 Although the differences in fruit composition were not large in any year (Table 1), delayed
257 maturity in cool climate regions can lead to sub-optimal total soluble solids (TSS) in fruit at harvest. It is
258 therefore essential to avoid yields above which a target °Brix maturity value (measuring the TSS content)

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are unlikely to be achieved (Greven et al. 2015). In 2009, as a consequence of the higher productivity of the 72-node vines, there was a small delay in fruit reaching the targeted maturity, evident from the significantly lower TSS at harvest. In 2010, maturation conditions were very favourable. No differences were found for TSS or °Brix between pruning treatments, with all fruit reaching 23 °Brix values (Table 1). No differences were found in juice pH or TA between the treatments in any of the years. In 2011, fruit ripening was slower but all fruit reached the target 20.5 °Brix threshold at the same time. Because in 2010 and 2011 the yields of both treatments were identical, these similarities in fruit maturity attributes between 48N and 72N vines were not unexpected (Greven et al. 2014). Despite both treatments showing lower yields in 2010, 72N with 50% more nodes laid down, dropped yield to the level of the 48N vines. This relative yield reduction for 72N vines between the first and second year of pruning conversion (Table 1) suggests a cumulative reduction of CHOs over time.

No differences in leaf area per vine or leaf area index (LAI) was found in 2009, between 48N- and 72N vines (Table 1) despite the significantly higher shoot number/vine for 72N vines. The additional nodes laid down did cause a reduced shoot number per node due to blind budding (Table 1). Yet, the 72N vines produced a 21% higher yield. However, in 2010 also no differences were found in LAI between 48N and 72N vines but this time around there was no difference in yield. We suggest the change in leaf/fruit ration between these two years was because 72N vines in 2010 developed from 72-node vines in 2009 instead of from 48-node vines in 2008. This forced the 72N vine into a new equilibrium between fruit and vegetative growth already from the second year onwards (Greven et al. 2014, Howell 2001).

Winter canopy assessment after harvest in 2009 showed a significant reduction in vine pruning weight and cane size and an increase in blind nodes in 72N vines (Table 1). This suggests priority partitioning of resources to fruit development early in the season, developing a higher crop on the 72N vines, at the expense of shoot vegetative development. In 2010 and 2011, despite the higher number of shoots, the total vine pruning weight for 72N vines was not different from that in 48N vines, resulting in a much lower individual cane weight (Table 1). As a consequence, there was no difference in the Ravaz

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Index (kg fruit/kg pruning wood) of the pruning treatments. Despite some significant differences between the treatments, in all years of the trial all vines stayed within 3-6 value which is the common Ravaz index value for Marlborough (Martin, pers. comm.).

Defoliation at harvest in 2009 did not affect the 2010 yield or yield components and did not affect fruit composition (Table 2). However, when this treatment was applied two years in a row, defoliated vines in 2011 showed significantly lower yields as a result of fewer berries/cluster and hence lower cluster weights (Table 2). Similar cumulative effects were reported by Holzapfel et al. (2006) for Semillon in the Riverina region in Australia, where defoliation reduced yield in the subsequent year by 21%, but the yield reduction reached 50% after two years of post-harvest defoliation. The result from the present study, however, contradicts the findings by Trought et al. (2011), where no differences in yield were reported after pruning vines only 10 days after harvest. It has been observed that under cool climate Marlborough conditions, vines tend to senesce soon after harvest (Bennett et al. 2005, Petrie et al. 2000, Trought et al. 2011).

Across all treatments, post-harvest defoliation in 2009 resulted in significantly lower shoot numbers per vine, an increase in blind buds, and lower pruning weight and cane weight in 2010 (Table 2). This does signal that post-harvest defoliation may reduce vine vegetative development in the following season. However, no differences in leaf layer number were found in any season after post-harvest defoliation (data not shown).

A cumulative effect on yield of defoliation over a number of years, when applied early in the season at or shortly after full bloom, has been shown by Candolfi-Vasconcelos and Koblet (1990). This cumulative effect of defoliation on yield has also been demonstrated by Holzapfel et al. (2006) in Riverina, Australia. The very high temperatures in Riverina result in fast fruit maturation and therefore a long period of leaves on vines to recharge the CHO reserves. In Marlborough, a cool climate region, this period is very short (Petrie et a. 2000). However, this work shows that even under those cool climate

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conditions the lack of CHO and N accumulation can have a significant effect on yield when occurring in consecutive years.

Surprisingly, only for a few parameters the defoliation and the pruning treatment effects were compounding. A slight interaction (P= 0.04) was found in 2010 where the shoots per vine for the defoliation treatment were only lower for 72N vines. While the trend was the same but not significant in 2010, in 2011, total vine % budburst decreased for all treatments when comparing 48N with 72N vines but more so for defoliated vines. The opposite was true for blind nodes per vine (Table 3). Yield components showed some interactions especially after three years of treatments. In 2011, defoliation reduced clusters/vine more for 48N than for 72N and berry weight was smallest for defoliated 72N vines. A similar but non-significant trend was found for cluster weight in 2010 and 2011 and clusters/vine and berry weight in 2010. Despite the treatment interactions observed in specific yield components, there was no interaction between defoliation and laid down nodes for yield/vine.

Pruning weight and cane mass reduced over time and lower cane mass may affect productivity in the following season.

Total nitrogen. Total N in the roots and trunk followed the same seasonal patterns during all three seasons of the experiment, varying the highest value between 0.8 and 1.7 mg/g dry matter (DM) for roots and between 0.3 and 0.8 mg/g DM for trunk. Nitrogen concentrations in both roots and trunk were highest just after budburst in early spring (five-leaf stage), after which they declined to a minimum at véraison. During the rest of the growing season N remained fairly low but increased sharply after harvest and was restored to close to annual maxima around leaf fall and remained at high concentrations until early spring the following year (Figures 5 and 6).

No difference was found between defoliated and foliated vines during any of the periods of low N between bloom and harvest (Figure 5). Dormant period N reserves were not monitored prior to the start of the experiment in the spring of the 2008-2009 season. During the dormant period before the start of the 2010 season, slightly lower N was measured in defoliated vines. However during the third and last season

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(2011), vine trunks submitted to, by now three years of post-harvest defoliation, had 50% lower N than of foliated vines due to a continued reduction of build-up of N reserves. This overall trend for lower vine N is likely to have been a contributing factor for the reduced fruitfulness of defoliated vines seen in 2011. Root N was less affected by defoliation than trunk N.

Changes in trunk total N were initially unaffected by the pruning treatment, but in the second season there was a slight tendency to a lower seasonal minimum N in the 72N vines (Figure 6). Differences in root N associated with pruning treatment were more marked, with 72N vines showing a progressively slower recovery of N in the post-véraison and dormant periods than observed for 48N vines.

The trends found in this study only partially correspond with South African studies with Chenin blanc (Conradie 1986), where N uptake was reported from bloom to véraison and after harvest. Figures 5 and 6 indicate a clear increase of N after harvest, but the pattern between bloom and véraison is for a decline rather than an increase. This difference is likely to be because our present study measured only N in roots and trunk and not N that was incorporated into the fresh vegetative parts and the developing fruit. This study and others (Conradie 1986, Mullins et al. 1992) clearly illustrate a reduction in permanent structure N until harvest, suggesting a strong demand for N by the developing canopy and fruit. Reduced N in the trunks of defoliated vines (Figure 6) may affect canopy development in the following season and cumulatively may reduce vine development, productivity and fruit quality. Additionally, Eltom et al. (2014) been suggested that lower cane mass as such may affect productivity the following season.

Over the three years of the trial it was shown that despite a sharp drop in N after the start of the growing season, every year the non-defoliated vines were able to replenish N to at least the pre-dormancy concentrations. However, post-harvest defoliation did result in a reduction of trunk N (Figure 6). Loescher et al. (1990) also found that late-season defoliation could result in nitrogen deficiency in the following season. Complete defoliation would prohibit nutrient resorption from the leaves and reallocation to storage, but would also have greatly reduced late-season nutrient uptake from the soil because of the elimination of transpiration. Lower spur nutrient contents following harvest defoliation of

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Semillion vines was also observed by Holzapfel et al. (2006) who proposed that both vegetative growth and yield the following years may have been more affected by the lower nitrogen status than carbohydrate status was. Peacock et al. (1989) showed that labeled N stored in roots during dormancy was remobilized early to support spring growth, with contribution from new-season N uptake being insignificant in new leaf tissue until bloom. Our data show a strong decline in N in both trunk and roots from early in the growing season until well past bloom. This decline was followed by an equally strong accumulation of N from the post-véraison period onwards throughout winter, until spring (Figures 5 and 6).

N in vine roots increased continuously from harvest, through the dormant period, until early in the next season. This suggests that vine root systems remain active in N uptake throughout winter in the Marlborough climate. Reduced N in roots occurred with high node and defoliation treatments. Both treatments could reasonably lead to reduced root development and activity in parallel with altered canopy responses, which could lead to small but progressive cumulative decline in root N

Carbohydrates. The total non-structural carbohydrates present in trunk and roots are available in soluble (sugars) and insoluble forms (starch). Starch is the stable form in storage tissues during dormancy and requires hydrolysis in spring before transport through the xylem as total soluble carbohydrates (TSC). From the outset, dynamic yearly changes in CHO were evident in both the root and trunk tissues. The total non-structural CHO was mainly made up of starch at most sampling time points, with TSC making up only 20% of CHO in the roots and 10-60% in the trunks (Figure 7). When comparing the root and trunk CHO, it is clear that the TSC dynamics were very similar, but with almost three times the concentration in the trunk during dormancy (85-90 mg/g DW) than in the root (25-35 mg/g DW), but with both tissues reaching very similar minimum concentrations (10-20 mg/g DW) during the growing season. This was not true for starch: despite the changes in concentration being similar and parallel, after harvest, trunk starch tended to be reduced faster than root starch was, and subsequently increased less rapidly after dormancy.

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Figures 7 and 8 show that root starch and trunk starch follow a similar and parallel trend with higher concentrations (120-160 mg/g DW) towards harvest and lower (40-100 mg/g DW) during dormancy and early spring growth, but both curves demonstrate considerable dynamic variability in CHO at any period.

Total soluble carbohydrates (TSC). TSC in both trunk and roots declined during the first year in the post-bloom period from lag phase to mid-ripening. During the two following years, TSC was slowly increasing from bloom to mid-ripening, thereafter recovering rapidly to reach a maximum concentration during vine dormancy. From harvest onwards, TSC in the roots were maintained at a constant but lower concentration than those in the trunk (Figures 7 and 8).

In the trunk, the TSC concentration increased strongly into the dormant period. Low CHO reserves have been shown to reduce winter hardiness (Wample and Bary 1992). The accumulation of sugars in the trunk approaching dormancy might therefore be attributed to the vine acclimation to low temperatures (Hamman Jr et al. 1996). These sugars were likely to have been converted from starch, as trunk starch decreased towards dormancy, or could have been the result of new assimilate from the leaf canopy during the reserve replenishment period (Figures 7 and 8). Early leaf drop or defoliation immediately after harvest therefore reduces a leaf supply of carbohydrate needed for the hardening off the shoots before winter. We suggest that this was compensated for by the stronger remobilization of reserve carbohydrates in the defoliated vines (Figures 7 and 8). Unfortunately, most of these carbohydrates are being lost as most of the canes are being pruned from the vines at winter pruning. The high TSC concentrations may be evidence of the trunk as a transition buffer pool between vine canes and root CHO storage at the onset of dormancy.

Starch. Trunk starch (Figures 7 and 8) was at its lowest point (20 mg/g DW) at the lag-phase after bloom, but increased rapidly until harvest, reaching 170 mg/g DW, a seven-fold increase from the minimum. Both root and trunk starch increased rapidly especially in the post-véraison period, to achieve relatively high concentrations by harvest. After harvest, starch in both trunk and root declined (Figures 7

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and 8). The decline in starch, which was more obvious in the roots, may be associated with the demand for carbohydrates to increase winter hardiness or for the development of new roots in autumn (Conradie and Bonnardot 2005, Coombe and Dry 1995, Williams 1996). Overall, the starch accumulation patterns showed very dynamic changes over quite short durations within the seasonal growth cycle, and demonstrate a highly responsive carbon economy. It is therefore probable that reserves can rapidly increase when growth demand from vine, fruit and root sinks alters during seasonal development. The shift in major competing sink activity at these times is the decline in the vegetative sink as shoot growth declines, as the fruit demand in that period remains high during ripening and maturation up to véraison, as shown by Coombe and Dry (1995). The CHO change on individual sample dates was much more consistent over the years in the trunks than in the roots. This could be because of issues with consistent root sampling compared with trunk sampling. However, over time clear patterns were seen between trunk and root, suggesting that the vine may sequester CHO reserves in the most accessible storage sink (the trunk) during the main growing season, with accumulation into the roots occurring when the major competitive sinks decline in their demands. For example, in the last phase of fruit development, the post-véraison ripening immediately before harvest, the crop sink has a relatively low demand for CHO, because significant starch accumulation can be seen to occur in both trunk and roots at this time. In agreement, Candolfi-Vasconcelos and co-workers (1994a) showed that roots rather than fruit are the priority sink for carbohydrates during the last stages of ripening.

From Figures 5, 6, 7 and 8, it can be seen that root nitrogen and root sugar followed the same trend but whereas root sugar reached its maximum at dormancy, after which it lowered rapidly, root N reached it maximum at the five-leaf stage or two months later. Trunk sugars and trunk N were found to be closely related and both reached their maximum concentration (85 and 0.8 mg/g DW resp.) around dormancy ($R^2 = 0.63$).

The annual dynamics of these two carbohydrate pools differed very little between the treatments. Our study shows that carbon sink concentration dynamics are highly responsive to changes in crop load

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and are also implicated in changes in canopy development, and may offer a partial explanation for the root starch patterns observed. We suggest that grapevine N and CHO partitioning favor the survival of the permanent structure over increasing vine size and yield. After harvest, in all the three years, starch declined in both root and trunk although somewhat more slowly in the roots during the second year.

After harvest, carbohydrates are mobilized into sugars and moved from the trunk to the roots, which are the most important sites of accumulation of carbohydrates in terms of vine reserves (Bates et al. 2002, Scholefield et al. 1978, Uys and Orffer 1983, Winkler and Williams 1945) These trends in CHO reserve pool dynamics, considered together, suggest that the trunk may function as a significant but transitional reserve pool between the root reserve and the rest of the vine. This is supported by the similarity in trunk CHO dynamics, seen across both pruning systems and defoliation treatments.

Starch concentrations were generally found to be lower in the roots of defoliated vines (Figure 7). The effect was increased (non-significantly) by the additional stress factor of an increased number of nodes retained. In all three years, after harvest, starch dropped rapidly in the roots but even more so in the trunks. These findings are similar to responses found in Shiraz vines in Wagga Wagga (Field et al. 2009) and in Semillon vines in Riverina (Holzapfel et al. 2006), both in Australia, where starch also declined but several weeks after harvest. The differences in time between the present work and the Australian work reflect how the harvest date was correlated with onset of leaf senescence and the length of growing season. In Marlborough, leaf fall is often experienced only a few weeks after harvest, while in the much warmer Australian wine regions, leaf fall is at least six weeks after harvest. However, in both regions starch build-up ceases with canopy senescence.

Neither defoliation (Figure 7) nor node number (Figure 8) treatments had an effect on trunk starch but both treatments affected root starch.

Responses to the post-harvest defoliation treatments were observed mainly in the roots, expressed as a reduction in root starch by up to 50%. In the trunk only a 20% reduction of starch was found but this happened simultaneously with an approximately 40% increase of TSC. This supports the concept of the

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trunk being a transitional accessible CHO reserve pool between the root system and the vine crown. It could be inferred that this is one of the mechanisms by which the vine responds to a major loss of leaf area (leaf defoliation) - by remobilizing reserve CHO (starch) from the roots. However, the reduced starch in the root in response to defoliation, as well as the reduction in starch in the root - but not in the trunk - following the 50% increase of retained nodes, suggest that the roots are probably the only true winter reserve pool, as found in Concord grape by Bates et al. (2002) and Pinot noir and Merlot by Zapata et al. (2004). The trunk may act as a transitional pool, although one that has considerable quantitative capacity, suggesting a major role in buffering CHO supply within the whole vine. CHO is needed for hardening off the shoots for winter (Wample and Bary 1992). With sufficient time after harvest and a large enough leaf area, the required CHO can be produced by the photosynthetically active leaves. Early leaf drop or defoliation immediately after harvest reduces a leaf supply of carbohydrate needed for the hardening off the shoots before winter. We suggest that this can be compensated for by the stronger remobilization of reserve carbohydrates in the defoliated vines (Figures 7 and 8). Most of these carbohydrates are being lost to the vine system, as most of the canes are being pruned from the vines at winter pruning.

In the absence of leaves, inorganic nitrogen acquired post defoliation or post leaf fall (Figures 5 and 6) can be sequestered in the root cell vacuoles or can be assimilated in the roots, using reserve carbohydrates as source of energy and carbon skeletons. N assimilation in roots is a costly process (Keller, 2015) and is probably a major cause of the decrease of root CHO observed between harvest and dormancy (Figures 7 and 8).

Although defoliation as well as the increased node number treatments reduced root starch significantly going into the winter period, all treatments reached a common minimum seasonal concentration occurring around bloom. Non-defoliated treatments therefore potentially had quantitatively greater carbohydrate reserves available for early development in the new season. Some vine responses reflected this, such as differences in blind bud proportions and changes in shoot number and size (Table 2). Although only after 3 years, also reductions in yield and yield components were found between

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defoliation and non-defoliation treatments (Table 2). No long-term depletion in CHO and N reserves pools occurred in response to combinations of pruning and defoliation treatments; rather, the effects were expressed as reduced vine yields and vegetative growth.

The reduced starch in the roots at the start of the 2011 season for 72N vines (Figure 8) and the reduction in shoot number, pruning weight and cane weight (Table 2) induced by defoliation treatment, are examples of vine responses to alterations in the carbon balance that affect ongoing vine productivity and fruit quality. Laying down 50% more nodes in the 72N treatment created the potential to increase crop by 50%. However, the extra crop load (yield) was achieved only in 2009, the first year of the treatment, and not in subsequent years, similar to reports by Greven et al. (2014).

In warm climates with long post-harvest photosynthetic activity, it has been shown that the CHO reserve build up during that period can be considerable (Field et al. 2009, Holzapfel et al. 2006, Smith and Holzapfel 2003, Williams 1996).This work showed that, contrary to what was suggested by Bennett and co-workers (Bennett et al. 2005, Trought et al. 2011), even under cool climate viticulture photosynthesis during the short post-harvest period provides a valuable contribution to the vine reserve pool. In its absence, sustainable high yields may be hard to maintain.

The present work now suggests that the ten days of leaves on the vines after harvest could be sufficient for a certain degree of nutrient retrieval into the vine reserves.

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Conclusions

Changes in CHO and N reserves were found to be very dynamic and were affected by different stages of vine development in response to defoliation and crop load differences. Defoliation or excessive crop loads did influence carbohydrate reserves in vines but only after several consecutive years of crop load and defoliation treatments did lower yields and poorer vegetative growth occur. However, the reductions in trunk and root reserves could be replenished during the next seasonal cycle of growth. This suggests that grapevine N and CHO partitioning favor vine permanent structure survival over increasing vine size or yield. Our work has shown that even the short-lived post-harvest canopy in cool climates contributes to the vine CHO pool.

In practical terms, the defoliation treatment as applied in this study can be equated to early leaf death caused by autumn frosts immediately post-harvest. Where frost protection systems are installed, we recommend that post-harvest frost protection should be carried out when vines are at risk of having low carbohydrate reserves. This would include young vines, vines that have been carrying heavy crops, and vines that have suffered early leaf drop in previous years. The need for post-harvest frost protection becomes increasingly important when vine reserve depleting effects accumulate over a number of seasons.

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Table 1 Fruit yield components, fruit composition and vine growth characteristics of Sauvignon blanc pruned to 48 (48N) or 72 nodes (72N) per vine.

	2009			2010			2011		
Treatment	48N	72N	Sign.	48N	72N	Sign.	48N	72N	Sign.
Fruit									
Yield (kg/vine)	12.7	15.4	***	9.3	9.8	ns	10.5	11.2	ns
Clusters/shoot	1.85	1.83	ns	1.60	1.43	*	1.48	1.51	ns
Cluster weight (g)	129	124	ns	118	109	*	146	123	***
Fruitfulness (g/shoot)	238	223	*	209	185	**	263	229	*
Berries/cluster	84	86	ns	76	80	ns	88	85	ns
Berry weight (g)	2.06	1.99	ns	1.98	1.89	*	1.88	1.83	ns
Total soluble solids (^{o}Brix)	20.5	19.9	*	23.5	23	ns	20.3	20.5	ns
Juice pH	2.88	2.86	ns	2.95	2.93	ns	3.01	2.99	ns
TA (g/L)	12.81	12.55	ns	11.83	12.26	ns	11.48	11.13	ns
Vine									
Budburst (%)[a]	99	88	***	89	93	ns	96	77	***
No. shoots/vine	52	66	***	44	53	***	48	55	***
Blind buds	9.5	17.2	***	9.8	17.2	***	10	22.6	***
LAI (m^2/m^2) [b]	3.1	3.3	ns	2.6	2.7	ns	3	3.3	ns
Leaf/fruit ratio (cm^2/g)	12.3	10.7	*	13.1	13.7	ns	15.0	14.7	ns
Pruning weight (kg/vine) [c]	2.4	2.2	*	2	2	ns	2.2	2.09	ns
Mean cane weight (g)	49	36	***	44	37	**	46.3	38	***
Ravaz Index	5.5	7.2	***	4.8	5	ns	4.75	5.37	**

Sign: *** = P <0.001, ** = P< 0.01, * = P<0.05, ns = P>0.05

[a] Vine % budburst = shoots per vine/retained count nodes per vine

[b] Leaf area index (LAI) and Leaf/fruit ratio only for defoliated vines

[c] Total pruning weight = cane + two-year-old wood weight.

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Table 2 Fruit yield, yield components and vine growth characteristics of Sauvignon blanc defoliated (Defol) or not defoliated (Fol) after harvest in 2009, 2010 and 2011.

	2009			2010			2011		
Treatment	Defol	Fol	Sign	Defol	Fol	Sign.	Defol	Fol	Sign.
Fruit									
Yield (kg/vine)		13.99		9.22	9.92	ns	10	11.64	****
Clusters/shoot		1.83		1.54	1.48	ns	1.60	1.57	ns
Cluster weight (g)		126		113	113	ns	127	143	*
Fruitfulness (g/shoot)		226		197	195	ns	233	260	**
Berries/cluster		[d]		78	78	ns	82	91	*
Berry weight (g)		2.02		1.92	1.94	ns	1.86	1.86	ns
Total soluble solids (Brix)		20.3		23.3	23.1	ns	20.9	19.9	ns
Juice pH		2.88		2.94	2.95	ns	3.01	2.99	ns
TA (g/L)		12.46		12.06	12.06	ns	10.87	11.73	ns
Vine									
Budburst (%) [a]	94	94	ns	92	91	ns	83	90	***
No. shoots/vine	59	59	ns	47	51	***	50	52	ns
Blind buds	13.9	13.4	ns	16.3	11.4	***	19	14	****
LAI (m^2/m^2) [b]	3.2			2.7			3.2		
Leaf/fruit ratio (cm^2/g)	11.5			13.4			14.9		
Pruning weight (kg/vine) [c]	2.3	2.2	ns	1.8	2.2	***	1.99	2.3	****
Cane weight (g)	43.0	42.5	ns	37.0	40.8	*	39.7	44.6	*
Ravaz Index	6.3	6.4	ns	5.2	4.6	*	5.04	5.08	ns

Sign: *** = P <0.001, ** = P< 0.01, * = P<0.05, ns = P>0.05

[a] Vine % budburst = shoots per vine/retained count nodes per vine

[b] Leaf area ratio (LAI) and Leaf/fruit ratio only for defoliated vines

[c] Total pruning weight = cane + two-year-old wood weight.

[d] Not measured

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Table 3 Interactions of defoliation and laid down nodes for fruit yield, yield components and vine growth characteristics of Sauvignon blanc in 2010 and 2011.

		48N		72N		
		Fol	Defol	Fol	Defol	Sign.
% Budburst	2010	99.8	93.9	89.6	79.8	ns
	2011	97.1[a]	94.1[a]	82.2[b]	72.1[c]	*
Blind nodes/vine	2010	7.7	11.5	14.5	20.3	ns
	2011	8.8[c]	11.3[c]	18.9[b]	26.3[a]	*
Total shoots	2010	50.8[b]	47.8[b]	67.1[a]	59.0[ab]	*
	2011	47.6	47.4	56.9	53.5	ns
Yield/vine (kg)	2010	9.3	9.3	10.3	9.2	ns
	2011	11.5	9.4	11.8	10.6	ns
Clusters/vine	2010	80.2	77.8	62.9	57.1	ns
	2011	76.4[b]	66.8[c]	87.3[a]	95.4[a]	*
Cluster weight (g)	2010	117.2	119.2	110.3	106.9	ns
	2011	150.2	142.5	135.6	110.7	ns
Berry weight (g)	2010	2	2	1.9	1.9	ns
	2011	1.9[ab]	1.9[a]	1.9[ab]	1.8[b]	*

Sign: *** = P <0.001, ** = P< 0.01, * = P<0.05, ns = P>0.05

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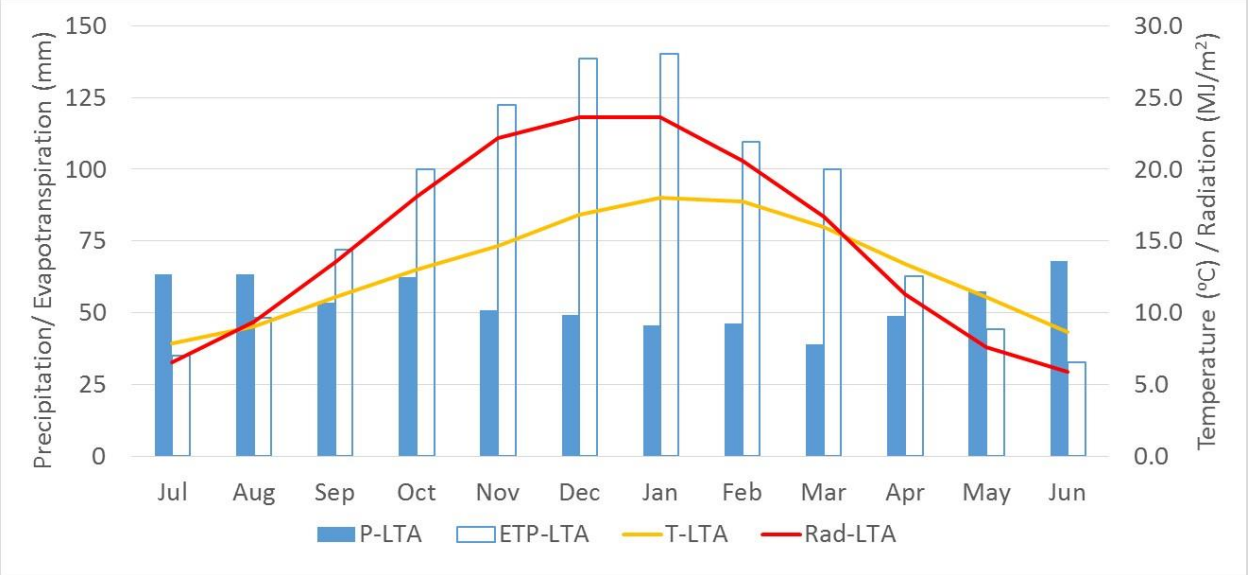

Figure 1 Marlborough long term average (LTA: 1984-2014) climate summary with monthly precipitation (P-LTA) and evapotranspiration (ETP-LTA) as well as daily average temperature (T-LTA) and daily radiation (MJ/m^2).

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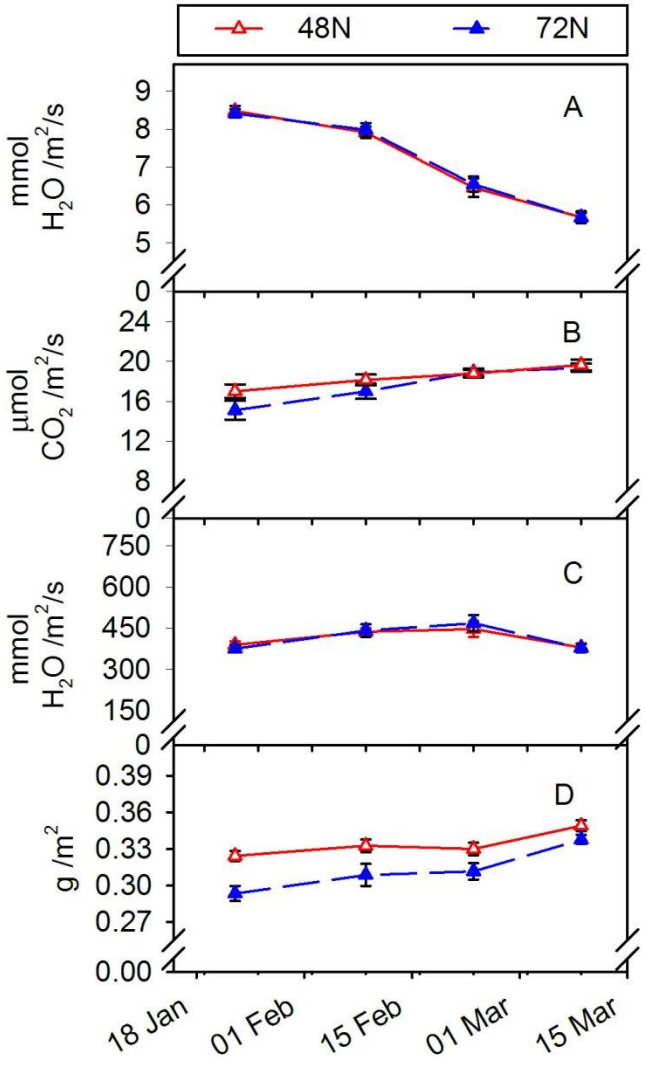

Figure 2 Effect of retained node number on Sauvignon blanc grapevine gas-exchange parameters and leaf chlorophyll content during the 2009 season for vines with 48 (48N) and 72 nodes (72N)retained at pruning. A: transpiration rate; B: photosynthetic rate; C: stomatal conductance to water vapor (g_s); D: leaf chlorophyll concentration. Vertical bars represent ±StError. Flowering: 12 December 2008; Véraison: 23 February 2009; Harvest: 15 April 2009.

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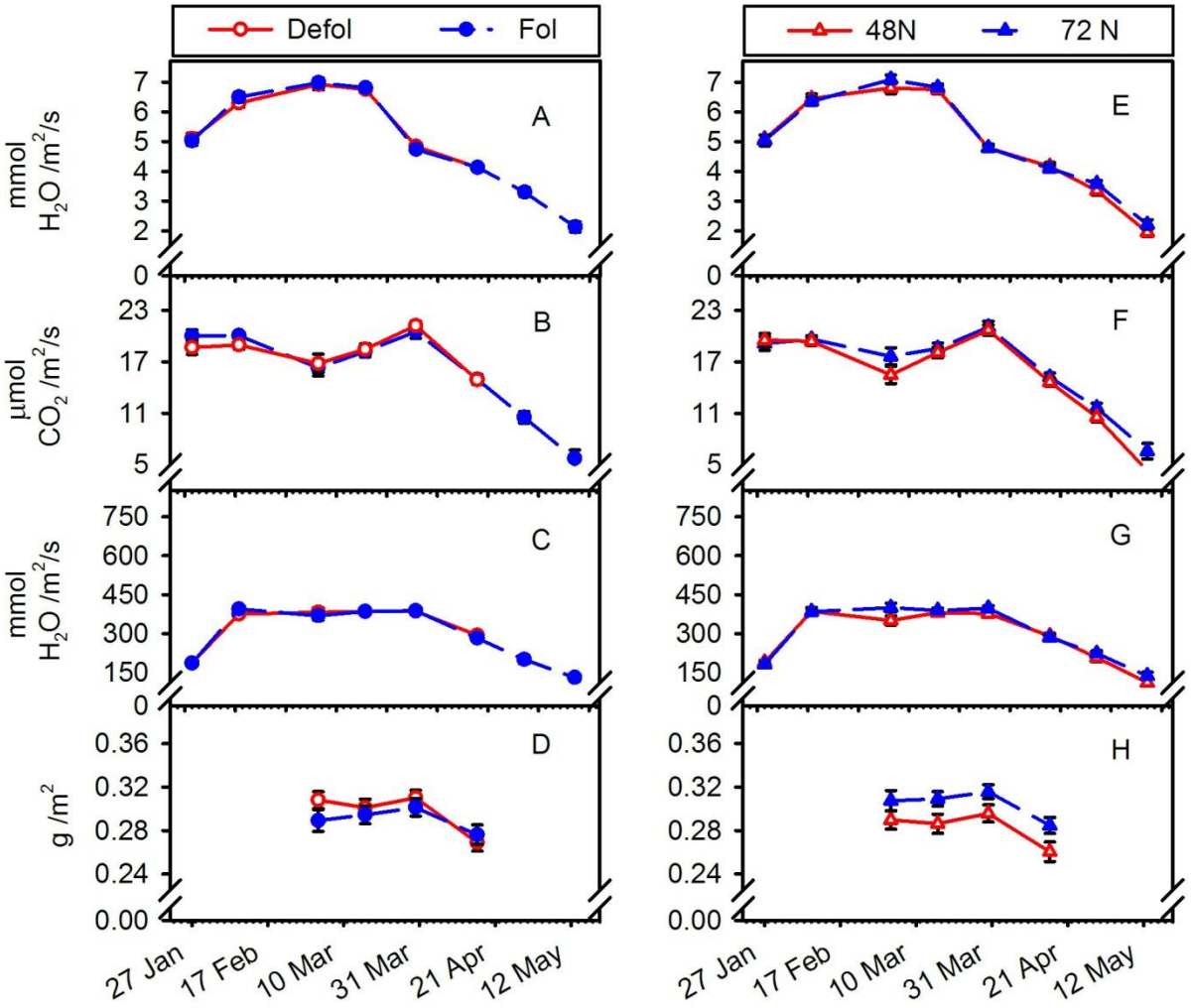

Figure 3 Gas exchange and chlorophyll content of Sauvignon blanc vines during the 2010 season. Defoliation with Fol = Foliated; Defol = Defoliated. Node number with 48 (48N) and 72 nodes (72N) retained. A/E: transpiration rate; B/F: photosynthetic rate; C/G: stomatal conductance (g_s); D/H: leaf chlorophyll concentration. Vertical bars represent ±StError. Flowering: 18 December 2009; Véraison: 22 February 2010; Harvest: 20 April 2010.

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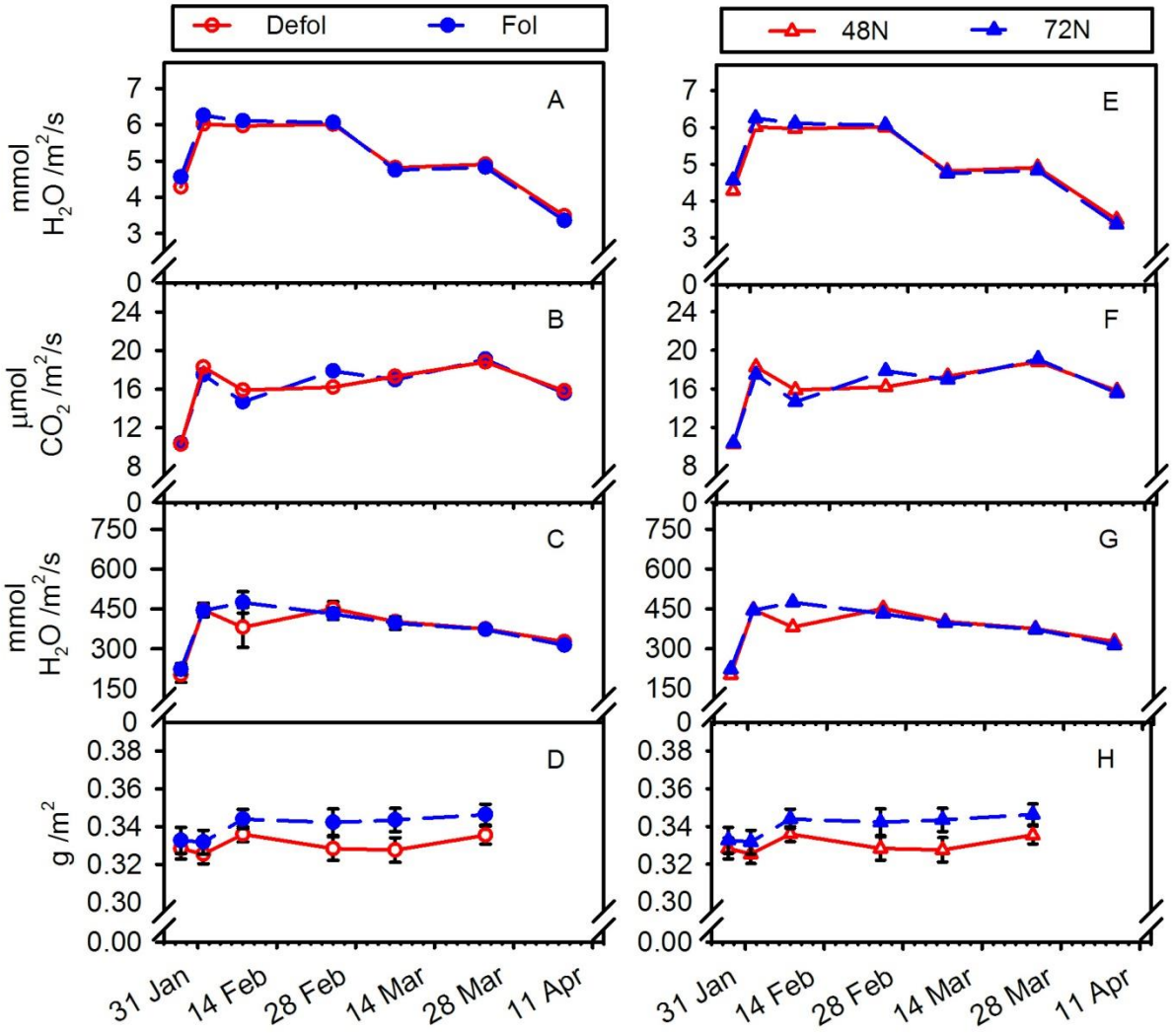

Figure 4 Gas exchange and chlorophyll content of Sauvignon blanc vines during the 2011 season. Defoliation with Fol = Foliated; Defol = Defoliated. Node number with 48 (48N) and 72 nodes (72N) retained. A/E: transpiration rate; B/F: photosynthetic rate; C/G: stomatal conductance (g_s); D/H: leaf chlorophyll concentration. Vertical bars represent ±StError. Flowering: 13 December 2010; Véraison: 15 February 2011; Harvest: 19 April 2011.

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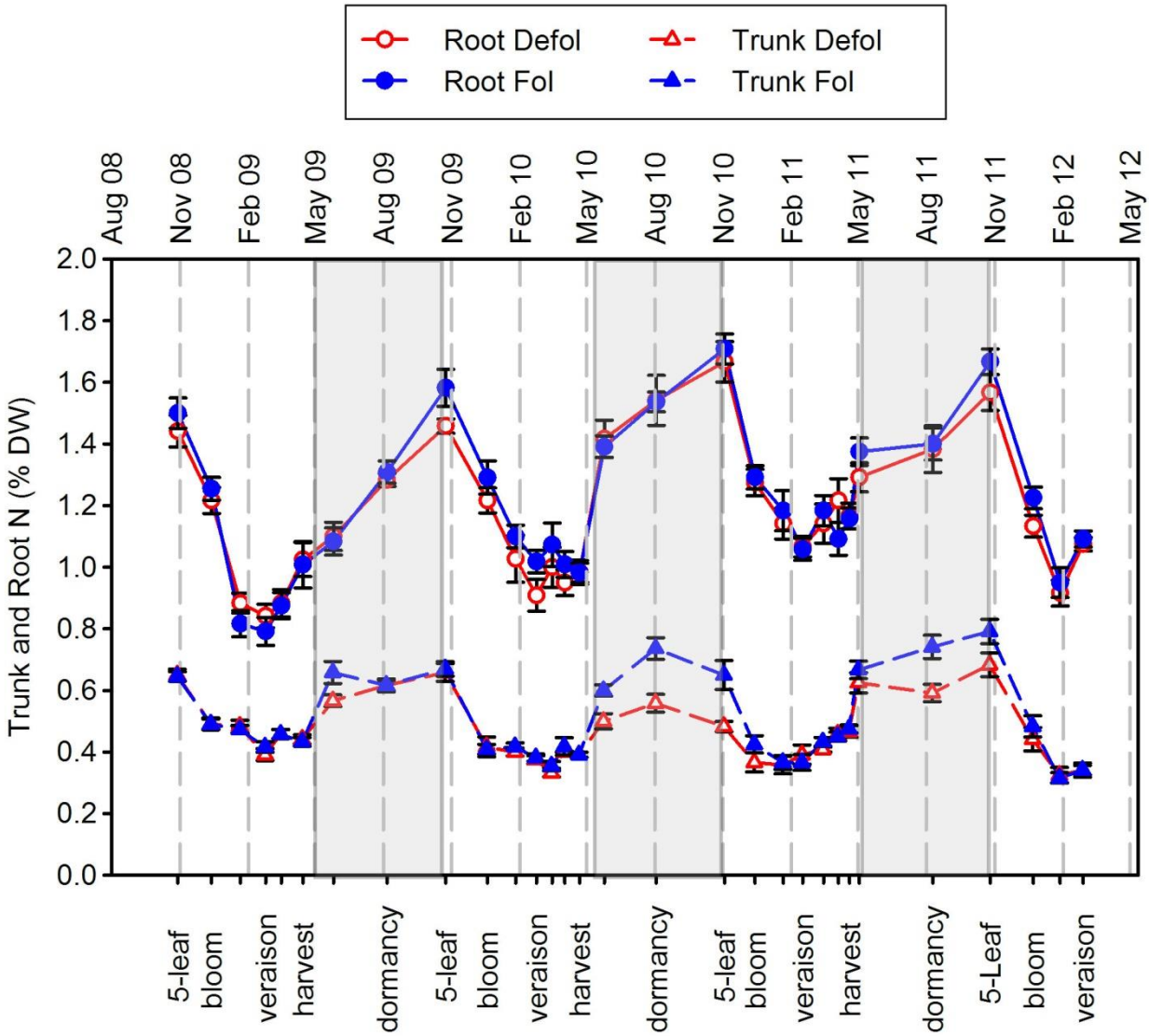

Figure 5 Total nitrogen (N) concentration measured in trunk and root tissue from Sauvignon blanc grapevines that were defoliated (Defol) or not (Fol) immediately after harvest (15 April 2009, 21 April 2010 and 19 April 2011). Error bars indicate ±StError.

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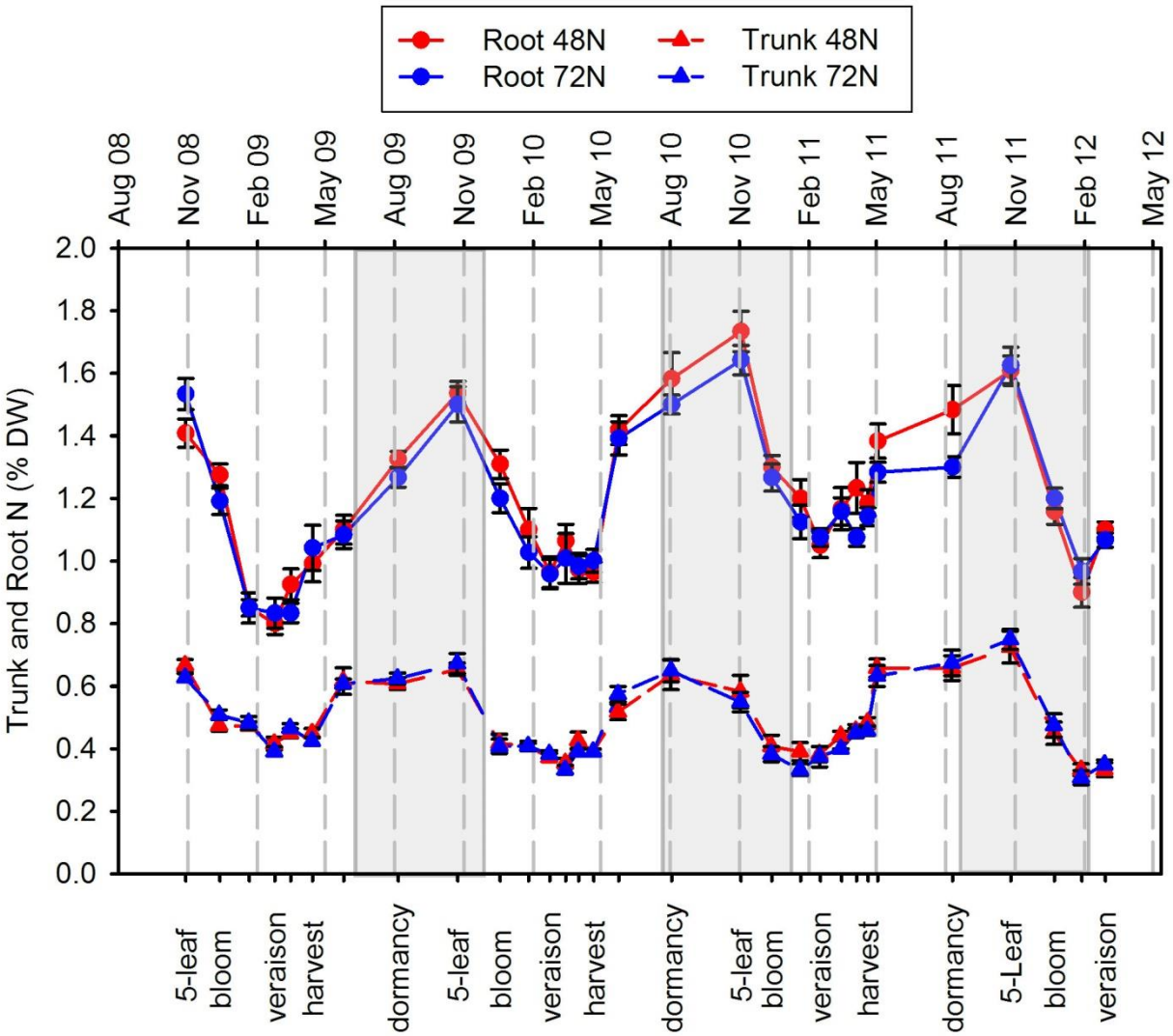

Figure 6 Total nitrogen (N) concentration measured in trunk and root tissue from Sauvignon blanc grapevines with 48 (48N) or 72 nodes (72N) retained at pruning, with error bars indicating ±StError.

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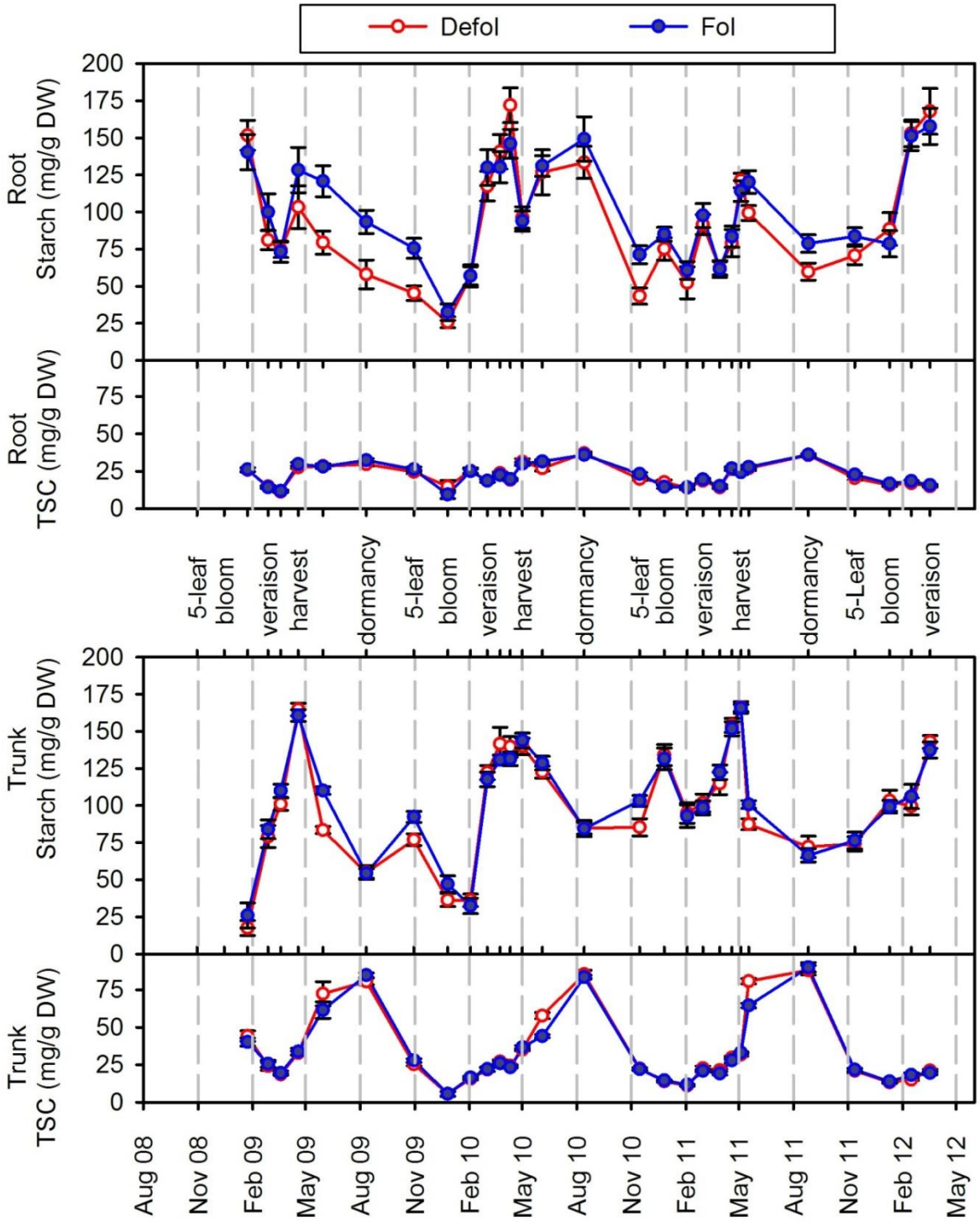

Figure 7 Total soluble carbohydrates (TSC) and Starch measured in root and trunk tissue from Sauvignon blanc grapevines that were defoliated (Defol) or not (Fol) immediately after harvest (15 April 2009, 21 April 2010 and 19 April 2011), with error bars indicating ±StError.

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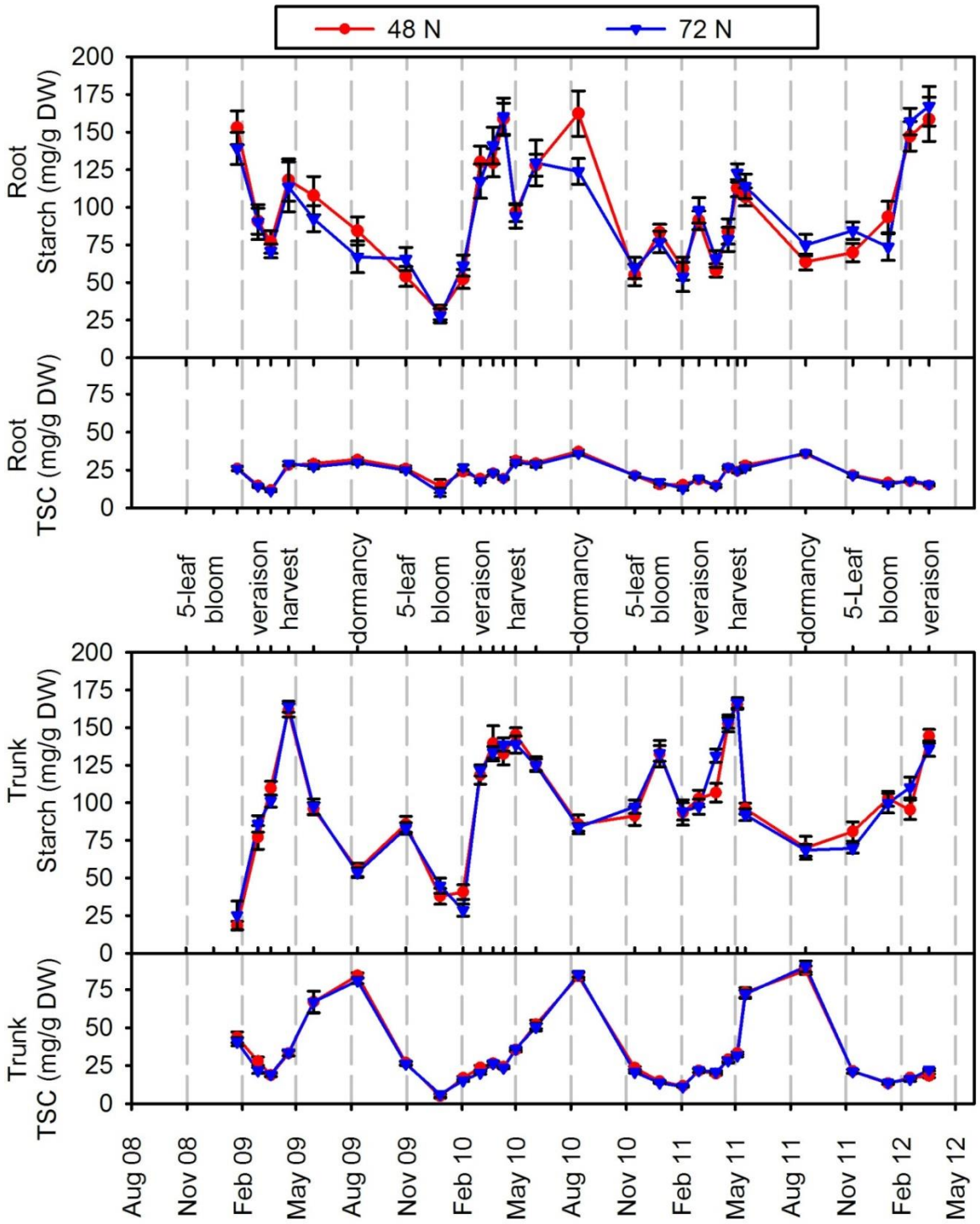

Figure 8 Total soluble carbohydrates (TSC) measured in root and trunk tissue from Sauvignon blanc grapevines with 48 or 72 nodes retained at pruning, with error bars indicating ±StError.