Received: 21 July 2020 | Accepted: 21 February 2021 | Published: 16 April 2021
DOI:10.20870/oeno-one.2021.55.2.4081

Effects of overliming on the nutritional status of grapevines with special reference to micronutrient content

Miguel Ángel Olego[1], Miguel Javier Quiroga[1], Mario Sánchez-García[2], Mateo Cuesta[1],
Jorge Cara-Jiménez[3] 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, Spain
[2] RGA Bioinvestigación S.L., Avenida de Portugal, 41, CP 24071, León, Spain
[3] Chemical and Environmental Bioprocess Engineering Group, Natural Resources Institute (IRENA),
Universidad de León, Avenida de Portugal, 41, CP 24071, León, Spain

**corresponding author*: molem@unileon.es

Associate editor: Luigi Bavaresco

ABSTRACT

Aluminium plays a central role in soil acidity, which is one of the main constraints on grape production in humid, northern temperate viticultural regions. To decrease the acidity of vineyard soil, it is usually amended with alkaline materials that provide conjugate bases to weak acids (liming). However, one practical consideration is the danger of overliming, which has potential implications in terms of yield reduction and decreased bioavailability of several mineral nutrients. The main aim of this study was to evaluate the effects of overliming using dolomitic lime on grapevines growing on acid soil. The effects on the topsoil fertility parameters (0–30 cm), petiole and berry nutrient levels, berry weight and must quality properties were studied in a vineyard planted with *Vitis vinifera* L. cv. Mencía for three years (2014–2016). Data analysis performed using a mixed model that took into account both random effects (year of sampling) and fixed effects (liming treatments) showed that overliming decreased the manganese content in both leaf and berry tissues. Until now, nothing was known about the effects of overliming on both vine nutritional status and harvest quality properties, thus this study fills an important knowledge gap.

KEYWORDS

aluminium, bioavailability, interaction, manganese, grape

INTRODUCTION

The acidity of acid-prone soils, which are naturally low in the barely hydrolytic cations K^+, Ca^{2+} and Mg^{2+}, can be enhanced, albeit inadequately, by applying various soil management practices (Havlin *et al.,* 2014). However, this degradation process can also be alleviated, or largely avoided, by applying effective cultivation practices which aim to reduce the exchangeable aluminium content below a critical threshold characteristic of each crop, as well as to maintain the soil pH above 5.5. Liming is one of the main methods used by farmers to enhance the fertility of acid soils, because it decreases the content of exchangeable Al^{3+} by replacing it with Ca^{2+} and Mg^{2+}, as well as the content of soluble Al^{3+} by precipitation with hydroxyl anions generated by hydrolysis of carbonates in soil solution (Olego *et al.,* 2016). Although the primary purpose of the liming of acid soils is to increase the soil pH through hydrolysis of carbonate anions, it is also used for its favourable effects on soil structure and its significant role in the control of certain plant pathogens (Havlin *et al.,* 2014).

When the soil pH drops, less Mg is held in exchangeable form due to a reduction in variable charge, while more is present in solution and thereby available for loss by leaching. Thus, increasing the cation exchange capacity without Mg addition reduces Mg saturation, and such lime-induced Mg deficiencies can be quite striking (McNaught *et al.,* 1973). Under these soil conditions, Mg deficiency occurs, which results in a reduction in leaf chlorophyll content and a change in the chlorophyll a:b ratio in favour of chlorophyll b (Farhat *et al.,* 2016). Although Mg decreases under different conditions, soil acidity is one of the most prevalent, causing a decrease in Mg availability to grapevines (a crop species whose pH for optimum growth, compared with other crops, ranges from moderately acidic (pH > 5.5) to slightly alkaline (pH < 7.5) (Havlin *et al.,* 2014), lower accumulation of Mg in seeds, marked inhibition of vine growth, acceleration of aging, and reduced productivity and quality in viticulture (Verbruggen and Hermans, 2013). Because of the reduced chlorophyll content in leaves, Mg deficiency manifests itself in the leaves as chlorosis, especially in older leaves, and it causes premature abscission (Zlámalová *et al.,* 2015). Very low soil pH (< 4.5) inhibits Mg^{2+} uptake by vines, and such acid soils tolerate higher leaching rates of Mg^{2+}, as well as higher concentrations of toxic ions, such as Al and Mn (Chen and Ma, 2013).

Treatment with dolomitic limestone is recommended for soils that are deficient in Mg^{2+}, but using it too frequently can result in high Mg^{2+}/K^+ ratios, and thus poor K^+ availability (Goulding, 2016). Dolomitic limestone mainly comprises mineral dolomite formed by a calcium and magnesium double carbonate ($CaMg(CO_3)_2$). Two important characteristics of this liming material are (i) its high neutralising capacity, which is higher than that of limestone because of the lower atomic weight of magnesium with respect to calcium, and (ii) its lower dissolution rate than that of calcite (Loeppert and Suarez, 1996).

The potential for adverse effects of acid soils on the growth, productivity and nutritional status of grapevines has been observed in diverse geographical regions; significant differences in the tolerance of grape cultivars to strongly acidic soils have been noted in shoot and root dry weights, along with root volume differences (Himelrick, 1991). With regard to the root system, roots suffering from Al^{3+} toxicity tend to become underdeveloped, thereby reducing nutrient uptake and increasing drought susceptibility (Sasaki *et al.,* 1996), and the actively growing root tip is the primary site and target of Al^{3+} stress in grapevine rootstocks (Cançado *et al.,* 2009). Specifically, in old vineyards growing on acid soils, Meyer *et al.* (1984) reported that the root system ends abruptly at the depth where the pH value drops, and the Al^{3+} concentration becomes relatively substantial. In this regard, 20 % of exchangeable Al can be considered as the a critical value for ensuring an adequate degree of base saturation (Fageria and Baligar, 2008), along with soil pH values above 5.5 (Weil and Brady, 2017).

Despite the beneficial effects of liming on soil acidity, inappropriate liming rates (*i.e.*, overliming) may result in deficiencies in micronutrients (Fageria and Baligar, 2008). Indeed, the detrimental effects of excess liming can include deficiencies in Fe, Mn, Cu and Zn (Davies, 1997; Moreira *et al.,* 2017). Although extensive research has been carried out on the effects of liming on the properties of acid soils (Quiroga *et al.,* 2017), overliming and its effects on soil fertility and grapevine nutrition have been scantly reported. Specifically, the impact of overliming on the micronutrient content of grape tissues, crop yield and must quality have not been investigated to date. The potential delay and even decrease in vine growth due to the large amounts of lime being, applied to acidic vineyard soils, and the resulting effect on harvest quality, should thus be taken into account in vineyard management for these edaphological conditions. We believe this is the first study to address this knowledge gap.

The main aim of this investigation was to study the short-term and long-term effects of overliming acidic vineyard soil with very low Mg content on bioavailable soil micronutrient levels, concentration ranges of mineral elements in petiole tissues and grape berries, berry weight and harvest quality characteristics.

MATERIALS AND METHODS

1. Study site

The study site was a commercial vineyard located approximately 550 metres above sea level within the protected designation of origin (PDO) Bierzo in the municipality of Cacabelos in León, Spain, at latitude 42°37'N and longitude 6°45'W (Figure 1). From a climatic point of view, this grape growing region is classified as region I (≤ 1,390 Celsius degree-days) based on the system devised by Amerine and Winkler (Jackson, 2020). For the years 2014, 2015 and 2016, the main meteorological data were as follows: an average temperature of 11.5, 12.5 and 12.1 ºC respectively; a reference evapotranspiration (FAO Penman-Monteith) of 915.1, 919.1 and 890.2 mm/yr^{-1} respectively; and rainfall of 605.2, 537.1 and 857.5 respectively (SIAR, 2020). From a bioclimatic point of view, the site would be classified as upper meso-Mediterranean based on the thermotype classification, and as subhumid according to the ombrotype classification (IGME, 1995). The soil under study corresponds to an Inceptisol according to the Soil Survey Manual (USDA, 2017). The parent material of the soils in the study area comprises Tertiary sediments (IGME, 1995). Fe oxyhydroxides are thus the commonest clay minerals in the vineyard soils developed on these Tertiary sediments (Fernández-Calviño *et al.,* 2009), with calcium minerals being almost completely absent.

The research was conducted on > 50-year-old *Vitis vinífera* L. cv. Mencía variety, grafted onto a Rupestris du Lot rootstock, which has been classified as highly sensitive to soil acidity (Fráguas, 1999). Rows were east-west oriented, and vines were spaced 0.5 and 0.6 m within and between rows respectively, with a resulting density of 33,333 vines/ha.

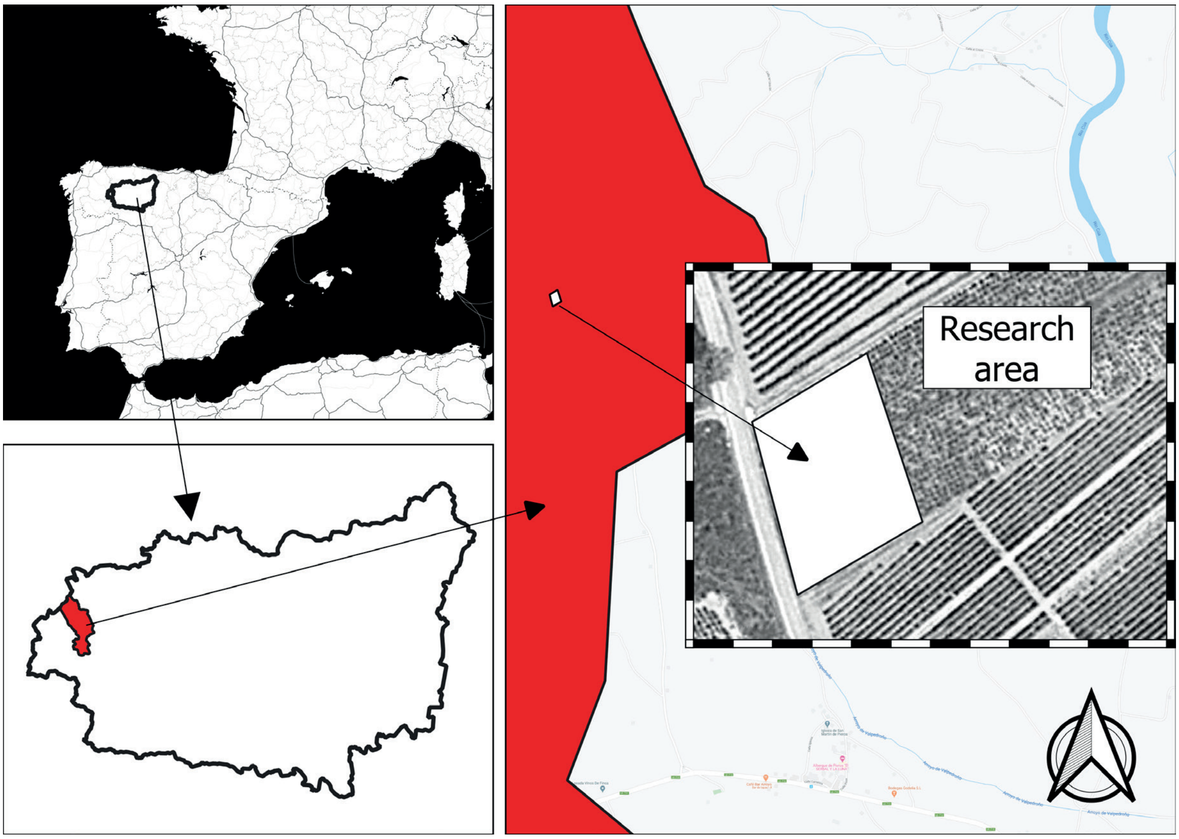

FIGURE 1. Location of the experimental site (north orientation is shown in the lower right corner).

Plants were head-trained with 3–4 arms, and 6–8 nodes per plant were retained at winter pruning, leaving a thumb-sized arm with two buds. The vineyard had no irrigation system support, and a no-tillage system was applied during the research period. Finally, no fertilisers or extra amendments other than those used in this research were applied to the soil under study.

2. Characterisation of the liming material and doses

The composition of the liming material used in this study was 31.1 % CaO and 18.4 % MgO, with a calcium carbonate equivalent (CCE) of 101.2 %. A hypothetical liming rate was calculated with the aim of decreasing the Al saturation of the effective cation exchange capacity (ECEC) to 20 %. Specifically, the lime requirement to ensure an adequate degree of base saturation (*i.e.*, 80 %) - as required in general by most annual and permanent crops (Fageria and Baligar, 2008) - was calculated using the known Cochrane's formula (Cochrane *et al.,* 1980); this yielded a value of about 4,120 kg CCE/ha, which corresponds to 4,000 kg of dolomitic lime/ha based on its CCE. Accordingly, it was decided to apply overliming doses which were three- and nine-fold the amount needed to achieve an Al saturation of the ECEC of 20 % (*i.e.*, 12,000 and 36,000 kg of dolomitic lime per ha respectively), which was the same irrespective of the different overliming doses. The ECEC corresponded to the arithmetic sum of the concentrations of exchangeable Ca, Mg, K and Al. Powdered dolomitic limestone was uniformly spread onto the entire surface of the subplots and manually incorporated by one-pass tillage at a depth of 10–15 cm in January 2014.

3. Experimental design

Four overliming treatments, with three replications per treatment, were applied: a control not treated with lime (C), liming with adequate dose (D), and overliming with three-fold (OD3) and nine-fold (OD9) doses. The study plot was split into 12 subplots with six vines in each (with two buffer vines, and one buffer row between subplots). Because of the homogeneity of the soil area under study (about 60 m^2), the treatment replications were distributed among the 12 subplots (about 2 m^2 each) in a completely random design with three treatments per row.

4. Soil sampling and analyses

Before the amendments were applied, agronomic characterisation of the acidic vineyard soil at a depth of 0–30 cm was carried out based on the following soil properties: texture, soil organic matter (SOM), soil pH in 0.01 M calcium chloride ($CaCl_2$) (pH), electrical conductivity (EC), Ca, Mg, K and Al content, and micronutrient content (Fe, Cu, Mn and Zn). After the amendments had been added in January 2014, the effects of liming on the following soil properties in each subplot were monitored for three years (2014, 2015 and 2016): pH, exchangeable Ca, Mg and Al, and micronutrient content. This monitoring was conducted by sampling the soil at a depth of 0–30 cm at the senescence phenological stage (end of leaf fall).

The soil samples (before and after liming) were collected using an auger. They were then sealed in plastic bags, transported to the laboratory and air-dried at room temperature. Next, they were disaggregated, passed through a 2-mm mesh sieve and then analysed. Textural classes according to the Unites States Department of Agriculture (USDA) were determined by the Bouyoucos hydrometer method (Bouyoucos, 1962). Next, analysis methods were applied to determine (i) the SOM by wet oxidation followed by titration with ferrous ammonium sulphate (MAPA, 1993), (ii) pH in 0.01 M $CaCl_2$ (pH) (CRISON micropH 2001; Jones, 2001), (iii) electrical conductivity at 25 °C (EC) in soil: water (1:2.5) suspension (CRISON conductimeter 522; MAPA, 1993), (iv) content of exchangeable cations (Ca, Mg and K) by extraction with successive aliquots of 1 M ammonium acetate ($NH_4C_2H_3O_2$; MAPA, 1993) followed by analysis of the displaced cations by atomic absorption spectrometry (AAS; Unicam SOLAAR 969), (v) exchangeable Al determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) using 1 M KCl as extraction solution (Little, 1964), and (vi) micronutrient levels (Fe, Cu, Mn, and Zn) extracted following the method of Lindsay and Norvell (1978) using a buffer solution of 0.005 M diethylenetriaminepentaacetic acid (DTPA) and 0.01 M calcium chloride ($CaCl_2$) buffered to pH 7.3 with subsequent determination in the extracts by AAS (Unicam SOLAAR 969).

5. Leaf sampling and analyses

The Ca, Mg, Fe, Mn, Cu and Zn content in petioles (Ca_P, Mg_P, Fe_P, Mn_P, Cu_P and Zn_P respectively) were monitored annually at the veraison phenological stage. Specifically, around 20 grape basal petioles located opposite the bunches were randomly collected per subplot each year. They were sealed in paper bags and transported to the laboratory.

The leaves were carefully rinsed with abundant deionised water, then dried for three days at 70 ºC (Bavaresco *et al.,* 2010). Next, they were wet-digested with an acidic mixture of perchloric, sulphuric and nitric acid at 420 ºC for 20 minutes (Calleja, 1978), and the nutrient contents in the extracts were determined by ICP-AES (iCAP^{TM} 7000 series, Thermo Scientific^{TM}).

6. Grape sampling and analyses

The grapes were sampled at harvest each year (during the second half of September), when degrees Brix readings taking in the vineyard were 22 ºBx or more. In each subplot, 100 grape berries were randomly chosen to determine their weight (W) and the must quality parameters. The grape must from each subplot was obtained manually from the 100 berries by gently pressing the grapes, using rubber gloves to avoid sample contamination. The following harvest quality properties of the must were determined: (i) real acidity (pH; CRISON micropH 2001), (ii) total soluble solids (TSS) measured using a refractometer (Zuzi Series 300), (iii) titratable acidity (TA) determined by titration of the grape must with sodium hydroxide (0.1 M) to an endpoint of pH 7 and expressed as the equivalent content of tartaric acid in g/l, and (iv) malic acid (MA) and tartaric acid (TcA) determined by enzymatic methods at 340 and 492 nm respectively (Analyzer BA400, BioSystems) (OIV, 2018). Bertoldi *et al.* (2011) suggested that grape micronutrients, such as Fe, Mn and Zn, mostly accumulate in the seeds, and Keller (2020) established that the skin can also contribute substantial amounts on a per berry basis; therefore, berry flesh was not considered in this work. The seeds and skins (the main micronutrient sinks) of 100 grapes were manually separated from the flesh and immediately dried at 60 ºC to constant weight. Ca, Mg, Fe, Mn, Cu and Zn in both dried seeds (hereafter referred to as Ca_S, Mg_S, Fe_S, Mn_S, Cu_S and Zn_S respectively) and skins (hereafter Ca_{Sk}, Mg_{Sk}, Fe_{Sk}, Mn_{Sk}, Cu_{Sk} and Zn_{Sk} respectively) were determined by ICP-AES (iCAP^{TM} 7000 series, Thermo Scientific^{TM}) after wet digestion with an acid mixture of perchloric, sulphuric and nitric acid at 420 ºC for 20 minutes (Calleja, 1978).

7. Statistical analysis

Statistical analyses were performed using R software (R Core Team, 2019). Several analyses of variance (ANOVAs) were carried out to study the effect of liming and overliming on C, D, OD3 and OD9 in terms of (i) soil chemical properties, (ii) petiole nutrient contents, (iii) grape yield and must quality properties, and (iv) nutrient contents of the grape tissue.

Mixed ANOVAs were used to determine whether the differences between liming treatments (T) were statistically significant and whether they depended on the year of sampling (Y), and to determine the interaction between the two. In this mixed design, the treatment factor was the between-group predictor, and the year factor was added as a random factor. To reveal the overall effect of each main effect and its interactions, a hierarchical multilevel model approach was used, in which models were built up with one predictor at a time from a baseline with no predictors other than the intercept. Factors in these nested models were added in the following order: no predictors, T, Y, and the interaction between T and Y. Maximum likelihood was used to compare the nested models using a variance analysis.

If the interaction between factors resulted in a significant effect, we did not interpret any main effects, because the higher-order interaction superseded it. In that case, the effect of treatment was split independently for each year of the research and studied using post hoc comparisons to determine which groups differed significantly. On the other hand, if the interaction between factors did not show a significant effect, the main effect of treatment dose was interpreted independently of the factor year, while the main effect of the factor year was ignored.

To carry out a mixed ANOVA, the hypotheses of univariate normal distribution and homoscedasticity of the data were tested in advance. However, an outlier analysis of the data was carried out beforehand. The hypothesis of univariate normality for every variable was tested using the Shapiro–Wilk test. Mixed ANOVA is fairly robust in terms of the error rate associated with violations of the assumption of homogeneity of variance (homoscedasticity) when sample sizes are equal (Field *et al.,* 2012), which was the case in the present study. However, when the assumptions of normality and homoscedasticity (equal variances) were violated, robust statistical methods were used, because violating these two assumptions is a serious practical concern (Mair and Wilcox, 2019). In such cases, Johansen’s formulation of the Welch–James test was implemented, using trimmed means and Winsorised variances (Villacorta, 2017).

When the likelihood ratio or Welch–James test of mixed ANOVAs was large enough to be statistically significant, post hoc comparisons were carried out to determine which groups significantly differed (at the *$P < 0.05$, **$P < 0.01$ or ***$P < 0.001$ level). These comparisons were done with Tukey's honest significance test and pairwise comparison for parametric and non-parametric mixed ANOVAs respectively. Additionally, when interactions between treatment and year was significant, the *F* ratio of the main effect of treatment for each year was evaluated previously to post hoc comparisons. Throughout this study, despite the mixed ANOVAs showing a significant effect of liming treatment on some of the study parameters, comparisons did not reveal any significant differences. A possible explanation for these finding is the control of the family-wise error rate (type I error rate) of the post hoc procedures.

RESULTS

1. Initial soil characterisation before liming and overliming

Table 1 shows the baseline characteristics of the acid soil under study (at 0–30 cm depth) before liming. The Al saturation of the ECEC was 58 % in the 0–30 cm layer (clearly higher than that which ensures an adequate degree of base saturation). Very low exchangeable Ca and Mg contents were also found. Conversely, the micronutrient (Fe, Mn, Cu and Zn) contents exceeded the levels considered suitable for soil fertility (Jones, 2001). It is very likely that the high Cu values were due to cupric-based fungicides that are frequently applied to the vineyards in this area.

TABLE 1. Average characteristics before liming in the 0–30 cm soil layer.

Soil parameter	Value
Sand (%)	32.1
Silt (%)	41.6
Clay (%)	26.2
Textural class (USDA)	Loam
pH	4.12
EC (dS/m)	0.04
SOM (%)	1.00
Ca (cmol(+)/kg)	0.85
Mg (cmol(+)/kg)	0.06
K (cmol(+)/kg)	0.25
Al (cmol(+)/kg)	1.60
Fe (mg/kg)	139
Mn (mg/kg)	39.7
Cu (mg/kg)	6.38
Zn (mg/kg)	3.36

2. Soil properties

The means and standard errors of the soil parameters for the treatment and control subplots throughout the three years of monitoring are shown in Figures 2 and 3. There are obvious differences in performance efficiency between liming doses, and OD9 stands out for its ability to drastically decrease Al levels, as well as to enhance Mg levels. Similarly, the effect of overliming on soil Mn levels is noteworthy.

Because of violations of parametric assumptions, robust mixed ANOVA methods were used for pH, Ca and Mg levels, and the hierarchical multilevel model for all other soil parameters.

TABLE 2. Analysis of variance performed on soil parameters (pH, Ca, Mg, Al, Fe, Mn, Cu and Zn) at leaf fall stage.

Soil parameter	W–J value (Treatment (T))	W–J value (Year (Y))	W–J value (T × Y)
pH	5.72 (*)	0.03	1.33
Ca	5.52 (*)	2.31	1.57
Mg	10.8 (**)	0.83	0.99
Soil parameter	**ML ratio (Treatment (T))**	**ML ratio (Year (Y))**	**ML ratio (T × Y)**
Al	41.4 (***)	3.68	24.4 (***)
Fe	3.11	2.65	10.6
Mn	23.2 (***)	6.06	1.08
Cu	10.3 (*)	3.77	7.02
Zn	0.97	1.51	16.7 (*)

The variability in the soil parameters pH, Ca and Mg was evaluated using robust mixed ANOVA (Welch–James (W–J) test), and Al, Fe, Mn, Cu and Zn were evaluated using the hierarchical multilevel model (maximum likelihood (ML) ratio). The results were significant at *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

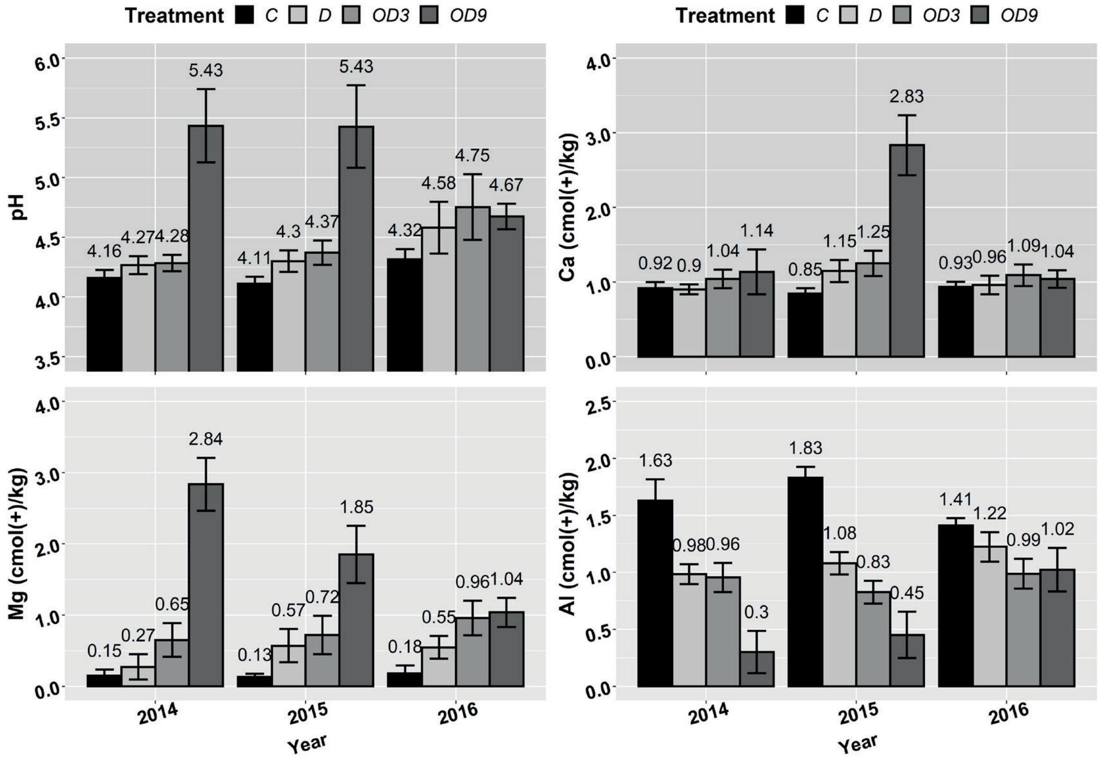

FIGURE 2. Mean values of soil parameters pH, Ca, Mg and Al for each treatment (2014–2016).

Treatments: control (C), liming dose (D) and overliming with three-fold dose (OD3) and nine-fold dose (OD9). Mean values are shown above the error bars. Error bars reflect the standard error (SE) of the mean (± 1 SE mean).

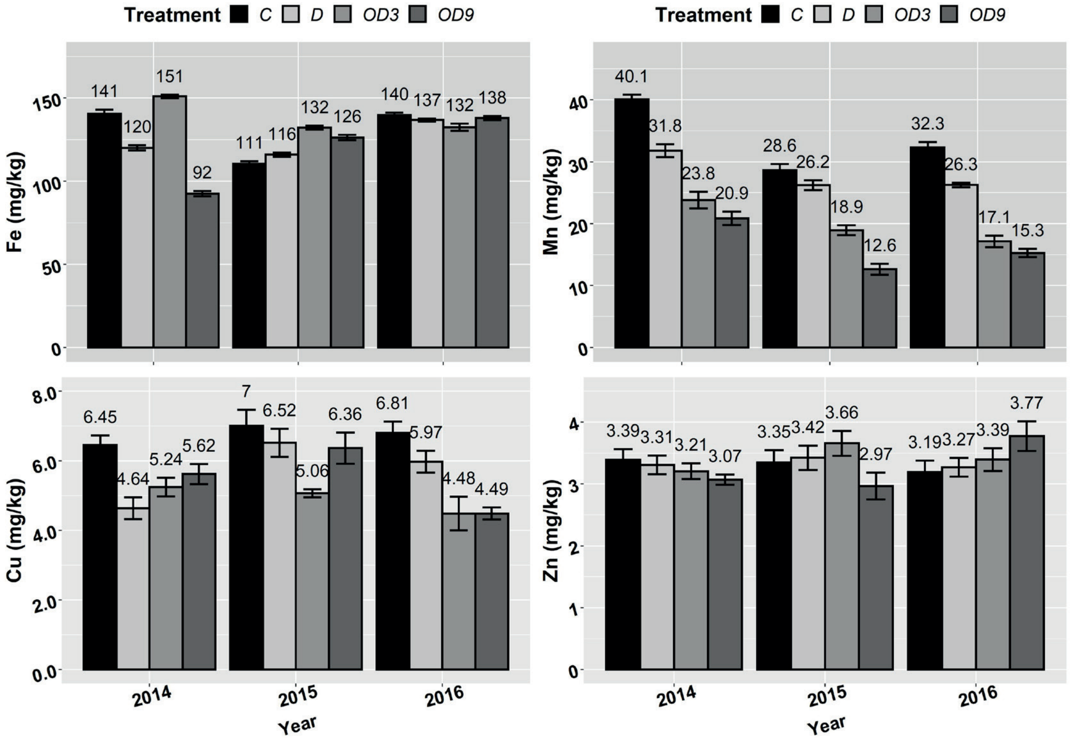

FIGURE 3. Mean values of soil parameters Fe, Mn, Cu and Zn for each treatment (2014–2016).

Treatments: control (C), liming dose (D) and overliming with three-fold dose (OD3) and nine-fold dose (OD9). Mean values are shown above the error bars. Error bars reflect the standard error (SE) of the mean (± 1 SE mean).

According to the mixed ANOVAs, there was a significant effect of liming treatment on the following soil properties: pH, Ca, Mg, Al, Mn and Cu. Moreover, the effect of year of sampling on each of the soil properties was non-significant (Table 2); indeed, the effect of liming treatment significantly changed with the year of sampling in terms of Zn and Al levels only, as revealed by the significant interaction between these factors (L × Y) (Table 2).

The post hoc comparisons revealed both significant increases (Mg: C < OD9 (*)) and decreases (Mn: C > OD3 (***); C > OD9 (***); D > OD9 (**)). Specifically, the Al comparisons were evaluated for each year of sampling (2014: C > D (*), C > OD3 (*), C > OD9 (***), D > OD9 (*), OD3 > OD9 (*); 2015: C > D (**), C > OD3 (**), C > OD9 (***), D > OD9 (*); 2016: non-significant differences). Regarding the Zn parameter, the significant interaction L × Y simply indicates that the year of sampling significantly changed the effect of treatments on this soil nutrient. Despite the significant effects of the liming treatment as identified by the mixed ANOVAs for pH, Ca and Cu, the comparisons did not reveal any significant differences. A plausible explanation for this is the control of the type I error rate in the post hoc procedures.

3. Petiole nutrient contents

Figure 4 shows the time evolution of the mean and standard error of the petiole nutrients levels (Ca_P, Mg_P, Fe_P, Mn_P, Cu_P and Zn_P) for both treatment (liming and overliming) and control subplots, throughout the three years of monitoring. The very high levels of Mn, as well as the low levels of Ca, are remarkable. On the other hand, in control subplots in particular petiole levels of Mg are in the critical range (Bavaresco *et al.,* 2010). Despite the very high Mn concentration in grapevine petioles observed at veraison (> 10 times higher than optimal), no foliar symptoms of toxicity were apparent.

As in the case of soil parameters, hierarchical multilevel models were used to investigate whether the differences between treatments were statistically significant and whether they depended on the year of sampling, as well as to study the interactions between these two factors (maximum likelihood ratio). However, whenever there were violations of parametric assumptions, robust mixed ANOVA methods were used (Welch–James (W–J) test; Table 3). According to the mixed ANOVAs, there was a significant effect of liming treatment on Mg_P, Mn_P and Zn_P. Additionally, year of sampling was significant for Ca_P, Fe_P, Cu_P and Zn_P, whereas the effect of liming treatments on Ca_P, Cu_P and Zn_P levels changed significantly with year of sampling (Table 3). Post hoc comparisons revealed both significant increases (Mg_P: C < OD3 (**), C < OD9 (**)) and decreases (Mn_P: C > OD3 (***), C > OD9 (***); D > OD3 (**)). The comparisons of Ca_P, Cu_P and Zn_P were evaluated for each year of sampling, with significant differences being found only in Ca_P (2015; C < OD9 (*)). For the nutrients Cu_P and Zn_P, the significance of the interaction L × Y simply indicates that the year of sampling significantly changed the effect of treatments on both of these petiole nutrients.

4. Berry weight, must quality and grape nutrient levels

The evolution of the berry weight (W), harvest quality parameters (pH, TSS, TA, MA and TcA) and grape nutrient levels (in seeds and skins) in the treatment and control subplots, was evaluated throughout the three years of monitoring. Figures 5, 6 and 7 show the time evolution of the mean and standard error of the harvest parameters, as well as nutrient content in grape skins and seeds for the treatment and control subplots respectively during the research period.

Again, multilevel hierarchical models and robust mixed ANOVA methods (Welch–James (W–J) test) were used (Tables 4 and 5). For berry weight and must quality parameters, none of the evaluated parameters required robust mixed ANOVA, while in the case of the berry nutrient levels, differences between the means of Ca_S, Fe_S, Cu_S and Zn_S, as well as Zn_{Sk}, were assessed using robust mixed ANOVA methods. According to the mixed ANOVAs, no liming treatment had significant effects on either berry weight or must quality parameters. As might be expected, year of sampling had a significant vintage effect on both berry weight and must quality parameters. Additionally, a significant interaction between liming and sampling year was found in terms of W, TA and MA.

The same mixed ANOVA procedures applied for berry weight and must quality were also applied for grape nutrient levels (using robust mixed ANOVA methods whenever violations of parametric assumptions appeared). According to the mixed ANOVAs, the liming treatment had a significant effect on nutrient content in seeds (Mg_S, Fe_S, Mn_S, Cu_S and Zn_S) and skins (Mg_{Sk}, and Mn_{Sk}).

TABLE 3. Analysis of variance performed on petiole nutrients (Ca_P, Mg_P, Fe_P, Mn_P, Cu_P and Zn_P) at veraison stage.

Petiole nutrient	W–J value (T)	W–J value (Y)	W–J value (T × Y)
Cu_P	4.43	16.6 (*)	4.33 (*)
Petiole nutrient	ML ratio (T)	ML ratio (Y)	ML ratio (T × Y)
Ca_P	5.48	11.3 (**)	14.3 (**)
Mg_P	11.8 (**)	2.10	2.98
Fe_P	7.44	13.5 (**)	4.12
Mn_P	23.7 (***)	0.90	2.89
Zn_P	11.6 (**)	15.1 (***)	21.9 (**)

The variability in the petiole nutrient Cu_P was evaluated using robust mixed ANOVA (Welch–James (W–J) test), and Ca_P, Mg_P, Fe_P, Mn_P and Zn_P were evaluated using the hierarchical multilevel model (maximum likelihood (ML) ratio). The results are significant when $^*p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ level.

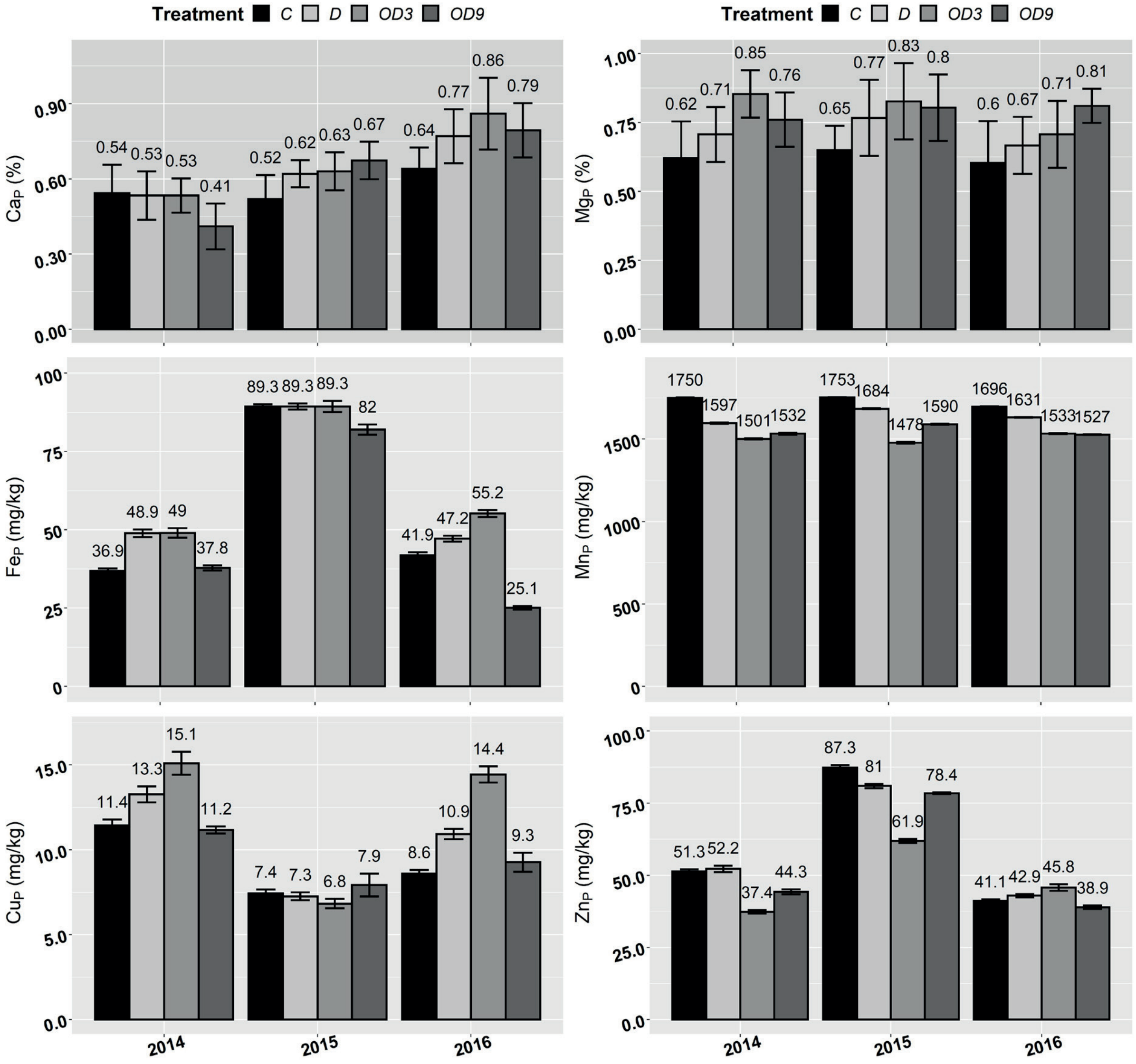

FIGURE 4. Mean values of petiole nutrients (Ca_P, Mg_P, Fe_P, Mn_P, Cu_P and Zn_P) for each treatment at veraison stage (2014–2016).

Treatments: control (C), liming dose (D) and overliming with three-fold dose (OD3) and nine-fold dose (OD9). Mean values are shown above the error bars. Error bars reflect the standard error (SE) of the mean (± 1 SE mean).

Additionally, year of sampling was significant for Ca_S, Fe_S, Mn_S, Zn_S, Cu_{sk} and Zn_{sk}, whereas the the year of sampling did not significantly change the effect of liming treatments in either grape tissue. Post hoc contrasts revealed significant differences in both seeds (Mg_S: C < OD3 (***), C < OD9 (***), D < OD3 (***), D < OD9 (***); Mn_S: C > OD3 (**), OD9 > OD3 (*); Fe_S: D < OD9 (*); Cu_S: C > OD3 (*); C > OD9 (***); OD3 > OD9 (*); Zn_S: C > OD3 (*), C > OD9 (*)) and skins (Mg_{Sk}: C < D (*), C < OD3 (***), C < OD9 (***); Mn_{Sk}: C > D (*); C > OD3 (***), C > OD9 (**), D > OD3 (*)). As in the case of the soil and petiole results, overliming appeared to have a remarkable effect on the Mg and Mn content of both grape tissues.

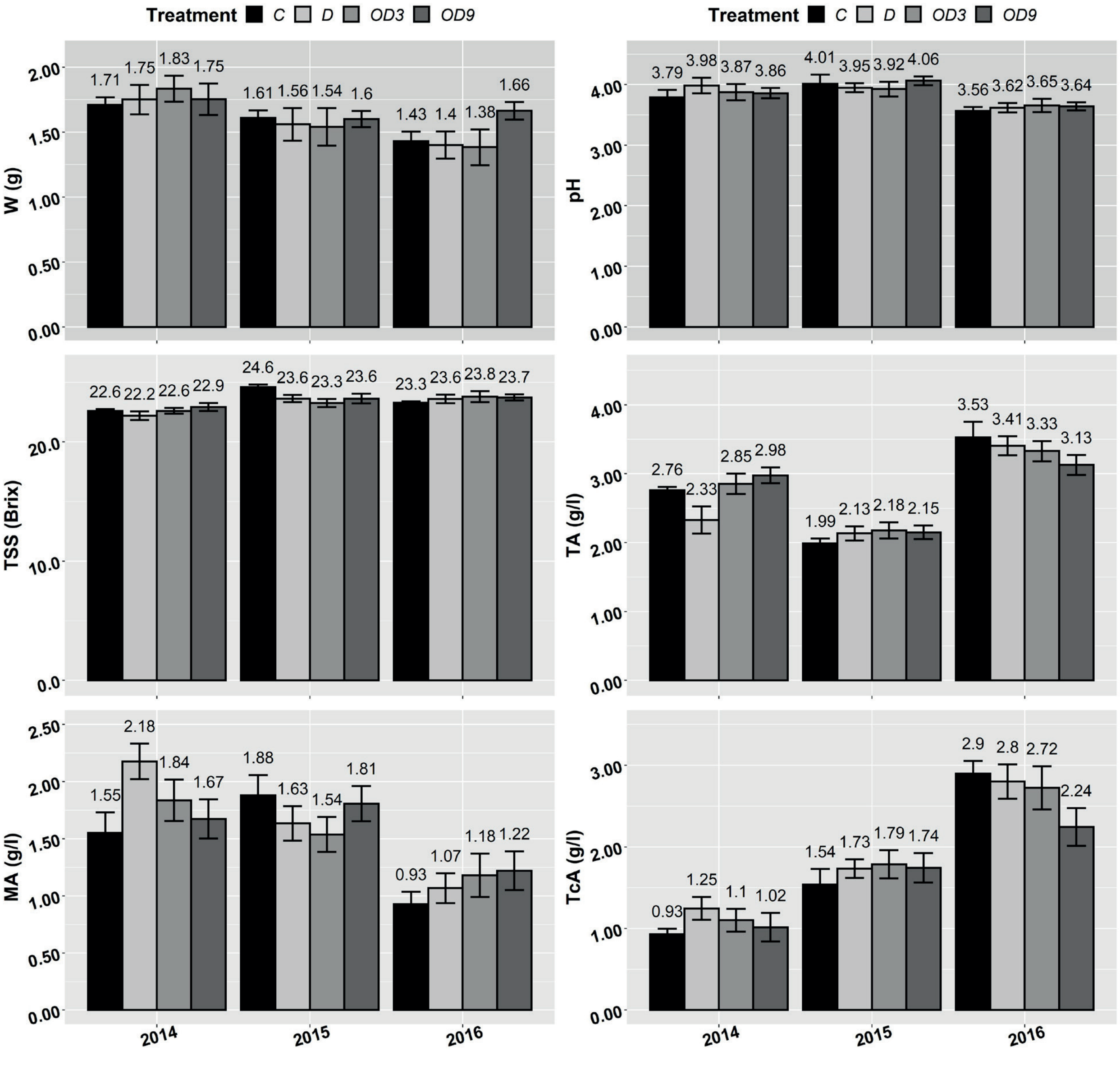

FIGURE 5. Mean values of harvest parameters (W, pH, TSS, TA, MA and TcA) for each treatment (2014–2016).

Treatments: control (C), liming dose (D) and overliming with three-fold dose (OD3) and nine-fold dose (OD9). Weight of berries (W) is expressed on per berry basis, and total acidity (TA) is expressed as g sulphuric acid per l. Mean values are shown above the error bars. Error bars reflect the standard error (SE) of the mean (± 1 SE mean).

TABLE 4. Analysis of variance performed on berry weight and must parameters (W, pH, TSS, TA, MA and TcA) at harvest.

Harvest parameter	ML ratio (T)	ML ratio (Y)	ML ratio (T × Y)
W	4.44	11.1 (**)	13.5 (*)
pH	2.13	12.6 (**)	8.50
TSS	0.88	7.23 (*)	4.63
TA	2.40	15.0 (***)	22.7 (***)
MA	2.03	11.6 (**)	17.7 (**)
TcA	3.35	15.1 (***)	8.89

The variability in all berry weight and must parameters was evaluated using a hierarchical multilevel model (maximum likelihood (ML) ratio). The results are significant when $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

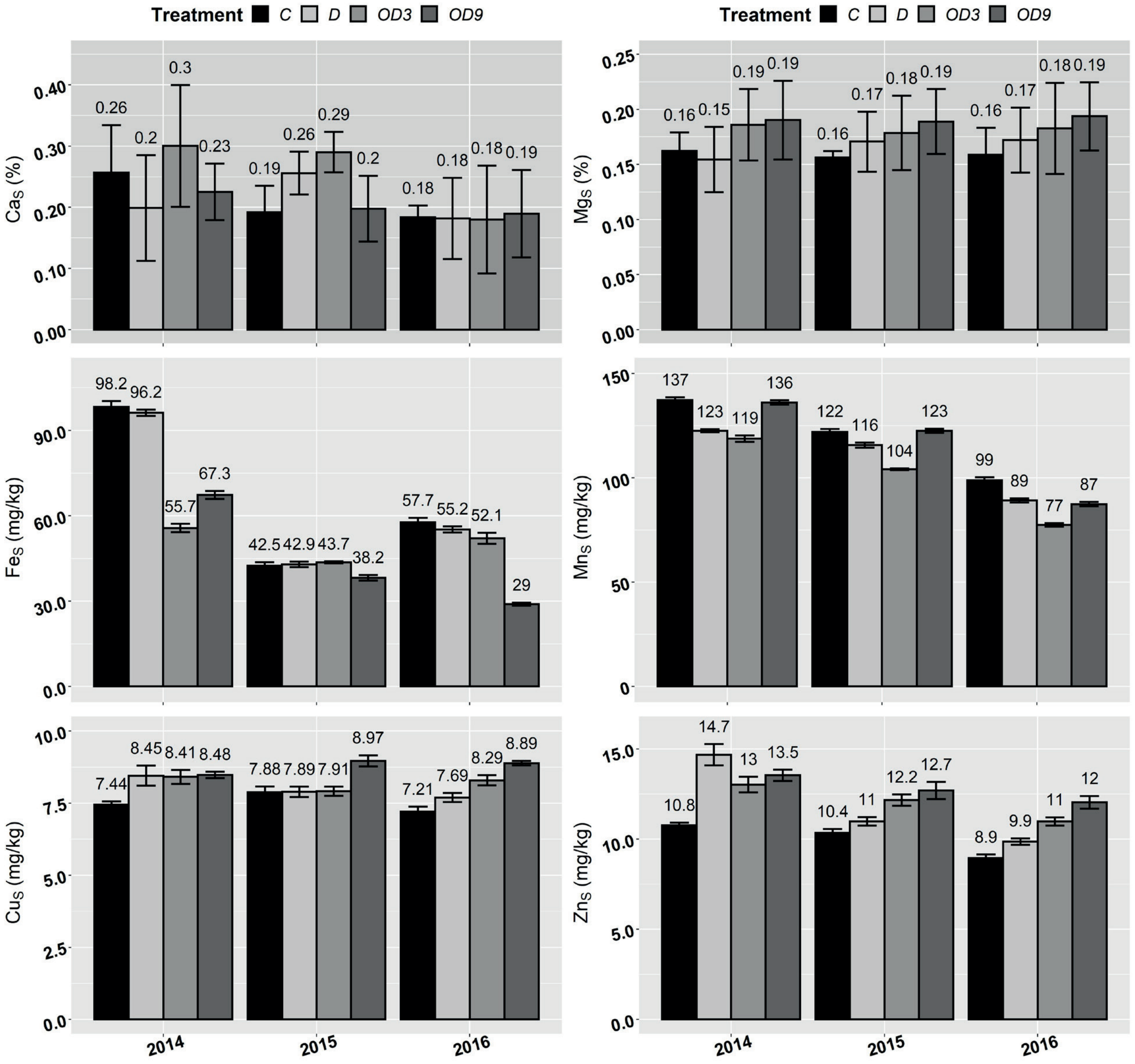

FIGURE 6. Mean values of nutrient levels in seeds (Ca_S, Mg_S, Fe_S, MnS, Cu_S and Zn_S) for each treatment at harvest time (2014–2016).

Treatments: control (C), liming dose (D) and overliming with three- (OD3) and nine-fold dose (OD9). Mean value are shown above error bars. Error bars reflect the standard error (SE) of the mean (± 1 SE mean).

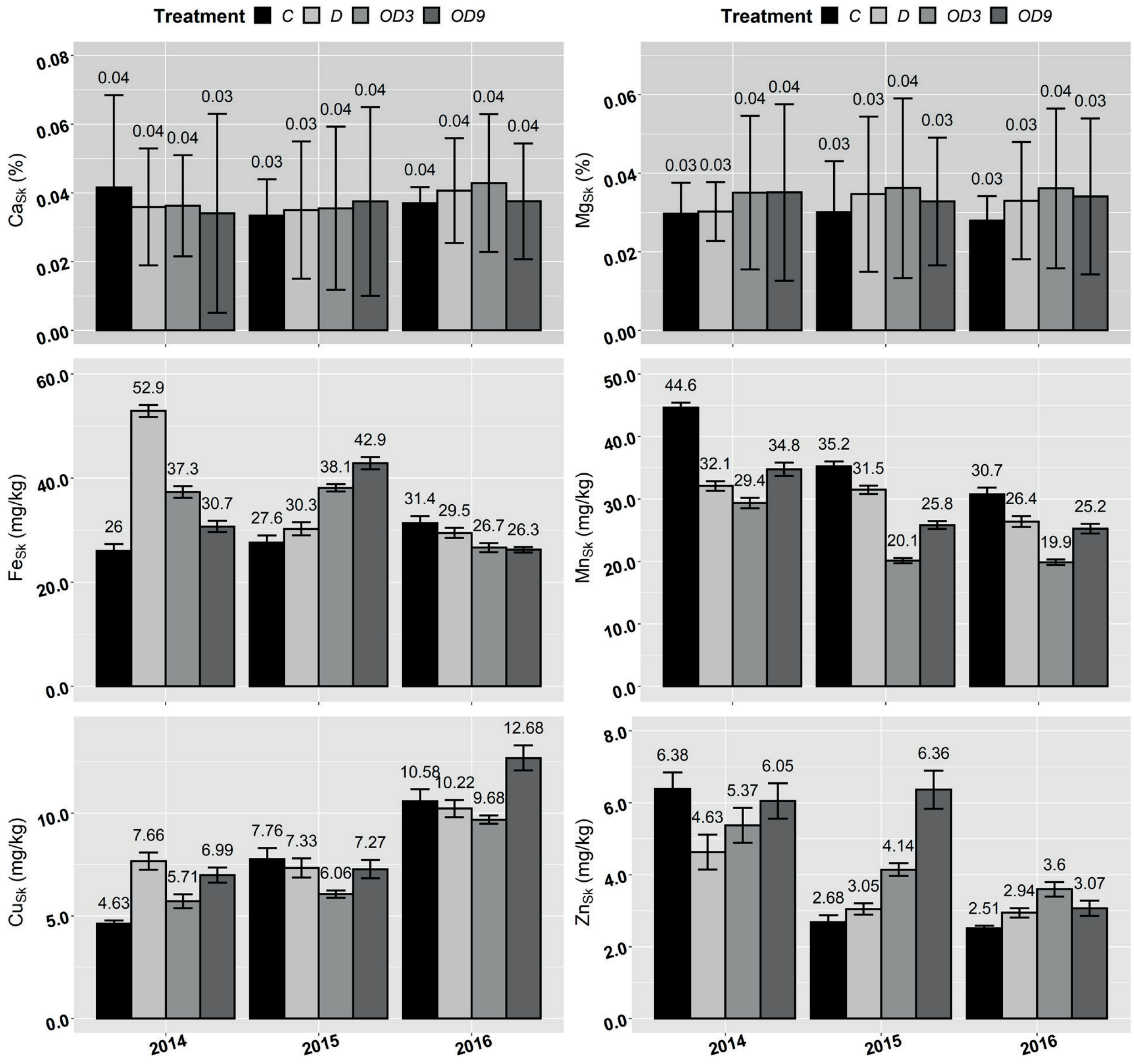

FIGURE 7. Mean values of nutrient levels in skins (Ca_{Sk}, Mg_{Sk}, Fe_{Sk}, Mn_{Sk}, Cu_{Sk} and Zn_{Sk}) for each treatment at harvest time (2014–2016).

Treatments: control (C), liming dose (D) and overliming with three-fold dose (OD3) and nine-fold dose (OD9). Mean values are shown above the error bars. Error bars reflect the standard error (SE) of the mean (± 1 SE mean).

TABLE 5. Factorial analysis of variance performed on nutrient level in grape tissues (seeds and skins) at harvest.

Grape nutrients in seeds (S)	W–J values (T)	W–J values (Y)	W–J values (T × Y)
CaS	1.33	6.10 (*)	1.82
FeS	4.60 (*)	11.3 (**)	1.71
CuS	35.8 (***)	1.01	2.98
ZnS	16.1 (**)	11.1 (*)	0.42
Grape nutrients in seeds (S)	ML ratios (T)	ML ratios (Y)	ML ratios (T × Y)
MgS	42.8 (***)	1.34	11.1
MnS	12.0 (**)	12.9 (**)	2.11
Grape nutrient in skins (Sk)	W–J value (T)	W–J value (Y)	W–J value (T × Y)
ZnSk	1.45	11.0 (*)	1.06
Grape nutrients in seeds (S)	ML ratios (T)	ML ratios (Y)	ML ratios (T × Y)
CaSk	0.84	5.07	11.9
MgSk	21.0 (***)	0.75	6.63
FeSk	2.63	3.30	12.1
MnSk	20.6 (***)	8.80	4.14
CuSk	5.26	11.3 (**)	7.46

The variability in the Ca, Fe, Cu and Zn levels in seeds, as well as Zn content in skins, was evaluated through robust mixed ANOVA (Welch–James (W–J) test). The variability in the levels of the other nutrients was evaluated through hierarchical multilevel model (maximum likelihood (ML) ratio). The results are significant when *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

DISCUSSION

As expected, on the acid vineyard soil, both liming and overliming were effective for decreasing acidity and improving the Ca and Mg content of the topsoil. In particular, overliming was more effective than liming for increasing soil Mg levels and decreasing soil Al levels. Firstly, there is no doubt that application of dolomite promoted a decrease in the water-soluble and exchangeable fractions of Mn as the dose of dolomite was increased. Specifically, overliming (in particular in the OD9 treatment) significantly decreased Mn levels, confirming that the degree of soil acidity is the factor which has the greatest influence on Mn content of soil and vines, with soil pH being the main parameter (Kalanquin *et al.,* 2013). Additionally, it should not be forgotten that interactions between soil nutrients affect their uptake and distribution (Rietra *et al.,* 2017). Specifically, the basic cations Mg^{2+} and Ca^{2+} moderate soil acidity and compete with Mn^{2+} for uptake by plants (Fernando and Lynch, 2015). The other soil micronutrients (Fe and Zn) were not significantly affected by liming, although overliming significantly decreased soil Cu levels. In this regard, overliming may induce soil micronutrient deficiencies more easily in other soils in which, in contrast to this study (Table 1), they have low bioavailability levels. On the other hand, one can hypothesise that both liming and overliming improve the soil structure, which in turn would improve root penetration and internal drainage (van Leeuwen *et al.,* 2018), resulting in better conditions for root vines to obtain nutrients and H_2O for optimum productivity.

In the third year of the investigation, both liming and overliming showed a decreased influence on soil properties (especially with regard to pH and levels of Ca, Mg, Al and Mn). Considering that the duration of the residual effect of liming can be estimated based on the decrease in Al saturation (this estimation is used to determine when additional liming amendments should be applied), the data obtained for the liming and overliming subplots clearly indicate that liming will become necessary again four years after the start of the research. Thus, contrary to expectations, the results of this study show that both liming and overliming have an identical residual effect (2–3 years) on soil parameters such as pH and Al content. However, the residual effect of overliming (OD3 and OD9) on soil Mg levels was clearly greater compared with the liming dose (D; Figure 2).

The current findings suggest that overliming (done in a natural way with an appropriate overliming dose) could be key to decreasing the amount of soluble Mn in vineyard soils in which Mn toxicity is a potential and serious problem, as was the case in the poorly aerated and acidic study soil.

This could be important, since vines can take up much more Mn than they require, and excess Mn can be extremely toxic (Pittman, 2005), inducing oxidative stress and resulting in symptoms such as stunted growth and necrotic lesions (Kochian *et al.,* 2004). Mn in its enzymatic role and naturally as a Lewis acid shows similar properties to Mg (the biochemical behaviour of Mn resembles that of Mg), so they might be expected to substitute each other in proteins and biochemical reactions (Schmidt and Husted, 2019). This could explain the large amounts of Mn found in the petiole tissues of the vines cultivated under extremely low levels of soil Mg in the current study.

Interestingly, there were also significant Mg_P increases and significant Mn_P decreases in the overliming compared with the control subplots (Figure 3). This reflects that, although nutrient storage in the woody parts of the vine makes it challenging to assess grapevine responses to amendment applications, the large increase in soil pH and Mg bioavailability caused by overliming with dolomitic limestone (particularly treatment OD9) counteracted this buffering effect. This is consistent with the fact that the liming dose (D) did not result in significant differences in petiole levels with respect to control subplots. However, since the tested vines experienced severe Mg deficiency, a fast response is clearly understandable. Cu levels in soil significantly decreased with overliming, as did soil Mn levels, but no such response was observed in thepetiole. While these data must be interpreted with caution, perhaps cupric-based fungicides applied during the present study are the principal reason for these findings.

Although Busenberg and Plummer (1989) suggested that the $CaCO_3$ component of dolomite dissolves faster than the $MgCO_3$ component, the results of the present study do not support this. Clearly, the soil Ca bioavailability level in this investigation only showed a marked increase for treatment OD9 in the second year after overliming (2015). This sharp increase in soil bioavailability Ca levels was not similarly reflected by an increase in Ca_P levels for liming and overliming with respect to control subplots; this suggests that nutrient movement and uptake by grapevines may depend on several interacting edaphic factors, including soil aeration and temperature, soil properties, cropping practices, rootstock and scion cultivar, as well as historical management practices (Kalcsits *et al.,* 2020). However, it should not be surprising that, even when Ca levels in soils are high, this is not the case for foliar and berry tissues, because Ca can be sequestered in vacuoles present in the root (Storey *et al.,* 2003). Additionally, microsite differences in soil pH are likely contribute to variable nutrient availability around the root system, which in turn could promote high nutrient content variability in different vine tissues (Pradubusuk and Davenport, 2011).

In general terms, our findings are consistent with data obtained by Bertoldi *et al.* (2011), who suggested that grape micronutrients such as Fe, Cu, Mn and Zn mostly accumulate in the seeds. However, Keller (2020) suggested that grape skins may also contribute substantial amounts of those micronutrients on a per berry basis. Our results indicate that the overliming of acid vineyard soils can decrease Mn levels in (both foliar and grape tissues of) vines. The correlation found between the Mg and Mn concentrations in both grape seeds and skins (Mg_S–Mg_{Sk}: 0.69 ($p < 0.05$); Mn_S–Mn_{Sk}: 0.78 ($p < 0.05$)) provides a good illustration of how both these tissues act similarly as sinks. In fact, the interaction between these two nutrients can be clearly seen in the case of the correlations found in grape tissues (Mg_S–Mn_{Sk}: –0.42 ($p < 0.05$); Mg_S–Mn_S: –0.28 ($p = 0.09$); Mn_S–Mg_{Sk}: –0.22 ($p = 0.20$)). Competition between Mg and Mn is apparent from the negative correlations found between Mn_P and Mg levels in grape tissues (Mn_P–Mg_S: –0.36 ($p < 0.05$); Mn_P–Mg_{Sk}: –0.51 ($p < 0.05$)); the opposite was found regarding the correlations between Mg_P and Mn levels in grape tissues (Mg_P–Mn_S: 0.48 ($p < 0.05$); Mg_P–Mn_{Sk}: 0.44 ($p < 0.05$)). These results suggest that Mg inhibits Mn transport from roots to both leaf and grape tissues, although it is unlikely that these two ions compete for common uptake sites in vines. In this sense, the substitution of Mg with Mn typically changes the catalytic rate of enzymes, and in many cases the functional role of the enzymes as well; RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) provides a good illustration of the extent to which differential binding of Mg and Mn changes the catalytic rate and substrate preference of a protein (Lilley *et al.,* 2003). The photosynthetic processes supported by this catalyst can thus be expected to be affected when vines have insufficient Mn foliar tissue levels (although this is not the case in the present study).

However, the role of Mn in metalloenzyme activation, along with the resulting impact on vine metabolism, is still poorly understood (Schmidt and Husted, 2019). Further studies are required to address the above questions.

Unlike Mn, it is somewhat surprising that both Cu and Zn levels in petioles and grape tissues tended to increase in limed and overlimed treatments compared to control subplots. In this regard, the micronutrient contents of grapevines depend on both their soil levels and the soil factors that regulate their availability; the effects of the latter factors and their relative degree of efficacy vary considerably depending on the micronutrient (Sillanpää, 1982). Our findings thus suggest differences in soil micronutrient behaviour in response to overliming. Specifically, one may hypothesise that overliming strongly enhances the oxidation reaction of Mn^{2+} to Mn^{4+} due to increased soil aeration (Alleoni *et al.,* 2005), thereby decreasing its bioavailability.

Neither liming nor overliming significantly affected yield grape or must quality. These results suggest that, although the practice of liming (and by extension, overliming) may affect harvest quality from morphological, physical, chemical and organoleptic points of view, the potential for extensive storage and mobilisation of nutrients within the woody parts of the vine may explain the delay in vine harvest response, even in the case of overliming. Anthocyanin levels in grapes, which have not been studied in this research, are apparently protected from degradation in cell vacuoles by high amounts of Mg or Mn (Sinilal *et al.,* 2011). It can therefore be assumed that if overliming had been carried out with a liming material lacking magnesium carbonate in its composition, a detrimental effect on the development of the colour of the grape could have occured. This is an important issue for future research.

Further work is required to establish overliming doses that could induce substantial changes in both quantitative and qualitative harvest parameters. Additionally, understanding nutrient partitioning in vines, and the effects of overliming on it, may be further elucidated by research into the relationship between soil and vine nutrient status, as well as nutrient–nutrient interactions (while also studying soil physical properties that affect mineral nutrient availability and thereby nutrient movement and uptake in grapevines (Cass, 2005)). Finally, the results of our study suggest that it may be possible to observe more obvious effects of overliming on coarse-textured soils, where lower buffer capacities and micronutrient levels are often present.

CONCLUSION

Liming and overliming with dolomitic limestone decreased soil exchangeable aluminium, improved the supply of magnesium and increased the soil pH in the acid soil cultivated with *Vitis vinífera* L. cv. Mencía, on which the present study was carried out. Our research did not detect any evidence of a decrease in berry weight and/or harvest quality as a result of overliming. Perhaps strong detrimental effects would have been observed in variable-charge vineyard soils, if overliming had increased pH values above those found in this study. One may hypothesise that clearer trends in both harvest and nutritional parameters will appear when studying younger vines and/or when using higher overliming doses.

The results of the current study reveal that overliming reduced uptake of manganese and its accumulation in both petioles and berries; however, no detrimental effects of overliming, such as deficient levels of manganese and any of the other evaluated micronutrients, were observed. Obviously, prior to overliming, the studied acid soil had high available levels of all the investigated micronutrients (in particular, manganese).

Acknowledgements: The authors are most grateful to Losada Vinos de Finca, S.A., for assisting with this research project.

REFERENCES

Alleoni, L.R.F., Cambri, M.A., & Caires, E. F. (2005). Atributos químicos de um Latossolo de Cerrado sob plantio direto de acordo com doses e formas de aplicação de calcário. *Revista Brasileira de Ciência do Solo, 29*(6), 923-934. https://doi.org/10.1590/S0100-06832005000600010

Bavaresco, L., Gatti, M., & Fregoni, M. (2010). Nutritional deficiencies. *In*: Delrot, S., Medrano, H., Or, E., Bavaresco, L., & Grando, S. (Eds.), *Methodologies and results in grapevine research* (pp. 165-191). Dordrecht, The Netherlands; Springer. https://doi.org/10.1007/978-90-481-9283-0_12

Bertoldi, D., Larcher, R., Bertamini, M., Otto, S., Concheri, G., & Nicolini, G. (2011). Accumulation and distribution pattern of macro- and microelements and trace elements in *Vitis vinifera* L. cv. Chardonnay berries. *Journal of Agricultural and Food Chemistry, 59*, 7224-7236. https://doi.org/10.1021/jf2006003

Bouyoucos, G. (1962). Hydrometer method improved for making particle size analyses of soils. *Agronomy Journal*, *544*, 464-465. https://doi.org/10.2134/agronj1962.00021962005400050028x

Busenberg, E., & Plummer, L. N. (1989). Thermodynamics of magnesian calcite solid–solutions at 25ºC and 1 atm total pressure. *Geochimica et Cosmochimica Acta*, *53*,1189-1208. https://doi.org/10.1016/0016-7037(89)90056-2

Calleja, A. (1978). La mineralización de muestras vegetales para el análisis de minerales por espectrofotometria y colorimetría. *Anales de la Facultad de Veterinaria de León*, *24*, 175-177.

Cançado, G. M. A., Ribeiro, A. P., Piñeros, M. A., Miyat, L. Y., Alvarenga, Á. A., Villa, F., Pasqual, M., & Purgatto, E. (2009). Evaluation of aluminium tolerance in grapevine rootstocks. *Vitis*, *48* (4), 167-173. https://doi.org/10.5073/vitis.2009.48.167-173

Cass, A. (2005). Effects of soil physical characteristics on mineral nutrient availability, movement, and uptake. In P. Christensen, & D.R. Smart, (Eds.), *Soil Environment and Vine Mineral Nutrition Symposium: Proceedings of the soil environment and vine mineral nutrition symposium* 2004 (pp. 3-11). San Diego: American Society for Enology and Viticulture.

Chen, Z., & Ma, J. (2013). Magnesium transporters and their role in Al tolerance in plants. *Plant Soil*, *368*, 51-56. https://doi.org/10.1007/s11104-012-1433-y

Cochrane, T. T., Salinas, J. G., & Sánchez, P. A. (1980). An equation for liming acid mineral soils to compensate crop aluminium tolerance. *Tropical Agriculture, 57*, 133-140.

Davies, B. E. (1997). Deficiencies and toxicities of trace elements and micronutrients in tropical soils: limitations of knowledge and future research needs. *Environmental Toxicology and Chemistry*, 16 (1), 75-83. https://doi.org/10.1002/etc.5620160108

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

Farhat, N., Elkhouni, A., Zorrig, W., Smaoui, A., Abdelly, C., & Rabhi, M. (2016). Effects of magnesium deficiency on photosynthesis and carbohydrate partitioning. *Acta Physiologiae Plantarum*, *38*:145. https://doi.org/10.1007/s11738-016-2165-z

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

Fernando, D. R. & Lynch, J. P. (2015). Manganese phytotoxicity: new light on an old problem. *Annals of Botany*, *116*, 313-319. https://doi.org/10.1093/aob/mcv111

Field, A., Miles, J., & Field, Z. (2012). *Discovering Statistics Using R* (1st ed.). SAGE Publications Ltd.

Fráguas, J. C. (1999). Tolerância de porta-enxertos de videira ao aluminio do solo. *Pesquisa Agropecuaria Brasileira*, *34*, 1193-1200. https://doi.org/10.1590/S0100-204X1999000700011

Goulding, K. W. T. (2016). Soil acidification and the importance of liming agricultural soils with particular reference to the United Kingdom. *Soil Use and Management*, *32*, 390-399. https://doi.org/10.1111/sum.12270

Havlin, J., Tisdale, S. L., Nelson, W. L., & Beaton, J. D. (2014). *Soil Fertility and Fertilizers* (8th ed.). Pearson.

Himelrick, D. G. (1991). Growth and nutritional responses of nine grape cultivars to low soil pH. *Hortscience*, 26 (3), 269-271. https://doi.org/10.21273/HORTSCI.26.3.269

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

Jackson, R. S. (2020). Wine Science: Principles and Applications. 5th ed (Academic Press: London, UK).

Jones, J. B. (2001). *Laboratory guide for conducting soil tests and plant analysis* (No. BOOK). CRC press. https://doi.org/10.1201/9781420025293

Kalanquin, D., Coste, M., Paricaud, T., Dubernet, M. & Fil, J. (2013). Le manganèse dans les vins, origine et teneurs. *Revue des oenologues et des techniques vitivinicoles et* oenologiques, *40* (149), 33-35.

Kalcsits, L., Lotze, E., Tagliavini, M., Hannam, K. D., Mimmo, T., Neilsen, D., Neilsen, G., Atkinson, D., Biasuz, E. C., Borruso, L., Cesco, S., Fallahi, E., Pii, Y., & Valverdi, N.A. (2020). Recent achievements and new research opportunities for optimizing macronutrient availability, acquisition, and distribution for perennial fruit crops. *Agronomy*, *10*, 1738; https://doi:10.3390/agronomy10111738

Keller, M. (2020). *The Science of Grapevines: Anatomy and Physiology*. Third Edition (Academic Press: London, UK).

Kochian, L. V., Hoekenga, O. A., & Piñeros, M. A. (2004). How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. *Annual Review of Plant Biology, 55*, 459-493. https://doi.org/10.1146/annurev.arplant.55.031903.141655

Lilley, R. M., Wang, X., Krausz, E., & Andrews, T. J. (2003). Complete spectra of the far-red chemiluminescence of the oxygenase reaction of Mn^{2+}-activated ribulose-bisphosphate carboxylase/oxygenase establish excited Mn^{2+} as the source. *The Journal of Biological Chemistry*, *278*, 16488-16493. https://doi.org/10.1074/jbc.M212402200Lindsay, W. L., & Norvell, W. A. (1978). Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society American* Journal, 42, 421-428. https://doi.org/10.2136/sssaj1978.03615995004200030009x

Little, I. (1964). The determination of exchangeable aluminium in soils. *Australian Journal of Soil Research, 2*, 76-82. https://doi.org/10.1071/SR9640076

Loeppert, R. H. & Suarez, D. L. (1996). Carbonate and Gypsum. *In*: Sparks, D. L., Page, A. L., Helmke, P. A., Loeppert, R. H., Soltanpour, P. N., & Tabatabai, M.A. (Eds.) *Methods of Soil Analysis Part III, Chemical Methods* (Soil Science Society of America: Madison, Wiscosin, USA) pp.437-474.

Mair, P., & Wilcox, R. (2019). Robust statistical methods in R using the WRS2 package. *Behavior Research Methods, 52*, 464-488. https://doi.org/10.3758/s13428-019-01246-w

MAPA (1993). *Métodos oficiales de análisis. Tomo III* (Secretaría General Técnica. Ministerio de Agricultura, Pesca y Alimentación: Madrid, Spain).

McNaught, K. F., Dorofaeff, F. D., & Karlovsky, J. (1973). Effect of some magnesium fertilisers on mineral composition of pasture on Horotiu sandy loam. *New Zealand Journal of Experimental Agriculture, 1*, 349-363. https://doi.org/10.1080/03015521.1973.10427926

Meyer, B. R., Roux, E., Le Renan, M. J., & Peisach, M. (1984). Pixe analysis for the study of toxic effects of aluminium in vines. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, 3* (1-3), 557-560. https://doi.org/10.1016/0168-583X(84)90438-5

Moreira, S. G., Prochnow, L. I., Pauletti, V., Silva, B. M., Kiehl, J. C., & Silva, C. G. M. (2017). Effect of liming on micronutrient availability to soybean grown in soil under different lengths of time under no tillage. *Acta Scientiarum. Agronomy, 39* (1), 89-97. https://doi.org/10.4025/actasciagron.v39i1.30691

OIV (2018). *Compendium of international methods of wine and must analysis. Volume 1* (International organization of wine and vine: Paris, France).

Olego, M. A., Visconti, F., Quiroga, M. J., de Paz, J. M., & Garzón-Jimeno, E. (2016). Assessing the effects of soil liming with dolomitic limestone and sugar foam on soil acidity, leaf nutrient contents, grape yield and must quality in a Mediterranean vineyard. *Spanish Journal of Agricultural Research*, *14*(2), e1102. http://dx.doi.org/10.5424/sjar/2016142-8406

Pittman, J. K. (2005). Managing the manganese: molecular mechanisms of manganese transport and homeostasis. *New Phytologist*, 167, 733-742. https://doi.org/10.1111/j.1469-8137.2005.01453.x

Pradubusuk, S., & Davenport, J. R. (2011). Seasonal distribution of micronutrients in mature Concord grape: boron, iron, manganese, copper, and zinc. *Journal of the American Society for Horticultural Science*, *136* (1), 69-77. https://doi.org/10.21273/JASHS.136.1.69

Quiroga, M. J., Olego, M. A., Sánchez-García, M., Medina, J. E., Visconti, F., Coque, J. J. R., & Garzón Jimeno, J. E. (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. *OENO One, 51* (4), 343. https://doi.org/10.20870/oeno-one.2017.51.4.2039

R Core Team (2019). *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing) http://www.R-project.org/ [accessed 06/2020].

Rietra, R. P. J. J., Heinen, M., Dimkpa, C. O., & Bindraban, P. S. (2017). Effects of nutrient antagonism and synergism on yield and fertilizer use efficiency. *Communications in soil science and plant analysis, V48* (16), 1895-1920. https://doi.org/10.1080/00103624.2017.1407429

Sasaki, M., Yamamoto, Y., & Matsumoto, H. (1996). Lignin deposition induced by aluminum in wheat (*Triticum aestivum*) roots. *Physiologia Plantarum, 96*, 193-198. https://doi.org/10.1111/j.1399-3054.1996.tb00201.x

Schmidt, S. B., & Husted, S. (2019). The Biochemical Properties of Manganese in Plants. *Plants, 8*, 381. https://doi.org/10.3390/plants8100381

SIAR (2020). *Sistema de Información Agroclimática para el Regadío.* http://eportal.mapa.gob.es/ /websiar/SeleccionParametrosMap.aspx?dst=1 [accessed 06/2020].

Sillanpää, M. (1982). *Micronutrients and the nutrient status of soils* (FAO: Roma, Italy).

Sinilal, B., Ovadia, R., Nissim-Levi, A., Perl, A., Carmeli-Weissberg, M., & Oren-Shamir, M. (2011). Increased accumulation and decreased catabolism of anthocyanins in red grape cell suspension culture following magnesium treatment. *Planta*, *234*, 61-71. https://doi.org/10.1007/s00425-011-1377-0

Storey, R., Jones, G., Schachtman, P., & Treeby, M. (2003). Calcium-accumulating cells in the meristematic region of grapevine root apices. *Functional Plant Biology, 30*, 719-727. https://doi.org/10.1071/FP02212

USDA (2017). *Soil Survey Manual. Agriculture Handbook No. 18.* (United States Department of Agriculture, Washington, DC, USA).

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

Verbruggen, N., & Hermans, C. (2013). Physiological and molecular responses to magnesium nutritional imbalance in plants. *Plant Soil, 368*, 87-99. https://doi.org/10.1007/s11104-013-1589-0

Villacorta, P. J. (2017). The welchADF package for robust hypothesis testing in unbalanced multivariate mixed models with heteroscedastic and non-normal data. *The R Journal*, *9*(2), 309-328. https://doi.org/10.32614/RJ-2017-049

Weil, R. R., & Brady, N.C. (2017). *The nature and properties of soils. 15th ed* (Pearson Education Limited: Harlow, UK).

Zlámalová, T., Elbl, J., Baroň, M., Bělíková, H., Lampíř, L., Hlušek, J., & Lošák, T. (2015). Using foliar applications of magnesium and potassium to improve yields and some qualitative parameters of vine grapes. *Plant, Soil and Environment*, *61*(10), 451-457. https://doi.org/10.17221/437/2015-PSE