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Understanding and managing nitrogen nutrition in grapevine: a review

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ABSTRACT

This review addresses the role of nitrogen (N) in vine balance and grape composition. It offers an integrative approach to managing grapevine N nutrition. Keeping in mind that N excess is just as detrimental to wine quality as N depletion, the control of grapevine N status, and ultimately must N composition, is critical for high-quality grape production. N fertilisation has been intensively used in the past century, despite plants absorbing only 30 to 40 % of applied N. By adapting plant material, soil management and vine balance to environmental conditions, it would be possible for grape growers to improve plant N use efficiency and minimise N input in the vineyard. Vineyard N management is a complex exercise involving a search for a balance between controlling vigour, optimising grape composition, regulating production costs and limiting pollution. The first part of this review describes grapevine N metabolism from root N uptake to vine development and grape ripening, including the formation of grape aroma compounds. The advantages and limits of methods available for measuring plant N status are addressed. The second part focuses on the parameters that influence grapevine N metabolism, distinguishing the impacts of environmental factors from those of vineyard management practices. Areas for further research are also identified.

KEYWORDS

nitrogen use efficiency, agronomic practices, physiology, partitioning, balance, leaf-to-fruit ratio, amino N, yeast assimilable nitrogen, vine, wine

ABBREVIATIONS

2-AAP: 2-aminoacetophenone
AA(s): amino acid(s)
AF: alcoholic fermentation
Atom % : atomic percentage
B: boron
C: carbon
CO_2: carbon dioxide
Cu: copper
DAP: diammonium phosphate
DMS: dimethyl sulphide
DW: dry weight
H_2S: hydrogen sulfide
MLF: malolactic fermentation
N: nitrogen
N_2 : dinitrogen
NH_3 : ammonia (gas)
NH_4^+ : ammonium
NO_2^- : nitrite
NO_3^- : nitrate
NUE: nitrogen use efficiency
S: sulphur
YAN: yeast assimilable nitrogen

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INTRODUCTION

Nitrogen (N) is a major nutrient for plants involved in many vital physiological processes. It is required in larger amounts than the other mineral nutrients and regulates plant vigour and development in the absence of water restriction. N was intensively applied to crops, mainly in the form of nitrate, during the twentieth century to increase production, regardless of the pollution resulting from crops using only 30-40 % of the fertiliser. In viticulture, optimum yield for high-quality grape is not the maximum allowed by the conditions of the vineyard. N fertilisation has consequently become a complex exercise in the search for a balance between optimising vigour and grape composition, controlling production costs and limiting pollution. Over the past decades, the application of N in vineyards has been reduced with the aim of adjusting vigour and yield. Moreover, the development of cover cropping has led to vines competing for N resources, which can be detrimental to the crop in some cases. This evolution of management practices has created situations with high grape N deficiencies, which can affect fermentation kinetics and wine flavours. White wines are particularly sensitive to grape N deficiency, as they can express a typical 'stress taste' often associated with strong bitterness, despite corrective winemaking techniques. Although several reviews about grapevine N metabolism have been published (Haynes, 1986; Wermelinger, 1991; Mengel and Pilbeam, 1992; Roubelakis-Angelakis and Kliewer, 1992; Loulakakis *et al*., 2009; Masclaux-Daubresse *et al*., 2010), the relationship between plant N status and grape composition is still not fully understood. The management of grapevine N status and, ultimately, grape N composition at harvest should be a prerequisite for grape production with a high-quality potential. The scope of this review is to compile state-of-the-art knowledge about grapevine N nutrition, ranging from plant biology to factors linked to N regulation. It will contribute to the implementation of sustainable practices in the vineyard. The first section focusses on N metabolism, with an emphasis on grapevine N requirement and monitoring. The mechanisms of N uptake, assimilation and efflux are addressed. The role of grape N in the formation of wine aroma is described. The second section gives a comprehensive description of the factors influencing grapevine N status. The agronomic parameters useful for growers to enhance N use efficiency and optimise grape composition, while minimising the use of fertilisers, are discussed. Perspectives for further research are also considered.

NITROGEN REQUIREMENTS AND MONITORING

1. Grape growing

N plays a key role in plant metabolism. As a macronutrient, it represents approximately 1.5 % of dry weight (% DW) of grapevine and enters the composition of key metabolites, such as proteins, amino acids (AAs), enzymes, DNA, RNA and chlorophyll.

1.1. Grapevine N requirements

The positive impact of N nutrition on biomass development is well known (Holzapfel and Treeby, 2007; Gatti *et al.*, 2018). The production of 1 kg of biomass requires from 20 to 50 g of N (Xu *et al.*, 2012). Grapevine N requirements are rather modest in comparison to non-perennial crops, even with high production objectives (Metay *et al.*, 2014), and have already been studied under different environmental conditions (Löhnertz, 1988; Porro *et al.*, 2007; Schreiner *et al.*, 2018). In the context of the sustainable production of 12 tons/ha of grape in cool climate, Löhnertz (1988) estimated the average grapevine N requirement to be 50 kg/ha per year (Table 1). This estimation ensures optimal vegetative growth, taking into account that only the grapes are exported from the vineyard; leaves are restored to the soil, as is the pruned wood in most vineyards.

TABLE 1. N allocation for Riesling at harvest (Löhnertz, 1988). Estimations for a yield of 12 tons/ha of grapes.

N allocation at harvest	Nitrogen kg/ha per year
Wood and roots	27
Grapes	23
Total exported and immobilised	50
Shoots	5
Leaves	37
Total	92

1.2. Symptoms of N deficiency and excess

N metabolism largely controls plant vigour and vegetative development (Metay *et al.*, 2014), and it also influences plant productivity and fruit composition. Both N deficiency and N excess have negative impacts on grapevine development and grape composition.

N deficiency results in weak vine growth, short inter-nodes, small and light-green to yellow leaves, low berry set, reduced long-term bud fruitfulness and yield (Guilpart *et al.*, 2014), reduced grape N content and possible delayed maturation (Schreiner *et al.*, 2018).

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N excess leads to high vigour, dense canopy, large dark-green leaves, extended vegetative growth period (competing with and delaying grape ripening) and increased grape sensitivity to fungal diseases (Thomidis *et al.*, 2016).

N status alters both vine production variables and grape composition to different degrees (Schreiner *et al.*, 2018). Vegetative growth is more constrained than reproductive growth as N status decreases, as illustrated in Figure 1.

1.3. Nitrogen seasonal cycle

Forecasting plant N status in perennial fruit crops requires an understanding of the seasonal plant N cycle. The N assimilation rate fluctuates depending on both the physiological stage (biotic parameters) and environmental conditions (abiotic parameters). Several reports have described grapevine seasonal N uptake and detailed N partitioning within the vine (Conradie, 1980; Conradie, 1991; Löhnertz, 1988; Wermelinger, 1991; Bates *et al.*, 2002; Zapata *et al.*, 2004a; Zapata *et al.*, 2004b; Treeby and Wheatley, 2006; Weyand and Schultz, 2006; Williams, 2015; Zufferey *et al.*, 2015; Schreiner, 2016; Holzapfel *et al.*, 2019). A model of seasonal changes in N content of grapevine tissues is shown in Figure 2.

Except in vineyards close to the equator where vines grow continuously, annual grapevine N requirement is usually concentrated in the vegetative period. Before the onset of winter - under the influence of seasonal changes in light and temperature - grapevines enter a phase in which metabolic activity is minimal and growth stops (Cookson *et al.,* 2013). Growth resumes at bud break, which is induced by increasing temperatures. Growth after bud break mainly depends on the vine's reserves in its storage organs (roots and wood), which have accumulated during the previous summer and autumn. During winter, the grapevine N reserves are mainly stored in the roots (about 75 % in dormant vines), in the form of AAs and proteins (Zapata *et al.,* 2004a; Zapata *et al.,* 2004b).

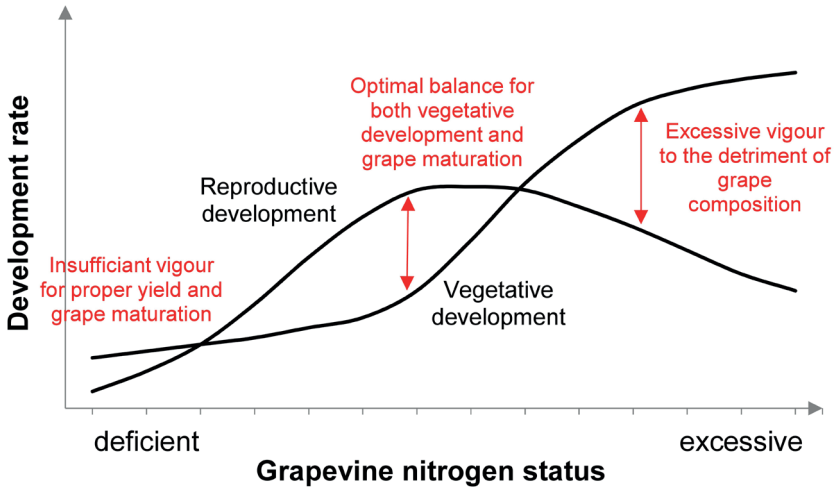


FIGURE 1. Hypothetical model of vegetative versus reproductive development rates as a function of grapevine N status.

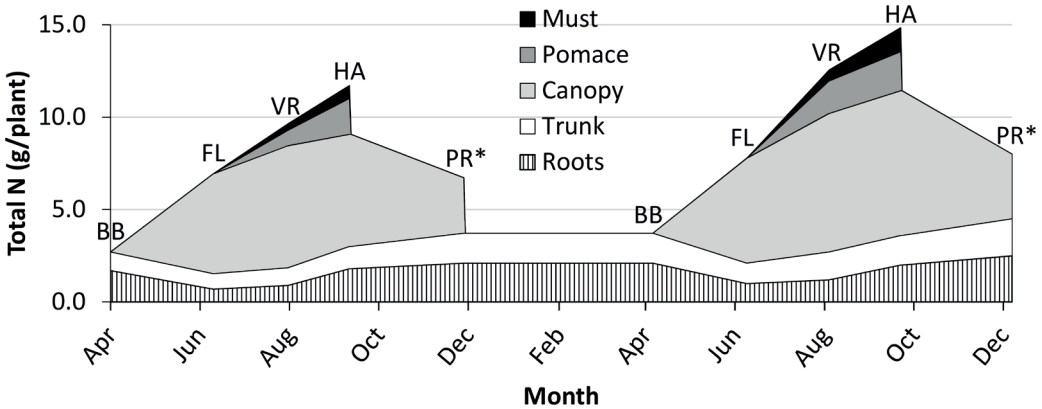


FIGURE 2. Changes in N content of plant parts in grapevines over two growing seasons.

Four-year-old potted Chasselas cv. (Verdenal *et al.*, unpublished data, 2017-2018). Letters designate major phenological stages: BB, budbreak; FL, flowering; VR, veraison; HA, fruit harvest; PR, pruning (*hypothetic values).

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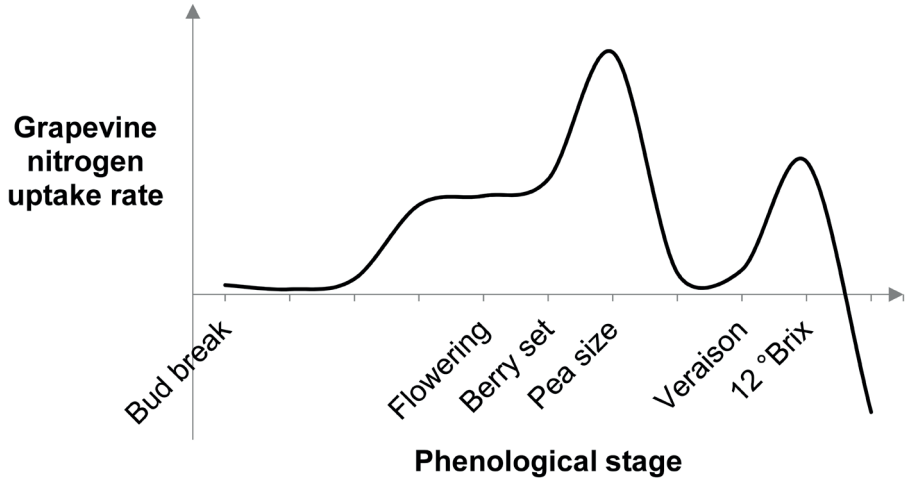


FIGURE 3. Annual evolution of the N uptake rate of grapevine (adapted from Löhnertz, 1988).

From bud break (phenological stage 07 on the BBCH scale) to the stage of 5-6 leaves (BBCH 53), N uptake remains low. N reserves from the roots and, to a lesser extent, from the wood are mobilised to support initial growth until root N uptake becomes sufficient around flowering (BBCH 65) (Zapata *et al.,* 2004a; Zufferey *et al.*, 2015). Soluble N in the storage organs reaches a maximum just before budbreak, and it decreases thereafter until the beginning of fruit growth (Wermelinger, 1991; Williams, 2015). After harvest, approximately 85 % of the increase in root and wood N reserves is due to N translocation from the leaves before leaf fall (Williams, 2015).

N uptake and AAs synthesis are necessary for the synthesis of proteins and enzymes, which are in turn required for the photosynthetic activity and other biochemical pathways related to plant development. Young leaves first behave as a sink for N compounds to ensure their own development; during the reproductive stage, leaves behave as a source of AAs for grape development and the refilling of reserves (Kant *et al.*, 2011). Substantial refilling of reserves can occur after harvest due to N relocation from the leaves prior to leaf fall. In warmer countries, the post-harvest period (from harvest to complete leaf fall) may last for up to four months, and N uptake during that period may contribute up to 30 % of the annual refilling of the N reserve (Conradie, 1992; Conradie, 2005). An increased supply of nitrogenous compounds is necessary for optimum flowering and berry development; grapes start accumulating N during the first growth stage, with major N uptake occurring from two weeks before flowering until four weeks after flowering (BBCH 65) (Figure 3) (Linsenmeier *et al.*, 2008; Holzapfel *et al.*, 2019). A lag phase is observed at the onset of grape ripening (veraison, BBCH 85), and then a second uptake peak occurs at the beginning of grape ripening (Löhnertz, 1988; Ribéreau-Gayon *et al.*, 2017). During ripening, NH_4^+ content decreases and organic N content increases in grape berries. Most of the berry N is imported in the form of glutamine (Keller, 2015), which is then converted in the berry into other AAs via transamination.

At the end of the vegetative period, some of the N migrates from the leaves to the roots. The refilling of root N reserves usually starts before grape maturity and continues until leaf fall (Holzapfel and Treeby, 2007; Rossouw *et al.*, 2017). The root N pool at the beginning of the vegetative season is related to the yield of the previous year and to vine age (Löhnertz, 1988).

2. Nitrogen monitoring

Grapevine N status not only influences plant vigour and yield, but also grape composition and subsequent wine quality. By monitoring plant N status, agronomic practices and fertilisation can be adjusted to meet production objectives. This section reviews the indicators of plant N status and highlights their advantages and drawbacks.

2.1. Soil analysis

N fertiliser recommendations are usually based on the soil measurement of mineral N; *i.e.*, the form in which N is directly available to plants. Mineral N is mostly present in soils as nitrate (NO_3^-), because NH_4^+ is quickly nitrified, except when soil pH is very low. Mineral N, however, represents only a small fraction of total soil N, and its amount varies significantly depending on the rates of N mineralisation, plant N uptake and soil N losses (*i.e.*, leaching, denitrification, erosion and gaseous emission). The size of the mineral N pool can vary from a few tenths of kilograms to a few hundreds of kg/ha.

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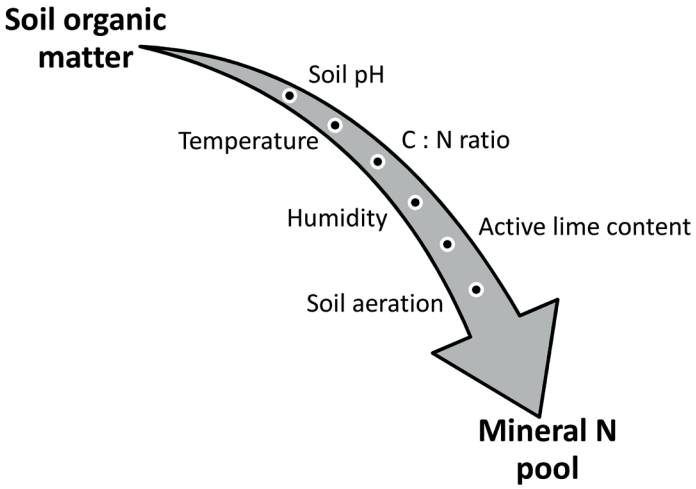


FIGURE 4. Factors influencing the mineralisation of soil organic matter.

Total N (mineral + organic) in the soil is not a good indicator of plant N status, because organic material needs to be broken down by soil microflora before being accessible to plants. Hence, the factors involved in the mineralisation of soil organic matter greatly influence the size of the mineral N pool available to the grapevine over time (Figure 4). Moreover, the sampling method used - particularly in terms of location and depth - can greatly affect results and interpretations. Consequently, a soil analysis can provide a baseline for N fertiliser management, but it is not sufficient on its own, as it does not reflect the dynamics of available soil N over the season. Moreover, it does not take into account grapevine N requirements, which also depend on yield and quality targets. Recommendations regarding N fertiliser supply can change on a yearly basis, especially with varying weather conditions (Van Cleemput *et al*., 2008).

2.2. Leaf and petiole analysis

Leaf petiole and leaf blade analyses can be used to monitor plant nutrition status during the season mainly for macro elements (N, phosphor, potassium, calcium, magnesium) (Gaudillère *et al.*, 2003), for which results are expressed in percent of dry weight (% DW). Leaf N concentration is well-correlated with the chlorophyll index (Spring, 1999). N content in the leaf blade is very different to that in the petiole: petiole N content is more sensitive to variations in N nutrition than leaf blade N content, which is more constant (Delas, 2010). Consequently, the chosen analysis (*i.e.*, on either the leaf blade or petiole, or both together) will greatly affect the results and require adapted interpretation thresholds (Table 2). The interpretation may be refined with the ratios of N/P and N/K (Crespy, 2007) (Table 3).

TABLE 2. Threshold values for the interpretation of grapevine leaf and petiole N content with regard to vine N status.

N concentration (% DW)	Very low	Adequate	Very high	Reference
Leaf blade + petiole	< 1.8	2.0 - 2.3	> 2.5	Spring and Verdenal (2017)
Petiole	< 0.4	0.4 - 0.6	> 0.6	Champagnol (1984)

Measurements are implemented at veraison on leaves (either leaf blade + petiole or petiole only) from the main shoots of the bunch area. Results are expressed as % DW.

TABLE 3. Thresholds for the ratios N/P and N/K for the interpretation of grapevine leaf and petiole analysis with regard to vine N status.

		Very low	Adequate	Very high
Leaf blade + petiole	N / P	< 9.7	10.7 - 12.8	> 13.9
	N / K	< 1.0	1.1 - 1.3	> 1.4
Petiole	N / P	< 2.5	2.5 - 3.5	> 3.5
	N / K	< 0.2	0.2 - 0.4	> 0.4

Measurements are implemented at veraison on adult leaves (either leaf blade + petiole or petiole only) from the bunch area. Results are expressed in % DW.

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The limitations of plant N assessment through tissue analysis for fertilisation purposes have long been acknowledged, and the interpretation of results should be carried out with care (Perez and Kliewer, 1982; Delas, 2010). The results are mainly used as a complement to other observations. Before making any decision on fertiliser application, it is recommended to complete the diagnosis with visual observations of plant morphology. High vigour, dense canopies and high yields are generally indicators of high vine N status. Leaf and petiole analyses are essentially used for research purposes to observe the impact of a particular practice on plant composition or to confirm a nutrition problem in the plant.

2.3. Chlorophyll index

Various tools have been developed for plant-based N status assessment. These are usually based on indirect and non-destructive measurements, such as chlorophyll concentration. Examples of hand-held chlorophyll meters used for diagnosis purposes are the N-Tester (Yara, Oslo, Norway), SPAD 502 (Konica Minolta, Nieuwegein, Netherlands) and Dualex (Force A, Orsay, France). Chlorophyll meter readings reflect the intensity of the green colour of the foliage, and are thus well-correlated with leaf chlorophyll and N concentrations (Spring and Zufferey, 2000; Cerovic *et al.*, 2015; Aranguren *et al.*, 2018; Vrignon-Brenas *et al.*, 2019). Therefore, chlorophyll content can be used to diagnose plant N status, making such readings effective tools for N monitoring. Knowledge of growth stage and sampling method is critical for a reliable estimation of grapevine N status in the vineyard. Interpretation thresholds have been proposed for measurements taken with the N-Tester for the cultivars Chasselas, Pinot noir and Gamay at the phenological stage of veraison (Table 4). Measurements taken earlier in the season are not recommended due to higher variability of the readings, since they are greatly influenced by cultivar, water status (*e.g.*, severe drought), deficiency of other nutrients (*e.g.*, magnesium, iron), disease symptoms on the leaves and canopy management (Cerovic *et al.*, 2015; Friedel *et al.*, 2020). Thresholds are currently lacking, but ideally, they should be available for every cultivar, and even for every cultivar-rootstock combination. Ongoing research is aiming to remotely characterise vine physiology and berry composition with the Normalised Difference Vegetation Index (NDVI) (Taskos *et al.*, 2015; Kotsaki *et al.,* 2020a; Kotsaki *et al.,* 2020b). The NDVI is well-adapted to assessing the spatial variability of vine N status, and it can fine-tune agronomic practices in specific areas within a vineyard. However, NDVI has the drawback of combining information; for example, leaf density (related to vine vigour, which does not depend on vine N status alone) and leaf colour intensity (related to vine N status and, to a lesser extent, the variety).

2.4. N isotope composition

N dynamics in grapevine can be monitored by analysing isotopes for research and development purposes. Elemental N has two stable isotopes (^{14}N and ^{15}N); *i.e.*, atoms with the same number of protons (seven protons for N) and different numbers of neutrons. Both are present in nature at the natural abundance of 99.634 and 0.366 atom % respectively (Deléens *et al.*, 1997). The stable N isotope composition of a sample is determined by isotope ratio mass spectrometry (IRMS). It is reported as a $\delta^{15}N$ value, which is the relative deviation of the sample heavy-to-light isotope ratio $^{15}N/^{14}N$ (R_{sample}) from an international reference ($R_{standard}$ of atmospheric N_2) (Coplen, 2011):

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

The δ unit is milliurey (mUr) as defined by the International System of Units (Coplen, 2011). A review (Santesteban *et al.*, 2014) and two studies (Durante *et al.*, 2016; Paolini *et al.*, 2016) have described variations in $^{15}N/^{14}N$ isotope ratios in

TABLE 4. Thresholds for the interpretation of N-Tester index with regard to vine N status for Chasselas, Pinot noir and Gamay.

N-Tester index			Corresponding grapevine N status
Chasselas	Pinot noir	Gamay	
< 420	< 460	< 380	Very low
420 - 460	460 - 500	380 - 430	Low
460 - 540	500 - 580	430 - 530	Normal
540 - 570	580 - 620	530 - 580	High
> 570	> 620	> 580	Very high

Measurements are implemented at veraison on adult leaves in the bunch area (Spring and Verdenal, 2017).

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natural abundance from soil to wine. Several isotope fractionations occur during the soil N cycle, and then to a lesser extent through grapevine N metabolism (Santesteban *et al.*, 2014). δ^{15}N values observed in plant tissues are mainly related to N source, with lower δ^{15}N values (*i.e.*, 0.2 mUr on average) for inorganic fertilisers than for organic matter (8.1 mUr on average) (Santesteban *et al.*, 2014). Grape δ^{15}N values are usually less than soil δ^{15}N values (Durante *et al.*, 2016). After grapevine N assimilation, a ^{15}N enrichment can be observed from roots (6.6 mUr on average) to must (33.7 mUr on average) (Verdenal *et al.*, 2020). N fractionation is related to several factors, such as water availability and fruit load. The water constraint that a grapevine can face during the vegetative season will negatively influence wine δ^{15}N values (Spangenberg and Zufferey, 2018). Conversely, fruit load will positively influence must δ^{15}N values; *i.e.*, from 19.5 mUr on average under low-yielding conditions to 33.7 mUr under high-yielding conditions (Verdenal *et al.*, 2020). Winemaking processes do not change δ^{15}N values from must to wine (Durante *et al.*, 2016). Despite multiple isotope fractionations from soil to grape, δ^{15}N values for leaves, grapes and wines conserve the variability of δ^{15}N found in the corresponding soil (Paolini *et al.*, 2016; Spangenberg and Zufferey, 2018).

In contrast to natural abundance, N labelling consists of applying an N source to the grapevine with a known ^{15}N abundance; *i.e.*, ^{15}N is artificially substantially enriched or depleted (*e.g.*, 10 atom %). Such a high concentration of ^{15}N is easily detectable and quantified in the plant organs. When studying N metabolism, this method allows the labelled N, which has accumulated in specific organs to be traced and quantified, and it provides an insight into the fate of crop-applied N in terms of its uptake, assimilation, distribution and release (Van Cleemput *et al*., 2008). Variations in the natural abundance of δ^{15}N and possible isotope fractionation are considered negligible compared to the ^{15}N content of the labelled source (Verdenal *et al.*, 2016a). Once the plant has assimilated the labelled N, each fraction of the plant can be analysed separately as described hereafter.

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

$$A\% = \frac{R}{R+1} \times 100 \quad (2)$$

Relative specific abundance (*RSA*, atom percent) is the proportion of newly incorporated N atoms originating from the labelling relative to total N in the sample (Cliquet *et al.*, 1990). The *RSA* also represents organ sink strength, which is independent of organ size (Deléens *et al.*, 1997):

$$RSA = \frac{A\%_{sample\ excess}}{A\%_{N\ supplied\ excess}} = \frac{A\%_{sample} - A\%_{non\ labelled\ control}}{A\%_{N\ supplied} - A\%_{non\ labelled\ control}} \quad (3)$$

The new N pool, which has originated from the labelling, can be quantified in each plant fraction and the partitioning (*% P*) can subsequently be calculated (Cliquet *et al.*, 1990):

$$\text{new N pool}_{fraction} = RSA_{fraction} \times NQ_{fraction} \quad (4)$$

where NQ is the total N quantity

$$\%\ P = \frac{\text{new N pool}_{fraction}}{\text{new N pool}_{whole\ plant}} \times 100 \quad (5)$$

The overall net N uptake can then be calculated:

$$\text{net N uptake} = \frac{\text{new N pool}_{whole\ plant}}{\text{labelled NQ}_{supplied}} \times 100 \quad (6)$$

Exclusively used for research purposes, the isotope labelling method has been applied on grapevine since the 1980s in order to study plant N metabolism (Conradie, 1983; Glad *et al.*, 1994; Morinaga *et al.*, 2003; Zapata *et al.*, 2004a; Zapata *et al.*, 2004b; Iandolino and Williams, 2014; Clarke *et al.*, 2015; Williams, 2015; Verdenal *et al.*, 2015; Verdenal *et al.*, 2016a; Verdenal *et al.*, 2020; Brunetto *et al.*, 2016; Hannam *et al.*, 2016).

2.5. Grape analysis

The analysis of grape N content at harvest gives an overall picture of plant N status over the entire season, including the ripening period, making it probably the most accurate indicator of grapevine N status. Conversely, the previously mentioned plant indicators (*i.e.*, leaf N content and chlorophyll index) are usually obtained at either the phenological stage of veraison (BBCH 85) or before. They consequently only give an integrative view of N metabolism until veraison. Van Leeuwen *et al.* (2000) have compared the performance of several indicators of grapevine N status. Both total N content and yeast assimilable N (YAN) in grape must were found to be correlated and highly responsive to fertilisation practices. YAN is the part of must N compounds that is assimilable by yeasts during alcoholic fermentation (AF), and it comprises ammonium (NH_4^+) and AAs (excluding proline and hydroxyproline); it also informs the winemaker about the must's fermentability. Low grape N concentration at harvest can be a sign of unbalanced vine nutrition. However, N fertilisation is not always the suitable solution. During grape development and ripening, berry N nutrition may be restrained by numerous biotic and

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abiotic factors, which may alter both N assimilation and partitioning in the plant, despite soil N abundance (Delas, 2010; Spring *et al.*, 2012). Consequently, low correlations are often observed between plant-based N indicators and grape N content at harvest, particularly if the grapevine N metabolism has been restrained during the ripening period. van Leeuwen *et al.* (2000) established a threshold at 180 mg/L of YAN, above which the grapevine N requirements are fulfilled and N fertilisation should be interrupted during the following year. This threshold may be lower in some situations for the production of red wine (van Leeuwen *et al.*, 2018). To interprete YAN at harvest as an indicator of plant N status, thresholds need to be determined. Since the YAN concentration is also related to grape variety, specific thresholds are required for each variety.

Early determination of must YAN content can potentially be used for the purpose of N fertilisation at the beginning of grape ripening, with the aim of increasing must YAN content at harvest. At veraison, grapes are already rich in N, mainly in the form of NH_4^+. YAN concentration generally decreases during grape ripening due to the decrease in NH_4^+, while AA concentration remains relatively stable (Nisbet *et al.*, 2014). A large database produced by the Agroscope Institute highlights the correlation between grape N content both at veraison and at harvest across 16 vintages (1997-2012), five cultivars and three experimental vineyards (240 data points, Lorenzini *et al.*, unpublished data, 1997-2012). Each year, ripening was monitored in selected plots of the main cultivars every week until harvest, as an indication of N for the grape growers. Approximately, 80 % of the situations had equivalent N concentrations at veraison and harvest (Figure 5). This confirmed the results of Nisbet *et al.* (2014), who also found a strong correlation between YAN content at veraison and at harvest ($r^2 = 0.82$). When initial N content was higher than 140 mg N/L, N content at harvest was still above that deficiency threshold in 70 % of the cases, and when initial N was deficient, N deficiency was confirmed at harvest in 90 % of the cases. N analysis at veraison is too variable for a precise prediction of N content at harvest, but it still gives a good indication of N deficiency.

To conclude, there is no unique indicator to determine vine N status. In most cases, the absence of universal thresholds is limiting, as the desired N status in both plant and grape is relative to grape variety, yield and production objectives. Plant N status can be assessed by both applying routine dosage of YAN at harvest and observing plant physiology (vigour, leaf colour and bud fruitfulness). With this information, N fertilisation and agronomic practices can be fine-tuned to obtain optimum plant N status. A combination of several indicators will increase the reliability of a diagnosis of vine N status.

3. Nitrogen metabolism

Grapevine N restriction affects fruit N accumulation, altering the abundance of certain AAs more than others, and thus changing the fruit AA profile (Schreiner *et al.,* 2014). Organic N solutions available in industry to manipulate AA concentrations in musts are still expensive, and they have less impact on wine

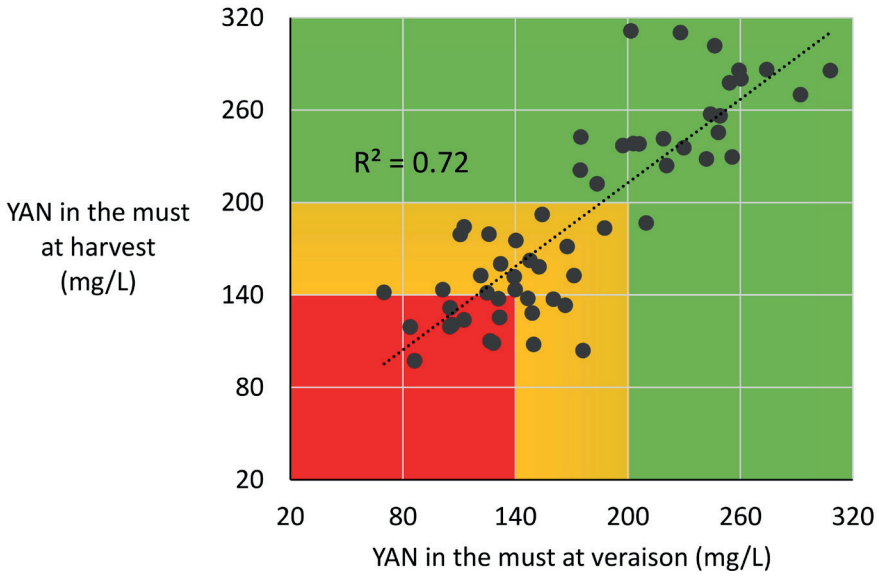


FIGURE 5. Linear regression between the concentrations of YAN at veraison (onset of ripening) and at harvest.
Data collected on Pinot noir from three different vineyards from 1997 to 2012. Risk of incomplete fermentation: green = none; orange = moderate; red = strong (Lorenzini *et al.*, unpublished data, 1997-2012, Agroscope, Switzerland).

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aromas than vineyard management practices. A wine sensory profile will mainly depend on the initial grape composition at harvest, which has to be managed at vineyard level, despite the substantial influence of the winemaking process (Gutiérrez-Gamboa *et al.*, 2019). In most vineyards, N availability is often limiting, which largely affects plant physiology, such as canopy expansion, root morphology, floral induction and seed dormancy (Hachiya and Sakakibara, 2016). A balanced grapevine N metabolism is thus required to achieve optimal N accumulation in the grapes and, ultimately, the desired wine flavour. Understanding N use efficiency (NUE) is critical for optimising the parameters involved in N metabolism to obtain both optimal production and composition of grapes at harvest, while reducing N fertilisation and environmental impacts (Masclaux-Daubresse *et al.*, 2010).

3.1. Nitrogen use efficiency

It is commonly admitted that nearly 60-70 % of N applied to crops through fertilisation is actually lost, mainly by soil leaching and by gaseous emission (Masclaux-Daubresse *et al.*, 2010; Reddy and Ulaganathan, 2015). Optimising grapevine N use with the aim of improving grape quality, while reducing the use of fertilisers and minimising N run-off into the environment, is critical for both the grower and the environment. The concept of NUE has been developed by several researchers (Lea and Azevedo, 2006; Masclaux-Daubresse *et al.*, 2010; Xu *et al.*, 2012). Crop NUE is usually represented by total yield produced per unit of fertiliser N applied (Xu *et al.*, 2012). The definition of NUE differs, however, depending on whether crops are cultivated for biomass or grain (Masclaux-Daubresse *et al.*, 2010). In the case of wine production, maximum grape yield is generally not the main target. Optimal grapevine NUE is not only a case of balancing N status between vegetative and reproductive growth, but also of favouring the accumulation in grapes of AAs and subsequent metabolites known to enhance wine quality (Schreiner *et al.*, 2018). Optimal NUE can also contribute to a reduction in N input, and thus environmental impact. NUE is the combination of two parameters: 1) assimilation efficiency (*i.e.*, uptake and assimilation), and 2) utilisation efficiency (*i.e.*, allocation and remobilisation) (Kant *et al.*, 2011). N uptake and N assimilation refer to two different processes: N uptake is the process of collecting inorganic N from the environment, from soil in particular; N assimilation is the formation of organic N compounds necessary for growth and development (*e.g.*, the AAs). In order to provide favourable conditions by adapting agricultural practices, it is first necessary to understand the agronomic traits that influence the efficiency of assimilation and utilisation; this would help to either enhance grape composition with the same N input, or maintain grape composition with lower N input (Kant *et al.*, 2011).

3.2. N uptake

Grapevines assimilate neither atmospheric dinitrogen (N_2) nor N bound to the organic matter present in the soil. Soil NO_3^- and NH_4^+ are the primary N source for grapevines, but they can also take up organic N (urea, AAs and peptides) to a lesser extent (Keller, 2015; Hachiya and Sakakibara, 2016).

Root uptake is an active process (energy consuming) which principally occurs in the fine roots (Zapata *et al.*, 2004b). NO_3^- uptake initially consists of a radial diffusion along both symplastic (interconnected cytoplasm) and apoplastic (intercellular spaces) routes: ions move through the root epidermis up to the endodermis. The endoderm plays a boundary role in the selection and regulation of ions. Energy from adenosine triphosphate consumption is used to 'pump' protons out of the root cells into the soil; protons diffuse back into the cells, carrying negatively-charged NO_3^- with them (Keller, 2015). The soil NO_3^- concentration is highly variable. The complex processes of active uptake by the roots allows the plant to adjust nutrient uptake according to its needs and to soil N availability. NO_3^- assimilation depends on both soil and plant N status and involves hormonal controls and interactions with carbon (C) metabolism and status. Root elongation is stimulated by soil N deficiency (Xu *et al.*, 2012). Numerous genes (> 20) are involved in regulating membrane transport (Morot-Gaudry *et al.*, 2017). N uptake rate is affected by root architecture, morphology and transporter activity on one hand, and by N form and concentration in the soil on the other (Xu *et al.*, 2012; Morot-Gaudry *et al.*, 2017).

Leaves can take up nutrients through their cuticle and stomata. Over the past decade, scientific progress has improved knowledge of plant response to foliar fertilisation, resulting in an increase of this practice in agriculture (Fernández and Eichert, 2009; Fernández and Brown, 2013). Leaf uptake is non-selective, in contrast to root uptake (Eichert, 2013). Nutrients penetrate the leaf cuticle and the stomata depending on the concentration gradient at the leaf surface. Janzen and Bruinsma (1989) demonstrated that up to 30 % of N present in wheat shoot tissues derives from atmospheric ammonia (NH_3). Furthermore, the application of foliar urea at veraison efficiently increases grape N content without influencing plant vigour, when all other management measures to optimise N status have failed or been insufficient (Lasa *et al.*, 2012; Hannam *et al.*, 2016). Urea is hydrophilic, and resulting N metabolites are easily transported from the leaves

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$$\underbrace{CO(NH_2)_2}_{\text{urea}} + H_2O \xrightarrow{\text{urease}} \underbrace{NH_3}_{\text{ammonia}} + \underbrace{H_2NCOOH}_{\text{carbamic acid}} \rightarrow 2NH_{3(gas)} + CO_{2(gas)} \quad (7)$$

to the sink organs. After application, urea is rapidly hydrolysed into NH_3 and carbon dioxide (CO_2) as follows (see equation 7 above) (Krogmeier *et al.*, 1989).

NH_3 cannot be directly assimilated by grapevine and will volatise into the atmosphere unless it reacts with water to form NH_4^+. The reaction depends on ambient temperature and humidity; wetter and cooler conditions are usually favourable for limiting NH_3 volatilisation and increasing foliar fertilisation efficiency.

$$NH_3 + H_2O \rightarrow NH_4^+ + HO^- \quad (8)$$

The combined formation of hydroxide (HO^-) raises the pH locally, which further increases NH_3 volatilisation. When foliar applications are necessary due to low vine N status, a supply of 10 to 20 kg N/ha is usually recommended at veraison, split into two to four weekly applications, to prevent symptoms of toxicity due to temporarily high concentrations of NH_3 and NH_4^+ (Figure 6) (Krogmeier *et al.*, 1989).



FIGURE 6. Leaf symptoms of NH_4^+ toxicity due to an excess of foliar urea.

3.3. Assimilation, transport and storage

Nitrate assimilation takes place in both the roots and leaves depending on N availability and supply (Llorens *et al.*, 2002). Once inside the root cells, nitrates can either be temporarily stored in the cell vacuoles for later use (buffer role), assimilated into organic compounds (*i.e.*, AAs), or transported to the leaves by the sap flow via the xylem vessels (Loulakakis *et al.*, 2009). Before assimilation, nitrates must be reduced into NH_4^+ in a two-step process: nitrate is first reduced to nitrite (NO_2^-) by the enzyme nitrate reductase, and then to ammonium by the enzyme nitrite reductase.

$$NO_3^- \xrightarrow{\text{nitrate reductase}} NO_2^- \xrightarrow{\text{nitrite reductase}} NH_4^+ \quad (9)$$

Xylem and phloem are efficient transport vessels in vascular plants. Xylem transports water and nutrients from the roots through the entire plant, while the phloem mainly transports organic compounds from the shoots and leaves to the rest of the plant. Glutamine and glutamic acid are the predominant AAs in the xylem sap, while arginine and glycine are predominant in the phloem (Gourieroux *et al.*, 2016). Over short distances, nutrients can also be simply diffused through unspecialised cell membranes and cytoplasm due to their charge (lipid and hydrophobic membranes) (Morot-Gaudry *et al.*, 2017). Figure 7 summarises N uptake and assimilation in grapevine.

In contrast to NO_3^-, NH_4^+ is toxic for plant tissues and is rapidly assimilated into AAs. Ammonium assimilation is catalysed by two enzymes: glutamine synthetase (GS) and glutamate synthase (GOGAT). The sequential action of the coupled GS/GOGAT has been found to play a predominant role in the assimilation of ammonium in higher plants (Loulakakis *et al.*, 2009).

$$\text{glutamate} + NH_4^+ \xrightarrow{\text{GS}} \text{glutamine} \quad (10)$$

$$\text{glutamine} + 2\ \text{oxoglutarate} \xrightarrow{\text{GOGAT}} 2\ \text{glutamates} \quad (11)$$

An alternative pathway for ammonium assimilation involves the enzyme glutamate dehydrogenase (GDH). The main role of GDH seems to be different, however, as the reaction can be reversed, thus oxidising glutamate (Keller, 2015).

$$NH_4^+ + 2\ \text{oxoglutarate} \xleftrightarrow{\text{GDH}} \text{glutamate} + H_2O \quad (12)$$

The accumulation of glutamine is the main source of organic N in grape; the synthesis of the other AAs occurs with the transfer of the glutamate amino group by different aminotransferases (Xu *et al.*, 2012; Ribéreau-Gayon *et al.*, 2017). AAs are the major form of organic N for transport and storage in the plant. The AAs are distributed throughout the entire plant via the phloem and the xylem. After harvest and before leaf fall, the major part of organic N is transferred and stored in the roots in the form of AAs - mostly arginine - and proteins (Zapata *et al.*, 2004a; Zapata *et al.*, 2004b).

3.4. N efflux

Net N uptake refers to total N influx minus total N efflux (Hachiya and Sakakibara, 2016). Plant N losses must be included in the N budget to avoid an overestimation of N losses in soil and an underestimation of plant N uptake (Xu *et al.*, 2012). Knowledge about the amount and composition of organic compounds released into the soil by plant roots is incomplete and

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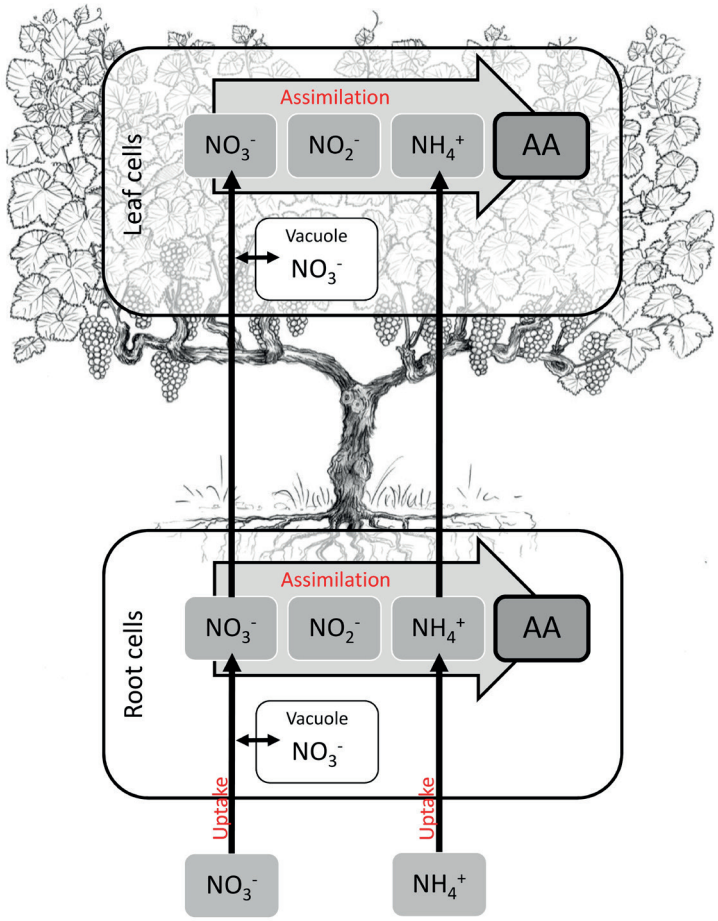


FIGURE 7. N uptake and assimilation in grapevine.
NO_3^-, nitrate; NO_2^-, nitrite; NH_4^+, ammonium; AA, amino acid.

not even available for grapevine, largely because of methodology limitations.

Nitrate, ammonium and AAs can be released by the roots into the soil, as a result of root activity and root life span. The rhizosphere is a site of intense interactions between roots and soil; organic components released from the roots influence the solubility and transport of nutrients and the decomposition of organic materials, as well as the activity and turnover of microorganisms (Reining *et al.*, 1995). Zapata *et al.* (2004a) showed that about 60 % of grapevine root N is lost from the perennial tissues between bud break and the onset of flowering. However, this amount does not correspond to the increase in N content in the annual tissues. This increase is only around 40 %, suggesting that approximately 20 % of the N reserve is lost early in the season via grapevine root necrosis (fine roots in particular) and to a lesser extent sap bleeding. Reining *et al.* (1995) investigated this issue in wheat: using a split-root experimental design with labelled N supply on one side, they showed that approximately 7 % of assimilated N was released into the soil of the unlabelled compartment. Merbach *et al.* (1999) confirmed the release of 5-6 % of ^{15}N previously assimilated by wheat, which represents 15 kg N ha^{-1} of N released by roots into the soil. Of the N exudates, 60 % was found in the soluble organic N pool and 9 % in the inorganic N pool (Janzen and Bruinsma, 1989). Ammonium efflux from the roots inhibits root cell elongation (Li *et al.*, 2010; Reddy and Ulaganathan, 2015). A nitrate efflux transporter has been identified in *Arabidopsis* roots, but its physiological role still needs to be determined (Xu *et al.*, 2012). Reddy and Ulaganathan (2015) have explained that plants release ammonium into the soil to maintain N homeostasis, because a high internal NH_4^+ concentration is toxic to the plant and reduces N uptake efficiency. The decomposition rate and the release of N compounds by *Quercus* fine roots are not only functions of environmental temperature, rainfall and humidity, but also of initial soil composition and root diameter (Usman *et al.*, 2000). In the case of *Pinus*, both the decomposition rate and the release of N compounds are negatively correlated to initial soil N content (Jing *et al.*, 2019). Changes in chemical traits of fine roots affect fine root decomposition to a greater extent than do changes in soil N availability (Gang *et al.*, 2019).

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To a lesser extent, photorespiration also induces N losses through the emission of NH_3 by leaves (Kumagai *et al.*, 2011). Differences in NH_3 losses between rice cultivars are a result of their different GS activities, which result in different capacities for the reassimilation of photorespiratory NH_3. Kumagai *et al.* (2011) also suggested that NH_3 emissions in rice leaves are not directly controlled by transpiration and stomatal conductance. The main factor for N losses (in the form of NH_3) from the aboveground parts is the excess of N accumulation in the tissues compared to N assimilation (Xu *et al.*, 2012). Leaf senescence is also a cause of N loss, even if most of the soluble N components are translocated to other organs via the phloem before leaf fall. However, the leaves fall on the ground and are a potential source of nutrients. Similar soil/roots and atmosphere/leaves interactions are likely in the case of grapevine, but their proportions are still unknown. Research on this subject is of critical importance to obtain a complete picture of N dynamics in grapevine.

3.5. Synergy between C and N metabolisms

The assimilation of NO_3^- and NH_4^+ into AAs is a dynamic process that is regulated by both internal factors (C and N metabolism) and external factors (environmental conditions) (Keller and Koblet, 1995). Besides water availability, C-N interaction is a cornerstone of optimal biomass production. Vrignon-Brenas *et al.* (2019) demonstrated the preponderant role of plant N status in C balance related to both gain and storage. Indeed, both biomass production and photosynthesis activity require N supply, which, in turn, depends on photosynthetised-C compounds for nitrate assimilation (Gauthier *et al.*, 2010). Stitt and Krapp (1999) published a detailed review describing the interaction between elevated CO_2 and N nutrition. Nitrate reduction requires a parallel C oxidation via the respiration process (Xu *et al.*, 2012). The C-skeletons and energy from starch and sucrose are essential for the biosynthesis of glutamine (Masclaux-Daubresse, 2010). In other words, C can be viewed as a substrate for N assimilation. Consequently, grapevine C status strongly influences N assimilation, which is fast when C status is high (Keller and Koblet, 1995). Conversely, under adverse environmental conditions, which restrict photosynthetic activity, N assimilation is reduced and AA synthesis is consequently limited. Higher N status stimulates both light-saturated photosynthesis activity and respiration rate. Under high N availability and proper light intensity, grapevine N demand is met, and assimilated N is accumulated in the root reserves, inducing lower N uptake (Keller, 2015). When subjected to low N supply and high irradiance, grapevine exhibited the highest root-to-shoot ratio (Grechi *et al.*, 2007). The regulation of N uptake and assimilation by photosynthesis ensures that N and C uptakes are correlated (Masclaux-Daubresse *et al.*, 2010).

4. Winemaking

4.1. Grape N composition and yeast assimilable N

Approximately 50 % of grape N is found in the seeds and skin, 8 % in the stem and 40 % in the must (Hernández-Orte *et al.*, 1999). Figure 8 illustrates the average must N composition at harvest. Free AAs are the main N form in the must, representing 60-80 % of total N (Aerny, 1996). There are two categories of free AAs depending on their molecule structure: AAs with a primary amine (-NH_2), representing 50-90 % of total AAs; AAs with a secondary amine (-NH-)

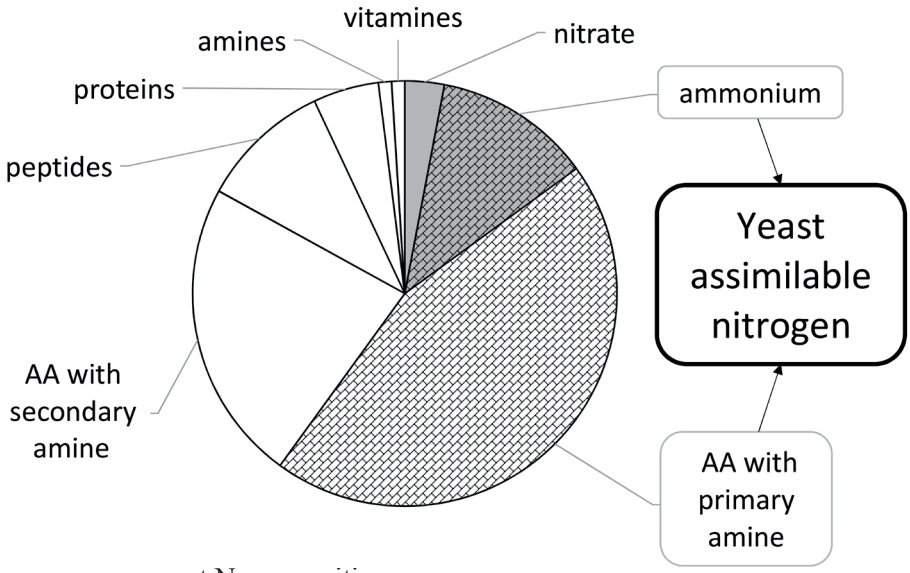


FIGURE 8. Average grape must N composition.
Grey = inorganic N; white = organic N; tiled pattern = yeast assimilable N; AA, amino acid.

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(Bell and Henschke, 2005). Other organic N forms are peptides (10-30 %), proteins (2-10 %) and trace amounts of vitamins, amines and nucleotides (< 5 %). Inorganic N forms are ammonium (5-20 %) and nitrate (< 5 %) (Henschke and Jiranek, 1993; Aerny, 1996; Bell and Henschke, 2005).

Yeasts play a major role in winemaking. For their growth and development, they assimilate soluble sugars, their major source of carbon, along with a mixture of nutrients, including lipids and N compounds (Ugliano and Henschke, 2009). Under the usual winemaking conditions, AAs with a secondary amine are not assimilable; *i.e.*, proline and hydroxyproline. Consequently, YAN is the sum of AAs with primary amine (organic) and ammonium (inorganic) (Figure 8).

$$YAN = AA(\text{primary amine}) + NH_4^+ \quad (13)$$

For oenological purposes, YAN is usually measured on a centrifuged must sample collected at harvest and does not consider the grape solids. YAN content is an indicator of the must fermentability and quality potential (Martínez-Gil *et al.*, 2012). Knowing YAN concentration in grapes before harvest can help winemakers to anticipate vinification conditions. Given the major role of YAN in winemaking, it is surprising that it is not always included in the must analyses to determine grape quality potential at harvest, along with the total soluble sugars, titratable acidity and pH. The assimilation order of the AAs during AF reflects both the initial must AA profile and the yeast strain preferences (Henschke and Jiranek, 1993). Yeasts select 'preferred' N sources that are rapidly assimilated into key components for their metabolism (Bell and Henschke, 2005; Crépin *et al*., 2017). However, Gobert *et al.* (2019) mentioned in their review that the 'preferred' and 'non-preferred' categories for YAN sources can widely vary depending on study conditions.

Oenological practices have major consequences for grape N extraction and, in turn, for must composition. N is present in the entire berry, but its distribution is uneven across berry fractions. Berry skin plays a central role in the synthesis of many compounds essential to wine quality, such as anthocyanins and aroma compounds (González-Barreiro *et al.*, 2015). During winemaking, the skin contact with must results in the extraction of the skin compounds and usually increases YAN content (Stines *et al.*, 2000). In the case of white wine making, cold racking is generally implemented before AF, and skins are not macerated in the must. Both actions are restrictive to YAN concentration in the must, which could explain why white wines are so sensitive to N restriction in the must.

4.2. Fermentation kinetics and must N correction

Must YAN concentration is often suboptimal, and this consequently restricts yeast growth and AF rate (Vilanova *et al.*, 2007: Hannam *et al.*, 2016). Below 200 mg YAN/L in the must, AF duration is negatively correlated to the concentration of YAN for a clarified must with average sugar concentration. Below 140 mg YAN/L, there is a major risk of stuck AF (Table 5)(Bell and Henschke, 2005; Torrea *et al.*, 2011). This threshold is lower in the case of red must, because grape N extraction is higher due to longer skin contact; for example, the Swiss cultivar Humagne rouge often has very low must YAN concentration at harvest (< 100 mg/L), and AF is still properly completed in most cases. Similar observations have been reported for Pinot noir (Schreiner *et al.*, 2018) and Merlot (Stockert *et al.*, 2013). The Australian Wine Research Institute recommends a minimum of 100 mg/L YAN for red must (AWRI, 2020).

TABLE 5. YAN concentration thresholds to guarantee proper alcoholic fermentation kinetics in white grape must.

Must YAN content (mg/L)	Risk of incomplete fermentation of clarified must
> 200	None
140 < … < 200	Moderate
< 140	Strong

To limit any risks related to must N deficiency, N supply to the must at the onset of AF - mainly in the form of diammonium phosphate (DAP) - has become a widespread practice. Bisson and Butzke (2000) recommended a YAN adjustment depending on °Brix degree: 200 mg/L at 21 °Brix, 250 mg/L at 23 °Brix, 300 mg/L at 25 °Brix and 350 mg/L at 27 °Brix. Martínez-Moreno *et al.* (2012) further demonstrated that the addition of a mixture of AAs increases AF kinetics and maximises sugar consumption more than DAP does. Rollero *et al.* (2016) highlighted the strong impact of yeast strain on the assimilation of N compounds and the formation of aromas during the AF.

Lactic acid bacteria require less N than yeasts during malo-lactic fermentation (MLF). N is assimilable to bacteria mainly in the form of AAs and, to a lesser extent, peptides (Ribéreau-Gayon *et al.*, 2017). A comparison of a wine's AA before and after MLF showed a very small decrease in their concentrations, probably due to the autolyse of

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yeasts and bacteria (Alcaide-Hidalgo *et al.*, 2007). Despite the observation of temporary N deficiency during AF due to rapid yeast growth, N deficiency rarely occurs at the end of AF and is not responsible for the difficulties in MLF kinetics.

4.3. Flavour development related to N metabolism

Wine flavours are the result of a complex mixture of volatile and non-volatile compounds. Their interactions have physicochemical effects on the release of aroma (Robinson *et al.*, 2014). It is beyond the scope of this review to describe all the grape and wine flavour-active compounds and their metabolisms; abundant literature can be found on this topic (Rapp and Mandery, 1986; Henschke and Jiranek, 1993; Bell and Henschke, 2005; Swiegers *et al.*, 2005; Dunlevy *et al.*, 2009; Styger *et al.*, 2011; Robinson *et al.*, 2014; González-Barreiro *et al.*, 2015; Alem *et al.*, 2019). This review focuses on the role of N - particularly AAs - in the formation of the flavour compounds and their precursors.

The characterisation of AA composition in grape is of major interest because AAs are precursors of a large number of metabolites in grape and wine, particularly volatile compounds (Jackson, 2008; Garde-Cerdán *et al.*, 2018). In terms of flavour development, the initial N pool contributes either directly or indirectly to the following (Figure 9):

- ⇨ Non-restricted C metabolism, involved in the synthesis of organic compounds responsible for varietal aromas, such as some aldehydes, terpenes and thiols;
- ⇨ The accumulation of aroma precursors (*i.e.*, glyco-, glutathione- or cysteine-conjugates) which release their flavour-active compounds via yeast metabolism; and
- ⇨ The accumulation of nutrients essential for yeast metabolism (*i.e.*, YAN). This greatly influences the biosynthesis of flavour constituents (*e.g.*, organic acids, higher alcohols, aldehydes and phenols) during the AF (Hernández-Orte *et al.*, 2006; Jackson, 2008).

Grape development and composition define the potential of wine aroma, which later develops during winemaking. Grape N accumulation starts with berry set. During the ripening phase (from veraison to harvest), the synergy between C and N metabolisms enhances AA accumulation and the biosynthesis of aroma compounds and their precursors. Hernández-Orte *et al.* (2002) demonstrated that the characteristic aroma of some varieties are partially related to the AA composition of the must. Martínez-Gil *et al.* (2012) confirmed that it is possible to estimate the concentration of esters in wines from the must N concentration. Grape aroma compounds can be found in either volatile ('free')

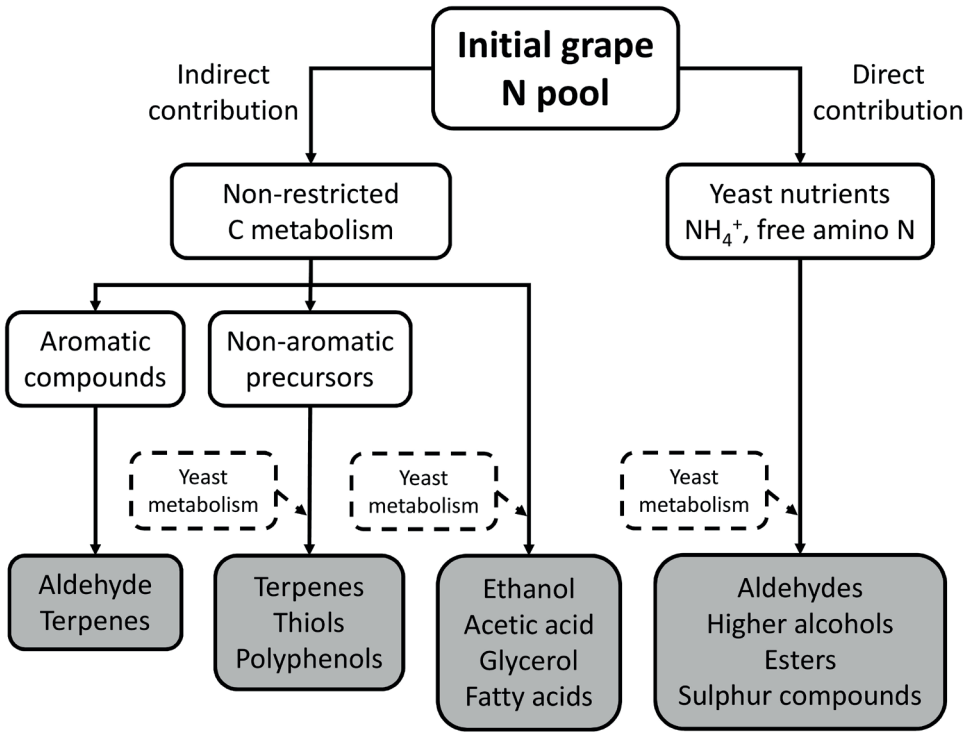


FIGURE 9. Contribution of the grape N pool to the biosynthesis of wine flavour-active compounds (in grey).

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or bound forms, such as glyco-, glutathione- and cysteine-conjugates (González-Barreiro *et al.*, 2015; Santamaría *et al.*, 2015). The bound form of these compounds is non-aromatic. As a result of the hydrolysis of glycoside, glutathione or cysteine, these compounds may then become volatile and thus aroma-active (Hjelmeland and Ebeler, 2015).

Terpenes, particularly monoterpenes and sesquiterpenes, are responsible for the characteristic aromas of varieties such as Gewürztraminer, Muscat and Riesling (Rapp and Versini, 1995; Robinson *et al.*, 2014). They are present in the grape in both free forms and non-aromatic glycoside precursors, and in variable proportions depending on the grape varieties. During winemaking, terpenes are released by the action of the glycosidase enzymes produced by grape, yeast and bacteria (Swiegers *et al.*, 2005). The presence of terpenes in wine is stimulated by higher YAN concentration in must (Hjelmeland and Ebeler, 2015).

Thiols (*e.g.*, mercaptohexanols) are another major group of wine aroma compounds, some of which give the characteristic aroma to varieties such as Sauvignon blanc and Petite Arvine. They are mainly present in the grape must as non-aromatic precursors. Helwi *et al.* (2016) demonstrated the positive impact of vine N status on the concentration of volatile thiols in wine through the increase in corresponding non-aromatic precursors in grape.

Methoxypyrazines are N compounds naturally present in berries and associated with 'bell pepper' aroma, characteristic of several varieties, in particular Cabernet-Sauvignon (González-Barreiro *et al.*, 2015). Their concentration decreases during grape ripening. However, vine N status does not influence the concentration of this metabolite in grape at harvest, which is affected by the modification of the bunch-zone microclimate (Robinson *et al.*, 2014; Helwi *et al.*, 2015).

Phenolic compounds form another diverse family related to the composition and concentration of grape AAs. The total phenolic content of grape must has been reported to be negatively correlated with the N treatment given to vines (Bell and Henschke, 2005; Choné *et al.*, 2006). However, Portu *et al*. (2015) reported increasing anthocyanin and flavonol concentration in wine after foliar treatment with phenylalanine. Phenylalanine is essential as a precursor in the flavonoid pathway for the synthesis of most phenolic compounds (Santamaría *et al.*, 2015). In contrast, the application of different forms of N (*i.e.*, urea, urea+sulphur and arginine) to Cabernet-Sauvignon decreased flavonoid concentration in wine (Gutiérrez-Gamboa *et al.*, 2017a). Similar results have been reported for Chasselas wine, for which suboptimal must YAN was correlated with increasing flavonol concentration in wine, but no effect on total phenol

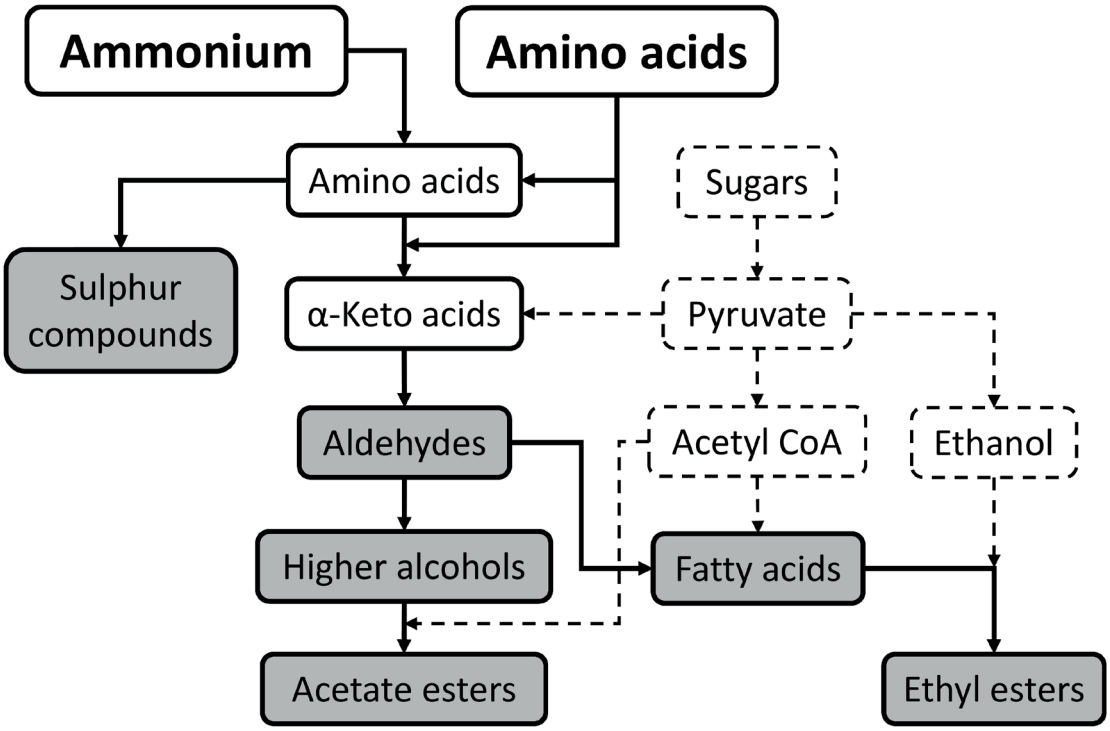


FIGURE 10. Synthesis of aroma-active compounds (in grey) through the yeast metabolism of AAs and ammonium.

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TABLE 6. Major AA metabolites in wine and their aroma characteristics.

AA	Chemical family	Compound	Concentration(mg/L)	Odour description	Odour threshold (mg/L)	References
Alanine	α-keto acid	α-ketopropionic acid				
	Aldehyde	Acetaldehyde	< 211	Fruity, rotting apple	100	1
Threonine	α-keto acid	α-ketobutyrate				
	Higher alcohol	1-propanol	13-125	Alcohol, fusel, sweet fruity, apple, pear	306	2, 3
		Methylglyoxal	0.1-1	Pungent	NR	1, 4
		Aminopropanol	NR	Fishy	NR	4
Valine	α-keto acid	α-ketoisovalerate				
	Aldehyde	Isobutyraldehyde	0.001-0.2	Apple, banana, melon, varnish, cheese	0.006	1, 4
	Higher alcohol	Isobutanol	9-174	Fruity, alcohol, solvent, green	40-75	4, 5, 6, 7
	Acetate ester	Isobutyl acetate	0.01-1.6	Banana, fruity	3.4 in 34 % spirit	4, 5, 3
	Fatty acid	Isobutyric acid	NR	Sweet, apple, rancid, butter, cheese	2.3	4, 6
	Ethyl ester	Ethyl isobutyrate	NR	Sweet, rubber	0.015 in 10 % ethanol	8, 9
Isoleucine	α-keto acid	α-keto-β-methylvaleate				
	Aldehyde	2-methylbutyraldehyde	0.003-0.1	Green (herbaceous), malty, fruity	0.016	1, 4
	Higher alcohol	2-methyl-1-butanol	15-150	Marzipan (almond), malty	1.2 in water	4, 7, 10
	Fatty acid	2-methylbutanoic acid	NR	Fruity, waxy, sweaty fatty acid	2.2 in water	4, 10
	Ethyl ester	Ethyl 2-methylbutanoate	0-0.9	Strawberry, pineapple	0.001 in 10 % ethanol	4, 9
Leucine	α-keto acid	α-ketoisocaproate				
	Aldehyde	Isovaleraldehyde	0.04-0.25	Fruity, nut, banana, apple, cheese, amylic	0.004	1, 4
	Higher alcohol	3-Methyl-1-butanol	45-490	Alcohol, nail polish, cheese	30	4, 5, 6, 8, 7
	Acetate ester	Isoamyl acetate	0.03-8.1	Banana, pear	0.03	4, 5, 6
	Fatty acid	Isovaleric acid	< 3	Rancid, cheese, rotten fruit, sweat	0.49 in water	4, 8, 10
	Ethyl ester	Ethyl isovalerate	0-0.7	Apple, fruity	0.0001 in water	4, 8, 10
Phenylalanine	α-keto acid	Phenylpyruvate				
	Aldehyde	Phenylacetaldehyde	0.0025-0.130	Floral	0.001	1
	Higher alcohol	2-Phenylethanol	4.0-197	Floral, rose, honey	10	5, 6, 8, 7
	Acetate ester	2-Phenylethyl acetate	0.01-4.5	Floral, rose, honey	0.25 in 10 % ethanol	5, 6, 8, 9
Methionine	α-keto acid	α-ketobutyrate				
	Aldehyde	3-methylthiopropanal	0.0005-0.080	Cooked potato, cabbage	0.250 in beer	1, 11
	Higher alcohol	3-methylthiopropanol	0.02-5	Cabbage, cooked vegetable	1	6, 7, 11
	Thiol	Methanethiol	NR	Rotten egg, cabbage	0.002-0.012 in beer	7, 11
	Ester	S-methyl thioacetate	0.002-0.016	Rotten vegetable	0.3 in beer	11

References: 1 : Waterhouse *et al.* (2016); 2 : Nykänen (1986); 3 : Miller (2019); 4 : Styger *et al.* (2011); 5 : Swiegers *et al.* (2005);6 : Gutiérrez-Gamboa *et al.* (2020a); 7 : Ribéreau-Gayon *et al.* (2017); 8 : Barbosa *et al*. (2009); 9 : Ong and Acree (1999); 10 : Czerny *et al.* (2008); 11 : Fleet (1993); NR : no reference.

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content was observed (Dienes-Nagy *et al.*, 2020). The effect of N nutrition on the phenolic compound content of grape is not yet fully understood and needs further investigation.

Winemaking strongly influences the development of wine aromas. Must N composition not only affects AF kinetics, but also the formation of aromatic compounds (Ugliano *et al.*, 2007; Styger *et al.*, 2011). The metabolism of yeasts releases a large number of aroma-active compounds; major volatile compounds derived from yeast metabolism include aldehydes, higher alcohols, esters and sulphur (S) compounds, all influencing wine flavour (Lambrechts and Pretorius, 2000; Santamaría *et al.*, 2015; Garde-Cerdán *et al.*, 2018) (Figure 10).

Ethanol, glycerol, fatty acids, acetic acid and carbon dioxide are only indirectly influenced by N metabolism. Crépin *et al.* (2017) studied aroma metabolism in *Saccharomyces cerevisiae*, and demonstrated that, contrary to what is generally acknowledged, only a limited fraction of the consumed AAs are directly incorporated by yeasts into proteins. Under the action of transaminases and deaminases, amine groups are collected from ammonium and AAs, and then are redistributed for *de novo* AA synthesis (Crépin *et al.*, 2017). The AAs can be further metabolised into higher alcohols through the Ehrlich pathway as follows (see equation 14 below) (Lilly *et al.*, 2006;Styger *et al.*, 2011):

The catabolism of AAs leads to the formation of α-keto acids and their corresponding aldehydes, which can be further reduced in 'higher alcohols' (Table 6).

The term higher alcohol refers to alcohols that possess more than two C atoms and have a higher molecular weight and boiling point than ethanol. Their concentration is usually positively correlated to must YAN concentration (Swiegers *et al.*, 2005). However, Henschke and Jiranek (1993) reported a negative correlation between the YAN concentration in must and the content of 2- and 3-methyl-1-butanol and 2-phenylethanol in wine. This may have resulted from the modified balance under N-deficient conditions between the reduced activity of the Ehrlich pathway and the increased activity of the biosynthetic pathway of branched-chain AAs from sugar metabolism (Swiegers *et al.*, 2005). At moderate concentrations (*i.e.*, below 300 mg/L), higher alcohols are desirable aroma compounds which contribute to the complexity of the wine fermentation bouquet. However, in high concentrations, 2- and 3-methyl-1-butanol has been shown to have a negative impact on wine bouquet, masking the fruity notes in red wine (Cameleyre *et al.*, 2015; de-la-Fuente-Blanco *et al.*, 2016).

The formation of esters is related to the availability of both higher alcohols and fatty acid precursors. In fact, two major groups of esters are formed during fermentation: the acetate esters and the ethyl esters (Figure 10). Acetyl-CoA is condensed with higher alcohols to form acetate esters, and fatty acids are condensed with ethanol to form ethyl esters as a result of enzymatically catalysed reactions (Bell and Henschke, 2005). Despite their formation not being directly related to AAs, their concentration in wine is often positively correlated to must N concentration (Bell and Henschke, 2005; Ugliano *et al.*, 2007; Barbosa *et al.*, 2009). Most esters contribute significantly to the fermentation bouquet. Acetate esters have been found in wine in a concentration range of 0-18.5 mg/L, often above their detection threshold (Swiegers *et al.*, 2005). Ethyl esters of branched chain fatty acids are only present in wine in concentrations below 1 mg/L. They are related to AAs, because they are formed from the oxidation of the aldehyde formed from α-keto acids during AA metabolism (Table 6). Swiegers *et al.* (2005) observed a synergy between grape and yeast metabolisms during the formation of characteristic ester profiles of grape varieties such as Chardonnay.

Suboptimal must YAN composition and concentration restrain yeast metabolism, including the sugar, N and S pathways. The production of both non-volatile and volatile metabolites is consequently affected and has sensory implications (Ugliano and Henschke, 2009). The increase in 2- and 3-methyl-1-butanol and 2-phenylethanol formation in these conditions demonstrates that modifications occur during yeast metabolism, and that there is also an increase in the formation of succinic acid and, consequently, in the succinic ester content of wine (Henschke and Jiranek, 1993; Garde-Cerdán and Ancín-Azpilicueta, 2008; Dienes-Nagy *et al.*, 2020). The formation of free hydrogen sulphide (H_2S) ('rotten egg') and mercaptan ('onion') can increase in the event of YAN starvation during AF, which is deleterious to the wine bouquet. H_2S is a by-product of the biosynthesis of S-containing compounds, including AAs, methionine and cysteine. N supplementation during AF rapidly suppresses the accumulation of H_2S (Henschke and Jiranek, 1993), which is highly reactive and takes part in the formation

$$\underbrace{RCH(NH_2)(COOH)}_{\text{amino acid}} \xrightarrow{\text{deamination}} \underbrace{RCO(COOH)}_{\text{α-keto acid}} \xrightarrow{\text{decarboxylation}} \underbrace{RCHO}_{\text{aldehyde}} \xrightarrow{\text{reduction}} \underbrace{RCH_2OH}_{\text{higher alcohol}} \quad (14)$$

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of other positive aroma-active S compounds, such as dimethyl sulphide (DMS) ('asparagus', 'truffle') (Swiegers *et al.*, 2005). Although DMS does not give fruity aromas, it is indirectly involved in their development in wine (De Royer Dupré *et al.*, 2014; Lytra *et al.*, 2014; Lytra *et al.*, 2016). The formation of 2-aminoacetophenone (2-APP) under low YAN conditions has been identified as being responsible for the atypical aging off-flavours in wines which are usually accompanied by an undesirable astringent and bitter flavour (Hoenicke *et al.*, 2002; Linsenmeier *et al.*, 2007). However, there is no clear correlation between the concentration of 2-AAP (or its precursor, indol-3-acetic acid) and the sensory perception of atypical aging. Schneider (2014) published a review about the atypical aging defect, discussing sensory discrimination, viticultural causes and oenological consequences, and thus illustrating the complexity of this problem. In contrast to N restriction, residual N in wine due to excessive supplementation can lead to precipitation (protein breakdown) and the formation of biogenic amines (allergen) and ethyl carbamate (carcinogenic) (Vincenzini *et al.*, 2017). N excess may also lead to the development of undesirable microorganisms, such as *Brettanomyces*, responsible for wine spoilage (Bell and Henschke, 2005).

Suboptimal must YAN is usually corrected in the cellar with the addition of N to prevent sluggish AF. Aroma production in wine is affected by both the timing of N addition and the composition of the N source (Seguinot *et al.*, 2018). The DAP supply to the must only increases the ammonium concentration, while a balanced must contains a complex mixture of ammonium and AAs. However, no clear correlation has been established between the impact of DAP supply and the wine sensory profile (Torrea *et al.*, 2011). Conversely, many studies have demonstrated the positive influence of adding AA directly to the must on the formation of volatile compounds and, ultimately, on the development of wine aroma (Hernández-Orte *et al.*, 2006; Garde-Cerdán and Ancín-Azpilicueta, 2008; Torrea *et al.*, 2011). Fairbairn *et al.* (2017) investigated the effects of single AAs additions on the production of major volatile compounds in wine, which resulted in a predictable production of aromatic compounds with linear correlations. However, these correlations were lost as the complexity of the N sources increased. The choice of N source also affects the formation of glycerol and organic acids (Ugliano and Henschke, 2009). Several studies have demonstrated that the following AAs have a positive influence on flavour development during AF: threonine, phenylalanine, alanine and aspartic acid (Hernández-Orte *et al.*, 2006).

Understanding the fate of N sources during winemaking and their impact on the development of wine flavours could certainly help improve NUE. Controlling the development of wine flavours would then be possible by modifying the amount, type and timing of N sources. Moreover, the production of grapes rich and naturally balanced in AA compounds offers the winemaker high potential for making good quality wine.

THE IMPACT OF ENVIRONMENTAL CONDITIONS AND AGRONOMIC PRACTICES

Research on wine flavours has focused on AF conditions, since the majority of wine flavour compounds appear during winemaking as a result of yeast and bacteria metabolism (Robinson *et al.*, 2014). However, since most of the substrates (particularly the N compounds) are grape-derived, the production of flavour compounds is strongly related to grape composition (Robinson *et al.*, 2014). Plant physiology and grape composition depend on climate conditions and soil characteristics before and during berry development; they can be managed to some extent by optimising agronomic practices (Masclaux-Daubresse *et al.*, 2010; Sweetman *et al.*, 2009). The following section reviews the parameters, which influence grapevine N metabolism, distinguishing between the impact of the environment inherent to the vineyard and the agronomic management practices of the grape grower.

1. The environmental conditions of the vineyard

There are environmental conditions specific to the vineyard site which impact plant water and nutrient uptake, as well as leaf gas exchange and photosynthetic activity. Water, N and C are the three major components that significantly affect plant N metabolism, apparently following Liebig's law of the minimum. Any factor that either directly or indirectly influences water, C or N availability to the plant will potentially affects its N metabolism. The impacts of environmental conditions on grapevine N metabolism are summarised in Figure 11.

1.1.Climate and soil

The influence of climate on the plant metabolism can be considered at a regional scale (macroclimate), vineyard scale (mesoclimate) or plant scale (microclimate). In long-term experiments, the climate is also considered in terms of the 'year' effect.

Edaphic conditions (*i.e.*, soil depth, structure, temperature, water availability, pH, organic matter

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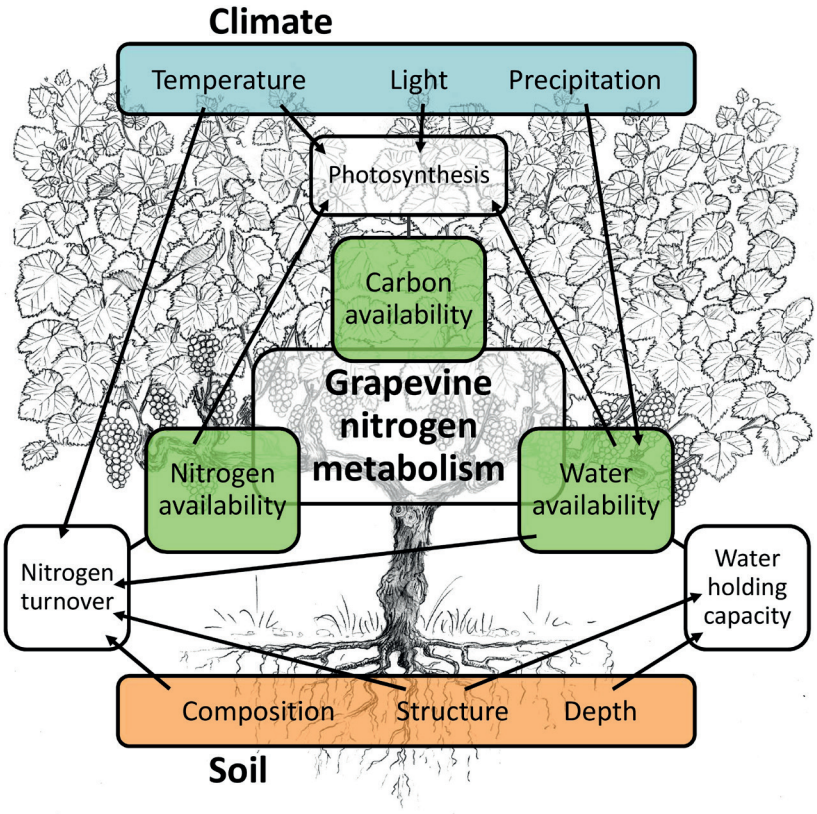


FIGURE 11. Impacts of environmental conditions (i.e., climate and soil) on grapevine N metabolism.

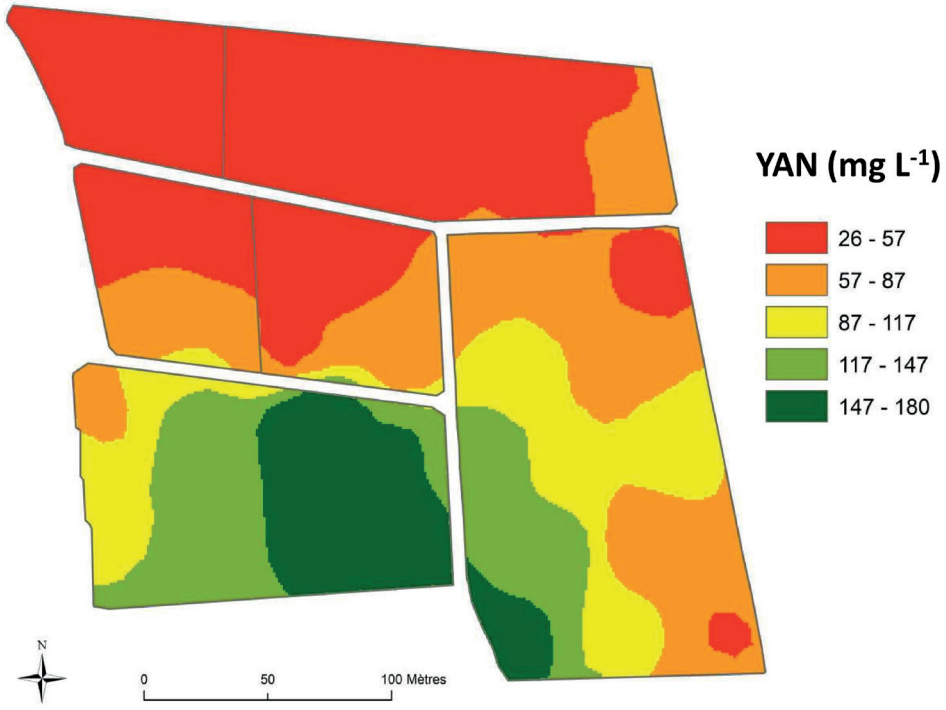


FIGURE 12. Variability of yeast assimilable N in grape must at harvest.
Map obtained by ordinary kriging method based on a regular grid of eight samples per ha. Merlot, 2018, Saint-Julien, Bordeaux, France (van Leeuwen *et al*., unpublished data).

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content, limestone content and C/N ratio) highly influence the soil N cycle (turnover) and the subsequent N availability to the vine (van Leeuwen *et al.*, 2000; Hardarson *et al.*, 2008; Masclaux-Daubresse *et al.*, 2010; Marschner and Rengel, 2012). Consequently, grapevine N status (represented by must YAN at harvest) can vary considerably over short distances due to soil heterogeneity. To optimise vineyard management, it is important to visualise this spatial variability, which can be obtained by measuring YAN on a regular grid in a vineyard (Figure 12).

Soil temperature plays a major role in plant N uptake and metabolism: high temperatures (without water restriction) increase soil microbial activity and thus enhance organic matter mineralisation (Molina and Smith, 1997); furthermore, they increase root growth (higher fine root density) and thus favour N uptake (Clarke *et al.*, 2015). Cold periods during springtime are a major cause of low N availability and uptake. However, excessively high air temperatures (*e.g.*, above 40 °C) can also limit root N assimilation, partly due to lower photosynthesis and lower C availability: in response to heat stress the plant limits water consumption by closing stomata, which in turn reduces photosynthesis activity (Zufferey *et al.*, 2017). Optimum temperature depends on grape variety, light intensity and phenological stage, and it is generally considered to be within the range of 10-35 °C (Hunter and Bonnardot, 2011; Keller, 2015). Temperatures out of this range can become a limiting factor for N metabolism. Global warming is a major concern in agriculture, as it also affects ambient CO_2 and solar radiation. It is generally projected that plant growth will increase under higher concentrations of ambient CO_2, due to improved photosynthetic activity (Tegeder, 2014). Because C metabolism and N metabolism are highly correlated, a higher concentration of C metabolites can improve N assimilation through the action of the enzymes GS/GOGAT; consequently, plant vigour will increase under unrestricted N availability. However, in many situations, restrictive N conditions can limit this increased capacity for using additional C (Stitt and Krapp, 1999).

Light is another factor that influences N metabolism. Poor weather conditions (*e.g.*, cloudy weather) can cause a decrease in N status, in response to reduced solar radiation (Keller, 2015). Light intensity influences photosynthesis rate and subsequent availability of C metabolites required for N assimilation (Masclaux-Daubresse *et al.*, 2010). Several studies have reported a correlation between grape exposure and the concentration of free aroma compounds or their bound glycosylated precursors (Bureau *et al.*, 2000; Marais *et al.*, 2001; Meyers *et al.*, 2013; Kwasniewski *et al.*, 2010). However, the relation between sunlight exposure and grape N content has not yet been clearly established.

Water and nutrients exist together in close association, because sufficient water availability (without waterlogging) will lead to nutrient solubilisation and facilitates plant N uptake and transport in the plant (Keller, 2005; Wang *et al.*, 2017). Vine water status depends on both climate-related factors (evapotranspiration and precipitation) and soil water holding capacity (van Leeuwen *et al.*, 2004). The best soils for viticulture induce both mild water restriction and non-limiting nutrient conditions (Fayolle *et al.*, 2019). Soil structure, texture and depth greatly affect water and nutrient availability for the plant, as they influence the soil water holding

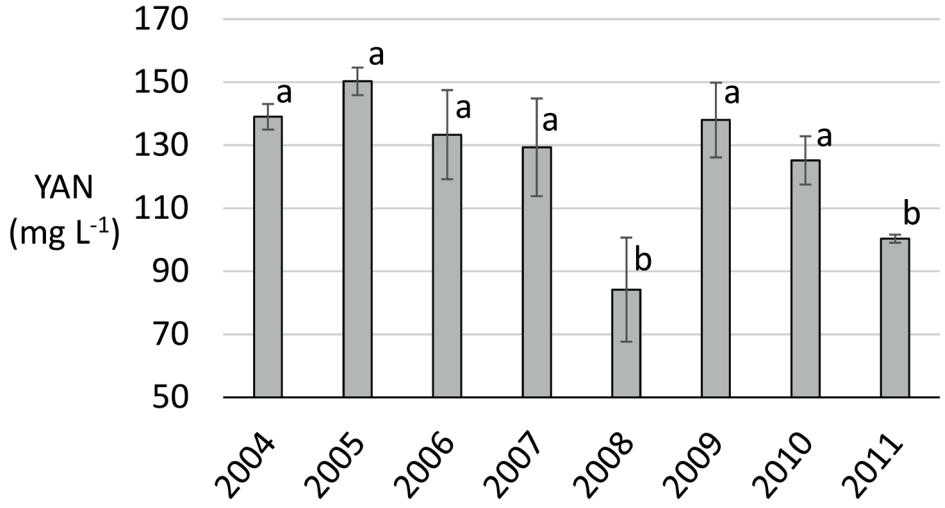


FIGURE 13. Year-to-year variability of YAN in grape must at harvest.

Average data from six vineyard blocks, located on three soils and planted with two grapevine varieties (Merlot and Cabernet franc) in Saint-Émilion, France (adapted from van Leeuwen *et al.*, 2004-2011, unpublished data).

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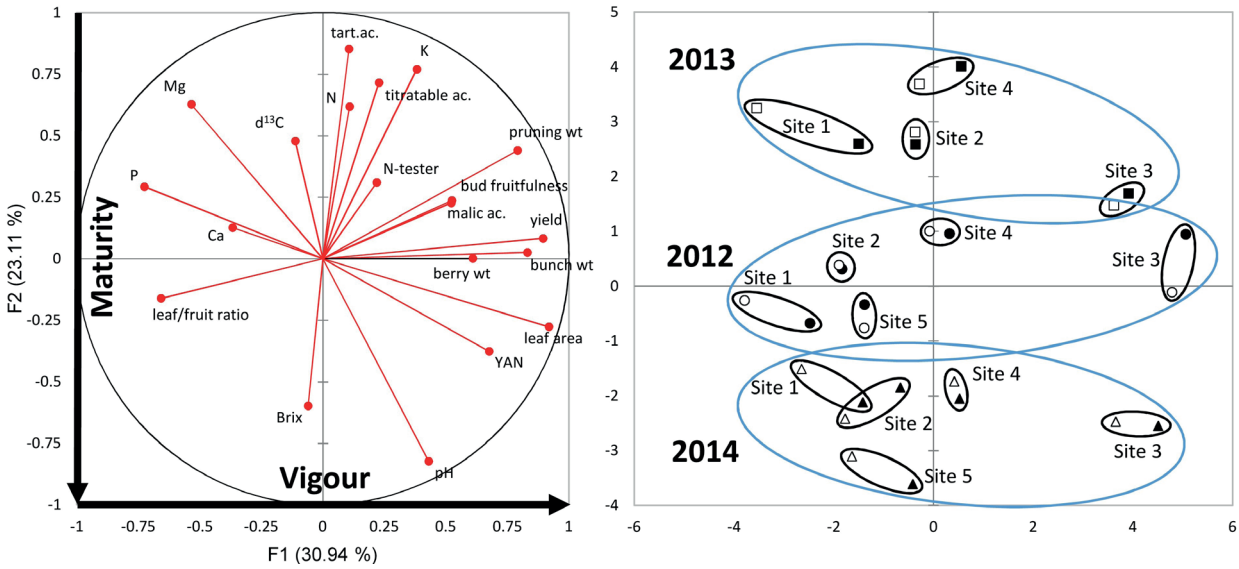


FIGURE 14. Impact of year, site and fertilisation on plant behaviour and must composition of the white cultivar, Doral (Chasselas × Chardonnay) in five vineyards (same plant material and agricultural practices) in a terroir study over three years in Switzerland.

White shapes = non-fertilised control treatment; black shapes = foliar urea supply at veraison (20 kg/ha of N) (adapted from Verdenal *et al.*, 2016b).

capacity and the potential for root development (van Leeuwen and Seguin, 2006). In shallow soils, grapevine often has low grape N concentration, usually attributed to limited root colonisation (Reynard *et al.*, 2011; Reynard *et al.*, 2012). Under non-limiting water conditions, the plant can easily absorb the mineral N required for its development. High plant sap flow is a result of high transpiration and photosynthesis (Zufferey and Murisier, 2007). However, water excess due to high quantities of precipitation may induce low N uptake, either because of soil N leaching or because of waterlogging, which reduces the amount of oxygen in the soil needed for microbial activity. Conversely, under hot and dry conditions (*i.e.*, during the growing season in summer), N availability decreases at the soil surface due to low water content. In these conditions, water is a limiting factor for microbial activity, N solubility, N mobility and N uptake (Marschner and Rengel, 2012). Grapevine can counterbalance lower N availability with higher organic N mobilisation from the root reserves, as has been shown in maize by Wang *et al.* (2017). Moreover, root growth is limited in these conditions. Excessive water restriction may further induce a lower rate of photosynthesis and a subsequent lower plant C status. Climatic water deficit (precipitations minus evapotranspiration) during vegetative development is consequently negatively correlated to the accumulation of YAN in grapes (Spring *et al.*, 2012). In an 8-year study combining six vineyards, three soil types and two cultivars, van Leeuwen *et al.* (unpublished data) observed a wide range of YAN values at harvest (from 80 to 150 mg/L) over the eight years (Figure 13). This variability was explained by the soil type (45 % of total variance explained), cultivar (17 %) and climatic conditions of each year (14 %). The two vintages 2008 and 2011 showed significantly lower YAN values. This was probably due to the particular climatic conditions of those years: spring 2008 was cool and rainy, while spring 2011 was warm and particularly dry Hernández-Orte *et al.* (1999) confirmed that the highest grape YAN accumulation was obtained in the years with mild temperatures and moderate rainfall during ripening.

The impacts of pedoclimatic conditions on berry composition was assessed by Echeverría *et al.* (2017), who found that the synthesis of primary compounds is mostly dependent on both the climate and the climate-soil interaction, while the synthesis of secondary compounds (*e.g.*, phenols) mostly depends on the source-sink relationship and the climate. These processes are regulated by both internal (C and N availability) and external factors (light, soil structure and composition, and soil microbiological activity) (Keller, 2015). A study by Verdenal *et al.* (2016) highlighted the strong overall impact of both climate and soil on grapevine N status. Five homogeneous plots of the white cultivar Doral (same plant material and agricultural practices) were chosen in different vineyards and were divided into control and N-fertilised treatments. Figure 14 shows the hierarchy of the three factors of discrimination; *i.e.*, year, site and fertilisation. First, the year (*i.e.*, climate) was the most variable and discriminating factor in terms of maturity and grape composition at harvest (*i.e.*, sugar content and acidity). Second, the soil

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had a very steady impact on grapevine vigour (*i.e.*, bud fruitfulness, leaf area, pruning weight, bunch weight, yield and YAN) with the same site differentiation every year of the study. Third, fertilisation had a relatively small and variable impact on grapevine physiology and grape composition, despite a considerable impact on must YAN concentration, which significantly improved the wine organoleptic profile ($R^2 = 0.70$). This example shows the hierarchy in the climate-soil-plant ecosystem and demonstrates the possibility of improving grape composition via cultural practices, despite the major influence of both the year-to-year variability of climatic conditions and spacial variability of soil composition.

1.2. Phenotypic plasticity

Dal Santo *et al.* (2016) and Dal Santo *et al.* (2018) focused on the phenotypic plasticity of grapevine and dissected the berry transcriptome in response to the environment. Using an innovative data mining and statistical method, they investigated the separate impacts of climate, soil and grape variety, as well as their interactions. They found that grapevine is highly sensitive to environmental conditions and is characterised by a broad phenotypic plasticity (Dal Santo *et al.*, 2016). In a study on *Arabidopsis*, Sakakibara *et al.* (2006) demonstrated that plants have the ability to sense their internal and external N status and to adapt to changing conditions by modifying their gene expression and morphology accordingly. Vines grown under low N and high irradiance conditions had the highest root-to-shoot ratios, and those grown under low irradiance and high N had the lowest (Grechi *et al.*, 2007). N deprivation was found to enhance root growth at the expense of aboveground growth, whereas canopy size was significantly greater under high N conditions (Grechi *et al.*, 2007). The plant can modify its root architecture, locally increasing root proliferation to reach nutrient-rich soil patches. The presence of nitrate stimulates the formation of lateral roots when it is applied to small sections of the primary roots (Lea and Azevedo, 2006). Leaves grown under low humidity (high vapour pressure deficit) have been found to be smaller than those grown under high humidity, even in the absence of soil water deficit (Keller, 2015). Canopy development and density ultimately affect the grape microclimate, particularly in terms of solar radiation interception. The grape AA profile of a given variety is generally similar from year to year, while AA concentration can vary widely (Hernández-Orte *et al.*, 1999).

The plant affects, in turn, the soil composition through the process of N uptake. The rhizosphere is locally alkalinised and acidified following the uptake of nitrate and ammonium respectively (Hachiya and Sakakibara, 2016). Microbial activity is inhibited by a lower pH, which affects the fraction of the cation-exchange capacity occupied by cations and subsequent soil fertility. The optimum pH for N uptake ranges from 5.5 to 8.0 (Longbottom, 2009). Plant nutrition can also be enhanced by symbiosis with soil microorganisms, such as mycorrhiza, which are considered as 'new organs' unifying root tissues with the fungus mycelium in a symbiotic relationship. Mycorrhiza have a high capacity for assimilating N in the soil, thus benefiting the plant 'host' (Trouvelot *et al.*, 2015). In return, the plant provides the fungus with photoassimilates. Such symbiosis concerns 95 % of plant species (Morot-Gaudry *et al.*, 2017). Krishna *et al.* (2005) confirmed that the inoculation of mycorrhiza increases grapevine N content, as well as many other metabolites, such as nitrate reductase, chlorophyll, phenolics and proline contents. Grapevine rootstocks differ very little in their ability to form mycorrhiza, but other factors, such as crop load and soil moisture, have a great influence on root colonisation by mycorrhiza (Schreiner, 2003). The mycorrhiza colonisation of grapevines has been found to be unaffected by the presence of a cover crop (Klodd *et al.*, 2016).

1.3. The concept of terroir

Understanding the impact of environmental conditions on plant N status helps make technical choices that will ensure and improve wine quality and sustainability. The International Organization of Vine and Wine defines the terroir as 'a concept that refers to an area in which collective knowledge of the interactions between the identifiable physical and biological environment and applied viti-vinicultural practices develops, providing distinctive characteristics for the products originating from this area. The terroir includes specific soil, topography, climate, landscape characteristics and biodiversity features' (Resolution OIV/VITI 333/2010). Vine growers must understand the intrinsic conditions of their vineyard in order to use the environmental conditions to their advantage (van Leeuwen *et al.*, 2018). In order to reach a desired crop quality, it is necessary to integrate the optimisation of NUE into management practices, thereby modulating the influence of the environmental conditions (Figure 15).

1.4. Agronomic choices

No vineyard would exist without human intervention (van Leeuwen and Seguin, 2006). Reynolds (2010) summarised the common goals of human agronomic practices in cool climate conditions in four points: 1) keep the fruits warm, 2) keep the leaves exposed to light, 3) achieve vine balance between vegetative and reproductive organs, and 4) avoid water stress.

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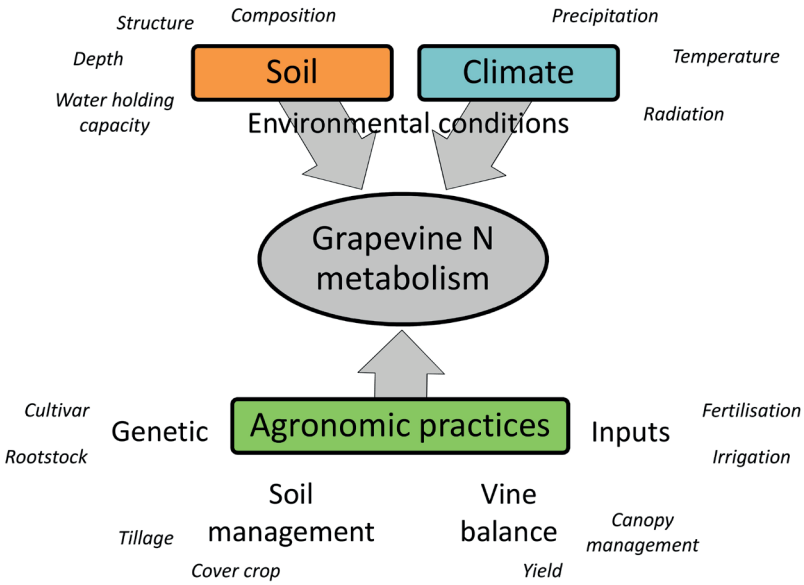


FIGURE 15. Illustration of the terroir concept, showing the influence of climate, soil and agronomic practices on grapevine N metabolism.

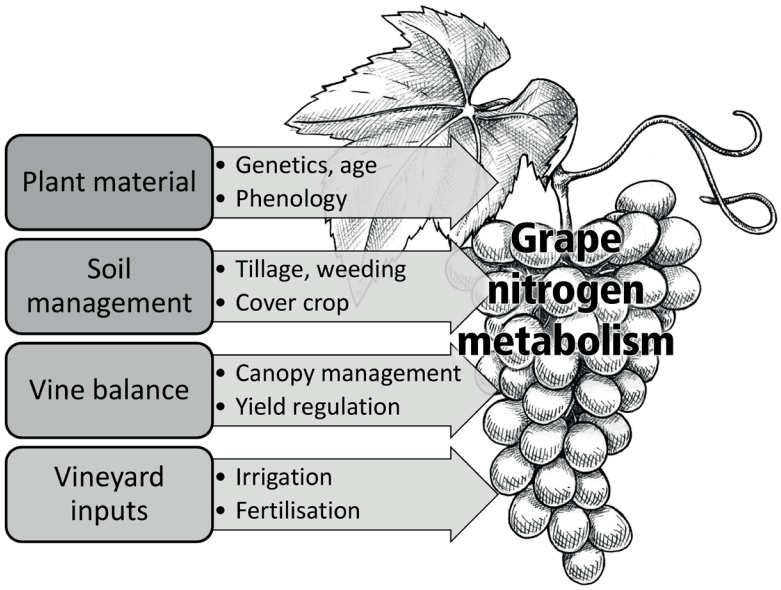


FIGURE 16. Agronomic practices influencing grape N metabolism.

However, there is no universal recipe, and vine growers must adapt their practices to their local environmental conditions in order to obtain optimal must composition. Habran *et al.* (2016) summarised the situation as follows: mild water deficit and moderate N availability can result in the metabolic synthesis of phenolic and aromatic compounds in berries, while surplus N can induce excessive vigour and exacerbate sensitivity to fungus. Consequently, N supply should be managed in such a way as to obtain a balance between vegetative and reproductive growth while preventing N deficiency. The objective is to optimise the grape N pool at veraison in order to enhance the biosynthesis of AAs and other aroma precursors in the must during grape maturation, while preserving vine balance and adequate ripening conditions. Several reviews have reported the influence of agricultural practices on the accumulation of aroma compounds and precursors in grapes (Poni *et al.*, 2018; Gutiérrez-Gamboa *et al.*, 2018), and on the development of aromas in wine (Robinson *et al.*, 2014; González-Barreiro *et al.*, 2015; Alem *et al.*, 2019). However, understanding how agronomic practices can specifically influence N metabolism would improve fruit quality control, as well as NUE and production sustainability (Boss *et al.*, 2014; González-Barreiro *et al.*, 2015). The following sections review the main agronomic choices that affect grape N metabolism. Four major factors are addressed: 1) plant material, 2) soil management, 3) vine balance, and 4) vineyard inputs (Figure 16).

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2. Plant material

Ensuring that planting material is adapted to vineyard environmental conditions is a prerequisite for the production of quality grapes, and involves making choices regarding the rootstock, variety and clone.

2.1. Genetics and age

Grape varieties genetically differ from each other in terms of concentration and composition of N compounds in their fruits. Genetics has a great impact on grapevine NUE. Plants use several ways to sense environmental and internal N status. One is nitrate concentration, which regulates a wide variety of metabolic processes, including N and C metabolism (Sakakibara *et al.*, 2006). The relative proportion of nitrate and ammonium in the soil influences N uptake. In rice, net nitrate uptake is inhibited by the presence of ammonium, compared to nitrate alone, while net ammonium uptake is enhanced by the presence of nitrate, compared to ammonium alone (Hachiya and Sakakibara, 2016). There is a general tendency across cultivars for increasing N uptake to induce lower leaf concentrations of K, P, Mg and boron (B) (Zamboni *et al.*, 2016). Under non-limiting water and nutrient conditions, a significant correlation usually appears between plant vigour, plant N status and grape N concentration, with variations depending on the plant material; *i.e.*, rootstock, variety and/or clone.

The influence of genetics on N metabolism has been highlighted between the two varieties, Merlot and Pinot noir (Zapata *et al.,* 2004b). In similar conditions, N uptake was higher in Pinot noir than in Merlot. Stines *et al.* (2000) suggested that the must AA profile is primarily genetically determined, whereas environmental conditions have a modifying effect. Several studies have shown a strong impact of grape varieties on the AA profile in grape must at harvest: the ratio of major AAs (proline, arginine, glutamine and histidine) to total AAs differed significantly across varieties (Hernández-Orte *et al.*, 1999; Stines *et al.,* 2000). Huang and Ough (1991) used the proline-to-arginine ratio to differentiate grape varieties. In Switzerland, a trial compared eight rootstocks over thirteen years, all grafted onto Pinot noir (clone RAC 12) and grown under homogeneous conditions (Spring *et al.*, 2016a). The 13-year average leaf N content varied from 2.0 to 2.4 % DW, depending on the rootstock. It was correlated with vigour and must YAN content. The average YAN concentration greatly varied (from 132 to 224 mg/L) as a function of the rootstock. To a lesser extent, clones of the same variety also influence N metabolism, which has been shown in two studies. The first study compared 19 clones of Pinot gris (grafted onto 3309C) over seven years, while the second study compared 17 clones of Petite Arvine (grafted onto 5BB) over nine years, all grown under homogeneous conditions (Spring *et al.*, 2016b; Spring *et al.*, 2018). The average must YAN at harvest varied from 100 to 145 mg/L for Pinot gris, and 195 to 240 mg N/L for Petite Arvine, depending on the clone. Besides sensitivity to soil N content, the root mechanisms involved in N uptake are strongly affected by the variety-rootstock combination, which opens possibilities for adjusting grape composition via choice of planting material (Tomasi *et al*., 2015; Habran *et al.*, 2016). Kant *et al.* (2011) reviewed the different genetic approaches for the improvement of NUE, starting with a description of the regulatory mechanisms involved in the plant response to N deficiency conditions. N uptake and remobilisation seem to be independently inherited traits; therefore, it is possible to combine favourable alleles when breeding for high NUE (Xu *et al.*, 2012).

Plant material has long-term repercussions on wine style and quality and it must be determined with care, since not every vineyard can produce any possible wine style. First, the plant material must be chosen according to local climate to guarantee full ripeness of the grapes at the end of the season (van Leeuwen and Seguin, 2006). Climatic indices, such as the heliothermal index (Huglin, 1978), or the Grapevine Sugar Ripeness model (Parker *et al.*, 2020) can be used for this purpose. Second, the plant material should be chosen according to soil N availability to guarantee balanced N nutrition. It should be kept in mind that grape N requirements are lower for red wine, compared to white wine, due to differences in the winemaking process; consequently, the producer might prefer to grow red varieties in vineyards, which have recurrent low N availability.

Moreover, grapevine age influences N metabolism. Using three white (Pinot blanc, Chasselas and Arvine) and three red cultivars (Gamay, Syrah and Humagne rouge), Zufferey and Maigre (2007) and Zufferey and Maigre (2008) compared the grapevine physiology and must composition of 4-8 years-old vines versus grapevines of 25 years of age and older. The young vines (< 8-years-old) were more susceptible to water stress and N deficiency due to their smaller and more superficial root system, and they had lower vigour, lower N status and lower grape YAN concentration. During the first years after planting, both root N reserves and N uptake restricted photosynthesis. Despite controlled and comparable yields, the red wines from older vines generally had higher quality aromas and a less astringent mouthfeel. Gamay wines showed no differences, which was probably due to the higher plasticity of the cultivar. No differences were found for white wines in terms of

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mouthfeel, and only a small preference for the aromas of wines from old vines was shown for Pinot blanc.

2.2. Maturity level

Grape maturity highly influences the berry AA profile. The accumulation of organic N and the formation of secondary metabolites within the berry, such as flavour-active compounds and their precursors, are affected by level of maturity (Hilbert *et al.*, 2003; Robinson *et al.*, 2014). Changes in AA profile during grape berry ripening have been demonstrated in several studies (Stines *et al.*, 2000; Hilbert *et al.*, 2003; Garde-Cerdán *et al.*, 2009; Garde-Cerdán *et al.*, 2018). Accumulation of grape YAN appears to differ significantly from other metabolites (González-Barreiro *et al.*, 2015). Berry N accumulation starts as soon as berry set starts (BBCH 71). At the onset of ripening (veraison, BBCH 85), the berry YAN pool is mainly composed of glutamine and NH_4^+, which both decline during grape ripening due to their conversion into other AAs (Stines *et al.*, 2000). Overall, NH_4^+ concentration decreases while free AA concentration usually increases (Garde-Cerdán *et al.*, 2018). Arginine accumulation in grape starts before veraison, while proline mostly accumulates during post-veraison (Stines *et al.*, 2000). The accumulation of both arginine and proline seem to be developmentally regulated (Stines *et al.*, 2000). Proline accumulation in vegetative tissues is often associated with osmotic stress during the post-veraison period (*e.g.*, high concentration of sugars). However, Stines *et al.* (2000) argued that proline accumulation is part of normal fruit development, as in many other plant species, while the factors influencing the proline-to-arginine ratio remain unknown.

To monitor grape ripening, parameters such as sugars usually provide the most basic information about quality potential (González-Barreiro *et al.*, 2015). A strong correlation was observed in the must between arginine accumulation and soluble sugar accumulation (Hernández-Orte *et al.*, 1999; Garde-Cerdán *et al.*, 2009). Garde-Cerdán *et al.* (2018) reported that technological maturity (*i.e.*, optimal sugar content) coincides with the highest concentration of organic N compounds at 25 °Brix. Hence, they introduced the term, 'nitrogenous maturity'. González-Barreiro *et al.* (2015) confirmed that maximum flavour-active compound content is reached at maturity and remains constant over the following weeks. They described the aroma development in red grape as follows: esters characterise the beginning of ripening period, aldehydes the middle and alcohols the end. Consequently, they suggested using the alcohol-to-aldehyde ratio to optimise on the harvest date and to maximise grape aroma. However, the accuracy of this index seems to be low for white varieties. The late formation of alcohols is desirable as they are precursors to the formation of esters in the presence of carboxylic acids during AF (González-Barreiro *et al.*, 2015). In view of the major role of must N (particularly YAN) in AF kinetics and in the development of wine flavour, must YAN concentration before and at harvest could be used as an indication of grape quality. In any case, must YAN should be routinely analysed for winemaking purposes, on the same basis as sugars and acids.

3. Soil management

Soil maintenance has a direct impact on grapevine root development and nutrition, with further consequences on must N composition and wine sensory profile (Bouzas-Cid *et al.*, 2018a). Proper soil maintenance guarantees sustainable soil fertility with proper N mineralisation and availability of mineral N for the plant. However, vineyard soil must be prepared before planting in order to relieve soil compaction and optimise soil structure. If necessary, an initial manuring can be applied. After planting, soils are usually managed through tillage, herbicides and/or cover crop.

Cover cropping is a common practice in vineyards which greatly affects soil N availability (Spring, 2001). The presence of a cover crop offers many advantages, such as reduced maintenance, reduced herbicide use, better soil stability, higher soil bearing capacity and permeability, and lower erosion. It also reduces plant N status and, consequently, overall grapevine vigour by limiting N availability (Tesic *et al.*, 2007; Reeve *et al.*, 2016). Depending on the cover-crop mixture, N competition between grapevine and cover crop can be exacerbated under low water availability (Celette *et al.*, 2009). The implantation of legume (*e.g.*, *Trifolium subterraneum*), which have the capacity of fixing N from the atmosphere, is an interesting alternative for limiting such competition (Spring, 2002). Both temporary and permanent cover crops decrease soil N mineralisation, due to a faster drying of the superficial soil layers (Celette *et al.*, 2009). Grapevines may adjust their root development to access deeper water resources, although deeper layers contain less mineral N (Celette *et al.*, 2009). Vegetative development is limited, thus improving the grape microclimate (better sun exposure and higher temperature) (Maigre and Aerny, 2001a; Reeve *et al.*, 2018). Lower N availability has been found to be related to a higher concentration of higher alcohols and phenolic compounds in wine (Choné *et al.*, 2001; Maigre and Aerny, 2001b). However, over four years of experimenting on Gamay, researchers found that the wines produced from vines with bare soil treatment were usually preferred to those from vines with cover crop treatment, due to

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FIGURE 17. Trial of *Hordeum murinum* as a cover crop. Sowing in 2007 and pictures taken in 2008. Epesses, Switzerland (Spring, 2008).

increased varietal aromas and reduced astringency (Maigre and Aerny, 2001b). It is difficult to control vine vigour exclusively via cover cropping. An excess of competition for N and water between the grapevines and the cover crop can damage the yield and the wine quality. In the 1980s, cover cropping was widely developed in Swiss vineyards and the winemakers started observing difficulties in AF kinetics, with the development of off-flavours, particularly in white wines. The lower N content in berries was explained by the reduced availability of soil N due to cover cropping (Gouthu *et al.*, 2012). Cover crop affects grapevine N status in the long-term, as it also affects the perennial reserve of N build-up necessary for the next year (Celette *et al.*, 2009; Gouthu *et al.*, 2012). Celette and Gary (2013) further showed that the dynamics of water and N availability for the grapevine are partially uncoupled.

The cover crop must be adapted to soil conditions, as there is no universal cover crop suitable for all vineyards. In a situation of excessive grapevine vigour, the use of a competitive cover crop can be an effective strategy for limiting vine growth and yield, although water availability and grape YAN content should be monitored (Reeve *et al.*, 2016). To minimise competition with grapevine, a temporary cover crop can otherwise be recommended. The cover crop can also be limited to the row spacing (80 % of the surface, weeding under the row) and even to every other row (only 40 % of the surface). The choice of the cover crop species is essential. The ideal cover crop species has the following characteristics (Delabays *et al.*, 2000): quick development, low vigour during summer, strong allelopathy towards other species, winter covering and frost resistance, and spontaneous seeding and regeneration. Ideally, the cover crop should grow during spring and autumn and dry during the summer, thus inducing lower competition for N and water and promoting grapevine development, as in the case of *Hordeum murinum* (Figure 17).

The use of the legume, *Trifolium subterraneum,* as a cover crop (every other row) increased the soil N content during the summer and increased the YAN content of Chasselas grapes at harvest in Switzerland (Spring, 2001). Consequently, AF was faster and the wines were significantly preferred (better aroma and mouthfeel, lower bitterness), in comparison to a mix of perennial and competitive grasses, such as *Festuca rubra, Festuca ovina, Poa pratensis, Poa compressa*, which reduced soil N availability (Spring, 2002). However, these results contradict those of Bouzas-Cid *et al.* (2018a) obtained from the cultivar, Mencia, under humid conditions in Spain. Depending on the environmental conditions, an adapted cover crop could be a sustainable solution for soil management and an option for modulating must composition and wine sensory profiles.

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4. Vine balance

Vine balance is a common term used to express the balance between the vegetative growth and reproductive development of a plant. A balanced vine has the appropriate capacity for producing fully ripened grapes, while building nutrient reserves for the following year (Howell, 2001; Lakso and Sacks, 2009). To reach this balance, both canopy size and crop load have to be controlled. Clingeleffer (2009) highlighted a trend over the last century towards lower planting density, larger canopy size and higher crop load per vine. Larger trellis systems have been created to accommodate the larger number of shoots (*e.g.*, Geneva double curtain).

4.1. Canopy management

Grapevine trellising and canopy management (*i.e.*, pruning, defoliation and hedge trimming) affect plant growth, fruit zone microclimate and consequently fruit composition (Azuma *et al.*, 2012). It can also affect N nutrition. Rühl and Clingeleffer (1993) observed that N accumulation in roots and wood can vary from 88 to 139 kg/ha, depending on the pruning system, with spur-pruning resulting in higher N accumulation than minimal-pruning. An ideal canopy maximises light interception and guarantees a non-limiting source of carbohydrates for the grapes through optimum photosynthesis activity. An abundance of carbohydrates contributes to non-limited N assimilation in leaves and roots. Light exposure enhances N reductase activity in leaves (Perez and Kliewer, 1982). A large canopy also guarantees adequate refilling of root N reserves, mainly in the form of AAs, in prevision for the following year (Zufferey *et al.*, 2015; Verdenal *et al.*, 2016a). Furthermore, an ideal canopy creates an optimal bunch microclimate, favouring the formation of secondary metabolites, such as phenolic compounds (Keller, 2015).

Plant N content and vigour are usually correlated (Verdenal *et al.*, 2020). An oversized canopy can, however, induce fruit N deficiency uncoupled from plant vigour (*i.e.*, due to improper canopy management), despite unlimited N resources for the plant (Spring *et al.*, 2012). A strong negative correlation between grape N concentration and canopy trimming height has been shown for Chasselas and Pinot noir, despite unchanged fruit load, as if the N content were 'diluted' within the volume of the biomass (Spring *et al.*, 2012). Verdenal *et al.* (2016a) observed that an oversized canopy (+31 % DW) induced a decrease in grape YAN concentration of up to 53 %. This situation can occur in vigorous grapevines in the absence of water restriction, and can strongly affect grape YAN concentration. Conversely, researchers found that a smaller canopy (due to either severe pruning, shorter height or removal of lateral shoots) induced higher grape YAN concentration, but then full ripeness was difficult to attain in unfavourable years due to restricted carbon supply (Weyand and Schultz, 2006; Spring *et al.*, 2012).

Leaf removal in the bunch area induces better light penetration through the canopy, thus increasing bunch exposure and promoting grape ripening. Early defoliation reduces methoxypyrazine accumulation in the grape (Ryona *et al.*, 2008; Serra-Stepke, 2010). Correlations between natural bunch exposure variability and the development of aromas is generally weaker than in situations in which differences are induced through imposed treatments, such as leaf removal (Meyers *et al.*, 2013). Kwasniewski *et al.* (2010) showed that the timing of leaf removal also had an impact on C_{13}-norisoprenoids in resulting wines. However, no constant relationship with grape N content could be highlighted across years and cultivars (Verdenal *et al.*, 2019).

4.2. Fruit load regulation

Bunch thinning (*i.e.*, crop load limitation by removing a proportion of fruits early in the season) is a worldwide practice for enhancing fruit maturation. Several studies have reported the influence of fruit load on C partitioning (Chaves, 1984; Morinaga *et al.*, 2003; Dai *et al.*, 2011; Dayer *et al.*, 2017), but it is still unclear how fruit load influences grape N accumulation and composition. Under high yield conditions, grape AAs originate in the leaves (Rossouw *et al.*, 2017). Root N reserves also play a major role in balancing grape N content. Root N accumulation in reserves is restricted by the presence of fruit before and after veraison (Rodriguez-Lovelle and Gaudillère, 2002; Rossouw *et al.*, 2017). In response to a higher fruit load, vines extract more C and N from reserves mainly located in the storage organs, to match the demand of the maturing fruits (Howell, 2001). Overproduction can potentially induce a significant reduction in N reserves in the long term, which may affect vigour, bud fruitfulness and even plant sustainability. As compensation, N uptake is generally higher under high-yielding conditions (Treeby and Wheatley, 2006). The modulation of both reserve N mobilisation and N uptake contributes to a relatively constant grape N concentration, despite a large crop load variation (Verdenal *et al.*, 2020). Grape AA profile has been found to change despite unchanged overall concentration, with yield conditions affecting certain AAs more than others (Figure 18) (Verdenal *et al.*, 2020). Several authors have confirmed changes in volatile compounds in response to bunch thinning (Rutan *et al.*, 2018; Wang *et al.*, 2019). Lin *et al.* (2018)

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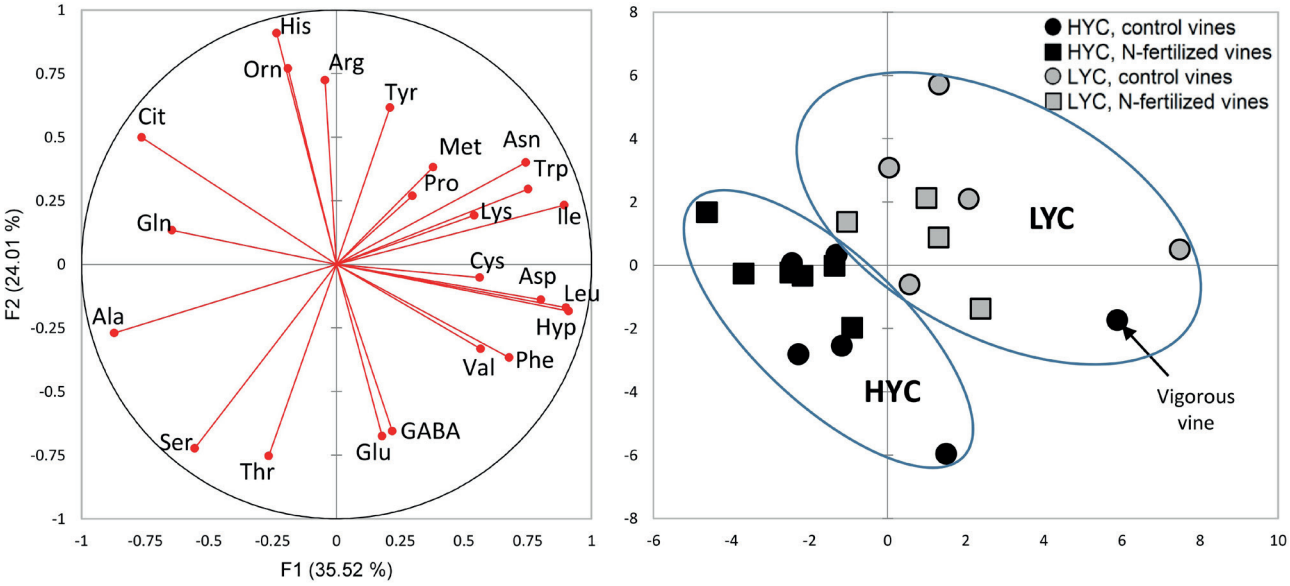


FIGURE 18. Impact of crop load on must AA composition. Principal component analysis (PCA) of must AA profiles (AA proportions in %) at harvest.

Black = high-yielding conditions (HYC, n = 12); grey = low-yielding conditions (LYC, n = 9); circles = control vines (n = 11); squares = N-fertilized vines (n = 10). The PCA discriminates the vines under HYC from those under LYC, independently of the fertilisation treatment. Chasselas, 2017, Pully, Switzerland (from Verdenal *et al*., 2020).

observed differential expressions of AA decarboxylase in relation to fruit load; *i.e.*, the enzyme regulating the concentration of aroma-active 2-phenylethanol. Based on this result, they further recommended a yield range at harvest for the cultivar Vidal for optimum aroma expression.

4.3. Leaf-to-fruit ratio

Production is at a maximum when the supply of resources equals or exceeds plant demand (Lawlor, 2002). In fact, several studies have shown an inconsistent impact of bunch thinning on fruit composition, highlighting the prevailing role of the leaf-to-fruit ratio (Jackson and Lombard, 1993; Keller *et al.*, 2005; Parker *et al.*, 2014; Parker *et al.,* 2015; Verdenal *et al.*, 2016b; Mawdsley *et al.*, 2018; Wang *et al.*, 2018). Indeed, bunch thinning may not alter the leaf area-to-fruit weight ratio enough to overcome carbon supply limitations (Reeve *et al.*, 2018). Howell (2001) wrote a detailed review on the growth-to-yield relationship for sustainable viticulture. Vine balance is usually understood in terms of the principles of vine C balance (Howell, 2001). It has been found that maintaining a sufficient leaf area-to-fruit weight ratio (above 1 m^2 of exposed leaf area per kg of fruit) promotes grape development and maturation by providing a non-limiting source of photosynthetic carbohydrates (Kliewer and Dokoozlian, 2005; Zufferey *et al.*, 2015; Gutiérrez-Gamboa *et al.*, 2019). Vine balance may also be expressed using the Ravaz index (*i.e.*, the fruit-to-pruning wood ratio) as the wood quantity is closely related to the leaf area (Howell, 2001). To summarise, under cool-climate conditions, a leaf-to-fruit ratio of 1.0 to 1.2 m^2/kg is recommended to promote both grape maturity and must YAN accumulation, while the root N reserve is replenished, which guarantees sustainability (Murisier and Zufferey, 1997; Verdenal *et al.*, 2016a).

4.4. Root restriction

Root restriction is an efficient method for controlling nutrient uptake and plant vigour, as it impacts both root development and activity. Root development can be limited by either root-zone limitation, partial root-zone drying or root pruning. Yang *et al.* (2007) studied the impact of root restriction on nitrate uptake kinetics using two pot sizes (2 and 12 L); they observed that root-zone limitation efficiently inhibited shoot and root development, while decreasing the amount of net N uptake. Root-zone limitation has further consequences on ascorbic acid and carotenoid pathways, among others, in plant metabolism (Leng *et al.*, 2017). Partial root-zone drying due to localised irrigation (50 % evapotranspiration) was found to limit both root development and canopy development, in comparison to both full irrigation (100 % evapotranspiration) and deficit irrigation (50 % evapotranspiration) (Santos *et al.*, 2005). Root pruning is a common practice in fruit production for limiting vigour; this practice affects the size of the root N reserve. Root

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pruning performed on grapevine after bud burst was shown to reduce both pruning weight (-8 %), petiole N content (-11 %) and must YAN content (-13 %) (Giese *et al*., 2015). However, the long-term impact of these practices on grapevine physiology is still unknown.

5. Vineyard inputs

5.1. Irrigation

Under limited water conditions, vine growers may irrigate their vineyards. Depending on the water constraint, quantity of water applied and timing of application, irrigation may influence soil N availability and plant N uptake, with further consequences on plant vigour and grape ripening (Keller, 2005; White *et al.*, 2007; Iandolino and Williams, 2014; Ortega-Heras *et al.*, 2014). Bouzas-Cid *et al.* (2018b) observed only minor variations in must AA concentration following irrigation treatments. However, their trial involved only a null to mild water restriction (average stem water-potential -0.63 MPa). The method of irrigation also influences N uptake. Drip versus furrow irrigation methods were compared in a trial (Williams, 2015). Plant N uptake was increased by only 12 % for furrow irrigation conditions, in comparison to 40 % for drip irrigation.

The amount of water the vine receives (from both rainfall and irrigation) and its temporal distribution affect the quality of red and white wines differently. For instance, deficit irrigation can be applied along with limited N supply to control vegetative development, yield and fruit composition (Keller, 2005). Zufferey *et al.* (2017, 2018) observed that the absence of water deficit negatively affects the quality of red wines (cv. Pinot noir), while it slightly enhances the quality of white wines (cv. Chasselas). Moreover, moderate water restriction is desirable when growing red grape (White *et al.*, 2007). Pinot noir wines produced from vines under moderate water restriction had a higher concentration of sugars, polyphenols and anthocyanins; they were thus found to be full-bodied, and to have better mouthfeel and higher-quality tannins (Zufferey *et al.*, 2017; Kotsaki *et al.*, 2020b). Conversely, irrigated Chasselas wines (no water restriction) were mostly preferred for their better mouthfeel and lower bitterness (Zufferey *et al.*, 2018). Moderate water restriction enhances grape maturation (Zufferey *et al.*, 2017), while it can also simultaneously induce lower N content in the plant and in must. Accumulations of C and N in grapes follow different pathways: under water restriction, non-structural reserve carbohydrate are remobilised, contributing to berry sugar accumulation, while fruit N accumulation can be affected due to lower N availability (Rossouw *et al.*, 2017; Zufferey *et al.*, 2018). However, it is not easy to separate the effect of water and N restriction in these trials.

5.2. Fertilisation

N fertilisation is an efficient practice for manipulating grape must composition, particularly in terms of pH, malic acid and potassium (Rühl *et al.*, 1992). N fertilisation purposely enhances N availability for the plant and increases N uptake. However, net N uptake from an applied fertiliser is usually as low as 30-40 %, mainly due to surface run-off, leaching or gaseous emissions (Van Cleemput *et al.*, 2008; Williams, 2015). Fertilisation efficiency largely depends on NUE (Porro *et al.*, 2010). The limiting factors for maximising NUE are different at high and low N supply, and NUE is generally higher under low N conditions (Xu *et al.*, 2012). The only consistent effect of vineyard N application on grape metabolites is an increase in total N compounds (Bell and Henschke, 2005). N fertilisation is usually applied to the soil surface between bud burst and flowering, which corresponds to the first period of high root N uptake. As a result, grapevine vegetative development and berry set are generally improved.

Excessive fertilisation is highly detrimental to both grape composition and grape sanitary status and to the environment. The negative impact on grape composition often manifests itself through an excessive increase in vigour. Many studies comparing different levels of N supply have demonstrated the negative consequences of excessive N supply on berry composition (Delas *et al.*, 1991; Hilbert *et al.*, 2003; Schreiner *et al.*, 2014; Soubeyrand *et al.*, 2014). In some cases, N supply was extremely high (*i.e.*, above 100 kg/ha), in which cases, vine vigour was exacerbated, while bud fruitfulness and leaf area increased. Berry set was lower and bunch rot sensitivity increased (both negatively affecting yield in extreme cases). Fruit maturity was delayed; the must at harvest contained less sugar, had higher concentrations of organic acids and a higher pH. Furthermore, it was found that, while progressively reducing the quantity of N supply, vegetative growth will decrease prior to a reduction in fruit load, thus further impacting must YAN (Schreiner *et al.*, 2014). It has been established that excessive N supply also induces lower anthocyanin and tannin content in red grapes, independently from phenylalanine content (Choné *et al.*, 2001; Hilbert *et al.*, 2003; Schreiner *et al.*, 2018). Further investigation is necessary to understand all the mechanisms related to N content and involved in the synthesis of polyphenols. One limiting factor is the higher C quantity required for N assimilation, to the detriment of the flavonoid pathway (Dai *et al.,* 2011;

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Soubeyrand *et al.*, 2018). Another negative factor related to flavonoid metabolism is the resulting excessive vigour of the canopy, which reduces fruit exposure to sunlight due to bunch shading (Stamatiadis *et al.*, 2007; Jackson, 2008). At a molecular level, genes involved in the flavonoid pathway (encoding phenylalanine ammonia-lyase, chalcone synthase, flavonoid30, 50hydroxylase, dihydroflavonol4reductase and leucoanthocyanidin dioxygenase) revealed a lower transcript level in berries under excessive N fertilisation (*i.e.*, 120 kg/ha of N), in comparison to a non-fertilised control treatment (Soubeyrand *et al.*, 2014).

Foliar fertilisation in viticulture has been implemented worldwide. A complete review has summarised the influence of foliar-fertiliser formulations and biostimulants (*i.e.*, elicitors and resistance inducers) on grape composition (Gutiérrez-Gamboa *et al.*, 2019). Amongst them, the application of urea at veraison is the most common, due to its low price and fast uptake by plants. Whether applied alone or with S (which facilitates urea uptake by the leaves), it efficiently increases the concentrations of NH_4^+, AAs, glycosides and glutathione in grapes (Lacroux *et al.*, 2008; Hannam *et al.*, 2016; Gutiérrez-Gamboa *et al.*, 2017a). Portu *et al.* (2015) even found a positive impact on anthocyanin and flavanol content, in opposition to the usual impact of soil N fertilisation. The direct addition of AAs on the canopy (*i.e.*, phenylalanine, proline and arginine) showed a lower efficiency (Garde-Cerdán *et al.*, 2014; Gutiérrez-Gamboa *et al.*, 2017a).

The localisation of fertilisation is also very important. N is usually applied to the soil before flowering. Soil fertilisation inevitably stimulates cover crop development, which consequently competes with the grapevine for access to water and nutrients (Maigre and Aerny, 2001a). The exclusive application of N under the row, instead of to the entire soil surface, significantly increases fertilisation efficiency, inducing lower competition and improved N uptake by the grapevine (Spring, 2003). In terms of foliar application, no differences have been found between applying urea exclusively to the top, bottom or entire canopy (Verdenal *et al.*, 2017). However, the authors recommended spreading urea over the entire canopy to limit the amount of urea per leaf surface unit, and to avoid necrosis symptoms due to a temporary excess of NH_4^+ in the leaves.

The timing of fertilisation can significantly influence the quantity of N uptake and N partitioning in the plant. Conradie (2005) summarises the different periods for optimum fertilisation efficiency, highlighting the impacts of climate, soil and plant genetics. For instance, in warmer countries such as South Africa, the long post-harvest period (several months) is effective for N application, while in cooler countries, little N is absorbed during that period (few weeks only) (Conradie, 1992). The application to soil of 60 kg/ha of N at berry set in N deficient vines was found to increase vigour and grape YAN content, as well as cysteine-conjugated compounds and glutathione, but it decreased phenolic compounds (Choné *et al.*, 2006). N supply was also found to increase grape aroma precursors; volatile thiols in wine were better preserved due to lower phenolic and higher glutathione levels (Choné *et al.*, 2006). Grapes benefit more from a late foliar N application than an application at the flowering stage (Porro *et al.*, 2010; Verdenal *et al.*, 2015). Foliar fertilisation during the period of veraison (in the form of urea) has often been shown to be a reliable and efficient way of increasing YAN concentration in must without affecting grapevine vigour (Nisbet *et al.*, 2014; Hannam *et al.,* 2016; Alem *et al.*, 2019; Gutiérrez-Gamboa *et al.*, 2019). It is particularly recommended for promoting the development of aromas in white and *rosé* wines. The impact of late foliar urea supply also improves the sensory profile of red wine, inducing a lower astringency (Reynard *et al*., 2012; Verdenal *et al.*, 2016c). Conversely, post-harvest N application has a negligible impact on grape YAN concentration in the following season (Holzapfel and Treeby, 2007).

Varying N applications according to vine N status across a vineyard block is an appropriate method of homogenising vine vigour, yield and grape composition. Vigour variations are generally related to vine N status and can be remotely determined using the NDVI. Using the NDVI, Gatti *et al.* (2018) applied three levels of fertilisation in their field trial depending on grapevine vigour and N status. Despite the fact that the NDVI is also related to other factors (*i.e.*, water availability and rootstock vigour), the homogeneity in terms of vigour was significantly increased within four years. This result should encourage further research on this important issue in vineyard management.

5.3. Other inputs

Copper (Cu) is widely used in viticulture, especially in organic production. It is the base component of the Bordeaux mixture used to control downy mildew. Copper formulations have been shown to affect grape AA concentration. Both the Bordeaux mixture and copper hydroxide decreased the content of AAs in grapes, compared to control samples (Garde-Cerdán *et al.*, 2017). Oliva *et al.* (2011) studied the impact of several fungicides (famoxadone, fenhexamid, fluquinconazole, kresoxim-methyl, quinoxyfen and trifloxystrobin) on grape N composition. These

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fungicides induced an overall lower N concentration with different quantitative and qualitative effects on grape AA composition, depending on the fungicide. It is not clear whether the impact of fungicide is due to a lower biosynthesis of AAs, or to a decrease in their precursors (Oliva *et al.*, 2011). Gutiérrez-Gamboa *et al.* (2019) have reviewed several studies, which have experimented on the use of biostimulants on grapevine. While chitosan, laminarin and yeast extracts decreased must AA content, methyl jasmonate, abscisic acid, riboflavin and seaweed extracts had a positive impact on AA accumulation in grape (Ju *et al.*, 2016; Garde-Cerdán *et al.*, 2017; Gutiérrez-Gamboa *et al.*, 2017b; González-Santamaría *et al.*, 2018; Gutiérrez-Gamboa *et al.*, 2020b). This list is not exhaustive, however.

To conclude, vineyard inputs greatly influence N availability for the plant, despite the risks of excessive supply and pollution of the environment. The variability of environmental conditions also play a major role in the efficiency of the input. An integrative view of the vineyard would be conducive to the sustainable optimisation of agronomic practices, in order to minimise the need for external inputs.

CONCLUSION AND PERSPECTIVES

This review emphasises the importance of N in viticulture and winemaking. Mineral N is assimilated into AAs, which are further involved in many metabolic pathways, from protein synthesis to the formation of grape aroma-active compounds. Grape AA content also influences the winemaking process, including both the fermentation kinetics and the development of wine flavours. Vineyard N status management should be based on the knowledge that N excess is as detrimental to wine quality as N depletion. Plant N demand is driven by vegetative development and N removal is related to crop load. While the amount of N exported from the vineyard is quite easy to establish, determining the soil mineral N availability is more complex, as it is influenced by environmental conditions. The influence of both the environment (*i.e.*, climate and soil) and plant genetics creates a myriad of unique situations to which growers must adapt their practices, in order to produce grapes of suitable quantity and quality.

The complexity of the processes involved requires an integrative approach to managing grapevine N nutrition. When necessary, N fertilisation can be carried out on the ground between bud burst and flowering to improve vegetative development, while a foliar application can be realised at veraison stage to enhance grape YAN concentration for winemaking purposes. Taking environmental conditions into account, the grape grower can also adapt plant material, soil management and vine balance to improve NUE and minimise N inputs in the vineyard. Grapevine N balance depends on canopy size, fruit load and annual replenishment of root N reserves. The major role of the roots in vine balance has been highlighted over the past decades, thanks to methods such as isotope labelling. The strong correlation between must YAN concentration and wine quality clearly shows a need for further research. Early assessment of grape N content during the season would help to justify late foliar N application in order to prevent grape YAN deficiency for winemaking. Recent research has shown that grape YAN content is a potential criterion for grape maturity and quality potential. It could also be a selective criterion for grapevine breeding. Further sustainable strategies for high-quality viticulture and wine production include improving plant material and fine-tuning agronomic practices to balance vine N status.

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