Year : 2021

Nitrogen dynamics and fertilization use efficiency in Vitis vinifera: carry-over effects of crop limitation

Verdenal Thibaut

Verdenal Thibaut, 2021, Nitrogen dynamics and fertilization use efficiency in Vitis vinifera: carry-over effects of crop limitation

Originally published at : Thesis, University of Lausanne

Posted at the University of Lausanne Open Archive http://serval.unil.ch
Document URN : urn:nbn:ch:serval-BIB_CCE2095826CD7

2021

Nitrogen dynamics and fertilization use efficiency in *Vitis vinifera*: carry-over effects of crop limitation

Thèse de doctorat

présentée à la Faculté des Géosciences et de l'Environnement
de l'Université de Lausanne

pour obtenir le grade de
Docteur en sciences de l'environnement

par

Thibaut Verdenal

Nitrogen dynamics and fertilization use efficiency in *Vitis vinifera*: carry-over effects of crop limitation

Thèse de doctorat

présentée à la Faculté des Géosciences et de l'Environnement
de l'Université de Lausanne

pour obtenir le grade de
Docteur en sciences de l'environnement

par

Thibaut Verdenal

Jury:

Prof. Johanna Marin-Carbonne, Univ. de Lausanne – Directeur

Prof. Cornelis van Leeuwen, Univ. de Bordeaux – Co-directeur

Dr. Samuel Jaccard, Univ. de Lausanne – Expert interne

Prof. Markus Keller, Washington State Univ. – Expert externe

Dr. Vivian Zufferey, Agroscope – Expert externe

Prof. Christian Kull, Univ. de Lausanne – Président du jury

Lausanne
2021

UNIL | Université de Lausanne
Faculté des géosciences et de l'environnement
bâtiment Géopolis bureau 4631

IMPRIMATUR

Vu le rapport présenté par le jury d'examen, composé de

Président de la séance publique :	M. le Professeur Christian Kull
Président du colloque :	M. le Professeur Christian Kull
Directrice de thèse :	Mme la Professeure Johanna Marin Carbonne
Co-directeur de thèse :	M. le Professeur Cornelis Van Leeuwen
Expert interne :	M. le Professeur Samuel Jaccard
Expert externe :	M. le Professeur Markus Keller
Expert externe :	M. le Docteur Vivian Zufferey

Le Doyen de la Faculté des géosciences et de l'environnement autorise l'impression de la thèse de

Monsieur Thibaut VERDENAL

Titulaire d'un
Diplôme d'ingénieur en agriculture
de l'Ecole supérieure d'Agriculture d'Angers

intitulée

Nitrogen dynamics and fertilization use efficiency in Vitis vinifera: carry-over effects of crop limitation

Lausanne, le 26 août 2021

Pour le Doyen de la Faculté des géosciences et de l'environnement

Professeur Christian Kull

Preface

One day, says the legend, there was a huge forest fire. All the animals were terrified and appalled, and they watched helplessly as the disaster unfolded. Only the little hummingbird was active, fetching a few drops with its beak to throw them on the fire. After a while, the armadillo, annoyed by this ridiculous agitation, said to him, "Hummingbird! You are not crazy? It is not with these drops of water that you will put out the fire!" And the hummingbird replied, "I know that, but I am doing my part."

Told by Pierre Rabhi from a Native American legend

This legend inspires in me a surge of solidarity to act responsibly to preserve our environment. Similar to the hummingbird's agitation, the subject of my thesis may sound insignificant to anyone out of this field, and yet a work of this nature is the result of multiple collaborations. I would not have succeeded alone in this task, and it is my duty and pleasure to acknowledge the significant contributions of the following individuals: Cornelis (Kees) van Leeuwen and Johanna Marin-Carbonne for their enlightened guidance throughout the writing of the thesis; Vivian Zufferey for his wise advice and constant support; Jean-Laurent Spring and Olivier Viret for allowing me to carry out this project in the context of my activity at Agroscope and for their helpful feedback; Ágnes Dienes-Nagy for her expertise in oenology and amino acid manipulation; Jorge E. Spangenberg for his involvement in the project and for sharing his expertise on isotope labelling; the Viticulture and Wine Analysis teams at Agroscope for their support and multiple contributions to this project and, in particular, Philippe Duruz and Laure Passot for their conscientious help in the field tasks; Jonas Siegrist and his team from Sol-Conseil for the supervision of sample drying and grinding; Sylvain Schnee for sharing his freeze dryer; and Christian Kull, Markus Keller, and Samuel Jaccard for their participation in the examining committee of my thesis. I also wish to express my love and gratitude to my wife and daughter, Shweta and Pauline, for their patience, personal sacrifice, and constant support during these four years.

This project was entirely financed by Agroscope. It is a continuation of the viticulture research program conducted at the Agroscope research station in Pully, Switzerland. Understanding the impact of our agricultural practices in relation to the plant and to the environmental conditions allows a better orientation of our technical choices with the aim of sustainability.

Pully, April 2021

Abstract

As an essential element for plant development, nitrogen (N) is used extensively since the twentieth century to increase production, although only 30–40% of the fertilizer is used by the crops. The rest of the fertilizer is usually lost to the environment. It is therefore essential to improve N use efficiency by the plant to minimize our ecological footprint. During wine production, N in grapes is also involved in alcoholic fermentation and in the development of aroma compounds, which both affect the quality of the wine. To prevent N deficiency in grapes, foliar N supply is usually applied at the beginning of fruit ripening in the form of urea. However, there is no universal recipe for optimal results. N metabolism in plants is fundamentally affected by environmental conditions and by our cultural practices, such as soil management, training systems, or vineyard inputs. Understanding the impact of these influencing factors allows us to better orient our technical choices with the objectives of quality and sustainability.

This thesis focuses on a common practice in viticulture, that is, crop limitation. It consists of removing grapes before the beginning of ripening to favor the maturation of the remaining fruits. We evaluated the impact of crop limitation on N distribution in the plant and on the efficiency of fertilization. For this purpose, a wide gradient of fruit load was set up in a homogeneous plot of Chasselas (*Vitis vinifera*), and foliar N was provided as ^{15}N-labelled urea. Isotope labelling identified the N in the fertilizer and provided a dynamic picture of its distribution in the plant over two consecutive years.

The close relationship between fruits and roots in the maintenance of plant N balance was highlighted. Leaf gas exchange rates were reduced in response to lower yield conditions, reducing C assimilation and increasing intrinsic water use efficiency. Fruit N concentration remained unchanged regardless of crop load. Moreover, the fruit amino acid profile varied with crop load, thus potentially affecting fruit aromas. Interestingly, the amino acids most affected by crop load were not the same as those affected by foliar N supply. The presence of fertilizer N in the plant in the following year had no carry-over effect on the plant vigor or grape N composition. Fertilization efficiency greatly varied in relation to crop load, with higher uptake rates under high-yield conditions. A significant amount of N was released by the plant into the soil during the fall and winter and was then assimilated again in the following year. N partitioning depended on both N species and N origin, either from the perennial reserves (mainly amino N) or from the seasonal uptake (mainly nitrate and ammonium). These findings demonstrate the impact of plant balance on fertilization efficiency and will contribute to the improvement of cultural practices in perennial crops.

Résumé

En tant qu'élément essentiel au développement des plantes, l'azote a été utilisé de manière intensive au cours du XX^{e} siècle, afin d'augmenter la production agricole. Cependant, les cultures n'utilisent que 30 à 40% de l'engrais, le reste étant généralement perdu dans l'environnement. Il est donc essentiel d'améliorer l'efficacité de l'utilisation de l'azote par la plante pour minimiser notre empreinte écologique. Lors de la production de vin, l'azote du raisin intervient dans le déroulement de la fermentation alcoolique et dans le développement des arômes du vin. Pour prévenir la carence en azote du raisin, une fertilisation est généralement réalisée au début de la maturation des fruits sous la forme d'urée foliaire. Le métabolisme de l'azote dans les plantes est fondamentalement affecté par les conditions environnementales et par nos pratiques culturales, telles que la gestion du sol, le système de conduite ou la fertilisation. Comprendre l'impact de ces facteurs d'influence permet de mieux orienter nos choix techniques dans un objectif de qualité et de durabilité.

Cette thèse se concentre sur une pratique courante en viticulture : la limitation de la récolte. Elle consiste à enlever des raisins avant la véraison pour favoriser la maturation des fruits restants. Nous avons évalué l'impact de cette pratique sur la distribution de l'azote dans la vigne et sur l'efficacité de la fertilisation. Pour cela, une parcelle homogène de Chasselas (*Vitis vinifera*) a subi un large gradient de charge en fruit et a reçu de l'azote foliaire marquée au ^{15}N. Le marquage isotopique a permis le suivi de la distribution l'azote foliaire dans la plante pendant deux années consécutives.

La relation étroite entre les fruits et les racines dans le maintien de l'équilibre de l'azote dans la plante a été mise en évidence. Les taux d'échange gazeux des feuilles ont été réduits en réponse à la limitation du rendement et l'efficience de l'eau a été améliorée. La concentration en azote des fruits est restée inchangée quelle que soit la charge en fruit. En revanche, le profil des acides aminés dans le moût a varié, ce qui a potentiellement affecté les arômes des fruits. Les acides aminés les plus affectés par la charge en fruit n'étaient pas les mêmes que ceux affectés par la fertilisation. La fertilisation foliaire n'a pas eu d'arrière-effet sur la vigueur ou la composition du moût dans l'année suivante. Le taux d'assimilation de l'azote était moins élevé dans des conditions de bas rendement. Une quantité importante d'azote a été libérée par la plante dans le sol avant la dormance et assimilée à nouveau l'année suivante. La distribution de l'azote dépendait à la fois de son espèce et de son origine, soit des réserves pérennes (principalement acides aminés), soit de l'absorption saisonnière (principalement nitrate et ammonium). Ces résultats démontrent l'impact de l'équilibre des plantes sur l'efficacité de la fertilisation et contribueront à l'amélioration des pratiques agronomiques dans les cultures pérennes.

Table of contents

List of abbreviations

2017-res-N	residual labelled N from 2017 N supply
2018-lab-N	new labelled N from 2018 N supply
2-AAP	2-aminoacetophenone
A	net C assimilation (gas exchange)
A%	absolute abundance (atom percent)
AF	alcoholic fermentation
Atom %	atomic percentage
C	carbon
C_a	ambient CO_2 (gas exchange)
C_i	internal leaf CO_2 (gas exchange)
CO_2	carbon dioxide
CQ	carbon quantity
CT	control treatment with no foliar N supply
Cu	copper
DAP	diammonium phosphate
DOY	day of year
DMS	dimethyl sulphide
DW	dry weight
E	transpiration (gas exchange)
FAA	free amino acid
FW	fresh weight
gsw	stomatal conductance (gas exchange)
H_2O	water
H_2S	hydrogen sulfide
HYC	high-yield conditions
LEA	leaf-exposed area
LYC	low-yield conditions
MLF	malolactic fermentation
N	nitrogen
N_2	dinitrogen
NH_3	ammonia (gas)
NH_4^+	ammonium
NO_2^-	nitrite
NO_3^-	nitrate
NQ	nitrogen quantity
NNP	new nitrogen pool
NUE	nitrogen use efficiency
OM	organic matter
%P	partitioning (percent)
PAN	primary amino nitrogen
RSA	relative specific abundance (atom percent)
S	sulphur
TA	titratable acidity
TLA	total leaf area
TN	total nitrogen
TOC	total organic carbon
total-lab-N	2017 and 2018 labeled nitrogen
TSL	total shoot length
TSS	total soluble sugars
YAN	yeast assimilable nitrogen

Introduction

Our society is constantly evolving, as are agricultural practices. Today, minimizing our ecological footprint is essential for future generations. Managing crop nutrition has become a challenge with respect to the environment, the producer, and the consumer.

Context of the study

Nitrogen (N) is an essential element for plant development and is required in a larger amount than any other nutrient applied to crops. Starting in the 1950s, the Third Agricultural Revolution, also called the Green Revolution, offered new hope with the introduction of technologies such as chemical fertilizers. Consequently, nitrate was extensively used to increase production, despite crops using only 30–40% of the fertilizer (e.g., Masclaux-Daubresse *et al.*, 2010). The remaining fertilizer was usually lost to the environment via leaching, denitrification, surface runoff, gaseous emissions, and microbial consumption (e.g., Kant *et al.*, 2011). The Swiss Federal Office for the Environment (OFEV) reported in 2019 that nitrate concentrations in groundwater exceeded the limit value of 25 mg L^{-1} in almost 15% of the monitoring stations in Switzerland. In areas where arable farming is the main activity, more than 40% of the monitoring stations exceeded that threshold (OFEV, 2019). Most nitrate emissions come from agriculture. Each year in Switzerland, more than 150,000 tons of nitrate are transferred from agricultural land to water, and excess N has remained at a high level since the beginning of the century (OFEV, 2019). The Swiss are sensitive to environmental matters, as illustrated by the referendum of June 13, 2021, entitled “For clean drinking water and healthy food.” Consumers are increasingly demanding products that are from sustainable production, and quality labels have multiplied on retail shelves. Sustainable agriculture combines respect for the environment, the producer, and the consumer. Consequently, agriculture has become a complex exercise consisting of optimizing quality, avoiding pollution, limiting costs, and reaching a production level which is no longer always the highest. As an example, in the past decades, agronomic practices in viticulture significantly evolved toward lower yields, fewer chemicals, and more cover crops. In this context, minimizing the need for N supply through the fine-tuning of cultural practices was found to be fundamental for sustainable agricultural development. Thus, managing plant N nutrition with precision became a challenge for producers.

In grape production (*Vitis vinifera*), N depletion is as detrimental as N excess to yield and fruit composition. N excess exacerbates plant vigor, increases sensitivity to fungal diseases, delays fruit ripening, and decreases phenolic compounds in particular anthocyanins, which are involved in the color

and mouth feeling of red wines. Conversely, N deficiency reduces yields and severely affects the winemaking process. For their growth and development during winemaking, the yeasts in the must assimilate a mixture of nutrients, including N compounds (Bell and Henschke, 2005). During alcoholic fermentation (AF), only primary amino acids (AAs) and ammonium are metabolized by the yeasts, and together they are called yeast assimilable N (YAN). Under YAN deficient conditions, the AF is slowed down and may even stop before the entire transformation of the sugars into alcohol by the yeast. AAs are also involved in the formation of wine flavor compounds and their precursors in grape must. Therefore, the concentration of YAN in grapes at harvest is a determinant for both the AF kinetics and the development of wine aromas. As a short-term solution to prevent grape YAN deficiency at harvest, foliar urea is commonly applied on the leaves at the onset of grape ripening, with the aim of improving fruit N status without either increasing plant vigor or delaying fruit ripening (Xia and Cheng, 2004; Hannam *et al.*, 2016). Grapevine N dynamics – that is, seasonal uptake and release – have been thoroughly studied in the past decades, allowing a good understanding of the plant N requirement (Conradie, 1991; Wermelinger, 1991; Bates *et al.*, 2002; Zapata *et al.*, 2004a; Weyand and Schultz, 2006; Loulakakis *et al.*, 2009; Masclaux-Daubresse *et al.*, 2010; Zufferey *et al.*, 2015; Schreiner, 2016; Holzapfel *et al.*, 2019). Even so, our understanding of the relationship between plant N status and fruit N composition remains incomplete. The concept of N use efficiency (NUE) represents the sum of both assimilation efficiency (uptake and assimilation) and utilization efficiency (allocation and remobilization) (Kant *et al.*, 2011). NUE is largely determined by environmental conditions (i.e., climate and soil), plant material, and management strategies (i.e., plant material genetics, soil management, plant development monitoring, and vineyard inputs) (Porro *et al.*, 2006; Habran *et al.*, 2016; Verdenal *et al.*, 2021). In other words, NUE could be managed to some extent through the optimization of agronomical practices in relation to environmental conditions and seasonal plant needs.

In grape production, the optimum yield is generally not the maximum allowed by the conditions of the vineyard, since overcropping may alter fruit ripening (e.g., changes of sugar and polyphenol accumulations) and subsequently reduce wine quality (Petrie and Clingeleffer, 2006; Rutan *et al.*, 2018). Crop load may be regulated via crop thinning, which consists of removing grapes before the onset of ripening in order to promote the maturation of the remaining fruits. Yet, crop thinning is often applied empirically and does not consistently improve either fruit composition or aroma development (Keller *et al.*, 2005; Mawdsley *et al.*, 2018; Wang *et al.*, 2018; Bubola *et al.*, 2020; Verdenal *et al.*, 2020). Alem *et al.* (2021) further demonstrated that crop thinning generally decreases the quantity of most carbon (C) compounds (i.e., sugars, organic acids, and glycosylated aroma compounds) accumulated in fruits.

Maintaining a balance between both vegetative and reproductive organs prevails over the consideration of the sole crop load to determine the physiological threshold for overcropping (Kliewer and Dokoozlian, 2005; Zufferey *et al.*, 2015). In most studies, the concept of vine balance is especially used in terms of C balance (Howell, 2001), although N balance is also of major importance. Understanding both the C and N dynamics of the plant in relation to the crop load is critical for the development of sustainable fertilization programs. In this context, a new approach is needed to model the impact of crop load on N distribution in the plant, with a particular focus on N accumulation in grapes.

Isotope labelling is a method that allows for the identification of chemical compounds and gives a dynamic picture of their distribution (Deléens *et al.*, 1997). Stable isotopes of a given chemical element have the same number of protons and a different number of neutrons. In nature, heavy isotopes (i.e., with an extra neutron, such as ^{15}N for the element N) are often present in traces, also called natural abundance (e.g., ^{15}N, 0.36 atom % of total N). Conversely to natural abundance, isotope labelling uses a chemical compound (e.g., urea CH_4N_2O) artificially enriched or depleted in a heavy isotope (e.g., 10 atom % ^{15}N). The modification of the heavy-to-light isotope ratio gives a special "fingerprint" to the chemical compound, which can be monitored and quantified in the plant over a period of time. The proportion of heavy-to-light isotopes can be analyzed with high precision using an elemental analysis–isotope ratio mass spectrometer (EA-IRMS). Using this method, a two-year trial was set in the context of this thesis.

Aims and relevance of the thesis

The aim of the thesis was to evaluate the incidence of crop limitation on N distribution in grapevine and N fertilization efficiency, with particular attention paid to the fruit N composition. We investigated the following three hypotheses:

1. A decrease in crop load affects N distribution in grapevine and induces carry-over effects in growth and fruit N composition in the following year.
2. A decrease in crop load decreases fertilization efficiency and the overall plant N uptake.
3. A decrease in crop load increases grape N content and modifies grape N composition.

These hypotheses can be summarized in one research question:

What effects does crop limitation have on grapevine nitrogen dynamics and fertilization use efficiency?

The thesis is structured as follows:

A general introduction presents a general overview of the context and the aim of the thesis. The research question is introduced and the hypotheses are listed.

- Chapter 1 presents a detailed literature review, which was published in 2021 in the journal *OENO One*. It highlights opportunities for growers to manage plant N metabolism through their cultural practices. The role of N in plant metabolism is also described from plant uptake to the formation of grape aromas. Methods to properly assess grapevine N status are addressed.

- Chapter 2 documents the two-year agronomic trial and describes the material and methods in detail, that is, the environmental conditions of the site, the experimental setup, the field measurement methods, the sample preparation, the isotope analysis method, the must analysis methods, and the data treatments.

- Chapter 3 presents the results from the first harvest. After one year under various yield conditions, N partitioning in the plant was assessed at harvest, and the impact of crop limitation on grape N composition was recorded and analyzed. These results were published in 2020 in the journal *Functional Plant Biology* and thus offered new insights into the capacities of perennial plants to modulate both N uptake and N reserve mobilization in response to crop limitation.

- Chapter 4 is a continuation of chapter 3 and focuses on the two-year dynamics of C and N. It also describes the dynamics of fertilizer N in the plant over two years in relation to crop limitation. The results presented have been submitted for publication in 2021 and demonstrate the high potential of crop limitation to control plant N use efficiency. Root development and activity appeared as key factors for understanding plant C and N dynamics.

A general conclusion provides the main conclusions and perspectives of this thesis.

1 Understanding and managing N metabolism: state of the art

Chapter 1 presents a detailed literature review, explaining the grapevine N requirements and how to assess vine N status. The role of N in the plant is described from plant uptake to the formation of grape aromas. The factors influencing plant N metabolism are categorized and explained. This review highlights opportunities for growers to manage plant N metabolism through their cultural practices and was published in 2021 in the journal *OENO One* of the International Viticulture and Enology Society (IVES).

1.1 Resume of the article

N plays a major role in plant metabolism and enters the composition of key metabolites, such as proteins, amino acids, chlorophyll, and DNA. Managing grapevine N nutrition first requires an understanding of plant N metabolism and its factors of variation. The goal of the following review was to compile the current knowledge on grapevine N nutrition, ranging from plant biology to factors related to N regulation. It will contribute to the implementation of sustainable practices in the vineyard.

Managing plant N nutrition is made up of two steps. The first step is to know the plant requirements. Annual grapevine N requirement is mainly related to both N storage in perennial plant parts and N export with grapes at harvest; leaves and pruning woods are usually restored to the ground in winter. N availability is correct when it corresponds to the needs of the plant. N excess usually exacerbates plant vigor, increases sensitivity to fungal diseases, and delays fruit ripening. Conversely, N deficiency reduces yields and affects the development of fruit aromas. Furthermore, in winemaking, the deficiency of yeast assimilable N in grapes may severely affect the fermentation kinetics and the wine flavor potential. Given the importance of N in crop yield and quality, N was intensively applied to crops in the last century, regardless that nearly 60–70% of fertilizer N was lost in the environment, mainly by soil leaching and gaseous emissions. This indicated that plant N monitoring is therefore essential to ensure that N availability matches crop needs. To this end, there are several methods for assessing the N status of plants, each with advantages and drawbacks. Soil analysis is not a good indicator of plant N status, since soil organic N mineralization fluctuates greatly with the environmental conditions over time. More reliable methods directly assess the N status of the plant *per se* (e.g., leaf analysis, chlorophyll index, must analysis). Combining routine N dosage in grapes at harvest and observations of plant physiology (e.g., vigor, leaf color, and bud fruitfulness) is a good practice when assessing plant N status.

The second step in managing plant N nutrition is to gain a comprehensive understanding of the factors of variation. Plant physiology and grape composition depend on the environmental conditions of the vineyard, that is, the climate and soil characteristics. The best soils for viticulture induce both slight water restriction and unconstrained nutrient conditions. Water availability leads to nutrient solubilization and facilitates plant nutrient uptake. By knowing the intrinsic conditions of their vineyard, growers can use the local environmental conditions to their advantage through the modulation of their cultivation practices. Four major factors of influence need to be addressed: 1) plant material genetics has a long-term impact on plant N use efficiency, and a suitable variety–rootstock combination must be selected; 2) soil management has a direct influence on plant root development and must be chosen according to the availability of water and nutrients; 3) vine balance must be adjusted to guarantee the production of fully ripened grapes, while building nutrient reserves for the following year; and 4) vineyard inputs must be kept to a minimum to limit the ecological footprint without compromising the quality of the harvest.

The article is organized in two parts: the first part focuses on N metabolism, with an emphasis on monitoring vine status. The mechanisms of plant N uptake, assimilation, and efflux are discussed. The role of vine N in wine aroma formation is described. The second part provides a complete description of the factors influencing plant N status. Agronomic parameters useful to improve N use efficiency and optimize grape composition, while minimizing the use of fertilizers, are discussed. Prospects for future research are also examined.

1.2 Article published in *OENO One*

Received: 29 June 2020 | Accepted: 3 November 2020 | Published: 4 January 2021
DOI:10.20870/oeno-one.2021.55.1.3866

OENO One
VINE AND WINE OPEN ACCESS JOURNAL

Understanding and managing nitrogen nutrition in grapevine: a review

Thibaut Verdenal[1], Ágnès Dienes-Nagy[1], Jorge E. Spangenberg[2], Vivian Zufferey[1], Jean-Laurent Spring[1], Olivier Viret[3], Johanna Marin-Carbonne[4] and Cornelis van Leeuwen[5]

[1]Agroscope Institute, Avenue Rochettaz 21, 1009 Pully, Switzerland
[2]Institute of Earth Surface Dynamics, Faculty of Geosciences and Environment, University of Lausanne, Switzerland
[3]Direction générale de l'agriculture, de la viticulture et des affaires vétérinaires (DGAV), 1110 Morges, Switzerland
[4]Earth Science Institute, Faculty of Geosciences and Environment, University of Lausanne, Switzerland
[5]EGFV, Univ. Bordeaux, Bordeaux Sciences Agro, Inrae, ISVV, F-33882 Villenave d'Ornon, France

*corresponding author: thibaut.verdenal@agroscope.admin.ch

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

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).

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).

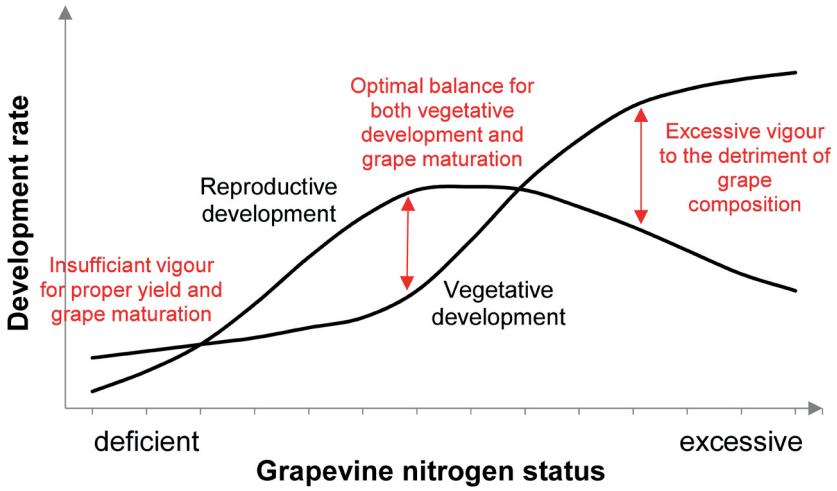


FIGURE 1. Hypothetical model of vegetative versus reproductive development rates as a function of grapevine N status.

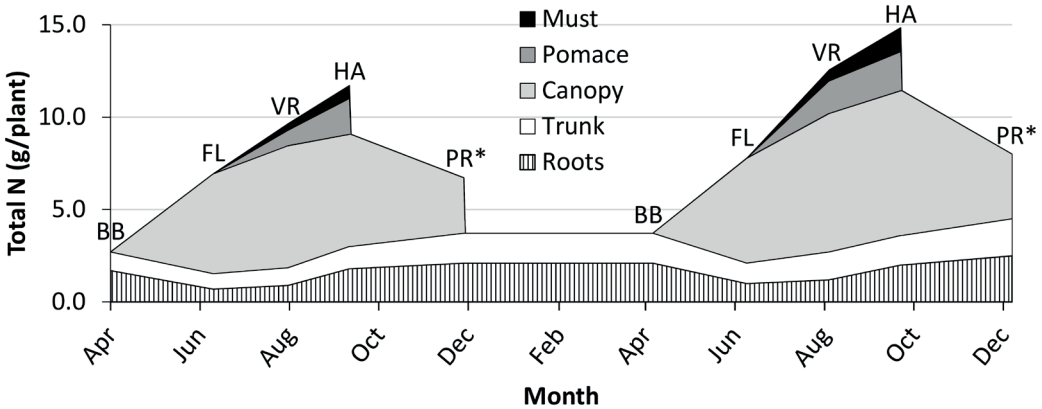


FIGURE 2. Changes in N content of plant parts in grapevines over two growing seasons.

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).

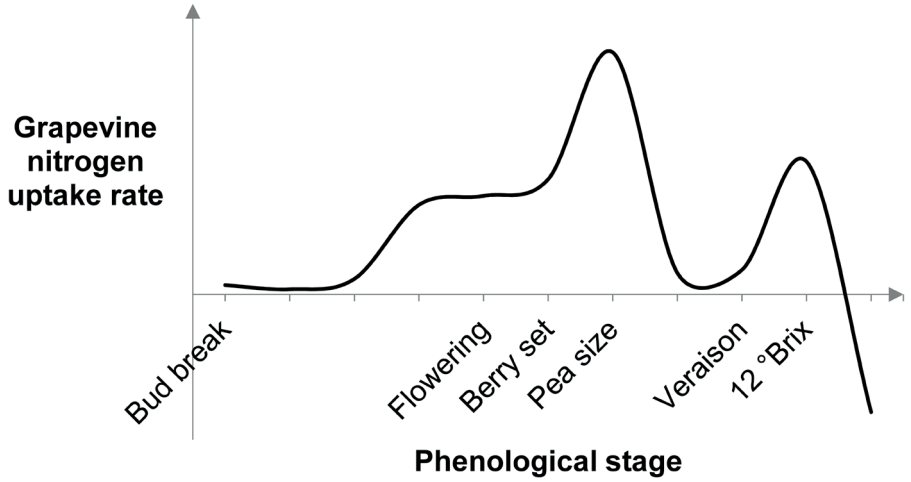


FIGURE 3. Annual evolution of the N uptake rate of grapevine (adapted from Löhnertz, 1988).

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.

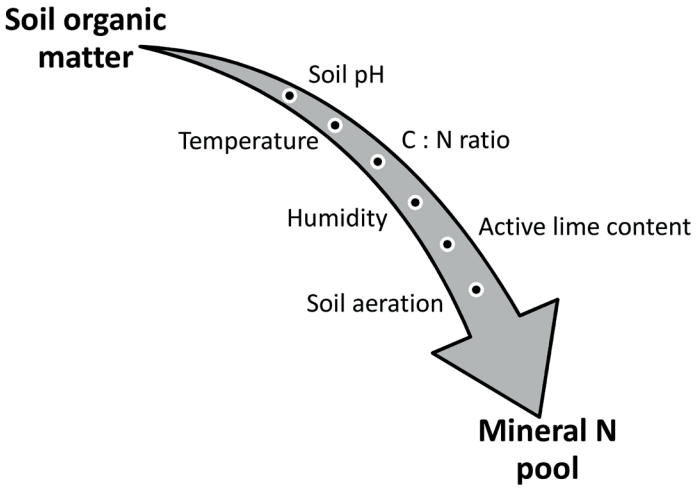


FIGURE 4. Factors influencing the mineralisation of soil organic matter.

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.

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).

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

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

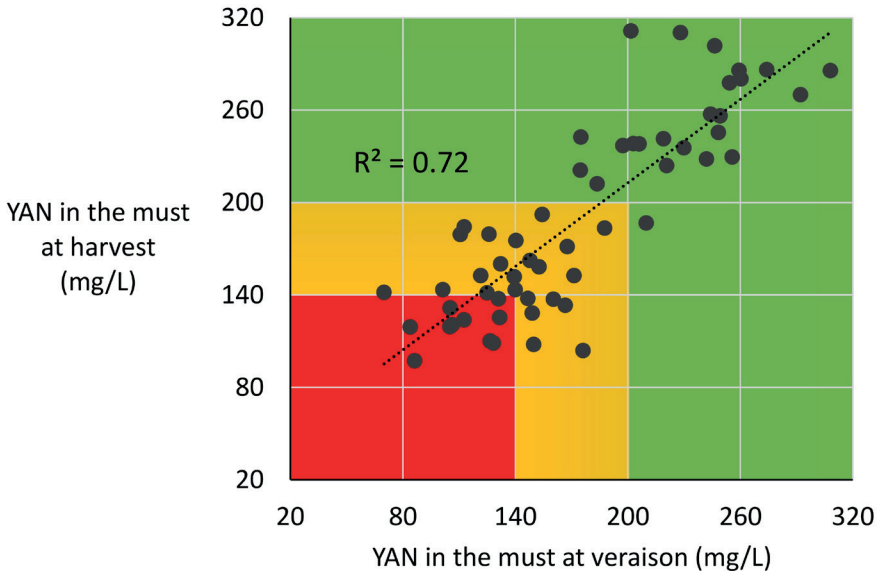


FIGURE 5. Linear regression between the concentrations of YAN at veraison (onset of ripening) and at harvest.
Data collected on Pinot noir from three different vineyards from 1997 to 2012. Risk of incomplete fermentation: green = none; orange = moderate; red = strong (Lorenzini *et al.*, unpublished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

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

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

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

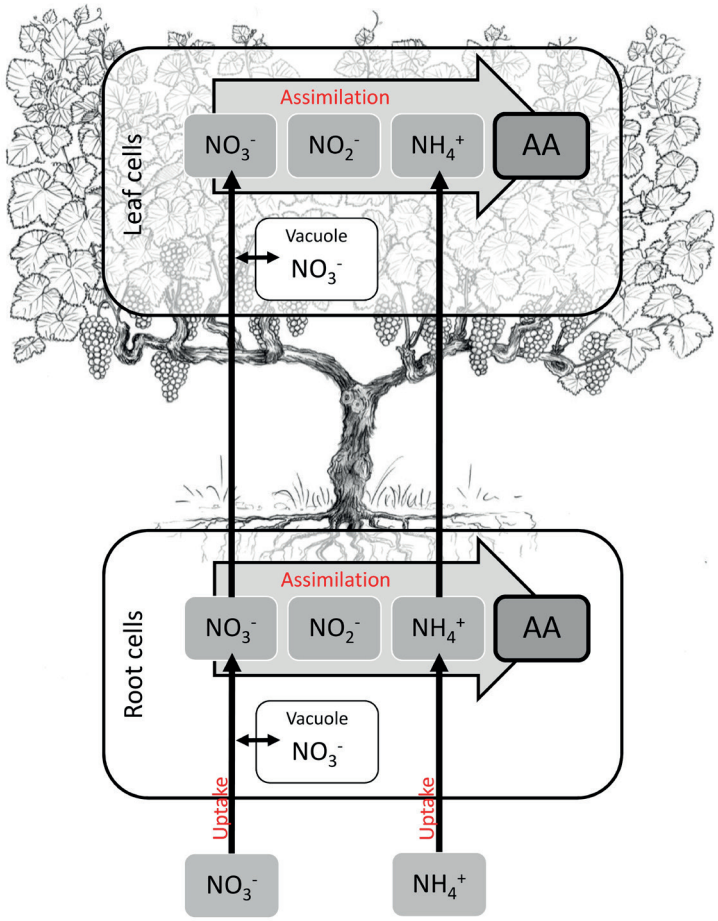


FIGURE 7. N uptake and assimilation in grapevine.
NO_3^-, nitrate; NO_2^-, nitrite; NH_4^+, ammonium; AA, amino acid.

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).

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-)

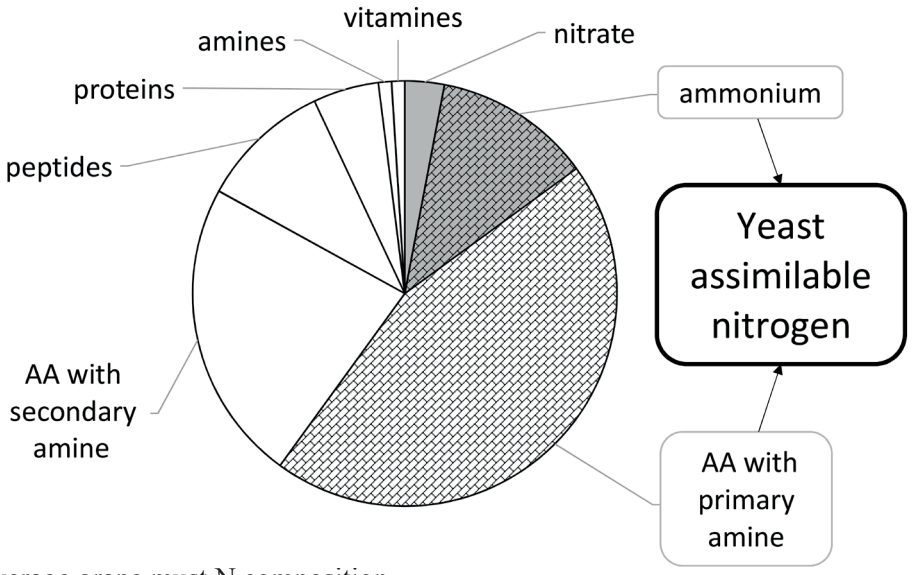


FIGURE 8. Average grape must N composition.
Grey = inorganic N; white = organic N; tiled pattern = yeast assimilable N; AA, amino acid.

(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

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')

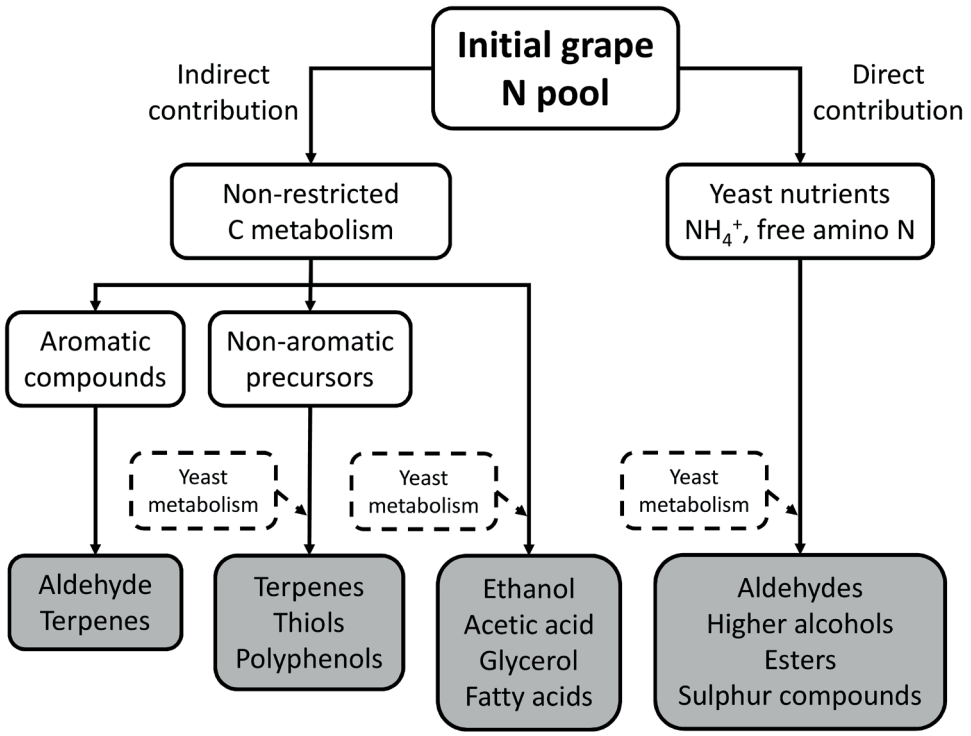


FIGURE 9. Contribution of the grape N pool to the biosynthesis of wine flavour-active compounds (in grey).

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

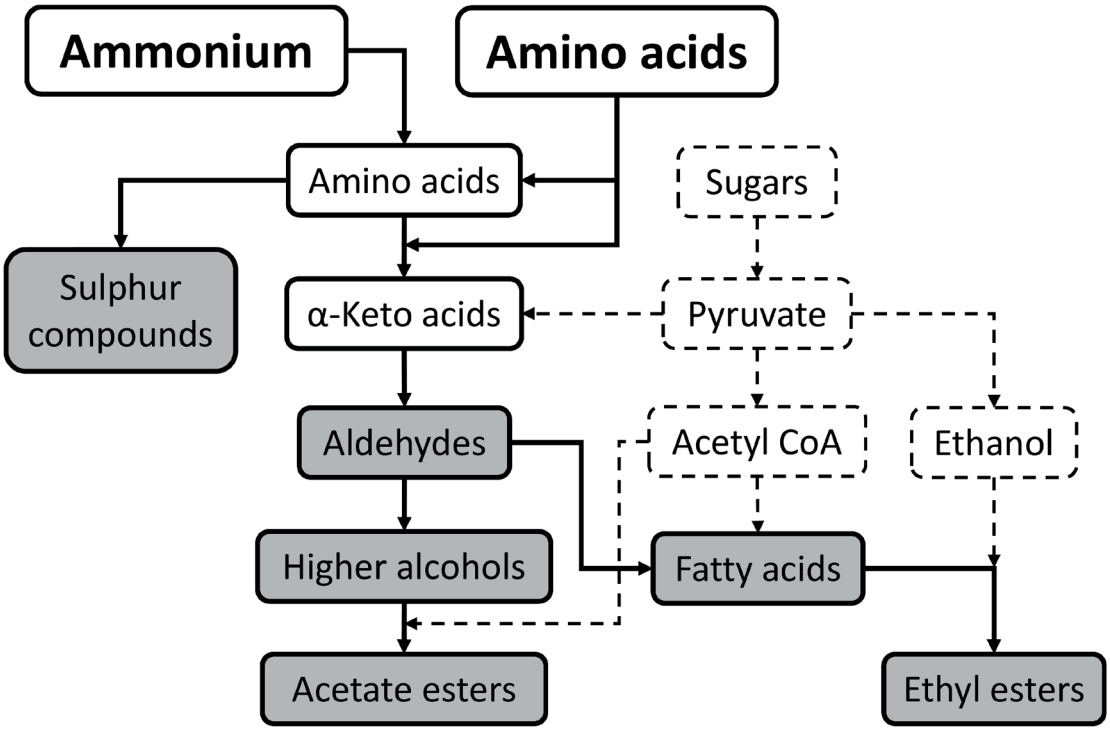


FIGURE 10. Synthesis of aroma-active compounds (in grey) through the yeast metabolism of AAs and ammonium.

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.

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

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

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

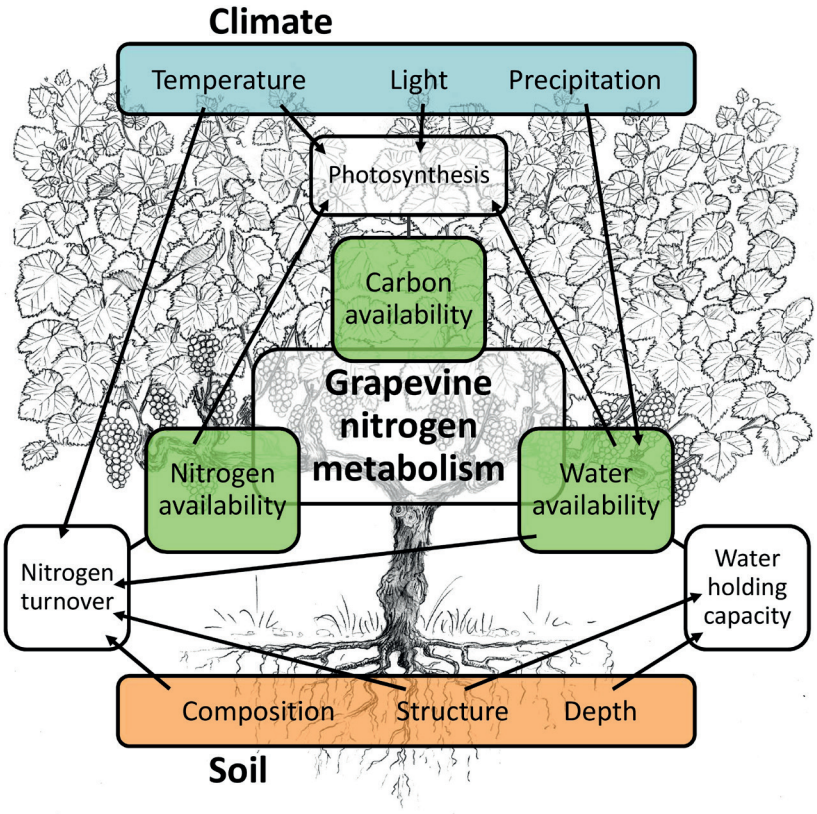


FIGURE 11. Impacts of environmental conditions (i.e., climate and soil) on grapevine N metabolism.

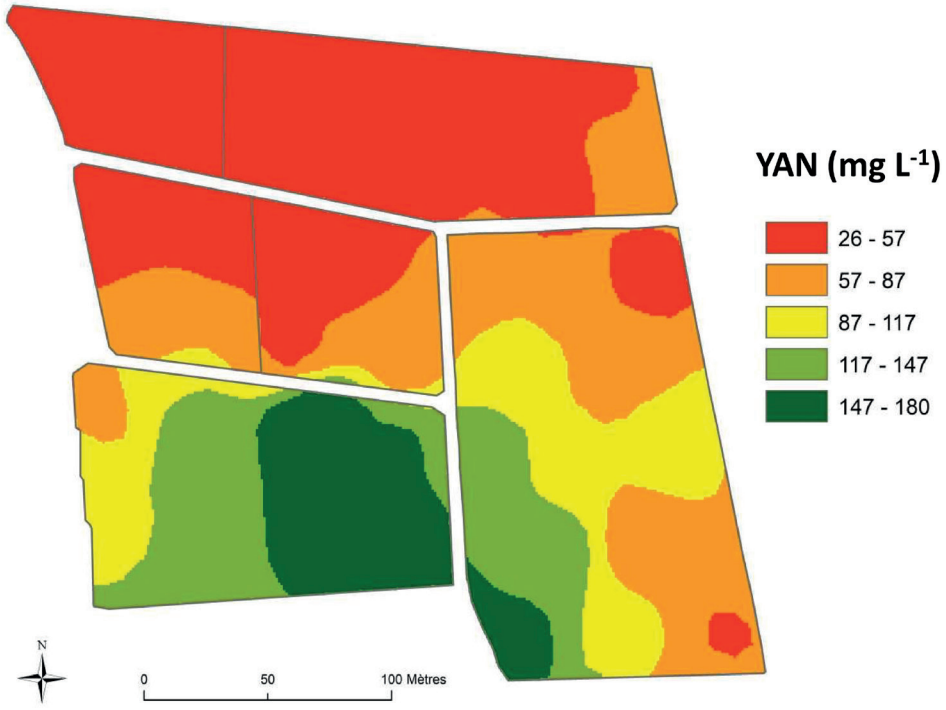


FIGURE 12. Variability of yeast assimilable N in grape must at harvest.
Map obtained by ordinary kriging method based on a regular grid of eight samples per ha. Merlot, 2018, Saint-Julien, Bordeaux, France (van Leeuwen *et al*., unpublished data).

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

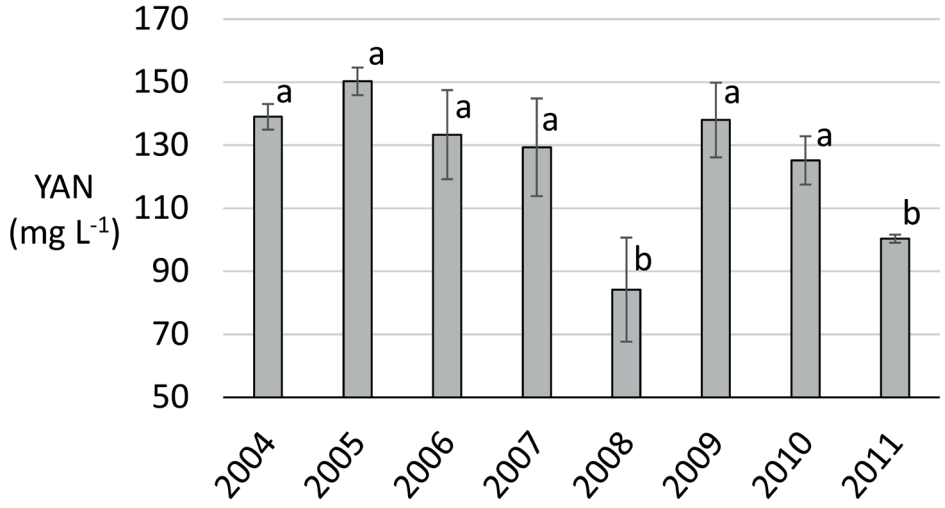


FIGURE 13. Year-to-year variability of YAN in grape must at harvest.

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).

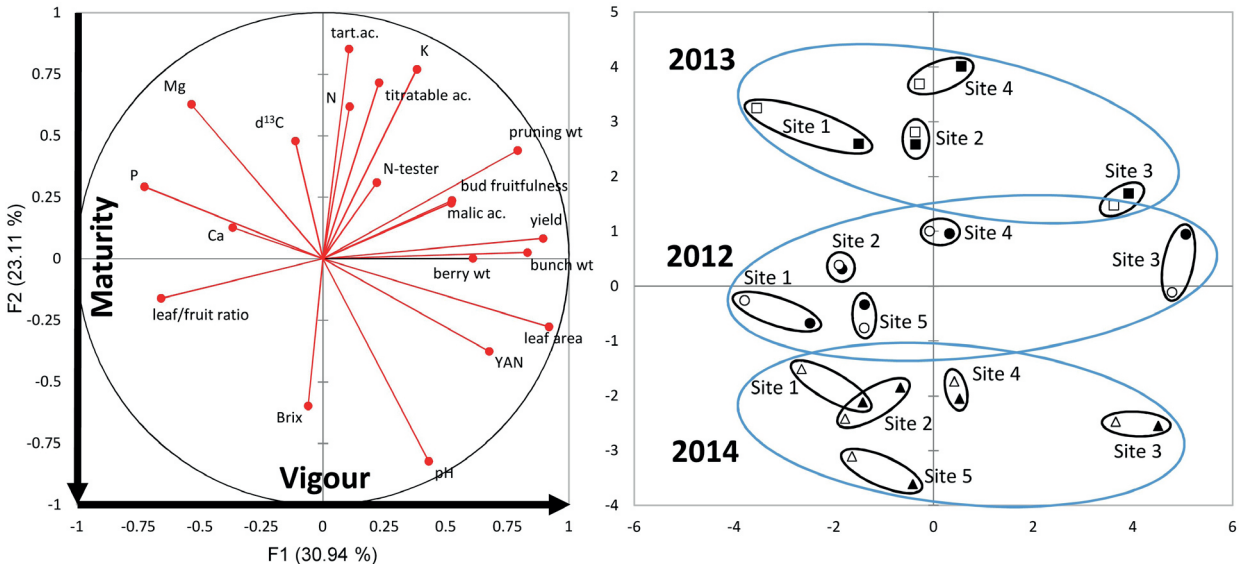


FIGURE 14. Impact of year, site and fertilisation on plant behaviour and must composition of the white cultivar, Doral (Chasselas × Chardonnay) in five vineyards (same plant material and agricultural practices) in a terroir study over three years in Switzerland.

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

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.

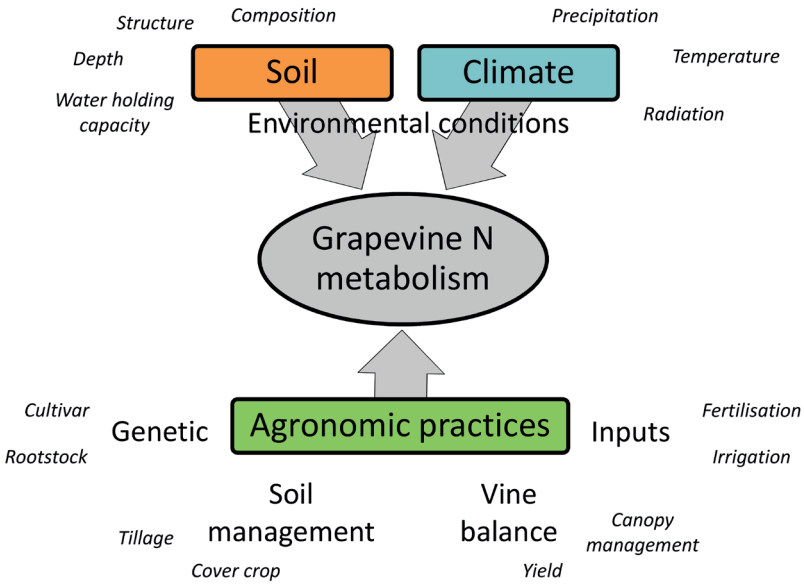


FIGURE 15. Illustration of the terroir concept, showing the influence of climate, soil and agronomic practices on grapevine N metabolism.

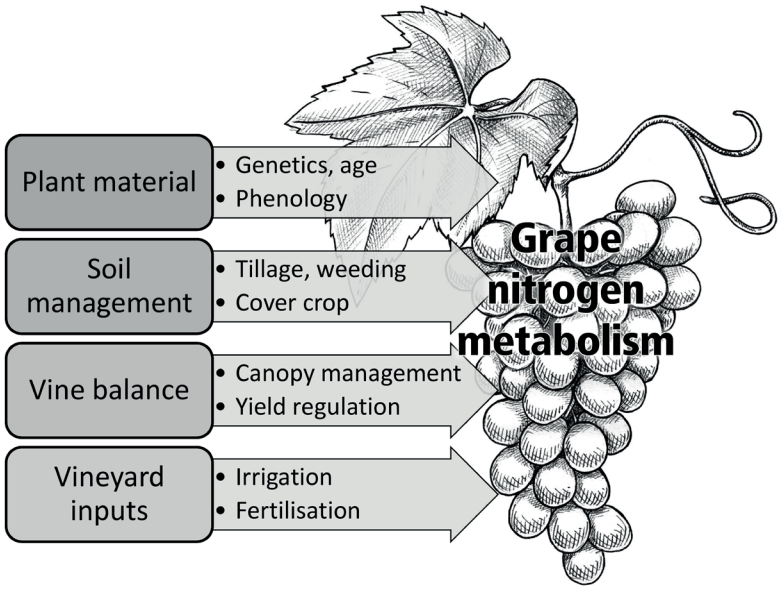


FIGURE 16. Agronomic practices influencing grape N metabolism.

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).

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

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

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.

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)

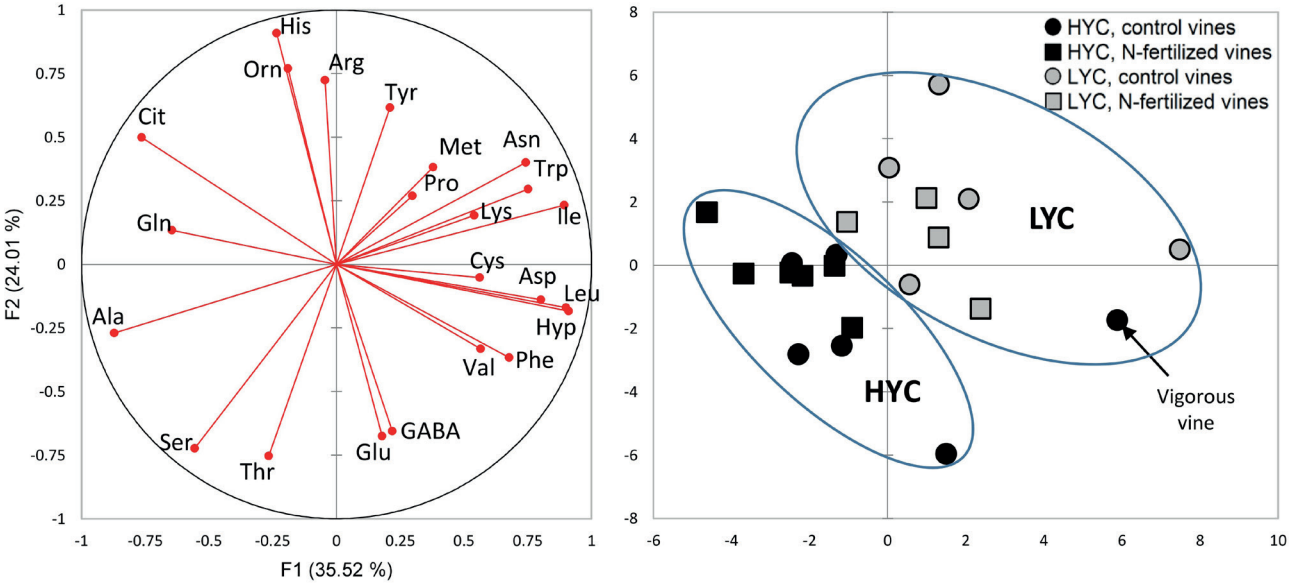


FIGURE 18. Impact of crop load on must AA composition. Principal component analysis (PCA) of must AA profiles (AA proportions in %) at harvest.

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

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;

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

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.

Acknowledgments: The authors would also like to thank Virginie Duquette for her artwork (www.gravir.ch). This research did not receive any specific funding.

REFERENCES

Aerny, J. (1996). Composés azotés des moûts et des vins. *Revue suisse de Viticulture, Arboriculture et Horticulture, 28*(3), 161-165.

Alcaide-Hidalgo, J. M., Moreno-Arribas, M. V., Martín-Álvarez, P. J., & Polo, M. C. (2007). Influence of malolactic fermentation, postfermentative treatments and ageing with lees on nitrogen compounds of red wines. *Food Chemistry, 103*(2), 572-581. doi:10.1016/j.foodchem.2006.09.002

Alem, H., Rigou, P., Schneider, R., Ojeda, H., & Torregrosa, L. (2019). Impact of agronomic practices on grape aroma composition: a review. *Journal of the Science of Food and Agriculture, 99*(3), 975-985. doi:10.1002/jsfa.9327

Aranguren, M., Castellón, A., & Aizpurua, A. (2018). Topdressing nitrogen recommendation in wheat after applying organic manures: the use of field diagnostic tools. *Nutrient Cycling in Agroecosystems, 110*(1), 89-103. doi:10.1007/s10705-017-9865-7

Autralian Wine Research Institute, 2020. Yeast Assimilable Nitrogen. https://www.awri.com.au/industry_support/winemaking_resources/wine_fermentation/yan/ visited on the 24/08/2020.

Azuma, A., Yakushiji, H., Koshita, Y., & Kobayashi, S. (2012). Flavonoid biosynthesis-related genes in grape skin are differentially regulated by temperature and light conditions. *Planta, 236*(4), 1067-1080. doi:10.1007/s00425-012-1650-x

Barbosa, C., Falco, V., Mendes-Faia, A., & Mendes-Ferreira, A. (2009). Nitrogen addition influences formation of aroma compounds, volatile acidity and ethanol in nitrogen deficient media fermented by *Saccharomyces cerevisiae* wine strains. *Journal of Bioscience and Bioengineering, 108*(2), 99-104. https://doi.org/10.1016/j.jbiosc.2009.02.017

Bates, T. R., Dunst, R. M., & Joy, P. (2002). Seasonal dry matter, starch, and nutrient distribution in 'Concord' grapevine roots. *HortSicence, 37*(2), 313-316. doi:10.21273/hortsci.37.2.313

Bell, S.-J., & Henschke, P. A. (2005). Implications of nitrogen nutrition for grapes, fermentation and wine. *Australian Journal of Grape and Wine Research, 11*, 242-295. doi:10.1111/j.1755-0238.2005.tb00028.x

Bisson, L. F., & Butzke, C. E. (2000). Diagnosis and rectification of stuck and sluggish fermentations. *American Journal of Enology and Viticulture, 51*(2), 168-177.

Boss, P. K., Bottcher, C., & Davies, C. (2014). Various Influences of Harvest Date and Fruit Sugar Content on Different Wine Flavor and Aroma Compounds. *American Journal of Enology and Viticulture, 65*(3), 341-353. doi:10.5344/ajev.2014.13137

Bouzas-Cid, Y., Trigo-Córdoba, E., Orriols, I., Falqué, E., & Mirás-Avalos, M. J. (2018a). Influence of soil management on the red grapevine (*Vitis vinifera* L.) Mencía must amino acid composition and wine volatile and sensory profiles in a humid region. *Beverages, 4*, 76. doi:10.3390/beverages4040076

Bouzas-Cid, Y., Díaz-Losada, E., Trigo-Córdoba, E., Falqué, E., Orriols, I., Garde-Cerdán, T., & Mirás-Avalos, J. M. (2018b). Effects of irrigation over three years on the amino acid composition of Albariño (*Vitis vinifera* L) musts and wines in two different terroirs. *Scientia Horticulturae, 227*, 313-325. https://doi.org/10.1016/j.scienta.2017.05.005

Brunetto, G., Ceretta, C. A., de Melo, G. W. B., Girotto, E., Ferreira, P. A. A., Lourenzi, C. R., da Rosa Couto R., Tassinaria, A., Knevitz Hammerschmitt R., da Silva, L. O. S., Lazzaretti B. P., de Souza Kulmann M. S. & Carranca, C., (2016). Contribution of nitrogen from urea applied at different rates and times on grapevine nutrition. *Scientia Horticulturae, 207*, 1-6. doi:10.1016/j.scienta.2016.05.002

Bureau, S. M., Baumes, R. L., & Razungles, A. J. (2000). Effects of vine or bunch shading on the glycosylated flavor precursors in grapes of *Vitis vinifera* L. cv. Syrah. *Journal of Agricultural and Food Chemistry, 48*(4), 1290-1297. doi:10.1021/jf990507x

Cameleyre, M., Lytra, G., Tempere, S., & Barbe, J.-C. (2015). Olfactory impact of higher alcohols on red wine fruity ester aroma expression in model solution. *Journal of Agricultural and Food Chemistry, 63*(44), 9777-9788. doi:10.1021/acs.jafc.5b03489

Celette, F., Findeling, A., & Gary, C. (2009). Competition for nitrogen in an unfertilized intercropping system: The case of an association of grapevine and grass cover in a Mediterranean climate. *European Journal of Agronomy, 30*(1), 41-51. doi:10.1016/j.eja.2008.07.003

Celette, F., & Gary, C. (2013). Dynamics of water and nitrogen stress along the grapevine cycle as affected by cover cropping. *European Journal of Agronomy, 45*, 142-152. https://doi.org/10.1016/j.eja.2012.10.001

Cerovic, Z. G., Ghozlen, N. B., Milhade, C., Obert, M., Debuisson, S., & Moigne, M. L. (2015). Nondestructive diagnostic test for nitrogen nutrition of grapevine (*Vitis vinifera* L.) based on dualex leaf-clip measurements in the field. *Journal of Agricultural and Food Chemistry, 63*(14), 3669-3680. doi:10.1021/acs.jafc.5b00304

Chaves, M. M. (1984). Photosynthesis and assimilate partition in fruiting and non-fruiting grapevine shoots. In C. Sybesma (Ed.), *Advances in photosynthesis research: proceedings of the VIth international congress on photosynthesis, Brussels, Belgium, August 1–6, 1983. Volume IV* (pp. 145-148). Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-94-017-4971-8_32

Choné, X., van Leeuwen, C., Chéry, P., & Ribéreau-Gayon, P. (2001). Terroir influence on water status and nitrogen status of non-irrigated Cabernet sauvignon (*Vitis vinifera* L.). Vegetative development, must and wine compositions (example of a Medoc top estate vineyard, Saint-Julien area, Bordeaux, 1997). *South African Journal of Enology and Viticulture, 22*(1), 8-15. doi:10.21548/22-1-2159

Choné, X., Lavigne-Cruège, V., Tominaga, T., van Leeuwen, C., Castagnède, C., Saucier, C., & Dubourdieu, D. (2006). Effect of vine nitrogen status on grape aromatic potential: falvor precursors (S-cysteine conjugates), glutathione and phenolic content in *Vitis vinifera* L. cv. Sauvignon blanc grape juice. *Journal International des Sciences de la Vigne et du Vin, 40*(1), 1-6. doi:10.20870/oeno-one.2006.40.1.880

Clarke, S. J., Lamont, K. J., Pan, H. Y., Barry, L. A., Hall, A., & Rogiers, S. Y. (2015). Spring root-zone temperature regulates root growth, nutrient uptake and shoot growth dynamics in grapevines. *Australian Journal of Grape and Wine Research, 21*(3), 479-489. doi:10.1111/ajgw.12160

Cliquet, J.-B., Deléens, E., Bousser, A., Martin, M., Lescure, J.-C., Prioul, J.-L., Mariotti, A., & Morot-Gaudry, J.-F., (1990). Estimation of carbon and nitrogen allocation during stalk elongation by ^{13}C and ^{15}N tracing in Zea mays L. *Plant Physiology, 92*(1), 79-87. doi:10.1104/pp.92.1.79

Clingeleffer, P. R. (2009). Influence of canopy management systems on vine productivity and fruit composition. In: *International symposium on grapevine canopy management*, California (Ed. University of California Davis), 13-20.

Conradie, W. J. (1980). Seasonal uptake of nutrients by Chenin blanc in sand culture. *South African Journal of Enology and Viticulture, 1*(1), 59-65. doi:10.21548/1-1-2414

Conradie, W. J. (1983). The uptake and distribution of ^{15}N enriched nitrate by three rootstock cultivars grafted to Chenin blanc. *South African Journal of Enology and Viticulture, 4*(2), 45-47. https://doi.org/10.21548/4-2-2369

Conradie, W. J. (1991). Distribution and translocation of nitrogen absorbed during early summer by two-year-old grapevines grown in sand culture. *American Journal of Enology and Viticulture, 42*(3), 180-190.

Conradie, W. J. (1992). Partitioning of nitrogen in grapevines during autumn and the utilisation of nitrogen reserves during the following growing season. *South African Journal of Enology and Viticulture, 13*(1), 45-51. https://doi.org/10.21548/13-1-2198

Conradie, W. J. (2005). *Partitioning of Mineral Nutrients and Timing of Fertilizer Applications for Optimum Efficiency.* Paper presented at the Soil Environment and Vine Mineral Nutrition, Davis.

Cookson, S. J., Clemente Moreno, M. J., Hevin, C., Nyamba Mendome, L. Z., Delrot, S., Trossat-Magnin, C., & Ollat, N. (2013). Graft union formation in grapevine induces transcriptional changes related to cell wall modification, wounding, hormone signalling, and secondary metabolism. *Journal of Experimental Botany, 64*(10), 2997-3008. doi:10.1093/jxb/ert144

Coplen, T. B. (2011). Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry, 25*(17), 2538-2560. doi:10.1002/rcm.5129

Crépin, L., Truong, N. M., Bloem, A., Sanchez, I., Dequin, S., & Camarasa, C. (2017). Management of Multiple Nitrogen Sources during Wine Fermentation by *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology, 83*(5), e02617-02616. doi:10.1128/aem.02617-16

Crespy, A. (2007). *Manuel pratique de fertilisation, qualité des moûts et des vins* (Oenoplurimedia Ed.).

Czerny, M., Christlbauer, M., Christlbauer, M., Fischer, A., Granvogl, M., Hammer, M., Hartl, C., Moran Hernandez, N., & Schieberle, P. (2008). Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. *European Food Research and Technology, 228*(2), 265-273. doi:10.1007/s00217-008-0931-x

Dai, Z. W., Ollat, N., Gomès, E., Decroocq, S., Tandonnet, J.-P., Bordenave, L., Pieri, P., Hilbert, G., Kappel, C., van Leeuwen C., Vivin, P. & Delrot, S., (2011). Ecophysiological, genetic, and molecular causes of variation in grape berry weight and composition: a review. *American Journal of Enology and Viticulture, 62*(4), 413-425. doi:10.5344/ajev.2011.10116

Dal Santo, S., Fasoli, M., Negri, S., D'Incà, E., Vicenzi, N., Guzzo, F., Tornielli, G. B., Pezzotti, M., & Zenoni, S., (2016). Plasticity of the berry ripening program in a white grape variety. *Frontiers in Plant Science, 7*(970). doi:10.3389/fpls.2016.00970

Dal Santo, S., Zenoni, S., Sandri, M., De Lorenzis, G., Magris, G., De Paoli, E., Di Gaspero, G., Del Fabbro, C., Morgante, M., Brancadoro, L., Grossi D., Fasoli, M.., Zuccolotto P., Tornielli G. B., & Pezzotti, M. (2018). Grapevine field experiments reveal the contribution of genotype, the influence of environment and the effect of their interaction (G×E) on the berry transcriptome. *The Plant Journal, 93*(6), 1143-1159. doi:10.1111/tpj.13834

Dayer, S., Peña, J. P., Gindro, K., Torregrosa, L., Voinesco, F., Martínez, L., Pietro, J. A. & Zufferey, V. (2017). Changes in leaf stomatal conductance, petiole hydraulics and vessel morphology in grapevine (*Vitis vinifera* cv. Chasselas) under different light and irrigation regimes. *Functional Plant Biology, 44*, 679-693. doi:10.1071/FP16041

Delabays, N., Spring, J. L., Ançay, A., Mosimann, E., & Schmid, A. (2000). Sélection d'espèces pour l'enherbement des cultures spéciales. *Revue suisse de Viticulture, Arboriculture et Horticulture, 32*(2), 95-104

de-la-Fuente-Blanco, A., Sáenz-Navajas, M.-P., & Ferreira, V. (2016). On the effects of higher alcohols on red wine aroma. *Food Chemistry, 210*, 107-114. https://doi.org/10.1016/j.foodchem.2016.04.021

Delas, J., Molot, C. & Soyer J.P. (1991). Effects of nitrogen fertilization and grafting on the yield and quality of the crop of *Vitis vinifera* cv. Merlot. International Symposium, Jun 1991, Seattle, United States.

Delas, J. (2010). *Fertilisation de la vigne, 2e edition*. Ed Féret, Bordeaux, 165 p.

Deléens, E., Morot-Gaudry, J.-F., Martin, F., Thoereux, A., & Gojon, A. (1997). Méthodologie ^{15}N. In *Assimilation de l'azote chez les plantes* (pp. 265-280). Paris: Inra.

De Royer Dupré, N., Schneider, R., Payan, J. C., Salançon, E., & Razungles, A. (2014). Effects of vine water status on dimethyl sulfur potential, ammonium, and amino acid contents in Grenache noir grapes (*Vitis vinifera*). *J Agric Food Chem, 62*(13), 2760-2766. doi:10.1021/jf404758g

Dienes-Nagy, Á., Marti, G., Breant, L., Lorenzini, F., Fuchsmann, P., Baumgartner, D., Zufferey, V., Spring, J.-L., Gindro, K., Viret, O., Wolfender, J.-L. & Rösti, J. (2020). Identification of putative chemical markers in white wine (Chasselas) related to nitrogen deficiencies in vineyards. *Oeno One, 54*(3), 583-599. doi:10.20870/oeno-one.2020.54.3.3285

Dunlevy, J. D., Kalua, C. M., Keyzers, R. A., & Boss, P. K. (2009). The Production of Flavour & Aroma Compounds in Grape Berries. *In* K. A. Roubelakis-Angelakis (Ed.), *Grapevine Molecular Physiology & Biotechnology* (pp. 293-340). Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-90-481-2305-6_11

Durante, C., & Bertacchini, L., Bontempo, L., Camin, F., Manzini, D., Lambertini, P., Marchetti, A., & Paolini, M. (2016). From soil to grape and wine: Variation of light and heavy elements isotope ratios. *Food Chemistry, 210*, 648-659. doi:10.1016/j.foodchem.2016.04.108

Echeverría, G., Ferrer, M., & Mirás-Avalos, J. (2017). Quantifying the relative impact of physical and human factors on the viticultural expression of terroir. *International Journal of Environmental & Agriculture Research, 3*(4), 12-25.

Eichert, T. (2013). *Foliar Nutrient Uptake - of Myths and Legends.* Paper presented at the VIIth ISon Mineral Nutrition of Fruit Crops, Bonn, Germany. https://doi.org/10.17660/ActaHortic.2013.984.5

Fairbairn, S., McKinnon, A., Musarurwa, H. T., Ferreira, A. C., & Bauer, F. F. (2017). The impact of single amino acids on growth and volatile aroma production by *Saccharomyces cerevisiae* Strains. *Frontiers in Microbiology, 8*(2554). doi:10.3389/fmicb.2017.02554

Fayolle, E., Follain, S., Marchal, P., Chéry, P., & Colin, F. (2019). Identification of environmental factors controlling wine quality: A case study in Saint-Emilion Grand Cru appellation, France. *Science of The Total Environment, 694*, 133718. https://doi.org/10.1016/j.scitotenv.2019.133718

Fernández, V., & Eichert, T. (2009). Uptake of hydrophilic solutes through plant leaves: current state of knowledge and perspectives of foliar fertilization. *Critical Reviews in Plant Sciences, 28*(1-2), 36-68. doi:10.1080/07352680902743069

Fernández, V., & Brown, P. H. (2013). From plant surface to plant metabolism: the uncertain fate of foliar-applied nutrients. *Frontiers in Plant Science, 4*(289). doi:10.3389/fpls.2013.00289

Fleet, G. H. (1993). *Wine microbiology and biotechnology*. London: Taylor and Francis.

Friedel, M., Hendgen, M., Stoll, M., & Löhnertz, O. (2020). Performance of reflectance indices and of a handheld device for estimating in-field the nitrogen status of grapevine leaves. *Australian Journal of Grape and Wine Research, 26*(2), 110-120. doi:10.1111/ajgw.12424

Gang, Q., Chang, S. X., Lin, G., Zhao, Q., Mao, B., & Zeng, D.-H. (2019). Exogenous and endogenous nitrogen differentially affect the decomposition of fine roots of different diameter classes of Mongolian pine in semi-arid northeast China. *Plant and Soil, 436*(1), 109-122. doi:10.1007/s11104-018-03910-0

Gaudillère, J.-P., Chantelot, E., Soyer, J.-P., Molot, C., & Milin, S. (2003). Leaf and must nitrogen content two complementary indicators of grapevine nitrogen status. *Journal International des Sciences de la Vigne et du Vin, 37*(2), 91-101. doi:10.20870/oeno-one.2003.37.2.943

Garde-Cerdán, T., & Ancín-Azpilicueta, C. (2008). Effect of the addition of different quantities of amino acids to nitrogen-deficient must on the formation of esters, alcohols, and acids during wine alcoholic fermentation. *LWT - Food Science and Technology, 41*(3), 501-510. doi:10.1016/j.lwt.2007.03.018

Garde-Cerdán, T., Lorenzo, C., Lara, J. F., Pardo, F., Ancin-Azpilicueta, C., & Salinas, M. R. (2009). Study of the evolution of nitrogen compounds during grape ripening. application to differentiate grape varieties and cultivated systems. *Journal of Agricultural and Food Chemistry, 57*, 2410-2419. doi:10.1021/jf8037049

Garde-Cerdán, T., Lopez, R., Portu, J., Gonzalez-Arenzana, L., Lopez-Alfaro, I., & Santamaria, P. (2014). Study of the effects of proline, phenylalanine, and urea foliar application to Tempranillo vineyards on grape amino acid content. Comparison with commercial nitrogen fertilisers. *Food Chemistry, 163*, 136-141. doi:10.1016/j.foodchem.2014.04.101

Garde-Cerdán, T., Mancini, V., Carrasco-Quiroz, M., Servili, A., Gutiérrez-Gamboa, G., Foglia, R., Pérez-Álvarez, E. P., & Romanazzi, G. (2017). Chitosan and laminarin as alternatives to copper for plasmopara viticola control: effect on grape amino acid. *Journal of Agricultural and Food Chemistry, 65*(34), 7379-7386. doi:10.1021/acs.jafc.7b02352

Garde-Cerdán, T., Gutiérrez-Gamboa, G., Fernández-Novales, J., Pérez-Álvarez, E. P., & Diago, M. P. (2018). Towards the definition of optimal grape harvest time in Grenache grapevines: Nitrogenous maturity. *Scientia Horticulturae, 239*, 9-16. doi:10.1016/j.scienta.2018.05.014

Gatti, M., Squeri, C., Garavani, A., Vercesi, A., Dosso, P., Diti, I., & Poni, S. (2018). Effects of variable rate nitrogen application on cv. Barbera performance: vegetative growth and leaf nutritional status. *American Journal of Enology and Viticulture, 69*(3), 196-209. doi:10.5344/ajev.2018.17084

Gauthier, P. P. G., Bligny, R., Gout, E., Mahé, A., Nogués, S., Hodges, M., & Tcherkez, G. G. B. (2010). In folio isotopic tracing demonstrates that nitrogen assimilation into glutamate is mostly independent from current CO_2 assimilation in illuminated leaves of Brassica napus. *New Phytologist, 185*(4), 988-999. doi:10.1111/j.1469-8137.2009.03130.x

Giese, W. G., Wolf, T. K., Velasco-Cruz, C., & Roberts, L. (2015). Cover crop and root pruning effects on the rooting pattern of SO4 rootstock grafted to Cabernet-Sauvignon. *American Journal of Enology and Viticulture*. doi:10.5344/ajev.2015.15066

Glad, C., Farineau, J., Regnard, J.-L., & Morot-Gaudry, J.-F. (1994). The relative contribution of nitrogen originating from two seasonal 15N supplies to the total nitrogen pool present in the bleeding sap and in whole *Vinifera* cv. Pinot noir grapevines at bloom time. *American Journal of Enology and Viticulture, 45*(3), 327-332.

Gobert, A., Tourdot-Maréchal, R., Sparrow, C., Morge, C., & Alexandre, H. (2019). Influence of nitrogen status in wine alcoholic fermentation. *Food Microbiology, 83*, 71-85. https://doi.org/10.1016/j.fm.2019.04.008

González-Barreiro, C., Rial-Otero, R., Cancho-Grande, B., & Simal-Gándara, J. (2015).

Wine aroma compounds in grapes: a critical review. *Critical Reviews in Food Science and Nutrition, 55*(2), 202-218. doi:10.1080/10408398.2011.650336

González-Santamaría, R., Ruiz-González, R., Nonell, S., Garde-Cerdán, T., & Pérez-Álvarez, E. P. (2018). Influence of foliar riboflavin applications to vineyard on grape amino acid content. *Food Chemistry, 240*, 601-606. doi:10.1016/j.foodchem.2017.07.115

Gourieroux, A. M., Holzapfel, B. P., Scollary, G. R., McCully, M. E., Canny, M. J., & Rogiers, S. Y. (2016). The amino acid distribution in rachis xylem sap and phloem exudate of *Vitis vinifera* 'Cabernet-Sauvignon' bunches. *Plant Physiology and Biochemistry, 105*, 45-54. doi:10.1016/j.plaphy.2016.04.010

Gouthu, S., Skinkis, P. A., Morre, J., Maier, C. S., & Deluc, L. G. (2012). Berry nitrogen status altered by cover cropping: Effects on berry hormone dynamics, growth and amino acid composition of Pinot noir. *Food Chemistry, 135*(1), 1-8. doi:10.1016/j.foodchem.2012.04.019

Grechi, I., Vivin, P., Hilbert, G., Milin, S., Robert, T., & Gaudillère, J. P. (2007). Effect of light and nitrogen supply on internal C:N balance and control of root-to-shoot biomass allocation in grapevine. *Environmental and experimental Botany, 59*, 139-149. doi:10.1016/j.envexpbot.2005.11.002

Guilpart, N., Metay, A., & Gary, C. (2014). Grapevine bud fertility and number of berries per bunch are determined by water and nitrogen stress around flowering in the previous year. *European Journal of Agronomy, 54*, 9-20. doi:10.1016/j.eja.2013.11.002

Gutiérrez-Gamboa, G., Garde-Cerdán, T., Portu, J., Moreno-Simunovic, Y., & Martínez-Gil, A. M. (2017a). Foliar nitrogen application in Cabernet-Sauvignon vines: Effects on wine flavonoid and amino acid content. *Food Research International, 96*, 46-53. doi:10.1016/j.foodres.2017.03.025

Gutiérrez-Gamboa, G., Portu, J., Santamaría, P., López, R., & Garde-Cerdán, T. (2017b). Effects on grape amino acid concentration through foliar application of three different elicitors. *Food Research International, 99*, 688-692. doi:10.1016/j.foodres.2017.06.022

Gutiérrez-Gamboa, G., Carrasco-Quiroz, M., Martínez-Gil, A. M., Pérez-Álvarez, E. P., Garde-Cerdán, T., & Moreno-Simunovic, Y. (2018). Grape and wine amino acid composition from Carignan noir grapevines growing under rainfed conditions in the Maule Valley, Chile: Effects of location and rootstock. *Food Research International, 105*, 344-352. doi:10.1016/j.foodres.2017.11.021

Gutiérrez-Gamboa, G., Romanazzi, G., Garde-Cerdán, T., & Pérez-Álvarez, E. P. (2019). A review of the use of biostimulants in the vineyard for improved grape and wine quality: effects on prevention of grapevine diseases. *J Sci Food Agric, 99*(3), 1001-1009. doi:10.1002/jsfa.9353

Gutiérrez-Gamboa, G., Garde-Cerdán, T., Rubio-Bretón, P., & Pérez-Álvarez, E. P. (2020a). Seaweed foliar applications at two dosages to Tempranillo blanco (*Vitis vinifera* L.) grapevines in two seasons: Effects on grape and wine volatile composition. *Food Research International, 130*, 108918. doi:10.1016/j.foodres.2019.108918

Gutiérrez-Gamboa, G., Garde-Cerdán, T., Rubio-Bretón, P., & Pérez-Álvarez, E. P. (2020b). Biostimulation to Tempranillo grapevines (*Vitis vinifera* L.) through a brown seaweed during two seasons: Effects on grape juice and wine nitrogen compounds. *Scientia Horticulturae, 264*, 109177. doi:10.1016/j.scienta.2020.109177

Habran, A., Commisso, M., Helwi, P., Hilbert, G., Negri, S., Ollat, N., Gomès, E., van Leeuwen, C., Guzzo, F., & Delrot, S. (2016). Roostocks/scion/nitrogen interactions affect secondary metabolism in the grape berry. *Frontiers in Plant Science, 7*(1134). doi:10.3389/fpls.2016.01134

Hachiya, T., & Sakakibara, H. (2016). Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. *Journal of Experimental Botany, 68*(10), 2501-2512. doi:10.1093/jxb/erw449

Hannam, K. D., Neilsen, G. H., Neilsen, D., Midwood, A. J., Millard, P., Zhang, Z., Thornton, B., & Steinke, D. (2016). Amino acid composition of grape (*Vitis vinifera* L.) juice in response to applications of urea to the soil or foliage. *American Journal of Enology and Viticulture, 67*(1), 47-55. doi:10.5344/ajev.2015.15015

Hardarson, G., Hood-Nowotny, R., Jensen, E. S., Peoples, M. B., Van Cleemput, O., van Kessel, C., … Zapata, F. (2008). Guidelines on nitrogen management in agricultural systems. *International Atomic Energy Agency, Vienna, IAEA-TCS-29*.

Haynes, R. J. (1986). Uptake and assimilation of mineral nitrogen by plants. In R. J. Haynes (Ed.), *Mineral nitrogen in the plant-soil system* (pp. 303-378). Orlando, Florida, USA: Academic Press. https://doi.org/10.1016/B978-0-12-334910-1.50010-8

Helwi, P., Habran, A., Guillaumie, S., Thibon, C., Hilbert, G., Gomès, E., Delrot, S., Darriet, P. & van Leeuwen, C. (2015). Vine nitrogen status does not have a direct impact on 2-methoxy-3-isobutylpyrazine in grape berries and wines. *J Agric Food Chem, 63*(44), 9789-9802. doi:10.1021/acs.jafc.5b03838

Helwi, P., Guillaumie, S., Thibon, C., Keime, C., Habran, A., Hilbert, G., Gomès, E., Darriet, P., Delrot, S., & van Leeuwen, C. (2016). Vine nitrogen status and volatile thiols and their precursors from plot to transcriptome level. *BMC Plant Biology, 16*(1), 173. doi:10.1186/s12870-016-0836-y

Henschke, P. A., & Jiranek, V. (1993). Yeasts – metabolism of nitrogen compounds. *In* G. H. Fleet (Ed.), *Wine microbiology and biotechnology* (pp. 77–164). Chur, Switzerland: Harwood Academic Publishers.

Hernández-Orte, P., Guitart, A., & Cacho, J. (1999). Changes in the concentration of amino acids during the ripening of *Vitis vinifera* Tempranillo variety from the *Denomination d'Origine* Somontano (Spain). *American Journal of Enology and Viticulture, 50*(2), 144-154.

Hernández-Orte, P., Cacho, J. F., & Ferreira, V. (2002). Relationship between varietal amino acid profile of grapes and wine aromatic composition. Experiments with model solutions and chemometric Study. *Journal of Agricultural and Food Chemistry, 50*(10), 2891-2899. doi:10.1021/jf011395o

Hernández-Orte, P., Ibarz, M. J., Cacho, J., & Ferreira, V. (2006). Addition of amino acids to grape juice of the Merlot variety: Effect on amino acid uptake and aroma generation during alcoholic fermentation. *Food Chemistry, 98*(2), 300-310. https://doi.org/10.1016/j.foodchem.2005.05.073

Hjelmeland, A. K., & Ebeler, S. E. (2015). Glycosidically bound volatile aroma compounds in grapes and wine: A Review. *American Journal of Enology and Viticulture, 66*(1), 1-11. doi:10.5344/ajev.2014.14104

Hilbert, G., Soyer, J.-P., Giraudon, J., Milin, S., & Gaudillère, J. P. (2003). Effects of nitrogen supply on must quality and anthocyanin accumulation in berries of cv. Merlot. *Vitis, 42*(2), 69-76.

Hoenicke, K., Simat, T. J., Steinhart, H., Christoph, N., Geßner, M., & Köhler, H.-J. (2002). 'Untypical aging off-flavor' in wine: formation of 2-aminoacetophenone and evaluation of its influencing factors. *Analytica chimica Acta, 458*(1), 29-37. http://dx.doi.org/10.1016/S0003-2670(01)01523-9

Holzapfel, B. P., & Treeby, M. T. (2007). Effects of timing and rate of N supply on leaf nitrogen status, grape yield and juice composition from Shiraz grapevines grafted to one of three different rootstocks. *Australian Journal of Grape and Wine Research, 13*(1), 14-22. doi:10.1111/j.1755-0238.2007.tb00067.x

Holzapfel, B. P., Smith, J. P., & Field, S. K. (2019). Seasonal vine nutrient dynamics and distribution of Shiraz grapevines. *Oeno One, 53*(2). doi:10.20870/oeno-one.2019.53.2.2425

Howell, G. S. (2001). Sustainable grape productivity and the growth-yield relationship: a review. *American Journal of Enology and Viticulture, 52*(3), 165-174.

Huang, Z., & Ough, C. S. (1991). Amino acid profiles of commercial grape juices and wines. *American Journal of Enology and Viticulture, 42*(3), 261-267.

Huglin, P., and Schneider, C., 1998. *Biologie et écologie de la vigne*. Lavoisier Tec. et Doc., Paris.

Hunter, J. J., & Bonnardot, V. (2011). Suitability of some climatic parameters for grapevine cultivation in South Africa, with focus on key physiological processes. *South African Journal of Enology and Viticulture, 32*(1), 18. doi:10.21548/32-1-1374

Iandolino, A. B., & Williams, L. E. (2014). Recovery of 15N labeled fertilizer by *Vitis vinifera* L. cv. Cabernet Sauvignon: effects of N fertilizer rates and applied water amounts. *American Journal of Enology and Viticulture, 65*(2), 189-196. doi:10.5344/ajev.2014.11019

Jackson, D. I., & Lombard, P. B. (1993). Environmental and management practices affecting grape composition and wine quality - a review. *American Journal of Enology and Viticulture, 44*(4), 409-430.

Jackson, R. S. (2008). *Wine Science* (3rd édition ed.). San Diego: Academic Press.

Janzen, H. H., & Bruinsma, Y. (1989). Methodology for the quantification of root and rhizosphere nitrogen dynamics by exposure of shoots to 15N-labelled ammonia. *Soil Biology and Biochemistry, 21*(2), 189-196. https://doi.org/10.1016/0038-0717(89)90094-1

Jing, H., Zhang, P., Li, J., Yao, X., Liu, G., & Wang, G. (2019). Effect of nitrogen addition on the decomposition and release of compounds from fine roots with different diameters: the importance of initial substrate chemistry. *Plant and Soil, 438*(1), 281-296. doi:10.1007/s11104-019-04017-w

Ju, Y.-L., Liu, M., Zhao, H., Meng, J.-F., & Fang, Y.-L. (2016). Effect of exogenous abscisic acid and methyl jasmonate on anthocyanin composition, fatty acids, and volatile compounds of Cabernet-Sauvignon (*Vitis vinifera* L.) grape berries. *Molecules, 21*, 1354. doi:10.3390/molecules21101354

Kant, S., Bi, Y.-M., & Rothstein, S. J. (2011). Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. *Journal of Experimental Botany, 62*(4), 1499-1509. doi:10.1093/jxb/erq297

Keller, M., & Koblet, W. (1995). Dry matter and leaf area partitioning, bud fertility and second season growth of *Vitis vinifera* L.: responses to nitrogen supply and limiting irradiance. *Vitis, 34*(2), 77-83.

Keller, M. (2005). Deficit irrigation and vine mineral nutrition. *American Journal of Enology and Viticulture, 56*(3), 267-283.

Keller, M., Mills, L. J., Wample, R. L., & Spayd, S. E. (2005). Cluster thinning effects on three deficit-irrigated *Vitis vinifera* cultivars. *American Journal of Enology and Viticulture, 56*(2), 91-103.

Keller, M. (2015). *The science of grapevines* (M. Keller Ed. 2nd edition): Elsevier Inc.

Kliewer, W. M., & Dokoozlian, N. (2005). Leaf area/crop weight ratios of grapevines: influence on fruit composition and wine quality. *American Journal of Enology and Viticulture, 56*(2), 170-181.

Klodd, A. E., Eissenstat, D. M., Wolf, T. K., & Centinari, M. (2016). Coping with cover crop competition in mature grapevines. *Plant and Soil, 400*(1), 391-402. doi:10.1007/s11104-015-2748-2

Kotsaki, E., Reynolds, A. G., Brown, R., Lee, H.-S., & Aubie, E. (2020a). Proximal sensing and relationships to soil and vine water status, yield, and berry composition in Ontario vineyards. *American Journal of Enology and Viticulture, 71*(2), 114-131. doi:10.5344/ajev.2019.19018

Kotsaki, E., Reynolds, A. G., Brown, R., Lee, H.-S., & Jollineau, M. (2020b). Spatial variability in soil and vine water status in Ontario vineyards: relationships to yield and berry composition. *American Journal of Enology and Viticulture, 71*(2), 132-148. doi:10.5344/ajev.2019.19019

Krishna, H., Singh, S. K., Sharma, R. R., Khawale, R. N., Grover, M., & Patel, V. B. (2005). Biochemical changes in micropropagated grape (*Vitis vinifera* L.) plantlets due to arbuscular-mycorrhizal fungi (AMF) inoculation during ex vitro acclimatization. *Scientia Horticulturae, 106*(4), 554-567. doi:10.1016/j.scienta.2005.05.009

Krogmeier, M. J., McCarty, G. W., & Bremner, J. M. (1989). Phytotoxicity of foliar-applied urea. *Proceedings of the National Academy of Sciences, 86*(21), 8189-8191. doi:10.1073/pnas.86.21.8189

Kumagai, E., Araki, T., Hamaoka, N., & Ueno, O. (2011). Ammonia emission from rice leaves in relation to photorespiration and genotypic differences in glutamine synthetase activity. *Annals of Botany, 108*(7), 1381-1386. doi:10.1093/aob/mcr245

Kwasniewski, M. T., Vanden Heuvel, J. E., Pan, B. S., & Sacks, G. L. (2010). Timing of cluster light environment manipulation during grape development affects C13 norisoprenoid and carotenoid concentrations in Riesling. *Journal of Agricultural and Food Chemistry, 58*(11), 6841-6849. doi:10.1021/jf904555p

Lacroux, F., Tregoat, O., van Leeuwen, C., Pons, A., Tominaga, T., Lavigne-Cruège, V., & Dubourdieu, D. (2008). Effect of foliar nitrogen and sulfur application on aromatic expression of *Vitis vinifera* L. cv. Sauvignon blanc. *Journal International des Sciences de la Vigne et du Vin, 42*(3), 125-132. doi:10.20870/oeno-one.2008.42.3.816

Lakso, A. and Sacks, G. (2009). Vine balance: what is it and how does it change over the season? In: *International symposium on grapevine canopy management, California* (Ed. University of California Davis), 21-25.

Lambrechts, M., & Pretorius, I. (2000). Yeast and its importance to wine aroma-a review. *South African Journal of Enology and Viticulture, 21*(1), 97-129. doi:10.21548/21-1-3560

Lasa, B., Menendez, S., Sagastizabal, K., Calleja-Cervantes, M., Irigoyen, I., Muro, J., Aparicio-Tejo, P., & Ariz, I. (2012). Foliar application of urea to "Sauvignon blanc" and "Merlot" vines: doses and time of application. *Plant Growth Regulation, 67*(1), 73-81. doi:10.1007/s10725-012-9667-5

Lawlor, D. W. (2002). Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *Journal of Experimental Botany, 53*(370), 773-787. doi:10.1093/jexbot/53.370.773

Lea, P. J., & Azevedo, R. A. (2006). Nitrogen use efficiency. 1. Uptake of nitrogen from the soil. *Annals of Applied Biology, 149*(3), 243-247. doi:10.1111/j.1744-7348.2006.00101.x

Leng, F., Tang, D., Lin, Q., Cao, J., Wu, D., Wang, S., & Sun, C. (2017). Transcriptomic analyses of ascorbic acid and carotenoid metabolites influenced by root restriction during grape berry development and ripening. *Journal of Agricultural and Food Chemistry, 65*(9), 2008-2016. doi:10.1021/acs.jafc.6b05322

Li, Q., Li, B.-H., Kronzucker, H. J., & Shi, W.-M. (2010). Root growth inhibition by NH_4^+ in Arabidopsis is mediated by the root tip and is linked to NH_4^+ efflux and GMPase activity. *Plant, Cell & Environment, 33*(9), 1529-1542. doi:10.1111/j.1365-3040.2010.02162.x

Lilly, M., Bauer, F. F., Styger, G., Lambrechts, M. G., & Pretorius, I. S. (2006). The effect of increased branched-chain amino acid transaminase activity in yeast on the production of higher alcohols and on the flavour profiles of wine and distillates. *FEMS Yeast Research, 6*(5), 726-743. doi:10.1111/j.1567-1364.2006.00057.x

Lin, Y., Jiang, C., Guo, Y., Li, K., Liu, Z., Lin, Z., Li, X., Yue, G., Fu, Q., Li, W., Zheng, J., Dou, Y., & Guo, X. (2018). Relationship between 2-phenylethanol content and differential expression of L-amino acid decarboxylases (AADC) in (*Vitis vinifera*) vidal wine grape at different loads. *Pakistan Journal of Botany, 50*(2), 661-666.

Linsenmeier, A., Rauhut, D., Kürbel, H., & Schubert, S. (2007). Untypical ageing off-flavour and masking effects due to long-term nitrogen fertilization. *Vitis-Geilweilerhof, 46*(1), 33-38.

Linsenmeier, A. W., Loos, U., & Löhnertz, O. (2008). Must composition and nitrogen uptake in a long-term trial as affected by timing of nitrogen fertilization in a cool-climate Riesling vineyard. *American Journal of Enology and Viticulture, 59*(3), 255-264.

Llorens, N., Arola, L., Bladé, C., & Mas, A. (2002). Nitrogen metabolism in a grapevine in vitro system. *Oeno One, 36*(3), 157-159. doi:10.20870/oeno-one.2002.36.3.968

Löhnertz, O. (1988). *Untersuchungen zum zeitlichen Verlauf der Nährstoffaufnahme bei Vitis vinifera (cv. Riesling).* Geisenheim, Universität Giessen, 228 p.

Longbottom, M. (2009). *Managing grapevine nutrition in a changing environment*: The Australian Wine Research Institute Ltd., p.144

Loulakakis, K. A., Morot-Gaudry, J. F., Velanis, C. N., Skopelitis, D. S., Moschou, P. N., Hirel, B., & Roubelakis-Angelakis, K. A. (2009). Advancements in nitrogen metabolism in grapevine. *In* K. A. Roubelakis-Angelakis (Ed.), *Grapevine Molecular Physiology & Biotechnology* (pp. 161-205). Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-90-481-2305-6_7

Lytra, G., Tempère, S., Zhang, S., Marchand, S., de Revel, G., & Barbe, J.-C. (2014). Olfactory impact of dimethyl sulfide on red wine fruity esters aroma expression in model solution. *Oeno One, 48*(1), 75-85. doi:10.20870/oeno-one.2014.48.1.1660

Lytra, G., Tempère, S., Marchand, S., de Revel, G., & Barbe, J.-C. (2016). How do esters and dimethyl sulphide concentrations affect fruity aroma perception of red wine? Demonstration by dynamic sensory profile evaluation. *Food Chemistry, 194*, 196-200. doi:10.1016/j.foodchem.2015.07.143

Maigre, D., & Aerny, J. (2001a). Enherbement permanent et fumure azotée sur cv. Gamay dans le Valais central. 1. Résultats agronomiques. *Revue suisse de Viticulture, Arboriculture et Horticulture, 33*(3), 145-150.

Maigre, D., & Aerny, J. (2001b). Enherbement et fumure azotée sur cv. Gamay dans le Valais central. 2. Résultats analytiques et organoleptiques. *Revue suisse de Viticulture, Arboriculture et Horticulture, 33*(4), 199-204.

Marais, J., Calitz, F., & Haasbroek, P. D. (2001). Relationship between microclimatic data, aroma component concentrations and wine quality parameters in the prediction of Sauvignon blanc wine quality. *South African Journal of Enology and Viticulture, 22*(1), 22. doi:10.21548/22-1-2161

Martínez-Gil, A. M., Garde-Cerdán, T., Lorenzo, C., Félix Lara, J., Pardo, F., & Rosario Salinas, M. (2012). Volatile compounds formation in alcoholic fermentation from grapes collected at 2 maturation stages: influence of nitrogen compounds and grape variety. *Journal of food science, 77*(1), C71-C79. doi:10.1111/j.1750-3841.2011.02441.x

Martínez-Moreno, R., Morales, P., Gonzalez, R., Mas, A., & Beltran, G. (2012). Biomass production and alcoholic fermentation performance of *Saccharomyces cerevisiae* as a function of nitrogen source. *FEMS Yeast Research, 12*(4), 477-485. doi:10.1111/j.1567-1364.2012.00802.x

Marschner, P., & Rengel, Z. (2012). Chapter 12 - Nutrient availability in soils. *In* P. Marschner (Ed.), *Marschner's Mineral Nutrition of Higher Plants (Third Edition)* (pp. 315-330). San Diego: Academic Press. doi:10.1016/B978-0-12-384905-2.00012-1

Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., & Suzuki, A. (2010). Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. *Annals of Botany, 105*(7), 1141-1157. doi:10.1093/aob/mcq028

Mawdsley, P. F. W., Dodson Peterson, J. C., & Casassa, L. F. (2018). Agronomical and Chemical Effects of the Timing of Cluster Thinning on Pinot Noir (Clone 115) Grapes and Wines. *Fermentation, 4*(3), 60. doi:10.3390/fermentation4030060

Mengel, K., & Pilbeam, D. J. (1992). Nitrogen metabolism of plants. *In: Proceedings of the phytochemical society of Europe* 33 New York: Oxford University Press.

Merbach, W., Mirus, E., Knof, G., Remus, R., Ruppel, S., Russow, R., Gransee, A., & Schulze, J. (1999). Release of carbon and nitrogen compounds by plant roots and their possible ecological importance⁺. *Journal of Plant Nutrition and Soil Science, 162*(4), 373-383.

Metay, A. l., Magnier, J., Guilpart, N., & Christophe, A. l. (2014). Nitrogen supply controls vegetative growth, biomass and nitrogen allocation for grapevine (cv. Shiraz) grown in pots. *Functional Plant Biology, 42*(1), 105. doi:10.1071/fp14062

Meyers, J. M., Sacks, G. L., & Vanden Heuvel J. E. (2013). Glycosylated aroma compound responses in 'Riesling' wine grapes to cluster exposure and vine yield. 23(5), 581. doi:10.21273/horttech.23.5.581

Miller, G. H. (2019). *Whiskey science*. Cham, Switzerland: Springer, 529 p. doi:10.1007/978-3-030-13732-8

Molina, J.-A. E., & Smith, P. (1997). Modeling carbon and nitrogen processes in soils. *In* D. L. Sparks (Ed.), *Advances in Agronomy* (Vol. 62, pp. 253-298): Academic Press. doi:10.1016/S0065-2113(08)60570-2

Morinaga, K., Imai, S., Yakushiji, H., & Koshita, Y. (2003). Effects of fruit load on partitioning of ^{15}N and ^{13}C, respiration, and growth of grapevine roots at different fruit stages. *Scientia Horticulturae, 97*, 239-253. doi:10.1016/S0304-4238(02)00199-1

Morot-Gaudry, J.-F., Moreau, F., Prat, R., Maurel, C., & Sentenac, H. (2017). *Biologie végétale: Nutrition et métabolisme-3e éd*: Dunod.

Murisier, F., & Zufferey, V. (1997). Rapport feuille-fruit de la vigne et qualité du raisin. *Revue suisse de Viticulture, Arboriculture et Horticulture, 29*(6), 355-362.

Nisbet, M. A., Martinson, T. E., & Mansfield, A. K. (2014). Accumulation and prediction of yeast assimilable nitrogen in New York winegrape cultivars. *American Journal of Enology and Viticulture, 65*(3), 325-332. doi:10.5344/ajev.2014.13130

Nykänen, L. (1986). Formation and occurrence of flavor compounds in wine and distilled alcoholic beverages. *American Journal of Enology and Viticulture, 37*(1), 84-96.

Oliva, J., Garde-Cerdán, T., Martínez-Gil, A. M., Rosario Salinas, M., & Barba, A. (2011). Fungicide effects on ammonium and amino acids of Monastrell grapes. *Food Chemistry, 129*(4), 1676-1680. doi:10.1016/j.foodchem.2011.06.030

Ong, P. K. C., & Acree, T. E. (1999). Similarities in the aroma chemistry of Gewürztraminer variety wines and lychee (Litchi chinesis Sonn.) fruit. *Journal of Agricultural and Food Chemistry, 47*(2), 665-670. doi:10.1021/jf980452j

Ortega-Heras, M., Pérez-Magariño, S., Del-Villar-Garrachón, V., González-Huerta, C., Moro Gonzalez, L. C., Guadarrama Rodríguez, A., . . . Martín de la Helguera, S. (2014). Study of the effect of vintage, maturity degree, and irrigation on the amino acid and biogenic amine content of a white wine from the Verdejo variety. *Journal of the Science of Food and Agriculture, 94*(10), 2073-2082. doi:10.1002/jsfa.6526

Paolini, M., Ziller, L., Bertoldi, D., Bontempo, L., Larcher, R., Nicolini, G., & Camin, F. (2016). $\delta^{15}N$ from soil to wine in bulk samples and proline. *Journal of Mass Spectrometry, 51*(9), 668-674. doi:10.1002/jms.3824

Parker, A. K., Hofmann, R. W., van Leeuwen, C., McLachlan, A. R. G., & Trought, M. C. T. (2014). Leaf area to fruit mass ratio determines the time of veraison in Sauvignon blanc and Pinot noir grapevines. *Australian Journal of Grape and Wine Research, 20*(3), 422-431. doi:10.1111/ajgw.12092

Parker, A. K., Hofmann, R. W., van Leeuwen, C., McLachlan, A. R. G., & Trought, M. C. T. (2015). Manipulating the leaf area to fruit mass ratio alters the synchrony of total soluble solids accumulation and titratable acidity of grape berries. *Australian Journal of Grape and Wine Research, 21*(2), 266-276. doi:10.1111/ajgw.12132

Parker, A. K., García de Cortázar-Atauri, I., Gény, L., Spring, J.-L., Destrac, A., Schultz, H., Molitor, D., Lacombe, T., Graça, A., Monamy, C., Stoll M., Storchi, P., Trought, M. C. T., Hofmann, R. W. & van Leeuwen, C. (2020). Temperature-based grapevine sugar ripeness modelling for a wide range of *Vitis vinifera* L. cultivars. *Agricultural and Forest Meteorology, 285-286*, 107902. https://doi.org/10.1016/j.agrformet.2020.107902

Petrie, P. R., & Clingeleffer, P. R. (2006). Crop thinning (hand versus mechanical), grape maturity and anthocyanin concentration: outcomes from irrigated Cabernet-Sauvignon (*Vitis vinifera* L.) in a warm climate. *Australian Journal of Grape and Wine Research, 12*(1), 21-29. doi:10.1111/j.1755-0238.2006.tb00040.x

Poni, S., Gatti, M., Palliotti, A., Dai, Z., Duchêne, E., Truong, T.-T., Ferrara, G., Stella Matarrese A. M. , Gallotta A., Bellincontro A., Mencarelli, F., & Tombesi, S. (2018). Grapevine quality: A multiple choice issue. *Scientia Horticulturae, 234*, 445-462. doi:10.1016/j.scienta.2017.12.035

Portu, J., Gonzalez-Arenzana, L., Hermosin-Gutierrez, I., Santamaria, P., & Garde-Cerdan, T. (2015). Phenylalanine and urea foliar applications to grapevine: effect on wine phenolic content. *Food Chemistry, 180*, 55-63. doi:10.1016/j.foodchem.2015.02.008

Porro, D., Dorigatti, C., Zatelli, A., Ramponi, M., Stefanini, M., & Policarpo, M. (2007). Partitioning of dry matter in grapevines during a season: estimation of nutrient requirement. *Proceedings of the XV International Symposium "GESCO 2007"*, Poreč – Croatia, Vol. 1: 403-411.

Porro, D., Stefanini, M., & Dorigatti, C. (2010). Nitrogen Foliar Uptake and Partitioning in "Cabernet Sauvignon" Gravevines. *Proceedings of the Proc. VIth IS on Mineral Nutrition*, Faro, Portugal. https://doi.org/10.17660/ActaHortic.2010.868.21

Rapp, A., & Mandery, H. (1986). Wine aroma. *Experientia, 42*(8), 873-884. doi:10.1007/bf01941764

Rapp, A., & Versini, G. (1995). Influence of nitrogen compounds in grapes on aroma compounds of wines. In C. George (Ed.), *Developments in Food Science* (Vol. 37, pp. 1659-1694): Elsevier. https://doi.org/10.1016/S0167-4501(06)80257-8

Reddy, M. M., & Ulaganathan, K. (2015). Nitrogen nutrition, its regulation and biotechnological approaches to improve crop productivity. *American Journal of Plant Sciences, 6*, 2745-2798. doi:10.4236/ajps.2015.618275

Reeve, A. L., Skinkis, P. A., Vance, A. J., Lee, J., & Tarara, J. M. (2016). Vineyard floor management influences 'Pinot noir' vine growth and productivity more than cluster thinning. *HortScience, 51*(10), 1233-1244. doi:10.21273/hortsci10998-16

Reeve, A. L., Skinkis, P. A., Vance, A. J., McLaughlin, K. R., Tomasino, E., Lee, J., & Tarara, J. M. (2018). Vineyard floor management and cluster thinning inconsistently affect 'Pinot noir' crop load, berry composition and wine quality. *HortScience, 53*(3), 318-328. doi:10.21273/hortsci12682-17

Reining, E., Merbach, W., & Knof, G. (1995). ^{15}N Distribution in wheat and chemical fractionation of root-borne ^{15}N in the soil. *Isotopes in Environmental and Health Studies, 31*(3-4), 345-349. doi:10.1080/10256019508036280

Reynard, J. S., Zufferey, V., Nicol, G. C., & Murisier, F. (2011). Soil parameters impact the vine-fruit-wine continuum by altering vine nitrogen status. *Journal International des Sciences de la Vigne et du Vin, 45*(4), 211-221. https://doi.org/10.20870/oeno-one.2011.45.4.1502

Reynard, J. S., Zufferey, V., Nicol, G. C., Pythoud, K., Aleid-Germanier, L., & Murisier, F. (2012). Adaptation du Gamaret aux terroirs viticoles vaudois. *Revue suisse de Viticulture, Arboriculture et Horticulture, 44*(5), 290-296.

Reynolds, A. G. (2010). Viticultural and vineyard management practices and their effects on grape and wine quality. In A. G. Reynolds (Ed.), *Managing Wine Quality* (pp. 365-444): Woodhead Publishing. https://doi.org/10.1533/9781845699284.3.365

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., & Lonvaud, A. (2017). *Traité d'œnologie* (7e edition ed. Vol. tome 1). Paris: Dunod.

Robinson, A. L., Boss, P. K., Solomon, P. S., Trengove, R. D., Heymann, H., & Ebeler, S. E. (2014). Origins of grape and wine aroma. Part 1. Chemical components and viticultural impacts. *American Journal of Enology and Viticulture, 65*(1), 1-24. doi:10.5344/ajev.2013.12070

Rodriguez-Lovelle, B., & Gaudillère, J. P. (2002). Carbon and nitrogen partitioning in either fruiting or non-fruiting grapevines: effects of nitrogen limitation before and after veraison. *Australian Journal of Grape and Wine Research, 8*(86), 86-94. doi:10.1111/j.1755-0238.2002.tb00216.x

Rollero, S., Mouret, J.-R., Sanchez, I., Camarasa, C., Ortiz-Julien, A., Sablayrolles, J.-M., & Dequin, S. (2016). Key role of lipid management in nitrogen and aroma metabolism in an evolved wine yeast strain. *Microbial Cell Factories, 15*(1), 32. doi:10.1186/s12934-016-0434-6

Rossouw, G. C., Smith, J. P., Barril, C., Deloire, A., & Holzapfel, B. P. (2017). Implications of the presence of maturing fruit on carbohydrate and nitrogen distribution in grapevines under postveraison water constraints. *Journal of the American Society for Horticultural Science, 142*(2), 71-84. doi:10.21273/JASHS03982-16

Roubelakis-Angelakis, K. A., & Kliewer, W. M. (1992). Nitrogen metabolims in grapevine. *In Horticultural reviews* (Vol. 14, pp. 407-452): John Wiley & Sons, Inc. https://doi.org/10.1002/9780470650523.ch9

Rühl, E. H., & Clingeleffer, P. R. (1993). Effect of minimal pruning and virus inoculation on the carbohydrate and nitrogen accumulation in Cabernet franc vines. *American Journal of Enology and Viticulture, 44*(1), 81-85.

Rühl, E., Fuda, A., & Treeby, M. (1992). Effect of potassium, magnesium and nitrogen supply on grape juice composition of Riesling, Chardonnay and Cabernet-Sauvignon [Victoria] vines. *Australian Journal of Experimental Agriculture, 32*(645-649). https://doi.org/10.1071/EA9920645

Rutan, T. E., Herbst-Johnstone, M., & Kilmartin, P. A. (2018). Effect of cluster thinning *Vitis vinifera* cv. Pinot noir on wine volatile and phenolic composition. *Journal of Agricultural and Food Chemistry, 66*(38), 10053-10066. doi:10.1021/acs.jafc.8b04062

Ryona, I., Pan, B. S., Intrigliolo, D. S., Lakso, A. N., & Sacks, G. L. (2008). Effects of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (*Vitis vinifera* L. Cv. Cabernet Franc). *Journal of Agricultural and Food Chemistry, 56*(22), 10838-10846. doi:10.1021/jf801877y

Sakakibara, H., Takei, K., & Hirose, N. (2006). Interactions between nitrogen and cytokinin in the regulation of metabolism and development. *Trends in Plant Science, 11*(9), 440-448. doi:10.1016/j.tplants.2006.07.004

Santamaría, P., Lopez, R., Portu, J., Gonzalez-Arenzana, L., Lopez-Alfaro, I., & Garde-Cerdan, T. (2015). Role of phenylalanine in viticulture and enology. *In:* Elliamson D (ed) *Phenylalanine. Dietary, sources, functions and health effects.* Nova Publisher, New York, pp 49–70

Santesteban, L., Miranda, C., Barbarin, I., & Royo, J. (2014). Application of the measurement of the natural abundance of stable isotopes in viticulture: a review. *Australian Journal of Grape and Wine Research, 21*(2), 157-167. doi:10.1111/ajgw.12124

Santos, T. P., Lopes, C. M., Rodrigues, M. L., De Souza, C. R., Ricardo-da-Silva, J. M., Maroco, J. P., Pereira, J.S., & Chaves, M. M. (2005). Effects of partial root-zone drying irrigation on cluster microclimate and fruit composition of field-grown Castelão grapevines. *Vitis, 44*(3), 117-125.

Schneider, V. (2014). Atypical aging defect: sensory discrimination, viticultural causes, and enological consequences. A review. *American Journal of Enology and Viticulture, 65*(3), 277-284. doi:10.5344/ajev.2014.14014

Schreiner, R. P. (2003). Mycorrhizal colonization of grapevine rootstocks under field conditions. *American Journal of Enology and Viticulture, 54*(3), 143-149.

Schreiner, R. P., Scagel, C. F., & Lee, J. (2014). N, P, and K supply to Pinot noir grapevines: impact on berry phenolics and free amino acids. *American Journal of Enology and Viticulture, 65*(1), 43-49. doi:10.5344/ajev.2013.13037

Schreiner, R. P. (2016). Nutrient uptake and distribution in young Pinot noir grapevines over two seasons. *American Journal of Enology and Viticulture, 67*(4), 436-448. doi:10.5344/ajev.2016.16019

Schreiner, R. P., Osborne, J., & Skinkis, P. A. (2018). Nitrogen requirements of Pinot noir based on growth parameters, must composition, and fermentation behavior. *American Journal of Enology and Viticulture, 69*(1), 45-58. doi:10.5344/ajev.2017.17043

Seguinot, P., Rollero, S., Sanchez, I., Sablayrolles, J.-M., Ortiz-Julien, A., Camarasa, C., & Mouret, J.-R. (2018). Impact of the timing and the nature of nitrogen additions on the production kinetics of fermentative aromas by *Saccharomyces cerevisiae* during winemaking fermentation in synthetic media. *Food Microbiology, 76*, 29-39. doi:10.1016/j.fm.2018.04.005

Serra-Stepke, I. M. (2010). *Effect of soil parameters and canopy structure on root growth and distribution.* (Master degree), Stellenbosch University, Stellenbosch, 71pp.

Soubeyrand, E., Basteau, C., Hilbert, G., van Leeuwen, C., Delrot, S., & Gomes, E. (2014). Nitrogen supply affects anthocyanin biosynthetic and regulatory genes in grapevine cv. Cabernet-Sauvignon berries. *Phytochemistry, 103*, 38-49. doi:10.1016/j.phytochem.2014.03.024

Soubeyrand, E., Colombié, S., Beauvoit, B., Dai, Z., Cluzet, S., Hilbert, G., Christel R., Christel R., Maneta-Peyret Lilly, Dieuaide-Noubhani M., Mérillon J.-M., Gibon, Y., Delrot S. & Gomès, E. (2018). Constraint-based modeling highlights cell energy, redox status and α-ketoglutarate availability as metabolic drivers for anthocyanin accumulation in grape cells under nitrogen limitation. *Frontiers in Plant Science, 9*(421). doi:10.3389/fpls.2018.00421

Spangenberg, J. E., & Zufferey, V. (2018). Changes in soil water availability in vineyards can be traced by the carbon and nitrogen isotope composition of dried wines. *Science of The Total Environment, 635*, 178-187. doi:10.1016/j.scitotenv.2018.04.078

Spring, J.-L. (1999). Indice *chlorophylliendu* feuillage et nutrition azotée du cépage Chasselas. *Revue suisse de Viticulture, Arboriculture et Horticulture, 31*(3), 141-145.

Spring, J.-L., & Zufferey, V. (2000). Intérêt de la détermination de l'indice chlorophyllien du feuillage en viticulture. *Revue suisse de Viticulture, Arboriculture et Horticulture, 32*(6), 323-328.

Spring, J. L. (2001). lnfluence du type d'enherbement sur le comportement de la vigne et la qualité des vins. Résultats d'un essai sur Chasselas dans le bassin lémanique. *Revue suisse de Viticulture, Arboriculture et Horticulture, 33*(5), 253-260.

Spring, J. L. (2002). Influence du type d'enherbement sur le comportement de la vigne et la qualité des vins. Résultats d'un essai sur Chasselas dans le bassin lémanique. 2. Résultats oenologiques. *Revue suisse de Viticulture, Arboriculture et Horticulture, 34*(2), 111-116.

Spring, J. L. (2003). Localisation de la fumure azotée sur l'intercep dans les vignes enherbées. *Revue suisse de Viticulture, Arboriculture et Horticulture, 35*(2), 113-119.

Spring, J. L., Verdenal, T., Zufferey, V., & Viret, O. (2012). Nitrogen dilution in excessive canopies of Chasselas and Pinot noir cvs. *Journal International des Sciences de la Vigne et du Vin, 46*(3), 233-240. doi:10.20870/oeno-one.2012.46.3.1520

Spring, J. L., Zufferey, V., Verdenal, T., & Viret, O. (2016a). Influence du porte-greffe sur le comportement du Pinot noir dans les conditions du Valais central. *Revue suisse de Viticulture, Arboriculture et Horticulture, 48*(2), 112-122.

Spring, J. L., Reynard, J. S., Zufferey, V., Verdenal, T., Duruz, P., & Viret, O. (2016b). Diversité intra-variétale et sélection clonale de la Petite Arvine. *Revue suisse de Viticulture, Arboriculture et Horticulture, 48*(3), 156-163.

Spring, J.-L., & Verdenal, T. (2017). Fertilisation en viticulture. *In:* S. Sinaj & W. Richner (Eds.), *Principes de fertilisation des cultures agricoles en Suisse (PRIF 2017)* (pp. 276).

Spring, J. L., Reynard, J. S., Zufferey, V., Verdenal, T., Duruz, P., Rösti, J., Lorenzini, F., & Favre, G. (2018). Nouveaux clones de Pinot gris sélectionnés à Agroscope. *Revue suisse de Viticulture, Arboriculture et Horticulture, 50*(3), 158-165.

Stamatiadis, S., Christofides, C., Tsdila, E., Taskos, D., & Schepers, J. S. (2007). Relationship of leaf stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) to biomass production in two fertilized Merlot vineyards. *American Journal of Enology and Viticulture, 58*(1), 67-74.

Stines, A. P., Grubb, J., Gockowiak, H., Henschke, P. A., Hoj, P. B., & van Heeswijck, R. (2000). Proline and arginine accumulation in developing berries of *Vitis vinifera* L. in Australian vineyards: Influence of vine cultivar, berry maturity and tissue type. *Australian Journal of Grape and Wine Research, 6*, 150-158. doi:10.1111/j.1755-0238.2000.tb00174.x

Stitt, M., & Krapp, A. (1999). The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant, Cell & Environment, 22*(6), 583-621. doi:10.1046/j.1365-3040.1999.00386.x

Stockert, C. M., Bisson, L. F., Adams, D. O., & Smart, D. R. (2013). Nitrogen status and fermentation dynamics for Merlot on two rootstocks. *American Journal of Enology and Viticulture, 64*(2), 195-202. doi:10.5344/ajev.2013.12065

Styger, G., Prior, B., & Bauer, F. F. (2011). Wine flavor and aroma. *Journal of Industrial Microbiology & Biotechnology, 38*(9), 1145. doi:10.1007/s10295-011-1018-4

Sweetman, C., Deluc, L. G., Cramer, G. R., Ford, C. M., & Soole, K. L. (2009). Regulation of malate metabolism in grape berry and other developing fruits. *Phytochemistry, 70*. doi:10.1016/j.phytochem.2009.08.006

Swiegers, J. H., Bartowsky, E. J., Henschke, P. A., & Pretorius, I. S. (2005). Yeast and bacterial modulation of wine aroma and flavour. *Australian Journal of Grape and Wine Research, 11*(2), 139-173. doi:10.1111/j.1755-0238.2005.tb00285.x

Taskos, D. G., Koundouras, S., Stamatiadis, S., Zioziou, E., Nikolaou, N., Karakioulakis, K., & Theodorou, N. (2015). Using active canopy sensors and chlorophyll meters to estimate grapevine nitrogen status and productivity. *Precision Agriculture, 16*(1), 77-98. doi:10.1007/s11119-014-9363-8

Tegeder, M. (2014). Transporters involved in source to sink partitioning of amino acids and ureides: opportunities for crop improvement. *Journal of Experimental Botany, 65*(7), 1865-1878. doi:10.1093/jxb/eru012

Tesic, D., Keller, M., & Hutton, R. J. (2007). Influence of vineyard floor management practices on grapevine vegetative growth, yield, and fruit composition. *American Journal of Enology and Viticulture, 58*(1), 1-11.

Thomidis, T., Zioziou, E., Koundouras, S., Karagiannidis, C., Navrozidis, I., & Nikolaou, N. (2016). Effects of nitrogen and irrigation on the quality of grapes and the susceptibility to *Botrytis* bunch rot. *Scientia Horticulturae, 212*, 60-68. doi:10.1016/j.scienta.2016.09.036

Tomasi, N., Monte, R., Varanini, Z., Cesco, S., & Pinton, R. (2015). Induction of nitrate uptake in Sauvignon Blanc and Chardonnay grapevines depends on the scion and is affected by the rootstock. *Australian Journal of Grape and Wine Research, 21*(2), 331-338. doi:10.1111/ajgw.12137

Torrea, D., Varela, C., Ugliano, M., Ancin-Azpilicueta, C., Leigh Francis, I., & Henschke, P. A. (2011). Comparison of inorganic and organic nitrogen supplementation of grape juice - Effect on volatile composition and aroma profile of a Chardonnay wine fermented with *Saccharomyces cerevisiae* yeast. *Food Chemistry, 127*(3), 1072-1083. doi:10.1016/j.foodchem.2011.01.092

Treeby, M. T., & Wheatley, D. M. (2006). Effect of nitrogen fertiliser on nitrogen partitioning and pool sizes in irrigated Sultana grapevines. *Australian Journal of Experimental Agriculture, 46*(9), 1207-1215. doi:10.1071/EA05238

Trouvelot, S., Bonneau, L., Redecker, D., van Tuinen, D., Adrian, M., & Wipf, D. (2015). Arbuscular mycorrhiza symbiosis in viticulture: a review. *Agronomy for Sustainable Development, 35*(4), 1449-1467. doi:10.1007/s13593-015-0329-7

Ugliano, M., Henschke, P. A., Herdrich, M. J., & Pretorius, I. S. (2007). Nitrogen management is critical for wine flavour and style. *Aus. N.Z. Wine Industry J., 22*(6), 24-30.

Ugliano, M., & Henschke, P. A. (2009). Yeasts and Wine Flavour. *In* M. V. Moreno-Arribas & M. C. Polo (Eds.), *Wine Chemistry and Biochemistry* (pp. 313-392). New York, : Springer. https://doi.org/10.1007/978-0-387-74118-5_17

Usman, S., Singh, S. P., Rawat, Y. S., & Bargali, S. S. (2000). Fine root decomposition and nitrogen mineralisation patterns in *Quercus leucotrichophora* and *Pinus roxburghii* forests in central Himalaya. *Forest Ecology and Management, 131*(1), 191-199. doi:https://doi.org/10.1016/S0378-1127(99)00213-3

Van Cleemput, O., Zapata, F., & Vanlauwe, B. (2008). Use of tracer technology in mineral fertilizer management. In *Guidelines on nitrogen management in agricultural systems* (Vol. 29, pp. 19-125). Vienna: International Atomic Energy Agency.

van Leeuwen, C., Friant, P., Soyer, J.-P., Molot, C., Chone, X., & Dubourdieu, D. (2000). L'intérêt du dosage de l'azote total et de l'azote assimilable dans le moût comme indicateur de la nutrition azotée de la vigne. *Journal International des Sciences de la Vigne et du Vin, 34*(2), 75-82.

van Leeuwen, C., Friant, P., Choné, X., Tregoat, O., Koundouras, S., & Dubourdieu, D. (2004). Influence of Climate, Soil and Cultivar on Terroir. *American Journal of Enology and Viticulture, 55*(3), 207-217.

van Leeuwen, C., & Seguin, G. (2006). The concept of terroir in viticulture. *Journal of Wine Research, 17*(1), 1-10. doi:10.1080/09571260600633135

van Leeuwen, C., Roby, J.-P., & de Rességuier, L. (2018). Soil-related terroir factors: a review. *Oeno One, 52*(2), 173-188. doi:10.20870/oeno-one.2018.52.2.2208

Verdenal, T., Spangenberg, J. E., Zufferey, V., Lorenzini, F., Spring, J. L., & Viret, O. (2015). Effect of fertilisation timing on the partitioning of foliar-applied nitrogen in *Vitis vinifera* cv. Chasselas: a ^{15}N labelling approach. *Australian Journal of Grape and Wine Research, 21*(1), 110-117. doi:10.1111/ajgw.12116

Verdenal, T., Spangenberg, J. E., Zufferey, V., Lorenzini, F., Dienes-Nagy, A., Gindro, K., Spring J.-L., Viret, O. (2016a). Leaf-to-fruit ratio affects the impact of foliar-applied nitrogen on N accumulation in the grape must. *Journal International des Sciences de la Vigne et du Vin, 50*(1), 23-33. doi:10.20870/oeno-one.2016.50.1.55

Verdenal, T., Zufferey, V., Spring, J. L., Rösti, J., Dienes-Nagy, A., Lorenzini, F., Wolfender, J.-L., Spangenberg, J., Burgo, S., Gindro, K, & Viret, O. (2016b). Complémentation foliaire azotée du cépage *Vitis vinifera* Doral dans le canton de Vaud. *Revue suisse de Viticulture, Arboriculture et Horticulture, 48*(4), 238-245.

Verdenal, T., Zufferey, V., Spring, J. L., Rösti, J., Dienes-Nagy, A., Lorenzini, F., Wolfender, J.-L., Spangenberg, J., Burgo, S., Gindro, K, Viret, O. (2016c). Complémentation foliaire azotée du cépage *Vitis vinifera* Doral dans le canton de Vaud. *Revue suisse de Viticulture, Arboriculture et Horticulture, 48*(4), 238-245.

Verdenal, T., Zufferey, V., Spring, J. L., Dienes-Nagy, A., Belcher, S., & Lorenzini, F. (2017). Impact de la zone d'application de l'urée foliaire sur la teneur en azote des raisins. *Revue suisse de Viticulture, Arboriculture et Horticulture, 49*(6), 354-359.

Verdenal, T., Zufferey, V., Dienes-Nagy, A., Bourdin, G., Gindro, K., Viret, O., & Spring, J.-L. (2019). Timing and intensity of grapevine defoliation: an extensive overview on five cultivars in Switzerland. *American Journal of Enology and Viticulture, 70*(4), 427-434. doi:10.5344/ajev.2019.19002

Verdenal, T., Spangenberg, J. E., Zufferey, V., Dienes-Nagy, Á., Viret, O., van Leeuwen, C., & Spring, J.-L. (2020). Impact of crop load on nitrogen uptake and reserve mobilisation in *Vitis vinifera*. *Functional Plant Biology, 47*, 744-756. doi:10.1071/FP20010

Vilanova, M., Ugliano, M., Varela, C., Siebert, T., Pretorius, I. S., & Henschke, P. A. (2007). Assimilable nitrogen utilisation and production of volatile and non-volatile compounds in chemically defined medium by *Saccharomyces cerevisiae* wine yeasts. *Applied Microbiology and Biotechnology, 77*(1), 145-157. doi:10.1007/s00253-007-1145-z

Vincenzini, M., Guerrini, S., Mangani, S., & Granchi, L. (2017). Amino acid metabolisms and production of biogenic amines and ethyl carbamate. *In* H. König, G. Unden, & J. Fröhlich (Eds.), *Biology of Microorganisms on Grapes, in Must and in Wine* (pp. 231-253). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-60021-5_10

Vrignon-Brenas, S., Metay, A., Leporatti, R., Gharibi, S., Fraga, A., Dauzat, M., Roland G., & Pellegrino, A. (2019). Gradual responses of grapevine yield components and carbon status to nitrogen supply. *Oeno One, 53*(2). doi:10.20870/oeno-one.2019.53.2.2431

Wang, Y., Janz, B., Engedal, T., & Neergaard, A. (2017). Effect of irrigation regimes and nitrogen rates on water use efficiency and nitrogen uptake in maize. *Agricultural Water Management, 179*, 271-276. doi:10.1016/j.agwat.2016.06.007

Wang, Y., He, Y.-N., Chen, W.-K., He, F., Chen, W., Cai, X.-D., Duan, C.-Q., & Wang, J. (2018). Effects of cluster thinning on vine photosynthesis, berry ripeness and flavonoid composition of Cabernet-Sauvignon. *Food Chemistry, 248*, 101-110. doi:10.1016/j.foodchem.2017.12.021

Wang, Y., He, Y.-N., He, L., He, F., Chen, W., Duan, C.-Q., & Wang, J. (2019). Changes in global aroma profiles of Cabernet-Sauvignon in response to cluster thinning. *Food Research International, 122*, 56-65. doi:10.1016/j.foodres.2019.03.061

Waterhouse, A. L., Sacks, G. L., & Jeffery, D. W. (2016). *Understanding wine chemistry*: Wiley-Blackwell. https://doi.org/10.1002/9781118730720

Wermelinger, B. (1991). *Nitrogen Dynamics in Grapevine: Physiology and Modeling.* Paper presented at the International Symposium on Nitrogen in Grapes and Wine, Seattle WA, USA.

Weyand, K. M., & Schultz, H. R. (2006). Long-term dynamics of nitrogen and carbohydrate reserves in woody parts of minimally and severely pruned Riesling vines in a cool climate. *American Journal of Enology and Viticulture, 57*(2), 172-182.

White, R., Balachandra, L., Edis, R., & Chen, D. (2007). The soil component of terroir. *Journal International des Sciences de la Vigne et du Vin, 41*(1), 9-18. https://doi.org/10.20870/oeno-one.2007.41.1.860

Williams, L. E. (2015). Recovery of ^{15}N Labeled Fertilizer by Thompson Seedless Grapevines: Effects of N Fertilizer Type and Irrigation Method. *American Journal of Enology and Viticulture, 66*, 509-517. doi:10.5344/ajev.2015.14115

Xu, G., Fan, X., & Miller, A. J. (2012). Plant nitrogen assimilation and use efficiency. *Annual Review of Plant Biology, 63*(1), 153-182. doi:10.1146/annurev-arplant-042811-105532

Zamboni, M., Garavani, A., Gatti, M., Vercesi, A., Parisi, M. G., Bavaresco, L., & Poni, S. (2016). Vegetative, physiological and nutritional behavior of new grapevine rootstocks in response to different nitrogen supply. *Scientia Horticulturae, 202*, 99-106. doi:10.1016/j.scienta.2016.02.032

Zapata, C., Deleens, E., Chaillou, S., & Magne, C. (2004a). Partitioning and mobilization of starch and N reserves in grapevine (*Vitis vinifera* L.). *J Plant Physiol, 161*(9), 1031-1040. doi:10.1016/j.jplph.2003.11.009

Zapata, C., Deléens, E., Chaillou, S., & Magné, C. (2004b). Mobilisation and distribution of starch and total N in two grapevine cultivars differing in their susceptibility to shedding. *Functional Plant Biology, 31*, 1127-1135. doi:10.1071/FP04028

Zufferey, V., & Maigre, D. (2007). Âge de la vigne. I. Influence sur le comportement physiologique des souches. *Revue suisse de Viticulture, Arboriculture et Horticulture, 39*(4), 257-261.

Zufferey, V., & Murisier, F. (2007). Assessment of plant hydraulics in grapevine on various "terroirs" in the canton de Vaud (Switzerland). *Journal International des Sciences de la Vigne et du Vin, 41*(2), 95-102. https://doi.org/10.20870/oeno-one.2007.41.2.854

Zufferey, V., & Maigre, D. (2008). Âge de la vigne II. Influence sur la qualité des raisins et des vins. *Revue suisse de Viticulture, Arboriculture et Horticulture, 40*(4), 241-245.

Zufferey, V., Murisier, F., Belcher, S., Lorenzini, F., Vivin, P., Spring, J. L., & Viret, O. (2015). Nitrogen and carbohydrate reserves in the grapevine (*Vitis vinifera* L. 'Chasselas'): the influence of the leaf to fruit ratio. *Vitis, 54*, 183-188. doi:10.5073/vitis.2015.54.183-188

Zufferey, V., Spring, J.-L., Verdenal, T., Dienes, A., Belcher, S., Lorenzini, F., Koestel F., Rösti, H., Gindro, K., Spangenberg, J., & Viret, O. (2017). The influence of water stress on plant hydraulics, gas exchange, berry composition and quality of Pinot noir wines in Switzerland. *Oeno One, 51*(1), 17-27. doi:10.20870/oeno-one.2017.51.1.1314

Zufferey, V., Verdenal, T., Dienes, A., Belcher, S., Lorenzini, F., Koestel, C., Gindro, K., Spangenberg, J.E., Viret, O. & Spring, J. L. (2018). The impact of plant water status on the gas exchange, berry composition and wine quality of Chasselas grapes in Switzerland. *Oeno One, 52*(4), 333-347. doi:10.20870/oeno-one.2018.52.4.2181

2 Material and methods

The adaptation of cultural practices represents a great potential to optimize plant N use efficiency while reducing the ecological footprint of crops. The research for this thesis focused on the impact of crop limitation on perennial crops, which is a common practice used in viticulture to promote grape maturation. For this purpose, a pot experiment was set up on grapevine to test the potential of crop limitation to manipulate plant N balance and fruit quality. This chapter presents the complete material and method of the trial: the environmental conditions of the experimental plot, the experimental setup, the field measurement methods, the excavation organization, the plant sample preparation, the isotope analysis method, the must analysis methods, and the data treatments.

2.1 Experimental conditions and plant material

2.1.1 Location and pedoclimatic conditions

The trial was conducted over two years (2017–2018) at the Agroscope experimental vineyard in Pully, Switzerland (46°30'45.8"N, 6°40'05.7"E). The local climate is temperate. During the first vine-growing season (April–October 2017), the total precipitation was 562 mm, and the daily mean temperature was 16.6°C. The 2018 climatic conditions were drier and hotter than 2017, with 412 mm of total precipitation and 17.8°C of average daily mean temperature from April through October (for data from the Swiss meteorological station in Pully, see Figure 2.1).

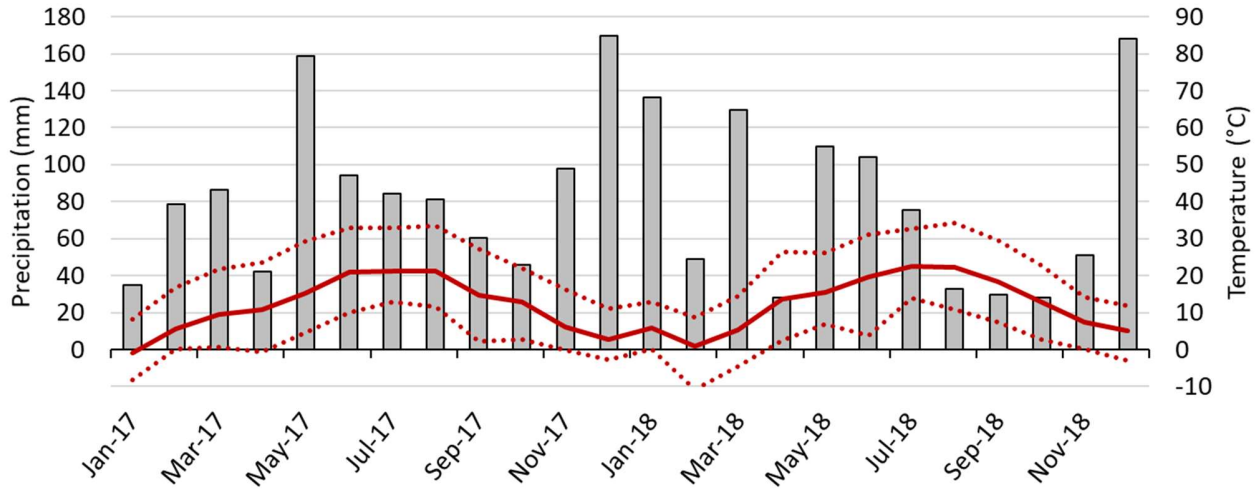


Figure 2.1. Monthly temperature and precipitation in Pully, Switzerland, from January 2017 to December 2018 (Swiss meteorological station in Pully)

The experiment was carried out in 90 L pots and under the field conditions (i.e., outdoors). Soil samples were collected in 2015 in the pots (four replicates) using a soil auger. The samples were analyzed at the laboratory Sol-Conseil (Gland, Switzerland) to determine both texture and composition. Soil samples were dried at 40°C for two days and sieved at 2 mm. The pH was determined via potentiometry, $CaCO_3$ was determined via the volume of CO_2 released after HCl addition, the soluble elements phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) were quantified via spectrometry after extraction in pure water (dilution ratio 1:10, one-hour stirring), and total N (TN) was quantified at the Institute of Earth Surface Dynamics (IDYST-UNIL) via IRMS (four replicates). The results are presented in Table 2.1.

Table 2.1. Soil texture and composition. Samples collected in the pots in 2015. Interpretations based on PRIF 2017 (Sinaj and Richner 2017)

Texture	Result	Interpretation
Clay (wt%)	15	
Silt (wt%)	38	Sandy Loam
Sand (wt%)	47	

Composition		Very low	Low	Ideal	High	Very high
pH	7.93			■		
OM (wt%)	1.75		■			
$CaCO_3$ total (wt%)	4.25		■			
TN (wt%)	0.10			■		
P (mg kg^{-1})	8.20			■		
K (mg kg^{-1})	25.24			■		
Ca (mg kg^{-1})	139.04			■		
Mg (mg kg^{-1})	11.35			■		

2.1.2 Plant material and seasonal phenology

Vitis vinifera L. Chasselas was chosen for this trial because it is the most planted cultivar and best representative of the studied region. It was grafted onto rootstock 3309 C and planted in 2013 in 90 L pots. Planting in pots ensured a good recovery of the root biomass at excavation, while the pot size allowed unconstrained root development. Before plantation, 225 pots were disposed of underground in three trenches/rows with a planting density of 8,330 vines ha^{-1} (1.5 × 0.8 m) and filled with the soil of

the trenches as growth media. The soil water-holding capacity was 11 L per pot, as estimated from soil texture (Saxton *et al.*, 1986). The stem water potential was monitored punctually during the two summers to prevent possible water restriction, using a pressure chamber (Model 600; PMS Instruments, Albany, NY, USA) (Scholander *et al.*, 1965). Vines were drip-irrigated twice in July (total 12 L per plant) for both seasons to maintain the stem water potential above –0.8 MPa. The vines were trained in a single Guyot trellis system, with 60 cm trunk height and 7 shoots per cane, following local vineyard practices. The canopy was trimmed at 120 cm above the trunk three times per season, that is, on day of year (DOY) 164, 191, and 215 in 2017 and on DOY 162, 183, and 218 in 2018. The dates of the main phenological stages were similar in 2017 and 2018 (Table 2.2): 50% bud burst (phenological scale BBCH 05, Lancashire *et al.* 1991) occurred on DOY 94 and 99, respectively; 50% flowering (BBCH 65) occurred on DOY 164 and 161; 50% veraison (i.e., the onset of grape ripening, BBCH 85) occurred on DOY 214 in both years; and harvest was performed on DOY 257 and 269, respectively, based on target soluble solids level.

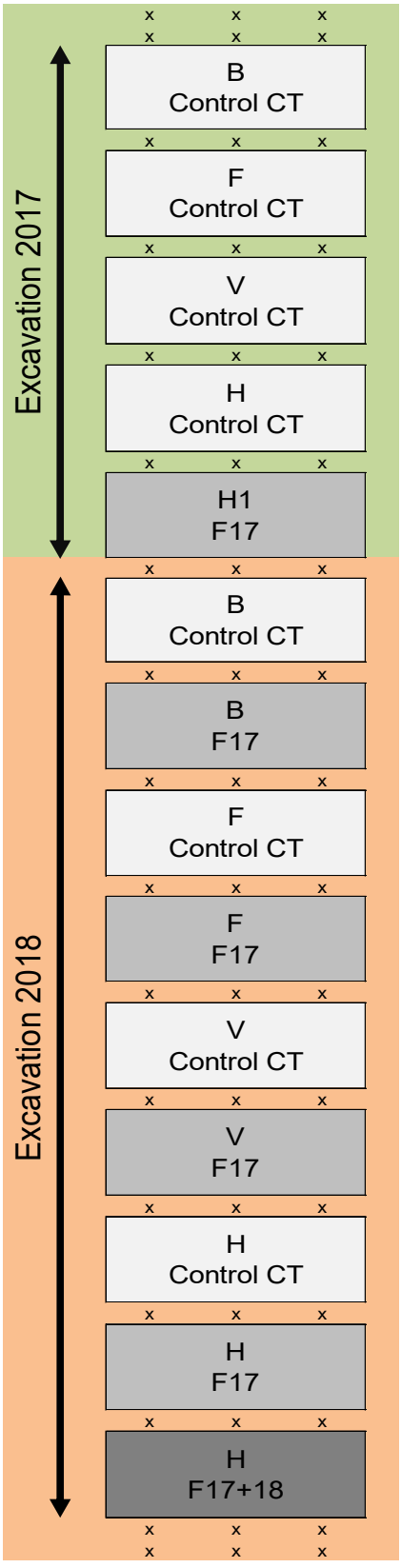
Table 2.2. Days of year for the principal phenological stage occurrences in both 2017 and 2018. Cultivar Chasselas, Pully, Switzerland.

Phenological stage	2017	2018
	DOY	
Bud burst	94	99
Flowering	164	161
Veraison	214	214
Harvest	257	269

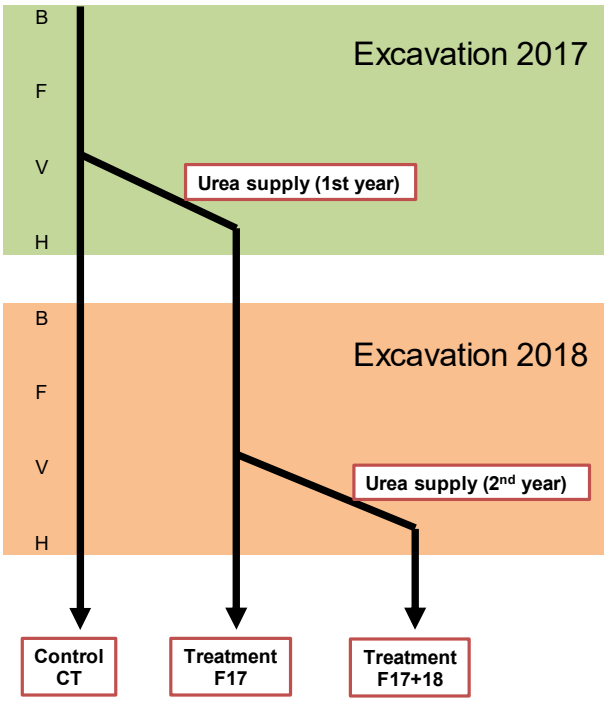
Despite homogeneity in terms of plant material and growing conditions, eight out of the 225 vines were identified as outliers (i.e., low vigor, low photosynthetic activity, low fruitfulness, low berry set, and incomplete winter cold hardening) and were discarded to optimize the homogeneous conditions of the trial.

2.2 Trial setup and factors of variation

The plants were organized into 14 homogeneous groups of 12 plants each (i.e. total 168 plants), and separated by the remaining plants used as buffers to minimize cross-contamination from the foliar N supply (Figure 2.2).



← **Figure 2.2.** Map of the field trial. Excavation times: B, bud burst; F, flowering; V, veraison; H, harvest; fertilization treatments: CT, non-fertilized control; F17, foliar N supply in 2017 only; F17+18, foliar N supply in both 2017 and 2018



↑ **Figure 2.3.** Timing of the implementation of N supply. B, bud burst; F, flowering; V, veraison; H, harvest. Fertilization treatments: CT, non-fertilized control; F17, foliar N supply in 2017 only; F17+18, foliar N supply in both 2017 and 2018

Two factors of variation were set in this trial, i.e., crop load and fertilization. In each group of 12 plants, the crop load treatment was set (i.e., two levels of crop load; six plants per level). The groups of 12 plants were destructively excavated at eight dates, corresponding to the four major phenological stages (i.e., 50% of each stage, bud break, flowering, veraison, and harvest) over two years, to assess the dynamics of total N and fertilizer N into the plant. For each excavation date, there were either one, two or three groups of vines excavated, corresponding to the number of fertilization levels (i.e., one to three fertilization levels; 12 plants per level). Each plant was considered a replicate. Each excavation date was statistically analyzed separately using one- or two-way ANOVA with interaction, as explained hereafter.

2.2.1 Crop limitation

In each group of 12 plants, a large crop load gradient was built by crop thinning at bunch closure (phenological stage BBCH 77; DOY 193 in 2017 and DOY 179 in 2018), keeping two to ten bunches per plant. Crop thinning in 2018 was based on the yield at harvest 2017 in order to maintain each plant under the same crop load treatment over the two consecutive seasons and promote cumulative responses. For statistical analyses, the groups of plants excavated before the 2017 crop thinning, that is, at bud break (1 group) and flowering (1 group), were considered homogeneous groups of plants, whereas the data from the other groups were split into two sub-groups of plants, that is, low-yield conditions (LYC) versus high-yield conditions (HYC). The threshold to split the groups of plants excavated in 2017 was 7.0 tons ha^{-1} at veraison (1 group, CT) and 13.0 tons ha^{-1} at harvest 2017 (2 groups, CT and F17), based on the median crop load by the time of excavation. The thresholds at bud break 2018 (2 groups) and flowering 2018 (2 groups) were based on the median crop load at harvest 2017. Due to a higher yield potential in 2018, the thresholds in the groups of plants excavated at veraison 2018 (2 groups) and at harvest 2018 (3 groups, CT, F17 and F17+18) were 12.5 tons ha^{-1} and 21.0 tons ha^{-1}, respectively.

2.2.2 Fertilization treatments

Three fertilization regimes were set: a control treatment (CT), a treatment with one foliar N supply in 2017 only (F17), and a treatment with foliar N supply in both 2017 and 2018 (F17+18). In 2017, the groups of vines corresponding to the treatments F17 and F17+18 each received 2.4 g N per plant (20 kg N ha^{-1}) in the form of ^{15}N-labelled urea (10 atom % ^{15}N; Sigma-Aldrich, Buchs, Switzerland), applied at veraison and split into four applications (DOY 199, 208, 214, and 226). In

2018, only the plants from the treatment F17+18 again received 2.4 g N of labelled urea each in the same conditions (DOY 198, 204, 211, and 219) (Figure 2.3). The labelled foliar urea was carefully applied on both sides of the entire canopy (dilution 3.44% w/v) with hand sprayers (Birshmeier, Stetten, Switzerland). No other fertilization occurred during the trial.

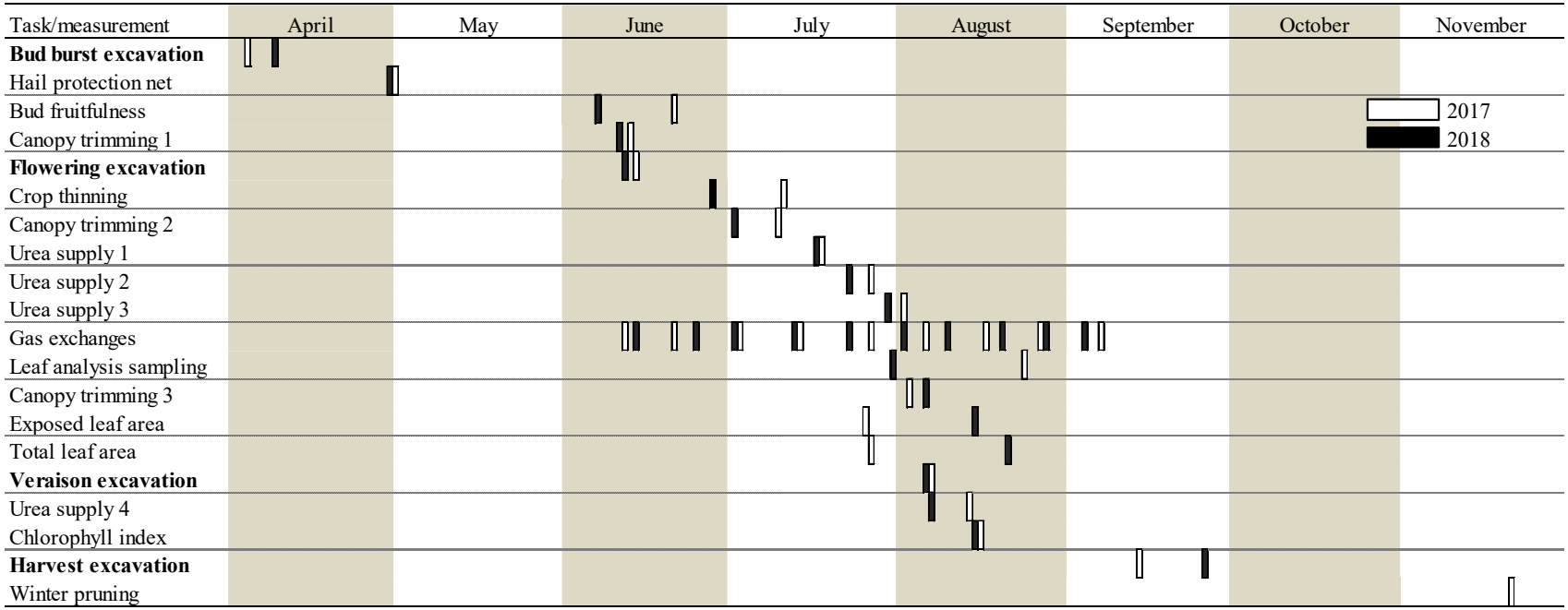
2.2.3 Excavation

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

2.3 Field measurements and sampling

The multiple vineyard tasks were accomplished with the help of the Agroscope viticulture team. Over the two years of the experiment, the plant's physiological development was measured for each treatment (i.e., fertilization × crop load), the plant nutrient status was assessed, and the leaf gas exchanges were monitored. The excavation of the plant and the preparation of the samples were carefully planned and executed in coordination with the vineyard team and the laboratories. The major field tasks and measurements were performed according to the timeline shown in Table 2.3.

Table 2.3. Timeline of the field tasks and measurements in both 2017 (in white) and 2018 (in black).



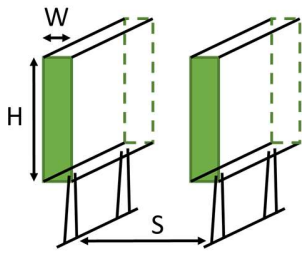
2.3.1 Plant physiological development

Pruning wood weight and bud fruitfulness

The winter pruning woods were collected and weighed vine per vine on DOY 325 in 2017 and then removed from the experimental plot. Bud fruitfulness was determined before crop limitation by counting both bunches and shoots per plant and was then expressed as the average number of bunches per shoot.

Light-exposed leaf area

The light-exposed leaf area (LEA, m^2 m^{-2} of ground) was measured both years on the fully developed canopy the same year as excavation (on DOY 237 in 2017 and on DOY 227 in 2018). The measure was carried out per groups of four vines. It was estimated using Carbonneau's method (1995), based on the canopy height (H), width (W), porosity (P), estimation of gap % in the canopy), and space between two rows (S), as follows (Figure 2.4):



$$LEA = \frac{(2H + W) \times (1 - P)}{S} \qquad (1)$$

Figure 2.4. Measurements required for the estimation of LEA.

Total leaf area

The total leaf area (TLA) per vine was assessed for both years on the vines excavated at harvest, with the non-destructive method of Mabrouk and Carbonneau (1996), based on the strong correlation between the length of a shoot and its total leaf area. The total shoot length (TSL, main shoot + laterals) was measured with a string, and the correlation equation in the context of this trial was determined as follows: 15 shoots from the buffer plants were collected on DOY 206 in 2017; shoot by shoot, TSL was measured and TLA was determined by scanning the leaves with a leaf area meter (LI-3100C, Li-COR Biosciences, Lincoln, NE, USA). As a result, equation (2) allowed the transformation of measured TSL into estimated TLA for both seasons (r = 0.98):

$$TLA = 14.4 \times TSL + 161.5 \qquad (2)$$

2.3.2 Plant nutrient status and photosynthesis activity

Leaf nutrient composition

The leaf mineral nutrients (i.e., total N, P, K, Ca, and Mg) were quantified in the groups of vines excavated at harvest and per yield condition. For this purpose, two main leaves (blade + petiole) per vine were collected from the median part of the canopy twice in 2017 (DOY 229 and 290) and once in 2018 (DOY 212). The samples were prepared and analyzed by the Sol-Conseil laboratory (Gland, Switzerland) as follows: the leaves were dried at 60°C and then powdered using a hammer mill (model 1974; Ammann, France), the plant material was then calcined and the ash was dissolved with hydrochloric acid, the extract was used for the determination of total elements (P, K, Ca, Mg) by inductively coupled plasma atomic emission spectroscopy (ICP-AES) and TN was determined using the Kjeldahl method (1883).

Chlorophyll index

The chlorophyll index reflects the intensity of the green color of the foliage and is well correlated with leaf chlorophyll and N concentrations (Cerovic et al., 2015). The chlorophyll index was measured after veraison (DOY 223 in 2017 and DOY 222 in 2018) in the median part of the canopy, in the groups of vines excavated at harvest and per yield condition on adult leaves from the median part of the canopy, using an infrared nondestructive method (N-Tester; Yara International, Paris, France).

Leaf gas exchanges

The leaf gas exchange rates were measured for both years approximately every 10 days from flowering to harvest, on sunny days from 12:00 PM to 03:00 PM, on the vines excavated at harvest. Net assimilation (A), transpiration (E), stomatal conductance (gsw), ambient CO_2 concentration (C_a), and internal CO_2 concentration (C_i) were determined nondestructively with a portable photosynthesis system (LI-6800; Li-COR Biosciences). At each measurement session, one measurement per vine was realized on one fully expanded leaf. During the measurements, the ambient conditions inside the LI-6800 leaf chamber were controlled by the system with preset parameters, as detailed in Table 2.4.

Table 2.4. Parameters of the Li-COR 6800 for the measurement of leaf gas exchanges in this trial.

Parameter	Setting
Air flow	700 µmol.s^{-1}
Relative humidity	Equal to ambient RH by the time of measurement, average 50%
Ambient CO_2	380 µmol.mol^{-1}
Fan speed	5,000 rpm
Light source	2,000 µmol.m^2.s^{-1}

2.3.3 Excavation and sample preparation

At excavation time, each vine was unearthed separately and split into four parts: roots, trunk (including wooden cane), canopy (including shoot trimmings collected during the same season), and grapes. The number of organs depended on the phenological stage by the time of excavation (e.g., only roots and trunks at bud burst). At both veraison and harvest stages, the grapes were weighed to determine the crop load (kg per plant) and then pressed manually to separate the liquids (must) from the solids (pomace). The shoots were pruned and collected with the leaves. Once unearthed, the roots and trunk were separated and washed with water. The five plant parts (roots, trunk, canopy, pomace, and must) were weighed to determine fresh weights (FWs). Must aliquots were taken for chemical (100 g) and stable isotope analysis (25 g). The plant parts (except must samples) were prepared at the Sol-Conseil laboratory where they were dried at 60°C until a constant weight for determination of the dry weight (DW) and were then ground with a hammer mill (model 1974; Ammann) to a fine powder. The musts aliquots were freeze dried at Agroscope where they were frozen in liquid N under gentle rotation and immediately put in a freeze-dryer (Alpha 1-4 LSC; Christ, Osterode am Harz, Germany) for 48 hours, and then weighed for determination of the DW.

A: Plantation in May 2013. B: Early canopy development in April 2017. C: Hail protection in 2017–2018. D: Labelled urea application by Thibaut Verdenal. E: Leaf area measure (LI-3100C, Li-COR Biosciences) by Nicolas Leclerc (intern Ecole Supérieure d'Agricultures, Angers). F: Leaf gas exchange measurement (LI-6800, Li-COR Biosciences).

G: Pomace and must separation after harvest. H: Must aliquots. I: Canopy removal before excavation by Elise Womelsdorf (intern PURPAN, Toulouse). J: 36 vines ready for excavation. K and L: Excavation. In order of appearance, Philippe Duruz (Agroscope), Thibaut Verdenal, Elise Womelsdorf, and Laure Passot (Bordeaux Sciences Agro intern).

M: Root systems after excavation. N: Root and trunk separation. In order of appearance, Thibaut Verdenal, Philippe Duruz, Elise Womelsdorf, and Laure Passot. O: Root and trunk ready for weighing. P: Trunks pre-cut before grinding in the hammer mill. Q: Must aliquots on the freeze-drier (Alpha 1-4 LSC; Christ). R: Plant fractions, dried and ground to a fine powder, ready for EA-IRMS.

2.4 Stable isotope analysis

Isotopes of a given chemical element have the same number of protons and a different number of neutrons. In this study, the stable isotope composition of both elemental C and N was analyzed (Figure 2.5). Elemental C has two stable isotopes – ^{12}C and ^{13}C – present in nature at a natural abundance of 98.89 and 1.11 atom %, respectively. Elemental N also has two stable isotopes – ^{14}N and ^{15}N – at a natural abundance of 99.63 and 0.37 atom %, respectively.

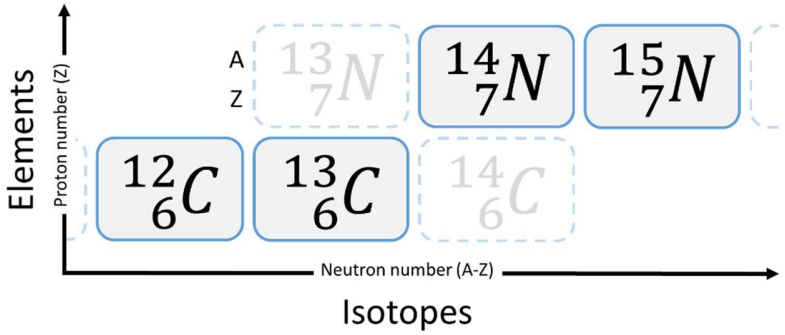


Figure 2.5. Representation of a few isotopes of the elements C and N. A, mass number; Z, proton number. Solid lines, stable isotopes; dashed-lines, radio-active isotopes (unstable).

In biological material, the relative abundance between the heavy and light isotopes of an element slightly but significantly varies in respect to several factors (e.g., climate and organic metabolism). These variations are due to the discrimination between heavy and light isotopes and the natural tendency of living organisms to prefer light isotopes in natural processes. For example, the ^{13}C-to-^{12}C ratio in plants globally is lower than that in the atmosphere due to photosynthesis, with small variations in discrimination; in this case, after assimilation, greater discrimination in favor of ^{13}C (i.e., higher ^{13}C-to-^{12}C ratio) means that the stomata of the plant were mostly closed, probably due to water restriction and drier climate conditions. The observation of these variations provides insights into several research fields with a wide range of applications: in geochemistry, it is useful in the study of the origin and cycling of C and organic matter in the biosphere and for the reconstruction of past climates; in archeology, it is widely used for the reconstruction of past diets; and in agriculture, it is used against fraud and for traceability of food origin. These examples of applications focus on the natural abundance of isotopes. In contrast, in this study, we used the particular method of isotope labelling: a source of labelled N – artificially enriched with ^{15}N – was given to the plants in the form of foliar N supply. The use of an unnatural isotope ratio as a tracer in the plant allowed the monitoring of plant N uptake and partitioning. Also, isotope labelling largely covered the variations due to natural isotope discrimination.

Conventionally, the stable isotope composition is reported as a δ value (i.e., $\delta^{13}C$ and $\delta^{15}N$), which is the relative deviation of the molar ratio (R) of the heaviest (^{i}E) to the lightest (^{j}E) isotopes (e.g., ^{13}C-to-^{12}C and ^{15}N-to-^{14}N) from an international standard (Coplen, 2011):

$$\delta^{i}E_{sample} = \frac{R\left(\frac{^{i}E}{^{j}E}\right)_{sample}}{R\left(\frac{^{i}E}{^{j}E}\right)_{standard}} - 1 \qquad (3)$$

The international reference standard for C isotopes is the Vienna Pee Dee Belemnite (VPDB). The original PDB sample was a sample of fossilized shells of an extinct organism called belemnite. The international reference standard for N isotopes is N_2 gas found in common air. The δ values were reported in milliurey (mUr) in conformity with the International System of Units (Brand and Coplen, 2012).

The stable C and N isotope compositions of plant parts were determined at IDYST-UNIL by EA-IRMS. Both the sample aliquots and the calibration standards were subjected to flash combustion on an elemental analyzer (Carlo Erba 1108; Fisons Instruments, Milan, Italy) connected with a continuous flow open split interface (ConFlo III; Thermo Fisher Scientific, Bremen, Germany) to an IRMS (Delta V Plus; Thermo Fisher Scientific, Bremen, Germany) (Figure 2.6). The calibration and normalization of both $\delta^{13}C$ and labelled $\delta^{15}N$ measurements to their international standards (i.e., VPDB and air-N_2, respectively) were realized with four reference materials manufactured in-house by Dr. Spangenberg (UREA 1, 2, 3, and 6) at different ^{13}C and ^{15}N abundances. These standards were prepared by mixing urea at natural C and N abundances with a labelled urea (99 atom % ^{13}C and 99 atom % ^{15}N; Sigma-Aldrich) at different ratios, as described in Spangenberg and Zufferey (2019). The in-house standards covered a wide range of abundances in ^{13}C (from natural abundance up to 370 mUr) and in ^{15}N (from natural abundance up to 1275 mUr). The calibration and normalization of the non-labelled $\delta^{15}N$ measurements to the air-N_2 scale were realized with both international (USGS-40, IAEA-600) and in-house standards (UNIL-Glycine, and then UNIL-UREA 2 after exhaustion of glycine). The standards used in this study were calibrated for both $\delta^{13}C$ and $\delta^{15}N$ measurements (Table 2.5).

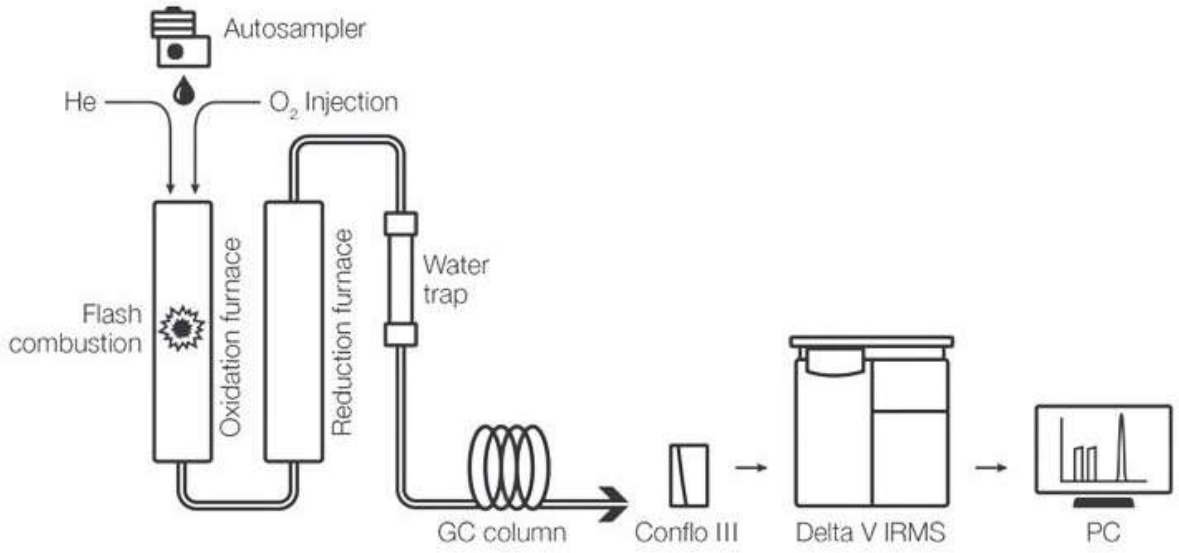


Figure 2.6. Schematic of the EA-IRMS system at IDYST-UNIL (source: www.thermofisher.com).

Table 2.5. $\delta^{13}C$ and $\delta^{15}N$ values of the standards used in this study for calibration of EA-IRMS, IDYST-UNIL (Brand *et al.*, 2014; Spangenberg and Zufferey, 2019).

Standard	$\delta^{13}C_{VPDB}$ (mUr)	$\delta^{15}N_{Air\text{-}N2}$ (mUr)
USGS-40	–26.39 ± 0.04	−4.52 ± 0.06
IAEA-600	–27.77 ± 0.04	0.91 ± 0.09
UNIL-Glycine	−26.02 ± 0.05	2.93 ± 0.09
UNIL-UREA 1	–43.89 ± 0.04	–1.39 ± 0.05
UNIL-UREA 2	–27.20 ± 0.05	49.94 ± 0.04
UNIL-UREA 3	–10.96 ± 0.05	101 ± 0.06
UNIL-UREA 6	–	1,275 ± 0.7

Both plant aliquots and calibration standards were weighed and sealed in tiny tin foils. The required quantities for a precise measurement varied depending on total organic C (TOC) and TN concentrations in the samples, related to the plant parts (Table 2.6). The measurement sessions contained 34 samples, each starting and ending with standards, as described in Table 2.6. In this study, 816 plant samples were analyzed by EA-IRMS for both $\delta^{13}C$ and $\delta^{15}N$, all performed in duplicate, for a total of 3,264 analyses (Table 2.7). The repeatability was better than 0.1 mUr (1 *SD*) for both $\delta^{13}C$ and $\delta^{15}N$ at natural abundance and better than 2 mUr for $\delta^{15}N$ in the ^{15}N-enriched samples.

Table 2.6. Aliquot and standard quantities used for EA-IRMS in this study, IDYST-UNIL.

EA-IRMS		N composition at natural abundance (δ^{15}N)	N composition in labelled samples (δ^{15}N)	C composition at natural abundance (δ^{13}C)
		µg	µg	µg
Sample aliquots	Roots	15,000	15,000	1,000–2,000
	Trunk	15,000	15,000	1,000–2,000
	Canopy	5,000–10,000	5,000–10,000	1,000–2,000
	Pomace	10,000–15,000	10,000–15,000	500
	Must	50,000	50,000	100–200
Standards before and after each analytical session		USGS-40 1,500–2,000	UREA 1 200–500	UREA 1 150–200
		IAEA-600 500–1,000	UREA 2 200–500	UREA 2 150–200
		UNIL-Glycine 500–1,000	UREA 6 200–500	UREA 3 150–200
				(2 × at random 1,500–2,000)

Table 2.7. Details of the samples analyzed by EA-IRMS at IDYST-UNIL.

	Timing	Plant number	Samples per plant	Total
2017	Bud burst	12	2	24
	Flowering	12	3	36
	Veraison	12	6	72
	Harvest	24	6	144
2018	Bud burst	24	2	48
	Flowering	24	3	72
	Thinned clusters	60	1	60
	Veraison	24	6	144
	Harvest	36	6	216
			Total	816

The TOC and TN concentrations (in wt%) were determined from the peak areas of the major isotopes with the calibrations used for δ^{13}C and δ^{15}N. The repeatability for the TOC and TN contents was greater than 0.2 wt%.

2.5 Grape must analysis

Must aliquots were sampled from the vines excavated at veraison and harvest, and then analyzed at the wine quality laboratory at Agroscope. After centrifugation, an infrared spectrometer (WineScan; FOSS NIR Systems, Hilleroed, Denmark) was used to determine the pH, total soluble solids (TSS), titratable acidity (TA), potassium (K), and contents of tartaric and malic acids, with the following associated errors (Table 2.8). The ammonium (NH_4^+) was quantified using an enzymatic test kit (Boehringer Mannheim GmbH, Mannheim, Germany). The primary amino N (PAN) concentration – excluding proline and hydroxyproline, which are not assimilable by yeasts in the fermentation conditions – was determined with the o-phthalaldehyde (OPA) method using the Primary Amino N kit (Bio Systems, Barcelona, Spain). The must YAN concentration was computed by adding the content of NH_4^+ and PAN, both expressed in mg N L^{-1} (Bell and Henschke, 2005).

Table 2.8. Grape must analyses and their associated errors.

Parameters	Range	Accuracy
Brix	6–26	0.2
pH	2.6–3.8	0.07
Titratable acidity (g L^{-1}, eq. tartaric ac.)	4.5–32.0	1.0
Tartaric acid (g L^{-1})	4.2–10.9	0.9
Malic acid (g L^{-1})	2.3–22.8	1.0
Ammonium (mg L^{-1})	0.08–80	0.4–0.8
Primary amino N (mg N L^{-1})	2–400	2%

To determine the free amino acid (FAA, in %) profiles of the grape musts, the aliquots were first diluted in water (1:100 dilution). FAAs were separately quantified by ultrahigh-performance liquid chromatography-mass spectrometry (UHPLC-MS), using an Infinity 1290 HPLC system connected with an electrospray interface (ESI) to a 6460C Triple Quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) (Figure 2.7).

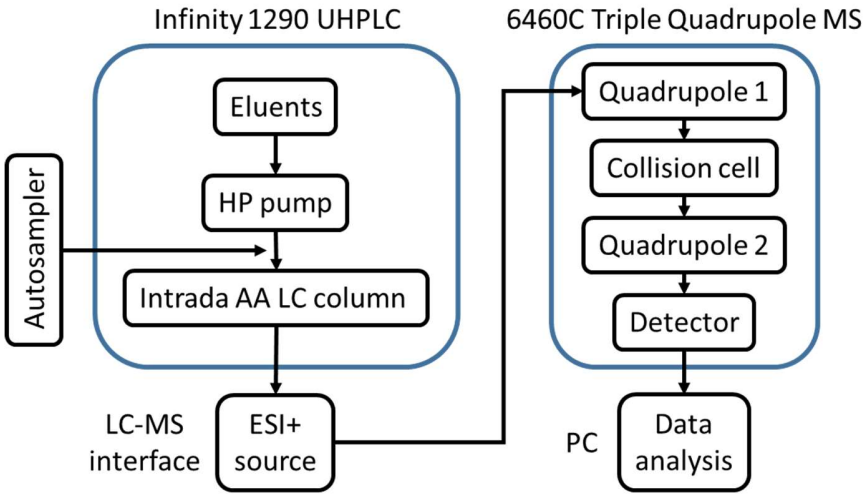


Figure 2.7. Schematic of the Agroscope UHPLC-MS system.

Chromatographic separation was performed on an Intrada amino acid column (50 x 3 mm, 3µm; Imtakt USA, Portland, OR, USA) with eluent A: THF/H_2O/CH_3CN/$HCOONH_4$[100mM]/HCOOH 75:12:9:4:0.3 and eluent B: $HCOONH_4$[100mM]. Each measurement session lasted 15 min, applying the following gradient of the eluents A and B at a flow rate of 0.6 mL min^{-1} (Table 2.9).

Table 2.9. Procedure of an UHPLC-MS measurement session,

Time (min)	Eluent A (%)	Eluent B (%)
0	100	0
3	100	0
6.5	86.5	13.5
7.5	20	80
11	20	80
15	100	0

MS detection was achieved in positive ionization mode using the multiple reaction monitoring (MRM) method for quantification. Due to the high variability of the FAA abundances, two groups of FAAs were formed regarding the threshold of 15 mmol L^{-1}, that is, low abundance and high abundance. Each group of FAAs was then analyzed by two methods using conditions optimized for their concentrations (Tables 2.10 and 2.11). The quantification of the FAAs with low abundance was realized with an injection volume of 1 µL of diluted must, whereas the quantification of the FAAs with high abundance was

realized with an injection volume of 0.1 μL. All the samples were therefore analyzed twice, that is, with both methods.

Table 2.10. Conditions of analysis for the AAs with low abundance. Injection of 1 μL of diluted must.

FAA < 15 mmol L^{-1}	Retention time (min)	Precursor ion	Product ion	Dwell time (ms)	Fragmentation energy (V)	Collision energy (V)
Tryptophan	1.8	205	188	20	88	6
Tyrosine	2.1	182	136	20	88	10
Isoleucine	2.4	132	86	20	65	6
Methionine	2.6	150	56	20	80	18
Leucine	2.7	132	86	20	65	6
Valine	3.4	118	72	20	60	6
Hydroxyproline	4.9	132	86	20	88	14
Aspartic acid	5.1	134	74	20	65	10
Glycine	6.0	76	30	20	40	6
Asparagine	6.2	133	74	20	75	14
Citrulline	6.6	176	70	20	83	26
Cystine	7.6	241	74	20	98	26
Histidine	9.1	156	83	20	93	26
Lysine	9.3	147	84	20	80	18
Ornithine	9.4	133	70	20	75	18

Table 2.11. Conditions of analysis for the AAs with high abundance. Injection of 0.1 μL of diluted must.

FAA > 15 mmol L^{-1}	Retention time (min)	Precursor ion	Product ion	Dwell time (ms)	Fragmentation energy (V)	Collision energy (V)
Phenylalanine	1.9	166	120	20	83	10
Glutamic acid	4.3	148	130	20	75	6
Proline	4.5	116	70	20	88	14
Threonine	4.8	120	56	20	70	14
Alanine	5.2	90	44	20	45	10
Serine	5.8	106	60	20	50	10
Glutamine	5.9	147	130	20	75	6
GABA	7.5	104	87	20	65	6
Arginine	10.0	175	70	20	113	22

FAAs were quantified using an external calibration curve prepared with standards (Sigma-Aldrich) for each FAA separately. A total of 60 samples were analyzed by UHPLC-MS in this study, all in duplicate. The repeatability of the values was better than 5% and 10% for both low and high abundances, respectively. Concentrations of each AA were reported in mg N L^{-1}.

2.6 Data treatment

The interpretation of the isotope analyses was based on the following method. The mineral content of each plant part was reported as Q (i.e., CQ for TOC quantity and NQ for TN quantity, in g) and calculated as below for NQ:

$$NQ_{part} = DW_{part} \times TN. \qquad (4)$$

The isotope-N molar ratio (R_{sample}) was calculated as follows:

$$R_{sample} = R_{air\text{-}N_2} \times (\delta^{15}N_{sample} + 1), \text{ where } R_{air\text{-}N_2} = 0.0036765. \qquad (5)$$

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

$$A\% = \frac{R}{R+1} \times 100. \qquad (6)$$

The relative specific abundance (RSA, atom %) represents the proportion of newly incorporated N atoms originating from the labelled source (e.g., fertilizer), compared to the total N quantity in the sample (Cliquet et al., 1990). The RSA also represents the organ sink strength, which is independent of the organ size (Deléens *et al.*, 1997):

$$RSA = \frac{A\%_{sample\ excess}}{A\%_{N\ supplied\ excess}} = \frac{A\%_{sample} - A\%_{non\text{-}labelled\ control}}{A\%_{N\ supplied} - A\%_{non\text{-}labelled\ control}}. \qquad (7)$$

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

$$NNP_{part} = RSA_{plant\ part} \times NQ_{plant\ part} \qquad (8)$$

$$\%P = \frac{new\ N\ pool_{plant\ part}}{new\ N\ pool_{whole\ plant}} \times 100. \qquad (9)$$

The overall net N uptake can then be calculated as follows:

$$\text{net N uptake} = \frac{\text{new N pool}_{\text{whole plant}}}{\text{total labelled N supplied}} \times 100. \qquad (10)$$

Considering the fertilizer as the ^{15}N-labelled source in the calculation of RSA (i.e., $A\%_{N\ supplied} = 10$ atom % ^{15}N) allowed estimating the net uptake and partitioning of the fertilizer N in the plant over the two vine-growing seasons. Alternatively, considering as the labelled source the initial N reserves present in the perennial parts of the plant at the onset of the second growing season (i.e., $A\%_{N\ supplied} = A\%_{(roots+trunk)}$, as affected by the residual labelled N from the 2017 N supply) theoretically allows estimating the partitioning of the perennial N reserves during the second season and to differentiate them from the seasonal root N uptake (non-labelled) in each plant part as follows:

$$NQ_{root\ uptake} = NQ_{total} - NQ_{reserves}. \qquad (11)$$

This calculation method was published in Verdenal *et al.* in 2021 (see Chapter 1).

Data were analysed using XLSTAT version 2020.5.1 software (Addinsoft, Paris, France). Each excavation date was considered as a separate plot and was subject to separate statistical analysis for the determination of the effects of the investigated factors, that is, crop load treatment (from bud break 2017), fertilization treatment (from harvest 2017), and their interaction. The significance of differences and interactions between treatments was assessed with one- or two-way ANOVA ($p < 0.05$), depending on the excavation date. A Newman-Keuls post hoc test performed to differentiate more than two groups. Regression analyses were used to highlight correlations between variables. Principal component analysis was used to evaluate the must FAA profiles.

3 Impact of crop limitation on N uptake and reserve mobilization

This chapter presents the results from the first harvest. After one year under various yield conditions, N partitioning was assessed at harvest, and the impact of crop limitation on grape N composition was shown. These results were published in 2020 in the journal *Functional Plant Biology* (CSIRO Publishing).

3.1 Resume of the article

The adaptation of cultural practices to improve NUE is a priority for the sustainable production of high-quality crops. A trial was set to study the impact of crop load on both N uptake and N reserve mobilization in grapevines. This article focuses on harvest 2017 of the trial. The findings highlight the great capacity of plants to adapt their N metabolism to external constraints. This confirms the possibility of monitoring NUE by adapting cultural practices, such as crop limitation in this case.

In response to a large crop load variation (0.5–2.5 kg m^{-2}), the roots were the most affected plant part and played a major role in the balance of fruit N content. Root development was reduced under high-yield conditions (–14% DW in the control), while canopy size was not affected. Root N reserves were highly solicited by the strong N-sink strength of maturing fruits. This suggests that several consecutive years of overproduction could affect plant capacity (i.e., vigor, bud fruitfulness, and potentially lifespan).

Fertilizer-N uptake was strongly affected by crop load. Foliar-N uptake was only 26% of the total amount applied under low-yield conditions and had no impact on fruit N concentration at harvest. Conversely, foliar-N uptake was 37% under high-yield conditions, and yeast assimilable N concentration was increased by 34%. The results suggested that soil N uptake by roots was also stimulated by higher-yield conditions. Plant N uptake largely contributed to fulfilling the high fruit N demand while limiting the mobilization of root N reserves. As a result of changes in N distribution and uptake, the N concentration in fruit remained unchanged despite the large crop loading gradient.

Despite unchanged N concentration in fruits, N composition was affected by crop load. Regardless of unrestrictive environmental conditions for fruit ripening, the fruit-free amino N profile was modified, which potentially altered the fruit aromas. In contrast, the impact of foliar N supply on N composition was negligible. In fact, fruit N composition appeared related to N partitioning rather than N uptake. This suggests that any parameter that influences plant N partitioning may potentially affect fruit N composition and subsequent aroma development.

In a search for fruit N balance, grapevines actively modulated root N reserve mobilization and fertilizer N uptake to maintain a uniform N concentration in the must. Crop limitation did not improve fruit N concentration but affected its composition (i.e., amino profile), suggesting a potential modification of the aroma profile. Does the crop load limitation always have a positive impact on grape composition and wine quality? This study encourages further research on NUE management via the modulation of cultural practices, with the aim of enhancing crop quality and sustainability.

3.2 Article published in *Functional Plant Biology*

CSIRO PUBLISHING

Functional Plant Biology
https://doi.org/10.1071/FP20010

Impact of crop load on nitrogen uptake and reserve mobilisation in *Vitis vinifera*

Thibaut Verdenal[A,E], *Jorge E. Spangenberg*[B], *Vivian Zufferey*[A], *Ágnes Dienes-Nagy*[A], *Olivier Viret*[C], *Cornelis van Leeuwen*[D] *and Jean-Laurent Spring*[A]

[A]Agroscope Institute, Avenue Rochettaz 21, 1009 Pully, Switzerland.
[B]Institute of Earth Surface Dynamics, University of Lausanne, 1015 Lausanne, Switzerland.
[C]Direction générale de l'agriculture, de la viticulture et des affaires vétérinaires, 1110 Morges, Switzerland.
[D]Ecophysiologie et Génomique Fonctionnelle de la Vigne (EGFV), Bordeaux Sciences Agro, Institut national de la recherche pour l'agriculture, l'alimentation et l'environnement (INRAE), Univ. Bordeaux, Institut des Sciences de la Vigne et du Vin (ISVV), 33882 Villenave d'Ornon, France.
[E]Corresponding author. Email: thibaut.verdenal@agroscope.admin.ch

Abstract. Nitrogen deficit affects both crop production and composition, particularly in crops requiring an optimal fruit N content for aroma development. The adaptation of cultural practices to improve N use efficiency (NUE) (i.e. N uptake, assimilation and partitioning) is a priority for the sustainable production of high-quality crops. A trial was set on potted grapevines (*Vitis vinifera* L. cv. Chasselas) to investigate the potential of crop limitation (via bunch thinning) to control plant NUE and ultimately fruit N composition at harvest. A large crop load gradient was imposed by bunch thinning (0.5–2.5 kg m^{-2}) and N traceability in the plant was realised with an isotope-labelling method (10 atom % ^{15}N foliar urea). The results indicate that the mobilisation of root reserves plays a major role in the balance of fruit N content. Fertiliser N uptake and assimilation appeared to be strongly stimulated by high-yielding conditions. Fertilisation largely contributed to fulfilling the high fruit N demand while limiting the mobilisation of root reserves under high yield conditions. Plants were able to modulate root N reserve mobilisation and fertiliser N uptake in function of the crop load, thus maintaining a uniform N concentration in fruits. However, the fruit free amino N profile was modified, which potentially altered the fruit aromas. These findings highlight the great capacity of plants to adapt their N metabolism to constraints, crop thinning in this case. This confirms the possibility of monitoring NUE by adapting cultural practices.

Additional keywords: crop thinning, foliar urea, grapevine, isotope labelling, N partitioning, reserve mobilisation.

Received 9 January 2020, accepted 16 March 2020, published online 12 June 2020

Introduction

Fruit composition, though partly determined by genotype and uncontrolled environmental conditions, can be managed to some extent through the optimisation of agricultural practices, such as vineyard floor management, fertilisation, canopy management and crop thinning, before and during fruit development (Masclaux-Daubresse *et al*. 2010; Sweetman *et al*. 2014; Alem *et al*. 2019). The effect of crop load on C assimilation and partitioning has been extensively studied (Chaves 1984; Morinaga *et al*. 2003; Dai *et al*. 2010). Although a higher crop load reduces root and trunk C reserves, it does not appear to affect the photoassimilation rate (Chaumont *et al*. 1994; Dayer *et al*. 2016; Reeve *et al*. 2016). In contrast to carbohydrates, it is still unclear how crop load influences N accumulation in fruits, even though N is essential for fruitfulness (number of bunches per shoot) and aroma development (Wang *et al*. 2007; Ojeda-Real *et al*. 2009; Schreiner *et al*. 2014). In grapevine (*Vitis vinifera* L.), the berry N concentration – particularly, yeast-assimilable N (YAN), including ammonium NH_4^+ and free amino N (FAN) – is a determining parameter for wine making, affecting both the alcoholic fermentation kinetics and the wine's organoleptic profile (Bell and Henschke 2005; Hannam *et al*. 2016).

Many studies have demonstrated that overcropping can delay fruit ripening (i.e. carbohydrate accumulation and acid degradation) and hence reduce fruit quality (Petrie and Clingeleffer 2006; Rutan *et al*. 2018). Therefore, crop thinning (i.e. limiting crop load by removing a proportion of fruits early in the season) has become a common practice to increase the source : sink ratio and enhance fruit maturation. Several studies have explained the impact of crop thinning on fruit composition, considering the leaf : fruit ratio as an indicator of balanced plants (Jackson and Lombard 1993; Keller *et al*.

2005; Mawdsley *et al*. 2018; Wang *et al*. 2018). In grapevines, a sufficient leaf : fruit ratio (above ~1 m^2 of exposed leaf area per kg of fruit) promotes fruit development and maturation by providing a nonlimiting source of photosynthetic carbohydrates (Kliewer and Dokoozlian 2005; Zufferey *et al*. 2015). However, an oversized canopy (caused by higher trimming height rather than higher vigour) modifies N partitioning in the plant and thus might induce a deficient N concentration in the fruits, despite proper resources being provided to the plant (Spring *et al*. 2012; Verdenal *et al*. 2016). It is known that under specific conditions, the pathways of C and N accumulation in some fruits are different. For example, under restricted water conditions, carbohydrates continue to accumulate in fruits through (partial) remobilisation of root reserves, whereas N concentration declines (Chaves 1984; Rossouw *et al*. 2017).

Predicting and modulating plant N status in perennial fruit crops requires an understanding of the seasonal movement of N within the plant. In the case of grapevine, 90% of the C reserves (mainly starch) and 75% of the N reserves (mainly amino acids) are stored in the roots of dormant vines (Bates *et al*. 2002; Zapata *et al*. 2004). C and N uptake is low for several weeks after bud burst. As a consequence, the root starch content decreases until early flowering and only then increases, with the photosynthetic carbohydrates provided from the leaves (Zapata *et al*. 2004). Similar to C, the root N reserves are the major source of N mobilised early in the season to support early shoot growth until root N uptake is sufficient to maintain growth near the flowering stage (Zapata *et al*. 2004; Schreiner 2016). Whole-vine N uptake is maximal before flowering (Schreiner 2016). The refilling of N reserves usually starts before fruit maturity and lasts until leaf senescence (Zufferey *et al*. 2015; Rossouw *et al*. 2017).

Plant N status depends on both N use efficiency (NUE) and N availability (Porro *et al*. 2010). NUE is the combination of the assimilation efficiency (which includes uptake and assimilation) and the utilisation efficiency (allocation and remobilisation) (Kant *et al*. 2011). NUE strongly depends on environmental and genetic factors. Plant growth is often limited in the natural environment by N availability, which restricts plant development (Hachiya and Sakakibara 2017). Such N restriction limits the accumulation of N in fruits, changing the fruits' FAN profile (Schreiner *et al*. 2014). Foliar urea application during veraison (i.e. the onset of fruit ripening when fruit starts accumulating total soluble sugars) increases fruit N content, thus improving their organoleptic character (Alem *et al*. 2019), without affecting plant vigour (Nisbet *et al*. 2014; Hannam *et al*. 2016; Gutiérrez-Gamboa *et al*. 2019). However, fertilisation efficiency largely depends on NUE. It has been estimated that 50–70% of N provided to crops is generally lost by leaching and volatilisation, depending on the conditions (Masclaux-Daubresse *et al*. 2010). Similar losses are reported for soil fertilisation, foliar fertilisation or both (Kant *et al*. 2011; Verdenal *et al*. 2016).

Therefore, improving NUE through the adaptation of agricultural practices is critical to enhance productivity and minimise N losses to the environment. In particular, assessment of the effect of crop load on the plant N source : sink relationship and fruit composition is essential for improving fruit quality, NUE and climate change adaptability (Boss *et al*. 2014; González-Barreiro *et al*. 2015). In this context, the aims of the study were (i) to identify how crop load strategies influence fruit N accumulation and composition, and (ii) to examine the impacts of crop load on fertiliser use efficiency and on the functional N balance between roots and fruits. These aims were accomplished by testing a large gradient of crop loads and by applying foliar ^{15}N-labelled urea to the potted white grapevine cultivar Chasselas.

Materials and methods

Experimental site

The experiment was conducted in 2017 at the Agroscope research station in Pully, Switzerland (46°30′45.8″N 6°40′05.7″E). The low-calcareous colluvial soil at the station developed on upper Oligocene (Chattian) molasse sedimentary rocks and is composed of clay (15wt %), silt (38wt %), sand (47wt %) and carbonates (4.3wt % equivalent $CaCO_3$). The soil pH was 7.9 and the humus content was 1.75%. Phosphorus (8.2 mg kg^{-1}), K (25.2 mg kg^{-1}) and Mg (11.4 mg kg^{-1}) were not deficient for vine growing. This soil was used as the growth medium in the pots. The soil water-holding capacity in the pot was 11 L. The climate in this region is classified as warm and temperate (Köppen–Geiger classification Cfb; Peel *et al*. 2007). During the grapevine growing season (April–October), the daily mean temperature ranged between 4.3°C (19 April) and 27.6°C (3 August), averaging 16.6°C; the total precipitation during that period was 562 mm (data from the Swiss meteorological station in Pully). An important amount of precipitation (252 mm) was received between 25 April and 6 June during the early stage of plant growth (before flowering). The plant water potential was measured regularly with a pressure chamber (Model 600, PMS Instruments) to prevent eventual water restriction (Scholander *et al*. 1965). The vines were drip-irrigated (6 L per plant) twice in July (10 and 17 July) when the stem water potential was below –0.8 MPa.

Plant material

Vitis vinifera L. cv. Chasselas cultivars were grafted onto 3309C rootstock and planted in 2013 in 90-L underground pots with a planting density of 8330 vines ha^{-1} (1.5 × 0.8 m). The pot size allowed the unconstrained development of the roots. Planting in pots was chosen to ensure good recovery of the root biomass. The vines were grown with a vertical shoot positioning system (single Guyot) with a trunk height of 60 cm and seven shoots per plant. In 2017, the phenological stages of bud burst (phenological stage 01 on the BBCH-scale, Lancashire *et al*. 1991), flowering (BBCH 65) and veraison (BBCH 85) occurred on the days of year (DOY) 84, 164 and 214 respectively. The canopy was trimmed to a height of 1.2 m and the lateral shoots were removed from the bunch area following common practices. Harvest was performed on DOY 257. Three out of the 24 vines were discarded from

the experiment because of outlier behaviour, such as unusually low fruitfulness, low berry set and small bunches, and poor plant development (vigour). These outlier vines had extremely low total N (TN) content (<0.5% DW) and YAN (<90 mg L^{-1}).

Crop load and ^{15}N labelling treatments

The plot was divided in two homogeneous blocks of 12 vines, namely the control and fertilised blocks. Each block consisted of three rows of four vines. Buffer vines separated the blocks to minimise fertiliser cross-contamination. In each block, three crop load conditions (one per row) were set by crop thinning at bunch closure (phenological stage BBCH 77, DOY 193, which is a standard time for crop thinning), maintaining two, five or eight bunches per plant, with the aim of building a large crop load gradient. For statistical purposes, the vines from each block were gathered in two groups of six vines each: low-yielding conditions (LYC) and high-yielding conditions (HYC), based on the yield per vine at harvest. Each vine was considered as a replicate. The threshold used to separate the two groups was set at 1.3 kg m^{-2}, which represents an average crop load for Chasselas in the region. The vines of the fertilised block received N during veraison (onset of maturation, BBCH 85) in four applications (DOY 199, 208, 214 and 226), for a total of 20 kg N ha^{-1} of ^{15}N-labelled urea (10 atom % ^{15}N, Sigma-Aldrich). The labelled foliar urea was carefully applied on both sides of the canopy with two hand-sprayers (Birchmeier). Besides the urea application in the fertilised treatment, the soil was the only source of nutrients.

Field measurements and sample preparation

For each vine row per treatment, the average chlorophyll index, the average light-exposed leaf area and average leaf mineral content were determined. The chlorophyll index was determined on primary leaves from the medial part of the canopy (n = 30, DOY 227) with an N-tester (Yara International) (Spring and Zufferey 2000). The leaf mineral content (i.e. total N, P, K, Ca and Mg) was determined by analysing the powder obtained from eight dried leaves (two per vine) sampled on DOY 236. Total N was determined by the Kjeldahl method (Method 5.3.2MV004, Sol-Conseil) and the other elements were determined by inductively coupled plasma–optical emission spectroscopy after acid digestion (Methods 5.3.2MV005, -6 and -7). The concentrations were expressed as % DW.

The light-exposed leaf area (m^2 m^{-2} of ground) was calculated on DOY 237 from the measured canopy height, width and porosity via the method of Carbonneau (1995) only once per treatment, since the percentage of holes could not be estimated for each vine separately. For each vine, the total leaf area was assessed via a nondestructive approach, based on the strong correlation between shoot length and total leaf area (Mabrouk and Carbonneau 1996). The correlation equation in the context of our experiment was determined as follows. Fifteen shoots from 15 different buffer plants were collected on DOY 206. The total shoot length (*TSL*, main shoot + laterals) was measured and the total leaf area was determined with a leaf area meter (LI-3100C, Li-Cor Biosciences). As a result, Eqn 1 allowed the estimation of the total leaf area (*TLA*) from the *TSL*:

$$TLA = 14.4 \times TSL + 161.5. \quad (1)$$

Leaf gas exchange was measured for one fully expanded leaf per vine on sunny days approximately every 10 days from flowering (BBCH 65, DOY 164) to harvest (BBCH 89, DOY 257). Photosynthesis (μmol m^{-2}), transpiration (mol m^{-2} s^{-1}), stomatal conductance (mol m^{-2} s^{-1}), ambient CO_2 concentration (μmol mol^{-1}) and internal CO_2 concentration (μmol mol^{-1}) were determined with a portable photosynthesis system (LI-6800, Li-Cor Biosciences). The shoot trimmings were collected three times (DOY 164, 191 and 215), weighed to determine the fresh weight (FW, g per vine) and then combined with the rest of canopy recovered at the time of excavation. Vine fruitfulness was determined before crop thinning and expressed as the number of bunches per shoot.

At harvest (DOY 257), the grape yield (kg m^{-2}) and the leaf : fruit ratio (light-exposed leaf area per kg of fruit) were determined per vine. The grapes were harvested and each vine was excavated separately and split into parts, including the roots, the trunk (including the cane), the canopy (including trimmings collected during the season) and the fruits. The grape bunches were pressed manually to separate the must from the pomace. The five plant parts (roots, trunk, canopy, pomace and must) were weighed to determine FW. Must aliquots were taken for chemical (100 g) and stable isotope analysis (25 g). The plant parts were dried in a 60°C oven until a constant weight, excluding the must, which was freeze-dried, for determination of the DW and were then powdered (<1300 μm).

Stable isotope analysis

The stable C and N isotope compositions of plant parts were determined by elemental analysis and isotope ratio MS with a Carlo Erba 1108 elemental analyser (Fisons Instruments) connected via a Conflo III interface to a Delta V Plus isotope ratio mass spectrometer (Thermo Fisher Scientific). The stable isotope compositions are reported in the δ notation (i.e. $\delta^{13}C$ and $\delta^{15}N$ values, in variations relative to international measurement standards) (Coplen 2011):

$$\delta^{i}E_{\text{sample}} = \frac{R\left(\frac{^{i}E}{^{j}E}\right)_{\text{sample}}}{R\left(\frac{^{i}E}{^{j}E}\right)_{\text{standard}}} - 1, \quad (2)$$

where R is the molar ratio of the heaviest (^{i}E) to the lightest (^{j}E) most abundant isotopes of chemical element E (e.g. ^{13}C : ^{12}C, ^{15}N : ^{14}N). The stable isotope standard for C is Vienna Pee Dee Belemnite limestone, and the standard for N is atmospheric molecular N (Coplen 2011). All isotopic analyses were performed in duplicate. The δ values are reported in milliurey (mUr) rather than ‰, in conformity with the International System of Units and according to the guidelines and recommendations of the International Union of Pure and Applied Chemistry (Coplen 2011; Brand 2011).

For calibration and normalisation of the measured isotopic ratios to the international scales (LSVEC lithium carbonate

scale for $\delta^{13}C$, atmospheric molecular N scale for $\delta^{15}N$), a three- to four-point calibration was used with international reference materials and six in-house urea standards (UNIL-Urea 1 to 6) at different ^{13}C and ^{15}N natural abundances and different ^{15}N enrichments (described in Spangenberg and Zufferey 2019). The $\delta^{13}C$ and $\delta^{15}N$ values of the in-house standards with natural ^{13}C and ^{15}N abundances were determined and normalised with the reference materials for glycines USGS64 ($\delta^{13}C$ = −40.81 mUr, $\delta^{15}n$ = 1.76 mUr), USGS65 ($\delta^{13}C$ = −20.29 mUr, $\delta^{15}n$ = 20.68 mUr) and USGS66 ($\delta^{13}C$ = −0.67 mUr, $\delta^{15}n$ = 40.83 mUr) as described by Schimmelmann *et al*. (2016). The ^{15}N-enriched standards were normalised with the reference materials USGS40 ($\delta^{15}n$ = –4.5 mUr), USGS41 ($\delta^{15}n$ = 47.6 mUr), USGS65, USGS66, IAEA 600 ($\delta^{15}n$ = 1.02 mUr), IAEA 310A ($\delta^{15}n$ = 47 mUr) and IAEA 310B ($\delta^{15}n$ = 245 mUr). For natural abundances, the repeatability and intermediate precision were better than 0.1 mUr (1 s.d.) for both $\delta^{13}C$ and $\delta^{15}N$. For the ^{15}N-enriched samples, the reproducibility of the $\delta^{15}N$ values was 2 mUr. The total organic C (TOC) and TN concentrations (in wt %) were determined from the peak areas of the major isotopes with the calibrations for $\delta^{13}C$ and $\delta^{15}N$. The repeatability was better than 0.2wt % for the TOC and TN contents.

Fruit composition

The fresh must aliquot for chemical analysis was centrifuged (2200*g*), the pH was measured and the content of total soluble solids (Brix), titratable acidity (expressed as g L^{-1} tartaric acid), and tartaric and malic acid contents (g L^{-1}) were determined by an infrared spectrophotometer (WineScan, FOSS NIR Systems).

Free amino acids were quantified (after 1 : 100 dilution of the aliquot) by ultrahigh-performance liquid chromatography–MS in an Infinity 1290 UPLC system connected to an Agilent 6460-C Triple Quadrupole LC-MS with electrospray positive ionisation (ESI^+) (Agilent Technologies). Chromatographic separation was performed on an Intrada AA column (50 × 3 mm, Imtakt) via the TI737E method detailed in the manufacturer's instructions. Detection was performed by multiple reaction monitoring. External calibration was performed using standards for each amino acid separately according to their abundance, either in the range of 1.5–15.0 µmol L^{-1} for amino acids below 3% abundance or in the range of 15.0–150.0 µmol L^{-1} for those above 3% abundance. Standards were prepared by dissolving amino acids in acidified water (0.2 M HCl). The repeatability of the values was better than 5% and 10% for low and high abundance respectively. The amino acid concentrations were reported in mg N L^{-1}. Ammonium was quantified with an enzymatic test kit (Boehringer Mannheim GmbH). The total FAN concentration was determined via the o-phthaldialdehyde (OPA) method using the Primary Amino Nitrogen kit (Bio Systems). Total YAN was computed by summing the NH_4^+ content (expressed in mg N L^{-1}) and primary FAN (excluding the secondary amino acids proline and hydroxyproline) (Bell and Henschke 2005).

Data treatment and statistical analysis

The N quantity (*NQ*, in g) in each organ was calculated as:

$$NQ_{\text{organ}} = DW_{\text{organ}} \times TN. \quad (3)$$

The abundance of ^{15}N (*A*%), which was the proportion of heavy isotopes per 100 atoms, was calculated as follows (Deléens *et al*. 1994):

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

The relative specific abundance (*RSA*, in %), which was the proportion of newly incorporated N atoms relative to total N atoms, was calculated as follows (Deléens *et al*. 1994):

$$RSA = \frac{A\%_{\text{sample\_enrichment}}}{A\%_{\text{nutrient\_enrichment}}} = \frac{A\%_{\text{sample}} - A\%_{\text{control}}}{A\%_{\text{nutrient}} - A\%_{\text{control}}}. \quad (5)$$

In our case, $A\%_{\text{nutrient}}$ = 10. The RSA represents the organ sink strength, which is independent of the organ size (Deléens *et al*. 1997):

The new N pool (*NNP*, in g) for each organ was calculated as follows:

$$NNP_{\text{organ}} = RSA_{\text{organ}} \times NQ_{\text{organ}}. \quad (6)$$

Thus, the percent proportion (%*P*) of new N in an organ, also called partitioning, was calculated as:

$$\%P_{organ} = \frac{NNP_{organ}}{NNP_{vine}} \times 100. \quad (7)$$

The results are presented as the average ± s.d. The statistical analysis was performed with XLSTAT ver. 2018.1.50011 (Addinsoft). The significance of the differences between treatments was evaluated with ANOVA ($P < 0.05$) and the Newman–Keuls *post hoc* test. Principal component analysis was used to evaluate the FAN composition.

Results

Vegetative growth and fruit development

From bud burst to harvest, the canopy reached 1145 ± 360 g per plant on average. A large yield gradient was obtained, spanning from a minimum of 0.5 to a maximum 2.5 kg m^{-2} (Table 1). Consequently, the leaf : fruit ratio varied from a minimum of 0.5 to a maximum of 2.4 m^2 kg^{-1}, depending on the crop load. The vigour was assessed by the canopy weight and was heterogeneous. The bunch and berry weights were correlated with vigour (r = 0.71, P = 0.015 and r = 0.70, P = 0.017 respectively). However, vigour was correlated with neither crop load nor fertilisation. Indeed, crop load was manually controlled by bunch thinning and urea was applied late in the season when the canopy was already developed.

No significant change arising from N fertilisation or crop load treatments was observed in terms of photosynthetic activity and gas exchange per unit of leaf area (Table S1, available as Supplementary Material to this paper). The

Table 1. Effect of crop load and urea treatment on the plant physiological parameters and grape must composition

Average ± 1 s.d. for Chasselas vines in 2017 at Pully, Switzerland. HYC, high-yielding conditions; LYC, low-yielding conditions; ns, nonsignificant; FAN, free amino N; YAN, yeast-assimilable N; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Variable		LYC		*P*-value	HYC		*P*-value	Control vines		*P*-value	N-fertilised vines		*P*-value	Interaction of yield condition × fertilisation
		Control vines ($n = 5$)	N-fertilised vines ($n = 4$)		Control vines ($n = 6$)	N-fertilised vines ($n = 6$)		LYC ($n = 5$)	HYC ($n = 6$)		LYC ($n = 4$)	HYC ($n = 6$)		
Plant measurements	Fruitfulness (bunches per shoot)	2.3 ± 0.3	1.9 ± 0.2	ns	2.2 ± 0.5	2.0 ± 0.3	ns	2.3 ± 0.3	2.2 ± 0.5	ns	1.9 ± 0.2	2.0 ± 0.3	ns	ns
	Yield (kg m^{-2})	0.8 ± 0.3	0.8 ± 0.2	ns	1.9 ± 0.4	1.6 ± 0.3	ns	0.9 ± 0.2	2.3 ± 0.4	***	0.9 ± 0.1	2.0 ± 0.4	**	ns
	Bunches per vine	3.2 ± 1.8	4.0 ± 3.4	ns	6.8 ± 2.6	6.0 ± 1.7	ns	3.2 ± 1.8	6.8 ± 2.6	*	4.0 ± 3.4	6.0 ± 1.7	ns	ns
	Bunch weight (g)	334 ± 138	305 ± 156	ns	370 ± 118	350 ± 81	ns	334 ± 138	370 ± 118	ns	306 ± 156	350 ± 81	ns	ns
	Berry weight (g)	3.4 ± 0.3	3.0 ± 0.3	ns	3.5 ± 0.3	3.4 ± 0.3	ns	3.4 ± 0.3	3.5 ± 0.3	ns	3.0 ± 0.3	3.4 ± 0.3	ns	ns
	Total leaf area (m^2 per plant)	1.8 ± 0.5	1.7 ± 0.7	ns	1.8 ± 0.4	2.1 ± 0.3	ns	1.8 ± 0.5	1.8 ± 0.4	ns	1.7 ± 0.7	2.1 ± 0.3	ns	ns
	Leaf : fruit ratio (m^2 kg^{-1})	1.7 ± 0.2	1.6 ± 0.2	ns	0.7 ± 0.5	0.7 ± 0.3	ns	1.7 ± 0.5	0.7 ± 0.2	***	1.6 ± 0.3	0.7 ± 0.2	***	ns
	Canopy weight (g per plant^{-1})	1095 ± 388	1026 ± 568	ns	1175 ± 332	1237 ± 262	ns	1095 ± 388	1175 ± 332	ns	1026 ± 569	1237 ± 262	ns	ns
Must composition at harvest	Total soluble solids (Brix)	19.0 ± 1.1	19.8 ± 0.2	ns	18.8 ± 0.8	19.7 ± 0.6	ns	19.0 ± 1.1	18.8 ± 0.8	ns	19.8 ± 0.2	19.7 ± 0.6	ns	ns
	pH	3.4 ± 0.1	3.4 ± 0.1	ns	3.3 ± 0.1	3.4 ± 0.0	ns	3.4 ± 0.1	3.3 ± 0.1	ns	3.4 ± 0.1	3.4 ± 0.0	ns	ns
	Titratable acidity (g L^{-1})	6.3 ± 0.4	5.9 ± 0.3	ns	6.4 ± 0.5	6.2 ± 0.2	ns	6.3 ± 0.4	6.4 ± 0.5	ns	5.9 ± 0.3	6.2 ± 0.2	ns	ns
	Tartaric acid (g L^{-1})	5.6 ± 0.2	5.6 ± 0.2	ns	5.6 ± 0.1	5.7 ± 0.2	ns	5.6 ± 0.2	5.6 ± 0.1	ns	5.6 ± 0.2	5.7 ± 0.2	ns	ns
	Malic acid (g L^{-1})	3.2 ± 0.2	2.9 ± 0.6	ns	3.2 ± 0.4	3.2 ± 0.3	ns	3.2 ± 0.2	3.2 ± 0.4	ns	2.9 ± 0.6	3.2 ± 0.3	ns	ns
	K (mg L^{-1})	1743 ± 156	1719 ± 250	ns	1630 ± 111	1702 ± 100	ns	1684 ± 156	1635 ± 111	ns	1646 ± 250	1650 ± 100	ns	ns
	NH_3 (mg L^{-1})	51 ± 13	41 ± 8	ns	38 ± 20	53 ± 14	ns	51 ± 13	38 ± 20	ns	41 ± 8	53 ± 14	ns	ns
	FAN (mg N L^{-1})	146 ± 51	155 ± 33	ns	131 ± 26	174 ± 19	**	146 ± 51	131 ± 26	ns	155 ± 33	174 ± 19	ns	ns
	YAN (mg N L^{-1})	188 ± 49	189 ± 35	ns	162 ± 40	217 ± 30	*	188 ± 49	162 ± 39	ns	189 ± 35	217 ± 30	ns	ns

average chlorophyll index was homogenous and independent of fertilisation and crop load (489 ± 22 at veraison). The leaf nutrient content was constant (averages: 2.15% DW TN, 0.2% DW P, 2.9% DW Ca and 0.2% DW Mg) and not restrictive for vine development, according to the thresholds defined for Chasselas (Sinaj and Richner 2017). In contrast, the leaf K concentration was strongly related to the bunch number ($r = -0.91$, $P = 0.013$). Leaf K was not restrictive under LYC (1.7% DW K for 2.2 ± 0.4 bunches per vine) and was lower and restrictive under high-yielding conditions (HYC, 1.2% DW K for 8.1 ± 1.5 bunches vine^{-1}). Leaf K was also positively correlated with bunch weight ($r = 0.90$, $P = 0.015$) and canopy weight ($r = 0.89$, $P = 0.018$) (data not shown).

Dry weight, total organic C, $\delta^{13}C$ and C : N ratio

The DW, TOC, TN and C and N isotope compositions of each plant part are statistically compared and presented in Table S2. The results are similar to the ones presented in others studies (Zapata *et al*. 2004; Schreiner 2016). For the control vines, the whole biomass was significantly higher under HYC than under LYC (Table 2). The largest difference was observed in the pomace and must DWs. Under HYC, the root DW was 27% lower and canopy DW was 8% higher than those under LYC; these differences were not significant because of vine-to-vine variability. Similar trends were observed in the N-fertilised vines. The whole-plant TOC was significantly lower under HYC; it decreased in grapes (must and pomace) and increased in the trunk, although there was no variation in the roots and canopy. No difference was observed between LYC and HYC in the N-fertilised vines. N fertilisation only affected the pomace DW and the root TOC. The $\delta^{13}C$ values varied insignificantly between a minimum of –29.2 mUr and a maximum of –28.0 mUr in all plant parts (organs and must) (Table 2). In the roots of the control vines, the C : N ratio was 16% higher in the vines under HYC than under LYC, whereas it was 27% lower under the urea treatment (Table 2). Under LYC, the must and the trunk were the plant parts with the highest C : N ratio at harvest. However, under HYC, the trunk had a lower C : N ratio (118 under HYC vs 159 under LYC) (Table S2). Differences in the C : N ratios in grapes for the different crop loads and fertilisation conditions were not significant because of the high vine-to-vine variability of TN and TOC.

Total N, NQ and $\delta^{15}N$

The canopy was the most concentrated plant part in terms of TN (1.4 and 1.3% DW under LYC and HYC respectively) and the must the least concentrated (0.3 and 0.2% DW under LYC and HYC respectively) (Table S2). In the control vines, only the TN in the trunk behaved differently between HYC and LYC compared with the other plant parts; the TN was 29% higher ($P = 0.033$) in the trunk of vines under HYC but there was no significant difference in the other plant parts (Table 3). In N-fertilised vines, only the roots had 20% more TN under HYC than under LYC (Table 3). For the vines under HYC, N fertilisation increased the TN by 34% ($P = 0.003$) and the NQ by 51% ($P = 0.023$) compared with the

Table 2. Effects and interactions of the crop load and N fertilisation on the DW, total organic C (TOC), C isotope composition ($\delta^{13}C$) and C : N ratio in the different plants parts at harvest
Average ± 1 s.d. for Chasselas vines at Pully in 2017. HYC, high-yielding conditions; LYC, low-yielding conditions; ns, nonsignificant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Variable	Organ	LYC			HYC			Control vines			N-fertilised vines			Interaction of
		Control vines ($n = 5$)	N-fertilised vines ($n = 4$)	*P*-value	Control vines ($n = 6$)	N-fertilised vines ($n = 6$)	*P*-value	LYC ($n = 5$)	HYC ($n = 6$)	*P*-value	LYC ($n = 4$)	HYC ($n = 6$)	*P*-value	yield condition × fertilisation
DW (g)	Roots	260 ± 25	265 ± 75	ns	216 ± 59	229 ± 16	ns	260 ± 25	216 ± 59	ns	265 ± 75	229 ± 16	ns	ns
	Trunk	291 ± 36	297 ± 62	ns	277 ± 49	300 ± 31	ns	291 ± 36	277 ± 49	ns	297 ± 62	300 ± 31	ns	ns
	Canopy	382 ± 122	385 ± 214	ns	413 ± 112	445 ± 84	ns	382 ± 122	413 ± 112	ns	385 ± 214	445 ± 84	ns	ns
	Pomace	75 ± 28	58 ± 4	ns	240 ± 61	149 ± 31	**	75 ± 28	240 ± 61	***	58 ± 4	149 ± 31	***	ns
	Must	117 ± 26	143 ± 41	ns	249 ± 53	288 ± 63	ns	117 ± 26	249 ± 53	***	143 ± 41	288 ± 63	**	ns
	Whole plant	1124 ± 140	1147 ± 307	ns	1394 ± 196	1411 ± 163	ns	1124 ± 140	1394 ± 196	*	1147 ± 307	1411 ± 163	ns	ns
TOC (% DW)	Roots	48.3 ± 0.9	48.2 ± 0.6	ns	49.5 ± 0.9	47.6 ± 0.8	**	48.3 ± 0.9	49.5 ± 0.9	ns	48.2 ± 0.6	47.6 ± 0.8	ns	**
	Trunk	46.7 ± 0.4	46.4 ± 0.9	ns	47.3 ± 0.5	46.7 ± 1.4	ns	46.7 ± 0.4	47.3 ± 0.5	*	46.4 ± 0.9	46.7 ± 1.4	ns	ns
	Canopy	45.4 ± 0.6	44.1 ± 2.1	ns	45.7 ± 1.0	45.4 ± 0.5	ns	45.4 ± 0.6	45.7 ± 1.0	ns	44.1 ± 2.1	45.4 ± 0.5	ns	ns
	Pomace	45.1 ± 0.5	43.7 ± 1.4	ns	43.2 ± 1.0	43.5 ± 0.7	ns	45.1 ± 0.5	43.2 ± 1.0	**	43.7 ± 1.4	43.5 ± 0.7	ns	ns
	Must	38.5 ± 0.4	37.7 ± 0.9	ns	37.4 ± 0.8	37.8 ± 0.6	ns	38.5 ± 0.4	37.4 ± 0.8	*	37.7 ± 0.9	37.8 ± 0.6	ns	*
	Whole plant	45.7 ± 0.2	45.4 ± 0.2	ns	44.2 ± 0.8	44.6 ± 0.4	ns	45.7 ± 0.2	44.2 ± 0.8	**	45.4 ± 0.2	44.6 ± 0.4	ns	ns
$\delta^{13}C$ (mUr)	Roots	–28.6 ± 0.3	–28.1 ± 0.6	ns	–28.4 ± 0.3	–28.1 ± 0.5	ns	–28.6 ± 0.3	–28.4 ± 0.3	ns	–28.1 ± 0.6	–28.1 ± 0.5	ns	ns
	Trunk	–28.2 ± 0.2	–28.4 ± 0.4	ns	–28.0 ± 0.1	-28.1 ± 0.3	ns	–28.2 ± 0.2	–28.0 ± 0.1	ns	–28.4 ± 0.4	–28.1 ± 0.3	ns	ns
	Canopy	–29.2 ± 0.7	–28.6 ± 0.1	ns	–29.0 ± 0.7	–29.1 ± 0.4	ns	–29.2 ± 0.7	–29.0 ± 0.7	ns	–28.6 ± 0.1	–29.1 ± 0.4	ns	ns
	Pomace	–28.8 ± 0.5	–29.1 ± 1.0	ns	–28.9 ± 0.8	-28.8 ± 0.2	ns	–28.8 ± 0.5	–28.9 ± 0.8	ns	–29.1 ± 1.0	–28.8 ± 0.2	ns	ns
	Must	–28.1 ± 0.6	–28.9 ± 1.2	ns	–28.1 ± 0.8	–28.5 ± 0.4	ns	–28.1 ± 0.6	–28.1 ± 0.8	ns	–28.9 ± 1.2	–28.5 ± 0.4	ns	ns
C : N ratio	Roots	64 ± 7	86 ± 4	***	74 ± 7	71 ± 2	ns	64 ± 7	74 ± 7	*	86 ± 4	71 ± 2	***	***
	Trunk	159 ± 39	111 ± 31	ns	117 ± 7	110 ± 16	ns	159 ± 39	117 ± 7	*	111 ± 31	110 ± 16	ns	ns
	Canopy	33 ± 3	35 ± 3	ns	35 ± 4	32 ± 3	ns	33 ± 3	35 ± 4	ns	35 ± 3	32 ± 3	ns	ns
	Pomace	44 ± 11	38 ± 2	ns	50 ± 6	37 ± 4	**	44 ± 11	50 ± 6	ns	38 ± 2	37 ± 4	ns	ns
	Must	150 ± 26	154 ± 39	ns	175 ± 32	129 ± 11	**	133 ± 52	175 ± 32	ns	154 ± 39	129 ± 11	ns	ns
	Whole plant	56 ± 8	54 ± 7	ns	59 ± 7	58 ± 12	ns	56 ± 8	59 ± 7	ns	61 ± 14	53 ± 5	ns	ns

Table 3. Effects and interactions of the crop load and N fertilisation on total N (TN), N isotope composition ($\delta^{15}N$) and N quantity (NQ) in the different plants parts at harvest
Average ± 1 s.d. for Chasselas vines at Pully in 2017. HYC, high-yielding conditions; LYC, low-yielding conditions; ns, nonsignificant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Variable	Organ	LYC			HYC			Control vines			N-fertilised vines			Interaction of
		Control vines ($n = 5$)	N-fertilised vines ($n = 4$)	*P*-value	Control vines ($n = 6$)	N-fertilised vines ($n = 6$)	*P*-value	LYC ($n = 5$)	HYC ($n = 6$)	*P*-value	LYC ($n = 4$)	HYC ($n = 6$)	*P*-value	yield condition × fertilisation
TN (% DW)	Roots	0.77 ± 0.13	0.56 ± 0.03	**	0.67 ± 0.07	0.67 ± 0.02	ns	0.77 ± 0.13	0.67 ± 0.07	ns	0.56 ± 0.03	0.67 ± 0.02	***	**
	Trunk	0.31 ± 0.09	0.44 ± 0.09	ns	0.40 ± 0.03	0.43 ± 0.06	ns	0.31 ± 0.09	0.40 ± 0.03	*	0.44 ± 0.09	0.43 ± 0.06	ns	ns
	Canopy	1.38 ± 0.13	1.34 ± 0.12	ns	1.33 ± 0.16	1.15 ± 0.13	ns	1.38 ± 0.13	1.33 ± 0.16	ns	1.34 ± 0.12	1.15 ± 0.13	ns	ns
	Pomace	1.09 ± 0.28	1.16 ± 0.10	ns	0.87 ± 0.11	1.19 ± 0.12	***	1.09 ± 0.28	0.87 ± 0.11	ns	1.16 ± 0.10	1.19 ± 0.12	ns	ns
	Must	0.35 ± 0.22	0.26 ± 0.07	ns	0.22 ± 0.04	0.29 ± 0.03	**	0.26 ± 0.04	0.22 ± 0.04	ns	0.26 ± 0.07	0.29 ± 0.03	ns	ns
	Whole plant	0.84 ± 0.13	0.77 ± 0.17	ns	0.77 ± 0.10	0.82 ± 0.07	ns	0.84 ± 0.13	0.77 ± 0.10	ns	0.77 ± 0.17	0.82 ± 0.07	ns	ns
$\delta^{15}N$ (mUr)	Roots	11 ± 5	2088 ± 344	***	7 ± 4	1408 ± 278	***	11 ± 5	7 ± 4	ns	2088 ± 344	1408 ± 278	ns	**
	Trunk	13 ± 5	1708 ± 473	***	12 ± 10	1411 ± 414	***	13 ± 5	12 ± 10	ns	1708 ± 473	1411 ± 414	ns	ns
	Canopy	13 ± 4	1874 ± 558	***	15 ± 9	2110 ± 312	***	13 ± 4	15 ± 9	ns	1874 ± 558	2110 ± 312	ns	ns
	Pomace	24 ± 10	2748 ± 490	***	27 ± 20	2295 ± 575	***	24 ± 10	27 ± 20	ns	2748 ± 490	2295 ± 575	ns	ns
	Must	19 ± 11	3444 ± 690	***	34 ± 20	2683 ± 710	***	19 ± 11	34 ± 20	ns	3444 ± 690	2683 ± 710	*	ns
NQ (g)	Roots	1.98 ± 0.45	1.47 ± 0.38	*	1.45 ± 0.41	1.54 ± 0.10	ns	1.98 ± 0.45	1.45 ± 0.41	*	1.47 ± 0.38	1.54 ± 0.10	ns	**
	Trunk	0.88 ± 0.15	1.33 ± 0.49	ns	1.12 ± 0.20	1.30 ± 0.24	ns	0.88 ± 0.15	1.12 ± 0.20	ns	1.33 ± 0.49	1.30 ± 0.24	ns	ns
	Canopy	5.36 ± 2.07	5.32 ± 3.37	ns	5.59 ± 2.25	9.24 ± 1.53	ns	5.36 ± 2.07	5.59 ± 2.25	ns	5.32 ± 3.37	6.51 ± 1.53	ns	ns
	Pomace	0.77 ± 0.17	0.67 ± 0.07	ns	2.12 ± 0.74	1.77 ± 0.42	ns	0.77 ± 0.17	2.12 ± 0.74	**	0.67 ± 0.07	1.77 ± 0.42	***	ns
	Must	0.40 ± 0.20	0.35 ± 0.02	ns	0.56 ± 0.19	0.84 ± 0.18	*	0.40 ± 0.20	0.56 ± 0.19	ns	0.35 ± 0.02	0.84 ± 0.18	***	*
	Whole plant	9.60 ± 2.49	9.15 ± 4.17	ns	10.84 ± 3.10	11.97 ± 1.99	ns	9.60 ± 2.49	10.84 ± 3.10	ns	9.15 ± 4.17	11.97 ± 1.99	ns	ns

control vines (Table 3, Fig. 1). No significant differences were observed in the vines under LYC as a result of fertilisation. These trends mimic those observed for the YAN content. HYC increased the NQ in grapes (particularly in the pomace), independent of N fertilisation. The NQ was lower by 27% in the roots of control vines under HYC compared with those under LYC. In the control vines, the NQ increased in grapes under HYC (P = 0.006), whereas it decreased in the roots (P = 0.026). In contrast, the NQ was not depleted in the roots of the N-fertilised vines. (Table 3). In the control vines, under both yield conditions, the $\delta^{15}N$ values increased gradually from the roots (7 ± 4 mUr) to grapes (34 ± 20 mUr). In the fertilised vines, the $\delta^{15}N$ values were lower in the must under HYC, through the variation was insignificant in the other plant parts.

Foliar N assimilation, relative specific abundance and partitioning

The fertiliser N uptake was 26% of the total amount applied in the vines under LYC and 37% in the vines under HYC (Fig. 2*a*). Indeed, the fertiliser N uptake was also a function of vine vigour (Fig. 2*b*). With nearly 20% of N originating from the urea application, the grapes (pomace + must) had the largest relative specific abundance (RSA) (i.e. the proportion of newly incorporated N atoms relative to total N atoms, in %) among all plant parts, regardless of the crop load (Table 4). The root RSA was 37% lower in the vines under HYC than under LYC (P = 0.009); there were no significant changes in the other plant parts. Under HYC, the new N pool was 41% higher for the whole plant (P = 0.023) and increased by 109% in grapes (pomace + must, P = 0.002), whereas it decreased by 27% in the roots (P = 0.063) and 11% in the trunk (P = 0.232) (Table 4, Fig. 3). Compared with LYC, the partitioning of new N under HYC was 50% lower in the roots and 39% lower in the trunk of the vines (both P = 0.001).

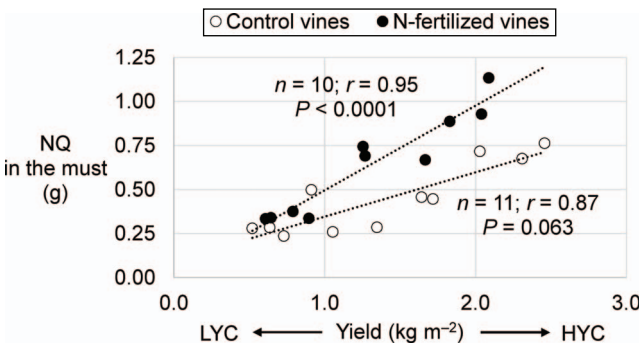


Fig. 1. Effect of N fertilisation on the total organic N quantity (NQ) in the must at harvest, in relation to the yield for Chasselas vines in 2017 at Pully, Switzerland.

Fruit composition

The total soluble solids (average 19.3 ± 0.8 Brix), titratable acidity (6.2 ± 0.4 g L^{-1}), tartaric acid (5.6 ± 0.2 g L^{-1}), malic acid (3.1 ± 0.4 g L^{-1}), potassium (1694 ± 148 mg L^{-1}),

Table 4. Effect of crop load on new N relative specific abundance (RSA), new N quantity (New N pool) and new N partitioning

Average ± 1 s.d. for Chasselas vines in 2017 at Pully, Switzerland. HYC, high-yielding conditions; LYC, low-yielding conditions; TN, total N; ns, nonsignificant; *, P < 0.05; **, P < 0.01; ***, P < 0.001

Variable	Organ	LYC (n = 4)	HYC (n = 6)	P-value
RSA (% TN)	Roots	8 ± 1	5 ± 1	**
	Trunk	6 ± 2	5 ± 2	ns
	Canopy	7 ± 2	8 ± 1	ns
	Pomace	10 ± 2	9 ± 2	ns
	Must	13 ± 3	10 ± 3	ns
	Whole plant	8 ± 2	7 ± 1	ns
New N pool (g)	Roots	0.11 ± 0.03	0.08 ± 0.02	ns
	Trunk	0.08 ± 0.02	0.07 ± 0.01	ns
	Canopy	0.32 ± 0.12	0.51 ± 0.15	ns
	Pomace	0.07 ± 0.01	0.15 ± 0.03	***
	Must	0.04 ± 0.01	0.08 ± 0.03	*
	Whole plant	0.63 ± 0.15	0.89 ± 0.14	*
Partitioning (%)	Roots	18 ± 3	9 ± 2	***
	Trunk	13 ± 1	8 ± 2	***
	Canopy	50 ± 8	57 ± 10	ns
	Pomace	12 ± 5	17 ± 4	ns
	Must	8 ± 3	10 ± 4	ns

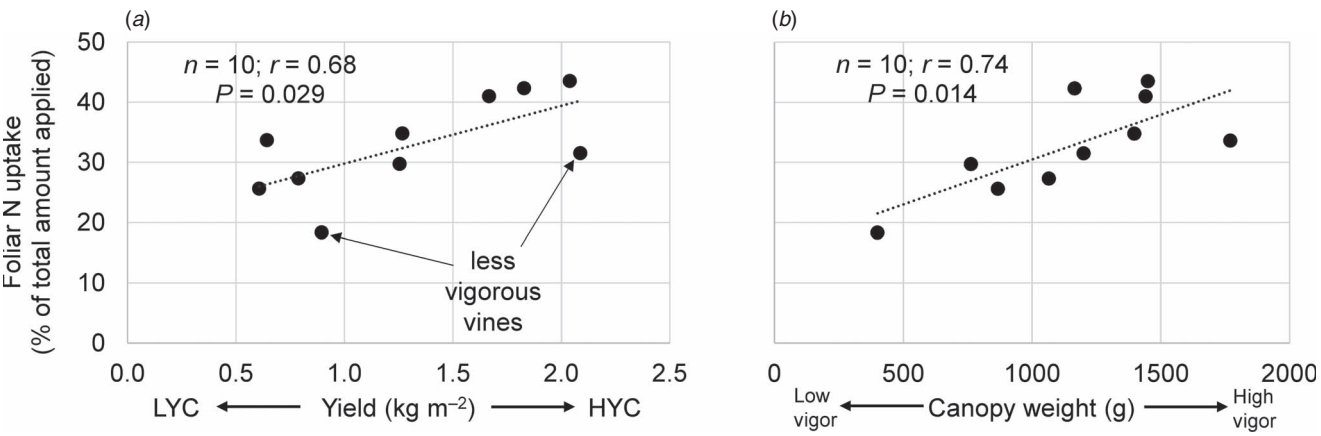


Fig. 2. Effect of (*a*) the yield and (*b*) the canopy weight on the uptake of fertiliser N applied at veraison for Chasselas vines in 2017 at Pully, Switzerland. LYC, low-yielding conditions; HYC, high-yielding conditions. The two dots below the trend line in (*a*) correspond to less vigorous grapevines.

ammonium (46 ± 6 mg L^{-1}) and amino acid (151 ± 35 mg N L^{-1}) levels and the pH (3.4 ± 0.1) remained uniform in the must despite different crop loads (Table 1). N fertilisation increased the must YAN concentration (+55 mg L^{-1}), particularly the FAN concentration (+43 mg N L^{-1}) in the HYC vines, although it had no effect on the vines under LYC (Table 1). The YAN concentration was correlated with plant vigour; the correlation was higher for N-fertilised vines (r = 0.82 vs r = 0.55 for the control vines; Fig. S1).

Amino acids in fruits

N fertilisation increased the total FAN concentration in the must (by 33%, P = 0.014) under HYC only (Table 1). The fertilised : control ratios of amino acid concentrations were calculated for each amino acid, including the nonassimilable proline and hydroxyproline (Fig. S2). The ratios under HYC were globally higher than 1.0, in contrast to the ratios under LYC (average 1.3 ± 0.2 under HYC and 1.0 ± 0.1 under LYC, P = 0.062). The differences between ratios under HYC and LYC were significant for arginine, aspartic acid, citrulline, histidine, tryptophan and tyrosine.

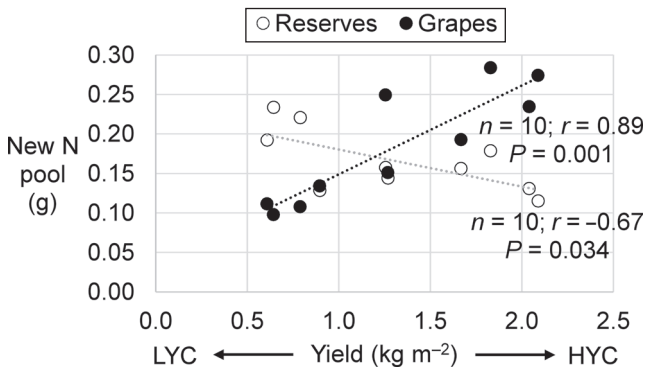


Fig. 3. Effect of the yield on new N quantity accumulated in the reserves (roots + trunk) and grapes (pomace + must) for Chasselas vines in 2017 at Pully, Switzerland. LYC, low-yielding conditions; HYC, high-yielding conditions.

N fertilisation had a small effect on the FAN profile, with an increase in the relative abundances of alanine and a decrease in the γ-amino-butyric acid and lysine contents (Table 5). The fruit load affected the must FAN profile without any impact on the total FAN concentration (Tables 1 and 5). The alanine, γ-amino-butyric acid, serine and threonine proportions were higher under HYC than under LYC, whereas the histidine, isoleucine, lysine, proline, tryptophan and tyrosine proportions were lower. Principal component analysis was used to assess the impact of fruit load and fertilisation on the FAN profiles better (Fig. 4). The principal component analysis of the relative amino acid abundance allowed a clear discrimination of the vines under LYC from the vines under HYC, independent of the fertilisation treatment (Fig. 4*b*).

Discussion

Relationship between vigour and plant N nutrition

Differences were observed between vines in terms of canopy weight, leaf area and bunch weight. This natural heterogeneity was independent of both experimental treatments for crop load (P = 0.402) and urea supply (P = 0.970) and did not affect the

Table 5. Effect of crop load and N fertilisation on the relative proportions of free amino acids (FAN profiles, %) in the must at harvest
Average ± 1 s.d. for Chasselas vines in 2017 at Pully, Switzerland. HYC, high-yielding conditions; LYC, low-yielding conditions; ns, nonsignificant; *, P < 0.05; **, P < 0.01; ***, P < 0.001

Amino acids (%)	Control vines (n = 11)	N-fertilized vines (n = 10)	P-value		LYC (n = 9)	HYC (n = 12)	P-value		Interaction of yield condition × fertilisation
Alanine	8.4 ± 1.8	10.0 ± 1.5	↑	**	7.9 ± 1.4	10.5 ± 1.3	↑	***	ns
Arginine	36.7 ± 3.5	36.2 ± 1.6		ns	37.9 ± 2.6	35.0 ± 2.1		*	ns
Asparagine	0.6 ± 0.2	0.5 ± 0.2		ns	0.7 ± 0.2	0.4 ± 0.2		**	ns
Aspartic acid	5.8 ± 1.4	5.0 ± 0.8		ns	5.9 ± 1.5	4.9 ± 0.8		ns	ns
Citrulline	1.7 ± 0.4	2.0 ± 0.3		ns	1.8 ± 0.3	1.9 ± 0.5		ns	*
γ-amino-butyric acid	4.1 ± 0.7	3.4 ± 0.4	↓	**	3.4 ± 0.5	4.1 ± 0.7	↑	*	ns
Glutamine	2.4 ± 0.2	2.5 ± 0.3		ns	2.4 ± 0.2	2.5 ± 0.3		ns	ns
Glutamic acid	10.4 ± 1.8	10.5 ± 1.2		ns	10.3 ± 1.4	10.6 ± 1.6		ns	ns
Histidine	2.3 ± 0.5	2.4 ± 0.3		ns	2.5 ± 0.4	2.2 ± 0.3	↓	*	ns
Hydroxy-proline	0.3 ± 0.1	0.3 ± 0.1		ns	0.3 ± 0.1	0.3 ± 0.1		ns	ns
Isoleucine	1.5 ± 0.2	1.5 ± 0.1		ns	1.6 ± 0.1	1.4 ± 0.1	↓	**	ns
Leucine	2.1 ± 0.3	1.9 ± 0.1		ns	2.0 ± 0.3	1.9 ± 0.3		ns	ns
Lysine	0.4 ± 0.1	0.3 ± 0.1	↓	*	0.4 ± 0.1	0.3 ± 0.1	↓	*	ns
Methionine	0.2 ± 0.0	0.2 ± 0.0		ns	0.2 ± 0.0	0.2 ± 0.0		ns	ns
Ornithine	0.8 ± 0.3	0.7 ± 0.2		ns	0.8 ± 0.3	0.7 ± 0.2		ns	ns
Phenylalanine	0.8 ± 0.2	0.8 ± 0.1		ns	0.8 ± 0.1	0.8 ± 0.2		ns	ns
Proline	14.5 ± 3.5	16.0 ± 3.2		ns	16.9 ± 3.1	13.6 ± 2.8	↓	*	ns
Serine	7.2 ± 1.2	7.3 ± 0.6		ns	6.7 ± 0.8	7.8 ± 0.8	↑	**	ns
Threonine	8.6 ± 1.3	8.8 ± 0.5		ns	8.1 ± 1.0	9.3 ± 0.7	↑	**	*
Tryptophan	1.6 ± 0.3	1.6 ± 0.3		ns	1.8 ± 0.3	1.4 ± 0.2	↓	**	ns
Tyrosine	1.4 ± 0.2	1.5 ± 0.1		ns	1.5 ± 0.2	1.4 ± 0.1	↓	**	ns
Valine	3.0 ± 0.3	3.0 ± 0.2		ns	3.0 ± 0.2	2.9 ± 0.3		ns	ns

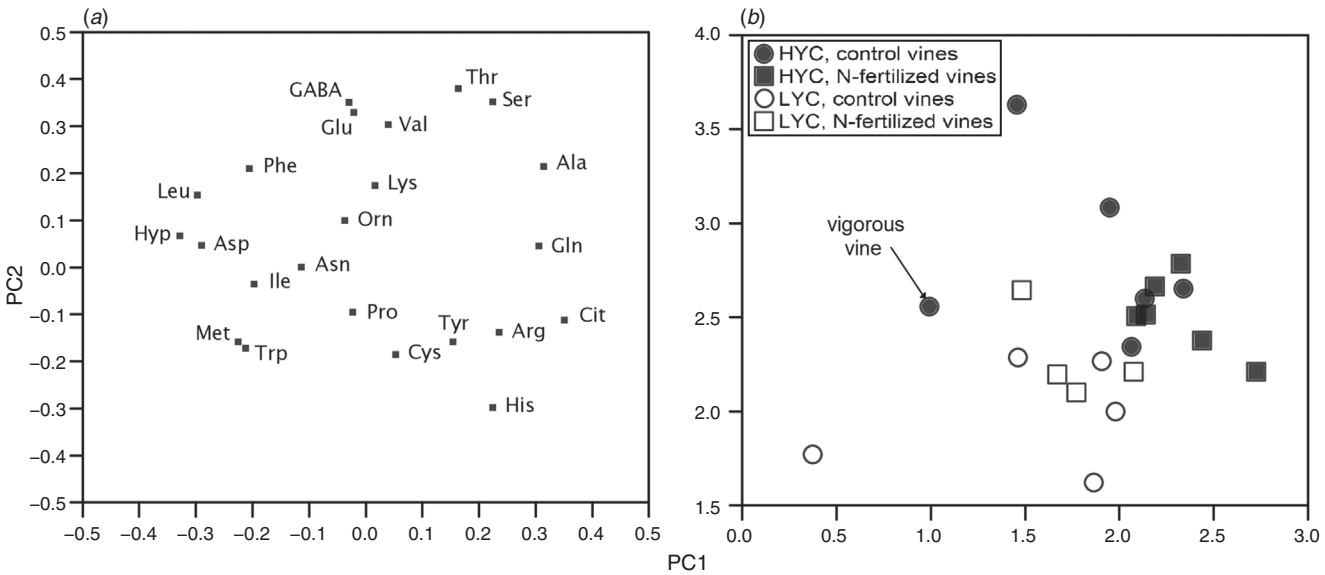


Fig. 4. Principal component analysis (PCA) of must amino acid profiles (amino acid proportions in %) at harvest for Chasselas vines in 2017 at Pully, Switzerland. (*a*) Correlations between variables; (*b*) observations (must free amino N (FAN) profiles): black, high-yielding conditions ($n = 12$); white, low-yielding conditions ($n = 9$); circles, control vines ($n = 11$); squares, N-fertilised vines ($n = 10$). Shorter distances between observations indicate similar FAN profiles. The PCA discriminates the vines under LYC from the vines under HYC, independent of the fertilisation treatment.

interpretations of the trial. Canopy weight was positively correlated with the fertiliser N uptake, N concentration and N quantity in the whole plant: more vigorous plants had higher YAN in grape must ($P = 0.077$ in the control treatment and $P = 0.004$ in the urea treatment, Fig. S1). This positive impact of N nutrition on plant growth and overall development has already been demonstrated by other researchers (Holzapfel and Treeby 2007; Gatti *et al*. 2018).

No impact of crop load on fruit N concentration or maturation

In 2017, the optimal climatic conditions (i.e. no water restriction, suitable temperature and sufficient luminosity) were conducive to proper fruit maturation in all treatments, as explained by Mawdsley *et al*. (2018). The $\delta^{13}C$ values indicate that the vines had sufficient water supply (Van Leeuwen *et al*. 2009). Despite the important variation of crop load between LYC and HYC (+155% in the control treatment; +117% in the urea treatment), the must TN content remained constant in both the control and urea treatments (Fig. S3). Despite the large crop load variation, all the vines reached full grape maturity in the same period and there was no differences in terms of total soluble solids, acidity, pH and YAN concentrations between the treatments, as shown in other studies (Keller *et al*. 2005; Wang *et al*. 2018), although the average leaf : fruit ratio in the HYC treatment was as low as 0.7 m^2 kg^{-1}. Unlike canopy oversizing, which induces a drop in terms of YAN concentration in the must (Spring *et al*. 2012), increasing the crop load did not affect YAN concentration. This result confirms the findings of Verdenal *et al*. (2016). Additionally, the must K concentration remained unchanged despite leaf K deficiency under HYC. Grapevines appeared to adapt their metabolism through the modulation of combined morphological and physiological mechanisms, as explained hereafter.

Limitation of root growth and smaller N reserves under HYC

The root DW was 17% and 14% lower under HYC than under LYC in the control and urea treatments respectively. This confirms the results from other research (Howell 2001; Morinaga *et al*. 2003). Morinaga *et al*. (2003) observed that under HYC, the growth of fine roots and lateral shoots is reduced, though the fine root respiration rate is higher. More C and N were mobilised from the trunk and root reserves under HYC to supply the maturing fruits (Howell 2001). C and N accumulation in the grapes appeared as a priority objective over root development and reserve refilling. Therefore, the TOC and TN contents increased in the fruits almost proportionally to the crop load, whereas root growth was consequently limited, along with the C and N storage capacity (Fig. S4).

In addition to limited root growth, the root N reserves were more solicited under HYC than under LYC: the NQ was 27% lower in the control treatment. Several studies mentioned that root N reserve accumulation is restricted by the presence of fruit before and after veraison (Rodriguez-Lovelle and Gaudillere 2002; Rossouw *et al*. 2017). This result suggests that several years of overproduction could potentially induce an important reduction in N reserves, which may affect vigour, bud fruitfulness and even the plant's lifespan.

Similar photosynthetic activity and higher leaf N assimilation under HYC

The photosynthetic activity was influenced by neither crop load nor urea application. This result confirms the findings

from Dayer *et al*. (2016), which showed no impact of crop load on CO_2 assimilation. The fertiliser N uptake was, on average, 42% higher under HYC than under LYC. RSA (a measure of N sink strength, independent of organ size) was the highest in grapes. When the crop load was greatly increased, the fruit N demand increased, consequently inducing modifications in N partitioning. These results confirm the findings from Verdenal *et al*. (2016), which suggested that increasing foliar N assimilation is a plant reaction to crop load variations to maintain fruit N concentrations. Foliar N assimilation was a function of both plant vigour and crop load.

Additionally, a higher crop load might also have stimulated soil N uptake in contrast to root growth. Stander *et al*. (2017) mentioned a similar correlation between both crop load and root sink activities in mandarin (*Citrus reticulata* Blanco) trees. This observation may explain why after N-labelling, the TN content was significantly higher in roots under HYC (+20%), whereas the RSA of new N was lower. This result suggests the possible presence of a nonlabelled N source, which can only be root N uptake from the soil.

Effect of crop load on the FAN profile

Despite a uniform overall concentration, the must FAN profile varied significantly in relation to the crop load, although the impact of urea supply was negligible. The primary : secondary amino acid ratio reflects the nutritional value of the must to yeasts, with the secondary amino acids being the nonassimilable proline and hydroxyproline (Bell and Henschke 2005). The index, which includes all amino acids, was significantly higher under HYC than under LYC (7.5 ± 1.7 and 6.0 ± 1.1 respectively, $P = 0.029$), indicating a higher nutritional value.

Several experiments have already demonstrated the impacts of bunch thinning: on N distribution in the grapevine (Zufferey *et al*. 2015; Rossouw *et al*. 2017), on global grapevine development and grape maturation (Keller *et al*. 2005), on respiration and growth rates (Morinaga *et al*. 2003), on the must composition on the must aroma profiles (Wang *et al*. 2018) and on the volatile and phenol composition of musts (Kok 2011; Rutan *et al*. 2018). The variable impact of crop thinning on volatile compounds and aroma development is mainly dependent on genotype and timing (Do *et al*. 2010; Alem *et al*. 2019) and could be either positive or negative. In fact, any parameters and/or practices affecting vine balance (climate conditions, plant vigour, canopy management, crop load, etc.) might affect aroma development. Consequently, an integrative point of view would be required to control and anticipate the development of the grapes' flavour-active compounds. Further research is still required to understand the mechanisms that balance the formation of secondary metabolite in grape in relation to FAN profiles.

Preservation of root N reserves through foliar urea supply

The uptake and the subsequent impact of foliar N fertilisation highly depended on the crop load. Fertilisation had no influence on the fruit YAN under LYC. However, the fertiliser N uptake was higher under HYC (Fig. 2); consequently, the fruit YAN was 34% higher ($P = 0.021$, Table 1). Under these conditions, the partitioning of new N was largely influenced by crop load: significantly smaller fractions of new N were allocated to the roots and trunk (−50% and −38%, respectively), whereas larger fractions tended to be allocated to the canopy and fruits (+14% and +35%, respectively). The positive impact of urea fertilisation on the must YAN content confirms many results from other studies (Dufourcq *et al*. 2009; Nisbet *et al*. 2014; Verdenal *et al*. 2015; Hannam *et al*. 2016). The newcontribution of this experiment is the positive correlation between NUE and the crop load.

In contrast to the control treatment, the urea supply maintained a root NQ that was unchanged despite variation in the crop load. Thus the urea supply allowed the N demand of fruits to be satisfied while preserving the root N reserves, potentially increasing plant sustainability under HYC. Reserve N refilling is essential for the following season growth (Holzapfel and Treeby 2007). The relationship between fruits and roots must be clarified to improve perennial fruit crop production, as root development and reserve capacity influence the following year's production.

To conclude, this experiment demonstrates the high potential of crop limitation to control plant NUE and ultimately fruit N composition at harvest. The results indicate that root development and activity are both key factors for understanding the mechanisms that balance plant N nutrition. Grapevines were in a constant search for fruit nutrition balance. They actively modulated root N reserve mobilisation and fertiliser N uptake to maintain a uniform N concentration in the must, despite crop load variations. Fertiliser N uptake and assimilation were strongly stimulated under HYC in answer to the higher fruit N demand and, consequently, preserved N reserves from excessive mobilisation and downsizing. Compared with HYC, LYC did not improve the YAN concentration in the must but only affected the FAN profile, suggesting a modification of the potential aroma profile. It is therefore questionable whether the crop load limitation always has a positive impact on the grapes' composition and ultimately on the wine quality. This study encourages further research on the potential of agricultural practices to monitor NUE, with the aim of enhancing crop quality and sustainability.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors thank the vineyard and laboratory teams of Agroscope and Sol-Conseil for their support in this project. Special thanks are given to Philippe Duruz and Laure Passot for their valuable work in the field and to Jonas Siegrist and Sylvain Schnee for their help in the preparation of the samples. This research did not receive any specific funding.

References

Alem H, Rigou P, Schneider R, Ojeda H, Torregrosa L (2019) Impact of agronomic practices on grape aroma composition: a review. *Journal of the Science of Food and Agriculture* **99**, 975–985. doi:10.1002/jsfa.9327

Bates TR, Dunst RM, Joy P (2002) Seasonal dry matter, starch, and nutrient distribution in 'Concord' grapevine roots. *HortScience* **37**(2), 313—316. doi:10.21273/HORTSCI.37.2.313

Bell S-J, Henschke PA (2005) Implications of nitrogen nutrition for grapes, fermentation and wine. *Australian Journal of Grape and Wine Research* **11**, 242–295. doi:10.1111/j.1755-0238.2005.tb00028.x

Boss PK, Bottcher C, Davies C (2014) Various influences of harvest date and fruit sugar content on different wine flavor and aroma compounds. *American Journal of Enology and Viticulture* **65**, 341–353. doi:10.5344/ajev.2014.13137

Brand WA (2011) New reporting guidelines for stable isotopes – an announcement to isotope users. *Isotopes in Environmental and Health Studies* **47**, 535–536. doi:10.1080/10256016.2011.645702

Carbonneau A (1995) La surface foliaire exposée potentielle. Guide pour sa mesure. *Le Progrès Agricole et Viticole* **112**, 204–212.

Chaumont M, Morot-Gaudry J-F, Foyer CH (1994) Seasonal and diurnal changes in photosynthesis and carbon partitioning in *Vitis vinifera* leaves in vines with and without fruit. *Journal of Experimental Botany* **45**(9), 1235–1243. doi:10.1093/jxb/45.9.1235

Chaves MM (1984) Photosynthesis and assimilate partition in fruiting and non-fruiting grapevine shoots. In. 'Advances in Photosynthesis Research: Proceedings of the VIth International Congress on Photosynthesis', 1–6 August 1983, Brussels, Belgium. (Ed C Sybesma) Volume IV. pp. 145–148. (Springer Netherlands: Dordrecht)

Coplen TB (2011) Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry* **25**, 2538–2560. doi:10.1002/rcm.5129

Dai ZW, Vivin P, Barrieu F, Ollat N, Delrot S (2010) Physiological and modelling approaches to understand water and carbon fluxes during grape berry growth and quality development: a review. *Australian Journal of Grape and Wine Research* **16**, 70–85. doi:10.1111/j.1755-0238.2009.00071.x

Dayer S, Prieto JA, Galat E, Peña JP (2016) Leaf carbohydrate metabolism in Malbec grapevines: combined effects of regulated deficit irrigation and crop load. *Australian Journal of Grape and Wine Research* **22**, 115–123. doi:10.1111/ajgw.12180

Deléens E, Cliquet J-B, Prioul J-L (1994) Use of ^{13}C and ^{15}N plant label near natural abundance for monitoring carbon and nitrogen partitioning. *Australian Journal of Plant Physiology* **21**, 133–146.

Deléens E, Morot-Gaudry J-F, Martin F, Thoereux A, Gojon A (1997) Méthodologie ^{15}N. In 'Assimilation de l'azote chez les plantes'. pp. 265–280. (Institut National de la Recherche Agronomique (INRA): Paris)

Do PT, Prudent M, Sulpice R, Causse M, Fernie AR (2010) The influence of fruit load on the tomato pericarp metabolome in a *Solanum chmielewskii* introgression line population. *Plant Physiology* **154**, 1128–1142. doi:10.1104/pp.110.163030

Dufourcq T, Charrier F, Poupault P, Schneider R, Gontier L, Serrano E (2009) Foliar spraying of nitrogen and sulfur at veraison: a viticultural technique to improve aromatic composition of white and rosés wines. In 'Proceedings of the 16th International GiESCO Symposium', Davis, USA). pp. 379–383. (UC Davis: Davis)

Gatti M, Squeri C, Garavani A, Vercesi A, Dosso P, Diti I, Poni S (2018) Effects of variable rate nitrogen application on cv. Barbera performance: vegetative growth and leaf nutritional status. *American Journal of Enology and Viticulture* **69**, 196–209. doi:10.5344/ajev.2018.17084

González-Barreiro C, Rial-Otero R, Cancho-Grande B, Simal-Gándara J (2015) Wine aroma compounds in grapes: a critical review. *Critical Reviews in Food Science and Nutrition* **55**, 202–218. doi:10.1080/10408398.2011.650336

Gutiérrez-Gamboa G, Romanazzi G, Garde-Cerdán T, Pérez-Álvarez EP (2019) A review of the use of biostimulants in the vineyard for improved grape and wine quality: effects on prevention of grapevine diseases. *Journal of the Science of Food and Agriculture* **99**, 1001–1009. doi:10.1002/jsfa.9353

Hachiya T, Sakakibara H (2017) Interactions between nitrate and ammonium in their uptake, allocation, assimilation and signaling in plants. *Journal of Experimental Botany* **68**, 2501–2512.

Hannam KD, Neilsen GH, Neilsen D, Midwood AJ, Millard P, Zhang Z, Thornton B, Steinke D (2016) Amino acid composition of grape (*Vitis vinifera* L.) juice in response to applications of urea to the soil or foliage. *American Journal of Enology and Viticulture* **67**, 47–55. doi:10.5344/ajev.2015.15015

Holzapfel BP, Treeby MT (2007) Effects of timing and rate of N supply on leaf nitrogen status, grape yield and juice composition from Shiraz grapevines grafted to one of three different rootstocks. *Australian Journal of Grape and Wine Research* **13**, 14–22. doi:10.1111/j.1755-0238.2007.tb00067.x

Howell GS (2001) Sustainable grape productivity and the growth–yield relationship: a review. *American Journal of Enology and Viticulture* **52**, 165–174.

Jackson DI, Lombard PB (1993) Environmental and management practices affecting grape composition and wine quality – a review. *American Journal of Enology and Viticulture* **44**, 409–430.

Kant S, Bi Y-M, Rothstein SJ (2011) Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. *Journal of Experimental Botany* **62**, 1499–1509. doi:10.1093/jxb/erq297

Keller M, Mills LJ, Wample RL, Spayd SE (2005) Cluster thinning effects on three deficit-irrigated *Vitis vinifera* cultivars. *American Journal of Enology and Viticulture* **56**, 91–103.

Kliewer WM, Dokoozlian N (2005) Leaf area/crop weight ratios of grapevines: influence on fruit composition and wine quality. *American Journal of Enology and Viticulture* **56**, 170–181.

Kok D (2011) Influences of pre-and post-veraison cluster thinning treatments on grape composition variables and monoterpene levels of *Vitis vinifera* L. cv. Sauvignon Blanc. *Journal of Food Agriculture and Environment* **9**(1), 22–26.

Lancashire PD, Bleiholder H, van den Boom T, Langelüddeke P, Stauss R, Weber E, Witzenberger A (1991) A uniform decimal code for growth stages of crops and weeds. *Annals of Applied Biology* **119**, 561–601. doi:10.1111/j.1744-7348.1991.tb04895.x

Mabrouk H, Carbonneau A (1996) Une méthode simple de détermination de la surface foliaire de la vigne (*Vitis vinifera* L.). *Le Progrès Agricole et Viticole* **113**, 392–398.

Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A (2010) Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. *Annals of Botany* **105**, 1141–1157. doi:10.1093/aob/mcq028

Mawdsley PFW, Dodson Peterson JC, Casassa LF (2018) Agronomical and chemical effects of the timing of cluster thinning on Pinot Noir (clone 115) grapes and wines. *Fermentation* **4**, 60doi:10.3390/fermentation4030060

Morinaga K, Imai S, Yakushiji H, Koshita Y (2003) Effects of fruit load on partitioning of ^{15}N and ^{13}C, respiration, and growth of grapevine roots at different fruit stages. *Scientia Horticulturae* **97**, 239–253. doi:10.1016/S0304-4238(02)00199-1

Nisbet MA, Martinson TE, Mansfield AK (2014) Accumulation and Prediction of Yeast Assimilable Nitrogen in New York Winegrape Cultivars. *American Journal of Enology and Viticulture* **65**, 325–332. doi:10.5344/ajev.2014.13130

Ojeda-Real LA, Lobit P, Cárdenas-Navarro R, Grageda-Cabrera O, Farías-Rodríguez R, Valencia-Cantero E, Macías-Rodríguez L (2009) Effect of nitrogen fertilization on quality markers of strawberry (*Fragaria* × *ananassa* Duch. cv. Aromas). *Journal of the Science of Food and Agriculture* **89**, 935–939. doi:10.1002/jsfa.3531

Peel MC, Finlayson BL, McMahon TA (2007) Updated world map of the Köppen–Geiger climate classification. *Hydrology and Earth System Sciences Discussions* **4**, 439–473. doi:10.5194/hessd-4-439-2007

Petrie PR, Clingeleffer PR (2006) Crop thinning (hand versus mechanical), grape maturity and anthocyanin concentration: outcomes from irrigated Cabernet Sauvignon (*Vitis vinifera* L.) in a warm climate. *Australian Journal of Grape and Wine Research* **12**, 21–29. doi:10.1111/j.1755-0238.2006.tb00040.x

Porro D, Stefanini M, Dorigatti C (2010) Nitrogen foliar uptake and partitioning in Cabernet Sauvignon grapevines. *Acta Horticulturae* **868**, 185–190.

Reeve AL, Skinkis PA, Vance AJ, Lee J, Tarara JM (2016) Vineyard floor management influences 'Pinot noir' vine growth and productivity more than cluster thinning. *HortScience* **51**, 1233–1244. doi:10.21273/HORTSCI10998-16

Rodriguez-Lovelle B, Gaudillere JP (2002) Carbon and nitrogen partitioning in either fruiting or non-fruiting grapevines: effects of nitrogen limitation before and after veraison. *Australian Journal of Grape and Wine Research* **8**, 86–94. doi:10.1111/j.1755-0238.2002.tb00216.x

Rossouw GC, Smith JP, Barril C, Deloire A, Holzapfel BP (2017) Implications of the presence of maturing fruit on carbohydrate and nitrogen distribution in grapevines under postveraison water constraints. *Journal of the American Society for Horticultural Science* **142**, 71–84. doi:10.21273/JASHS03982-16

Rutan TE, Herbst-Johnstone M, Kilmartin PA (2018) Effect of cluster thinning *Vitis vinifera* cv. Pinot Noir on wine volatile and phenolic composition. *Journal of Agricultural and Food Chemistry* **66**, 10053–10066. doi:10.1021/acs.jafc.8b04062

Schimmelmann A, Qi H, Coplen TB, Brand WA, Fong J, Meier-Augenstein W, Kemp HF, Toman B, Ackermann A, Assonov S, Aerts-Bijma AT, Brejcha R, Chikaraishi Y, Darwish T, Elsner M, Gehre M, Geilmann H, Gröning M, Hélie J-F, Herrero-Martín S, Meijer HAJ, Sauer PE, Sessions AL, Werner RA (2016) Organic reference materials for hydrogen, carbon, and nitrogen stable isotope-ratio measurements: caffeines, n-alkanes, fatty acid methyl esters, glycines, l-valines, polyethylenes, and oils. *Analytical Chemistry* **88**, 4294–4302. doi:10.1021/acs.analchem.5b04392

Scholander PF, Bradstreet ED, Hemmingsen EA, Hammel HT (1965) Sap pressure in vascular plants. *Science* **148**, 339–346. doi:10.1126/science.148.3668.339

Schreiner RP (2016) Nutrient uptake and distribution in young Pinot Noir grapevines over two seasons. *American Journal of Enology and Viticulture* **67**, 436–448. doi:10.5344/ajev.2016.16019

Schreiner RP, Scagel CF, Lee J (2014) N, P, and K supply to Pinot Noir grapevines: impact on berry phenolics and free amino acids. *American Journal of Enology and Viticulture* **65**, 43–49. doi:10.5344/ajev.2013.13037

Sinaj S, Richner W (2017) 'Principes de fertilisation des cultures agricoles en Suisse (PRIF 2017).' Recherche Agronique Suisse 8 (6). Publication spéciale.

Spangenberg JE, Zufferey V (2019) Carbon isotope compositions of whole wine, wine solid residue, and wine ethanol, determined by EA/IRMS and GC/C/IRMS, can record the vine water status—a comparative reappraisal. *Analytical and Bioanalytical Chemistry* **411**, 2031–2043. doi:10.1007/s00216-019-01625-4

Spring J-L, Zufferey V (2000) Intérêt de la détermination de l'indice chlorophyllien du feuillage en viticulture. *Revue Suisse de Viticulture, d'Arboriculture et d'Horticulture* **32**, 323–328.

Spring J-L, Verdenal T, Zufferey V, Viret O (2012) Nitrogen dilution in excessive canopies of Chasselas and Pinot Noir cvs. *Journal International des Sciences de la Vigne et du Vin* **46**, 233–240. doi:10.20870/oeno-one.2012.46.3.1520

Stander OPJ, Barry GH, Cronjé PJR (2017) Fruit-load-induced starch accumulation causes leaf chlorosis in "off" 'Nadorcott' mandarin trees. *Scientia Horticulturae* **222**, 62–68. doi:10.1016/j.scienta.2017.05.019

Sweetman C, Sadras VO, Hancock RD, Soole KL, Ford CM (2014) Metabolic effects of elevated temperature on organic acid degradation in ripening *Vitis vinifera* fruit. *Journal of Experimental Botany* **65**, 5975–5988. doi:10.1093/jxb/eru343

Van Leeuwen C, Tregoat O, Choné X, Bois B, Pernet D, Gaudillere JP (2009) Vine water status is a key factor in grape ripening and vintage quality for red Bordeaux wine. How can it be assessed for vineyard management purposes? *Journal International des Sciences de la Vigne et du Vin* **43**, 121–134. doi:10.20870/oeno-one.2009.43.3.798

Verdenal T, Spangenberg JE, Zufferey V, Lorenzini F, Spring JL, Viret O (2015) Effect of fertilisation timing on the partitioning of foliar-applied nitrogen in *Vitis vinifera* cv. Chasselas: a ^{15}N labelling approach. *Australian Journal of Grape and Wine Research* **21**(1), 110–117. doi:10.1111/ajgw.12116

Verdenal T, Spangenberg JE, Zufferey V, Lorenzini F, Dienes-Nagy A, Gindro K, Spring JL, Viret O (2016) Leaf-to-fruit ratio affects the impact of foliar-applied nitrogen on N accumulation in the grape must. *Journal International des Sciences de la Vigne et du Vin* **50**, 23–33. doi:10.20870/oeno-one.2016.50.1.55

Wang Y-T, Huang S-W, Liu R-L, Jin J-Y (2007) Effects of nitrogen application on flavor compounds of cherry tomato fruits. *Journal of Plant Nutrition and Soil Science* **170**, 461–468. doi:10.1002/jpln.200700011

Wang Y, He Y-N, Chen W-K, He F, Chen W, Cai X-D, Duan C-Q, Wang J (2018) Effects of cluster thinning on vine photosynthesis, berry ripeness and flavonoid composition of Cabernet Sauvignon. *Food Chemistry* **248**, 101–110. doi:10.1016/j.foodchem.2017.12.021

Zapata C, Deleens E, Chaillou S, Magne C (2004) Partitioning and mobilization of starch and N reserves in grapevine (*Vitis vinifera* L.). *Plant Physiology* **161**, 1031–1040. doi:10.1016/j.jplph.2003.11.009

Zufferey V, Murisier F, Belcher S, Lorenzini F, Vivin P, Spring JL, Viret O (2015) Nitrogen and carbohydrate reserves in the grapevine (*Vitis vinifera* L. 'Chasselas'): the influence of the leaf to fruit ratio. *Vitis* **54**, 183–188.

Handling Editor: Ulrike Mathesius

10.1071/FP20010_AC

Supplementary Material: *Functional Plant Biology*

Supplementary Material

Impact of crop load on nitrogen uptake and reserve mobilisation in *Vitis vinifera*

Thibaut Verdenal[A,E], *Jorge E. Spangenberg*[B], *Vivian Zufferey*[A], *Ágnes Dienes-Nagy*[A], *Olivier Viret*[C], *Cornelis van Leeuwen*[D] *and Jean-Laurent Spring*[A]

[A]Agroscope Institute, Avenue Rochettaz 21, 1009 Pully, Switzerland.

[B]Institute of Earth Surface Dynamics, University of Lausanne, 1015 Lausanne, Switzerland.

[C]Direction générale de l'agriculture, de la viticulture et des affaires vétérinaires, 1110 Morges, Switzerland.

[D] Ecophysiologie et Génomique Fonctionnelle de la Vigne (EGFV), Bordeaux Sciences Agro, Institut national de la recherche pour l'agriculture, l'alimentation et l'environnement (INRAE), Univ. Bordeaux, Institut des Sciences de la Vigne et du Vin (ISVV), 33882 Villenave d'Ornon, France.

[E]Corresponding author. Email: thibaut.verdenal@agroscope.admin.ch

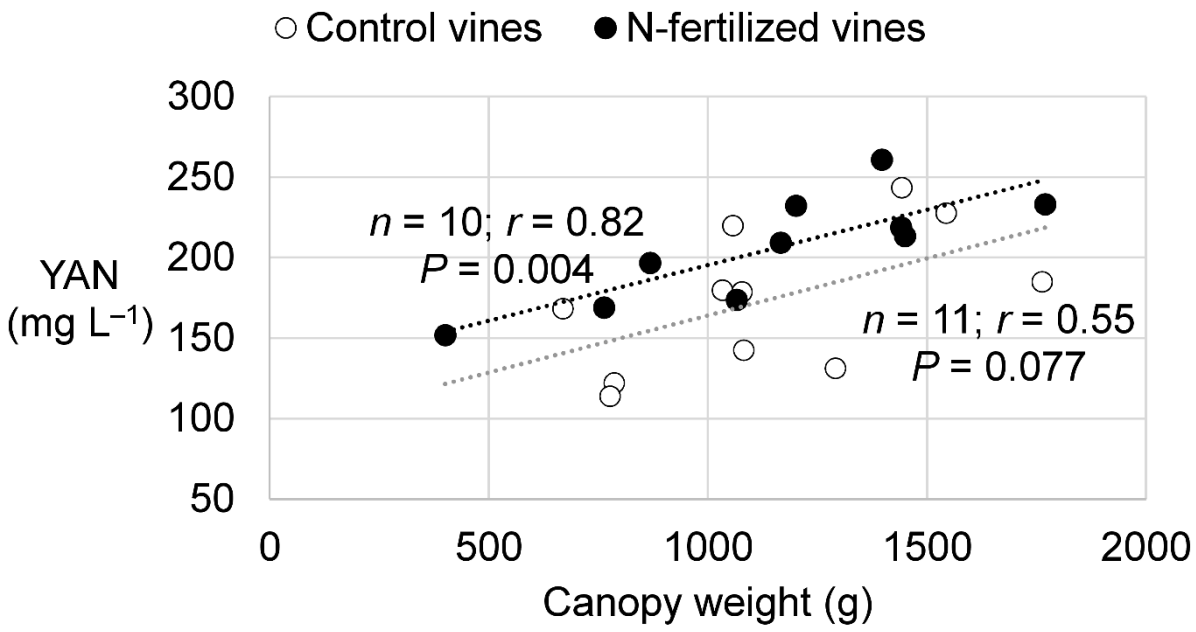


Fig. S1. Effect of canopy weight on YAN concentration in grape must, with and without foliar-N fertilization. Chasselas vines, 2017, Pully, Switzerland.

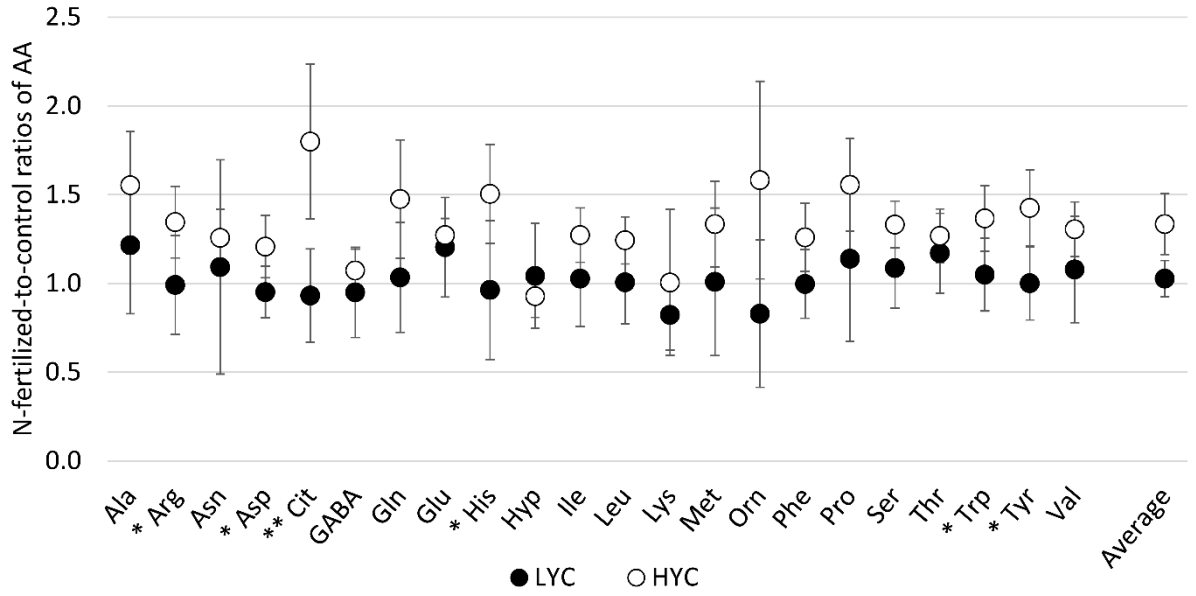


Fig. S2. N-fertilized-to-control ratios of amino acid concentrations in the must under both high yield (HYC, $n = 12$) and low-yield (LYC, $n = 9$) conditions. Values ± 1 SD, * $P < 0.05$; ** $P < 0.01$.

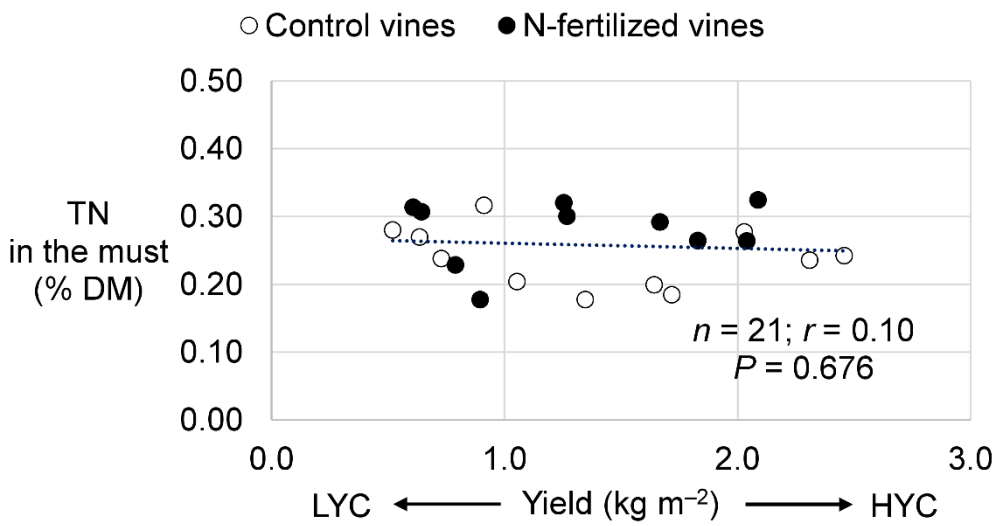


Fig. S3. Effect of crop load on total nitrogen (TN) concentration in grape must (% dry weight), in both control and urea treatments. LYC low yield condition; HYC high yield condition. Chasselas vines, 2017, Pully, Switzerland.

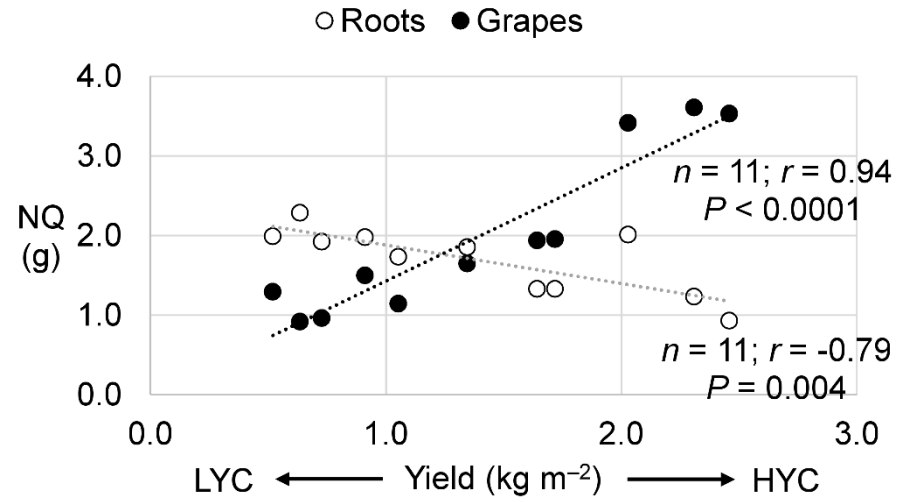


Fig. S4. Effect of crop load on N quantity (g) in grapes and in roots. LYC low yield condition; HYC high yield condition. Chasselas vines, 2017, Pully, Switzerland

Table S1. Effect of crop load on the leaf gas exchanges, i.e. photosynthesis (A), transpiration (E), stomatal conductance (gsw), ambient CO_2 concentration (Ca) and internal CO_2 concentration (Ci) Average ± 1 s.d. Chasselas vines, 2017, Pully, Switzerland. HYC, high-yielding conditions; LYC, low-yielding conditions; ns, non significant

Variable	Control vines (n = 11)	N-fertilized vines (n = 10)	P-value	LYC (n = 9)	HYC (n = 12)	P-value	Interaction yield condition × fertilisation
E (mmol m^{-2} s^{-1})	5.6 ± 0.4	5.8 ± 0.6	ns	5.8 ± 0.6	5.6 ± 0.3	ns	ns
A (µmol m^{-2} s^{-1})	15.2 ± 0.8	15.4 ± 1.0	ns	15.3 ± 1.0	15.2 ± 0.9	ns	ns
C_a (µmol mol^{-1})	331.2 ± 2.5	330.4 ± 3.5	ns	330.7 ± 3.5	331 ± 2.7	ns	ns
C_i (µmol mol^{-1})	231.3 ± 4.3	229 ± 7.5	ns	230.9 ± 8.0	229.4 ± 4.4	ns	ns
gws (mol m^{-2} s^{-1})	0.302 ± 0.032	0.304 ± 0.043	ns	0.309 ± 0.051	0.297 ± 0.022	ns	ns

Table S2. Dry weights (DW), total nitrogen (TN), nitrogen isotope composition ($\delta^{15}N$), nitrogen quantity (NQ), total organic carbon (TOC), carbon isotope composition ($\delta^{13}C$), and C/N ratio, in the different plants parts at harvest without urea supply (control treatment) under both low and high yield conditions (LYC and HYC)

Chasselas vines, Pully, 2017. HYC, high-yielding conditions; LYC, low-yielding conditions; mean values (average ± 1 s.d.) within the same row followed by different letters are significantly different (Newman-Keuls, $P < 0.05$). ns, non significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Harvest - LYC						
	Roots	Trunk	Canopy	Pomace	Must	*P*-value
DW (g)	260 ± 25 b	291 ± 36 b	382 ± 122 a	75 ± 28 c	117 ± 26 c	***
DW (%)	59.8 ± 2.1 a	58.2 ± 1 a	35.2 ± 1.6 b	26 ± 1.7 c	20.6 ± 1.1 d	***
TN (% DW)	0.8 ± 0.1 c	0.3 ± 0.1 d	1.4 ± 0.1 a	1.1 ± 0.3 b	0.3 ± 0 d	***
$\delta^{15}N$ (mUr)	10.8 ± 4.8	12.7 ± 5.2	12.9 ± 4.3	24.3 ± 9.6	19.5 ± 11.1	ns
NQ (g)	2 ± 0.2 b	0.9 ± 0.1 b	5.4 ± 2.1 a	0.8 ± 0.2 b	0.4 ± 0.2 b	***
TOC (%DW)	48.3 ± 0.9 a	46.7 ± 0.4 b	45.4 ± 0.6 c	45.1 ± 0.5 c	38.5 ± 0.4 d	***
$\delta^{13}C$ (mUr)	–28.6 ± 0.3 ab	–28.2 ± 0.2 a	–29.2 ± 0.7 b	–28.8 ± 0.5 ab	–28.1 ± 0.6 a	*
Ratio C/N	64 ± 7 b	159 ± 39 a	33 ± 3 b	44 ± 11 b	150 ± 26 a	***

Harvest - HYC						
	Roots	Trunk	Canopy	Pomace	Must	*P*-value
DW (g)	216 ± 59 b	277 ± 49 b	413 ± 112 a	240 ± 61 b	249 ± 53 b	***
DW (%)	58.7 ± 2 a	55.9 ± 6.3 a	35.2 ± 0.9 b	23.8 ± 0.6 c	20.2 ± 1 d	***
TN (% DW)	0.7 ± 0.1 c	0.4 ± 0 d	1.3 ± 0.2 a	0.9 ± 0.1 b	0.2 ± 0 e	***
$\delta^{15}N$ (mUr)	6.6 ± 4.1 b	12 ± 9.6 ab	15.1 ± 9.3 ab	27 ± 20.2 ab	33.7 ± 20 a	*
NQ (g)	1.4 ± 0.4 b	1.1 ± 0.2 b	5.6 ± 2.3 a	2.1 ± 0.7 b	0.6 ± 0.2 b	***
TOC (%DW)	49.5 ± 0.9 a	47.3 ± 0.5 b	45.7 ± 0.4 c	43.2 ± 1 d	37.4 ± 0.8 e	***
$\delta^{13}C$ (mUr)	–28.4 ± 0.3	–28 ± 0.1	–28.9 ± 0.7	–28.9 ± 0.8	–28.1 ± 0.8	ns
Ratio C/N	74 ± 7 c	118 ± 7 b	35 ± 4 d	50 ± 6 d	175 ± 32 a	***

4 Carbon and nitrogen dynamics and fertilization use efficiency over two years

Keeping in mind the impact of crop limitation on N partitioning and grape N composition at harvest, it is necessary to evaluate the carry-over effects of crop limitation in the following year. This chapter focuses on the two-year dynamics of C and N. It also describes the dynamics of fertilizer N in the plant over two years in relation to crop limitation. This chapter was accepted for publication in 2021 as an original research article in the *Australian Journal of Grape and Wine Research* of the Australian Society of Viticulture and Oenology (ASVO).

4.1 Resume of the article

Knowing the impact of cultural practices on C and N dynamics in perennial plants is critical for improving N use efficiency and reducing the ecological footprint. Plant physiology and grape composition were monitored over two consecutive years (i.e., 2017 and 2018). The environmental conditions – particularly water and N availability – were non-restrictive to grapevine development and conditioned the results of this trial. A significant amount of N was released by the roots into the soil before winter rest and was assimilated again in the following year (n+1). Net N uptake should be seen as the sum of total N influx and total N efflux. A large yield gradient was achieved via bunch thinning in both years. Despite a homogeneous N supply in the fertilized treatment, fertilizer N uptake varied greatly in relation to crop load in both years: foliar N supply promoted a higher fruit N concentration at harvest under high-yield conditions, while the gain was not significant under low-yield conditions. These results demonstrate the importance of adapting fertilization programs to cultural practices. This important finding explains why, in some situations, foliar-N supply does not efficiently improve fruit N concentration and may instead contribute to environmental pollution. Fertilizer N distribution in the plant was affected by crop load, particularly in fruits and perennial reserves, while it remained constant in the canopy. To the detriment of the roots, 40% of fertilizer N was located in the fruits at harvest under high-yield conditions, versus only 24% under low-yield conditions. The hierarchy in N-sink strength between plant organs was highlighted, with the abundance of fertilizer N (relative to total N) decreasing gradually from the fruits to the roots and trunk.

Plants regulated C and N uptake in relation to the demand for ripening fruits, instead of promoting a stronger vigor to the plant in response to crop limitation. Leaf gas exchange rates were lower in relation to crop limitation, thus reducing C and N uptake and increasing water use efficiency. Both C and N quantities in fruits were reduced proportionally to crop limitation, while their concentrations were

unchanged. The roots were the plant fraction that benefited the most from crop limitation, highlighting the close coordination of C and N metabolites between fruits and roots. However, crop limitation affected certain amino acids more than others, thus potentially affecting the fruit aroma profile. Interestingly, the amino acids most affected by crop limitation were not the same as the ones affected by foliar N supply. The quantity of residual fertilizer N in the perennial reserves at the end of the year was negatively correlated to the crop load. In year n+1, residual fertilizer N in the plant had no carry-over effect, either on vegetative parameters or on grape composition, in comparison to the control treatment. N partitioning in year n+1 depended on both N species and N origin, either from the perennial reserves (mainly amino N) or from the seasonal foliar uptake (2018-lab-N, mainly NH_4^+ from urea assimilation).

This trial demonstrated the high potential of crop limitation to control plant N use efficiency. Root development and activity appeared as key factors for understanding plant C and N dynamics. Grapevines were in constant search for nutrient balance between organs, while fruits showed the strongest N-sink strength. These findings illustrate the impact of plant balance on fertilization efficiency and will contribute to the improvement of cultural practices and to the development of precise nutrition models in perennial crops.

4.2 Introduction

N is an essential element for plant development and is required in a larger amount than any other nutrient applied to crops. During the twentieth century, nitrate (NO_3^-) was intensively used to increase production, despite crops using only 30% to 40% of the fertilizer (Masclaux-Daubresse *et al.*, 2010). The remaining fertilizer was usually lost to the environment via leaching, denitrification, surface runoff, gaseous emissions, and microbial consumption (e.g., Kant *et al.*, 2011). Thus, understanding the dynamics behind nutrient uptake, transport, storage, and remobilization is crucial for quantifying the nutrient budget and adjusting cultural practices, in particular for perennial crops. Therefore, minimizing the need for N supply through the fine-tuning of cultural practices is fundamental for sustainable agricultural development.

In grape production (*Vitis vinifera* L.), N depletion is as detrimental as N excess to yield and fruit composition. N excess exacerbates plant vigor, increases sensitivity to fungal diseases, and delays fruit ripening. Conversely, N deficiency reduces yields and severely affects the winemaking process. Bell and Henschke (2005) detailed the implications of N nutrition for grape, fermentation, and wine quality. In their review, they explained the significant role of grape YAN (i.e., primary free amino acids and NH_4^+) in fermentation kinetics and formation of flavor-active compounds in wine. Grapevine N dynamics, that

is, seasonal uptake and release, have been thoroughly studied in the past decades, providing a good understanding of the plant requirement in nutrients (Conradie, 1991; Wermelinger, 1991; Bates *et al.*, 2002; Zapata *et al.*, 2004; Weyand and Schultz, 2006; Loulakakis *et al.*, 2009; Masclaux-Daubresse *et al.*, 2010; Zufferey *et al.*, 2015; Schreiner, 2016; Holzapfel *et al.*, 2019). Even so, our understanding of the relationship between plant N status and fruit N composition remains incomplete. The concept of NUE represents the sum of both assimilation efficiency (uptake and assimilation) and utilization efficiency (allocation and remobilization) (Kant *et al.*, 2011). The NUE is largely determined by environmental conditions (i.e., climate and soil), plant material, and management strategies (i.e., plant material genetics, soil management, plant development monitoring, and vineyard inputs) (Porro *et al.*, 2006; Habran *et al.*, 2016; Verdenal *et al.*, 2021). Fruit N management is a multi-sided exercise in the search for balance between controlling yield and optimizing fruit composition, while limiting environmental impact.

In grape production, the optimum yield is generally not the maximum allowed by the conditions of the vineyard, since overcropping may delay fruit ripening (i.e., slower sugar accumulation) and alter subsequent wine quality (Petrie and Clingeleffer, 2006; Rutan *et al.*, 2018). Crop load may be regulated via crop thinning, which consists of removing grapes before the onset of ripening in order to promote the maturation of the remaining fruits. However, crop thinning does not consistently improve fruit composition or aroma development (Keller *et al.*, 2005; Mawdsley *et al.*, 2018; Wang *et al.*, 2018; Bubola *et al.*, 2020; Verdenal *et al.*, 2020). Alem *et al.* (2021) further demonstrated that crop thinning generally decreases the quantity of most C compounds (i.e., sugars, organic acids, and glycosylated aroma compounds) accumulated in fruits. Maintaining a balance between both vegetative and reproductive organs prevails over the consideration of the sole crop load to determine the physiological threshold for overcropping (Kliewer and Dokoozlian, 2005; Zufferey *et al.*, 2015). In most studies, the concept of vine balance is primarily used in terms of C (Howell, 2001), although N balance is also considered to be of major importance. Understanding both C and N dynamics, including storage, remobilization, and final fate, while taking into account N demand, is critical for the development of sustainable fertilization programs (Muhammad *et al.*, 2020). In a previous article, we demonstrated that the mobilization of root N reserves plays a major role in fruit N balance (Verdenal *et al.*, 2020). The ability of perennial crops to accumulate N reserves in roots and trunk has implications for plant vigor and production over the following years. In this article, the carry-over effect of both crop thinning and N supply on plant DW, as well as on C and N dynamics over two consecutive vine-growing seasons, is addressed. The effects on fruit composition and fertilizer use efficiency are also highlighted. Our findings were the result of the implementation of a large crop load gradient and the use of a ^{15}N-labelling approach on the white

grapevine cultivar Chasselas (*Vitis vinifera* L.). Advantages and limitations of the ^{15}N-labelling method to differentiate the reserve N mobilization from the seasonal root N uptake from the soil are discussed.

4.3 Materials and methods

4.3.1 Experimental site

The trial was conducted over two years (2017–2018) at the Agroscope experimental site in Pully, Switzerland (46°30'45.8"N, 6°40'05.7"E). The local climate is temperate. During the first vine-growing season (April–October 2017), the total precipitation was 562 mm, and the daily mean temperature was 16.6°C. The 2018 climatic conditions were drier and hotter than 2017, with 412 mm of total precipitation and 17.8°C of average daily mean temperature from April through October (data from the Swiss meteorological station in Pully). The low-calcareous colluvial soil of the site was composed of 47 wt.% sand, 38 wt.% silt, and 15 wt.% clay. The soil contained 1.75 wt.% of organic matter, 0.10 wt.% total N (TN), 4.3 wt.% carbonates (eq. $CaCO_3$), and the pH was 7.9. Phosphorus (P, 8.2 mg kg^{-1}), potassium (K, 25.2 mg kg^{-1}) and magnesium (Mg, 11.4 mg kg^{-1}) were not restrictive for vine growing.

4.3.2 Plant material

Vitis vinifera L. Chasselas was grafted onto rootstock 3309 C and planted in 2013 in 90 L pots. Planting in pots ensured a good recovery of the root biomass when the vines were uprooted for analyses, while the pot size allowed an unconstrained development of the roots. Before plantation, 225 pots were randomly disposed of underground with a planting density of 8,330 vines ha^{-1} (1.5 × 0.8 m) and filled with the soil of the trenches as a growth medium. The soil water-holding capacity was 11 L per pot. The plant water potential was monitored to prevent possible water restriction using a pressure chamber (Model 600; PMS Instruments, Albany, NY, USA) (Scholander *et al.* 1965). Vines were drip-irrigated twice in July for both seasons (i.e., total 12 L water per plant and per year) to maintain a stem water potential above –0.8 MPa (no water deficit). Vines were trained in a single Guyot trellis system, with 60 cm trunk height and seven shoots per cane. The canopy was trimmed at 120 cm above the trunk three times per season: on the day of year (DOY) 164, 191, and 215 in 2017; and on DOY 162, 183, and 218 in 2018. The dates of the main phenological stages were similar between 2017 and 2018: 50% bud break (phenological scale BBCH 05; Lancashire *et al.* 1991) occurred on DOY 94 and 99, respectively; 50% flowering (BBCH 65) occurred on DOY 164 and 161; 50% veraison (i.e., the onset of grape ripening, BBCH 85) occurred on DOY 214 in both years; and harvest was performed on DOY 257 and 269, respectively. At the end of 2017, winter pruning was completed, and the shoots were removed from the

experimental plot. Despite homogeneity of the entire plot in terms of plant material and growing conditions, eight out of the 225 vines were identified as outliers (i.e., low vigour, low photosynthetic activity, low fruitfulness, low berry set, and incomplete winter cold hardening) and were discarded to optimize the homogeneous conditions of the trial.

4.3.3 Crop load and ^{15}N-labelling treatments

Crop load treatment

In each group of 12 plants, a large crop load gradient was built by crop thinning at bunch closure (phenological stage BBCH 77; DOY 193 in 2017 and DOY 179 in 2018), keeping two to ten bunches per plant. Crop thinning in 2018 was based on the yield at harvest 2017 in order to maintain each plant under the same crop load treatment over the two consecutive seasons and promote cumulative responses. For statistical analyses, the groups of plants excavated before the 2017 crop thinning, that is, at bud break (1 group) and flowering (1 group), were considered homogeneous groups of plants, whereas the data from the other groups were split into two sub-groups of plants, that is, low-yield conditions (LYC) versus high-yield conditions (HYC). The threshold to split the groups of plants excavated in 2017 was 7.0 tons ha^{-1} at veraison (1 group, CT) and 13.0 tons ha^{-1} at harvest 2017 (2 groups, CT and F17), based on the median crop load by the time of excavation. The thresholds at bud break 2018 (2 groups) and flowering 2018 (2 groups) were based on the median crop load at harvest 2017. Due to a higher yield potential in 2018, the thresholds in the groups of plants excavated at veraison 2018 (2 groups) and at harvest 2018 (3 groups, CT, F17 and F17+18) were 12.5 tons ha^{-1} and 21.0 tons ha^{-1}, respectively.

Fertilization treatment

Three fertilization levels were set: a control treatment (CT); a treatment with one fertilization in 2017 only (F17); and a treatment with fertilization in both 2017 and 2018 (F17 + 18). In 2017, the groups of 12 plants corresponding to the treatments F17 and F17 + 18 each received 2.4 g N per plant (20 kg N ha^{-1}) in the form of ^{15}N-labelled urea (10 atom % ^{15}N; Sigma-Aldrich, Buchs, Switzerland), applied on the leaves at veraison and split into four applications (DOY 199, 208, 214, and 226). In 2018, only the plants from the treatment F17 + 18 again received 2.4 g of ^{15}N-labelled urea in the same conditions (DOY 198, 204, 211 and 219). The labelled foliar urea was carefully applied plant by plant on both sides of the canopy (dilution 3.44% w/v) with hand-sprayers (Spray-matic 1.25, Birshmeier, Stetten, Switzerland). No other fertilization occurred during the trial.

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

4.3.4 Field measurements and sample preparation

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

The light-exposed leaf area (m^2 m^{-2} of ground) was measured, based on the canopy height, width, and porosity, measured after veraison the year of excavation (on DOY 237 in 2017 and on DOY 227 in 2018), as described by Carbonneau (1995). The total leaf area (TLA) per vine was assessed with the non-destructive method of Mabrouk and Carbonneau (1996), based on the strong correlation between the length of a shoot and its TLA. To determine this equation in our context, 15 shoots from 15 different buffer plants were collected on DOY 206 in 2017. The total shoot length (TSL, main shoot + laterals) was measured, and the TLA was determined with a leaf area meter (LI-3100C; Li-Cor Biosciences, Lincoln, NE, USA). As a result, equation (1) allowed the transformation of measured TSL into estimated TLA for both seasons (r = 0.98):

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

The leaf mineral nutrient composition (i.e., total N, P, K, Ca, and Mg) was determined from the dry extracts of two adult leaves (blade + petiole) per vine just after veraison (DOY 229 in 2017 and DOY 212 in 2018) and at leaf fall (DOY 290 in 2017 only) (Sol-Conseil Laboratory, Gland, Switzerland). The chlorophyll index was measured in 2018 at DOY 222 using an infrared non-destructive method on adult leaves from the median part of the canopy (N-Tester; Yara International, Paris, France); this method reflects the intensity of the green colour of the canopy and is thus well correlated to leaf N concentration

(van Leeuwen *et al.* 2000; Aranguren *et al.* 2018). In both 2017 and 2018, leaf gas exchanges were measured approximately every 10 days between flowering (BBCH 65) and harvest (BBCH 89), on sunny days between 12:00 PM and 03:00 PM, on the plants excavated at harvest and on one fully expanded leaf per vine: net assimilation (A), transpiration (E), stomatal conductance (gsw), internal CO_2 concentration (C_i), and intrinsic water use efficiency (WUE_i) were determined non-destructively with a portable photosynthesis system (LI-6800; Li-Cor Biosciences). During the measurements, the ambient conditions inside the LI-6800 leaf chamber were controlled by the system with the following pre-set parameters: air flow, 700 µmol.s^{-1}; relative humidity, 50 %; ambient CO_2, 380 µmol.mol^{-1}; fan speed, 5000 rpm; and light source, 2000 µmol.m^2.s^{-1}.

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

4.3.5 Stable isotope analysis and fruit composition

The C and N isotope compositions were analysed by elemental analysis/isotope ratio mass spectrometry. A Carlo Erba 1108 elemental analyser (Fisons Instruments, Milan, Italy) was coupled with a Conflo III interface to a Delta V Plus isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) operated under continuous helium flow, as detailed in Spangenberg and Zufferey (2018). The calibration and normalization of the measured $\delta^{13}C$ values to the standard Vienna Pee Dee Belemnite (VPDB) was performed with international and in-house reference materials at different ^{13}C at natural abundance ($\delta^{13}C$ values in Brand *et al*. 2014 and Spangenberg *et al.*, 2010 respectively). The calibration and normalization of the $\delta^{15}N$ measurements to the international Air-N_2 scale was realized with a dedicated set of six in-house urea standards with different ^{15}N enrichments, covering the $\delta^{15}N$ range of –1.39 to 1275 mUr. The preparation of these standards is detailed in Spangenberg and Zufferey (2019). The stable isotope composition of each sample was reported as δ value (i.e. $\delta^{13}C$ and $\delta^{15}N$), which is the relative deviation

of the molar ratio (R) of the heaviest (^{i}E) to the lightest (^{j}E) isotopes (e.g. $^{13}C : {}^{12}C$, $^{15}N : {}^{14}N$) from an international standard (Coplen 2011):

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

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

4.3.6 Data treatment

The data treatments were realized based on the method detailed in Verdenal *et al.* (2021). The mineral content of each plant fraction was reported as Q (i.e., CQ for TOC quantity and NQ for TN quantity, in g) and calculated as below for NQ:

$$NQ_{fraction} = DW_{fraction} \times TN. \quad (3)$$

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

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

The RSA (in atom percent) represents the proportion of newly incorporated N atoms originating from the labelled source (e.g., fertilizer), compared with the TN quantity in the sample (Cliquet et al. 1990). The RSA also represents the organ sink strength, which is independent of the organ size (Deléens *et al.*, 1997):

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

The NNP (in g), originating from the labelled source, may be quantified in each plant fraction and the %P subsequently calculated (Cliquet *et al.*, 1990).

$$NNP_{fraction} = RSA_{fraction} \times NQ_{fraction} \quad (6)$$

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

The overall net N uptake can then be calculated:

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

Considering the fertilizer as the ^{15}N-labelled source in the calculation of RSA (i.e., $A\%_{N\ supplied}$ = 10 atom % ^{15}N) allowed estimating the partitioning of the fertilizer N assimilated by the plant over the two vine-growing seasons. Alternatively, considering as the labelled source the initial N reserves present in the perennial fractions of the plant at the onset of the second growing season (i.e., $A\%_{N\ supplied} = A\%_{(roots+trunk)}$, as affected by the residual labelled N from the 2017 N supply) theoretically allowed estimating the partitioning of the N reserves during the second season and to differentiate them from the seasonal root N uptake (non-labelled) in each plant fraction as follows:

$$NQ_{root\ uptake} = NQ_{total} - NQ_{reserves} \quad (9)$$

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

Data were analysed using XLSTAT version 2020.5.1 software (Addinsoft, Paris, France). Each date of excavation was subject to separate statistical analysis for the determination of the effects of the investigated factors, that is, crop load treatment (from bud break 2017), fertilization treatment (from harvest 2017), and their interaction. Only the statistical results of the excavation at harvest 2018 are presented in details in this article, since we had to select the main results only and the grape composition

at harvest are the major (and most significant) results for grape production and winemaking. The significance of differences and interactions between treatments was assessed with one- or two-way ANOVA ($p < 0.05$), depending on the excavation date. A Newman-Keuls post hoc test performed to differentiate more than two groups. Principal component analysis was used to evaluate the must FAA profiles.

4.4 Results

4.4.1 Vegetative growth and development

A large yield gradient was achieved via bunch thinning in both consecutive years 2017 and 2018 (Table 4.1). As a result, the 2017 yield varied on average from 0.8 kg m^{-2} under LYC to 1.9 kg m^{-2} under HYC. The 2018 yield capacity was higher, and the yield varied on average from 1.2 kg m^{-2} under LYC to 3.2 kg m^{-2} under HYC. Crop load affected neither the bunch weight (an average of 343 ± 114 g in 2017 and 469 ± 132 g in 2018) nor the pruning weight (average 723 ± 314 g in the winter of 2017–2018). The 2018 average light-exposed leaf area was 1.4 ± 0.1 m^2 per m^2 of soil. The 2018 average TLA was 2.0 ± 0.4 m^2 per plant. Leaf-to-fruit ratio (i.e., TLA to crop load ratio) was highly affected by crop load in both years and was particularly low in 2018 under HYC (average 0.7 m^2 kg^{-1} compared with 1.8 m^2 kg^{-1} under LYC). The average chlorophyll index at veraison 2018 was homogeneous in the whole plot at 470 ± 17, indicating an adequate leaf N content regardless of fertilization and crop load. No N deficiency symptoms could be observed on the leaves of the control treatment. Average N content in leaves (blade + petiole) at veraison 2017 was adequate at 2.15% DW (2.15% in the control and 2.16% in the fertilized treatment), while 2018 leaf N content was lower – but not deficient – at 1.83% DW (1.79% in the control and 1.86% in the fertilized treatment). The other 2018 nutrient contents were adequate with 0.20% P, 1.85% K, 2.53% Ca, and 0.20% Mg, regardless of the treatment. Foliar N supply at veraison had no impact on the vegetative observations or on the yield components in either year.

Table 4.1. Two-year field measurements as a function of foliar N supply and crop load. Chasselas cultivar, 2018, at Pully Switzerland.

Year	Variable	Foliar N supply			Crop load			Interaction fertilization × crop load
		0 kg ha^{-1}	20 kg ha^{-1}	*p*-value	LYC	HYC	*p*-value	
2017 (n = 21)	Fruitfulness (bunches per shoot)	2.3	2.0	ns	2.1	2.2	ns	ns
	Bunches per vine	5.2	5.2	ns	3.8	6.7	*	ns
	Bunch weight (g)	354	332	ns	320	369	ns	ns
	Yield (kg m^{-2})	1.4	1.3	ns	0.8	1.9	***	ns
	Total leaf area (m² per plant)	1.8	1.9	ns	1.8	2.0	ns	ns
	Leaf-to-fruit ratio (m² TLA kg^{-1})	1.7	1.7	ns	2.3	1.0	**	ns
2018 (n = 22)	Winter pruning weight (g plant^{-1})	749	694	ns	739	705	ns	ns
	Fruitfulness (bunches per shoot)	2.1	2.1	ns	2.0	2.2	ns	ns
	Bunches per vine	5.7	6.1	ns	3.3	8.4	***	ns
	Bunch weight (g)	482	447	ns	437	492	ns	ns
	Yield (kg m^{-2})	2.1	2.3	ns	1.2	3.2	***	ns
	Total leaf area (m² per plant)	1.9	2.0	ns	1.8	2.1	ns	ns
	Leaf-to-fruit ratio (m² TLA kg^{-1})	1.2	1.3	ns	1.8	0.7	***	ns

Note. HYC, high-yielding conditions; LYC, low-yielding conditions; TLA, total leaf area; ns, non-significant; *p < 0.05; **p < 0.01; ***p < 0.001.

The 2018 seasonal photosynthesis activity was globally higher in the period from flowering to veraison (DOY 165 to 204, average $A_{Jun–Jul}$ = 14.8 µmol CO_2 m^{-2} s^{-1}) in comparison with the period from veraison to harvest (DOY 214 to 247, average $A_{Aug–Sep}$ = 10.3 µmol CO_2 m^{-2} s^{-1}) (Table 4.2). The gsw values varied from 259 mmol m^{-2} s^{-1} on DOY 165 down to 112 mmol mol^{-1} on DOY 240. Consequently, WUE_i increased gradually from flowering to harvest (average $WUE_{Jun–Jul}$ = 66 and $WUE_{Aug–Sep}$ = 73). The fertilized vines punctually had lower gas exchanges than the control vines (i.e., DOY 176 and 194; data not shown). However, these differences could not be related to fertilization treatment, as urea supply occurred later in the season (i.e., from DOY 198 to 219) and no difference was observed after foliar N supply. Crop thinning significantly reduced the gas exchange rates on DOY 176 and had an overall impact on them over the entire period of flowering to harvest, with lower E (average –6%), lower A (–4%), lower gsw (–8%), lower C_i (–2%), and subsequently higher WUE_i (+ 6%).

Table 4.2. Foliar gas exchange rates measured from June to September 2018 as a function of DOY and crop load for the Chasselas cultivar, 2018, at Pully Switzerland.

2018 (n = 22)	E (mmol H_2O m^{-2} s^{-1})			A (µmol CO_2 m^{-2} s^{-1})			gsw (mmol m^{-2} s^{-1})			C_i (µmol mol^{-1})			WUE_i (A*1000/gsw)		
DOY	LYC	HYC	*p*-value	LYC	HYC	*p*-value	LYC	HYC	*p*-value	LYC	HYC	*p*-value	LYC	HYC	*p*-value
165	4.4	4.4	ns	17.0	17.0	ns	267	257	ns	236	232	ns	64	67	ns
176	5.1	6.3	***	14.2	16.2	*	207	268	**	221	239	*	75	61	*
183	6.7	7.4	ns	14.9	15.7	ns	246	286	ns	236	245	ns	62	56	ns
194	6.2	6.8	ns	13.6	14.8	ns	202	235	ns	224	232	ns	71	65	ns
204	5.8	6.0	ns	12.1	12.5	ns	178	184	ns	229	228	ns	69	69	ns
214	5.6	5.3	ns	13.1	12.6	ns	221	212	ns	245	243	ns	60	62	ns
222	4.6	4.7	ns	10.1	10.7	ns	139	152	ns	223	228	ns	75	72	ns
232	4.3	4.5	ns	10.0	10.1	ns	124	135	ns	212	222	ns	82	76	ns
240	3.5	3.5	ns	9.0	8.6	ns	113	115	ns	216	223	ns	82	78	ns
247	2.9	3.3	ns	9.2	10.3	ns	132	155	ns	236	240	ns	72	68	ns
Average	4.9	5.2	*	12.3	12.8	*	183	200	**	228	233	**	71	67	**

Note. Mean values within the same row followed by different letters are significantly different (Newman-Keuls, $p < 0.05$). DOY, day of year; E, transpiration; A, net assimilation; Ca, ambient CO_2; Ci, intercellular CO_2; gsw, stomatal conductance; WUE_i, intrinsic water use efficiency; LYC, low-yield conditions; HYC, high-yield conditions; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

4.4.2 Fruit composition

In comparison with the control treatment, foliar N supply did not affect grape maturation (i.e., TSS and TA contents), but it improved the must YAN concentration (average gain + 34 mg N L^{-1}), particularly in terms of PAN (+27 mg N L^{-1}) (Table 4.3). Foliar N supply also increased the K concentration (+134 mg L^{-1}). Conversely to foliar N supply, crop thinning highly affected grape maturation in 2018: TSS concentration and pH were lower under HYC, while TA, tartaric acid, malic acid, and NH_4^+ concentrations increased. Similarly, to 2017, the variations of must PAN and YAN at the 2018 harvest as a function of crop load were not significant.

Table 4.3. Must composition of the vines excavated at harvest 2018, as a function of foliar N supply and crop load for the Chasselas cultivar, 2018, at Pully Switzerland.

Variable	Foliar N supply in 2018			Crop load			Interaction fertilization × crop load
	0 kg ha^{-1} (n = 12)	20 kg ha^{-1} (n = 10)	p-value	LYC (n = 10)	HYC (n = 12)	p-value	
TSS (Brix)	20.2	20.3	ns	21.4	19.1	***	ns
pH	3.56	3.65	ns	3.68	3.53	**	ns
TA (g L^{-1})	4.7	4.8	ns	4.3	5.1	***	ns
Tartaric acid (g L^{-1})	5.2	5.3	ns	5.1	5.5	**	ns
Malic acid (g L^{-1})	2.3	2.5	ns	2.1	2.6	**	ns
Potassium (mg L^{-1})	1,808	1,938	*	1,925	1,820	ns	ns
NH_3 (mg L^{-1})	12	21	ns	10	23	*	ns
PAN (mg N L^{-1})	82	109	*	96	94	ns	ns
YAN (mg N L^{-1})	92	126	*	104	113	ns	ns

Note. HYC, high-yielding conditions; LYC, low-yielding conditions; TSS, total soluble sugars; TA, titratable acidity; PAN, primary amino nitrogen; YAN, yeast assimilable nitrogen; ns, non-significant; *p < 0.05; **p < 0.01; ***p < 0.001.

As an indicator of vine balance, the leaf-to-fruit ratio was highly correlated (p < 0.0001) to both sugar content and TA in the must at harvest 2018 (r = 0.82 and r = –0.78, respectively, Figure 4.1). The must TSS varied from 19.1 °Bx under HYC up to 21.4 °Bx under LYC, and TA varied from 5.1 g L^{-1} under HYC down to 4.3 g L^{-1} under LYC. In other words, crop thinning significantly improved grape ripening in 2018, as shown by the variation of the maturity index (TSS-to-TA ratio) as a function of crop load (r = –0.81 and p < 0.0001; Figure 4.2). The maturity index was not affected in 2017 (p = 0.171), probably due to the smaller yield gradient and the less restrictive leaf-to-fruit ratio under HYC (1.0 m^2 kg^{-1} in 2017 versus 0.7 m^2 kg^{-1} in 2018). The must YAN concentration at harvest 2018 was improved by the fertilization treatment and reached, on average, 126 ± 33 mg L^{-1} in comparison with 92 ± 34 mg L^{-1} in the control treatment. However, the impact of foliar N supply on the YAN concentration was related to the crop load: it was insignificant under LYC (p = 0.204), while it was significant under HYC (p = 0.032), similarly to 2017. Independently from crop load, plant vigor (i.e., canopy weight) increased with must YAN concentration at harvest under both control (p = 0.005) and fertilization treatments (p = 0.006).

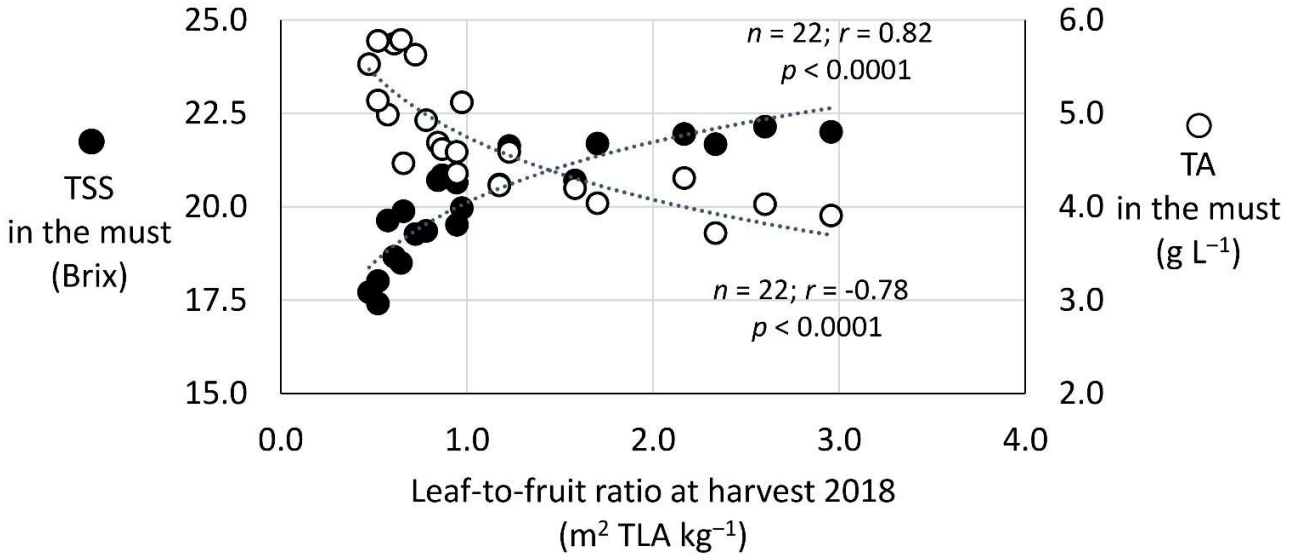


Figure 4.1. Impact of the leaf-to-fruit ratio on the concentrations of TSS and TA in the must at harvest 2018. TLA, total leaf area. Chasselas cultivar, 2018, Pully Switzerland.

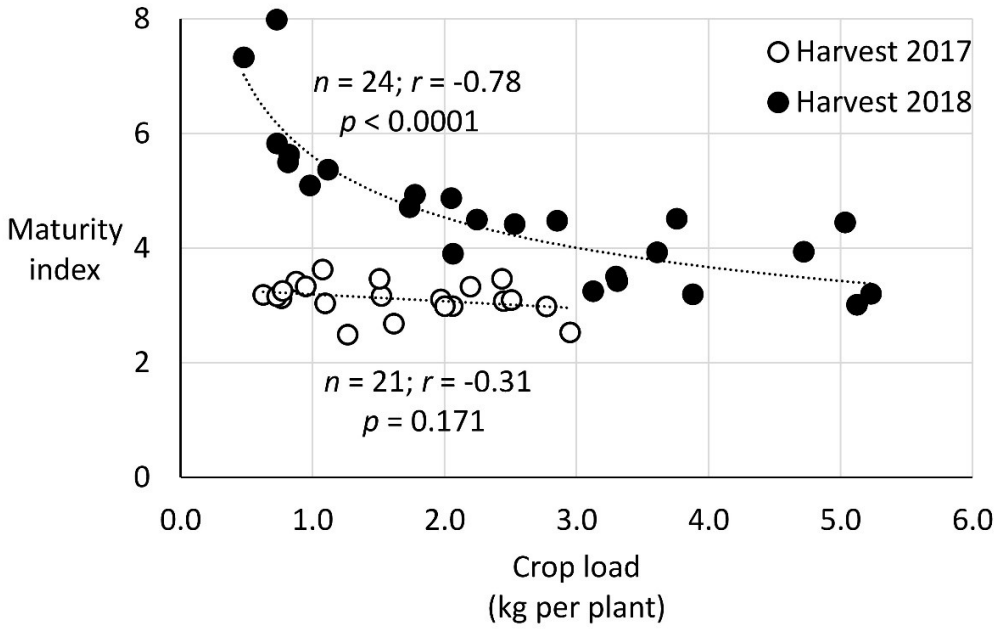


Figure 4.2. Impact of crop load on the grape maturity index (TSS-to-TA ratio) at harvests 2017 and 2018. TSS, total soluble sugars in Brix degree; TA, titratable acidity as equivalent to tartaric acid. Chasselas cultivar, 2018, Pully Switzerland.

The year had a major influence on most of the individual FAA quantities, while the total FAA quantity remained similar between the 2017 and 2018 harvests (115 ± 30 mg N L^{-1} in 2017 and 107 ± 34 mg N L^{-1} in 2018) (Table 4.4). Foliar N supply increased the concentration of most individual FAA as well as of the total FAA. Conversely, crop thinning had no significant impact on the total FAA quantity and only increased the concentration of a few major FAAs, including glutamic acid and proline.

Table 4.4. Must amino acid concentrations (mg N L^{-1}) at harvest 2018, as a function of year, foliar N supply, and crop load for the Chasselas cultivar, 2018, at Pully Switzerland.

Amino acids (mg N L^{-1})	Year		*p*-value	2018 fertilization		*p*-value	Crop load		*p*-value	Interaction fertilization × crop load
	2017 (*n* = 21)	2018 (*n* = 22)		0 kg ha^{-1} (*n* = 23)	20 kg ha^{-1} (*n* = 20)		LYC (*n* = 21)	HYC (*n* = 22)		
Ala	9.7	8.3	ns	7.6	10.4	**	8.6	9.4	ns	ns
Arg	37.1	24.1	***	26.3	34.9	**	30.6	30.6	ns	ns
Asn	0.5	0.5	ns	0.5	0.6	ns	0.5	0.5	ns	ns
Asp	5.2	5.4	ns	4.8	5.8	**	5.3	5.3	ns	*
Cit	2.0	0.8	***	1.1	1.7	**	1.4	1.4	ns	ns
GABA	3.7	8.0	***	5.5	6.2	ns	5.8	5.9	ns	ns
Gln	2.5	1.0	***	1.5	2.0	*	1.7	1.8	ns	ns
Glu	10.2	9.8	ns	9.2	10.8	*	11.1	8.9	**	ns
His	2.4	1.9	ns	1.9	2.5	*	2.3	2.1	ns	ns
Hyp	0.3	0.5	***	0.3	0.4	ns	0.4	0.3	ns	ns
Ile	1.5	1.8	*	1.5	1.8	*	1.8	1.5	ns	ns
Leu	1.9	1.1	***	1.4	1.6	ns	1.6	1.4	ns	ns
Lys	0.3	0.3	ns	0.3	0.3	ns	0.4	0.3	*	ns
Met	0.2	0.8	***	0.5	0.5	ns	0.6	0.5	*	ns
Orn	0.8	0.4	***	0.5	0.7	ns	0.7	0.6	ns	ns
Phe	0.8	1.1	***	0.9	1.0	ns	1.0	0.9	ns	ns
Pro	14.9	23.8	**	17.0	21.7	ns	25.1	13.6	***	ns
Ser	7.4	6.3	*	6.0	7.7	**	6.6	7.0	ns	ns
Thr	8.8	7.1	**	6.6	9.3	***	7.4	8.5	ns	ns
Trp	1.6	1.2	**	1.3	1.5	*	1.5	1.3	ns	*
Tyr	1.5	0.9	***	1.0	1.3	*	1.2	1.1	ns	ns
Val	3.0	2.4	**	2.4	2.9	*	2.9	2.5	ns	ns
TOTAL	115	107	ns	99	126	**	119	106	ns	ns

Note. HYC, high-yielding conditions; LYC, low-yielding conditions; ns, non-significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

In terms of FAA profile, crop thinning and foliar N supply affected different FAAs, except GABA and threonine (Table 4.5). LYC decreased the proportions of alanine, GABA, glutamine, serine, and threonine, while it increased the proportion of proline. Foliar N supply increased the proportions of citrulline and threonine, while it decreased the proportions of GABA, hydroxyproline, leucine, lysine, methionine, and phenylalanine (Table 4.5).

Table 4.5. Must amino acid profiles (% of total amino N) at harvest 2018, as a function of year, fertilization, and crop load for the Chasselas cultivar, 2018, at Pully Switzerland.

Amino acids (%)	Year		*p*-value	2018 fertilization		*p*-value	Crop load		*p*-value	Interaction fertilization × crop load
	2017 (n = 21)	2018 (n = 22)		0 kg ha^{-1} (n = 23)	20 kg ha^{-1} (n = 20)		LYC (n = 21)	HYC (n = 22)		
Ala	8.2	7.8	ns	7.7	8.2	ns	7.1	8.8	***	ns
Arg	31.7	21.7	***	25.6	27.8	ns	25.4	28.0	ns	ns
Asn	0.4	0.5	ns	0.5	0.4	ns	0.5	0.4	ns	ns
Asp	4.6	5.4	ns	5.2	4.8	ns	4.7	5.3	ns	ns
Cit	1.7	0.7	***	1.0	1.3	***	1.2	1.2	ns	ns
GABA	3.2	7.7	***	6.0	5.0	**	4.9	6.1	***	**
Gln	2.1	0.9	***	1.5	1.6	ns	1.4	1.6	*	ns
Glu	9.0	9.4	ns	9.7	8.7	ns	9.6	8.8	ns	ns
His	2.0	1.8	**	1.8	1.9	ns	1.9	1.9	ns	ns
Hyp	0.2	0.4	***	0.4	0.3	*	0.3	0.3	ns	ns
Ile	1.3	1.7	***	1.5	1.4	ns	1.5	1.5	ns	ns
Leu	1.7	1.0	***	1.4	1.3	**	1.3	1.3	ns	ns
Lys	0.3	0.3	ns	0.3	0.3	*	0.3	0.3	ns	ns
Met	0.2	0.8	***	0.5	0.4	***	0.5	0.5	ns	ns
Orn	0.7	0.4	***	0.5	0.5	ns	0.5	0.5	ns	ns
Phe	0.7	1.1	***	1.0	0.8	**	0.9	0.9	ns	ns
Pro	12.7	21.0	***	17.2	16.6	ns	21.0	12.7	***	ns
Ser	6.4	6.0	ns	6.2	6.1	ns	5.6	6.8	***	ns
Thr	7.7	6.5	**	6.7	7.5	*	6.2	7.9	***	ns
Trp	1.4	1.1	**	1.3	1.2	ns	1.3	1.3	ns	ns
Tyr	1.2	0.8	***	1.0	1.0	ns	1.0	1.0	ns	ns
Val	2.6	2.2	***	2.4	2.3	ns	2.4	2.3	ns	ns

Note. HYC, high-yielding conditions; LYC, low-yielding conditions; ns, non-significant; *p < 0.05; **p < 0.01; ***p < 0.001.

The musts were discriminated as a function of their FAA profiles using a principal component analysis (PCA; Figure 4.3). The variables crop load (in kg per plant) and maturity index were added to the PCA as supplementary variables. The PCA showed that the must FAA profiles at harvest were discriminated first by the year and then by the combination of both crop load and grape maturity. Since the 2017 maturity index was constant, the crop load was the main factor of discrimination in that year. No discrimination was observed for N-fertilized versus control vines. Proline was highly correlated with TSS in terms of both quantity (r = 0.71; P < 0.0001) and proportion of total FAA (r = 0.86; P < 0.0001).

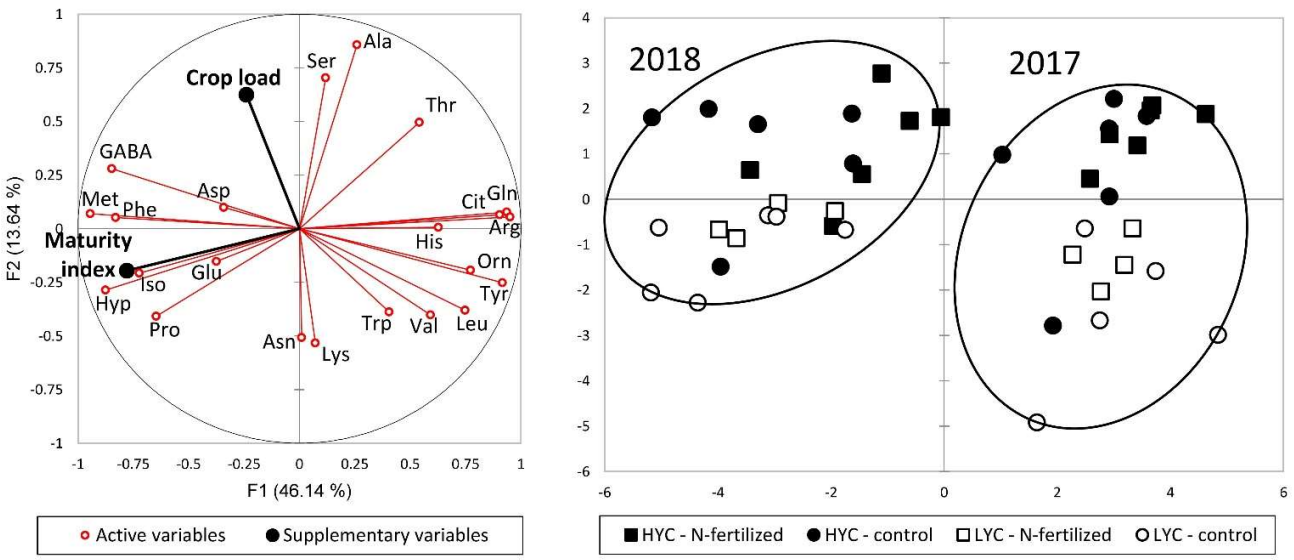


Figure 4.3. Discrimination of the musts at harvests 2017 and 2018, as a function of their amino N profiles. The supplementary data crop load (kg per plant) and maturity index (TSS-to-TA ratio) did not influence the cloud. LYC: low-yield conditions; HYC: high-yield conditions. Chasselas cultivar, 2018, at Pully, Switzerland.

4.4.3 C and N dynamics

Table 4.6 summarizes the variations of DW, TOC, δ^{13}C, CQ, TN, δ^{15}N, and NQ in the plant parts at harvest under both LYC and HYC and in the absence of foliar N supply. On average (both LYC and HYC together), the DW varied from 70% in the roots to 21% in the must at harvest. The trunk and roots had the highest TOC concentration (average 47 DW), while the must had the lowest (average 38% DW). The δ^{13}C values varied significantly among the plant parts, from –29.8 mUr in the pomace to –27.8 mUr in the must, on average. Under HYC, the canopy and must had both the highest CQ (i.e., 274 and 229 g, respectively), while under LYC, the must CQ was low (97 g) and represented only 40% of the canopy CQ. The TN was highest in the canopy and pomace (average 0.9% DW) and lowest in the must (average 0.2% DW) independent of the crop load. The highest δ^{15}N values were in the must (average 73 mUr) and the canopy and pomace (average 26 mUr). On average, the δ^{15}N values were higher in 2018 than in 2017, that is, 45 mUr and 16 mUr, respectively. When compared with the other plant parts, canopy NQ was the highest under both HYC and LYC at 5.5 g and 4.9 g, respectively.

Table 4.6. DW and C and N composition of the different plant parts at harvest 2018, without fertilization, under both low- and high-yield conditions for the Chasselas cultivar, 2018, Pully Switzerland.

		Roots	Trunk	Canopy	Pomace	Must	*p*-value
HYC	DW (g)	300 ± 62 b	395 ± 19 b	614 ± 213 a	228 ± 60 b	604 ± 158 a	***
	DW (%)	68.0 ± 2.7 a	60.5 ± 1.4 b	40.2 ± 1.9 c	27.6 ± 1.5 d	20.5 ± 1.5 e	***
	TOC (% DW)	47.7 ± 1.1 a	46.7 ± 0.7 a	44.6 ± 0.5 b	43.4 ± 0.9 c	37.8 ± 1.0 d	***
	δ^{13}C (mUr)	–29.0 ± 0.3 c	–28.6 ± 0.2 b	–28.9 ± 0 bc	–30.1 ± 0.4 d	–28.2 ± 0.3 a	***
	CQ (g)	143 ± 28 cd	185 ± 11 bc	274 ± 94 a	99 ± 27 d	229 ± 62 ab	***
	TN (% DW)	0.5 ± 0.1 b	0.4 ± 0.0 c	0.9 ± 0.2 a	0.9 ± 0.1 a	0.2 ± 0.0 d	***
	δ^{15}N (mUr)	47.0 ± 17 ab	44.1 ± 29.4 ab	28.6 ± 28 b	23.7 ± 21.5 b	74.7 ± 39.3 a	*
	NQ (g)	1.5 ± 0.1 b	1.4 ± 0.2 b	5.5 ± 1.5 a	2.2 ± 0.7 b	1.0 ± 0.4 b	***
LYC	DW (g)	323 ± 70 b	357 ± 41 b	547 ± 157 a	80 ± 26 c	255 ± 109 b	***
	DW (%)	70.9 ± 3.9 a	61.4 ± 1.4 b	42.2 ± 2.4 c	29.4 ± 1.6 d	21.4 ± 1.0 e	***
	TOC (% DW)	47.2 ± 1.1 a	46.2 ± 0.2 b	44.7 ± 0.7 c	42.8 ± 0.9 d	38.1 ± 0.5 e	***
	δ^{13}C (mUr)	–28.7 ± 0.2 bc	–28.4 ± 0.1 b	–28.7 ± 0.5 bc	–29.4 ± 0.7 c	–27.4 ± 0.8 a	***
	CQ (g)	152 ± 31 b	165 ± 20 b	244 ± 70 a	34 ± 11 d	97 ± 41 c	***
	TN (% DW)	0.5 ± 0 b	0.4 ± 0.1 b	0.9 ± 0.2 a	0.9 ± 0.1 a	0.1 ± 0.0 c	***
	δ^{15}N (mUr)	42.8 ± 21.0 ab	61.2 ± 37.0 b	25.5 ± 7.7 b	22.5 ± 7.3 b	72.0 ± 30.1 a	*
	NQ (g)	1.6 ± 0.4 b	1.3 ± 0.3 b	4.9 ± 2.1 a	0.7 ± 0.2 b	0.4 ± 0.1 b	***

Note. HYC, high-yielding conditions; LYC, low-yielding conditions; DW, dry weight; TOC, total organic carbon; CQ, carbon quantity; TN, total nitrogen; NQ, nitrogen quantity; ns, non-significant; *p < 0.05; **p < 0.01; ***p < 0.001.

Carbon

The impact of foliar N supply and crop load on C distribution is summarized in Table 4.7. Foliar N supply had a negligible effect on TOC, CQ, and δ^{13}C: only the trunk showed –2% in TOC and +0.5 mUr in δ^{13}C in the fertilized treatment. Conversely, the whole plant TOC was affected by crop thinning, with a 3% increase under LYC in comparison with HYC, despite a 1% decrease in the pomace. CQ was highly affected by crop thinning: in comparison with HYC, CQ decreased under LYC in the canopy (–21%) and grapes (–58%), while it increased in the roots (+ 17%). The δ^{13}C values slightly increased under LYC in both grapes and roots. While not influenced by crop thinning, the C:N ratio decreased in the grapes (i.e., pomace + must) due to N supply.

Table 4.7. Impact of fertilization and crop load on the carbon concentration (TOC) and quantity (CQ), carbon isotope composition ($\delta^{13}C$), and C:N ratio for the Chasselas cultivar, 2018, at Pully Switzerland.

Variable	Plant fraction	2018 fertilization			Crop load			Interaction fertilization × crop load
		0 kg ha^{-1} (n = 12)	20 kg ha^{-1} (n = 10)	p-value	LYC (n = 10)	HYC (n = 12)	p-value	
TOC (% DW)	Roots	47.6	47.3	ns	47.2	47.7	ns	ns
	Trunk	47.2	46.1	***	46.6	46.7	ns	ns
	Canopy	44.4	44.7	ns	44.6	44.6	ns	ns
	Thinned bunches	44.8	44.8	ns	44.9	44.7	ns	ns
	Pomace	42.4	42.5	ns	42.0	42.9	*	ns
	Must	38.1	37.7	ns	37.8	38.0	ns	ns
	Whole plant	43.9	43.7	ns	44.3	43.3	***	ns
CQ (g)	Roots	134	118	ns	136	116	*	*
	Trunk	179	167	ns	168	178	ns	*
	Canopy	284	302	ns	258	328	*	ns
	Thinned bunches	16	13	ns	18	11	ns	ns
	Pomace	63	68	ns	38	93	***	ns
	Must	168	167	ns	99	237	***	ns
	Whole plant	845	835	ns	716	964	***	ns
$\delta^{13}C$ (mUr)	Roots	–28.8	–28.9	ns	–28.7	–29.0	*	ns
	Trunk	–28.3	–28.8	**	–28.5	–28.6	ns	ns
	Canopy	–29.2	–29.1	ns	–29.1	–29.2	ns	ns
	Thinned bunches	–29.4	–29.6	ns	–29.3	–29.7	*	ns
	Pomace	–29.7	–29.5	ns	–29.3	–29.9	**	ns
	Must	–27.9	–27.7	ns	–27.6	–28.0	*	ns
	Whole plant	–28.7	–28.7	ns	–28.6	–28.8	ns	ns
C:N ratio	Roots	83	74	ns	82	75	ns	ns
	Trunk	124	114	ns	117	121	ns	ns
	Canopy	47	43	ns	47	44	ns	ns
	Thinned bunches	28	28	ns	29	27	ns	ns
	Pomace	53	43	**	50	46	ns	ns
	Must	250	185	*	219	215	ns	ns
	Whole plant	74	64	ns	69	68	ns	ns

Note. HYC, high-yielding conditions; LYC, low-yielding conditions; ns, non-significant; *p < 0.05; **p < 0.01; ***p < 0.001.

The CQ distribution in the plant was monitored over two seasons (Figure 4.4). The differences in CQ between LYC and HYC were mainly related to crop size: the share of grape CQ in the whole plant was higher under HYC in both seasons. Despite a lower CQ in the roots under HYC in comparison with LYC, the kinetics of CQ in the reserves (roots + trunk) were similar in both seasons, with a decrease from bud burst to flowering, an increase from flowering to veraison, and then a slower increase after veraison until leaf fall. A global increase in the C reserves in the perennial parts (root + trunk) was observed at harvest 2018 (+ 26%) in comparison with harvest 2017, independent of the crop load. Both trunk and canopy CQ distributions were similar either under LYC or under HYC. Under HYC, grape CQ at harvest was equivalent to canopy CQ in both the 2017 and 2018 seasons.

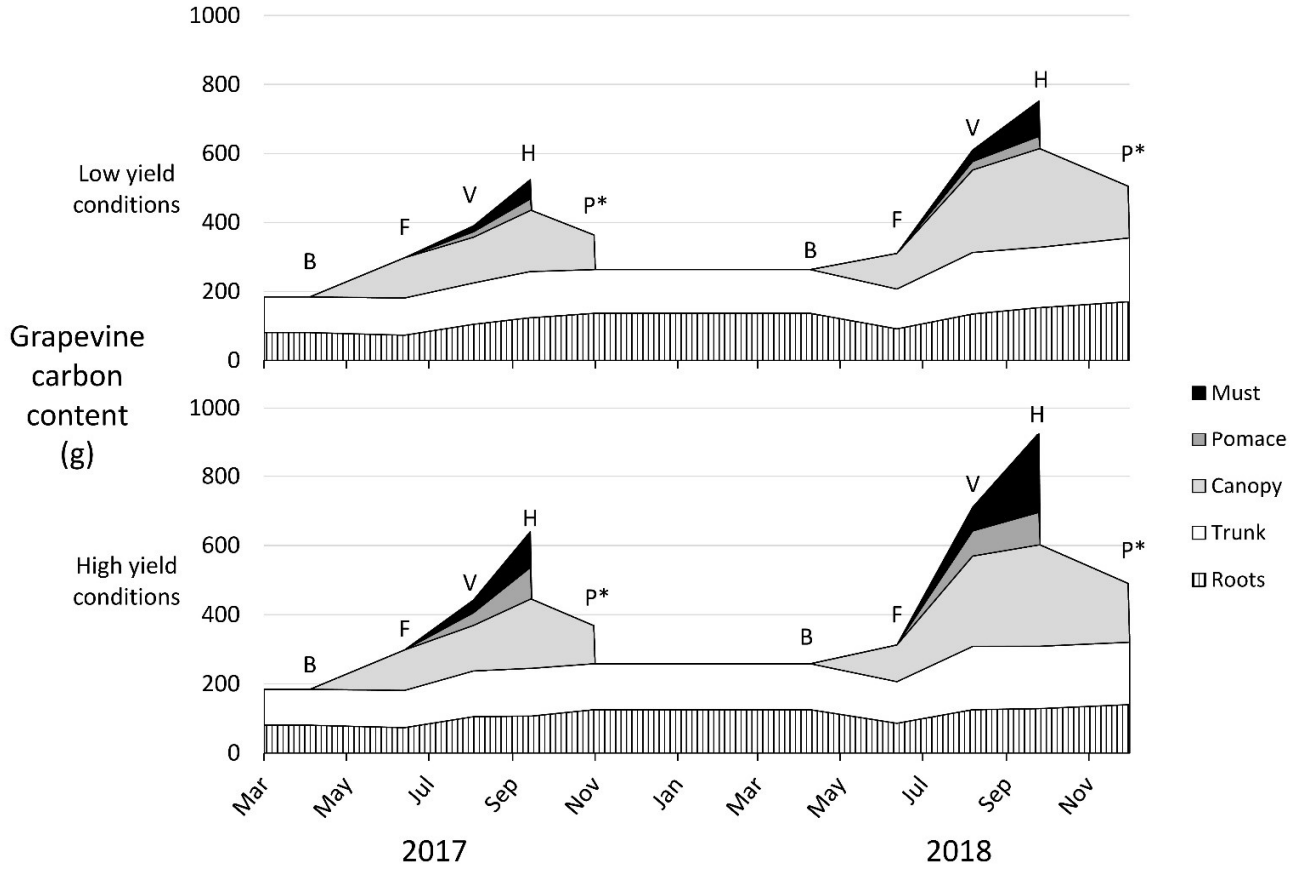


Figure 4.4. C kinetics from March 2017 to December 2018 as a function of crop load. B, bud burst; F, flowering; V, veraison; H, harvest; P, pruning; * extrapolated data. Chasselas cultivar, 2018, at Pully, Switzerland.

Dry Weight and Nitrogen

The changes in DW and N distribution as a function of foliar N supply and crop load are summarized in Table 4.8. In comparison with bud burst 2017, the reserves present in the roots and trunks at bud burst 2018 were higher by 55% for the DW. Without affecting the plant DW, foliar N supply efficiently increased TN concentration in the grapes (+23%). Conversely, crop thinning highly affected the whole plant DW (–27% under LYC in comparison with HYC), with a large decrease in the grapes (–58%) and canopy (–21%) and an increase in the roots (+19%). The overall vine capacity decreased with crop thinning. Crop thinning had no significant impact on TN. Consequently, NQ varied proportionally to DW, with a significant loss in the whole plant under LYC (– 27%), mainly due to the lower crop load.

Table 4.8. Impact of fertilization and crop load on the DW, TN, NQ for the Chasselas cultivar, 2018, at Pully Switzerland.

Variable	Plant fraction	2018 fertilization		*p*-value	Crop load		*p*-value	Interaction fertilization × crop load
		0 kg ha^{-1} (n = 12)	20 kg ha^{-1} (n = 10)		LYC (n = 10)	HYC (n = 12)		
DW (g)	Roots	282	249	ns	288	243	*	*
	Trunk	380	362	ns	359	382	ns	*
	Canopy	638	676	ns	579	736	*	ns
	Thinned bunches	36	29	ns	41	25	ns	ns
	Pomace	149	158	ns	90	217	***	ns
	Must	440	445	ns	261	623	***	ns
	Whole plant	1,925	1,919	ns	1618	2226	***	ns
TN (% DW)	Roots	0.60	0.66	ns	0.59	0.67	ns	ns
	Trunk	0.38	0.41	ns	0.40	0.39	ns	ns
	Canopy	0.98	1.07	ns	0.98	1.06	ns	ns
	Thinned bunches	1.60	1.64	ns	1.58	1.66	ns	ns
	Pomace	0.82	1.00	**	0.86	0.96	ns	ns
	Must	0.16	0.21	*	0.18	0.20	ns	ns
	Whole plant	0.62	0.70	ns	0.66	0.66	ns	ns
NQ (g)	Roots	1.67	1.64	ns	1.69	1.62	ns	*
	Trunk	1.46	1.47	ns	1.43	1.51	ns	*
	Canopy	6.35	7.22	ns	5.73	7.83	*	ns
	Thinned bunches	0.57	0.48	ns	0.64	0.41	ns	ns
	Pomace	1.24	1.65	ns	0.77	2.12	***	ns
	Must	0.74	1.03	ns	0.47	1.29	***	ns
	Whole plant	12.03	13.48	ns	10.72	14.78	*	ns

Note. HYC, high-yielding conditions; LYC, low-yielding conditions; ns, non-significant; *p < 0.05; **p < 0.01; ***p < 0.001.

The distribution of NQ was monitored over two seasons (Figure 4.5). The differences in NQ between LYC and HYC were mainly due to both grapes and canopy: the share of grape and canopy NQ at harvest was lower under LYC, particularly in 2018 (–38%). At harvest, a major part of N was located in the canopy (i.e., 53% of NQ under HYC and 52% under LYC). The NQ in the reserves (roots + trunk) were similar in both seasons, with a decrease from bud burst to flowering and a refilling from flowering to leaf fall. A global increase in the N reserves in the perennial parts (root + trunk) was observed between the 2017 and 2018 harvests and was greater under LYC than under HYC (i.e., +16% and +12%, respectively). Under LYC, 30% of N was located in the reserves at harvest, while 10% migrated to the grapes. Conversely, under HYC, 20% of N remained in the reserves at harvest, while 20% migrated to the grapes.

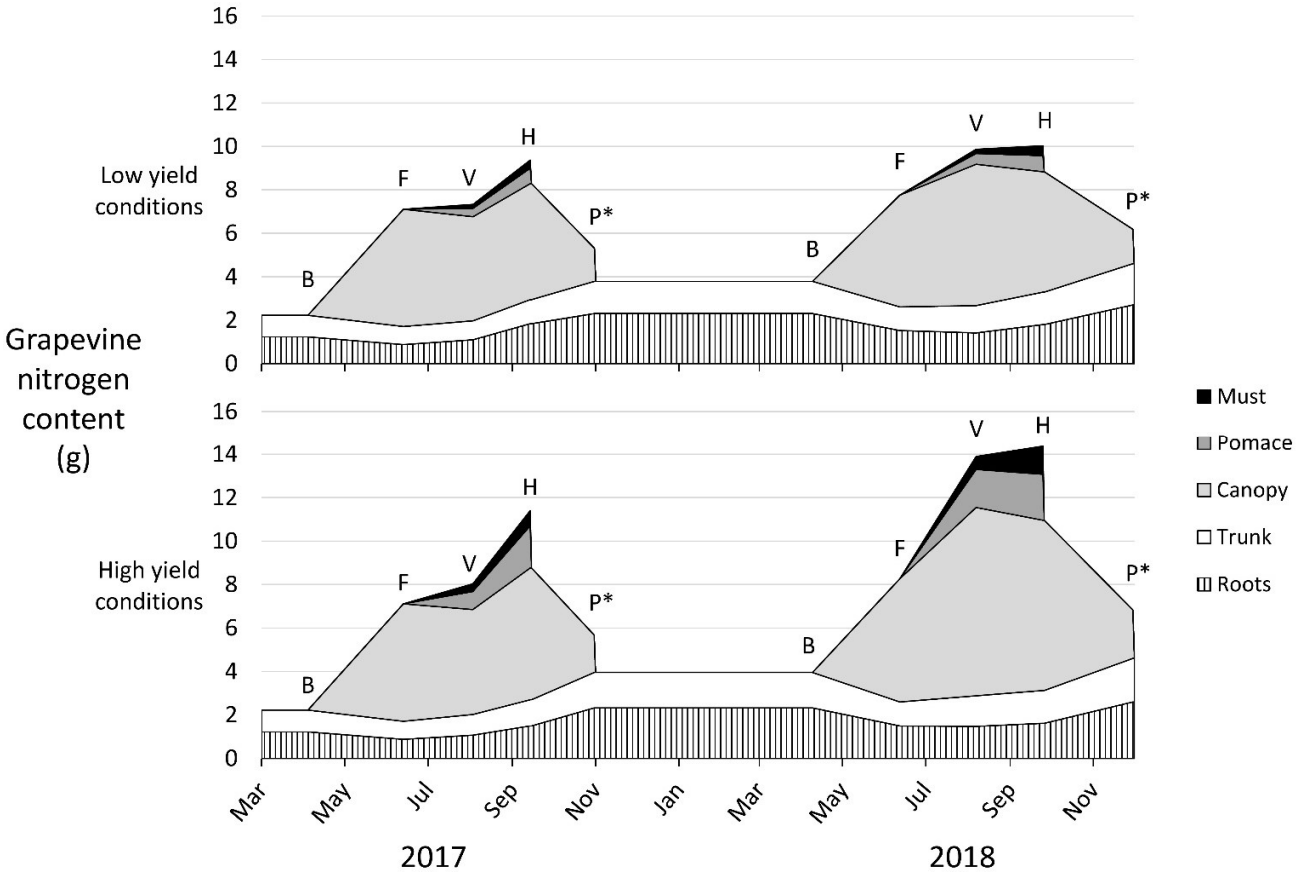


Figure 4.5. N kinetics from March 2017 to November 2018 as a function of crop load. B, bud burst; F, flowering; V, veraison; H, harvest; P, pruning; *extrapolated data. Chasselas cultivar, Pully, Switzerland.

4.4.4 Fertilization and N uptake

Table 4.9 shows the RSA, quantity, and partitioning at harvest 2018 of 1) residual labelled N from the 2017 foliar N supply (2017-res-N), 2) new labelled N from the 2018 foliar N supply (2018-lab-N), and 3) accumulation of both 2017 and 2018 labelled N (total-lab-N). At harvest 2018, 2017-res-N RSA was relatively constant throughout the plant (average 3.7%). Conversely, 2018-lab-N RSA greatly varied across plant parts: the grapes had the highest RSA (up to 10.4% in must under LYC). The accumulation of both 2017 and 2018 labelled N exacerbated the differences between LYC and HYC: the total-lab-N RSA was overall higher in all plant parts under LYC. The quantities of total-lab-N under LYC were globally higher in the perennial reserves (198 mg, + 48%) and lower in the grapes (175 mg, –52%), while it remained constant in the canopy (average 547 ± 83 mg) and in the whole plant (average 1,014 ± 179 mg). In terms of partitioning, the portions of both 2017-res-N and 2018-lab-N located in the canopy were

similar: 55% of total-lab-N was located on average in the canopy, independent of the crop load. Under LYC, total-lab-N was higher in the perennial reserves (+69%) and lower in the grapes (–41%) compared with HYC. Up to 33% of total-lab-N was located in the grapes under HYC at harvest 2018.

Table 4.9. Relative specific abundance (RSA), quantity (mg), and partitioning (%) of residual labelled N from 2017 foliar N supply, new labelled N from 2018 foliar N supply, and the accumulation of both 2017 and 2018 N supplies at harvest 2018, as a function of crop load for the Chasselas cultivar, Pully Switzerland.

Variable	Plant fraction	2017 Residual labelled N			2018 New labelled N			2017 + 2018 Total labelled N		
		LYC (n = 6)	HYC (n = 6)	p-value	LYC (n = 4)	HYC (n = 6)	p-value	LYC (n = 10)	HYC (n = 12)	p-value
RSA (% TN)	Roots	3.6	3.1	ns	2.5	1.1	**	6.1	4.2	**
	Trunk	3.2	2.6	*	3.5	2.0	**	6.7	4.6	***
	Canopy	4.0	3.5	ns	5.7	3.9	ns	9.7	7.4	*
	Thinned bunches	4.7	4.0	ns	0.0	0.0	ns	4.7	4.0	ns
	Pomace	3.8	3.2	ns	9.2	6.1	**	13.0	9.3	***
	Must	5.1	3.5	ns	10.4	7.7	*	15.5	11.2	**
	Whole plant	3.9	3.3	ns	5.5	4.5	ns	9.4	7.8	ns
Labelled N pool (mg)	Roots	68	46	ns	35	19	*	103	65	**
	Trunk	49	36	*	45	33	ns	95	69	**
	Canopy	232	216	ns	297	342	ns	529	558	ns
	Thinned bunches	31	15	ns	0	0	ns	31	15	ns
	Pomace	26	55	**	77	152	*	103	207	**
	Must	24	32	ns	48	122	*	72	154	*
	Whole plant	431	401	ns	502	667	ns	934	1,068	ns
Labelled N partitioning (%)	Roots	16	11	*	7	3	**	11	6	**
	Trunk	12	9	ns	10	5	**	10	7	**
	Canopy	53	54	ns	59	52	ns	57	53	ns
	Thinned bunches	7	4	ns	0	0	ns	3	1	***
	Pomace	6	14	**	15	22	*	11	19	**
	Must	6	8	ns	9	18	**	8	14	**

Note. RSA, relative specific abundance; HYC, high-yielding conditions; LYC, low-yielding conditions; ns, non-significant; *p < 0.05; **p < 0.01; ***p < 0.001.

The isotope labelling method allowed the estimation of fertilized N assimilated by the plant out of the total N applied in 2017, in 2018, and over the two years. Foliar N uptake (in relation to the total quantity supplied) varied as a function of the year, with 34% in 2017 and 25% in 2018. Over the two years of the experiment, average labelled N uptake was 29% and varied as a function of crop thinning (i.e., 34% under HYC versus 25 under LYC, p < 0.0001; Figure 4.6).

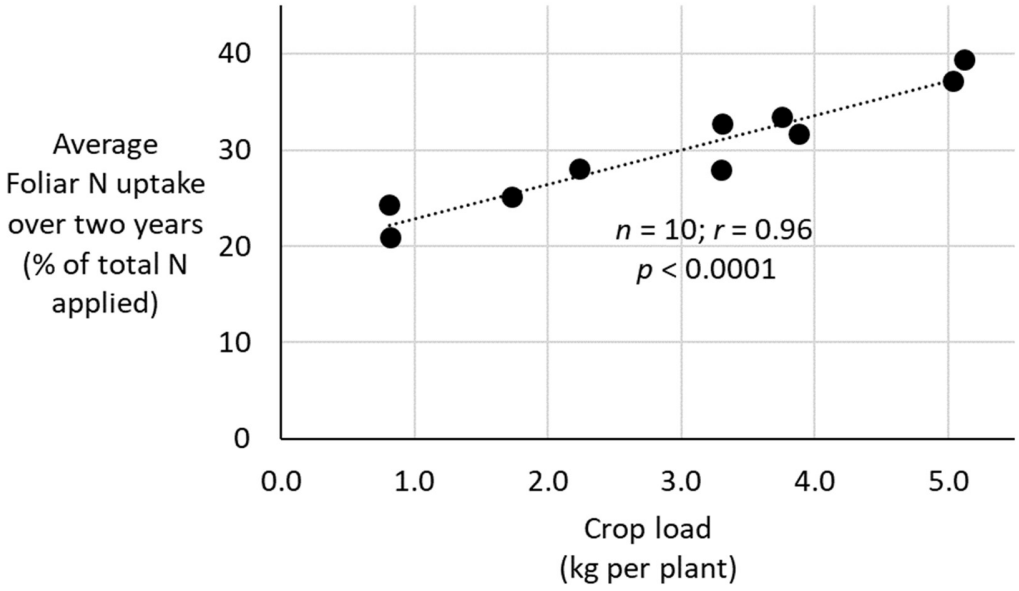


Figure 4.6. Total foliar N uptake over two years as a function of crop load. Chasselas cultivar, 2017 and 2018, at Pully, Switzerland.

The evolution of 2017-res-N was monitored from harvest 2017 to winter pruning 2018 (Figure 4.7). Because the quantity of 2017-res-N varied as a function of crop load, its distribution in the plant during the following year (2018) is shown as a percentage of the 2017-res-N measured in the whole plant at harvest 2017 (i.e., 0.95 g N under HYC and 0.68 g N under LYC). In 2017, labelled N contained in grapes (i.e., 21% under LYC and 26% under HYC) was exported at harvest. Similarly, labelled N contained in shoots (i.e., 15% under LYC and 13% under HYC) was exported at winter pruning. Before leaf fall, approximately 25% of the leaf N was relocated to the reserves (roots + trunk), and the rest returned to the soil, either directly via leaf fall or through root effluxes. During winter 2017–2018, 33% of 2017-res-N was still in the plant reserves under LYC versus 23% under HYC. The following season, the perennial reserves showed a decrease in 2017-res-N from bud burst to harvest. From harvest to winter pruning, the reserves refilled again due to leaf N relocation before leaf fall. The increase of 2017-res-N in the whole plant from bud burst to veraison suggests a de novo uptake of labelled N from the soil, which would correspond to the 2017-res-N released previously. The 2017-res-N pool reached a maximum at veraison 2018 of 69% under LYC versus 54% under HYC. The similar amount of 2017-res-N present in grapes at both veraison and harvest suggests that the accumulation of labelled N in the grapes mostly occurred before veraison under both yield conditions.

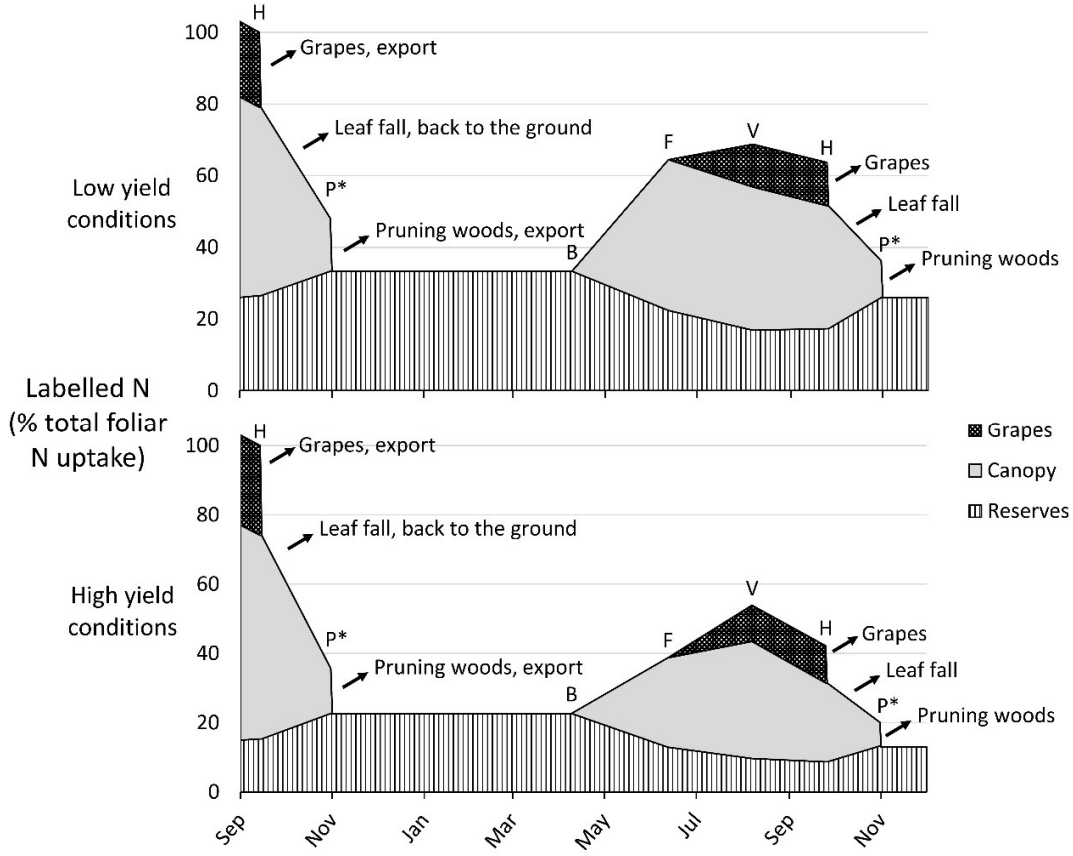


Figure 4.7. Kinetics of residual labelled N from 2017 foliar N supply (2017-res-N) as a percentage of total foliar N uptake. B, bud burst; F, flowering; V, veraison; H, harvest; P, pruning; * extrapolated data. Chasselas cultivar, 2017 and 2018, at Pully, Switzerland.

At harvest 2018, 63% of the initial labelled N was still found in the plant under LYC: 17% in the roots and trunk, 34% in the canopy, and 12% in the grapes. Conversely, under HYC, only 43% of initial labelled N was found: 9% in the roots and trunk, 23% in the canopy, and 11% in the grapes. Grape and pruning wood exports occurred at harvest and winter pruning similarly to 2017. During the second winter, 26% of 2017-res-N was still in the plant reserves under LYC, versus only 13% under HYC. This tendency could easily be extrapolated over the following years. Over two years, the partitioning of 2017-res-N showed a balance between both reserves (roots + trunk) and grapes (pomace + must) as a function of crop load (Figure 4.8). Canopy labelled N content remained relatively stable at 55% of total 2017-res-N on average over all the range of yield conditions. Under HYC, the share of labelled N located in the grapes increased drastically (+25% of total labelled N) to the detriment of the reserve labelled N content (+25%).

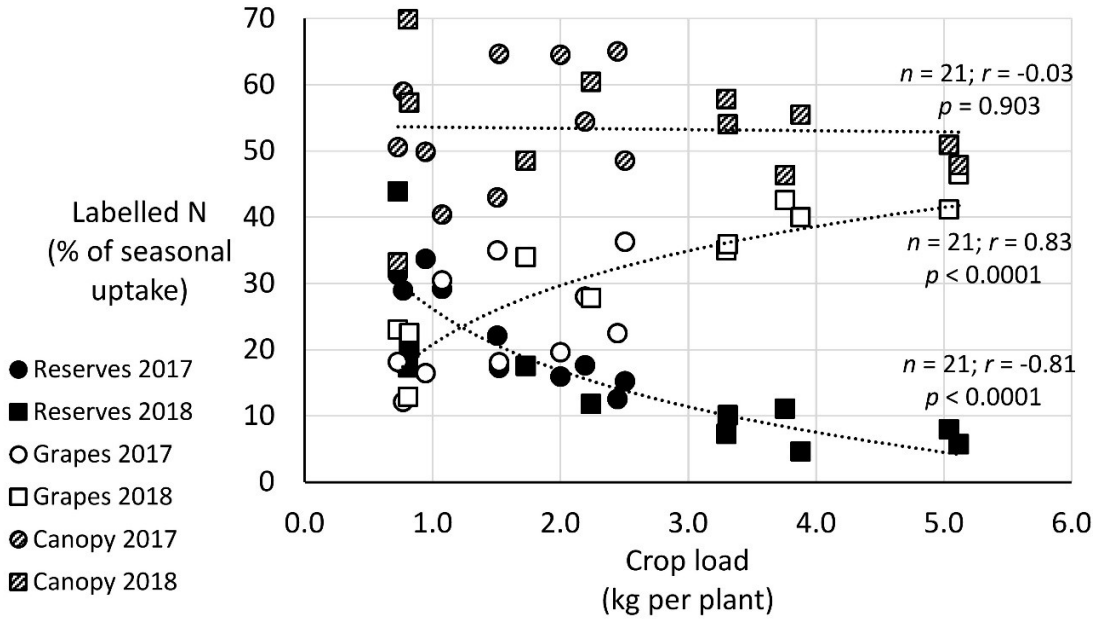


Figure 4.8. Partitioning of labelled N at both 2017 and 2018 harvests, as a function of crop load. Chasselas cultivar, Pully, Switzerland.

4.5 Discussion

4.5.1 Environmental conditions, plant growth, and nutrient seasonal cycle

The environmental conditions and the initial plant N status were non-restrictive and conditioned the results of this trial. The environmental conditions were conducive to unrestricted vegetative development (i.e., neither water nor nutrient restriction). The average must $\delta^{13}C$ values at harvest were lower than the threshold of –26 mUr, suggesting unlimited water supply to the vines during fruit ripening (van Leeuwen *et al.*, 2009). Average leaf composition and chlorophyll index measured at the onset of fruit ripening indicated no severe N deficiency even in the nonfertilized treatment, according to the thresholds published for the grape cultivar Chasselas under the Swiss cool climate (Spring and Verdenal, 2017). The perennial parts of the vines gained, on average, 55% DW in one year, which is a quite substantial growth rate for five-year-old vines. In both seasons, the photosynthesis rate gradually declined from flowering until harvest, following ordinary seasonal patterns as described previously (Keller *et al.,* 2001; Zufferey *et al.*, 2018). The DW, C, and N seasonal dynamics were in accordance with other studies on perennial crops (Zapata *et al.*, 2004b; Zufferey *et al.*, 2015; Schreiner, 2016; Muhammad *et al.*, 2020): C and N contents in perennial fractions were greatest from leaf fall to bud burst and lowest at flowering.

From bud burst to flowering, root N uptake was low and N demand – due to intense vegetative growth – was mainly supported by the mobilization of root and wood reserves. Reserve refilling occurred mostly during fruit ripening and substantially after harvest, due to N relocation from the canopy before leaf fall. Under the hot climate conditions of South Africa and Australia and under irrigation, Conradie (1980, 1991) and Holzapfel *et al.* (2019) observed a more important N uptake during the post-harvest period, which lasts several months in these climates. Wermelinger (1991) suggested that more than 40% of the leaf N on grapevine is translocated before leaf fall from the senescent leaves to the perennial plant fractions. In our trial, 5–10% of leaf N was relocated to the grapes until harvest and then 25–30% to the perennial reserves until leaf fall. A significant share of leaf N was released to the ground. As demonstrated by Khalsa *et al.* (2016), in *Prunus dulcis,* leaf litter decomposition led to a greater mineral N pool. The leaves contained, on average, 0.83% DW of N at leaf fall 2017, which represented a 60% decrease in comparison with the leaf N content at the onset of fruit ripening in the same year (2.15% DW). This observation does not exclude the hypothesis of root N leaching: N return to the ground could be a combination of both leaf fall and root efflux; the relative part of each cannot be demonstrated with the present experimental setup. Most studies about nutrient dynamics do not consider root N efflux in their models. In fact, the plant N cycle should be seen as open. Total N uptake is the sum of total N influx and total N efflux (Hachiya and Sakakibara, 2017). Considering plant N efflux as part of the plant N budget would prevent both an overestimation of N losses and an underestimation of N uptake (Xu *et al.*, 2012). As an example, *Triticum aestivum* released 5–6% of the N previously assimilated, which represents 15 kg N ha^{-1} released by roots to the ground (Merbach *et al.*, 1999). Unfortunately, studies addressing grapevine root efflux are scarce.

4.5.2 Crop load affected fertilizer N efficiency

The absorption of nutrients by leaves has been acknowledged since the nineteenth century (e.g., Fernández *et al.*, 2020). It is currently an accepted practice for crop fertilization. Foliar urea (20 kg N ha^{-1}) was applied on the leaves at the onset of grape ripening, with the aim of improving fruit N status without either increasing plant vigor or delaying fruit ripening (Xia and Cheng, 2004; Hannam *et al.*, 2016). As expected, foliar N supply efficiently increased fruit N concentration during the season of its application. Foliar N supply did not affect either $\delta^{13}C$ or plant vigor. This is in contradiction with the findings of Taskos *et al.* (2020), who observed – on both cultivars Cabernet sauvignon and Xinomavro – that leaf N content was positively correlated to leaf gas exchange rates and negatively correlated to WUE_i. Their results suggest that the large amount of soil-applied ammonium nitrate (i.e., 120 kg N ha^{-1}) greatly promoted plant vigor, with A increasing more slowly than gsw. However, no measurement of

plant development (e.g., crop load, leaf area, and pruning weight) was carried out in this trial to corroborate this hypothesis. Like crop load, foliar N supply affected certain FAAs more than others, thus potentially affecting fruit aroma profile. This is consistent with findings from Schreiner *et al.* (2014) on the grape cultivar Pinot noir. Interestingly, the FAAs that were the most affected by foliar N supply were not the same as the ones affected by crop load. However, the impact of foliar N supply on grapes' FAA profile was small in relation to both the year and the crop load (Figure 4.3).

Despite a homogeneous N supply in the entire plot, fertilizer N uptake varied greatly in relation to crop load, as mentioned in other studies (Morinaga *et al.*, 2003; Treeby and Wheatley, 2006; Verdenal *et al.*, 2016a). It was, on average, 29% ± 8% of total N applied, with higher uptake rates under HYC (i.e., 34% versus 25% under LYC; Figure 4.6). Foliar N supply promoted a higher fruit YAN concentration at harvest under HYC (+55 mg L^{-1} in 2017, $p = 0.021$; +54 mg L^{-1} in 2018, $p = 0.032$), while the gain was not significant under LYC (+1 mg L^{-1} in 2017, $p = 0.986$; +14 mg L^{-1} in 2018, $p = 0.460$). This can be explained by the lower fertilizer uptake rate under LYC, regardless of the year. In other words, the fertilization efficiency greatly varied according to crop load. This important finding explains why, in some situations, foliar N supply does not efficiently improve fruit N concentration and may potentially cause environmental contamination.

Once assimilated, fertilizer N was not homogeneously distributed in the plant. The RSA varied in function of the plant fractions, decreasing gradually from the fruits to the roots and wood, showing a hierarchy in N-sink strength among the plant fractions. Fertilizer N content was also affected by crop load in both grapes and perennial reserves, while it remained constant in the canopy in both years. To the detriment of the roots and wood, 40% of fertilizer N was located in the fruits at harvest under HYC, versus only 24% under LYC. The quantity of N exported from the vineyard is related to the amount of grape harvested, inducing higher fertilizer N loss under HYC. After harvest 2017, a share of 2017-res-N was relocated from the canopy to the perennial reserves before leaf fall and was subsequently redistributed to the whole plant during 2018. In comparison with the control treatment, the presence of 2017-res-N in the plant in 2018 had no carry-over effect either on vegetative parameters or on grape composition. The TN and NQ remained unchanged at bud burst 2018, regardless of either crop load or fertilization. This result confirms that foliar urea supply at the onset of fruit ripening is a good practice for short-term fruit N correction (Hannam *et al.* 2016). Conversely to the fluctuation of 2018-lab-N RSA between plant fractions, 2017-res-N RSA was constant in the whole plant in 2018 regardless of plant fractions and crop load. These results suggest that the N partitioning depended on both N species and N

origin, either from the perennial reserves (2017-res-N, mainly FAA) or from the seasonal uptake (2018-lab-N, mainly NH_4^+) (Keller, 2020). Both 2017 and 2018 foliar N supplies contributed to the accumulation of fertilizer N in the plant without increasing either NQ or TN, suggesting that soil N uptake was related to the initial plant N reserve.

The ^{15}N-labelling method allowed quantifying fertilizer N uptake and tracking its distribution and redistribution into the plant. In this trial, the foliar urea supplied at veraison 2017 was the unique source of labelled N. Thus, the calculation of RSA (i.e., $A\%_{N\ supplied}$ = 10 atom % ^{15}N) allowed us to estimate the partitioning of fertilizer N assimilated by the plant over the two growing seasons (Figure 4.7). Alternatively, labelling a particular plant fraction (e.g., perennial fraction) would have allowed studying the distribution of nutrients originating from that fraction only (Bowen and Zapata, 1991). In this trial, at the onset of the second season, the perennial fraction (roots + trunk) of the fertilized plants still contained 2017-res-N. Thus, considering the N pool initially present in the perennial fraction as the unique source of labelled N (i.e., $A\%_{N\ supplied} = A\%_{(roots+trunk)}$), as affected by 2017-res-N, theoretically allowed us to estimate the partitioning of the N reserves during the second season, differentiating them from the unlabelled seasonal root N uptake. However, as demonstrated in this trial, a considerable portion of labelled N was released to the soil at the end of the growing season, either through leaf fall or root leaching. It was then assimilated again in the second year. Consequently, the perennial plant fractions were not the only source of labelled N in the second year, which prevented the differentiation of reserve N mobilization from soil N uptake in the context of our trial. To address this issue, plants would need to be transplanted before the second season in new soil, not containing any labelled N.

4.5.3 Crop load affected C and N dynamics as well as grape composition

Most of the aboveground vegetative parameters (i.e., leaf area, bunch weight, pruning woods) were not influenced by crop thinning during the trial period. Crop thinning did not promote a stronger vigor of the canopy in response to grape sink reduction. This is in agreement with Keller *et al.* (2005), but in contradiction with Morinaga *et al.* (2003), who observed a higher vegetative development under LYC. In our trial, canopy DW under LYC was less than under HYC at the end of the second year, similar to the findings of Bowen *et al.* (2011). The non-restrictive conditions of our trial in terms of water and nutrients, even under HYC, may explain why the vines showed a higher capacity under HYC. The supply of resources exceeded the demand and allowed the maximal production set by genetic potential to be reached (Lawlor, 2002). Conversely, in respect to vigor, the plant capacity is defined by the total annual growth of a grapevine and is an indicator of the net resource gain from the environment (Keller, 2020).

Several studies have already reported the influence of crop load on C partitioning in grapevine (e.g., Morinaga *et al.*, 2003; Zapata *et al.*, 2004; Zufferey *et al.*, 2015). In response to crop thinning, plants required less C from the perennial fractions to meet the demand for ripening fruits (Howell, 2001). In this trial, this relationship was confirmed, and the close relation between C and N metabolisms was highlighted. Both CQ and NQ in fruits were reduced proportionally due to crop thinning, while their concentrations were unchanged by crop load (Figure 4.9). Moreover, in the roots, DW and CQ increased by 19% and 17% DW, respectively, while NQ increased insignificantly. The roots were the plant fraction most affected by crop thinning, highlighting the close coordination of C and N metabolites between grapes and roots. Similarly, Stander *et al.* (2017) observed that higher fruit load limited root growth on mandarin trees (*Citrus reticulate*). As a major carbohydrate sink in the plant, the fruit load disturbs the balance between fruit ripening and root growth by limiting the allocation of C and N metabolites to roots.

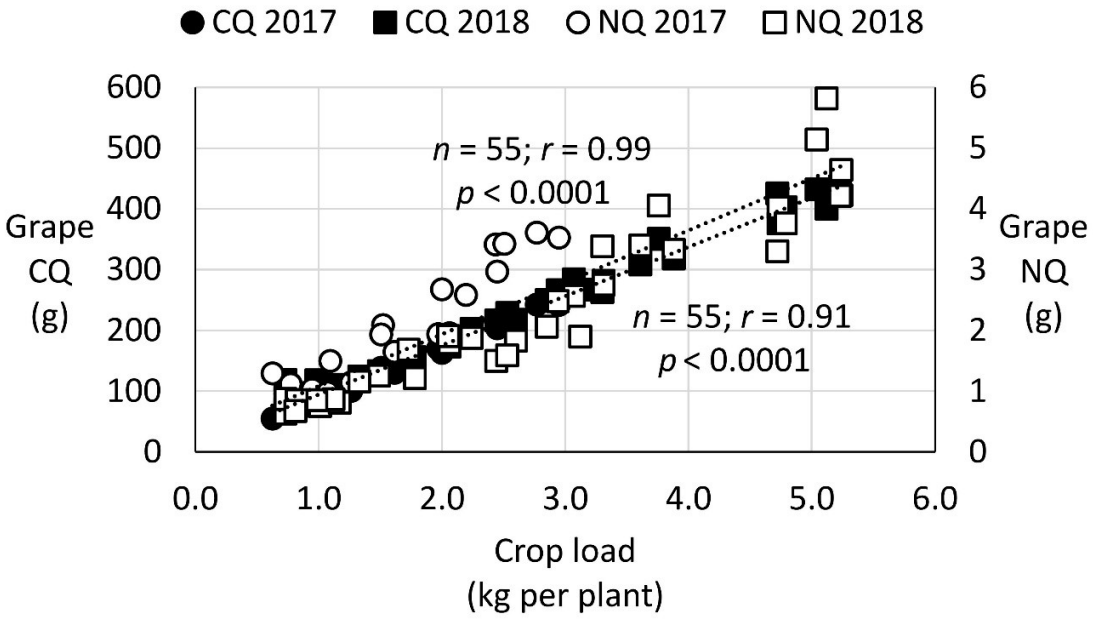


Figure 4.9. CQ and NQ in grapes as a function of crop load at harvests 2017 and 2018. Chasselas cultivar, Pully, Switzerland.

The impact of crop load on the gas exchange rates may vary greatly from negative to positive, depending on the crop and on the environmental conditions (Lin *et al.*, 2018). In our trial, lower fruit C and N demand induced by crop thinning was most probably the reason for lower leaf gas exchange rates. Seasonal photosynthesis activity was globally reduced by crop thinning, although the differences between LYC and HYC were not always significant on a daily time step. The lower leaf gas exchange rates under LYC were mainly due to a lower gsw, which promoted a higher WUE_i (i.e., A/gsw) and,

subsequently, a higher $\delta^{13}C$ in all plant fractions, particularly in fruits and roots. The positive correlation between $\delta^{13}C$ and WUE_i was already established by Livingston *et al.* (1999) on white spruce (*Picea glauca*). These authors described WUE_i as an indicator of the compromise between photosynthesis and transpiration. Krapp *et al.* (1993) explained this phenomenon by the removal of importing organs (sink), resulting in a gradual inhibition of photosynthesis in the exporting leaves (source). This "sink regulation" of photosynthesis is usually associated with a higher carbohydrate content in the leaves, as carbohydrates may cause a feedback inhibition of photosynthesis (Krapp *et al.*, 1993; Wang *et al.*, 2018). Wang *et al.* (2018) further demonstrated that the earlier crop thinning is carried out, the greater the down regulation of photosynthesis. In our study, the lower gas exchange rates under LYC induced a lower uptake of both C and N. This was probably due to the close relation between both C and N metabolisms: C assimilation rate requires N supply, which depends, in turn, on the availability of C compounds for nitrate assimilation (Lawlor, 2002; Gauthier *et al.*, 2010; Vrignon-Brenas *et al.*, 2019; Keller, 2020). Similarly, Alem *et al.* (2021) demonstrated that crop thinning resulted in significantly lower accumulation (in quantity, not concentration) of most metabolites in fruits (e.g., soluble sugars, organic acids, glycosylated aroma precursors). The relation between C and N dynamics varies according to crop load, but is also influenced by genetics and environmental conditions (Lawlor, 2002). In 2018, the plants showed signs of overcropping under HYC: the share of CQ and NQ allocated to the perennial plant fraction was smaller, and fruit ripening was delayed in comparison with LYC. C availability was probably the cornerstone, due to an unbalanced ratio between canopy size and crop load, that is, the source-to-sink ratio. Kliewer and Dokoozlian (2005) have shown on grapevine that a minimum of 1.0 m^2 of leaf area per kg of fruit is required for complete fruit maturation. In our trial, under HYC and with the plant leaf area limited by trellising and hedging, photosynthesis activity was insufficient to fulfill the fruit demand of carbohydrates, and berry ripeness was subsequently delayed. The leaf-to-fruit ratio was limiting under HYC (i.e., 0.7 $m^2\ kg^{-1}$), resulting in a loss in TSS (average –5 °Bx) and a gain in TA (average + 0.8 $g\ L^{-1}$) at harvest 2018 in comparison with LYC, confirming previous findings (Bubola *et al.*, 2020; Sivilotti *et al.*, 2020). However, crop thinning had no significant impact on 2017 fruit ripening, perhaps due to the lower yield potential that year and the non-limiting leaf-to-fruit ratio (1.0 $m^2\ kg^{-1}$ under HYC). The contrast between 2017 and 2018 demonstrates the inconsistent impact of crop load on fruit ripening, as already established (Keller *et al.*, 2005; Reeve *et al.*, 2016; Rutan *et al.*, 2018). An excessive leaf-to-fruit ratio may not be desirable either: in conditions similar to this trial, the increase of canopy size – via higher canopy trimming height – guaranteed fruit ripeness, but also induced a lower N concentration in the whole plant, particularly in fruits. This mechanism could be considered a "dilution" in the volume of the biomass, with negative consequences on fermentation kinetics of the grape must and subsequently

on wine quality (Verdenal *et al.*, 2016a). The management of vine balance either via crop thinning or canopy trimming affects plant N metabolism in different ways, showing the complexity of managing the plant source-to-sink balance.

Fruit N concentration remained constant regardless of crop load. Despite major differences observed in vine balance and fruit maturation, berry YAN concentration remained unchanged. Howell (2001) explained that plants extract less C and N from their perennial reserves in response to lower crop load, to match the demand for maturing fruits. The plant N-sink strength showed a hierarchy among the plant organs, and fruits seemed to have priority over the roots, which is common in perennial crops (Morinaga *et al.*, 2003; González-Real *et al.*, 2008). This finding demonstrates that crop thinning is not an efficient practice for controlling fruit N concentration. However, fruit FAA proportions were changed, potentially affecting wine aromas (Figure 4.3). Crop thinning induced lower proportions of alanine and threonine, theoretically responsible for fruity, but also rotten, fishy, and pungent aromas (Verdenal *et al.*, 2021). Proline increased with crop thinning, but being a non-assimilable FAA for yeast, proline variations have little influence on the aroma potential. Further research on grapevine, including winemaking followed by sensory analysis, would be required to measure the real impact of crop thinning on grape and wine aromas. Several authors have mentioned the impact of crop load on fruit volatile compounds (Rutan *et al.*, 2018; Wang *et al.*, 2019). Lin *et al.* (2018) even recommended a yield range at harvest for optimal aroma composition on the grape cultivar Vidal under the environmental conditions of Liaoning, China. The impact of crop thinning on both grape maturation and must amino N composition could be observed from the onset of ripening (Figures 4.10 and 4.11). This suggests that the accumulation of metabolites in fruits was influenced by crop load as early as berry formation. This confirms results from other studies (Keller et al., 2005; Wang *et al.*, 2018), showing that an earlier crop thinning results in a greater impact on grape composition at harvest.

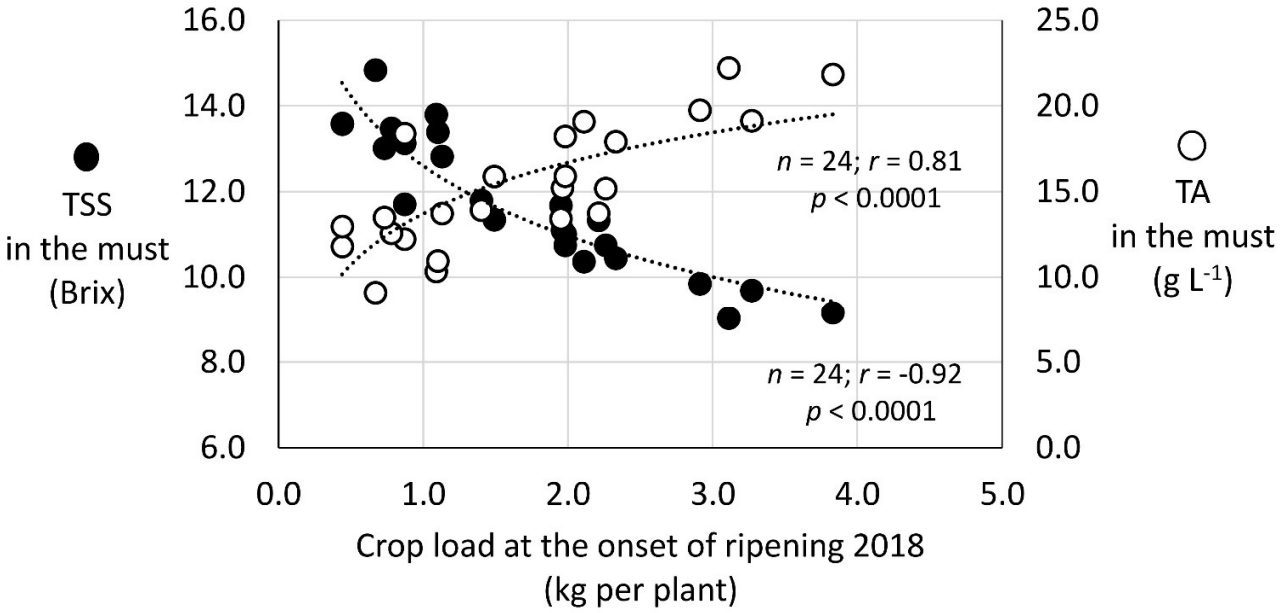


Figure 4.10. Impact of the crop load on the concentrations of TSS and TA in the must at the onset of ripening 2018. Chasselas cultivar, 2018, Pully Switzerland.

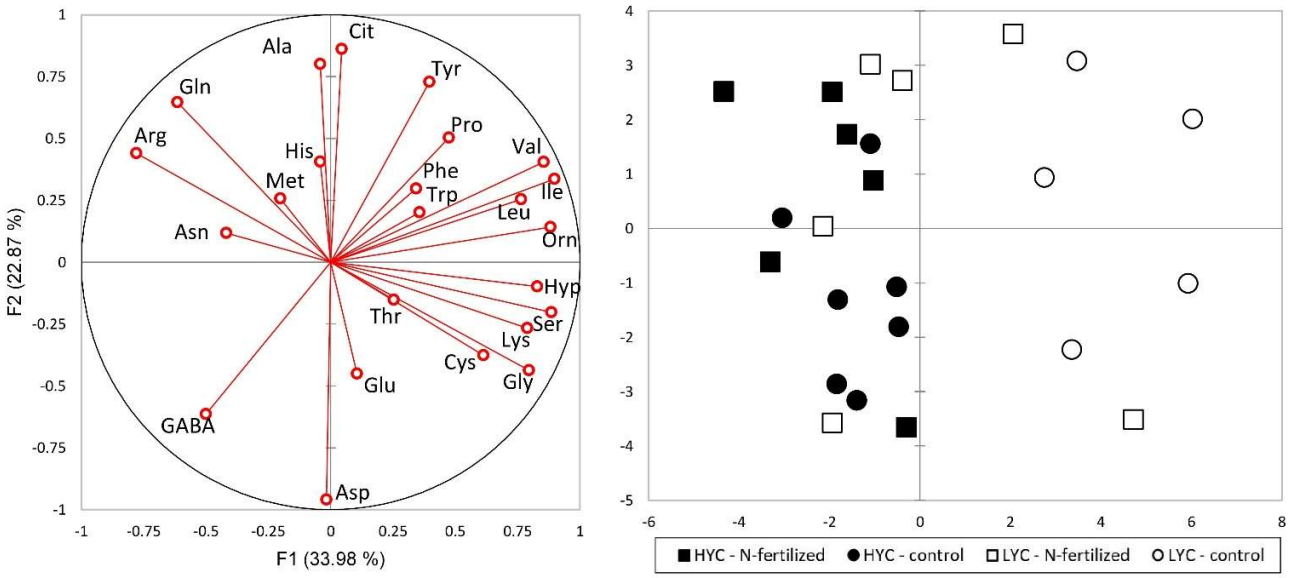


Figure 4.11. Discrimination of the musts at the onset of ripening 2018, as a function of their amino N profiles. LYC, low-yield conditions; HYC, high-yield conditions. Chasselas cultivar, 2018, at Pully Switzerland.

Conclusion and perspectives

This two-year trial enabled us to draw conclusions on the impacts of both crop limitation and fertilization on plant metabolism.

Conclusions

Regarding crop limitation, we conclude the following:

- In the absence of external environmental constraints, crop limitation does not promote stronger vigor of the plant. Grapevines modulate their metabolism: both CQ and NQ in fruits are reduced proportionally to crop limitation, while their concentrations remain unchanged. Leaf gas exchange rates are reduced accordingly: LYC induces a higher WUE_i (i.e., A/gsw), similarly to a car, which consumes less fuel at a lower speed for the same distance.
- A hierarchy in N-sink strength among the plant fractions exists: vines prioritize C and N accumulation in the grapes over root development and reserve refilling. Despite the large variation in crop load, the TN content of the grapes remains constant at the expense of the root N content. The extra solicitation of root N reserves under HYC suggests that several years of overproduction could potentially induce an important reduction in N reserves, which may affect plant vigor and bud fruitfulness in the long term.
- The crop load has no impact on the YAN concentration in the must, but it affects its composition, that is, the proportions of FAAs. Certain FAAs are more affected than others, thus potentially affecting the fruit aroma potential. It is therefore questionable whether the crop load limitation always has a positive impact on the grapes' composition and ultimately on the wine quality. Interestingly, the FAAs that are the most affected by crop load are not the same as the ones affected by foliar N supply. However, the impact of foliar N supply on grapes' FAA profile is negligible in comparison to the impact of both the year and the crop load.
- We confirmed that the leaf-to-fruit ratio is a relevant parameter to guarantee proper grape ripening: an insufficient leaf-to-fruit ratio (i.e., below 1 m^2 kg^{-2} of fruit) is detrimental to the accumulation of carbohydrates in grapes. However, the variation of the leaf-to-fruit ratio in the context of our study (i.e., with constant leaf area and variable fruit load) had no effect on grape N concentration.
- A significant part of the labelled N is lost to the soil during the fall and winter, which is most likely a combination of leaf fall and root efflux. Root N leaching may play a more important role

than we currently think in the N cycle of the vine. Part of the N exudated may be reassimilated the following year.

- Crop limitation affects the accumulation of metabolites in fruits from the beginning of ripening and potentially even before. This result suggests the presence of a carry-over effect from the previous year's crop load.

About ^{15}N-labelled fertilization, we can draw the following conclusions:

- We confirmed that the application of foliar urea at the onset of grape ripening efficiently improves fruit N status without either increasing plant vigor or delaying fruit ripening. The presence of residual fertilizer N in the plant in the following year has no carry-over effect on either vegetative parameters or grape composition. This result confirms that foliar urea supply at the onset of fruit ripening is a good practice for short-term fruit N correction.
- The fertilization efficiency (i.e., the gain in YAN in the grapes in relation to the quantity of fertilizer applied) greatly varies according to crop load: N uptake is strongly stimulated under HYC in answer to higher fruit N demand. This important finding explains why, in some situations, foliar N supply does not efficiently improve fruit N concentration and may potentially cause environmental pollution.
- N partitioning depends on both N species and N origin: N originating from the perennial reserves (mainly FAA) is homogeneously distributed throughout the plant, while N originating from the seasonal uptake (mainly NH_4^+) is distributed following the hierarchy in N-sink strength between plant parts, giving priority to the fruits.

Our two-year trial highlighted the impact of crop limitation and foliar N supply on plant N metabolism. It demonstrated that plants are able to modulate both root N reserve mobilization and mineral N uptake in relation to crop load, thus maintaining a stable N concentration in the fruits. The roots were the plant fraction most affected by crop limitation, emphasizing the close coordination of C and N metabolites between grapes and roots. As a major sink for both C and N, the fruit load disturbed the balance between fruit ripening and root growth by limiting the allocation of C and N metabolites to the roots under HYC. These results indicate that root activity is a key factor for understanding the mechanisms that balance plant N nutrition and that the fruit-to-root ratio could be a relevant parameter for accurate N management in perennial crops. Since quantifying the roots is impossible under field conditions, accurate physiological models are required, which would include root activity and lifespan.

Advantages and limits of the methodology

Environmental conditions play a major role in plant metabolism. In our trial, they were conducive to unrestricted development of the plants. This situation was an advantage in observing the physiological behavior of the plants under the isolated influence of crop limitation.

Planting in large pots was a good solution for an easy excavation. Buried pots limited water loss by maintaining a lower temperature and therefore lower evaporation. The use of pots allowed us to control the root environment, that is, the variability of soil depth, soil nutrients, and water. However, a few roots could still escape out of the pots. The top of the pots must be approximately 5 cm above the soil level to prevent the roots from coming out of the pots. Paul Schreiner (Oregon State University, US) advised us to use a copper screen at the bottom of the pot in future work, which is very efficient in preventing root escape by the bottom drain holes.

Despite the homogeneity of the plantation, the plants showed some variability in terms of vegetative development. However, these variations were not related to the variation factors (i.e., crop limitation and foliar N supply) and were instead attributed to hazard. If this type of trial were developed again, it would probably be worth considering increasing the number of replicates per treatment (e.g., from six to eight). More replicates would also have allowed winemaking from the different treatments in quantities sufficient for sensory analyses. During the data analysis of the distributions of C and N in the plant throughout the two years of the trial, it became clear that a fifth date of excavation each year would have been useful, that is, just after winter pruning. This would have prevented hypothetical extrapolations.

^{15}N-labelling was shown to be a powerful method to quantify fertilizer N uptake and to track its distribution into the plant. The EA-IRMS measures were particularly precise with the use of the UNIL-IDYST in-house standards, which covered a large range of ^{15}N abundances. In this trial, the foliar urea supplied at veraison 2017 was the unique source of labelled N. Hence, it was possible to monitor its partitioning over the two growing seasons. N-labelling largely covered the small variations in isotope ratios due to natural discrimination.

Alternatively, it was not possible to differentiate the reserve N mobilization (labelled) from the soil N uptake (non-labelled) in 2018. Labelling a particular plant fraction theoretically allows the study of the distribution of nutrients originating from that fraction only. In our trial, at the onset of 2018, the perennial fraction (roots + trunk) of the fertilized plants still contained residual labelled N from foliar N supply 2017. Thus, considering the N pool initially present in the perennial fraction as the unique source of

labelled N (i.e. $A\%_{N\ supplied} = A\%_{(roots+trunk)}$, as affected by 2017-res-N) would allow the estimation of the distribution of N reserves during the second year, differentiating them from the unlabelled seasonal root N uptake. However, as demonstrated, a considerable share of labelled N was released into the soil at the end of 2017 through either leaf fall or root leaching. It was then assimilated again in the second year. Consequently, the perennial plant fractions were not the only source of labelled N in 2018, which prevented the differentiation of reserve N mobilization from soil N uptake in the context of our trial. To solve this problem, it would be necessary to export the leaves after leaf fall and before they decompose, simply by blowing them off the plot and collecting them. By doing so, the labelled N assimilated in the second year would necessarily come from the root exudates of the previous year. Another possibility for completely eradicating the external source of labelled N would be to transplant the vines before the second season to a new soil that does not contain labelled N, with the disadvantage of disturbing the root system.

Perspectives

This experiment demonstrated the high potential of crop limitation to modulate plant N balance. It provided some answers but also raised new questions.

Environmental conditions (climate and soil) have a dominant impact on plant physiology and fruit composition. Thus, any restriction, such as water, nutrients, or anything else, would have potentially affected the interpretation of this study. In order to complete the understanding of the influence of crop load on plant metabolism, it would be interesting to reiterate this trial under restrictive conditions. This would probably unravel some metabolic correlations that are masked under non-restrictive conditions. For example, one could empirically expect, under water restriction, that HYC would induce a lower N concentration in grapes than LYC. This would thus be in contradiction with the present study due to different environmental conditions. Another example is the increase of atmospheric CO_2 related to climate changes: it would probably increase the plant capacity in the absence of water and N constraints, and therefore raise the overcropping threshold. The vines could bear a higher crop load while maintaining a sustainable plant N balance. Optimization of cultural practices in accordance with environmental conditions is key to sustainable crop production.

Crop limitation affected the FAA profile of the must and potentially affected the wine flavors. Following the application of ^{15}N-labelled fertilizer, the analysis of the isotope ratios of each AA separately in the must at harvest would probably identify the metabolic pathways that promoted the formation of certain

AAs more than others. In terms of winemaking, fermentation kinetics should not be affected by crop limitation, as demonstrated in our study, since the YAN concentration remained unchanged. However, without winemaking, we were not able to confirm the positive effect of crop limitation on the wine flavors. Trials including winemaking should be conducted to study the consequences of this practice on the final product, that is, the wine.

Fertilizer use efficiency is highly affected by crop limitation. As detailed in chapter 2, the use of elicitors could potentially improve plant N uptake. As an example, the combination of N and sulfur has already been shown to be more effective than N alone in improving N concentration in grapes. Our trial was carried out on the white cultivar Chasselas, which has a high natural yield potential. A severe crop limitation is a current practice in Switzerland to restrain Chasselas below its full capacity of production and to respect the production quotas. Our trial did not allow the determination of an ideal crop load. However, considering our results, it seems possible to cultivate Chasselas with a larger canopy and a lower planting density in order to accommodate a larger number of shoots and grapes per vine. This would reduce the production cost while maintaining the plant N balance. In fact, the variability of genetics among cultivars, rootstocks, and even clones should be taken into consideration.

Crop limitation is a practice commonly used to promote grape maturation. It would be interesting to contrast it to other vineyard practices, such as canopy management or root pruning. Unlike canopy oversizing, which induces a drop in YAN concentration in the must, limiting the crop load does not affect the YAN concentration. The management of vine balance either via crop limitation or canopy trimming affects plant N metabolism in different ways, showing the complexity of managing the plant source-to-sink balance. Our findings demonstrated the importance of the fruit-to-root balance. A threshold for a maximum fruit-to-root ratio would be required to ensure a sufficient C and N reserve refill for the following year, similar to the threshold for a minimum leaf-to-fruit ratio, which guarantees complete grape maturation. In the case of N management, we should probably even consider the “leaf-to-fruit-to-root” ratio. However, since quantifying the roots is impossible under field conditions, the development of accurate models, which include root activity and lifespan, is required.

Plant N metabolism requires more attention for the development of precise nutrition models. Root activity plays a major role in the net plant N uptake, but data about grapevine root N metabolism are scarce. Trials should be conducted to evaluate grapevine root activity and their lifespan, as well as the amount of nutrients released into the rhizosphere in one season. Currently, software, such as STICS (INRAe, France), is able to simulate the water, C, and N balances of various types of crops. STICS

simulates crop functioning at a daily time step at the field scale for an average plant, with input variables related to climate, soil, and crop management. Introducing our results in the model would probably increase its accuracy in relation to both crop load and foliar N supply.

The plant integrates the influence of the climate, genetics, and cultural practices. Consequently, an integrative point of view is required to anticipate and manage N accumulation in grapes. Our results provide new insights into the understanding of plant N metabolism and will contribute to the improvement of cultural practices. We have demonstrated the importance of adapting fertilization programs according to cultural practices. This study encourages further research on the potential of cultural practices to monitor NUE, with the aim of enhancing crop quality and sustainability.

References

Aerny, J. (1996). Composés azotés des moûts et des vins. *Revue suisse de Viticulture, Arboriculture et Horticulture, 28*(3), 161-165.

Alcaide-Hidalgo, J. M., Moreno-Arribas, M. V., Martín-Álvarez, P. J., & Polo, M. C. (2007). Influence of malolactic fermentation, postfermentative treatments and ageing with lees on nitrogen compounds of red wines. *Food Chemistry, 103*(2), 572-581. doi:10.1016/j.foodchem.2006.09.002

Alem, H., Rigou, P., Schneider, R., Ojeda, H., & Torregrosa, L. (2019). Impact of agronomic practices on grape aroma composition: a review. *Journal of the Science of Food and Agriculture, 99*(3), 975-985. doi:10.1002/jsfa.9327

Alem, H., Ojeda, H., Rigou, P., Schneider, R., & Torregrosa, L. (2021). The reduction of plant sink/source does not systematically improve the metabolic composition of Vitis vinifera white fruit. *Food Chemistry, 345*, 128825. doi:10.1016/j.foodchem.2020.128825

Aranguren, M., Castellón, A., & Aizpurua, A. (2018). Topdressing nitrogen recommendation in wheat after applying organic manures: the use of field diagnostic tools. *Nutrient Cycling in Agroecosystems, 110*(1), 89-103. doi:10.1007/s10705-017-9865-7

Autralian Wine Research Institute, 2020. Yeast Assimilable Nitrogen. https://www.awri.com.au/industry_support/winemaking_resources/wine_fermentation/yan/ visited on the 24/08/2020.

Azuma, A., Yakushiji, H., Koshita, Y., & Kobayashi, S. (2012). Flavonoid biosynthesis-related genes in grape skin are differentially regulated by temperature and light conditions. *Planta, 236*(4), 1067-1080. doi:10.1007/s00425-012-1650-x

Barbosa, C., Falco, V., Mendes-Faia, A., & Mendes-Ferreira, A. (2009). Nitrogen addition influences formation of aroma compounds, volatile acidity and ethanol in nitrogen deficient media fermented by *Saccharomyces cerevisiae* wine strains. *Journal of Bioscience and Bioengineering, 108*(2), 99-104. doi:10.1016/j.jbiosc.2009.02.017

Bates, T. R., Dunst, R. M., & Joy, P. (2002). Seasonal dry matter, starch, and nutrient distribution in 'Concord' grapevine roots. *HortSicence, 37*(2), 313-316. doi:10.21273/hortsci.37.2.313

Bell, S.-J., & Henschke, P. A. (2005). Implications of nitrogen nutrition for grapes, fermentation and wine. *Australian Journal of Grape and Wine Research, 11*, 242-295. doi:10.1111/j.1755-0238.2005.tb00028.x

Bisson, L. F., & Butzke, C. E. (2000). Diagnosis and rectification of stuck and sluggish fermentations. *American Journal of Enology and Viticulture, 51*(2), 168-177.

Boss, P. K., Bottcher, C., & Davies, C. (2014). Various Influences of Harvest Date and Fruit Sugar Content on Different Wine Flavor and Aroma Compounds. *American Journal of Enology and Viticulture, 65*(3), 341-353. doi:10.5344/ajev.2014.13137

Bouzas-Cid, Y., Trigo-Córdoba, E., Orriols, I., Falqué, E., & Mirás-Avalos, M. J. (2018a). Influence of soil management on the red grapevine (*Vitis vinifera* L.) Mencía must amino acid composition and wine volatile and sensory profiles in a humid region. *Beverages, 4*, 76. doi:10.3390/beverages4040076

Bouzas-Cid, Y., Díaz-Losada, E., Trigo-Córdoba, E., Falqué, E., Orriols, I., Garde-Cerdán, T., & Mirás-Avalos, J. M. (2018b). Effects of irrigation over three years on the amino acid composition of Albariño (*Vitis vinifera* L) musts and wines in two different terroirs. *Scientia Horticulturae, 227*, 313-325. doi:10.1016/j.scienta.2017.05.005

Bowen, G. D., & Zapata, F. (1991). Efficiency in uptake and use of nitrogen by plants. In: *International Symposium on the Use of Stable Isotopes in Plant Nutrition, Soil Fertility and Environmental Studies*, International Atomic Energy Agency (IAEA), Vienna, Austria.

Bowen, G.D., & Zapata, F. (1991). Efficiency in uptake and use of nitrogen by plants.

Bowen, P., Bogdanoff, C., Usher, K., Estergaard, B., & Watson, M. (2011). Effects of Irrigation and Crop Load on Leaf Gas Exchange and Fruit Composition in Red Winegrapes Grown on a Loamy Sand. *American Journal of Enology and Viticulture, 62*, 9-22. doi:10.5344/ajev.2010.10046

Brand, W. A., Coplen, T. B., Vogl, J., Rosner, M., & Prohaska, T. (2014). Assessment of international reference materials for isotope-ratio analysis (IUPAC Technical Report). *Pure and Applied Chemistry, 86*(3), 425-467.

Brunetto, G., Ceretta, C. A., de Melo, G. W. B., Girotto, E., Ferreira, P. A. A., Lourenzi, C. R., da Rosa Couto R., Tassinaria, A., Knevitz Hammerschmitt R., da Silva, L. O. S., Lazzaretti B. P., de Souza Kulmann M. S. & Carranca, C., (2016). Contribution of nitrogen from urea applied at different rates and times on grapevine nutrition. *Scientia Horticulturae, 207*, 1-6. doi:10.1016/j.scienta.2016.05.002

Bubola, M., Rusjan, D., & Lukić, I. (2020). Crop level vs. leaf removal: Effects on Istrian Malvasia wine aroma and phenolic acids composition. *Food Chemistry, 312*, 126046. 10.1016/j.foodchem.2019.126046

Bureau, S. M., Baumes, R. L., & Razungles, A. J. (2000). Effects of vine or bunch shading on the glycosylated flavor precursors in grapes of *Vitis vinifera* L. cv. Syrah. *Journal of Agricultural and Food Chemistry, 48*(4), 1290-1297. doi:10.1021/jf990507x

Cameleyre, M., Lytra, G., Tempere, S., & Barbe, J.-C. (2015). Olfactory impact of higher alcohols on red wine fruity ester aroma expression in model solution. *Journal of Agricultural and Food Chemistry, 63*(44), 9777-9788. doi:10.1021/acs.jafc.5b03489

Celette, F., Findeling, A., & Gary, C. (2009). Competition for nitrogen in an unfertilized intercropping system: The case of an association of grapevine and grass cover in a Mediterranean climate. *European Journal of Agronomy, 30*(1), 41-51. doi:10.1016/j.eja.2008.07.003

Celette, F., & Gary, C. (2013). Dynamics of water and nitrogen stress along the grapevine cycle as affected by cover cropping. *European Journal of Agronomy, 45*, 142-152. doi:10.1016/j.eja.2012.10.001

Cerovic, Z. G., Ghozlen, N. B., Milhade, C., Obert, M., Debuisson, S., & Moigne, M. L. (2015). Nondestructive diagnostic test for nitrogen nutrition of grapevine (*Vitis vinifera* L.) based on dualex leaf-clip measurements in the field. *Journal of Agricultural and Food Chemistry, 63*(14), 3669-3680. doi:10.1021/acs.jafc.5b00304

Champagnol, F. (1984). *Eléments de physiologie de la vigne et de viticulture générale*: Montpellier.

Chaves, M. M. (1984). Photosynthesis and assimilate partition in fruiting and non-fruiting grapevine shoots. In C. Sybesma (Ed.), *Advances in photosynthesis research: proceedings of the VIth international congress on photosynthesis, Brussels, Belgium, August 1–6, 1983. Volume IV* (pp. 145-148). Dordrecht: Springer Netherlands. doi:10.1007/978-94-017-4971-8_32

Choné, X., van Leeuwen, C., Chéry, P., & Ribéreau-Gayon, P. (2001). Terroir influence on water status and nitrogen status of non-irrigated Cabernet sauvignon (*Vitis vinifera* L.). Vegetative development, must and wine compositions (example of a Medoc top estate vineyard, Saint-Julien area, Bordeaux, 1997). *South African Journal of Enology and Viticulture, 22*(1), 8-15. doi:10.21548/22-1-2159

Choné, X., Lavigne-Cruège, V., Tominaga, T., van Leeuwen, C., Castagnède, C., Saucier, C., & Dubourdieu, D. (2006). Effect of vine nitrogen status on grape aromatic potential: falvor precursors (S-cysteine conjugates), glutathione and phenolic content in *Vitis vinifera* L. cv. Sauvignon blanc grape juice. *Journal International des Sciences de la Vigne et du Vin, 40*(1), 1-6. doi:10.20870/oeno-one.2006.40.1.880

Clarke, S. J., Lamont, K. J., Pan, H. Y., Barry, L. A., Hall, A., & Rogiers, S. Y. (2015). Spring root-zone temperature regulates root growth, nutrient uptake and shoot growth dynamics in grapevines. *Australian Journal of Grape and Wine Research, 21*(3), 479-489. doi:10.1111/ajgw.12160

Cliquet, J.-B., Deléens, E., Bousser, A., Martin, M., Lescure, J.-C., Prioul, J.-L., Mariotti, A., & Morot-Gaudry, J.-F., (1990). Estimation of carbon and nitrogen allocation during stalk elongation by ^{13}C and ^{15}N tracing in Zea mays L. *Plant Physiology, 92*(1), 79-87. doi:10.1104/pp.92.1.79

Clingeleffer, P. R. (2009). Influence of canopy management systems on vine productivity and fruit composition. In: *International symposium on grapevine canopy management*, California (Ed. University of California Davis), 13-20.

Conradie, W. J. (1980). Seasonal uptake of nutrients by Chenin blanc in sand culture. *South African Journal of Enology and Viticulture, 1*(1), 59-65. doi:10.21548/1-1-2414

Conradie, W. J. (1983). The uptake and distribution of ^{15}N enriched nitrate by three rootstock cultivars grafted to Chenin blanc. *South African Journal of Enology and Viticulture, 4*(2), 45-47. doi:10.21548/4-2-2369

Conradie, W. J. (1991). Distribution and translocation of nitrogen absorbed during early summer by two-year-old grapevines grown in sand culture. *American Journal of Enology and Viticulture, 42*(3), 180-190.

Conradie, W. J. (1992). Partitioning of nitrogen in grapevines during autumn and the utilisation of nitrogen reserves during the following growing season. *South African Journal of Enology and Viticulture, 13*(1), 45-51. doi:10.21548/13-1-2198

Conradie, W. J. (2005). *Partitioning of Mineral Nutrients and Timing of Fertilizer Applications for Optimum Efficiency.* Paper presented at the Soil Environment and Vine Mineral Nutrition, Davis.

Cookson, S. J., Clemente Moreno, M. J., Hevin, C., Nyamba Mendome, L. Z., Delrot, S., Trossat-Magnin, C., & Ollat, N. (2013). Graft union formation in grapevine induces transcriptional changes related to cell wall modification, wounding, hormone signalling, and secondary metabolism. *Journal of Experimental Botany, 64*(10), 2997-3008. doi:10.1093/jxb/ert144

Coplen, T. B. (2011). Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry, 25*(17), 2538-2560. doi:10.1002/rcm.5129

Crépin, L., Truong, N. M., Bloem, A., Sanchez, I., Dequin, S., & Camarasa, C. (2017). Management of Multiple Nitrogen Sources during Wine Fermentation by *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology, 83*(5), e02617-02616. doi:10.1128/aem.02617-16

Crespy, A. (2007). *Manuel pratique de fertilisation, qualité des moûts et des vins* (Oenoplurimedia Ed.).

Czerny, M., Christlbauer, M., Christlbauer, M., Fischer, A., Granvogl, M., Hammer, M., Hartl, C., Moran Hernandez, N., & Schieberle, P., (2008). Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. *European Food Research and Technology, 228*(2), 265-273. doi:10.1007/s00217-008-0931-x

Dai, Z. W., Ollat, N., Gomès, E., Decroocq, S., Tandonnet, J.-P., Bordenave, L., Pieri, P., Hilbert, G., Kappel, C., van Leeuwen C., Vivin, P. & Delrot, S., (2011). Ecophysiological, genetic, and molecular causes of variation in grape berry weight and composition: a review. *American Journal of Enology and Viticulture, 62*(4), 413-425. doi:10.5344/ajev.2011.10116

Dal Santo, S., Fasoli, M., Negri, S., D'Incà, E., Vicenzi, N., Guzzo, F., Tornielli, G. B., Pezzotti, M., & Zenoni, S., (2016). Plasticity of the berry ripening program in a white grape variety. *Frontiers in Plant Science, 7*(970). doi:10.3389/fpls.2016.00970

Dal Santo, S., Zenoni, S., Sandri, M., De Lorenzis, G., Magris, G., De Paoli, E., Di Gaspero, G., Del Fabbro, C., Morgante, M., Brancadoro, L., Grossi D., Fasoli, M.., Zuccolotto P., Tornielli G. B., & Pezzotti, M. (2018). Grapevine field experiments reveal the contribution of genotype, the influence of environment and the effect of their interaction (G×E) on the berry transcriptome. *The Plant Journal, 93*(6), 1143-1159. doi:10.1111/tpj.13834

Dayer, S., Peña, J. P., Gindro, K., Torregrosa, L., Voinesco, F., Martínez, L., Pietro, J. A. & Zufferey, V. (2017). Changes in leaf stomatal conductance, petiole hydraulics and vessel morphology in grapevine (*Vitis vinifera* cv. Chasselas) under different light and irrigation regimes. *Functional Plant Biology, 44*, 679-693. doi:10.1071/FP16041

Delabays, N., Spring, J. L., Ançay, A., Mosimann, E., & Schmid, A. (2000). Sélection d'espèces pour l'enherbement des cultures spéciales. *Revue suisse de Viticulture, Arboriculture et Horticulture, 32*(2), 95-104.

de-la-Fuente-Blanco, A., Sáenz-Navajas, M.-P., & Ferreira, V. (2016). On the effects of higher alcohols on red wine aroma. *Food Chemistry, 210*, 107-114. doi:10.1016/j.foodchem.2016.04.021

Delas, J., Molot, C. & Soyer J.P. (1991). Effects of nitrogen fertilization and grafting on the yield and quality of the crop of Vitis vinifera cv. Merlot. International Symposium, Jun 1991, Seattle, United States.

Delas, J., (2010). *Fertilisation de la vigne, 2e edition*. Ed Féret, Bordeaux, 165 p.

Deléens, E., Morot-Gaudry, J.-F., Martin, F., Thoereux, A., & Gojon, A. (1997). Méthodologie ^{15}N. In *Assimilation de l'azote chez les plantes* (pp. 265-280). Paris: Inra.

De Royer Dupré, N., Schneider, R., Payan, J. C., Salançon, E., & Razungles, A. (2014). Effects of vine water status on dimethyl sulfur potential, ammonium, and amino acid contents in Grenache noir grapes (*Vitis vinifera*). *J Agric Food Chem, 62*(13), 2760-2766. doi:10.1021/jf404758g

Dienes-Nagy, Á., Marti, G., Breant, L., Lorenzini, F., Fuchsmann, P., Baumgartner, D., Zufferey, V., Spring, J.-L., Gindro, K., Viret, O., WolfenderJ.-L. & Rösti, J. (2020). Identification of putative chemical markers in white wine (Chasselas) related to nitrogen deficiencies in vineyards. *Oeno One, 54*(3), 583-599. doi:10.20870/oeno-one.2020.54.3.3285

Dunlevy, J. D., Kalua, C. M., Keyzers, R. A., & Boss, P. K. (2009). The Production of Flavour & Aroma Compounds in Grape Berries. *In* K. A. Roubelakis-Angelakis (Ed.), *Grapevine Molecular Physiology & Biotechnology* (pp. 293-340). Dordrecht: Springer Netherlands. doi:10.1007/978-90-481-2305-6_11

Durante, C., & Bertacchini, L., Bontempo, L., Camin, F., Manzini, D., Lambertini, P., Marchetti, A., & Paolini, M. (2016). From soil to grape and wine: Variation of light and heavy elements isotope ratios. *Food Chemistry, 210*, 648-659. doi:10.1016/j.foodchem.2016.04.108

Echeverría, G., Ferrer, M., & Mirás-Avalos, J. (2017). Quantifying the relative impact of physical and human factors on the viticultural expression of terroir. *International Journal of Environmental & Agriculture Research, 3*(4), 12-25.

Eichert, T. (2013). *Foliar Nutrient Uptake - of Myths and Legends.* Paper presented at the VIIth ISon Mineral Nutrition of Fruit Crops, Bonn, Germany. doi:10.17660/ActaHortic.2013.984.5

Fairbairn, S., McKinnon, A., Musarurwa, H. T., Ferreira, A. C., & Bauer, F. F. (2017). The impact of single amino acids on growth and volatile aroma production by *Saccharomyces cerevisiae* Strains. *Frontiers in Microbiology, 8*(2554). doi:10.3389/fmicb.2017.02554

Fayolle, E., Follain, S., Marchal, P., Chéry, P., & Colin, F. (2019). Identification of environmental factors controlling wine quality: A case study in Saint-Emilion Grand Cru appellation, France. *Science of The Total Environment, 694*, 133718. doi:10.1016/j.scitotenv.2019.133718

Fernández, V., & Eichert, T. (2009). Uptake of hydrophilic solutes through plant leaves: current state of knowledge and perspectives of foliar fertilization. *Critical Reviews in Plant Sciences, 28*(1-2), 36-68. doi:10.1080/07352680902743069

Fernández, V., & Brown, P. H. (2013). From plant surface to plant metabolism: the uncertain fate of foliar-applied nutrients. *Frontiers in Plant Science, 4*(289). doi:10.3389/fpls.2013.00289

Fernández, V., Gil-Pelegrín, E., & Eichert, T. (2020). Foliar water and solute absorption: an update. *The Plant Journal*. doi:10.1111/tpj.15090

Fleet, G. H. (1993). *Wine microbiology and biotechnology*. London: Taylor and Francis.

Friedel, M., Hendgen, M., Stoll, M., & Löhnertz, O. (2020). Performance of reflectance indices and of a handheld device for estimating in-field the nitrogen status of grapevine leaves. *Australian Journal of Grape and Wine Research, 26*(2), 110-120. doi:10.1111/ajgw.12424

Gang, Q., Chang, S. X., Lin, G., Zhao, Q., Mao, B., & Zeng, D.-H. (2019). Exogenous and endogenous nitrogen differentially affect the decomposition of fine roots of different diameter classes of Mongolian pine in semi-arid northeast China. *Plant and Soil, 436*(1), 109-122. doi:10.1007/s11104-018-03910-0

Gaudillère, J.-P., Chantelot, E., Soyer, J.-P., Molot, C., & Milin, S. (2003). Leaf and must nitrogen content two complementary indicators of grapevine nitrogen status. *Journal International des Sciences de la Vigne et du Vin, 37*(2), 91-101. doi:10.20870/oeno-one.2003.37.2.943

Garde-Cerdán, T., & Ancín-Azpilicueta, C. (2008). Effect of the addition of different quantities of amino acids to nitrogen-deficient must on the formation of esters, alcohols, and acids during wine alcoholic fermentation. *LWT - Food Science and Technology, 41*(3), 501-510. doi:10.1016/j.lwt.2007.03.018

Garde-Cerdán, T., Lorenzo, C., Lara, J. F., Pardo, F., Ancin-Azpilicueta, C., & Salinas, M. R. (2009). Study of the evolution of nitrogen compounds during grape ripening. application to differentiate grape varieties and cultivated systems. *Journal of Agricultural and Food Chemistry, 57*, 2410-2419. doi:10.1021/jf8037049

Garde-Cerdán, T., Lopez, R., Portu, J., Gonzalez-Arenzana, L., Lopez-Alfaro, I., & Santamaria, P. (2014). Study of the effects of proline, phenylalanine, and urea foliar application to Tempranillo vineyards on grape amino acid content. Comparison with commercial nitrogen fertilisers. *Food Chemistry, 163*, 136-141. doi:10.1016/j.foodchem.2014.04.101

Garde-Cerdán, T., Mancini, V., Carrasco-Quiroz, M., Servili, A., Gutiérrez-Gamboa, G., Foglia, R., Pérez-Álvarez, E. P., & Romanazzi, G. (2017). Chitosan and laminarin as alternatives to copper for plasmopara viticola control: effect on grape amino acid. *Journal of Agricultural and Food Chemistry, 65*(34), 7379-7386. doi:10.1021/acs.jafc.7b02352

Garde-Cerdán, T., Gutiérrez-Gamboa, G., Fernández-Novales, J., Pérez-Álvarez, E. P., & Diago, M. P. (2018). Towards the definition of optimal grape harvest time in Grenache grapevines: Nitrogenous maturity. *Scientia Horticulturae, 239*, 9-16. doi:10.1016/j.scienta.2018.05.014

Gatti, M., Squeri, C., Garavani, A., Vercesi, A., Dosso, P., Diti, I., & Poni, S. (2018). Effects of variable rate nitrogen application on cv. Barbera performance: vegetative growth and leaf nutritional status. *American Journal of Enology and Viticulture, 69*(3), 196-209. doi:10.5344/ajev.2018.17084

Gauthier, P. P. G., Bligny, R., Gout, E., Mahé, A., Nogués, S., Hodges, M., & Tcherkez, G. G. B. (2010). In folio isotopic tracing demonstrates that nitrogen assimilation into glutamate is mostly independent from current CO_2 assimilation in illuminated leaves of Brassica napus. *New Phytologist, 185*(4), 988-999. doi:10.1111/j.1469-8137.2009.03130.x

Giese, W. G., Wolf, T. K., Velasco-Cruz, C., & Roberts, L. (2015). Cover crop and root pruning effects on the rooting pattern of SO4 rootstock grafted to Cabernet-Sauvignon. *American Journal of Enology and Viticulture*. doi:10.5344/ajev.2015.15066

Glad, C., Farineau, J., Regnard, J.-L., & Morot-Gaudry, J.-F. (1994). The relative contribution of nitrogen originating from two seasonal 15N supplies to the total nitrogen pool present in the bleeding sap and in whole *Vinifera* cv. Pinot noir grapevines at bloom time. *American Journal of Enology and Viticulture, 45*(3), 327-332.

Gobert, A., Tourdot-Maréchal, R., Sparrow, C., Morge, C., & Alexandre, H. (2019). Influence of nitrogen status in wine alcoholic fermentation. *Food Microbiology, 83*, 71-85. doi:10.1016/j.fm.2019.04.008

González-Barreiro, C., Rial-Otero, R., Cancho-Grande, B., & Simal-Gándara, J. (2015). Wine aroma compounds in grapes: a critical review. *Critical Reviews in Food Science and Nutrition, 55*(2), 202-218. doi:10.1080/10408398.2011.650336

González-Real, M. M., Baille, A., & Liu, H. Q. (2008). Influence of fruit load on dry matter and N-distribution in sweet pepper plants. *Scientia Horticulturae, 117*, 307-315. doi:10.1016/j.scienta.2008.05.026

González-Santamaría, R., Ruiz-González, R., Nonell, S., Garde-Cerdán, T., & Pérez-Álvarez, E. P. (2018). Influence of foliar riboflavin applications to vineyard on grape amino acid content. *Food Chemistry, 240*, 601-606. doi:10.1016/j.foodchem.2017.07.115

Gourieroux, A. M., Holzapfel, B. P., Scollary, G. R., McCully, M. E., Canny, M. J., & Rogiers, S. Y. (2016). The amino acid distribution in rachis xylem sap and phloem exudate of *Vitis vinifera* 'Cabernet-Sauvignon' bunches. *Plant Physiology and Biochemistry, 105*, 45-54. doi:10.1016/j.plaphy.2016.04.010

Gouthu, S., Skinkis, P. A., Morre, J., Maier, C. S., & Deluc, L. G. (2012). Berry nitrogen status altered by cover cropping: Effects on berry hormone dynamics, growth and amino acid composition of Pinot noir. *Food Chemistry, 135*(1), 1-8. doi:10.1016/j.foodchem.2012.04.019

Grechi, I., Vivin, P., Hilbert, G., Milin, S., Robert, T., & Gaudillère, J. P. (2007). Effect of light and nitrogen supply on internal C:N balance and control of root-to-shoot biomass allocation in grapevine. *Environmental and experimental Botany, 59*, 139-149. doi:10.1016/j.envexpbot.2005.11.002

Guilpart, N., Metay, A., & Gary, C. (2014). Grapevine bud fertility and number of berries per bunch are determined by water and nitrogen stress around flowering in the previous year. *European Journal of Agronomy, 54*, 9-20. doi:10.1016/j.eja.2013.11.002

Gutiérrez-Gamboa, G., Garde-Cerdán, T., Portu, J., Moreno-Simunovic, Y., & Martínez-Gil, A. M. (2017a). Foliar nitrogen application in Cabernet-Sauvignon vines: Effects on wine flavonoid and amino acid content. *Food Research International, 96*, 46-53. doi:10.1016/j.foodres.2017.03.025

Gutiérrez-Gamboa, G., Portu, J., Santamaría, P., López, R., & Garde-Cerdán, T. (2017b). Effects on grape amino acid concentration through foliar application of three different elicitors. *Food Research International, 99*, 688-692. doi:10.1016/j.foodres.2017.06.022

Gutiérrez-Gamboa, G., Carrasco-Quiroz, M., Martínez-Gil, A. M., Pérez-Álvarez, E. P., Garde-Cerdán, T., & Moreno-Simunovic, Y. (2018). Grape and wine amino acid composition from Carignan noir grapevines growing under rainfed conditions in the Maule Valley, Chile: Effects of location and rootstock. *Food Research International, 105*, 344-352. doi:10.1016/j.foodres.2017.11.021

Gutiérrez-Gamboa, G., Romanazzi, G., Garde-Cerdán, T., & Pérez-Álvarez, E. P. (2019). A review of the use of biostimulants in the vineyard for improved grape and wine quality: effects on prevention of grapevine diseases. *J Sci Food Agric, 99*(3), 1001-1009. doi:10.1002/jsfa.9353

Gutiérrez-Gamboa, G., Garde-Cerdán, T., Rubio-Bretón, P., & Pérez-Álvarez, E. P. (2020a). Seaweed foliar applications at two dosages to Tempranillo blanco (*Vitis vinifera* L.) grapevines in two seasons: Effects on grape and wine volatile composition. *Food Research International, 130*, 108918. doi:10.1016/j.foodres.2019.108918

Gutiérrez-Gamboa, G., Garde-Cerdán, T., Rubio-Bretón, P., & Pérez-Álvarez, E. P. (2020b). Biostimulation to Tempranillo grapevines (*Vitis vinifera* L.) through a brown seaweed during two seasons: Effects on grape juice and wine nitrogen compounds. *Scientia Horticulturae, 264*, 109177. doi:10.1016/j.scienta.2020.109177

Habran, A., Commisso, M., Helwi, P., Hilbert, G., Negri, S., Ollat, N., Gomès, E., van Leeuwen, C., Guzzo, F., & Delrot, S. (2016). Roostocks/scion/nitrogen interactions affect secondary metabolism in the grape berry. *Frontiers in Plant Science, 7*(1134). doi:10.3389/fpls.2016.01134

Hachiya, T., & Sakakibara, H. (2016). Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. *Journal of Experimental Botany, 68*(10), 2501-2512. doi:10.1093/jxb/erw449

Hannam, K. D., Neilsen, G. H., Neilsen, D., Midwood, A. J., Millard, P., Zhang, Z., Thornton, B., & Steinke, D. (2016). Amino acid composition of grape (*Vitis vinifera* L.) juice in response to applications of urea to the soil or foliage. *American Journal of Enology and Viticulture, 67*(1), 47-55. doi:10.5344/ajev.2015.15015

Hardarson, G., Hood-Nowotny, R., Jensen, E. S., Peoples, M. B., Van Cleemput, O., van Kessel, C., … Zapata, F. (2008). Guidelines on nitrogen management in agricultural systems. *International Atomic Energy Agency, Vienna, IAEA-TCS-29*.

Haynes, R. J. (1986). Uptake and assimilation of mineral nitrogen by plants. In R. J. Haynes (Ed.), *Mineral nitrogen in the plant-soil system* (pp. 303-378). Orlando, Florida, USA: Academic Press. doi:10.1016/B978-0-12-334910-1.50010-8

Helwi, P., Habran, A., Guillaumie, S., Thibon, C., Hilbert, G., Gomès, E., Delrot, S., Darriet, P. & van Leeuwen, C. (2015). Vine nitrogen status does not have a direct impact on 2-methoxy-3-isobutylpyrazine in grape berries and wines. *J Agric Food Chem, 63*(44), 9789-9802. doi:10.1021/acs.jafc.5b03838

Helwi, P., Guillaumie, S., Thibon, C., Keime, C., Habran, A., Hilbert, G., Gomès, E., Darriet, P., Delrot, S., & van Leeuwen, C. (2016). Vine nitrogen status and volatile thiols and their precursors from plot to transcriptome level. *BMC Plant Biology, 16*(1), 173. doi:10.1186/s12870-016-0836-y

Henschke, P. A., & Jiranek, V. (1993). Yeasts – metabolism of nitrogen compounds. *In* G. H. Fleet (Ed.), *Wine microbiology and biotechnology* (pp. 77–164). Chur, Switzerland: Harwood Academic Publishers.

Hernández-Orte, P., Guitart, A., & Cacho, J. (1999). Changes in the concentration of amino acids during the ripening of *Vitis vinifera* Tempranillo variety from the *Denomination d'Origine* Somontano (Spain). *American Journal of Enology and Viticulture, 50*(2), 144-154.

Hernández-Orte, P., Cacho, J. F., & Ferreira, V. (2002). Relationship between varietal amino acid profile of grapes and wine aromatic composition. Experiments with model solutions and chemometric Study. *Journal of Agricultural and Food Chemistry, 50*(10), 2891-2899. doi:10.1021/jf011395o

Hernández-Orte, P., Ibarz, M. J., Cacho, J., & Ferreira, V. (2006). Addition of amino acids to grape juice of the Merlot variety: Effect on amino acid uptake and aroma generation during alcoholic fermentation. *Food Chemistry, 98*(2), 300-310. doi:10.1016/j.foodchem.2005.05.073

Hjelmeland, A. K., & Ebeler, S. E. (2015). Glycosidically bound volatile aroma compounds in grapes and wine: A Review. *American Journal of Enology and Viticulture, 66*(1), 1-11. doi:10.5344/ajev.2014.14104

Hilbert, G., Soyer, J.-P., Giraudon, J., Milin, S., & Gaudillère, J. P. (2003). Effects of nitrogen supply on must quality and anthocyanin accumulation in berries of cv. Merlot. *Vitis, 42*(2), 69-76.

Hoenicke, K., Simat, T. J., Steinhart, H., Christoph, N., Geßner, M., & Köhler, H.-J. (2002). 'Untypical aging off-flavor' in wine: formation of 2-aminoacetophenone and evaluation of its influencing factors. *Analytica chimica Acta, 458*(1), 29-37. doi:10.1016/S0003-2670(01)01523-9

Holzapfel, B. P., & Treeby, M. T. (2007). Effects of timing and rate of N supply on leaf nitrogen status, grape yield and juice composition from Shiraz grapevines grafted to one of three different rootstocks. *Australian Journal of Grape and Wine Research, 13*(1), 14-22. doi:10.1111/j.1755-0238.2007.tb00067.x

Holzapfel, B. P., Smith, J. P., & Field, S. K. (2019). Seasonal vine nutrient dynamics and distribution of Shiraz grapevines. *Oeno One, 53*(2). doi:10.20870/oeno-one.2019.53.2.2425

Howell, G. S. (2001). Sustainable grape productivity and the growth-yield relationship: a review. *American Journal of Enology and Viticulture, 52*(3), 165-174.

Huang, Z., & Ough, C. S. (1991). Amino acid profiles of commercial grape juices and wines. *American Journal of Enology and Viticulture, 42*(3), 261-267.

Huglin, P., and Schneider, C., 1998. *Biologie et écologie de la vigne*. Lavoisier Tec. et Doc., Paris.

Hunter, J. J., & Bonnardot, V. (2011). Suitability of some climatic parameters for grapevine cultivation in South Africa, with focus on key physiological processes. *South African Journal of Enology and Viticulture, 32*(1), 18. doi:10.21548/32-1-1374

Iandolino, A. B., & Williams, L. E. (2014). Recovery of ^{15}N labeled fertilizer by *Vitis vinifera* L. cv. Cabernet sauvignon: effects of N fertilizer rates and applied water amounts. *American Journal of Enology and Viticulture, 65*(2), 189-196. doi:10.5344/ajev.2014.11019

Jackson, D. I., & Lombard, P. B. (1993). Environmental and management practices affecting grape composition and wine quality - a review. *American Journal of Enology and Viticulture, 44*(4), 409-430.

Jackson, R. S. (2008). *Wine Science* (3rd édition ed.). San Diego: Academic Press.

Janzen, H. H., & Bruinsma, Y. (1989). Methodology for the quantification of root and rhizosphere nitrogen dynamics by exposure of shoots to ^{15}N-labelled ammonia. *Soil Biology and Biochemistry, 21*(2), 189-196. doi:10.1016/0038-0717(89)90094-1

Jing, H., Zhang, P., Li, J., Yao, X., Liu, G., & Wang, G. (2019). Effect of nitrogen addition on the decomposition and release of compounds from fine roots with different diameters: the importance of initial substrate chemistry. *Plant and Soil, 438*(1), 281-296. doi:10.1007/s11104-019-04017-w

Ju, Y.-L., Liu, M., Zhao, H., Meng, J.-F., & Fang, Y.-L. (2016). Effect of exogenous abscisic acid and methyl jasmonate on anthocyanin composition, fatty acids, and volatile compounds of Cabernet-Sauvignon (*Vitis vinifera* L.) grape berries. *Molecules, 21*, 1354. doi:10.3390/molecules21101354

Kant, S., Bi, Y.-M., & Rothstein, S. J. (2011). Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. *Journal of Experimental Botany, 62*(4), 1499-1509. doi:10.1093/jxb/erq297

Keller, M., & Koblet, W. (1995). Dry matter and leaf area partitioning, bud fertility and second season growth of *Vitis vinifera* L.: responses to nitrogen supply and limiting irradiance. *Vitis, 34*(2), 77-83.

Keller, M. (2005). Deficit irrigation and vine mineral nutrition. *American Journal of Enology and Viticulture, 56*(3), 267-283.

Keller, M., Mills, L. J., Wample, R. L., & Spayd, S. E. (2005). Cluster thinning effects on three deficit-irrigated *Vitis vinifera* cultivars. *American Journal of Enology and Viticulture, 56*(2), 91-103.

Keller, M. (2015). *The science of grapevines* (M. Keller Ed. 2nd edition): Elsevier Inc.

Keller, M. (2020). *The science of grapevines* (M. Keller Ed. 3rd edition): Elsevier Inc.

Khalsa, S. D. S., Almanza, C. A., Brown, P. H., & Smart, D. R. (2016). Leaf litter C and N cycling from a deciduous permanent crop. *Soil Science and Plant Nutrition, 62*, 271-276. doi:10.1080/00380768.2016.1188413

Kliewer, W. M., & Dokoozlian, N. (2005). Leaf area/crop weight ratios of grapevines: influence on fruit composition and wine quality. *American Journal of Enology and Viticulture, 56*(2), 170-181.

Klodd, A. E., Eissenstat, D. M., Wolf, T. K., & Centinari, M. (2016). Coping with cover crop competition in mature grapevines. *Plant and Soil, 400*(1), 391-402. doi:10.1007/s11104-015-2748-2

Kotsaki, E., Reynolds, A. G., Brown, R., Lee, H.-S., & Aubie, E. (2020a). Proximal sensing and relationships to soil and vine water status, yield, and berry composition in Ontario vineyards. *American Journal of Enology and Viticulture, 71*(2), 114-131. doi:10.5344/ajev.2019.19018

Kotsaki, E., Reynolds, A. G., Brown, R., Lee, H.-S., & Jollineau, M. (2020b). Spatial variability in soil and vine water status in Ontario vineyards: relationships to yield and berry composition. *American Journal of Enology and Viticulture, 71*(2), 132-148. doi:10.5344/ajev.2019.19019

Krapp, A., Hofmann, B., Schäfer, C., & Stitt, M. (1993). Regulation of the expression of rbcS and other photosynthetic genes by carbohydrates: a mechanism for the 'sink regulation' of photosynthesis? *The Plant Journal, 3*, 817-828. doi: 10.1111/j.1365-313X.1993.00817.x

Krishna, H., Singh, S. K., Sharma, R. R., Khawale, R. N., Grover, M., & Patel, V. B. (2005). Biochemical changes in micropropagated grape (*Vitis vinifera* L.) plantlets due to arbuscular-mycorrhizal fungi (AMF) inoculation during ex vitro acclimatization. *Scientia Horticulturae, 106*(4), 554-567. doi:10.1016/j.scienta.2005.05.009

Krogmeier, M. J., McCarty, G. W., & Bremner, J. M. (1989). Phytotoxicity of foliar-applied urea. *Proceedings of the National Academy of Sciences, 86*(21), 8189-8191. doi:10.1073/pnas.86.21.8189

Kumagai, E., Araki, T., Hamaoka, N., & Ueno, O. (2011). Ammonia emission from rice leaves in relation to photorespiration and genotypic differences in glutamine synthetase activity. *Annals of Botany, 108*(7), 1381-1386. doi:10.1093/aob/mcr245

Kwasniewski, M. T., Vanden Heuvel, J. E., Pan, B. S., & Sacks, G. L. (2010). Timing of cluster light environment manipulation during grape development affects C13 norisoprenoid and carotenoid concentrations in Riesling. *Journal of Agricultural and Food Chemistry, 58*(11), 6841-6849. doi:10.1021/jf904555p

Lacroux, F., Tregoat, O., van Leeuwen, C., Pons, A., Tominaga, T., Lavigne-Cruège, V., & Dubourdieu, D. (2008). Effect of foliar nitrogen and sulfur application on aromatic expression of *Vitis vinifera* L. cv. Sauvignon blanc. *Journal International des Sciences de la Vigne et du Vin, 42*(3), 125-132. doi:10.20870/oeno-one.2008.42.3.816

Lakso, A. and Sacks, G. (2009). Vine balance: what is it and how does it change over the season? In: *International symposium on grapevine canopy management, California* (Ed. University of California Davis), 21-25.

Lambrechts, M., & Pretorius, I. (2000). Yeast and its importance to wine aroma-a review. *South African Journal of Enology and Viticulture, 21*(1), 97-129. doi:10.21548/21-1-3560

Lasa, B., Menendez, S., Sagastizabal, K., Calleja-Cervantes, M., Irigoyen, I., Muro, J., Aparicio-Tejo, P., & Ariz, I. (2012). Foliar application of urea to "Sauvignon blanc" and "Merlot" vines: doses and time of application. *Plant Growth Regulation, 67*(1), 73-81. doi:10.1007/s10725-012-9667-5

Lawlor, D. W. (2002). Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *Journal of Experimental Botany, 53*(370), 773-787. doi:10.1093/jexbot/53.370.773

Lea, P. J., & Azevedo, R. A. (2006). Nitrogen use efficiency. 1. Uptake of nitrogen from the soil. *Annals of Applied Biology, 149*(3), 243-247. doi:10.1111/j.1744-7348.2006.00101.x

Leng, F., Tang, D., Lin, Q., Cao, J., Wu, D., Wang, S., & Sun, C. (2017). Transcriptomic analyses of ascorbic acid and carotenoid metabolites influenced by root restriction during grape berry development and ripening. *Journal of Agricultural and Food Chemistry, 65*(9), 2008-2016. doi:10.1021/acs.jafc.6b05322

Li, Q., Li, B.-H., Kronzucker, H. J., & Shi, W.-M. (2010). Root growth inhibition by NH_4^+ in Arabidopsis is mediated by the root tip and is linked to NH_4^+ efflux and GMPase activity. *Plant, Cell & Environment, 33*(9), 1529-1542. doi:10.1111/j.1365-3040.2010.02162.x

Lilly, M., Bauer, F. F., Styger, G., Lambrechts, M. G., & Pretorius, I. S. (2006). The effect of increased branched-chain amino acid transaminase activity in yeast on the production of higher alcohols and on the flavour profiles of wine and distillates. *FEMS Yeast Research, 6*(5), 726-743. doi:10.1111/j.1567-1364.2006.00057.x

Lin, Y., Jiang, C., Guo, Y., Li, K., Liu, Z., Lin, Z., Li, X., Yue, G., Fu, Q., Li, W., Zheng, J., Dou, Y., & Guo, X. (2018). Relationship between 2-phenylethanol content and differential expression of L-amino acid decarboxylases (AADC) in (*Vitis vinifera*) vidal wine grape at different loads. *Pakistan Journal of Botany, 50*(2), 661-666.

Linsenmeier, A., Rauhut, D., Kürbel, H., & Schubert, S. (2007). Untypical ageing off-flavour and masking effects due to long-term nitrogen fertilization. *VITIS-GEILWEILERHOF-, 46*(1), 33-38.

Linsenmeier, A. W., Loos, U., & Löhnertz, O. (2008). Must composition and nitrogen uptake in a long-term trial as affected by timing of nitrogen fertilization in a cool-climate Riesling vineyard. *American Journal of Enology and Viticulture, 59*(3), 255-264.

Livingston, N. J., Guy, R. D., Sun, Z. J., & Ethier, G. J. (1999). The effects of nitrogen stress on the stable carbon isotope composition, productivity and water use efficiency of white spruce (Picea glauca (Moench) Voss) seedlings. *Plant, Cell & Environment, 22*, 281-289. doi:10.1046/j.1365-3040.1999.00400.x

Llorens, N., Arola, L., Bladé, C., & Mas, A. (2002). Nitrogen metabolism in a grapevine in vitro system. *Oeno One, 36*(3), 157-159. doi:10.20870/oeno-one.2002.36.3.968

Löhnertz, O. (1988). *Untersuchungen zum zeitlichen Verlauf der Nährstoffaufnahme bei Vitis vinifera (cv. Riesling).* Geisenheim, Universität Giessen, 228 p.

Longbottom, M. (2009). *Managing grapevine nutrition in a changing environment*: The Australian Wine Research Institute Ltd., p.144

Loulakakis, K. A., Morot-Gaudry, J. F., Velanis, C. N., Skopelitis, D. S., Moschou, P. N., Hirel, B., & Roubelakis-Angelakis, K. A. (2009). Advancements in nitrogen metabolism in grapevine. *In* K. A. Roubelakis-Angelakis (Ed.), *Grapevine Molecular Physiology & Biotechnology* (pp. 161-205). Dordrecht: Springer Netherlands. doi:10.1007/978-90-481-2305-6_7

Lytra, G., Tempère, S., Zhang, S., Marchand, S., de Revel, G., & Barbe, J.-C. (2014). Olfactory impact of dimethyl sulfide on red wine fruity esters aroma expression in model solution. *Oeno One, 48*(1), 75-85. doi:10.20870/oeno-one.2014.48.1.1660

Lytra, G., Tempère, S., Marchand, S., de Revel, G., & Barbe, J.-C. (2016). How do esters and dimethyl sulphide concentrations affect fruity aroma perception of red wine? Demonstration by dynamic sensory profile evaluation. *Food Chemistry, 194*, 196-200. doi:10.1016/j.foodchem.2015.07.143

Maigre, D., & Aerny, J. (2001a). Enherbement permanent et fumure azotée sur cv. Gamay dans le Valais central. 1. Résultats agronomiques. *Revue suisse de Viticulture, Arboriculture et Horticulture, 33*(3), 145-150.

Maigre, D., & Aerny, J. (2001b). Enherbement et fumure azotée sur cv. Gamay dans le Valais central. 2. Résultats analytiques et organoleptiques. *Revue suisse de Viticulture, Arboriculture et Horticulture, 33*(4), 199-204.

Marais, J., Calitz, F., & Haasbroek, P. D. (2001). Relationship between microclimatic data, aroma component concentrations and wine quality parameters in the prediction of Sauvignon blanc wine quality. *South African Journal of Enology and Viticulture, 22*(1), 22. doi:10.21548/22-1-2161

Martínez-Gil, A. M., Garde-Cerdán, T., Lorenzo, C., Félix Lara, J., Pardo, F., & Rosario Salinas, M. (2012). Volatile compounds formation in alcoholic fermentation from grapes collected at 2 maturation stages: influence of nitrogen compounds and grape variety. *Journal of food science, 77*(1), C71-C79. doi:10.1111/j.1750-3841.2011.02441.x

Martínez-Moreno, R., Morales, P., Gonzalez, R., Mas, A., & Beltran, G. (2012). Biomass production and alcoholic fermentation performance of *Saccharomyces cerevisiae* as a function of nitrogen source. *FEMS Yeast Research, 12*(4), 477-485. doi:10.1111/j.1567-1364.2012.00802.x

Marschner, P., & Rengel, Z. (2012). Chapter 12 - Nutrient availability in soils. *In* P. Marschner (Ed.), *Marschner's Mineral Nutrition of Higher Plants (Third Edition)* (pp. 315-330). San Diego: Academic Press. doi:10.1016/B978-0-12-384905-2.00012-1

Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., & Suzuki, A. (2010). Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. *Annals of Botany, 105*(7), 1141-1157. doi:10.1093/aob/mcq028

Mawdsley, P. F. W., Dodson Peterson, J. C., & Casassa, L. F. (2018). Agronomical and Chemical Effects of the Timing of Cluster Thinning on Pinot Noir (Clone 115) Grapes and Wines. *Fermentation, 4*(3), 60. doi:10.3390/fermentation4030060

Mengel, K., & Pilbeam, D. J. (1992). *Nitrogen metabolism of plants. In: Proceedings of the phytochemical society of Europe* 33 New York: Oxford University Press.

Merbach, W., Mirus, E., Knof, G., Remus, R., Ruppel, S., Russow, R., Gransee, A., & Schulze, J. (1999). Release of carbon and nitrogen compounds by plant roots and their possible ecological importance+. *Journal of Plant Nutrition and Soil Science, 162*(4), 373-383.

Metay, A. l., Magnier, J., Guilpart, N., & Christophe, A. l. (2014). Nitrogen supply controls vegetative growth, biomass and nitrogen allocation for grapevine (cv. Shiraz) grown in pots. *Functional Plant Biology, 42*(1), 105. doi:10.1071/fp14062

Meyers, J. M., Sacks, G. L., & Heuvel, J. E. V. (2013). Glycosylated aroma compound responses in 'Riesling' wine grapes to cluster exposure and vine yield. 23(5), 581. doi:10.21273/horttech.23.5.581

Miller, G. H. (2019). *Whiskey science*. Cham, Switzerland: Springer, 529 p. doi:10.1007/978-3-030-13732-8

Molina, J.-A. E., & Smith, P. (1997). Modeling carbon and nitrogen processes in soils. *In* D. L. Sparks (Ed.), *Advances in Agronomy* (Vol. 62, pp. 253-298): Academic Press. doi:10.1016/S0065-2113(08)60570-2

Morinaga, K., Imai, S., Yakushiji, H., & Koshita, Y. (2003). Effects of fruit load on partitioning of ^{15}N and ^{13}C, respiration, and growth of grapevine roots at different fruit stages. *Scientia Horticulturae, 97*, 239-253. doi:10.1016/S0304-4238(02)00199-1

Morot-Gaudry, J.-F., Moreau, F., Prat, R., Maurel, C., & Sentenac, H. (2017). *Biologie végétale: Nutrition et métabolisme-3e éd*: Dunod.

Muhammad, S., Sanden, B. L., Lampinen, B. D., Smart, D. R., Saa, S., Shackel, K. A., & Brown, P. H. (2020). Nutrient Storage in the Perennial Organs of Deciduous Trees and Remobilization in Spring – A Study in Almond (Prunus dulcis) (Mill.) D. A. Webb [Original Research]. *Frontiers in Plant Science, 11*(658). doi:10.3389/fpls.2020.00658

Murisier, F., & Zufferey, V. (1997). Rapport feuille-fruit de la vigne et qualité du raisin. *Revue suisse de Viticulture, Arboriculture et Horticulture, 29*(6), 355-362.

Nisbet, M. A., Martinson, T. E., & Mansfield, A. K. (2014). Accumulation and prediction of yeast assimilable nitrogen in New York winegrape cultivars. *American Journal of Enology and Viticulture, 65*(3), 325-332. doi:10.5344/ajev.2014.13130

Nykänen, L. (1986). Formation and occurrence of flavor compounds in wine and distilled alcoholic beverages. *American Journal of Enology and Viticulture, 37*(1), 84-96.

OFEV, Federal Office for the Environment (2019). État et évolution des eaux souterraines en Suisse: résultats de l'observation nationale des eaux souterraines NAQUA, état 2016. Office fédéral de l'environnement (ed.), Berne. *État de l'environnement 1901*, 144 p.

Oliva, J., Garde-Cerdán, T., Martínez-Gil, A. M., Rosario Salinas, M., & Barba, A. (2011). Fungicide effects on ammonium and amino acids of Monastrell grapes. *Food Chemistry, 129*(4), 1676-1680. doi:10.1016/j.foodchem.2011.06.030

Ong, P. K. C., & Acree, T. E. (1999). Similarities in the aroma chemistry of Gewürztraminer variety wines and lychee (Litchi chinesis Sonn.) fruit. *Journal of Agricultural and Food Chemistry, 47*(2), 665-670. doi:10.1021/jf980452j

Ortega-Heras, M., Pérez-Magariño, S., Del-Villar-Garrachón, V., González-Huerta, C., Moro Gonzalez, L. C., Guadarrama Rodríguez, A., Villanueva Sanchez, S., Gallo González, R., & Martín de la Helguera, S. (2014). Study of the effect of vintage, maturity degree, and irrigation on the amino acid and biogenic amine content of a white wine from the Verdejo variety. *Journal of the Science of Food and Agriculture, 94*(10), 2073-2082. doi:10.1002/jsfa.6526

Paolini, M., Ziller, L., Bertoldi, D., Bontempo, L., Larcher, R., Nicolini, G., & Camin, F. (2016). $\delta^{15}N$ from soil to wine in bulk samples and proline. *Journal of Mass Spectrometry, 51*(9), 668-674. doi:10.1002/jms.3824

Parker, A. K., Hofmann, R. W., van Leeuwen, C., McLachlan, A. R. G., & Trought, M. C. T. (2014). Leaf area to fruit mass ratio determines the time of veraison in Sauvignon blanc and Pinot noir grapevines. *Australian Journal of Grape and Wine Research, 20*(3), 422-431. doi:10.1111/ajgw.12092

Parker, A. K., Hofmann, R. W., van Leeuwen, C., McLachlan, A. R. G., & Trought, M. C. T. (2015). Manipulating the leaf area to fruit mass ratio alters the synchrony of total soluble solids accumulation and titratable acidity of grape berries. *Australian Journal of Grape and Wine Research, 21*(2), 266-276. doi:10.1111/ajgw.12132

Parker, A. K., García de Cortázar-Atauri, I., Gény, L., Spring, J.-L., Destrac, A., Schultz, H., Molitor, D., Lacombe, T., Graça, A., Monamy, C., Stoll M., Storchi, P., Trought, M. C. T., Hofmann, R. W. & van Leeuwen, C. (2020). Temperature-based grapevine sugar ripeness modelling for a wide range of *Vitis vinifera* L. cultivars. *Agricultural and Forest Meteorology, 285-286*, 107902. doi:10.1016/j.agrformet.2020.107902

Petrie, P. R., & Clingeleffer, P. R. (2006). Crop thinning (hand versus mechanical), grape maturity and anthocyanin concentration: outcomes from irrigated Cabernet Sauvignon (Vitis vinifera L.) in a warm climate. *Australian Journal of Grape and Wine Research, 12*, 21-29. doi:10.1111/j.1755-0238.2006.tb00040.x

Perez, J. R., & Kliewer, W. M. (1982). Influence of light regime and nitrate fertilization on nitrate reductase activity and concentrations of nitrate and arginine in tissues of three cultivars of grapevines. *American Journal of Enology and Viticulture, 33*(2), 86-93.

Poni, S., Gatti, M., Palliotti, A., Dai, Z., Duchêne, E., Truong, T.-T., Ferrara, G., Matarrese, A. M. S., Gallotta, A., Bellincontro, A., Mencarelli, F., & Tombesi, S. (2018). Grapevine quality: A multiple choice issue. *Scientia Horticulturae, 234*, 445-462. doi:10.1016/j.scienta.2017.12.035

Portu, J., Gonzalez-Arenzana, L., Hermosin-Gutierrez, I., Santamaria, P., & Garde-Cerdan, T. (2015). Phenylalanine and urea foliar applications to grapevine: effect on wine phenolic content. *Food Chemistry, 180*, 55-63. doi:10.1016/j.foodchem.2015.02.008

Porro, D., Stefanini, M., Dorigatti, C., Ziller, L., Camin, F., & Policarpo, M. (2006). Nitrogen partitioning in different aerial organs of grapevine in relation to timing and foliar application [Vitis vinifera L.; Trentino]. *Italian Horticultural Society, 13*(3), 74-79.

Porro, D., Stefanini, M., & Dorigatti, C. (2010). *Nitrogen Foliar Uptake and Partitioning in "Cabernet Sauvignon" Gravevines.* Paper presented at the Proc. VIth IS on Mineral Nutrition of Fruit Crops, Faro, Portugal. doi:10.17660/ActaHortic.2010.868.21

Rapp, A., & Mandery, H. (1986). Wine aroma. *Experientia, 42*(8), 873-884. doi:10.1007/bf01941764

Rapp, A., & Versini, G. (1995). Influence of nitrogen compounds in grapes on aroma compounds of wines. In C. George (Ed.), *Developments in Food Science* (Vol. 37, pp. 1659-1694): Elsevier. doi:10.1016/S0167-4501(06)80257-8

Reddy, M. M., & Ulaganathan, K. (2015). Nitrogen nutrition, its regulation and biotechnological approaches to improve crop productivity. *American Journal of Plant Sciences, 6*, 2745-2798. doi:10.4236/ajps.2015.618275

Reeve, A. L., Skinkis, P. A., Vance, A. J., Lee, J., & Tarara, J. M. (2016). Vineyard floor management influences 'Pinot noir' vine growth and productivity more than cluster thinning. *HortScience, 51*(10), 1233-1244. doi:10.21273/hortsci10998-16

Reeve, A. L., Skinkis, P. A., Vance, A. J., McLaughlin, K. R., Tomasino, E., Lee, J., & Tarara, J. M. (2018). Vineyard floor management and cluster thinning inconsistently affect 'Pinot noir' crop load, berry composition and wine quality. *HortScience, 53*(3), 318-328. doi:10.21273/hortsci12682-17

Reining, E., Merbach, W., & Knof, G. (1995). ^{15}N Distribution in wheat and chemical fractionation of root-borne ^{15}N in the soil. *Isotopes in Environmental and Health Studies, 31*(3-4), 345-349. doi:10.1080/10256019508036280

Reynard, J. S., Zufferey, V., Nicol, G. C., & Murisier, F. (2011). Soil parameters impact the vine-fruit-wine continuum by altering vine nitrogen status. *Journal International des Sciences de la Vigne et du Vin, 45*(4), 211-221. doi:10.20870/oeno-one.2011.45.4.1502

Reynard, J. S., Zufferey, V., Nicol, G. C., Pythoud, K., Aleid-Germanier, L., & Murisier, F. (2012). Adaptation du Gamaret aux terroirs viticoles vaudois. *Revue suisse de Viticulture, Arboriculture et Horticulture, 44*(5), 290-296.

Reynolds, A. G. (2010). Viticultural and vineyard management practices and their effects on grape and wine quality. In A. G. Reynolds (Ed.), *Managing Wine Quality* (pp. 365-444): Woodhead Publishing. doi:10.1533/9781845699284.3.365

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., & Lonvaud, A. (2017). *Traité d'œnologie* (7^{e} edition ed. Vol. tome 1). Paris: Dunod.

Robinson, A. L., Boss, P. K., Solomon, P. S., Trengove, R. D., Heymann, H., & Ebeler, S. E. (2014). Origins of grape and wine aroma. Part 1. Chemical components and viticultural impacts. *American Journal of Enology and Viticulture, 65*(1), 1-24. doi:10.5344/ajev.2013.12070

Rodriguez-Lovelle, B., & Gaudillère, J. P. (2002). Carbon and nitrogen partitioning in either fruiting or non-fruiting grapevines: effects of nitrogen limitation before and after veraison. *Australian Journal of Grape and Wine Research, 8*(86), 86-94. doi:10.1111/j.1755-0238.2002.tb00216.x

Rollero, S., Mouret, J.-R., Sanchez, I., Camarasa, C., Ortiz-Julien, A., Sablayrolles, J.-M., & Dequin, S. (2016). Key role of lipid management in nitrogen and aroma metabolism in an evolved wine yeast strain. *Microbial Cell Factories, 15*(1), 32. doi:10.1186/s12934-016-0434-6

Rossouw, G. C., Smith, J. P., Barril, C., Deloire, A., & Holzapfel, B. P. (2017). Implications of the presence of maturing fruit on carbohydrate and nitrogen distribution in grapevines under postveraison water constraints. *Journal of the American Society for Horticultural Science, 142*(2), 71-84. doi:10.21273/JASHS03982-16

Roubelakis-Angelakis, K. A., & Kliewer, W. M. (1992). Nitrogen metabolims in grapevine. *In Horticultural reviews* (Vol. 14, pp. 407-452): John Wiley & Sons, Inc. doi:10.1002/9780470650523.ch9

Rühl, E. H., & Clingeleffer, P. R. (1993). Effect of minimal pruning and virus inoculation on the carbohydrate and nitrogen accumulation in Cabernet franc vines. *American Journal of Enology and Viticulture, 44*(1), 81-85.

Rühl, E., Fuda, A., & Treeby, M. (1992). Effect of potassium, magnesium and nitrogen supply on grape juice composition of Riesling, Chardonnay and Cabernet-Sauvignon [Victoria] vines. *Australian Journal of Experimental Agriculture, 32*(645-649). doi:10.1071/EA9920645

Rutan, T. E., Herbst-Johnstone, M., & Kilmartin, P. A. (2018). Effect of cluster thinning *Vitis vinifera* cv. Pinot noir on wine volatile and phenolic composition. *Journal of Agricultural and Food Chemistry, 66*(38), 10053-10066. doi:10.1021/acs.jafc.8b04062

Ryona, I., Pan, B. S., Intrigliolo, D. S., Lakso, A. N., & Sacks, G. L. (2008). Effects of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (*Vitis vinifera* L. Cv. Cabernet Franc). *Journal of Agricultural and Food Chemistry, 56*(22), 10838-10846. doi:10.1021/jf801877y

Sakakibara, H., Takei, K., & Hirose, N. (2006). Interactions between nitrogen and cytokinin in the regulation of metabolism and development. *Trends in Plant Science, 11*(9), 440-448. doi:10.1016/j.tplants.2006.07.004

Santamaría, P., Lopez, R., Portu, J., Gonzalez-Arenzana, L., Lopez-Alfaro, I., & Garde-Cerdan, T. (2015). Role of phenylalanine in viticulture and enology. *In:* Elliamson D (ed) *Phenylalanine. Dietary, sources, functions and health effects.* Nova Publisher, New York, pp 49–70

Santesteban, L., Miranda, C., Barbarin, I., & Royo, J. (2014). Application of the measurement of the natural abundance of stable isotopes in viticulture: a review. *Australian Journal of Grape and Wine Research, 21*(2), 157-167. doi:10.1111/ajgw.12124

Santos, T. P., Lopes, C. M., Rodrigues, M. L., De Souza, C. R., Ricardo-da-Silva, J. M., Maroco, J. P., Pereira, J.S., & Chaves, M. M. (2005). Effects of partial root-zone drying irrigation on cluster microclimate and fruit composition of field-grown Castelão grapevines. *Vitis, 44*(3), 117-125.

Schneider, V. (2014). Atypical aging defect: sensory discrimination, viticultural causes, and enological consequences. A review. *American Journal of Enology and Viticulture, 65*(3), 277-284. doi:10.5344/ajev.2014.14014

Schreiner, R. P. (2003). Mycorrhizal colonization of grapevine rootstocks under field conditions. *American Journal of Enology and Viticulture, 54*(3), 143-149.

Schreiner, R. P., Scagel, C. F., & Lee, J. (2014). N, P, and K supply to Pinot noir grapevines: impact on berry phenolics and free amino acids. *American Journal of Enology and Viticulture, 65*(1), 43-49. doi:10.5344/ajev.2013.13037

Schreiner, R. P. (2016). Nutrient uptake and distribution in young Pinot noir grapevines over two seasons. *American Journal of Enology and Viticulture, 67*(4), 436-448. doi:10.5344/ajev.2016.16019

Schreiner, R. P., Osborne, J., & Skinkis, P. A. (2018). Nitrogen requirements of Pinot noir based on growth parameters, must composition, and fermentation behavior. *American Journal of Enology and Viticulture, 69*(1), 45-58. doi:10.5344/ajev.2017.17043

Seguinot, P., Rollero, S., Sanchez, I., Sablayrolles, J.-M., Ortiz-Julien, A., Camarasa, C., & Mouret, J.-R. (2018). Impact of the timing and the nature of nitrogen additions on the production kinetics of fermentative aromas by *Saccharomyces cerevisiae* during winemaking fermentation in synthetic media. *Food Microbiology, 76*, 29-39. doi:10.1016/j.fm.2018.04.005

Serra-Stepke, I. M. (2010). *Effect of soil parameters and canopy structure on root growth and distribution.* (Master degree), Stellenbosch University, Stellenbosch, 71pp.

Sinaj S. & Richner W. (2017). Principes de fertilisation des cultures agricoles en Suisse (PRIF 2017). *Recherche Agronomique Suisse, 8*, publication spéciale, 276 pp.

Sivilotti, P., Falchi, R., Vanderweide, J., Sabbatini, P., Bubola, M., Vanzo, A., Lisjak, K., Peterlunger, E., & Herrera, J. C. (2020). Yield reduction through cluster or selective berry thinning similarly modulates anthocyanins and proanthocyanidins composition in Refosco dal peduncolo rosso (Vitis vinifera L.) grapes. *Scientia Horticulturae, 264*, 109166. doi:10.1016/j.scienta.2019.109166

Soubeyrand, E., Basteau, C., Hilbert, G., van Leeuwen, C., Delrot, S., & Gomes, E. (2014). Nitrogen supply affects anthocyanin biosynthetic and regulatory genes in grapevine cv. Cabernet-Sauvignon berries. *Phytochemistry, 103*, 38-49. doi:10.1016/j.phytochem.2014.03.024

Soubeyrand, E., Colombié, S., Beauvoit, B., Dai, Z., Cluzet, S., Hilbert, G., Christel R., Christel R., Maneta-Peyret Lilly, Dieuaide-Noubhani M., Mérillon J.-M., Gibon, Y., Delrot S. & Gomès, E. (2018). Constraint-based modeling highlights cell energy, redox status and α-ketoglutarate availability as metabolic drivers for anthocyanin accumulation in grape cells under nitrogen limitation. *Frontiers in Plant Science, 9*(421). doi:10.3389/fpls.2018.00421

Spangenberg, J. E., & Zufferey, V. (2018). Changes in soil water availability in vineyards can be traced by the carbon and nitrogen isotope composition of dried wines. *Science of The Total Environment, 635*, 178-187. doi:10.1016/j.scitotenv.2018.04.078

Spring, J.-L. (1999). Indice *chlorophylliendu* feuillage et nutrition azotée du cépage Chasselas. *Revue suisse de Viticulture, Arboriculture et Horticulture, 31*(3), 141-145.

Spring, J.-L., & Zufferey, V. (2000). Intérêt de la détermination de l'indice chlorophyllien du feuillage en viticulture. *Revue suisse de Viticulture, Arboriculture et Horticulture, 32*(6), 323-328.

Spring, J. L. (2001). lnfluence du type d'enherbement sur le comportement de la vigne et la qualité des vins. Résultats d'un essai sur Chasselas dans le bassin lémanique. *Revue suisse de Viticulture, Arboriculture et Horticulture, 33*(5), 253-260.

Spring, J. L. (2002). Influence du type d'enherbement sur le comportement de la vigne et la qualité des vins. Résultats d'un essai sur Chasselas dans le bassin lémanique. 2. Résultats oenologiques. *Revue suisse de Viticulture, Arboriculture et Horticulture, 34*(2), 111-116.

Spring, J. L. (2003). Localisation de la fumure azotée sur l'intercep dans les vignes enherbées. *Revue suisse de Viticulture, Arboriculture et Horticulture, 35*(2), 113-119.

Spring, J. L., Verdenal, T., Zufferey, V., & Viret, O. (2012). Nitrogen dilution in excessive canopies of Chasselas and Pinot noir cvs. *Journal International des Sciences de la Vigne et du Vin, 46*(3), 233-240. doi:10.20870/oeno-one.2012.46.3.1520

Spring, J. L., Zufferey, V., Verdenal, T., & Viret, O. (2016a). Influence du porte-greffe sur le comportement du Pinot noir dans les conditions du Valais central. *Revue suisse de Viticulture, Arboriculture et Horticulture, 48*(2), 112-122.

Spring, J. L., Reynard, J. S., Zufferey, V., Verdenal, T., Duruz, P., & Viret, O. (2016b). Diversité intra-variétale et sélection clonale de la Petite Arvine. *Revue suisse de Viticulture, Arboriculture et Horticulture, 48*(3), 156-163.

Spring, J.-L., & Verdenal, T. (2017). Fertilisation en viticulture. *In:* S. Sinaj & W. Richner (Eds.), *Principes de fertilisation des cultures agricoles en Suisse (PRIF 2017)* (pp. 276).

Spring, J. L., Reynard, J. S., Zufferey, V., Verdenal, T., Duruz, P., Rösti, J., Lorenzini, F., & Favre, G. (2018). Nouveaux clones de Pinot gris sélectionnés à Agroscope. *Revue suisse de Viticulture, Arboriculture et Horticulture, 50*(3), 158-165.

Stamatiadis, S., Christofides, C., Tsdila, E., Taskos, D., & Schepers, J. S. (2007). Relationship of leaf stable isotopes (δ^{13}C and δ^{15}N) to biomass production in two fertilized Merlot vineyards. *American Journal of Enology and Viticulture, 58*(1), 67-74.

Stander, O. P. J., Barry, G. H., & Cronjé, P. J. R. (2017). Fruit-load-induced starch accumulation causes leaf chlorosis in “off” ‘Nadorcott’ mandarin trees. *Scientia Horticulturae, 222*, 62-68. doi:10.1016/j.scienta.2017.05.019

Stines, A. P., Grubb, J., Gockowiak, H., Henschke, P. A., Hoj, P. B., & van Heeswijck, R. (2000). Proline and arginine accumulation in developing berries of *Vitis vinifera* L. in Australian vineyards: Influence of vine cultivar, berry maturity and tissue type. *Australian Journal of Grape and Wine Research, 6*, 150-158. doi:10.1111/j.1755-0238.2000.tb00174.x

Stitt, M., & Krapp, A. (1999). The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant, Cell & Environment, 22*(6), 583-621. doi:10.1046/j.1365-3040.1999.00386.x

Stockert, C. M., Bisson, L. F., Adams, D. O., & Smart, D. R. (2013). Nitrogen status and fermentation dynamics for Merlot on two rootstocks. *American Journal of Enology and Viticulture, 64*(2), 195-202. doi:10.5344/ajev.2013.12065

Styger, G., Prior, B., & Bauer, F. F. (2011). Wine flavor and aroma. *Journal of Industrial Microbiology & Biotechnology, 38*(9), 1145. doi:10.1007/s10295-011-1018-4

Sweetman, C., Deluc, L. G., Cramer, G. R., Ford, C. M., & Soole, K. L. (2009). Regulation of malate metabolism in grape berry and other developing fruits. *Phytochemistry, 70*. doi:10.1016/j.phytochem.2009.08.006

Swiegers, J. H., Bartowsky, E. J., Henschke, P. A., & Pretorius, I. S. (2005). Yeast and bacterial modulation of wine aroma and flavour. *Australian Journal of Grape and Wine Research, 11*(2), 139-173. doi:10.1111/j.1755-0238.2005.tb00285.x

Taskos, D. G., Koundouras, S., Stamatiadis, S., Zioziou, E., Nikolaou, N., Karakioulakis, K., & Theodorou, N. (2015). Using active canopy sensors and chlorophyll meters to estimate grapevine nitrogen status and productivity. *Precision Agriculture, 16*(1), 77-98. doi:10.1007/s11119-014-9363-8

Tegeder, M. (2014). Transporters involved in source to sink partitioning of amino acids and ureides: opportunities for crop improvement. *Journal of Experimental Botany, 65*(7), 1865-1878. doi:10.1093/jxb/eru012

Tesic, D., Keller, M., & Hutton, R. J. (2007). Influence of vineyard floor management practices on grapevine vegetative growth, yield, and fruit composition. *American Journal of Enology and Viticulture, 58*(1), 1-11.

Thomidis, T., Zioziou, E., Koundouras, S., Karagiannidis, C., Navrozidis, I., & Nikolaou, N. (2016). Effects of nitrogen and irrigation on the quality of grapes and the susceptibility to *Botrytis* bunch rot. *Scientia Horticulturae, 212*, 60-68. doi:10.1016/j.scienta.2016.09.036

Tomasi, N., Monte, R., Varanini, Z., Cesco, S., & Pinton, R. (2015). Induction of nitrate uptake in Sauvignon Blanc and Chardonnay grapevines depends on the scion and is affected by the rootstock. *Australian Journal of Grape and Wine Research, 21*(2), 331-338. doi:10.1111/ajgw.12137

Torrea, D., Varela, C., Ugliano, M., Ancin-Azpilicueta, C., Leigh Francis, I., & Henschke, P. A. (2011). Comparison of inorganic and organic nitrogen supplementation of grape juice - Effect on volatile composition and aroma profile of a Chardonnay wine fermented with *Saccharomyces cerevisiae* yeast. *Food Chemistry, 127*(3), 1072-1083. doi:10.1016/j.foodchem.2011.01.092

Taskos, D., Zioziou, E., Nikolaou, N., Doupis, G., & Koundouras, S. (2020). Carbon isotope natural abundance (δ13C) in grapevine organs is modulated by both water and nitrogen supply. *Oeno One, 54*, 1183-1199. doi:10.20870/oeno-one.2020.54.4.3600

Treeby, M. T., & Wheatley, D. M. (2006). Effect of nitrogen fertiliser on nitrogen partitioning and pool sizes in irrigated Sultana grapevines. *Australian Journal of Experimental Agriculture, 46*(9), 1207-1215. doi:10.1071/EA05238

Trouvelot, S., Bonneau, L., Redecker, D., van Tuinen, D., Adrian, M., & Wipf, D. (2015). Arbuscular mycorrhiza symbiosis in viticulture: a review. *Agronomy for Sustainable Development, 35*(4), 1449-1467. doi:10.1007/s13593-015-0329-7

Ugliano, M., Henschke, P. A., Herdrich, M. J., & Pretorius, I. S. (2007). Nitrogen management is critical for wine flavour and style. *Aus. N.Z. Wine Industry J., 22*(6), 24-30.

Ugliano, M., & Henschke, P. A. (2009). Yeasts and Wine Flavour. *In* M. V. Moreno-Arribas & M. C. Polo (Eds.), *Wine Chemistry and Biochemistry* (pp. 313-392). New York, : Springer. doi:10.1007/978-0-387-74118-5_17

Usman, S., Singh, S. P., Rawat, Y. S., & Bargali, S. S. (2000). Fine root decomposition and nitrogen mineralisation patterns in *Quercus leucotrichophora* and *Pinus roxburghii* forests in central Himalaya. *Forest Ecology and Management, 131*(1), 191-199. doi:10.1016/S0378-1127(99)00213-3

van Cleemput, O., Zapata, F., & Vanlauwe, B. (2008). Use of tracer technology in mineral fertilizer management. In *Guidelines on nitrogen management in agricultural systems* (Vol. 29, pp. 19-125). Vienna: International Atomic Energy Agency.

van Leeuwen, C., Friant, P., Soyer, J.-P., Molot, C., Chone, X., & Dubourdieu, D. (2000). L'intérêt du dosage de l'azote total et de l'azote assimilable dans le moût comme indicateur de la nutrition azotée de la vigne. *Journal International des Sciences de la Vigne et du Vin, 34*(2), 75-82.

van Leeuwen, C., Friant, P., Choné, X., Tregoat, O., Koundouras, S., & Dubourdieu, D. (2004). Influence of Climate, Soil and Cultivar on Terroir. *American Journal of Enology and Viticulture, 55*(3), 207-217.

van Leeuwen, C., & Seguin, G. (2006). The concept of terroir in viticulture. *Journal of Wine Research, 17*(1), 1-10. doi:10.1080/09571260600633135

Van Leeuwen, C., Tregoat, O., Choné, X., Bois, B., Pernet, D., & Gaudillere, J. P. (2009). Vine water status is a key factor in grape ripening and vintage quality for red bordeaux wine. How can it be assessed for vineyard management purposes ? *Journal International des Sciences de la Vigne et du Vin*, *43*, 121-134.

van Leeuwen, C., Roby, J.-P., & de Rességuier, L. (2018). Soil-related terroir factors: a review. *Oeno One, 52*(2), 173-188. doi:10.20870/oeno-one.2018.52.2.2208

Verdenal, T., Spangenberg, J. E., Zufferey, V., Lorenzini, F., Spring, J. L., & Viret, O. (2015). Effect of fertilisation timing on the partitioning of foliar-applied nitrogen in *Vitis vinifera* cv. Chasselas: a ^{15}N labelling approach. *Australian Journal of Grape and Wine Research, 21*(1), 110-117. doi:10.1111/ajgw.12116

Verdenal, T., Spangenberg, J. E., Zufferey, V., Lorenzini, F., Dienes-Nagy, A., Gindro, K., Spring J.-L., Viret, O. (2016a). Leaf-to-fruit ratio affects the impact of foliar-applied nitrogen on N accumulation in the grape must. *Journal International des Sciences de la Vigne et du Vin, 50*(1), 23-33. doi:10.20870/oeno-one.2016.50.1.55

Verdenal, T., Zufferey, V., Spring, J. L., Rösti, J., Dienes-Nagy, A., Lorenzini, F., Wolfender, J.-L., Spangenberg, J., Burgo, S., Gindro, K, & Viret, O. (2016b). Complémentation foliaire azotée du cépage Vitis vinifera Doral dans le canton de Vaud. *Revue suisse de Viticulture, Arboriculture et Horticulture, 48*(4), 238-245.

Verdenal, T., Zufferey, V., Spring, J. L., Dienes-Nagy, A., Belcher, S., & Lorenzini, F. (2017). Impact de la zone d'application de l'urée foliaire sur la teneur en azote des raisins. *Revue suisse de Viticulture, Arboriculture et Horticulture, 49*(6), 354-359.

Verdenal, T., Zufferey, V., Dienes-Nagy, A., Bourdin, G., Gindro, K., Viret, O., & Spring, J.-L. (2019). Timing and intensity of grapevine defoliation: an extensive overview on five cultivars in Switzerland. *American Journal of Enology and Viticulture, 70*(4), 427-434. doi:10.5344/ajev.2019.19002

Verdenal, T., Spangenberg, J. E., Zufferey, V., Dienes-Nagy, Á., Viret, O., van Leeuwen, C., & Spring, J.-L. (2020). Impact of crop load on nitrogen uptake and reserve mobilisation in *Vitis vinifera*. *Functional Plant Biology, 47*, 744-756. doi:10.1071/FP20010

Verdenal, T., Dienes-Nagy, Á., Spangenberg, J. E., Zufferey, V., Spring, J.-L., Viret, O., Marin-Carbonne, J., & van Leeuwen, C. (2021). Understanding and managing nitrogen nutrition in grapevine: a review. *Oeno One, 55*, 1-44. doi:10.20870/oeno-one.2021.55.1.3866

Vilanova, M., Ugliano, M., Varela, C., Siebert, T., Pretorius, I. S., & Henschke, P. A. (2007). Assimilable nitrogen utilisation and production of volatile and non-volatile compounds in chemically defined medium by *Saccharomyces cerevisiae* wine yeasts. *Applied Microbiology and Biotechnology, 77*(1), 145-157. doi:10.1007/s00253-007-1145-z

Vincenzini, M., Guerrini, S., Mangani, S., & Granchi, L. (2017). Amino acid metabolisms and production of biogenic amines and ethyl carbamate. *In* H. König, G. Unden, & J. Fröhlich (Eds.), *Biology of Microorganisms on Grapes, in Must and in Wine* (pp. 231-253). Cham: Springer International Publishing. doi:10.1007/978-3-319-60021-5_10

Vrignon-Brenas, S., Metay, A., Leporatti, R., Gharibi, S., Fraga, A., Dauzat, M., Roland G., & Pellegrino, A. (2019). Gradual responses of grapevine yield components and carbon status to nitrogen supply. *Oeno One, 53*(2). doi:10.20870/oeno-one.2019.53.2.2431

Wang, Y., Janz, B., Engedal, T., & Neergaard, A. d. (2017). Effect of irrigation regimes and nitrogen rates on water use efficiency and nitrogen uptake in maize. *Agricultural Water Management, 179*, 271-276. doi:10.1016/j.agwat.2016.06.007

Wang, Y., He, Y.-N., Chen, W.-K., He, F., Chen, W., Cai, X.-D., & C.-Q., Duan (2018). Effects of cluster thinning on vine photosynthesis, berry ripeness and flavonoid composition of Cabernet-Sauvignon. *Food Chemistry, 248*, 101-110. doi:10.1016/j.foodchem.2017.12.021

Wang, Y., He, Y.-N., He, L., He, F., Chen, W., Duan, C.-Q., & Wang, J. (2019). Changes in global aroma profiles of Cabernet-Sauvignon in response to cluster thinning. *Food Research International, 122*, 56-65. doi:10.1016/j.foodres.2019.03.061

Waterhouse, A. L., Sacks, G. L., & Jeffery, D. W. (2016). *Understanding wine chemistry*: Wiley-Blackwell. doi:10.1002/9781118730720

Wermelinger, B. (1991). *Nitrogen Dynamics in Grapevine: Physiology and Modeling.* Paper presented at the International Symposium on Nitrogen in Grapes and Wine, Seattle WA, USA.

Weyand, K. M., & Schultz, H. R. (2006). Long-term dynamics of nitrogen and carbohydrate reserves in woody parts of minimally and severely pruned Riesling vines in a cool climate. *American Journal of Enology and Viticulture, 57*(2), 172-182.

White, R., Balachandra, L., Edis, R., & Chen, D. (2007). The soil component of terroir. *Journal International des Sciences de la Vigne et du Vin, 41*(1), 9-18. doi:1010.20870/oeno-one.2007.41.1.860

Williams, L. E. (2015). Recovery of ^{15}N Labeled Fertilizer by Thompson Seedless Grapevines: Effects of N Fertilizer Type and Irrigation Method. *American Journal of Enology and Viticulture, 66*, 509-517. doi:10.5344/ajev.2015.14115

Xia, G., & Cheng, L. (2004). Foliar urea application in the fall affects both nitrogen and carbon storage in young "Concord" grapevines grown under a wide range of nitrogen supply. *Journal of the American Society for Horticultural Science*, *129*, 653-659.

Xu, G., Fan, X., & Miller, A. J. (2012). Plant nitrogen assimilation and use efficiency. *Annual Review of Plant Biology, 63*(1), 153-182. doi:10.1146/annurev-arplant-042811-105532

Zamboni, M., Garavani, A., Gatti, M., Vercesi, A., Parisi, M. G., Bavaresco, L., & Poni, S. (2016). Vegetative, physiological and nutritional behavior of new grapevine rootstocks in response to different nitrogen supply. *Scientia Horticulturae, 202*, 99-106. doi:10.1016/j.scienta.2016.02.032

Zapata, C., Deleens, E., Chaillou, S., & Magne, C. (2004a). Partitioning and mobilization of starch and N reserves in grapevine (*Vitis vinifera* L.). *J Plant Physiol, 161*(9), 1031-1040. doi:10.1016/j.jplph.2003.11.009

Zapata, C., Deléens, E., Chaillou, S., & Magné, C. (2004b). Mobilisation and distribution of starch and total N in two grapevine cultivars differing in their susceptibility to shedding. *Functional Plant Biology, 31*, 1127-1135. doi:10.1071/FP04028

Zufferey, V., & Maigre, D. (2007). Âge de la vigne. I. Influence sur le comportement physiologique des souches. *Revue suisse de Viticulture, Arboriculture et Horticulture, 39*(4), 257-261.

Zufferey, V., & Murisier, F. (2007). Assessment of plant hydraulics in grapevine on various "terroirs" in the canton de Vaud (Switzerland). *Journal International des Sciences de la Vigne et du Vin, 41*(2), 95-102. doi:10.20870/oeno-one.2007.41.2.854

Zufferey, V., & Maigre, D. (2008). Âge de la vigne II. Influence sur la qualité des raisins et des vins. *Revue suisse de Viticulture, Arboriculture et Horticulture, 40*(4), 241-245.

Zufferey, V., Murisier, F., Belcher, S., Lorenzini, F., Vivin, P., Spring, J. L., & Viret, O. (2015). Nitrogen and carbohydrate reserves in the grapevine (*Vitis vinifera* L. 'Chasselas'): the influence of the leaf to fruit ratio. *Vitis, 54*, 183-188. doi:10.5073/vitis.2015.54.183-188

Zufferey, V., Spring, J.-L., Verdenal, T., Dienes, A., Belcher, S., Lorenzini, F., Koestel F., Rösti, H., Gindro, K., Spangenberg, J., & Viret, O. (2017). The influence of water stress on plant hydraulics, gas exchange, berry composition and quality of Pinot noir wines in Switzerland. *Oeno One, 51*(1), 17-27. doi:10.20870/oeno-one.2017.51.1.1314

Zufferey, V., Verdenal, T., Dienes, A., Belcher, S., Lorenzini, F., Koestel, C., Gindro, K., Spangenberg, J.E., Viret, O. & Spring, J. L. (2018). The impact of plant water status on the gas exchange, berry composition and wine quality of Chasselas grapes in Switzerland. Oeno One, 52(4), 333-347. doi:10.20870/oeno-one.2018.52.4.2181

Appendixes

Appendix I – Raw data, vines excavated in 2017: Field measurements, dry weight, and carbon and nitrogen composition

Appendix II – Raw data, vines excavated in 2018: Field measurements, dry weight, and carbon and nitrogen composition

Appendix III – Raw data: Amino acids in musts in 2017

Appendix IV – Raw data: Proportions of amino acids in musts in 2017

Appendix V – Raw data: Must amino acids in musts in 2018

Appendix VI – Raw data: Proportions of amino acids in musts in 2018

Appendix I - Raw data
Vines excavated in 2017

Excavation in 2017						Field measurements 2017								Must analyses 2017									1 Roots													
Blocks 1-5						bunches per shoot	#	g	m^2 m^{-2} of soil	m^2 m^{-2} of soil	kg per vine	g	$kg\ m^{-2}$	Brix		$g\ L^{-1}$	$g\ L^{-1}$	$g\ L^{-1}$	$mg\ L^{-1}$	$mg\ L^{-1}$	$mg\ L^{-1}$	$mg\ L^{-1}$	g	%	wt.% DW	mUr	g	wt.% DW	mUr	g		%		g	%
block	row	rep	Excavation time	Fertilization treatment	Yield condition	Bud fruitfulness	Bunches per vines	Canopy weight	Total leaf area	Light-exposed leaf area	Crop load	Bunch weight	Yield	Total soluble sugars	pH	Titratable acidity	Tartaric acid	Malic acid	K	NH_3	N-alpha aminé	YAN	1 DW	1 % DW	1 TOC	1 $\delta^{13}C$	1 CQ	1 TN	1 $\delta^{15}N$	1 NQ	1 R	1 A%	1 RSA	1 NNP	1 %P
1	A	1	Bud burst	C	no group																		168	65	47.4	-29.1	79.5	0.71	7	1.19					
1	A	2	Bud burst	C	no group																		202	68	49.8	-28.6	100.5	0.65	4	1.32					
1	A	3	Bud burst	C	no group																		216	68	47.7	-28.6	103.2	0.77	5	1.66					
1	A	4	Bud burst	C	no group																		240	68	47.9	-28.5	115.1	0.72	2	1.73					
1	B	1	Bud burst	C	no group																		139	67	48.3	-28.3	67.0	0.73	2	1.01					
1	B	2	Bud burst	C	no group																		172	69	47.5	-27.8	81.5	0.75	3	1.29					
1	B	3	Bud burst	C	no group																		135	70	47.9	-28.4	64.5	0.64	3	0.86					
1	B	4	Bud burst	C	no group																		146	70	47.4	-28.4	69.3	0.73	2	1.07					
1	C	1	Bud burst	C	no group																		121	72	46.7	-28.8	56.4	0.69	2	0.83					
1	C	2	Bud burst	C	no group																		141	70	47.5	-27.7	66.9	0.80	1	1.13					
1	C	3	Bud burst	C	no group																		168	70	49.7	-28.2	83.3	0.68	1	1.14					
1	C	4	Bud burst	C	no group																		162	68	48.6	-27.9	79.0	0.91	2	1.48					
2	A	1	Flowering	C	no group																		233	58	48.1	-28.6	112.2	0.68	3	1.59					
2	A	2	Flowering	C	no group																		195	58	48.6	-28.2	94.8	0.52	4	1.02					
2	A	3	Flowering	C	no group																		161	59	49.4	-28.9	79.5	0.55	3	0.89					
2	A	4	Flowering	C	no group																		139	59	48.6	-29.0	67.3	0.53	5	0.74					
2	B	1	Flowering	C	no group																		121	63	48.4	-28.7	58.6	0.52	2	0.63					
2	B	2	Flowering	C	no group																		148	62	47.7	-27.9	70.5	0.53	2	0.78					
2	B	3	Flowering	C	no group																		121	59	46.9	-28.9	56.6	0.76	2	0.92					
2	B	4	Flowering	C	no group																		141	63	47.5	-28.4	67.2	0.63	3	0.89					
2	C	1	Flowering	C	no group																		113	62	47.7	-28.7	54.0	0.63	2	0.71					
2	C	2	Flowering	C	no group																		121	61	47.4	-28.6	57.2	0.55	2	0.67					
2	C	3	Flowering	C	no group																		151	55	51.1	-28.9	77.0	0.58	2	0.87					
2	C	4	Flowering	C	no group																		181	62	47.0	-28.3	84.9	0.45	2	0.82					
3	A	1	Veraison	C	LYC	1.9	2	1302			0.6	288	0.5	11.7	2.8	20.1	8.0	13.7	1339	78	57	122	254	67	47.7	-28.1	121.4	0.47	9	1.20					
3	A	2	Veraison	C	LYC	2.0	2	1974			0.6	304	0.5	11.8	2.8	20.1	7.9	14.0	1474	122	103	203	216	65	47.4	-28.3	102.2	0.56	11	1.20					
3	A	3	Veraison	C	LYC	1.9	2	679			0.3	152	0.3	9.2	2.7	27.8	9.4	19.7	1580	79	55	120	270	72	45.2	-27.8	122.0	0.47	17	1.26					
3	A	4	Veraison	C	LYC	2.0	3	1018			0.5	166	0.4	11.1	2.8	23.0	8.5	16.3	1352	169	106	245	233	64	47.6	-28.3	111.0	0.50	15	1.16					
3	B	1	Veraison	C	HYC	1.7	5	987			0.8	169	0.7	11.2	2.8	20.9	8.3	14.2	1317	102	81	165	238	61	48.8	-28.0	116.0	0.52	4	1.23					
3	B	2	Veraison	C	HYC	2.7	5	830			1.0	190	0.8	10.7	2.7	22.1	8.6	14.8	1288	52	41	83	214	67	46.8	-27.5	100.2	0.41	5	0.88					
3	B	3	Veraison	C	LYC	2.0	6	670			0.7	123	0.6	10.6	2.8	23.7	8.8	16.8	1518	180	89	238	142	67	46.9	-28.7	66.7	0.48	5	0.69					
3	B	4	Veraison	C	HYC	2.0	5	1589			1.5	305	1.3	11.5	2.8	19.8	7.8	13.9	1342	152	123	248	190	57	47.3	-28.3	89.8	0.51	5	0.97					
3	C	1	Veraison	C	HYC	2.5	9	1617			2.1	238	1.8	10.0	2.8	22.0	8.0	15.8	1443	143	76	194	244	59	47.2	-27.9	115.1	0.62	3	1.52					
3	C	2	Veraison	C	HYC	1.9	9	756			1.0	108	0.8	11.3	2.7	22.3	9.1	14.9	1498	62	42	94	165	68	48.3	-28.4	79.9	0.42	2	0.70					
3	C	4	Veraison	C	HYC	2.3	9	832			0.8	94	0.7	11.2	2.7	22.8	9.2	15.1	1270	54	32	76	278	68	45.9	-27.1	127.4	0.41	2	1.13					
4	A	1	Harvest	C	LYC	2.0	2	1443	2.5	1.3	0.6	312	0.5	19.6	3.5	6.2	5.4	3.4	1876	50	202	243	281	60	49.2	-28.6	138.3	0.71	14	1.99					
4	A	2	Harvest	C	LYC	2.1	2	1033	1.4	1.3	0.8	382	0.6	19.5	3.4	6.3	5.7	2.9	1638	46	142	180	280	56	47.5	-28.5	132.8	0.82	9	2.28					
4	A	4	Harvest	C	LYC	2.6	2	1544	2.2	1.3	1.1	547	0.9	18.8	3.5	6.2	5.7	3.4	1915	59	179	228	220	60	49.2	-28.9	108.2	0.90	17	1.98					
4	B	1	Harvest	C	LYC	2.7	6	786	1.6	1.2	1.3	211	1.1	17.1	3.2	6.9	5.8	3.2	1547	67	67	122	260	61	48.1	-28.2	125.2	0.67	8	1.73					
4	B	2	Harvest	C	LYC	2.2	4	669	1.4	1.2	0.9	219	0.7	19.7	3.4	5.8	5.4	3.0	1739	32	142	168	259	62	47.6	-28.6	123.1	0.74	5	1.92					
4	B	3	Harvest	C	HYC	2.0	3	1082	1.3	1.2	1.6	539	1.3	17.5	3.3	6.5	5.7	3.2	1729	43	107	143	313	61	48.7	-28.2	152.3	0.59	6	1.85					
4	B	4	Harvest	C	HYC	2.0	5	1763	2.6	1.2	2.4	487	2.0	19.6	3.5	5.6	5.5	2.8	1793	38	154	185	254	59	49.6	-28.1	125.9	0.79	8	2.01					
4	C	1	Harvest	C	HYC	2.3	9	1058	1.7	1.3	2.8	308	2.3	19.0	3.3	6.4	5.6	3.1	1537	70	162	220	175	60	51.2	-28.1	89.6	0.70	2	1.23					
4	C	2	Harvest	C	HYC	1.7	6	1292	1.9	1.3	2.0	328	1.6	19.4	3.3	6.3	5.4	3.0	1624	17	117	131	209	59	49.5	-28.2	103.5	0.64	4	1.33					
4	C	3	Harvest	C	HYC	2.3	9	1078	1.8	1.3	3.0	328	2.5	18.1	3.2	7.2	5.8	3.9	1588	45	142	179	146	57	48.8	-28.9	71.5	0.63	5	0.93					
4	C	4	Harvest	C	HYC	3.1	9	777	1.5	1.3	2.1	229	1.7	19.2	3.2	6.4	5.6	3.0	1508	15	102	114	200	56	49.0	-28.6	97.8	0.66	14	1.33					
5	A	2	Harvest	F17	LYC	2.0	3	868	1.5	1.2	0.7	243	0.6	19.8	3.4	6.3	5.6	3.2	1785	35	168	197	211	60	48.6	-28.1	102.5	0.56	2494	1.18	0.0128	1.27	0.09	0.11	18
5	A	3	Harvest	F17	LYC	1.7	2	1066	1.9	1.2	0.9	474	0.8	19.8	3.4	5.9	5.9	2.6	1757	32	147	174	359	61	47.7	-28.5	171.2	0.53	2063	1.89	0.0113	1.11	0.08	0.15	22
5	A	4	Harvest	F17	LYC	2.0	2	1770	2.6	1.2	0.8	386	0.6	19.5	3.5	6.0	5.6	3.4	1964	50	192	233	290	61	47.8	-28.6	138.3	0.59	2139	1.70	0.0115	1.14	0.08	0.14	17
5	B	1	Harvest	F17	HYC	1.7	5	1441	2.2	1.3	2.0	400	1.7	19.5	3.4	6.5	5.8	3.5	1761	54	174	219	244	62	46.9	-28.5	114.6	0.67	1528	1.64	0.0093	0.92	0.06	0.09	10
5	B	2	Harvest	F17	HYC	2.0	6	1450	2.3	1.3	2.4	408	2.0	19.3	3.4	6.3	5.7	3.4	1753	55	168	213	233	59	47.6	-28.5	110.7	0.70	1146	1.63	0.0079	0.78	0.04	0.07	7
5	B	3	Harvest	F17	HYC	2.0	6	1201	2.4	1.3	2.5	418	2.1	18.9	3.4	6.1	5.4	3.2	1601	64	179	232	216	58	48.0	-28.4	103.6	0.68	973	1.48	0.0073	0.72	0.04	0.05	7
5	B	4	Harvest	F17	LYC	2.0	4	1398	2.2	1.3	1.5	380	1.3	19.7	3.5	6.2	5.9	3.3	1841	68	205	261	249	60	46.5	-28.2	115.7	0.66	1555	1.63	0.0094	0.93	0.06	0.09	11
5	C	2	Harvest	F17	HYC	2.6	9	1166	2.1	1.1	2.2	244	1.8	20.2	3.3	6.1	5.7	2.9	1585	46	172	209	211	61	48.3	-27.5	102.0	0.67	1638	1.41	0.0097	0.96	0.06	0.09	8
5	C	3	Harvest	F17	LYC	1.8	6	764	1.6	1.1	1.5	251	1.3	20.4	3.4	5.9	5.7	2.7	1668	29	145	169	220	60	48.3	-27.6	106.4	0.67	1607	1.47	0.0096	0.95	0.06	0.09	12
5	C	4	Harvest	F17	LYC	2.0	9	401	0.9	1.1	1.1	119	0.9	19.9	3.3	5.5	5.4	2.2	1370	46	114	152	199	60	48.8	-27.3	97.1	0.56	1655	1.12	0.0098	0.97	0.06	0.07	16

Appendix I - Raw data
Vines excavated in 2017

Excavation in 2017						2 Trunk																		3 Canopy																		4 Pomace																	
Blocks 1-5						g	%	wt. % DW	mUr	g	wt. % DW	mUr	g		%		g		%					g	%	wt. % DW	mUr	g	wt. % DW	mUr	g		%					g	%	wt. % DW	mUr	g	wt. % DW	mUr	g		%					g	%
block	row	rep	Excavation time	Fertilization treatment	Yield condition	2 DW	2 % DW	2 TOC	2 δ^{13}C	2 CQ	2 TN	2 δ^{15}N	2 NQ	2 R	2 A%	2 RSA	2 NNP	2 %P	3 DW	3 % DW	3 TOC	3 δ^{13}C	3 CQ	3 TN	3 δ^{15}N	3 NQ	3 R	3 A%	3 RSA	3 NNP	3 %P	4 DW	4 % DW	4 TOC	4 δ^{13}C	4 CQ	4 TN	4 δ^{15}N	4 NQ	4 R	4 A%	4 RSA	4 NNP	4 %P
1	A	1	Bud burst	C	no group	227	59	49.4	-28.3	112.2	0.48	6	1.09																																														
1	A	2	Bud burst	C	no group	204	60	47.9	-28.8	97.7	0.39	4	0.80																																														
1	A	3	Bud burst	C	no group	237	59	49.5	-28.1	117.3	0.41	4	0.98																																													
1	A	4	Bud burst	C	no group	303	59	48.3	-28.2	146.6	0.43	2	1.31																																													
1	B	1	Bud burst	C	no group	222	60	47.9	-28.3	106.1	0.51	1	1.14																																													
1	B	2	Bud burst	C	no group	254	59	48.0	-27.9	122.0	0.50	1	1.27																																													
1	B	3	Bud burst	C	no group	184	58	48.9	-27.7	89.8	0.47	1	0.85																																													
1	B	4	Bud burst	C	no group	189	58	46.8	-28.3	88.4	0.48	1	0.90																																													
1	C	1	Bud burst	C	no group	148	60	49.1	-28.3	72.7	0.48	1	0.71																																													
1	C	2	Bud burst	C	no group	219	59	48.4	-27.1	106.0	0.53	1	1.17																																													
1	C	3	Bud burst	C	no group	197	60	49.1	-27.9	96.6	0.44	1	0.86																																													
1	C	4	Bud burst	C	no group	174	60	48.2	-27.5	83.9	0.54	1	0.94																																													
2	A	1	Flowering	C	no group	296	58	47.4	-28.4	140.5	0.39	3	1.14						447	28	45.2	-29.4	201.8	2.32	4	10.35																																										
2	A	2	Flowering	C	no group	259	57	47.5	-28.2	123.1	0.39	3	1.00						301	24	45.1	-28.6	135.7	2.35	4	7.07																																										
2	A	3	Flowering	C	no group	242	55	47.0	-28.6	113.9	0.40	3	0.97						315	27	45.0	-29.3	141.9	2.19	4	6.90																																										
2	A	4	Flowering	C	no group	188	54	47.6	-28.9	89.2	0.35	4	0.65						233	29	45.7	-29.3	106.6	2.29	4	5.34																																										
2	B	1	Flowering	C	no group	186	58	48.5	-28.2	90.3	0.36	3	0.67						278	35	45.8	-29.5	127.2	1.67	3	4.63																																										
2	B	2	Flowering	C	no group	213	58	46.4	-27.9	98.6	0.32	2	0.68						272	33	45.4	-29.3	123.4	1.79	3	4.87																																										
2	B	3	Flowering	C	no group	224	55	47.6	-28.0	106.6	0.43	2	0.97						278	32	46.4	-29.1	128.8	2.32	3	6.45																																										
2	B	4	Flowering	C	no group	263	55	47.7	-28.1	125.4	0.36	3	0.96						242	30	46.2	-28.8	111.7	2.02	4	4.88																																										
2	C	1	Flowering	C	no group	179	58	47.1	-28.1	84.3	0.36	2	0.64						214	27	45.3	-29.5	96.6	2.02	3	4.32																																										
2	C	2	Flowering	C	no group	258	55	45.6	-28.5	117.3	0.33	2	0.85						207	31	45.3	-29.3	93.9	1.81	3	3.75																																										
2	C	3	Flowering	C	no group	193	55	47.5	-28.7	91.6	0.37	2	0.72						135	25	44.8	-29.5	60.6	2.41	3	3.27																																										
2	C	4	Flowering	C	no group	225	58	48.1	-28.0	108.3	0.31	2	0.70						167	30	45.7	-29.1	76.5	1.82	2	3.05																																										
3	A	1	Veraison	C	LYC	281	55	48.0	-28.1	135.1	0.33	6	0.94						356	27	44.9	-29.7	159.7	1.24	12	4.42																																										
3	A	2	Veraison	C	LYC	255	51	42.7	-28.1	108.6	0.38	9	0.96						488	25	45.3	-28.2	220.9	1.89	14	9.20																																										
3	A	3	Veraison	C	LYC	248	56	47.4	-27.6	117.7	0.31	15	0.76						182	27	44.2	-30.3	80.4	1.34	20	2.43																																										
3	A	4	Veraison	C	LYC	300	53	48.2	-28.4	144.4	0.34	14	1.03						275	27	44.6	-29.2	122.7	1.74	25	4.77																																										
3	B	1	Veraison	C	HYC	226	51	47.0	-27.8	106.3	0.39	5	0.88						292	30	45.1	-29.0	131.4	1.42	29	4.13																																										
3	B	2	Veraison	C	HYC	277	53	47.8	-27.3	132.6	0.31	5	0.85						244	29	44.2	-28.6	108.0	1.27	16	3.10																																										
3	B	3	Veraison	C	LYC	198	50	47.5	-28.1	94.2	0.32	5	0.64						174	26	45.2	-29.3	78.8	1.81	12	3.15																																										
3	B	4	Veraison	C	HYC	255	50	46.7	-28.0	119.2	0.37	5	0.94						378	24	44.7	-27.5	169.1	1.97	10	7.43																																										
3	C	1	Veraison	C	HYC	326	51	46.7	-27.8	152.4	0.37	3	1.22						408	25	43.6	-28.5	178.0	1.89	9	7.71																																										
3	C	2	Veraison	C	HYC	251	55	46.7	-28.0	117.1	0.30	3	0.76						212	28	43.9	-28.6	93.1	1.49	13	3.16																																										
3	C	4	Veraison	C	HYC	353	55	47.4	-27.4	167.3	0.31	3	1.08						247	30	44.6	-28.5	110.1	1.39	12	3.42																																										
4	A	1	Harvest	C	LYC	309	59	46.5	-28.1	144.0	0.34	13	1.06						499	35	44.9	-29.4	224.3	1.57	13	7.85																																										
4	A	2	Harvest	C	LYC	315	57	46.2	-28.4	145.7	0.26	13	0.81						383	37	45.9	-29.1	175.6	1.28	13	4.92																																										
4	A	4	Harvest	C	LYC	300	57	47.2	-28.3	141.5	0.27	21	0.80						505	33	46.3	-28.4	233.8	1.40	19	7.09																																										
4	B	1	Harvest	C	LYC	303	59	47.0	-27.8	142.4	0.24	9	0.72						280	36	45.2	-28.9	126.8	1.42	7	3.98																																										
4	B	2	Harvest	C	LYC	227	59	46.7	-28.3	105.9	0.45	8	1.02						240	36	45.0	-30.2	108.0	1.23	12	2.95																																										
4	B	3	Harvest	C	HYC	251	60	47.6	-27.9	119.4	0.40	7	1.01						369	34	45.4	-29.7	167.6	1.32	14	4.89																																										
4	B	4	Harvest	C	HYC	344	57	47.5	-28.0	163.4	0.43	19	1.48						611	35	45.5	-29.8	277.7	1.63	12	9.93																																										
4	C	1	Harvest	C	HYC	207	43	47.6	-27.9	98.7	0.44	4	0.91						378	36	46.2	-28.5	174.6	1.13	9	4.28																																										
4	C	2	Harvest	C	HYC	317	57	47.1	-28.0	149.4	0.38	6	1.20						449	35	45.6	-28.7	204.8	1.28	11	5.75																																										
4	C	3	Harvest	C	HYC	282	59	47.7	-28.2	134.7	0.38	7	1.08						394	37	46.1	-28.3	181.9	1.31	11	5.18																																										
4	C	4	Harvest	C	HYC	260	58	46.5	-28.0	120.7	0.39	29	1.01						274	35	45.5	-28.2	124.5	1.29	34	3.54																																										
5	A	2	Harvest	F17	LYC	275	58	46.2	-28.6	127.0	0.48	1683	1.31	0.0099	0.98	0.06	0.08	13	318	37	45.7	-28.7	145.3	1.37	1909	4.36	0.0107	1.06	0.07	0.31	51	58	27	43.4	-29.8	25.0	1.09	2814	0.63	0.0140	1.38	0.10	0.07	11
5	A	3	Harvest	F17	LYC	328	60	45.8	-28.4	150.5	0.46	1339	1.51	0.0086	0.85	0.05	0.08	12	412	39	46.2	-28.3	190.1	1.27	1674	5.23	0.0098	0.97	0.06	0.33	50	53	30	45.2	-29.7	24.1	1.23	2574	0.66	0.0131	1.30	0.10	0.06	10
5	A	4	Harvest	F17	LYC	362	58	45.8	-28.7	165.7	0.51	1427	1.84	0.0089	0.88	0.05	0.10	12	661	37	46.1	-28.7	304.6	1.50	1293	9.89	0.0084	0.84	0.05	0.48	59	62	26	44.5	-29.1	27.5	1.26	2219	0.78	0.0118	1.17	0.08	0.06	8
5	B	1	Harvest	F17	HYC	298	60	44.1	-28.2	131.4	0.52	1086	1.56	0.0077	0.76	0.04	0.06	6	494	34	46.0	-28.7	227.1	1.41	2434	6.98	0.0126	1.25	0.09	0.63	64	174	25	44.3	-28.8	77.0	1.15	1887	2.00	0.0106	1.05	0.07	0.14	14
5	B	2	Harvest	F17	HYC	327	57	46.5	-28.5	152.2	0.43	1167	1.42	0.0080	0.79	0.04	0.06	6	522	36	46.1	-29.2	240.7	1.47	2367	7.68	0.0124	1.22	0.09	0.68	65	169	25	43.6	-28.8	73.6	1.21	2010	2.04	0.0111	1.09	0.07	0.15	15
5	B	3	Harvest	F17	HYC	324	59	47.7	-27.9	154.3	0.47	1094	1.52	0.0077	0.76	0.04	0.06	8	432	36	45.8	-29.8	197.6	1.40	1632	6.03	0.0097	0.96	0.06	0.37	49	181	25	44.0	-28.7	79.5	1.27	2023	2.29	0.0111	1.10	0.08	0.17	23
5	B	4	Harvest	F17	LYC	270	59	46.3	-28.3	125.3	0.40	1239	1.08	0.0082	0.82	0.05	0.05	6	503	36	46.0	-29.1	231.3	1.56	1843	7.85	0.0105	1.03	0.07	0.54	65	104	26	43.0	-28.9	44.7	1.34	1834	1.39	0.0104	1.03	0.07	0.10	11
5	C	2	Harvest	F17	HYC	325	59	47.5	-27.7	154.5	0.39	1936	1.28	0.0108	1.07	0.07	0.09	9	427	37	46.7	-29.1	199.1	1.60	2162	6.85	0.0116	1.15	0.08	0.55	54	145	27	43.7	-28.9	63.4	1.17	2783	1.69	0.0139	1.37	0.10	0.18	17
5	C	3	Harvest	F17	LYC	256	59	47.9	-27.9	122.6	0.37	1946	0.96	0.0108	1.07	0.07	0.07	10	295	39	47.1	-28.4	138.7	1.25	2222	3.70	0.0118	1.17	0.08	0.31	43	120	27	42.5	-28.5	51.1	0.99	3232	1.19	0.0156	1.53	0.12	0.14	20
5	C	4	Harvest	F17	LYC	222	58	47.7	-27.8	105.6	0.30	2383	0.67	0.0124	1.23	0.09	0.06	14	148	37	47.2	-28.4	70.1	1.23	2620	1.82	0.0133	1.31	0.10	0.18	40	60	29	41.9	-27.7	25.1	1.07	3385	0.64	0.0161	1.59	0.13	0.08	18

Appendix I - Raw data
Vines excavated in 2017

Excavation in 2017						5 Must												7 Whole plant														
Blocks 1-5						g	%	wt.% DW	mUr	g	wt.% DW	mUr	g	%			g	%	g	wt.% DW	mUr	g	%			g	%					
block	row	rep	Excavation time	Fertilization treatment	Yield condition	5 DW	5 % DW	5 TOC	5 δ^{13}C	5CQ	5 TN	5 δ^{15}N	5 NQ	5 R	5 A%	5 RSA	5 NNP	5 %P	7 DW	7 % DW	7 TOC	7 δ^{13}C	7 CQ	7 TN	7 δ^{15}N	7 NQ	7 R	7 A%	7 RSA	7 NNP	7 %P
1	A	1	Bud burst	C	no group														395	61	48.5	-28.6	191.7	0.58	7	2.29					
1	A	2	Bud burst	C	no group														406	64	48.9	-28.7	198.3	0.52	4	2.12					
1	A	3	Bud burst	C	no group														453	63	48.7	-28.3	220.5	0.58	4	2.64					
1	A	4	Bud burst	C	no group														544	63	48.1	-28.3	261.7	0.56	2	3.04					
1	B	1	Bud burst	C	no group														360	62	48.0	-28.3	173.1	0.60	2	2.15					
1	B	2	Bud burst	C	no group														426	63	47.8	-27.8	203.5	0.60	2	2.55					
1	B	3	Bud burst	C	no group														318	63	48.5	-28.0	154.3	0.54	2	1.71					
1	B	4	Bud burst	C	no group														335	63	47.1	-28.3	157.7	0.59	2	1.97					
1	C	1	Bud burst	C	no group														269	66	48.0	-28.5	129.1	0.57	1	1.53					
1	C	2	Bud burst	C	no group														360	63	48.1	-27.4	172.9	0.64	1	2.30					
1	C	3	Bud burst	C	no group														364	64	49.3	-28.0	179.8	0.55	1	2.00					
1	C	4	Bud burst	C	no group														337	64	48.4	-27.7	163.0	0.72	1	2.42					
2	A	1	Flowering	C	no group														976	44	46.6	-28.9	454.5	1.34	3	13.08					
2	A	2	Flowering	C	no group														755	44	46.8	-28.3	353.6	1.20	4	9.09					
2	A	3	Flowering	C	no group														718	44	46.7	-29.0	335.3	1.22	4	8.75					
2	A	4	Flowering	C	no group														559	45	47.0	-29.1	263.1	1.20	4	6.73					
2	B	1	Flowering	C	no group														585	48	47.2	-28.9	276.1	1.01	3	5.93					
2	B	2	Flowering	C	no group														632	48	46.3	-28.5	292.5	1.00	2	6.32					
2	B	3	Flowering	C	no group														622	45	46.9	-28.7	292.0	1.34	3	8.35					
2	B	4	Flowering	C	no group														646	47	47.1	-28.5	304.3	1.04	3	6.73					
2	C	1	Flowering	C	no group														506	46	46.4	-28.9	235.0	1.12	2	5.67					
2	C	2	Flowering	C	no group														585	48	45.9	-28.8	268.4	0.90	2	5.27					
2	C	3	Flowering	C	no group														479	47	47.9	-29.0	229.1	1.01	2	4.86					
2	C	4	Flowering	C	no group														573	51	47.0	-28.4	269.7	0.80	2	4.56					
3	A	1	Veraison	C	LYC	47	13	36.7	-27.8	17.2	0.29	8	0.14						980	44	46.2	-28.7	452.9	0.72	9	7.09					
3	A	2	Veraison	C	LYC	59	13	37.5	-27.5	22.1	0.46	12	0.27						1051	39	44.7	-28.2	470.2	1.15	12	12.04					
3	A	3	Veraison	C	LYC	22	11	36.3	-28.5	8.0	0.35	16	0.08						743	52	45.6	-28.4	338.5	0.64	17	4.75					
3	A	4	Veraison	C	LYC	46	12	36.7	-26.8	16.8	0.40	15	0.18						879	45	46.4	-28.6	407.6	0.85	18	7.51					
3	B	1	Veraison	C	HYC	73	12	36.9	-27.0	26.9	0.35	6	0.25						879	42	46.1	-28.2	405.6	0.81	13	7.10					
3	B	2	Veraison	C	HYC	79	12	37.5	-26.8	29.7	0.31	7	0.24						876	44	45.7	-27.7	399.8	0.65	8	5.69					
3	B	3	Veraison	C	LYC	64	12	35.7	-27.5	22.8	0.38	5	0.24						622	41	45.5	-28.6	282.5	0.85	7	5.26					
3	B	4	Veraison	C	HYC	131	12	35.5	-27.0	46.7	0.44	6	0.58						1044	34	44.8	-27.8	467.7	1.06	7	11.07					
3	C	1	Veraison	C	HYC	168	11	35.9	-27.3	60.2	0.36	11	0.60						1275	36	44.5	-28.1	567.5	0.98	7	12.48					
3	C	2	Veraison	C	HYC	87	13	36.6	-27.3	31.6	0.29	5	0.25						778	28	34.9	-22.2	271.1	0.62	6	4.78					
3	C	4	Veraison	C	HYC	63	12	40.1	-26.5	25.2	0.30	4	0.19						1004	47	45.8	-27.6	460.0	0.64	5	6.42					
4	A	1	Harvest	C	LYC	95	21	38.1	-28.3	36.4	0.28	14	0.71						1226	45	45.8	-28.8	561.4	0.96	14	11.74					
4	A	2	Harvest	C	LYC	105	21	38.9	-27.7	41.0	0.27	12	0.28						1141	45	45.7	-28.6	521.6	0.78	13	8.92					
4	A	4	Harvest	C	LYC	157	21	38.8	-27.9	61.0	0.32	39	0.50						1261	41	46.0	-28.4	580.4	0.90	23	11.36					
4	B	1	Harvest	C	LYC	127	19	38.7	-27.7	49.3	0.20	15	0.26						1087	45	45.5	-28.3	494.9	0.70	10	7.57					
4	B	2	Harvest	C	LYC	99	22	38.0	-29.0	37.6	0.24	17	0.24						907	47	45.4	-29.1	411.5	0.76	11	6.85					
4	B	3	Harvest	C	HYC	161	19	38.4	-27.8	61.8	0.18	28	0.29						1250	43	45.5	-28.5	569.6	0.75	13	9.40					
4	B	4	Harvest	C	HYC	259	21	37.0	-27.5	95.7	0.28	44	0.72						1749	39	44.8	-28.6	783.8	0.96	21	16.83					
4	C	1	Harvest	C	HYC	286	20	36.4	-27.3	104.3	0.24	11	0.68						1360	34	44.5	-28.0	605.5	0.74	9	10.03					
4	C	2	Harvest	C	HYC	229	20	36.8	-28.0	84.4	0.20	13	0.46						1399	37	42.7	-27.2	596.8	0.70	10	9.82					
4	C	3	Harvest	C	HYC	315	20	38.1	-28.8	120.1	0.24	45	0.76						1421	39	46.1	-29.9	655.8	0.78	18	11.06					
4	C	4	Harvest	C	HYC	242	21	37.3	-29.4	90.2	0.18	62	0.45						1185	39	43.9	-28.7	520.5	0.66	40	7.82					
5	A	2	Harvest	F17	LYC	107	21	38.6	-29.7	41.1	0.31	3681	0.33	0.0172	1.69	0.14	0.05	7	968	46	45.6	-28.7	440.8	0.81	2221	7.81	0.0118	1.17	0.08	0.61	100
5	A	3	Harvest	F17	LYC	164	22	37.4	-29.4	61.4	0.23	3199	0.38	0.0154	1.52	0.12	0.04	7	1316	48	45.4	-28.6	597.2	0.73	1923	9.66	0.0107	1.06	0.07	0.65	100
5	A	4	Harvest	F17	LYC	111	21	38.2	-29.4	42.4	0.31	2638	0.34	0.0134	1.32	0.10	0.03	4	1485	45	45.7	-28.7	678.5	0.98	1629	14.55	0.0097	0.96	0.06	0.81	100
5	B	1	Harvest	F17	HYC	229	19	37.5	-29.0	85.8	0.29	2069	0.67	0.0113	1.12	0.08	0.05	5	1438	41	44.2	-28.6	635.9	0.89	1877	12.86	0.0106	1.05	0.07	0.98	100
5	B	2	Harvest	F17	HYC	351	21	37.0	-28.7	129.8	0.26	2355	0.93	0.0123	1.22	0.09	0.08	8	1602	39	44.1	-28.8	707.0	0.85	1905	13.70	0.0107	1.06	0.07	1.04	100
5	B	3	Harvest	F17	HYC	350	20	38.4	-28.6	134.4	0.32	2397	1.13	0.0125	1.23	0.09	0.10	13	1501	39	44.6	-28.8	669.3	0.83	1646	12.45	0.0097	0.96	0.06	0.76	100
5	B	4	Harvest	F17	LYC	230	22	38.0	-28.6	87.5	0.30	2166	0.69	0.0116	1.15	0.08	0.06	7	1357	42	44.6	-28.7	604.5	0.93	1724	12.64	0.0100	0.99	0.06	0.83	100
5	C	2	Harvest	F17	HYC	335	21	38.5	-27.9	129.1	0.26	3268	0.89	0.0157	1.54	0.12	0.11	11	1444	41	45.2	-28.4	652.6	0.84	2364	12.18	0.0124	1.22	0.09	1.01	100
5	C	3	Harvest	F17	LYC	232	22	37.7	-28.2	87.5	0.32	3843	0.74	0.0178	1.75	0.14	0.11	15	1123	42	44.2	-27.6	495.9	0.70	2451	7.91	0.0126	1.24	0.09	0.71	100
5	C	4	Harvest	F17	LYC	190	22	36.5	-27.2	69.3	0.18	4257	0.34	0.0193	1.90	0.16	0.05	12	819	44	44.8	-27.7	367.3	0.56	2757	4.58	0.0138	1.36	0.10	0.44	100

Appendix II - Raw data
Vines excavated in 2018

Excavation in 2018 — Blocks 6-14

Field measurements 2017

block	row	rep	Excavation time	Fertilization treatment	Yield condition	Bud fruitfulness 17 (bunches per shoot)	Bunches per vines 17 (#)	Winter pruning weight 17 (g)	Canopy weight 17 (g)	Crop load 17 (kg per vine)	Bunch weight (g)	Canopy-to-fruit ratio (g/g)	Yield 17 ($kg\ m^{-2}$)
6	A	1	Bud burst	C	LYC	2.0	2	480	903	0.6	300	1.5	0.50
6	A	2	Bud burst	C	HYC	2.1	2	1070	1823	1.6	800	1.1	1.33
6	A	3	Bud burst	C	LYC	2.0	2	600	1090	0.1	35	15.6	0.06
6	A	4	Bud burst	C	LYC	2.3	2	750	1324	0.7	370	1.8	0.62
6	B	1	Bud burst	C	HYC	2.1	4	1400	2338	1.7	418	1.4	1.39
6	B	2	Bud burst	C	LYC	2.0	5	680	1215	1.4	282	0.9	1.18
6	B	3	Bud burst	C	HYC	2.1	5	870	1511	2.2	440	0.7	1.83
6	B	4	Bud burst	C	HYC	2.1	5	580	1059	1.6	318	0.7	1.33
6	C	1	Bud burst	C	HYC	1.9	7	930	1605	2.6	374	0.6	2.18
6	C	2	Bud burst	C	HYC	2.1	6	540	996	1.7	287	0.6	1.43
6	C	3	Bud burst	C	HYC	2.3	9	1040	1776	3.3	361	0.5	2.71
6	C	4	Bud burst	C	LYC	1.8	5	570	1043	0.8	152	1.4	0.63
7	A	1	Bud burst	F17	LYC	2.1	3	530	981	0.7	233	1.4	0.58
7	A	2	Bud burst	F17	LYC	2.5	3	750	1324	1.3	420	1.1	1.05
7	A	3	Bud burst	F17	LYC	1.9	3	880	1527	1.0	337	1.5	0.84
7	A	4	Bud burst	F17	LYC	1.8	3	520	965	0.5	150	2.1	0.38
7	B	1	Bud burst	F17	HYC	1.7	5	790	1386	1.5	290	1.0	1.21
7	B	2	Bud burst	F17	HYC	2.1	6	810	1418	1.7	287	0.8	1.43
7	B	3	Bud burst	F17	LYC	1.4	5	650	1168	0.9	172	1.4	0.72
7	B	4	Bud burst	F17	LYC	2.4	6	750	1324	1.2	195	1.1	0.98
7	C	1	Bud burst	F17	HYC	1.8	7	600	1090	2.1	294	0.5	1.72
7	C	2	Bud burst	F17	HYC	2.0	8	990	1698	2.6	328	0.6	2.18
7	C	3	Bud burst	F17	HYC	2.0	7	720	1277	2.2	309	0.6	1.80
7	C	4	Bud burst	F17	HYC	2.2	8	670	1199	2.5	315	0.5	2.10
8	A	1	Flowering	C	LYC	2.1	2	1310	2198	0.9	460	2.4	0.77
8	A	2	Flowering	C	LYC	2.5	4	490	918	0.8	193	1.2	0.64
8	A	3	Flowering	C	HYC	1.9	3	660	1184	1.3	440	0.9	1.10
8	A	4	Flowering	C	LYC	1.5	2	550	1012	0.5	265	1.9	0.44
8	B	1	Flowering	C	HYC	2.2	6	780	1371	1.4	235	1.0	1.18
8	B	2	Flowering	C	HYC	2.2	5	900	1558	2.4	470	0.7	1.96
8	B	3	Flowering	C	HYC	2.1	6	940	1620	1.6	272	1.0	1.36
8	B	4	Flowering	C	LYC	1.7	6	750	1324	0.9	150	1.5	0.75
8	C	1	Flowering	C	LYC	1.5	6	460	872	0.9	157	0.9	0.78
8	C	2	Flowering	C	HYC	2.1	9	750	1324	3.4	376	0.4	2.82
8	C	3	Flowering	C	HYC	2.3	9	580	1059	1.6	177	0.7	1.33
8	C	4	Flowering	C	HYC	1.7	7	450	856	1.3	189	0.6	1.10
9	A	1	Flowering	F17	LYC	2.0	3	490	918	0.6	213	1.4	0.53
9	A	3	Flowering	F17	LYC	2.1	3	460	872	0.6	213	1.4	0.53
9	A	4	Flowering	F17	LYC	2.0	2	450	856	0.5	270	1.6	0.45
9	B	1	Flowering	F17	LYC	1.7	6	840	1464	1.1	177	1.4	0.88
9	B	2	Flowering	F17	HYC	1.5	6	700	1246	1.3	208	1.0	1.04
9	B	3	Flowering	F17	LYC	1.7	6	700	1246	0.8	135	1.5	0.68
9	B	4	Flowering	F17	LYC	1.9	6	680	1215	0.9	150	1.3	0.75
9	C	1	Flowering	F17	HYC	1.7	7	450	856	1.6	226	0.5	1.32
9	C	2	Flowering	F17	HYC	1.9	8	530	981	1.3	159	0.8	1.06
9	C	3	Flowering	F17	HYC	1.5	6	480	903	2.2	362	0.4	1.81
9	C	4	Flowering	F17	HYC	1.9	10	720	1277	2.8	284	0.4	2.37
10	A	1	Veraison	C	LYC	2.1	3	530	981	0.7	243	1.3	0.6
10	A	2	Veraison	C	LYC	2.5	4	550	1012	0.7	180	1.4	0.6
10	A	3	Veraison	C	LYC	2.3	2	260	560	0.4	220	1.3	0.4
10	A	4	Veraison	C	LYC	2.0	3	390	762	0.7	233	1.1	0.6
10	B	1	Veraison	C	LYC	2.0	5	750	1324	1.4	270	1.0	1.1
10	B	2	Veraison	C	HYC	2.0	5	750	1324	0.8	164	1.6	0.7
10	B	3	Veraison	C	HYC	1.9	5	840	1464	0.9	178	1.6	0.7
10	B	4	Veraison	C	LYC	1.7	6	740	1308	1.4	237	0.9	1.2
10	C	1	Veraison	C	HYC	2.1	8	850	1480	1.9	238	0.8	1.6
10	C	2	Veraison	C	HYC	2.0	7	650	1168	1.9	274	0.6	1.6
10	C	3	Veraison	C	HYC	2.1	9	520	965	1.9	211	0.5	1.6
10	C	4	Veraison	C	HYC	1.7	7	500	934	1.8	253	0.5	1.5
11	A	1	Veraison	F17	LYC	2.2	3	890	1542	1.4	470	1.1	1.2
11	A	2	Veraison	F17	LYC	2.7	3	470	887	1.0	330	0.9	0.8
11	A	3	Veraison	F17	LYC	2.3	4	440	840	1.1	285	0.7	1.0
11	A	4	Veraison	F17	LYC	2.7	3	380	747	0.7	223	1.1	0.6
11	B	1	Veraison	F17	HYC	2.0	5	1750	2884	2.4	484	1.2	2.0
11	B	2	Veraison	F17	HYC	1.9	5	1120	1901	2.1	422	0.9	1.8
11	B	3	Veraison	F17	HYC	2.2	5	1080	1839	1.7	338	1.1	1.4
11	B	4	Veraison	F17	LYC	2.2	5	820	1433	0.8	166	1.7	0.7
11	C	1	Veraison	F17	HYC	2.0	9	490	918	3.0	330	0.3	2.5
11	C	2	Veraison	F17	HYC	2.5	7	720	1277	3.0	429	0.4	2.5
11	C	3	Veraison	F17	LYC	1.7	8	460	872	1.3	163	0.7	1.1
11	C	4	Veraison	F17	HYC	2.1	9	860	1496	4.0	444	0.4	3.3
12	A	1	Harvest	C	LYC	2.0	3	480	903	0.6	207	1.5	0.5
12	A	2	Harvest	C	LYC	1.7	3	490	918	0.8	250	1.2	0.6
12	A	3	Harvest	C	HYC	2.1	2	550	1012	1.1	570	0.9	1.0
12	A	4	Harvest	C	HYC	2.2	3	680	1215	1.1	370	1.1	0.9
12	B	1	Harvest	C	LYC	2.0	6	930	1605	1.0	168	1.6	0.8
12	B	2	Harvest	C	HYC	2.1	6	1060	1808	1.5	253	1.2	1.3
12	B	3	Harvest	C	HYC	2.0	6	1000	1714	1.7	282	1.0	1.4
12	B	4	Harvest	C	LYC	1.9	4	1040	1776	0.7	165	2.7	0.6
12	C	1	Harvest	C	HYC	2.2	8	610	1106	2.3	290	0.5	1.9
12	C	2	Harvest	C	LYC	1.2	5	360	716	1.4	272	0.5	1.1
12	C	3	Harvest	C	HYC	2.0	8	650	1168	1.5	181	0.8	1.2
13	A	1	Harvest	F17	LYC	2.0	4	450	856	0.9	230	0.9	0.8
13	A	2	Harvest	F17	HYC	2.1	2	520	965	1.3	640	0.8	1.1
13	A	3	Harvest	F17	HYC	2.0	3	720	1277	1.3	427	1.0	1.1
13	A	4	Harvest	F17	LYC	2.0	2	710	1262	1.4	690	0.9	1.2
13	B	1	Harvest	F17	HYC	2.0	6	1180	1995	2.6	432	0.8	2.2
13	B	2	Harvest	F17	LYC	2.1	7	930	1605	1.0	137	1.7	0.8
13	B	3	Harvest	F17	LYC	1.8	5	1150	1948	1.5	290	1.3	1.2
13	B	4	Harvest	F17	LYC	1.9	4	1280	2151	1.1	265	2.0	0.9
13	C	1	Harvest	F17	HYC	2.1	8	390	762	1.9	238	0.4	1.6
13	C	2	Harvest	F17	HYC	1.7	8	410	794	1.6	194	0.5	1.3
13	C	3	Harvest	F17	HYC	1.9	9	810	1418	3.1	341	0.5	2.6
13	C	4	Harvest	F17	LYC	2.0	8	440	840	1.3	159	0.7	1.1
14	A	1	Harvest	F17+18	HYC	1.9	3	840	1464	1.4	457	1.1	1.1
14	A	4	Harvest	F17+18	LYC	2.2	2	230	513	0.7	370	0.7	0.6
14	B	1	Harvest	F17+18	HYC	2.1	5	1300	2182	2.1	428	1.0	1.8
14	B	2	Harvest	F17+18	LYC	1.0	4	850	1480	1.1	285	1.3	1.0
14	B	3	Harvest	F17+18	LYC	2.3	6	990	1698	1.3	217	1.3	1.1
14	B	4	Harvest	F17+18	LYC	1.9	6	410	794	1.1	190	0.7	1.0
14	C	1	Harvest	F17+18	HYC	1.6	5	590	1074	1.7	334	0.6	1.4
14	C	2	Harvest	F17+18	HYC	2.0	8	550	1012	2.7	331	0.4	2.2
14	C	3	Harvest	F17+18	HYC	2.1	7	740	1308	3.5	506	0.4	3.0
14	C	4	Harvest	F17+18	HYC	2.0	5	410	794	2.0	406	0.4	1.7

Field measurements 2018 / Must analyses 2018

block	row	rep	Bud fruitfulness 18 (bunches per shoot)	Bunches per vines 18 (#)	Canopy weight 18 (g)	Total leaf area ($m^2\ m^{-2}$ of sc)	Light-exposed leaf area ($m^2\ m^{-2}$ of sc)	Crop load 18 (kg per vine)	Bunch weight (g)	Exposed Leaf-to-fruit ratio (m^2/kg)	Total Leaf-to-fruit ratio (m^2/kg)	Canopy-to-fruit ratio (g/g)	Yield 18 ($kg\ m^{-2}$)	Total soluble sugars (Brix)	pH	Titratable acidity ($g\ L^{-1}$)	Tartaric acid ($g\ L^{-1}$)	Malic acid ($g\ L^{-1}$)	K ($mg\ L^{-1}$)	NH_3 ($mg\ L^{-1}$)	N-alpha aminé ($mg\ L^{-1}$)	YAN ($mg\ L^{-1}$)
6	A	1																				
6	A	2																				
6	A	3																				
6	A	4																				
6	B	1																				
6	B	2																				
6	B	3																				
6	B	4																				
6	C	1																				
6	C	2																				
6	C	3																				
6	C	4																				
7	A	1																				
7	A	2																				
7	A	3																				
7	A	4																				
7	B	1																				
7	B	2																				
7	B	3																				
7	B	4																				
7	C	1																				
7	C	2																				
7	C	3																				
7	C	4																				
8	A	1	2.3		1661																	
8	A	2	1.4		862																	
8	A	3	2.4		1472																	
8	A	4	2.3		1838																	
8	B	1	1.9		1359																	
8	B	2	2.1		1479																	
8	B	3	2.0		1642																	
8	B	4	2.0		1317																	
8	C	1	1.7		1003																	
8	C	2	2.4		1584																	
8	C	3	2.4		1239																	
8	C	4	2.6		893																	
9	A	1	1.6		1544																	
9	A	3	2.0		1201																	
9	A	4	2.3		1619																	
9	B	1	2.0		1218																	
9	B	2	2.1		1273																	
9	B	3	2.0		1318																	
9	B	4	2.0		1355																	
9	C	1	2.6		1299																	
9	C	2	2.4		1313																	
9	C	3	2.7		1236																	
9	C	4	2.7		1548																	
10	A	1	2.4	2	1800			0.9	435			2.1	0.7	13.1	3.1	12.2	6.4	8.3	1694	70	95	152
10	A	2	2.7	2	1679			1.1	545			1.5	0.9	13.8	3.1	10.4	6.3	6.6	1629	41	79	112
10	A	3	1.9	3	567			0.4	147			1.3	0.4	13.6	3.1	11.8	6.6	7.5	1646	26	49	70
10	A	4	1.6	2	1387			0.8	390			1.8	0.7	13.5	3.0	12.6	6.8	8.0	1573	40	63	96
10	B	1	1.9	5	1418			1.4	280			1.0	1.2	11.8	3.0	13.9	7.2	9.1	1711	47	53	91
10	B	2	1.9	5	1844			2.0	392			0.9	1.6	11.1	3.0	15.2	7.4	10.3	1835	79	61	126
10	B	3	2.0	5	1665			2.0	390			0.9	1.6	11.7	3.0	13.4	6.5	9.2	1596	82	80	147
10	B	4	1.9	5	1457			1.5	298			1.0	1.2	11.4	2.9	15.9	6.9	10.9	1603	46	51	89
10	C	1	2.2	10	1941			3.1	311			0.6	2.6	9.0	2.9	22.2	8.5	16.0	1957	188	73	228
10	C	2	2.0	10	1492			2.3	233			0.6	1.9	10.5	2.9	17.9	8.0	12.3	1863	75	57	118
10	C	3	1.7	10	1585			2.0	198			0.8	1.6	10.8	2.9	18.2	8.1	12.4	1782	74	52	113
10	C	4	1.6	10	1399			2.0	198			0.7	1.7	11.0	3.0	15.9	7.7	10.6	1794	76	51	114
11	A	1	2.0	2	3283			1.1	565			2.9	0.9	12.8	3.1	13.7	6.6	9.8	1844.6	126	115	218
11	A	2	1.5	2	935			0.4	220			2.1	0.4	13.6	2.9	13.0	6.9	8.1	1517.0	8	33	40
11	A	3	2.4	2	1468			1.1	550			1.3	0.9	13.4	3.1	11.0	6.1	7.1	1498.4	54	89	133
11	A	4	3.0	2	743			0.7	335			1.1	0.6	14.8	3.2	9.1	6.2	5.3	1477.1	54	89	134
11	B	1	2.2	10	2950			3.8	383			0.8	3.2	9.2	3.0	21.8	7.9	16.5	2047.4	286	109	345
11	B	2	2.0	5	2148			2.3	452			1.0	1.9	10.8	3.0	15.2	7.0	10.7	1816.0	120	88	186
11	B	3	1.9	5	2077			2.2	442			0.9	1.8	11.3	3.0	13.8	7.0	9.1	1675.3	68	72	128
11	B	4	1.6	2	1786			0.7	365			2.4	0.6	13.0	3.0	13.5	6.7	9.1	1730.3	48	71	110
11	C	1	2.3	10	1322			2.1	211			0.6	1.8	10.4	2.9	19.1	8.1	13.2	1875.3	55	42	87
11	C	2	2.3	10	2259			3.3	327			0.7	2.7	9.7	3.0	19.2	7.4	14.0	1944.9	118	64	161
11	C	3	2.0	5	1369			0.9	174			1.6	0.7	11.7	2.9	18.4	7.8	12.9	1917.0	66	55	110
11	C	4	2.6	10	1900			2.9	291			0.7	2.4	9.9	2.9	19.8	7.2	14.7	1877.7	124	72	174
12	A	1	2.1	2	802	1.7	1.4	1.5	745	1.1	1.4	0.5	1.2	19.7	3.5	4.3	5.0	1.7	1713	23	97	115
12	A	2	2.4	2	1709	2.0	1.4	1.3	665	1.3	1.8	1.3	1.1	21.4	3.6	3.7	4.4	1.6	1737	7	86	92
12	A	3	2.3	5	1803	1.9	1.4	3.1	614	0.5	0.7	0.6	2.6	20.2	3.6	4.5	5.2	1.9	1706	13	95	105
12	A	4	2.7	5	1187	1.6	1.4	2.6	520	0.6	0.7	0.5	2.2	19.3	3.5	4.4	5.1	1.9	1588	12	73	83
12	B	1	2.3	2	1378	2.0	1.4	1.0	505	1.7	2.4	1.4	0.8	18.6	3.4	5.1	5.6	2.0	1614	21	73	90
12	B	2	2.6	10	1657	2.1	1.4	5.2	524	0.3	0.5	0.3	4.4	18.2	3.4	5.2	5.7	2.1	1524	32	85	112
12	B	3	2.0	10	1562	1.7	1.4	4.7	473	0.4	0.4	0.3	3.9	18.2	3.3	5.6	5.7	2.3	1438	31	82	107
12	B	4	2.1	2	1554	1.8	1.4	1.2	585	1.4	1.8	1.3	1.0	19.6	3.6	4.5	5.0	2.1	1748	12	86	96
12	C	1	2.0	11	1999	2.0	1.5	4.8	435	0.4	0.5	0.4	4.0	18.8	3.4	5.6	5.5	2.7	1655	16	85	99
12	C	2	1.7	5	1053	1.7	1.5	2.4	488	0.7	0.8	0.4	2.0	20.4	3.4	4.9	5.3	2.1	1580	2	53	54
12	C	3	2.0	6	1939	2.0	1.5	2.9	490	0.6	0.8	0.7	2.4	20.4	3.4	4.7	5.4	1.9	1618	10	75	83
13	A	1	1.8	2	1390	1.6	1.5	1.1	560	1.6	1.7	1.2	0.9	21.7	3.6	4.0	4.9	1.5	1775	6	67	72
13	A	2	2.7	5	1602	2.3	1.5	2.9	570	0.6	0.9	0.6	2.4	19.5	3.6	4.4	5.1	2.1	1806	10	76	83
13	A	3	2.4	5	2198	2.4	1.5	3.6	722	0.5	0.8	0.6	3.0	19.4	3.6	4.9	5.2	2.9	1955	21	115	132
13	A	4	2.0	4	2074	1.7	1.5	2.1	515	0.9	1.0	1.0	1.7	20.0	3.6	5.1	5.1	3.1	1903	27	130	152
13	B	1	2.7	10	1824	2.1	1.4	5.2	523	0.3	0.5	0.3	4.4	17.7	3.4	5.5	5.9	2.5	1682	40	90	123
13	B	2	2.2	2	817	1.4	1.4	0.7	365	2.3	2.3	1.1	0.6	21.7	3.7	3.7	4.8	1.6	1801	3	70	72
13	B	3	2.2	5	1713	2.0	1.4	2.1	410	0.8	1.2	0.8	1.7	20.6	3.7	4.2	5.3	2.2	2016	11	85	94
13	B	4	2.1	3	1740	2.3	1.4	1.8	593	0.9	1.6	1.0	1.5	20.7	3.7	4.2	4.8	2.5	2030	13	116	126
13	C	1	1.8	10	1091	1.6	1.4	3.1	312	0.5	0.6	0.3	2.6	18.7	3.3	5.8	5.6	2.6	1485	8	50	57
13	C	2	1.8	10	1283	1.8	1.4	2.5	253	0.7	0.8	0.5	2.1	20.7	3.5	4.7	5.4	1.9	1683	0	43	43
13	C	3	2.0	10	2003	2.3	1.4	4.7	472	0.4	0.6	0.4	3.9	19.6	3.5	5.0	5.5	2.4	1807	6	74	79
13	C	4	1.5	2	1544	1.8	1.4	1.0	490	1.7	2.2	1.6	0.8	22.0	3.6	4.3	5.0	2.0	1752	0	67	68
14	A	1	2.4	5	2573	2.7	1.3	3.8	752	0.4	0.9	0.7	3.1	20.9	3.7	4.6	5.0	2.8	2017	26	147	169
14	A	4	2.3	2	1114	2.0	1.3	0.8	410	1.9	3.0	1.4	0.7	22.0	3.9	3.9	5.0	2.0	2175	10	108	116
14	B	1	2.0	10	2224	2.8	1.4	5.0	504	0.3	0.7	0.4	4.2	19.9	3.7	4.5	5.3	2.5	2057	25	124	145
14	B	2	2.0	3	1329	1.8	1.4	0.8	270	2.1	2.6	1.6	0.7	22.2	3.8	4.0	5.0	1.9	1999	4	93	96
14	B	3	2.1	5	1420	1.8	1.4	2.2	448	0.7	0.9	0.6	1.9	20.7	3.6	4.6	5.4	2.2	1856	17	110	123
14	B	4	2.0	5	1192	1.8	1.4	1.7	346	1.0	1.2	0.7	1.4	21.6	3.6	4.6	5.3	2.2	1856	7	104	110
14	C	1	2.3	6	2284	1.4	1.5	3.3	550	0.5	0.5	0.7	2.7	18.0	3.6	5.1	5.4	3.0	1936	41	133	167
14	C	2	2.0	9	2090	2.1	1.5	3.9	431	0.5	0.6	0.5	3.2	18.5	3.5	5.8	5.7	3.3	1909	26	83	104
14	C	3	2.0	10	2370	2.2	1.5	5.1	512	0.4	0.5	0.5	4.3	17.4	3.5	5.8	5.9	3.1	1788	60	125	174
14	C	4	2.0	11	1356	2.0	1.5	3.3	301	0.5	0.7	0.4	2.8	19.3	3.4	5.6	5.8	2.7	1717	13	69	80

1 Roots

block	row	rep	1 DW (g)	1 % DW (%)	1 TOC (wt.% DW)	1 $\delta^{13}C$ (mUr)	1 CQ (g)	1 TN (wt.% DW)	1 $\delta^{15}N$ (mUr)	1 NQ (g)	1 R	1 A% (%)	1 RSA	1 NNP (g)	1 %P (%)
6	A	1	267	65	45.7	-28.0	122.2	0.82	25	2.18					
6	A	2	272	63	46.0	-27.7	125.1	0.91	8	2.47					
6	A	3	466	64	45.7	-27.7	213.0	0.70	9	3.24					
6	A	4	660	61	45.2	-27.5	298.1	0.79	52	3.17					
6	B	1	302	61	45.9	-27.6	138.5	0.96	21	2.89					
6	B	2	224	63	46.5	-28.3	104.0	0.84	12	1.87					
6	B	3	298	65	46.7	-27.8	139.3	0.93	10	2.77					
6	B	4	257	64	46.1	-28.0	118.6	0.79	16	2.03					
6	C	1	273	63	44.8	-27.7	122.3	0.75	12	2.06					
6	C	2	319	61	45.5	-27.5	145.3	0.75	10	2.39					
6	C	3	270	64	45.2	-27.5	122.0	0.96	6	2.60					
6	C	4	210	61	44.5	-27.7	93.6	0.91	36	1.92					
7	A	1	307	62	43.6	-28.2	133.8	0.78	1400	2.40	0.0088	0.87	0.05	0.13	65
7	A	2	266	63	43.2	-27.9	115.0	1.06	2068	2.81	0.0113	1.12	0.08	0.22	63
7	A	3	267	61	44.3	-27.9	118.1	0.96	1662	2.56	0.0098	0.97	0.06	0.16	60
7	A	4	260	63	45.9	-28.1	119.3	0.87	1679	2.25	0.0098	0.98	0.06	0.14	66
7	B	1	309	60	45.1	-28.3	139.6	0.82	1579	2.54	0.0095	0.94	0.06	0.15	64
7	B	2	253	61	46.0	-28.0	116.5	0.83	2176	2.10	0.0117	1.15	0.08	0.17	63
7	B	3	193	62	44.6	-27.9	86.3	0.89	1741	1.72	0.0101	1.00	0.06	0.11	64
7	B	4	215	60	46.0	-28.0	98.9	0.84	1679	1.80	0.0098	0.98	0.06	0.11	62
7	C	1	261	63	45.8	-27.8	119.7	0.67	1277	1.75	0.0084	0.83	0.05	0.08	57
7	C	2	261	63	44.9	-27.8	117.2	0.85	1897	2.23	0.0106	1.05	0.07	0.16	62
7	C	3	276	65	45.2	-28.2	124.8	0.94	1358	2.60	0.0087	0.86	0.05	0.13	63
7	C	4	209	60	45.6	-28.5	95.3	1.03	1507	2.15	0.0092	0.91	0.06	0.12	62
8	A	1	282	49	46.8	-28.4	131.7	1.05	41	2.95					
8	A	2	174	53	46.5	-29.6	80.9	0.61	14	1.06					
8	A	3	314	70	45.7	-30.0	143.3	0.80	13	2.50					
8	A	4	185	48	44.7	-30.1	82.7	0.89	36	1.66					
8	B	1	157	49	47.4	-29.7	74.4	0.87	28	1.37					
8	B	2	249	51	45.9	-29.4	114.0	0.89	13	2.20					
8	B	3	162	48	46.1	-29.4	74.6	0.91	13	1.48					
8	B	4	203	50	46.4	-29.3	94.1	0.85	22	1.71					
8	C	1	183	47	46.9	-29.5	86.0	0.65	31	1.19					
8	C	2	132	48	46.0	-29.7	60.9	0.97	14	1.28					
8	C	3	164	47	47.8	-29.6	78.5	0.62	15	1.02					
8	C	4	146	52	46.9	-29.1	68.2	0.59	101	0.85					
9	A	1	229	46	46.9	-29.8	107.5	0.81	1896	1.86	0.0106	1.05	0.07	0.13	29
9	A	3	195	48	46.2	-29.9	90.3	0.60	2309	1.16	0.0122	1.20	0.09	0.10	19
9	A	4	193	46	47.1	-30.0	91.2	0.78	1966	1.50	0.0109	1.08	0.07	0.11	21
9	B	1	219	47	46.2	-29.8	101.3	0.75	1583	1.65	0.0095	0.94	0.06	0.10	27
9	B	2	179	43	46.2	-29.4	82.6	0.87	1932	1.56	0.0108	1.07	0.07	0.11	27
9	B	3	120	47	47.0	-30.2	56.4	0.93	1826	1.12	0.0104	1.03	0.07	0.08	20
9	B	4	179	48	45.8	-29.3	81.8	0.77	1289	1.38	0.0084	0.83	0.05	0.07	17
9	C	1	130	48	45.1	-30.0	58.5	0.77	1552	1.00	0.0094	0.93	0.06	0.06	17
9	C	2	206	48	46.1	-29.8	95.0	0.66	1337	1.35	0.0086	0.85	0.05	0.07	19
9	C	3	162	50	65.3	-29.7	105.8	0.70	1700	1.14	0.0099	0.98	0.06	0.07	18
9	C	4	163	46	47.0	-29.9	76.5	1.08	1292	1.76	0.0084	0.84	0.05	0.08	25
10	A	1	279	75	46.1	-29.1	128.5	0.42	38	1.16					
10	A	2	267	74	46.1	-29.0	123.1	0.45	13	1.19					
10	A	3	239	80	46.1	-28.4	109.9	0.45	9	1.08					
10	A	4	326	77	45.3	-29.0	147.6	0.44	12	1.43					
10	B	1	195	78	46.9	-29.3	91.6	0.44	22	0.86					
10	B	2	225	76	47.2	-29.0	106.3	0.48	11	1.07					
10	B	3	245	77	46.3	-29.2	113.5	0.50	13	1.23					
10	B	4	285	76	47.1	-29.0	134.0	0.44	21	1.24					
10	C	1	197	71	47.8	-29.2	94.2	0.58	65	1.15					
10	C	2	306	72	47.2	-29.2	144.4	0.41	13	1.27					
10	C	3	359	72	48.5	-28.4	174.1	0.47	6	1.70					
10	C	4	277	75	47.2	-28.3	130.7	0.50	18	1.39					
11	A	1	280	66	46.6	-28.9	130.4	0.70	1115	1.96	0.0078	0.77	0.04	0.08	20
11	A	2	320	72	46.6	-29.2	149.3	0.50	1363	1.60	0.0087	0.86	0.05	0.08	16
11	A	3	344	71	46.2	-28.7	158.9	0.47	1268	1.62	0.0083	0.83	0.05	0.07	11
11	A	4	377	73	46.1	-28.7	174.0	0.51	1093	1.92	0.0077	0.76	0.04	0.08	18
11	B	1	244	61	47.6	-29.4	116.4	0.95	783	2.31	0.0066	0.65	0.03	0.06	13
11	B	2	224	69	48.4	-28.9	108.4	0.74	835	1.66	0.0067	0.67	0.03	0.05	9
11	B	3	297	68	46.8	-28.4	139.1	0.56	1076	1.67	0.0076	0.76	0.04	0.06	11
11	B	4	284	71	46.7	-29.0	132.5	0.50	852	1.43	0.0068	0.68	0.03	0.04	11
11	C	1	268	72	46.1	-28.7	123.5	0.47	563	1.26	0.0057	0.57	0.02	0.02	7
11	C	2	250	70	46.0	-29.2	114.8	0.59	761	1.47	0.0065	0.64	0.03	0.04	8
11	C	3	281	71	47.3	-28.9	132.7	0.46	962	1.29	0.0072	0.72	0.03	0.04	11
11	C	4	295	68	46.7	-29.1	138.0	0.60	925	1.76	0.0071	0.70	0.03	0.06	11
12	A	1	393	75	45.5	-28.5	178.6	0.46	26	1.81					
12	A	2	348	73	47.9	-28.9	166.5	0.53	18	1.83					
12	A	3	330	71	46.3	-29.3	153.0	0.48	28	1.58					
12	A	4	413	71	47.2	-28.6	194.7	0.40	41	1.66					
12	B	1	307	73	46.8	-28.7	144.0	0.46	51	1.42					
12	B	2	241	66	48.8	-29.0	117.7	0.57	31	1.37					
12	B	3	272	65	48.5	-29.2	131.9	0.54	51	1.48					
12	B	4	359	69	47.6	-28.5	170.7	0.55	48	1.98					
12	C	1	266	66	48.6	-28.7	129.3	0.61	73	1.62					
12	C	2	209	65	48.1	-29.0	100.8	0.49	71	1.02					
12	C	3	279	69	46.7	-29.2	130.3	0.57	57	1.58					
13	A	1	366	72	47.6	-28.9	174.0	0.44	871	1.61	0.0069	0.68	0.03	0.05	13
13	A	2	298	70	47.2	-28.7	140.4	0.48	1086	1.44	0.0077	0.76	0.04	0.06	15
13	A	3	252	67	47.9	-28.8	120.8	0.84	1077	2.12	0.0076	0.76	0.04	0.08	14
13	A	4	338	69	46.6	-28.8	157.5	0.84	924	2.83	0.0071	0.70	0.03	0.09	19
13	B	1	217	64	47.6	-29.3	103.1	0.68	631	1.48	0.0060	0.60	0.02	0.03	10
13	B	2	342	73	46.8	-28.6	160.0	0.51	891	1.73	0.0070	0.69	0.03	0.06	19
13	B	3	246	73	46.5	-28.5	114.4	0.65	1282	1.61	0.0084	0.83	0.05	0.08	14
13	B	4	313	73	47.4	-28.6	148.6	0.54	1091	1.69	0.0077	0.76	0.04	0.07	13
13	C	1	224	68	48.6	-28.9	108.9	0.54	646	1.21	0.0061	0.60	0.02	0.03	10
13	C	2	219	73	48.7	-28.9	106.4	0.52	850	1.13	0.0068	0.68	0.03	0.03	9
13	C	3	221	68	48.4	-28.5	106.9	0.59	884	1.30	0.0069	0.69	0.03	0.04	10
13	C	4	347	73	48.1	-28.5	167.0	0.54	897	1.89	0.0070	0.69	0.03	0.06	16
14	A	1	265	70	46.6	-29.0	123.7	0.98	1098	2.59	0.0077	0.77	0.01	0.02	4
14	A	4	227	73	47.4	-28.7	107.4	0.68	1358	1.55	0.0087	0.86	0.01	0.02	7
14	B	1	323	66	47.6	-28.8	153.5	0.58	1179	1.89	0.0080	0.79	0.01	0.02	3
14	B	2	245	71	46.9	-28.5	115.2	0.52	1763	1.29	0.0102	1.01	0.03	0.04	8
14	B	3	298	69	47.4	-28.7	141.0	0.62	1460	1.83	0.0090	0.90	0.02	0.03	5
14	B	4	230	70	47.0	-29.3	108.1	0.54	1944	1.25	0.0108	1.07	0.04	0.05	9
14	C	1	200	62	48.1	-29.1	96.3	0.70	1108	1.40	0.0077	0.77	0.01	0.01	3
14	C	2	223	62	48.1	-29.0	107.3	0.64	974	1.43	0.0073	0.72	0.00	0.01	1
14	C	3	233	60	47.0	-29.3	109.6	0.87	1112	2.04	0.0078	0.77	0.01	0.02	2
14	C	4	244	71	46.7	-29.0	114.2	0.58	1326	1.41	0.0086	0.85	0.02	0.03	4

2 Trunk

block	row	rep	2 DW (g)	2 % DW (%)	2 TOC (wt.% DW)	2 $\delta^{13}C$ (mUr)	2 CQ (g)	2 TN (wt.% DW)	2 $\delta^{15}N$ (mUr)	2 NQ (g)	2 R	2 A% (%)	2 RSA	2 NNP (g)	2 %P (%)
6	A	1	190	57	44.5	-27.7	84.6	0.66	23	1.26					
6	A	2	313	57	46.4	-28.0	145.3	0.68	8	2.12					
6	A	3	268	57	46.6	-27.9	124.8	0.53	8	1.42					
6	A	4	577	59	45.2	-27.1	260.4	0.53	50	1.79					
6	B	1	325	58	43.7	-27.2	141.8	0.61	19	1.98					
6	B	2	300	56	43.9	-27.9	131.6	0.50	8	1.50					
6	B	3	314	57	44.6	-27.8	139.9	0.64	11	2.00					
6	B	4	292	57	44.5	-27.4	130.0	0.55	21	1.62					
6	C	1	301	57	41.8	-27.3	125.7	0.48	10	1.45					
6	C	2	299	57	43.8	-26.9	131.2	0.48	10	1.44					
6	C	3	368	58	44.5	-27.2	163.7	0.59	5	2.15					
6	C	4	196	55	46.0	-27.5	89.9	0.49	36	0.97					
7	A	1	274	56	45.7	-27.7	125.3	0.50	1301	1.38	0.0085	0.84	0.05	0.07	35
7	A	2	342	56	45.8	-27.6	156.4	0.58	1771	1.97	0.0102	1.01	0.07	0.13	37
7	A	3	311	55	44.6	-27.2	138.7	0.59	1566	1.83	0.0094	0.93	0.06	0.11	40
7	A	4	205	57	44.9	-27.9	92.0	0.59	1630	1.21	0.0097	0.96	0.06	0.07	34
7	B	1	282	56	45.2	-28.0	127.5	0.58	1367	1.63	0.0087	0.86	0.05	0.08	36
7	B	2	302	57	44.2	-27.3	133.3	0.55	1636	1.65	0.0097	0.96	0.06	0.10	37
7	B	3	200	58	44.5	-27.7	89.3	0.57	1441	1.14	0.0090	0.89	0.05	0.06	36
7	B	4	229	57	45.4	-27.7	104.1	0.62	1310	1.41	0.0085	0.84	0.05	0.07	38
7	C	1	275	56	45.2	-27.0	124.4	0.44	1396	1.20	0.0088	0.87	0.05	0.06	43
7	C	2	301	56	45.7	-27.6	137.4	0.50	1699	1.50	0.0099	0.98	0.06	0.10	38
7	C	3	290	57	43.0	-27.4	124.8	0.56	1293	1.63	0.0084	0.84	0.05	0.08	37
7	C	4	228	57	45.6	-27.7	104.2	0.58	1477	1.33	0.0091	0.90	0.06	0.07	38
8	A	1	342	50	45.4	-29.5	155.3	0.55	108	1.89					
8	A	2	215	51	45.2	-29.6	97.3	0.38	30	0.81					
8	A	3	278	51	45.4	-29.7	126.3	0.44	31	1.21					
8	A	4	277	48	45.0	-29.5	124.7	0.42	46	1.16					
8	B	1	249	49	45.4	-29.2	112.9	0.44	42	1.10					
8	B	2	280	50	45.1	-29.2	126.4	0.41	17	1.16					
8	B	3	257	48	45.9	-29.2	117.9	0.47	18	1.21					
8	B	4	253	50	46.1	-29.2	116.8	0.43	19	1.08					
8	C	1	197	49	45.4	-29.5	89.2	0.39	36	0.77					
8	C	2	272	49	45.2	-29.3	122.9	0.38	17	1.03					
8	C	3	289	51	46.5	-29.0	134.4	0.35	18	1.02					
8	C	4	191	51	45.3	-29.1	86.7	0.39	141	0.75					
9	A	1	262	50	45.6	-29.3	119.5	0.41	1234	1.08	0.0082	0.81	0.05	0.05	11
9	A	3	276	49	45.4	-29.7	125.2	0.37	1996	1.02	0.0110	1.09	0.07	0.08	15
9	A	4	273	49	46.4	-28.1	126.6	0.40	1472	1.09	0.0091	0.90	0.05	0.06	11
9	B	1	251	50	45.8	-29.1	114.8	0.48	1188	1.20	0.0080	0.80	0.04	0.05	15
9	B	2	223	49	46.2	-29.0	102.9	0.40	1288	0.88	0.0084	0.83	0.05	0.04	10
9	B	3	265	48	46.0	-29.4	121.7	0.45	1285	1.18	0.0084	0.83	0.05	0.06	14
9	B	4	198	49	40.2	-28.8	79.5	0.41	1355	0.82	0.0087	0.86	0.05	0.04	11
9	C	1	286	50	46.2	-29.5	132.3	0.39	1064	1.13	0.0076	0.75	0.04	0.04	13
9	C	2	289	50	46.5	-29.2	134.5	0.36	1123	1.05	0.0078	0.77	0.04	0.04	12
9	C	3	222	50	45.6	-29.3	101.2	0.45	1217	1.00	0.0081	0.81	0.04	0.04	11
9	C	4	316	49	46.1	-29.1	145.7	0.47	942	1.49	0.0071	0.71	0.03	0.05	16
10	A	1	386	63	47.0	-29.1	181.1	0.35	153	1.36					
10	A	2	457	65	47.0	-29.0	215.0	0.34	59	1.56					
10	A	3	284	67	48.4	-27.6	137.7	0.25	60	0.72					
10	A	4	374	65	46.8	-28.9	174.9	0.30	31	1.12					
10	B	1	386	64	47.7	-28.6	184.0	0.28	30	1.09					
10	B	2	372	63	46.7	-28.9	173.7	0.34	58	1.25					
10	B	3	367	63	46.4	-29.1	170.6	0.33	25	1.20					
10	B	4	414	66	46.4	-28.9	192.3	0.31	38	1.29					
10	C	1	363	61	47.2	-28.8	171.4	0.35	66	1.29					
10	C	2	418	64	46.7	-29.1	195.6	0.31	46	1.31					
10	C	3	396	64	46.7	-28.7	184.9	0.32	32	1.26					
10	C	4	328	64	47.4	-28.2	155.8	0.34	28	1.10					
11	A	1	434	58	47.1	-28.5	204.5	0.43	713	1.88	0.0063	0.63	0.03	0.05	12
11	A	2	372	66	46.3	-28.8	172.1	0.31	1288	1.16	0.0084	0.83	0.05	0.05	11
11	A	3	317	64	46.1	-28.7	146.0	0.36	1199	1.15	0.0081	0.80	0.04	0.05	8
11	A	4	361	66	47.0	-28.3	169.5	0.37	1130	1.32	0.0078	0.78	0.04	0.05	13
11	B	1	446	61	46.3	-29.1	206.5	0.52	628	2.32	0.0060	0.59	0.02	0.05	11
11	B	2	383	63	47.7	-28.7	182.7	0.41	704	1.58	0.0063	0.62	0.02	0.04	7
11	B	3	444	61	46.3	-28.5	205.5	0.34	877	1.53	0.0069	0.69	0.03	0.05	8
11	B	4	423	64	47.6	-28.6	201.3	0.33	885	1.38	0.0069	0.69	0.03	0.04	11
11	C	1	360	63	46.9	-28.6	168.9	0.34	660	1.21	0.0061	0.61	0.02	0.03	8
11	C	2	405	61	47.4	-28.9	192.3	0.36	712	1.45	0.0063	0.63	0.03	0.04	7
11	C	3	354	63	47.2	-28.8	167.0	0.30	989	1.07	0.0073	0.73	0.04	0.04	9
11	C	4	398	62	46.4	-29.0	184.6	0.37	845	1.47	0.0068	0.67	0.03	0.04	8
12	A	1	365	63	46.1	-28.5	168.4	0.38	77	1.38					
12	A	2	415	61	46.4	-28.4	192.5	0.42	90	1.76					
12	A	3	383	62	47.1	-28.9	180.3	0.43	84	1.65					
12	A	4	409	62	47.7	-28.7	195.2	0.38	67	1.54					
12	B	1	345	61	46.2	-28.3	159.5	0.39	96	1.34					
12	B	2	394	60	46.6	-28.7	183.7	0.33	16	1.31					
12	B	3	366	59	46.2	-28.2	169.2	0.33	22	1.20					
12	B	4	358	62	46.3	-28.3	165.4	0.36	25	1.27					
12	C	1	422	60	47.0	-28.5	198.4	0.33	58	1.41					
12	C	2	301	60	45.7	-28.6	137.7	0.28	18	0.85					
12	C	3	396	61	45.7	-28.5	181.3	0.35	17	1.39					
13	A	1	411	61	47.1	-28.2	193.3	0.41	962	1.68	0.0072	0.72	0.04	0.06	16
13	A	2	353	61	47.0	-28.2	165.7	0.39	770	1.39	0.0065	0.65	0.03	0.04	10
13	A	3	434	59	46.5	-28.8	201.7	0.43	792	1.88	0.0066	0.65	0.03	0.05	9
13	A	4	323	61	46.4	-28.5	150.0	0.47	655	1.50	0.0061	0.60	0.02	0.04	7
13	B	1	382	59	47.5	-28.7	181.2	0.39	518	1.50	0.0056	0.55	0.02	0.03	8
13	B	2	381	62	47.8	-28.0	182.0	0.38	911	1.46	0.0070	0.70	0.03	0.05	16
13	B	3	380	61	47.4	-27.8	180.0	0.39	861	1.47	0.0068	0.68	0.03	0.05	9
13	B	4	417	62	46.7	-28.1	195.0	0.38	881	1.57	0.0069	0.69	0.03	0.05	10
13	C	1	318	60	47.8	-28.3	152.0	0.32	687	1.03	0.0062	0.62	0.02	0.03	9
13	C	2	336	61	46.6	-28.9	156.9	0.34	811	1.15	0.0067	0.66	0.03	0.03	9
13	C	3	400	61	47.9	-27.9	191.6	0.35	760	1.39	0.0065	0.64	0.03	0.04	9
13	C	4	424	63	47.4	-28.6	201.1	0.37	1025	1.55	0.0074	0.74	0.04	0.06	15
14	A	1	430	60	45.3	-28.8	194.9	0.47	1350	2.02	0.0086	0.86	0.02	0.05	7
14	A	4	285	62	46.2	-28.7	131.6	0.43	1811	1.21	0.0103	1.02	0.04	0.04	13
14	B	1	447	59	46.0	-28.6	205.5	0.41	1332	1.82	0.0086	0.85	0.02	0.04	5
14	B	2	343	60	45.7	-28.5	156.8	0.40	1754	1.36	0.0101	1.00	0.03	0.05	9
14	B	3	385	61	46.8	-28.7	180.1	0.39	1665	1.50	0.0098	0.97	0.03	0.05	7
14	B	4	305	60	45.5	-29.0	138.6	0.39	1875	1.20	0.0106	1.05	0.04	0.05	9
14	C	1	357	57	46.9	-29.0	167.1	0.41	992	1.46	0.0073	0.73	0.01	0.02	4
14	C	2	409	60	46.1	-29.1	188.3	0.38	1056	1.55	0.0076	0.75	0.01	0.02	4
14	C	3	390	58	46.4	-28.8	181.2	0.42	1251	1.64	0.0083	0.82	0.02	0.03	4
14	C	4	330	61	46.8	-28.6	154.4	0.39	1483	1.27	0.0091	0.90	0.03	0.04	6

Appendix II - Raw data

Vines excavated in 2018

Excavation in 2018 — Blocks 6-14

3 Canopy

block	row	rep	Excavation time	Fertilization treatment	Yield condition	3 DW	3 % DW	3 TOC	3 $\delta^{13}C$	3 CQ	3 TN	3 $\delta^{15}N$	3 NQ	3 R	3 A%	3 RSA	3 NNP	3 %P
						g	%	wt.% DW	mUr	g	wt.% DW	mUr	g		%		g	%
6	A	1	Bud burst	C	LYC													
6	A	2	Bud burst	C	HYC													
6	A	3	Bud burst	C	LYC													
6	A	4	Bud burst	C	LYC													
6	B	1	Bud burst	C	HYC													
6	B	2	Bud burst	C	LYC													
6	B	3	Bud burst	C	HYC													
6	B	4	Bud burst	C	HYC													
6	C	1	Bud burst	C	HYC													
6	C	2	Bud burst	C	HYC													
6	C	3	Bud burst	C	HYC													
6	C	4	Bud burst	C	LYC													
7	A	1	Bud burst	F17	LYC													
7	A	2	Bud burst	F17	LYC													
7	A	3	Bud burst	F17	LYC													
7	A	4	Bud burst	F17	LYC													
7	B	1	Bud burst	F17	HYC													
7	B	2	Bud burst	F17	HYC													
7	B	3	Bud burst	F17	LYC													
7	B	4	Bud burst	F17	LYC													
7	C	1	Bud burst	F17	HYC													
7	C	2	Bud burst	F17	HYC													
7	C	3	Bud burst	F17	HYC													
7	C	4	Bud burst	F17	HYC													
8	A	1	Flowering	C	LYC	295	18	45.2	-29.3	133.2	2.85	25	8.39					
8	A	2	Flowering	C	LYC	161	19	43.6	-29.7	70.2	2.22	15	3.57					
8	A	3	Flowering	C	HYC	267	18	41.9	-29.5	111.8	2.18	15	5.82					
8	A	4	Flowering	C	LYC	306	17	43.5	-29.3	132.9	2.19	33	6.68					
8	B	1	Flowering	C	HYC	242	18	44.5	-29.0	107.5	2.30	35	5.55					
8	B	2	Flowering	C	HYC	256	17	44.5	-28.7	113.9	2.01	12	5.14					
8	B	3	Flowering	C	HYC	275	17	44.1	-29.5	121.2	2.75	13	7.55					
8	B	4	Flowering	C	LYC	237	18	44.8	-29.5	106.2	2.62	17	6.20					
8	C	1	Flowering	C	LYC	169	17	43.2	-29.3	72.8	2.26	45	3.80					
8	C	2	Flowering	C	HYC	274	17	44.4	-28.9	121.9	2.29	13	6.29					
8	C	3	Flowering	C	HYC	227	18	43.9	-29.5	99.7	1.91	12	4.33					
8	C	4	Flowering	C	HYC	159	18	44.3	-29.9	70.6	2.09	111	3.33					
9	A	1	Flowering	F17	LYC	257	17	44.8	-29.2	115.1	2.09	1348	5.38	0.0086	0.86	0.05	0.27	60
9	A	3	Flowering	F17	LYC	208	17	43.5	-29.7	90.4	2.05	2180	4.26	0.0117	1.16	0.08	0.34	66
9	A	4	Flowering	F17	LYC	282	17	43.1	-29.7	121.5	2.19	1574	6.18	0.0095	0.94	0.06	0.36	68
9	B	1	Flowering	F17	LYC	215	18	43.0	-28.7	92.2	2.24	1202	4.81	0.0081	0.80	0.04	0.21	59
9	B	2	Flowering	F17	HYC	222	17	44.2	-28.7	98.1	1.99	1636	4.41	0.0097	0.96	0.06	0.27	64
9	B	3	Flowering	F17	LYC	212	16	44.5	-29.3	94.1	2.48	1326	5.24	0.0085	0.85	0.05	0.26	66
9	B	4	Flowering	F17	LYC	249	18	44.0	-29.0	109.5	2.18	1377	5.43	0.0087	0.87	0.05	0.28	72
9	C	1	Flowering	F17	HYC	244	19	44.0	-29.2	107.1	1.98	1308	4.82	0.0085	0.84	0.05	0.23	70
9	C	2	Flowering	F17	HYC	237	18	44.9	-29.6	106.5	2.07	1353	4.91	0.0086	0.86	0.05	0.24	69
9	C	3	Flowering	F17	HYC	216	18	44.0	-29.1	95.3	2.27	1578	4.92	0.0095	0.94	0.06	0.29	71
9	C	4	Flowering	F17	HYC	271	17	44.1	-28.7	119.3	2.04	960	5.53	0.0072	0.72	0.03	0.19	59
10	A	1	Veraison	C	LYC	582	32	47.0	-29.5	273.6	1.28	40	7.43					
10	A	2	Veraison	C	LYC	541	32	45.8	-29.4	248.0	1.35	15	7.30					
10	A	3	Veraison	C	LYC	195	34	46.3	-28.6	90.6	1.20	15	2.35					
10	A	4	Veraison	C	LYC	460	33	45.8	-29.4	210.7	1.10	18	5.04					
10	B	1	Veraison	C	LYC	436	31	45.7	-29.1	199.1	1.08	46	4.69					
10	B	2	Veraison	C	HYC	534	29	45.0	-29.1	240.0	1.38	16	7.37					
10	B	3	Veraison	C	HYC	490	29	45.6	-29.4	223.5	1.61	17	7.91					
10	B	4	Veraison	C	LYC	480	33	46.2	-29.0	221.5	1.11	40	5.33					
10	C	1	Veraison	C	HYC	531	27	44.8	-29.5	237.5	1.85	62	9.80					
10	C	2	Veraison	C	HYC	529	35	45.7	-29.5	241.7	0.92	14	4.85					
10	C	3	Veraison	C	HYC	500	32	44.3	-29.3	221.7	1.32	12	6.58					
10	C	4	Veraison	C	HYC	446	32	46.3	-29.4	206.1	1.15	24	5.12					
11	A	1	Veraison	F17	LYC	942	29	45.1	-29.1	424.3	1.02	598	9.59	0.0059	0.58	0.02	0.20	51
11	A	2	Veraison	F17	LYC	313	33	46.2	-29.6	144.6	0.83	2169	2.59	0.0116	1.15	0.08	0.21	41
11	A	3	Veraison	F17	LYC	503	34	46.7	-28.9	235.0	1.51	1578	7.57	0.0095	0.94	0.06	0.44	66
11	A	4	Veraison	F17	LYC	237	32	45.6	-28.9	107.8	1.67	1605	3.94	0.0096	0.95	0.06	0.23	54
11	B	1	Veraison	F17	HYC	720	24	44.8	-29.0	322.3	1.90	559	13.71	0.0057	0.57	0.02	0.27	55
11	B	2	Veraison	F17	HYC	586	27	45.3	-29.3	265.2	2.04	897	11.95	0.0070	0.69	0.03	0.38	69
11	B	3	Veraison	F17	HYC	639	31	45.7	-28.6	292.0	1.36	1188	8.66	0.0080	0.80	0.04	0.37	63
11	B	4	Veraison	F17	LYC	571	32	45.2	-29.0	258.1	1.11	1099	6.35	0.0077	0.77	0.04	0.25	65
11	C	1	Veraison	F17	HYC	411	31	46.6	-29.1	191.7	1.83	971	7.52	0.0072	0.72	0.03	0.26	72
11	C	2	Veraison	F17	HYC	667	30	45.4	-29.5	302.7	1.72	848	11.49	0.0068	0.67	0.03	0.35	66
11	C	3	Veraison	F17	LYC	416	30	45.6	-29.5	189.3	1.52	1288	6.33	0.0084	0.83	0.05	0.30	71
11	C	4	Veraison	F17	HYC	581	31	44.8	-29.6	260.1	1.28	1083	7.43	0.0077	0.76	0.04	0.29	53
12	A	1	Harvest	C	LYC	363	45	45.1	-28.5	163.6	0.66	34	2.38					
12	A	2	Harvest	C	LYC	716	42	44.2	-29.0	316.4	0.77	15	5.51					
12	A	3	Harvest	C	HYC	720	40	44.7	-28.9	321.8	0.96	15	6.92					
12	A	4	Harvest	C	HYC	510	43	45.5	-28.9	231.9	0.76	21	3.89					
12	B	1	Harvest	C	LYC	580	42	45.5	-28.0	264.0	0.77	32	4.45					
12	B	2	Harvest	C	HYC	637	38	44.4	-28.9	283.1	1.40	13	8.90					
12	B	3	Harvest	C	HYC	597	38	44.5	-28.9	265.7	0.95	19	5.66					
12	B	4	Harvest	C	LYC	669	43	44.9	-28.7	300.7	1.20	26	8.00					
12	C	1	Harvest	C	HYC	840	42	44.3	-28.9	371.7	0.82	86	6.90					
12	C	2	Harvest	C	LYC	406	39	43.7	-29.1	177.6	1.03	21	4.19					
12	C	3	Harvest	C	HYC	768	40	44.1	-28.9	338.3	0.80	18	6.13					
13	A	1	Harvest	F17	LYC	575	41	45.6	-28.9	262.3	0.80	1155	4.61	0.0079	0.79	0.04	0.20	52
13	A	2	Harvest	F17	HYC	661	41	44.9	-28.8	296.9	0.73	1015	4.81	0.0074	0.74	0.04	0.18	46
13	A	3	Harvest	F17	HYC	804	37	44.5	-29.4	358.0	1.30	817	10.46	0.0067	0.66	0.03	0.31	53
13	A	4	Harvest	F17	LYC	855	41	44.2	-28.9	378.0	1.24	710	10.63	0.0063	0.62	0.03	0.27	55
13	B	1	Harvest	F17	HYC	680	37	44.9	-29.6	305.5	1.12	632	7.64	0.0060	0.60	0.02	0.17	51
13	B	2	Harvest	F17	LYC	325	40	43.9	-29.4	142.6	0.94	1196	3.05	0.0081	0.80	0.04	0.13	45
13	B	3	Harvest	F17	LYC	646	38	44.8	-28.7	289.6	1.12	1111	7.27	0.0078	0.77	0.04	0.30	56
13	B	4	Harvest	F17	LYC	747	43	43.9	-29.4	327.6	0.98	1071	7.30	0.0076	0.76	0.04	0.29	57
13	C	1	Harvest	F17	HYC	442	41	44.7	-29.3	197.6	1.01	970	4.45	0.0072	0.72	0.04	0.16	58
13	C	2	Harvest	F17	HYC	501	39	43.2	-29.8	216.5	0.99	1352	4.99	0.0086	0.86	0.05	0.25	63
13	C	3	Harvest	F17	HYC	781	39	44.3	-29.0	346.3	0.85	965	6.63	0.0072	0.72	0.04	0.23	54
13	C	4	Harvest	F17	LYC	640	41	44.2	-29.2	283.0	0.68	1324	4.36	0.0085	0.85	0.05	0.21	54
14	A	1	Harvest	F17+18	HYC	1090	42	45.8	-28.8	499.0	0.88	1770	9.60	0.0102	1.01	0.03	0.30	46
14	A	4	Harvest	F17+18	LYC	467	42	44.9	-29.1	210.1	1.19	1952	5.56	0.0109	1.07	0.03	0.19	57
14	B	1	Harvest	F17+18	HYC	867	39	44.2	-29.3	383.2	1.55	1764	13.46	0.0102	1.01	0.03	0.42	51
14	B	2	Harvest	F17+18	LYC	560	42	44.8	-28.8	251.3	0.83	2978	4.66	0.0146	1.44	0.07	0.34	70
14	B	3	Harvest	F17+18	LYC	589	41	44.2	-29.0	260.3	1.08	2717	6.38	0.0137	1.35	0.06	0.40	60
14	B	4	Harvest	F17+18	LYC	488	41	44.8	-29.5	218.5	0.92	2563	4.47	0.0131	1.29	0.06	0.26	49
14	C	1	Harvest	F17+18	HYC	696	30	44.6	-29.5	310.3	1.31	1595	9.15	0.0095	0.95	0.02	0.23	58
14	C	2	Harvest	F17+18	HYC	848	41	44.5	-29.3	377.7	0.92	1996	7.81	0.0110	1.09	0.04	0.32	55
14	C	3	Harvest	F17+18	HYC	905	38	44.2	-28.7	400.4	0.96	2283	8.70	0.0121	1.19	0.05	0.45	48
14	C	4	Harvest	F17+18	HYC	550	41	44.8	-29.4	246.4	1.15	2332	6.30	0.0123	1.21	0.05	0.34	54

4 Pomace and 5 Must (blocks 6–9: no data)

block	row	rep	4 DW	4 % DW	4 TOC	4 $\delta^{13}C$	4 CQ	4 TN	4 $\delta^{15}N$	4 NQ	4 R	4 A%	4 RSA	4 NNP	4 %P	5 DW	5 % DW	5 TOC	5 $\delta^{13}C$	5 CQ	5 TN	5 $\delta^{15}N$	5 NQ	5 R	5 A%	5 RSA	5 NNP	5 %P
			g	%	wt.% DW	mUr	g	wt.% DW	mUr	g		%		g	%	g	%	wt.% DW	mUr	g	wt.% DW	mUr	g		%		g	%
10	A	1	51	21	44.2	-30.0	22.7	0.98	62	0.50						89	15	36.4	-28.4	32.3	0.27	43	0.24					
10	A	2	62	22	44.7	-30.3	27.8	0.94	20	0.58						118	15	37.1	-28.4	43.9	0.22	11	0.26					
10	A	3	25	26	45.0	-28.2	11.1	0.88	25	0.22						48	15	36.9	-26.2	17.6	0.15	9	0.07					
10	A	4	50	21	43.3	-29.5	21.7	0.83	23	0.42						74	15	38.7	-27.3	28.7	0.22	14	0.16					
10	B	1	72	23	45.6	-29.8	32.8	1.02	37	0.73						139	13	38.0	-27.5	52.6	0.16	27	0.23					
10	B	2	115	20	45.8	-29.7	52.5	1.07	17	1.23						159	12	37.4	-27.2	59.6	0.24	296	0.39					
10	B	3	128	19	44.6	-29.5	57.0	1.03	19	1.32						159	13	37.5	-27.4	59.6	0.30	194	0.48					
10	B	4	95	20	44.2	-29.7	42.0	0.97	26	0.92						119	13	39.8	-27.3	47.4	0.17	41	0.20					
10	C	1	247	15	44.6	-30.1	110.2	1.21	61	3.00						151	10	36.5	-27.8	54.9	0.43	279	0.65					
10	C	2	138	20	46.0	-30.2	63.4	1.07	16	1.47						188	12	36.2	-27.7	67.9	0.22	17	0.42					
10	C	3	116	20	45.7	-29.2	53.2	1.01	12	1.17						164	12	37.6	-26.5	61.6	0.24	45	0.39					
10	C	4	114	21	46.2	-29.2	52.5	1.03	28	1.17						173	13	37.4	-26.1	64.7	0.26	439	0.45					
11	A	1	62	22	44.9	-29.6	27.7	1.15	700	0.71	0.0062	0.62	0.02	0.02	4	113	14	36.4	-27.9	41.3	0.40	890	0.45	0.0069	0.69	0.03	0.01	4
11	A	2	25	24	44.9	-30.3	11.2	0.76	2006	0.19	0.0111	1.09	0.07	0.01	3	47	15	36.7	-28.4	17.2	0.11	1914	0.05	0.0107	1.06	0.07	0.00	1
11	A	3	80	20	43.5	-28.5	34.7	0.94	1374	0.75	0.0087	0.87	0.05	0.04	6	99	15	36.8	-26.9	36.5	0.25	2016	0.25	0.0111	1.10	0.07	0.02	3
11	A	4	41	24	43.9	-28.8	17.9	0.90	1421	0.37	0.0089	0.88	0.05	0.02	4	61	13	37.6	-26.4	23.0	0.23	1293	0.14	0.0084	0.84	0.05	0.01	2
11	B	1	210	21	47.6	-30.1	100.1	1.55	568	3.26	0.0058	0.57	0.02	0.06	13	287	10	37.7	-27.9	108.2	0.65	534	1.86	0.0056	0.56	0.02	0.03	7
11	B	2	140	20	44.9	-30.3	62.8	1.16	806	1.62	0.0066	0.66	0.03	0.05	8	178	12	37.8	-28.5	67.3	0.30	745	0.54	0.0064	0.64	0.03	0.01	3
11	B	3	172	18	45.9	-29.3	78.8	1.00	1037	1.72	0.0075	0.74	0.04	0.06	11	158	13	36.9	-27.7	58.3	0.22	965	0.35	0.0072	0.72	0.03	0.01	2
11	B	4	53	21	43.7	-29.2	23.0	0.89	903	0.47	0.0070	0.69	0.03	0.02	4	65	15	37.6	-27.4	24.5	0.23	827	0.15	0.0067	0.67	0.03	0.00	1
11	C	1	135	19	45.3	-30.3	61.3	0.97	793	1.31	0.0066	0.65	0.03	0.04	10	160	12	36.3	-28.0	58.1	0.18	715	0.29	0.0063	0.63	0.03	0.01	2
11	C	2	203	20	46.2	-30.1	94.0	1.20	817	2.44	0.0067	0.66	0.03	0.07	14	233	11	37.2	-27.6	86.8	0.32	773	0.75	0.0065	0.65	0.03	0.02	4
11	C	3	48	22	44.6	-30.4	21.4	0.93	1256	0.45	0.0083	0.82	0.05	0.02	5	78	13	37.9	-27.8	29.6	0.23	1185	0.18	0.0080	0.80	0.04	0.01	2
11	C	4	197	19	44.9	-30.7	88.6	1.11	944	2.18	0.0071	0.71	0.03	0.07	13	197	11	35.9	-28.6	70.8	0.31	890	0.62	0.0069	0.69	0.03	0.02	4
12	A	1	80	31	43.7	-29.3	34.8	1.00	21	0.80						252	21	38.7	-26.3	97.7	0.18	93	0.46					
12	A	2	69	31	43.6	-28.4	30.3	1.15	29	0.80						243	23	38.5	-28.1	93.5	0.15	113	0.36					
12	A	3	209	28	42.4	-29.7	88.5	0.88	12	1.83						499	22	39.2	-28.4	195.5	0.15	124	0.74					
12	A	4	133	29	43.8	-30.4	58.3	0.92	19	1.23						437	21	36.3	-28.7	158.7	0.14	63	0.60					
12	B	1	75	27	41.6	-29.3	31.0	0.78	31	0.58						144	20	38.1	-27.1	54.8	0.12	41	0.17					
12	B	2	272	29	44.7	-30.4	121.7	1.08	13	2.95						779	19	38.6	-28.3	300.6	0.22	72	1.69					
12	B	3	309	26	43.2	-30.1	133.4	0.97	15	3.01						655	19	37.1	-28.3	242.6	0.16	32	1.02					
12	B	4	54	30	42.8	-29.7	22.9	0.90	14	0.48						201	21	37.6	-27.4	75.7	0.16	61	0.32					
12	C	1	233	28	44.1	-30.2	102.5	1.01	67	2.35						788	20	38.0	-28.0	299.7	0.18	119	1.41					
12	C	2	123	29	42.3	-30.3	52.0	0.84	18	1.03						435	22	37.8	-28.3	164.3	0.11	52	0.46					
12	C	3	210	26	42.5	-29.4	89.3	0.81	15	1.70						468	22	37.8	-27.7	176.6	0.17	38	0.78					
13	A	1	112	29	40.4	-28.9	45.4	0.57	1204	0.64	0.0081	0.80	0.04	0.03	7	167	24	38.8	-27.3	64.9	0.14	1130	0.23	0.0078	0.78	0.04	0.01	2
13	A	2	187	27	42.6	-29.9	79.9	0.74	1028	1.38	0.0075	0.74	0.04	0.05	13	454	22	37.5	-28.7	170.0	0.15	970	0.68	0.0072	0.72	0.04	0.02	6
13	A	3	189	28	42.5	-30.1	80.3	0.95	829	1.78	0.0067	0.67	0.03	0.05	9	599	21	38.1	-28.6	228.5	0.27	934	1.61	0.0071	0.71	0.03	0.05	9
13	A	4	106	30	43.8	-29.8	46.5	0.98	679	1.05	0.0062	0.61	0.02	0.03	5	340	20	37.2	-28.3	126.5	0.22	699	0.74	0.0062	0.62	0.03	0.02	4
13	B	1	268	28	43.6	-30.2	116.8	0.97	615	2.59	0.0059	0.59	0.02	0.06	17	831	20	38.5	-28.4	319.8	0.19	641	1.62	0.0060	0.60	0.02	0.04	11
13	B	2	43	29	41.9	-29.5	18.0	0.69	1244	0.30	0.0082	0.82	0.05	0.01	5	273	24	36.8	-27.7	100.5	0.12	1612	0.33	0.0096	0.95	0.06	0.02	7
13	B	3	159	27	42.2	-29.0	67.1	0.83	1089	1.31	0.0077	0.76	0.04	0.05	10	311	22	38.6	-27.5	120.1	0.19	1891	0.59	0.0106	1.05	0.07	0.04	8
13	B	4	69	27	41.3	-29.0	28.4	0.87	911	0.60	0.0070	0.70	0.03	0.02	4	336	23	37.9	-27.3	127.5	0.18	1540	0.61	0.0093	0.93	0.06	0.03	7
13	C	1	178	27	43.2	-29.9	76.8	0.78	875	1.38	0.0069	0.68	0.03	0.04	16	484	20	39.3	-27.6	190.2	0.11	844	0.51	0.0068	0.67	0.03	0.02	6
13	C	2	135	28	42.7	-30.2	57.7	0.76	1041	1.03	0.0075	0.74	0.04	0.04	10	454	22	37.6	-28.3	170.7	0.12	1586	0.56	0.0095	0.94	0.06	0.03	8
13	C	3	293	26	42.0	-29.7	123.3	0.81	953	2.37	0.0072	0.71	0.03	0.08	19	768	22	39.4	-27.7	302.5	0.12	872	0.93	0.0069	0.68	0.03	0.03	7
13	C	4	49	31	43.0	-30.0	21.2	0.90	1130	0.44	0.0078	0.78	0.04	0.02	5	258	24	37.3	-28.1	96.5	0.16	1480	0.41	0.0091	0.90	0.05	0.02	6
14	A	1	267	29	42.5	-29.8	113.5	1.08	2531	2.88	0.0130	1.28	0.06	0.18	28	618	22	38.4	-28.5	237.3	0.19	3077	1.18	0.0150	1.48	0.08	0.10	15
14	A	4	68	28	41.7	-28.5	28.3	0.88	3178	0.60	0.0154	1.51	0.08	0.05	15	132	24	37.8	-27.0	50.0	0.19	3761	0.25	0.0175	1.72	0.09	0.02	7
14	B	1	246	29	43.1	-29.5	106.0	1.11	2435	2.74	0.0126	1.25	0.06	0.16	20	873	21	37.3	-27.7	325.9	0.27	2881	2.40	0.0143	1.41	0.07	0.18	21
14	B	2	43	30	41.2	-29.8	17.7	0.95	3339	0.41	0.0160	1.57	0.09	0.04	7	153	24	38.0	-27.8	58.3	0.17	3962	0.26	0.0182	1.79	0.10	0.03	5
14	B	3	119	29	43.8	-29.3	52.2	0.98	3430	1.17	0.0163	1.60	0.09	0.11	16	403	22	37.3	-27.3	150.4	0.17	4199	0.70	0.0191	1.88	0.11	0.08	12
14	B	4	133	27	41.2	-29.4	54.8	0.82	3726	1.10	0.0174	1.71	0.10	0.11	22	279	23	38.2	-27.8	106.4	0.21	4235	0.58	0.0192	1.89	0.11	0.06	12
14	C	1	150	27	43.6	-29.7	65.4	1.24	1856	1.86	0.0105	1.04	0.04	0.07	18	524	19	37.6	-27.7	197.3	0.29	2112	1.52	0.0114	1.13	0.04	0.07	17
14	C	2	216	26	43.2	-29.8	93.4	0.96	2462	2.08	0.0127	1.26	0.06	0.13	22	599	20	37.5	-27.8	224.9	0.21	3055	1.24	0.0149	1.47	0.08	0.10	18
14	C	3	284	27	42.9	-29.7	121.6	1.20	2626	3.39	0.0133	1.32	0.07	0.23	24	741	19	37.6	-27.6	278.6	0.33	3179	2.42	0.0154	1.51	0.09	0.21	22
14	C	4	193	27	43.3	-30.0	83.8	0.98	2822	1.89	0.0141	1.39	0.07	0.14	23	533	21	37.1	-27.7	197.8	0.16	3442	0.86	0.0163	1.61	0.10	0.08	13

Appendix II - Raw data

Vines excavated in 2018

Excavation in 2018 Blocks 6-14						6 Grapes													7 Whole plant													8 Bunch thinning												
						g	%	wt.% DW	mUr	g	wt.% DW	mUr	g		%		g	%	g	%	wt.% DW	mUr	g	wt.% DW	mUr	g		%		g	%	g	%	wt.% DW	mUr	g	wt.% DW	mUr	g		%		g	%
block	row	rep	Excavation time	Fertilization treatment	Yield condition	6 DW	6 % DW	6 TOC	6 $\delta^{13}C$	6 CQ	6 TN	6 $\delta^{15}N$	6 NQ	6 R	6 A%	6 RSA	6 NNP	6 %P	7 DW	7 % DW	7 TOC	7 $\delta^{13}C$	7 CQ	7 TN	7 $\delta^{15}N$	7 NQ	7 R	7 A%	7 RSA	7 NNP	7 %P	8 DW	8 % DW	8 TOC	8 $\delta^{13}C$	8 CQ	8 TN	8 $\delta^{15}N$	8 NQ	8 R	8 A%	8 RSA	8 NNP	8 %P
6	A	1	Bud burst	C	LYC														457	62	45.2	-27.9	206.7	0.75	24	3.44																		
6	A	2	Bud burst	C	HYC														585	60	46.2	-27.9	270.3	0.79	8	4.59																		
6	A	3	Bud burst	C	LYC														733	62	46.1	-27.8	337.8	0.63	8	4.66																		
6	A	4	Bud burst	C	LYC														1237	60	45.2	-27.3	558.5	0.67	51	8.26																		
6	B	1	Bud burst	C	HYC														626	59	44.7	-27.4	280.2	0.78	20	4.87																		
6	B	2	Bud burst	C	LYC														524	59	45.0	-28.1	235.7	0.64	10	3.37																		
6	B	3	Bud burst	C	HYC														612	61	45.6	-27.8	279.2	0.78	10	4.77																		
6	B	4	Bud burst	C	HYC														549	60	45.2	-27.7	248.6	0.66	19	3.65																		
6	C	1	Bud burst	C	HYC														574	60	43.2	-27.5	248.0	0.61	11	3.51																		
6	C	2	Bud burst	C	HYC														618	59	44.7	-27.2	276.4	0.62	10	3.83																		
6	C	3	Bud burst	C	HYC														637	61	44.8	-27.3	285.7	0.75	5	4.76																		
6	C	4	Bud burst	C	LYC														406	58	45.2	-27.6	183.5	0.71	36	2.88																		
7	A	1	Bud burst	F17	LYC														581	59	44.6	-28.0	259.1	0.65	1354	3.78	0.0087	0.86	0.05	0.19	100													
7	A	2	Bud burst	F17	LYC														608	59	44.6	-27.7	271.4	0.79	1902	4.78	0.0107	1.06	0.07	0.34	100													
7	A	3	Bud burst	F17	LYC														577	58	44.5	-27.6	256.8	0.76	1610	4.39	0.0096	0.95	0.06	0.26	100													
7	A	4	Bud burst	F17	LYC														465	60	45.5	-28.0	211.3	0.75	1657	3.47	0.0098	0.97	0.06	0.21	100													
7	B	1	Bud burst	F17	HYC														591	58	45.2	-28.2	267.1	0.70	1478	4.17	0.0091	0.90	0.06	0.23	100													
7	B	2	Bud burst	F17	HYC														555	59	45.0	-27.6	249.8	0.68	1883	3.76	0.0106	1.05	0.07	0.26	100													
7	B	3	Bud burst	F17	LYC														394	60	44.6	-27.8	175.6	0.73	1588	2.86	0.0095	0.94	0.06	0.17	100													
7	B	4	Bud burst	F17	LYC														444	58	45.7	-27.9	203.0	0.72	1489	3.22	0.0091	0.91	0.06	0.18	100													
7	C	1	Bud burst	F17	HYC														537	59	45.5	-27.4	244.1	0.55	1338	2.95	0.0086	0.85	0.05	0.15	100													
7	C	2	Bud burst	F17	HYC														562	60	45.3	-27.7	254.6	0.66	1791	3.73	0.0103	1.02	0.07	0.25	100													
7	C	3	Bud burst	F17	HYC														566	61	44.1	-27.8	249.6	0.75	1325	4.23	0.0085	0.85	0.05	0.21	100													
7	C	4	Bud burst	F17	HYC														437	59	45.6	-28.1	199.5	0.80	1492	3.48	0.0092	0.91	0.06	0.19	100													
8	A	1	Flowering	C	LYC														919	39	45.7	-29.1	420.3	1.44	61	13.23																		
8	A	2	Flowering	C	LYC														550	42	45.1	-29.6	248.3	0.99	21	5.44																		
8	A	3	Flowering	C	HYC														859	48	44.4	-29.7	381.4	1.11	19	9.53																		
8	A	4	Flowering	C	LYC														768	36	44.3	-29.5	340.3	1.24	38	9.50																		
8	B	1	Flowering	C	HYC														647	37	45.6	-29.2	294.8	1.24	36	8.02																		
8	B	2	Flowering	C	HYC														784	40	45.2	-29.1	354.3	1.08	14	8.50																		
8	B	3	Flowering	C	HYC														693	36	45.3	-29.4	313.7	1.48	15	10.24																		
8	B	4	Flowering	C	LYC														693	39	45.8	-29.3	317.1	1.30	19	8.99																		
8	C	1	Flowering	C	LYC														549	38	45.2	-29.4	248.0	1.05	37	5.76																		
8	C	2	Flowering	C	HYC														679	36	45.0	-29.2	305.7	1.27	15	8.60																		
8	C	3	Flowering	C	HYC														680	39	46.0	-29.3	312.6	0.94	15	6.37																		
8	C	4	Flowering	C	HYC														496	41	45.4	-29.4	225.5	0.99	119	4.93																		
9	A	1	Flowering	F17	LYC														748	37	45.7	-29.4	342.1	1.11	1476	8.32	0.0091	0.90	0.05	0.45	100													
9	A	3	Flowering	F17	LYC														679	39	45.1	-29.8	305.9	0.95	2143	6.45	0.0116	1.14	0.08	0.52	100													
9	A	4	Flowering	F17	LYC														748	37	45.3	-29.2	339.3	1.17	1638	8.78	0.0097	0.96	0.06	0.53	100													
9	B	1	Flowering	F17	LYC														685	39	45.0	-29.2	308.2	1.12	1319	7.66	0.0085	0.85	0.05	0.36	100													
9	B	2	Flowering	F17	HYC														623	36	45.5	-29.0	283.6	1.10	1597	6.85	0.0095	0.95	0.06	0.42	100													
9	B	3	Flowering	F17	LYC														596	37	45.7	-29.5	272.2	1.26	1408	7.54	0.0089	0.88	0.05	0.39	100													
9	B	4	Flowering	F17	LYC														625	37	43.3	-29.0	270.9	1.22	1345	7.63	0.0086	0.85	0.05	0.38	100													
9	C	1	Flowering	F17	HYC														659	38	45.2	-29.5	297.9	1.05	1250	6.95	0.0083	0.82	0.05	0.33	100													
9	C	2	Flowering	F17	HYC														733	39	45.9	-29.5	336.1	1.00	1257	7.31	0.0083	0.82	0.05	0.35	100													
9	C	3	Flowering	F17	HYC														600	38	50.4	-29.3	302.3	1.18	1477	7.06	0.0091	0.90	0.05	0.40	100													
9	C	4	Flowering	F17	HYC														749	37	45.6	-29.1	341.4	1.17	1024	8.77	0.0074	0.74	0.04	0.33	100													
10	A	1	Veraison	C	LYC	140	17	39.2	-29.0	55.0	0.53	50	0.75						1439	46	46.0	-29.3	661.5	0.79	71	11.42						52	9	44.7	-29.4	23.3	1.41	41	0.73					
10	A	2	Veraison	C	LYC	181	18	39.7	-29.1	71.8	0.46	14	0.84						1523	46	45.5	-29.2	692.8	0.79	27	12.05						78	10	44.9	-29.1	34.9	1.50	14	1.16					
10	A	3	Veraison	C	LYC	72	19	39.6	-26.9	28.7	0.40	14	0.29						811	57	46.4	-28.1	375.7	0.58	29	4.66						20	14	44.9	-28.3	8.9	1.14	20	0.23					
10	A	4	Veraison	C	LYC	124	17	40.6	-28.2	50.5	0.47	18	0.58						1333	51	45.5	-29.1	605.8	0.66	20	8.83						49	10	45.6	-29.1	22.2	1.37	19	0.67					
10	B	1	Veraison	C	LYC	210	16	40.6	-28.3	85.4	0.46	31	0.96						1255	45	45.6	-28.8	572.3	0.64	35	7.99						27	11	44.9	-29.2	12.1	1.45	36	0.39					
10	B	2	Veraison	C	HYC	274	15	40.9	-28.2	112.1	0.59	179	1.62						1430	42	45.0	-28.9	643.5	0.82	57	11.72						25	10	44.5	-30.2	11.3	1.63	16	0.41					
10	B	3	Veraison	C	HYC	287	15	40.7	-28.3	116.5	0.63	116	1.80						1418	43	44.9	-29.1	636.4	0.89	38	12.59						28	10	44.1	-29.8	12.4	1.62	16	0.45					
10	B	4	Veraison	C	LYC	214	16	41.7	-28.4	89.4	0.52	34	1.12						1414	48	45.7	-28.9	646.7	0.66	35	9.27						21	11	44.5	-29.3	9.4	1.33	30	0.28					
10	C	1	Veraison	C	HYC	398	13	41.5	-29.2	165.1	0.92	143	3.65						1489	38	44.9	-29.2	668.6	1.07	85	15.91						1	11	44.1	-30.2	0.4	1.87	64	0.02					
10	C	2	Veraison	C	HYC	325	15	40.3	-28.8	131.3	0.58	17	1.88						1590	46	45.2	-29.2	717.9	0.60	23	9.47						11	10	44.6	-29.9	5.0	1.44	14	0.16					
10	C	3	Veraison	C	HYC	280	15	41.0	-27.6	114.9	0.56	31	1.56						1541	46	45.3	-28.6	698.0	0.73	19	11.18						6	10	43.8	-30.1	2.4	1.42	11	0.08					
10	C	4	Veraison	C	HYC	287	16	40.9	-27.3	117.2	0.57	276	1.63						1345	45	45.6	-28.4	612.8	0.69	77	9.34						7	10	44.8	-29.2	2.9	1.54	30	0.10					
11	A	1	Veraison	F17	LYC	175	17	39.4	-28.5	69.0	0.66	823	1.16	0.0067	0.67	0.03	0.03	9	1902	39	45.2	-28.9	860.4	0.84	728	15.92	0.0064	0.63	0.03	0.40	100	72	9	45.1	-28.6	32.3	1.86	793	1.33	0.0066	0.65	0.03	0.04	9
11	A	2	Veraison	F17	LYC	72	18	39.6	-29.0	28.4	0.33	1946	0.24	0.0108	1.07	0.07	0.02	3	1200	51	45.9	-29.2	550.9	0.61	1680	7.28	0.0099	0.98	0.06	0.50	100	124	12	45.7	-29.7	56.6	1.38	2288	1.70	0.0121	1.19	0.08	0.14	29
11	A	3	Veraison	F17	LYC	179	17	39.8	-27.6	71.3	0.56	1730	1.00	0.0100	0.99	0.06	0.06	10	1398	47	45.5	-28.6	635.8	0.87	1435	12.16	0.0090	0.89	0.05	0.67	100	55	11	44.7	-29.0	24.7	1.47	1581	0.81	0.0095	0.94	0.06	0.05	7
11	A	4	Veraison	F17	LYC	102	17	40.1	-27.4	40.9	0.50	1344	0.51	0.0086	0.85	0.05	0.03	6	1120	55	45.7	-28.5	511.6	0.74	1258	8.32	0.0083	0.82	0.05	0.43	100	43	11	45.0	-29.4	19.4	1.45	1664	0.63	0.0098	0.97	0.06	0.04	9
11	B	1	Veraison	F17	HYC	497	15	41.9	-28.8	208.3	1.03	548	5.12	0.0057	0.57	0.02	0.10	20	1910	35	44.7	-29.0	854.7	1.23	601	23.53	0.0059	0.59	0.02	0.48	100	3	10	43.5	-28.1	1.3	2.25	622	0.07	0.0060	0.59	0.02	0.00	0
11	B	2	Veraison	F17	HYC	318	15	40.9	-29.3	130.2	0.68	772	2.16	0.0065	0.65	0.03	0.06	11	1544	39	45.4	-29.1	701.3	1.16	815	17.95	0.0067	0.66	0.03	0.55	100	33	9	45.0	-29.8	14.9	1.78	916	0.59	0.0070	0.70	0.03	0.02	3
11	B	3	Veraison	F17	HYC	330	16	41.6	-28.5	137.1	0.63	1003	2.07	0.0074	0.73	0.04	0.07	13	1758	41	45.2	-28.5	795.0	0.83	1057	14.67	0.0076	0.75	0.04	0.60	100	48	10	44.6	-29.4	21.3	1.54	1244	0.74	0.0082	0.82	0.05	0.03	6
11	B	4	Veraison	F17	LYC	118	17	40.4	-28.2	47.5	0.53	861	0.62	0.0068	0.68	0.03	0.02	5	1440	47	45.8	-28.9	659.5	0.73	971	10.47	0.0072	0.72	0.03	0.39	100	45	10	44.8	-29.7	20.0	1.53	1191	0.68	0.0081	0.80	0.04	0.03	8
11	C	1	Veraison	F17	HYC	295	15	40.4	-29.0	119.4	0.54	750	1.61	0.0064	0.64	0.03	0.04	12	1341	44	45.2	-28.9	606.4	0.87	757	11.69	0.0065	0.64	0.03	0.36	100	7	12	44.7	-29.4	2.9	1.50	954	0.10	0.0072	0.71	0.03	0.00	1
11	C	2	Veraison	F17	HYC	436	15	41.4	-28.8	180.8	0.73	793	3.20	0.0066	0.65	0.03	0.09	17	1775	39	45.0	-29.1	797.9	1.01	792	17.89	0.0066	0.65	0.03	0.53	100	16	10	44.6	-29.5	7.3	1.73	914	0.28	0.0070	0.70	0.03	0.01	2
11	C	3	Veraison	F17	LYC	126	16	40.4	-28.8	50.9	0.50	1212	0.63	0.0081	0.81	0.04	0.03	7	1187	48	45.9	-29.1	544.8	0.80	1115	9.49	0.0078	0.77	0.04	0.42	100	11	10	44.5	-29.7	4.8	1.62	1398	0.18	0.0088	0.87	0.05	0.01	2
11	C	4	Veraison	F17	HYC	394	15	40.4	-29.7	159.4	0.71	917	2.80	0.0070	0.70	0.03	0.09	17	1768	40	44.5	-29.4	786.8	0.85	964	15.08	0.0072	0.72	0.03	0.55	100	100	30	44.7	-30.0	44.8	1.61	1052	1.61	0.0075	0.75	0.04	0.06	11
12	A	1	Harvest	C	LYC	332	24	39.9	-27.1	132.5	0.38	76	1.26						1512	51	44.3	-28.2	669.6	0.51	52	7.72						59	11	44.7	-29.0	26.5	1.50	33	0.89					
12	A	2	Harvest	C	LYC	313	24	39.6	-28.2	123.8	0.37	95	1.16						1861	48	44.6	-28.7	830.7	0.62	46	11.48						69	9	45.5	-29.7	31.5	1.76	16	1.22					
12	A	3	Harvest	C	HYC	708	24	40.1	-28.8	284.1	0.36	91	2.57						2201	42	43.9	-28.9	966.3	0.62	53	13.66						60	9	45.4	-29.4	27.2	1.58	11	0.95					
12	A	4	Harvest	C	HYC	570	23	38.1	-29.1	217.1	0.32	52	1.83						1940	46	44.1	-28.9	856.4	0.49	44	9.53						39	10	45.5	-29.7	17.6	1.56	16	0.60					
12	B	1	Harvest	C	LYC	218	22	39.3	-27.8	85.8	0.34	38	0.75						1499	49	45.0	-28.2	675.1	0.58	51	8.72						48	10	45.1	-29.5	21.8	1.56	34	0.76					
12	B	2	Harvest	C	HYC	1051	21	40.2	-28.8	422.3	0.44	57	4.64						1981	36	43.2	-28.9	855.3	0.58	39	11.50						42	9	45.8	-29.7	19.4	1.55	11	0.66					
12	B	3	Harvest	C	HYC	964	21	39.0	-28.9	376.0	0.42	27	4.03						2214	37	42.9	-28.8	949.7	0.57	27	12.60						15	11	45.4	-30.0	6.9	1.60	26	0.24					
12	B	4	Harvest	C	LYC	255	23	38.7	-27.9	98.6	0.32	51	0.81						1695	48	44.8	-28.5	760.0	0.76	34	12.93						55	10	44.9	-29.1	24.6	1.57	28	0.86					
12	C	1	Harvest	C	HYC	1020	22	39.4	-28.5	402.2	0.37	107	3.76						2558	39	43.2	-28.6	1105.9	0.54	88	13.83						10	9	45.0	-29.3	4.4	1.48	72	0.14					
12	C	2	Harvest	C	LYC	558	23	38.8	-28.7	216.4	0.27	44	1.50						1496	40	42.9	-28.9	642.3	0.53	36	7.88						22	11	45.1	-29.8	9.7	1.51	18	0.33					
12	C	3	Harvest	C	HYC	678	24	39.2	-28.3	266.0	0.37	31	2.48						2150	42	43.2	-28.7	928.8	0.56	27	12.01						29	10	45.1	-29.3	13.0	1.53	15	0.44					
13	A	1	Harvest	F17	LYC	280	26	39.4	-27.9	110.3	0.31	1160	0.86	0.0079	0.79	0.04	0.04	10	1674	49	45.3	-28.6	759.0	0.56	1054	9.42	0.0076	0.75	0.04	0.38	100	42	10	44.8	-29.0	19.0	1.54	1437	0.66	0.0090	0.89	0.05	0.03	9
13	A	2	Harvest	F17	HYC	641	23	39.0	-29.0	249.9	0.32	987	2.06	0.0073	0.73	0.04	0.07	19	2009	42	43.7	-28.8	878.2	0.52	979	10.53	0.0073	0.72	0.04	0.39	100	56	9	45.0	-29.8	25.3	1.49	1225	0.84	0.0082	0.81	0.04	0.04	10
13	A	3	Harvest	F17	HYC	788	22	39.2	-29.0	308.8	0.43	909	3.40	0.0070	0.70	0.03	0.11	19	2329	39	43.5	-29.1	1011.9	0.80	874	18.69	0.0069	0.68	0.03	0.58	100	51	9	44.8	-29.0	22.7	1.64	949	0.83	0.0072	0.71	0.03	0.03	5
13	A	4	Harvest	F17	LYC	446	23	38.8	-28.6	173.0	0.40	695	1.79	0.0062	0.62	0.02	0.04	9	2063	43	43.8	-28.7	903.0	0.89	739	18.44	0.0064	0.64	0.03	0.50	100	100	9	44.6	-28.7	44.6	1.68	826	1.68	0.0067	0.67	0.03	0.05	10
13	B	1	Harvest	F17	HYC	1099	22	39.7	-28.8	436.6	0.38	634	4.21	0.0060	0.60	0.02	0.10	28	2404	36	43.2	-29.1	1038.2	0.64	616	15.27	0.0059	0.59	0.02	0.34	100	26	10	45.2	-29.5	11.9	1.67	732	0.44	0.0064	0.63	0.03	0.01	3
13	B	2	Harvest	F17	LYC	316	24	37.5	-27.9	118.5	0.20	1562	0.63	0.0094	0.93	0.06	0.04	12	1396	50	44.2	-28.5	617.8	0.52	1133	7.33	0.0078	0.78	0.04	0.30	100	33	10	45.0	-29.5	14.8	1.39	1474	0.46	0.0091	0.90	0.05	0.02	8
13	B	3	Harvest	F17	LYC	470	24	39.8	-28.0	187.2	0.40	1620	1.90	0.0096	0.95	0.06	0.11	22	1759	44	44.3	-28.3	778.6	0.71	1219	12.57	0.0082	0.81	0.04	0.53	100	17	9	44.7	-28.4	7.4	1.93	1291	0.32	0.0084	0.84	0.05	0.02	3
13	B	4	Harvest	F17	LYC	405	23	38.5	-27.6	155.9	0.30	1433	1.21	0.0089	0.89	0.05	0.06	13	1944	47	44.0	-28.6	854.8	0.66	1113	12.74	0.0078	0.77	0.04	0.50	100	62	9	44.8	-29.5	27.7	1.55	1218	0.96	0.0082	0.81	0.04	0.04	9
13	C	1	Harvest	F17	HYC	662	22	40.3	-28.2	267.0	0.29	852	1.90	0.0068	0.68	0.03	0.06	22	1648	41	44.1	-28.6	726.3	0.52	824	8.62	0.0067	0.67	0.03	0.27	100	2	11	44.1	-30.0	0.8	1.74	1037	0.03	0.0075	0.74	0.04	0.00	0
13	C	2	Harvest	F17	HYC	589	24	38.8	-28.7	228.4	0.27	1461	1.59	0.0090	0.90	0.05	0.09	22	1650	42	43.0	-29.1	710.1	0.54	1214	8.92	0.0081	0.81	0.04	0.39	100	4	11	44.3	-30.6	2.0	1.41	1352	0.06	0.0086	0.86	0.05	0.00	1
13	C	3	Harvest	F17	HYC	1061	23	40.1	-28.2	425.7	0.31	894	3.30	0.0070	0.69	0.03	0.11	25	2474	38	43.5	-28.5	1075.7	0.52	895	12.81	0.0070	0.69	0.03	0.43	100	11	10	45.5	-29.8	5.2	1.64	1233	0.19	0.0082	0.81	0.05	0.01	2
13	C	4	Harvest	F17	LYC	308	25	38.2	-28.4	117.6	0.28	1424	0.85	0.0089	0.88	0.05	0.04	11	1746	50	44.7	-28.8	780.8	0.52	1185	9.05	0.0080	0.80	0.04	0.39	100	27	10	45.2	-29.6	12.1	1.50	1392	0.40	0.0088	0.87	0.05	0.02	5
14	A	1	Harvest	F17+18	HYC	885	24	39.7	-28.9	350.8	0.46	2912	4.05	0.0144	1.42	0.08	0.31	47	2732	41	43.8	-28.9	1195.4	0.71	1986	19.33	0.0110	1.09	0.04	0.65	100	61	8	44.2	-29.1	27.1	1.74	778	1.07	0.0065	0.65	-0.01	0.00	0
14	A	4	Harvest	F17+18	LYC	200	25	39.1	-27.5	78.3	0.43	3563	0.85	0.0168	1.65	0.09	0.07	23	1225	48	44.7	-28.7	548.0	0.80	2035	9.81	0.0112	1.10	0.04	0.33	100	46	10	44.5	-29.2	20.7	1.39	956	0.65	0.0072	0.71	-0.01	0.00	0
14	B	1	Harvest	F17+18	HYC	1119	23	38.6	-28.1	431.9	0.46	2783	5.14	0.0139	1.37	0.07	0.36	44	2779	39	42.6	-28.6	1184.6	0.82	2030	22.73	0.0111	1.10	0.04	0.83	100	24	9	44.3	-29.6	10.4	1.80	950	0.42	0.0072	0.71	-0.01	0.00	0
14	B	2	Harvest	F17+18	LYC	196	25	38.7	-28.2	75.9	0.34	3826	0.67	0.0177	1.74	0.10	0.07	13	1366	49	44.5	-28.6	608.4	0.61	2546	8.34	0.0130	1.29	0.06	0.49	100	21	10	43.9	-29.2	9.2	1.71	1183	0.36	0.0080	0.80	0.00	0.00	0
14	B	3	Harvest	F17+18	LYC	522	24	38.8	-27.8	202.6	0.36	4023	1.87	0.0185	1.81	0.11	0.20	30	1839	44	43.8	-28.5	805.6	0.67	2622	12.28	0.0133	1.31	0.06	0.67	100	46	10	46.7	-29.6	21.4	1.53	1018	0.70	0.0074	0.74	-0.01	0.00	0
14	B	4	Harvest	F17+18	LYC	412	24	39.2	-28.3	161.2	0.41	4071	1.68	0.0186	1.83	0.11	0.18	34	1459	44	43.7	-29.0	637.4	0.62	2723	8.99	0.0137	1.35	0.06	0.53	100	25	10	44.8	-29.9	11.0	1.62	1130	0.40	0.0078	0.78	-0.01	0.00	0
14	C	1	Harvest	F17+18	HYC	674	21	39.0	-28.2	262.8	0.50	2055	3.38	0.0112	1.11	0.04	0.14	37	1961	35	43.4	-28.9	851.7	0.82	1576	16.06	0.0095	0.94	0.03	0.39	100	34	9	44.5	-29.3	15.3	1.98	571	0.68	0.0058	0.57	-0.02	0.00	0
14	C	2	Harvest	F17+18	HYC	816	22	39.0	-28.3	318.4	0.41	2897	3.33	0.0143	1.41	0.08	0.25	44	2300	39	43.2	-28.9	994.0	0.62	2047	14.20	0.0112	1.11	0.04	0.57	100	5	10	44.5	-29.7	2.4	1.66	963	0.09	0.0072	0.72	0.00	0.00	0
14	C	3	Harvest	F17+18	HYC	1025	21	39.0	-28.2	400.1	0.57	3026	5.82	0.0148	1.46	0.08	0.47	50	2564	36	42.7	-28.6	1096.1	0.72	2310	18.37	0.0122	1.20	0.05	0.94	100	11	9	45.0	-30.2	4.9	1.58	815	0.17	0.0067	0.66	-0.01	0.00	0
14	C	4	Harvest	F17+18	HYC	726	23	38.8	-28.3	281.6	0.38	3277	2.75	0.0157	1.55	0.09	0.25	40	1859	41	43.1	-28.8	800.6	0.64	2412	11.87	0.0125	1.24	0.06	0.62	100	9	10	45.3	-30.3	4.0	1.52	1006	0.13	0.0074	0.73	0.00	0.00	0

Appendix III - Raw data

Amino acids in grape must in 2017 (mg L^{-1})

block	row	rep	Excavation time	Fertilization treatment	Yield condition	Ac. Aspartique	Ac. Glutamique	Alanine	Arginine	Asparagine	Citrulline	Cystine	GABA	Glutamine	Glycine	Histidine	Hydroxy-proline	Isoleucine	Leucine	Lysine	Méthionine	Ornithine	Phenylalanine	Proline	Serine	Threonine	Tryptophane	Tyrosine	Valine
3	A	1	Veraison	C	LYC	7.2	2.9	3.4	10.4	0.2	0.4	0.0	0.3	2.2	0.0	0.8	0.0	0.3	0.6	0.0	0.0	0.6	0.3	0.7	4.0	3.0	0.2	0.4	0.7
3	A	2	Veraison	C	LYC	6.3	4.3	5.9	18.3	0.3	1.5	0.0	0.2	6.0	0.0	1.4	0.0	0.6	0.9	0.1	0.1	0.7	0.4	1.2	7.9	4.0	0.4	0.9	1.0
3	A	3	Veraison	C	LYC	4.9	1.9	1.5	8.3	0.2	0.4	0.0	0.2	3.1	0.0	0.6	0.0	0.3	0.4	0.0	0.0	0.3	0.3	0.3	3.5	2.1	0.2	0.4	0.5
3	A	4	Veraison	C	LYC	5.8	2.5	4.0	18.2	0.3	1.4	0.0	0.4	5.6	0.0	1.3	0.0	0.5	0.8	0.1	0.1	0.6	0.4	0.7	5.9	3.5	0.4	0.8	0.8
3	B	1	Veraison	C	HYC	4.4	2.8	2.5	13.8	0.2	0.9	0.0	0.4	3.6	0.0	0.6	0.0	0.4	0.8	0.1	0.0	0.4	0.4	0.6	5.1	3.5	0.3	0.5	0.8
3	B	2	Veraison	C	HYC	5.3	2.4	1.3	5.9	0.1	0.1	0.0	0.2	1.2	0.0	1.1	0.0	0.3	0.5	0.2	0.0	0.6	0.2	0.4	3.7	2.3	0.2	0.4	0.5
3	B	3	Veraison	C	LYC	5.8	2.7	2.4	15.5	0.3	0.9	0.0	0.4	4.1	0.0	1.0	0.0	0.5	0.8	0.0	0.0	0.4	0.4	0.6	5.8	4.4	0.3	0.8	0.7
3	B	4	Veraison	C	HYC	5.5	3.5	4.3	16.3	0.4	1.1	0.0	0.5	4.6	0.0	0.9	0.0	0.5	0.8	0.0	0.1	0.5	0.4	0.7	5.6	4.2	0.4	0.7	0.8
3	C	1	Veraison	C	HYC	4.4	2.9	3.8	16.0	0.3	0.8	0.0	0.5	3.0	1.0	1.0	0.0	0.4	0.8	0.0	0.1	0.4	0.5	0.5	5.4	4.1	0.3	0.8	0.8
3	C	2	Veraison	C	HYC	4.0	2.6	1.4	7.6	0.0	0.3	0.0	0.3	1.7	0.0	0.3	0.0	0.2	0.4	0.0	0.0	0.1	0.2	0.4	2.9	2.7	0.2	0.4	0.4
3	C	4	Veraison	C	HYC	4.2	3.0	0.8	5.4	0.0	0.1	0.0	0.3	0.8	0.0	0.5	0.0	0.1	0.3	0.1	0.0	0.1	0.1	0.3	2.2	1.8	0.1	0.2	0.3
4	A	1	Harvest	C	LYC	6.4	10.8	12.1	55.7	0.9	3.0	0.0	4.8	3.4	0.0	3.6	0.3	2.1	2.6	0.6	0.3	1.4	1.2	22.8	8.8	10.0	2.2	2.1	4.1
4	A	2	Harvest	C	LYC	5.3	9.6	5.6	37.8	0.5	1.5	0.0	4.0	2.1	0.0	2.7	0.3	1.5	1.9	0.4	0.2	0.7	0.8	14.3	5.4	7.2	1.7	1.4	2.6
4	A	4	Harvest	C	LYC	6.9	10.4	8.6	52.4	0.8	3.0	0.0	3.6	3.0	0.0	3.9	0.3	2.0	2.4	0.6	0.3	1.7	1.0	16.6	7.8	9.1	2.4	2.3	3.6
4	B	1	Harvest	C	LYC	3.7	4.8	2.1	13.9	0.3	0.5	0.0	1.6	1.0	0.0	0.9	0.2	0.7	1.0	0.2	0.1	0.3	0.4	6.3	2.3	2.6	0.9	0.5	1.1
4	B	2	Harvest	C	LYC	6.0	9.8	8.3	32.4	0.6	1.8	0.0	3.3	2.2	0.0	2.1	0.2	1.4	1.9	0.4	0.2	0.6	0.8	19.5	7.7	8.4	1.3	1.4	3.2
4	B	3	Harvest	C	HYC	3.9	7.0	5.1	23.0	0.6	0.7	0.0	3.5	1.3	0.0	1.2	0.3	1.2	1.8	0.2	0.2	0.4	0.6	9.5	4.2	6.6	1.1	1.0	2.3
4	B	4	Harvest	C	HYC	5.5	11.1	11.6	38.3	0.4	2.1	0.0	3.9	2.6	0.0	2.6	0.3	1.5	2.0	0.5	0.2	0.9	0.7	18.2	7.2	8.6	1.4	1.4	3.1
4	C	1	Harvest	C	HYC	4.5	10.2	10.5	40.7	0.4	2.3	0.0	4.3	2.7	0.0	2.6	0.3	1.5	1.9	0.2	0.2	0.7	0.7	11.3	8.3	9.9	1.5	1.4	2.9
4	C	2	Harvest	C	HYC	4.2	10.1	7.8	25.2	0.2	1.2	0.0	3.6	1.7	0.0	1.7	0.2	1.0	1.3	0.4	0.1	0.8	0.6	8.4	7.2	7.2	0.9	1.0	2.1
4	C	3	Harvest	C	HYC	4.0	7.7	9.4	32.8	0.3	1.5	0.0	4.5	2.3	0.0	1.6	0.2	1.3	1.7	0.2	0.2	0.4	0.7	7.8	7.1	9.4	1.2	1.2	2.6
4	C	4	Harvest	C	HYC	3.9	8.5	5.9	19.8	0.3	0.8	0.0	3.0	1.5	0.0	0.9	0.2	0.9	1.3	0.2	0.1	0.2	0.7	9.1	5.8	6.7	0.9	0.8	2.1
5	A	2	Harvest	F17	LYC	5.6	12.6	10.2	42.9	0.7	2.1	0.0	3.4	3.0	0.0	2.8	0.2	1.7	1.9	0.4	0.2	1.0	0.8	15.8	7.4	9.0	1.7	1.5	3.2
5	A	3	Harvest	F17	LYC	5.6	10.3	7.9	34.8	0.6	1.8	0.0	2.6	2.2	0.0	2.4	0.3	1.6	2.0	0.3	0.2	0.8	0.9	20.0	7.1	8.9	1.6	1.7	3.3
5	A	4	Harvest	F17	LYC	6.2	13.3	12.1	49.8	1.2	2.3	0.0	4.4	3.0	0.0	3.7	0.4	2.0	2.5	0.5	0.3	1.0	1.0	27.1	8.3	10.5	2.3	1.8	4.2
5	B	1	Harvest	F17	HYC	5.2	10.1	13.5	41.8	0.5	2.3	0.0	4.4	2.6	0.0	2.7	0.2	1.7	2.3	0.3	0.3	0.9	0.9	18.7	9.1	11.0	1.6	1.7	3.7
5	B	2	Harvest	F17	HYC	4.5	11.9	12.3	39.4	0.4	2.4	0.0	3.9	3.0	0.0	2.6	0.2	1.4	1.9	0.2	0.2	0.9	0.7	13.7	8.8	10.1	1.5	1.5	2.9
5	B	3	Harvest	F17	HYC	4.4	11.0	13.6	42.6	0.4	2.5	0.0	4.4	2.8	0.0	2.6	0.2	1.6	2.1	0.2	0.2	0.9	1.0	13.9	8.7	10.8	1.9	1.6	3.2
5	B	4	Harvest	F17	LYC	6.2	12.4	17.2	48.5	0.5	3.8	0.0	4.6	4.2	0.0	3.5	0.2	1.8	2.3	0.4	0.3	1.5	0.8	18.9	10.2	11.5	1.7	2.0	3.8
5	C	2	Harvest	F17	HYC	6.0	12.2	12.1	39.6	0.5	2.6	0.0	3.6	3.1	0.0	2.6	0.3	1.6	2.0	0.1	0.2	0.8	1.0	19.8	8.8	9.9	1.7	1.5	3.3
5	C	3	Harvest	F17	LYC	5.1	11.7	9.4	29.9	0.4	1.9	0.0	3.6	2.2	0.0	1.9	0.2	1.3	1.7	0.4	0.2	0.5	0.7	14.9	7.5	8.0	1.3	1.3	2.8
5	C	4	Harvest	F17	LYC	4.2	7.6	5.5	25.0	0.3	1.1	0.0	2.6	1.5	0.0	1.2	0.3	1.0	1.4	0.3	0.1	0.2	0.6	9.5	4.9	6.5	1.5	1.1	2.0

Appendix IV - Raw data

Proportions of amino acids in the must in 2017

block	row	rep	Excavation time	Fertilization treatment	Yield condition	Ac. Aspartique	Ac. Glutamique	Alanine	Arginine	Asparagine	Citrulline	Cystine	GABA	Glutamine	Glycine	Histidine	Hydroxy-proline	Isoleucine	Leucine	Lysine	Méthionine	Ornithine	Phenylalanine	Proline	Serine	Threonine	Tryptophane	Tyrosine	Valine
3	A	1	Veraison	C	LYC	18.7	7.6	8.8	26.9	0.5	1.1	0.1	0.7	5.7	0.0	2.0	0.0	0.9	1.5	0.0	0.1	1.4	0.7	1.7	10.2	7.9	0.6	1.1	1.7
3	A	2	Veraison	C	LYC	10.1	6.9	9.4	29.2	0.5	2.5	0.0	0.4	9.7	0.0	2.3	0.0	0.9	1.4	0.2	0.1	1.1	0.6	1.9	12.6	6.3	0.7	1.5	1.6
3	A	3	Veraison	C	LYC	16.6	6.6	5.0	28.3	0.7	1.3	0.1	0.6	10.7	0.0	2.2	0.0	0.9	1.5	0.0	0.1	1.0	0.9	1.2	12.0	7.1	0.5	1.4	1.6
3	A	4	Veraison	C	LYC	10.7	4.6	7.4	33.6	0.6	2.6	0.0	0.8	10.4	0.0	2.3	0.0	0.9	1.4	0.2	0.1	1.1	0.8	1.3	10.8	6.5	0.7	1.6	1.5
3	B	1	Veraison	C	HYC	10.4	6.7	5.8	32.6	0.6	2.0	0.1	1.0	8.5	0.0	1.5	0.0	1.0	1.8	0.2	0.1	1.1	0.9	1.4	12.2	8.3	0.8	1.3	1.8
3	B	2	Veraison	C	HYC	19.7	9.0	4.7	21.9	0.5	0.5	0.1	0.6	4.5	0.0	4.2	0.0	1.0	1.8	0.6	0.1	2.1	0.9	1.4	13.9	8.5	0.7	1.4	1.8
3	B	3	Veraison	C	LYC	12.1	5.6	5.0	32.3	0.6	2.0	0.1	0.9	8.4	0.0	2.0	0.0	0.9	1.6	0.1	0.1	0.9	0.9	1.3	12.0	9.1	0.6	1.8	1.5
3	B	4	Veraison	C	HYC	10.6	6.7	8.3	31.4	0.7	2.1	0.0	1.0	8.9	0.0	1.7	0.0	0.9	1.6	0.0	0.1	0.9	0.7	1.4	10.9	8.1	0.7	1.4	1.6
3	C	1	Veraison	C	HYC	9.2	6.0	8.0	33.4	0.7	1.7	0.0	1.1	6.2	2.2	2.1	0.0	0.9	1.7	0.1	0.2	0.8	1.0	1.0	11.2	8.6	0.7	1.6	1.6
3	C	2	Veraison	C	HYC	15.1	10.0	5.5	29.1	0.0	1.1	0.1	1.2	6.3	0.0	1.1	0.0	0.9	1.7	0.0	0.1	0.2	0.8	1.6	11.0	10.3	0.8	1.4	1.6
3	C	4	Veraison	C	HYC	20.5	14.3	3.8	26.4	0.2	0.6	0.1	1.2	4.0	0.0	2.3	0.0	0.7	1.4	0.3	0.0	0.3	0.6	1.4	10.4	8.7	0.6	0.9	1.3
4	A	1	Harvest	C	LYC	4.0	6.8	7.6	35.0	0.6	1.9	0.0	3.0	2.1	0.0	2.3	0.2	1.3	1.6	0.3	0.2	0.9	0.8	14.3	5.5	6.3	1.4	1.3	2.6
4	A	2	Harvest	C	LYC	4.9	8.9	5.2	35.2	0.5	1.4	0.0	3.7	1.9	0.0	2.5	0.3	1.4	1.8	0.4	0.2	0.7	0.7	13.3	5.0	6.7	1.6	1.3	2.5
4	A	4	Harvest	C	LYC	4.8	7.3	6.0	36.7	0.6	2.1	0.0	2.5	2.1	0.0	2.8	0.2	1.4	1.7	0.4	0.2	1.2	0.7	11.6	5.5	6.4	1.7	1.6	2.5
4	B	1	Harvest	C	LYC	8.2	10.5	4.6	30.6	0.7	1.1	0.1	3.5	2.1	0.0	1.9	0.4	1.5	2.3	0.5	0.2	0.7	0.9	13.9	5.0	5.7	2.0	1.2	2.5
4	B	2	Harvest	C	LYC	5.3	8.6	7.3	28.6	0.5	1.6	0.0	2.9	2.0	0.0	1.9	0.2	1.3	1.6	0.3	0.2	0.5	0.7	17.1	6.8	7.4	1.2	1.2	2.8
4	B	3	Harvest	C	HYC	5.2	9.2	6.7	30.4	0.8	1.0	0.0	4.6	1.8	0.0	1.6	0.4	1.6	2.3	0.3	0.2	0.6	0.8	12.5	5.6	8.8	1.5	1.3	3.0
4	B	4	Harvest	C	HYC	4.4	8.9	9.4	30.9	0.4	1.7	0.0	3.1	2.1	0.0	2.1	0.2	1.2	1.6	0.4	0.2	0.7	0.6	14.6	5.8	6.9	1.2	1.1	2.5
4	C	1	Harvest	C	HYC	3.8	8.6	8.8	34.1	0.3	1.9	0.0	3.6	2.3	0.0	2.2	0.2	1.2	1.6	0.2	0.2	0.6	0.6	9.5	7.0	8.3	1.2	1.2	2.5
4	C	2	Harvest	C	HYC	4.9	11.7	9.0	29.0	0.2	1.4	0.0	4.1	2.0	0.0	2.0	0.2	1.2	1.5	0.4	0.1	0.9	0.6	9.6	8.3	8.3	1.0	1.2	2.4
4	C	3	Harvest	C	HYC	4.0	7.8	9.6	33.6	0.3	1.5	0.0	4.6	2.3	0.0	1.6	0.2	1.3	1.7	0.2	0.2	0.4	0.7	8.0	7.2	9.6	1.2	1.2	2.6
4	C	4	Harvest	C	HYC	5.3	11.6	8.0	26.9	0.4	1.1	0.0	4.1	2.0	0.0	1.3	0.3	1.2	1.8	0.3	0.2	0.3	1.0	12.3	7.8	9.1	1.3	1.0	2.8
5	A	2	Harvest	F17	LYC	4.3	9.8	8.0	33.5	0.6	1.6	0.0	2.7	2.3	0.0	2.2	0.2	1.3	1.5	0.3	0.2	0.8	0.6	12.3	5.8	7.0	1.3	1.2	2.5
5	A	3	Harvest	F17	LYC	4.8	8.8	6.8	29.8	0.5	1.5	0.0	2.2	1.8	0.0	2.1	0.2	1.3	1.8	0.2	0.2	0.7	0.7	17.1	6.1	7.6	1.4	1.5	2.8
5	A	4	Harvest	F17	LYC	3.9	8.4	7.6	31.5	0.8	1.5	0.0	2.8	1.9	0.0	2.3	0.3	1.3	1.6	0.3	0.2	0.7	0.6	17.2	5.3	6.7	1.5	1.2	2.6
5	B	1	Harvest	F17	HYC	3.9	7.5	9.9	30.9	0.4	1.7	0.0	3.3	1.9	0.0	2.0	0.2	1.3	1.7	0.2	0.2	0.6	0.6	13.8	6.7	8.1	1.2	1.3	2.7
5	B	2	Harvest	F17	HYC	3.6	9.6	9.9	31.7	0.3	1.9	0.0	3.1	2.4	0.0	2.1	0.2	1.2	1.6	0.2	0.2	0.7	0.5	11.0	7.0	8.1	1.2	1.2	2.3
5	B	3	Harvest	F17	HYC	3.3	8.5	10.4	32.6	0.3	1.9	0.0	3.4	2.1	0.0	2.0	0.2	1.2	1.6	0.1	0.2	0.7	0.7	10.6	6.7	8.3	1.5	1.2	2.4
5	B	4	Harvest	F17	LYC	4.0	8.0	11.0	31.0	0.3	2.4	0.0	2.9	2.7	0.0	2.2	0.1	1.1	1.5	0.3	0.2	1.0	0.5	12.1	6.6	7.4	1.1	1.3	2.4
5	C	2	Harvest	F17	HYC	4.5	9.1	9.1	29.7	0.4	2.0	0.0	2.7	2.4	0.0	2.0	0.2	1.2	1.5	0.1	0.2	0.6	0.7	14.9	6.6	7.4	1.3	1.1	2.5
5	C	3	Harvest	F17	LYC	4.8	11.0	8.8	28.0	0.4	1.8	0.0	3.3	2.1	0.0	1.8	0.2	1.2	1.6	0.4	0.2	0.5	0.6	13.9	7.0	7.5	1.2	1.2	2.7
5	C	4	Harvest	F17	LYC	5.4	9.7	7.0	31.8	0.3	1.4	0.0	3.3	1.9	0.0	1.5	0.3	1.3	1.8	0.4	0.2	0.3	0.8	12.1	6.3	8.2	1.9	1.4	2.6

Appendix V - Raw data

Amino acids in grape must in 2018 (mg L^{-1})

block	row	rep	Excavation time	Fertilization treatment	Yield condition	Ac. Aspartique	Ac. Glutamique	Alanine	Arginine	Asparagine	Citrulline	Cystine	GABA	Glutamine	Glycine	Histidine	Hydroxyproline	Isoleucine	Leucine	Lysine	Methionine	Ornithine	Phenylalanine	Proline	Serine	Threonine	Tryptophane	Tyrosine	Valine
10	A	1	Veraison	C	LYC	10.7	8.5	11.9	21.3	0.6	2.8	0.0	1.4	4.8	3.1	2.8	0.1	1.5	1.1	0.6	1.0	1.6	1.0	2.4	12.1	8.9	0.6	1.4	1.9
10	A	2	Veraison	C	LYC	11.3	6.3	10.0	17.3	0.5	1.8	0.0	1.4	2.9	4.2	2.6	0.1	1.4	1.0	0.6	0.9	1.6	1.1	2.1	11.8	8.1	0.7	1.4	1.9
10	A	3	Veraison	C	LYC	10.2	4.8	2.8	9.3	0.3	0.6	0.0	1.7	1.5	2.0	1.3	0.1	0.7	0.5	0.3	0.5	0.8	0.5	1.0	6.2	4.5	0.5	0.7	0.9
10	A	4	Veraison	C	LYC	9.5	5.5	5.2	13.4	0.3	1.1	0.0	1.4	2.3	2.0	1.7	0.1	0.9	0.7	0.3	0.7	1.0	0.6	1.3	6.8	6.5	0.5	0.9	1.2
10	B	1	Veraison	C	LYC	10.1	4.7	4.2	10.8	0.4	0.9	0.0	1.7	2.0	3.2	1.7	0.1	0.9	0.6	0.4	0.0	1.1	0.5	0.8	8.2	5.4	0.5	0.9	1.1
10	B	2	Veraison	C	HYC	9.6	4.2	3.7	12.9	0.3	0.8	0.0	1.9	2.6	1.0	1.3	0.0	0.6	0.5	0.2	0.0	0.6	0.4	0.5	4.6	5.0	0.4	0.6	0.7
10	B	3	Veraison	C	HYC	9.9	5.8	7.3	17.9	0.4	1.6	0.0	1.5	3.5	1.2	1.8	0.0	0.8	0.6	0.3	0.8	0.9	0.5	0.8	6.2	6.6	0.6	0.9	1.1
10	B	4	Veraison	C	LYC	8.3	3.7	3.6	10.7	0.2	0.6	0.0	1.5	1.7	1.1	1.2	0.0	0.5	0.5	0.2	0.5	0.4	0.4	0.5	4.4	5.3	0.3	0.6	0.7
10	C	1	Veraison	C	HYC	9.5	4.6	4.0	15.7	0.4	0.8	0.0	1.8	3.3	1.2	1.8	0.0	0.6	0.5	0.2	0.6	0.6	0.6	0.6	5.1	4.7	0.4	0.8	0.8
10	C	2	Veraison	C	HYC	9.3	3.6	4.3	12.0	0.3	0.7	0.0	2.4	2.0	1.5	1.3	0.0	0.6	0.5	0.3	0.5	0.6	0.4	0.5	6.0	5.2	0.3	0.6	0.8
10	C	3	Veraison	C	HYC	10.6	4.8	3.2	9.5	0.2	0.6	0.0	1.3	1.8	1.1	1.1	0.0	0.5	0.4	0.2	0.5	0.4	0.3	0.5	4.2	4.5	0.4	0.5	0.6
10	C	4	Veraison	C	HYC	9.1	3.9	3.1	10.3	0.3	0.7	0.0	2.6	1.6	1.3	1.2	0.0	0.5	0.4	0.3	0.4	0.5	0.3	0.4	3.9	4.4	0.4	0.5	0.6
11	A	1	Veraison	F17	LYC	12.2	10.6	15.6	23.2	0.7	2.9	0.0	2.1	7.1	2.7	2.6	0.1	1.2	0.8	0.5	1.1	1.6	0.7	2.7	10.1	6.8	0.9	1.5	1.7
11	A	2	Veraison	F17	LYC	8.7	5.4	2.2	5.3	0.2	0.4	0.0	0.6	0.8	2.0	0.9	0.0	0.5	0.4	0.2	0.4	0.6	0.3	1.0	4.8	3.2	0.4	0.5	0.7
11	A	3	Veraison	F17	LYC	10.3	6.4	10.0	18.0	0.3	2.1	0.0	1.3	3.3	1.0	1.8	0.0	1.0	0.7	0.3	1.0	0.8	0.6	1.9	6.4	7.9	0.6	1.0	1.4
11	A	4	Veraison	F17	LYC	10.5	7.3	8.4	17.0	0.3	1.9	0.0	1.0	3.6	1.2	2.0	0.1	1.1	0.9	0.3	1.0	0.9	0.7	3.9	7.5	8.7	0.9	1.1	1.7
11	B	1	Veraison	F17	HYC	10.0	5.2	6.3	23.7	0.6	1.4	0.0	2.9	5.6	1.1	2.5	0.0	0.8	0.6	0.3	0.8	0.7	0.7	1.4	6.4	5.2	0.6	1.2	0.9
11	B	2	Veraison	F17	HYC	9.7	6.6	7.3	19.8	0.5	1.8	0.0	1.8	4.0	1.1	2.1	0.0	0.8	0.7	0.3	0.8	0.8	0.6	1.2	5.7	5.5	0.6	1.0	1.1
11	B	3	Veraison	F17	HYC	9.6	6.0	6.9	14.7	0.4	1.4	0.0	2.1	2.9	1.2	1.5	0.0	0.8	0.6	0.3	0.7	0.8	0.4	1.0	5.4	5.8	0.5	0.9	1.0
11	B	4	Veraison	F17	LYC	10.5	6.3	6.7	13.6	0.3	1.2	0.0	1.6	2.6	0.6	1.4	0.0	0.7	0.5	0.2	0.7	0.6	0.3	0.9	5.3	5.4	0.4	0.7	0.9
11	C	1	Veraison	F17	HYC	8.1	3.6	2.3	7.7	0.2	0.3	0.0	1.7	1.0	1.2	0.9	0.0	0.4	0.4	0.2	0.4	0.4	0.3	0.4	4.1	3.5	0.3	0.4	0.5
11	C	2	Veraison	F17	HYC	8.8	4.4	4.7	13.6	0.3	0.6	0.0	2.3	2.4	1.3	1.4	0.0	0.5	0.4	0.2	0.6	0.4	0.6	0.5	4.7	4.3	0.3	0.7	0.7
11	C	3	Veraison	F17	LYC	9.2	6.2	3.7	10.0	0.3	0.6	0.0	1.9	1.9	1.3	1.0	0.0	0.5	0.4	0.2	0.5	0.5	0.3	0.6	4.6	4.0	0.3	0.5	0.6
11	C	4	Veraison	F17	HYC	8.0	4.9	5.3	16.1	0.4	0.9	0.0	1.8	3.2	0.8	1.8	0.0	0.7	0.6	0.2	0.6	0.6	0.7	0.6	5.9	5.1	0.4	0.9	0.9
12	A	1	Harvest	C	LYC	3.7	10.4	8.6	25.0	0.9	1.0	0.0	6.2	1.7	1.4	2.4	0.6	2.4	1.4	0.4	1.2	0.7	1.2	39.0	6.8	7.0	1.9	1.3	3.2
12	A	2	Harvest	C	LYC	4.2	13.9	7.1	19.4	0.3	0.6	0.0	6.6	1.2	0.9	1.7	0.5	2.0	1.1	0.3	1.1	0.4	1.0	29.6	5.4	6.2	1.0	0.9	2.7
12	A	3	Harvest	C	HYC	4.8	11.5	7.8	22.3	0.4	0.7	0.0	6.5	1.2	1.2	1.9	0.5	1.9	1.1	0.4	1.0	0.5	1.2	23.0	6.8	7.5	1.2	0.9	2.6
12	A	4	Harvest	C	HYC	3.6	7.5	5.2	16.7	0.4	0.3	0.0	6.2	0.8	0.7	1.5	0.4	1.6	1.0	0.3	0.7	0.2	1.0	14.7	4.2	5.3	1.1	0.7	1.9
12	B	1	Harvest	C	LYC	3.7	9.5	7.0	13.0	0.2	0.3	0.0	6.5	1.2	1.1	1.5	0.4	1.8	1.2	0.3	1.0	0.4	1.3	14.1	5.5	4.0	1.6	0.8	2.2
12	B	2	Harvest	C	HYC	4.8	5.9	6.5	20.7	0.4	0.4	0.0	6.7	1.4	0.8	1.9	0.3	1.6	1.0	0.3	0.8	0.4	1.2	9.1	5.8	7.0	1.2	0.8	2.0
12	B	3	Harvest	C	HYC	5.8	6.9	6.8	17.0	0.4	0.3	0.0	5.8	1.7	0.9	1.5	0.2	1.3	0.8	0.2	0.7	0.2	1.2	8.1	6.3	6.8	1.0	0.6	1.8
12	B	4	Harvest	C	LYC	4.8	12.0	6.0	20.9	0.7	0.4	0.0	5.5	1.1	1.0	1.7	0.6	2.0	1.2	0.4	1.0	0.5	1.0	20.3	5.6	5.8	1.3	0.8	2.3
12	C	1	Harvest	C	HYC	5.3	7.2	8.2	18.4	0.4	0.3	0.0	8.0	1.6	0.8	1.6	0.3	1.4	0.8	0.3	0.7	0.3	1.3	10.5	6.5	6.3	1.0	0.7	1.7
12	C	2	Harvest	C	LYC	4.4	9.7	4.2	8.6	0.2	0.1	0.0	5.3	0.7	0.6	0.8	0.2	0.7	0.4	0.1	0.5	0.2	0.8	7.4	3.9	2.7	0.6	0.4	1.0
12	C	3	Harvest	C	HYC	4.4	9.8	6.5	15.9	0.3	0.5	0.0	6.4	1.1	0.6	1.3	0.4	1.2	0.8	0.2	0.7	0.3	0.8	12.8	5.2	4.7	0.8	0.6	1.6
13	A	1	Harvest	F17	LYC	3.3	12.5	5.4	11.7	0.3	0.3	0.0	6.0	0.7	1.1	1.3	0.5	1.5	1.0	0.3	0.8	0.3	0.9	29.5	4.8	3.8	1.0	0.8	2.2
13	A	2	Harvest	F17	HYC	4.5	9.5	6.3	15.6	0.4	0.4	0.0	6.8	0.8	0.8	1.4	0.5	1.7	1.1	0.3	0.9	0.3	1.1	17.3	4.6	5.3	1.0	0.7	2.1
13	A	3	Harvest	F17	HYC	5.9	8.4	10.7	32.4	0.7	0.8	0.0	10.8	1.7	0.8	2.4	0.4	2.0	1.0	0.3	0.8	0.6	1.2	19.0	6.8	7.9	1.4	1.1	2.4
13	A	4	Harvest	F17	LYC	5.4	9.9	11.5	38.5	0.9	1.2	0.0	10.3	1.3	0.8	3.1	0.5	2.8	1.3	0.4	1.1	0.6	1.4	27.0	7.0	9.2	1.6	1.3	3.2
13	B	1	Harvest	F17	HYC	4.9	5.5	8.0	21.2	0.4	0.4	0.0	7.7	1.0	1.1	1.9	0.3	1.6	1.0	0.3	0.8	0.3	1.2	7.4	6.8	7.9	1.1	0.8	2.1
13	B	2	Harvest	F17	LYC	4.4	10.3	5.8	11.5	0.4	0.3	0.0	6.3	0.8	1.0	1.3	0.4	1.9	1.4	0.3	1.1	0.3	1.4	37.5	6.0	3.9	1.0	0.7	2.6
13	B	3	Harvest	F17	LYC	4.9	10.8	7.6	20.5	0.4	0.7	0.0	7.8	0.8	0.6	1.7	0.5	1.5	0.9	0.3	0.8	0.4	0.9	19.9	5.0	5.5	1.2	0.7	1.9
13	B	4	Harvest	F17	LYC	5.0	14.9	11.0	28.4	0.5	0.9	0.0	9.7	1.3	0.9	2.4	0.6	2.4	1.4	0.4	1.3	0.6	1.4	37.7	7.3	8.5	1.4	1.2	3.2
13	C	1	Harvest	F17	HYC	3.8	4.5	4.5	9.0	0.2	0.1	0.0	5.5	0.4	0.6	0.7	0.2	0.7	0.5	0.1	0.4	0.1	0.7	6.3	3.6	3.1	0.7	0.3	0.9
13	C	2	Harvest	F17	HYC	3.7	6.0	4.0	6.3	0.2	0.1	0.0	4.8	0.3	0.6	0.7	0.2	0.6	0.5	0.1	0.4	0.1	0.6	9.5	3.7	1.8	0.6	0.3	0.9
13	C	3	Harvest	F17	HYC	4.6	7.0	6.4	16.2	0.3	0.3	0.0	8.2	0.6	0.6	1.3	0.3	1.1	0.7	0.2	0.6	0.3	0.8	9.2	4.5	4.9	0.9	0.6	1.4
13	C	4	Harvest	F17	LYC	5.1	11.0	5.5	12.0	0.3	0.3	0.0	5.7	0.7	0.9	1.1	0.4	1.4	0.9	0.2	0.9	0.2	1.2	25.0	5.5	4.1	0.8	0.7	2.0
14	A	1	Harvest	F17+18	HYC	7.1	11.5	13.5	41.9	1.0	1.6	0.0	11.4	1.6	1.8	3.6	0.9	3.2	1.7	0.6	1.2	0.9	1.8	44.8	10.9	12.0	1.9	1.6	4.3
14	A	4	Harvest	F17+18	LYC	5.0	11.5	8.8	26.2	0.5	1.2	0.0	10.1	0.7	0.9	2.4	0.7	2.4	1.4	0.1	0.8	0.6	1.4	67.5	7.4	7.8	1.4	1.2	3.6
14	B	1	Harvest	F17+18	HYC	6.0	9.0	10.5	35.5	0.6	1.5	0.0	10.5	1.1	1.1	2.7	0.5	2.3	1.2	0.4	0.9	0.5	1.1	20.8	7.1	9.8	1.6	1.1	2.7
14	B	2	Harvest	F17+18	LYC	6.5	13.0	7.3	21.1	0.5	0.8	0.0	6.7	0.8	0.7	1.7	0.5	1.9	1.0	0.3	0.9	0.5	1.2	31.5	6.4	5.8	1.1	0.8	2.2
14	B	3	Harvest	F17+18	LYC	5.2	10.7	9.1	29.5	0.5	1.3	0.0	8.9	1.1	0.8	2.2	0.5	2.2	1.3	0.3	1.0	0.6	1.0	26.1	6.5	8.8	1.4	1.1	2.8
14	B	4	Harvest	F17+18	LYC	5.0	14.0	9.4	25.4	0.4	1.2	0.0	8.8	1.0	1.0	1.8	0.5	1.9	1.1	0.4	0.9	0.6	1.1	32.5	6.4	7.2	1.3	1.0	2.5
14	C	1	Harvest	F17+18	HYC	7.9	8.8	10.3	41.2	0.6	1.7	0.0	8.2	1.7	0.9	2.9	0.4	2.0	1.0	0.3	0.7	0.6	1.0	10.1	7.7	10.7	1.3	1.0	2.4
14	C	2	Harvest	F17+18	HYC	8.4	9.2	9.4	30.9	0.5	1.0	0.0	7.2	1.4	1.1	2.0	0.3	1.7	0.9	0.2	0.7	0.4	1.0	9.8	7.3	9.7	1.2	0.8	2.0
14	C	3	Harvest	F17+18	HYC	7.2	8.2	9.9	37.1	0.7	1.1	0.0	7.5	1.6	1.1	2.7	0.4	2.1	1.2	0.3	0.9	0.4	1.3	9.6	8.3	11.5	1.5	1.0	2.4
14	C	4	Harvest	F17+18	HYC	5.5	6.7	5.5	15.2	0.4	0.3	0.0	6.5	0.6	1.2	1.1	0.4	1.1	0.7	0.2	0.6	0.3	0.8	9.6	4.9	5.7	0.9	0.6	1.4

Appendix VI - Raw data

Proportions of amino acids in the must in 2018

block	row	rep	Excavation time	Fertilization treatment	Yield condition	Ac. Aspartique	Ac. Glutamique	Alanine	Arginine	Asparagine	Citrulline	Cystine	GABA	Glutamine	Glycine	Histidine	Hydroxyproline	Isoleucine	Leucine	Lysine	Methionine	Ornithine	Phenylalanine	Proline	Serine	Threonine	Tryptophane	Tyrosine	Valine
10	A	1	Veraison	C	LYC	10.5	8.3	11.6	20.9	0.5	2.7	0.0	1.4	4.7	3.1	2.7	0.1	1.5	1.1	0.6	1.0	1.6	0.9	2.3	11.9	8.7	0.6	1.4	1.9
10	A	2	Veraison	C	LYC	12.4	6.9	10.9	19.0	0.5	2.0	0.0	1.6	3.2	4.6	2.8	0.1	1.5	1.1	0.7	1.0	1.8	1.2	2.3	13.0	8.9	0.7	1.5	2.1
10	A	3	Veraison	C	LYC	19.8	9.4	5.4	18.1	0.5	1.2	0.0	3.3	3.0	3.8	2.5	0.1	1.3	0.9	0.6	1.0	1.6	0.9	1.9	12.0	8.8	1.0	1.3	1.7
10	A	4	Veraison	C	LYC	14.9	8.6	8.2	21.0	0.4	1.7	0.0	2.2	3.7	3.1	2.6	0.1	1.4	1.0	0.5	1.1	1.5	0.9	2.0	10.6	10.2	0.9	1.4	1.8
10	B	1	Veraison	C	LYC	16.6	7.8	7.0	17.9	0.6	1.5	0.0	2.8	3.4	5.3	2.8	0.2	1.4	1.1	0.7	0.0	1.9	0.9	1.4	13.6	8.9	0.8	1.5	1.8
10	B	2	Veraison	C	HYC	18.3	8.0	7.0	24.7	0.6	1.5	0.0	3.7	4.9	2.0	2.5	0.1	1.1	0.9	0.4	0.0	1.1	0.7	1.0	8.7	9.6	0.8	1.2	1.4
10	B	3	Veraison	C	HYC	14.0	8.1	10.3	25.3	0.5	2.3	0.0	2.1	4.9	1.7	2.5	0.1	1.2	0.9	0.4	1.1	1.3	0.7	1.1	8.7	9.3	0.9	1.3	1.5
10	B	4	Veraison	C	LYC	17.6	7.9	7.6	22.8	0.4	1.2	0.0	3.2	3.7	2.3	2.5	0.1	1.1	1.0	0.5	1.1	0.9	0.9	1.1	9.4	11.3	0.7	1.2	1.5
10	C	1	Veraison	C	HYC	16.2	7.9	6.8	26.8	0.7	1.4	0.0	3.1	5.7	2.1	3.1	0.1	1.0	0.8	0.4	1.0	1.0	0.9	1.0	8.7	8.0	0.7	1.3	1.3
10	C	2	Veraison	C	HYC	17.3	6.6	7.9	22.4	0.5	1.3	0.0	4.5	3.8	2.8	2.4	0.1	1.1	0.9	0.5	1.0	1.1	0.8	0.9	11.1	9.7	0.6	1.2	1.5
10	C	3	Veraison	C	HYC	22.4	10.2	6.7	20.1	0.5	1.2	0.0	2.7	3.9	2.4	2.3	0.1	1.0	0.8	0.5	1.0	0.9	0.6	1.0	8.8	9.5	0.8	1.1	1.4
10	C	4	Veraison	C	HYC	19.5	8.3	6.6	22.1	0.6	1.5	0.0	5.6	3.4	2.8	2.6	0.1	1.0	0.8	0.5	0.9	1.0	0.7	0.9	8.4	9.4	0.8	1.1	1.4
11	A	1	Veraison	F17	LYC	11.2	9.7	14.3	21.2	0.6	2.7	0.0	2.0	6.5	2.4	2.4	0.1	1.1	0.7	0.4	1.0	1.5	0.7	2.5	9.2	6.2	0.8	1.4	1.6
11	A	2	Veraison	F17	LYC	21.8	13.7	5.5	13.5	0.5	1.0	0.0	1.6	2.1	5.0	2.3	0.1	1.3	0.9	0.6	1.0	1.6	0.8	2.6	12.1	8.0	0.9	1.3	1.7
11	A	3	Veraison	F17	LYC	13.2	8.2	12.9	23.0	0.4	2.6	0.0	1.7	4.2	1.2	2.3	0.1	1.3	1.0	0.3	1.3	1.0	0.7	2.5	8.2	10.2	0.8	1.3	1.8
11	A	4	Veraison	F17	LYC	12.8	8.8	10.3	20.7	0.4	2.3	0.0	1.2	4.4	1.5	2.4	0.1	1.4	1.1	0.3	1.2	1.1	0.8	4.8	9.2	10.6	1.1	1.4	2.1
11	B	1	Veraison	F17	HYC	12.7	6.6	8.0	30.1	0.8	1.8	0.0	3.6	7.1	1.3	3.2	0.0	1.0	0.8	0.4	1.0	0.9	0.9	1.7	8.1	6.5	0.8	1.5	1.2
11	B	2	Veraison	F17	HYC	13.1	9.0	9.9	26.7	0.6	2.5	0.0	2.4	5.4	1.5	2.8	0.1	1.1	0.9	0.4	1.1	1.1	0.8	1.6	7.7	7.5	0.8	1.4	1.5
11	B	3	Veraison	F17	HYC	14.8	9.2	10.7	22.8	0.6	2.1	0.0	3.2	4.5	1.8	2.3	0.1	1.2	1.0	0.4	1.1	1.2	0.6	1.5	8.3	9.0	0.8	1.3	1.6
11	B	4	Veraison	F17	LYC	17.0	10.3	10.9	22.0	0.5	2.0	0.0	2.6	4.2	1.0	2.2	0.0	1.2	0.9	0.3	1.2	0.9	0.5	1.5	8.5	8.8	0.7	1.1	1.5
11	C	1	Veraison	F17	HYC	21.0	9.3	5.9	20.1	0.5	0.8	0.0	4.5	2.6	3.2	2.4	0.0	1.1	0.9	0.5	1.1	1.0	0.8	1.1	10.6	9.1	0.7	1.1	1.4
11	C	2	Veraison	F17	HYC	16.3	8.1	8.8	25.2	0.6	1.1	0.0	4.2	4.5	2.4	2.7	0.1	1.0	0.8	0.4	1.1	0.8	1.0	0.9	8.8	8.0	0.6	1.3	1.3
11	C	3	Veraison	F17	LYC	18.8	12.6	7.5	20.4	0.5	1.3	0.0	3.8	4.0	2.7	2.0	0.1	1.1	0.8	0.4	0.9	1.0	0.6	1.3	9.3	8.1	0.6	0.9	1.3
11	C	4	Veraison	F17	HYC	13.3	8.0	8.8	26.7	0.7	1.5	0.0	2.9	5.2	1.4	3.0	0.0	1.2	0.9	0.4	1.0	1.0	1.2	0.9	9.7	8.5	0.7	1.5	1.5
12	A	1	Harvest	C	LYC	2.9	8.1	6.7	19.5	0.7	0.8	0.0	4.8	1.4	1.1	1.9	0.5	1.9	1.1	0.3	0.9	0.6	0.9	30.4	5.3	5.5	1.4	1.0	2.5
12	A	2	Harvest	C	LYC	3.9	12.9	6.5	18.0	0.3	0.6	0.0	6.1	1.1	0.8	1.6	0.4	1.8	1.0	0.3	1.0	0.3	0.9	27.4	5.0	5.8	0.9	0.8	2.5
12	A	3	Harvest	C	HYC	4.5	10.8	7.3	20.8	0.4	0.6	0.0	6.1	1.2	1.1	1.8	0.4	1.8	1.0	0.4	0.9	0.5	1.1	21.5	6.4	7.0	1.1	0.9	2.4
12	A	4	Harvest	C	HYC	4.8	9.8	6.8	22.0	0.5	0.4	0.0	8.2	1.1	0.9	1.9	0.5	2.1	1.3	0.3	1.0	0.3	1.3	19.4	5.5	6.9	1.5	0.9	2.4
12	B	1	Harvest	C	LYC	4.7	12.1	9.0	16.5	0.3	0.4	0.0	8.3	1.6	1.5	2.0	0.5	2.2	1.5	0.3	1.2	0.5	1.6	18.0	7.0	5.1	2.0	1.0	2.9
12	B	2	Harvest	C	HYC	6.0	7.3	8.0	25.6	0.5	0.4	0.0	8.3	1.7	1.0	2.3	0.4	2.0	1.2	0.3	0.9	0.4	1.5	11.3	7.2	8.6	1.4	1.0	2.5
12	B	3	Harvest	C	HYC	7.6	9.0	8.9	22.3	0.5	0.4	0.0	7.6	2.3	1.2	1.9	0.3	1.7	1.1	0.3	0.9	0.3	1.5	10.6	8.3	8.9	1.3	0.8	2.3
12	B	4	Harvest	C	LYC	5.0	12.4	6.2	21.5	0.8	0.5	0.0	5.7	1.1	1.1	1.8	0.6	2.0	1.2	0.4	1.1	0.5	1.1	20.9	5.8	6.0	1.3	0.8	2.3
12	C	1	Harvest	C	HYC	6.3	8.6	9.8	22.1	0.5	0.4	0.0	9.6	1.9	1.0	2.0	0.3	1.7	0.9	0.3	0.8	0.4	1.5	12.6	7.8	7.5	1.2	0.9	2.0
12	C	2	Harvest	C	LYC	8.2	18.2	7.8	16.1	0.4	0.2	0.0	10.0	1.3	1.2	1.4	0.4	1.3	0.8	0.3	0.9	0.4	1.5	13.8	7.2	5.0	1.1	0.7	1.8
12	C	3	Harvest	C	HYC	5.8	12.7	8.5	20.7	0.4	0.6	0.0	8.4	1.4	0.8	1.7	0.5	1.6	1.0	0.3	0.9	0.4	1.0	16.7	6.8	6.1	1.1	0.7	2.1
13	A	1	Harvest	F17	LYC	3.7	13.9	6.0	13.0	0.3	0.4	0.0	6.6	0.8	1.3	1.5	0.6	1.6	1.1	0.4	0.9	0.4	1.0	32.8	5.3	4.3	1.1	0.8	2.4
13	A	2	Harvest	F17	HYC	5.4	11.4	7.5	18.7	0.5	0.4	0.0	8.1	1.0	0.9	1.7	0.6	2.0	1.3	0.3	1.0	0.3	1.3	20.8	5.6	6.4	1.2	0.9	2.5
13	A	3	Harvest	F17	HYC	5.0	7.0	9.0	27.1	0.6	0.7	0.0	9.1	1.4	0.7	2.0	0.4	1.7	0.8	0.3	0.7	0.5	1.0	15.9	5.7	6.6	1.1	0.9	2.0
13	A	4	Harvest	F17	LYC	3.8	7.0	8.2	27.4	0.6	0.8	0.0	7.3	0.9	0.6	2.2	0.4	2.0	1.0	0.3	0.8	0.4	1.0	19.2	5.0	6.5	1.2	0.9	2.3
13	B	1	Harvest	F17	HYC	5.9	6.5	9.5	25.3	0.5	0.4	0.0	9.2	1.2	1.3	2.2	0.4	1.9	1.2	0.3	0.9	0.4	1.5	8.9	8.1	9.4	1.4	0.9	2.6
13	B	2	Harvest	F17	LYC	4.4	10.2	5.8	11.4	0.4	0.3	0.0	6.3	0.8	1.0	1.2	0.4	1.9	1.3	0.3	1.1	0.3	1.3	37.3	6.0	3.9	1.0	0.7	2.5
13	B	3	Harvest	F17	LYC	5.1	11.4	8.0	21.5	0.4	0.7	0.0	8.2	0.9	0.7	1.7	0.5	1.6	0.9	0.3	0.8	0.4	0.9	20.9	5.2	5.8	1.3	0.7	2.0
13	B	4	Harvest	F17	LYC	3.5	10.5	7.7	19.9	0.4	0.6	0.0	6.8	0.9	0.7	1.7	0.4	1.7	1.0	0.3	0.9	0.4	1.0	26.5	5.1	6.0	1.0	0.8	2.3
13	C	1	Harvest	F17	HYC	8.1	9.5	9.6	19.1	0.5	0.2	0.0	11.6	0.9	1.3	1.5	0.5	1.4	1.0	0.3	0.9	0.3	1.5	13.3	7.6	6.6	1.5	0.7	2.0
13	C	2	Harvest	F17	HYC	8.0	13.0	8.8	13.7	0.3	0.2	0.0	10.4	0.6	1.2	1.4	0.5	1.3	1.0	0.2	0.9	0.3	1.4	20.6	8.1	3.9	1.3	0.7	2.0
13	C	3	Harvest	F17	HYC	6.5	9.8	9.1	22.8	0.4	0.5	0.0	11.6	0.8	0.8	1.8	0.4	1.6	0.9	0.3	0.9	0.4	1.2	13.0	6.4	6.8	1.3	0.8	1.9
13	C	4	Harvest	F17	LYC	5.9	12.8	6.4	14.0	0.4	0.3	0.0	6.6	0.8	1.1	1.3	0.5	1.6	1.1	0.3	1.0	0.3	1.3	29.2	6.4	4.7	1.0	0.8	2.3
14	A	1	Harvest	F17+18	HYC	3.9	6.4	7.5	23.2	0.5	0.9	0.0	6.3	0.9	1.0	2.0	0.5	1.8	0.9	0.3	0.7	0.5	1.0	24.8	6.0	6.7	1.0	0.9	2.4
14	A	4	Harvest	F17+18	LYC	3.1	7.0	5.4	16.0	0.3	0.7	0.0	6.2	0.4	0.6	1.5	0.4	1.5	0.9	0.1	0.5	0.3	0.8	41.2	4.5	4.8	0.8	0.7	2.2
14	B	1	Harvest	F17+18	HYC	4.7	7.0	8.2	27.6	0.5	1.2	0.0	8.1	0.9	0.8	2.1	0.4	1.8	0.9	0.3	0.7	0.4	0.9	16.1	5.5	7.6	1.3	0.9	2.1
14	B	2	Harvest	F17+18	LYC	5.8	11.5	6.5	18.6	0.4	0.7	0.0	6.0	0.7	0.6	1.5	0.4	1.6	0.9	0.3	0.8	0.4	1.0	27.8	5.6	5.1	1.0	0.7	2.0
14	B	3	Harvest	F17+18	LYC	4.2	8.7	7.4	24.0	0.4	1.0	0.0	7.3	0.9	0.7	1.8	0.4	1.8	1.0	0.3	0.8	0.5	0.8	21.3	5.3	7.2	1.2	0.9	2.3
14	B	4	Harvest	F17+18	LYC	4.0	11.2	7.5	20.3	0.3	0.9	0.0	7.1	0.8	0.8	1.5	0.4	1.5	0.8	0.3	0.7	0.5	0.9	25.9	5.1	5.7	1.0	0.8	2.0
14	C	1	Harvest	F17+18	HYC	6.4	7.1	8.3	33.3	0.5	1.4	0.0	6.6	1.4	0.8	2.4	0.3	1.6	0.8	0.3	0.6	0.5	0.8	8.2	6.2	8.7	1.1	0.8	2.0
14	C	2	Harvest	F17+18	HYC	7.8	8.6	8.8	28.8	0.4	1.0	0.0	6.7	1.3	1.0	1.9	0.3	1.6	0.9	0.2	0.7	0.4	0.9	9.2	6.9	9.0	1.1	0.8	1.9
14	C	3	Harvest	F17+18	HYC	6.1	6.9	8.4	31.5	0.6	0.9	0.0	6.4	1.4	0.9	2.3	0.3	1.7	1.0	0.3	0.7	0.4	1.1	8.2	7.0	9.8	1.3	0.8	2.1
14	C	4	Harvest	F17+18	HYC	7.9	9.5	7.9	21.7	0.6	0.4	0.0	9.3	0.9	1.7	1.6	0.5	1.6	1.0	0.3	0.8	0.4	1.1	13.6	6.9	8.1	1.2	0.8	2.0

Appendix VII - Raw data
Leaf gas exchanges 2017

vine	fertilization	crop load	date	mmol m^{-2} s^{-1} E	µmol m^{-2} s^{-1} A	mmol m^{-2} s^{-1} gsw	µmol mol^{-1} C_i	A*1000/gsw WUE_{inst}
3 A 1	C	LYC	13.06.2017	5.4	16.0	296	224	54
3 A 2	C	LYC	13.06.2017	5.4	17.3	346	227	50
3 A 3	C	LYC	13.06.2017	5.9	15.4	359	243	43
3 A 4	C	LYC	13.06.2017	6.1	17.2	370	232	46
4 A 1	C	LYC	13.06.2017	6.5	17.9	412	234	43
4 A 2	C	LYC	13.06.2017	4.7	12.8	226	230	57
4 A 4	C	LYC	13.06.2017	6.5	18.4	405	229	45
5 A 2	F17	LYC	13.06.2017	6.6	18.8	404	226	47
5 A 3	F17	LYC	13.06.2017	5.9	16.5	344	231	48
5 A 4	F17	LYC	13.06.2017	6.5	18.7	406	227	46
3 B 1	C	HYC	13.06.2017	6.4	17.2	462	247	37
3 B 2	C	HYC	13.06.2017	6.1	17.7	336	219	53
3 B 3	C	LYC	13.06.2017	5.5	14.6	293	234	50
3 B 4	C	HYC	13.06.2017	6.4	18.5	394	226	47
4 B 1	C	LYC	13.06.2017	6.2	18.2	327	213	56
4 B 2	C	LYC	13.06.2017	6.3	16.3	361	236	45
4 B 3	C	HYC	13.06.2017	6.6	20.1	473	229	42
4 B 4	C	HYC	13.06.2017	6.7	18.2	384	226	47
5 B 1	F17	HYC	13.06.2017	5.9	18.1	321	212	56
5 B 2	F17	HYC	13.06.2017	6.0	18.0	340	219	53
5 B 3	F17	HYC	13.06.2017	6.1	19.2	394	222	49
5 B 4	F17	LYC	13.06.2017	6.5	17.9	435	238	41
3 C 1	C	HYC	13.06.2017	6.5	17.4	399	235	44
3 C 2	C	HYC	13.06.2017	5.5	14.9	274	225	55
3 C 4	C	HYC	13.06.2017	5.2	13.7	262	233	52
4 C 1	C	HYC	13.06.2017	6.0	15.7	365	243	43
4 C 2	C	HYC	13.06.2017	6.1	17.8	329	217	54
4 C 3	C	HYC	13.06.2017	6.2	17.8	336	218	53
4 C 4	C	HYC	13.06.2017	6.8	18.1	392	228	46
5 C 2	F17	HYC	13.06.2017	6.5	18.3	389	227	47
5 C 3	F17	LYC	13.06.2017	6.4	17.4	360	227	48
5 C 4	F17	LYC	13.06.2017	3.7	13.2	146	175	91
3 A 1	C	LYC	22.06.2017	6.1	12.7	240	231	53
3 A 2	C	LYC	22.06.2017	7.0	15.5	317	227	49
3 A 3	C	LYC	22.06.2017	7.2	18.6	349	213	53
3 A 4	C	LYC	22.06.2017	6.7	14.9	286	226	52
4 A 1	C	LYC	22.06.2017	8.0	19.1	443	228	43
4 A 2	C	LYC	22.06.2017	4.6	11.3	148	198	77
4 A 4	C	LYC	22.06.2017	7.1	15.3	317	231	48
5 A 2	F17	LYC	22.06.2017	7.4	14.5	326	240	44
5 A 3	F17	LYC	22.06.2017	9.4	20.7	516	226	40
5 A 4	F17	LYC	22.06.2017	8.2	17.5	384	227	46
3 B 1	C	HYC	22.06.2017	7.5	17.2	394	234	44
3 B 2	C	HYC	22.06.2017	5.8	13.0	229	226	57
3 B 3	C	LYC	22.06.2017	5.8	12.9	260	238	50
3 B 4	C	HYC	22.06.2017	6.7	17.0	322	220	53
4 B 1	C	LYC	22.06.2017	5.9	14.3	238	216	60
4 B 2	C	LYC	22.06.2017	6.7	15.5	287	222	54
4 B 3	C	HYC	22.06.2017	8.8	19.0	433	225	44
4 B 4	C	HYC	22.06.2017	8.4	19.8	423	218	47
5 B 1	F17	HYC	22.06.2017	7.9	18.5	359	215	52
5 B 2	F17	HYC	22.06.2017	6.5	13.2	264	235	50
5 B 3	F17	HYC	22.06.2017	8.1	20.4	391	208	52
5 B 4	F17	LYC	22.06.2017	8.1	17.5	402	231	44
3 C 1	C	HYC	22.06.2017	7.4	16.6	358	230	47
3 C 2	C	HYC	22.06.2017	5.7	13.3	232	224	57
3 C 4	C	HYC	22.06.2017	5.9	12.8	239	231	54
4 C 1	C	HYC	22.06.2017	6.7	14.3	284	232	50
4 C 2	C	HYC	22.06.2017	7.2	13.9	317	243	44
4 C 3	C	HYC	22.06.2017	6.8	13.9	307	241	45
4 C 4	C	HYC	22.06.2017	6.6	11.0	277	259	40
5 C 2	F17	HYC	22.06.2017	7.9	17.8	381	226	47
5 C 3	F17	LYC	22.06.2017	7.0	15.6	307	226	51
5 C 4	F17	LYC	22.06.2017	4.8	13.5	171	189	79

Appendix VII - Raw data
Leaf gas exchanges 2017

vine	fertilization	crop load	date	mmol m^{-2} s^{-1} E	µmol m^{-2} s^{-1} A	mmol m^{-2} s^{-1} gsw	µmol mol^{-1} C_i	A*1000/gsw WUE_{inst}
3 A 1	C	LYC	04.07.2017	4.2	12.0	235	243	51
3 A 2	C	LYC	04.07.2017	4.5	12.3	254	245	49
3 A 3	C	LYC	04.07.2017	4.7	13.9	294	243	47
3 A 4	C	LYC	04.07.2017	4.0	12.5	230	237	54
4 A 1	C	LYC	04.07.2017	5.8	15.7	344	238	46
4 A 2	C	LYC	04.07.2017	5.0	16.9	276	210	61
4 A 4	C	LYC	04.07.2017	5.7	17.0	341	227	50
5 A 2	F17	LYC	04.07.2017	6.4	16.7	376	236	44
5 A 3	F17	LYC	04.07.2017	6.8	17.4	399	235	44
5 A 4	F17	LYC	04.07.2017	5.2	13.3	276	241	48
3 B 1	C	HYC	04.07.2017	4.8	14.1	291	240	49
3 B 2	C	HYC	04.07.2017	5.3	16.8	320	224	53
3 B 3	C	LYC	04.07.2017	5.2	17.5	370	231	47
3 B 4	C	HYC	04.07.2017	5.2	13.8	300	244	46
4 B 1	C	LYC	04.07.2017	4.8	15.6	249	212	63
4 B 2	C	LYC	04.07.2017	4.8	13.4	279	243	48
4 B 3	C	HYC	04.07.2017	3.9	10.3	189	242	55
4 B 4	C	HYC	04.07.2017	5.9	17.8	389	232	46
5 B 1	F17	HYC	04.07.2017	5.3	16.9	304	219	56
5 B 2	F17	HYC	04.07.2017	5.6	16.2	316	227	51
5 B 3	F17	HYC	04.07.2017	6.2	20.4	427	220	48
5 B 4	F17	LYC	04.07.2017	5.7	16.4	350	234	47
3 C 1	C	HYC	04.07.2017	4.8	12.1	269	252	45
3 C 2	C	HYC	04.07.2017	3.7	11.2	187	231	60
3 C 4	C	HYC	04.07.2017	3.8	10.2	191	244	54
4 C 1	C	HYC	04.07.2017	4.6	15.0	240	214	63
4 C 2	C	HYC	04.07.2017	4.5	15.9	221	196	72
4 C 3	C	HYC	04.07.2017	5.1	13.3	325	254	41
4 C 4	C	HYC	04.07.2017	5.2	13.2	269	240	49
5 C 2	F17	HYC	04.07.2017	5.7	16.5	333	229	50
5 C 3	F17	LYC	04.07.2017	5.4	14.1	307	243	46
5 C 4	F17	LYC	04.07.2017	4.8	13.8	282	240	49
3 A 1	C	LYC	15.07.2017	4.1	14.4	214	209	67
3 A 2	C	LYC	15.07.2017	4.2	12.3	216	232	57
3 A 3	C	LYC	15.07.2017	4.4	14.8	223	209	66
3 A 4	C	LYC	15.07.2017	4.5	14.4	285	237	51
4 A 1	C	LYC	15.07.2017	5.1	14.6	294	236	50
4 A 2	C	LYC	15.07.2017	4.3	13.7	244	229	56
4 A 4	C	LYC	15.07.2017	4.5	14.1	220	214	64
5 A 2	F17	LYC	15.07.2017	5.2	15.1	300	234	50
5 A 3	F17	LYC	15.07.2017	5.0	14.4	277	232	52
5 A 4	F17	LYC	15.07.2017	5.4	16.7	293	217	57
3 B 1	C	HYC	15.07.2017	4.1	13.0	204	219	64
3 B 2	C	HYC	15.07.2017	3.8	13.4	200	213	67
3 B 3	C	LYC	15.07.2017	2.9	11.5	131	187	88
3 B 4	C	HYC	15.07.2017	3.8	14.1	200	205	70
4 B 1	C	LYC	15.07.2017	3.8	15.1	216	203	70
4 B 2	C	LYC	15.07.2017	3.7	13.9	192	202	73
4 B 3	C	HYC	15.07.2017	4.1	13.8	228	222	60
4 B 4	C	HYC	15.07.2017	4.4	14.0	235	223	60
5 B 1	F17	HYC	15.07.2017	4.1	14.8	207	201	71
5 B 2	F17	HYC	15.07.2017	4.4	14.0	261	232	54
5 B 3	F17	HYC	15.07.2017	4.7	15.5	274	223	57
5 B 4	F17	LYC	15.07.2017	4.9	13.4	258	236	52
3 C 1	C	HYC	15.07.2017	4.4	13.4	241	231	55
3 C 2	C	HYC	15.07.2017	4.5	14.6	265	228	55
3 C 4	C	HYC	15.07.2017	4.5	14.6	252	224	58
4 C 1	C	HYC	15.07.2017	4.4	14.7	245	220	60
4 C 2	C	HYC	15.07.2017	4.7	15.8	301	229	52
4 C 3	C	HYC	15.07.2017	4.0	12.8	216	227	59
4 C 4	C	HYC	15.07.2017	4.3	12.9	242	237	53
5 C 2	F17	HYC	15.07.2017	5.5	16.2	336	233	48
5 C 3	F17	LYC	15.07.2017	4.0	11.6	187	226	62
5 C 4	F17	LYC	15.07.2017	4.8	13.6	272	239	50
3 A 1	C	LYC	28.07.2017	6.1	15.5	335	237	46
3 A 2	C	LYC	28.07.2017	6.7	15.9	336	233	47
3 A 3	C	LYC	28.07.2017	7.3	17.0	374	231	46
3 A 4	C	LYC	28.07.2017	7.4	17.8	370	224	48
4 A 1	C	LYC	28.07.2017	8.1	17.8	401	229	44
4 A 2	C	LYC	28.07.2017	6.7	14.9	281	225	53
4 A 4	C	LYC	28.07.2017	7.4	18.7	393	222	48
5 A 2	F17	LYC	28.07.2017	7.5	17.9	374	224	48
5 A 3	F17	LYC	28.07.2017	7.5	16.2	392	241	41
5 A 4	F17	LYC	28.07.2017	7.4	16.4	371	234	44
3 B 1	C	HYC	28.07.2017	6.9	17.1	349	225	49
3 B 2	C	HYC	28.07.2017	5.9	14.2	279	233	51
3 B 3	C	LYC	28.07.2017	5.1	12.4	236	237	53
3 B 4	C	HYC	28.07.2017	7.3	18.3	364	219	50

Appendix VII - Raw data
Leaf gas exchanges 2017

vine	fertilization	crop load	date	mmol m^{-2} s^{-1} E	µmol m^{-2} s^{-1} A	mmol m^{-2} s^{-1} gsw	µmol mol^{-1} C_i	A*1000/gsw WUE_{inst}
4 B 1	C	LYC	28.07.2017	7.3	18.4	386	223	48
4 B 2	C	LYC	28.07.2017	6.7	16.7	297	215	56
4 B 3	C	HYC	28.07.2017	5.9	16.1	266	211	61
4 B 4	C	HYC	28.07.2017	6.3	15.9	283	218	56
5 B 1	F17	HYC	28.07.2017	6.2	16.0	299	223	53
5 B 2	F17	HYC	28.07.2017	5.0	14.4	191	193	75
5 B 3	F17	HYC	28.07.2017	7.3	17.8	350	219	51
5 B 4	F17	LYC	28.07.2017	6.1	14.3	241	217	59
3 C 1	C	HYC	28.07.2017	5.6	13.2	245	231	54
3 C 2	C	HYC	28.07.2017	6.4	14.9	276	225	54
3 C 4	C	HYC	28.07.2017	6.0	13.0	256	236	51
4 C 1	C	HYC	28.07.2017	6.9	17.4	346	223	50
4 C 2	C	HYC	28.07.2017	6.3	15.4	291	225	53
4 C 3	C	HYC	28.07.2017	7.7	17.2	389	232	44
4 C 4	C	HYC	28.07.2017	6.7	16.4	319	223	52
5 C 2	F17	HYC	28.07.2017	6.6	17.0	300	214	57
5 C 3	F17	LYC	28.07.2017	5.7	14.7	241	215	61
5 C 4	F17	LYC	28.07.2017	5.8	15.7	258	212	61
3 A 1	C	LYC	07.08.2017					
3 A 2	C	LYC	07.08.2017					
3 A 3	C	LYC	07.08.2017					
3 A 4	C	LYC	07.08.2017					
4 A 1	C	LYC	07.08.2017	6.2	16.7	395	240	42
4 A 2	C	LYC	07.08.2017	5.0	12.3	255	245	48
4 A 4	C	LYC	07.08.2017	5.6	14.0	339	251	41
5 A 2	F17	LYC	07.08.2017	5.5	14.5	362	252	40
5 A 3	F17	LYC	07.08.2017	6.1	16.3	383	241	43
5 A 4	F17	LYC	07.08.2017	5.8	17.3	351	227	49
3 B 1	C	HYC	07.08.2017					
3 B 2	C	HYC	07.08.2017					
3 B 3	C	LYC	07.08.2017					
3 B 4	C	HYC	07.08.2017					
4 B 1	C	LYC	07.08.2017	5.2	16.5	293	219	56
4 B 2	C	LYC	07.08.2017	4.7	14.4	255	226	56
4 B 3	C	HYC	07.08.2017	5.8	16.3	387	242	42
4 B 4	C	HYC	07.08.2017	6.0	15.9	364	241	44
5 B 1	F17	HYC	07.08.2017	5.9	17.4	347	225	50
5 B 2	F17	HYC	07.08.2017	5.2	15.2	318	237	48
5 B 3	F17	HYC	07.08.2017	5.4	16.7	288	215	58
5 B 4	F17	LYC	07.08.2017	5.5	15.6	314	232	50
3 C 1	C	HYC	07.08.2017					
3 C 2	C	HYC	07.08.2017					
3 C 4	C	HYC	07.08.2017					
4 C 1	C	HYC	07.08.2017	5.0	13.9	287	240	48
4 C 2	C	HYC	07.08.2017	5.3	15.5	347	242	45
4 C 3	C	HYC	07.08.2017	4.5	13.1	218	223	60
4 C 4	C	HYC	07.08.2017	5.6	14.7	311	239	47
5 C 2	F17	HYC	07.08.2017	5.1	15.9	301	227	53
5 C 3	F17	LYC	07.08.2017	5.3	15.2	286	228	53
5 C 4	F17	LYC	07.08.2017	4.0	12.1	201	228	60
3 A 1	C	LYC	18.08.2017					
3 A 2	C	LYC	18.08.2017					
3 A 3	C	LYC	18.08.2017					
3 A 4	C	LYC	18.08.2017					
4 A 1	C	LYC	18.08.2017	6.5	14.8	382	250	39
4 A 2	C	LYC	18.08.2017	6.7	13.2	393	264	34
4 A 4	C	LYC	18.08.2017	6.3	14.4	366	252	39
5 A 2	F17	LYC	18.08.2017	6.5	12.2	320	259	38
5 A 3	F17	LYC	18.08.2017	6.7	14.8	359	246	41
5 A 4	F17	LYC	18.08.2017	7.6	15.6	395	245	39
3 B 1	C	HYC	18.08.2017					
3 B 2	C	HYC	18.08.2017					
3 B 3	C	LYC	18.08.2017					
3 B 4	C	HYC	18.08.2017					
4 B 1	C	LYC	18.08.2017	5.6	14.5	296	236	49
4 B 2	C	LYC	18.08.2017	5.8	14.3	309	240	46
4 B 3	C	HYC	18.08.2017	6.1	14.7	346	245	43
4 B 4	C	HYC	18.08.2017	6.4	12.1	332	263	36
5 B 1	F17	HYC	18.08.2017	7.1	15.9	357	237	44
5 B 2	F17	HYC	18.08.2017	6.4	14.0	298	239	47
5 B 3	F17	HYC	18.08.2017	7.0	16.6	377	235	44
5 B 4	F17	LYC	18.08.2017	6.0	14.1	250	223	56
3 C 1	C	HYC	18.08.2017					
3 C 2	C	HYC	18.08.2017					
3 C 4	C	HYC	18.08.2017					
4 C 1	C	HYC	18.08.2017	5.6	12.0	280	254	43
4 C 2	C	HYC	18.08.2017	5.9	13.6	298	244	46
4 C 3	C	HYC	18.08.2017	7.0	17.1	438	243	39
4 C 4	C	HYC	18.08.2017	6.3	16.2	305	223	53
5 C 2	F17	HYC	18.08.2017	6.4	13.8	315	245	44
5 C 3	F17	LYC	18.08.2017	6.3	13.3	275	239	48
5 C 4	F17	LYC	18.08.2017	5.5	13.4	235	226	57

Appendix VII - Raw data
Leaf gas exchanges 2017

vine	fertilization	crop load	date	mmol m^{-2} s^{-1} E	µmol m^{-2} s^{-1} A	mmol m^{-2} s^{-1} gsw	µmol mol^{-1} C_i	A*1000/gsw WUE_{inst}
3 A 1	C	LYC	29.08.2017					
3 A 2	C	LYC	29.08.2017					
3 A 3	C	LYC	29.08.2017					
3 A 4	C	LYC	29.08.2017					
4 A 1	C	LYC	29.08.2017	7.2	17.2	364	228	47
4 A 2	C	LYC	29.08.2017	6.3	16.0	299	222	54
4 A 4	C	LYC	29.08.2017	5.9	14.2	277	232	51
5 A 2	F17	LYC	29.08.2017	7.2	15.5	339	235	46
5 A 3	F17	LYC	29.08.2017	6.3	13.2	300	247	44
5 A 4	F17	LYC	29.08.2017	7.1	15.2	364	243	42
3 B 1	C	HYC	29.08.2017					
3 B 2	C	HYC	29.08.2017					
3 B 3	C	LYC	29.08.2017					
3 B 4	C	HYC	29.08.2017					
4 B 1	C	LYC	29.08.2017	5.9	14.4	245	218	59
4 B 2	C	LYC	29.08.2017	6.6	15.3	295	226	52
4 B 3	C	HYC	29.08.2017	6.1	13.9	252	225	55
4 B 4	C	HYC	29.08.2017	6.2	14.5	296	234	49
5 B 1	F17	HYC	29.08.2017	4.9	12.0	176	211	68
5 B 2	F17	HYC	29.08.2017	4.9	12.9	180	203	72
5 B 3	F17	HYC	29.08.2017	5.3	13.4	212	215	63
5 B 4	F17	LYC	29.08.2017	4.8	12.2	172	206	71
3 C 1	C	HYC	29.08.2017					
3 C 2	C	HYC	29.08.2017					
3 C 4	C	HYC	29.08.2017					
4 C 1	C	HYC	29.08.2017	3.9	10.8	137	199	79
4 C 2	C	HYC	29.08.2017	6.9	14.6	314	237	46
4 C 3	C	HYC	29.08.2017	5.3	12.3	209	226	59
4 C 4	C	HYC	29.08.2017	6.3	15.4	276	219	56
5 C 2	F17	HYC	29.08.2017	5.7	14.2	228	213	62
5 C 3	F17	LYC	29.08.2017	5.3	13.0	200	214	65
5 C 4	F17	LYC	29.08.2017	4.1	11.3	134	189	84
3 A 1	C	LYC	08.09.2017					
3 A 2	C	LYC	08.09.2017					
3 A 3	C	LYC	08.09.2017					
3 A 4	C	LYC	08.09.2017					
4 A 1	C	LYC	08.09.2017	4.7	16.6	348	255	48
4 A 2	C	LYC	08.09.2017	3.8	15.1	246	237	61
4 A 4	C	LYC	08.09.2017	3.0	12.0	173	231	69
5 A 2	F17	LYC	08.09.2017	4.0	14.5	279	253	52
5 A 3	F17	LYC	08.09.2017	3.5	13.4	230	246	58
5 A 4	F17	LYC	08.09.2017	4.0	14.4	266	250	54
3 B 1	C	HYC	08.09.2017					
3 B 2	C	HYC	08.09.2017					
3 B 3	C	LYC	08.09.2017					
3 B 4	C	HYC	08.09.2017					
4 B 1	C	LYC	08.09.2017	3.3	12.3	225	255	54
4 B 2	C	LYC	08.09.2017	3.8	14.8	253	242	59
4 B 3	C	HYC	08.09.2017	3.4	13.9	237	244	59
4 B 4	C	HYC	08.09.2017	3.9	14.3	278	255	51
5 B 1	F17	HYC	08.09.2017	3.6	13.1	258	259	51
5 B 2	F17	HYC	08.09.2017	2.7	9.7	168	255	58
5 B 3	F17	HYC	08.09.2017	3.2	12.7	203	241	62
5 B 4	F17	LYC	08.09.2017	3.4	14.3	236	241	60
3 C 1	C	HYC	08.09.2017					
3 C 2	C	HYC	08.09.2017					
3 C 4	C	HYC	08.09.2017					
4 C 1	C	HYC	08.09.2017	3.4	13.2	233	249	57
4 C 2	C	HYC	08.09.2017	3.8	15.5	275	245	56
4 C 3	C	HYC	08.09.2017	3.9	14.7	270	249	54
4 C 4	C	HYC	08.09.2017	3.8	14.3	264	251	54
5 C 2	F17	HYC	08.09.2017	3.7	15.2	241	234	63
5 C 3	F17	LYC	08.09.2017	3.9	15.6	259	237	60
5 C 4	F17	LYC	08.09.2017	3.9	15.9	289	246	55

Appendix VIII - Raw data
Leaf gas exchanges 2018

vine	fertilization	crop load	date	mmol m^{-2} s^{-1} E	µmol m^{-2} s^{-1} A	mmol m^{-2} s^{-1} gsw	µmol mol^{-1} C_i	A*1000/gsw WUE_{inst}
12 A 1	C	LYC	14.06.2018	3.7	15.5	196	215	79
12 A 2	C	LYC	14.06.2018	3.3	15.0	170	201	88
12 A 3	C	HYC	14.06.2018	3.7	14.7	200	225	73
12 A 4	C	HYC	14.06.2018	4.2	14.2	288	266	49
13 A 1	F17	LYC	14.06.2018	4.7	18.2	292	237	62
13 A 2	F17	HYC	14.06.2018	4.4	15.4	253	244	61
13 A 3	F17	HYC	14.06.2018	4.4	17.1	253	231	68
13 A 4	F17	LYC	14.06.2018	4.3	17.0	251	231	68
14 A 1	F17+18	HYC	14.06.2018	4.6	17.9	269	231	67
14 A 4	F17+18	LYC	14.06.2018	4.2	16.4	233	227	70
12 B 1	C	LYC	14.06.2018	4.5	17.7	277	236	64
12 B 2	C	HYC	14.06.2018	4.3	17.4	257	230	68
12 B 3	C	HYC	14.06.2018	4.6	16.8	271	240	62
12 B 4	C	LYC	14.06.2018	4.3	16.6	267	240	62
13 B 1	F17	HYC	14.06.2018	4.5	16.9	277	241	61
13 B 2	F17	LYC	14.06.2018	4.7	17.3	295	245	59
13 B 3	F17	LYC	14.06.2018	3.9	16.3	254	239	64
13 B 4	F17	LYC	14.06.2018	3.9	15.3	215	228	71
14 B 1	F17+18	HYC	14.06.2018	4.3	16.3	220	221	74
14 B 2	F17+18	LYC	14.06.2018	4.7	17.6	288	240	61
14 B 3	F17+18	LYC	14.06.2018	4.4	17.6	265	231	67
14 B 4	F17+18	LYC	14.06.2018	4.2	17.2	264	235	65
12 C 1	C	HYC	14.06.2018	5.1	19.3	313	236	62
12 C 2	C	LYC	14.06.2018	4.8	18.2	287	235	63
12 C 3	C	HYC	14.06.2018	3.8	14.7	204	228	72
13 C 1	F17	HYC	14.06.2018	5.0	18.8	301	235	63
13 C 2	F17	HYC	14.06.2018	4.8	18.3	330	249	55
13 C 3	F17	HYC	14.06.2018	3.9	17.7	210	203	84
13 C 4	F17	LYC	14.06.2018	4.7	17.1	323	255	53
14 C 1	F17+18	HYC	14.06.2018	4.6	15.1	268	252	56
14 C 2	F17+18	HYC	14.06.2018	4.0	16.4	217	218	76
14 C 3	F17+18	HYC	14.06.2018	3.9	15.6	223	230	70
14 C 4	F17+18	HYC	14.06.2018	4.5	17.8	258	227	69
12 A 1	C	LYC	25.06.2018	4.9	14.3	188	217	76
12 A 2	C	LYC	25.06.2018	5.9	14.8	273	253	54
12 A 3	C	HYC	25.06.2018	5.7	12.9	232	252	56
12 A 4	C	HYC	25.06.2018	7.1	18.3	326	243	56
13 A 1	F17	LYC	25.06.2018	5.8	15.1	229	231	66
13 A 2	F17	HYC	25.06.2018	6.7	17.9	282	232	63
13 A 3	F17	HYC	25.06.2018	5.6	14.0	229	242	61
13 A 4	F17	LYC	25.06.2018	6.3	17.3	281	236	62
14 A 1	F17+18	HYC	25.06.2018	6.5	16.8	297	245	57
14 A 4	F17+18	LYC	25.06.2018	2.6	10.8	88	149	124
12 B 1	C	LYC	25.06.2018	6.3	16.2	274	241	59
12 B 2	C	HYC	25.06.2018	6.2	17.5	272	231	64
12 B 3	C	HYC	25.06.2018	6.2	15.6	250	236	63
12 B 4	C	LYC	25.06.2018	6.4	17.9	260	223	69
13 B 1	F17	HYC	25.06.2018	7.0	17.2	295	241	58
13 B 2	F17	LYC	25.06.2018	5.6	15.5	231	230	67
13 B 3	F17	LYC	25.06.2018	5.9	14.3	229	238	63
13 B 4	F17	LYC	25.06.2018	6.3	15.4	247	237	62
14 B 1	F17+18	HYC	25.06.2018	5.7	15.5	263	244	59
14 B 2	F17+18	LYC	25.06.2018	5.1	13.2	217	245	61
14 B 3	F17+18	LYC	25.06.2018	5.2	15.4	213	223	72
14 B 4	F17+18	LYC	25.06.2018	3.5	10.4	117	203	89
12 C 1	C	HYC	25.06.2018	6.2	15.8	244	233	65
12 C 2	C	LYC	25.06.2018	6.6	17.3	277	234	63
12 C 3	C	HYC	25.06.2018	6.9	18.5	277	225	67
13 C 1	F17	HYC	25.06.2018	6.8	15.9	280	244	57
13 C 2	F17	HYC	25.06.2018	6.3	17.4	245	220	71
13 C 3	F17	HYC	25.06.2018	7.0	17.7	280	231	63
13 C 4	F17	LYC	25.06.2018	7.1	17.5	314	245	56
14 C 1	F17+18	HYC	25.06.2018	5.9	13.7	244	250	56
14 C 2	F17+18	HYC	25.06.2018	6.1	16.3	278	243	59
14 C 3	F17+18	HYC	25.06.2018	6.5	16.6	286	243	58
14 C 4	F17+18	HYC	25.06.2018	5.9	16.1	242	230	66
12 A 1	C	LYC	02.07.2018	3.0	8.5	91	199	93
12 A 2	C	LYC	02.07.2018	7.5	18.2	325	242	56
12 A 3	C	HYC	02.07.2018	7.6	16.6	342	257	49
12 A 4	C	HYC	02.07.2018	8.3	16.8	370	261	45
13 A 1	F17	LYC	02.07.2018	5.8	12.6	196	237	64
13 A 2	F17	HYC	02.07.2018	8.4	17.5	354	253	49
13 A 3	F17	HYC	02.07.2018	7.7	17.0	327	250	52
13 A 4	F17	LYC	02.07.2018	7.8	16.7	305	246	55
14 A 1	F17+18	HYC	02.07.2018	7.7	14.7	287	254	51
14 A 4	F17+18	LYC	02.07.2018	5.0	12.2	156	216	78
12 B 1	C	LYC	02.07.2018	5.2	11.4	197	251	58

Appendix VIII - Raw data
Leaf gas exchanges 2018

vine	fertilization	crop load	date	mmol m^{-2} s^{-1} E	µmol m^{-2} s^{-1} A	mmol m^{-2} s^{-1} gsw	µmol mol^{-1} C_i	A*1000/gsw WUE_{inst}
12 B 2	C	HYC	02.07.2018	8.4	19.8	371	243	53
12 B 3	C	HYC	02.07.2018	7.2	16.5	280	239	59
12 B 4	C	LYC	02.07.2018	5.8	13.5	227	245	59
13 B 1	F17	HYC	02.07.2018	7.9	17.8	357	253	50
13 B 2	F17	LYC	02.07.2018	5.7	11.5	189	243	61
13 B 3	F17	LYC	02.07.2018	8.7	19.1	366	246	52
13 B 4	F17	LYC	02.07.2018	7.8	16.0	323	256	49
14 B 1	F17+18	HYC	02.07.2018	8.2	16.7	328	251	51
14 B 2	F17+18	LYC	02.07.2018	7.1	15.5	249	235	62
14 B 3	F17+18	LYC	02.07.2018	6.0	14.1	199	224	71
14 B 4	F17+18	LYC	02.07.2018	7.2	15.6	261	239	60
12 C 1	C	HYC	02.07.2018	7.5	16.9	295	242	57
12 C 2	C	LYC	02.07.2018	6.6	16.9	268	233	63
12 C 3	C	HYC	02.07.2018	7.8	17.3	331	250	52
13 C 1	F17	HYC	02.07.2018	6.7	15.9	252	234	63
13 C 2	F17	HYC	02.07.2018	5.9	15.2	204	217	74
13 C 3	F17	HYC	02.07.2018	6.4	16.0	265	240	60
13 C 4	F17	LYC	02.07.2018	6.9	16.7	278	238	60
14 C 1	F17+18	HYC	02.07.2018	7.9	15.4	290	249	53
14 C 2	F17+18	HYC	02.07.2018	6.9	12.7	235	252	54
14 C 3	F17+18	HYC	02.07.2018	7.7	15.1	299	255	51
14 C 4	F17+18	HYC	02.07.2018	7.0	14.8	233	233	64
12 A 1	C	LYC	13.07.2018	4.0	12.0	135	200	89
12 A 2	C	LYC	13.07.2018	6.6	15.7	256	237	61
12 A 3	C	HYC	13.07.2018	6.5	13.2	244	253	54
12 A 4	C	HYC	13.07.2018	8.0	17.7	344	250	52
13 A 1	F17	LYC	13.07.2018	7.7	17.0	273	232	62
13 A 2	F17	HYC	13.07.2018	8.0	17.3	308	242	56
13 A 3	F17	HYC	13.07.2018	7.6	17.2	271	230	63
13 A 4	F17	LYC	13.07.2018	8.0	17.3	327	248	53
14 A 1	F17+18	HYC	13.07.2018	8.2	16.2	306	249	53
14 A 4	F17+18	LYC	13.07.2018	3.8	9.8	100	186	98
12 B 1	C	LYC	13.07.2018	5.6	14.4	193	217	75
12 B 2	C	HYC	13.07.2018	6.6	12.8	240	254	53
12 B 3	C	HYC	13.07.2018	6.9	15.1	246	237	62
12 B 4	C	LYC	13.07.2018	5.9	12.9	216	245	60
13 B 1	F17	HYC	13.07.2018	8.4	18.5	334	241	55
13 B 2	F17	LYC	13.07.2018	6.6	14.7	230	234	64
13 B 3	F17	LYC	13.07.2018	6.3	12.5	200	239	62
13 B 4	F17	LYC	13.07.2018	7.3	15.7	262	239	60
14 B 1	F17+18	HYC	13.07.2018	5.3	11.4	150	219	76
14 B 2	F17+18	LYC	13.07.2018	6.7	13.9	215	232	65
14 B 3	F17+18	LYC	13.07.2018	6.1	14.5	190	213	76
14 B 4	F17+18	LYC	13.07.2018	4.7	9.0	120	222	75
12 C 1	C	HYC	13.07.2018	7.7	17.0	290	239	58
12 C 2	C	LYC	13.07.2018	5.9	14.7	191	213	77
12 C 3	C	HYC	13.07.2018	5.8	13.7	195	225	70
13 C 1	F17	HYC	13.07.2018	6.8	16.0	240	227	67
13 C 2	F17	HYC	13.07.2018	5.9	14.8	187	209	79
13 C 3	F17	HYC	13.07.2018	5.9	13.4	192	226	70
13 C 4	F17	LYC	13.07.2018	6.2	14.6	199	218	74
14 C 1	F17+18	HYC	13.07.2018	6.5	12.7	199	235	64
14 C 2	F17+18	HYC	13.07.2018	7.9	16.3	276	238	59
14 C 3	F17+18	HYC	13.07.2018	7.1	13.4	231	244	58
14 C 4	F17+18	HYC	13.07.2018	4.5	9.8	130	222	76
12 A 1	C	LYC	23.07.2018	3.0	8.6	83	180	104
12 A 2	C	LYC	23.07.2018	6.3	14.3	204	224	70
12 A 3	C	HYC	23.07.2018	6.5	12.0	207	246	58
12 A 4	C	HYC	23.07.2018	6.6	14.7	227	232	65
13 A 1	F17	LYC	23.07.2018	4.7	10.8	142	222	76
13 A 2	F17	HYC	23.07.2018	6.8	13.7	220	237	62
13 A 3	F17	HYC	23.07.2018	6.3	14.5	207	224	70
13 A 4	F17	LYC	23.07.2018	5.6	11.0	164	233	67
14 A 1	F17+18	HYC	23.07.2018	5.9	10.8	166	236	65
14 A 4	F17+18	LYC	23.07.2018	4.4	10.5	130	213	81
12 B 1	C	LYC	23.07.2018	7.2	15.8	243	229	65
12 B 2	C	HYC	23.07.2018	6.0	13.4	189	224	71
12 B 3	C	HYC	23.07.2018	7.4	14.1	242	242	58
12 B 4	C	LYC	23.07.2018	5.5	11.6	163	226	71
13 B 1	F17	HYC	23.07.2018	6.6	13.1	208	236	63
13 B 2	F17	LYC	23.07.2018	7.0	13.1	226	244	58
13 B 3	F17	LYC	23.07.2018	5.5	10.6	157	233	68
13 B 4	F17	LYC	23.07.2018	5.5	10.1	160	241	63
14 B 1	F17+18	HYC	23.07.2018	5.0	9.6	134	228	71
14 B 2	F17+18	LYC	23.07.2018	6.2	13.3	199	230	67
14 B 3	F17+18	LYC	23.07.2018	6.0	11.9	182	235	65
14 B 4	F17+18	LYC	23.07.2018	7.0	15.0	222	226	68
12 C 1	C	HYC	23.07.2018	7.5	15.1	265	244	57
12 C 2	C	LYC	23.07.2018	7.2	15.0	228	229	66
12 C 3	C	HYC	23.07.2018	5.9	12.2	168	222	72
13 C 1	F17	HYC	23.07.2018	7.2	14.9	245	238	61
13 C 2	F17	HYC	23.07.2018	6.7	12.7	197	234	64
13 C 3	F17	HYC	23.07.2018	5.2	11.7	144	210	81
13 C 4	F17	LYC	23.07.2018	6.3	13.5	185	220	73
14 C 1	F17+18	HYC	23.07.2018	6.7	14.0	225	237	62
14 C 2	F17+18	HYC	23.07.2018	5.4	12.9	165	214	78
14 C 3	F17+18	HYC	23.07.2018	4.7	9.8	145	236	68
14 C 4	F17+18	HYC	23.07.2018	5.0	12.1	147	208	82
12 A 1	C	LYC	02.08.2018	6.2	14.8	315	265	47
12 A 2	C	LYC	02.08.2018	5.3	11.8	234	264	50

Appendix VIII - Raw data
Leaf gas exchanges 2018

vine	fertilization	crop load	date	mmol m^{-2} s^{-1} E	µmol m^{-2} s^{-1} A	mmol m^{-2} s^{-1} gsw	µmol mol^{-1} C_i	A*1000/gsw WUE_{inst}
12 A 3	C	HYC	02.08.2018	5.4	12.8	223	250	58
12 A 4	C	HYC	02.08.2018	6.7	15.8	323	259	49
13 A 1	F17	LYC	02.08.2018	7.3	16.3	307	250	53
13 A 2	F17	HYC	02.08.2018	6.3	14.8	271	251	55
13 A 3	F17	HYC	02.08.2018	6.0	11.0	236	270	47
13 A 4	F17	LYC	02.08.2018	6.6	14.0	277	259	51
14 A 1	F17+18	HYC	02.08.2018	5.7	11.6	208	254	56
14 A 4	F17+18	LYC	02.08.2018	5.2	12.0	196	245	61
12 B 1	C	LYC	02.08.2018	4.6	11.1	196	255	57
12 B 2	C	HYC	02.08.2018	4.6	12.1	170	229	71
12 B 3	C	HYC	02.08.2018	5.9	12.8	252	261	51
12 B 4	C	LYC	02.08.2018	4.4	11.0	160	235	69
13 B 1	F17	HYC	02.08.2018	6.5	16.1	317	257	51
13 B 2	F17	LYC	02.08.2018	6.3	15.0	296	258	51
13 B 3	F17	LYC	02.08.2018	5.0	11.5	185	244	62
13 B 4	F17	LYC	02.08.2018	5.6	13.3	221	245	60
14 B 1	F17+18	HYC	02.08.2018	4.1	9.3	140	241	66
14 B 2	F17+18	LYC	02.08.2018	6.0	14.4	230	239	62
14 B 3	F17+18	LYC	02.08.2018	4.4	12.4	164	222	76
14 B 4	F17+18	LYC	02.08.2018	5.0	10.4	188	257	55
12 C 1	C	HYC	02.08.2018	6.4	15.8	279	246	57
12 C 2	C	LYC	02.08.2018	5.3	14.2	213	233	67
12 C 3	C	HYC	02.08.2018	4.8	13.0	197	237	66
13 C 1	F17	HYC	02.08.2018	6.0	15.5	259	243	60
13 C 2	F17	HYC	02.08.2018	6.2	15.6	261	242	60
13 C 3	F17	HYC	02.08.2018	4.5	12.3	159	219	77
13 C 4	F17	LYC	02.08.2018	5.3	13.4	203	236	66
14 C 1	F17+18	HYC	02.08.2018	4.1	9.4	136	236	69
14 C 2	F17+18	HYC	02.08.2018	6.1	15.1	241	238	63
14 C 3	F17+18	HYC	02.08.2018	5.1	11.4	193	250	59
14 C 4	F17+18	HYC	02.08.2018	3.4	9.5	118	219	81
12 A 1	C	LYC	10.08.2018	5.8	14.3	216	233	66
12 A 2	C	LYC	10.08.2018	5.4	12.3	195	241	63
12 A 3	C	HYC	10.08.2018	6.8	15.0	280	252	54
12 A 4	C	HYC	10.08.2018	6.0	14.4	242	244	60
13 A 1	F17	LYC	10.08.2018	3.4	7.6	99	226	76
13 A 2	F17	HYC	10.08.2018	5.2	11.7	170	232	69
13 A 3	F17	HYC	10.08.2018	5.2	11.5	172	235	67
13 A 4	F17	LYC	10.08.2018	6.4	11.8	228	259	52
14 A 1	F17+18	HYC	10.08.2018	4.3	9.2	126	228	73
14 A 4	F17+18	LYC	10.08.2018	4.5	10.6	136	219	78
12 B 1	C	LYC	10.08.2018	4.8	12.6	173	225	73
12 B 2	C	HYC	10.08.2018	4.8	10.9	161	235	68
12 B 3	C	HYC	10.08.2018	5.4	11.8	184	240	64
12 B 4	C	LYC	10.08.2018	4.3	9.9	136	228	73
13 B 1	F17	HYC	10.08.2018	5.9	13.0	223	247	58
13 B 2	F17	LYC	10.08.2018	5.5	12.9	184	228	70
13 B 3	F17	LYC	10.08.2018	3.8	8.7	112	222	78
13 B 4	F17	LYC	10.08.2018	4.6	10.3	138	224	75
14 B 1	F17+18	HYC	10.08.2018	3.0	7.0	74	196	95
14 B 2	F17+18	LYC	10.08.2018	5.6	11.3	165	230	69
14 B 3	F17+18	LYC	10.08.2018	3.3	8.0	84	194	95
14 B 4	F17+18	LYC	10.08.2018	4.6	9.5	125	223	75
12 C 1	C	HYC	10.08.2018	5.6	12.5	191	235	66
12 C 2	C	LYC	10.08.2018	5.7	11.7	196	246	60
12 C 3	C	HYC	10.08.2018	4.2	9.8	135	229	73
13 C 1	F17	HYC	10.08.2018	4.6	11.6	158	225	74
13 C 2	F17	HYC	10.08.2018	4.3	10.3	151	236	68
13 C 3	F17	HYC	10.08.2018	4.1	10.5	127	212	83
13 C 4	F17	LYC	10.08.2018	4.4	11.4	142	215	80
14 C 1	F17+18	HYC	10.08.2018	5.6	12.3	170	224	72
14 C 2	F17+18	HYC	10.08.2018	6.2	14.0	203	227	69
14 C 3	F17+18	HYC	10.08.2018	4.2	8.3	135	249	61
14 C 4	F17+18	HYC	10.08.2018	4.0	8.6	117	228	74
12 A 1	C	LYC	20.08.2018	2.6	7.0	72	194	97
12 A 2	C	LYC	20.08.2018	4.4	9.3	134	234	69
12 A 3	C	HYC	20.08.2018	5.2	11.6	175	236	66
12 A 4	C	HYC	20.08.2018	4.8	11.6	164	230	71
13 A 1	F17	LYC	20.08.2018	5.6	13.0	179	223	73
13 A 2	F17	HYC	20.08.2018	4.9	11.3	148	220	76
13 A 3	F17	HYC	20.08.2018	4.4	9.7	130	226	74
13 A 4	F17	LYC	20.08.2018	5.9	10.7	183	248	58
14 A 1	F17+18	HYC	20.08.2018	5.1	9.4	146	241	64
14 A 4	F17+18	LYC	20.08.2018	3.7	8.2	94	206	87
12 B 1	C	LYC	20.08.2018	3.9	10.2	114	201	89
12 B 2	C	HYC	20.08.2018	6.3	13.3	219	242	61
12 B 3	C	HYC	20.08.2018	4.3	9.6	129	226	74
12 B 4	C	LYC	20.08.2018	4.6	9.8	145	237	67
13 B 1	F17	HYC	20.08.2018	5.0	9.6	163	251	59
13 B 2	F17	LYC	20.08.2018	3.4	9.0	94	193	96
13 B 3	F17	LYC	20.08.2018	3.8	9.6	108	203	89

Appendix VIII - Raw data
Leaf gas exchanges 2018

vine	fertilization	crop load	date	mmol m^{-2} s^{-1} E	µmol m^{-2} s^{-1} A	mmol m^{-2} s^{-1} gsw	µmol mol^{-1} C_i	A*1000/gsw WUE_{inst}
13 B 4	F17	LYC	20.08.2018	5.0	12.1	152	213	80
14 B 1	F17+18	HYC	20.08.2018	5.0	11.7	142	207	83
14 B 2	F17+18	LYC	20.08.2018	3.9	8.9	112	219	79
14 B 3	F17+18	LYC	20.08.2018	4.1	9.2	114	214	81
14 B 4	F17+18	LYC	20.08.2018	4.4	10.6	121	202	88
12 C 1	C	HYC	20.08.2018	5.8	12.4	187	234	66
12 C 2	C	LYC	20.08.2018	4.8	12.9	148	200	87
12 C 3	C	HYC	20.08.2018	4.8	12.0	148	212	81
13 C 1	F17	HYC	20.08.2018	4.1	10.8	127	208	85
13 C 2	F17	HYC	20.08.2018	3.6	9.6	110	206	87
13 C 3	F17	HYC	20.08.2018	4.7	11.7	144	212	81
13 C 4	F17	LYC	20.08.2018	3.8	9.7	110	203	89
14 C 1	F17+18	HYC	20.08.2018	5.9	12.7	190	232	67
14 C 2	F17+18	HYC	20.08.2018	4.6	10.9	136	215	80
14 C 3	F17+18	HYC	20.08.2018	3.7	7.2	105	238	69
14 C 4	F17+18	HYC	20.08.2018	3.1	7.1	84	213	85
12 A 1	C	LYC	28.08.2018	0.7	3.0	21	132	142
12 A 2	C	LYC	28.08.2018	3.8	11.0	139	220	79
12 A 3	C	HYC	28.08.2018	4.2	10.8	159	237	68
12 A 4	C	HYC	28.08.2018	3.7	10.9	154	234	71
13 A 1	F17	LYC	28.08.2018	2.6	7.4	77	196	96
13 A 2	F17	HYC	28.08.2018	3.8	9.1	125	231	73
13 A 3	F17	HYC	28.08.2018	3.7	8.7	125	237	69
13 A 4	F17	LYC	28.08.2018	5.3	10.4	200	262	52
14 A 1	F17+18	HYC	28.08.2018	4.6	9.0	156	255	57
14 A 4	F17+18	LYC	28.08.2018	3.4	9.6	107	203	90
12 B 1	C	LYC	28.08.2018	3.1	10.1	110	201	91
12 B 2	C	HYC	28.08.2018	3.2	9.4	112	213	84
12 B 3	C	HYC	28.08.2018	3.4	8.6	118	233	73
12 B 4	C	LYC	28.08.2018	3.7	10.5	139	225	76
13 B 1	F17	HYC	28.08.2018	3.7	8.0	119	242	67
13 B 2	F17	LYC	28.08.2018	3.3	10.4	116	203	90
13 B 3	F17	LYC	28.08.2018	2.7	7.3	87	217	84
13 B 4	F17	LYC	28.08.2018	3.2	8.5	110	226	77
14 B 1	F17+18	HYC	28.08.2018	2.3	6.4	60	181	106
14 B 2	F17+18	LYC	28.08.2018	4.5	10.9	149	227	73
14 B 3	F17+18	LYC	28.08.2018	2.7	8.2	85	194	97
14 B 4	F17+18	LYC	28.08.2018	3.3	7.5	100	228	76
12 C 1	C	HYC	28.08.2018	5.2	12.4	203	245	61
12 C 2	C	LYC	28.08.2018	4.7	12.7	178	228	71
12 C 3	C	HYC	28.08.2018	3.7	10.9	140	221	78
13 C 1	F17	HYC	28.08.2018	2.6	8.1	88	203	92
13 C 2	F17	HYC	28.08.2018	3.7	10.9	128	208	85
13 C 3	F17	HYC	28.08.2018	2.9	8.0	93	213	86
13 C 4	F17	LYC	28.08.2018	3.3	9.1	107	212	85
14 C 1	F17+18	HYC	28.08.2018	4.7	11.1	158	232	70
14 C 2	F17+18	HYC	28.08.2018	5.3	12.4	186	235	67
14 C 3	F17+18	HYC	28.08.2018	1.9	4.6	58	227	80
14 C 4	F17+18	HYC	28.08.2018	2.5	6.5	78	218	84
12 A 1	C	LYC	04.09.2018	1.7	7.7	75	191	102
12 A 2	C	LYC	04.09.2018	4.7	14.3	258	254	55
12 A 3	C	HYC	04.09.2018	3.3	9.8	161	253	61
12 A 4	C	HYC	04.09.2018	4.4	13.1	230	253	57
13 A 1	F17	LYC	04.09.2018	2.2	7.5	102	237	74
13 A 2	F17	HYC	04.09.2018	3.2	9.7	155	250	63
13 A 3	F17	HYC	04.09.2018	3.9	11.6	196	252	59
13 A 4	F17	LYC	04.09.2018	3.8	9.6	180	265	53
14 A 1	F17+18	HYC	04.09.2018	4.1	9.0	190	275	47
14 A 4	F17+18	LYC	04.09.2018	2.6	9.3	115	223	80
12 B 1	C	LYC	04.09.2018	3.0	11.7	153	225	77
12 B 2	C	HYC	04.09.2018	3.6	10.9	176	250	62
12 B 3	C	HYC	04.09.2018	2.8	9.3	136	242	69
12 B 4	C	LYC	04.09.2018	3.3	12.1	167	231	72
13 B 1	F17	HYC	04.09.2018	2.6	8.6	123	241	70
13 B 2	F17	LYC	04.09.2018	2.5	8.3	118	242	70
13 B 3	F17	LYC	04.09.2018	2.5	8.4	108	228	78
13 B 4	F17	LYC	04.09.2018	2.3	8.2	100	221	82
14 B 1	F17+18	HYC	04.09.2018	2.1	7.6	89	217	86
14 B 2	F17+18	LYC	04.09.2018	4.1	12.0	207	253	58
14 B 3	F17+18	LYC	04.09.2018	2.5	9.8	102	196	97
14 B 4	F17+18	LYC	04.09.2018	3.2	7.8	134	259	58
12 C 1	C	HYC	04.09.2018	3.9	11.4	197	255	58
12 C 2	C	LYC	04.09.2018	4.4	13.7	239	252	58
12 C 3	C	HYC	04.09.2018	2.7	9.9	127	225	78
13 C 1	F17	HYC	04.09.2018	3.6	12.9	193	239	67
13 C 2	F17	HYC	04.09.2018	3.8	14.1	197	229	71
13 C 3	F17	HYC	04.09.2018	2.5	9.5	112	215	85
13 C 4	F17	LYC	04.09.2018	2.8	9.6	137	240	70
14 C 1	F17+18	HYC	04.09.2018	3.3	10.7	146	231	73
14 C 2	F17+18	HYC	04.09.2018	3.5	10.2	177	258	58
14 C 3	F17+18	HYC	04.09.2018	2.7	8.2	118	242	69
14 C 4	F17+18	HYC	04.09.2018	3.7	11.7	164	232	71