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Profiling of physiological responses and quality aspects in *Vitis vinifera* L. as influenced by aspects of N application

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"Der Gelehrte studiert die Natur nicht, weil das etwas Nützliches ist. Er studiert sie, weil er daran Freude hat, und er hat Freude daran, weil sie so schön ist. Wenn die Natur nicht so schön wäre, so wäre es nicht der Mühe wert, sie kennen zu lernen, und das Leben wäre nicht wert, gelebt zu werden."

Henri Poincaré (1854-1912, franz. Mathematiker und Astronom)

Contents

List of manuscripts

Subsequent manuscripts of the present cumulative doctoral thesis have already been published, are accepted for publication or have the status "submitted":

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Lang C.P., Merkt N., Geilfus C.M., Graeff-Hönninger S., Simon J., Rennenberg H., Zörb C. (2018) Interactions between grapevines and trees: Effects on water relations, nitrogen nutrition, and wine. Archives of Agronomy and Soil Science 65 (2), 224-239. https://doi.org/10.1080/03650340.2018.1493197.

List of relevant abbreviations

AF system	Agroforestry system
ATF	Amino transporter family
GOGAT	Glutamate synthase
GS	Glutamine synthetase
HATS	High-affinity transporters
cHATS	Constitutive high-affinity transporters
iHATS	Inducible high-affinity transporters
LATS	Low-affinity transporters
cLATS	Constitutive low-affinity transporters
iLATS	Inducible low-affinity transporters
MA	Malic acid
MIPs	Major intrinsic proteins
N	Nitrogen
NH_4^+	Ammonium
NiR	Nitrite reductase
NO_3^-	Nitrate
NR	Nitrate reductase
NRA	Nitrate reductase activity
RU140	Rootstock variety Ruggerie 140

SO4	Rootstock variety Selection Oppenheim 4
TTA	Tartaric acid
UT	Urea transporter
UV radiation	Ultraviolet radiation
$\delta^{15}N$	Normalized ratio of the stable nitrogen isotopes ^{15}N to ^{14}N

CHAPTER 1

General Introduction

Chapter 1: General Introduction

From an economic point of view, the grapevine (*Vitis vinifera* L.) is one of the most important cultivated fruit crops worldwide because of its multiple uses in the food industry such as in the production of wine, juice or beverages (Ali et al. 2010). The International Organization of Vine and Wine (OIV 2018) reports, that, in 2017, 7.5 mha of the world´s surface area were cultivated with grapevines. Europe has the largest vineyard area in the world and produces about 62% of the world's wine (OIV 2018). The remainder is used for table grape or raisin production. Viticulture is an important production branch of agriculture in Germany with an acreage of about 100000 ha (BMEL 2019). Nevertheless, viticulture and the wine industry together represent a competitive business, with success depending on the final product - the quality of the wine.

Not only the geographical origin, which is often referred to with the French term *'terroir',* but also the environmental conditions, the biotic and abiotic factors, the viticultural practices, the genotype and the complex interaction of these factors have important influences on the quality of wine (Jackson and Lombard 1993; Conde et al. 2007). In particular, the winegrowing practices can be controlled by the winemaker who can thus influence the quality of the product. In this context, plant nutrition by fertilizer application in the vineyard is an important quality-defining aspect. The macronutrient nitrogen (N) is the most abundant soil-derived nutrient for grapevines. It is of upmost importance for the growth of grapevines and the production of berry quality. Inadequate use of N may have undesirable consequences for plant development and thus for the quality of wine (Bell and Henschke 2005).

1.2 Plant nutrient nitrogen (N)

The macronutrient nitrogen (N) makes up the largest part of the Earth's atmosphere (79%) and is the fourth abundant element in the cellular biomass of plants (Robertson and Vitousek 2009; Stein and Klotz 2016). Furthermore, it is the second most required nutrient by plants, their dry matter consisting of about 1.5% N (Marschner 1997). The nutrient is an important constituent of many plant components such as primary and secondary metabolites, proteins, nucleic and amino acids and coenzymes (reviewed by O'Brien et al. 2016). The availability of N is one of the major aspects that determines plant growth and quality, development, primary production and productivity in most agricultural cropping systems

(Robertson and Vitousek 2009; Kiba and Krapp 2016). Therefore, its application is a fundamental aspect of modern and intensive agricultural crop production systems worldwide (Andrews et al. 2013). Plants use N in multiple processes: uptake, assimilation, translocation, recycling and remobilization (Masclaux-Daubresse et al. 2010). Plants can take up N from the soil through their root system by absorption through the plasma membrane as inorganic (nitrate and ammonium) or organic (urea, amino acids, peptides) forms or by mycorrhizae, that are associated with roots. Nitrate (NO_3^-) and ammonium (NH_4^+) are considered to be the most important N-forms for plants and grapevines (Loulakakis et al. 2009; Andrews et al. 2013; Kiba and Krapp 2016). Nitrate uptake involves several steps. The first step is the reduction from nitrate (NO_3^-) to nitrite (NO_2^-) driven by the enzyme nitrate reductase (NR), followed by the second step, the reduction to ammonium (NH_4^+) driven by the enzyme nitrate reductase (NiR). Ammonium (NH_4^+) is assimilated to glutamine and glutamate by the enzymes glutamine synthetase (GS) and glutamate synthase (GOGAT) (Goel and Singh 2015; Balotf et al. 2016). The amino acids synthesized from this step (glutamine and glutamate) are considered to be N donors for all other amino acids (Bungard et al. 1999). Urea can be taken up via the enzyme urease or by active transporter systems (UT) (Witte 2011) (symmetrized in Figure 1).

The uptake of N is an active process via membrane-located transporters, which can be divided in two transporter families: the low-affinity transporters (LATS) and the high-affinity transporters (HATS). Various subfamilies with several differing members exist, depending on the N-form and influx; these transporter families can be differentiated into co-existing inducible (iHATS / iLATS) or constitutive (cHAT / cLATS) systems. LATS typically operate at external nitrate concentrations of > 0.5 mM, whereas HATS operate at external nitrate concentrations of < 0.5 mM (Forde 2000; Noguero and Lacombe 2016).

Nitrate can be stored or assimilated within roots and / or transported via the xylem to organs of sink such as leaves, flowers or fruits. Furthermore, it can be stored in the shoot, although the storage is determined by genotype, plant tissue and environmental conditions. Nevertheless, the biologically availability of nitrate in the soil is low (Andrews at al. 2013) and therefore fundamentally affects plant performance. Non-legume plants need about 20 - 50 g N, taken up by the root, to produce 1 kg dry biomass. However, in intensive agricultural crop production, it is described, at the same time, approximately 50% - 70% of the applied N is not used by the plant and therefore lost in the soil (Robertson and Vitousek 2009; reviewed by Hirel et al. 2011). An unadjusted supply of N can have dramatic effects on agricultural crop production and cause severe environmental damage.

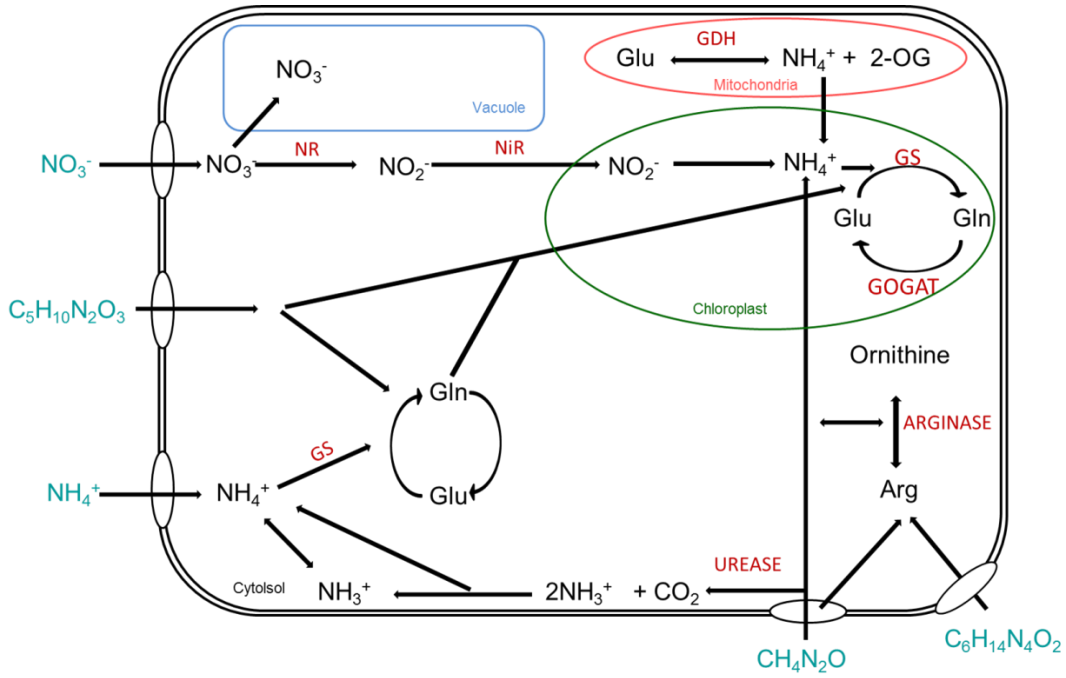

Fig. 1: Schematic representation of the enzymatic mechanisms of nitrogen uptake and assimilation in higher plants based on various N-forms used in this thesis (blue). The involved enzymes are shown (red); NR (nitrate reductase), NiR (nitrite reductase), GS (glutamine synthetase), GOGAT (glutamate synthase), GDH (glutamate dehydrogenase), UREASE and ARGINASE. The figure is a summary of given information modified after Mérigout et al. (2008); Witte (2011); Goel and Singh (2015).

1.3 Wine quality and influencing factors

The quality of wine is a multifactorial construct that has to fulfil several requirements and characteristics (Young and Viver 2010) based on national and international wine law, on the winemaker him/her-self and on the consumer, who determines quality through extrinsic and intrinsic factors by its individual preferences and demands. A holistic quality definition is thus difficult to achieve (Charters and Pettigrew 2007). Since no universal system exists for classifying wine quality grades, wines are often divided into different groups characterized by, for example, carbon dioxide content, alcohol content, colour and stylistic, varietal or geographic origin (Jackson 2008). According to German wine law, wines produced in Germany have to be divided into two groups: table wine and quality wine with various subgroups (Tab. 1) (BMJV 2019).

The chemical composition of grapes and / or berries is often associated with wine quality and can be influenced and characterized by many components, such as variety, genotype and physiological aspects, *e.g.* clonal selection of scion and / or rootstock. Environmental determinants including macro- and micro climate, soil and

water conditions and biotic and abiotic factors, and viticultural practices such as canopy and fertilization management and harvesting protocols affect quality (reviewed by Jackson and Lombard 1993; Conde et al. 2007). Furthermore, differences exist in quality between vintages, specific vineyards and geographical origin or *terroir* (Jackson 2008). The attributes influenced by these factors are mainly primary and secondary metabolites including flavour and aroma compounds (Table 2). The main primary metabolites in grapes and wine are sugars, amino acids, biogenic amines, polysaccharides, alcohols and organic acids. The main secondary metabolites in grapes and wine are phenols, phenylpropanoids, flavonoids, stilbenoids and antioxidants (Ali et al. 2010). These are synthesized during growth and development or during fermentation by the oenological parameters of the wine. Flavour and aroma are produced by a complex mixture of hundreds of compounds (Robinson et al. 2014) and can be further evaluated by various features of post-fermentation treatments (Styger et al. 2011). Furthermore, aroma can be differentiated according their origin; a) aroma originating from grapes, b) aroma built up during fermentation and c) aroma resulting from gaining and post-bottle treatment (Rapp and Mandery 1986). The concentrations of such compounds range from several mg L^{-1} to a few ng L^{-1} (Conde et al. 2007) and the threshold for having an influence on taste is about 1% (Rapp and Mandery 1986). Nevertheless, quality is determined by many interacting factors and a combination of these defines the optimum.

Table 1: Wine quality grades for German wine from the grape variety *Vitis vinifera* L. according to German wine law (BMJV 2019). Quality classes increases from top to bottom.

Quality grade	**Subunit grade**
Table wine (*Landwein*)	
Quality wine (*Qualitätswein*)	
	Kabinett
	Spätlese
	Auslese
	Beerenauslese
	Trockenbeerenauslese
	Eiswein

Table 2: Table of functional and chemical compounds in grapes and wine. Aliphatic and aromatic compounds are listed according Jackson (2008); Ilc et al. (2016).

Compound class	
Acetales	
Acids (fixed)	
	Fatty acids
	Volatile acids
Alcohols	
	Higher (fusel) alcohols
Aliphatic alcohols	
Carbonyls	
	Aldehydes
	Aliphatic aldehydes
	Ketones
	Aliphatic ketones
Carboxylic acids	
Esters	
Aliphatic esters	
Lactones	
Nitrogen compounds & nitrogen containing volatiles	
	Amides
	Amines
	α-Amino acids
	Pyrazines
	Pyridines
C_{13} Norisoprenoids	
Phenolic compounds * & volatile phenols	
Sugar	
Sulfur compounds & sulfur containing volatiles	
	Thiols & Thiolester
	Thiolanes
	Thiazoles
Terpenes	
Water	

* Subclasses of phenolic compounds are listed separately

1.4 Grapevine N status and its correlation to wine quality

Nitrogen is the most important nutrient for grapevines (Bell and Henschke 2005) and is the one most commonly missing in the grapevine, although it is the one most widely used in the vineyard to increase yield, productivity and quality (Kiba and Krapp 2016). A major role is played by N in many biological functions and processes that directly or indirectly trigger grapevine physiology, such as the sink : source relationship, vegetative and generative growth and various metabolic pathways (Bell and Henschke 2005). In addition to growth and development, sensitivities to fungal diseases, flower and fruit growth and berry ripening and maturation are all particularly affected (Conde et al. 2007). The nutrient determines the composition and concentration of berry components that mainly contribute to wine quality (Bell und Henschke 2005). Furthermore, N is also very important during fermentation from must to wine. It regulates the fermentation kinetics through yeast growth, the resulting by-products and the chemical and sensory properties of the wine (Figure 2) (Mendes-Ferreira et al. 2011). Many nitrogenous components are metabolized by microorganisms during fermentation and ensure a normal alcoholic fermentation process. These so-called fermentable N compounds can vary between 100 - 200 mg L^{-1} and are more pronounced in red wine than in white wine (Conde et al. 2007). One of the most important representatives of the N-containing components in berries, must and wine is the group of phenolic compounds (Table 3). The range and concentration of these secondary metabolites determines flavour and aroma (Jackson and Lombard 1993). They define sensory properties such as astringency and, to lesser extent, bitterness (Mazerolles et al 2010). Furthermore, phenolic components are crucial for the organoleptic properties, appearance, fragrance, mouth-feel and colour of wine and thus determine its aroma bouquet (Teixeira et al. 2013). Phenolic compounds are mainly located in berry pulp, seeds and stem but can also be synthesized from microbial and oak sources (Kennedy 2008; Ali et al. 2010). Nitrogen might have a huge impact on aroma expression with decreased aroma precursors such as phenolic compounds. This can be influenced by viticultural practices such as soil N fertilization (Choné et al. 2006; Portu et al. 2015). However, these findings cannot be generalized because of contrasting results. On the one hand, N fertilization increases the proportion of quality-giving metabolites but, on the other hand, an excessively high N dose leads to an altered source : sink ratio and thus to a reduction in other secondary metabolites (Portu et al. 2015).

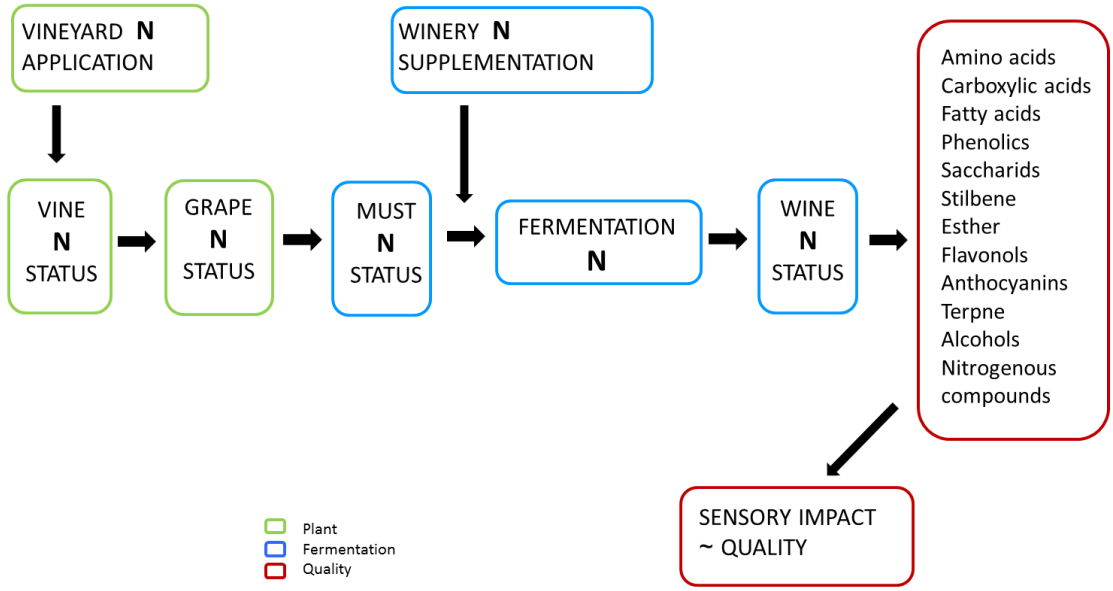

Figure 2: Schematic representation of the main factors that influence the N status of the grapevine. Various levels, starting from vineyard application to the resulting wine, are shown. Green boxes represent the plant level, blue boxes represent the fermentation level and red boxes represent the quality level. Figure is modified after Bell and Henschke (2005).

Table 3: Classification of phenolic compounds based on Vardhan and Shukla (2017).

Phenolic compounds	
Simple Phenolics	**Polyphenolics**
Phenolic acids	**Flavonoids**
Coumarins	Flavonols
	Flavones
	Isoflavones
	Flavanones
	Falvanol
	Anthocyanins
	Chalcones
	Non flavonoids
	Stilbenes
	Lignans
	Tannins

1.5 Agroforestry systems (AF)

Agroforestry systems (AF) are defined as land-use systems and technologies that deliberately combine woody perennials such as trees, shrubs, palms or bamboos with agricultural crops and / or animals on the same land unit (Lundgren and

Raintree 1983). Based on Nair (1985), several systems can be distinguished. In the present work, the focus is on the agrisilvicultural system, consisting of vines and woody perennials. These traditional systems were widely used in many Mediterranean countries of Europe such as Spain, Greece and Southern France (Eichhorn et al. 2006). The mixed cultivation of woody perennials and agricultural crops allows them to influence each other in many ways that are mostly positive and include economic aspects, such as timber, food and biomass production, soil fertility and nutrient cycling, erosion control, altered microclimate and increased biodiversity (Torralba et al. 2016). The enrichment of the soil with N brings great advantages and can be achieved either by biological N_2 fixation or by the release and recycling of above and below ground organic matter (Jose 2009).

Nevertheless, in such a system, competition occurs between species for resources such as nutrients, light and space. In addition, interactions take place between the species, allelopathy or shading (Jose et al. 2004). However, the ways in which they all affect the grapevine physiology and the quality of the wine remain uncertain.

1.6 Objectives of the study

In a vineyard system, N is an essential macronutrient that directly or indirectly triggers vegetative and generative growth. The N-form and its amount have a significant impact on must and wine quality traits such as primary and secondary metabolites (Choné et al. 2006; Portu et al. 2015). However, the knowledge concerning N assimilation into the vine, berry and the resulting wine in relation to the various N-forms is limited. Nitrate and ammonium are considered to be the predominant N-forms in the soil, although, urea is the most commonly used N fertilizer on the global scale (Witte, 2011). The amino acid glutamine is the most important physiological N-form of transport in the vine and the amino acid arginine is the most important N-form of storage in the vine (Alleweldt and Merkt 1992; Bell and Henschke 2005). Another important factor is the rootstock, which is not only a storage organ, but also actively involved in nutrient uptake (Ollat et al. 2016). Only a few studies of N uptake and allocation capacity in various grapevine rootstocks based on the different N-forms have been published. In particular, with regard to amino acids and their consequent effects on berry and wine quality, no published reports are available. The aim of this work was to examine the N allocation capacity of two different rootstocks in response to different N-forms. Subsequently, N allocation capacity was studied in a grafted grapevine with regard to different

N-forms and to different N amounts and the oenological parameters in the must (Chapter 2).

Ammonium and amino acids are considered as inhibitors for NR, but the relevant data is inconsistent and no information is currently available for grapevine rootstocks. Therefore, studies were carried out on the influence of the different N-forms on key N enzymes in the grapevine rootstock SO4 at various times. At the enzymatic level, NR was measured in diverse plant organs. Furthermore, the enzymes NR, NiR and GS were measured at the transcriptional level (Chapter 3).

The aroma and sensory experiences produced by wine are mainly related to the presence of the primary and secondary metabolites it contains, such as phenolic compounds. A change in the metabolic expression pattern in the grapevine can be caused by the application of different N-forms. However, little information exists about metabolic changes resulting from treatment with the different N-forms in field trials. A metabolic fingerprint analysis is a powerful tool for studying initial general changes. Therefore, a metabolite analysis with a focus on phenolic components in the grapevine leaf and wine was carried out with regard to various N-forms. Furthermore, sensory analyses performed by a trained tasting panel were used to investigate the change in the aroma profile of the wine (Chapter 4).

In a separate, more applied project, the changes in plant physiology associated with growing conditions and the related changes in wine quality were examined. Little is known about N uptake by a grapevine grown in an AF system consisting of trees and grapevines, and the resulting wine quality. Thus, in Chapter 5, an isotopic discriminant analysis was performed to measure uptake capacity by using labelled $\delta^{15}N$. Furthermore, the oenological parameters and the sensory aspects of the wine were analysed.

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CHAPTER 2

Different nitrogen (N) forms affect responses to N form and N supply of rootstocks and grafted grapevines

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Research Article

Different nitrogen (N) forms affect responses to N form and N supply of rootstocks and grafted grapevines

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ABSTRACT

Rootstocks play an important role in the cultivation of grapevines. In addition to the uptake and storage of nutrients, rootstocks and their root system affect the growth and metabolite composition of the berries. Nitrogen can be taken up in various forms, such as nitrate, ammonium or amino acids or even small peptides, and is of considerable importance in vigor control and in yield and berry quality. Amino acids in the must adjust fermentation kinetics, constitute a major source for yeast and affect vine metabolism. In the present study, two different experiments were undertaken; nitrate, ammonium, urea, arginine and glutamine at various doses (0; 0.5; 1.0; 3.0 g N/plant) were used to fertilize (*i*) two hydroponically grown rootstock varieties (Ru140 and SO4) and (*ii*) grafted grapevines of *Vitis vinifera* L *cv.* Regent (rootstock SO4) grown in pots. Accumulation capabilities, generative growth and berry quality were examined. It can be assed that the preferred N form is rootstock-variety-dependent. We demonstrated that grapevines were able to take up nitrogen in the form of amino acids; (arginine to a greater extent than glutamine). Although, growth was reduced, nitrogen content and nitrate reductase activity were comparable for nitrate, ammonium and urea nutrition. In terms of berry quality, only minor differences between the N forms applied were identified. An economic optimum in terms of vine and berry quality was detected. Excessive amounts of nitrogen seemed to lead to the increased growth of green plant tissue. Berry yield increased with increasing nitrogen supply but slightly decreased at the highest dosage, whereas quality parameters such as must pH increased and the total acid concentration was reduced.

1. Introduction

Rootstocks play a key role in the nutrient uptake of grapevines depending on the availability of the nutrients and environmental conditions. Hence, they provide the basis for grape berry mineral nutrition and the biochemical composition of berry metabolites. Furthermore rootstocks are directly involved in the process and efficiency of water and nutrient translocation to the upper parts of the plant. They are also decisively responsible for the storage of inorganic nutrients and photosynthate-based compounds [1,2]. Additionally, rootstocks stimulate the reproductive growth and yield of the grapevine [3]. The scion and its vegetative growth are intensively controlled by the rootstock, not only with regard to nutrient and water uptake, but also for its transport, hormone regulation and signalling [4]. Moreover, the use of grafted rootstocks in well-managed vineyards is indispensable, because of the resistance of the rootstocks to phylloxera. Therefore, more than 80% of the vineyards worldwide contain vines grafted by a scion of the European vine species *Vitis vinifera* onto a rootstock either as a single American *Vitis* species or as interspecific hybrids between *V. rupestris, V. berlandieri, V. labrusca, V. rotundifolia or V riparia* [5,6,2].

Nitrogen (N) is one of the most limiting nutrients for growth and development, and is also the most important macronutrient in the grapevine [7]. Additionally, it is the nutrient most likely to be unsatisfactory for grapevines, despite its being the most commonly applied nutrient to vineyards [8] in doses of ca. 40 - 80 kg ha^{-1} a^{-1}. N application has a considerable effect on the control of vigor, yield and berry quality as well as on the sink to source relationship of grapevines [1,9]. The ratio of vegetative to generative growth (equivalent to the sink: source ratio) is of great importance to the grapevines. The ratio influences solute transport and accumulation in the grape berry resulting in an alteration in berry growth and a change in the chemical composition of the berry, the must and the wine [10,11].

The assimilation of N can occur in various parts of the plant, such as the roots, trunk, stem, leaves and berries [12]. Consequently, N is found

Abbreviations: Ru140, rootstock Ruggeri 140; SO4, rootstock Selction Oppenheim 4; AM, ammonium; Arg, arginine; CaN, calcium nitrate; Gln, glutamine; N, nitrogen; NRA, nitrate reductase activity; UR, urea; TA, total acidity

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in various forms, such as nitrate, ammonium, amino acids, proteins and small peptides in the plant organs [1]. Whereas ammonium and nitrate are the most commonly available and the predominant N forms in soil, the availability and the magnitude for the uptake and allocation of N is a multifactorial process. The N uptake efficiency depends on the environmental conditions, genetic variability, developmental stages and physiological status of the rootstock and the scion [8,13]. According to Wermelinger et al. [12], N assimilates and remains in the root or in the woody perennial parts of the grapevine not only as reserves for storage or metabolic purposes, but also for grapevine development and metabolism. The vast majority of the nitrogenous compounds are transported *via* the xylem to the upper parts of the plant. Amino acids, built from N sources, are crucial for the quality of the berry and hence the wine itself, *e.g.* for the YAN = yeast-assimilable nitrogen, which is of upmost importance for the fermentation kinetics of the must and therefore contributes to the wine quality [7]. The amino acids glutamine and arginine play a key role in grapevine physiology. Glutamine, which is mainly stored in the shoot, is known to be the main transport form for N and account for more than 85% of the xylem N [14,15]. The amino acid arginine is considered to be the major storage compound for N in the vine [16]. Arginine is thought to be produced from glutamine or glutamate in the storage tissue of the grapevine and is converted back to glutamine for remobilization [15].

N uptake mechanisms have been well-studied over the last few decades but little is known about the absorption capacities and allocation opportunities of amino acids as N sources in grapevine, in particular in terms of the physiological responses of grapevines to amino acids as a fertilizer that may originate from the microbial decay of leaves or pruning remnants or from pomace returned back into the vine rows as an organic fertilizer. In this study, we have investigated (1) whether the grapevine has the capability of accumulating different forms of N, namely nitrate, ammonium, urea, glutamine and arginine, (2) the way that accumulation is influenced by the different N forms; and (3) whether berry quality is influenced by the N form. To test these problem experimentally, we applied different N forms in different dosages (0; 0.5; 1.0; 3.0 g N/ plant) to vines. Two different experiments were established. In the first experiment (Exp.1), two different rootstock varieties, namely 'Ruggeri 140′ and 'Selection Oppenheim 4′ were grown hydroponically under controlled conditions in nutrient solution containing different N sources in equal concentrations. Rootstock 'Ruggeri 140′ and 'Selection Oppenheim 4′ were chosen because the two rootstocks vary according to their N supply and subsequently in their growth performance. Based on the literature 'Ruggeri 140′ is characterized as a vine that exhibits strong vigor and that is well suited to dry and calcareous soils, although its demand for N fertilization is low. In contrast, 'Selection Oppenheim 4′ exhibits medium vigor and requires soils that are not prone to dryness. Its rootstock responds sensitively to N deficiency [15,17]

A second experiment (Exp.2) was established in pots in order to detect any effects of different N accumulations and their influences on a grafted (rootstock SO4) grapevine variety *Vitis vinifera* L. *cv*. Regent. The rootstock SO4 was chosen because it is often cultivated in Germany (experimental site) and France and therefore of interest for the trial site. Furthermore, the quality of these berries as affected by various N forms and dosages was analysed. The hydroponics experiment (Exp.1) serves as a basic element and reveals the first findings in plant physiology regarding the nitrogen allocation of amino acids. The soil experiment (Exp.2) can be seen as an extension to Exp.1; a transfer of the findings and initial basic results from the hydroponic culture to soil-grown plants.

2. Materials and methods

2.1. Experiment I (hydroponics)

2.1.1. Plant growth conditions

One bud cuttings from the two grapevine rootstocks 140 Ruggeri (Ru140) (*Vitis berlandieri x Vitis rupestris*) and Selection Oppenheim 4 (SO4) (*Vitis berlandieri x Vitis riparia*) were pre-cultivated in a sand bed in a greenhouse. Before, being transferred into plastic pots containing of 4.5 L hydroponic solution, the rootstocks were thinned out to one shoot and were placed in 10 mM $CaSO_4$ for 2 days to accelerate wound closure. Six biological replicates (six pots) with each having one plant per pot were cultivated per treatment. The concentration of the nutrient solution was one-fourth at the start of the experiment and was increased to one-half after three days and to full strength after another three days. The full-strength nutrient solution had the following concentration: 0.5 mM KH_2PO_4, 0.7 mM K_2SO_4, 0.65 mM $MgSO_4$, 100 µM Fe-Sequestren, 20 µM H_3BO_3, 3 µM $MnSO_4$, 5 µM $ZnSO_4$, 0.4 µM $CuSO_4$, 0.05 µM $(NH_4)_6Mo_7O_{24}$ and 22pp potassium water glass (K_2SiO_3). The rootstocks were treated with five different N forms, namely calcium nitrate, ammonium sulphate, urea, arginine and glutamine. The N treatments had the following concentrations: 2 mM $Ca(NO_3)_2$, 2 mM $(NH_4)_2SO_4$, 2 mM CH_4N_2O, 1 mM $C_6H_{14}N_4O_2$ (arginine) and 2 mM $C_5H_{10}N_2O_3$ (glutamine). All plants, other than the one that was treated with $Ca(NO_3)_2$, additionally recieved 2 mM $CaSO_4$ to adjust sulphate or calcium concentrations. The nutrient solutions were renewed once per week in the first two weeks, following which it was renewed every second day. Rootstocks were cultivated for seven weeks in a greenhouse (ambient temperature °C).

2.1.2. Physiological data and yield determination

Each pot was weighed before and after solution change to measure the quantity of nutrient solution that had been taken up from the rootstock. Furthermore, a sample of 20 mL from every nutrient solution (before and after solution change) was collected to measure the quantity of N taken up by the plant from the nutrient solution. The samples were immediately frozen and stored at −20 °C until analysis. At harvest, rootstocks were dipped in dH_2O for 20 s. to clear roots of any remaining nutrient solution. Plants were divided into their different organs. The youngest leaves (five apical fully developed leaves) and the medium mature leaves were immediately frozen in liquid N. The samples were ground to a fine powder by using liquid N and stored at -80 °C for further analysis. Shoot, wood and the remaining leaves (five basal physiologically active leaves) were collected and oven-dried at 80 °C and ground to a fine powder, following which the samples were stored in a desiccator until analysis.

2.1.3. Enzyme extraction and assessment of nitrate reductase activity (NRA)

About 150–200 mg of frozen leaf powder [n = 6 technical replicates] (from the youngest leaves and medium mature leaves) were weighed in 2 mL reaction tubes, following which 800 µL buffer solution 1 (100 mM Tris/HCl pH 8.0, 1 mM EDTA, 10 mM cysteine) were added and vortexed vigorously. The mixture was centrifuged for 10 min at 18 700 *xg* at 4 °C. The aqueous phase was then isolated and directly placed on ice. Subsequently, 400 µL of the supernatant was pipetted into two test tubes and, each was mixed with 600 µL of buffer solution 2 (100 mM phosphate buffer pH 7.0, 100 mM KNO_3, 1 mM NADH + H^+). The reaction in one tube (test tube 1) was immediately stopped by adding 200 µL of 1 M $C_4H_6O_4Zn$ and incubated on ice for 30 min. The second tube (test tube 2) was incubated for 30 min. at 30 °C, the reaction was stopped by the addition of 200 µL of 1 M $C_4H_6O_4Zn$. Both test tubes were centrifuged for 2 min. at 18 700 *xg* and room temperature. Aliquots of 500 µL of the supernatants were transferred to new tubes and mixed with 500 µL of 1% (w/v) sulphanilamide in 25% HCl and 500 µL of 0.02% (w/v) N-(1-naphthyl)

ethylenediaminedihydrochloride. Test tubes were vortexed vigorously before being incubated for 15 min at RT. The absorbance of both test tubes was measured at 540 nm according a standard curve (50 mM of KNO_2) with a plate reader (Tristar^2 Multimode Reader LB942, Berthold Technologies, Bad Wildbad, Germany). The concentration of NO_2^- was calculated by the subtraction of the value of test tube 2 from that of test tube 1. Values of NRA are expressed as nitrate reductase (NR) activity µmol NO_3^- /g^{-1} FW / h^{-1}.

2.1.4. Nitrogen content in hydroponics

For the analysis of N, we used 7.5–8.5 mg of ovendried sample material [n = 5 technical replicates]. The analysis was carried out in an N analyser system, based on the method of pyro-chemiluminescence (Antek 7000 Element Analyzer, Antek Instruments GmbH, Düsseldorf, Germany).

2.2. Experiment II (soil experiment)

2.2.1. Plant growth conditions

In 2015, 120 two-year-old graftings of *Vitis vinifera* L. *cv.* Regent on rootstock SO4 were planted in Mitscherlich pots with 6.6 kg soil, consisting of 50% clay, 45% sand and 5% turf. The vines were kept in the greenhouse for the 2015 season under maintenance fertilisation of 1.0 g/ plant HARKAPHOS BLAU 15 + 10 + 15(2) (COMPO EXPERT, Krefeld, Germany) and 0.1 g /plant FETRILON COMBI (BASF, Ludwigshafen, Germany) every two weeks. Vines were thinned to one shoot and hibernated outside the greenhouse. At the beginning of the second season in May 2016, the plants were transferred into the greenhouse (13–23 °C) and, for each plant, the vertically trained shoot was pruned to 30–40 cm with at least two remaining inflorescences. During cultivation, grapevine shoots were cut at 1.30 m and laterals were removed regularly.

2.2.2. Nitrogen treatment of the grapevines

Nitrogen was applied at 17th June 2016, prior to flowering (BBCH 19). It was supplied as $Ca(NO_3)_2$, $(NH_4)_2SO_4$, CH_4N_2O or $C_6H_{14}N_4O_2$. Ammonium sulphate and urea (PIAGRAN 46) were applied with nitrification inhibitors (SKW Stickstoffwerke Piesteritz GmbH, Lutherstadt Wittenberg). Nitrogen treatments were applied in four different quantities: 0 g N/plant, 0.5 g N/plant, 1.0 g N/plant and 3.0 g N/plant (referred as N0, N0.5, N1.0, N3.0) and in eight biological replicates per treatment. In total, we carried out ten different treatment combinations (N0; CaN-N0.5; CaN-N1.0; CaN-N3.0; AM-N0.5; AM-N1.0; AM-N3.0; UR-N0.5; UR-N1.0; UR-N3.0; Arg-N0.5; Arg-N1.0; Arg-N3.0). The treatment with nil N fertilization (N0) is here referred to as the 'control'.

2.2.3. Physiological data and yield determination

Berries were harvested on 23rd to 24th September 2016. The total weight [g] of the grape bunches per plant [n = 8 technical replicates] was determined after separation of the berries and stems. Must yield [g] was documented. Leaves and shoots were sampled as described above

2.2.4. Nitrogen content

The method of Dumas [18] was used for the analysis of N in ovendried material of leaves and shoots. The analysis was performed with an elemental analyser (Vario MACRO cube CHNS, Elementar Analysensysteme GmbH, Hanau, Germany).

2.2.5. Must analysis

Titratable acidity (TA) and pH were analysed by means of a titrator (TitroLine easy, Schott, Mainz). Total soluble solids (°Brix; TSS) were measured with a refractometer (Opton, Zeiss, Germany). Total phenolic content was measured with a spectrophotometer by using the Folin-Ciocalteu method [19]. Total acid, subdivided into tartaric acid and malic acid, and sugar were determined with a high performance liquid chromatography (HPLC) (Merck-Hitachi, Darmstadt, Germany). Potassium phosphate (20 mM, pH 1.5) was used as the mobile phase with a flow rate of 1 mL min^{-1} and detection at 210 nm. The employed column was a Synergi™ 4 µm Hydro-RP 80 Å, LC column 250 x 4.6 mm, Ea (separation column).

2.3. Statistical analysis and data visualization

The two experiments, namely; hydroponics (1) and soil (2) experiments, were analysed separately by using SAS software (version 9.4, Cary, North Carolina, U.S.A.). For both experiments, a MIXED- MODEL with a Kenward-Roger test was used at a significance level of $p \leq 0.05$. Log-transformed and thus inverse-transformed data were empolyed for all parameters, except for the analysis of N of leaves and shoots. For the statistical test of the soil experiment, a sqrt-transformation and thus inverse-transformed data were used for the parameters pH, total acid and must weight. The following abbreviations were used: calcium nitrate, CaN; ammonium sulphate, AM; urea, UR; arginine, Arg; glutamine, Gln.

3. Results

3.1. Experiment I (hydroponics)

3.1.1. Vegetative growth of the rootstocks Ru140 and SO4

The biomass of the youngest leaves from the rootstock variety Ru140 (Fig. 1, black bars) was significantly different between AM, Arg and Gln on one hand and between CaN and Gln and between UR and Gln on the other. The N form AM had the significantly highest biomass production (mean; 1.0 g FW^{-1} /plant) and the amino acid Gln had the significantly lowest biomass production (mean; 0.3 g FW^{-1} /plant). Within the rootstock variety SO4, the N form CaN (mean; 2.3 g FW^{-1} /plant) had the significantly highest biomass and the amino acid Arg (mean; 0.6 g FW^{-1} /plant) has the significantly lowest biomass. Biomasses of SO4 and Ru140 only showed significant differences between CaN, AM and UR on one hand and both amino acids on the other. This was consistent in young, middle and old leaves and in the shoots (Fig. 1a–d, grey bars).

Fig. 1e shows the biomass allocation of the roots. SO4 root growth was significantly affected in the order of the highest growth with CaN > AM, UR > amino acids. Ru140 root biomass allocation was similar. For every leaf type and for every plant organ (Fig. 1a–e), the rootstock variety SO4 had significantly higher biomass allocation as compared with the rootstock variety Ru140.

3.1.2. Root to shoot ratio and leaf to shoot ratio

The root: shoot ratio of the rootstocks Ru140 und SO4, when cultivated with different N forms, showed significantly higher ratios when the amino acids Arg and Gln were applied compared with the N forms CaN, AM and UR applied. The rootstock Ru140 showed a significantly higher ratio compared with rootstock SO4 (Table 1).

3.1.3. Nitrogen content of the different plant organs with respect to nitrogen forms applied

The N content [% N DW^{-1}] of the leaves of the rootstocks Ru140 and SO4 did not show significant differences, within different N forms (Fig. 2). A tendency, with SO4 having consistently the higher N content compared with Ru140 was detected (Fig. 2a).

The shoots showed similar trends with a higher N content of SO4 over Ru140 (Fig. 2b). In both genotypes, N nutrition with amino acids led to significantly lower N contents in the shoots (Fig. 2b). The N content in the wood were similar following all N treatments and in both genotypes (Fig. 2c).

3.1.4. Nitrate reductase activity (NRA) of the rootstocks Ru140 and SO4

The NRA in the young leaves of the rootstock Ru140 exhibited no

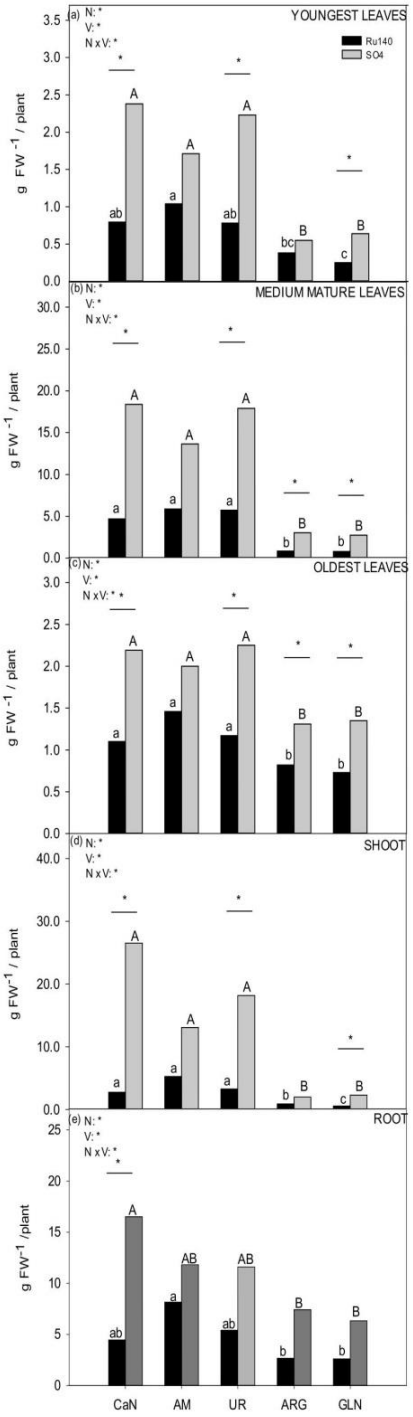

Fig. 1. Fresh weight [g FW $^{-1}$ / plant], following treatment with the five different Nforms (N) (CaN, AM, UR, Arg, Gln) in the plant organs of the two rootstock varieties (V) Ru140 (black) and SO4 (grey); (a), medium mature laves (b), oldest leaves (c), shoot (d) and root (e). Bars represent inverse-transformed adjusted means (n = 6). Lower case letters indicate significant differences between the various nitrogen forms within the variety Ru140, capital letters indicate significant differences between the various nitrogen forms within the variety SO4. Asterisks indicate significant differences within one Nform and between the two rootstock varieties. MIXED MODELS results are included in each diagram: (*) $p \leq 0.05$.

Table 1
Root to shoot ratio of the two rootstock varieties Ru140 and SO4 in response to five different Nforms (N) (CaN, AM, UR, Arg, Gln). The first blocks represent the different N forms. The average values of the rootstock are shown at the end of every block (separated line). Capital letters indicate significant differences between the rootstocks; lower case letters indicate significant differences between the different N supply treatments. Data are adjusted means (n = 6); MIXED MODELS $p \leq 0.05$.

Rootstock	Treatment	Root : Shoot
Ru 140	**CaN**	1.73 b
	AM	1.61 b
	UR	1.69 b
	Arg	3.53 a
	Gln	5.00 a
	Ø	2.42 A
SO4	**CaN**	0.61 b
	AM	0.84 b
	UR	0.63 b
	Arg	3.51 a
	Gln	2.50 a
	Ø	1.23 B

significant difference when cultivated with different N forms (Fig. 3a). In comparison, the rootstock SO4 showed significant higher NRA with AM nutrition (mean; 0.01 µmol NO_3^-/g FW/h^{-1}) and significantly lower NRA with Gln nutrition (mean; 0.003 µmol NO_3^- /g FW/h^{-1}). Moreover, the NRA of Ru140 with Gln nutrition is approximately four times higher in comparison with that of SO4 whereas the situation with AM nutrition is *vice versa* (Fig. 3a). The medium mature leaves demonstrated a similar pattern with no significant differences under all N treatments of Ru140 but a difference in SO4 with a higher NRA for CaN and for both amino acids treatments (Fig. 3b). The NRA of UR is rootstock dependent with a ten times higher NRA in Ru140 (Fig. 3b). However, no significant difference was detected in the NRA between the different Nforms when compared with each other independently from leaf type or rootstock variety (Fig. 3c).

3.2. Experiment II - soil experiment

3.2.1. Vegetative growth of Regent in response to different Nforms and quantities

The biomass allocation of the grapevine leaves showed a significant difference when cultivated with different N supply treatments (Fig. 4a). The treatment N0 without any N fertilization had the significantly lowest biomass allocation (mean; 4.1 g FW^{-1} / plant). The treatment N1.0 led to the significantly highest biomass allocation (mean; 5.3 g FW^{-1} / plant). Within the shoots the treatment N3.0 had the significantly lowest biomass allocation (mean; 27.0 g FW^{-1} / plant).

The biomass allocation of the grapevine leaves, when cultivated with the different N forms showed significant differences. The N form UR had the significantly highest biomass allocation (mean; 5.6 g FW^{-1} / plant) and the N0 treatment without any N had the significant lowest biomass allocation (mean; 4.1 g FW^{-1} / plant). The biomass allocation of the grapevine shoots exhibited no significant difference when cultivated with the different N forms (Fig. 4b).

3.2.2. Nitrogen content of Regent with regard to the different N forms and quantities

The N content in leaves and shoots depended on the N quantity in the nutrient solution. The higher the applied N amount (N3.0 mean; 2.1% N DW^{-1}), the higher was the N content in the leaves; however, no significant difference was detected between N0 (mean; 1.3% N DW^{-1}) and N1.0 (mean; 1.5% N DW^{-1}). A similar pattern can be seen in the shoots. The N0 treatment had the significantly lowest N content (mean; 0.3% N DW^{-1}) and the N3.0 treatment had the significantly highest N content (mean; 0.8% N DW^{-1}) (Fig. 5a). A significant increase in the N

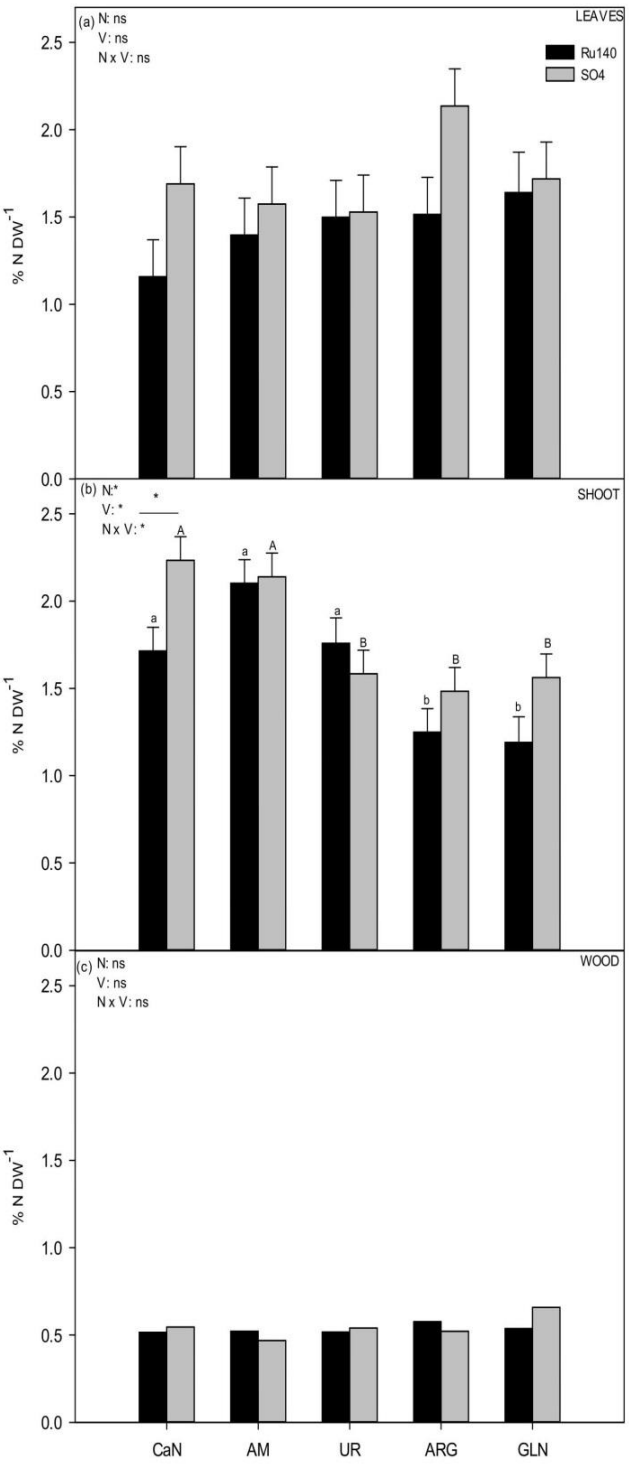

Fig. 2. Nitrogen content [%N DW^{-1}] after treatment with the five different Nforms(N) (CaN, AM, UR, Arg, Gln) in the following plant organs of the two rootstock varieties (V) Ru140 (black) and SO4 (grey); leaves (a), shoot (b) and wood (c). Bars represent adjusted means ± SE (n = 6). Lower case letters indicate significant differences between the various nitrogen forms within the variety Ru140, capital letters indicate significant differences between the various nitrogen forms within the variety SO4. Asterisks indicate significant differences within one Nform and between the two rootstock varieties. MIXED MODELS results are included in each diagram: ns: not significant, (*) $p \leq 0.05$.

content in the leaves and shoots attributable the different N treatments was detected (Fig. 5b). The N forms CaN, AM and UR exhibited a significantly higher N content and the amino acid Arg a significantly lower N content. In the shoots, the N form CaN had the significantly highest N content and the N0 treatment had the significantly lowest N content (Fig. 5b).

3.2.3. Generative growth with regard to different nitrogen forms and quantities

The total yield of a grape bunch and the resulting berry yield (minus stem) were not influenced by the N form (Table 2). Again, the N0 treatment had a significantly lower yield compared with all N treatments. The amount of N applied increased the total yield of a grape

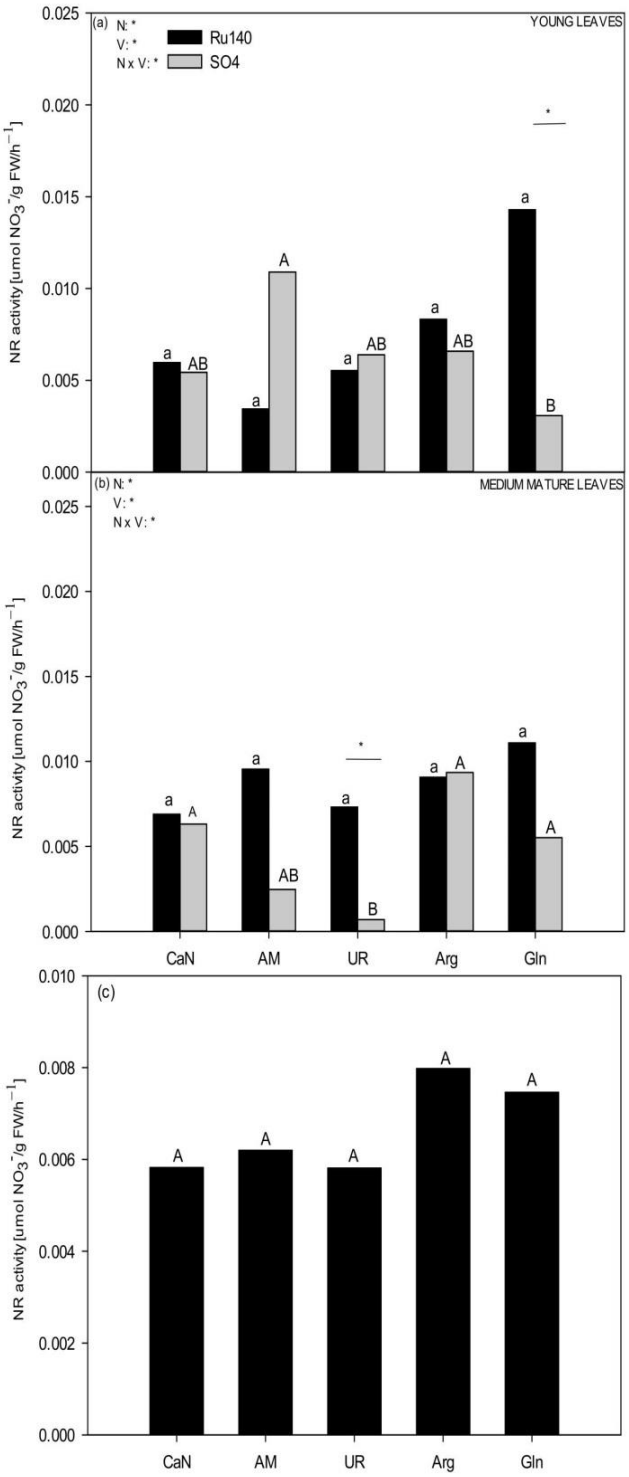

Fig. 3. Nitrate reductase activity (NRA) [μmol NO_3^- /g FW/h^{-1}] following treatment with the five different Nforms (N) (CaN, AM, UR, Arg, Gln) in leaf types in the two rootstock varieties (V) Ru140 (black) and SO4 (grey); young leaves (a), medium mature leaves (b). A comparison between the different N forms independent of the leaf types is shown in (c). Bars represent the inverse-transformed adjusted means (n = 6). Lower case letters indicate significant differences between the various nitrogen forms within the variety Ru140, capital letters indicate significant differences between the various nitrogen forms within the variety SO4. Asterisks indicate significant differences within one Nform and between the two rootstock varieties. MIXED MODELS results are included in each diagram: (*) $p \leq 0.05$.

bunch and the resulting berry yield. The N3.0 treatment, however, gave slight but significant lower yield values (grape bunch mean; 152.8 g FW^{-1} / plant; berry yield mean; 148.7 g FW^{-1} / plant) compared with the N0.5 and N1.0 treatments.

3.2.4. Ratio of vegetative growth to generative growth

The ratio of the vegetative growth to generative growth ratio in Regent cultivated with different N forms showed the significantly highest ratio within the N0 treatment (without any N). However, no significance was seen compared with UR. The amino acid Arg exhibited

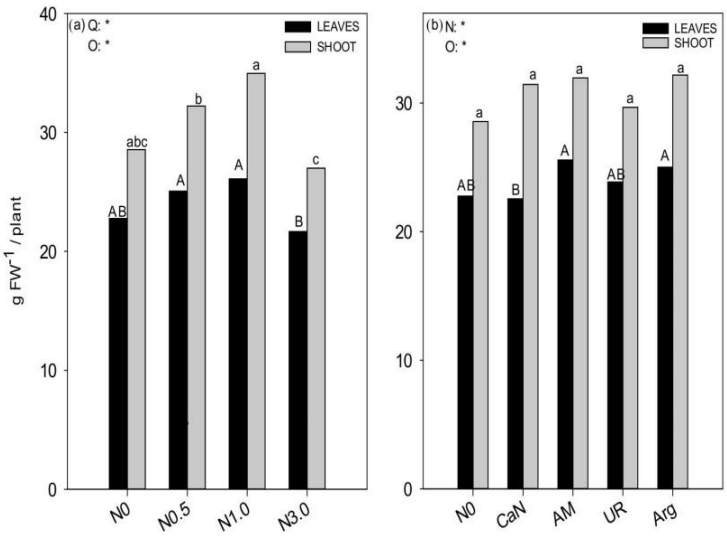

Fig. 4. Fresh weight [g FW $^{-1}$ / plant] of *Vitis vinifera* L. *cv.* Regent in the plant organs (O) of leaves and shoots, in response to four different nitrogen supply treatments (Q) (N0, N0.5, N1.0, N3.0) (a) and five different Nforms (N) (CaN, AM, UR, Arg, Control) (b). Bars represent the inverse-transformed adjusted means (n = 8). Capital letters indicate significant differences within the leaves and lower case letters indicate significant differences within the shoot. MIXED MODELS results are included in each diagram: (*) $p \leq 0.05$.

the significantly lowest vegetative to generative ratio but no significant difference was apparent compared with CaN, AM, or UR. With increasing N quantity (from N0.5 to N3.0), the ratio of vegetative growth to generative growth increased, except in the case of Arg. The N0 treatment had the significantly highest vegetative to generative ratio (mean; 0.56) (Table 3).

3.2.5. Quality of the grapevine berries with regard to different nitrogen forms and quantities

The must pH was not influenced by N forms, but the N quantity had significant influences; the higher the applied N amount, the higher was the pH value in the berries. The N0 treatment had the significantly lowest pH value (Table 4). The total acid concentrations significantly decreased with increasing N quantities. The N0 treatment had significantly higher acid concentrations (mean; 8.3 g L^{-1}), and the AM treatment has significantly lower acid concentrations (mean; 7.2 g L^{-1}). These results were inverse in terms of tartaric acid concentrations; here, the N3.0 treatment resulted in significant acid concentrations (mean; 6.2 g L^{-1}). The tartaric acid concentration was also influenced by the applied N form. Arg treatment led to significantly higher acid concentrations (mean; 6.7 g L^{-1}) and AM led to significantly lower acid concentrations (mean; 6.2 g L^{-1}). With regard to the malic acid concentration, the N0 gave significantly the highest acid concentration (mean; 4.6 g L^{-1}) and the N form CaN exhibited significantly lower malic acid concentrations (mean; 3.9 g L^{-1}). The concentration of phenolic compounds of the must increase with higher N application. The N0 treatment had, however, significantly higher concentrations (mean; 0.34 g L^{-1}). The N forms UR (mean; 0.20 g L^{-1}) and Arg (mean; 0.21 g L^{-1}) lead to significantly lower concentration of phenolic compounds. A comparable pattern with higher concentrations after zero N fertilization was detected in the must weight. The must weight is an important trait for grape ripening. It significantly decreased with increasing N quantities. The N0 treatment, gave significantly higher must weight (mean; 23.3° Brix). The N forms CaN and UR delay ripening and gave a significantly lower must weight (Table 4).

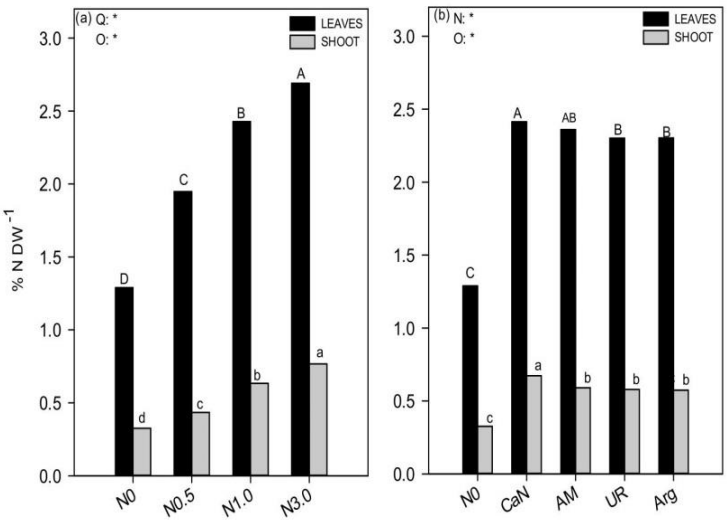

Fig. 5. Nitrogen content [%N DW $^{-1}$] of *Vitis vinifera* L. *cv.* Regent in the plant organs (O) of leaves and shoots, in response to four different nitrogen supply treatments (Q) (N0, N0.5, N1.0, N3.0) (a) and five different Nforms (N) (CaN, AM, UR, Arg, Control) (b). Bars represent the inverse transformed adjusted means (n = 8). Capital letters indicate significant differences within the leaves and lower case letters indicate significant differences within the shoot. MIXED MODELS results are included in each diagram: (*) $p \leq 0.05$.

Table 2
Total yield of grape bunch [g FW^{-1} / plant] and berry yield [g FW^{-1} / plant] of *Vitis vinifera* L. *cv.* Regent in response to four different Nforms (CaN, AM, UR, Arg) and four different nitrogen supply treatments (N0, N0.5, N1.0, N3.0). The first four blocks represent the adjusted means of the respective N form in combination with the supply treatments. The average values within one N form are shown at the end of every block (separated line). The last block represents the estimates of a comparison between the N supply treatments. Capital letters indicate significant differences between the N forms; lower case letters indicate significant differences between the different supply treatments within one N form. Bold italic capitals indicate significant differences between N supply treatments. Data are adjusted means ± SE (n = 8); MIXED MODELS $p \leq 0.05$.

Treatment	Total yield (bunch) [g FW^{-1} / plant]	Berry yield [g FW^{-1} / plant]
CaN		
N0.5	225.2 ± 17.9 a	216.1 ± 16.6 a
N1.0	237.3 ± 17.8 a	224.7 ± 16.5 a
N3.0	127.1 ± 17.4 b	121.1 ± 16.1 b
Ø	196.6 ± 11.4 A	187.3 ± 10.4 A
AM		
N0.5	206.8 ± 17.9 a	196.8 ± 16.6 a
N1.0	193.1 ± 17.6 a	184.1 ± 16.4 a
N3.0	170.3 ± 17.7 a	162.9 ± 16.4 a
Ø	190.1 ± 11.4 A	181.3 ± 10.4 A
UR		
N0.5	203.3 ± 17.4 a	193.9 ± 16.1 a
N1.0	225.7 ± 17.6 a	216.0 ± 16.3 a
N3.0	118.7 ± 17.4 b	110.7 ± 17.1 b
Ø	182.6 ± 11.5 A	178.3 ± 10.7 A
Arg		
N0.5	187.8 ± 17.6 a	179.4 ± 16.3 a
N1.0	213.9 ± 17.7 a	207.9 ± 16.4 a
N3.0	194.9 ± 17.5 a	185.7 ± 16.3 a
Ø	199.5 ± 11.5 A	191.0 ± 10.49 A
(N0)	104.9 ± 18.78 B b ***C***	96.3 ± 16.0 B b ***C***
N0.5	205.8 ± 10.6 ***A***	196.6 ± 9.6 ***A***
N1.0	218.0 ± 10.6 ***A***	208.2 ± 9.6 ***A***
N3.0	152.8 ± 10.5 ***B***	148.7 ± 9.6 ***B***

Table 3
Vegetative growth to generative growth ratio of *Vitis vinifera* L. *cv.* Regent in response to four different Nforms (CaN, AM, UR, Arg) and four different nitrogen supply treatments (N0, N0.5, N1.0, N3.0). The first four blocks represent the adjusted means of the respective N form in combination with the supply treatments. The average values within one N form are shown at the end of every block (separated line). The last block represents the estimates of a comparison between the N supply treatments. Capital letters indicate significant differences between the N forms; lower case letters indicate significant differences between the different supply treatments within one N form. Bold italic capitals indicate significant differences between N supply treatments. Data are adjusted means (n = 8); MIXED MODELS $p \leq 0.05$.

Treatment	Vegetative growth : Generative growth
CaN	
N0.5	0.28 bc
N1.0	0.25 b
N3.0	0.40 ac
Ø	0.31 B
AM	
N0.5	0.30 b
N1.0	0.32 b
N3.0	0.34 ab
Ø	0.32 B
UR	
N0.5	0.30 b
N1.0	0.27 b
N3.0	0.53 a
Ø	0.36 AB
Arg	
N0.5	0.30 b
N1.0	0.30 b
N3.0	0.30 b
Ø	0.30 B
(N0)	0.56 A a ***A***
N0.5	0.30 ***B***
N1.0	0.28 ***B***
N3.0	0.39 ***A***

4. Discussion

4.1. Vegetative growth and nitrogen content of grapevines in response to different nitrogen forms and quantities

In terms of vigor both grapevine rootstocks responded with a significant growth difference to the N forms tested (Fig. 1). The biomass of all green plant parts was significantly greater following treatment with CaN, AM and UR compared with the two amino acids. In detail, the data indicated that calcium nitrate (CaN) was the preferred N form for the rootstock SO4 and ammonium sulphate (AM) was the preferred N form for rootstock Ru140 (Fig. 1a–e). Therefore, the preferred N form, (in this case either nitrate or ammonium), is variety-dependent. Nitrate and ammonium are the major sources for inorganic N of the plant [20]. Whereas nitrate is readily mobile in the xylem and can be included into vacuoles as a storage pool, ammonium has to be integrated into amino acids or amides to be available for the plant. The uptake of N in the form of nitrate or ammonium is an active process that is controlled by plasma-membrane-localized transporters in the root [20,21]. In higher plants two transporter families for nitrate uptake are present, *i.e.* low affinity transporters (LATS) NRT1/PTR and high affinity transporters (HATS) NRT2 [22,23], whereas the uptake of ammonium is mediated by the high affinity transporters of the AMT/MEP/Rh (AMT) subfamily [21]. The situation of these transporter activities in the grapevine remains unclear. The transporter families seem to be differentially regulated or are present in different rootstock genotypes of the grapevine. Initial results of Tomasi et al. [24] indicate that in the grapevine, NRT1 transporters are sustained by LATS and NRT2 transporters are sustained by HATS. This needs to be elucidated in a further study.

Root to shoot ratios can often characterize plants responses to changed nutritional status [25]. Our results demonstrate an increased root to shoot ratio when the amino acids Arg and Gln are applied. This indicates that the rootstock may can enhances its root growth to increase its absorption capacity for Arg and Gln and presumably to raise transporter density per root unit. The sink to source relationship would change towards a greater sink capacity. Therefore, roots may have a higher priority for photosynthate accumulation [25]. This is valid for both rootstock varieties, but for Ru140 to a greater extent than for SO4. However, Jiao et al. [26] demonstrated that amino acids as a sole N source can stimulate root growth by using the higher concentration of free amino acids (converted to available amino acids and N-containing compounds) in the roots that can supply carbon skeleton and N for growth and development.

Our data show that none of the applied N forms, including both amino acids (in equal amounts), influence the N content in leaves (Fig. 2a). This implies that the leaves are able to take up N from all the different N sources at a similar rate. However, as stated above, the rootstocks exhibit less growth when N is applied as amino acids. Moreover, the N content in the wood is not affected (Fig. 2c). This is explainable on the basis that wood serves as a storage organ for remobilized N from former growth periods and therefore is presumably not affected by the N treatments in the two experiments.

The nitrate reductase activity (NRA) was influenced by leaf age and variety (Fig. 3a and b). This observation is consistent with Marschner [27] who indicates that the maximum NRA in plants occurs when the leaf expands and that NRA is very low in fully expanded leaves. The

Table 4
Estimated values of the chemical attributes of berries *Vitis vinifera* L. *cv*. Regent in response to four different Nforms (CaN, AM, UR, Arg) and four different nitrogen supply treatments (N0, N0.5, N1.0, N3.0). The first four blocks represent the estimates of the respective N form in combination with the supply treatments. The average values within one N form are shown at the end of every block (separated line). The last block represents the estimates of a comparison between the N supply treatments. Capital letters indicate significant differences between the N forms; lower case letters indicate significant differences between the different supply treatments within one N form. Bold italic capitals indicate significant differences between N supply treatments. Data are adjusted means ± SE (n = 8); MIXED MODELS $p \leq 0.05$. In transformed data, SE is not shown.

Treatment	pH	Total acid [g L^{-1}]	Tararic acid [g L^{-1}]	Malic acid [g L^{-1}]	Phenols [g L^{-1}]	Must weight [° Brix]
CaN						
N0.5	3.2 b	7.4 ab	6.6 ± 0.23 a	3.6 ± 0.2 a	0.27 ± 0.03 ab	22.0 ac
N1	3.2 b	8.0 a	6.7 ± 0.22 a	4.0 ± 0.2 a	0.22 ± 0.03 b	17.5 c
N3	3.4 a	6.9 b	5.8 ± 0.22 b	4.0 ± 0.2 a	0.31 ± 0.02 a	22.1 ac
Ø	3.3 A	7.4 ABC	6.4 ± 0.2 AB	3.9 ± 0.1 C	0.26 ± 0.01 A	20.5 B
AM						
N0.5	3.3 a	7.7 a	6.2 ± 0.3 a	3.5 ± 0.2 a	0.21 ± 0.03 b	22.6 a
N1	3.3 a	7.0 b	6.2 ± 0.3 a	3.4 ± 0.2 a	0.26 ± 0.03 ab	22.3 a
N3	3.4 a	6.9 b	6.4 ± 0.3 a	3.1 ± 0.2 a	0.27 ± 0.03 ab	20.9 a
Ø	3.3 A	7.2 C	6.2 ± 0.2 B	3.3 ± 0.1 B	0.25 ± 0.01 AC	20.5 AB
UR						
N0.5	3.1 b	8.1 a	6.7 ± 0.3 a	3.9 ± 0.2 a	0.17 ± 0.03 b	22.1 ab
N1	3.2 b	7.7 a	6.9 ± 0.3 a	3.4 ± 0.2 ab	0.19 ± 0.03 b	19.8 b
N3	3.5 a	6.1 b	6.0 ± 0.3 b	2.9 ± 0.2 b	0.23 ± 0.03 b	22.6 a
Ø	3.3 A	7.3 ABC	6.6 ± 0.2 AB	3.4 ± 0.1 B	0.20 ± 0.01 B	21.5 B
ARG						
N0.5	3.1 b	8.4 a	6.8 ± 0.2 a	3.8 ± 0.2 a	0.18 ± 0.03 b	22.7 a
N1	3.3 a	7.2 bc	6.5 ± 0.3 a	3.5 ± 0.2 ab	0.21 ± 0.02 bc	21.9 a
N3	3.4 a	7.0 b	6.7 ± 0.2 a	3.3 ± 0.2 b	0.25 ± 0.02 ac	21.6 a
Ø	3.2 A	7.5 BC	6.7 ± 0.2 A	3.5 ± 0.1 B	0.21 ± 0.01 BC	21.5 AB
CONTROL (N0)	3.2 A ab ***A***	8.3 A ac ***AB***	6.3 ± 0.3 AB ab ***AB***	4.6 ± 0.1 A bc ***C***	0.34 ± 0.02 A a ***A***	23.3 A a ***A***
N0.5	3.3 ***A***	7.9 ***A***	6.6 ± 0.1 ***A***	3.1 ± 0.1 ***A***	0.21 ± 0.01 ***B***	22.0 ***AB***
N1	3.2 ***A***	7.5 ***B***	6.6 ± 0.1 ***A***	3.6 ± 0.1 ***AB***	0.22 ± 0.01 ***B***	22.0 ***B***
N3	3.4 ***B***	6.7 ***C***	6.2 ± 0.1 ***B***	3.4 ± 0.1 ***B***	0.26 ± 0.01 ***A***	20.2 ***B***

NRA under amino acid nutrition does not differ significantly from that under other N forms (Fig. 3c). This finding contrasts with the findings of Baloft [28]. Glutamine (and ammonium) is considered as an NRA repressor [29,30]. However, several researchers have reported no changes of NRA attributable to amino acid nutrition; these ideas have been reviewed by Srivastava [31].

Based on our results, grapevines are able to take up N in the form of the two amino acids, although arginine is taken up to a greater extent than glutamine. One reason might be that arginine is a major N form for storage and transport in sink tissues [30,32,33] and the most important storage compound in the grapevine [16]. Amino acid transporters (APC and ATF) in the vascular systems of the plants transport molecules from sites of primary assimilation to organs with nutritional needs, such as developing leaves, meristems and reproductive organs, which are not a part of the N assimilation system [34]. Nitrogen availability is a trigger for root growth and can modulate architecture and thus influences N uptake by increasing absorptive root surface [23]. Thus, the reduced vigor and reduced NRA of the vines when amino acids were applied might also lead to a similar N content together with a reduced total N uptake. The N content in these plants is similar but the plants are smaller and the total N accumulation is lower compared with plants supplied with AM or CaN.

Obviously with increasing N application, biomass allocation of leaves and shoots as well as N content in these plant organs increased (Fig. 5a and b). Other studies [1,35–37] have found that additional N leads to an increased N content in grapevine tissues. Interestingly, our results (*i.e.* Fig. 4a) indicate an optimum N amount, namely N = 1.0 g/L^{-1}, in the soil per plant when cultivated in a pot. The high N dose (N3.0) induces a saturation of the available N in the green plant parts and in the berries and results in the accumulation and storage of N in the shoots and leaves. These findings are consistent with those of Bell and Henschke [7] who have concluded that high levels of N increases and disrupts vine growth and balance leading to detrimental impacts on berry yield and possibly influencing the composition of grape and berry metabolites. In contrast Loulakakis and Roubelakis-Angelakis [38] argue that a high supply of nitrate is innocuous and that N can be stored in green plant tissues without any deleterious effect.

4.2. Yield and quality effects of applied nitrogen forms and quantities

Berry yield increased with increasing N supply but slightly decreased with the highest dosage (N3.0). These results demonstrate, similar to the vegetative growth data (Fig. 2), that an optimum for N supply exists for grapevines and a negative effect takes place with further increase. The N source-to-sink relationship is obviously negatively influenced by excessive N [7,39]. If sink and source compete for carbohydrates, this may lead to a limitation of photosynthates in growing tissues and might result in reduced growth or reduced vigor, thereby changing the quality of the berries and subsequently of the wine. Some quality influences of a changed leaf to fruit ratio have been reviewed by Jackson and Lombard [9]. With increasing N quantities, the ratio of vegetative to generative growth (which equate with the sink to source ratio) changed to a higher vegetative biomass (sink) (Table 3). This would further indicate that, with increasing N quantities, even more photosyntates such as starch and sugar remain in the leaves and, therefore, the berry yield may decrease further. The different N- forms do not influence this sink to source relationship, although greater vegetative growth is dependent on whether the plant is fertilized. The plants fertilized with the N form Arg show a similar vegetative to generative growth ratio compared with the other N forms, confirming that these amino acid can be taken up by the plant root and that subsequently N is assimilated into the grape. The biochemical composition of the berries is influenced by the N form and by the N quantity (Table 4). The higher the N dose applied, the lower the total acid, malic acid and tartaric acid, which are important factors for grape quality (wine stability and impacts on taste) [40]. These acids also play an important role in berry ripening, with a higher N supply delaying the maturation of grapevines and therefore prolonging the ripening

process.

The lower the N dose applied the higher the total phenolic compounds in the must (Tab .4). Low N status in grapevines limits aroma precursors and glutathione synthesis is reduced, resulting in increased phenolic compounds in the must and may negatively impact wine quality [41,42].

This observation is consistent with the studies of Hilbert et al. [43] and Conde et al. [44] influencing the must acidity by the adjustment of the N supply to the vine may serve as a controlling element effecting the wine quality.

5. Conclusions

The rootstock exerts a great impact on the plant performance of a grapevine. The preference of the grapevine rootstocks Ru140 and SO4 for a specific N form as a N source is variety-dependent. Nitrate, ammonium and urea and the amino acids Arg and Gln are taken up by the vine roots but vigor and nitrate reductase activity is lower if the vines grow with amino acids. The amount of applied N has a greater impact on biomass allocation and the N content of Regent compared with the supply of other N forms, whereas growth and berry yield increase with an increasing N supply. An optimal N amount has been detected and excess supply leads to yield reductions. A transition in the sink : source relationship was detected when the optimum N amount is exceeded. Excessive amounts of N lead to the increased growth of green parts and result in the lower availability of other nutrients or carbohydrates for quality production in the berries and, therefore, in the wine. In terms of wine quality, the total must acidity decreases together with the main organic acids, whereas the concentration of phenolic compounds and must weight increases.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.plantsci.2018.10.004.

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Appendix for Chapter 2

Supplemental Data

Nitrogen concentration in nutrient solution

The analysis of Gln and Arg by α-amino-N was done according to (Lie 1973) by the use of a colorimetric method. This based on an oxidative decarboxylation and development of CO_2, ammonia and an aldehyde. Afterwards ninhydrin reacted with ammonia via the formation of a blue complex which can be measured photometrically.

The analysis of NO_3^- was done for all different nutrient solutions. The colorimetric method was done according to a German DIN norm (DIN ISO 38405-9: 2011-09) – protocol. Following addition was made: KNO_3 for standard, and the incubation took place in an ultrasonic bath. The method is based on a reaction of nitrate ions, which are dissolved in a phosphoric and sulfuric solution, with 2,6-dimethylphenol to 4 nitro-dimethylphenol. The reaction product can be detected photometrically at 324 nm.

S.1: (a) Nitrate (NO_3^-) [mg / ml] content and (b) α- amino N content [mg / ml] in the different nutrient solutions (CaN, AM, UR, ARG and GLN) before plant cultivation (PC) and after two days of plant cultivation (PC) within the different rootstock varieties Ru140 and SO4.Capital letters indicate significant differences between before plant cultivation (Before PC) and after plant cultivation (After PC) within one rootstock variety (Ru140 & SO4). Shown data are estimates ± SE (n=1 Before PC; n=8 After PC); MIXED MODELS $p \geq 0.05$.

(a)

Sample	Nitrate (NO_3^-) [mg / ml]	
	Before PC	After PC
CaN		
Ru140	278.11 ± 21.13 A	287.20 ± 8.91 A
SO4	278.11 ± 21.13 A	288.78 ± 8.91 A
AM		
Ru140	1.84 ± 21.13 A	8.90 ± 8.91 A
SO4	1.84 ± 21.13 A	3.82 ± 8.91 A
UR		
Ru140	1.71 ± 21.13 A	17.05 ± 8.91 A
SO4	1.71 ± 21.13 A	16.30 ± 8.91 A
ARG		
Ru140	(')-2.24 ± 21.13 A	47.03 ± 8.91 B
SO4	(')-2.24 ± 21.13 A	36.56 ± 8.91 B
GLN		
Ru140	2.10 ± 21.13 A	26.27 ± 8.91 A
SO4	2.10 ± 21.13 A	50.49 ± 8.91 B

(b)

Sample	α- amino N [mg / ml]	
	Before PC	After PC
ARG		
Ru140	3.54 ± 1.14 A	3.33 ± 0.53 A
SO4	3.54 ± 1.14 A	2.73 ± 0.43 A
GLN		
Ru140	10.53 ± 5.22 A	4.19 ± 0.92 A
SO4	10.53 ± 5.22 A	2.88 ± 0.63 B

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CHAPTER 3

Expression of key enzymes for nitrogen assimilation in grapevine rootstock in response to N-form and timing

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Expression of key enzymes for nitrogen assimilation in grapevine rootstock in response to N-form and timing

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Expression of key enzymes for nitrogen assimilation in grapevine rootstock in response to N-form and timing

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Abstract

Background and Aims: Rootstocks play an essential role in grapevine scion growth and development. They influence water and nutrient uptake and affect biomass allocation of the scion and the grape berry composition connected with wine quality. Nitrogen (N) can be taken up by the roots in various forms such as nitrate, ammonium or urea. These N-forms and their possible differential assimilation directly and indirectly influence grapevine vegetative and generative growth. N assimilation is driven by N acquiring enzymes such as nitrate reductase (NR), nitrite reductase (NiR) and glutamine synthetase (GS). This assimilation physiology can be influenced by factors such as light conditions or substrate availability.

Methods: Hydroponically grown grapevine rootstocks were fertilized with various N-forms, namely calcium nitrate (CaN), ammonium (AM), urea (UR), and glutamine (Gln). The transcript expression of the enzymes NR, NiR and GS and the enzymatic nitrate reductase activity (eNRA) were examined at various time points (0 h, 3 h, and 6 h) after N application.

Results and Conclusion: The data suggest that the grapevine rootstock SO4 has the ability to assimilate the amino acid Gln. Furthermore, AM, UR, and in some organs Gln, can regulate the co-enzymes NR and NiR, both of which function as activators of the NO_3^- assimilation process. The eNRA is clearly defined by the plant organ.

Supporting Information available online

Key words: gene expression / grapevine / nitrogen assimilation / rootstock

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1 Introduction

The grafting of woody perennials is a common agronomic practice used worldwide to improve growth and productivity and to deal with several biotic and abiotic factors. Nowadays, more than 70 crop species are grafted (*Warschefsky* et al., 2016). In grapevines, the complex interactions between the rootstock and scion play essential roles with regard to several environmental conditions, such as mineral nutrition, water uptake, and pest and disease control, and for grape berry composition. European scion varieties *Vitis vinifera* are grafted onto American rootstock varieties or interspecific hybrids of *Vitis* species such as *V. berlandieri, V. riparia, V. rupestris, V. rotundifolia*, or *V. amurensis* (*Arrigo* and *Arnold*, 2007) to avoid devastating phylloxera infection. Rootstocks are not only major organs for nutrient storage; they are also actively involved in nutrient and water uptake. Moreover, rootstocks form a linkage between the roots in the soil and the above ground part of the plant (*Ollat* et al., 2016). This rootstock–scion combination affects scion growth and development and *vice versa* and, in particular, the translocation of nutrients into the vegetative and generative plant organs (*Medici* et al., 2017). Furthermore, plant vigor, hormonal regulation, and long-distance signaling between various plant parts are influenced by the rootstock (*Zhang* et al., 2016).

Nitrogen (N) is an essential macronutrient in the grapevine and is one of the most limiting nutrients for plant growth and development. N application directly and indirectly triggers grapevine growth and development, the resulting grape berry composition and, thus, the must composition and fermentation kinetics of the wine (*Bell* and *Henschke*, 2005). N is an important precursor for plant components, such as primary and secondary metabolites, proteins, and nucleic acids, and for aroma compounds (*Downey* et al., 2006; *Tomasi* et al., 2015). Nitrate and ammonium are considered as the predominant N sources for higher plants and occur in various concentrations in the soil (*Xu* et al., 2012). Many plants have the ability to take up N in organic form, especially as amino acids that come primarily from the organic matter in the soil. The significance of this uptake and / or absorption is still unclear (*Andersson* and *Berggren*, 2005). This becomes increasingly obvious and commonplace in regions where N supply is limited in the system (*Schimel* and *Bennett*, 2004). Glutamine is an important signaling molecule for plants and is considered a donor for the synthesis of other amino acids, nucleotides and other important nitrogen-containing components. Thus, glutamine can play an essential role in the growth and development of plants; this has already been demonstrated in rice (*Kan* et al., 2015). However, former results have shown that

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other N-forms, such as urea and amino acids, can also be assimilated by the vine (*Lang* et al., 2018). The uptake and assimilation of these different N-forms in grapevines depend largely on the genetic prerequisite and on the developmental status of the plant (*Roubelakis-Angelakis* and *Kliewer*, 1992). An overview of the N uptake and assimilation process in plant cells of higher plants and therefore also of grapevines is shown in Fig. 1. The first step in the assimilation of N is the reduction of nitrate (NO_3^-) to nitrite (NO_2^-), catalyzed by the enzyme nitrate reductase (NR, EC 1.7.1.1 / 1.7.1.2 / 1.7.1.3), and the following reduction to ammonium (NH_4^+) by the enzyme nitrate reductase (NiR, EC 1.7.7.1) in the cytosol. NH_4^+ is assimilated by the enzymes glutamine synthetase (GS, EC 6.3.1.2) and glutamate synthase, which is also called glutamine 2-oxoglutarate aminotransferase (GOGAT, EC 1.4.1.14), to produce various amino acids by the incorporation of glutamine (Gln) and glutamate (Glu) in the chloroplast and the cytosol (*Goel* and *Singh*, 2015; *Balotf* et al., 2016). The assimilation of urea (CH_4N_2O) either can be directly mediated by the enzyme urease (EC, 3.5.1.5) to form NH_4^+ or can be mediated *via* the ornithine pathway and / or during the catabolism of purins or ureides (*Kojima* et al., 2006; *Witte*, 2011). The enzyme glutamate dehydrogenase (GDH, EC 1.4.1.3) is involved in NH_4^+ assimilation and catalyzes the synthesis of glutamate (Glu) from NH_4^+ and 2-oxoglutarate (*Goel* and *Singh*, 2015). The review of *Lillo* and *Appenroth* (2001) suggests that the regulation of NR is adjusted by several environmental stimuli and internal factors, with light and nitrate affecting the enzyme induction strongly. Water availability, salt concentration, *e.g.*, calcium or NaCl concentration, plant hormones and plant exposure to such conditions can also have an effect (reviewed by *Reda* et al., 2011; *O'Brien* et al., 2016). *Hunter* and *Ruffner* (1997) have reported a circadian regulation of nitrate reductase activity. Different rootstock varieties vary considerably according to their capability for taking up and accumulating N (*Lang* et al., 2018). Furthermore, the different rootstock varieties are adapted to disparate environmental and agricultural conditions, *e.g.*, location, soil type, water, and nutrient availability. Despite the uptake and assimilation processes of N in grapevines having been elaborated at the transcriptional level in detail over the last few decades, little is known based on the various N-forms, about the regulation of the gene expression of NR, and the downstream enzymes NiR and GS in rootstocks. The rootstock 'Selektion Oppenheim' (SO4) is one of the most widely used rootstocks in Germany and has been chosen for this study, based on its variety characteristics of vigorous growth, good root development, and sensitive response to N deficiency (*Keller* et al., 2001; *Bundessortenamt*, 2015).

We have investigated the expression of NR, NiR, and GS in rootstocks treated with various forms of N, namely calcium nitrate, ammonium sulfate, urea, and glutamine at both the transcript and enzyme levels. Rootstocks were grown hydroponically under controlled conditions in nutrient solution with equal N concentrations for three weeks to establish growth. Thereafter, the rootstocks did not receive N for 6 days (recovery period). Leaves and root samples were harvested at two time points (3 h, 6 h) after treatment and from the respective control (0 h). According to our knowledge, this is the first time that the rootstock SO4 has been examined in a gene expression analysis. The direct link between transcript analysis and

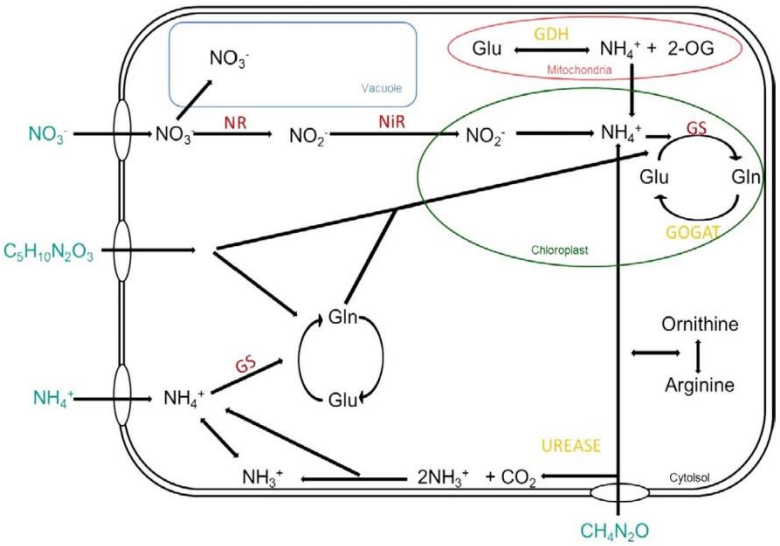

Figure 1: Schematic representation of the enzymatic mechanisms of nitrogen uptake and assimilation in higher plants. N-forms: CaN, AM, UR, and Gln (blue) were applied in the experiment. Involved enzymes are shown in yellow: NR (nitrate reductase), NiR (nitrite reductase), GS (glutamine synthase), GOGAT (glutamate synthase), GDH (glutamate dehydrogenase), UREASE. Enzymes referred to this study are labelled in red. Modified after *Mérigout* et al. (2008), *Witte* (2011), and *Goel* and *Singh* (2015).

enzyme expression is difficult to demonstrate, yet analysis of both, enzyme amount and activity are important for a better understanding of the biosynthesis of N in rootstocks. The aim of this study was to answer the following specific questions: (1) are any inducing or repressing effects apparent in the transcript expression of NR, NiR, and GS in response to different N-forms and N recovery; (2) do plant organs and especially the roots have any influence on the enzymatic nitrate reductase activity (eNRA)?

2 Material and methods

2.1 Plant cultivation and harvest

One-bud cuttings from the grapevine rootstock Selection Oppenheim (SO4) (*Vitis berlandieri x Vitis riparia*) were precultivated in order to allow the development of roots and shoots in a sand bed in the greenhouse. Before being transferred into plastic pots, cuttings were cultivated in a climatic chamber under controlled conditions (20–22°C) rootstocks were thinned to one shoot and placed in 10 mM $CaSO_4$ for two days to promote adaptation. Four biological replicates were cultivated per treatment. Every pot contained 4.5 L aerated nutrient solution (pH-values of nutrient solution see supplemental Tab. S1). The concentration of the nutrient solution was one-fourth at the beginning of the experiment and was increased by increment to one-half and full strength every third day. Full strength nutrient solution had the following composition: 0.5 mM KH_2PO_4, 0.7 mM K_2SO_4, 0.65 mM $MgSO_4$, 100 µM Fe-sequestren, 20 µM H_3BO_3, 3 µM $MnSO_4$, 5 µM $ZnSO_4$, 0.4 µM $CuSO_4$, 0.05 µM $(NH_4)_6Mo_7O_{24}$ and 22 ppm potassium water glass (K_2SiO_3). The rootstocks were treated with four different N-forms under the following conditions; 2 mM calcium nitrate ($Ca(NO_3)_2$), 2 mM ammonium sulphate [$(NH_4)_2SO_4$], 2 mM urea (CH_4N_2O), and 2 mM glutamine ($C_5H_{10}N_2O_3$). All plants, except those treated with $Ca(NO_3)_2$, were additionally given 2 mM $CaSO_4$ to adjust sulphate or calcium concentrations. Nutrient solutions were renewed every third day for three weeks. After that period, no N was applied for 6 days, in which the nutrient solution was renewed every second day.

At harvest, nutrient solution was set again to full strength of N in all pots. Thereafter, various leaf fractions and roots were harvested at the starting point (0 h) and at 3 h and 6 h after N application. Additionally, a sample of 20 mL from each nutrient solution variant was collected for each time point (0 h, 3 h, 6 h) and subsequently stored at –20°C. Leaves were divided according to age; young leaves (four to five apical fully developed leaves) and old leaves all being photosynthetically active leaves. Root tips were dipped in dH_2O to clear them from adhering nutrient solution. Immediately after harvest, samples were frozen in liquid nitrogen. All samples were ground to a fine powder and stored at –80°C until analysis. The following abbreviations are used throughout: calcium nitrate (CaN), ammonium (AM), urea (UR), and glutamine (Gln).

2.2 Enzymatic determination of nitrate reductase activity (eNRA)

Enzymatic assay for both leaf fractions and roots (n = 3 technical replicates for each biological replicate) was carried out based on the established method for grapevines described previously (*Lang* et al., 2018). Values of eNRA are expressed as nitrate reductase (NR) activity µmol NO_2^- g^{-1} FW h^{-1}.

2.3 RNA isolation, preparation, and transcript expression measurements

Total RNA (RNA_{tot}) was isolated from young leaves by the method of *Fort* et al. (2008) by use of CTAB precipitation with minor modifications (incubation time, volumes, additional precipitation).

RNA purity and yield were determined by spectrophotometry (in a NanoDrop, Thermo Scientific, Waltham, MA, USA). Measurements were conducted at the wavelength ratios of A_{260}/A_{230} and A_{260}/A_{280}. mRNA was purified on Dynabeads mRNA Purification Kit (AMBION Life Technologies, Thermo Fischer Scientific, Waltham, MA, USA) according to the manufacturer's instructions. For cDNA synthesis, cleaned RNA_{tot} was reverse transcribed by using the SuperScript VILO cDNA Synthesis Kit (Invitrogen, Thermo Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The cDNA was used as a template for qRT-PCR measurements with the iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. The conditions for qRT-PCR amplifications were: 95°C for 0:30 s, 95°C for 0:15 s, 40 cycles of 0:15 s at 60°C, 95°C for 0:10 s, 65°C for 0:05 s and 5 cycles at 95°C. Three technical replicates were measured per enzyme in each biological replicate.

Primers (Tab. 1) were designed based on the NCBI Primer-BLAST database (https://www.ncbi.nlm.nih.gov/) and integrated Primer-BLAST software, which uses the algorithm of Primer3 software (http://primer3plus.com/cgi-bin/dev/primer3plus.cgi). The target amplicons were 80 to 200 bp in length. Subsequently, the amplicon of the corresponding primer pairs were analyzed according to secondary structures, such as hairpins, and to self-dimers and hetero-dimers with Oligo Analyzer software from Integrated DNA Technologies, Inc. (https://eu.idtdna.com/calc/analyzer). The following primers were designed: UBQ (ubiquitin) (XM_002274238.4) and ACT (actin) (XM_002282480.4) as reference genes; NR (XM_019226724.1), NiR (KF747767.1), and GS1.1 (XM_002274103.3) as genes of interest. Alignments for the different nucleotide sequences of one gene were designed by Clustal Omega of EMBL-EBI (https://www.ebi.ac.uk/Tools/msa/clustalo/). To confirm that the amplicon was of the expected size, a 1.5%-agarose gel analysis of the PCR products was run followed by staining with ethidium bromide (see supplemental Fig. S1). Amplified DNA was sequenced in both orientations by automated DNA sequencing (Eurofins Genomics Germany GmbH, Ebersberg, Germany). Sequence data were compared with corresponding Genbank sequences in the NCBI Primer-BLAST database (https://www.ncbi.nlm.nih.gov/). Only correct amplicons were taken into account.

Table 1: Sequence of primers used for qRT-PCR analysis; (a) reference genes UBQ and ACT; (b) genes of interest NR, NiR and GS1.1.

Genes name	Abbreviation	Accession number	Orientation	Sense 5′–3′ sequence	Product length (bp)
a) Reference gene					
Ubiquitin	UBQ	XM_002274238.4	Forward	CTGAGTCCGATACCGCAGTTG	113
			Reverse	CTAGGTTCCGAAACCCTGAGAT	
Actin	ACT	XM_002282480.4	Forward	GCCATGTATGTTGCCATTCAGGC	169
			Reverse	CAGTGAGGTCACGTCCAGCAAG	
b) Gene of Interest					
Nitrate Reductase	NR	XM_002274103.3	Forward	CGAAAATGAACCCGATTGGAAA	101
			Reverse	ATCCAATTATCCGCAGTTCCTT	
Nitrite Reductase	NiR	KF747767.1	Forward	CGTGGGAATACAGCCTTCACTA	164
			Reverse	TTGGGACTAAAGAACCGCCTAC	
Glutamine Synthetase Isoform 1	GS 1.1	XM_002274103.3	Forward	TGGTGTTCCAGATACATCCTTG	112
			Reverse	TAGTTAGTGTGACATCCTGCAC	

2.4 Statistics

The experimental design was completely randomized for testing N-forms and included split plots for testing time points. Both experiments were conducted with four biological replications (n = 4) each, and the results were expressed as adjusted means ± SE.

The qRT-PCR data of the gene expression measurement were analyzed by Bio-Rad CFX 96 Manager 3.1 Software (Bio-Rad, Hercules, CA, USA). Data were calculated according to *Pfaffl* (2001) and *Pfaffl* et al. (2002). The fold change of the normalized expression levels is shown in Fig. 3. Red lines indicate ± 50% change compared with the control. If this threshold was exceeded, the transcript expression was defined as changed or influenced; if the threshold was not exceeded, we did not consider that a transcript expression change had taken place. Enzymatic measurements were analyzed by SAS software (version 9.4, Cary, North Carolina, USA). A MIXED MODEL with a Kenward–Roger test was determined with a significance level of $p \leq 0.05$.

3 Results

3.1 Transcript expression of key enzymes in response to various N-forms

Compared with that of the control (0 h), the transcript expression of the enzymes NR and NiR changed after CaN application, as detected by its exceeding the red line (Fig. 2a). In detail, the relative expression of NR decreased 3-fold and 10-fold after 3 h and 6 h of CaN application, respectively. NiR decreased 2-fold and 11-fold after 3 h and 6 h after CaN application, respectively. The relative expression of enzyme GS 1.1 did not exceed the red line.

When treated with AM, the relative expression of enzyme NR increased 22-fold and 13-fold after 3 h and 6 h of application compared with the control (0 h). The relative expression of the enzyme NiR increased 23-fold and 15-fold after 3 h and 6 h of AM application. The enzyme GS 1.1 does not exceed the red line (Fig. 2b).

Compared with the control (0 h), the relative expression of the enzyme NR increased 15-fold after 3 h when treated with UR and the relative expression of NiR increased 17-fold after 3 h of UR application. Furthermore, the relative expression of the enzyme GS 1.1 increased 2.5-fold after 6 h of UR application (Fig. 2c).

The relative expression of the enzyme NR increased 3-fold after 3 h of Gln application and the relative expression of NiR increased by 2-fold after 3 h of Gln application compared with the control (0 h). The relative expression of the enzyme GS 1.1 decreased 3-fold after 6 h of Gln application (Fig. 2d).

3.2 Enzymatic NR activity (eNRA) in response to various N-forms

3.2.1 Interaction of time point and N-form

In young leaves, a significant change of eNRA was only detected for the N-form AM compared with all other N-forms. The eNRA after 3 h was significantly reduced compared with that at 0 h and 6 h. In old leaves, UR was the only N-form without significant changes in the activity measured at the different time points. All other N-forms increased in their activity after 3 h and decrease after 6 h but did not drop below the control (0 h). In root tips, a significant reduction in the eNRA from 0 h > 3 h > 6 h was detected in the N-forms AM, UR, and Gln (Tab. 2).

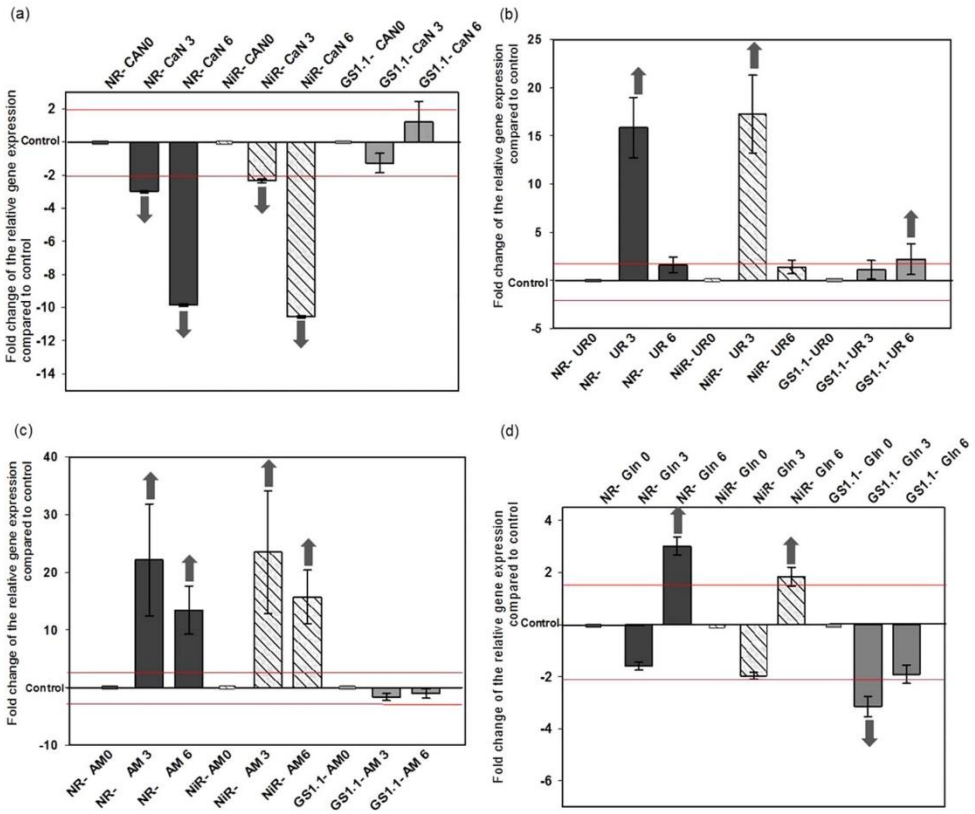

Figure 2: Relative gene expression as fold change compared with control for nitrate reductase (NR, dark grey), nitrite reductase (NiR, light grey striped), and glutamine synthetase isoform 1 (GS1.1, grey) of young leaves on plants of grapevine rootstock SO4. Rootstocks treated with different N-forms: (a) CaN, (b) AM, (c) UR, and (d) Gln. Bars represent gene expression normalized to two reference genes (ACT & UBQ) by using the Pfaffl-method. Red lines represent a ± 50% change compared with the control.

3.2.2 eNRA in plant organs after application time

In young leaves, the N-form AM led to the significantly highest eNRA in general compared with all other N-forms. In old leaves, the N-form Gln resulted in the significantly highest eNRA and the N-form UR in the significantly lowest eNRA. Within root tips, the N-form CaN produced the significantly highest eNRA and the N-form UR the significantly lowest eNRA (Fig. 3a). The eNRA was significantly affected by the various N-forms, regardless of the plant organ in the order of the highest eNRA: AM > CaN > Gln > UR (Tab. 3a). No significant changes were detected in young leaves at the different time points. In old leaves, the eNRA significantly increased after 3 h but further decreased after 6 h after N application. In the root tips, the eNRA significantly decreased continuously from 0 h > 3 h > 6 h (Fig. 3b). The eNRA was not affected by the time point, regardless of the plant organ (Tab. 3b).

4 Discussion

4.1 Inducing effects on the transcript expression in response to various N-forms

Nitrogen is taken up from the soil to the sink organs mainly young leaves, flowers, or fruits, by incorporation into the roots and is transferred within the plant *via* xylem (*Crawford* and *Glass*, 1998). The enzymes NR, NiR, and GS are involved in the first steps in the N assimilation of plants (reviewed by *Andrews* et al., 2013).

In several studies, the addition of external N has been reported to increase the expression of the co-regulated enzymes NR and NiR (*Srivastava*, 1980; *Li* et al., 1995; *Cao* et al., 2008). However, in the present experiment, the expression of NR and NiR was reduced in the presence of exogenously applied CaN. This was much more pronounced after

Table 2: Interaction of time point and N-form: enzymatic nitrate reductase activity (eNRA; μmol NO_2^- g^{-1} FW h^{-1}) of the rootstock SO4 in the various plant organs: young leaves, old leaves, and root tips in response to four different N-forms (CaN, AM, UR, Gln) at different time points. Inverse-transformed adjusted means ± SE (n = 4) are shown. Lower case letters indicate significant differences between the different time points within one N-form and within one plant organ. MIXED MODELS $p \leq 0.05$.

Plant organ	Treatment	0 h	3 h	6 h
YOUNG LEAVES	CaN	0.0246 ± 0.004	0.0217 ± 0.004	0.0203 ± 0.004
	AM	0.0364 ± 0.004 a	0.0213 ± 0.004 b	0.0359 ± 0.004 a
	UR	0.0215 ± 0.004	0.0210 ± 0.004	0.0130 ± 0.004
	Gln	0.0198 ± 0.004	0.0237 ± 0.004	0.0214 ± 0.004
OLD LEAVES	CaN	0.0100 ± 0.002 b	0.0180 ± 0.003 a	0.0151 ± 0.002 ab
	AM	0.0109 ± 0.002 b	0.0206 ± 0.003 a	0.0127 ± 0.002 b
	UR	0.0115 ± 0.002	0.0210 ± 0.002	0.0100 ± 0.002
	Gln	0.0102 ± 0.002 b	0.0202 ± 0.003 a	0.0162 ± 0.003 ab
ROOT TIPS	CaN	0.0207 ± 0.003	0.0235 ± 0.003	0.0158 ± 0.002
	AM	0.0231 ± 0.003 a	0.0188 ± 0.003 ab	0.0143 ± 0.002 b
	UR	0.0242 ± 0.003 a	0.0122 ± 0.002 b	0.0112 ± 0.002 b
	Gln	0.0238 ± 0.003 a	0.0130 ± 0.002 b	0.0118 ± 0.002 b

Table 3: Enzymatic nitrate reductase activity (eNRA) [μmol NO_2^- g^{-1} FW h^{-1}] of the rootstock SO4 in response to (a) four different N-forms (CaN, AM, UR, Gln) and (b) three different time points (0, 3, 6 h). Inverse-transformed adjusted means are shown ± SE (n = 4). Lower case letters indicate significant differences. MIXED MODELS $p \leq 0.05$.

Treatment	Enzymatic nitrate reductase activity (eNRA) (μmol NO_2^- g^{-1} FW h^{-1})
(a)	
CaN	0.0184 a
AM	0.0191 a
UR	0.0140 b
Gln	0.0172 ab
(b)	
0 h	0 0178 a
3 h	0 0178 a
6 h	0.0155 a

6 h than after 3 h (Fig. 2a). Plants were previously grown for 6 days without N treatment and then re-fertilized (recovery-period). Excessive levels of nitrogen intake can reduce NR (reviewed by *Balotf* et al., 2016). The preliminary data suggest that the amount of N was too high and therefore the activity was reduced.

Contrary observations were made under the treatments AM and UR, and to a limited extent under the treatment with Gln. Additionally, the gene expression under NR and NiR was more pronounced after 3 h than after 6 h (Fig. 2b–d). Nevertheless, ammonium and amino acids are considered to be neutral or inhibiting agents of NR activity (*Li* et al., 1995). These observations were not confirmed in the present experiments on grapevine rootstocks. However, AM, UR, and Gln do indeed have an increasing effect on the expression of NR and NiR (Fig. 2). The expression of GS increased by 2-fold after 6 h compared with the control, when plants were treated with UR and decreased by 3-fold after 6 h compared with the control, when plants were treated with Gln (Fig. 2c, d). Urea uptake is generally mediated by the enzyme urease or by active transporters and leads to the production of NH_4^+ (*Mérigout* et al., 2008; *Witte*, 2011). Therefore, more NH_4^+ is available for the synthesis of GS and more N can be incorporated by the assimilation process. This may indicate that these N-forms have a stimulating effect on the NO_3^- assimilation pathway in the grapevine rootstock SO4. Similar results for NH_4^+ have been reported by *Bungard* et al. (1999) for *Clematis vitalba* (lianas plant, like the grapevine). GS links assimilation with the transport of N in the plant (*Paczek* et al., 2002). Since GS, in most cases, is not changed (below threshold, Fig. 2), except for UR and Gln treatments, we consider that the time course of 6 h for the further transport of N is too short. This could be the case after UR application, because of the increased synthesis of AM.

4.2 Roots have an influence on eNRA

The eNRA is a gene-expression-triggered process and is thus a less expressed characteristic than those of enzymes themselves (Fig. 3, Tabs. 2 and 3). The data show analogies with gene expression, but the eNRA is noticeably different in the individual plant organs (Fig. 3a). The differences at the various time points are more pronounced within the single plant organs (Fig. 3b). The activity in the roots decreases with

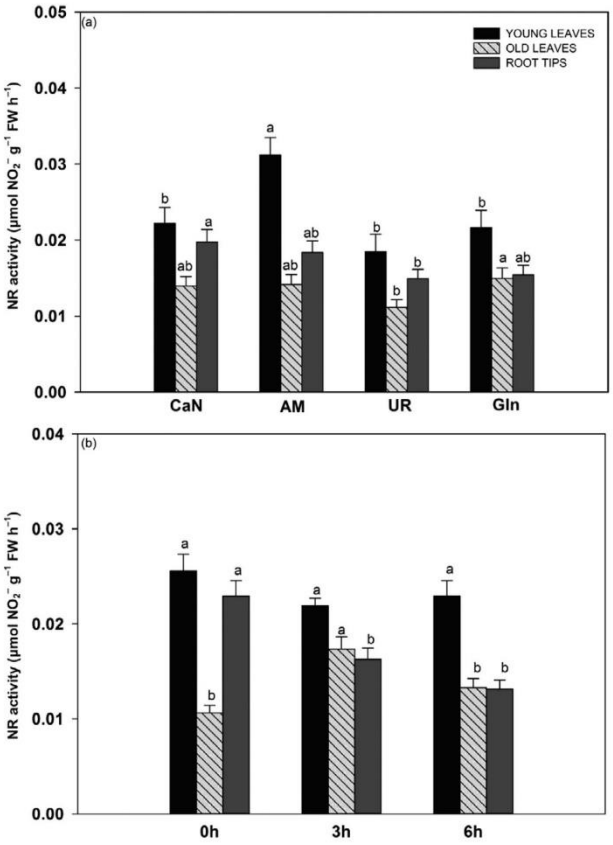

Figure 3: Enzymatic nitrate reductase activity (NRA; µmol NO_2^- g^{-1} FW h^{-1}) in various plant organs of the grapevine rootstock SO4, namely young leaves (black), old leaves (light grey striped), and root tips (grey) in response to four different applied N-forms (CaN, AM, UR, Gln) (a) and at various time points (b). Bars represent inverse-transformed adjusted means ± SE (n = 4). Lower case letters indicate significant differences between various N-forms (a) or between time points (b) within one plant organ. MIXED MODELS $p \leq 0.05$.

increasing duration (0 > 3 > 6), since the N has been transferred to the leaves. A similar pattern is present in the old leaves: first the activity increases as N is delivered *via* the roots and then the eNRA decreases as N is further transferred into the young leaves.

Roots, in particular, have a higher eNRA, because they are directly in contact with the nutrient and N assimilation occurs there (*Reda* et al., 2011). Nitrogen is subsequently taken up *via* high-affinity transporter systems (HATS) or low-affinity transporter systems (LATS) (*Garnett* et al., 2009). At the enzymatic level, eNRA is highest when the plants are supplied the N-forms CaN and AM (Fig. 3a) and, therefore, these are the preferred N-forms for the grapevine (*Goel* and *Singh*, 2015). Moreover, on the application of UR and Gln, a considerably increased eNRA is measurable, reflecting that N is actively absorbed and assimilated from the amino acid form and from urea. This further supports the above-mentioned hypothesis that UR and Gln contribute markedly to the NO_3^- assimilation pathway.

The results presented here suggest that the grapevine rootstock SO4 is able to assimilate all N-forms including the amino acid Gln. Furthermore, the N-forms AM, UR, and Gln have inducing effects on the transcript expression of NR and NiR. However, the eNRA is reduced after application of UR. The plant root can positively influence the eNRA and, thus, the assimilation rate of N of the grapevine rootstock with a consequent effect on the growth and development of the scion.

5 Conclusion

Grapevine rootstocks have a high impact on the N assimilation rate and hence the provision of nitrogen to grapevine scions. The fertilization of grapevines with various N-forms, such as nitrate, ammonium, urea, or glutamine leads to different patterns in N assimilation. The amino acid Gln can indeed be assimilated in rootstock SO4. Furthermore, AM, UR, and in some cases Gln can enhance the regulation of the enzymes NR and NiR. The eNRA is differently affected in roots and leaves. The bulk of N is assimilated early during the first 3 h of treatment. Nevertheless, the amount of N applied to the SO4 rootstock may have an impact on transcript analysis. This hypothesis should be explored in further experiments.

Acknowledgments

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Appendix for Chapter 3

Supplemental Data

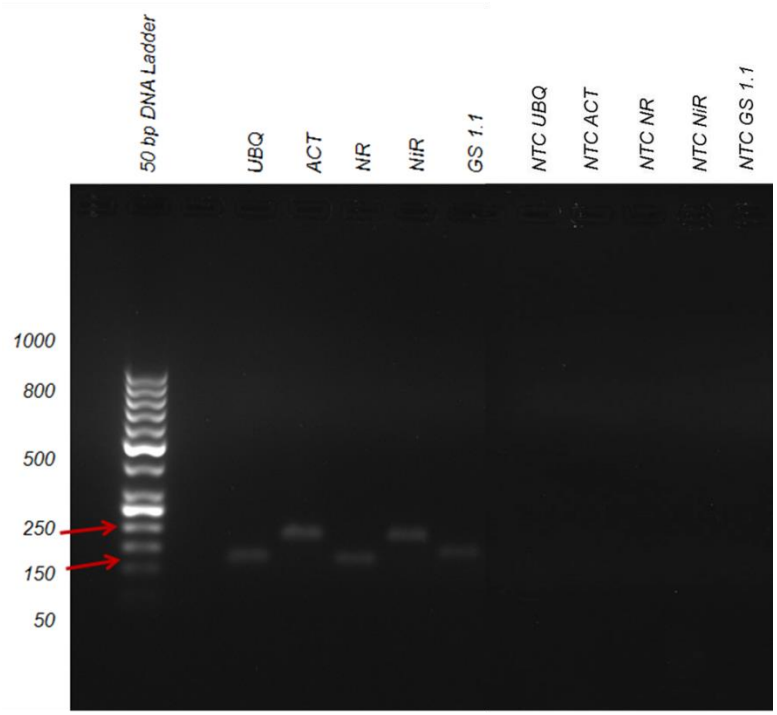

Figure S1: Agarose gel electrophoresis of qRT-PCR primer amplicon. qRT-PCR products were separated on a 1.5%-agarose / x 0.5 TBE gel and stained with ethidium bromide. Lane 1: Gene Ruler 50 bp DNA Ladder ready to use (Thermo Scientific), lanes 2 ,3: amplicons of the two reference genes (UBT & ACT), lanes 4-6: amplicons of the genes of interest (NR, NiR, GS 1.1) lanes 7-11: no template control (NTC) amplicons of all genes.

Table S1: pH-values of all different nutrient solutions at all time points.

N-form	Timepoint	pH
CaN	0 h	6.9
AM	0 h	5.0
UR	0 h	6.7
Gln	0 h	5.8
CaN	3 h	6.7
AM	3 h	4.1
UR	3 h	7.0
Gln	3 h	6.3
CaN	6 h	6.7
AM	6 h	4.1
UR	6 h	7.0
Gln	6 h	6.3

Table S2: Sequencing data of the real-time RT-PCR. The primer pairs are shown in column 1. The corresponding DNA sequencing results and their description are shown in columns 3 -6. All sequences were aligned against NCBI´s reference mRNA (BLAST). The Genbank accession number is shown in column 2.

Primer pair used for qRT-PCR	Genbank accession number	Sequencing summary of qRT-PCR	Description	Query coverag. (%) Per ident (%)	E-value
a) Reference gene					
Vv.UBQ	XM_002274238.4	TTTNNTCGAWTCGAKGAAANTTMTCCSGWTMTMTGAWWCAMSCAACTGCRGKATCGGANCTCAGRGTTTCGGAACCTAGA	PREDICTED: Vitis vinifera nitrate reductase [NADH]-like (LOC100264320), transcript variant X1, mRNA No further BLAST result found	53/74(72%)	6.00E-05
Vv.ACT	XM_002282480.4	CATGTATGTTGCCATTCAGGCNGTTCTCTCTCTATATGCCAGTGGTCGTACAACTGGTATTGTANCTGGATTCTGGTGATGGTGTGAGTCACACTGTGCCAATTTATGAAGGTTATGCCCTTCCCCATGCTATCCTTCGTCTTGACCTTGCTGGACGTGACCTCACTG	Vitis vinifera actin-7 (LOC100232866), mRNA No further BLAST result found	162/168(96%)	2.00E-71
b) Gene of Interest					
Vv.NR	XM_002274103.3	TNGAGCCGTCCGTCTTRSMCTYRCGAGACSAARKMWYTSNNATCGGRTWCATTKTC	PREDICTED: Vitis vinifera nitrate reductase [NADH]-like (LOC100264320), transcript variant X1, mRNA	47/47(100%)	1.00E-14
Vv.NiR	NM_001281265.1	TCAAWGATCATGGCGTACATGCCTGCCACAAMRMANGGMASATTYSRMTTCAMTCKGCWAGTTAGTGMRGTKCTKTAKTCCCACRA	Vitis labrusca x Vitis vinifera nitrite reductase mRNA, partial cds No further BLAST result found	86/131(66%)	4E-11
Vv.GS 1.1	XM_002274103.3	CACTTTTTCGTCTAKTGSTYTSRMTCGAGTGAGASCAMAACCMMTAGMKKGTGWCTGKWATKSTCKCAAGGATGTATCTGGAACACYAACTAA	PREDICTED: Vitis vinifera glutamine synthetase leaf isozyme, chloroplastic (LOC100242605), mRNA No further BLAST result found	56/79(71%)	5.00E-07

CHAPTER 4

Different forms of nitrogen application affect metabolite patterns in grapevine leaves and the sensory of wine

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Research article

Different forms of nitrogen application affect metabolite patterns in grapevine leaves and the sensory of wine

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ABSTRACT

The quality of grapevine berries, must and wine is influenced by environmental and viticultural inputs and their complex interactions. Aroma and flavour are decisive for quality and are mainly determined by primary and secondary metabolites. In particular, phenolic compounds contribute to berry and wine quality. The influence of various nitrogen forms on *i)* the composition of phenolic compounds in leaves and wine and; *ii)* the resulting wine quality were studied in a vineyard system. Must and wine quality was evaluated by chemical analysis and sensory testing. Metabolomic profiling was also performed. Aroma and sensory profile were significantly changed by the application of nitrogen in contrast to no nitrogen fertilisation. The levels of 33 metabolites in leaves and 55 metabolites in wine were significantly changed altered by fertilisation with the various nitrogen forms. In leaves, more metabolites were increased by the use of calcium nitrate or ammonium but were decreased by the use of urea. In terms of wine, the used nitrogen forms decreased more metabolites compared with no fertilisation.

1. Introduction

From an economic point of view, the grapevine (*Vitis vinifera* L.) is one of the most important fruit species cultivated worldwide because of their numerous uses in the food production industry, such as grape juice, wine and other beverages (Ali et al., 2009). The quality of the grapevine and its resulting juice and wine is determined by several organoleptic properties, which are attributes of grape variety, fermentation conditions, *terroir*, environmental and viticultural inputs and of the complex interaction of these factors (Jackson and Lombard, 1993; Alañón et al., 2015). Aroma and flavour are decisive quality traits that are mainly determined by primary and secondary metabolites. Primary metabolites, which include sugars, amino acids, biogenic amides, polysaccharides, alcohols and organic acids, are directly involved in the growth, development and reproduction of grapevines. Secondary metabolites comprise a large selection of species-specific chemicals with more than 85 000 compounds. One of the major representatives of these secondary metabolites in grapevines are phenolic compounds (Verpoorte, 2000; Ali et al., 2009). Phenols are the most important contributors to fruit and wine quality, especially for sensory properties astringency and, to a lesser extent, bitterness (Mazerolles et al., 2010). Their range and concentrations are important determinates of flavour and aroma (Jackson and Lombard, 1993). Phenolic compounds also affect flavour, appearance, taste, mouth-feel, fragrance and colour, all off which also define the aroma bouquet of a wine. In addition to their organoleptic properties, phenols provide protection against environmental challenges (Teixeira et al., 2013) and are the main substrates for juice and wine oxidation (Jackson, 2008; Kennedy, 2008; Keller, 2010). Phenols are often present in the leaf epidermis, whereas in the berry, phenolics are mainly produced in the skin and seeds and are influenced by the grape variety, the vinification process, and the degradation and polymerisation that occurs during wine ageing (Winkel-Shirley, 2002; Keller, 2010). The major phenolic compounds in grapevines are the flavonoids such as flavones, flavonols, flavanones, flavan-3-ols and anthocyanins and the non-flavonoids such as phenylpropanoids, volatile phenols and stilbenes (Jackson, 2008; Teixeira et al., 2013).

Nitrogen (N) is an important nutrient both for grapevine growth and for berry quality formation. The N status of a grapevine influences the composition and concentration of the quality components of the berry and, therefore, contributes mainly to wine quality (Bell and Henschke, 2005). Agricultural practices such as N fertilisation can also affect the accumulation of secondary metabolites (Downey et al., 2006; Jezek et al., 2018). Phenolic compounds and aroma precursors are especially influenced by variations in N supply to grapevines (Choné et al., 2006; Portu et al., 2015a). Field trials in vineyards are necessary to evaluate these processes properly and in detail.

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CHAPTER 4:
Different forms of nitrogen application affect metabolite patterns in grapevine leaves and the sensory of wine

Table 1
Oenological parameters of the four experimental musts in response to three different N-forms (CaN, AM, UR) and a control during the two experimental years 2015 and 2016. Capital letters indicate significant differences between the two experimental years 2015 and 2016; lower case letters indicate significant differences between the different N-forms and the control. Data are adjusted means ± SE (n = 4); MIXED MODELS $p \leq 0.05$.

Treatment	pH	Total acid [g L^{-1}]	Tartaric acid [g L^{-1}]	Malic acid [g L^{-1}]	Must weight [° Brix]
2015 A					
CaN	3.39 ± 0.06 a	7.33 ± 0.18	5.87 ± 0.21	4.61 ± 0.13	21.3 ± 2.08
AM	3.26 ± 0.05 ab	7.61 ± 0.18	5.78 ± 0.21	4.89 ± 0.13	21.5 ± 2.10
UR	3.22 ± 0.05 b	7.61 ± 0.17	6.01 ± 0.21	4.81 ± 0.13	21.0 ± 2.17
control	3.24 ± 0.05 ab	7.69 ± 0.18	5.80 ± 0.21	4.86 ± 0.13	21.0 ± 2.11
2016 B					
CaN	3.56 ± 0.06	5.90 ± 0.18	2.07 ± 0.21	2.83 ± 0.13 b	23.8 ± 2.16
AM	3.57 ± 0.06	5.85 ± 0.18	2.01 ± 0.21	3.52 ± 0.13 a	24.0 ± 2.09
UR	3.52 ± 0.06	5.84 ± 0.18	2.20 ± 0.21	3.49 ± 0.13 a	23.8 ± 2.14
control	3.59 ± 0.06	5.84 ± 0.18	2.13 ± 0.21	3.41 ± 0.13 a	23.8 ± 2.06

Table 2
Oenological parameters of the four experimental wines in response to three different N-forms (CaN, AM, UR) and a control during the two experimental years 2015 and 2016. Capital letters indicate significant differences between the two experimental years 2015 and 2016; lower case letters indicate significant differences between the different N-forms and the control. Data are adjusted means ± SE (n = 4); MIXED MODELS $p \leq 0.05$.

Treatment	pH	Total acid [g L^{-1}]	Tartaric acid [g L^{-1}]	Malic acid [g L^{-1}]	Lactic acid [g L^{-1}]	Alcohol [g L^{-1}]
2015 A						
CaN	3.1 ± 0.1	6.5 ± 0.4	2.0 ± 0.04 ab	3.1 ± 0.11	0.88 ± 0.1	83 ± 4.4
AM	3.1 ± 0.1	6.6 ± 0.4	1.9 ± 0.04 b	3.4 ± 0.12	0.85 ± 0.1	87 ± 4.6
UR	3.0 ± 0.1	6.5 ± 0.3	2.1 ± 0.04 a	3.5 ± 0.12	0.79 ± 0.1	81 ± 4.3
control	3.1 ± 0.1	6.6 ± 0.4	2.0 ± 0.04 ab	3.3 ± 0.12	0.81 ± 0.1	85 ± 4.5
2016 B						
CaN	3.6 ± 0.1	5.3 ± 0.2	1.4 ± 0.04 b	2.9 ± 0.12 b	0.86 ± 0.1 a	110 ± 5.8
AM	3.6 ± 0.1	5.4 ± 0.2	1.4 ± 0.04 b	3.1 ± 0.12 a	0.67 ± 0.1 b	106 ± 1.3
UR	3.6 ± 0.1	5.5 ± 0.2	1.6 ± 0.04 a	3.2 ± 0.12 a	0.68 ± 0.1 b	109 ± 5.8
control	3.6 ± 0.1	5.5 ± 0.3	1.5 ± 0.04 a	3.1 ± 0.12 a	0.59 ± 0.1 b	108 ± 5.7

Metabolic profiling is a method for the identification and quantification of as many pre-defined small molecule metabolites as possible occurring within a system, and generally associated with a specific metabolic pathway, whereas metabolic fingerprinting is a high-throughput screening tool for samples each having a different biological status or origin (Dunn and Ellis, 2005; Cozzolino, 2016). Grapevine metabolomic studies can be used to provide insights into a wide domain of flavour and aroma components in berries and wine or in their associated grapevine physiology. Moreover, changes can be identified in phenolic compounds and their related shikimate or phenylpropanoid pathways based on agricultural practices.

The little information that is available, concerning the impact of different N forms in the vineyard system and their effect on the grapevine metabolome of leaves and wine is conflicting. Furthermore, data on phenolic compounds and their effect on aroma and flavour composition are rare. We have studied the influence of various N-forms such as calcium nitrate, ammonium and urea in a vineyard experiment with grafted grapevines, namely *Vitis vinifera* L. *cv.* Regent on rootstock *cv.* SO4. Our metabolomic fingerprint analysis of grapevine leaves and wine provides a first overview of the way in which many phenolic compounds can be detected and influenced by N supply. Not only have must and wine quality been analysed, but also a sensory profile for the resulting wine was conducted. The influences of various N-forms on *i*) general phenolic compounds in leaves and wine and; *ii*) wine quality have been investigated in this study.

2. Materials and methods

2.1. Plant growth conditions and experimental design

Field experiments run from June 2015 to October 2016 in a vineyard located at the campus of the University of Hohenheim, Stuttgart, Germany (Long. 48° 42′ 29.149″N Lat. 9° 12′ 42.25″E). Grapevines with good resistance against powdery and downy mildew, namely *Vitis vinifera* L. *cv.* Regent, were grafted onto the rootstock SO4 (Selection Oppenheim 4). Vines were spaced 1.40 m within the row and 1.80 m between rows. The experimental design of the vineyard was a fully randomised block design with 16 blocks in total; these were divided into four treatments and four biological replications. Each block consisted of four grapevines. The treatments consisted of three different N forms; calcium nitrate ($Ca(NO_3)_2$), ammonium sulfate ($(NH_4)_2SO_4$) and urea (CH_4N_2O). In the cases of ammonium sulfate and urea, nitrification inhibitors (SKW Stickstoffwerke Piesteritz GmbH, Lutherstadt Wittenberg, Germany) were employed. The fourth treatment lacked N and was considered as the control. The following abbreviations are used throughout: calcium nitrate (CaN), ammonium (AM), urea (UR) and control. All N forms were applied in liquid solution, with same amount of water without N being applied to the control. The applied N amount was 60 kg N ha^{-1} and was calculated with regard to the block size (35.3 m^2). Treatment occurred in June 2015 and May 2016, at BBCH stage 53.

2.2. Leaf harvest for metabolite profiling

Samples were collected on 15th October 2016, one day after the grape harvest. In all 16 plots, one whole cane at the two-year-old shoot was chosen from four vines each. At this shoot, the youngest fully expanded leaves in a healthy and green stage where harvested and immediately frozen in liquid nitrogen. For further analysis, samples were ground up under liquid nitrogen and stored at −80 °C.

2.3. Juice and wine samples

Vitis vinifera L. *cv.* Regent grapes were harvested by hand-picking on 25th September in the experimental year 2015 and on 14th October in the experimental year 2016. Because of the technical requirements of

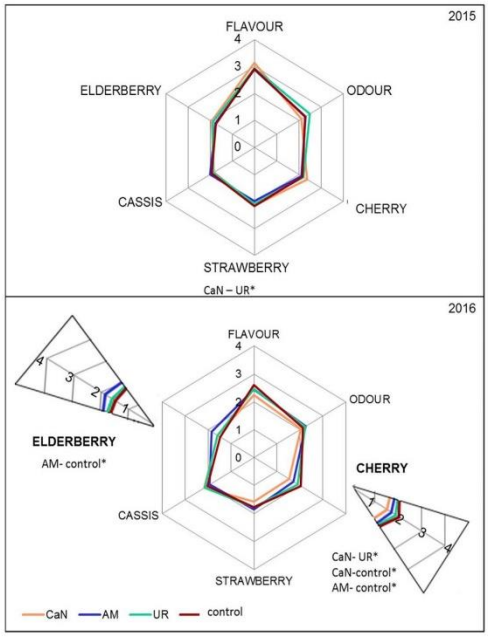

Fig. 1. Spider plots of the sensory profile of the experimental wines from *Vitis vinifera* L. *cv.* Regent in response to three different N-forms (CaN ; AM ; UR) and a control ; during the two experimental years 2015 and 2016. Bold aroma attributes indicate significant differences between these aroma attributes in the two years 2015 and 2016. Asterisks indicate significant differences between different N-forms within one aroma attribute and within one year. Inverse-transformed adjusted means are shown (n = 4); MIXED MODELS $p \leq 0.05$.

the following vinification process, each of the 16 blocks was harvested separately. Four vines from each block were harvested and the same vines as those for leaf sampling were used. Grapes were processed separately. 16 different wines were each produced in the two experimental years giving a total of 32 wines. Must weight (total soluble solids in °Brix), pH and total acid (TA) of the must were analysed. Vilification was performed at the viticulture unit of the Department of Quality of Plant Products, Institute of Crop Science at the University of Hohenheim, Stuttgart, Germany. Before the berries were pressed by using a hydraulic press, they were squeezed to scratch the skin. Must samples of approximately 40 mL were taken and immediately frozen for further analyses. Particulates (sedimented skin residues and pulp), were removed from the must samples by centrifugation for 5 min at 14.000 × *g*. After pressing, 2 g L^{-1} bentonite was added and the must was inoculated with 0.3 g L^{-1} yeast (Anchor Vin, 2000; *S. cerevisiae*). At the end of fermentation, wines were separated from the yeast and sulfured with 200 mg L^{-1} potassium disulfite ($K_2S_2O_5$). All wines were stored in bottles in the wine cellar of the institute at 12 °C ambient temperature before being tasted.

2.3.1. Oenological parameters in must and wine

The must and the wine were analysed for both experimental years. The total soluble solids (°Brix) were measured with a refractometer (Opton, Zeiss, Germany). Total acid (TA) and pH were measured by means of a titrator (TiroLine easy, Schott, Mainz). The parameters lactic acid (LA), tartaric acid (TTA) and malic acid (MA) (only for wine) and the alcohol content were determined by high performance liquid chromatography (HPLC) (Merck-Hitachi, Darmstadt, Germany; column oven: Knauer Berlin, Germany). For analysis of the organic acids in the must and wine, potassium phosphate (20 mM, pH 1.5) at a flow rate of 1 mL min^{-1} and detection at 210 nm UV was used as the mobile phase. The utilised separation column was a Synergi™ 4 µm Hydro-RP 80 Å, LC column 250 × 4.6 mm, Ea, (Phenomenex, Aschaffenburg, Germany). For the analysis of the alcohol content in the wine, sulfuric acid (isocratic, 0.05 N) at a flow rate of 0.5 mL min^{-1} and detection at 210 nm UV was used as the mobile phase. The utilised separation column was a Phenomenex Rezex™ ROA-Organic Acid H^+ (8%), LC column 300 × 7.8 mm, Ea, (Phenomenex, Aschaffenburg, Germany) and the utilised precolumn was a Phenomenex SecurityGuard Cartridge, Carbo-H 4 × 3.0 mm (Phenomenex, Aschaffenburg, Germany).

2.3.2. Wine sensory analysis

Sensory analysis of the wine was conducted from both experimental years 2015 and 2016. The wines were evaluated twice by a trained tasting panel during four sessions at the institute. During the first two sessions on different days, participants evaluated the wine of the experimental year 2015 (14 participants at the 1st session and 13 participants at the 2nd session). During the last two sessions (again on different days), participants evaluated the wine of the experimental year 2016 (12 participants at the 3rd session and 10 participants at the 4th session). In total, 32 wines were tested for intensity (four different wines in four replications) in random order. Wines were served at ambient temperature and in clear glasses. Water was provided. The panellists were given defined aroma attributes for evaluation. In total, six attributes, namely four for aroma (cherry, strawberry, cassis and elderberries) and two for flavour/odour were used. These aroma attributes were scored on a five-point scale, with '0' representing non-characteristic or non-existent intensity and '5' representing a high or extreme intensity. During the whole sensory session, the panellists had access to commercial aroma attributes, for comparison purposes.

2.4. Metabolite extraction of the leaf samples

Fresh, ground leaf material (a pooled sample of all biological replicates) from the vintage 2016, (approximately 500 mg) was mixed with 1 mL pre-cooled methanol:water solution (80:20 v/v). Three technical replicates per N-form of treatment were conducted. The samples were homogenised by being shaken vigorously for 5 min and were then stored at −20 °C overnight for protein precipitation. Each sample was centrifuged (Heraeus Pico 21 centrifuge, Thermo Fisher Scientific, Waltham, MA, USA) for 10 min at 14 0.000 × *g* at 4 °C. The supernatant was filtered through a 0.22 µm sterile filter (Rotilabo syringe filter PVDF sterile, pore size 0.22 µm, Carl Roth GmbH, Karlsruhe, Germany) and stored at −20 °C until LC-MS analysis.

2.4.1. Metabolite extraction of wine samples

Wine samples from the vintage 2016 were taken from bottles one day before measurement. From each of the 16 wines, three technical replicates were taken and filtered through a 0.2 µm sterile filter (Rotilabo syringe filter PVPF sterile, pore size 0.22 µm, Carl Roth GmbH, Karlsruhe, Germany). Samples were stored at 6 °C until LC-MS analysis.

2.5. UHPLC-MS analysis

UHPLC-MS analysis was performed by using an Agilent 1290 Ultra Performance Liquid Chromatography system coupled to a Q-Exactive Plus Mass Spectrometer (Thermo Fischer Scientific, Waltham, MA, USA). Analyte separation was achieved by a Waters ACQUITY CSH C18 column (1.7 µm, 2.1 µm × 150 mm); the mobile phases were acetonitrile and water each with 0.2% formic acid for leaf samples and methanol and water each with 0.2% formic acid for wine samples. The column temperature was set to 40 °C and the auto sampler temperature was set to 10 °C. The injection volumes used were 3 µL for leaf samples

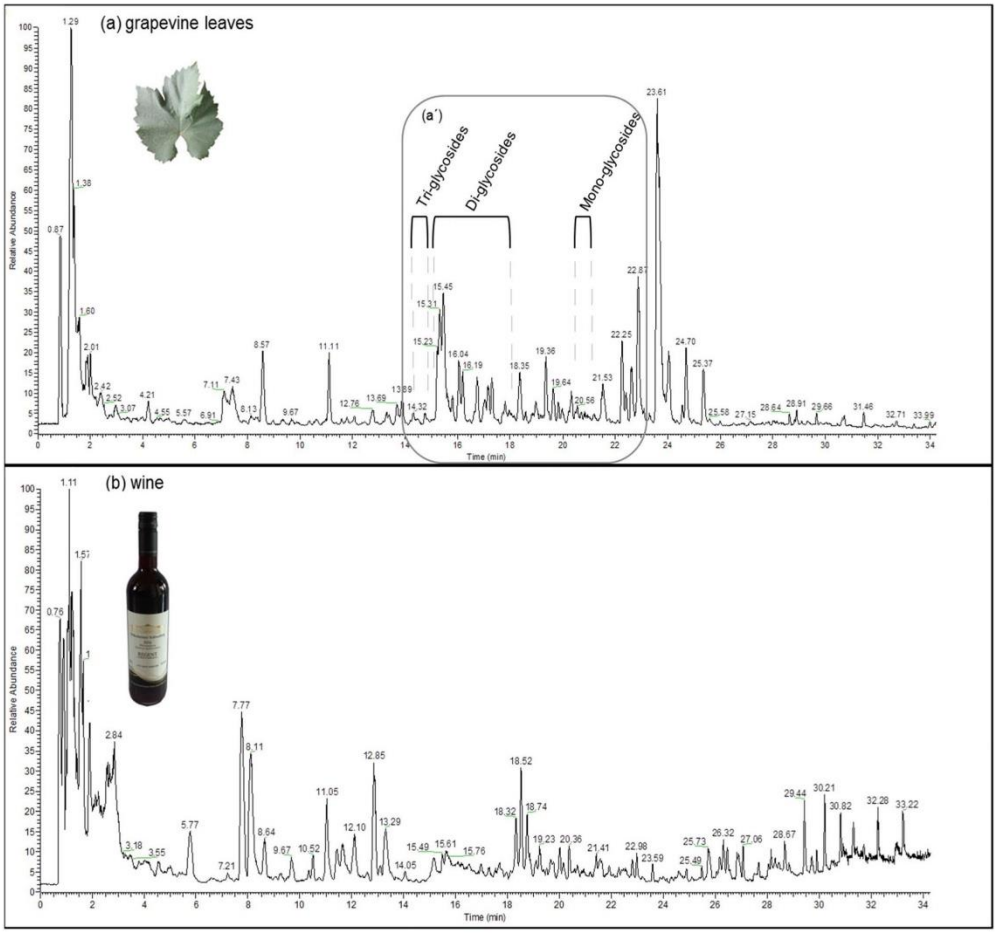

Fig. 2. UHPLC-ESI-MS chromatograms under ESI + ionisation mode of the metabolic extracts from (a) grapevine leaves, control as example and (b) wine, UR as example. The insert (a') highlights the parts of the chromatogram where different kaempferol and quercetin glycosides (mono-, di-, or tri-glycosylation) are detected.

and 4 µL for wine samples. The flow rate for the separation mode of the leaf samples was set to 0.30 mL min^{-1} and the separation mode for the wine samples was set to 0.35 mL min^{-1}. Mass spectrometry analysis was performed in ESI + and ESI- ionisation modes by using a Xcalibur version 4.0.27.42. Data of the ESI + mode are shown.

2.6. Metabolite profiling and data processing

Data for the metabolomics workflow were analysed by using Compound Discoverer software 3.0 (Thermo Fischer Scientific, Waltham, MA, USA). The metabolic annotation was based on the defined four levels of metabolic identification given by Sumner et al. (2007). Individual compounds were assigned based on exact mass, isotope pattern or either by ChemSpider (formula or exact mass) or by an in-house mass list of common grapevine compounds based on peer-reviewed research articles. A tentative annotation of metabolites without a standard was based on spectral features (mass deviation < 5 ppm of the theoretical value, on isotopic pattern fit and at least one indicative fragment ion in the MS/MS spectrum), on literature information concerning chromatographic properties and mass spectra records from external databases such as HMDB, KEGG and MassBank and on an internal database including wine metabolites based on peer reviewed literature. MS/MS data were also used for the further support of the annotation of a few tentative marker metabolites. Thereafter, two filter steps were carried out: the first filter settings were specified in the program itself (Suppl. Table. 1) and the second filter settings were based on experience (review of molecular formula, molecular mass, retention time and peak formation) and on peer-reviewed literature. Two additional filter criteria were added for the annotation of tentative phenolic compounds; the molecular formula had to have a C-H-O backbone, and the structural formula proposed by the software required an aromatic ring (C6 carbon structure).

To obtain an overview about all regulated compounds, the results from the grapevine leaves and wine were depicted in volcano plots (Suppl. Fig. 1.1 for grapevine leaves and Suppl. Fig. 1.2 for wine).

Table. 3a

Regulated tentative compounds in grapevine leaves of *Vitis vinifera* L. *cv.* Regent during the experimental year 2016 by using UHPLC-ESI-MS in positive ionisation mode. Relative ratios of the generated Log^2 fold changes and *p*-values of the three different N - forms (CaN, AM, UR) and a control are shown. Significance is indicated by colour coding of the Log^2 fold changes (significance = ≥ +1/≥ −1) and the *p*-values (p ≤ 0.05); ANOVA (pooled samples n = 3).

-2.01 → -3.00	1.01 → -2.00	0.00 → 0.99	1.00 → 2.00	2.01 → 3.00
1.0-0.05	0.05-0.01	0.01-0.005	0.005-0.001	0.001-0.0005	0.0005-0

Metabolite annotation	Formula	Molecular Mass [*m/z*]	RT [min]	Log^2 Fold						*p*-value					
				CaN / control	AM / control	UR / control	CaN / AM	CaN /UR	AM / UR	CaN / control	AM / control	UR / control	CaN / AM	CaN /UR	AM / UR
Acetophenone	C_8H_8O	120.0578	1.39	-1.35	-0.93	0.36	-0.42	-1.72	-1.30	0.00019	0.00152	0.24122	0.23247	0.00004	0.00020
Tyramine	$C_8H_{11}NO$	137.0842	1.40	-1.34	-0.93	0.36	-0.41	-1.71	-1.30	0.00019	0.00149	0.23788	0.24170	0.00004	0.00019
UNKNOWN*		170.0192	3.69	1.15	0.50	0.31	0.66	0.85	0.19	0.00057	0.05463	0.18980	0.02290	0.00709	0.81014
Tentative compound	$C_{13}H_{22}O_2$	210.1621	17.78	1.30	0.82	0.60	0.48	0.70	0.22	0.00044	0.00892	0.09229	0.10330	0.00989	0.37972
Tentative compound	$C_{18}H_{28}O$	260.2140	36.63	0.88	1.02	0.44	-0.14	0.44	0.59	0.00151	0.00157	0.14732	0.99998	0.03146	0.03312
Tentative compound	$C_{18}H_{33}O$	278.2246	35.07	1.15	0.94	0.47	0.20	0.67	0.47	0.00011	0.00028	0.02983	0.72549	0.00401	0.01527
UNKNOWN*		295.1634	3.58	-1.05	-1.54	-1.69	0.48	0.63	0.15	0.00154	0.00025	0.00007	0.34454	0.03959	0.43953
Tentative compound	$C_{21}H_{34}O_4$	350.2458	32.86	1.20	0.95	0.92	0.24	0.27	0.03	0.03000	0.00043	0.00129	0.00152	0.71101	0.62688
Tentative compound	$C_{21}H_{36}O_4$	352.2615	35.47	1.38	1.10	0.42	0.28	0.96	0.68	0.00004	0.00018	0.03909	0.29993	0.00073	0.00630
Tentative compound	$C_{21}H_{36}O_4$	352.2615	36.98	1.38	1.22	0.64	0.16	0.74	0.58	0.00004	0.00013	0.00816	0.46336	0.00259	0.01768
Tentative compound	$C_{19}H_{32}O_7$	372.2149	17.78	1.20	0.76	0.47	0.44	0.73	0.29	0.00024	0.00705	0.07312	0.05419	0.00541	0.37090
UNKNOWN*		377.1588	21.44	0.61	-1.62	-1.77	2.22	2.37	0.15	0.05085	0.00007	0.00005	0.00001	0.00001	0.94722
UNKNOWN*		413.2263	8.65	1.11	0.66	0.41	0.45	0.70	0.25	0.00020	0.00895	0.14255	0.03123	0.00240	0.25906
UNKNOWN*		435.2836	17.78	1.36	0.82	0.59	0.54	0.77	0.24	0.00003	0.00111	0.02248	0.01226	0.00069	0.13930
Tentative phenolic compound	$C_{25}H_{28}O_7$	438.1683	29.24	1.93	1.23	0.74	0.70	1.19	0.49	0.00000	0.00005	0.00305	0.00381	0.00006	0.01204
UNKNOWN*		454.1632	27.44	1.54	1.33	0.58	0.21	0.96	0.75	0.00003	0.00010	0.00655	0.31827	0.00162	0.01554
UNKNOWN*		479.2005	1.39	-1.22	-0.75	0.41	-0.48	-1.63	-1.16	0.00024	0.00290	0.15842	0.14799	0.00003	0.00025
ASN-LYS-SER-TYR	$C_{22}H_{34}N_6O_6$	510.2443	36.63	0.89	1.07	0.43	-0.17	0.46	0.64	0.00262	0.00124	0.20784	0.90647	0.04377	0.01747
Tentative compound	$C_{27}H_{46}O_6$	514.3146	36.63	0.95	1.10	0.46	-0.15	0.49	0.63	0.00125	0.00111	0.13701	0.99943	0.02678	0.02292
Tentative phenolic compound	$C_{31}H_{28}O_{13}$	608.1533	24.07	-1.69	-0.93	-1.29	-0.76	-0.39	0.36	0.00003	0.00127	0.00022	0.01037	0.11975	0.34983
Kaempferol-di-hexoside	$C_{27}H_{30}O_{16}$	610.1540	16.64	-1.65	-1.50	-1.75	-0.15	0.09	0.24	0.00017	0.00028	0.00009	0.92972	0.87082	0.55652
Kaempferol-di-glucoside	$C_{27}H_{30}O_{16}$	610.1541	17.48	-1.49	-1.36	-1.61	-0.13	0.12	0.25	0.00057	0.00085	0.00030	0.97819	0.90266	0.71614
Quercetin- di-hexoside	$C_{27}H_{30}O_{17}$	626.1483	15.43	-1.00	-0.69	-1.27	-0.31	0.27	0.58	0.00529	0.02779	0.00144	0.60674	0.68989	0.15803
Quercetin- di-glucoside-galctoside	$C_{27}H_{30}O_{17}$	626.1486	16.03	-0.75	-0.62	-1.19	-0.13	0.44	0.57	0.00711	0.01716	0.00046	0.90339	0.14208	0.05491
UNKNOWN*		630.2692	30.99	1.79	1.35	0.45	0.44	1.34	0.90	0.00010	0.00085	0.05895	0.17949	0.00200	0.03669
Quercetin-hexoside-glucuronide	$C_{27}H_{28}O_{18}$	640.1281	20.71	-0.75	-0.75	-1.24	0.00	0.50	0.50	0.00068	0.00036	0.00003	0.91902	0.03465	0.08424
UNKNOWN*		652.1981	8.62	1.11	0.81	0.20	0.30	0.91	0.61	0.00625	0.02762	0.71678	0.68414	0.02546	0.12370
UNKNOWN*		676.3672	35.07	1.02	1.03	0.32	-0.01	0.70	0.71	0.00031	0.00036	0.08037	0.99813	0.00708	0.00878
UNKNOWN*		693.3936	35.47	1.70	1.48	0.74	0.22	0.96	0.74	0.00003	0.00009	0.00793	0.43326	0.00160	0.01086
UNKNOWN*		721.4248	35.07	1.01	1.10	0.46	-0.09	0.55	0.64	0.00071	0.00037	0.02434	0.90519	0.07050	0.02756
Kaempferol-tri-glucoside	$C_{33}H_{40}O_{21}$	772.2065	14.28	-1.79	-1.04	-1.68	-0.74	-0.11	0.63	0.00001	0.00079	0.00002	0.00371	0.76711	0.01265
UNKNOWN*		791.3216	25.76	1.13	0.77	0.38	0.36	0.75	0.39	0.00122	0.01677	0.16082	0.21172	0.02206	0.41822
UNKNOWN*		833.0926	19.21	-1.15	-0.44	-0.09	-0.71	-1.06	-0.35	0.00037	0.02974	0.59024	0.02271	0.00138	0.18223

*UNKNOWN metabolites – not identified, tentative molecular mass indicated, tentative phenolic compounds were annotated based on two more filter criteria: C-H-O backbone and aromatic ring (C6 carbon structure).

Table. 3b

Regulated tentative phenolic compounds in grapevine leaves of *Vitis vinifera* L. *cv.* Regent during the experimental year 2016 by using UHPLC-ESI-MS in positive ionisation mode. Relative ratios of the generated Log^2 fold changes and as *p*-values of the three different N - forms (CaN, AM, UR) and a control are indicated. Significance is shown by colour coding of the Log^2 fold changes (significance = ≥ +1/≥ −1) and the *p*-values (p ≤ 0.05); ANOVA (pooled samples n = 3).

-2.01 → -3.00	1.01 → -2.00	0.00 → 0.99	1.00 → 2.00	2.01 → 3.00
1.0-0.05	0.05-0.01	0.01-0.005	0.005-0.001	0.001-0.0005	0.0005-0

Metabolite annotation	Formula	Molecular Mass [*m/z*]	RT [min]	Log^2 Fold						*p*-value					
				CaN / control	AM / control	UR / control	CaN / AM	CaN / UR	AM / UR	CaN / control	AM / control	UR / control	CaN / AM	CaN / UR	AM / UR
Tentative phenolic compound	$C_{25}H_{26}O_7$	438.1683	29.24	1.93	1.23	0.74	0.70	1.19	0.49	0.00000	0.00005	0.00305	0.00381	0.00006	0.01204
Tentative phenolic compound	$C_{31}H_{28}O_{13}$	608.1533	24.07	-1.69	-0.93	-1.29	-0.76	-0.39	0.36	0.00003	0.00127	0.00022	0.01037	0.11975	0.34983
FLAVONOLS															
Kaempferol-di-hexoside	$C_{27}H_{30}O_{16}$	610.1540	16.64	-1.65	-1.50	-1.75	-0.15	0.09	0.24	0.00017	0.00028	0.00009	0.92972	0.87082	0.55652
Kaempferol-diglucoside	$C_{27}H_{30}O_{16}$	610.1541	17.48	-1.49	-1.36	-1.61	-0.13	0.12	0.25	0.00057	0.00085	0.00030	0.97819	0.90266	0.71614
Quercetin- di-galactoside	$C_{27}H_{30}O_{17}$	626.1483	15.43	-1.00	-0.69	-1.27	-0.31	0.27	0.58	0.00529	0.02779	0.00144	0.60674	0.68989	0.15803
Quercetin- di-glucoside-galctoside	$C_{27}H_{30}O_{17}$	626.1486	16.03	-0.75	-0.62	-1.19	-0.13	0.44	0.57	0.00711	0.01716	0.00046	0.90339	0.14208	0.05491
Quercetin-hexoside-glucuronide	$C_{27}H_{28}O_{18}$	640.1281	20.71	-0.75	-0.75	-1.24	0.00	0.50	0.50	0.00068	0.00036	0.00003	0.91902	0.03465	0.08424
Kaempferol-tri-glucoside	$C_{33}H_{40}O_{21}$	772.2065	14.28	-1.79	-1.04	-1.68	-0.74	-0.11	0.63	0.00001	0.00079	0.00002	0.00371	0.76711	0.01265

Tentative phenolic compounds were annotated based on two more filter criteria: C-H-O backbone and aromatic ring (C6 carbon structure).

Table. 4a
Regulated tentative compounds in wine of *Vitis vinifera* L. *cv.* Regent during the experimental year 2016 by using UHPLC-ESI-MS in positive ionisation mode. Relative ratios of the generated Log^2 fold changes and *p*-values of the three different N - forms (CaN, AM, UR) and a control are shown. Significance is indicated by colour coding of the Log^2 fold changes (significance = ≥ +1/≥ −1) and the *p*-values (p ≤ 0.05); ANOVA (pooled samples n = 3).

Metabolite annotation	Formula	Molecular Mass [m/z]	RT [min]	Log^2 Fold						*p*-value					
				CaN / control	AM / control	UR / control	CaN / AM	CaN / UR	AM / UR	CaN / control	AM / control	UR / control	CaN / AM	CaN / UR	AM / UR
Tentative compound	$C_{10}H_6N$	143.0945	1.23	0.24	-0.7	-1.01	0.94	1.25	0.31	0.07439	0.00014	0.00001	0.00002	2.2805E-06	0.04561
Tentative compound	$C_{10}H_7NO$	157.0526	18.61	0.3	-0.76	0.45	1.05	-0.15	-1.21	0.00133	2.1393E-06	0.00004	1.1191E-07	0.01694	2.8272E-08
Tentative compound	$C_7H_{11}NO_3$	157.0737	11.01	-1.2	-0.41	-0.67	-0.79	-0.53	0.26	9.14E-10	2.6049E-06	3.9946E-08	1.4965E-08	2.7269E-07	0.00007
Tentative compound	$C_4H_{12}NO_6P$	169.0498	9.70	-1.64	-0.64	0.6	-0.99	-2.24	-1.24	9.9925E-09	0.00002	0.00003	2.4477E-07	1.2986E-09	7.4271E-08
Tentative compound	$C_8H_{10}O_4$	170.0576	9.70	-1.87	-0.74	0.68	-1.13	-2.55	-1.42	2.2651E-09	2.0404E-06	2.0734E-06	3.9757E-08	5.324E-11	1.1581E-08
Tentative compound	$C_5H_5N_7O$	179.0555	10.99	-1.2	-0.39	-0.62	-0.81	-0.58	0.23	1.3423E-06	0.00494	0.00012	0.00002	0.00050	0.02586
UNKNOWN*		186.0638	6.26	-0.72	-1.21	-0.64	0.49	-0.08	-0.57	0.00893	0.00015	0.01696	0.02010	0.95963	0.01051
Tentative compound	$C_8H_{16}N_2O_3$	188.1159	2.97	0.92	1.09	-0.50	-0.17	1.42	1.59	9.044E-07	2.3281E-07	0.00016	0.08993	2.9039E-08	1.6578E-08
Tentative compound	$C_7H_{19}P_3$	196.0709	20.54	-0.17	-0.59	0.79	0.42	-0.96	-1.38	0.46406	0.00146	0.00006	0.00907	0.00002	1.4145E-06
Tentative compound	$C_8H_{14}N_2O_4$	202.0949	12.82	-0.23	-0.87	0.29	0.64	-0.52	-1.16	0.15654	0.00015	0.01577	0.00156	0.00092	8.0276E-06
Tentative compound	$C_{11}H_{14}O_4$	210.0889	20.98	1.64	0.41	-1.51	1.22	3.15	1.92	1.0537E-07	0.00181	2.0071E-07	1.7306E-06	1.4258E-09	2.4929E-08
Tentative compound	$C_{10}H_7N_7$	225.0762	24.40	0.29	-0.54	-0.84	0.83	1.13	0.3	0.06397	0.00052	0.00008	0.00004	9.1481E-06	0.22193
Tentative compound	$C_{14}H_{22}O_3$	238.1565	27.09	-0.3	-0.79	0.24	0.49	-0.54	-1.03	0.00292	1.1583E-06	0.00987	0.00003	0.00005	1.3821E-07
Tentative compound	$C_{12}H_{24}N_2O_3$	244.1784	14.87	0.82	0.46	-0.38	0.37	1.20	0.84	2.9053E-06	0.00029	0.00256	0.00080	1.91E-07	5.2279E-06
Tentative compound	$C_{13}H_{27}PS$	246.1576	10.59	1.03	0.76	-0.37	0.27	1.40	1.13	9.9556E-07	9.5723E-06	0.00276	0.01330	7.7117E-08	5.3552E-07
Tentative compound	$C_{13}H_{26}N_2O_3$	258.1939	18.36	0.74	0.36	-0.44	0.38	1.18	0.80	2.3549E-07	0.00007	0.00001	0.00004	9.2797E-09	1.0252E-07
UNKNOWN*		261.0421	9.76	0.95	-0.28	-0.81	1.24	1.76	0.53	1.7519E-06	0.02056	0.00001	2.6479E-07	1.9524E-08	0.00029
UNKNOWN*		268.9679	11.24	-0.2	0.55	-0.6	-0.76	0.4	1.15	0.00549	2.3947E-06	1.9124E-06	2.1743E-07	0.00004	1.1875E-08
UNKNOWN*		274.0008	11.00	-1.2	-0.47	-0.73	-0.73	-0.47	0.26	0.00002	0.01900	0.00039	0.00057	0.03202	0.03924
UNKNOWN*		276.0775	7.20	-0.35	0.29	-0.95	-0.64	0.60	1.24	0.00252	0.00327	2.5731E-06	0.00002	0.00010	1.8932E-07
UNKNOWN*		288.0164	11.00	-1.16	-0.33	-0.58	-0.84	-0.58	0.25	6.608E-07	0.00184	0.00006	0.00002	0.00029	0.02490
UNKNOWN*		288.0411	11.24	-0.23	0.47	-0.66	-0.71	0.43	1.13	0.00707	0.00008	0.00002	3.498E-06	0.00069	1.282E-07
Tentative compound	$C_{15}H_{12}O_6$	288.0629	17.85	0.93	1.14	-0.17	-0.21	1.10	1.31	2.4608E-07	4.2641E-08	0.01554	0.01278	4.4708E-08	1.6888E-08
Tentative compound	$C_6H_{11}N_3O_6S$	289.0369	9.76	1.37	-0.18	-0.62	1.55	1.99	0.44	5.2904E-07	0.03647	0.00004	1.044E-07	1.6585E-08	0.00085
(±) Epicatechin	$C_{15}H_{14}O_6$	290.0785	17.85	1.04	1.3	-0.18	-0.26	1.22	1.48	6.6609E-09	1.2797E-09	0.00139	0.00018	2.0289E-09	2.933E-10
Tentative compound	$C_{16}H_{12}O_7$	316.0580	18.71	-0.04	-0.7	0.81	0.66	-0.85	-1.51	0.92616	0.00476	0.00880	0.01025	0.00412	0.00006
UNKNOWN*		316.0725	17.59	-0.08	0.67	-0.57	-0.75	0.49	1.24	0.15623	2.1938E-07	7.0402E-07	7.5159E-08	2.3332E-06	3.4526E-09
Tentative compound	$C_{14}H_{27}N_3O_5$	317.1946	4.74	0.69	-0.36	0.19	1.04	0.50	-0.55	8.4952E-08	0.00001	0.00195	6.6385E-09	1.3011E-06	6.4114E-07
Tentative compound	$C_{15}H_{14}O_8$	322.0685	14.39	1.01	0.32	-1.7	0.69	2.71	2.02	1.2006E-07	0.00076	5.3588E-09	3.0858E-06	3.3751E-11	1.3767E-09
Tentative compound	$C_{17}H_{26}N_2O_4$	322.1888	19.44	0.79	0.23	-0.35	0.56	1.14	0.58	1.7757E-06	0.01986	0.00108	0.00002	8.774E-08	0.00004
Tentative compound	$C_{16}H_{31}N_3O_4$	329.2308	15.84	0.96	0.48	1.09	0.49	-0.13	-0.61	1.4306E-06	0.00040	8.096E-07	0.00016	0.67204	0.00006
UNKNOWN*		331.2101	15.92	0.72	-0.32	0.3	1.04	0.42	-0.62	1.5177E-08	9.2658E-06	0.00001	1.5468E-09	8.4778E-07	3.9678E-08
Tentative compound	$C_{15}H_{28}N_3O_5$	331.2101	11.72	0.84	-0.2	0.27	1.04	0.57	-0.47	6.3227E-08	0.00292	0.00055	1.6294E-08	1.6406E-06	8.2437E-06
UNKNOWN*		349.1367	9.70	-2.14	-0.75	0.74	-1.39	-2.88	-1.49	1.613E-09	3.3829E-06	2.9998E-06	2.1455E-08	4.114E-11	1.5262E-08
Tentative compound	$C_{12}H_{21}N_3O_8$	349.1370	1.46	0.65	0.51	-0.89	0.15	1.54	1.40	0.00011	0.00107	8.3186E-06	0.15110	1.0274E-07	3.1749E-07
UNKNOWN*		354.0921	9.70	-1.90	-0.74	0.67	-1.16	-2.57	-1.41	1.432E-09	1.6163E-06	2.0802E-06	2.5797E-08	2.836E-11	1.0746E-08
UNKNOWN*		356.1254	25.90	-0.52	-0.81	0.31	0.29	-0.83	-1.12	0.00441	0.00014	0.05485	0.04115	0.00017	0.00001
Tentative compound	$C_{19}H_{36}N_2O_4$	357.2622	19.72	0.66	-0.41	0.16	1.07	0.5	-0.57	6.1874E-06	0.00008	0.03022	6.903E-08	0.00007	6.4798E-06
Tentative compound	$C_{20}H_{37}N_3O_4$	367.1526	15.49	0.99	0.28	-0.23	0.71	1.22	0.51	3.3675E-07	0.00581	0.00266	3.8044E-06	3.5455E-08	0.00003
UNKNOWN*		370.0661	9.70	-2.11	-0.77	0.69	-1.34	-2.8	-1.46	5.8756E-10	9.3067E-07	2.7873E-06	1.5465E-08	1.0273E-11	9.7997E-09
UNKNOWN*		385.0215	9.70	-2.09	-0.82	0.86	-1.26	-2.95	-1.68	3.41E-09	2.4084E-06	2.8648E-06	7.1551E-08	1.1255E-10	1.3524E-08
Tentative compound	$C_{20}H_{38}N_3O_4$	385.2935	22.90	0.74	-0.44	0.24	1.18	0.5	-0.68	0.00007	0.00443	0.04471	2.7147E-06	0.00152	0.00015
UNKNOWN*		407.1723	17.22	0.23	1.02	0.91	-0.79	-0.68	0.11	0.00046	8.9237E-09	1.717E-08	3.4328E-08	1.5089E-07	0.01919
UNKNOWN*		434.0354	9.70	-2.51	-0.83	0.82	-1.68	-3.33	-1.65	1.529E-08	0.00004	0.00005	4.5901E-07	2.6422E-09	1.7801E-07
UNKNOWN*		466.1041	14.13	-0.02	0.47	-1.3	-0.49	1.28	1.77	0.99631	0.00004	1.7063E-08	0.00004	1.6444E-08	2.7939E-09
UNKNOWN*		468.1239	18.15	-1.25	-0.71	0.29	-0.54	-1.54	-1.00	9.7472E-09	5.1E-07	0.00066	5.4086E-06	2.7684E-09	3.1459E-08
Quercetin-glucoronide	$C_{21}H_{18}O_{13}$	478.0743	22.21	-0.75	0.39	0.81	-1.14	-1.56	-0.42	0.00016	0.00202	0.00006	3.1874E-06	4.6557E-07	0.02109
UNKNOWN*		501.2792	16.10	0.08	-0.57	0.45	0.65	-0.37	-1.02	0.62183	0.00001	0.00010	6.6569E-06	0.00029	1.4259E-07
UNKNOWN*		566.1568	19.92	-0.04	0.44	-0.91	-0.48	0.87	1.35	0.93862	0.00093	3.0022E-06	0.00052	4.0985E-06	1.2853E-07
UNKNOWN*		623.1777	18.43	-0.24	0.39	-0.62	-0.62	0.38	1.01	0.01930	0.00038	0.00001	0.00002	0.00031	2.9982E-07
Isorhamnetin-caffeoyl-conjugate	$C_{31}H_{28}O_{14}$	624.1472	22.07	0.29	0.5	-0.93	-0.21	1.22	1.43	0.10079	0.00460	0.00023	0.17739	0.00003	6.0664E-06
UNKNOWN*		682.1697	22.49	0.26	0.37	-0.76	-0.11	1.02	1.13	0.04783	0.00403	0.00002	0.30801	2.3623E-06	9.1598E-07
UNKNOWN*		686.2024	9.70	-4.3	-2.12	1.66	-2.18	-5.96	-3.78	4.1063E-06	0.00072	0.00249	0.00059	2.5573E-07	8.9853E-06
UNKNOWN*		692.1707	22.49	0.18	0.33	-0.75	-0.15	0.93	1.08	0.05696	0.00180	0.00001	0.10171	1.9848E-06	5.1994E-07
UNKNOWN*		788.1697	18.79	-0.35	-0.46	-1.13	0.12	0.78	0.67	0.19325	0.03054	0.00006	0.57682	0.00045	0.00176

*UNKNOWN metabolites – not identified, tentative molecular mass indicated; tentative phenolic compounds were annotated based on two more filter criteria: C-H-O backbone and aromatic ring (C6 carbon structure).

2.7. Statistical analysis

Data were analysed by using SAS software (version 9.4, Cary, NC, U.S.A.). A MIXED MODEL with a Kenward-Roger test and a significance level of $p \leq 0.05$ were employed. Log-transformed and thus inverse-transformed data were used for the parameters pH_{must} and $alcohol_{wine}$. For the sensory analysis of the wine, every aroma compound was analysed separately and compared between the N-forms within one year. Log-transformed and thus inverse-transformed data were used for the aroma attributes odour, cherry, strawberry, cassis and elderberry.

Table. 4b

Regulated tentative phenolic compounds in wine of *Vitis vinifera* L. *cv*. Regent during the experimental year 2016 by using UHPLC-ESI-MS in positive ionisation mode. Relative ratios of the generated Log^2 fold changes and *p*-values of the three different N - forms (CaN, AM, UR) and a control are shown. Significance is indicated by colour coding of the Log^2 fold changes (significance = ≥ +1/≥ −1) and the *p*-values ($p \leq 0.05$); ANOVA (pooled samples n = 3).

-3.01 → -4.00 | -2.01 → -3.00 | -1.01 → -2.00 | 0.00 → 0.99 | 1.00 → 2.00 | 2.01 → 3.00 | 3.01 → 4.00

1.0-0.05 | 0.05-0.01 | 0.01-0.005 | 0.005-0.001 | 0.001-0.0005 | 0.0005-0

Metabolite annotation	Formula	Molecular Mass [m/z]	RT [min]	Log^2 Fold CaN /control	AM / control	UR / control	CaN / AM	CaN / UR	AM / UR	*p*-value CaN /control	AM / control	UR / control	CaN / AM	CaN /UR	AM / UR
Tentative phenolic compound	$C_{11}H_{14}O_4$	210.0889	20.98	1.64	0.41	-1.51	1.22	3.15	1.92	1.0537E-07	0.00181	2.0071E-07	1.731E-06	1.426E-09	2.493E-08
Tentative phenolic compound	$C_{15}H_{12}O_6$	288.0629	17.85	0.93	1.14	-0.17	-0.21	1.10	1.31	2.4608E-07	4.2641E-08	0.01554	0.01278	4.471E-08	1.689E-08
Tentative phenolic compound	$C_{16}H_{12}O_7$	316.0580	18.71	-0.04	-0.7	0.81	0.66	-0.85	-1.51	0.92616	0.00476	0.00880	0.01025	0.00412	0.00006
Tentative phenolic compound	$C_{15}H_{14}O_6$	322.0685	14.39	1.01	0.32	-1.7	0.69	2.71	2.02	1.2006E-07	0.00076	5.3588E-09	3.086E-06	3.375E-11	1.377E-09
FLAVANOLS (FLAVAN -3-OLS)															
(±) Epicatechin	$C_{15}H_{14}O_6$	290.0785	17.85	1.04	1.3	-0.18	-0.26	1.22	1.48	6.6609E-09	1.2797E-09	0.00139	0.00018	2.029E-09	2.933E-10
FLAVONOLS															
Quercetin-glucoronide	$C_{21}H_{18}O_{13}$	478.0743	22.21	-0.75	0.39	0.81	-1.14	-1.56	-0.42	0.00016	0.00202	0.00006	3.187E-06	4.656E-07	0.02109
Isorhamnetin-caffeoyl-conjugate	$C_{31}H_{28}O_{14}$	624.1472	22.07	0.29	0.5	-0.93	-0.21	1.22	1.43	0.10079	0.00460	0.00023	0.17739	0.00003	6.066E-06

Tentative phenolic compounds were annotated based on two more filter criteria: C-H-O backbone and aromatic ring (C6 carbon structure).

For the data analysis of leaf and wine metabolomics, an analysis of variance (ANOVA) and a Tukey HSD test (posthoc test) with a *p*-value of $p \leq 0.05$ were carried out by using Compound Discoverer 3.0 Software (Thermo Fischer Scientific, Waltham, MA, USA). The corresponding principal component analyses (PCA; PC1 *vs* PC2), the volcano plots (Log^2-FoldChange: 0.5) and the heatmaps were performed with the same Software; $p \leq 0.05$. The heatmaps were based on a Pearson distance function and the hierarchical trees were calculated by the average linkage method the normalised data were scaled before clustering.

3. Results

3.1. Oenological parameters of must and wine in response to different N-forms

No significant differences were detected in the oenological parameters of the must, except for pH (Table 1). The N-form CaN led to significantly higher pH values (mean: 3.4) and the N-form UR led to significantly lower pH values (mean: 3.2). In total the values of the chemical attributes were higher in 2015 compared with 2016, except for pH and must weight. Only a few significant changes were detectable in the oenological parameters of the wine (Table 2). In the wine of 2015, the acid concentration was influenced by the N-forms. UR treatment led to significantly higher concentrations (mean. 2.1) and AM led to significantly lower concentrations (mean: 1.9) in TTA. In the experimental year 2016 the chemical attributes TTA, MA and LA were significantly influenced by the different N-forms (Table 2); UR lead to significantly higher acid concentrations (mean: 1.6 TTA; 3.2 MA) and CaN resulted in significantly lower concentrations (mean: 1.4 TTA; 2.9 MA). The N-form CaN (mean: 0.86) was associated with significantly higher concentrations of LA in comparison with the other N-forms. In total, the concentrations of the oenological parameters in 2015 were higher compared with 2016, except for pH and alcohol.

The spider plots of the aroma profile of the various wines in 2015 (Fig. 1) showed a significant change between the N-forms CaN and UR in the expression of the aroma attribute strawberry. The aroma profile of the wine 2016 exhibited significant changes between the N-forms AM and the control with regards to the expression of the aroma attribute elderberry as well as between the N-forms CaN-UR, CaN-control and AM-control with respect to the aroma attribute cherry.

3.2. Metabolic responses in grapevine leaves and wine of the different N-forms

Fig. 2 shows the UHPLC-ESI-MS analyses of extracted compounds (metabolites) from grapevine leaves (Fig. 2a) and wine (Fig. 2b).

In total, 5166 features in grapevine leaves were detected by the Compound Discoverer software, of which 37 were significantly changed in abundance (Log^2 fold changes ≥ 1 and p-value ≤ 0.05, Suppl. Table. 1). Eight of these compounds belonged to the group of polyphenols (Tables 3a and 3b). In addition, 355 unregulated phenolic compounds were detected (see supplemental data Suppl. Table. 2.1). In wine, 5521 features were detected in total, 55 of which were changed in abundance (Suppl. Table. 1). Seven of these compounds belonged to the group of polyphenols (Tables 4a and b) and 125 unregulated phenolic compounds were detected (data see supplemental Suppl. Table. 1.2). UHPLC-ESI-MS data are summarised in Tables 3a and b and , Tables 4a and b and in the supplementary material (Suppl. Tables 2.1 and 2.2).

A principal component analysis (PCA, scores plot) of the grapevine leaves and of the wine was performed to detect the impact of the different N-forms on the leaves and the wine metabolome (Fig. 4a andb and). The purpose was to classify the data into their variability by means of so-called principal components and thereby identify patterns in the dataset (Ringnér, 2008; Næs et al., 2010). The technical replicates (triplicates of each N-form, pooled QC samples) were clustered together in a shared colour plot (heatmap, Fig. 4a andb and) or in a one-point cloud (PCA, Fig. 3ab and), indicating high reproducibility of the analytical method applied. The PCA of the grapevine leaves (Fig. 3a) revealed that all N-forms could be separated from the control by principal component one. Principal component two clearly separated UR from the other N-forms and the control. The PCA of the wine (Fig. 3b) revealed that all N-forms were separated from the control. However, principal component two clearly separated the N-forms CaN and AM. The Venn diagrams (Fig. 5a and b) also illustrated the pattern indicated by the PCA. CaN showed the highest change of metabolite profile compared with the control, both in leaves and in wine. In the order of the highest number of regulated tentative metabolites was: CaN - control > AM - control > UR – control. However, when CaN was compared with other N-forms, this pattern for leaves slightly changed to; CaN - UR > AM - UR > CaN - UR and for wine to; AM - UR > CaN - UR > CaN - UR. This indicates that the N-forms CaN and AM had similar effects on the metabolic profile of leaves and wine.

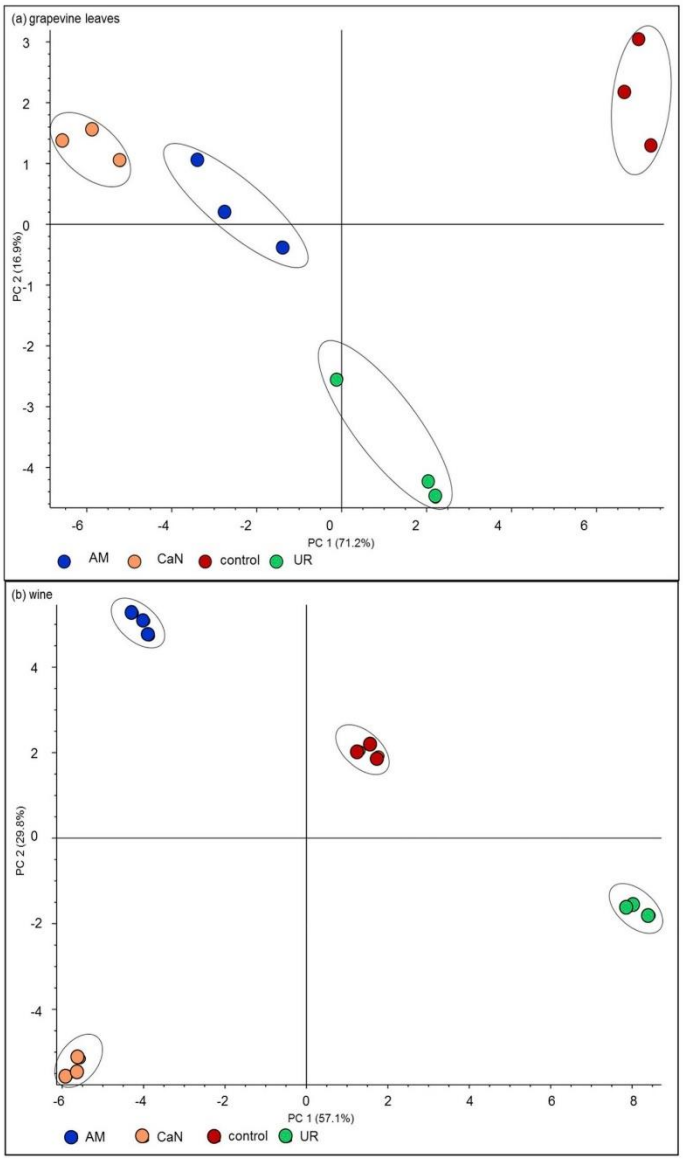

Fig. 3. Principal component analysis (PCA; PC1 vs. PC2) in (a) leaves and (b) wine were generated from normalised data of regulated compounds of the three different forms CaN ( ); AM ( ) and UR ( ) and a control ( ); nitrogen patterns are marked with circles, (pooled samples n = 3); $p \leq 0.05$.

3.3. Metabolic profiling of phenolic compounds

The peak detection data of all differentially abundant compounds are summarised in Table 3a for grapevine leaves and in Table 4a for wine. However, since we focused on phenolic compounds, these were listed in separate tables (Tables 3b and 4b). We detected 8 significantly changed phenolic components in the leaves, accounting for 24% of all regulated metabolites (Table 3b). Six phenolic compounds could be assigned to the group of flavonols, including three kaempferol and three quercetin derivatives based on the precise *m/z* value, the deduced sum formula and the fragmentation spectrum. For two phenolic compounds (tentative phenolic compounds, based on the filter criteria) only the molecular formula and the molecular mass [*m/z*] is provided, since the fragmentation spectrum did not provide sufficient information for unambiguous identification. The glycosylation of the detected kaempferol and quercetin derivatives decreased with increasing retention time (insert Fig. 2 a') and the glycosides eluted in the order Tri > Di > Mono. Notably, only one phenolic compound increased in abundance compared to the control based on the N treatment. In contrast, all 6 flavonols were significantly decreased in abundance by N treatment,

especially when the vines were treated with UR. Kaempferol derivatives decreased under all forms of N fertilisation compared with the control (Table 3b).

In the wine, we to detected 7 phenolic compounds that changed in abundance accounting for 13% of all changed metabolites. Three of them were identified and assigned, namely the flavanol (±) epicatechin and the flavonols quercetin and isorhamnetin. A highly significant increase in the abundance of the tentative phenolic compounds, and of (±) epicatechin could be seen when vines were treated with CaN and AM in comparison with the control. On the other hand, the N-form UR caused a highly significant decrease in the abundance of the phenolic compounds in comparison with the control. The flavonols showed no change after any N fertilisation compared with the control. On evaluating the differences between the various N-forms, we observed that, under CaN treatment, most of the identified phenolic compounds increased in abundance compared with treatment with other N-forms.

4. Discussion

4.1. Quality influences on must and wine in response to different N-forms

Acid and pH are two of the most important quality factors in grapes and wine. In addition to wine stability and microbiological control, both parameters have an influence on organoleptic parameters (Torija et al., 2003; Ali et al., 2009). The typical pH range for white wines and red wines are 3.0–3.4 and 3.3–3.7, respectively. Lower values within this range in musts are preferred because pH increases during or after fermentation (Waterhouse et al., 2016). The UR treatment increased the concentration of TTA (Table 2), which might have a positive effect on the fermentation process. Similar results have been reported by Portu et al. (2015a). Our results indicate that CaN increases the concentration of LA. During alcoholic fermentation, malolactic fermentation conducted by lactic acid bacteria produces LA (Cappello et al., 2017). We have studied the influence of fertilised versus non-fertilised (control) vines and of the N-forms CaN and UR on wine (Fig. 1). The two mentioned N-forms showed the highest difference in the evaluation of the aroma attributes. The results of the oenological parameters and the sensory evaluation seem to support the controversially discussed hypothesis that N fertilisation does indeed influence the sensory attributes as reviewed by Bell and Henschke, 2005. However, the N-forms CaN and AM also appear to influence the wine sensory profile, leading to individual fruity tasting nuances. Furthermore, a difference can be seen between the experimental years. The vintage effect and thus the unique climate conditions are known significantly to influence fruity aroma composition (Robinson et al., 2014).

4.2. Metabolic changes in grapevine leaves and wine

The metabolite profiling of grapevine leaves and wine, suggests that

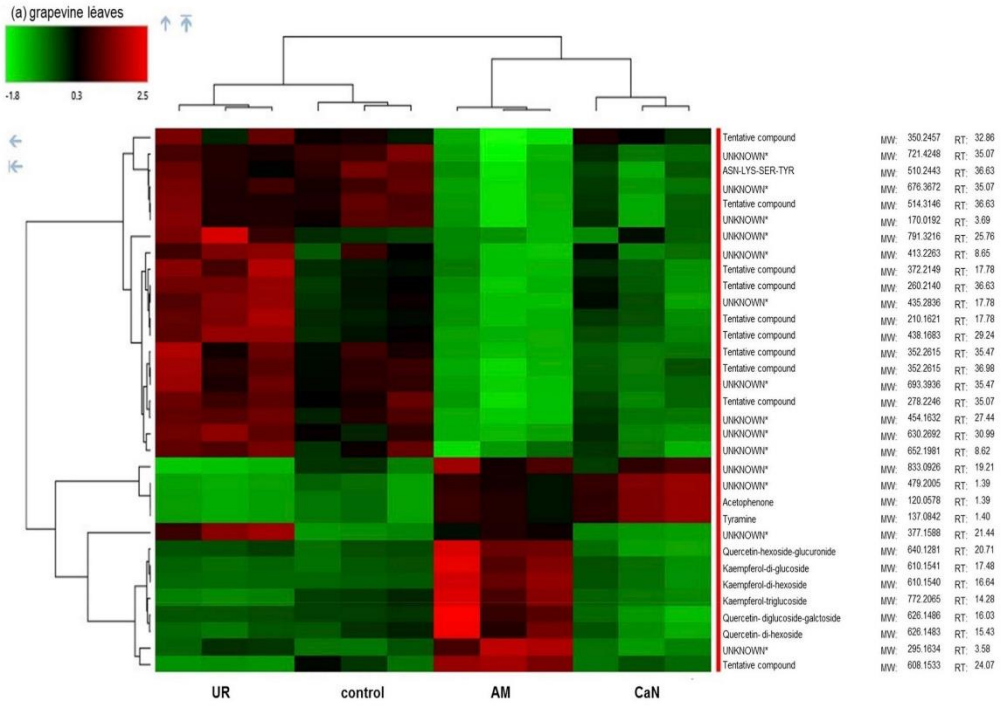

Fig. 4. Clustered heatmaps of the regulated compounds of (a) grapevine leaves and (b) wine of *Vitis vinifera* L. *cv.* Regent during the experimental year 2016. Individual metabolites are represented by rows and N treatments are represented by columns (CaN, AM, UR and a control). Heatmap visualisation of metabolomic differences is based on the relative amount (by area) in a given N sample. Green: downregulation of the metabolite; red: upregulation of the metabolite. Hierarchial clustering was formed by Pearson's distance function. Hierarchial trees were calculated by the average linkage method (pooled samples n = 3); $p \leq 0.05$. Each rectangle represents the relative amount (by area) of a particular compound in a given N sample (CaN, AM, UR and control). The dendogram, represents the distance or similarity between samples. Clusters, define a site characteristic reaction pattern between the N-forms or between the compounds. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

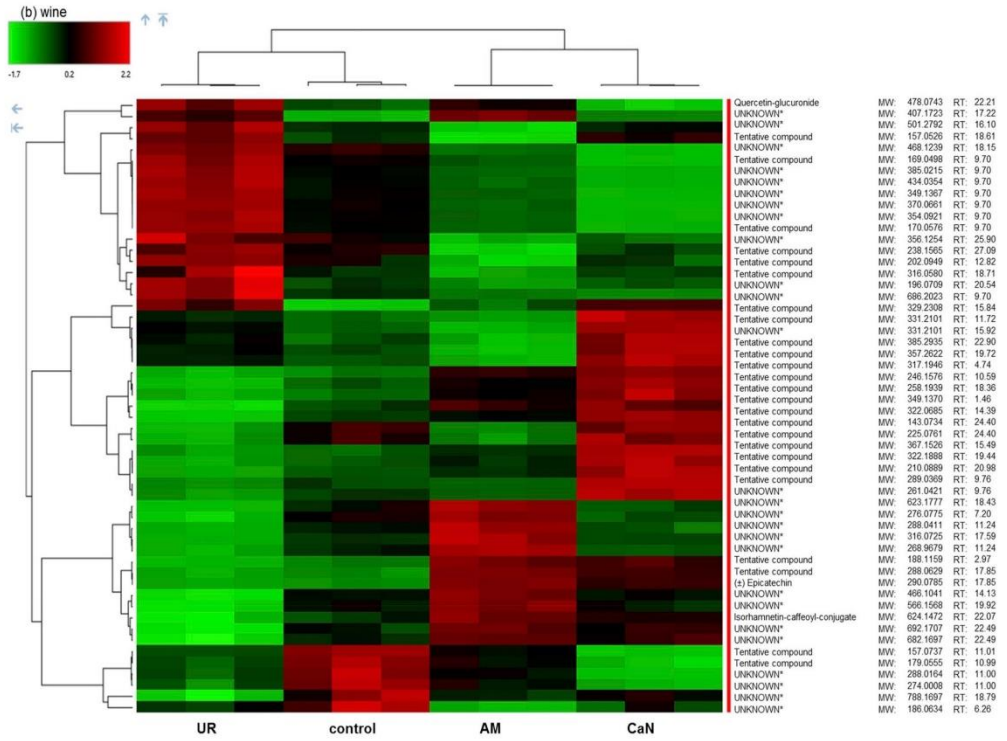

Fig. 4. (*continued*)

fertilisation with CaN, AM or UR has a significant influence on the grapevine metabolome. In general, CaN had the greatest impact on the metabolite profile in leaves and in wine (Fig. 5a and b), followed by AM and UR. After AM and UR treatment, the tentative phenolic compounds, show a comparable pattern (Tables 3b and 4b). As mentioned above, the N-forms CaN and AM are similar in their regulation pattern. Notably, UR treatment mainly leads to a decreased abundance of metabolites in leaves and wine. In contrast, CaN and AM mainly increase the abundance of metabolites in leaves but, decrease the metabolites in wine, especially the tentative phenolic compounds. Nevertheless, the extent of the abundance change was dependent on the compound itself. Leser and Treutter (2005) have measured a significant reduction of phenolic compounds upon treatment with increasing N amounts and conclude a reduction in flavonoid biosynthesis because of high N supply in apple leaves. Portu et al. (2015b) have found similar results in wine. The annotated polyphenols in the leaves and wine (Tables 3b and 4b) are mainly flavonoids and not only belong to a subgroup of flavonols, involved in the co-pigmentation process of anthocyanins in red berries, but also have a high antioxidant and free radical scavenging activity. Flavonols in leaves and berries can also serve as UV protectors (Castillo-Muñoz et al., 2007; De Rosso et al., 2014) that can be increased by the N fertilisation rate. (±) Epicatechin belongs to the flavanols (flavan -3-ols), also named condensed tannins; they are important contributors to wine stability and organoleptic properties such as body and mouth-feel (Downey et al., 2006; Teixeira et al., 2013).

Nitrogen also increases vegetative growth, which in turn has an influence on the ripening of the grapes and thus has an effect on quality. Delayed maturity affects the biochemical composition of the berry (Lang et al., 2018) and thus affects the formation of aroma compounds and taste. These effects are based on changes in flavonoid metabolism attributable to a possible imbalance or a competition for sugar between leaf and berry (Braidot et al., 2008). This implies that a higher supply of N might shift the sink: source ratio in favour of plant biomass and that therefore less sugar is available for the berry. As a result, less aroma precursors accumulate in the berries. Since, in the present experiment, the same amount of total N was applied in all N treatments, we consider that the differences in the metabolic profile, especially the phenolic compounds, are based on the different uptake or utilisation capacity of the different N-forms. Nitrate and ammonium are major N sources for the grapevine (Lang et al., 2018; Loulakakis and Roubelakis-Angelakis, 2001). Their uptake is an active process driven by root-based membrane transporters such as NRT for nitrate or AMT for ammonium (Goel and Singh, 2015). In contrast to nitrate and ammonium, the uptake process for urea is still under debate; it can be absorbed both actively and passively by the plant (Witte, 2011). In their study on the effects of the growth conditions of grapevines, including defoliation, Rossouw et al. (2018) showed that polyphenols and other products of the shikimate pathway are affected through N availability. Therefore, we suggest that UR might be available to a lower extent for the vine root compared with CaN and AM. In addition, we assume that the different N-forms may trigger the induction of specific phenolic biosynthesis such as the shikimate and the phenylpropanoid pathways, to a different metabolic extent.

To our surprise, a higher number of metabolites in wine were affected by the different N-forms than in the leaves. We think that this is because large part of the N that occur in must and, that is used for fermentation, the so-called yeast-assimilable nitrogen (YAN), is metabolised by the yeast. Therefore, more significant differences are

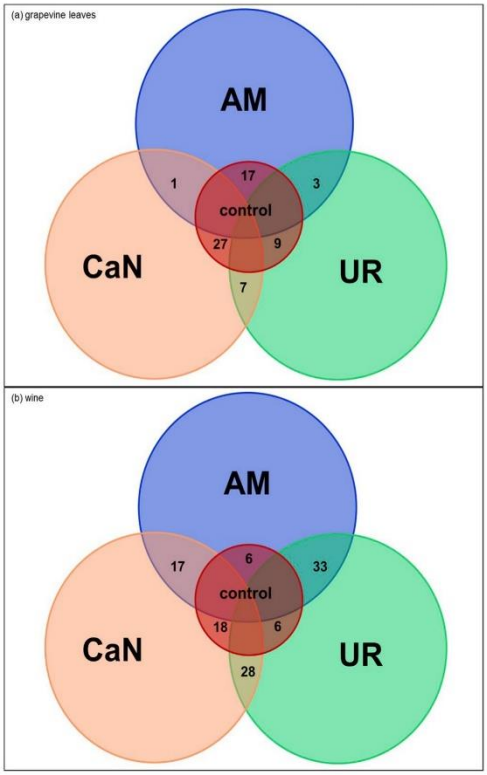

Fig. 5. Venn diagram of regulated compounds of (a) leaves and (b) wine in response to measured significant differences of various N-forms. Numbers include the distribution of all regulated compounds detected in all N comparisons CaN/control, AM/control, UR/control, CaN/AM, CaN/UR, AM/UR.

detectable, especially in the N forms CaN and AM, because of their better plant availability. We assume that CaN and AM provide significantly more N for the yeast cultures than UR. However, as rosé wines were used in the present work, the number of detectable phenolic compounds is lower compared with those in red wine but is higher than those in white wine (Sun et al., 2007). Furthermore, the maceration stage was shortened and thus less polyphenols were able to diffuse from the berry skin into the must. In general, a comparison between leaf and wine might be difficult as the wine is berry juice, which has gone through several processes. Nevertheless, in our experiment, we have been able to detect an influence of fertilisation with different the N-forms on the vines with respect to the phenol composition of the wine and thus their influence on the organoleptic and sensory properties of the wine.

5. Conclusion

Fertilisation with different N-forms such as calcium nitrate, ammonium or urea influences the metabolite profile in grapevine leaves and the sensory attributes of the resulting wine. The quality of must and wine and, the sensory profile of the wine, show greater changes between fertilised and non-fertilised vines and between the N-forms CaN and UR. The metabolic changes found in leaves are less pronounced than those in wine. The N-forms CaN and AM are similar in their regulation pattern. UR mainly decreases the metabolites and phenolic compounds in leaves and in wine, whereas CaN and AM mainly decreases the phenolic compounds in the leaves but increases phenolic compounds and the tentative phenolic compounds of wine. These phenolic components influence the organoleptic and sensory properties of wine. Based on our results, we conclude that CaN and AM fertilisation might have a positive effect on wine quality compared with the application of UR.

Contribution

Conceptualization: Christian Zörb, Jens Pfannstiel, Nikolaus Merkt. Data curation: Carina Paola Lang. Formal analysis: Carina Paola Lang, Christian Zörb. Investigation: Carina Paola Lang. Methodology: Carina Paola Lang, Nikolaus Merkt, Iris Klaiber, Jens Pfannstiel. Software: Carina Paola Lang, Iris Klaiber. Supervision: Christian Zörb, Nikolaus Merkt. Validation: Carina Paola Lang, Christian Zörb. Visualisation: Carina Paola Lang, Iris Klaiber. Writing- original draft: Carina Paola Lang. Writing-review & editing: Christian Zörb, Nikolaus Merkt, Jens Pfannstiel.

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Abbreviations used

AM	ammonium
CaN	calcium nitrate
C:N ratio	carbon: nitrogen ratio
ESI	electrospray ionisation
HPLC	high performance liquid chromatography
LA	lactic acid
LC-MS	liquid chromatography mass spectrometry
MA	malic acid
N	nitrogen
PC	principal component
PCA	principal component analysis
SO4	rootstock Selection Oppenheim 4
TA	total acid
TIC	total ion chromatogram
TTA	tartaric acid
UHPLC	ultra high performance liquid chromatography
UR	urea
YAN	yeast-assimilable nitrogen

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.09.009.

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Appendix for Chapter 4

Supplemental Data

Suppl. Table 1: Filter conditions of the metabolomic profiling data workflow performed with Compound Discoverer software 3.0 by Thermo Fischer Scientific.

Filter parameter	Setting
Background	Is false
Norm. Area	Has any value in at least 3 files
p-value	Is less than or equal to 0.05 in ratio (CaN) / (control) OR Is less than or equal to 0.05 in ratio (AM) / (control) OR Is less than or equal to 0.05 in ratio (UR) / (control) OR Is less than or equal to 0.05 in ratio (CaN) / (AM) OR Is less than or equal to 0.05 in ratio (CaN) / (UR) OR Is less than or equal to 0.05 in ratio (AM) / (UR)
Checked compound	Is true
MS^2 spectrum	Is required
Log^2 Fold Change	Is true at a value of -1 to +1
Area Max	>150000

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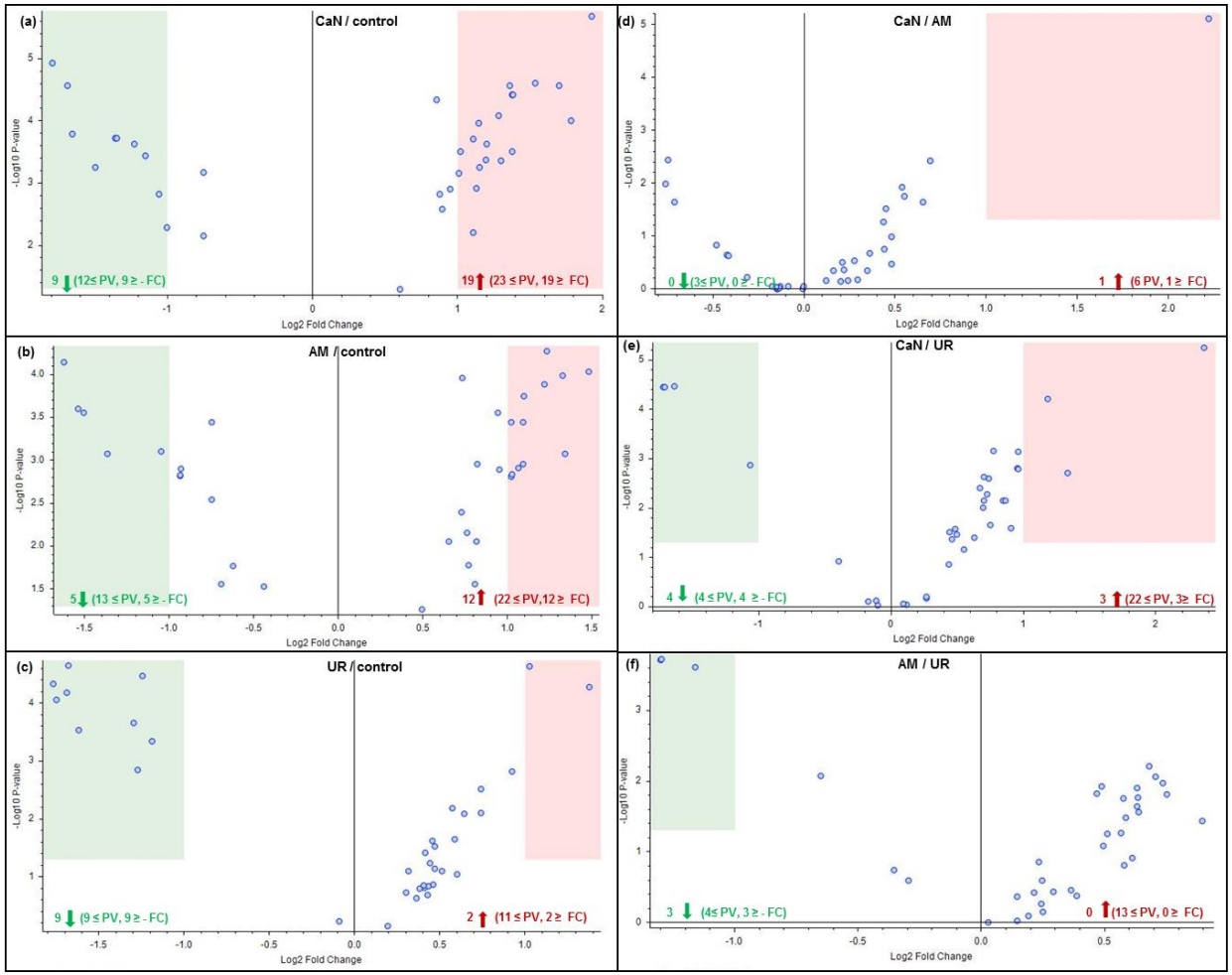

Suppl. Fig. 1.1: Volcano plots of the regulated compounds in grapevine leaves of *Vitis vinifera* L *cv*. Regent in response to different N-forms. Shown are the Log^2 fold changes of the measured differences; CaN / control (a); AM / control (b); UR / control (c); CaN / AM (d); CaN / UR (e); AM / UR (f). ANOVA (pooled samples n=3*); p*-value (PV) $p \leq 0.05$; Log^2 Fold Change (FC): 1.0.

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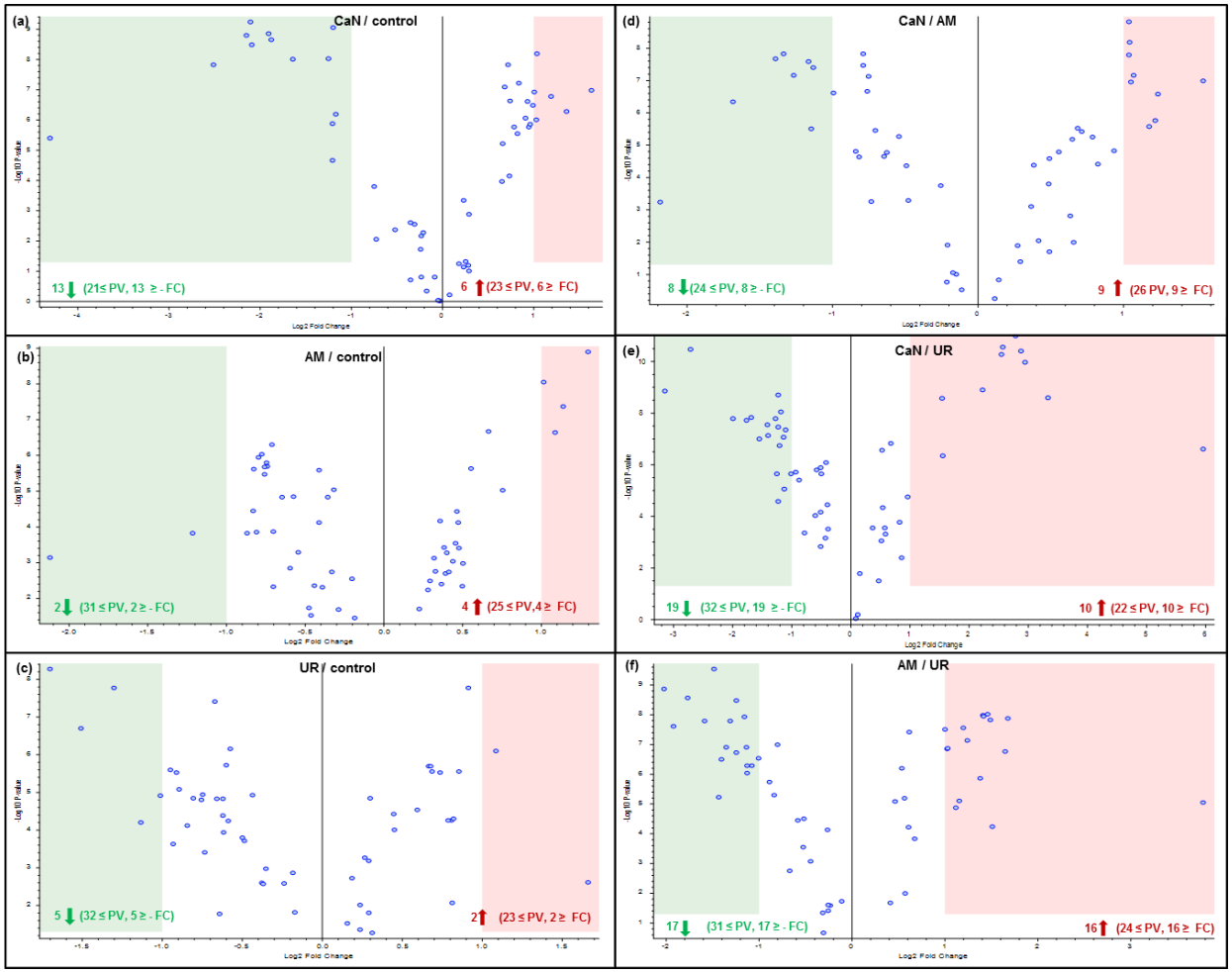

Suppl. Fig. 1.2: Volcano plots of the regulated compounds in wine of *Vitis vinifera* L *cv*. Regent in response to different N-forms. Shown are the Log^2 fold changes of the measured differences; CaN / control (a); AM / control (b); UR / control (c); CaN / AM (d); CaN / UR (e); AM / UR (f). ANOVA (pooled samples n=3*); p*-value (PV) $p \leq 0.05$; Log^2 Fold Change (FC): 1.0.

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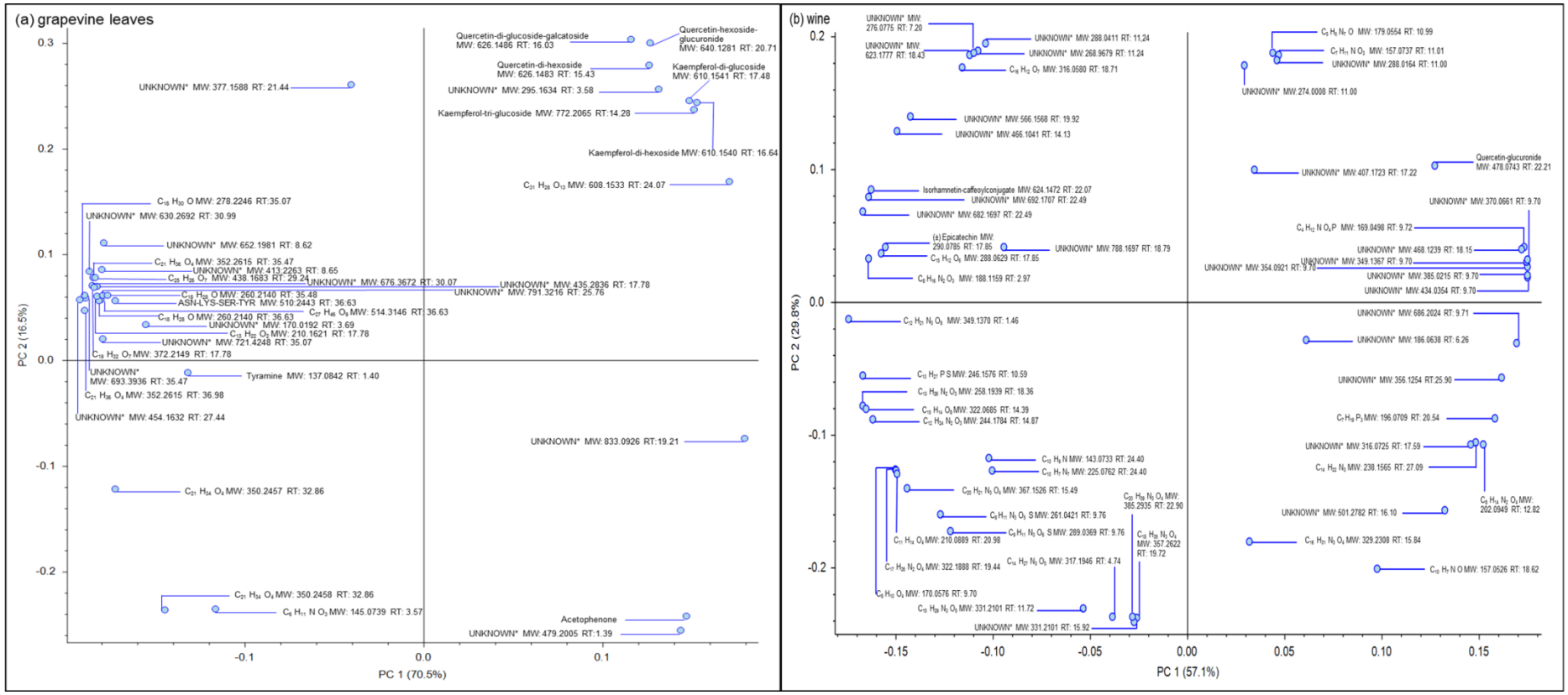

Suppl. Fig. 2: Loading plots of the Principal Component Analysis (PCA; PC1 vs. PC2) of grapevine leaves (a) and wine (b). The blue dots indicate all tentative regulated compounds; (pooled samples n=3); $p \leq 0.05$.

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Suppl. Tab. 2.1: Tentative unregulated phenolic compounds in grapevine leaves of *Vitis vinifer*a L. *cv.* Regent during the experimental year 2016 by using UHPLC-ESI-MS in positive ionisation mode. Relative ratios of the generated Log^2 fold changes and *p*-values of the three different N - forms (CaN, AM, UR) and a control are shown. Significance: the Log^2 fold changes (significance = ≥ +1 / ≥ -1) and the p-values ($p \leq 0.05$); ANOVA (pooled samples n = 3).

Metabolite annotation	Formula	Molecular Weight [*m/z*]	RT [min]	Log^2 Fold						*p*-value					
				CaN / control	AM / control	UR / control	CaN / AM	CaN / UR	AM / UR	CaN / control	AM / control	UR / control	CaN / AM	CaN / UR	AM / UR
Tentative phenolic compound	$C_7 H_6 O_3$	138.0318	13.69	-0.56	-0.23	-0.43	-0.33	-0.12	0.20	0.00035	0.05887	0.00195	0.01101	0.39958	0.10916
Tentative phenolic compound	$C_7 H_6 O_3$	138.0319	12.07	0.08	0.29	0.15	-0.21	-0.07	0.14	0.99825	0.59264	0.98637	0.50257	0.95722	0.77564
Tentative phenolic compound	$C_9 H_6 O_3$	162.0318	11.29	-0.21	0.07	0.20	-0.27	-0.41	-0.13	0.10593	0.85284	0.84332	0.31128	0.03340	0.42019
Tentative phenolic compound	$C_{10} H_{10} O_2$	162.0681	11.59	0.26	0.26	-0.18	0.00	0.44	0.44	0.41524	0.63981	0.27700	0.97278	0.02897	0.05236
Tentative phenolic compound	$C_{10} H_{10} O_2$	162.0681	34.22	-0.74	-0.21	0.52	-0.53	-1.26	-0.73	0.12335	0.75326	0.99211	0.44936	0.08371	0.60068
Tentative phenolic compound	$C_{10} H_{10} O_2$	162.0681	24.01	-0.09	-0.05	-0.41	-0.03	0.32	0.36	0.62605	0.50526	0.00883	0.99591	0.04663	0.06361
Tentative phenolic compound	$C_{10} H_{10} O_2$	162.0681	33.99	-0.67	-0.23	0.52	-0.44	-1.19	-0.75	0.12717	0.62912	0.99897	0.58004	0.10473	0.55220
Tentative phenolic compound	$C_{10} H_{10} O_2$	162.0682	14.87	0.29	0.25	0.23	0.04	0.06	0.02	0.78364	0.90602	0.87579	0.99221	0.99707	0.99982
Tentative phenolic compound	$C_{10} H_{10} O_2$	162.0682	32.69	-0.67	-0.65	0.13	-0.02	-0.80	-0.78	0.14305	0.44950	0.96942	0.80788	0.25946	0.68915
Tentative phenolic compound	$C_{11} H_{14} O$	162.1045	16.75	0.62	0.36	0.08	0.27	0.55	0.28	0.00758	0.15689	0.95332	0.19890	0.01480	0.30996
Tentative phenolic compound	$C_{11} H_{14} O$	162.1046	23.30	0.59	0.27	-0.05	0.32	0.64	0.32	0.02684	0.50079	0.97821	0.20592	0.04630	0.71684
Tentative phenolic compound	$C_{11} H_{14} O$	162.1046	16.06	0.43	0.25	-0.01	0.18	0.44	0.25	0.03993	0.41619	0.99347	0.36787	0.02789	0.30497
Tentative phenolic compound	$C_9 H_8 O_3$	164.0475	14.46	-0.30	0.06	0.24	-0.37	-0.54	-0.17	0.03010	0.86206	0.99307	0.08984	0.02096	0.73108
Tentative phenolic compound	$C_9 H_8 O_3$	164.0475	11.81	0.30	0.21	0.01	0.09	0.28	0.19	0.37793	0.82403	0.98788	0.82983	0.25369	0.65414
Tentative phenolic compound	$C_9 H_8 O_3$	164.0475	13.90	0.22	0.21	-0.07	0.01	0.29	0.27	0.71436	0.94028	0.66228	0.95444	0.19222	0.36972
Tentative phenolic compound	$C_9 H_8 O_3$	164.0475	2.05	0.49	0.32	0.09	0.17	0.40	0.23	0.01994	0.22783	1.00000	0.35924	0.01945	0.22241
Tentative phenolic compound	$C_{11} H_{16} O$	164.1201	16.05	0.48	0.29	0.10	0.19	0.37	0.19	0.01651	0.20775	0.99956	0.32968	0.01899	0.23851
Tentative phenolic compound	$C_{11} H_{16} O$	164.1203	16.75	0.65	0.46	0.09	0.19	0.56	0.37	0.01301	0.12278	0.82734	0.41996	0.04175	0.37851
Tentative phenolic compound	$C_9 H_{10} O_3$	166.0631	11.78	-0.17	-0.14	-0.47	-0.03	0.30	0.33	0.81372	0.53119	0.08400	0.95188	0.28098	0.51594
Tentative phenolic compound	$C_9 H_{10} O_3$	166.0632	8.98	0.10	-0.16	-0.47	0.26	0.58	0.32	0.73337	0.95749	0.10744	0.46205	0.02508	0.21309
Tentative phenolic compound	$C_8 H_8 O_4$	168.0424	13.94	0.15	0.29	0.01	-0.14	0.14	0.28	0.99983	0.62320	0.93065	0.58102	0.95195	0.32663
Tentative phenolic compound	$C_8 H_8 O_4$	168.0424	25.60	0.21	0.28	0.07	-0.07	0.14	0.21	0.48730	0.35964	0.99426	0.99282	0.62197	0.47717
Tentative phenolic compound	$C_{11} H_{10} O_2$	174.0682	23.32	0.25	0.22	-0.07	0.03	0.31	0.29	0.55745	0.70316	0.87745	0.99323	0.23257	0.32516
Tentative phenolic compound	$C_{11} H_{10} O_2$	174.0683	24.22	0.23	0.22	-0.15	0.01	0.38	0.37	0.64742	0.78249	0.59632	0.99421	0.13863	0.19479
Tentative phenolic compound	$C_9 H_6 O_4$	178.0267	11.37	0.44	0.47	-0.06	-0.03	0.49	0.53	0.08637	0.21261	0.98682	0.91251	0.05429	0.13564
Tentative phenolic compound	$C_9 H_6 O_4$	178.0267	14.78	0.46	0.45	-0.11	0.01	0.57	0.56	0.19169	0.24575	0.81073	0.99750	0.05529	0.07195
Tentative phenolic compound	$C_9 H_6 O_4$	178.0267	12.52	0.39	0.42	0.01	-0.03	0.39	0.42	0.13120	0.09333	0.89550	0.99456	0.04917	0.03500

Suppl. Tab. 2.1 (*continued*)

Tentative phenolic compound	$C_9 H_6 O_4$	178.0267	11.58	0.61	0.50	0.06	0.11	0.55	0.44	0.05078	0.27268	0.99791	0.63447	0.03968	0.21658
Tentative phenolic compound	$C_{10} H_{10} O_3$	178.0630	8.58	0.38	0.31	-0.06	0.07	0.44	0.37	0.03048	0.07228	0.99624	0.92425	0.02270	0.05342
Tentative phenolic compound	$C_{10} H_{10} O_3$	178.0631	20.03	-0.12	-0.31	-0.54	0.19	0.42	0.23	0.94550	0.26978	0.04566	0.51200	0.09840	0.59687
Tentative phenolic compound	$C_9 H_8 O_4$	180.0423	11.29	-0.22	0.06	0.19	-0.27	-0.40	-0.13	0.11825	0.73847	0.86204	0.44807	0.03950	0.33665
Tentative phenolic compound	$C_{10} H_{12} O_3$	180.0787	15.33	0.27	0.43	0.08	-0.17	0.19	0.36	0.92205	0.31218	0.97349	0.61785	0.73119	0.17998
Tentative phenolic compound	$C_{10} H_{12} O_3$	180.0787	16.03	0.31	0.36	0.01	-0.05	0.30	0.35	0.11505	0.11453	0.95965	1.00000	0.05782	0.05756
Tentative phenolic compound	$C_{12} H_{10} O_2$	186.0682	8.12	0.43	0.42	0.29	0.01	0.14	0.13	0.23752	0.30424	0.68284	0.99727	0.77941	0.87046
Tentative phenolic compound	$C_{12} H_{10} O_2$	186.0682	23.32	0.26	0.18	-0.13	0.08	0.39	0.31	0.64678	0.79855	0.64785	0.99168	0.15721	0.22981
Tentative phenolic compound	$C_{13} H_{16} O$	188.1202	17.71	0.36	0.28	0.07	0.07	0.28	0.21	0.20328	0.56390	0.96678	0.82724	0.10852	0.34052
Tentative phenolic compound	$C_{13} H_{16} O$	188.1202	16.75	0.67	0.44	0.12	0.23	0.55	0.32	0.00924	0.11143	0.90965	0.33391	0.02213	0.27245
Tentative phenolic compound	$C_{13} H_{16} O$	188.1203	16.06	0.51	0.29	0.00	0.22	0.51	0.29	0.02118	0.27428	0.99949	0.31880	0.01826	0.23788
Tentative phenolic compound	$C_{13} H_{16} O$	188.1203	17.43	0.65	0.46	0.04	0.19	0.61	0.41	0.04122	0.35206	0.99911	0.44494	0.03430	0.29915
Tentative phenolic compound	$C_7 H_{12} O_6$	192.0635	1.66	-0.49	-0.13	0.13	-0.36	-0.62	-0.26	0.01212	0.28203	0.88098	0.17935	0.00478	0.10414
Tentative phenolic compound	$C_{11} H_{12} O_3$	192.0787	19.09	0.38	0.11	-0.17	0.27	0.55	0.28	0.04214	0.99266	0.28922	0.06152	0.00341	0.20336
Tentative phenolic compound	$C_{10} H_{10} O_4$	194.0580	16.57	0.60	0.04	0.29	0.56	0.31	-0.25	0.06635	0.99530	0.24833	0.09196	0.77775	0.33303
Tentative phenolic compound	$C_{10} H_{10} O_4$	194.0580	16.06	0.36	0.24	0.20	0.12	0.16	0.04	0.11040	0.39417	0.45782	0.77219	0.70103	0.99911
Tentative phenolic compound	$C_{10} H_{10} O_4$	194.0581	14.31	0.28	0.24	0.18	0.04	0.11	0.06	0.77318	0.92045	0.99493	0.98596	0.88398	0.97848
Tentative phenolic compound	$C_{12} H_{16} O_3$	208.1100	34.22	-0.60	-0.25	0.43	-0.35	-1.03	-0.68	0.15848	0.69766	0.99998	0.60007	0.15063	0.67719
Tentative phenolic compound	$C_{12} H_{16} O_3$	208.1100	33.99	-0.58	-0.22	0.50	-0.36	-1.08	-0.72	0.17458	0.67446	0.99987	0.66293	0.15875	0.63566
Tentative phenolic compound	$C_{12} H_{16} O_3$	208.1100	16.01	0.32	0.22	0.02	0.10	0.30	0.19	0.54367	0.98696	0.92901	0.72554	0.27068	0.79118
Tentative phenolic compound	$C_{13} H_{20} O_2$	208.1463	16.93	1.21	0.81	0.47	0.40	0.74	0.34	0.01035	0.06610	0.19208	0.55706	0.22569	0.86823
Tentative phenolic compound	$C_{13} H_{20} O_2$	208.1464	19.17	0.76	0.31	0.26	0.44	0.50	0.06	0.00793	0.18227	0.28404	0.17995	0.11304	0.98601
Tentative phenolic compound	$C_{13} H_{20} O_2$	208.1465	13.18	0.83	0.39	0.32	0.44	0.51	0.07	0.02055	0.66272	0.77370	0.10476	0.07860	0.99676
Tentative phenolic compound	$C_{13} H_{20} O_2$	208.1465	17.05	0.51	0.19	-0.07	0.32	0.57	0.25	0.06015	0.74133	0.93245	0.24812	0.02640	0.42366
Tentative phenolic compound	$C_{13} H_{20} O_2$	208.1465	16.65	0.17	-0.08	-0.38	0.25	0.55	0.30	0.36095	0.97822	0.15020	0.55561	0.01328	0.08679
Tentative phenolic compound	$C_{13} H_{20} O_2$	208.1465	22.68	-0.25	-0.48	-0.14	0.23	-0.11	-0.35	0.56123	0.17689	0.56612	0.77857	1.00000	0.77397
Tentative phenolic compound	$C_{13} H_{20} O_2$	208.1465	10.99	0.66	0.32	-0.05	0.34	0.70	0.36	0.03800	0.55138	0.97865	0.25325	0.02221	0.35876
Tentative phenolic compound	$C_{13} H_{20} O_2$	208.1465	10.35	0.61	0.34	0.67	0.26	-0.07	-0.33	0.43425	0.76427	0.45824	0.92578	0.99996	0.94046
Tentative phenolic compound	$C_{13} H_{20} O_2$	208.1465	18.86	0.19	-0.04	-0.22	0.23	0.41	0.18	0.49697	0.90665	0.07037	0.22139	0.00949	0.17874
Tentative phenolic compound	$C_{13} H_{20} O_2$	208.1465	19.65	0.81	0.47	0.15	0.34	0.66	0.32	0.00258	0.13683	0.91715	0.06609	0.00553	0.31929
Tentative phenolic compound	$C_{13} H_{20} O_2$	208.1465	18.60	0.76	0.19	0.01	0.57	0.76	0.18	0.00287	0.47081	0.88038	0.01950	0.00125	0.18876
Tentative phenolic compound	$C_{13} H_{20} O_2$	208.1465	19.37	0.72	0.52	0.12	0.20	0.60	0.41	0.01479	0.15736	0.99974	0.38182	0.01317	0.13940
Tentative phenolic compound	$C_{13} H_{20} O_2$	208.1465	14.22	0.88	0.38	-0.20	0.49	1.07	0.58	0.00057	0.11447	0.22741	0.01139	0.00008	0.00646
Tentative phenolic compound	$C_{13} H_{20} O_2$	208.1465	13.89	0.77	0.53	-0.14	0.25	0.91	0.66	0.00714	0.06271	0.99992	0.42587	0.00770	0.06823
Tentative phenolic compound	$C_{11} H_{14} O_4$	210.0891	20.02	-0.19	-0.38	-0.62	0.19	0.42	0.23	0.83868	0.16039	0.02941	0.45880	0.09441	0.63786
Tentative phenolic compound	$C_{11} H_{14} O_4$	210.0892	25.09	0.58	0.53	1.00	0.05	-0.42	-0.47	0.13881	0.20997	0.00732	0.98962	0.21580	0.14282

Suppl. Tab. 2.1 (*continued*)

Tentative phenolic compound	$C_{11}H_{14}O_4$	210.0892	32.72	-0.55	-0.39	0.65	-0.16	-1.20	-1.04	0.18539	0.55885	0.99278	0.79837	0.12824	0.42043
Tentative phenolic compound	$C_{13}H_{22}O_2$	210.1621	17.32	0.67	0.36	0.14	0.31	0.53	0.22	0.01992	0.49762	0.97642	0.15424	0.03466	0.71959
Tentative phenolic compound	$C_{10}H_{12}O_5$	212.0685	8.61	-0.15	-0.16	-0.60	0.01	0.45	0.44	0.89830	0.95933	0.08491	0.99692	0.22041	0.16816
Tentative phenolic compound	$C_{10}H_{12}O_5$	212.0685	9.39	-0.06	0.00	-0.48	-0.06	0.43	0.49	0.97734	0.99044	0.08078	0.99958	0.14106	0.12223
Tentative phenolic compound	$C_{10}H_{12}O_5$	212.0685	9.71	0.12	0.01	-0.46	0.10	0.58	0.47	0.90847	0.91165	0.04168	0.57962	0.01679	0.10461
Tentative phenolic compound	$C_{11}H_{16}O_4$	212.1049	16.77	0.21	0.21	0.65	0.00	-0.44	-0.44	0.76807	0.88292	0.20451	0.99447	0.63207	0.49835
Tentative phenolic compound	$C_{11}H_{16}O_4$	212.1050	14.87	0.37	0.29	0.57	0.07	-0.20	-0.28	0.32106	0.58464	0.19094	0.94663	0.97708	0.78570
Tentative phenolic compound	$C_{13}H_{24}O_2$	212.1777	22.41	0.20	-0.01	0.11	0.22	0.09	-0.13	0.27840	0.99990	0.36496	0.25667	0.99603	0.33816
Tentative phenolic compound	$C_{13}H_{14}O_3$	218.0944	16.04	0.08	-0.14	-0.12	0.22	0.19	-0.03	0.96139	0.51786	0.48644	0.78117	0.74984	0.99992
Tentative phenolic compound	$C_{13}H_{14}O_3$	218.0944	17.00	0.34	0.32	0.01	0.02	0.33	0.31	0.08828	0.17000	0.99423	0.96370	0.06221	0.12060
Tentative phenolic compound	$C_{13}H_{14}O_3$	218.0944	17.80	0.31	0.30	-0.03	0.01	0.34	0.33	0.30456	0.34247	0.87997	0.99967	0.11300	0.12902
Tentative phenolic compound	$C_{13}H_{14}O_3$	218.0945	20.34	0.00	0.14	-0.16	-0.14	0.16	0.30	0.98037	0.99948	0.47937	0.95972	0.68635	0.42455
Tentative phenolic compound	$C_{13}H_{14}O_3$	218.0945	19.63	0.04	0.15	-0.12	-0.11	0.17	0.28	0.96154	0.98988	0.47983	0.86159	0.74263	0.33941
Tentative phenolic compound	$C_{13}H_{14}O_3$	218.0945	20.81	0.43	0.42	0.00	0.00	0.43	0.43	0.07688	0.12071	0.97871	0.98777	0.04445	0.06982
Tentative phenolic compound	$C_{13}H_{14}O_3$	218.0945	21.55	0.31	0.37	-0.02	-0.06	0.33	0.40	0.20652	0.15346	0.86303	0.99604	0.07011	0.05154
Tentative phenolic compound	$C_{13}H_{14}O_3$	218.0945	20.61	-0.11	-0.09	-0.64	-0.03	0.52	0.55	0.81952	0.31220	0.02415	0.75537	0.08169	0.31647
Tentative phenolic compound	$C_{15}H_{22}O$	218.1672	21.20	1.20	0.11	-0.49	1.09	1.69	0.59	0.00056	0.78809	0.10041	0.00148	0.00005	0.02707
Tentative phenolic compound	$C_{13}H_{16}O_3$	220.1100	24.01	-0.06	-0.06	-0.39	0.00	0.33	0.33	0.56495	0.55849	0.01065	1.00000	0.06681	0.06793
Tentative phenolic compound	$C_{13}H_{20}O_3$	224.1413	22.36	0.68	0.23	0.15	0.45	0.52	0.08	0.09006	0.74526	0.94465	0.35258	0.19267	0.96392
Tentative phenolic compound	$C_{13}H_{20}O_3$	224.1413	19.47	0.77	0.40	0.20	0.37	0.57	0.20	0.01161	0.31817	0.96037	0.15035	0.02219	0.55005
Tentative phenolic compound	$C_{13}H_{20}O_3$	224.1413	19.95	0.62	0.35	0.08	0.28	0.54	0.27	0.02079	0.31230	0.96902	0.27535	0.03834	0.51983
Tentative phenolic compound	$C_{13}H_{20}O_3$	224.1413	13.07	0.44	0.33	0.05	0.11	0.39	0.28	0.08621	0.45038	0.99998	0.61119	0.09081	0.46896
Tentative phenolic compound	$C_{13}H_{20}O_3$	224.1413	16.75	0.67	0.36	0.11	0.30	0.56	0.25	0.00582	0.15329	0.94107	0.15280	0.01191	0.32142
Tentative phenolic compound	$C_{13}H_{20}O_3$	224.1413	18.31	0.35	0.24	0.03	0.11	0.32	0.21	0.15142	0.37895	0.99823	0.89235	0.19006	0.45813
Tentative phenolic compound	$C_{13}H_{20}O_3$	224.1413	16.05	0.49	0.29	0.04	0.20	0.45	0.25	0.01945	0.23504	0.99997	0.34155	0.01837	0.22217
Tentative phenolic compound	$C_{13}H_{20}O_3$	224.1413	15.00	0.67	0.42	0.02	0.25	0.66	0.40	0.00738	0.09694	0.99999	0.30374	0.00714	0.09340
Tentative phenolic compound	$C_{13}H_{20}O_3$	224.1413	14.54	0.43	0.30	-0.02	0.13	0.45	0.32	0.07027	0.36734	0.94591	0.62916	0.03279	0.18245
Tentative phenolic compound	$C_{13}H_{20}O_3$	224.1413	14.77	0.31	0.09	-0.25	0.21	0.56	0.34	0.27305	0.96898	0.53114	0.46466	0.03912	0.32052
Tentative phenolic compound	$C_{14}H_{10}O_3$	226.0631	25.64	0.01	0.19	-0.17	-0.17	0.19	0.36	0.79113	0.99681	0.39519	0.68223	0.87632	0.30920
Tentative phenolic compound	$C_{11}H_{14}O_5$	226.0842	15.62	-0.18	-0.18	-0.54	0.00	0.36	0.36	0.66269	0.42974	0.01255	0.97081	0.06227	0.11489
Tentative phenolic compound	$C_{13}H_{22}O_3$	226.1569	20.31	0.56	0.23	0.09	0.33	0.47	0.14	0.05318	0.48388	0.78593	0.39862	0.19740	0.94285
Tentative phenolic compound	$C_{13}H_{22}O_3$	226.1569	17.06	0.30	0.17	-0.03	0.13	0.33	0.20	0.08022	0.70509	1.00000	0.34909	0.08247	0.71582
Tentative phenolic compound	$C_{13}H_{22}O_3$	226.1569	16.65	0.42	0.11	-0.13	0.31	0.55	0.24	0.07515	0.80577	0.73180	0.25919	0.01770	0.28511
Tentative phenolic compound	$C_{13}H_{22}O_3$	226.1571	18.86	0.27	-0.03	-0.19	0.29	0.45	0.16	0.39090	0.97886	0.16703	0.24093	0.01609	0.27971
Tentative phenolic compound	$C_{14}H_{12}O_3$	228.0787	11.11	0.11	0.29	-0.05	-0.19	0.15	0.34	0.97297	0.50944	0.49502	0.31289	0.72772	0.07500
Tentative phenolic compound	$C_{14}H_{12}O_3$	228.0788	34.54	-0.30	-0.23	0.43	-0.07	-0.73	-0.66	0.30052	0.55525	0.99581	0.94673	0.22536	0.43917

Suppl. Tab. 2.1 (*continued*)

Tentative phenolic compound	$C_{14}H_{12}O_3$	228.0788	21.47	0.11	0.19	0.04	-0.08	0.07	0.15	0.98661	0.99288	0.96219	0.93026	0.99880	0.87728
Tentative phenolic compound	$C_{14}H_{12}O_3$	228.0789	24.56	0.10	0.10	0.07	0.00	0.03	0.03	0.98826	0.99919	0.99938	0.99744	0.99696	1.00000
Tentative phenolic compound	$C_{14}H_{16}O_3$	232.1101	23.30	0.67	0.34	0.06	0.34	0.62	0.28	0.01539	0.26874	0.90869	0.24019	0.03801	0.57378
Tentative phenolic compound	$C_{15}H_{22}O_2$	234.1620	17.32	0.64	0.36	0.23	0.28	0.41	0.13	0.04943	0.62026	0.98771	0.27487	0.07775	0.79499
Tentative phenolic compound	$C_{15}H_{22}O_2$	234.1620	30.01	0.72	0.52	0.18	0.20	0.54	0.34	0.11901	0.66974	0.96928	0.51476	0.21832	0.89001
Tentative phenolic compound	$C_{15}H_{22}O_2$	234.1621	37.41	-0.23	-0.10	0.01	-0.14	-0.24	-0.10	0.08108	0.72754	0.99550	0.33515	0.05879	0.60087
Tentative phenolic compound	$C_{15}H_{22}O_2$	234.1621	29.82	-0.05	0.18	-0.14	-0.23	0.08	0.32	0.44069	0.94710	0.45438	0.22670	0.99999	0.23511
Tentative phenolic compound	$C_{15}H_{22}O_2$	234.1621	26.72	0.10	-0.22	-0.23	0.32	0.33	0.01	0.95807	0.32226	0.69060	0.16826	0.42480	0.88372
Tentative phenolic compound	$C_{13}H_{16}O_4$	236.1050	21.55	0.32	0.37	-0.03	-0.05	0.35	0.40	0.19458	0.16797	0.82403	0.99951	0.05838	0.05013
Tentative phenolic compound	$C_{15}H_{12}O_3$	240.0788	13.39	0.00	0.44	0.00	-0.44	0.00	0.44	0.95008	0.35168	0.98914	0.17744	0.99596	0.23817
Tentative phenolic compound	$C_{15}H_{28}O_2$	240.2090	30.55	1.04	0.42	-0.26	0.62	1.30	0.68	0.00034	0.12844	0.75883	0.00525	0.00014	0.03193
Tentative phenolic compound	$C_{15}H_{28}O_2$	240.2090	28.08	0.68	-0.14	-0.70	0.82	1.38	0.56	0.03461	0.65639	0.02755	0.00721	0.00047	0.14358
Tentative phenolic compound	$C_{14}H_{10}O_4$	242.0581	17.42	0.20	0.34	0.01	-0.14	0.19	0.33	0.59189	0.45082	0.99515	0.99296	0.46675	0.34342
Tentative phenolic compound	$C_{11}H_{14}O_6$	242.0792	11.04	0.08	-0.09	-0.48	0.18	0.57	0.39	0.95852	0.60579	0.02238	0.35645	0.01158	0.13186
Tentative phenolic compound	$C_{11}H_{14}O_6$	242.0792	10.71	-0.03	-0.13	-0.60	0.10	0.57	0.48	1.00000	0.69379	0.02273	0.68201	0.02208	0.10745
Tentative phenolic compound	$C_{15}H_{14}O_3$	242.0944	31.69	0.20	0.09	-0.29	0.10	0.48	0.38	0.86482	0.79676	0.52567	0.99882	0.20673	0.16983
Tentative phenolic compound	$C_{15}H_{14}O_3$	242.0944	31.90	-0.12	0.12	0.00	-0.24	-0.12	0.12	0.70383	0.99945	0.76777	0.76455	0.99935	0.82425
Tentative phenolic compound	$C_{15}H_{14}O_3$	242.0945	30.65	-0.14	-0.03	-0.07	-0.11	-0.07	0.03	0.33228	0.92598	0.69711	0.63929	0.88892	0.95823
Tentative phenolic compound	$C_{15}H_{14}O_3$	242.0945	30.17	-0.50	-0.07	-0.12	-0.43	-0.38	0.05	0.12836	0.77649	0.58982	0.44160	0.62300	0.98557
Tentative phenolic compound	$C_{15}H_{14}O_3$	242.0945	28.56	0.29	0.21	-0.09	0.08	0.37	0.30	0.79614	0.99512	0.87608	0.90017	0.39949	0.76463
Tentative phenolic compound	$C_{14}H_{14}O_4$	246.0893	11.11	0.10	0.28	-0.09	-0.18	0.19	0.37	0.99941	0.40108	0.48229	0.34983	0.54346	0.05335
Tentative phenolic compound	$C_{15}H_{18}O_3$	246.1257	31.47	-0.30	-0.13	-0.05	-0.18	-0.25	-0.08	0.39580	0.94476	0.82184	0.68560	0.85009	0.98790
Tentative phenolic compound	$C_{15}H_{18}O_3$	246.1258	21.73	0.08	0.15	-0.07	-0.07	0.15	0.22	0.97910	0.98969	0.87493	0.89877	0.98258	0.72667
Tentative phenolic compound	$C_{15}H_{18}O_3$	246.1258	27.41	-0.09	0.05	0.11	-0.14	-0.20	-0.06	0.34865	0.89833	0.95190	0.70539	0.61081	0.99813
Tentative phenolic compound	$C_{14}H_{16}O_4$	248.1050	17.69	0.20	-0.15	-0.37	0.35	0.57	0.22	0.68992	0.90574	0.39094	0.34532	0.08825	0.74675
Tentative phenolic compound	$C_{14}H_{16}O_4$	248.1050	21.07	0.07	-0.08	-0.30	0.15	0.36	0.21	0.86981	0.94613	0.44040	0.58628	0.16787	0.73422
Tentative phenolic compound	$C_{14}H_{16}O_4$	248.1050	21.94	0.35	0.05	-0.45	0.30	0.80	0.50	0.19770	0.99963	0.08798	0.22545	0.00451	0.07659
Tentative phenolic compound	$C_{14}H_{16}O_4$	248.1050	17.85	0.39	-0.28	-0.29	0.67	0.68	0.01	0.35218	0.70713	0.41028	0.08148	0.03730	0.94259
Tentative phenolic compound	$C_{14}H_{16}O_4$	248.1050	18.28	0.26	-0.12	-0.57	0.38	0.83	0.45	0.47242	0.90055	0.06546	0.20324	0.00836	0.17049
Tentative phenolic compound	$C_{14}H_{16}O_4$	248.1050	17.54	0.02	-0.31	-0.42	0.33	0.44	0.11	0.90406	0.46739	0.23433	0.20314	0.09264	0.93924
Tentative phenolic compound	$C_{15}H_{20}O_3$	248.1414	27.24	0.49	0.12	-0.01	0.37	0.51	0.13	0.10937	0.94811	0.99732	0.22780	0.14280	0.98504
Tentative phenolic compound	$C_{15}H_{12}O_4$	256.0736	28.02	-0.27	-0.31	-0.78	0.03	0.50	0.47	0.15390	0.06381	0.00190	0.92047	0.04034	0.09762
Tentative phenolic compound	$C_{14}H_{10}O_5$	258.0530	14.20	0.18	0.39	0.00	-0.21	0.19	0.40	0.94513	0.46580	0.91843	0.76333	0.65545	0.21366
Tentative phenolic compound	$C_{15}H_{14}O_4$	258.0895	25.03	0.13	0.10	0.03	0.03	0.10	0.07	0.98900	0.80355	0.95755	0.93085	0.99745	0.97575
Tentative phenolic compound	$C_{14}H_{12}O_5$	260.0686	20.65	0.33	0.22	0.13	0.12	0.21	0.09	0.19417	0.87486	0.90996	0.48849	0.44238	0.99971
Tentative phenolic compound	$C_{14}H_{16}O_5$	264.0998	11.11	0.06	0.26	-0.07	-0.21	0.13	0.34	0.90295	0.63521	0.47002	0.30306	0.83313	0.09676

Suppl. Tab. 2.1 (*continued*)

Tentative phenolic compound	$C_{15}H_{20}O_4$	264.1363	13.31	-0.11	0.18	0.07	-0.29	-0.18	0.11	0.30721	0.93160	0.96391	0.13946	0.52614	0.72093
Tentative phenolic compound	$C_{15}H_{20}O_4$	264.1363	13.63	-0.15	0.27	-0.04	-0.42	-0.11	0.31	0.17987	0.86750	0.57634	0.06158	0.77070	0.23585
Tentative phenolic compound	$C_{15}H_{12}O_5$	272.0685	24.01	0.13	0.08	-0.02	0.05	0.15	0.10	0.85919	0.98306	0.90057	0.97230	0.49638	0.73117
Tentative phenolic compound	$C_{15}H_{12}O_5$	272.0685	21.56	0.17	0.36	0.02	-0.20	0.14	0.34	0.94164	0.49797	0.99394	0.80177	0.84804	0.37520
Tentative phenolic compound	$C_{15}H_{12}O_5$	272.0685	20.92	0.41	0.48	0.00	-0.07	0.41	0.48	0.27520	0.32606	0.97582	0.99905	0.15995	0.19220
Tentative phenolic compound	$C_{15}H_{12}O_5$	272.0686	26.63	-0.51	-0.06	-0.18	-0.45	-0.33	0.12	0.00862	0.61552	0.22611	0.04662	0.15788	0.82092
Tentative phenolic compound	$C_{16}H_{16}O_4$	272.1049	13.40	0.00	0.43	0.00	-0.44	-0.01	0.43	0.94244	0.36558	0.99089	0.17837	0.99290	0.25435
Tentative phenolic compound	$C_{15}H_{14}O_5$	274.0842	27.20	0.10	0.23	0.25	-0.12	-0.15	-0.03	0.94852	0.70793	0.89702	0.94353	0.99842	0.97821
Tentative phenolic compound	$C_{15}H_{14}O_5$	274.0842	10.89	0.10	-0.07	-0.39	0.17	0.49	0.32	0.94158	0.82478	0.04652	0.52280	0.02146	0.15579
Tentative phenolic compound	$C_{15}H_{14}O_5$	274.0842	25.60	0.26	0.35	0.12	-0.09	0.15	0.23	0.26626	0.28633	0.94589	0.99993	0.50585	0.53618
Tentative phenolic compound	$C_{14}H_{16}O_6$	280.0947	8.58	0.29	0.34	0.01	-0.05	0.28	0.33	0.07863	0.14733	1.00000	0.96824	0.07867	0.14741
Tentative phenolic compound	$C_{15}H_{24}O_5$	284.1624	20.31	-0.05	-0.04	0.14	-0.01	-0.19	-0.18	0.99385	1.00000	0.57941	0.99498	0.72046	0.58877
Tentative phenolic compound	$C_{15}H_{10}O_6$	286.0479	24.06	-0.10	-0.11	0.06	0.01	-0.16	-0.17	0.53383	0.35893	0.98806	0.98339	0.71025	0.51321
Tentative phenolic compound	$C_{15}H_{10}O_6$	286.0479	25.37	-0.03	0.09	-0.15	-0.12	0.12	0.24	0.56983	0.99895	0.60419	0.64769	0.99991	0.68230
Tentative phenolic compound	$C_{15}H_{10}O_6$	286.0479	24.71	-0.38	-0.36	-0.58	-0.03	0.20	0.23	0.34310	0.28377	0.09830	0.99864	0.78954	0.86172
Tentative phenolic compound	$C_{17}H_{18}O_4$	286.1205	21.58	0.41	0.27	-0.10	0.14	0.51	0.37	0.11663	0.73432	0.95627	0.44699	0.05744	0.46033
Tentative phenolic compound	$C_{17}H_{18}O_4$	286.1206	17.82	0.44	0.30	-0.10	0.13	0.54	0.40	0.25315	0.61518	0.96201	0.86072	0.13259	0.37047
Tentative phenolic compound	$C_{14}H_{22}O_6$	286.1418	17.51	-0.39	-0.24	-0.50	-0.15	0.11	0.26	0.29987	0.38559	0.06529	0.99665	0.69239	0.57734
Tentative phenolic compound	$C_{15}H_{12}O_6$	288.0635	13.69	-0.64	-0.34	-0.28	-0.30	-0.36	-0.06	0.00007	0.00796	0.01071	0.00660	0.00496	0.99534
Tentative phenolic compound	$C_{15}H_{14}O_7$	288.0635	17.00	-0.57	-0.24	-0.09	-0.33	-0.48	-0.15	0.00003	0.00410	0.38252	0.00273	0.00010	0.03802
Tentative phenolic compound	$C_{15}H_{12}O_6$	288.0635	16.46	0.38	0.36	0.04	0.02	0.34	0.32	0.04096	0.12155	0.85198	0.86477	0.12660	0.35205
Tentative phenolic compound	$C_{15}H_{12}O_6$	288.0635	21.49	0.31	0.26	0.17	0.05	0.14	0.09	0.77484	0.42989	0.99420	0.91610	0.88962	0.55902
Tentative phenolic compound	$C_{15}H_{12}O_6$	288.0635	21.20	-0.15	0.06	0.05	-0.21	-0.20	0.00	0.64901	0.98287	0.89540	0.83888	0.95838	0.98586
Tentative phenolic compound	$C_{15}H_{12}O_6$	288.0635	17.42	0.21	0.33	0.03	-0.12	0.18	0.30	0.70313	0.56488	0.99108	0.99421	0.54327	0.41606
Tentative phenolic compound	$C_{15}H_{14}O_6$	290.0791	16.99	-0.46	-0.18	-0.16	-0.28	-0.30	-0.02	0.01011	0.29556	0.40694	0.14077	0.09731	0.99307
Tentative phenolic compound	$C_{15}H_{14}O_6$	290.0792	13.69	-0.56	-0.24	-0.42	-0.32	-0.14	0.17	0.00053	0.09433	0.00581	0.01238	0.21351	0.24357
Tentative phenolic compound	$C_{19}H_{16}O_3$	292.1093	17.05	0.13	0.12	-0.38	0.00	0.51	0.50	0.92375	0.99353	0.18776	0.98295	0.07946	0.13173
Tentative phenolic compound	$C_{19}H_{16}O_3$	292.1093	15.54	0.48	0.36	-0.28	0.13	0.76	0.63	0.02790	0.31462	0.06901	0.35716	0.00077	0.00566
Tentative phenolic compound	$C_{11}H_{18}O_9$	294.0953	15.81	0.36	0.25	0.21	0.11	0.15	0.04	0.19587	0.63110	0.87928	0.75204	0.48629	0.96040
Tentative phenolic compound	$C_{11}H_{18}O_9$	294.0953	20.81	0.69	0.61	0.16	0.08	0.54	0.45	0.09890	0.10589	0.50328	0.99996	0.60804	0.63524
Tentative phenolic compound	$C_{19}H_{18}O_3$	294.1257	22.65	0.18	0.08	-0.28	0.10	0.46	0.36	0.50302	0.99933	0.15782	0.44185	0.02067	0.18589
Tentative phenolic compound	$C_{19}H_{18}O_3$	294.1259	23.10	-0.40	-0.62	-1.65	0.22	1.25	1.03	0.15151	0.00550	0.00000	0.14508	0.00002	0.00013
Tentative phenolic compound	$C_{12}H_{22}O_8$	294.1317	12.78	0.33	0.40	-0.01	-0.07	0.35	0.42	0.74499	0.54200	0.85765	0.98197	0.33751	0.21057
Tentative phenolic compound	$C_{12}H_{22}O_8$	294.1317	13.40	0.23	0.44	-0.04	-0.21	0.27	0.48	0.94429	0.39912	0.77126	0.69055	0.47195	0.11179
Tentative phenolic compound	$C_{16}H_{24}O_5$	296.1626	28.84	0.82	0.26	0.37	0.57	0.45	-0.12	0.00166	0.40046	0.05656	0.01250	0.09293	0.50467
Tentative phenolic compound	$C_{16}H_{24}O_5$	296.1626	20.66	0.44	0.13	0.17	0.30	0.26	-0.04	0.05195	0.66451	0.39265	0.25983	0.48196	0.95287

Suppl. Tab. 2.1 (*continued*)

Tentative phenolic compound	$C_{15}H_8O_7$	300.0270	22.61	0.08	-0.07	0.18	0.15	-0.10	-0.25	0.84104	0.55391	0.25838	0.20638	0.64858	0.03908
Tentative phenolic compound	$C_{15}H_8O_7$	300.0271	22.88	-0.02	-0.10	-0.14	0.08	0.12	0.04	0.72072	0.23020	0.06727	0.73040	0.28862	0.81178
Tentative phenolic compound	$C_{15}H_8O_7$	300.0273	22.26	0.07	0.08	0.11	0.00	-0.04	-0.04	0.99952	0.99820	0.35218	0.99992	0.39996	0.42829
Tentative phenolic compound	$C_{15}H_{10}O_7$	302.0427	16.03	-0.74	-0.53	-0.99	-0.21	0.25	0.46	0.01742	0.06519	0.00359	0.77899	0.61919	0.20396
Tentative phenolic compound	$C_{15}H_{10}O_7$	302.0428	21.02	0.23	0.21	0.00	0.02	0.23	0.21	0.78598	0.98966	0.99924	0.91744	0.71946	0.97209
Tentative phenolic compound	$C_{15}H_{10}O_7$	302.0428	22.25	0.17	0.11	0.21	0.06	-0.04	-0.09	0.69828	0.87915	0.69676	0.98234	1.00000	0.98195
Tentative phenolic compound	$C_{15}H_{10}O_7$	302.0428	22.61	0.21	0.04	-0.03	0.17	0.24	0.07	0.90281	0.98983	0.94512	0.76546	0.63066	0.99441
Tentative phenolic compound	$C_{15}H_{10}O_7$	302.0428	22.86	-0.10	-0.16	-0.35	0.06	0.24	0.19	0.90285	0.58715	0.11951	0.92156	0.29740	0.59711
Tentative phenolic compound	$C_{15}H_{10}O_7$	302.0428	20.54	0.00	0.00	-0.66	0.00	0.66	0.66	0.96115	0.77252	0.02125	0.96067	0.04130	0.08162
Tentative phenolic compound	$C_{15}H_{10}O_7$	302.0428	20.71	-0.43	-0.46	-1.10	0.04	0.67	0.63	0.01775	0.01084	0.00021	0.98114	0.01706	0.02833
Tentative phenolic compound	$C_{15}H_{12}O_7$	304.0584	14.20	0.17	0.31	-0.06	-0.13	0.23	0.36	0.99148	0.85568	0.68383	0.95435	0.52690	0.29204
Tentative phenolic compound	$C_{15}H_{12}O_7$	304.0584	18.95	0.07	-0.13	-0.14	0.21	0.21	0.00	0.99961	0.94381	0.72344	0.96771	0.77690	0.95542
Tentative phenolic compound	$C_{15}H_{12}O_7$	304.0584	13.77	0.23	0.29	-0.17	-0.07	0.40	0.47	0.64956	0.63470	0.56195	0.99999	0.12782	0.12318
Tentative phenolic compound	$C_{15}H_{12}O_7$	304.0584	12.70	-0.67	0.20	-0.05	-0.87	-0.62	0.25	0.02095	1.00000	0.76432	0.02070	0.08225	0.75964
Tentative phenolic compound	$C_{15}H_{12}O_7$	304.0585	23.37	-0.14	-0.43	-0.21	0.29	0.07	-0.22	0.95684	0.30849	0.79667	0.54483	0.97389	0.77467
Tentative phenolic compound	$C_{15}H_{14}O_7$	306.0741	8.17	-0.38	0.07	-0.11	-0.45	-0.27	0.17	0.01163	0.73099	0.22217	0.04820	0.21993	0.70542
Tentative phenolic compound	$C_{15}H_{14}O_7$	306.0741	12.71	-0.65	0.07	-0.04	-0.72	-0.61	0.11	0.00736	0.99144	0.50252	0.01059	0.05240	0.65853
Tentative phenolic compound	$C_{13}H_{12}O_9$	312.0482	15.23	0.38	0.61	-0.46	-0.23	0.83	1.06	0.70861	0.26537	0.35490	0.79904	0.08252	0.02304
Tentative phenolic compound	$C_{19}H_{20}O_4$	312.1363	19.84	0.28	0.15	0.02	0.13	0.27	0.13	0.09037	0.90871	0.88538	0.22509	0.03271	0.54165
Tentative phenolic compound	$C_{16}H_{12}O_7$	316.0586	25.87	-0.24	-0.06	-0.26	-0.17	0.02	0.19	0.24091	0.69993	0.29091	0.76902	0.99879	0.84119
Tentative phenolic compound	$C_{16}H_{12}O_7$	316.0586	24.75	-0.21	-0.12	-0.27	-0.09	0.06	0.15	0.43514	0.57439	0.26210	0.99295	0.97409	0.90121
Tentative phenolic compound	$C_{16}H_{12}O_7$	316.0586	25.05	-0.30	-0.29	-0.56	-0.01	0.26	0.27	0.25366	0.24115	0.06296	0.99998	0.74963	0.77008
Tentative phenolic compound	$C_{16}H_{12}O_7$	316.0586	24.36	-0.01	-0.02	0.00	0.00	-0.01	-0.01	0.73255	0.66050	0.96242	0.99914	0.93999	0.89642
Tentative phenolic compound	$C_{17}H_{16}O_6$	316.0949	24.38	0.07	0.30	0.08	-0.23	-0.01	0.22	0.94218	0.62484	0.85388	0.34389	0.99469	0.25373
Tentative phenolic compound	$C_{18}H_{20}O_5$	316.1312	17.14	0.45	0.15	-0.08	0.31	0.53	0.22	0.03006	0.54171	0.78260	0.20749	0.00851	0.17027
Tentative phenolic compound	$C_{18}H_{20}O_5$	316.1312	18.97	0.34	0.20	-0.25	0.14	0.59	0.45	0.11215	0.56974	0.28893	0.58937	0.00801	0.04596
Tentative phenolic compound	$C_{18}H_{20}O_5$	316.1313	18.29	0.23	0.12	-0.42	0.11	0.64	0.53	0.43499	0.94848	0.08621	0.72343	0.00975	0.04066
Tentative phenolic compound	$C_{18}H_{20}O_5$	316.1313	19.09	0.36	0.11	-0.05	0.25	0.41	0.16	0.04866	0.90888	0.81710	0.12345	0.01461	0.46127
Tentative phenolic compound	$C_{18}H_{20}O_5$	316.1314	19.56	0.13	0.11	-0.35	0.03	0.49	0.46	0.96014	0.99998	0.02413	0.96837	0.01257	0.02289
Tentative phenolic compound	$C_{15}H_{10}O_8$	318.0378	20.19	0.24	0.47	0.01	-0.23	0.23	0.46	0.94671	0.74451	0.93737	0.96196	0.69221	0.43451
Tentative phenolic compound	$C_{15}H_{10}O_8$	318.0378	20.47	0.34	0.48	-0.14	-0.15	0.48	0.63	0.80128	0.82483	0.54382	0.99996	0.18000	0.19215
Tentative phenolic compound	$C_{15}H_{10}O_8$	318.0378	21.48	-0.16	0.19	-0.22	-0.35	0.06	0.41	0.28412	0.99997	0.31920	0.30010	0.99969	0.33666
Tentative phenolic compound	$C_{15}H_{12}O_8$	320.0533	16.15	0.30	0.34	0.09	-0.04	0.21	0.25	0.30178	0.29787	0.97563	1.00000	0.48636	0.48100
Tentative phenolic compound	$C_{15}H_{12}O_8$	320.0533	19.96	-0.07	0.17	0.04	-0.25	-0.12	0.13	0.64109	0.75997	0.99647	0.20470	0.52527	0.86196
Tentative phenolic compound	$C_{15}H_{14}O_8$	322.0690	12.71	-0.56	0.21	0.00	-0.77	-0.56	0.20	0.01553	0.99919	0.55802	0.01844	0.10182	0.62904
Tentative phenolic compound	$C_{20}H_{22}O_4$	326.1517	22.65	0.17	0.08	-0.28	0.10	0.45	0.36	0.53575	0.99923	0.18585	0.46955	0.02646	0.22008

Suppl. Tab. 2.1 (*continued*)

Tentative phenolic compound	$C_{20}H_{22}O_4$	326.1518	23.10	-0.40	-0.62	-1.65	0.22	1.26	1.03	0.15507	0.00537	0.00000	0.13783	0.00002	0.00013
Tentative phenolic compound	$C_{19}H_{20}O_5$	328.1312	18.29	0.37	0.23	-0.29	0.14	0.66	0.52	0.36142	0.75599	0.80052	0.87304	0.10767	0.29787
Tentative phenolic compound	$C_{19}H_{22}O_5$	330.1468	18.16	0.33	0.30	0.27	0.03	0.06	0.02	0.07791	0.39312	0.18359	0.63793	0.92460	0.93058
Tentative phenolic compound	$C_{19}H_{22}O_5$	330.1468	24.01	-0.01	0.03	-0.36	-0.04	0.35	0.38	0.82658	0.85573	0.01964	0.99990	0.06456	0.05904
Tentative phenolic compound	$C_{19}H_{22}O_5$	330.1468	19.97	0.20	0.05	-0.28	0.15	0.48	0.34	0.55607	0.99976	0.25327	0.60340	0.03846	0.22634
Tentative phenolic compound	$C_{19}H_{22}O_5$	330.1468	19.84	0.29	0.13	-0.06	0.16	0.34	0.19	0.28320	0.99466	0.54157	0.38115	0.04183	0.41777
Tentative phenolic compound	$C_{19}H_{22}O_5$	330.1468	18.29	0.43	0.16	-0.28	0.27	0.71	0.44	0.26958	0.99955	0.57273	0.30810	0.04292	0.51563
Tentative phenolic compound	$C_{19}H_{22}O_5$	330.1468	21.58	0.35	0.21	-0.07	0.14	0.42	0.29	0.12126	0.86038	0.94689	0.34330	0.05670	0.57570
Tentative phenolic compound	$C_{15}H_{16}O_9$	340.0798	11.58	0.55	0.45	0.05	0.10	0.49	0.39	0.04036	0.23833	0.99787	0.60087	0.03155	0.18819
Tentative phenolic compound	$C_{15}H_{16}O_9$	340.0798	11.38	0.51	0.41	-0.06	0.09	0.57	0.47	0.05192	0.20509	0.93407	0.76234	0.02305	0.09129
Tentative phenolic compound	$C_{20}H_{22}O_5$	342.1469	24.01	-0.07	0.00	-0.39	-0.07	0.32	0.39	0.40355	0.93196	0.00797	0.71916	0.07571	0.01728
Tentative phenolic compound	$C_{19}H_{22}O_6$	346.1419	21.79	0.48	0.26	-0.27	0.22	0.76	0.53	0.03516	0.53909	0.31078	0.24252	0.00313	0.04608
Tentative phenolic compound	$C_{16}H_{18}O_9$	354.0952	16.96	0.09	0.25	-0.35	-0.16	0.44	0.60	0.97907	0.67706	0.26679	0.87258	0.15908	0.05518
Tentative phenolic compound	$C_{20}H_{22}O_6$	358.1418	28.31	-0.63	-0.06	-0.63	-0.56	0.00	0.56	0.00118	0.73971	0.00219	0.00380	0.94234	0.00754
Tentative phenolic compound	$C_{20}H_{22}O_6$	358.1419	25.12	0.14	0.06	-0.16	0.08	0.31	0.23	0.87286	1.00000	0.32033	0.86967	0.11675	0.32335
Tentative phenolic compound	$C_{20}H_{24}O_6$	360.1574	22.06	0.20	-0.07	-0.36	0.27	0.56	0.29	0.60477	0.99972	0.43827	0.55502	0.08194	0.48365
Tentative phenolic compound	$C_{20}H_{18}O_7$	370.1054	13.40	0.01	0.46	-0.02	-0.45	0.03	0.48	0.93443	0.35878	0.97530	0.16814	0.99798	0.21270
Tentative phenolic compound	$C_{19}H_{32}O_7$	372.2149	17.78	1.20	0.76	0.47	0.44	0.73	0.29	0.00024	0.00705	0.07312	0.05419	0.00541	0.37090
Tentative phenolic compound	$C_{17}H_{20}O_{10}$	384.1060	10.89	0.10	-0.06	-0.45	0.16	0.56	0.40	0.93433	0.94818	0.10185	0.68959	0.04474	0.21288
Tentative phenolic compound	$C_{17}H_{24}O_{10}$	388.1373	15.62	-0.12	-0.01	-0.56	-0.11	0.44	0.55	0.56543	0.96016	0.00242	0.82740	0.01267	0.00430
Tentative phenolic compound	$C_{19}H_{32}O_8$	388.2098	19.17	0.90	0.39	0.35	0.51	0.55	0.04	0.00281	0.26899	0.24974	0.03628	0.03930	0.99993
Tentative phenolic compound	$C_{19}H_{32}O_8$	388.2100	17.05	0.23	0.01	-0.24	0.22	0.47	0.25	0.22469	0.99290	0.63165	0.31649	0.04095	0.48665
Tentative phenolic compound	$C_{20}H_{22}O8$	390.1317	17.77	0.18	0.04	-0.13	0.14	0.31	0.17	0.68914	0.94295	0.17508	0.39571	0.03650	0.35885
Tentative phenolic compound	$C_{20}H_{22}O8$	390.1317	24.56	-0.01	0.04	0.12	-0.05	-0.13	-0.08	0.73681	0.95373	0.99478	0.95214	0.60378	0.87335
Tentative phenolic compound	$C_{21}H_{14}O_8$	394.0671	16.08	0.16	0.04	-0.02	0.11	0.18	0.07	0.09684	0.48660	0.99652	0.61752	0.07214	0.38488
Tentative phenolic compound	$C_{23}H_{24}O_6$	396.1578	23.43	-0.06	0.02	-0.46	-0.08	0.40	0.48	0.31833	0.89693	0.00619	0.66626	0.07540	0.01515
Tentative phenolic compound	$C_{21}H_{22}O_8$	402.1318	28.01	-0.33	-0.30	-0.83	-0.02	0.51	0.53	0.27174	0.18892	0.00862	0.99219	0.12996	0.18968
Tentative phenolic compound	$C_{17}H_{24}O_{11}$	404.1322	9.68	-0.06	0.05	-0.83	-0.11	0.77	0.88	0.99981	0.62152	0.71625	0.66551	0.75862	0.99811
Tentative phenolic compound	$C_{24}H_{30}O_6$	414.2044	35.45	0.79	0.22	-0.15	0.57	0.94	0.36	0.21347	0.49099	0.81692	0.90081	0.59599	0.92848
Tentative phenolic compound	$C_{19}H_{28}O_{10}$	416.1687	17.21	0.55	0.13	-0.03	0.41	0.58	0.17	0.00608	0.93248	0.99883	0.01296	0.00508	0.88074
Tentative phenolic compound	$C_{20}H_{18}O_{10}$	418.0904	25.64	-0.79	-0.52	-0.76	-0.27	-0.03	0.24	0.01332	0.08041	0.03454	0.58656	0.89380	0.92896
Kaempferol-pentoside	$C_{20}H_{18}O_{10}$	418.0905	26.01	-0.82	-0.60	-0.80	-0.23	-0.02	0.21	0.02672	0.07941	0.03012	0.86247	0.99974	0.89777
Tentative phenolic compound	$C_{22}H_{26}O_8$	418.1629	21.52	0.65	-0.07	-0.27	0.72	0.91	0.20	0.01684	0.95596	0.37251	0.00869	0.00200	0.63243
Tentative phenolic compound	$C_{25}H_{26}O_6$	422.1725	17.05	0.36	0.22	-0.50	0.14	0.85	0.72	0.54316	0.79506	0.57042	0.96564	0.09984	0.18906
Tentative phenolic compound	$C_{22}H_{30}O_8$	422.1944	23.92	0.52	0.10	-0.42	0.41	0.93	0.52	0.08967	0.99630	0.30619	0.12099	0.00694	0.23264
Tentative phenolic compound	$C_{20}H_{24}O_{10}$	424.1348	16.20	0.38	0.08	-0.03	0.30	0.41	0.11	0.00600	0.83228	0.97475	0.01795	0.01014	0.97132

Suppl. Tab. 2.1 (*continued*)

Tentative phenolic compound	$C_{20}H_{24}O_{10}$	424.1349	15.64	0.31	0.27	0.03	0.04	0.28	0.24	0.03334	0.09501	0.77861	0.87652	0.12728	0.34190
Tentative phenolic compound	$C_{20}H_{24}O_{10}$	424.1349	15.81	0.28	0.21	0.02	0.06	0.26	0.20	0.12510	0.34015	0.99848	0.87314	0.10028	0.27923
Tentative phenolic compound	$C_{20}H_{24}O_{10}$	424.1349	14.76	0.40	0.20	-0.23	0.19	0.63	0.44	0.12908	0.76283	0.39405	0.45661	0.01268	0.10763
Keampferol-rhamnoside	$C_{21}H_{20}O_{10}$	432.1058	24.06	-0.05	0.00	0.09	-0.05	-0.14	-0.09	0.97113	0.99757	0.91403	0.99421	0.99629	0.96546
Quercetin-pentoside	$C_{20}H_{18}O_{11}$	434.0854	24.12	-0.17	-0.03	-0.39	-0.14	0.22	0.36	0.48084	0.78110	0.43660	0.94383	0.99974	0.91667
Tentative phenolic compound	$C_{21}H_{22}O_{10}$	434.1192	28.65	-0.29	0.10	0.09	-0.39	-0.38	0.01	0.09752	0.99997	0.99794	0.09181	0.07620	0.99911
Tentative phenolic compound	$C_{21}H_{22}O_{10}$	434.1193	21.24	0.10	0.13	-0.20	-0.03	0.31	0.34	0.97379	0.99999	0.37223	0.96923	0.58438	0.36149
Tentative phenolic compound	$C_{21}H_{22}O_{10}$	434.1214	21.56	0.43	0.45	0.09	-0.03	0.33	0.36	0.01713	0.00828	0.67353	0.94261	0.08430	0.03858
Tentative phenolic compound	$C_{21}H_{22}O_{10}$	434.1215	24.01	0.18	0.11	-0.03	0.07	0.21	0.14	0.45846	0.97635	0.89086	0.67771	0.18924	0.69275
Tentative phenolic compound	$C_{23}H_{20}O_{9}$	440.1089	15.64	0.47	0.25	0.13	0.22	0.34	0.12	0.00584	0.20115	0.56778	0.11609	0.03415	0.82011
Tentative phenolic compound	$C_{23}H_{20}O_{9}$	440.1089	15.81	0.23	0.12	0.13	0.11	0.10	-0.01	0.25494	0.94647	0.87499	0.48715	0.59949	0.99658
Tentative phenolic compound	$C_{22}H_{18}O_{10}$	442.0905	21.78	-0.33	-0.02	-0.15	-0.30	-0.18	0.13	0.06836	0.63458	0.52281	0.35466	0.44867	0.99681
Quercetin-rhamnoside	$C_{21}H_{20}O_{11}$	448.1007	22.24	0.25	0.23	0.15	0.02	0.10	0.08	0.49512	0.58676	0.89412	0.99813	0.86553	0.92881
Kaempferol-hexoside	$C_{21}H_{20}O_{11}$	448.1010	24.70	-0.37	-0.44	-0.54	0.07	0.17	0.10	0.26314	0.13835	0.07814	0.96218	0.81361	0.97576
Kaempferol-hexoside	$C_{21}H_{20}O_{11}$	448.1010	24.05	-0.21	-0.26	-0.14	0.05	-0.07	-0.12	0.26285	0.11059	0.56094	0.91745	0.91088	0.59270
Kaempferol-hexoside	$C_{23}H_{28}O_{9}$	448.1714	21.73	0.14	0.27	-0.15	-0.13	0.29	0.42	0.99669	0.97279	0.68349	0.91984	0.79360	0.45337
Tentative phenolic compound	$C_{21}H_{22}O_{11}$	450.1164	21.49	0.48	0.37	0.11	0.11	0.37	0.26	0.10935	0.33778	0.83413	0.83296	0.33674	0.77272
Tentative phenolic compound	$C_{21}H_{22}O_{11}$	450.1167	23.37	-0.08	-0.46	-0.17	0.38	0.09	-0.29	0.92169	0.18933	0.62018	0.41672	0.92332	0.74904
Tentative phenolic compound	$C_{24}H_{20}O_{9}$	452.1110	26.85	0.11	0.22	0.06	-0.11	0.04	0.16	0.99973	0.81976	0.99924	0.77529	0.99627	0.87610
Tentative phenolic compound	$C_{24}H_{20}O_{9}$	452.1110	27.12	0.19	0.27	0.05	-0.08	0.14	0.22	0.93977	0.72493	0.99928	0.95945	0.96934	0.78992
Tentative phenolic compound	$C_{24}H_{20}O_{9}$	452.1111	23.88	-0.03	0.19	-0.08	-0.22	0.05	0.27	0.79351	0.57874	0.83176	0.19216	0.99981	0.21345
Tentative phenolic compound	$C_{28}H_{22}O_{6}$	454.1418	29.79	-0.07	0.15	0.57	-0.22	-0.64	-0.42	0.82090	0.97073	0.47126	0.97099	0.91305	0.70922
Tentative phenolic compound	$C_{28}H_{22}O_{6}$	454.1418	30.66	0.00	0.84	-0.14	-0.83	0.14	0.97	0.86502	0.00358	0.99264	0.00933	0.95618	0.00496
Tentative phenolic compound	$C_{25}H_{26}O_{8}$	454.1632	27.44	1.54	1.33	0.58	0.21	0.96	0.75	0.00003	0.00010	0.00655	0.31827	0.00162	0.01554
Tentative phenolic compound	$C_{23}H_{20}O_{10}$	456.1035	20.92	0.34	0.33	0.15	0.02	0.20	0.18	0.08021	0.12133	0.83268	0.99047	0.25600	0.36973
Tentative phenolic compound	$C_{23}H_{20}O_{10}$	456.1035	21.56	0.42	0.45	0.16	-0.03	0.26	0.29	0.00398	0.00169	0.31094	0.87907	0.04657	0.01685
Tentative phenolic compound	$C_{21}H_{24}O_{10}$	458.1192	25.60	-0.02	0.03	-0.17	-0.05	0.15	0.20	0.99095	0.91749	0.40846	0.79294	0.55682	0.18178
Tentative phenolic compound	$C_{23}H_{24}O_{10}$	460.1372	25.98	0.19	0.10	-0.40	0.09	0.59	0.51	0.67522	0.98379	0.18650	0.85695	0.03761	0.11443
Kaempferol-glucuronide	$C_{21}H_{18}O_{12}$	462.0802	25.36	-0.26	-0.03	-0.16	-0.23	-0.10	0.12	0.10000	0.96262	0.58282	0.19302	0.53166	0.83701
Quercetin-hexoside	$C_{21}H_{20}O_{12}$	464.0959	22.60	0.23	0.08	0.01	0.15	0.22	0.07	0.70258	0.99914	0.96402	0.62967	0.44841	0.98579
Quercetin-hexoside	$C_{21}H_{20}O_{12}$	464.0960	22.86	0.02	-0.04	-0.17	0.06	0.19	0.13	0.99842	0.93066	0.43440	0.97057	0.51621	0.75628
Tentative phenolic compound	$C_{22}H_{24}O_{11}$	464.1299	27.85	0.05	0.21	0.15	-0.16	-0.10	0.06	0.99994	0.97213	0.97251	0.98100	0.98130	1.00000
Quercetin-hexoside	$C_{23}H_{28}O_{10}$	464.1664	16.57	-0.12	0.03	-0.03	-0.15	-0.09	0.06	0.73877	0.99775	0.85327	0.63907	0.99539	0.76515
Tentative phenolic compound	$C_{21}H_{24}O_{12}$	468.1249	17.16	-0.17	-0.03	0.12	-0.14	-0.29	-0.15	0.31134	0.87001	0.99928	0.69367	0.26626	0.81370
Tentative phenolic compound	$C_{21}H_{24}O_{12}$	468.1249	18.05	-0.23	-0.38	-0.05	0.15	-0.18	-0.33	0.20638	0.07326	0.95549	0.87643	0.39150	0.14892
Tentative phenolic compound	$C_{21}H_{24}O_{12}$	468.1249	17.89	-0.30	-0.14	-0.02	-0.17	-0.28	-0.12	0.16704	0.63272	0.99781	0.68651	0.21269	0.73175

Suppl. Tab. 2.1 (*continued*)

Quercetin-glucuronide	$C_{21}H_{18}O_{13}$	478.0752	23.60	-0.01	0.14	0.07	-0.15	-0.08	0.06	0.53063	0.98180	0.94315	0.73432	0.82904	0.99756
Quercetin-glucuronide	$C_{22}H_{22}O_{12}$	478.1115	24.74	-0.14	-0.21	-0.44	0.07	0.30	0.23	0.66769	0.27320	0.10312	0.84520	0.46355	0.88814
Isorhamnetin-hexoside	$C_{22}H_{22}O_{12}$	478.1117	25.05	-0.18	-0.17	-0.41	-0.01	0.23	0.23	0.35265	0.22886	0.07235	0.98547	0.65973	0.83845
Myricetin-hexoside	$C_{21}H_{20}O_{13}$	480.0909	20.47	0.30	0.41	-0.11	-0.11	0.41	0.52	0.68750	0.71667	0.63837	0.99994	0.16977	0.18245
Myricetin-hexoside	$C_{21}H_{20}O_{13}$	480.0909	20.19	0.29	0.42	0.08	-0.13	0.21	0.34	0.84218	0.70058	0.99891	0.99218	0.77266	0.62170
Myricetin-hexoside	$C_{21}H_{20}O_{13}$	480.0910	20.73	0.10	-0.13	-0.47	0.22	0.57	0.35	0.97851	0.99631	0.35421	0.99786	0.54609	0.45323
Tentative phenolic compound	$C_{24}H_{18}O_{11}$	482.0848	20.69	-0.09	-0.03	-0.97	-0.06	0.88	0.94	0.95947	0.92065	0.00514	0.99898	0.00952	0.01139
Tentative phenolic compound	$C_{21}H_{24}O_{13}$	484.1195	17.80	0.20	0.13	0.02	0.07	0.18	0.11	0.98453	0.99763	0.92582	0.99829	0.99311	0.97211
Isorhamnetin-glucuronide	$C_{22}H_{20}O_{13}$	492.0909	25.87	-0.09	-0.10	-0.34	0.01	0.24	0.24	0.36523	0.54868	0.08063	0.98161	0.68628	0.48371
Quercetin-metyl-glucuronide	$C_{22}H_{20}O_{13}$	492.0909	25.58	0.95	0.22	-0.41	0.73	1.37	0.64	0.01434	0.84377	0.16432	0.04406	0.00088	0.05201
Myricetin-glucuronide	$C_{21}H_{18}O_{14}$	494.0700	19.62	0.35	0.44	-0.20	-0.09	0.56	0.65	0.98643	0.80605	0.99193	0.62637	0.92609	0.92251
Myricetin-glucuronide	$C_{21}H_{18}O_{14}$	494.0701	21.48	-0.03	0.28	-0.18	-0.31	0.15	0.46	0.67056	0.97873	0.40959	0.45945	0.95902	0.25365
Tentative phenolic compound	$C_{25}H_{28}O_{11}$	504.1611	18.12	0.24	0.28	-0.35	-0.05	0.59	0.63	0.50884	0.26146	0.17685	0.94041	0.02349	0.01107
Tentative phenolic compound	$C_{24}H_{30}O_{12}$	510.1719	14.33	0.19	0.32	-0.09	-0.13	0.28	0.41	1.00000	0.78946	0.64313	0.77971	0.65378	0.22191
Tentative phenolic compound	$C_{27}H_{46}O_{9}$	514.3146	36.63	0.95	1.10	0.46	-0.15	0.49	0.63	0.00125	0.00111	0.13701	0.99943	0.02678	0.02292
Tentative phenolic compound	$C_{25}H_{40}O_{11}$	516.2576	20.24	0.57	0.46	0.07	0.11	0.50	0.39	0.05343	0.17938	0.99452	0.82265	0.07534	0.24835
Tentative phenolic compound	$C_{24}H_{30}O_{13}$	526.1668	12.94	0.17	0.35	0.01	-0.18	0.16	0.34	0.99268	0.99971	0.86473	0.98300	0.95592	0.82349
Tentative phenolic compound	$C_{27}H_{30}O_{11}$	530.1766	18.97	0.41	0.25	-0.20	0.16	0.62	0.45	0.01541	0.22073	0.19970	0.29208	0.00109	0.01052
Tentative phenolic compound	$C_{27}H_{30}O_{11}$	530.1771	19.55	0.11	0.11	-0.44	0.00	0.55	0.55	0.99860	1.00000	0.02697	0.99868	0.02186	0.02686
Luteolin-malonyl-glucoside	$C_{24}H_{22}O_{14}$	534.1016	26.16	-0.42	-0.32	0.26	-0.10	-0.68	-0.58	0.00251	0.00942	0.24519	0.70957	0.00030	0.00086
Luteolin-malonyl-glucoside	$C_{24}H_{22}O_{14}$	534.1016	26.68	-0.36	-0.30	0.14	-0.07	-0.50	-0.44	0.00504	0.01153	0.81925	0.91172	0.00181	0.00386
Tentative phenolic compound	$C_{26}H_{30}O_{12}$	534.1760	21.62	0.60	0.31	0.27	0.29	0.33	0.04	0.00008	0.00830	0.01271	0.00852	0.00563	0.98685
Tentative phenolic compound	$C_{26}H_{32}O_{12}$	536.1875	26.50	0.52	0.11	0.03	0.41	0.49	0.08	0.28835	0.99995	0.99918	0.26995	0.33839	0.99776
Tentative phenolic compound	$C_{27}H_{32}O_{12}$	548.1874	18.29	0.18	0.11	-0.40	0.07	0.58	0.51	0.82794	0.97318	0.07369	0.97126	0.02239	0.04070
Tentative phenolic compound	$C_{27}H_{32}O_{12}$	548.1874	18.67	0.31	0.18	-0.31	0.12	0.61	0.49	0.24343	0.86117	0.39720	0.59787	0.02396	0.14414
Tentative phenolic compound	$C_{27}H_{32}O_{12}$	548.1875	19.09	0.27	0.12	-0.11	0.15	0.39	0.24	0.47959	0.96513	0.46946	0.73343	0.06452	0.26972
Tentative phenolic compound	$C_{27}H_{32}O_{12}$	548.1875	18.97	0.30	0.19	-0.23	0.11	0.53	0.42	0.05608	0.55296	0.11230	0.35755	0.00192	0.01684
Quercetin-glucuronide	$C_{24}H_{22}O_{15}$	550.0963	24.55	-0.30	-0.21	-0.38	-0.09	0.08	0.17	0.08035	0.12220	0.11314	0.99010	0.99456	0.99993
Tentative phenolic compound	$C_{27}H_{28}O_{13}$	560.1513	24.37	-0.39	-0.52	-0.97	0.13	0.58	0.44	0.63386	0.21194	0.10372	0.78027	0.49681	0.95236
Tentative phenolic compound	$C_{28}H_{34}O_{12}$	562.2031	19.47	0.26	0.21	-0.35	0.05	0.61	0.56	0.53148	0.89055	0.29590	0.89695	0.04277	0.11358
Tentative phenolic compound	$C_{28}H_{34}O_{12}$	562.2031	17.81	0.33	0.24	-0.06	0.09	0.40	0.30	0.17889	0.35053	0.81379	0.95230	0.05192	0.10789
Tentative phenolic compound	$C_{28}H_{34}O_{12}$	562.2031	18.16	0.58	0.40	0.20	0.17	0.38	0.21	0.03721	0.17294	0.69916	0.70176	0.17405	0.63417
Tentative phenolic compound	$C_{28}H_{34}O_{12}$	562.2032	18.29	0.34	0.13	-0.30	0.20	0.64	0.43	0.41319	0.98038	0.38573	0.61158	0.04186	0.24049
Tentative phenolic compound	$C_{28}H_{34}O_{12}$	562.2034	19.98	0.07	-0.19	-0.43	0.26	0.50	0.24	0.76975	0.99436	0.27615	0.63482	0.07290	0.37437
Tentative phenolic compound	$C_{28}H_{34}O_{12}$	562.2034	19.84	0.31	0.16	-0.02	0.15	0.33	0.18	0.19562	0.84213	0.69696	0.53102	0.04173	0.28969
Tentative phenolic compound	$C_{27}H_{32}O_{13}$	564.1823	17.98	0.03	-0.11	-0.61	0.13	0.64	0.51	0.93388	0.99974	0.10441	0.90482	0.04576	0.11797

Suppl. Tab. 2.1 (*continued*)

Tentative phenolic compound	$C_{27}H_{32}O_{13}$	564.1823	16.98	-0.11	-0.02	-0.58	-0.09	0.47	0.56	0.92218	0.99845	0.11235	0.86108	0.26187	0.08983
Tentative phenolic compound	$C_{27}H_{32}O_{13}$	564.1847	28.02	-0.30	-0.34	-0.75	0.04	0.44	0.40	0.09790	0.04922	0.00294	0.95977	0.10861	0.21249
Tentative phenolic compound	$C_{29}H_{34}O_{12}$	574.2031	18.27	0.16	-0.21	-0.69	0.37	0.85	0.48	0.63595	0.58176	0.00567	0.12991	0.00138	0.03195
Tentative phenolic compound	$C_{29}H_{34}O_{12}$	574.2032	22.07	0.58	0.02	-0.23	0.56	0.81	0.25	0.17730	0.98395	0.51460	0.28263	0.02390	0.34545
Kaempferol-coumaryl-conjugate	$C_{30}H_{26}O_{12}$	578.1428	16.76	0.01	0.20	-0.12	-0.20	0.13	0.32	0.46988	0.97254	0.44714	0.28341	0.99996	0.26745
Kaempferol-coumaryl-conjugate	$C_{30}H_{26}O_{12}$	578.1428	19.47	-0.08	0.09	-0.06	-0.17	-0.02	0.15	0.23581	0.97931	0.33040	0.38215	0.99304	0.51259
Kaempferol-coumaryl-conjugate	$C_{30}H_{26}O_{12}$	578.1430	22.09	-0.14	0.07	-0.18	-0.21	0.04	0.25	0.43213	0.99353	0.68999	0.31817	0.96177	0.54664
Kaempferol-coumaryl-conjugate	$C_{30}H_{26}O_{12}$	578.1432	13.28	-0.28	0.08	-0.37	-0.35	0.09	0.44	0.63224	0.99877	0.08764	0.71404	0.43755	0.10777
Kaempferol-rutinoside	$C_{30}H_{26}O_{13}$	594.1379	16.69	-0.33	0.07	-0.26	-0.40	-0.06	0.33	0.03276	0.99833	0.10044	0.02616	0.85414	0.07983
Kaempferol-rutinoside	$C_{30}H_{26}O_{13}$	594.1380	14.29	-0.11	0.29	-0.15	-0.40	0.04	0.45	0.51211	0.95908	0.51538	0.28917	1.00000	0.29143
Kaempferol-rutinoside	$C_{30}H_{26}O_{13}$	594.1380	11.69	-0.06	0.20	-0.11	-0.26	0.05	0.31	0.65131	0.99899	0.66774	0.72754	0.99999	0.74342
Kaempferol-rutinoside	$C_{27}H_{30}O_{15}$	594.1589	24.06	-0.08	-0.07	0.10	-0.01	-0.19	-0.18	0.47735	0.65057	0.82196	0.98808	0.16061	0.24623
Quercetin-feruloyl-conjugate	$C_{26}H_{28}O_{16}$	596.1381	21.01	0.14	0.24	0.10	-0.10	0.05	0.14	0.98514	0.93711	0.98637	0.99529	1.00000	0.99466
Quercetin-feruloyl-conjugate	$C_{26}H_{28}O_{16}$	596.1381	21.27	0.22	0.22	0.13	0.00	0.10	0.10	0.82586	0.74524	0.95593	0.99847	0.98366	0.95380
Kaempferol-di-hexoside	$C_{30}H_{26}O_{14}$	610.1329	15.90	-0.34	0.07	-0.03	-0.41	-0.31	0.10	0.06292	0.99933	0.85038	0.07456	0.19361	0.90002
Kaempferol-di-hexoside	$C_{27}H_{30}O_{16}$	610.1540	16.64	-1.65	-1.50	-1.75	-0.15	0.09	0.24	0.00017	0.00028	0.00009	0.92972	0.87082	0.55652
Kaempferol-di-hexoside	$C_{27}H_{30}O_{16}$	610.1541	17.48	-1.49	-1.36	-1.61	-0.13	0.12	0.25	0.00057	0.00085	0.00030	0.97819	0.90266	0.71614
Quercetin-rhamnoside-hexoside	$C_{27}H_{30}O_{16}$	610.1541	22.25	0.19	0.18	0.14	0.02	0.05	0.03	0.54250	0.85340	0.77917	0.93416	0.97154	0.99860
Isorhamnetin-rhamnoside-hexoside	$C_{28}H_{32}O_{16}$	624.1696	24.36	-0.05	-0.10	-0.05	0.06	0.01	-0.05	0.92233	0.53921	0.99774	0.86713	0.96952	0.63915
Quercetin-di-hexoside	$C_{27}H_{30}O_{17}$	626.1483	15.43	-1.00	-0.69	-1.27	-0.31	0.27	0.58	0.00529	0.02779	0.00144	0.60674	0.68989	0.15803
Quercetin-di-hexoside	$C_{27}H_{30}O_{17}$	626.1486	16.03	-0.75	-0.62	-1.19	-0.13	0.44	0.57	0.00711	0.01716	0.00046	0.90339	0.14208	0.05491
Myricetin-rhamnoside-hexoside	$C_{27}H_{30}O_{17}$	626.1488	20.16	0.43	0.53	0.14	-0.10	0.28	0.39	0.69833	0.64914	0.98299	0.99973	0.87724	0.83857
Quercetin-di-hexoside	$C_{27}H_{30}O_{17}$	626.1489	20.00	-0.62	-0.52	-0.97	-0.11	0.35	0.45	0.04141	0.09357	0.00745	0.93584	0.59891	0.31613
Quercetin-hexoside-glucuronide	$C_{27}H_{28}O_{18}$	640.1279	16.81	-0.60	-0.58	-0.91	-0.03	0.31	0.34	0.00381	0.00771	0.00069	0.93808	0.45532	0.22584
Quercetin-hexoside-glucuronide	$C_{27}H_{28}O_{18}$	640.1281	20.71	-0.75	-0.75	-1.24	0.00	0.50	0.50	0.00068	0.00036	0.00003	0.91902	0.03465	0.08424
Quercetin-rhamnoside-feruloyl-conjugate	$C_{30}H_{26}O_{16}$	642.1223	22.65	0.09	-0.16	-0.60	0.26	0.70	0.44	0.99679	0.54184	0.07895	0.43647	0.05926	0.48253
Quercetin-rhamnoside-feruloyl-conjugate	$C_{30}H_{26}O_{16}$	642.1226	21.65	0.27	0.32	-0.26	-0.05	0.53	0.58	0.50040	0.55365	0.18125	0.99962	0.02356	0.02699
Quercetin-rhamnoside-feruloyl-conjugate	$C_{30}H_{26}O_{16}$	642.1228	24.23	0.25	-0.22	-0.63	0.46	0.88	0.41	0.85299	0.69696	0.20645	0.29862	0.06786	0.70930
Myricetin-hexoside-glucuronide	$C_{27}H_{28}O_{19}$	656.1229	21.47	-0.10	0.13	0.07	-0.23	-0.17	0.06	0.66217	0.99106	0.98972	0.50376	0.49631	1.00000

Suppl. Tab. 2.1 (*continued*)

Kaempferol-glucuronide-feruloyl-conjugate	$C_{37}H_{30}O_{16}$	730.1537	20.06	-0.50	-0.16	-0.25	-0.35	-0.25	0.10	0.00385	0.36202	0.12147	0.03770	0.12044	0.84123
Tentative phenolic compound	$C_{39}H_{32}O_{15}$	740.1741	19.31	-0.41	0.12	-0.22	-0.53	-0.19	0.34	0.18533	0.94182	0.59430	0.08526	0.76527	0.32102
Tentative phenolic compound	$C_{39}H_{32}O_{15}$	740.1742	19.52	0.21	0.36	-0.15	-0.15	0.36	0.51	0.84849	0.39699	0.71654	0.82477	0.30835	0.09623
Tentative phenolic compound	$C_{39}H_{32}O_{15}$	740.1743	20.96	0.21	0.29	0.01	-0.09	0.19	0.28	0.96733	0.80290	0.99880	0.96721	0.92987	0.72649
Kaempferol-glucuronide-feruloyl-conjugate	$C_{37}H_{30}O_{17}$	746.1484	18.15	-0.65	-0.10	-0.19	-0.55	-0.47	0.08	0.00617	0.41188	0.39935	0.05549	0.05762	0.99999
Kaempferol-glucuronide-feruloyl-conjugate	$C_{37}H_{30}O_{17}$	746.1484	17.97	-0.55	-0.12	-0.25	-0.43	-0.30	0.13	0.00693	0.28645	0.20078	0.09646	0.14093	0.99251
Quercetin-hexoside-rutinoside	$C_{33}H_{40}O_{21}$	772.2064	16.22	0.15	0.13	0.10	0.02	0.05	0.04	0.99010	0.96187	0.91116	0.99775	0.98340	0.99789
Kaempferol-tri-hexoside	$C_{33}H_{40}O_{21}$	772.2065	14.28	-1.79	-1.04	-1.68	-0.74	-0.11	0.63	0.00001	0.00079	0.00002	0.00371	0.76711	0.01265
Quercetin-tri-hexoside	$C_{33}H_{40}O_{22}$	788.2015	13.99	-0.45	-0.22	-0.52	-0.23	0.07	0.30	0.01648	0.21141	0.01314	0.32376	0.99810	0.26094
Quercetin-di-hexoside-glucuronide	$C_{33}H_{38}O_{23}$	802.1807	14.81	-0.64	-0.44	-0.86	-0.20	0.22	0.42	0.00133	0.01031	0.00037	0.37510	0.61088	0.06879

Suppl. Tab. 2.2: Tentative unregulated phenolic compounds in wine of *Vitis vinifer*a L. *cv.* Regent during the experimental year 2016 by using UHPLC-ESI-MS in positive ionisation mode. Relative ratios of the generated Log^2 fold changes and p-values of the three different N - forms (CaN, AM, UR) and a control are shown. Significance: the Log^2 fold changes (significance = ≥ +1 / ≥ -1) and the p-values ($p \leq 0.05$); ANOVA (pooled samples n = 3).

Metabolite annotation	Formula	Molecular Weight [*m/z*]	RT [min]	Log^2 Fold					*p*-value					
				CaN / control	AM / control	UR / control	CaN / AM	CaN / UR	AM / UR	CaN / control	AM / control	UR / control	CaN / AM	CaN /UR	AM / UR
Tentative phenolic compound	$C_{32}H_{30}O_{14}$	638.1627	22.48	0.20	0.23	-0.71	-0.03	0.91	0.94	0.00738	0.00104	6.71E-07	0.38871	7.7E-08	4.2E-08
Tentative phenolic compound	$C_{13}H_{20}O_5$	256.1306	17.67	-0.06	0.00	0.06	-0.06	-0.12	-0.06	0.05470	0.99349	0.22384	0.03808	0.00336	0.31233
Tentative phenolic compound	$C_{10}H_8O_4$	192.0421	22.25	0.06	0.10	-0.07	-0.03	0.13	0.17	0.98665	0.87539	0.86136	0.71184	0.96779	0.46599
Tentative phenolic compound	$C_{14}H_{18}O_2$	218.1303	21.52	0.16	-0.03	0.03	0.19	0.13	-0.06	0.36286	0.97462	0.99681	0.21426	0.45815	0.92414
Tentative phenolic compound	$C_9H_{10}O_5$	198.0526	17.58	-0.17	-0.18	0.10	0.02	-0.27	-0.28	0.17941	0.16202	0.32892	0.99984	0.01425	0.01291
Tentative phenolic compound	$C_{12}H_{16}O$	176.1198	26.19	-0.51	-0.55	-0.51	0.04	0.00	-0.04	0.00086	0.00134	0.00105	0.97449	0.99726	0.99583
Tentative phenolic compound	$C_{16}H_{12}O_7$	316.0578	18.62	-0.24	-0.08	-0.40	-0.16	0.16	0.32	0.01789	0.35182	0.00814	0.20954	0.92914	0.09129
Tentative phenolic compound	$C_{11}H_{12}O_2$	176.0835	21.38	-0.02	-0.07	-0.10	0.05	0.08	0.03	0.83751	0.17536	0.09388	0.49356	0.29151	0.96802
Tentative phenolic compound	$C_{15}H_{12}O_8$	320.0527	16.29	-0.17	-0.07	-0.28	-0.10	0.11	0.21	0.00181	0.28780	7.48E-05	0.01991	0.03941	0.00041
Tentative phenolic compound	$C_{16}H_{16}O_7$	320.0891	17.74	-0.03	0.02	-0.04	-0.05	0.01	0.06	0.48047	0.98458	0.67129	0.32081	0.98440	0.48117
Tentative phenolic compound	$C_8H_8O_5$	184.0374	10.21	-0.06	-0.04	-0.27	-0.03	0.21	0.23	0.96801	0.99999	0.46293	0.97316	0.70791	0.47678

Suppl. Tab. 2.2 (*continued*)

Tentative phenolic compound	$C_{13}H_{10}O_2$	198.0678	22.10	0.12	0.09	-0.20	0.03	0.32	0.29	0.01176	0.09179	0.00389	0.48491	0.00006	0.00022
Tentative phenolic compound	$C_{15}H_{12}O_6$	288.0629	21.68	-0.07	-0.32	-0.17	0.24	0.10	-0.15	0.99560	0.01454	0.13355	0.01969	0.18229	0.43148
Tentative phenolic compound	$C_{16}H_{12}O_7$	316.0579	17.08	-0.12	-0.08	0.27	-0.04	-0.39	-0.35	0.37563	0.22340	0.00503	0.97503	0.00073	0.00048
Tentative phenolic compound	$C_{28}H_{22}O_6$	454.1410	22.10	0.07	0.05	-0.26	0.02	0.33	0.31	0.66470	0.73638	0.15208	0.99915	0.02979	0.03567
Tentative phenolic compound	$C_9H_{10}O_5$	198.0526	21.10	-0.24	-0.34	-0.27	0.09	0.03	-0.07	0.00345	0.00057	0.00368	0.40722	0.99994	0.38232
Tentative phenolic compound	$C_{11}H_{12}O_4$	208.0732	20.07	0.12	0.04	-0.06	0.08	0.18	0.10	0.29354	0.99937	0.25719	0.33987	0.01811	0.22030
Tentative phenolic compound	$C_{13}H_{18}O_2$	206.1304	18.13	0.03	0.02	0.07	0.01	-0.04	-0.05	0.98571	0.67070	0.99999	0.84655	0.98214	0.65541
Tentative phenolic compound	$C_{12}H_{18}O_3$	210.1253	20.92	-0.13	-0.65	0.07	0.52	-0.20	-0.72	0.05251	5.62E-05	0.30860	1.7E-05	0.00438	1.01E-06
Tentative phenolic compound	$C_9H_8O_4$	180.0420	21.10	-0.30	-0.34	-0.32	0.04	0.02	-0.02	0.00501	0.00216	0.00746	0.89040	0.98787	0.73802
Tentative phenolic compound	$C_{13}H_{14}O_3$	218.0940	20.92	-0.06	-0.04	-0.14	-0.02	0.08	0.10	0.74021	0.93728	0.13679	0.96737	0.49985	0.29477
Tentative phenolic compound	$C_8H_6O_4$	166.0264	16.29	-0.22	-0.25	0.14	0.03	-0.36	-0.39	0.01516	0.00868	0.06888	0.97235	0.00049	0.00033
Tentative phenolic compound	$C_7H_4O_5$	168.0057	4.14	0.15	0.25	0.17	-0.09	-0.02	0.08	0.97361	0.86277	0.83833	0.98319	0.97481	0.99994
Tentative phenolic compound	$C_{13}H_{20}O_3$	224.1408	17.74	-0.12	-0.06	-0.01	-0.06	-0.11	-0.05	0.00356	0.04387	0.61673	0.29200	0.01736	0.24827
Tentative phenolic compound	$C_9H_6O_2$	146.0366	13.49	-0.60	-0.14	-0.06	-0.45	-0.54	-0.08	6.38E-06	0.20091	0.54833	2.7E-05	1.5E-05	0.83652
Tentative phenolic compound	$C_9H_{12}O_4$	184.0733	18.61	0.31	0.25	-0.19	0.07	0.50	0.44	0.00869	0.01151	0.63552	0.99609	0.00201	0.00257
Tentative phenolic compound	$C_{16}H_{12}O_7$	316.0579	17.95	-0.08	-0.02	0.17	-0.06	-0.25	-0.19	0.28694	0.87883	0.01828	0.64570	0.00169	0.00697
Tentative phenolic compound	$C_{27}H_{32}O_{14}$	580.1785	20.59	0.00	-0.15	-0.34	0.16	0.34	0.19	1.00000	0.09153	0.00220	0.09451	0.00225	0.08135
Tentative phenolic compound	$C_{13}H_{14}O_3$	218.0940	18.69	0.12	0.01	0.07	0.11	0.05	-0.06	0.74121	0.99935	0.98485	0.80303	0.90239	0.99558
Tentative phenolic compound	$C_{11}H_{14}O_5$	226.0838	23.12	-0.20	-0.13	-0.07	-0.07	-0.13	-0.06	0.05312	0.45686	1.00000	0.42322	0.05249	0.45263
Tentative phenolic compound	$C_{13}H_{18}O_2$	206.1304	18.43	-0.06	-0.08	-0.05	0.01	-0.01	-0.03	0.99963	0.88818	0.99208	0.84621	0.98083	0.96980
Tentative phenolic compound	$C_{13}H_{18}O_2$	206.1304	18.00	-0.02	-0.05	0.02	0.03	-0.04	-0.07	0.55631	0.89467	1.00000	0.90984	0.56097	0.89790
Tentative phenolic compound	$C_{16}H_{10}O_6$	298.0472	18.61	-0.39	-0.31	-0.25	-0.08	-0.14	-0.06	0.00154	0.01715	0.01236	0.27447	0.37249	0.99432
Tentative phenolic compound	$C_9H_6O_{23}$	162.0314	21.68	0.51	0.26	-0.11	0.24	0.62	0.37	0.00030	0.00801	0.62289	0.06663	0.00010	0.00182
Tentative phenolic compound	$C_{15}H_{14}O_6$	290.0785	16.50	-0.01	0.08	0.09	-0.09	-0.10	-0.01	0.99954	0.59150	0.43416	0.65075	0.48761	0.99009
Tentative phenolic compound	$C_{10}H_{10}O_2$	162.0678	19.82	0.14	0.08	0.01	0.06	0.13	0.07	0.45253	0.53915	0.92190	0.99824	0.20915	0.26025
Tentative phenolic compound	$C_{13}H_{14}O_3$	218.0940	19.23	0.27	0.06	0.05	0.21	0.22	0.01	0.01571	0.67822	0.99971	0.07605	0.01775	0.72831
Tentative phenolic compound	$C_{17}H_{14}O_8$	346.0683	22.37	-0.16	-0.08	-0.22	-0.08	0.06	0.14	0.72503	0.68405	0.35464	0.99984	0.88997	0.91719
Tentative phenolic compound	$C_{15}H_{12}O_5$	272.0680	24.04	-0.03	0.05	-0.11	-0.08	0.08	0.16	0.97684	0.67458	0.06714	0.87654	0.03824	0.01385
Tentative phenolic compound	$C_{11}H_{16}O$	164.1199	23.62	-0.25	-0.46	-0.35	0.21	0.10	-0.11	0.03672	0.00243	0.01466	0.22539	0.90453	0.50713
Tentative phenolic compound	$C_{20}H_{22}O_5$	342.1463	19.81	0.11	0.05	-0.03	0.06	0.14	0.08	0.10170	0.83891	0.99981	0.31192	0.11352	0.87343
Tentative phenolic compound	$C_{14}H_{14}O_6$	278.0787	21.01	-0.79	-0.17	0.07	-0.62	-0.86	-0.24	1.46E-05	0.26364	0.27682	6.1E-05	4.4E-06	0.01747
Tentative phenolic compound	$C_{16}H_{18}O_7$	322.1048	22.81	-0.12	0.16	-0.35	-0.28	0.23	0.51	0.44524	0.29413	0.00577	0.03369	0.04669	0.00067
Tentative phenolic compound	$C_{13}H_{18}O_2$	206.1304	19.14	0.09	-0.07	0.05	0.16	0.04	-0.12	0.20915	0.74781	0.17090	0.05100	0.99872	0.04137
Tentative phenolic compound	$C_{12}H_{12}O_2$	188.0834	24.71	0.06	0.07	0.12	-0.01	-0.06	-0.05	0.33463	0.12710	0.04780	0.88330	0.52104	0.89652
Tentative phenolic compound	$C_9H_{12}O_4$	184.0733	15.20	-0.52	-0.50	0.03	-0.02	-0.55	-0.53	0.00022	0.00062	0.93814	0.69291	0.00013	0.00036
Tentative phenolic compound	$C_{16}H_{12}O_7$	316.0578	19.08	-0.11	-0.63	0.30	0.53	-0.41	-0.93	0.76744	9.5E-06	0.00099	1.8E-05	0.00037	3.4E-07

Suppl. Tab. 2.2 (*continued*)

Tentative phenolic compound	$C_{21}H_{20}O_8$	400.1154	20.78	-0.02	-0.32	-0.47	0.30	0.45	0.15	0.99680	0.08744	0.01975	0.11633	0.02604	0.71745
Tentative phenolic compound	$C_{11}H_{12}O$	160.0886	21.86	-0.10	-0.09	0.09	-0.01	-0.19	-0.18	0.89255	0.65964	0.55344	0.96426	0.24209	0.12826
Tentative phenolic compound	$C_{20}H_{22}O_5$	342.1463	23.65	-0.02	0.03	-0.05	-0.04	0.03	0.08	0.90899	0.83488	0.95194	0.99781	0.65477	0.55537
Tentative phenolic compound	$C_{12}H_{12}O_4$	220.0731	20.06	0.18	-0.06	0.08	0.24	0.10	-0.14	0.06277	0.31758	0.58723	0.00526	0.36521	0.05341
Tentative phenolic compound	$C_{18}H_{20}O_5$	316.1306	20.40	0.20	0.11	0.08	0.09	0.12	0.03	0.01226	0.16184	0.46329	0.31358	0.10190	0.83760
Tentative phenolic compound	$C_{16}H_{14}O6$	302.0785	11.65	-0.47	-0.18	-0.39	-0.29	-0.08	0.21	0.00238	0.23305	0.00338	0.03451	0.98963	0.05274
Tentative phenolic compound	$C_{17}H_{14}O_8$	346.0682	19.59	-0.25	-0.33	-0.07	0.08	-0.18	-0.26	0.02434	0.01098	0.71098	0.93081	0.11038	0.04770
Tentative phenolic compound	$C_{10}H_{12}O_3$	180.0784	18.92	0.04	0.05	0.00	-0.01	0.04	0.05	0.54228	0.34556	0.98110	0.97634	0.74855	0.52580
Tentative phenolic compound	$C_{13}H_{18}O$	190.1355	20.72	0.12	0.14	0.34	-0.01	-0.22	-0.20	0.93441	0.82913	0.35994	0.99274	0.66094	0.80480
Tentative phenolic compound	$C_{10}H_{12}O_2$	164.0835	22.33	-0.02	-0.03	-0.10	0.01	0.08	0.07	1.00000	0.98551	0.57456	0.98404	0.56816	0.76235
Tentative phenolic compound	$C_{15}H_{10}O_7$	302.0425	19.30	-0.16	-0.17	-0.16	0.01	0.00	-0.01	0.43458	0.89554	0.83351	0.80848	0.87479	0.99882
Tentative phenolic compound	$C_{20}H_{22}O_4$	326.1513	22.01	0.24	0.01	-0.01	0.23	0.25	0.02	0.00297	0.68442	0.99614	0.01206	0.00384	0.79988
Tentative phenolic compound	$C_{10}H_{12}O_2$	164.0835	20.41	0.01	0.03	0.01	-0.02	0.00	0.02	0.99987	0.99816	0.87692	0.99486	0.90420	0.79954
Tentative phenolic compound	$C_{12}H_{14}O$	174.1043	17.66	-0.69	-0.48	-0.11	-0.22	-0.58	-0.37	1.48E-07	3.11E-06	0.05334	0.00121	6.8E-07	2.13E-05
Tentative phenolic compound	$C_{15}H_{12}O_6$	288.0629	22.65	-0.37	-0.12	0.01	-0.25	-0.38	-0.13	3.34E-05	0.07226	0.98468	0.00039	4.3E-05	0.11774
p-Coumaric acid	$C_9H_8O_3$	164.0471	19.27	0.25	0.18	-0.01	0.07	0.26	0.19	0.00259	0.03251	0.99632	0.27134	0.00203	0.02425
Tentative phenolic compound	$C_{12}H_{18}O_3$	210.1253	21.25	-0.02	-0.59	0.17	0.57	-0.19	-0.76	1.00000	0.00243	0.48604	0.00248	0.47611	0.00050
Tentative phenolic compound	$C_{15}H_{12}O_7$	304.0578	17.56	-0.13	-0.43	0.28	0.30	-0.41	-0.71	0.31084	0.00056	0.01453	0.00435	0.00150	1.91E-05
Tentative phenolic compound	$C_{11}H_{14}O_2$	178.0991	22.33	0.01	-0.07	-0.19	0.07	0.20	0.12	0.96290	0.99578	0.41763	0.89582	0.23205	0.53135
Tentative phenolic compound	$C_8H_8O_3$	152.0472	8.49	0.11	0.05	0.07	0.06	0.04	-0.02	0.08879	0.53425	0.27879	0.53395	0.83540	0.94108
Tentative phenolic compound	$C_{20}H_{22}O_6$	358.1411	23.05	0.04	0.01	0.00	0.02	0.04	0.01	0.53557	0.96382	0.99993	0.79234	0.56634	0.97475
Tentative phenolic compound	$C_{12}H_{18}O_3$	210.1252	24.69	-0.32	-0.67	0.11	0.35	-0.43	-0.78	0.02955	9.38E-05	0.27854	0.00318	0.00245	2.15E-05
Kaempferol-di-hexoside	$C_{30}H_{26}O_{12}$	578.1420	13.56	-0.17	0.15	-0.31	-0.32	0.14	0.46	0.00105	0.00043	1.82E-05	4.2E-06	0.00593	3.7E-07
Tentative phenolic compound	$C_{15}H_{12}O_6$	288.0629	20.60	-0.25	-0.17	-0.10	-0.08	-0.15	-0.07	0.00073	0.00652	0.18880	0.28809	0.00982	0.14032
Tentative phenolic compound	$C_{23}H_{24}O_{13}$	508.1212	22.78	-0.12	-0.15	-0.24	0.02	0.12	0.09	0.05552	0.02684	0.00044	0.95167	0.01593	0.03242
Tentative phenolic compound	$C_8H_4O_3$	148.0159	21.68	0.44	0.36	-0.06	0.08	0.50	0.42	1.67E-05	2.81E-05	0.79158	0.87277	9.2E-06	1.49E-05
Tentative phenolic compound	$C_{15}H_{12}O_7$	304.0578	21.60	-0.30	-0.02	-0.05	-0.27	-0.25	0.03	0.00349	0.99487	0.39844	0.00465	0.03005	0.51771
Tentative phenolic compound	$C_9H_6O_2$	146.0366	17.77	0.18	0.71	0.05	-0.53	0.13	0.66	0.37260	0.01227	0.90493	0.13311	0.72596	0.03039
Tentative phenolic compound	$C_{12}H_{18}O_2$	206.1304	17.74	-0.08	-0.03	-0.03	-0.05	-0.05	0.00	0.25301	0.78697	0.89322	0.70146	0.57104	0.99513
Tentative phenolic compound	$C_{12}H_{14}O_4$	222.0888	24.63	0.62	-0.08	-0.22	0.70	0.84	0.14	9.06E-05	0.95595	0.17216	6.1E-05	1.6E-05	0.33264
Tentative phenolic compound	$C_{12}H_{18}O$	178.1355	25.75	-0.28	-0.52	-0.31	0.24	0.03	-0.21	0.01614	6.43E-05	0.00645	0.00308	0.89122	0.00730
Tentative phenolic compound	$C_{11}H_{12}O_3$	192.0784	8.56	0.21	-0.12	-0.22	0.33	0.43	0.10	0.00018	0.02313	0.00027	1.1E-05	1.2E-06	0.01816
Tentative phenolic compound	$C_8H_6O_3$	150.0314	21.60	-0.32	-0.29	-0.58	-0.03	0.26	0.29	0.00192	0.00414	3.89E-05	0.90986	0.01156	0.00502
Tentative phenolic compound	$C_8H_8O_5$	184.0369	11.68	0.03	-0.16	0.02	0.19	0.01	-0.18	0.98279	0.76623	0.98094	0.92417	0.88062	0.55992
Tentative phenolic compound	$C_{16}H_{14}O_7$	318.0733	21.60	-0.37	-0.28	-0.64	-0.10	0.27	0.36	0.00094	0.00500	4.4E-05	0.49339	0.03542	0.00508
Tentative phenolic compound	$C_{15}H_{12}O_7$	304.0578	19.28	-0.16	-0.06	-0.03	-0.10	-0.13	-0.03	0.01145	0.50049	0.77407	0.08551	0.04230	0.95700

Suppl. Tab. 2.2 (*continued*)

Tentative phenolic compound	$C_9 H_4 O_5$	192.0055	15.18	-0.21	-0.10	-0.05	-0.11	-0.16	-0.05	0.00025	0.09190	0.16295	0.00464	0.00284	0.97516
Tentative phenolic compound	$C_{10} H_8 O_3$	176.0471	19.10	0.09	0.39	-0.11	-0.30	0.20	0.50	0.99979	0.12146	0.18309	0.10831	0.20447	0.00557
Tentative phenolic compound	$C_{10} H_8 O_3$	176.0471	20.08	0.14	0.02	-0.05	0.12	0.19	0.07	0.34150	0.93249	0.56737	0.15760	0.05517	0.87534
Tentative phenolic compound	$C_{15} H_{12} O_6$	288.0629	14.12	0.69	0.71	0.00	-0.02	0.69	0.71	6.14E-08	4.04E-08	0.94783	0.62844	5.1E-08	3.45E-08
Tentative phenolic compound	$C_{10} H_{10} O_4$	194.0577	20.07	0.16	-0.01	0.00	0.17	0.16	-0.01	0.28466	0.74046	0.77636	0.06974	0.07676	0.99988
Tentative phenolic compound	$C_{10} H_8 O_3$	176.0471	24.63	0.59	-0.04	-0.16	0.63	0.75	0.12	2.36E-07	0.36400	0.00127	0.00000	2.5E-08	0.010072
Tentative phenolic compound	$C_{14} H_{16} O_3$	232.1096	24.93	-0.31	-0.28	0.09	-0.03	-0.40	-0.37	0.01036	0.02891	0.25797	0.86818	0.00097	0.00224
Tentative phenolic compound	$C_{31} H_{28} O_{13}$	608.1521	22.52	0.13	0.31	-0.68	-0.18	0.81	0.99	0.18981	0.00108	2.55E-06	0.01599	8.2E-07	1.19E-07
Tentative phenolic compound	$C_9 H_6 O_2$	146.0366	19.27	0.26	0.19	-0.03	0.06	0.29	0.22	0.00468	0.02320	0.80943	0.62408	0.00166	0.00717
(+) Catechin	$C_{15} H_{14} O_6$	290.0786	14.12	0.85	0.93	0.01	-0.08	0.84	0.92	9.05E-07	4.02E-07	0.95841	0.40166	1.2E-06	5.11E-07
Tentative phenolic compound	$C_{20} H_{20} O_8$	388.1151	19.72	0.20	-0.09	0.06	0.30	0.14	-0.15	0.52896	0.18257	0.99487	0.02554	0.66089	0.13151
Tentative phenolic compound	$C_{20} H_{20} O_8$	388.1151	20.06	0.14	-0.13	0.03	0.27	0.11	-0.16	0.19778	0.05542	0.88042	0.00305	0.48858	0.02000
Tentative phenolic compound	$C_{27} H_{32} O_{14}$	580.1785	20.78	-0.05	-0.28	-0.39	0.23	0.34	0.11	0.99814	0.02416	0.00136	0.03049	0.00163	0.16818
Tentative phenolic compound	$C_{16} H_{14} O_7$	318.0735	17.28	-0.17	-0.39	0.40	0.23	-0.57	-0.79	0.03166	0.00087	0.00187	0.07113	6.7E-05	8.6E-06
Tentative phenolic compound	$C_{11} H_{12} O$	160.0886	24.69	-0.19	-0.21	0.08	0.02	-0.27	-0.29	0.65218	0.71935	0.55678	0.99931	0.12698	0.15020
Tentative phenolic compound	$C_{13} H_{20} O_5$	256.1306	18.15	0.16	-0.03	0.08	0.19	0.08	-0.11	0.17974	0.72200	0.95362	0.04075	0.34976	0.44359
Tentative phenolic compound	$C_{14} H_{16} O_4$	248.1045	19.73	0.26	0.16	-0.23	0.10	0.49	0.39	0.00043	0.00245	0.00208	0.41082	6E-06	1.68E-05
Tentative phenolic compound	$C_{18} H_{20} O_5$	316.1306	19.26	0.13	0.01	-0.01	0.12	0.14	0.02	0.39054	0.98040	1.00000	0.58439	0.38256	0.97769
Tentative phenolic compound	$C_{12} H_{18} O_3$	210.1253	24.93	-0.20	-0.74	0.07	0.54	-0.27	-0.81	0.30486	0.00443	0.73672	0.05400	0.07456	0.00135
Tentative phenolic compound	$C_{15} H_{12} O_7$	304.0578	16.91	-0.07	-0.40	0.25	0.33	-0.32	-0.65	0.82352	0.00057	0.00192	0.00140	0.00076	6.79E-06
Tentative phenolic compound	$C_{12} H_{16} O$	176.1198	23.62	-0.31	-0.41	-0.38	0.10	0.07	-0.03	0.00658	0.00288	0.00396	0.90133	0.97475	0.99268
Tentative phenolic compound	$C_7 H_6 O_4$	154.0264	16.20	-0.25	-0.01	-0.37	-0.24	0.12	0.36	0.00184	0.94641	9.96E-05	0.00344	0.06344	0.00016
Tentative phenolic compound	$C_9 H_8 O_3$	164.0471	13.48	-0.61	-0.09	-0.02	-0.52	-0.59	-0.07	8.1E-07	0.10071	0.52442	3.2E-06	1.6E-06	0.59307
Tentative phenolic compound	$C_8 H_8 O_5$	184.0371	5.36	-0.06	-0.11	0.11	0.04	-0.17	-0.22	0.91396	0.45418	0.14593	0.80307	0.05882	0.01684
Tentative phenolic compound	$C_9 H_8 O_3$	164.0471	25.52	-0.15	-0.11	-0.12	-0.04	-0.03	0.01	0.00797	0.03198	0.01233	0.73152	0.98584	0.89256
Tentative phenolic compound	$C_9 H_6 O_2$	146.0366	18.94	-0.03	-0.12	0.23	0.09	-0.26	-0.35	0.99999	0.46659	0.27024	0.47895	0.26194	0.03259
Tentative phenolic compound	$C_{17} H_{14} O_8$	346.0682	20.04	-0.36	-0.29	-0.06	-0.07	-0.30	-0.23	0.00106	0.00417	0.99994	0.63788	0.00112	0.00445
Tentative phenolic compound	$C_{24} H_{24} O_{12}$	504.1262	20.85	-0.10	-0.09	-0.53	-0.01	0.43	0.44	0.90368	0.64121	0.00096	0.94951	0.00201	0.00372
Tentative phenolic compound	$C_8 H_6 O_4$	166.0264	1.55	-0.27	-0.05	0.03	-0.22	-0.30	-0.08	0.06443	0.40939	0.91997	0.54393	0.15569	0.74619
Tentative phenolic compound	$C_{21} H_{30} O_{10}$	442.1835	17.96	-0.07	-0.09	-0.10	0.02	0.03	0.01	0.55148	0.49864	0.28390	0.99963	0.93600	0.96158
Tentative phenolic compound	$C_{10} H_{12} O_5$	212.0681	16.29	-0.17	-0.20	0.27	0.03	-0.44	-0.47	0.00366	0.00257	0.00142	0.99004	1.7E-05	1.38E-05
Tentative phenolic compound	$C_{12} H_{16} O$	176.1198	26.36	-0.28	-0.29	-0.31	0.01	0.03	0.02	0.09983	0.06217	0.07357	0.98603	0.99613	0.99934
Tentative phenolic compound	$C_7 H_4 O_5$	168.0057	3.81	0.04	0.08	-0.09	-0.04	0.13	0.17	0.99842	0.99983	0.94158	0.99506	0.97708	0.91820
Tentative phenolic compound	$C_{11} H_{10} O_7$	254.0421	21.55	-0.14	-0.51	0.15	0.37	-0.29	-0.66	0.01332	5.05E-06	0.05484	8.7E-05	0.00038	9.79E-07
Kaempferol-rutinoside	$C_{30} H_{26} O_{13}$	594.1365	21.95	0.17	0.38	-0.58	-0.21	0.75	0.96	0.16126	0.00407	0.00012	0.09648	2E-05	3.65E-06
Tentative phenolic compound	$C_9 H_6 O_3$	162.0314	23.20	0.75	0.08	-0.06	0.67	0.81	0.14	3.32E-05	0.26780	0.76059	0.00016	0.00002	0.06877

Suppl. Tab. 2.2 (*continued*)

Tentative phenolic compound	$C_9 H_8 O_4$	180.0419	11.48	0.13	0.13	-0.32	0.00	0.45	0.45	0.95928	0.65526	0.04368	0.89816	0.02218	0.00889
Tentative phenolic compound	$C_9 H_8 O_3$	164.0472	15.34	0.08	0.15	0.24	-0.07	-0.16	-0.09	0.69442	0.18108	0.00975	0.65788	0.04377	0.22488
Tentative phenolic compound	$C_{17} H_{14} O_8$	346.0683	22.78	-0.15	-0.12	-0.22	-0.04	0.07	0.10	0.25718	0.40120	0.00768	0.98290	0.12153	0.07320
Tentative phenolic compound	$C_{13} H_{14} O_3$	218.0940	20.53	0.31	0.13	0.04	0.19	0.27	0.09	0.00169	0.21275	0.39985	0.02511	0.01279	0.95660
Jasmonic acid C	$C_{12} H_{18} O_3$	210.1253	23.62	-0.17	-0.35	-0.21	0.18	0.04	-0.14	0.17056	0.00397	0.13742	0.08846	0.99851	0.11028
Tentative phenolic compound	$C_8 H_8 O_4$	168.0421	19.83	-0.04	0.04	-0.28	-0.09	0.24	0.32	0.30998	0.13107	5.18E-06	0.00993	1.7E-05	1.36E-06
Tentative phenolic compound	$C_{10} H_{14} O_4$	198.0889	18.37	-0.18	-0.14	0.18	-0.05	-0.36	-0.32	0.71860	0.54868	0.30568	0.98933	0.07135	0.04636

CHAPTER 5

Interaction between grapevines and trees; effects on water relations, nitrogen nutrition, and wine

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Interaction between grapevines and trees: effects on water relations, nitrogen nutrition, and wine

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Interaction between grapevines and trees: effects on water relations, nitrogen nutrition, and wine quality

Abstract

Agroforestry systems (AF) consisting of grapevines and trees, may lead to resource competition for water and nutrients. This study aimed to evaluate the impact of a combined cultivation on water relations, nitrogen nutrition and the resulting wine quality.

^{15}N-labeled inorganic nitrogen (N) sources were used to quantify net N uptake capacity. N content and δ^{15}N natural abundance were analysed as integrating parameters of N nutrition. Leaf water potential (ψ_{leaf}) was determined to evaluate the water status of grapevines. Wine quality was evaluated by chemical and sensory analyses. In result, AF system reduced leaf water potential and increased net N uptake capacity in grapevines. However, chemical composition and sensory quality of the wine were not significantly affected in the present system consisting of Riesling, Sauvignon Blanc, oak and poplar.

Nitrogen availability of grapevines was favourable and water relations were improved, whereas wine quality was similar when grown with trees or without. Trees were able to reduce water and nitrogen losses without negative effects on wine quality.

This work provides information on benefits and limits for intercropping of trees and grapevines in terms of performance of grapevines and wine quality compared to traditional vineyard systems.

Keywords: agroforestry, grapevine, nitrogen, water, wine

Introduction

Agroforestry systems (AF) are land-use systems that combine woody perennials with agricultural crops, animals or both on the same unit of land (Lundgren and Raintree 1983). In the present study, we focused on an agri-silvicultural system, consisting of vines as a woody perennial crop and trees, which was traditionally used in southern Europe, such as Italy, Portugal, Spain, Greece and France; and was called *Piantata* or

Vitis arbusta in Italy, or *Joualle* in France (Altieri and Nicholls 2002; Eichhorn et al. 2006; Nerlich et al. 2013). The combined cultivation of vine with woody perennials gives rise to interspecific interactions, being either competitive or synergetic. For example trees have the potential to build a physical barrier for weeds and insects, alter microclimate, raise biodiversity, enhance soil fertility or even improve air and water quality (Jose 2009). On the other hand, trees may act as competitors for resources such as light, space, nutrients and/or water (Jose et al. 2004; Bainard et al. 2011), and it was shown that especially water and nitrogen (N) availability are strongly linked to each other (Hu et al. 2013). Nitrogen is an important growth-promoting nutrient for trees (Rennenberg and Dannenmann 2015). In vine, N availability influences not only yield and growth, but is of utmost importance for the concentration of amino acids and N-containing secondary metabolites in berries that are also relevant for the wine quality. Furthermore, yeast-assimilable nitrogen (YAN) is important for the fermentation of the must because it influences yeast growth and fermentation kinetics (Bell and Henschke 2005). Aroma compounds do not only arise from must, but also originate from products of yeast metabolism, especially from sugar and N compounds present in the grapes (Mendes-Ferreira et al. 2011). Water supply strongly determines vine phenology and grape ripening (van Leeuwen et al. 2009). A shortage in water supply may have adverse effects on the development of grapevines and quality formation of the wine (Keller 2005; Chaves et al. 2010; Lovisolo et al. 2010). On the other hand, a moderate lack of water may lead to the adaptive accumulation of metabolites such as phenols or anthocyanins, favourable for vine quality and sensory features (Ribéreau-Gayon et al. 2006; Deluc et al. 2009; Lovisolo et al. 2016). Nitrogen nutrition and water availability are interlinked, because water acts as a solvent for N compounds in the soil, facilitating

uptake from the soil solution into the root. However, it is not known whether a competition between vines and trees can lead to impaired water and N nutrition in AF systems, especially if the tree species has high in water demand, and whether this results in quality changes of the vines and the resulting wine.

In the present study we investigated whether an agri-silvicultural AF system, consisting of vine (*Vitis vinifera* L. *cv.* Riesling or *cv*. Sauvignon Blanc) and poplar (*Populus alba* or *Populus tremula x. P. alba*) or oak (*Quercus petraea*) trees, was associated with impaired water relations and N nutrition of the grapevines, and if this AF system altered the quality of the wine. These two tree species were chosen because they greatly differ in terms of resource needs, *e.g.* oak has low, and poplar has high water- and N requirements. To this aim we used δ^{15}N-labelled organic and inorganic N sources to quantify net N uptake capacity (nNUC), while leaf water potential (ψ_{leaf}) and δ^{13}C abundance were determined to evaluate the water status of the vines. Moreover, the wine quality in terms of sugar, phenols and quality-determining acids was measured and the sensory profile as well as flavour and odour were evaluated. With this experiment we evaluated whether the water and N supply to the vines, as well as wine quality, was more affected in comparison of the AF system with poplar or oak.

Material and Methods

Plant material and experimental conditions

The field experiment was conducted in 2013 and 2015 in a 0.50-ha experimental vineyard in Ayl, Rhineland-Palatinate, Germany (Long. 49°37'N, Lat. 006°32'E), and consisted of grapevines and tress grown in an agroforestry (AF) system. The AF system was established in 2007 when oaks were three years old and poplars were one year old. The soil is classified as a hortic anthrosol with a skeleton fraction of 20-30

% and 15 % clay. Grapevines *Vitis vinifera* L. *cv*. Riesling (R) and *Vitis vinifera* L. *cv*. Sauvignon Blanc (S) (both grafted on rootstock Selection Oppenheim 4 (SO4)) in a wine nursery and one year old at planting were arranged as monoculture (control group), and as a mixed cropping system with oak (*Quercus petraea*) (RO, SO) or poplar (*Populus tremula x. P. alba*) (RP, SP). In addition trees were also planted as monoculture (O, P) as controls. Imperfections (population losses based on accretion problems) in the existing tree population of *P. tremula x. P. alba* were filled with trees of *P. alba*. Trees were pruned periodically to a height of 3 m. In total the vineyard was divided into 36 plots (12 m x 10 m; see supplemental data, S.1). Treatments included monocultures of each species (15 trees and 25 vines per plot, respectively, four replicates each), and combinations of vines and trees in every variation (mixed cropping systems, five replicates each). The set-up was a fully randomized experimental block design with an inclination of 26.6 %. Rows were planted in a SE / ESE direction with spacing of 2 m. The spacing between trees and vines among one row was 4 m and the spacing within and depending on the necessary space for tree and vine growth (Fig. 1). Annual precipitation [mm], average temperature [°C] and sunshine duration [h] from 2013 and 2015 are given in the supplemental data (S.2). Data were taken from the nearest official weather station ‘Trier Petrisberg’ (Long. 49°45'N, Lat. 006°40'E), of the German Meteorological Service.

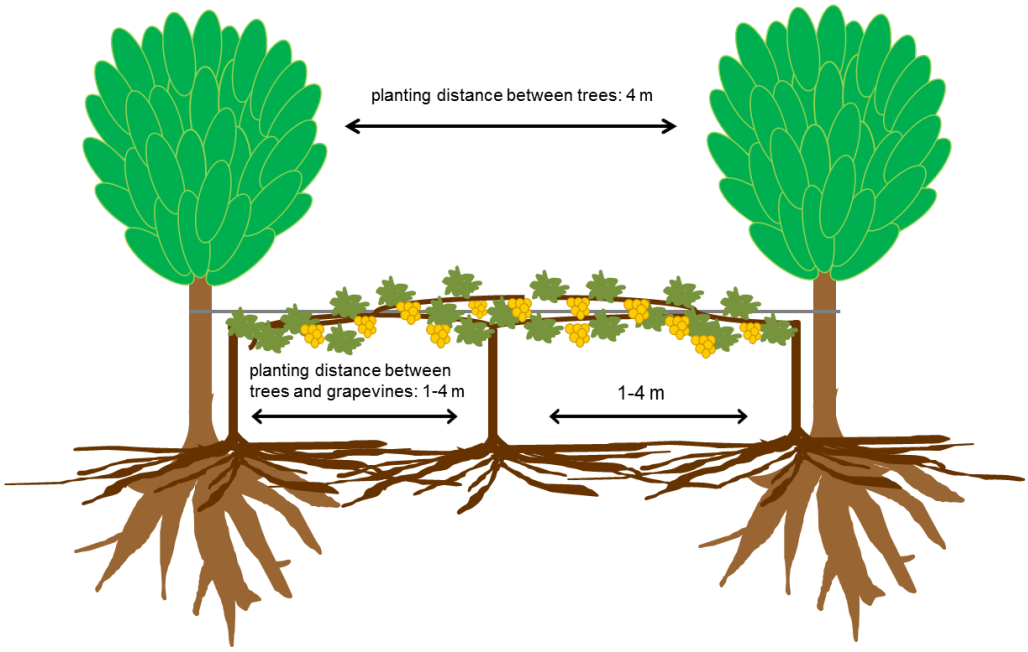

Fig.1: Schematic view of the cultivated agroforestry system. Section of a vine row and trees. The planting distance between trees is 4 m. The planting distance between trees and vines is 1-4m, depending on cultivation as monoculture or mixed cropping system.

Leaf Water Potential Measurements

Leaf water potential (ψ_{leaf}) of grapevines was measured in September 2013, at BBCH 85 -89, using a Scholander pressure chamber (Scholander et al. 1965). The measurements took place pre-dawn. The date was chosen, because at this stage of development, berries started to soften and had a high water requirement. In every plot, four fully expanded vines were randomly chosen. From these vines always the youngest fully expanded apical leaf were sampled. Values are expressed in MPa.

Determination of nitrate, ammonium, arginine and glutamine net uptake capacity in grapevine roots

N net uptake capacity was determined by ^{15}N-tracer labelling experiments as previously described for beech (*Fagus sylvatica*) and spruce (*Picea abies*) (Gessler et al. 1998). Since grapevines have a cyclical N demand and uptake with a maximum between bloom and pea-size (June-July) (Hanson and Howell 1995), uptake studies were conducted in July 2015. Samples of grapevine fine root (five fine roots per plant, located in the upper 5-10 cm of the soil) were taken from all cropping systems between 10 am and 2 pm to avoid diurnal variation (Gessler et al. 2002). Six biological replicates [n =6] were analysed per plot. The fine roots were carefully dug free from soil, by using a small scraper and a brush. Coarse dirt was removed and intact roots were incubated for 2 h in an artificial soil solution with the following nutrient composition: 100 µM KNO_3, 1 µM NH_4Cl, 25 µM Gln (glutamine), 10 µM Arg (arginine), 10 µM $AlCl_3$, 90 µM $CaCl_2$, 7 µM $FeSO_4$, 50 µM KCl, 6 µM K_2HPO_4, 24 µM $MnCl_2$, 20 µM NaCl and 70 µM $MgCl_2$. Five nutrient solutions containing different labelled N sources were used: ammonium ($^{15}NH_4^+$) or nitrate ($^{15}NO_3^-$) as inorganic N forms, or glutamine ($^{15}N^{13}C$-Gln) or arginine ($^{15}N^{13}C$-Arg) as organic N forms. The fifth solution was used as control and did not contain labelled N. After incubation, the roots were cut off from the vines and washed twice with 0.5 M $CaCl_2$ solution to remove adhering nutrients and carefully blotted dry. For later analyses of ^{15}N, roots were dried for two days at 60°C and ground at 25.5 s^{-1} for 45 seconds using a vibrating tube mill (MM 301, Retsch, Haan, Germany). Fresh and dry weights were documented. Net N uptake capacities (nmol N g^{-1} fw h^{-1}) were calculated from the incorporation of ^{15}N into the root material according to the equation published by Kreuzwieser et al. (2002):

$$\text{Net N uptake} = (({}^{15}N_I - {}^{15}N_n) \times Nt_{ot} \times dw \times 105) / (MW \times fw \times t),$$

where $^{15}N_I$ and $^{15}N_n$ are the atom% of ^{15}N in labelled (N_I, labelled) and non-labelled (N_n, natural abundance) roots, respectively; N_{tot} is the total N percentage, MW the molecular weight of ^{15}N, dw is dry weight and t is the time of exposure.

Leaf sampling

In July 2015 leaf samples were collected in all 36 plots, from two randomly selected vines as well as from two randomly selected trees. Leaves were chosen based on age, habitus and diseases, in order to collect uniform sample material. For each vine, the tenth apical leaf was harvested from a healthy shoot (Alleweldt et al. 1982). For consistent sampling of the trees, the fifth leaf of two individual second order branches were harvested. These leaves were chosen because they represent fully developed leaves with the highest rate of photosynthesis. The samples were frozen in dry ice, ground to a fine powder in liquid N_2, and stored at -80°C until further analyses. For element and stable isotope analyses, aliquots of the powder were dried for two days at 60°C.

Element and stable isotope analyses of C and N in leaf and root tissues

Total carbon (C) and N concentrations as well as ^{13}C and ^{15}N abundance were determined in oven-dried, finely ground leaf (1.4-2.0 mg) and root (1.5-2.2 mg) material using an elemental analyser (NC 2500, CE Instrument, Milan, Italy) coupled via a Conflo II Interface to an isotope-ratio mass spectrometer (Finnigan MAT GmbH, Bremen, Germany). A working standard (glutamic acid) was calibrated against the primary standards of the U.S. Geological Survey USGS 40 and USGS 41 for quantification of $\delta^{13}C$ abundance and USGS 25 and USGS 41 for quantification

of $\delta^{15}N$ abundance (Qi et al. 2003; Coplen et al. 2006). The working standard was analysed after every 10th sample to account for potential instrument drift over time as reported by Simon et al. (2011).

Wine samples and processing

Grapes were hand-harvested on October 1st (Sauvignon Blanc) and on October 22th (Riesling) 2013, respectively, when the local defined must weight was reached. All grape bunches were harvested from each plot, but due to technical limitations, only one wine was produced from the four replicate plots of each cropping system. Vinification was done at the 'Dept. of Quality of Plant Products', Institute of Crop Science at the University of Hohenheim, Stuttgart, Germany. A total yield of between 9 L and 14 L of must was collected from each cultivation system by squeezing the berries using a hydraulic press. After pressing, 2 g L^{-1} bentonite was added. After 24 h of cooling at 2 °C in a cold store, musts were separated from trub and enriched with 20 g L^{-1} sucrose. Thiamine and the wine yeast nutrient 'NutriVin' (Anchor, Johannesburg, South Africa) were added. The musts were inoculated with 0.3 g L^{-1} yeast (Anchor Vin 2000; *S. cerevisiae*) and rested till the end of fermentation, when wines were separated from the sedimented yeast and sulphured with 200 mg L^{-1} potassium disulphide ($K_2S_2O_5$). While Sauvignon Blanc wines showed a satisfactory natural purification during sedimentation of the trub, Riesling samples had to be filtered before filling in bottles. Wines were stored in bottles in the wine cellar (12°C ambient temperature) of the University of Hohenheim for 1.5 years before tasting. Sauvignon Blanc was not available in 2015.

Wine analyses

Wine analyses of the vintage 2013 were conducted after the vinification process. pH and total acids (Schmitt 1983) were measured with a titrator (TitroLine easy, Schott, Mainz). Phenolics were determined spectrophotometrically using the Folin-Ciocalteu reagent according to Singleton et al. (1999). Sugars, mainly fructose and glucose, lactic acid, tartaric acid and malic acid were analysed by high performance liquid chromatography (HPLC), (Merck-Hitachi, Darmstadt, Germany). The determination of the different sugars by HPLC is based on Mast et al. (2015). For the determination of the different acids, sulphuric acid (50 mM) was used as the mobile phase with a flow rate of 0.5 ml min^{-1}. Detection was made at 210nm. Phenomenex SecurityGuard Cartridges, Carbo-H 4 x 3.0mm as precolumn and Phenomenex Rezex™ ROA-Organic Acid H^+ (8%), LC Column 300 x 7.8 mm, Ea as separation column were used.

Wine sensory analysis

We were able to conduct descriptive sensory analysis of the wine Riesling and Sauvignon Blanc only of 2013 using a trained tasting panel consisting of 10-12 persons. The technical repetition of the wine samples at another day is necessary to account for daily variation in the sensory perception of each panel member. The six wines were tested for intensity in two replications at random at ambient temperature. For evaluation of the Sauvignon Blanc variations, the panellists were given a total of nine defined attributes, seven for aroma (cassis, green pepper, green grass, passion fruit, asparagus, gooseberry and lemon) and two for flavour/odour (intensity; high/low). Twelve attributes, ten for aroma (pineapple, apple, pear, cassis, petrol, honey, mint, peach, rose and lemon) and two for flavour/odour (intensity; high/low)

were used for evaluation of the Riesling variations. For scoring an established four - point scale was provided, with 0 for non-characteristic intensity and 4 for high/extreme intensity.

Statistical analysis

Statistical tests of the data were performed using SAS software (version 9.4, Cary, North Carolina, U.S.A.). A MIXED MODEL, a Kenward-Roger-test with a correction after Tukey-Kramer ($p \leq 0.05$) was used. Studied factors were N source and cropping system. All chemical attributes were analysed separately, and pH values were log-transformed before analysis. For sensory analyses, each aroma attribute was separately analysed and compared between the different wine samples. Sauvignon Blanc and Riesling monocultures served as control for their respective AF systems. Principal component analysis (PCA) was carried out by using the program XLSTAT (https://www.xlstat.com/de/).

Results

Leaf water potential Ψ_{leaf} of grapevine in dependency to the AF

Vitis vinifera L. *cv.* Riesling and *cv.* Sauvignon Blanc had different leaf water potential (Ψ_{leaf}) when grown in the studied AF system (mean R = -0.30; S = -0.31) Fig. 2. Leaf water potential of Riesling was significantly reduced in the AF system by 26.0 % (RO) and 28.7 % (RP), respectively, compared to the monoculture (Fig. 2a), while there was no significant effect of the different AF for Sauvignon Blanc (Fig. 2b).

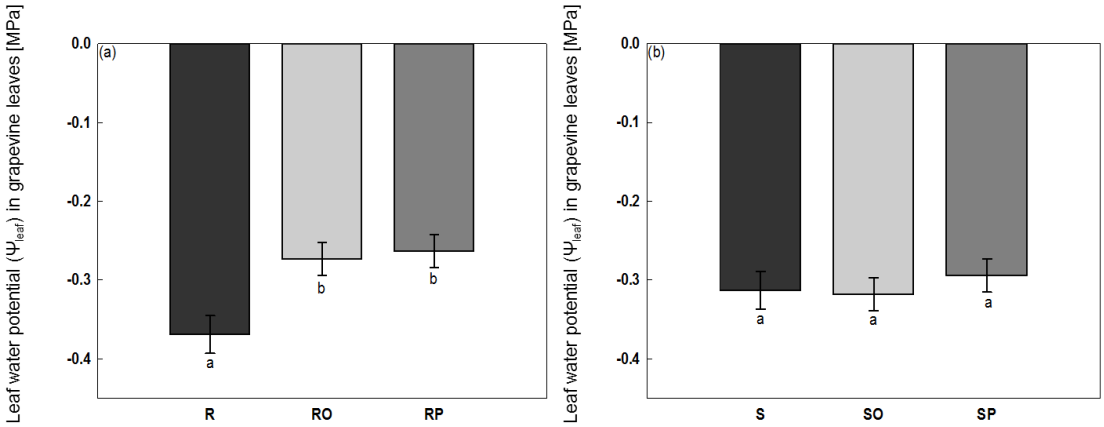

Fig.2: Leaf water potential (Ψ_{leaf}) [MPa] in grapevine leaves of the six different cultivation systems (a) Riesling (R;), Riesling/oak (RO;), Riesling/poplar (RP;) and (b) Sauvignon Blanc (S;); Sauvignon Blanc/oak (SO;); Sauvignon Blanc/poplar (SP;). Bars represent means ± SE (single copping n=16, mixed cropping n=20). Cultivation systems of Riesling cropping systems and Sauvignon Blanc cropping systems were analysed separately. Different letters indicate significant differences; MIXED MODELS, $p \leq 0.05$.

Total leaf N- and C-concentration and C:N Ratio

The total leaf N concentration was increased in both varieties when grown together with oak, RO raised by 22.5 % and SO raised by 23.4 %, compared to the monoculture (Fig. 3a and b). No significant differences were found for total C concentration comparing the AF systems of Riesling (Fig. 3c) and Sauvignon Blanc (Fig. 3d). Despite the observed increase in total leaf N concentrations, no significant effects were observed for C:N ratios in both grapevine varieties (R Fig. 3e and S Fig. 3f).

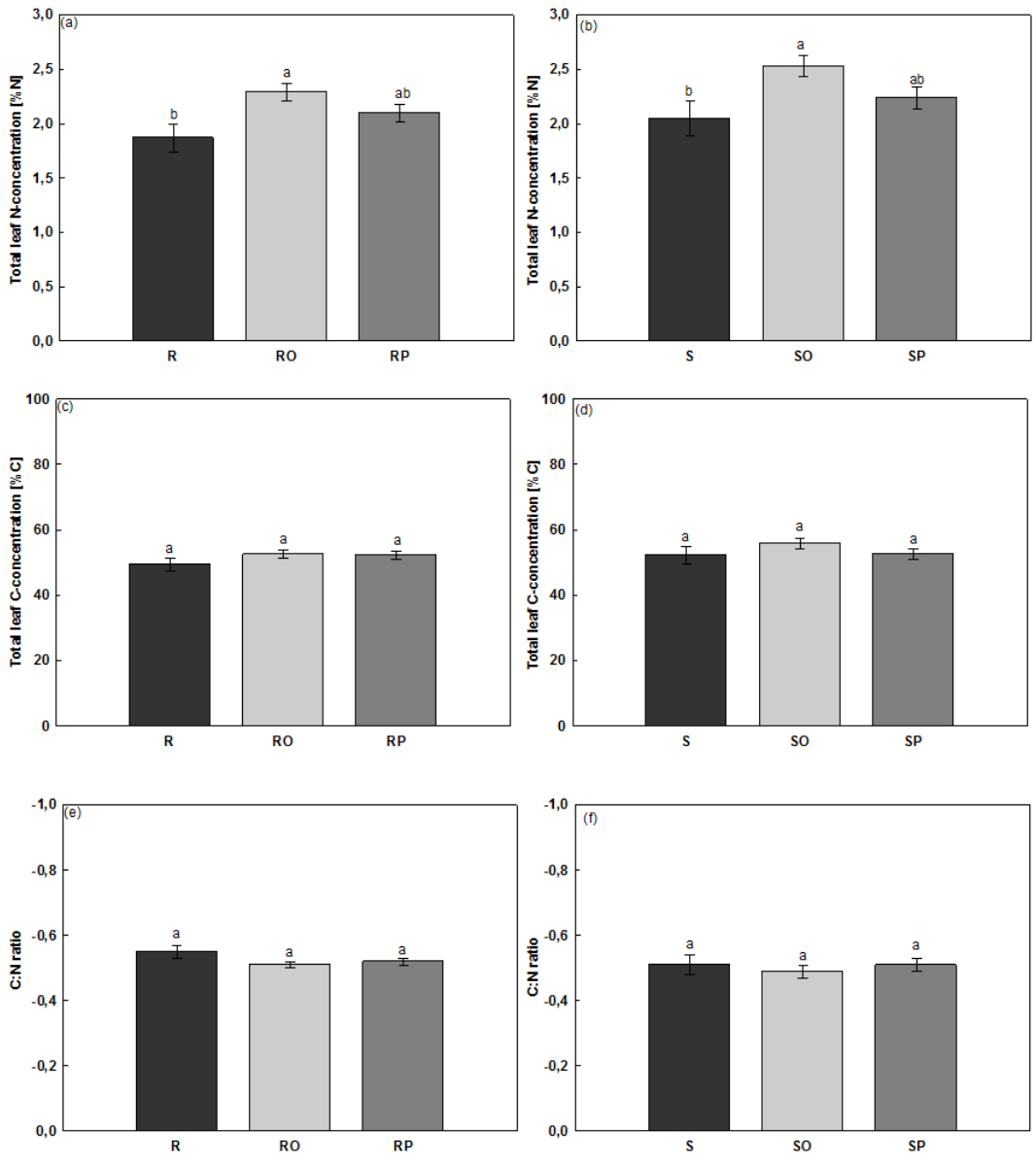

Fig.3: Total N-concentration [%N], total C-concentration [%C] in leaves of the six different cultivation systems (a) & (b) [%N] and (c) & (d) [%C] and C:N ratio in leaves (e) & (f); R (■); RO(■);RP (■) and S (■); SO (■); SP (■). Bars represents means ± SE (single copping n=8, mixed cropping n=20). Riesling cropping systems and Sauvignon Blanc cropping systems were analysed separately. Different letters indicate significant differences; MIXED MODELS, $p \leq 0.05$.

Isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$

The $\delta^{15}N$ abundances were significantly higher compared to atmospheric N (mean $\delta^{15}N‰$ = zero) for both Riesling (mean $\delta^{15}N‰$ = 2.403) and Sauvignon Blanc (mean $\delta^{15}N‰$ =1.270) monocultures (Fig. 4c and 4d). They were reduced in all AF systems, but the extent of the reduction was higher when grown with oak compared to poplar (mean $\delta^{15}N‰$ RO: = 0.079; RP: = 0.818; SO: = 0.290; SP: = 0.713).

The isotopic signatures in the discrimination of $\delta^{13}C$ have no significant differences, neither in a monoculture (mean $\delta^{13}C‰$ R; S: = -26.23), nor in an AF system (mean $\delta^{13}C‰$ RO; RP; SO; SP: = -26.63) (Figs. 4a and 4b).

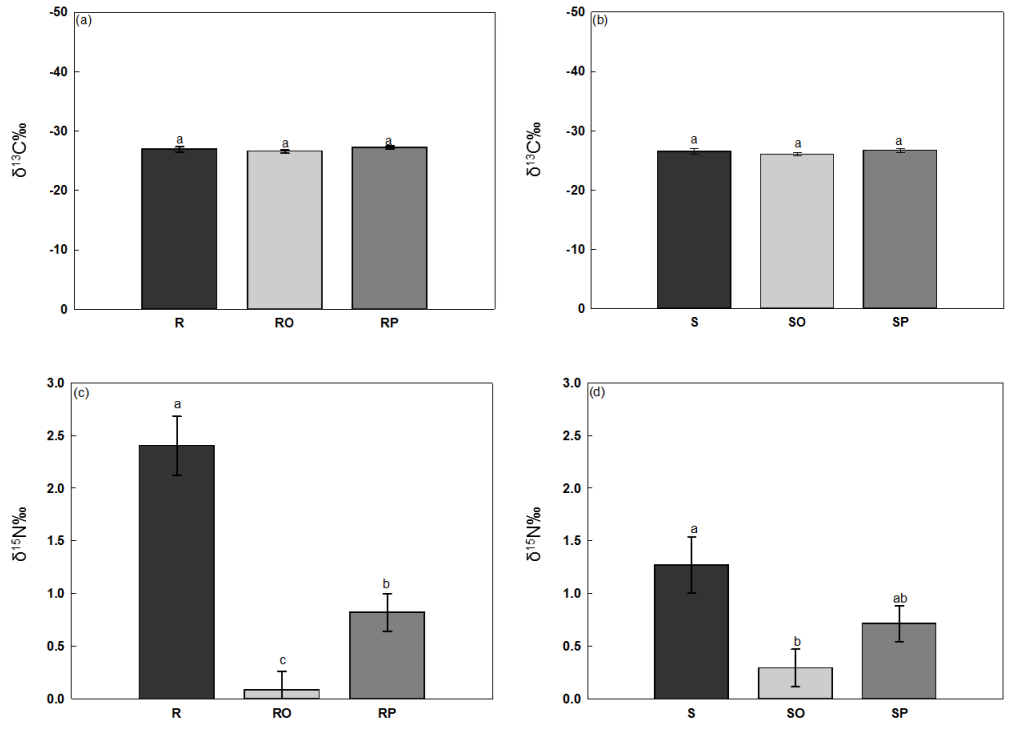

Fig. 4: Carbon (a) & (b) isotope composition [$\delta^{13}C‰$] in and nitrogen (c) & (d) isotope composition [$\delta^{15}N‰$] in leaves of the six different cultivation systems (a) & (c) R (■); RO (■); RP (■) and (b) & (d) S (■); SO (■); SP (■). Bars represent means ± SE (single copping n=8, mixed cropping n=20). Riesling cropping systems and Sauvignon Blanc cropping systems were analysed separately. Different letters indicate significant differences; MIXED MODELS, $p \leq 0.05$. Exposition period: 2 h.

Net uptake capacity in fine roots of different nitrogen forms

Across all AF systems, Riesling had significantly higher nNUC for NO_3^- (mean 16.5 nmol N g^{-1} fw h^{-1}) compared to NH_4^+ (mean 6.4 nmol N g^{-1} fw h^{-1}) (Fig. 5a). Even though the effects of the cropping systems (R; RO; RP) were not statistically significant, there was a tendency for the cultivation system RP (mean 16.6 nmol N g^{-1} fw h^{-1}) to have the highest net uptake capacity for both NO_3^- and NH_4^+ (Fig. 5a).

For Sauvignon Blanc, the nNUC across all systems (S; SO; SP) for NO_3^- (mean 14.5 nmol N g^{-1} fw h^{-1}) was slightly, but not significantly, higher than that for NH_4^+ (mean 7.5 nmol N g^{-1} fw h^{-1}). The highest nNUC values were observed in SO, with a significantly higher nNUC for NO_3^-, compared to NH_4^+ (Fig. 5b).

Regarding organic N forms, mean nNUC values across all systems were higher for Arg (214.1 (R) and 145.9 (S) nmol N g^{-1} fw h^{-1}) compared to Gln (61.4 (R) and 48.3 (S) nmol N g^{-1} fw h^{-1}) for both varieties (Fig. 5c and 5d). A significant difference between the cropping system was only seen for Riesling where nNUC for Arg was higher in the monoculture compared to the RO mixed cropping system.

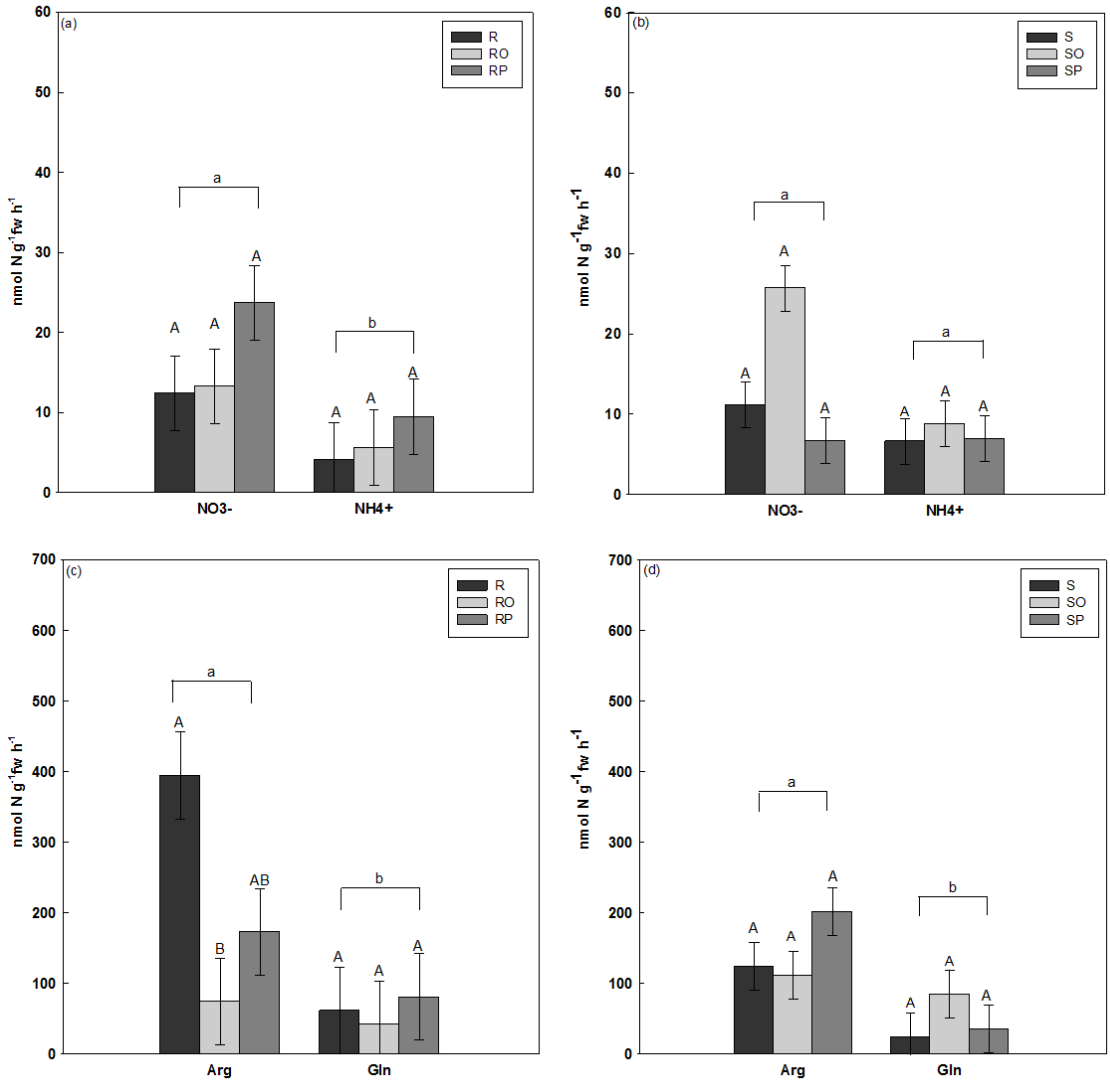

Fig. 5: Net nitrogen uptake capacity [nmol N g^{-1}fw h^{-1}] in grapevine roots of the four different nitrogen forms (a) & (b) [NO_3^-, NH_4^+] and (c) & (d) [Arg, Gln]. Measured in the six different cultivation systems (a) &(c) R (■); RO (■); RP (■) and (b) & (d) S (■); SO (■); SP (■). Bars represent means ± SE (single copping n=8, mixed cropping n=20). Riesling cropping systems and Sauvignon Blanc cropping systems were analysed separately. Lower case letters indicate significant differences within a nitrogen form; capital letters indicate significant differences within a cultivation system between different N-forms, MIXED MODELS, $p \leq 0.05$. Exposition period: 2 h.

Wine quality

Only few significant changes in wine composition were detected for the different AF systems (Table I). Compared to the Riesling monoculture (R), total acid concentration was increased in RO (R vs. RO), and lactic acid concentration and sugars decreased in RP (R vs. RP). For Sauvignon Blanc, the combination with oak (SO) resulted in lower pH values and increased sugar concentrations (S vs. SO), while total acid concentration was reduced in SP (S vs SP) (Table I). A principal component analysis (PCA) Biplot provides a visualization of the two principal components by identifying groups (Ringnér 2008). In the present study, Riesling and its AF clustered away from Sauvignon Blanc and its AF (Fig. 6). The separation was based on the loadings of the second PC. Furthermore, oak had the highest impact (longest cluster distance from the respective monoculture) on the chemical composition of both wine varieties. Overall, the PCA indicated that the changes of the chemical attributes of the Riesling wines were mainly influenced by tartaric and lactic acid, while differences for Sauvignon Blanc wines were mostly caused by malic acid, total acid and sugars. The sensory analyses of the different wines indicated no significant changes in the aroma attributes when grapevine was grown in combination with trees, neither for Riesling (Fig 7a), nor for Sauvignon Blanc (Fig. 7b). Only slight tendencies for differences in a few aroma attributes (e.g. mint and odour) were detected.

Table I.: Mean values of chemical attributes of the six experimental wines made from grapes of the different cultivation systems. Mixed cropping systems of the Riesling and Sauvignon Blanc varieties were separated analysed. Riesling and Sauvignon Blanc act as control. Significant differences (Riesling n= 2, Sauvignon Blanc n=1) are marked with an asterisk (MIXED MODELS, $p \leq 0.05$). (R, Riesling; RO, Riesling/oak; RP, Riesling/poplar; S, Sauvignon Blanc; SO, Sauvignon Blanc/oak; SP, Sauvignon Blanc /poplar). ND = not detectable.

Sample	pH	Total acid [g L^{-1}]	Tataric acid [g L^{-1}]	Malic acid [g L^{-1}]	Lactic acid [g L^{-1}]	Sugar [g L^{-1}]	Phenols [g L^{-1}]
R	2.51	11.31	5.03	3.33	2.73	3.74	0.148
RO	2.37	11.91*	5.43	3.25	2.52	3.19	0.125
RP	2.53	11.15	5.10	3.09	2.32*	2.75*	0.099
S	2.66	13.19	5.48	4.22	1.76	3.43	ND
SO	1.74*	12.86	4.91	4.51	1.70	4.10*	ND
SP	2.52	12.47*	5.19	3.94	1.90	3.55	ND

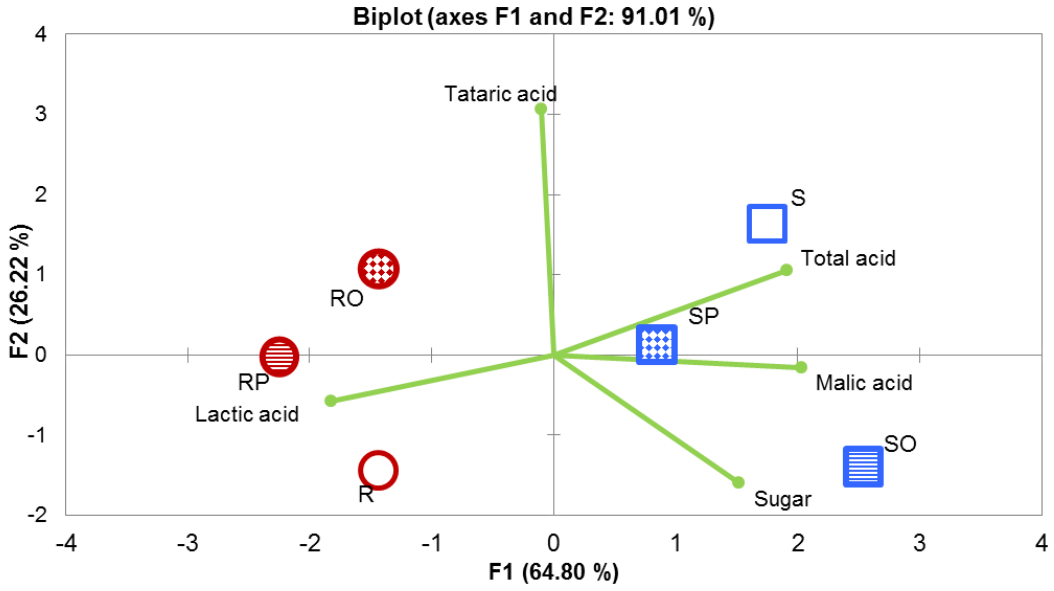

Fig. 6: Principal component analysis Biplot (PCA; F1 vs F2) of the chemical attributes of the six experimental wines from grapes of the different cultivation systems (R (), Riesling; RO (), Riesling/oak; RP (), Riesling/poplar; S (), Sauvignon Blanc; SO (), Sauvignon Blanc/oak; SP (), Sauvignon Blanc/poplar).

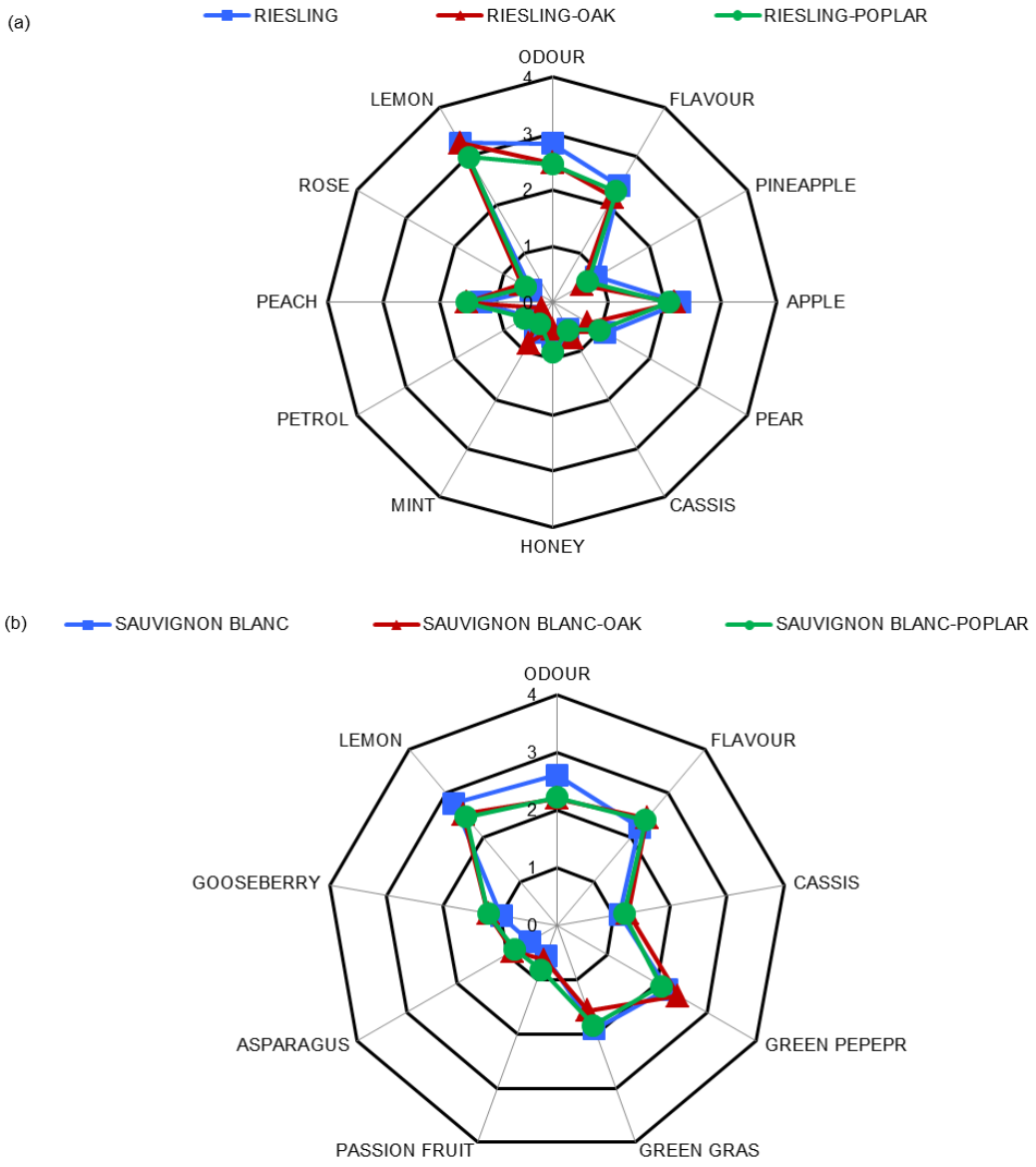

Fig. 7: Aroma and sensory descriptions for the matured wines, of the different cultivation systems (a) (■) Riesling, (▲) Riesling/oak; (●) Riesling/poplar and (b) (■) Sauvignon Blanc; (▲) Sauvignon Blanc/oak; (●) Sauvignon Blanc/poplar, as determined by the tasting panel. Mean values are shown for the two appointments (n=10; 12). Riesling and Sauvignon Blanc act as control.

Discussion

Cultivation of grapevine in an AF can improve water relations at drought

The measured water status of the grapevine cultivar Riesling was affected by the AF. Cultivation with oak and poplar increased leaf water potential (Ψ_{leaf}) in Riesling but not in Sauvignon Blanc (Fig. 2a and 2b), which seems to be a benefit for Riesling when grown in an AF. According to Deloire et al. (2004), there is a good relationship between the water status of plants, measured in terms of the leaf water potential (Ψ_{leaf}) and the available water reserves in the soil area occupied by the roots. A reduction in leaf water potential (Ψ_{leaf}) reflects lower availability of water in the soil or can even be an indication of water stress (Schultz 2003; Deloire et al. 2004). However, severe water stress did not occur during the data collection period, (see supplemental data, S.2). Grapevines close their stomata to reduce water loss; however, a prolonged closure leads to a reduced photosynthesis, reduced sugar accumulation and finally resulting in a reduced wine quality (Santos et al. 2007).

There was no significant difference in leaf water potential (Ψ_{leaf}) between the tree species used in this study. There was no competition for water in the AF systems, but rather the opposite, since the mixed cropping combination RO lead to a significant reduction in leaf water potential and, therefore, to an easing of competition for water. Apparently, water relations of the grapevine cultivar Riesling can be improved by AF under these conditions. According to Bayala and Wallace (1996), trees may affect the availability of water to crops in an AF by improving soil physiological properties, reducing runoff and soil surface evaporation as well as intercepting rain. Trees have a deep root system with a consistent framework of large perennial roots and many short-lived branch roots (Pallardy 2008). By contrast, roots of grapevines are mostly located in the top 60 cm of the soil, these fine roots do most of the water and N

acquisition (Jackson 2008). Cannell et al. (1996) suggested a 'biophysical hypothesis' for agroforestry research. They assumed that beneficial effects by growing trees in combination with crops only occur when the trees were able to acquire resources like water, light and nutrients that the crops would otherwise not acquire. Overall, the present results indicated that, tree roots could provide Riesling grapevine roots with water from deeper soil layers. These phenomena is a so-called 'hydraulic lift', a process, of passive soil - water movement from deep - moist to shallow - drier soil layers, driven by the water potential gradient (Caldwell et al. 1998). In addition, trees can act as windbreakers and shielding the soil from radiation and wind (Bayala and Wallace 1996). Therefore, trees slow the movement of wind and air circulation, leading to reduced evaporative, while, the distribution and utilization of water is improved (Davis and Norman 1988; Jose et al. 2004).

The $\delta^{13}C$ method is an integrating measure for characterizing the water supply from the time of development to the time of harvest of the plant material studied (Gaudillere 2002). It is determined by the gradient of CO_2 in the atmosphere and the intercellular CO_2 concentration of the leaves (C_i/C_a). This ratio is mainly influenced by water availability (Farquhar et al. 1989). Our results did not show significant differences in the abundance of $\delta^{13}C$ in the grapevine leaves between the cultivation types (Fig. 4a and 4b). This finding supports our conclusion from Fig. 2, that there was no competition for water in the studied AF system. Moreover, these results clarify that, the overall water availability during the vegetation period was sufficient for the grapevines.

Cultivation in AF can improve N nutrition of grapevines

When Riesling and Sauvignon Blanc grow with trees, the total leaf N concentration significantly increased in combination with oak and slightly increased in combination with poplar (Figs. 3a and 3b). The nitrogen isotope composition [δ^{15}N‰] of the leaves demonstrated (Figs. 4c and 4d) that the monoculture of both Riesling and Sauvignon Blanc had the highest abundances. This is an indicator for the amount of the isotope, which was taken up by the plant (Robinson 2001). At the first glance, this is contradictory to the net uptake capacities of N that were reduced in the AF systems, whereas the N concentrations of the leaves were enhanced. So far, we just can hypothesize about this discrepancy. The differences in nNUC may not reflect the actual uptake of organic and inorganic N compounds due to different availabilities in the soil. The different N contents could result from different uptake capacities. Furthermore, environmental factors have major impacts on the uptake of N by the roots. Our measurements took place at one time point in summer, but the leaves developed earlier in the vegetation period. Therefore, we cannot exclude that net uptake capacities were different between monocultures and AF system at other time points and different N-pools, with different N forms and quantities were built throughout the year. Finally, the N content of leaves in a perennial woody plant greatly depends on stored resources in the plant that are mobilized in spring (Dickson 1989; Millard and Grelet 2010). Therefore, the N content of leaves often reflects the net uptake capacities of the previous year. The net N uptake capacity of the current year rather determines the extent at which storage pools in the stem are refilled.

What can be concluded from the differences in leaf $\delta^{15}N$ abundance between monocultures and agroforestry system cultivation of grapevines?

The N isotope composition [$\delta^{15}N‰$] of the leaves is an indicator for the origin of N acquired by the plant (Robinson et al. 2000). From the results it may be concluded that the N taken up from the soil was less subjected to biological N_2 fixation by free living soil-microbes, when the grapevine was grown in combination with trees. However, the differences observed may also result from (a) different N isotope fractionation processes during uptake, transport and metabolite transformation of soil N sources (Schmidt et al. 2015) and/or (b) differences in the availability and use of inorganic versus organic N compounds in the soil (Näsholm et al. 2009). In addition, plants can change their preference for different N forms (e.g. NO_3^- versus NH_4^+) with different nitrogen isotope composition in response to environmental conditions, but also to management practices, such as fertilization or harvest, that can shift to proportionate uptake rates of NO_3^- and NH_4^+ (Högberg 1997; Pardo et al. 2002). However, the origin of the N isotopes and the $\delta^{15}N$ of the major N compounds in the xylem sap, which were taken up by the plant for N assimilation, remain unknown.

Water and nitrogen consumption of grapevine is not influenced by agroforestry cultivation

Water and fertilizer management, especially for N, are strongly linked to each other, in a way, that changes in one parameter will affect the efficiency of the other. This means, the more water is available for plants, the more available nitrogen can be taken up by the roots and therefore be retrievable for the plant. From a comparison of Figs. 2a, 3a and 3b, this conclusion can be supported. However the extension is variety dependent. In the mixed cropping varieties of Riesling in combination with oak and poplar, the leaf water potential (Ψ_{leaf}) was significantly lower (Figs. 2a) and

simultaneously the total leaf N-concentration was higher; similar tendencies can be seen in Sauvignon Blanc but the results are not statistically significant.

The C:N ratio in figures 3e and 3f describe the proportion of carbon (C) and N in leaves of the six different AF systems studied. The smaller the ratio, the more N is available. This implies that the combination with the smallest C:N ratio, had the highest net N uptake, a change in water or nitrogen supply results in a C: N ratio imbalance (Chen et al. 2015). According to our results no statistical differences were detected between monoculture and mixed cropping systems, again disproving a competition for nitrogen and water. The concluding physiological influences and changes of trees on and grapevines in relation to water and nitrogen, as summarized in Figs. 2-5, are shown as a schematic Figure 8.

Chemical attributes of Riesling and Sauvignon Blanc are slightly changed when grown in an AF system

The influence of the trees on the wine is variety dependent (Table I). The PCA in Fig. 6 indicates that these changes were minor (RO: total acid, RP: lactic acid and sugar; SO: pH and sugar, SP: total acid) when AF systems were compared to monocultures. Trees influenced mainly sugar and acid concentration of the wine. Nevertheless, the results indicate that the sugar to acids balance, that primary contributes to flavour (Liu et al. 2006; Conde et al. 2007), is influenced by both tree varieties. Tartaric and malic acid account for two-third or even more of all organic acids in grapes determining the pH of wine (Kliewer 1966; Waterhouse et al. 2016). To conclude, after this first hint, more studies have to be done to exactly clarify which tree may influence which wine parameter. The aroma and sensory evaluation of Riesling (Fig. 7a) and Sauvignon Blanc (Fig. 7b), indicate only small tendencies towards an increase or decrease of single aroma compounds. Habran et al. (2016)

reported that a mild water deficit and moderate N availability affected berry metabolism towards the synthesis of phenolic and aroma compounds. Chapman et al. (2005) and Habran et al. (2016) found that vegetal aroma contributes, especially bell-pepper, is reduced under water deficit whereas fruity aroma descriptors were higher under these conditions. However, this can not be proven because there was no water stress for the vines, and there were no significant differences detected in tasting. Only tendencies prove small changes in the aroma components of the wine (Fig. 7). Several hundreds of volatile compounds contribute to wine aroma, with concentrations ranging from several mg/L to a few ng/L, sometimes even less (Francis and Newton 2005; Conde et al. 2007). The olfactory threshold and the perception of these compounds can vary considerably. Many complex mechanisms that are involved in the development of aroma; these may include biochemical, cultural and enzymatic factors, as well as viticultural management practices during growth, processing and fermentation (González-Barreiro et al. 2015). This is the reason, why aromas are difficult to study (Francis and Newton 2005; Ribéreau-Gayon et al. 2006). As a quintessence only slightly differences of the chemical attributes and the aroma components of the wines were detected in the AF systems (Table I; Figs. 6 and 7).

Conclusions

Mixed cropping systems of grapevines (*Vitis vinifera* L. *cv.* Riesling and *Vitis vinifera* L. *cv.* Sauvignon Blanc) with oak (*Quercus petraea*) and poplar (*Populus tremula x P. alba*) revealed that the presence of the trees increased leaf water potential Ψ_{leaf} of the neighboured Riesling but not of Sauvignon Blanc. Furthermore, N availability and acquisition by grapevines increased with cultivation in

combination with trees in agroforestry systems. We conclude from these outcomes that, at least under the conditions of this study, there was no competition in this type of agri-silvicultural system with regard to water and N in two different years. Rather, the different plant species supported each other in their net N uptake capacity. In addition, trees do not significantly affect quality-associated chemical attributes of the wines and their related quality. The sensory attributes of the wines were similarly good in both cultivation systems. These findings suggest that an agri-silvicultural system could be useful for practical implementation towards a resource-preserving production of high quality wine.

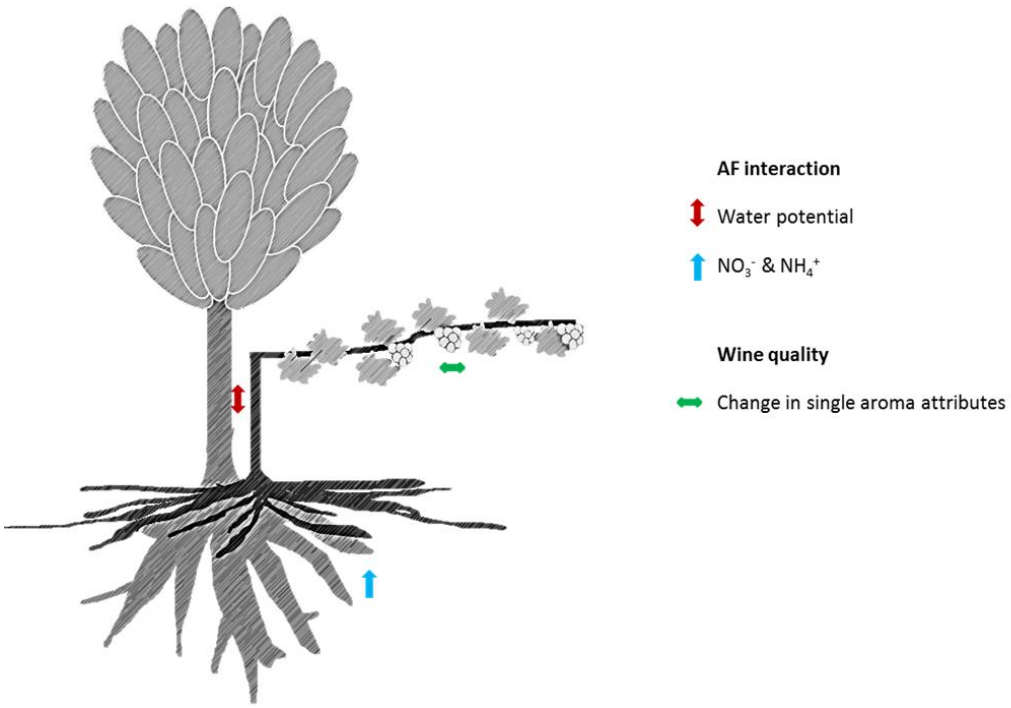

Fig. 8: Schematized conclusion of the effects of trees on the grapevine in an agri-silvicultural system. Nitrate and ammonium is increased in mixexd cropping system; no difference in water potential, therefore no water stress in mixed cropping system; comparable wine quality in both cropping systems. Abbreviations; blue arrow, increase in absorption level; red arrow, change in both directions; green arrow, no change.

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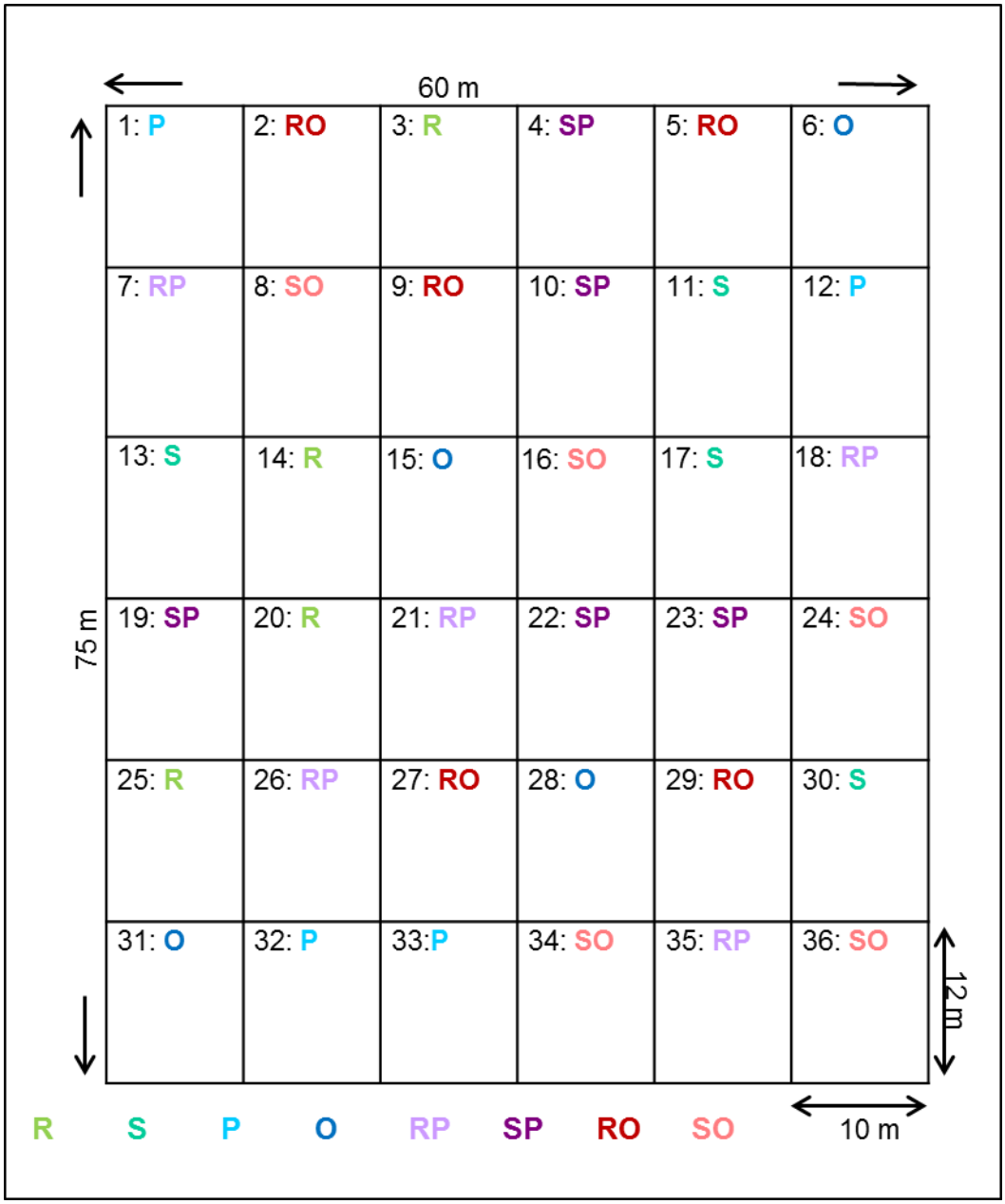
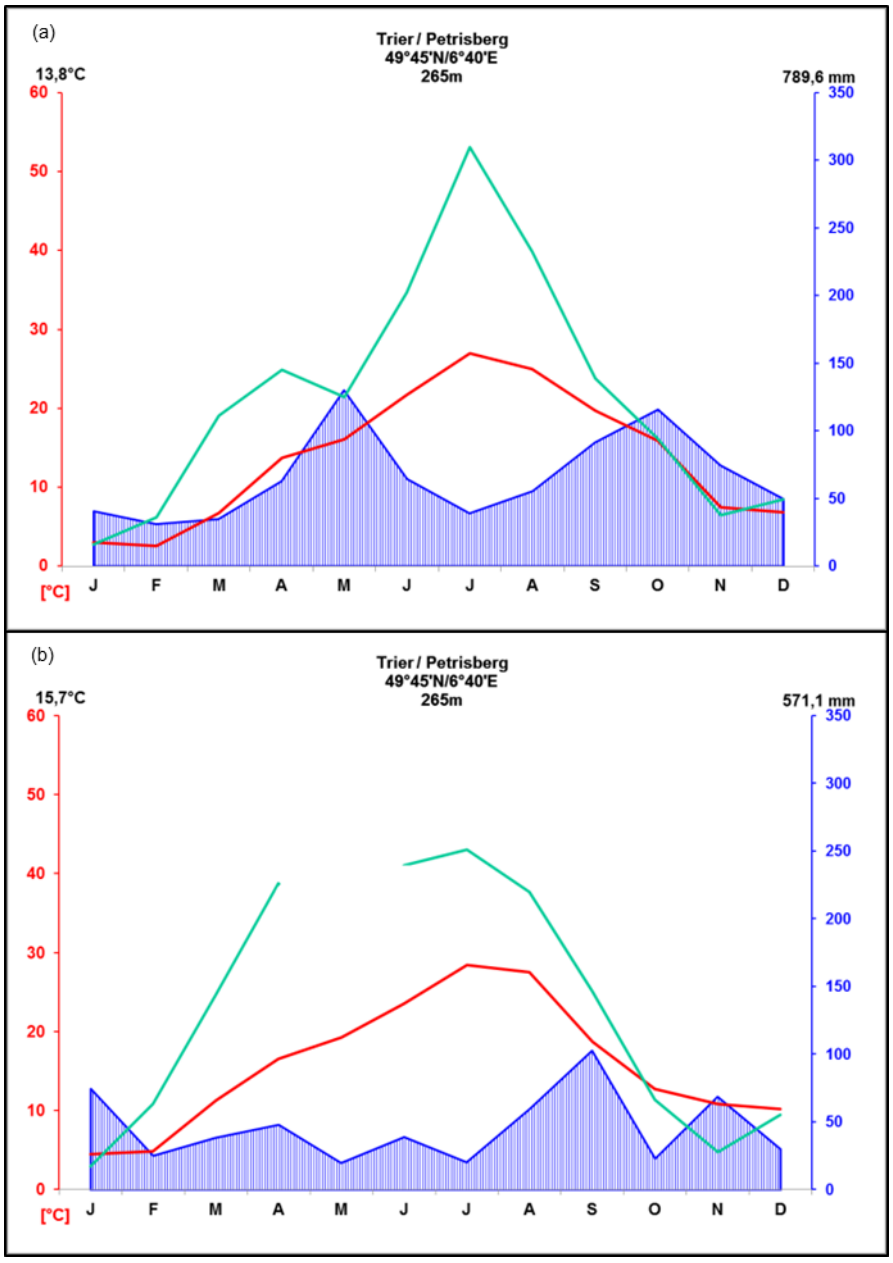
Supplemental material



S.1: Experimental design of the vineyard in Ayl (a), divided in 36 plots with cultivation systems, monoculture and mixed cropping. (**R**), Riesling; (**RO**), Riesling/oak; (**RP**), Riesling/poplar; (**S**), Sauvignon Blanc; (**SO**), Sauvignon Blanc/oak; (**SP**), Sauvignon Blanc/poplar; (**O**), oak; (**P**), poplar.



S.2: Annual precipitation [mm] ( ), average temperature [°C] ( ) and sunshine duration [h] ( ) at closest official location in Trier/Pertrisberg from the experimental years 2013 (a) and 2015 (b). Time period of the measurement was January till December.

CHAPTER 6

General Discussion

Chapter 6: General Discussion

Nitrogen (N) influences vegetative growth, which determines generative growth, and it significantly influences the quality of berries and wine. During the fermentation of must to wine, N is indispensable for the formation of flavour and aroma. These components are decisive for the sensory properties of a wine.

However, an excessive amount of N can also have opposing effects and thus can reduce quality (Bell and Henschke 2005). The grapevine is capable of assimilating various N-forms such as nitrate and ammonium. Amino acids are considered to be potential precursors for N-containing molecules and can be used in the assimilation of the plant (Ortiz-Lopez et al. 2000). Quality is a fundamental factor for wine. Therefore, a better understanding of an adapted quality-defining N fertilization strategy in the vineyard is essential.

In this work, the allocation of various N-forms and their effects on the quality of berries and wine were investigated by means of; hydroponics (Chapters 2, 3), pot trials (Chapter 2) and vineyard trials (Chapters 4, 5). Plant physiological and quality factors, plus the sensory aspects of wine, were also studied following the various N-form treatments.

6.1 Nitrogen allocation in grapevines in response to the different N-forms

The grapevines in the hydroponics and pot experiments were treated with 4 mM total N. The grapevines in the field experiment were fertilized with a quantity of 60 kg N ha^{-1}, calculated in relation to the size of each experimental block in the vineyard. The following N-forms were used; nitrate (NO_3^-), ammonium (NH_4^+), urea, arginine and glutamine. For ammonium and urea nitrate inhibitors were used (ammonium sulphate and PIAGRAN 46).

Grapevine rootstocks and grafted grapevines are able to assimilate N from all five different N-forms offered. The two rootstocks SO4 (Selection Oppenheim 4) and RU140 (Ruggerie 140) showed a different preference for a specific N-from as an N source. In total, the two rootstocks showed similar assimilation patterns but at different levels. The N-forms NO_3^- and NH_4^+ were the preferred N sources. This was detectable with regard to the vegetative growth and N content (Chapter 2, Figs 1 and 2) and according to the metabolic and sensory analyses (Chapter 4). The N-form urea showed similar but mainly reduced physiological growth patterns (biomass, N content and NRA; Chapter 2) compared with NO_3^- and NH_4^+. However, this could only be determined for the physiological growth parameters of the

grapevine. The uptake of N in the plant can occur either by HATS or by LATS, depending on the external N concentration (Noguero and Lacombe 2016). The transporters can be significantly upregulated by high N amounts (Cochetel et al. 2017). Urea is taken up either by active transporters (UT) or by passive by major intrinsic proteins (MIPs), although this is still under debate. These transporters act within a gradient system, in both directions (Wang et al. 2008; Witte 2011). Therefore, based on the results, N uptake and assimilation under urea treatment are reduced compared with that after treatments of NO_3^- or NH_4^+. The subsequent N availability influences and changes the N transport system. An additional consideration is that urea has to be hydrolysed to ammonia and carbon dioxide and further to NH_4^+ before it can be assimilated (Sirko and Brodzik 2000). This is an energy-intense process and reduces the possibility of taking up N in the same amounts to those after NO_3^- or NH_4^+ treatment. Nitrogen uptake is induced in the root cells and then further translocated into organs of sink via the xylem system. The enzymes NR, NiR and GS represent the initial steps in the N assimilation pathway of the plant and thus are ultimately involved in plant growth (Orsel et al. 2002; Nunes-Nesi et al. 2010). Amino acids and NH_4^+ are potential inhibitors of NR (Caboche and Rouzé 1990; Li et al. 1995) thereby reducing the N assimilation and uptake by the plant. The enzymatic NR activity (NRA) and also the transcript expression of NR and the co-regulated NiR showed an increase in their expression when NH_4^+ was applied (Chapter 2, Fig. 3; Chapter 4 Fig.3 and Tab. 2). Furthermore, the assimilation ability of amino acids and NH_4^+ could be confirmed by means of the N content in leaves (Chapter 2 Figs. 2 and 5). These results may provide initial evidence that the N assimilation pathway in grapevines can be stimulated by the addition of NH_4^+. Comparable results have been found by Bungard et al. (1999) for *Clematis vitalba* (also a liana plant), but not for tobacco or barley. Thus, the stimulation effect of NH_4^+ on N metabolism is species-dependent and should therefore be further investigated.

The above experiment demonstrated that the two amino acids arginine and glutamine were assimilated by the grapevine and transported to the leaves (Chapter 2, Figs 1, 2, 4 and 5). Both amino acids stimulate NRA (Chapter 2, Fig. 3) and the transcript expression of NR and NiR (Chapter 3, Fig. 3). Amino acids can also be taken up by high or low transport systems (ATF - amino acid transporter family) that can be further divided into several subfamilies. Nevertheless, little is known about the regulation of the uptake of amino acids (Ortiz-Lopez et al. 2000; Tegeder and Rentsch 2010). Only a few studies have shown that the amino acid transporters of various plant organs are stimulated by N (Tegeder et al. 2007, Tegeder and Rentsch

2010). Based on our studies, we can now assume that this also applies to the grapevine, but to variable degrees depending on the amino acid form.

In addition to the N-form, the amount of N applied has a great influence on the N assimilation of grapevines. With increasing amounts, both the growth and the N content increases, but if the N amount is too high, vegetative growth is significantly reduced (Chapter 2, Fig. 4). Similar results have been obtained in the studies of Zerihun and Treeby (2002) and Hilbert et al (2003). Increasing vine growth can lead to a change in the sink : source relationship and results in a changed canopy density and microclimate. When a vine becomes overgrown and experiences uncontrolled vegetative growth, it leads to saturation and further to the high accumulation and storage of N in plant tissues (reviewed by Bell and Henscke 2005).

The rootstocks SO4 and RU140 differ in their ability to absorb the different N-forms. Comparable results have been found by Keller et al. (2001). Based on our results and peer-reviewed references (Keller et al. 2001), the rootstock SO4 reacts more sensitively to N applications. Thus, the rootstock SO4 was defined as an approp model for N assimilation processes and was further used in the experiments described in Chapters 2-5.

6.2 Grapevine yield is affected by different N amounts

In contrast to vegetative growth, generative growth is unaffected by the N-form (Chapter 2, Tab. 2). Similar results have been reported by Brunetto et al. (2013) based on various sources of N fertilization in grapevines.

The amount of N strongly influences berry growth. With increasing N amounts, growth increases to a certain threshold; however, if this is exceeded, the additional N leads to a reduction of the yield. Excessive levels of N cause a significant reduction in berry yield. When grapevines are overfertilized, vegetative growth increases and a competitive situation arises between vegetative and generative growth (Portu et al. 2015). This might be related to the sink : source relationship. In addition, competition for assimilate translocation between leaves and berries might result (Delgado et al. 2004). Competition for carbohydrates and further photosynthates alters the N distribution and N availability for the berries and thus also the metabolic pathways responsible for the synthesis of taste and aroma (Bravdo and Hepner 1987). Higher proportion metabolites such as sugar and starch remain in the vegetative parts of the plant and are less available for the berries, thereby reducing berry yield and quality.

6.3 Quality components of must and wine change in response to different N-forms and amounts

6.3.1 Oenological parameters

The oenological parameters of must and wine make up an important part of wine quality. They can influence the vinification process and partly define the quality level of wine. The pH and acid contents are influenced by both the amount of N and the N-form (Chapter 2, Tab. 4 and Chapter 4, Tabs. 1 and 2). Nevertheless, the N amount was seen to have a higher influence compared with the N-form. The highest differences were obtained between NO_3^- and urea and the zero application without additional N. With increasing N amount, the pH increased and the acid content decreased. The optimal pH for white wines lies within a range of 3.0 - 3.4 and for red wine within a range of 3.3 - 3.7. The examined pH values of the musts occurred in the lower range but were still acceptable (Chapter 4). Low pH levels are desirable because they increase during fermentation (Waterhouse et al. 2016). The pH determines the amount and strength of the acidity and the mineral content (Conde et al. 2007). The total acid is mainly determined by the 'fixed acids' of which tartaric acid (TAA) and malic acid (MA) account for about 90% (Jackson 2008). Although TTA and MA were somewhat low in the pot trial compared with the field trial, they were still within the prescribed range by Waterhouse et al. (2016) (TTA; 2 - 6 g L^{-1}, MA; 2 - 7 g L^{-1}). Both acids decreased with the increasing amount of N. Application with urea and NO_3^- had the highest impact on pH and acidy content in must and wine (Chapter 2, Tab. 4 and Chapter 4, Tabs. 1 and 2). Urea mainly increases the total amount of acids, especially TTA and lowers the pH value. Nitrate reduces the MA content and raises the pH value. Acid and pH are considered to be the most important chemical parameters in must and wine and highly contribute to their organoleptic properties. In addition, they are extremely important for the microbial and chemical stability of the wine and the fermentation process (Torija et al. 2003).

The must weight is a reflection of the maturity of the grapes and thus the time of harvest. The higher the must weight, the riper the fruits and the better the quality because of the increased stored assimilates (sugar, amino acids, nitrogen components). Increased N amounts lead to decreased must weights (Chapter 2, Tab. 4). Excessive amounts of N thus delayed the maturity of the berries and this can lead to strong quality losses.

On the one hand, an increased amount of N lead to a lower acid content, which is an indication that more assimilates such as sugar, are stored and that the berry is in an advanced stage of maturity. On the other hand, an increased amount of N results

in lower must weight, which in turn indicates a retarded berry's maturity. Nevertheless, these observations were not consistent and were highly variable within the different investigated years. The same can be seen in the numerous contrasting and variable study results. The influence of N on individual chemical parameters in wine has been controversially discussed (Spayd et al. 1994; Hilbert et al. 2003; Brunetto et al. 2013). Nevertheless, the present results clearly show a significant effect of N application on acidity content, pH and must weight and thus should not be disregarded.

6.3.2 Phenolic content

In addition to their favourable defence abilities against abiotic and biotic stresses such as UV radiation or attack by parasites and pathogens, phenolics contribute largely to the organoleptic properties of wine and thus are important contributors to grape and wine quality (Dai and Mumper 2010; Teixeira et al. 2013). They influence the colour, astringency, bitterness, taste and mouthfeel of a wine (Mazerolles et al. 2010). The total phenolic content increased with increasing N amount, after N treatments, but was highest under the zero application without N (Chapter 2, Tab. 4). Phenolic content in grape berries varies with vintage and, with environmental and viticultural conditions (Kennedy 2008). Both, high and low levels of N have an influence on the phenolic content of grapes (Hilbert et al. 2003; Portu et al. 2015). The content of total phenolics in must and wine is highly dependent on the variety. In red wines, a high content of total phenolics is preferred as they increase the proportion of secondary metabolites such as tannins or the colouring anthocyanins strongly. White wines are more likely to be negatively affected by excessively high total phenolic levels, as they cause a lower glutathione content and thus fewer aroma precursors attributable to the control of oxidative spoilage (Choné et al. 2006; Kritzinger et al. 2013).

In addition, the phenolic content in must and wine is highly dependent on the N-form offered. In the case of urea application, the phenolic content is significantly reduced but is significantly increased when NH_3^- and NH_4^+ are applied (Chapter 2, Tab. 4). This pattern is also clearly visible in the metabolite profile. The tentative phenolic compounds, measured within the metabolic profiling, of both leaves and wine are significantly reduced in abundance when urea is applied but increase when NH_3^- or NH_4^+ is applied (Chapter 4, Tabs. 4b and 5b).

As discussed in sections 6.2 and 6.3.1, the maturation of the berries, the chemical and oenological parameters and, thus, quality is strongly dependent on vegetative growth. An altered N supply leads to a competition for nutrients and thus to a changed sink : source ratio. If biomass production is increased more than berry growth, then the storage of high quality metabolites is disrupted. This can result in a reduction of the aroma precursors that accumulate in the berry. The alteration of the metabolite profile and especially of the tentative phenolic compounds might be attributable to a change in N assimilation and thus to an altered N distribution in the grapevine when urea is applied. As shown and discussed in the hydroponic experiments (Chapter 2 and Chapter 6.1), the assimilation of urea in the roots is reduced in comparison with NO_3^- and NH_4^+. Therefore, urea might be available to a lower extent to the grapevine, compared with NO_3^- and NH_4^+, and thus fewer phenolic compounds accumulate in the berries.

6.4 Aroma and sensory profile

Wine aroma is described as a complex equilibrium of compounds that constantly interact with each other (Cañas et al. 2018). Mainly primary and secondary metabolites and, more precisely, volatile and non-volatile compounds alter wine aroma (Bell and Henschke 2005; Francis and Newton 2005). Environmental conditions, genotype and viticultural practices can have a huge impact on aroma formation in grapes and wine (reviewed by Hernandez-Orte et al. 2015; Robinson et al. 2014).

The influence of N fertilization on the aroma profile and sensory effects in wine is a matter of controversy (Bell and Henschke 2005; González-Barreiro et al. 2015). The present results are similarly contentious (Chapter 4, Fig. 1). On one hand, a significant increase in individual aroma attributes influenced by the N-form is apparent. Differences between the N-forms can be observed; NO_3^- and urea show the highest differences. On the other hand, the change also depends on whether N application even took place (zero application), *i.e.* NO_3^- or NH_4^+ treatment compared with the control treatment. The results show a similar pattern for NO_3^- and urea application as that previously discussed (Chapter 6). The N-forms NO_3^- and urea have the highest impact, clearly suggesting that aroma formation and thus the sensory aspect of wine is dependent on N availability and N assimilation level in the grapevine. Numerous aroma components in the berry are responsible for flavour and aroma in must and wine, some of these substances are directly or indirectly

linked to the N availability for the grapevine. During fermentation, some of these substances are metabolized and are considered as precursor compounds for aroma (Bell and Henschke 2005). The influence can be both positive and negative, since the individual components are regulated differently.

6.5 Nitrogen and water availability in AF systems have no negative effect on the quality of the wine

Chapter 5 describes an additional project that is difficult to relate to the experiments of the previous chapters because it involves a completely different cropping system (agroforestry system) and the associated influences of the tree on the growth and physiology of the grapevine. However, as some similar points to the association between N and wine quality arise, therefore wine quality is discussed here from the point of view of an intercropping cultivation system.

In an AF system, competitive circumstances or interactions often occur between cultivated crops. These interactions can be both positive and negative (Jose et al. 2004). Water and nutrients, especially N, are of upmost importance for viticultural production (Keller 2005; Stefanelli et al. 2010). However, the preliminary results showed no competition for water or N under the conditions studied. Furthermore, the mixed cropping system enhanced water, N availability and N acquisition to the grapevine, but this was variety-dependent (Chapter 5, Figs. 2-5). Environmental conditions and viticultural practices clearly determine the quality of the wine (reviewed by Hernandez-Orte et al. 2015). According to van Leeuwen et al. (2009) and Bell and Henscke (2005), water and N are among the most important components for berry quality and thus also for the quality of wine. Since no competition for N or water was observed, we can assume that these differences are attributable to the trees, their possible allelopathic effects and the release of allelochemicals. The present results indicate that the trees have a small influence on the chemical parameters in the wine, mainly with regard to pH and acidity, but no clear pattern is seen (Chapter 5, Tab. 1). Furthermore, no significant differences, just tendencies, in the aroma and sensory evaluation were determined. This could be because of the lack of N competition. The discussion in Chapter 6.3.3 leading to the conclusion that N has a controversial influence on the formation of aroma and sensory in wine is hereby supported.

Although in the Roman Empire the intercropping cultivation system of grapevines and trees was a very traditional cultivation form, nevertheless, it is almost forgotten

today. In addition to resource-saving cultivation, multiple uses of tree cultivation and increase of biodiversity, this type of cultivation can show new marketing strategies for the highly competitive viticulture.

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CHAPTER 7

Summary

Summary

Viticulture and the vinification of vines (*Vitis vinifera L*.) to wine is an important branch in agriculture world-wide. Berry quality and the associated wine quality are the driving factors here. Nitrogen (N) is the most important plant nutrient for the grapevine. In addition to its influence on vegetative and generative growth, it determines significantly the metabolite composition and the oenological parameters of the grape berry. Nitrogen is present in various forms, such as nitrate, ammonium or amino acid, in the individual plant organs and is used differently by the grapevine. Grapevines are believed to have the ability to assimilate N in various forms, which in turn may affect the quality of berries and the resulting wine.

For a better understanding of the effects of N on berry and wine quality, knowledge of which N-form can be assimilated by the vine and the way that this affects oenological parameters and quality-giving metabolites is essential. To this end, several investigations were carried out at various test levels, starting with hydroponic experiments, a pot experiment and a further field experiment, and on the matured wine. The various N-forms of nitrate, ammonium, urea and the amino acids arginine and glutamine were applied, following which the plant-physiological reactions of the grapevine and quality-determining parameters in berry and wine were measured. Furthermore, a metabolite profile with a focus on phenolic components was prepared and a sensory analysis of the wine was performed.

The grapevines in the hydroponics and pot experiments were treated with 4 mM total N. The grapevines in the field experiment were fertilized with 60 kg N ha^{-1}, calculated in relation to the block size. The rootstocks SO4 and RU140 showed similar patterns of N assimilation with respect to the N-form but differed significantly with regard to the level of growth and N content among all N-forms. The N-sensitive rootstock SO4 reacted more strongly than the rootstock RU140 and, therefore, SO4 was used for further experiments. This suggests that grapevines are able to assimilate the amino acids glutamine and arginine, as also shown by the enzymatic nitrate reductase activity and the increased abundance of the transcripts of nitrate reductase and nitrite reductase. Nevertheless, the N-forms NO_3^- and NH_4^+ were preferentially assimilated. The assimilation under urea treatment was significantly reduced. In addition to the N-form, the amount of N applied had an influence on N assimilation in the grapevine. With increasing amounts, the vegetative and generative growth increased up to a threshold. However, if this threshold was exceeded, both were significantly reduced. If the grapevine is overfertilized, the

sink : source ratio changes, which will lead to a change in the biomass production and furthermore to a saturation and storage of N. In addition, competition for assimilates occurs, this alters the N distribution and N availability within the plant and the berries. The N-form has no influence on berry yield.

The oenological and chemical parameters of the must and the wine are of enormous importance for product quality. The key components include pH and acidity, which contribute significantly to the organoleptic properties of wine. Both factors are influenced by the N-form and the amount of N offered. As the amount of N increases, the pH increases and the acidity decreases. The N-forms NO_3^- and urea and, the zero application (without additional N) show the highest influences. The must weight is a defining factor reflecting the berry's maturity and thus the time of harvest. As the amount of N increases, the must weight decreases. On the one hand, an increased N amount leads to lower acidity in the berry, indicating that more sugar is being stored and that the berry is in an advanced stage of maturity. On the other hand, an increased N amount leads to a decreasing must weight, which leads further to a maturation delay. The total phenolic content increases with increasing N amount, but is highest following zero N application. Tentative phenols measured in the metabolite profile are markedly down-regulated after urea treatment and are upregulated with NO_3^- following NH_4^+ treatment. This result might arise from reduced N assimilation in the root and thus reduced N availability for the berries.

The influence of N on the aroma and sensory aspects of wine is controversial. The individual aroma attributes show both an increase and a decrease in their intensity attributable to N, mainly urea and NO_3^-. A marked influence between N-treated vines and the zero application is also apparent. However, these contrasting results clearly show that aroma and thus the sensory characteristics of wine can be influenced both positively and negatively.

The results of the aroma and sensory evaluation in the agroforestry system underline once again the controversial influence of N on the sensory features of wine; no significant influence was measured.

In summary, N has a significant influence on the vegetative and generative growth of the grapevine. The influence of N can be both positive and negative and is in part directly or indirectly linked to wine quality and should therefore not be ignored.

CHAPTER 8

Zusammenfassung

Zusammenfassung

Der Weinbau und der Ausbau von Weinreben (*Vitis vinifera* L.) zu Wein ist ein bedeutender Zweig in der Landwirtschaft weltweit. Die Beerenqualität und die damit verbundene Weinqualität gelten hierbei als treibende Faktoren. Stickstoff (N) ist der wichtigste Pflanzennährstoff für die Weinrebe. Neben seinem Einfluss auf das vegetative und generative Wachstum, bestimmt er maßgeblich die Metabolitenzusammensetzung und die oenologischen Parameter der Weinbeere. Stickstoff liegt in diversen Formen, zB. als Nitrat, Ammonium oder Aminosäuren in einzelnen Pflanzenorgangen vor und wird von der Weinrebe unterschiedlich genutzt. Es wird vermutet, dass die Rebe die Fähigkeit besitzt, N in verschiedenen Formen zu assimilieren, das sich wiederrum auf die Beeren- und Weinqualität auswirkt.
Für ein besseres Verständnis, wie N die Beeren- und Weinqualität beeinflusst, ist es von enormer Bedeutung zu wissen, welche N-Form von der Rebe assimiliert werden kann. Um dies zu erreichen, wurden mehrere Versuche auf verschiedenen Versuchsebenen, von der Hydrokultur, über einen Topfversuch, bis hin zum Feldversuch und dem ausgebauten Wein, durchgeführt. Die N-Formen Nitrat, Ammonium, Harnstoff sowie die Aminosäuren Arginin und Glutamin wurden appliziert und pflanzenphysiologische Reaktionen der Weinrebe sowie qualitätsbestimmende Parameter in Beere und Wein gemessen. Des Weiteren wurde ein Metabolitenprofil mit dem Fokus auf phenolische Komponenten erstellt und eine sensorische Analyse des Weins durchgeführt.

In den hydroponischen Kulturen als auch im Topfversuch wurden die Weinreben und die Unterlagsreben mit 4 mM reinem N behandelt. Im Feldversuch wurden die Weinreben mit 60 kg N ha^{-1} versorgt und auf die jeweilige Parzellengröße berechnet. Die Unterlagsreben SO4 und RU140 zeigten gleiche Muster in der N-Assimilation in Bezug auf Biomassebildung und N-Gehalt unter allen N-Formen, unterschieden sich jedoch deutlich in der Höhe der Ausprägung. Die N sensitive Unterlage SO4 reagierte stärker als die Unterlage RU140, daher wurde SO4 auch für die weiteren Versuche verwendet. Es konnte gezeigt werden, dass Weinreben in der Lange sind, auch die Aminosäuren Glutamin und Arginin zu assimilieren. Die enzymatische Nitratreduktaseaktivität sowie die gesteigerte Abundanz der Transkripte von Nitratreduktase und Nitritreduktase zeigten dies ebenfalls. Dennoch wurden die N-Formen NO_3^- und NH_4^+ präferiert assimiliert, deutlich verringert war die Assimilation unter Harnstoff. Neben der N-Form hatte vor allem die Menge an appliziertem N einen Einfluss auf die N-Assimilation. Mit steigender N-Menge,

stiegen vegetatives und generatives Wachstum bis zu einem Schwellenwert an. Wurde dieser Schwellenwert jedoch überschritten, wurde beides signifikant reduziert. Wird die Weinrebe überdüngt, kommt es zu einer Veränderung im sink : source Verhältnis, dies führt zu einer Veränderung in der Biomassebildung und weiterhin zu einer Übersättigung und Einlagerung von N. Des Weiteren kommt es zu einer Konkurrenzsituation um Assimilate, dies verändert die N-Verteilung und die N-Verfügbarkeit in der Pflanze und in den Beeren. Die N-Form hatte auf den Beerenertrag keinen Einfluss.

Die oenologischen und chemischen Parameter des Mostes und des Weins sind von enormer Bedeutung für die Qualität. Zu den wichtigsten Komponenten gehören der pH-Wert und der Säuregehalt, sie tragen maßgeblich zu den organoleptischen Eigenschaften des Weins bei. Beide Faktoren wurden von der N-Form und der angebotenen N-Menge beeinflusst. Mit steigender N-Menge, stieg der pH-Wert und der Säuregehalt sank. Die N-Formen NO_3^- und Harnstoff, sowie die unbehandelte Variante (keine N Applikation) zeigten den größten Einfluss. Das Mostgewicht gilt als definierender Faktor für die Reife der Beeren und bestimmt somit den Lesezeitpunkt. Mit steigender N-Menge sank das Mostgewicht. Einerseits führt eine erhöhte N-Menge zu einem geringeren Säuregehalt in der Beere, dies ist ein Indikator dafür, dass mehr Zucker eingelagert wird und die Beere sich in einem fortgeschrittenen Reifestadium befindet. Andererseits führt eine erhöhte N-Menge zu einem sinkenden Mostgewicht, das zu einer Reifeverzögerung führt.

Der Gesamtphenolgehalt stieg mit steigender N-Menge, doch war er am höchsten bei der unbehandelten Variante. Die Phenole im Metabolitenprofil waren deutlich runterreguliert bei einer Behandlung mit Harnstoff und hochreguliert bei einer Behandlung mit NO_3^- und NH_4^+. Dies könnte aus einer verringerten N-Assimilation in der Wurzel und einer damit verringerten N-Verfügbarkeit für die Beeren resultieren.

Der Einfluss von N auf das Aroma und die Sensorik im Wein ist ein kontrovers diskutiertes Thema. Die einzelnen Aromaattribute zeigen sowohl eine Erhöhung, als auch eine Verringerung der Intensität durch den Einfluss von N, hauptsächlich durch Harnstoff und NO_3^-. Außerdem gibt es einen deutlichen Einfluss, zwischen N behandelten Weinreben und der unbehandelten Variante. Diese kontrastierenden Ergebnisse zeigen jedoch deutlich, dass Aroma und somit auch die Sensorik von Wein sowohl positiv als auch negativ beeinflussbar ist.

Die Ergebnisse der Sensorik im Agroforstsystem unterstreichen nochmals den kontroversen Einfluss von N auf die Sensorik, es konnte kein signifikanter Einfluss gemessen werden.

Zusammenfassend kann man sagen, dass Stickstoff einen deutlichen Einfluss auf das vegetative und generative Wachstum der Weinrebe hat. Diese Einflüsse können sowohl positiv als auch negativ (Überdüngung) sein und sind teilweise direkt oder indirekt mit der Weinqualität verbunden und sollten deshalb nicht außer Acht gelassen werden.

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Curriculum Vitae

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…….....

(Place, date) (Signature)

Affidavit

pursuant to Sec. 8(2) of the University of Hohenheim's doctoral degree regulations for Dr.sc.agr.

1. I hereby declare that I independently completed the doctoral thesis submitted on the topic

Profiling of physiological responses and quality aspects in *Vitis vinifera* L. as influenced by aspects of N application.

2. I only used the sources and aids documented and only made use of permissible assistance by third parties. In particular, I properly documented any contents which I used - either by directly quoting or paraphasing - from other works.

3. I did not accept any assistance from a commercial doctoral agency or consulting firm.

4. I am aware of the meaning of this affidavit and the criminal penalties of an incorrect or incomplete affidavit.

I hereby confirm the correctness of the above declaration. I hereby affirm in lieu of oath that I have, to the best of my knowledge, declared nothing but the truth and have not omitted any information.

…….....

(Place, date) (Signature)

Affidavit

Information

The University of Hohenheim requires an affidavit declaring that the academic work was done independently in order to credibly claim that the doctoral candidate independently completed the academic work.

Because the legislative authorities place particular importance on affidavits, and because affidavits can have serious consequences, the legislative authorities have placed criminal penalties on the issuance of a false affidavit. In the case of wilful (that is, with the knowledge of the person issuing the affidavit) issuance of a false affidavit, the criminal penalty includes a term of imprisonment for up to three years or a fine.

A negligent issuance (that is, an issuance although you should have known that the affidavit was false) is punishable by a term of imprisonment for up to one year or a fine.

The respective regulations can be found in Sec. 156 StGB (Criminal Code) (false affidavit) and in Sec. 161 StGB (negligent false oath, negligent false affidavit).

Sec. 156 StGB: False Affidavit:
Issuing a false affidavit to an authority body responsible for accepting affidavits or perjury under reference to such an affidavit shall be punishable with a term of imprisonment up to three years or with a fine.

Sec. 161 StGB: Negligent False Oath, Negligent False Affidavit:
Subsection 1: If one of the actions described in Secs. 154 and 156 is done negligently, the action shall be punishable by a term of imprisonment of up to one year or a fine.

Subsection 2: Impunity shall apply if the perpetrator corrects the false information in a timely manner. The regulations in Sec. 158 (2) and (3) apply mutatis mutandis.

The German original version of this affidavit is solely valid; all other versions are merely informative.

I have taken note of the information on the affidavit.

……....

(Place, date) (Signature)