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Nitrogen dynamics and fertilisation use efficiency: carry-over effect of crop limitation

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Abstract

Background and Aims: Knowing the impact of cultural practices on nitrogen (N) dynamics in perennial crops is critical to promote N use efficiency. This study focused on the impact of crop regulation on the plant N dynamics, on the fruit N composition, and on the N fertilisation use efficiency.

Methods and Results: A large crop load gradient was set in a homogeneous plot of the grape cultivar Chasselas. Fertilisation in the form of ¹⁵N-labelled foliar urea allowed the measurement of N uptake and partitioning among plant fractions. Dry mass, carbon, and N dynamics were assessed over two consecutive seasons. Crop regulation did not affect grape N concentration at harvest. Both N uptake and root N mobilisation were reduced in response to crop regulation. Fertilisation efficiency was higher under high-yield conditions in terms of N uptake and grape N accumulation. The carry-over effects of crop regulation in the following year were highlighted.

Conclusions: Crop regulation strongly affects the overall plant N cycle, that is, uptake, distribution and release. Crop regulation improves must sugar concentration at harvest, while N concentration remained unchanged. The efficiency of N fertilisation varies greatly with crop load, which limits the interest of fertilisation under low-yield conditions.

Significance of the Study: These results contribute to the development of accurate nutrition models and sustainable cultural practices.

Keywords: ¹⁵N isotope labelling, amino acids, foliar urea, nitrogen use efficiency, reserve mobilisation, yield

Introduction

Nitrogen (N) is an essential element for plant development and is required in a larger amount than any other nutrient applied to crops. During the twentieth century, nitrate (NO₃⁻) was intensively used to increase production, despite crops using only 30–40% of the fertiliser applied (Masclaux-Daubresse et al. 2010). The remaining fertiliser is usually lost to the environment via leaching, denitrification, surface runoff, gaseous emissions, and microbial consumption (e.g. Kant et al. 2011). The Office Fédéral de l'Environnement (2019) reported that nitrate concentration in groundwater exceeded the limit value of 25 mg/L in almost 15% of the monitoring stations in Switzerland. Understanding the dynamics behind nutrient uptake, transport, storage, and remobilisation is crucial for quantifying the nutrient budget and adjusting cultural practices, in particular for perennial crops. Therefore, minimising the need for N fertilisation through the fine-tuning of cultural practices is fundamental for sustainable agricultural development.

In grape production (*Vitis vinifera* L.), N depletion is as detrimental as N excess to yield and fruit composition. Nitrogen exacerbates plant vigour, increases sensitivity to fungal diseases, and delays fruit ripening (Schreiner

et al. 2018). Conversely, N deficiency reduces plant vigour, bud fruitfulness, and consequently yield. Moreover, N deficiency severely affects the winemaking process. Bell and Henschke (2005) detailed the implications of N nutrition for grape, fermentation, and wine quality. In their review, they explained the significant role of grape yeast assimilable N (YAN), that is primary free amino acids and ammonium (NH₄⁺), in fermentation kinetics and formation of flavour-active compounds in wine. Grapevine N dynamics, that is, seasonal uptake and release, have been thoroughly studied in the past decades, allowing a good understanding of the plant requirement in nutrients (Conradie 1991, Wermelinger 1991, Zapata et al. 2004, Weyand and Schultz 2006, Loulakakis et al. 2009, Masclaux-Daubresse et al. 2010, Zufferey et al. 2015, Schreiner et al. 2018, Holzapfel et al. 2019). Even so, our understanding of the relationship between plant N status and fruit N composition remains incomplete. The concept of N use efficiency (NUE) represents the sum of both assimilation efficiency (uptake and assimilation) and utilisation efficiency (allocation and remobilisation) (Kant et al. 2011). The NUE is largely determined by environmental conditions (i.e. climate and soil), plant material, and management strategies (i.e. plant material genetics, soil management, plant development monitoring, and vineyard inputs) (Porro et al. 2006, Habran et al. 2016, Verdenal et al. 2021). Fruit N management is a multi-sided exercise in the search for balance between

*We declare that our research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

controlling yield and optimising fruit composition, while limiting environmental impact.

In grape production, the optimum yield (i.e. fruit quantity produced per unit of land surface) is generally not the maximum allowed by the conditions of the vineyard, since overcropping may delay fruit ripening (i.e. slower sugar accumulation) and alter subsequent wine quality (Petrie and Clingeleffer 2006, Rutan et al. 2018). Crop load (i.e. fruit quantity per plant) may be regulated downward via crop thinning, which consists of removing grapes before the onset of ripening in order to promote the maturation of the remaining fruit. Crop thinning, however, does not consistently improve fruit composition or aroma development (Keller et al. 2005, Mawdsley et al. 2018, Wang et al. 2018, Bubola et al. 2020, Verdenal et al. 2020). Alem et al. (2021) further demonstrated that crop thinning generally decreases the quantity of most carbon (C) compounds (i.e. sugars, organic acids, and glycosylated aroma compounds) accumulated in fruit. Maintaining a balance between both vegetative and reproductive organs prevails over the consideration of the sole crop load to determine the physiological threshold for overcropping (Kliewer and Dokoozlian 2005, Zufferey et al. 2015). In most studies, the concept of vine balance is primarily used in terms of C (Howell 2001), although N balance is also considered to be of major importance. Understanding both C and N dynamics, including storage, remobilisation, and final fate, while taking into account N demand, is critical for the development of sustainable fertilisation programs (Muhammad et al. 2020). In a previous article, we demonstrated over one season that the mobilisation of N from the perennial fractions of the plant plays a major role in fruit N balance (Verdenal et al. 2020). The ability of perennial crops to accumulate N reserves in roots and wood suggested implications for plant vigour and production over the following years. This experiment continued for 2 years, and the present manuscript focuses on the carryover impact of crop regulation on the plant C and N dynamics, on the fruit N composition, and on the N fertiliser use efficiency. Our findings were the result of the implementation of a large crop load gradient over 2 years and the use of a ^{15}N -labelling approach on the white grapevine cultivar Chasselas (*V. vinifera* L.).

Materials and methods

Experimental site

The trial was conducted over 2 years (2017–2018) at the Agroscope experimental site in Pully, Switzerland (46°30'45.8"N, 6°40'05.7"E). The local climate is temperate. During the first growing season (April–October 2017), the precipitation was 562 mm, and the daily mean temperature was 16.6°C. The 2018 climatic conditions were drier and hotter than 2017, with 412 mm of precipitation and an average daily mean temperature of 17.8°C from April through October (data from the Swiss meteorological station in Pully). The low-calcareous colluvial soil of the site was composed of 47 mass (m)% sand, 38 m% silt, and 15 m% clay. The soil contained 1.75 m% of organic matter, 0.10 m% total N (TN), 4.3 m% carbonates (eq. CaCO_3), and the pH was 7.9. Phosphorus (P, 8.2 mg/kg), potassium (K, 25.2 mg/kg), and magnesium (Mg, 11.4 mg/kg) were not restrictive for vine growing.

Plant material

Chasselas was grafted onto rootstock 3309 C and planted in 2013 in 90 L pots. The use of pots ensured a good recovery

of the root biomass when the vines were excavated for analysis, while the large pot size provided sufficient soil volume for the development of root system biomass. Prior to planting, 225 pots were randomly placed in trenches with a planting density of 8330 vines/ha (1.5 × 0.8 m) and filled with the soil of the trenches as a growth medium. The soil water-holding capacity was 11 L per pot. Vines were rainfed with a back-up irrigation. The drip-irrigation system was installed and used twice in July in each season to avoid stem water potential dropping below -0.8 MPa during periods of low rainfall. The stem water potential was measured with a pressure chamber (Model 600, PMS Instruments, Albany, NY, USA) (Scholander et al. 1965). Vines were trained in a single Guyot trellis system, with 60 cm trunk height and seven shoots per cane. The canopy was trimmed at 120 cm above the top of the trunk three times per season: on the day of the year (DOY) 164, 191, and 215 in 2017; and on DOY 162, 183, and 218 in 2018. The date of the main phenological stages was similar between 2017 and 2018: 50% budburst [phenological scale BBCH 05 (Lancashire et al. 1991)] occurred on DOY 94 and 99, respectively; 50% flowering (BBCH 65) occurred on DOY 164 and 161; 50% veraison (i.e. the onset of grape ripening, BBCH 85) occurred on DOY 214 in both years; and harvest was performed on DOY 257 and 269, respectively. At the end of 2017, winter pruning was completed, and the shoots were removed from the experimental plot. Despite homogeneity of the entire plot in terms of plant material and growing conditions, eight out of the 225 vines were identified as outliers (i.e. low vigour, low photosynthetic activity, low fruitfulness, low berry set, and incomplete winter cold hardening) and were discarded to optimise the homogeneous conditions of the trial.

Crop load and ^{15}N labelling treatments

The plants were organised into 14 homogeneous groups of 12 plants each (i.e. total 168 plants), and separated by the remaining plants used as buffers to minimise cross-contamination from the fertilisation. Two factors of variation were set in this trial, that is, crop load and fertilisation. In each group of 12 plants, the crop load treatment was set (i.e. two levels of crop load, six plants per level). The groups of 12 plants were destructively excavated at eight dates, corresponding to the four major phenological stages (i.e. 50% of each stage, budburst, flowering, veraison and harvest) over 2 years, to assess the dynamics of total N and fertiliser N into the plant. For each excavation date, there were either one, two or three groups of vines excavated, corresponding to the number of fertilisation levels (i.e. one to three fertilisation levels, 12 plants per level). Each plant was considered a replicate. Each excavation date was statistically analysed separately using one- or two-way ANOVA with interaction, as explained hereafter.

Crop load treatment

In each group of 12 plants, a large crop load gradient was established by crop thinning at bunch closure (phenological stage BBCH 77; DOY 193 in 2017 and DOY 179 in 2018), keeping two to ten bunches per plant. Crop thinning in 2018 was based on the yield at harvest 2017 in order to maintain each plant under the same yield condition over the two consecutive seasons and promote cumulative responses. For statistical analysis, the groups of plants excavated before the 2017 crop thinning, that is, at budburst (one group) and flowering (one group), were considered

homogeneous groups of plants, whereas the data from the other groups were split into two sub-groups of plants, that is, low-yield conditions (LYC) versus high-yield conditions (HYC). The threshold to split the groups of plants excavated in 2017 was 0.7 kg/m² at veraison (one group, CT) and 1.3 kg/m² at harvest 2017 (two groups, CT and F17), based on the median yield by the time of excavation. The thresholds at budburst 2018 (two groups) and flowering 2018 (two groups) were based on the median yield at harvest 2017. Due to a higher yield potential in 2018, the thresholds in the groups of plants excavated at veraison 2018 (two groups) and at harvest 2018 (three groups, CT, F17, and F17+18) were 1.25 and 2.1 kg/m², respectively.

Fertilisation treatment

Three fertilisation levels were set: (i) a Control treatment (CT); (ii) a treatment with one fertilisation in 2017 only (F17); and (iii) a treatment with fertilisation in both 2017 and 2018 (F17+18). In 2017, the groups of 12 plants corresponding to the treatments F17 and F17+18 each received 2.4 g N/plant (20 kg N/ha) in the form of ¹⁵N-labelled urea (10 atom % ¹⁵N) (Sigma-Aldrich, Buchs, Switzerland), applied on the leaves at veraison and split into four applications (DOY 199, 208, 214, and 226). In 2018, only the plants from the treatment F17+18 again received 2.4 g of ¹⁵N-labelled urea under the same conditions (DOY 198, 204, 211, and 219). The labelled foliar urea was carefully applied plant by plant on both sides of the canopy (dilution 3.44% w/v) with hand-sprayers (Spray-matic 1.25, Birshmeier, Stetten, Switzerland). No other fertilisation occurred during the trial.

Each group of 12 plants was destructively excavated at once at one of the four major phenological stages described previously over the two seasons. For each excavation date, the number of plants excavated (i.e. 12, 24, or 36) was related to the fertilisation levels at that date (i.e. one, two or three): before veraison 2017, only one group of vines per excavation date (CT); between veraison 2017 and veraison 2018, two groups per excavation date (CT and F17); and after veraison 2018, three groups per excavation date (i.e. CT, F17, and F17+18). Consequently, a group of 12 vines (CT) was excavated at each stage from budburst 2017 to harvest 2018 (total eight groups); a group of treatment F17 was excavated at each stage starting from harvest 2017 (i.e. after 2017 urea application) to harvest 2018 (five groups); and a group of treatment F17+18 was excavated only at harvest 2018 (i.e. after 2018 urea application; one group).

Field measurements and sample preparation

The field measurements and sample preparation were conducted as described in Verdenal et al. (2020). The winter pruning wood was collected and weighed on a per vine basis on DOY 325 in 2017 and then removed from the experimental plot. Vine fruitfulness was determined before crop thinning and expressed as the average number of bunches per shoot.

The total leaf area (TLA) per vine was assessed with the non-destructive method of Mabrouk and Carbonneau (1996), based on the strong correlation between the length of a shoot and its TLA. To determine this equation in our context, 15 shoots from 15 different buffer plants were collected on DOY 206 in 2017. The total shoot length (TSL, main shoot + laterals) was measured, and the TLA was determined with a leaf area meter (LI-3100C; LI-COR Biosciences, Lincoln,

NE, USA). As a result, Equation 1 allowed the transformation of measured TSL into estimated TLA for both seasons ($r = 0.98$):

$$\text{TLA} = 14.4 \times \text{TSL} + 161.5 \quad (1)$$

The leaf macronutrient composition, total N, P, K, Ca, and Mg, was determined from the dry extracts of two adult leaves (blade + petiole) per vine just after veraison (DOY 229 in 2017 and DOY 212 in 2018) and at leaf fall (DOY 290 in 2017 only) (Sol-Conseil Laboratory, Gland, Switzerland), and then compared to the thresholds published for the grape cultivar Chasselas under the Swiss cool climate (Spring and Verdenal 2017). The chlorophyll index was measured in 2018 at DOY 222 using an infrared non-destructive method on adult leaves from the median part of the canopy (N-Tester, Yara International, Paris, France); this method reflects the intensity of the green colour of the canopy and is thus well correlated to leaf N concentration (van Leeuwen et al. 2000, Aranguren et al. 2018). In both 2017 and 2018, leaf gas exchange was measured approximately every 10 days between flowering (BBCH 65) and harvest (BBCH 89), on sunny days between 1200 and 1500, on the plants excavated at harvest and on one fully expanded leaf per vine: net assimilation (A), transpiration (E), stomatal conductance (g_s), internal CO₂ concentration (C_i), and intrinsic water use efficiency (WUE_i) were determined non-destructively with a portable photosynthesis system (LI-6800, LI-COR Biosciences). During the measurements, the ambient conditions inside the LI-6800 leaf chamber were controlled by the system with the following pre-set parameters: air flow, 700 μmol/s; relative humidity, 50%; ambient CO₂, 380 μmol/mol; fan speed, 5000 rpm; and light source, 2000 μmol/(m² · s).

Each vine was excavated separately and split into four fractions: roots, trunk (including wooden cane), canopy (including shoot trimmings collected during the same season), and grapes. The number of organs depended on the phenological stage by the time of excavation, for example only roots and trunk at budburst. At both veraison and harvest stages, the grapes were weighed to determine the crop load (kg/plant) and then pressed manually to separate the liquid phase (must) from the solid phase (pomace). The five plant fractions (roots, trunk, canopy, pomace, and must) were weighed to determine fresh mass (FM). Must aliquots were taken for chemical (100 g) and stable isotope (25 g) analysis. The plant fractions were dried at 60°C until a constant mass, while the musts were freeze-dried. Dry mass could be determined for all samples. The samples for isotope analysis were ground to fine powder, except for the must samples.

Stable isotope analysis

The C and N isotope composition was analysed by elemental analysis/isotope ratio mass spectrometry. A Carlo Erba 1108 elemental analyser (Fisons Instruments, Milan, Italy) was coupled with a ConFlo III interface to a Delta V Plus isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) operated under continuous helium flow, as detailed in Spangenberg and Zufferey (2018). The calibration and normalisation of the measured δ¹³C values to the standard Vienna Pee Dee Belemnite (VPDB) were performed with international and in-house reference materials at different ¹³C at natural abundance [δ¹³C values in Brand et al. (2014) and Spangenberg et al. (2010), respectively].

The calibration and normalisation of the $\delta^{15}\text{N}$ measurements to the international Air- N_2 scale were realised with a dedicated set of six in-house urea standards with different ^{15}N enrichment, covering the $\delta^{15}\text{N}$ range of -1.39 to 1275 mUr. The preparation of these standards is detailed in Spangenberg and Zufferey (2019). The stable isotope composition of each sample was reported as δ value (i.e. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), which is the relative deviation of the molar ratio (R) of the heaviest (^iE) to the lightest (^jE) isotopes (e.g. $^{13}\text{C}:^{12}\text{C}$, $^{15}\text{N}:^{14}\text{N}$) from an international standard (Coplen 2011) (Equation 2):

$$\delta^{15}\text{N}_{\text{sample}} = \frac{R\left(\frac{^{15}\text{N}}{^{14}\text{N}}\right)_{\text{sample}}}{R\left(\frac{^{15}\text{N}}{^{14}\text{N}}\right)_{\text{standard}}} - 1 \quad (2)$$

The δ values were reported in milliurey (mUr) as recommended by the International System of Units (Brand 2011). All the isotope analyses were in duplicate. The repeatability was better than 0.1 mUr (1 SD) for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at natural abundance and better than 2 mUr for $\delta^{15}\text{N}$ in ^{15}N -enriched samples. The concentration of total organic C (TOC) and of total N (TN) [in mass (m)%] was determined from the total area of the major isotopes with the same calibrations used for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The repeatability for the concentration of TOC and TN was better than 0.2 m%.

Fruit composition

Fruit composition was analysed in aliquots of centrifuged fresh must collected from the vines excavated at veraison and harvest. The pH, TSS, TA, potassium (K), and concentration of tartaric and malic acids were determined with a WineScan infrared spectrometer (FOSS NIR Systems, Hillerød, Denmark). The ammonium (NH_4^+) was quantified using an enzymatic test kit (Boehringer Mannheim, Mannheim, Germany). The primary amino N (PAN) concentration—excluding proline and hydroxyproline, which are not assimilable by yeasts under fermentation conditions—was determined with the OPA method using the Primary Amino Nitrogen kit (Bio Systems, Barcelona, Spain). The must YAN concentration was computed by summing the concentrations of NH_4^+ and PAN, both expressed in mg N/L (Bell and Henschke 2005).

To determine the free amino acid (FAA) profiles (in %) of the grape musts, the FAAs were separately quantified in the must aliquots by ultrahigh-performance liquid chromatography (UHPLC)/MS using an Infinity 1290 HPLC system connected with an electrospray interface (ESI) to a 6460C Triple Quadrupole MS (Agilent Technologies, Santa Clara, CA, USA). Liquid chromatography was achieved with an Intra amino acid column (50×3 mm; Imtakt, Portland, OR, USA), following the methods detailed in Verdenal et al. (2020). Detection was achieved by multiple reaction monitoring. The calibration was done with standards for each AA separately according to their abundance. The repeatability of the values was better than 5 and 10% for low and high abundance, respectively. The concentration of FAA was reported in mg N/L.

Data treatment

Data were treated as detailed in Verdenal et al. (2021). The concentration of C and N of each plant fraction was reported

as Q (i.e. CQ for TOC quantity and NQ for TN quantity, in g) and calculated as below for NQ (Equation 3):

$$\text{NQ}_{\text{fraction}} = \text{DW}_{\text{fraction}} \times \text{TN} \quad (3)$$

The absolute abundance of ^{15}N [atom percent (A%)] is the proportion of heavy isotopes per 100 N atoms (Cliquet et al. 1990) (Equation 4):

$$\text{A\%} = \frac{R}{R+1} \times 100 \quad (4)$$

The relative specific abundance (RSA, in A%) represents the proportion of newly incorporated N atoms originating from the labelled source (e.g. fertiliser), compared with the TN quantity in the sample (Cliquet et al. 1990). The RSA also represents the organ sink strength, which is independent of the organ size (Deléens et al. 1997) (Equation 5):

$$\text{RSA} = \frac{\text{A\%}_{\text{sample excess}}}{\text{A\%}_{\text{N supplied excess}}} = \frac{\text{A\%}_{\text{sample}} - \text{A\%}_{\text{non-labelled control}}}{\text{A\%}_{\text{N supplied}} - \text{A\%}_{\text{non-labelled control}}} \quad (5)$$

The new N pool (NNP, in g), originating from the labelled source, may be quantified in each plant fraction and the partitioning (%P) subsequently calculated (Cliquet et al. 1990) (Equations 6 and 7).

$$\text{NNP}_{\text{fraction}} = \text{RSA}_{\text{fraction}} \times \text{NQ}_{\text{fraction}} \quad (6)$$

$$\%P = \frac{\text{new N pool}_{\text{fraction}}}{\text{new N pool}_{\text{whole plant}}} \times 100 \quad (7)$$

The overall net N uptake can then be calculated (Equation 8):

$$\text{net N uptake} = \frac{\text{new N pool}_{\text{whole plant}}}{\text{labelled NQ}_{\text{supplied}}} \times 100 \quad (8)$$

In the last group of plants F17+18, the differentiation of the new labelled N in 2018 (2018-lab-N) from the residual labelled N from the 2017 fertilisation (2017-res-N) was realised as follows: the calculation of RSA for each plant fraction was done using A% measured in the group F17 excavated at the same date as the initial abundance before labelling (instead of the natural abundance). The accumulation of both 2017 and 2018 fertilisations was estimated by adding 2017-res-N and 2018-lab-N (total-lab-N). Consequently, $\text{A\%}_{\text{sample excess}}$ was directly related to 2018-lab-N. The statistical analysis of the impact of 2018 fertilisation was done by comparing the two groups of plants F17 and F17+18 excavated at harvest 2018, that is F17 as the non-fertilised treatment in 2018 and F17+18 as the fertilised treatment.

Data were analysed using XLSTAT version 2020.5.1 software (Addinsoft, Paris, France). Each date of excavation was subject to separate statistical analysis for the determination of the effects of the investigated factors, that is, crop load treatment (from budburst 2017), fertilisation treatment (from harvest 2017), and their interaction. Only the statistical results of the excavation at harvest 2018 are presented in detail as the grape composition at harvest is the major (and most significant) result for grape production and winemaking. The significance of differences and interactions between treatments was assessed with one- or two-way

Table 1. Two-year field measurements as a function of foliar N fertilisation in the same year and crop load.

Year	Variable	N fertilisation			Crop load			Interaction fertilisation × crop load
		0 kg/ha	20 kg/ha	P-value	LYC	HYC	P-value	
2017 (<i>n</i> = 21)	Fruitfulness (bunches per shoot)	2.3	2.0	n.s.	2.1	2.2	n.s.	n.s.
	Bunches per vine	5.2	5.2	n.s.	3.8	6.7	*	n.s.
	Bunch mass (g)	354	332	n.s.	320	369	n.s.	n.s.
	Yield (kg/m ²)	1.4	1.3	n.s.	0.8	1.9	***	n.s.
	Total leaf area (m ² /plant)	1.8	1.9	n.s.	1.8	2.0	n.s.	n.s.
	Leaf-to-fruit ratio (m ² TLA/kg)	1.7	1.7	n.s.	2.3	1.0	**	n.s.
2018 (<i>n</i> = 22)	Winter pruning mass (g/plant)	749	694	n.s.	739	705	n.s.	n.s.
	Fruitfulness (bunches per shoot)	2.1	2.1	n.s.	2.0	2.2	n.s.	n.s.
	Bunches per vine	5.7	6.1	n.s.	3.3	8.4	***	n.s.
	Bunch mass (g)	482	447	n.s.	437	492	n.s.	n.s.
	Yield (kg/m ²)	2.1	2.3	n.s.	1.2	3.2	***	n.s.
	Total leaf area (m ² /plant)	1.9	2.0	n.s.	1.8	2.1	n.s.	n.s.
	Leaf-to-fruit ratio (m ² TLA/kg)	1.2	1.3	n.s.	1.8	0.7	***	n.s.

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant. Data from the vines excavated at both harvests 2017 (treatments CT and F17) and 2018 (F17 and F17+18); mean values within the same row followed by different letters are significantly different (Newman–Keuls). HYC, high-yielding conditions; LYC, low-yielding conditions; TLA, total leaf area.

ANOVA ($P < 0.05$), depending on the excavation date. A Newman–Keuls post-hoc test was performed to differentiate more than two groups. Principal component analysis (PCA) was used to discriminate the musts at harvests 2017 and 2018, as a function of their FAA profiles. The supplementary data crop load (kg/plant) and maturity index (TSS-to-TA ratio) did not influence the cloud.

Results

Vegetative growth and development

A large fruit load gradient was achieved via bunch thinning in both consecutive years 2017 and 2018 (Table 1). As a result, the 2017 yield varied on average from 0.8 kg/m² under LYC to 1.9 kg/m² under HYC. The 2018 yield capacity was higher, and the yield varied on average from 1.2 kg/m² under LYC to 3.2 kg/m² under HYC (Table 1). Crop regulation affected neither the bunch mass (an average of 343 ± 114 g in 2017 and 469 ± 132 g in 2018) nor the pruning mass (average 723 ± 314 g in the winter of 2017/18). The 2018 average TLA was 2.0 ± 0.4 m²/plant. Leaf-to-fruit ratio was highly affected by crop load in both

years and was particularly low in 2018 under HYC (0.7 m²/kg compared with 1.8 m²/kg under LYC, Table 1). Foliar N fertilisation at veraison had no impact on the vegetative observations or on the yield components in either year (Table 1). The average chlorophyll index at veraison 2018 was homogeneous in the whole plot at 470 ± 17. No N deficiency symptoms could be observed on the leaves of the Control treatment. Average leaf N concentration at veraison 2017 was adequate at 2.15% DM on average (2.15% in the Control and 2.16% in the fertilised treatment), while 2018 leaf N concentration was lower—but not deficient—at 1.83% DM on average (1.79% in the Control and 1.86% in the fertilised treatment). The concentration of other nutrients in 2018 was adequate with an average of 0.20% P, 1.85% K, 2.53% Ca, and 0.20% Mg, regardless of the treatment (data not shown).

In both seasons, photosynthesis (*A*) activity was generally higher in the period from flowering to veraison [DOY 165–204, average $A_{Jun-Jul} = 14.8 \mu\text{mol CO}_2/(\text{m}^2 \cdot \text{s})$] in comparison with the period from veraison to harvest [DOY 214–247, average $A_{Aug-Sep} = 10.3 \mu\text{mol CO}_2/(\text{m}^2 \cdot \text{s})$] (Table 2). The stomatal conductance (g_s) values followed the same trend as

Table 2. Foliar gas exchange rates as a function of day of year and crop load.

2018 (<i>n</i> = 22)	<i>E</i> [mmol H ₂ O/(m ² · s)]			<i>A</i> [μmol CO ₂ /(m ² · s)]			<i>g_s</i> [mmol/(m ² · s)]			<i>C_i</i> (μmol/mol)			<i>WUE_i</i> (<i>A</i> × 1000/ <i>g_s</i>)		
	LYC	HYC	P-value	LYC	HYC	P-value	LYC	HYC	P-value	LYC	HYC	P-value	LYC	HYC	P-value
DOY															
165	4.4	4.4	n.s.	17.0	17.0	n.s.	267	257	n.s.	236	232	n.s.	64	67	n.s.
176	5.1	6.3	***	14.2	16.2	*	207	268	**	221	239	*	75	61	*
183	6.7	7.4	n.s.	14.9	15.7	n.s.	246	286	n.s.	236	245	n.s.	62	56	n.s.
194	6.2	6.8	n.s.	13.6	14.8	n.s.	202	235	n.s.	224	232	n.s.	71	65	n.s.
204	5.8	6.0	n.s.	12.1	12.5	n.s.	178	184	n.s.	229	228	n.s.	69	69	n.s.
214	5.6	5.3	n.s.	13.1	12.6	n.s.	221	212	n.s.	245	243	n.s.	60	62	n.s.
222	4.6	4.7	n.s.	10.1	10.7	n.s.	139	152	n.s.	223	228	n.s.	75	72	n.s.
232	4.3	4.5	n.s.	10.0	10.1	n.s.	124	135	n.s.	212	222	n.s.	82	76	n.s.
240	3.5	3.5	n.s.	9.0	8.6	n.s.	113	115	n.s.	216	223	n.s.	82	78	n.s.
247	2.9	3.3	n.s.	9.2	10.3	n.s.	132	155	n.s.	236	240	n.s.	72	68	n.s.
Average	4.9	5.2	*	12.3	12.8	*	183	200	**	228	233	**	71	67	**

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant. Data measured from June to September 2018 on the vines excavated at harvest 2018 (treatments F17 and F17+18). *A*, net assimilation; *C_i*, intercellular CO₂; DOY, day of year; *E*, transpiration; *g_s*, stomatal conductance; HYC, high-yield conditions; LYC, low-yield conditions; *WUE_i*, intrinsic water use efficiency.

Table 3. Must composition at harvest as a function of foliar N fertilisation in the same year and crop load.

Variable	N fertilisation			Crop load			Interaction fertilisation × crop load
	0 kg/ha (n = 12)	20 kg/ha (n = 10)	P-value	LYC (n = 10)	HYC (n = 12)	P-value	
TSS (°Brix)	20.2	20.3	n.s.	21.4	19.1	***	n.s.
pH	3.56	3.65	n.s.	3.68	3.53	**	n.s.
TA (g/L)	4.7	4.8	n.s.	4.3	5.1	***	n.s.
Tartaric acid (g/L)	5.2	5.3	n.s.	5.1	5.5	**	n.s.
Malic acid (g/L)	2.3	2.5	n.s.	2.1	2.6	**	n.s.
Potassium (mg/L)	1808	1938	*	1925	1820	n.s.	n.s.
NH ₃ (mg/L)	12	21	n.s.	10	23	*	n.s.
PAN (mg N/L)	82	109	*	96	94	n.s.	n.s.
YAN (mg/L)	92	126	*	104	113	n.s.	n.s.

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant. Data measured on the vines excavated at harvest 2018 (treatments F17 and F17+18). HYC, high-yielding conditions; LYC, low-yielding conditions; PAN, primary amino nitrogen; YAN, yeast assimilable nitrogen.

A (i.e. DOY 165–204, average $g_{s\text{Jun-Jul}} = 233 \text{ mmol/m}^2 \cdot \text{s}$) in comparison with the period from veraison to harvest (DOY 214–247, average $g_{s\text{Aug-Sep}} = 150 \text{ mmol/m}^2 \cdot \text{s}$) (Table 2). Consequently, water use efficiency (WUE_i) increased gradually from flowering to harvest (average $WUE_{\text{Jun-Jul}} = 66$ and $WUE_{\text{Aug-Sep}} = 73$) (Table 2). Crop thinning significantly reduced gas exchange rates on DOY 176 and had an overall impact on them over the entire period of flowering to harvest, with lower E (average -6%), lower A (-4%), lower g_s (-8%), lower C_i (-2%), and subsequently higher WUE_i ($+6\%$) (Table 2).

Fruit composition

In comparison with the Control treatment, fertilisation did not affect grape maturation (i.e. TSS and TA concentration), but it improved the must YAN concentration (average gain

+34 mg N/L), particularly in terms of PAN (+27 mg N/L) (Table 3). Fertilisation also increased the K concentration (+134 mg/L). In contrast to fertilisation, crop thinning greatly affected grape maturation in 2018: TSS and pH were lower under HYC, while TA, and the concentration of tartaric acid, malic acid, and NH₄⁺ increased (Table 3). Similarly to 2017, the variation of must PAN and YAN at the 2018 harvest as a function of crop load was not significant.

As an indicator of vine balance, the leaf-to-fruit ratio was correlated ($P < 0.0001$) to both sugar concentration and TA in the must at harvest 2018 ($r = 0.82$ and $r = -0.78$, respectively). The must TSS varied from 19.1°Brix under HYC up to 21.4°Brix under LYC, and TA varied from 5.1 g/L under HYC down to 4.3 g/L under LYC (Table 3). The must YAN concentration at harvest was increased by the fertilisation treatment, that is, average $126 \pm 33 \text{ mg/L}$ in

Table 4. Amino acid profiles in the must at harvest, as a function of year, foliar N fertilisation in the same year and crop load.

Amino acids (%)	Amino acid profile (% total amino N)									Interaction fertilisation × crop load
	Year		P-value	N fertilisation		P-value	Crop load		P-value	
	2017 (n = 21)	2018 (n = 22)		0 kg/ha (n = 23)	20 kg/ha (n = 20)		LYC (n = 21)	HYC (n = 22)		
Alanine	8.2	7.8	n.s.	7.7	8.2	n.s.	7.1	8.8	***	n.s.
Arginine	31.7	21.7	***	25.6	27.8	n.s.	25.4	28.0	n.s.	n.s.
Asparagine	0.4	0.5	n.s.	0.5	0.4	n.s.	0.5	0.4	n.s.	n.s.
Aspartic acid	4.6	5.4	n.s.	5.2	4.8	n.s.	4.7	5.3	n.s.	n.s.
Citrulline	1.7	0.7	***	1.0	1.3	***	1.2	1.2	n.s.	n.s.
γ-Aminobutyric acid	3.2	7.7	***	6.0	5.0	**	4.9	6.1	***	**
Glutamine	2.1	0.9	***	1.5	1.6	n.s.	1.4	1.6	*	n.s.
Glutamic acid	9.0	9.4	n.s.	9.7	8.7	n.s.	9.6	8.8	n.s.	n.s.
Histidine	2.0	1.8	**	1.8	1.9	n.s.	1.9	1.9	n.s.	n.s.
Hydroxyproline	0.2	0.4	***	0.4	0.3	*	0.3	0.3	n.s.	n.s.
Isoleucine	1.3	1.7	***	1.5	1.4	n.s.	1.5	1.5	n.s.	n.s.
Leucine	1.7	1.0	***	1.4	1.3	**	1.3	1.3	n.s.	n.s.
Lysine	0.3	0.3	n.s.	0.3	0.3	*	0.3	0.3	n.s.	n.s.
Methionine	0.2	0.8	***	0.5	0.4	***	0.5	0.5	n.s.	n.s.
Ornithine	0.7	0.4	***	0.5	0.5	n.s.	0.5	0.5	n.s.	n.s.
Phenylalanine	0.7	1.1	***	1.0	0.8	**	0.9	0.9	n.s.	n.s.
Proline	12.7	21.0	***	17.2	16.6	n.s.	21.0	12.7	***	n.s.
Serine	6.4	6.0	n.s.	6.2	6.1	n.s.	5.6	6.8	***	n.s.
Threonine	7.7	6.5	**	6.7	7.5	*	6.2	7.9	***	n.s.
Tryptophan	1.4	1.1	**	1.3	1.2	n.s.	1.3	1.3	n.s.	n.s.
Tyrosine	1.2	0.8	***	1.0	1.0	n.s.	1.0	1.0	n.s.	n.s.
Valine	2.6	2.2	***	2.4	2.3	n.s.	2.4	2.3	n.s.	n.s.

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant. Data from the vines excavated at harvest 2017 (treatments CT and F17) and 2018 (F17 and F17+18). HYC, high-yielding conditions; LYC, low-yielding conditions.

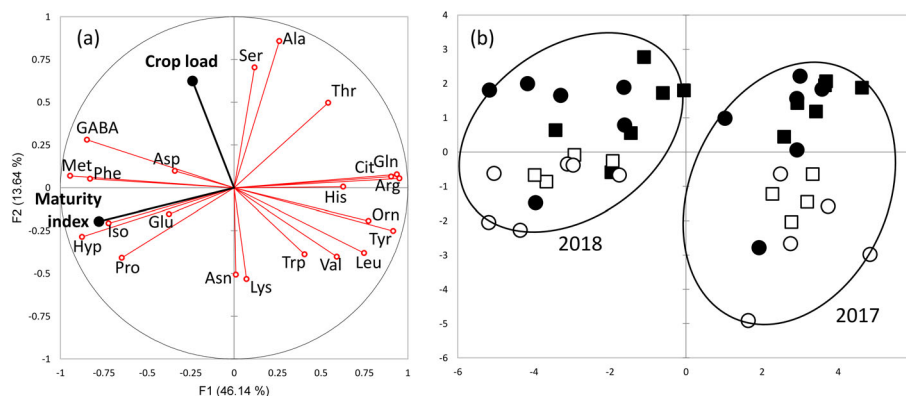


Figure 1. Principal component analysis (PCA) of the amino N profiles of the musts at harvests 2017 and 2018. (a) Variables: correlations between amino acid concentrations. The supplementary data crop load (kg/plant) and maturity index (TSS-to-TA ratio) are not used to calculate the coordinates of the amino acids. (b) Observations: shorter distances between observations indicates similar amino N profiles. Data measured on the vines excavated at both harvests 2017 (treatments CT and F17) and 2018 (treatments F17 and F17+18). Low-yield conditions, Control (○) and fertilised (□); high-yield conditions, Control (●) and fertilised (■).

comparison with 92 ± 34 mg/L in the Control treatment (Table 3). The impact of fertilisation, however, on the YAN concentration was insignificant under LYC ($P = 0.204$), while it was significant under HYC ($P = 0.032$), similar to 2017 (Verdenal et al. 2020). Independently from crop load, plant vigour (i.e. canopy mass) increased with must YAN concentration at harvest under both Control ($P = 0.005$) and fertilisation treatments ($P = 0.006$).

The year had a major influence on the concentration of most of the individual FAAs, while the total FAA concentration remained similar between the 2017 and 2018 harvests (115 ± 30 mg N/L in 2017 and 107 ± 34 mg N/L in 2018). Fertilisation increased the concentration of most individual FAAs as well as of the total FAAs. Conversely, crop thinning had no significant impact on the total FAA concentration and only increased the concentration of a few major FAAs, that is, glutamic acid and proline (data not shown).

In terms of FAA profile, crop thinning and fertilisation affected different FAAs, except for γ -aminobutyric acid and threonine (Table 4). The LYC decreased the proportion of alanine, γ -aminobutyric acid, glutamine, serine, and threonine, while it increased the proportion of proline. Fertilisation increased the proportion of citrulline and threonine, while it decreased the proportion of γ -aminobutyric acid, hydroxyproline, leucine, lysine, methionine, and phenylalanine (Table 4). Proline was highly correlated with TSS in terms of both quantity and proportion of total FAA. The musts were discriminated as a function of their FAA profiles using a (PCA) (Figure 1). The variables crop load (in kg/plant) and maturity index were added to the PCA as supplementary variables (Figure 1). The PCA showed that the must FAA profiles at harvest were discriminated first by the year and then by the combination of both crop load and grape maturity. Since the 2017 maturity index was constant, the crop load was the main factor of discrimination in that year (Figure 1). No discrimination was observed for N-fertilised versus Control vines.

Carbon

Fertilisation had a negligible effect on TOC, CQ, and $\delta^{13}\text{C}$: only the trunk showed -2% in TOC and $+0.5$ mUr in $\delta^{13}\text{C}$ in the fertilised treatment (Table 5). Conversely, the whole plant TOC was affected by crop thinning, with a 3% increase under LYC in comparison with HYC, despite

a 1% decrease in the pomace (Table 5). The CQ was highly affected by crop thinning: in comparison with HYC, CQ was increased in the roots ($+17\%$) under LYC, while it was decreased in the canopy (about 21%) and grapes (i.e. pomace + must, -58%). The $\delta^{13}\text{C}$ values slightly increased under LYC in both grapes and roots (Table 5). While not influenced by crop thinning, the C:N ratio decreased in the grapes (i.e. pomace + must) due to N fertilisation.

The CQ distribution in the plant was monitored over two seasons (Figure 2). The difference in CQ between LYC and HYC was mainly related to crop size: the share of grape CQ in the whole plant was higher under HYC in both seasons. Despite a lower CQ in the roots at harvest under HYC in comparison with LYC (Table 5), the kinetics of CQ in the perennial fractions (roots + trunk) were similar in both seasons, with a decrease from budburst to flowering, an increase from flowering to veraison, and then a slower increase after veraison until pruning (Figure 2). A global increase in the C content in the perennial fractions (root + trunk) was observed at harvest 2018 ($+26\%$) in comparison with harvest 2017, independently from the crop load (Figure 2). The distribution of both trunk and canopy CQ was similar either under LYC or under HYC. Under HYC, grape CQ at harvest was equivalent to canopy CQ in both the 2017 and 2018 seasons.

Dry mass and nitrogen

The composition of the plant fractions was compared in the Control treatment (data not shown). The DM varied from 70% in the roots to 21% in the must at harvest. The TN was highest in the canopy and pomace (average 0.9% DM) and lowest in the must (average 0.2% DM) independently from the crop load. The $\delta^{15}\text{N}$ values were the highest in the must (average 73 mUr) and the lowest in the canopy and pomace (average 26 mUr). When compared with the other plant fractions, canopy NQ was the highest under both HYC and LYC at 5.5 and 4.9 g, respectively.

In comparison with budburst 2017, the roots and wood DM at budburst 2018 was higher by 55%. Without affecting the plant DM, fertilisation increased TN concentration in the grapes ($+23\%$) (Table 6). Conversely, crop thinning affected the whole plant DM (-27% under LYC in comparison with HYC), with a large decrease in the grapes (-58%) and

Table 5. Impact of foliar N fertilisation in the same year and crop load on the carbon concentration and quantity, carbon isotope composition and C:N ratio.

Variable	Plant fraction	N fertilisation			Crop load			Interaction fertilisation × crop load
		0 kg/ha (n = 12)	20 kg/ha (n = 10)	P-value	LYC (n = 10)	HYC (n = 12)	P-value	
Carbon concentration (TOC) (% DM)	Roots	47.6	47.3	n.s.	47.2	47.7	n.s.	n.s.
	Trunk	47.2	46.1	***	46.6	46.7	n.s.	n.s.
	Canopy	44.4	44.7	n.s.	44.6	44.6	n.s.	n.s.
	Thinned bunches	44.8	44.8	n.s.	44.9	44.7	n.s.	n.s.
	Pomace	42.4	42.5	n.s.	42.0	42.9	*	n.s.
	Must	38.1	37.7	n.s.	37.8	38.0	n.s.	n.s.
	Whole plant	43.9	43.7	n.s.	44.3	43.3	***	n.s.
Carbon quantity (CQ) (g)	Roots	134	118	n.s.	136	116	*	*
	Trunk	179	167	n.s.	168	178	n.s.	*
	Canopy	284	302	n.s.	258	328	*	n.s.
	Thinned bunches	16	13	n.s.	18	11	n.s.	n.s.
	Pomace	63	68	n.s.	38	93	***	n.s.
	Must	168	167	n.s.	99	237	***	n.s.
	Whole plant	845	835	n.s.	716	964	***	n.s.
Carbon isotope composition ($\delta^{13}C$) (mUr)	Roots	-28.8	-28.9	n.s.	-28.7	-29.0	*	n.s.
	Trunk	-28.3	-28.8	**	-28.5	-28.6	n.s.	n.s.
	Canopy	-29.2	-29.1	n.s.	-29.1	-29.2	n.s.	n.s.
	Thinned bunches	-29.4	-29.6	n.s.	-29.3	-29.7	*	n.s.
	Pomace	-29.7	-29.5	n.s.	-29.3	-29.9	**	n.s.
	Must	-27.9	-27.7	n.s.	-27.6	-28.0	*	n.s.
	Whole plant	-28.7	-28.7	n.s.	-28.6	-28.8	n.s.	n.s.
C:N ratio	Roots	83	74	n.s.	82	75	n.s.	n.s.
	Trunk	124	114	n.s.	117	121	n.s.	n.s.
	Canopy	47	43	n.s.	47	44	n.s.	n.s.
	Thinned bunches	28	28	n.s.	29	27	n.s.	n.s.
	Pomace	53	43	**	50	46	n.s.	n.s.
	Must	250	185	*	219	215	n.s.	n.s.
	Whole plant	74	64	n.s.	69	68	n.s.	n.s.

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant. Data measured on the vines excavated at harvest 2018 (treatments F17 and F17+18). HYC, high-yielding conditions; LYC, low-yielding conditions.

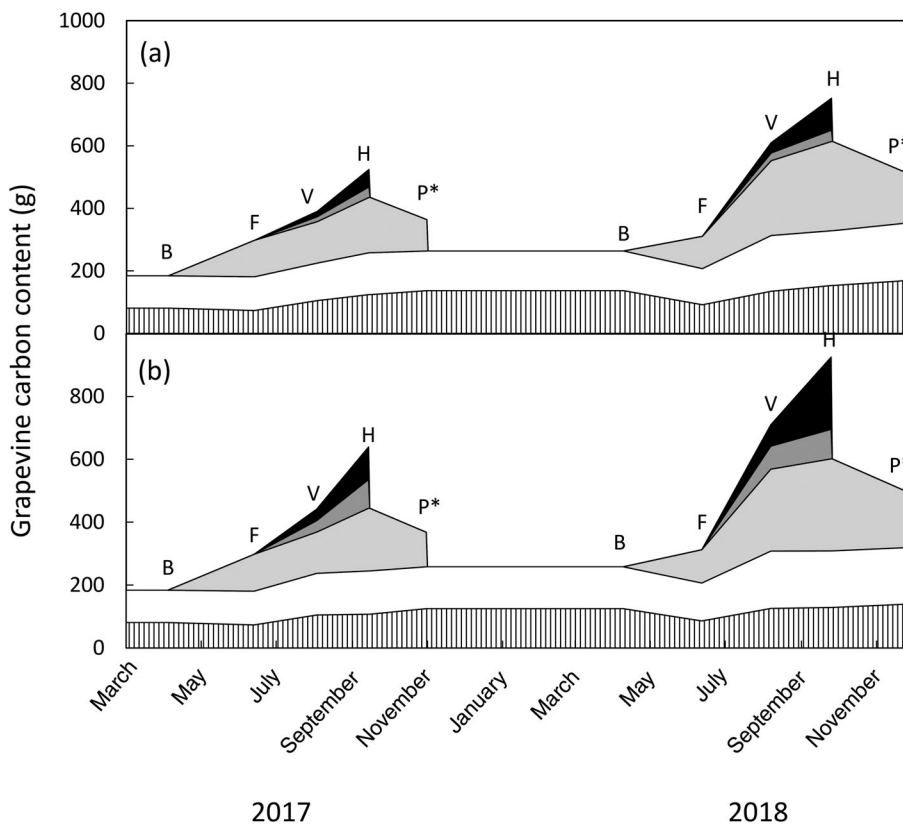


Figure 2. Effect of crop load on the dynamics of carbon distribution from March 2017 to December 2018; (a) low-yield conditions and (b) high-yield conditions. Data measured on the vines excavated at each phenological stage (treatment CT) over 2 years. B, buddburst; F, flowering; V, veraison; H, harvest; P, pruning; *, extrapolated data. Must (■), pomace (▒), canopy (░), trunk (□) and roots (▨).

Table 6. Impact of foliar N fertilisation in the same year and of crop load on the dry mass, nitrogen concentration and quantity of plant fractions.

Variable	Plant fraction	N fertilisation			Crop load			Interaction fertilisation × crop load
		0 kg/ha (n = 12)	20 kg/ha (n = 10)	P-value	LYC (n = 10)	HYC (n = 12)	P-value	
Dry mass (DM) (g)	Roots	282	249	n.s.	288	243	*	*
	Trunk	380	362	n.s.	359	382	n.s.	*
	Canopy	638	676	n.s.	579	736	*	n.s.
	Thinned bunches	36	29	n.s.	41	25	n.s.	n.s.
	Pomace	149	158	n.s.	90	217	***	n.s.
	Must	440	445	n.s.	261	623	***	n.s.
	Whole plant	1925	1919	n.s.	1618	2226	***	n.s.
Nitrogen composition (TN) (% DM)	Roots	0.60	0.66	n.s.	0.59	0.67	n.s.	n.s.
	Trunk	0.38	0.41	n.s.	0.40	0.39	n.s.	n.s.
	Canopy	0.98	1.07	n.s.	0.98	1.06	n.s.	n.s.
	Thinned bunches	1.60	1.64	n.s.	1.58	1.66	n.s.	n.s.
	Pomace	0.82	1.00	**	0.86	0.96	n.s.	n.s.
	Must	0.16	0.21	*	0.18	0.20	n.s.	n.s.
	Whole plant	0.62	0.70	n.s.	0.66	0.66	n.s.	n.s.
Nitrogen quantity (NQ) (g)	Roots	1.67	1.64	n.s.	1.69	1.62	n.s.	*
	Trunk	1.46	1.47	n.s.	1.43	1.51	n.s.	*
	Canopy	6.35	7.22	n.s.	5.73	7.83	*	n.s.
	Thinned bunches	0.57	0.48	n.s.	0.64	0.41	n.s.	n.s.
	Pomace	1.24	1.65	n.s.	0.77	2.12	***	n.s.
	Must	0.74	1.03	n.s.	0.47	1.29	***	n.s.
	Whole plant	12.03	13.48	n.s.	10.72	14.78	*	n.s.

Data measured on the vines excavated at harvest 2018 (treatments F17 and F17+18); *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant. HYC, high-yielding conditions; LYC, low-yielding conditions.

canopy (−21%) and an increase in the roots (+19%) (Table 6). The overall vine capacity decreased with crop thinning. Crop thinning had no significant impact on TN. Consequently, NQ varied proportionally to DM, with a

significant loss in the whole plant under LYC (−27%), mainly due to the lower crop load (Table 6).

The distribution of NQ was monitored over two seasons (Figure 3). The difference in NQ between LYC and HYC was

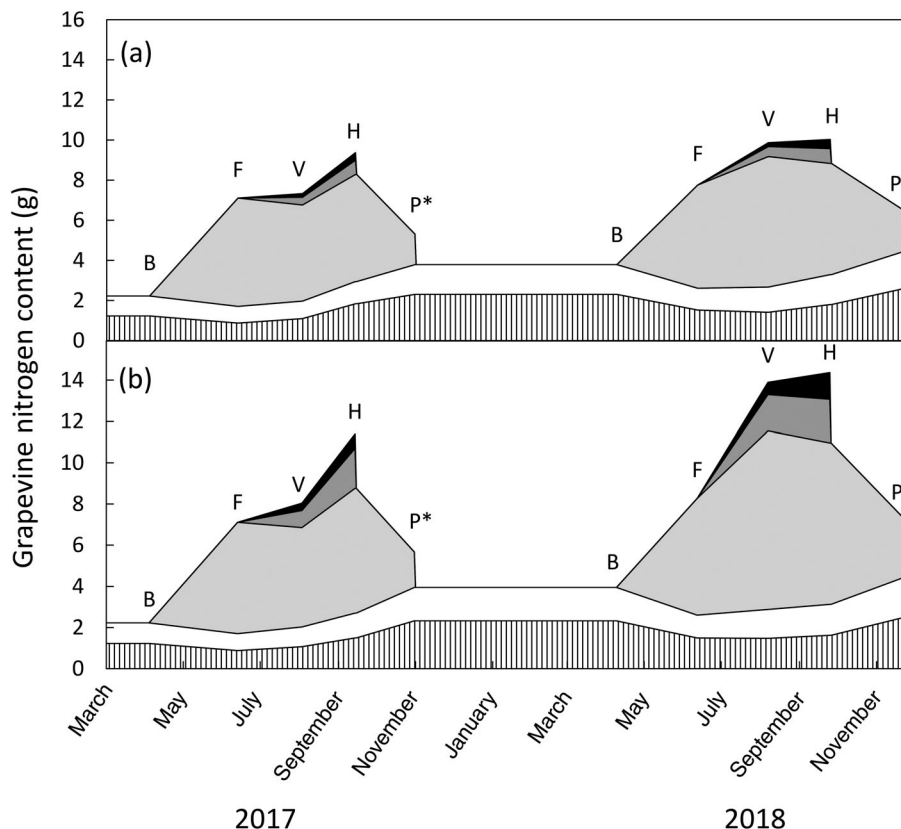


Figure 3. Effect of crop load on the dynamics of nitrogen distribution from March 2017 to November 2018; (a) low-yield conditions and (b) high-yield conditions. Data measured on the vines excavated at each phenological stage (treatment CT) over 2 years. B, budburst; F, flowering; V, veraison; H, harvest; P, pruning; *extrapolated data. Must (■), pomace (▣), canopy (▤), trunk (□) and roots (▨).

Table 7. Relative specific abundance, quantity of labelled N pool and partitioning of residual labelled N from 2017 fertilisation, new labelled N from 2018 fertilisation and the accumulation of both 2017 and 2018 fertilisations (total-lab-N) at harvest 2018, as a function of crop load.

Variable	Plant fraction	2017-res-N			2018-lab-N			Total-lab-N		
		LYC (n = 6)	HYC (n = 6)	P-value	LYC (n = 4)	HYC (n = 6)	P-value	LYC (n = 10)	HYC (n = 12)	P-value
Relative specific abundance (RSA) (% TN)	Roots	3.6	3.1	n.s.	2.5	1.1	**	6.1	4.2	**
	Trunk	3.2	2.6	*	3.5	2.0	**	6.7	4.6	***
	Canopy	4.0	3.5	n.s.	5.7	3.9	n.s.	9.7	7.4	*
	Thinned bunches	4.7	4.0	n.s.	0.0	0.0	n.s.	4.7	4.0	*
	Pomace	3.8	3.2	n.s.	9.2	6.1	**	13.0	9.3	***
	Must	5.1	3.5	n.s.	10.4	7.7	*	15.5	11.2	**
	Whole plant	3.9	3.3	n.s.	5.5	4.5	n.s.	9.4	7.8	n.s.
Labelled N pool (mg)	Roots	68	46	n.s.	35	19	*	103	65	**
	Trunk	49	36	*	45	33	n.s.	95	69	**
	Canopy	232	216	n.s.	297	342	n.s.	529	558	n.s.
	Thinned bunches	31	15	n.s.	0	0	n.s.	31	15	n.s.
	Pomace	26	55	**	77	152	*	103	207	**
	Must	24	32	n.s.	48	122	*	72	154	*
	Whole plant	431	401	n.s.	502	667	n.s.	934	1068	n.s.
Labelled N partitioning (%)	Roots	16	11	*	7	3	**	11	6	**
	Trunk	12	9	n.s.	10	5	**	10	7	**
	Canopy	53	54	n.s.	59	52	n.s.	57	53	n.s.
	Thinned bunches	7	4	n.s.	0	0	n.s.	3	1	***
	Pomace	6	14	**	15	22	*	11	19	**
	Must	6	8	n.s.	9	18	**	8	14	**

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant. Data measured on the vines excavated at harvest 2018 (treatments F17 and F17+18). HYC, high-yielding conditions; LYC, low-yielding conditions; RSA, relative specific abundance; TN, total nitrogen.

mainly due to both grapes and canopy: the share of grape and canopy NQ at harvest was lower under LYC, particularly in 2018 (−38%) (Table 6). At harvest, a major part of N was located in the canopy (i.e. 53% of NQ under HYC and 52% under LYC). The NQ in the perennial fractions (roots + trunk) was similar in both seasons, with a decrease from budburst to flowering and an increase from flowering to pruning. A global increase of NQ in the perennial fractions (root + trunk) was observed between the 2017 and 2018 harvests and was greater under LYC than under HYC (i.e. +16% and +12%, respectively). Under LYC, 30% of NQ was located in the reserves at harvest, while 10% migrated to the grapes. Conversely, under HYC, 20% of NQ remained in the perennial fractions at harvest, while 20% migrated to the grapes (Figure 3).

Fertilisation and N uptake

At harvest 2018, 2017-res-N RSA was relatively constant throughout the plant (average 3.7%) (Table 7). Conversely, 2018-lab-N RSA varied greatly across plant fractions: the grapes had the highest RSA (up to 10.4% in must under LYC). The accumulation of both 2017 and 2018 labelled N exacerbated the differences between LYC and HYC: the total-lab-N RSA was higher overall in all plant fractions under LYC (Table 7). The quantity of total-lab-N under LYC was generally higher in the perennial fractions (roots + wood; 198 mg; +48%) and lower in the grapes (pomace + must; 175 mg; −52%), while it remained constant in the canopy (average 547 ± 83 mg) and in the whole plant (average 1014 ± 179 mg). In terms of partitioning, the proportion of both 2017-res-N and 2018-lab-N located in the canopy was similar: 55% of total-lab-N was located on average in the canopy, independently from crop load (Table 7). Under LYC, total-lab-N was higher in the perennial fractions (+69%) and lower in the grapes (−41%) compared with HYC. Up to 33% of total-lab-N was located in the grapes under HYC at harvest 2018 (Table 7).

The isotope labelling method allowed the estimation of fertilised N assimilated by the plant out of the total N applied in 2017, in 2018, and over the 2 years. Fertilised N uptake (in relation to the total quantity supplied) varied as a function of the year, with 34% in 2017 and 25% in 2018. Over the 2 years of the experiment, average labelled N uptake was 29% and varied as a function of crop regulation (i.e. 34% under HYC vs 25% under LYC, $P < 0.0001$) (Figure 4).

The dynamics of 2017-res-N in the plant were monitored from harvest 2017 to winter pruning 2018. Because the quantity of 2017-res-N varied as a function of crop load, its distribution in the plant during the following year (2018) is shown as a proportion of the 2017-res-N measured in the whole plant at harvest 2017 (i.e. 0.95 g N under HYC and 0.68 g N under LYC) (Figure 5). In 2017, labelled N

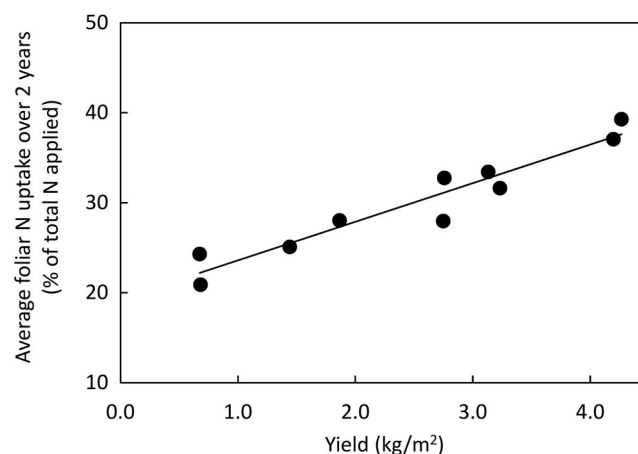


Figure 4. Effect of yield on the 2-year average foliar N uptake following foliar N fertilisation at veraison. Data measured on the vines excavated at both harvests 2017 (treatment F17) and 2018 (treatment F17+18). $n = 10$, $r = 0.96$, $P < 0.0001$.

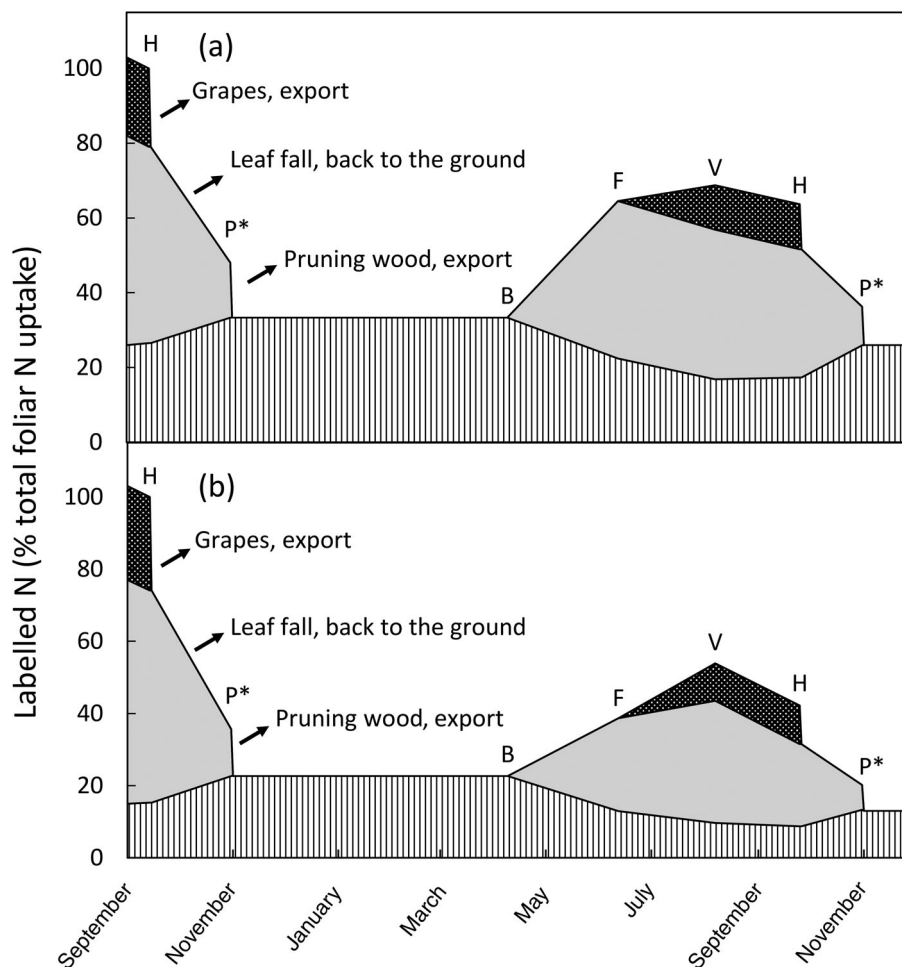


Figure 5. Effect of crop load on the dynamics of residual labelled N from 2017 fertilisation (2017-res-N) as a proportion of total foliar N assimilation; (a) low-yield conditions and (b) high-yield conditions. Data measured on the vines excavated at each phenological stage 2017 from September 2017 to November 2018 (treatments F17). B, buddburst; F, flowering; V, veraison; H, harvest; P, pruning; *extrapolated data. Grapes (must + pomace) (▨), canopy (■) and reserves (roots + trunk) (▩).

contained in grapes (i.e. 21% under LYC and 26% under HYC) was exported at harvest. Similarly, labelled N contained in shoots (i.e. 15% under LYC and 13% under HYC) was exported at winter pruning. Before leaf fall, approximately 25% of the leaf N was relocated to the perennial fractions (roots + trunk), and the rest returned to the soil, either directly via leaf fall or through root leaching. During winter 2017/2018, 33% of 2017-res-N was still in the plant perennial fractions under LYC, versus 23% under HYC (Figure 5). The following season, the perennial fraction showed a decrease in 2017-res-N from buddburst to harvest. From harvest to winter pruning, the perennial fractions increased again due to leaf N relocation before pruning. The increase of 2017-res-N in the whole plant from buddburst to veraison suggests a de-novo uptake of labelled N from the soil, which would correspond to the 2017-res-N released previously. The 2017-res-N pool reached a maximum at veraison 2018 of 69% under LYC versus 54% under HYC (Figure 5). The amount of 2017-res-N present in grapes was similar at both veraison and harvest under both yield levels. At harvest 2018, 63% of the initial labelled N was still found in the plant under LYC: 17% in the roots and wood, 34% in the canopy, and 12% in the grapes. Conversely, under HYC, only 43% of initial labelled N was found: 9% in the roots and wood, 23% in the canopy, and 11% in the grapes (Figure 5). Export of grape and pruning wood occurred at

harvest and winter pruning similarly to 2017. During the second winter, 26% of 2017-res-N was still in the plant perennial fractions under LYC, versus only 13% under HYC. This tendency could easily be extrapolated over the following years. Over 2 years, the partitioning of 2017-res-N

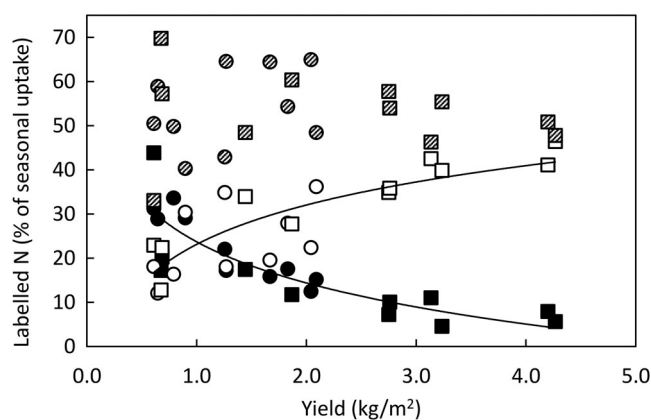


Figure 6. Effect of yield on the partitioning of labelled N at harvest, following foliar N fertilisation at veraison. Data measured on the vines excavated at both harvests 2017 (treatment F17) and 2018 (treatment F17 +18). Grapes [2017 (○), 2018 (□)] ($n = 21$, $r = 0.83$, $P < 0.0001$), canopy [2017 (⊙), 2018 (⊚)] ($n = 21$, $r = 0.83$, $P < 0.0001$) and reserves [2017 (●), 2018 (■)] ($n = 21$, $r = 0.81$, $P < 0.0001$).

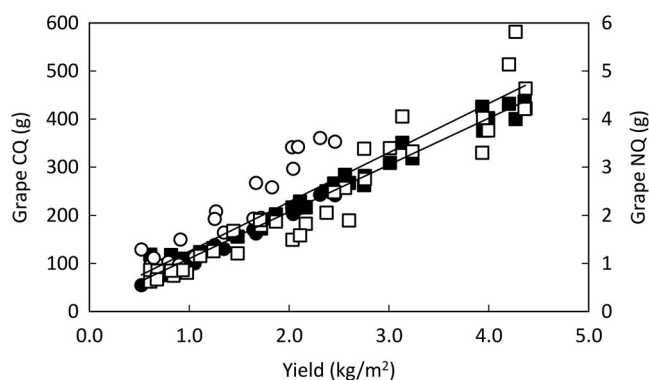


Figure 7. Effect of yield on carbon quantity (CQ) [2017 (●), 2018 (■)] and nitrogen quantity (NQ) [2017 (○), 2018 (□)] in grapes at harvest. Data measured on the vines excavated at harvest 2017 (treatment CT and F17) and 2018 (treatment CT, F17 and F17+18). CQ, $n = 55$, $r = 0.99$, $P < 0.0001$; NQ, $n = 55$, $r = 0.91$, $P < 0.0001$.

showed a balance between both perennial fractions (roots + trunk) and grapes (pomace + must) as a function of crop load (Figure 6). Canopy labelled N content remained relatively stable at 55% of total 2017-res-N on average overall range of yield conditions. Under HYC, the share of labelled N located in the grapes increased strongly (+25% of total labelled N) to the detriment of the reserve labelled N content (−25%) (Figure 6).

Discussion

Environmental conditions, plant growth, and nutrient seasonal cycle

The environmental conditions of soil and climate were conducive to unrestricted vegetative development (i.e. neither water nor nutrient restriction). The average must $\delta^{13}\text{C}$ values at harvest were lower than the threshold of -26 mUr, suggesting unlimited water supply to the vines during fruit ripening (van Leeuwen et al. 2009). Average leaf composition and chlorophyll index measured at the onset of fruit ripening indicated no severe N deficiency even in the non-fertilised treatment, according to the thresholds published for the grape cultivar Chasselas under the Swiss cool climate (Spring and Verdenal 2017). The perennial fractions of the vines gained on average 55% DM in 1 year, which is a quite substantial growth rate for 5-year-old vines. In both seasons, the photosynthesis rate gradually declined from flowering until harvest, as described previously (Keller et al. 2001, Zufferey et al. 2018).

The DM, C, and N seasonal dynamics were in accordance with other studies on grapevines and other perennial crops (Zapata et al. 2004, Zufferey et al. 2015, Schreiner 2016, Muhammad et al. 2020): C and N content in perennial fractions was greatest from leaf fall to budburst and lowest at flowering. From budburst to flowering, root N uptake was low and N demand—due to intense vegetative growth—was mainly supported by the mobilisation of root and wood reserves. The refilling of reserves occurred mostly during fruit ripening and substantially after harvest, due to N relocation from the canopy before leaf fall. Under the hot climate conditions of South Africa and Australia, Conradie (1980, 1991) and Holzapfel et al. (2019) observed a more important N uptake during the postharvest period, which lasts several months under these climates. Wermelinger (1991) suggested that more than 40% of the leaf N on grapevine is translocated before leaf fall from the

senescent leaves to the perennial plant fractions. In our trial, 5–10% of leaf N was relocated to the grapes until harvest and then 25–30% to the perennial fractions until leaf fall. A significant share of leaf N was released to the ground and potentially increased the soil mineral N pool. Khalsa et al. (2016) demonstrated in *Prunus dulcis* that leaf litter decomposition led to a larger mineral N pool. In our trial, the leaves contained, on average, 0.83% DM of N at leaf fall 2017, which represented a 60% decrease in comparison with the leaf N content at the onset of fruit ripening in the same year (2.15% DM). This observation does not exclude the hypothesis of root N leaching: N return to the ground could be a combination of both leaf fall and root efflux, which could not be demonstrated with the present experimental setup. Most studies about nutrient dynamics do not consider root N efflux in their models. In fact, the plant N cycle should be seen as open. Total N uptake is the sum of total N influx and total N efflux (Hachiya and Sakakibara 2017). Considering plant N efflux as part of the plant N budget would prevent both an overestimation of N losses and an underestimation of N uptake (Xu et al. 2012). As an example, *Triticum aestivum* released 5–6% of the N previously assimilated, which represents 15 kg N/ha released by roots to the soil (Merbach et al. 1999). Unfortunately, studies of grapevine root efflux are scarce.

Crop load affected fertiliser N efficiency

The absorption of nutrients by leaves has been acknowledged since the nineteenth century (e.g. Fernández et al. 2021), and foliar application is a widely accepted method of fertilisation. Foliar urea (20 kg N/ha) was applied on the leaves at the onset of grape ripening, with the aim of improving fruit N status without either increasing plant vigour or delaying fruit ripening (Hannam et al. 2016). As expected, foliar fertilisation efficiently increased fruit N concentration during the season of its application. Nitrogen fertilisation did not affect either $\delta^{13}\text{C}$ or plant vigour, but was positively correlated to WUE_i . This result contrasts with the findings of Taskos et al. (2020), who observed—on both cultivars Cabernet Sauvignon and Xinomavro—that leaf N concentration was negatively correlated to WUE_i . Their results suggest that the large amount of soil-applied ammonium nitrate (i.e. 120 kg N/ha) greatly promoted plant vigour, with A increasing more slowly than g_s . However, N form (i.e. ammonium nitrate vs urea), and application location (i.e. ground vs foliar) might partially explain the contrast between the two experiments. Like crop load, fertilisation affected certain FAAs more than others, thus potentially affecting fruit aroma profile. This is consistent with findings from Schreiner et al. (2014) on the grape cultivar Pinot Noir. Interestingly, the FAAs which were the most affected by fertilisation were not the same as the ones affected by crop load. The impact of fertilisation, however, on the FAA profile of grapes was small in relation to both the year and the crop load (Figure 1).

Despite a homogeneous N supply in the entire plot, fertiliser N uptake varied greatly in relation to crop load, as observed in other studies (Morinaga et al. 2003, Treeby and Wheatley 2006, Verdenal et al. 2016). It was, on average, $29 \pm 8\%$ of total N applied, with a higher uptake rate under HYC (i.e. 34% vs 25% under LYC; Figure 4). Fertilisation promoted a higher fruit YAN concentration at harvest under HYC (+55 mg/L in 2017, $P = 0.021$; +54 mg/L in 2018, $P = 0.032$), while the gain was not significant under LYC (+1 mg/L in 2017, $P = 0.986$; +14 mg/L in 2018,

$P = 0.460$). This can be explained by the lower fertiliser uptake rate under LYC, regardless of the year. In other words, the fertilisation efficiency greatly varied according to crop load. This important finding explains why, in some situations, foliar N fertilisation does not efficiently improve fruit N concentration and may potentially cause environmental contamination.

Once assimilated, fertiliser N was not homogeneously distributed in the plant. The RSA varied across the plant fractions, decreasing gradually from the fruit to the roots and wood, showing a hierarchy in N sink strength among the plant fractions. Fertiliser N content was also affected by crop load in both grapes and perennial fractions, while it remained constant in the canopy in both seasons. To the detriment of the roots and wood, 40% of fertiliser N was located in the fruit at harvest under HYC, versus only 24% under LYC. The quantity of N exported from the vineyard is related to the amount of grape harvested, inducing higher fertiliser N loss under HYC. After harvest 2017, a share of 2017-res-N was relocated from the canopy to the perennial fractions before pruning and was subsequently redistributed to the whole plant during 2018. In comparison with the Control treatment, the presence of 2017-res-N in the plant in 2018 had no carryover effect either on vegetative parameters or on grape composition. The TN and NQ remained unchanged at budburst 2018, regardless of either crop load or fertilisation. This result confirms that foliar urea supply at the onset of fruit ripening is a good practice for short-term fruit N correction (Hannam et al. 2016). Conversely to the fluctuation of 2018-lab-N RSA between plant fractions, 2017-res-N RSA was constant in the whole plant in 2018 regardless of plant fractions and crop load. These results suggest that N partitioning depended on both N species and N origin, either from the perennial reserves (2017-res-N, mainly FAA) or from the seasonal foliar uptake (2018-lab-N, mainly NH_4^+ from urea assimilation) (Keller 2020). Fertilisation in both 2017 and 2018 contributed to the accumulation of fertiliser N in the plant without increasing either NQ or TN, suggesting that soil N uptake was related to the initial plant N reserve.

The ^{15}N -labelling method allowed the quantifying of fertiliser N uptake and the tracking of its distribution and redistribution into the plant. Implementation of efficient N management in perennial crops requires knowledge of N uptake patterns and allocation by considering a multi-year production cycle (Khalsa et al. 2020). In this trial, the foliar urea supplied at veraison 2017 was the unique source of labelled N. Thus, the calculation of RSA (i.e. $A\%_{\text{N supplied}} = 10 \text{ atom } \% \text{ } ^{15}\text{N}$) allowed us to estimate the partitioning of fertiliser N assimilated by the plant over the two growing seasons (Figure 5). Alternatively, labelling a particular plant fraction such as the perennial fraction would have allowed studying the distribution of nutrients originating exclusively from that fraction, while differentiating from other origins (Bowen and Zapata 1991). In this trial, at the onset of the second season, the perennial fraction (roots + wood) of the fertilised plants still contained 2017-res-N. Thus, considering the N pool initially present in the perennial fraction as the unique source of labelled N [i.e. $A\%_{\text{N supplied}} = A\%_{(\text{roots}+\text{trunk})}$], as affected by 2017-res-N), theoretically allowed an estimate of the partitioning of the N from the perennial fractions during the second season, differentiating them from the unlabelled seasonal root N uptake. As demonstrated in this trial, however, a considerable portion of labelled N was released to the soil at the

end of the growing season, either through leaf fall or root leaching. It was then available for re-assimilation in the second season. Khalsa et al. (2020) highlighted the long-term N cycling in a California almond orchard and confirmed the role of N recycling from the tree biomass—leaf litter and root turnover—to the soil organic matter. Consequently, the perennial plant fractions were not the only source of labelled N in the second year, which prevented the differentiation of reserve N mobilisation from soil N uptake in the context of our trial. To assess this issue experimentally, plants would need to be transplanted before the second season in new soil, not containing any labelled N.

Crop load affected N dynamics and grape composition

Most of the aboveground vegetative parameters, such as leaf area, bunch mass, and pruning wood, were not influenced by crop thinning during the trial period. Crop regulation did not promote a stronger vigour of the canopy in response to crop thinning. This is in agreement with Keller et al. (2005), but in contradiction with Morinaga et al. (2003), who observed a higher vegetative development under LYC. In our trial, canopy DM under LYC was smaller than under HYC at the end of the second year, similar to the findings of Bowen et al. (2011). The non-restrictive conditions of our trial in terms of water and nutrients, even under HYC, may explain why the vines showed a higher capacity under HYC. The supply of resources exceeded the demand and allowed the maximal production set by genetic potential to be reached (Lawlor 2002). Conversely, in respect to vigour, the plant capacity is defined by the total annual growth of a grapevine and is an indicator of the net resource gain from the environment (Keller 2020). Several studies have already reported the influence of crop load on C partitioning in grapevine (Morinaga et al. 2003, Zapata et al. 2004, Zufferey et al. 2015). In this trial, crop thinning significantly improved grape ripening in 2018, as shown by the variation of the maturity index (TSS-to-TA ratio) as a function of crop load ($r = -0.81$ and $P < 0.0001$). The maturity index was not affected in 2017 ($P = 0.171$), probably due to the smaller difference in crop load between the treatments and the less restrictive leaf-to-fruit ratio under HYC ($1.0 \text{ m}^2/\text{kg}$ in 2017 vs $0.7 \text{ m}^2/\text{kg}$ in 2018). In response to crop thinning, plants required less C from the perennial fractions to meet the demand for ripening fruits (Howell 2001). In this trial, this relationship was confirmed, and the close relationship between the metabolism of C and N was highlighted. Both CQ and NQ in fruits were reduced proportionally to crop thinning, while their concentration was unchanged by crop load (Figure 7). In the roots, DM and CQ increased by 19 and 17%, respectively, while NQ increased insignificantly. The roots were the plant fraction most affected by crop thinning, highlighting the close coordination of C and N metabolites between grapes and roots. Howell (2001) already mentioned the development of fruits at the expense of vegetative tissues, particularly the roots. Stander et al. (2017) also observed that higher crop load limited root growth on mandarin trees (*Citrus reticulata*). As a major carbohydrate sink in the plant, the fruit load disturbs the balance between fruit ripening and root growth by limiting the allocation of C and N metabolites to roots.

The impact of crop load on the gas exchange rate may vary greatly from negative to positive, depending on the crop and on the environmental conditions (Lin et al. 2018). In our trial, lower fruit C and N demand induced by crop thinning was the likely reason for lower leaf gas exchange rate. Seasonal photosynthesis activity was reduced by crop

thinning, although the differences between LYC and HYC were not always significant on a daily time step. The lower leaf gas exchange rates under LYC were mainly due to a lower g_s , which promoted a higher WUE_i (i.e. A/g_s) and subsequently, a higher $\delta^{13}C$ in all plant fractions, particularly in fruit and roots. The positive correlation between $\delta^{13}C$ and WUE_i was already established by Livingston et al. (1999) on white spruce (*Picea glauca*). These authors described WUE_i as an indicator of the compromise between photosynthesis and transpiration. Krapp et al. (1993) explained this phenomenon by the removal of importing organs (sink), resulting in a gradual inhibition of photosynthesis in the exporting leaves (source). This 'sink regulation' of photosynthesis is usually associated with a higher carbohydrate content in the leaves, as carbohydrates may cause a feedback inhibition of photosynthesis (Krapp et al. 1993, Wang et al. 2018). Wang et al. (2018) further demonstrated that the earlier crop thinning is carried out, the greater the downregulation of photosynthesis. In our study, the lower gas exchange rate under LYC induced a lower uptake of both C and N. This was probably due to the close relation between both C and N metabolism: C assimilation rate requires N supply, which depends, in turn, on the availability of C compounds for nitrate assimilation (Lawlor 2002, Gauthier et al. 2010, Vripon-Brenas et al. 2019, Keller 2020). Alem et al. (2021) also demonstrated that crop thinning resulted in a significantly lower accumulation of most metabolites in the whole crop (e.g. soluble sugars, organic acids, glycosylated aroma precursors). The relationship between C and N dynamics varies according to crop load, but is also influenced by genetics and environmental conditions (Lawlor 2002). Under HYC, the plants showed signs of overcropping: availability of C was probably the driver, due to an unbalanced ratio between canopy size and crop load, that is, the source-to-sink ratio. Kliewer and Dokoozlian (2005) have shown on grapevine that a minimum of 1.0–1.5 m² leaf area/kg fruit is required for complete grape maturation. This threshold was confirmed with the cultivar Chasselas by Zufferey et al. (2015). In our trial, under HYC and with the plant leaf area limited by trellising and hedging, photosynthesis activity was insufficient to fulfil the fruit demand of carbohydrates, and berry ripening was subsequently delayed. The leaf-to-fruit ratio was limiting under HYC (i.e. 0.7 m/kg), resulting in a significant loss in TSS (average -1.3°Brix) at harvest 2018 in comparison with LYC, confirming previous findings (Bubola et al. 2020, Sivilotti et al. 2020). Crop thinning, however, had no significant impact on 2017 fruit ripening, perhaps due to the lower yield potential that year and the non-limiting leaf-to-fruit ratio (1.0 m²/kg under HYC). The contrast between 2017 and 2018 demonstrates the inconsistent impact of crop load on fruit ripening, as already established (Keller et al. 2005, Reeve et al. 2016, Rutan et al. 2018). An excessive leaf-to-fruit ratio may not be desirable either: in conditions similar to this trial, the increase of canopy size—via higher canopy trimming height—guaranteed fruit ripeness, but also induced a lower N concentration in the whole plant, particularly in fruit. This mechanism could be considered a 'dilution' due to the higher volume of the biomass, with negative consequences on fermentation kinetics and subsequently on wine quality (Verdenal et al. 2016). The management of vine balance either via crop thinning or canopy trimming affects plant N metabolism in different ways, showing the complexity of managing the plant source-to-sink balance.

Fruit N concentration remained constant regardless of crop load. The similar amount of 2017-res-N present in grapes at both veraison and harvest suggests that the accumulation of labelled N in the grapes mostly occurred before veraison. Despite major differences observed in vine balance and fruit maturation, berry YAN concentration remained unchanged. Howell (2001) explained that plants extract less N from their perennial reserves in response to lower crop load, to match the demand from ripening fruits. The plant N sink strength showed a hierarchy among the plant organs, and fruits appeared to have priority over the roots, which is common in perennial crops (Morinaga et al. 2003, González-Real et al. 2008). This finding demonstrates that crop thinning is not an efficient practice for controlling fruit N concentration. The fruit FAA proportion, however, was changed, potentially affecting wine aroma (Figure 1). Crop thinning induced a lower proportion of alanine and threonine, theoretically responsible for fruity, but also rotten, fishy, and pungent aromas (Verdenal et al. 2021). Being a non-assimilable FAA for yeast, variation in proline has little influence on the aroma potential. Further research on grapevine, including winemaking followed by sensory analysis, would be required to measure the real impact of crop thinning on grape and wine aroma. Several authors have mentioned the impact of crop load on fruit volatile compounds (Rutan et al. 2018, Wang et al. 2019). Lin et al. (2018) even recommended a yield range at harvest for optimal aroma composition on the grape cultivar Vidal under the environmental conditions of Liaoning, China. The impact of crop thinning on both grape maturation and must amino N composition could be observed from the onset of ripening (data not shown). This suggests that the accumulation of N metabolites in fruit was influenced by crop load as early as berry formation. This confirms results from other studies (Keller et al. 2005, Wang et al. 2018), showing that an earlier crop thinning results in a greater impact on grape composition at harvest.

Conclusion

This study highlights the strong influence of crop regulation on the grapevine N dynamics, on the grape N composition, and on the N fertiliser use efficiency. It demonstrates the high potential of crop regulation to control plant NUE. Intra- and inter-seasonal N dynamics were highlighted over two seasons. Root development and activity appeared as key factors for understanding plant N dynamics. Grapevines exhibited a dynamic nutrient balance between organs, while fruit showed the strongest N sink strength. Consequently, under HYC, fruit N accumulation occurred to the detriment of root development and storage. The efficiency of fertiliser use was highly affected by crop load. Under LYC, no effect of fertilisation was observed on fruit composition. These results demonstrate the importance of adapting fertilisation programs according to cultural practices. Plant N release, that is either leaf fall or root leaching, and reassimilation played a major role in the net plant N uptake and require more attention in the development of precise nutrition models. As shown in this trial, all these findings must be considered in relation to the environmental conditions of climate and soil that have a dominant impact on plant physiology and fruit composition.

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