OENO One
VINE AND WINE OPEN ACCESS JOURNAL
Volume 51 > Number 3 > 2017

Effects of liming on soil properties, leaf tissue cation composition and grape yield in a moderately acid vineyard soil. Influence on must and wine quality

Miguel Javier Quiroga[1], Miguel Ángel Olego[2*], Mario Sánchez-García[1], Jesús Esteban Medina[1], Fernando Visconti[3], Juan José Rubio Coque[1] and José Enrique Garzón Jimeno[1]

[1]Instituto de Investigación de la Viña y el Vino (IIVV). Universidad de León. Avenida de Portugal, 41, CP 24071, León, España
[2]RGA Bio-Investigación, SL. Laboratorio 29. Instituto de Recursos Naturales. Avenida de Portugal, 41, CP 24071, León, España
[3]Instituto Valenciano de Investigaciones Agrarias-IVIA, Centro para el Desarrollo de la Agricultura Sostenible, Crta. Moncada-Náquera km 4.5, CP 46113 Moncada, Valencia, España

Abstract

Aims: Soil acidity decreases soil fertility and grapevine growth. Aluminum toxicity has been recognized as one of the most common causes of reduced grape yields in acid vineyard soils. The main aim of this study was to evaluate the effect of two liming materials, i.e. dolomitic lime and sugar foam, on a vineyard cultivated in an acid soil.

Methods and results: The effects were studied in two soil layers (0-30 and 30-60 cm), as well as on leaf nutrient contents, grape yield, and must and wine quality properties, in a vineyard dedicated to *Vitis vinifera* L. cv. Mencía cultivation. The data management and analysis were carried out using ANOVA.

Conclusion: Sugar foam was more efficient than dolomitic limestone as liming material since it induced the highest decrease in soil acidity properties at the same calcium carbonate equivalent dose. Effects of liming on leaf nutrient contents, grape yield, and must and wine quality properties were barely observed.

Significance and impact of the study: Until recently, little was known about the effects of liming on both vine nutritional status and must/wine quality properties. Thus, this research fills an important knowledge gap.

Keywords: soil acidity, Mencía, veraison, aluminum saturation, total acidity

Abbreviations used (in alphabetical order)

%Al/CECe: aluminum saturation of the effective cation exchange capacity
A: absorbance
AAS: atomic absorption spectroscopy
AF: alcoholic fermentation
ANOVA: analysis of variance
C: control
C1: contrast 1
C2: contrast 2
Cab: calcium concentration in blades
Caff: caffeic acid
Cag: calcium concentration in grape seeds
Cap: calcium concentration in petioles
Cat: (+)-catechin
CCE: calcium carbonate equivalent
CEC: cation exchange capacity
CECe: effective cation exchange capacity
CI: confidence interval
C.I.: color index
Cou: p-coumaric acid
Epi: (-)-epicatechin
Fer: trans-ferulic acid
Gall: gallic acid
GY: grape yield
ICP-AES: inductively coupled plasma atomic emission spectroscopy
Kb: potassium concentration in blades
Kg: potassium concentration in grape skins
Kp: potassium concentration in petioles
Malv: malvidin-3-O-glucoside
Mgb: magnesium concentration in blades
Mgg: magnesium concentration in grape seeds
Mgp: magnesium concentration in petioles
pH: real acidity
pH_{KCl}: pH in KCl 1N
pH_W: pH in water
Resv: trans-resveratrol
S: percentage of base saturation
SF: sugar foam
SOM: soil organic matter
SPE: solid phase extraction
ta: tartaric acid
TA: total acidity
TAnth: total anthocyanin content
Tc: tannin concentration
TPI: total polyphenol index
TSS: total soluble solids
W100: weight of 100 berries

manuscript received 4th December 2016 - accepted 8th March 2017
doi: 10.20870/oeno-one.2017.51.4.2039

*Corresponding author: molem@unileon.es

OENO One, 2017, **51**, 4, 342-362

Introduction

With an approximate land area affected by soil acidity of 4 billion ha, representing about 30% of the total ice-free land area of the world (Sumner and Noble, 2003), this edaphic characteristic is one of the most yield-limiting factors for crop production as a result of high Al^{3+} levels and low availability of basic ions in both surface and subsurface soil.

Liming is one of the main methods used by farmers to enhance the fertility of acid soils because it decreases the contents of exchangeable Al^{3+} through replacement with Ca^{2+} and Mg^{2+}, and also the contents of soluble Al^{3+} by precipitation with the hydroxyl anions generated by carbonate hydrolysis in the soil solution. Additionally, liming has beneficial effects on soil structure since it improves soil structural stability (Lanyon, 2001; Hansen and Cass, 2002), and it plays a significant role in the control of certain plant pathogens (Havlin *et al.*, 2014). The effectiveness of liming materials depends on (i) their neutralizing power, which is accounted for by their calcium carbonate equivalent (CCE), and (ii) their fineness (Edmeades and Ridley, 2003; Álvarez *et al.*, 2009). However, soil has a huge buffering capacity that is able to diminish the effects of all kinds of amendments, and the soil acidification process is accelerated by factors such as acid rain and excess application of ammonium-based inorganic nitrogen fertilizers (Zheng, 2010).

Liming is usually performed before vineyard implantation, increasing the soil pH and cation exchange capacity (CEC) values (Joris *et al.*, 2012). Appropriate soil liming after vineyard planting is a high-potential strategy to minimize the toxic effects of Al in established vineyards. However, some common liming materials, such as limestone ($CaCO_3$), used in agriculture to increase yield potential lose their effectiveness in vineyards established on acid soils due to the low solubility and mobility of carbonate in the soil profile, the liming effects being usually limited to the lime application/incorporation sites (Soratto and Crusciol, 2008). Among certain strategies used to ameliorate subsurface soil acidity, it can be cited the replacement of lime by the surface application of more soluble materials (Castro and Crusciol, 2013).

Dolomitic limestone and sugar foams are two materials often used for liming. Dolomitic limestone comprises mainly the mineral dolomite, which is made of a calcium and magnesium double carbonate ($CaMg(CO_3)_2$). Two important characteristics of this liming material are (i) its high neutralizing capacity, which is higher than that of limestone because of the lower atomic weight of magnesium compared to calcium, and (ii) its low dissolution rate, which is approximately 100-fold lower than that of calcite (Loeppert and Suarez, 1996). Sugar foams are sugar beet-manufacturing residues, which arise from the purification-flocculation of colloid matter in the beet extract by treatment with lime and carbon dioxide (Vidal *et al.*, 2006). This industrial by-product can be used to correct soil acidity and Al phytotoxicity in acid soils because of its high content in active lime. In addition, this liming material contains abundant organic matter and several essential micronutrients (Vidal *et al.*, 1997).

Although the application of sugar foams to the soil is a common practice used worldwide, especially in the last 20 or 30 years due to the improvements observed in some soil properties (Sikora and Azad, 1993), little is known about its effects on the quantity and quality of grapes cultivated on acid soils. Sugar foams may effectively counteract the symptoms of aluminum toxicity in plants, which brings about poor growth and low yields. Specifically, in old vineyards on acid soils, the root system ends abruptly at the depth where the pH value drops and the Al^{3+} concentration becomes relatively important (Meyer *et al.*, 1984). In the same way, Cançado *et al.* (2009) reported that, as in many other plant species, the primary site and target of Al stress in grapevine rootstocks is confined to the actively growing root tip, revealed by a severe inhibition of root growth. Moreover, Kirchhof *et al.* (1991), in their research on *Vitis vinifera* cv. Chardonnay, showed how acidic soil conditions, mainly in the subsoil, depressed growth of vine root system. Finally, Wooldridge *et al.* (2010), on a mixture of cv. Pinot noir and Chardonnay with several rootstock vines cultivated on acid soils, found a more reduced root stress when compared to those cultivated on unlimed soils.

Although extensive research has been carried out on the effects of liming on the properties of acid soils, its effects on nutrient distribution in vine vegetative parts (leaf blades and petioles) and grape berries as well as on crop yield and must and wine quality are still not fully understood. So far the influence of soil liming on Ca, Mg and K contents in grape berries has not been investigated. This is an important knowledge gap because these elements remarkably affect grape juice quality and therefore the vinification process. In the present work, we analyzed the effects of adding two different lime amendments, i.e. dolomitic limestone and sugar foam, on (i) soil acidity, (ii) concentration ranges of Ca, Mg and K in leaf tissues, i.e. blades and petioles, and grape berries, (iii) grape

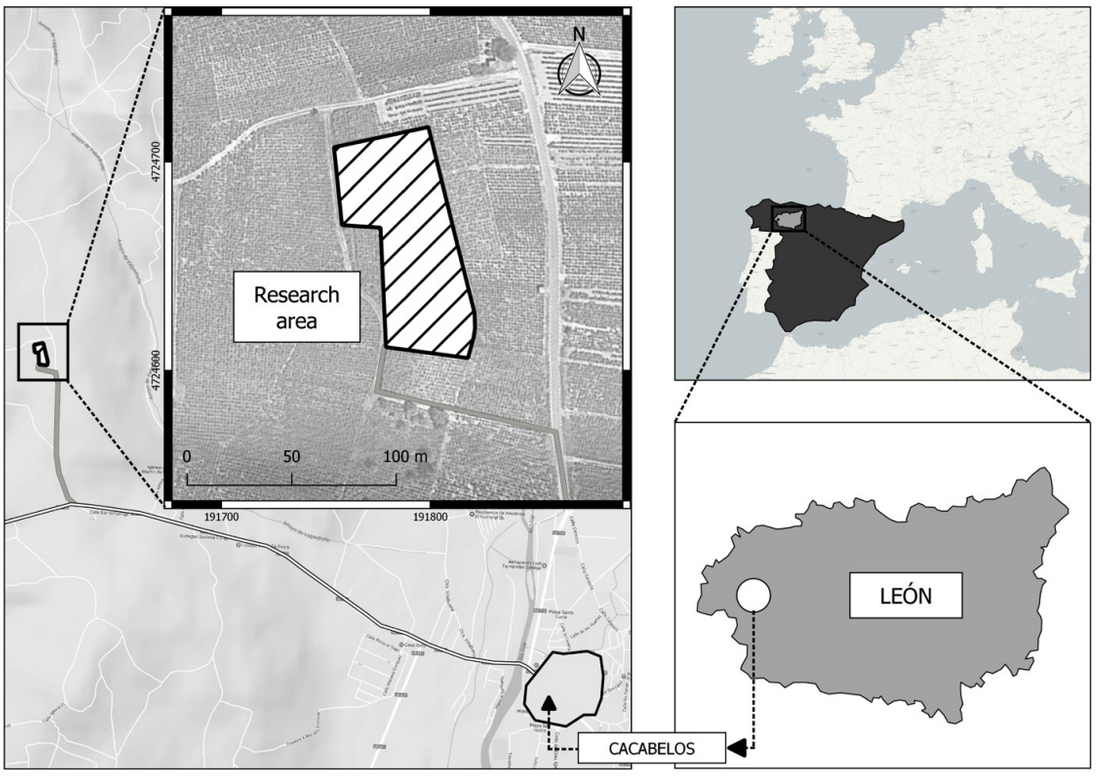

Figure 1. Map of the location of the research area (latitude and longitude are showed in Universal Transverse Mercator coordinates).

yield and, finally, (iv) must and wine quality characteristics.

Materials and methods

1. Study site

A commercial vineyard located 556 meters above sea level in the municipality of Cacabelos (León; Spain; 42°36'N lat and 6°45'W long) was selected as the study site (Figure 1). From a climatic point of view, the grape growing region would be classified as Region I (≤ 1,390 Celsius degree-days) based on the classification system of Amerine and Winkler (Jackson, 2014). The mean reference evapotranspiration (FAO Penman-Monteith) and mean rainfall were, respectively, 922 and 616 mm yr^{-1} for 2000-2011 (SIAR, 2012). From a bioclimatic point of view, the site would be classified as upper meso-Mediterranean based on the «thermotypes» classification and subhumid based on the «ombrotypes» classification (IGME, 1995). The soil under study corresponded to an Inceptisol, suborder Xerept, group Haploxerept according to Soil Survey Staff (2010). The parent material of the soils in the study area is made of Tertiary sediments (IGME, 1995). Accordingly, Fe oxyhydroxides are the most common clay minerals in the vineyard soils developed on these Tertiary sediments (Fernández-Calviño *et al.*, 2009), from which calcium minerals are almost completely absent.

2. Experimental treatments

The research was conducted on a 60-year-old *Vitis vinifera* L. cv. Mencía vineyard over three consecutive seasons (2009, 2010 and 2011), and the planting density was 4,166 (1.5 m × 1.6 m) plants per hectare. The plants had been grafted on Rupestris du Lot rootstock, which is considered as highly sensitive to soil acidity (Fráguas, 1999). Planting lines displayed a north-south orientation. The trellising system involved head training, with 4-5 arms per plant. Winter pruning left thumb-sized arms with two buds. The vineyard had no irrigation system. No fertilizers or extra amendments other than those used in this research were applied during the study period. The liming factor was applied at three levels (control (C), dolomitic limestone (DL) and sugar foam (SF)), with three replications per level. The study plot was split into nine 450-m^2 subplots. The treatment replications were distributed among the nine subplots in a completely random design.

OENO One, 2017, **51**, 4, 342-362

Table 1. Chemical composition of the liming materials expressed on a dry matter basis.

	Ca	Mg	Na	K	Al	Fe	Mn	Cu	Zn	OM	CCE
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(g kg^{-1})	(g kg^{-1})
Dolomitic limestone	222	111	0,89	2,91	9529	10483	361	12	26	0	1012
Sugar foam	289	8,75	0,3	0,75	2469	1420	121	12	32	79	758

OM, organic matter; CCE, calcium carbonate equivalent.

Table 2. Average characteristics before liming in the 0-30 and 30-60 cm soil layers.

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Textural class (USDA)		pH_W	pH_{KCl}
º	17,5	52,2	30,3	Sandy clay loam		5,01	3,94
30-60	21,5	48,2	30,3	Clay loam		4,98	3,98
	EC	SOM	P	Ca	Mg	K	
	(dS m^{-1})	(%)	(mg kg^{-1})	(cmol(+) kg^{-1})	(cmol(+) kg^{-1})	(cmol(+) kg^{-1})	
0-30	0,04	2,14	10,2	1,95	0,52	0,22	
30-60	0,05	1,61	7,87	1,98	0,52	0,12	
	Al	Fe	Mn	Cu	Zn	S	
	(cmol(+) kg-1)	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(%)	
0-30	1,58	127	18,9	5,82	1,33	62,9	
30-60	1,38	121	19,7	2,5	1,04	65,4	

pH_W, pH in water; pH_{KCl}, pH in KCl 1N; EC, electrical conductitvity; SOM, soil organic matter; dS, deciSiemens; S, percentage of base saturation.

3. Characterization of the liming materials and liming dose

The composition of the two liming materials used in this study (Villa, 2005) is shown in Table 1. The dolomitic limestone exhibited a higher Mg content than the sugar foam, whereas this latter presented a higher organic matter content. The high Ca content of the sugar foam is due mainly to the presence of Ca in the form of slaked lime ($Ca(OH)_2$) and, to a lesser extent, as carbonate ($CaCO_3$) (Espejo, 2001). The slaked lime progressively reacts with atmospheric carbon dioxide to produce $CaCO_3$. This carbonation occurs at a rate that depends on the aggregate size and porosity, and water content of the sugar foam.

The liming rates were established with the aim of decreasing the aluminum saturation of the effective cation exchange capacity (%Al/CECe) down to 20%. Achieving a %Al/CECe of 20% ensures an adequate degree of base saturation, i.e. 80%, required by most crop plant species (Fageria and Baligar, 2008). Specifically, the lime requirement was calculated using the known Cochrane's formula (Cochrane *et al.*, 1980) and resulted to be 2,720 kg CCE ha^{-1}, which corresponded to 2,800 kg of dolomitic lime ha^{-1} and 3,900 kg of sugar foam ha^{-1} according to the CCE contents of each material (Table 1). The dolomitic limestone was in a powdery state, whereas the sugar foam consisted of aggregates of variable size, which were manually disaggregated before incorporation into the soil. The liming materials were uniformly spread onto the entire surface of the subplots and incorporated with one-pass tillage in November 2008.

4. Soil sampling and analyses

Before the amendments were added, the following soil properties were evaluated at two soil depths (0-30 and 30-60 cm) using the methods indicated below: texture, soil organic matter (SOM), pH in water (pH_W), pH in KCl 1N (pH_{KCl}), electrical conductivity (EC), phosphorus, Ca, Mg, K and Al contents, the percentage of base saturation (S) and the micronutrient contents (Fe, Cu, Mn, and Zn) (Table 2).

After the amendments were applied in late November 2008, the effects of liming on the following soil properties were monitored: pH_W, S, and exchangeable Ca, Mg, K and Al contents. This monitoring was conducted by sampling the soil at 0-30 and 30-60 cm depths at the veraison stage, which occurs in August in the study area.

The soil samples were collected using an auger, transported to the laboratory in plastic bags and air-dried at room temperature. Next, they were

disaggregated to pass through a 2-mm mesh sieve and analyzed. Textural classes according to USDA were determined by the Bouyoucos hydrometer method (Bouyoucos, 1962). Official methods of analysis (MAPA, 1993) were used for the determination of (i) SOM by wet oxidation followed by titration with ferrous ammonium sulfate, (ii) pH in a soil:water (1:2.5) suspension, (iii) EC at 25°C (EC_{25}) in a soil:water (1:2.5) suspension, and (iv) the content of exchangeable cations (Ca, Mg, and K) by successive extraction with ammonium acetate 1M ($NH_4C_2H_3O_2$) and subsequent analysis of the displaced cations by atomic absorption spectrometry (AAS). The exchangeable Al was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) using KCl 1M as the extraction solution (Little, 1964). The micronutrients (Fe, Cu, Mn, and Zn) were extracted following the method of Lindsay and Norvell (1978) using a buffer solution of diethylenetriaminepentaacetic acid (DTPA) 0.005 M and calcium chloride ($CaCl_2$) 0.01 M buffered to pH 7.3 and determined by AAS. The P levels were determined by UV-visible spectroscopy after successive extraction with sodium bicarbonate 0.5 M at pH 8.5 following the method proposed by Olsen *et al.* (1954). CECe was obtained from the arithmetic sum of the concentrations of the exchangeable Ca, Mg, K and Al. S was calculated by dividing the sum of Ca, Mg and K by the CECe.

5. Leaf sampling and analyses

The Ca, Mg and K contents in blades (Cab, Mgb and Kb, respectively) and petioles (Cap, Mgp, and Kp, respectively) were annually monitored along with the soil properties at the veraison stage. Specifically, 30 basal leaves opposite grape bunches were randomly collected per subplot each year in early August. The leaves were sealed in plastic bags, transported to the laboratory, carefully rinsed with abundant deionized water, and then dried for three days at 70°C (Bavaresco *et al.*, 2010). Then, they were wet digested with an acid mixture of perchloric, sulphuric and nitric acid at 420 °C for 20 minutes (Calleja, 1978), and the cation contents in the extracts determined by ICP-AES.

6. Grape sampling and analyses

The grapes were sampled at harvest every year on 12th, 20th and 11th September of 2009, 2010 and 2011, respectively. The harvested grapes from each subplot were weighted to determine yield (GY). Next, 300 grape berries per subplot were randomly chosen to determine the weight of 100 berries (W100) and must quality. In order to calculate W100, 100 of these berries were weighted. The grape must of each subplot was obtained manually from the 300 berries by gently pressing the grapes, using rubber gloves to avoid sample contamination. In the must obtained, the following quality properties were determined: (i) real acidity (pH), (ii) total soluble solids (TSS), which were measured using a refractometer, and (iii) total acidity (TA), which was determined by titration of the grape must with sodium hydroxide (0.1 N) to an endpoint of pH 7, and expressed as the equivalent content of tartaric acid (ta) in g l^{-1} (OIV, 2014).

The content of each nutrient in grape berries must be investigated in the berry part that acts as its main reservoir. Thus, the concentrations of Ca and Mg were studied in grape seeds (Cag and Mgg, respectively), whereas K content (Kg) was studied in grape skins (Conde *et al.*, 2007; Keller, 2010). The seeds and skins from 100 grapes were manually separated from the flesh and immediately dried at 60°C to constant weight. Ca and Mg in dried seeds and K in dried skins were determined by ICP-AES after wet digestion with an acid mixture of perchloric, sulphuric and nitric acid at 420 °C for 20 minutes (Calleja, 1978).

7. Winemaking process

Winemaking was performed in three consecutive vintages (2009-2011) for all of the biological replicates. The damaged grape clusters (those broken or with visual microbial alterations) were discarded in order to avoid undesirable contamination and degradation compounds. Selected clusters were de-stemmed, crushed and macerated for 24-48 hours at 10°C (with 0.05 g l^{-1} of SO_2) in 1,000-l stainless steel (AISI 3162B) tanks with cooling jacket (Industrias Céspedes e Hijos, SL, Salvatierra de Miño, Spain). After correcting total acidity (6.5 g l^{-1} of tartaric acid), the grape extract was inoculated with 0.1 g l^{-1} of previously hydrated commercial yeast *Saccharomyces bayanus* P51 and *Saccharomyces bayanus* Levuline FB (AZ3 Oeno SL, Hernani, Gipuzkoa, Spain). Alcoholic fermentation (AF) was carried out at 25-28°C. The juice was drawn and pumped over the cap daily during the fermentation period. Fermentation was considered completed when both density reached approximately 0.90 g ml^{-1} (measured using a hydrometer) and glucose and fructose were consumed (measured by enzymatic analysis).

At the end of AF, the wine was transferred to another tank and left on its lees for 24-48 hours. After that, the wine was transferred again to another tank and

OENO One, 2017, **51**, 4, 342-362

left on its light lees for 6-8 weeks, removing the wine periodically with nitrogen gas. After that, the wine was transferred into 225-l medium toast, fine grain French oak barrels (for economic reasons, 2nd use in 2009 and 3rd use in 2010 and 2011) where malolactic fermentation took place. The wine was maintained for 12 months in these barrels.

8. Tannin concentration and wine color analyses

Tannin concentration (Tc, in g l^{-1}) was determined by the method proposed by Glories (1988, cited by Ribéreau-Gayon *et al.*, 2006), whereas hue, color index (C.I.), total polyphenol index (TPI) and total anthocyanin content (TAnth, in mg l^{-1}) were determined by absorbance measurements (A, in nm) in UV-visible spectroscopy (Ruiz Hernández, 2002). More specifically, hue was evaluated as the quotient of A_{420} and A_{520}, C.I. as the summation of A_{420}, A_{520} and A_{620} multiplied by the quotient of factor dilution and optical path, TPI as the multiplication between A_{280} and the factor dilution and, finally, TAnth as the multiplication between A_{520}, the conversion factor 22.76 and the factor dilution. Samples were collected both before and after must AF.

9. Wine phenolic compounds

The following phenolic compounds were measured in red wine samples by high-performance liquid chromatography (HPLC) after both AF and ageing time (12 months in French barrels): gallic acid (Gall), caffeic acid (Caff), p-coumaric acid (Cou), (+)-catechin (Cat), (-)-epicatechin (Epi), trans-resveratrol (Resv), malvidin-3-O-glucoside (Malv) and trans-ferulic acid (Fer).

Chemicals and phenolic standard solutions: Gallic acid, trans-caffeic acid, (+)-catechin hydrate, (-)-epicatechin, trans-ferulic acid and trans-resveratrol were supplied by Sigma Aldrich (St. Louis, MO, USA); cyanidin-3-O-lathyroside chloride, malvidin-3-O-glucoside chloride and 2-cumaric acid were supplied by Extrasynthèse (Lyon, France). Water used in the analysis was generated from a Milli-Q water purification system (Wasserlab, Barbatáin, Spain). Acetic acid, methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Wine samples were centrifuged at 3500 rpm for five minutes prior to any extraction.

Solid-phase extraction procedure (SPE): SPE was carried out according to the protocol described by Pérez-Magariño *et al.* (2008). All cartridges (HLB 6 cc 200 mg) were supplied by Oasis®. The sorbent type used (N-vinylpyrrolidone – divinylbenzene copolymer; average particle size/amount of 30 µm/200 mg) was supplied by Waters (Barcelona, Spain).

A manifold system (Waters, Barcelona, Spain) was used for SPE. All cartridges were conditioned by rinsing with 3 ml of methanol and 3 ml of Milli-Q water. The wine sample to be extracted (2 ml) was acidified with 1 N H_2SO_4 (0.5 ml) prior to loading onto the conditioned cartridge. All cartridges were washed with 5 ml of Milli-Q water. The retained phenolic compounds were eluted with 5 ml of ethyl acetate, followed by 5 ml of methanol. The fractions were collected separately to ascertain whether some of the phenolic compounds were eluted during the washing step. The collected fractions were evaporated also separately to dryness in a SAVANT Speed Vac Concentrator model SVC100H vacuum evaporator (T < 35°C) (Farmingdale, New York, USA) and immediately dissolved in a known volume (1 ml) of methanol/water (20:80).

HPLC analyses of phenolic compounds: The extracts obtained were analyzed with a Waters Alliance 2695 model, with a 2487 Dual λ Absorbance Detection System. Each extract was analyzed separately and previously filtered through 0.45-µm nylon filters (Symta, Madrid, Spain). The injection volume was 10 µl for each fraction. The chromatographic separation was carried out on a reverse-phase XBridgeTM-C18 column (250 mm × 4.6 mm i.d., 5 µm particle size) provided by Waters (Barcelona, Spain) and thermostated at 25°C. The chromatographic conditions were modified based on the method proposed by Pérez-Magariño *et al.* (2008). The solvents were (A) water/acetic acid (98:2 v/v) and (B) water/acetonitrile/acetic acid (78:20:2 v/v/v). The gradient was linear at a flow rate of 1 ml/min from 0 to 25% solvent B for 15 minutes, from 25 to 70% B for 20 minutes, and from 70 to 100% B for 25 minutes, then isocratic for 5 minutes, and after that from 100 to 0% B for 5 minutes and, finally, isocratic for 20 minutes.

The phenolic compounds analyzed were identified by comparing their retention times and UV-vis spectra with their respective standards. Cyanidin-3-O-lathyroside chloride was used as external standard. The quantification of the different phenolic compounds was carried out at 520 nm for Malv and 280 nm for the rest of phenolic compounds. The samples were thermostated at 4°C. The quantification of the different phenolic compounds was carried out applying each calibration line constructed using the corresponding standards. The linear calibration of all compounds was satisfactory with squared-R values above 0.99.

10. Comparisons between the liming treatments

Statistical analyses were performed using R software (R Core Team, 2015). Several analyses of variance (ANOVAs) were carried out to study the effect of liming, with three levels or groups, namely control, dolomitic limestone and sugar foam, on (i) the soil chemical properties (pH_W, S, Ca, Mg, K and Al), (ii) the leaf nutrient contents (Cab, Mgb, Kb, Cap, Mgp and Kp), (iii) the grape yield components (GY and W_{100}), (iv) the grape must quality properties (pH, TSS, TA), (v) the grape nutrient contents (Cag, Mgg and Kg), (vi) the tannin concentration (Tc), (vii) the wine color parameters (hue, C.I., TPI and TAnth) and, finally, (viii) the wine phenolic compound content.

In the ANOVAs of the soil chemical properties, the soil depth was also taken into account as a block factor with two levels or groups, i.e. shallow soil (0-30 cm) and deep soil (30-60 cm). In all the ANOVAs the year of sampling with three levels (2009, 2010 and 2011) was also included as a block factor. Therefore, a three-way ANOVA (liming, soil depth and sampling year) was used for each one of the soil properties, whereas a two-way ANOVA (liming and sampling year) was used for the leaf and grape nutrient contents, as well as for grape yield, must quality properties, tannin concentration and wine color measurements. Since the wine ageing conditions changed throughout the investigation period, all phenolic compounds were studied as a function of year and liming directly.

In case the liming factor presented a significant effect on any of the properties, the corresponding "between groups" variance was split into two independent variance contributions or main effects, namely the effect of just liming and the effect of the specific material used to lime. Given the experimental design, these are the only two possible causes leading to a significant effect of the liming factor. To evaluate if either or both effects explain a likely significant ANOVA, two orthogonal contrasts were planned beforehand. Through contrast 1 (C1), liming was compared against no liming, i.e. the dolomitic limestone and sugar foam levels of the liming factor were jointly compared against the control. Through contrast 2 (C2), the liming materials were compared against each other, i.e. sugar foam versus dolomitic limestone. These orthogonal contrasts allowed us to measure the effect sizes in a standardized way.

To carry out an ANOVA the hypotheses of univariate normal distribution and homoscedasticity of the data have to be tested in advance. The univariate normality hypotheses for every variable were tested using the Kolmogorov-Smirnov test. A confidence level of P=0.05 was used to evaluate the significance of the test. All soil, leaf (blades and petioles), yield, must and wine parameters presented univariate normality. Additionally, the ANOVA is fairly robust in terms of the error rate associated to violations of the assumption of homogeneity of variance (homoscedasticity) when sample sizes are equal (Field, 2009) as occurs in the present study.

Results

1. Soil characterization before liming

The baseline characteristics of the two soil depths before liming are shown in Table 2. The Al content was 1.58 ± 0.89 cmol(+) kg^{-1} (95% CI in the 0-30 cm layer, and 1.38 ± 0.57 cmol(+) kg^{-1} (95% CI) in the 30-60 cm layer. These exchangeable Al contents are over the 20% limit in both depth intervals. The 20% limit is considered the highest Al saturation that most plants can tolerate (Fageria and Baligar, 2008). The important exchangeable soil acidity in the plot is further indicated by the larger-than-one differences between pH_W and pH_{KCl} at both soil depths. Low exchangeable Ca, K and Mg contents were also found, as well as low P content. Conversely, the micronutrients (Fe, Mn, and Cu) exceeded the levels considered suitable for soil fertility. The high Cu values were likely due to cupric-based fungicides that are frequently applied to the local vineyards.

2. Soil properties

The time evolution of pH_W, Ca, Mg, K, Al and S levels for the treatment and control subplots throughout the three years of monitoring is shown in Figures 2 and 3. Apparently, there are some differences in performance efficiency between both liming materials. More specifically, sugar foam seemed to decrease Al levels more than dolomitic limestone.

An ANOVA was used to investigate if the differences between liming treatments were statistically significant, and furthermore, if they depended on the soil depth, the year of sampling, and the interactions between both. According to the ANOVA, there was a significant effect ($P<0.05$) of liming in all soil properties (Table 3). By contrast, the effect of soil depth was not significant ($P>0.05$) in any of the soil properties. Additionally, the effect of liming did not significantly change with the soil depth, which was revealed by the non-significant interaction ($P>0.05$) between liming and soil depth factors (L x D). There was a significant effect ($P<0.05$) of year of sampling

OENO One, 2017, **51**, 4, 342-362

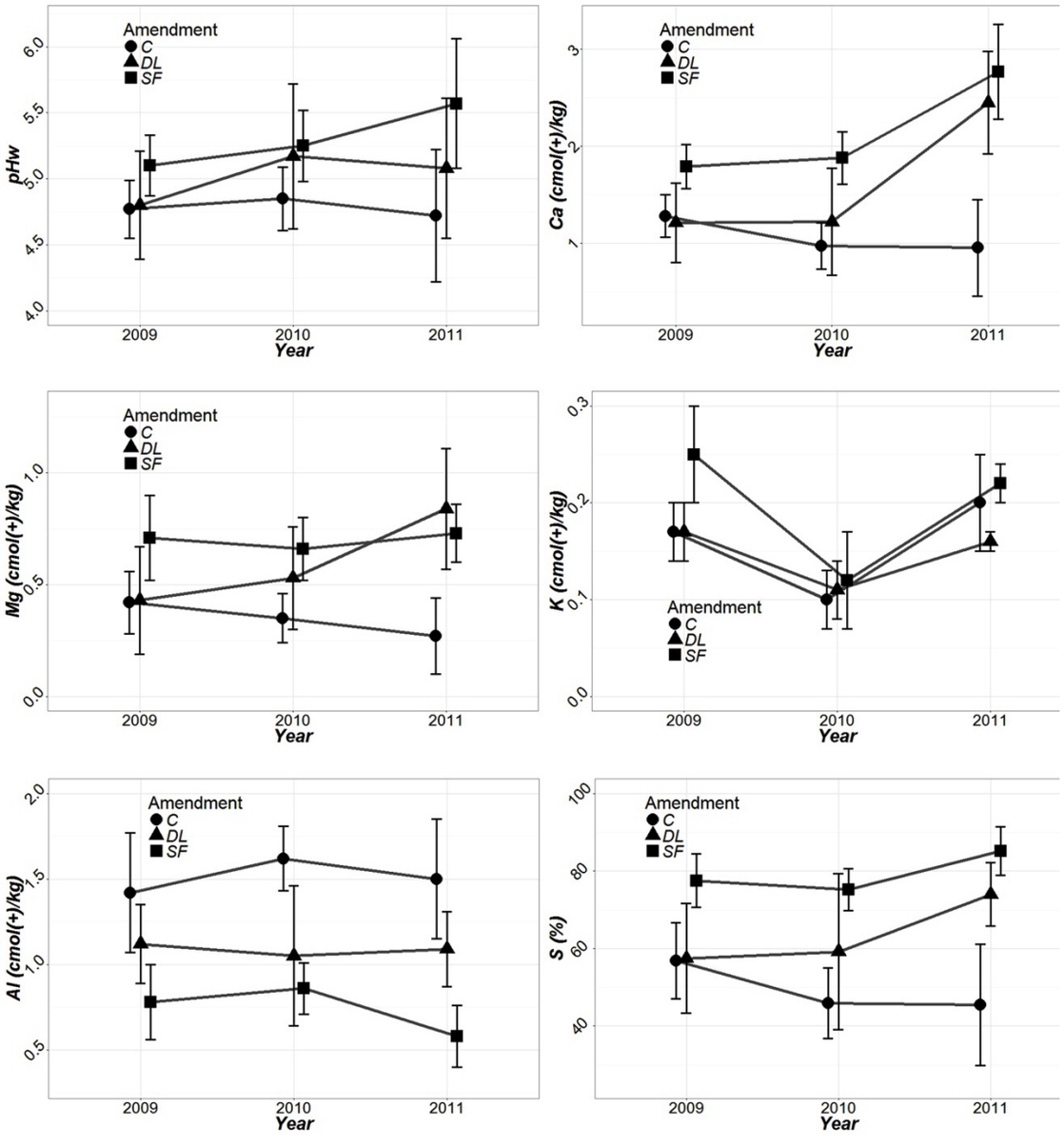

Figure 2. Temporal evolution of pHW, Ca, Mg, K, Al and S over three years (2009-2011) after liming at 0-30 cm soil depth.

Standard errors are shown as bars (± 1 SE mean). C: control; DL: dolomitic limestone; S: sugar foam.

on exchangeable Ca and K levels. Even though the interactions between liming and year of sampling (L x Y), depth and year of sampling (D x Y), and all three factors (L x D x Y) were non-significant in all soil properties, Ca and K were studied as a function of year and liming, whereas pH_W, Mg, Al and S levels were studied as a function of just liming. To know if there were significant differences between liming materials, the "between groups" variance was split into two summands through two contrasts (Table 4).

The first contrast (C1) revealed that just liming significantly increased pH_W, S, Ca (in the year 2011) and Mg, and significantly decreased Al levels. The second contrast (C2) revealed that sugar foam significantly increased pH_W, S, Ca, and Mg, and decreased Al levels significantly more than dolomitic limestone. Specifically, at the end of the investigation, liming (C1) made the average Al levels decreased by 0.71 ± 0.26 cmol(+) kg^{-1} (95% CI), whereas it made the average Ca and Mg levels increased by 1.44 ± 0.43 cmol(+) kg^{-1} (95% CI) and

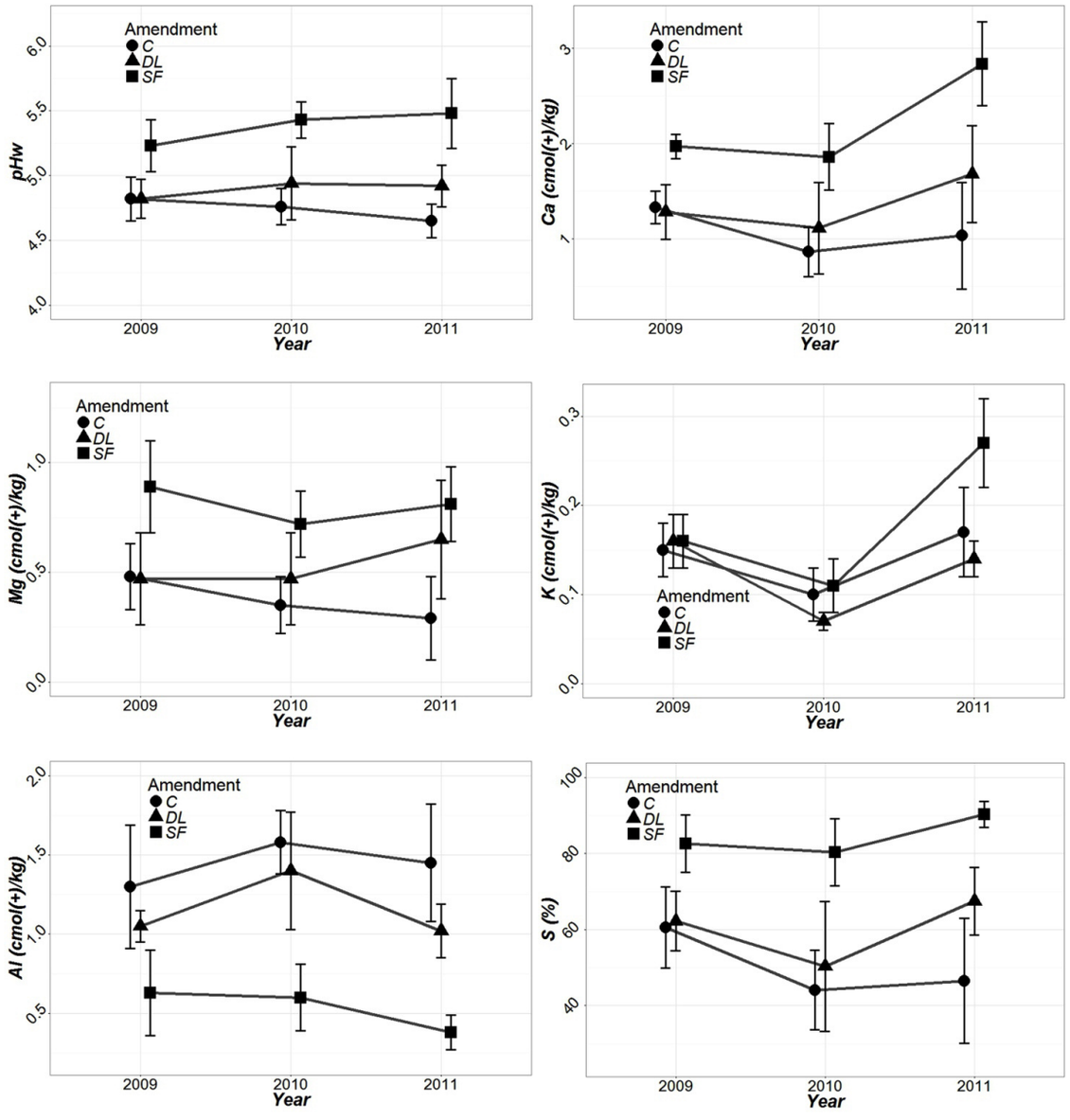

Figure 3. Temporal evolution of pHW, Ca, Mg, K, Al and S over three years (2009-2011) after liming at 30-60 cm soil depth.

Standard errors are shown as bars (± 1 SE mean). C: control; DL: dolomitic limestone; S: sugar foam.

0.47 ± 0.16 cmol(+) kg^{-1} (95% CI), respectively. These increments in both exchangeable cations (Ca and Mg), as well as the decrease in Al saturation caused by liming, were summed up by the increase in S. Specifically, at the end of the investigation, the average S level increase in limed subplots was 33.2 ± 11.9% (95% CI) compared to control subplots. Similarly, the different effect of the liming material sugar foam in comparison to dolomitic limestone (C2) was observed in pH_W and S, which increased by 0.52 ± 0.51 (95% CI) and 17.0 ± 16.4 (95% CI), respectively, and Al, which decreased by 0.57 ± 0.81 (95% CI). The inverse relationship between levels of Ca, Mg and S on the one hand and Al on the other can be observed in Figure 4.

3. Leaf nutrient contents

Means and standard errors of blade and petiole nutrient contents, arranged by liming material, are shown in Figure 5. ANOVA was used to determine if the slight differences between liming treatments were statistically significant, and furthermore, if they depended on the year of sampling and the interactions between these two factors. According to the ANOVA, there were no significant effects of the liming factor on any of the nutrient contents in both

OENO One, 2017, **51**, 4, 342-362

Table 3. Factorial analysis of variance performed on soil properties at veraison stage.

Factor	df_M	df_R	pH_W		Ca		Mg		K		Al		S	
			F	P-value	F	P-value	F	P-value	F	P-value	F	P-value	F	P-value
Liming (L)	2	36	13.9	***	12.3	***	6.25	**	3.77	*	14.9	***	12.4	***
Year (Y)	2		1.09	0.35	4.23	*	0.30	0.75	11.8	***	0.72	0.49	1.06	0.36
Depth (D)	1		0.09	0.76	0.11	0.74	0.06	0.82	1.27	0.27	0.28	0.60	0.03	0.87
L × Y	4		0.86	0.5	1.60	0.2	0.82	0.52	0.52	0.72	0.21	0.93	0.67	0.62
L × D	2		0.39	0.68	0.33	0.72	0.32	0.73	0.03	0.97	0.39	0.68	0.22	0.80
Y× D	2		0.3	0.74	0.23	0.8	0.16	0.85	0.39	0.68	0.11	0.9	0.13	0.88
L × D × Y	4		0.13	0.97	0.23	0.92	0.04	0.99	0.86	0.50	0.17	0.96	0.06	0.99

*significant at the P<0.05 level; **significant at the P<0.01 level; ***significant at the P<0.001 level; df_M, degrees of freedom of the model; df_R, degrees of freedom of the residuals of the model. pH_W, pH in water; S, percentage of bas saturation; Ca, Mg, K and Al (exchangeable Ca, Mg, K and Al, respectively).

Table 4. Effects and effect sizes of liming against control (C1), and liming with sugar foam against liming with dolomitic limestone (C2), on soil properties at veraison stage.

Factor	df_M	df_R	pH_W		Ca		Mg		K		Al		S	
			F	P-value	F	P-value	F	P-value	F	P-value	F	P-value	F	P-value
Liming (L)	2	36	13.9	***	12.3	***	6.25	**	3.77	*	14.9	***	12.4	***
Year (Y)	2		1.09	0.35	4.23	*	0.30	0.75	11.8	***	0.72	0.49	1.06	0.36
Depth (D)	1		0.09	0.76	0.11	0.74	0.06	0.82	1.27	0.27	0.28	0.60	0.03	0.87
L × Y	4		0.86	0.5	1.60	0.2	0.82	0.52	0.52	0.72	0.21	0.93	0.67	0.62
L × D	2		0.39	0.68	0.33	0.72	0.32	0.73	0.03	0.97	0.39	0.68	0.22	0.80
Y× D	2		0.3	0.74	0.23	0.8	0.16	0.85	0.39	0.68	0.11	0.9	0.13	0.88
L × D × Y	4		0.13	0.97	0.23	0.92	0.04	0.99	0.86	0.50	0.17	0.96	0.06	0.99

*significant at the P<0.05 level; **significant at the P<0.01 level; ***significant at the P<0.001 level. pH_W, pH in water; S, percentage of base saturation; Ca, Mg, K and Al (exchangeable Ca, Mg, K and Al, respectively); $Mean_d$ (mean differences between control and amendments for contrast 1, and between sugar foam and dolomite for contrast 2); df_R (degrees of freedom for the residuals of the model); r (effect size).

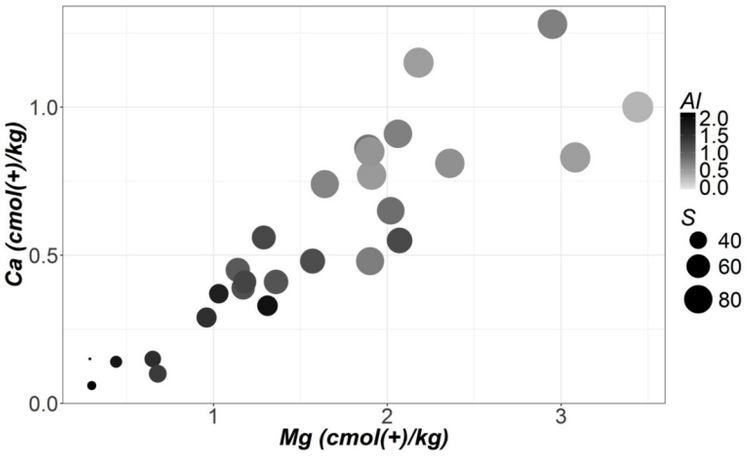

Figure 4. Relationship between Ca, Mg, Al and S levels in soil as a function of just liming. Al in cmol(+) kg-1; S (percentage of base saturation) in %.

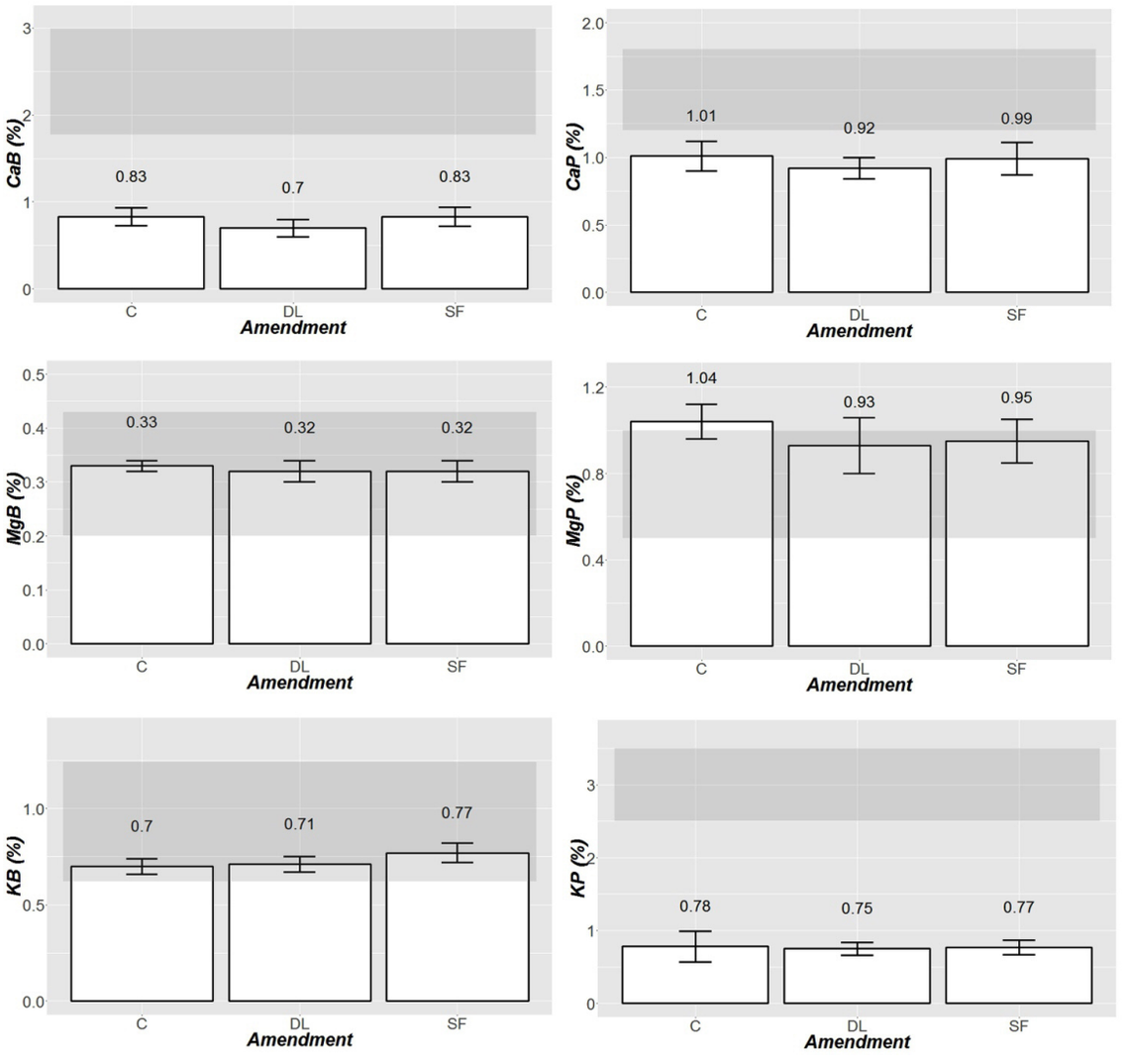

Figure 5. Bar graphs of blade (Cab, Mgb and Kb) and petiole (Cap, Mgp and Kp) element composition at veraison (2009-2011).

Average values are displayed within bar graphs. Standard errors are shown as bars (± 1 SE mean). The shaded areas in the graphs illustrate the concentration ranges that are optimal for adequate vine growth at veraison according to Fregoni (2005). C: control; DL: dolomitic limestone; SF: sugar foam.

blades and petioles (Table 5). Interestingly, there was a significant effect ($P<0.05$) of year of sampling on Ca and Mg content in blades and petioles, as well as on K content in blades. Additionally, the interaction between liming and sampling year (L x Y) was non-significant ($P>0.05$) in all the nutrients evaluated.

4. Yield, must quality and grape nutrient contents

Means and standard errors of grape nutrient contents (Cag, Mgg and Kg), yield parameters (GY and W100) and must quality properties (pH, TA and TSS) in the treatment and control subplots are shown in Table 6.

According to the ANOVA, liming had no significant effects on any of the nutrient contents. Interestingly, there was a significant effect of year of sampling on Mgg ($P<0.05$) and Kg ($P<0.001$). The non-significant interaction between liming and sampling year (L x Y) ($P<0.05$) revealed that the effect of sampling year on grape nutrient contents did not change with liming. Additionally, there were no significant effects of the liming factor on any of the yield parameters and must quality properties except for TA ($P<0.01$). In the year 2011, both C1 and C2 were significant ($P<0.05$) in the above parameter. Interestingly, there was a significant effect of year of sampling on pH ($P<0.01$) as well as on W100 and

OENO One, 2017, **51**, 4, 342-362

Table 5. Factorial analysis of variance performed on calcium, magnesium and potassium content in blades and petioles at veraison stage.

Factor	df_M	df_R	Cab (%)		Mgb (%)		Kb (%)		Cap (%)		Mgp (%)		Kp (%)	
			F	P-value	F	P-value	F	P-value	F	P-value	F	P-value	F	P-value
Liming (L)	2		1,31	0,29	0,2	0,82	1,86	0,18	0,49	0,62	0,79	0,47	0,02	0,98
Year (Y)	2	18	18,8	***	4,45	*	15,2	***	51,8	***	4,43	*	3,14	0,07
L × Y	4		0,18	0,95	0,14	0,97	0,55	0,7	0,31	0,87	0,13	0,97	0,66	0,63

*significant at the P<0.05 level; ***significant at the P<0.001 level; df_M, degrees of freedom of the model; df_R, degrees of freedom for the residuals of the model; Cab, Mgb and Kb (calcium, magnesium and potassium contents in blades respectively); Cap, Mgp, and Kp (calcium, magnesium and potassium contents in petioles, respectively).

Table 6. Means and stardand errors (SE) of yield parameters, must quality properties and grape nutrient contents at harvest time.

Year	Amendment	GY (kg ha^{-1})		W100 (g)		pH		TA (g ta l^{-1})		TSS (ºBaumé)		Cag (%)		Mgg (%)		Kg (%)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
	C	6156	1211	226	14,8	3,45	0,08	5,5	0,19	12,9	0,07	0,22	0,02	0,14	0	2,4	0,44
2009	DL	6912	1560	214	13	3,47	0,05	5,35	0,48	12,9	0,33	0,24	0,03	0,15	0,01	1,77	0,15
	SF	7041	1863	229	6,66	3,45	0,02	5,73	0,15	13	0	0,28	0,01	0,15	0,01	1,81	0,2
	C	6409	1384	175	11,6	3,3	0,04	5,28	0,15	12,3	0,07	0,33	0,02	0,15	0,01	1,03	0,1
2010	DL	6985	1095	178	12,6	3,31	0,04	5,33	0,08	12,6	0,35	0,37	0,05	0,16	0,01	1,22	0,2
	SF	8234	1840	189	3,65	3,36	0,02	5,53	0,09	12,9	0,18	0,27	0,02	0,15	0	1,39	0,24
	C	4050	700	194	10,2	3,42	0,05	3,4	0,21	13	0,2	0,3	0,05	0,14	0,01	1,18	0,07
2011	DL	5155	753	203	7,47	3,41	0	3,9	0,13	13	0,12	0,31	0,03	0,16	0,01	0,98	0,15
	SF	5157	1555	202	10,5	3,38	0,01	5,04	0,42	12,7	0,18	0,25	0,01	0,15	0,01	1,18	0,06

GY (grape yield); W100 (weight of 100 berries); TA (total acidity expressed as the equivalent content of tartaric acid (ta) in g l^{-1}); TSS (total soluble solids); Cag, Mgg and Kg (calcium, magnesium and potassium contents in grapes, respectively).

TA (P<0.001). It is also interesting to note the absence of significant interactions (P>0.05) between liming and sampling year (L x Y). Although there are no significant differences with the control subplots in terms of productivity, an increasing trend in grape yields could be observed in limed subplots. Specifically, just liming with either sugar foam or dolomite limestone increased grape yields by nearly 30% at the end of the experiment (2011). These results might be important to farmers in economic terms.

5. Tannin concentration, color measurements and phenolic compounds in wines

There was a significant effect (P<0.05) of sampling year, as well as liming and sampling year (L x Y), on tannin and all wine color parameters measured both at the beginning and the end of AF. Based on the foregoing, means and standard errors of those parameters arranged by liming material and sampling year are shown in Table 7, and were studied as a function of both factors (Table 8). While many significant differences have appeared because of liming, the results obtained only showed one consistent trend: liming significantly increased hue after AF. No particular trend before and after AF was found with liming treatments compared with control. Interestingly, TAnth levels measured before AF in both control and liming treatments in the 2010 vintage were very different against the other two vintages (2009 and 2011). It was not possible to associate this fact to any particular cause.

Data for phenolic compounds (Gall, Caff, Cou, Cat, Epi, Resv, Malv and Fer), arranged by liming material and sampling year, are shown in Table 9. Due to the differences in ranges of measurement for each of the phenolic compounds, all the measures were scaled prior to be plotted (Figure 6).

The results obtained by applying one-way ANOVAs revealed that significant differences (P<0.05) existed in most of the quantified phenolic compounds. However, the results showed only two consistent trends: liming significantly (P<0.05) increased Gall and Cat after ageing throughout the experiment. Similarly to tannin concentration and color measurements, no particular trend before and after

Table 7. Means and standard errors (SE) of tannin concentration and color measurements before and after alcoholic fermentation (AF) in must and wines respectively.

Parameter	Year	Amendment	Before AF		After AF	
			Mean	SE	Mean	SE
Tc (g l^{-1})	2009	C	1,91	0,02	3,17	0,06
		DL	2,3	0,02	3,19	0,01
		SF	2,22	0,01	2,98	0,01
	2010	C	2,33	0,02	3,26	0,04
		DL	2,35	0,01	3,33	0,03
		SF	2,2	0,01	3,21	0,01
	2011	C	3,21	0,01	2,71	0,02
		DL	3,01	0,01	3,36	0,01
		SF	2,88	0,01	3,33	0,01
Hue	2009	C	0,66	0,01	0,66	0,01
		DL	0,51	0,01	0,69	0,01
		SF	0,61	0,01	0,71	0,01
	2010	C	0,94	0,03	0,55	0,01
		DL	1,3	0,04	0,55	0,01
		SF	1,31	0,08	0,56	0,01
	2011	C	1,13	0,02	0,58	0,01
		DL	0,92	0,01	0,59	0,01
		SF	1,06	0,02	0,6	0,01
C.I.	2009	C	6,4	0,01	18,1	0,09
		DL	4,23	0,04	22,6	0,03
		SF	5,03	0,04	24,3	0,09
	2010	C	1,6	0,1	12,3	0,03
		DL	1,5	0,06	12,5	0,03
		SF	2,37	0,07	11,8	0,01
	2011	C	20,2	0,22	16,1	0,06
		DL	8,87	0,26	15,5	0,03
		SF	14,4	0,09	15,1	0,03
TPI	2009	C	32,1	0,19	71,9	0,06
		DL	33,9	0,01	75,8	0,03
		SF	27,1	0,01	71,9	0,07
	2010	C	12	0,09	74,9	0,03
		DL	10,6	0,06	75,3	0,01
		SF	12	0,12	74,7	0,09
	2011	C	34,2	0,09	87	0,01
		DL	22,6	0,01	87,2	0,03
		SF	27,5	0,09	86,3	0,03
TAnth (mg l^{-1})	2009	C	498	0,99	901	2,13
		DL	660	1,01	969	1,74
		SF	395	1	878	0,77
	2010	C	63	0,62	899	4,36
		DL	50	0,23	931	1
		SF	61,2	1,26	934	0,73
	2011	C	390	1,3	1129	0,64
		DL	298	0,66	1126	0,77
		SF	348	0,66	1083	0,01

Tc (tannin concentration); C.I. (color index); TPI (total phenol index); TAnth (total anthocyanin content).

OENO One, 2017, **51**, 4, 342-362

Table 8. Effects and effect sizes of liming against control (C1) and liming with sugar foam against liming with dolomitic limestone (C2) on tannin concentration and color measurements before and after alcoholic fermentation (AF) in must and wines respectively.

Parameter	Year	Contrast	df_R	Before AF				After AF			
				$Mean_d$	t-ratio	P-value	r	$Mean_d$	t-ratio	P-value	r
Tc (g l^{-1})	2009	C1	6	0,35	14,8	***	0,99	-0,09	-2,03	0,09	#
		C2		-0,08	-3,06	*	0,78	-0,21	-4,17	**	0,86
	2010	C1		-0,06	-4,38	**	0,87	0,01	0,26	0,8	#
		C2		-0,15	-9,74	***	0,97	-0,12	-3,37	*	0,81
	2011	C1		-0,27	-23,9	***	0,99	0,63	30,5	***	1
		C2		-0,13	-9,7	***	0,97	-0,03	-1,4	0,21	#
Hue	2009	C1		-0,1	-15,8	***	0,99	0,04	6	***	0,93
		C2		0,1	13,4	***	0,98	0,03	3,46	*	0,82
	2010	C1		0,37	5,66	**	0,92	0,01	>100	***	1
		C2		0,01	0,13	0,9	#	0,01	>100	***	1
	2011	C1		-0,14	-7,82	***	0,95	0,02	>100	***	1
		C2		0,14	6,94	***	0,94	0,01	>100	***	1
C.I.	2009	C1		-1,77	-53	***	1	5,38	59	***	1
		C2		0,8	20,8	***	0,99	1,63	15,5	***	0,99
	2010	C1		0,33	3,54	*	0,82	-0,2	-6	***	0,93
		C2		0,87	7,96	***	0,96	-0,67	-17,3	***	0,99
	2011	C1		-8,55	-34,4	***	1	-0,77	-14,6	***	0,99
		C2		5,5	19,2	***	0,99	-0,4	-6,57	***	0,94
TPI	2009	C1		-1,57	-11,9	***	0,98	1,95	29,3	***	1
		C2		-6,8	-44,9	***	1	-3,83	-49,8	***	1
	2010	C1		-0,75	-6,64	***	0,94	0,05	0,75	0,48	#
		C2		1,37	10,5	***	0,97	-0,63	-8,23	***	0,96
	2011	C1		-9,17	-104	***	1	-0,22	-6,5	***	0,94
		C2		4,93	48,4	***	1	-0,9	-23,4	***	0,99
TAnth (mg l^{-1})	2009	C1		30	24,5	***	1	22,4	11,1	***	0,98
		C2		-266	-188	***	1	-90,3	-38,7	***	1
	2010	C1		-7,42	-7,35	***	0,95	32,8	10,2	***	0,97
		C2		11,2	9,58	***	0,97	3,43	0,93	0,39	#
	2011	C1		-67,1	-59,2	***	1	-24,3	-34,5	***	1
		C2		50,1	38,3	***	1	-42,4	-52,2	***	1

*significant at the P<0.05 level; **significant at the P<0.01 level; ***significant at the P<0.001 level. Tc (tannin concentration); C.I. (color index); TPI (total phenol index); TAnth (total anthocyanin content); $Mean_d$ (mean differences between control and amendments for contrast 1, and between sugar foam and dolomite for contrast 2); df_R (degrees of freedom for the residuals of the model); r (effect size).

ageing was found with liming treatments compared with control.

Discussion

In the Haploxerept acid soil studied in this work, liming was effective in decreasing soil acidity, and specifically, sugar foam was more effective than dolomitic limestone. These results agree with those of García Navarro *et al.* (2009) who, in their assays on Anthrosols and Luvisols (FAO, 2006), reported that the application of sugar foam on dryland crops produced a positive impact on soil fertility through an increase in soil pH_W and exchangeable Ca contents. Additionally, although liming materials were incorporated with one-pass tillage at a soil depth of 20-30 cm, no significant differences in soil properties were found between the arable and the underlying soil layer (0-30 and 30-60 cm).

In spite of the higher CCE of dolomitic limestone compared to sugar foam, the latter was more efficient

Table 9. Effects and effect sizes of liming against control (C1) and liming with sugar foam against liming with dolomitic limestone (C2) on wine phenolic compounds after alcoholic fermentation (AF) and after ageing.

Parameter	Year	Contrast	df_R	Before AF				After AF			
				$Mean_d$	t-ratio	P-value	r	$Mean_d$	t-ratio	P-value	r
Tc (g l^{-1})	2009	C1	6	0,35	14,8	***	0,99	-0,09	-2,03	0,09	#
		C2		-0,08	-3,06	*	0,78	-0,21	-4,17	**	0,86
	2010	C1		-0,06	-4,38	**	0,87	0,01	0,26	0,8	#
		C2		-0,15	-9,74	***	0,97	-0,12	-3,37	*	0,81
	2011	C1		-0,27	-23,9	***	0,99	0,63	30,5	***	1
		C2		-0,13	-9,7	***	0,97	-0,03	-1,4	0,21	#
Hue	2009	C1		-0,1	-15,8	***	0,99	0,04	6	***	0,93
		C2		0,1	13,4	***	0,98	0,03	3,46	*	0,82
	2010	C1		0,37	5,66	**	0,92	0,01	>100	***	1
		C2		0,01	0,13	0,9	#	0,01	>100	***	1
	2011	C1		-0,14	-7,82	***	0,95	0,02	>100	***	1
		C2		0,14	6,94	***	0,94	0,01	>100	***	1
C.I.	2009	C1		-1,77	-53	***	1	5,38	59	***	1
		C2		0,8	20,8	***	0,99	1,63	15,5	***	0,99
	2010	C1		0,33	3,54	*	0,82	-0,2	-6	***	0,93
		C2		0,87	7,96	***	0,96	-0,67	-17,3	***	0,99
	2011	C1		-8,55	-34,4	***	1	-0,77	-14,6	***	0,99
		C2		5,5	19,2	***	0,99	-0,4	-6,57	***	0,94
TPI	2009	C1		-1,57	-11,9	***	0,98	1,95	29,3	***	1
		C2		-6,8	-44,9	***	1	-3,83	-49,8	***	1
	2010	C1		-0,75	-6,64	***	0,94	0,05	0,75	0,48	#
		C2		1,37	10,5	***	0,97	-0,63	-8,23	***	0,96
	2011	C1		-9,17	-104	***	1	-0,22	-6,5	***	0,94
		C2		4,93	48,4	***	1	-0,9	-23,4	***	0,99
TAnth (mg l^{-1})	2009	C1		30	24,5	***	1	22,4	11,1	***	0,98
		C2		-266	-188	***	1	-90,3	-38,7	***	1
	2010	C1		-7,42	-7,35	***	0,95	32,8	10,2	***	0,97
		C2		11,2	9,58	***	0,97	3,43	0,93	0,39	#
	2011	C1		-67,1	-59,2	***	1	-24,3	-34,5	***	1
		C2		50,1	38,3	***	1	-42,4	-52,2	***	1

significant at the P<0.05 level;significant at the P<0.01 level; ***significant at the P<0.001 level. pH_W, pH in water; S, percentage of base saturation; gallic acid (Gall), Caffeic acid (Caff), p-Coumaric acid (Cou), (+)-catechin (Cat), (-)-epicatechin (Epi), trans-resveratrol (Resv), malvidin-3-O-glucoside (Malv) and trans-ferulic acid (Fer); $Mean_d$ (mean differences between control and amendments for contrast 1, and between sugar foam and dolomite for contrast 2); df_R (degrees of freedom for the residuals of the model); r (effect size).

as liming material than the former. This remarkable higher efficiency of sugar foam over dolomitic limestone could be explained by the higher solubility of $Ca(OH)_2$ over $CaMg(CO_3)_2$, which is roughly three orders of magnitude higher. Similarly, the higher exchangeable Mg in soils limed with sugar foam compared to dolomitic limestone subplots could be explained by the higher solubility of $Mg(OH)_2$ compared to $CaMg(CO_3)_2$. These results agree with those of Augustin *et al.* (1997), who investigated the chemical reactions of magnesium fertilizers when they are applied to the soil, and suggested that magnesium fertilizers can be grouped into four classes according to their dissolution rates: $MgSO_4$ > MgO ≈ **$Mg(OH)_2$** > slag lime ≈ **dolomitic limestone** ≈ magnesite > basalt. These results differ from the previous findings of Vidal *et al.* (2006) in their comparative study of several liming materials (sugar foam, dolomitic lime, calcium carbonate and gypsum) developed from acid soils under laboratory conditions. The latter authors, based on the results for the leachate properties, reported that limed soils with

OENO One, 2017, **51**, 4, 342-362

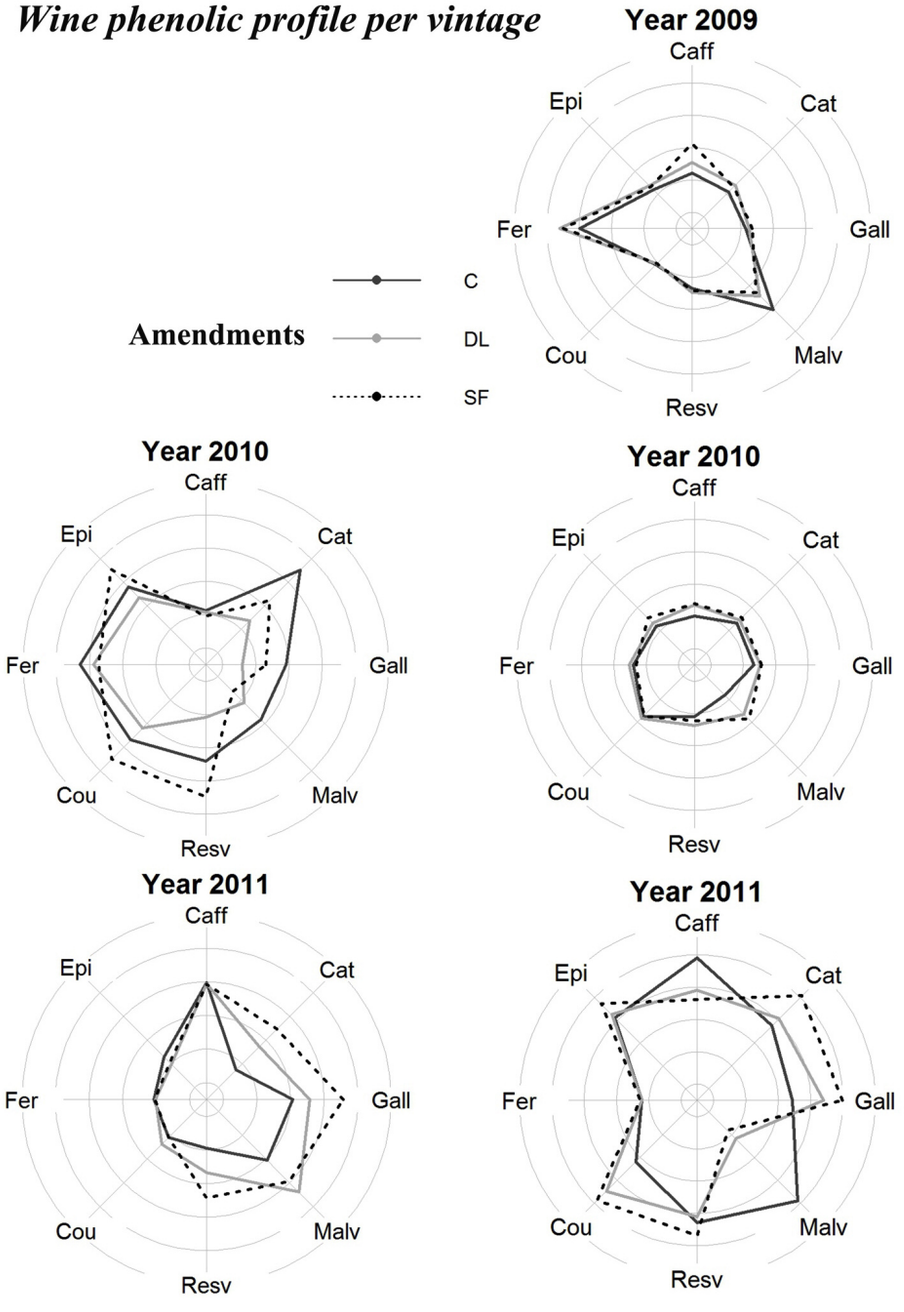

Figure 6. Wine phenolic profiles of Mencía wines in response to liming treatments per vintage before (left) and after (right) the ageing process in French oak barrels.

Gallic acid (Gall), caffeic acid (Caff), p-coumaric acid (Cou), (+)-catechin (Cat), (-)-epicatechin (Epi), trans-resveratrol (Resv), malvidin-3-O-glucoside (Malv) and trans-ferulic acid (Fer). C: control; DL: dolomitic limestone; SF: sugar foam.

dolomitic limestone showed significantly more exchangeable Mg than those treated with sugar foam. This indicates the need for caution when comparing data derived from laboratory and field studies.

Although chemical soil analysis indicates the potential availability of some nutrients that vine roots may take up, leaf analysis is often the most reliable method of assessing vine nutritional status. Moreover, because the vine integrates the variability in the soil explored by its roots, leaf analysis is often the basis of the fertilizer recommendation programs for vines. However, in the present study, data from soil testing and leaf analysis have indicated that there is no clear relationship between them. Although blades have higher sensitivity to detect individual macronutrient deficiencies or excesses (Romero *et*

al., 2014), results in blade nutritional diagnosis also have not provided any further evidence supporting any significant effect of liming on tissue cation composition at veraison.

Assuming that liming did not significantly increase yield grape, an increasing trend has been found in limed subplots. The increase sequence in yield data (C□DL□SF) is consistent with those obtained for Ca and Mg data (C□DL□SF), as well as for Al data (C□DL□SF). In view of the foregoing considerations, soil liming may be advisable close to the vine root system. However, in many established vineyards the use of lime could be difficult as a result of the existence of trellis systems.

Although soils that are rich in Ca promote the accumulation of sugar (Jiang and Zhang, 2012), the findings of the current study did not show any significant increase in this parameter. Despite the fact that liming did not cause significant differences in yield quality properties, it is interesting to note that TA decreased more in control than in limed subplots (particularly during the last two years) (Figure 7), and more when sugar foam was used. A possible explanation for these results may be related to the grape K content, which, when excessive, may decrease must TA resulting in an increase in must pH (Conde *et al.*, 2007). The higher K content in leaf tissues showed by limed subplots (although not significant) does not seem to be in agreement with a reduction in K uptake in limed soils. However, this issue could originate from the combined effect of rainfall during the annual vegetative cycle and liming. Thus, Chatonnet (2005) reported that late rainfall close to harvest leads to fast K uptake by vine roots, which drops must TA without necessarily affecting must pH. In 2011, an abnormal rainy August (34.6 mm) and rainfall (8.2 mm) ten days before harvest occurred. Although these conditions are similar to those reported by Chatonnet, no significant differences in Kg were found in this research and several questions remain unanswered.

As reported by Yokotsuka *et al.* (1999), the addition of limestone to the native soil significantly influenced both tannin concentration and color parameters in Mencía wines in every vintage of the experiment; however, there was a weak consistency in the trends observed. This, together with the results obtained in blades and petioles as well as in grapes and yield, suggests that the specific physiological characteristics of the combination of rootstock and scion, along with marked nutrient storage in the woody parts of the vine, make assessing grapevine responses to amendment application particularly challenging (Jackson, 2014; Olego and Garzón, 2014). However, since soil chemistry is an important

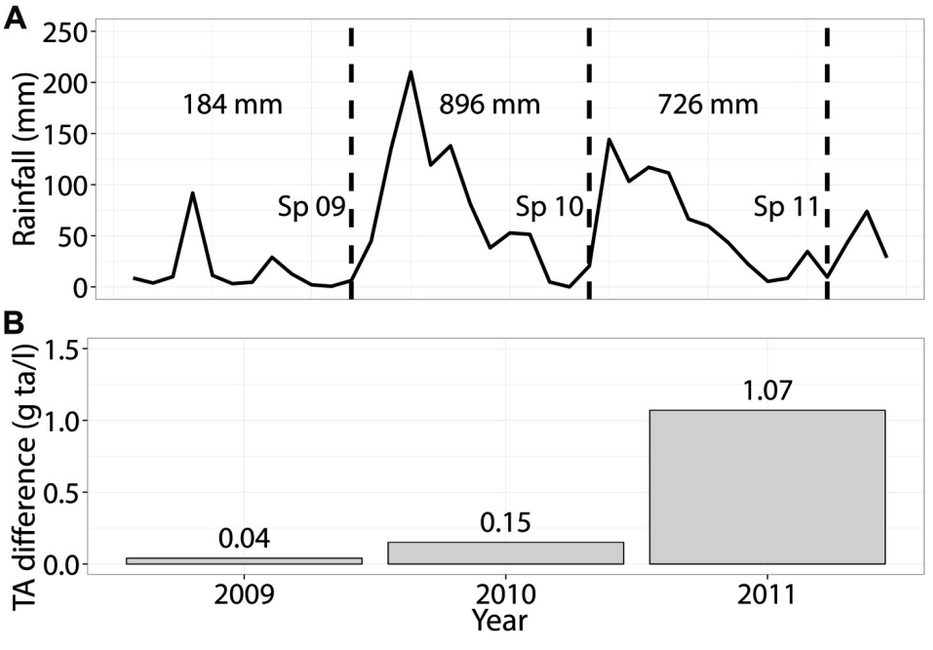

Figure 7. Monthly rainfall (top) and differences in total acidity (TA) between control and limed subplots (bottom), during the period of investigation.

Dashed lines correspond to months of harvest (Sp: September).

factor in terroir, vine nutrition can be instrumental to the specific growth pattern of the vine and thus cause a specific canopy architecture and therefore a ripening pattern (Goode, 2013).

Wine quality is the result of a complex set of interactions (terroir effect), which include soil variables. With respect to the vine's productive system, soil and climate represent the two physical elements fundamental to understand the concept of terroir (van Leeuwen and Seguin, 2006); compared to climate, soil is an even more complex system. Thus, the impact of the pedologic environment on wine quality is difficult to analyze singularly and separately from the rest of the productive system (Seguin, 1986). Accordingly, it is difficult to study the effects of soils that have been artificially modified to improve yield on grape and wine composition, because the soil influences hydro and mineral nutrition and the functioning of the root system. Although phenolic compounds are heavily influenced by the vintage, soil type, mesoclimate and cultural practices (Nadal, 2010), as for the measurements of tannin concentration and color parameters, no definite trends (with consistency before and after wine ageing) in phenolic compounds have been found in the present study as a consequence of liming. Caution must be taken in interpreting the influence of soil amendments on phenolic compounds, as climatic differences between vintages added uncertainty to their interpretation. Additionally, phenolic compounds in wines varied between seasons, regardless of variations in winemaking practices (i.e. different age in the French oak barrels used per vintage) which also could affect wine composition (Cohen and Kennedy, 2010). However, although the composition of wine is heavily dependent on the winemaking technique (Cortell *et al.*, 2008), understanding the impacts of soil types on the phenolic compounds in grapes and wines is necessary for developing an effective viticulture management program (Li *et al.*, 2011).

Conclusion

As expected, liming with either sugar foam or dolomitic limestone decreased soil exchangeable aluminum, improved supply of calcium and magnesium, and increased soil pH and base saturation in acid Haploxerept cultivated with *Vitis vinifera* L. cv. Mencía. However, sugar foam behaved as a more efficient liming material than dolomitic limestone. This fact may be explained by the higher solubility of sugar foam calcium and magnesium components in the soil. The reduced efficiency of dolomitic limestone might be partly offset by a greater durability.

Although the increasing trend in grape yield with liming, more pronounced with sugar foam than with dolomitic limestone, was not significant, this might have an important economic impact. Finally, no significant trends in properties related to must and wine quality were found. Maybe more evident trends in these must and wine properties would have appeared using higher liming doses or extending the monitoring span to more years. Further research needs to examine more closely the impact of the changes in soil composition on wine quality.

Acknowledgements : This work was funded by the "Excelentísima Diputación Provincial de León". We are specially grateful to "Losada Vinos de Finca, S.A." for assistance in the research project.

References

Álvarez E., Viadé A. and Fernández-Marcos M.L., 2009. Effect of liming with different sized limestone on the forms of aluminium in a Galician soil (NW Spain). *Geoderma*, **152**, 1-8. doi:10.1016/j.geoderma.2009.04.011

Augustin A., Mindrup M. and Meiwes K.J., 1997. Soil chemistry, 255-273. In: *Magnesium deficiency in forest ecosystems*. Kluwer academic publishers, Dordrecht, The Netherlands.

Bavaresco L., Gatti M. and Fregoni M., 2010. Nutritional deficiencies, 165-191. In: *Methodologies and results in grapevine research*. Springer, Dordrecht, The Netherlands.

Bouyoucos G., 1962. Hydrometer method improved for making particle size analyses of soils. *Agron. J.*, **54**, 464-465. doi:10.2134/agronj1962.00021962005400050028x

Calleja A., 1978. La mineralización de muestras vegetales para el análisis de minerales por espectrofotometría y colorimetría. *An. Fac. Vet. León*, **24**, 175-177.

Cançado G.M.A., Ribeiro A.P., Piñeros M.A., Miyata L.Y., Alvarenga Á.A., Villa F., Pasqual M. and Purgatto E., 2009. Evaluation of aluminium tolerance in grapevine rootstocks. *Vitis*, **48**, 167-173.

Castro G.S.A. and Crusciol C.A.C., 2013. Effects of superficial liming and silicate application on soil fertility and crop yield under rotation. *Geoderma*, **195-196**, 234-242. doi:10.1016/j.geoderma.2012.12.006

Chatonnet P., 2005. Origen, importancia y factores de variación de la acidez y del pH: visión general de la problemática de la disminución de la acidez de los vinos, 9-23. In: *Gestión de pH en el vino de calidad*. Fundación para la cultura del vino, Madrid, Spain.

Cochrane T.T., Salinas J.G. and Sánchez P.A., 1980. An equation for liming acid mineral soils to compensate crop aluminium tolerance. *Trop. Agric.*, **57**, 133-140.

Cohen S.D. and Kennedy J.A., 2010. Plant metabolism and the environment: implications for managing phenolics. *Crit. Rev. Food Sci. Nutr.*, **50**, 620-643. doi:10.1080/10408390802603441

Conde C., Silva P., Fontes N., Dias A.C.P., Tavares R.M., Sousa M.J., Agasse A., Delrot S. and Gerós H., 2007. Biochemical changes throughout grape berry development and fruit and wine quality. *Food*, **1**, 1-22.

Cortell J.M., Sivertsen H.K., Kennedy J.A. and Heymann H., 2008. Influence of vine vigor on Pinot noir fruit composition, wine chemical analysis, and wine sensory attributes. *Am. J. Enol. Vitic.*, **59**, 1-10.

Edmeades D.C. and Ridley A.M., 2003. Using lime to ameliorate topsoil and subsoil acidity, 297-336. In: *Handbook of soil acidity*. Marcel Dekker Inc., New York, USA. doi:10.1201/9780203912317.ch11

Espejo R., 2001. El uso de la espuma de azucarería, fosfoyeso y residuos dolomíticos de convertidor como enmendantes de suelos ácidos, 377-386. In: *Proceedings of the First International Congress on the Management of Organic Waste in Rural Land Grange Areas*. Universidad de Navarra ed., Pamplona, Spain.

Fageria N.K. and Baligar V.C., 2008. Ameliorating soil acidity of tropical oxisols by liming for sustainable crop production. *Adv. Agron.*, **99**, 345-399. doi:10.1016/S0065-2113(08)00407-0

FAO, 2006. *World reference base for soil resources. World Soil Resources Reports No. 103*. FAO, Rome, Italy.

Fernández-Calviño D., Nóvoa-Muñoz J.C., Díaz-Raviña M. and Arias-Estévez M., 2009. Copper accumulation and fractionation in vineyard soils from temperate humid zone (NW Iberian Peninsula). *Geoderma*, **153**, 119-129. doi:10.1016/j.geoderma.2009.07.024

Field A., 2009. *Discovering statistics using SPSS, 3rd ed*. SAGE, London, The United Kingdom.

Fráguas J.C., 1999. Tolerância de porta-enxertos de videira ao aluminio do solo. *Pesq. Agropec. Bras.*, **34**, 1193-1200. doi:10.1590/S0100-204X1999000700011

Fregoni M., 2005. *Viticoltura di qualitá*. Tecniche Nuove, Milano, Italia.

García Navarro F.J., Amorós Ortiz-Villajos J.A., Sánchez-Jiménez C.J., Bravo Martín-Consuegra S., Márquez Cubero E. and Jiménez Ballesta R., 2009. Application of sugar foam to red soils in a semiarid Mediterranean environment. *Environ. Earth Sci.*, **59**, 603-611. doi:10.1007/s12665-009-0058-9

Goode J., 2013. *Wine science. The application of science in winemaking, 2nd ed*. Mitchell Beazley – Octopus Publishing Group, London, The United Kingdom.

Hansen D. and Cass A., 2002. Soil amelioration trials at Lyndoch, 18-24. In: *Sustainable viticultural production: optimising soil resources*. CSIRO, Melbourne, Australia.

Havlin J.L., Tisdale S.L., Nelson W.L. and Beaton J.D., 2014. *Soil fertility and fertilizers. An introduction to nutrient management, 8th ed*. Pearson, New Jersey, USA.

IGME, 1995. *Atlas del medio natural de la provincia de León*. Instituto Tecnológico Geominero de España, Madrid, Spain.

Jackson R.S., 2014. *Wine science: principles and applications, 4th ed*. Academic Press, London, The United Kingdom.

Jiang B. and Zhang Z.W., 2012. Comparison on phenolic compounds and antioxidant properties of cabernet sauvignon and merlot wines from four wine grape-growing regions in China. *Molecules*, **17**, 8804-8821. doi:10.3390/molecules17088804

Joris H.A.W., da Fonseca A.F., Asami V.Y., Briedis C., Borszowskei P.R. and Garbuio F.J., 2012. Adsorçao de metais pesados após calagem superficial em um Latossolo Vermelho sob sistema de plantio direto. *Rev. Cienc. Agron.*, **43**, 1-10. doi:10.1590/S1806-66902012000100001

Keller M., 2010. *The science of grapevines: anatomy and physiology*. Academic Press, London, The United Kingdom.

Kirchhof G., Blackwell J. and Smart R.E., 1991. Growth of vineyard roots into segmentally ameliorated acidic subsoils. *Plant Soil*, **134**, 121-126. doi:10.1007/BF00010724

Lanyon D.M., 2001. Coalescence of a water stable soil. Doctoral Thesis, University of Melbourne.

Li Z., Pan Q., Jin Z., Mu L. and Duan C., 2011. Comparison on phenolic compounds in *Vitis vinifera* cv. Cabernet Sauvignon wines from five wine-growing regions in China. *Food Chem.*, **125**, 77-83. doi:10.1016/j.foodchem.2010.08.039

Lindsay W.L. and Norvell W.A., 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci. Soc. Am. J.*, **42**, 421-428. doi:10.2136/sssaj1978.03615995004200030009x

Little I., 1964. The determination of exchangeable aluminium in soils. *Aust. J. Soil Res.*, **2**, 76-82. doi:10.1071/SR9640076

Loeppert R.H. and Suarez D.L., 1996. Carbonate and gypsum, 437-474. In: *Methods of soil analysis Part III, Chemical methods*. Soil Science Society of America, Madison, USA.

MAPA, 1993. *Métodos oficiales de análisis, tomo III*. Ministerio de Agricultura, Pesca y Alimentación, Secretaría General Técnica, Madrid, España.

Meyer B.R., Le Roux E., Renan M.J. and Peisach M., 1984. PIXE analysis for the study of toxic effects of aluminium in vines. *Nucl. Instrum. Methods Phys. Res. B*, **3**, 557-560. doi:10.1016/0168-583X(84)90438-5

Nadal M., 2010. Phenolic maturity in red grapes, 389-409. In: *Methodologies and results in grapevine research*. Springer, Dordrecht, The Netherlands.

OIV, 2014. *Compendium of international methods of wine and must analysis. Volume 1.* International organization of wine and vine, Paris, France.

Olego M.A. and Garzón J.E., 2014. Predictive modelling of magnesium concentration in grapevine petioles as a basis for liming recommendations in vineyard acid soils. *Vitis*, **53**, 29-32.

Olsen S.R., Cole, C.V., Watanabe F.S. and Dean L.A., 1954. *Estimation of available phosphorus in soils by extraction with sodium bicarbonate, circular 939*. U.S.D.A., Washington, D.C., USA.

Pérez-Magariño S., Ortega-Heras M. and Cano-Mozo E., 2008. Optimization of a solid-phase extraction method using copolymer sorbents for isolation of phenolic compounds in red wines and quantification by HPLC. *J. Agric. Food Chem.*, **56**, 11560-11570. doi:10.1021/jf802100j

R Core Team, 2015. R: A language and environment for statistical computing website. R Foundation for Statistical Computing. URL: http://www.R-project.org/ [Accessed 2016].

Ribéreau-Gayon P., Glories Y., Maujean A. and Dubourdieu D., 2006. *Handbook of enology. Volume 2. The chemistry of wine. Stabilization and treatments, 2nd ed*. Wiley and Sons, Chichester, The United Kingdom. doi:10.1002/0470010398

Romero I., Benito A., Dominguez N., García-Escudero E. and Martin I., 2014. Leaf blade and petiole nutritional diagnosis for *Vitis vinifera* L. cv. 'Tempranillo' by deviation from optimum percentage method. *Span. J. Agric. Res.*, **12**, 206-214. doi:10.5424/sjar/2014121-4308

Ruiz Hernández M., 2002. *La crianza del vino tinto desde la perspectiva vitícola, 1st ed.* Mundi-Prensa, Madrid, Spain.

Seguin G., 1986. Terroirs and pedology of wine growing. *Experientia*, **42**, 861-873. doi:10.1007/BF01941763

SIAR, 2012. Sistema de Información Agroclimática para el Regadío. Ministerio de Agricultura, Pesca y Alimentación, Madrid. URL: http://www.magrama.gob.es/siar/ [Accessed 2012]

Sikora L.J. and Azad M.I., 1993. Effect of compost-fertilizer combinations on wheat yields. *Compost Sci. Util.*, **1**, 93-96. doi:10.1080/1065657X.1993.10757878

Soil Survey Staff, 2010. *Keys to soil taxonomy, 11th ed*. USDA-Natural Resources Conservation Service, Washington, D.C., USA.

Soratto R.P. and Crusciol C.A.C., 2008. Dolomite and phosphogypsum surface application effects on annual crops nutrition and yield. *Agron. J.*, **100**, 261-270. doi:10.2134/agrojnl2007.0120

Sumner M.E. and Noble A.D., 2003. Soil acidification: the world story, 1-28. In: *Handbook of soil acidity*. Marcel Dekker, New York, USA.

van Leeuwen C. and Seguin G., 2006. The concept of terroir in viticulture. *J. Wine Res.*, **17**, 1-10. doi:10.1080/09571260600633135

Vidal M., Urbano P., López A., Blázquez R. and Roquero C., 1997. Usefulness and efficiency of the waste of the sugar foam used as liming matter, 116-125. In: *Proceedings of the 11th International World Fertilizer Congress, Vol. III*. International Scientific Centre of Fertilizers, Braunschweig, Germany.

Vidal M., Garzón E., García V. and Villa E., 2006. Differentiating the amending effects of calcareous materials applied to acid soils by use of optimal scaling procedures. *Agrochimica*, **50**, 132-147.

Villa E., 2005. Incidencia de la aplicación de espumas de azucarería y otras enmiendas calizas sobre la producción de biomasa. Mejora a corto plazo de los condicionantes agronómicos de los suelos ácidos de raña del norte de León. Doctoral Thesis, Universidad de León.

Wooldridge J., Louw P.J.E. and Conradie W.J., 2010. Effects of rootstock on grapevine performance, petiole and must composition, and overall wine score of *Vitis vinifera* cv. Chardonnay and Pinot noir. *S. Afr. J. Enol. Vitic.*, **31**, 45-48. http://dx.doi.org/10.21548/31-1-1399

Yokotsuka K., Nagao A., Nakazawa K. and Sato M., 1999. Changes in anthocyanins in berry skins of Merlot and Cabernet-Sauvignon grapes grown in two soils modified with limestone or oyster shell versus a native soil over two years. *Am. J. Enol. Vitic.*, **50**, 1-12.

Zheng S.J., 2010. Crop production on acidic soils: overcoming aluminium toxicity and phosphorus deficiency. *Ann. Bot.*, **106**, 183-184. doi:10.1093/aob/mcq134