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Soil and grapevine leaf quality in organic vineyards of different ages in DO Rioja-Alavesa, northern Spain

Calidad del suelo y de la hoja de la vid en viñedos ecológicos de diferentes edades en la D.O. Rioja-Alavesa, norte de España
Qualidade do solo e das folhas de videira em vinhas biológicas de diferentes idades no DO Rioja-Alavesa, norte de Espanha

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ABSTRACT

The soil from three organically cultivated plots in *Rioja Alavesa* vineyards, specifically in Lanciego (Álava, Spain), and the foliage of their vines were analyzed. The aim of this study was to determine differences in soil and grapevine quality between different aged vineyards. The first 20 centimeters of the soil were sampled and leaves were collected during the growing season. The results show that the quality of the soil in the three plots was optimal and did not differ from reported values of soils from traditionally cultivated plots. The only element found at a lower concentration in the three plots and the leaves was iron. Organic cultivation of vineyards is a viable mode of cultivation and could help reduce greenhouse gas emissions and contamination by pesticides and fertilizers.

RESUMEN

Se analizó el suelo y el follaje de tres parcelas cultivadas orgánicamente en viñedos de la Rioja Alavesa, *concretamente en Lanciego (Álava, España). El objetivo de este estudio fue determinar las diferencias de calidad del suelo y de la vid entre viñedos de diferentes edades. Se tomaron muestras de los primeros 20 centímetros del suelo y se recogieron las hojas durante el período vegetativo. Los resultados muestran que la calidad del suelo en las tres parcelas era óptima y no difería de la de los suelos de las parcelas cultivadas tradicionalmente. El único elemento que se encontró en menor concentración en las tres parcelas y en las hojas fue el hierro. El cultivo orgánico de viñedos es un modo de cultivo viable y podría ayudar a reducir las emisiones de gases de efecto invernadero y la contaminación por pesticidas y fertilizantes.*

RESUMO

Foram analisados os solos e as folhas das videiras de três parcelas de vinhas cultivadas organicamente (modo biológico) na região de Rioja Alavesa, *concretamente em Lanciego (Álava). O objetivo deste estudo foi o de determinar as diferenças na qualidade do solo e da videira entre as vinhas de diferentes idades. Foram amostrados os primeiros 20 centímetros do solo e foram colhidas as folhas das videiras durante o período vegetativo. Os resultados mostraram que a qualidade do solo nas três parcelas era ótima e não diferiu da dos solos das parcelas tradicionalmente cultivadas. O ferro foi o único elemento químico que apresentou menor concentração nas três parcelas e nas folhas. A cultura biológica (orgânica) das vinhas é um modo de cultivo viável e poderia ajudar a reduzir as emissões de gases com efeito de estufa e a contaminação por pesticidas e fertilizantes.*

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1. Introduction

Grapevines have been cultivated for around 8000 years (McGovern et al. 2017). Soil provides water and nutrients to the vineyards. Grapevines are one of the most important crops in terms of income (Anderson 2001) and thus vineyards have come under pressure to increase productivity and become more efficient (Galati et al. 2015). In response to this challenge, organic farming is being developed in some areas of small estates with appropriate environmental conditions, with the aim of restoring species diversity and implementing methods used in the past, focusing on the production of high quality wines and respect for the environment. Many studies in vineyards have focused on soil erosion (Boix-Fayos et al. 2006; Burns et al. 2016; Prosdocimi et al. 2016; Napoli et al. 2017), reflecting the need to evaluate the impact of land abandonment and land use change and farming on soil erosion and degradation (Tarolli et al. 2014, 2015; Comino et al. 2017). Other studies have analyzed the impact of different management strategies on the characteristics of vineyard soils (López-Piñeiro et al. 2013) and soil degradation (Biddoccu et al. 2017), soil as a yeast reservoir and its effect on wine fermentation (Ramírez et al. 2020), the impact of biochar on soil properties (Giagnoni et al. 2019), the effect of mechanical tools on soil status (Novara et al. 2019; Pijl et al. 2019), water availability and capacity (Coulouma et al. 2020), soil losses using remote sensing (Baiamonte et al. 2019) and the soil microbial community (Chou et al. 2018; Vadakattu et al. 2019; Di Giacinto et al. 2020; Zhao et al. 2020). Many studies on vineyards have been carried out in Mediterranean areas (Olego et al. 2016; Okur et al. 2016; Rodrigo-Comino et al. 2018), where topography and hereditary subdivision of plots often results in estates less than 1 ha in sizes and so facilitates the adoption of organic farming. A few studies have analysed the physicochemical properties of soils in organic vineyards in small estates (Costantini et al. 2015; Okur et al. 2016; Morelli et al. 2019) but only in the short term after planting the vines. The current study compared areas with similar characteristics but with different aged vines that were planted at different times. Only a few studies have analyzed the effect of the long-term application of organic compounds, but on micro-organisms, not soil physico-chemical properties (Mackie et al. 2013).

The leaf characteristics of grapevines are very important and influence grape characteristics and wine quality (Johnson et al. 2003). Some studies have analyzed grapevine leaf characteristics (Zarco-Tejada et al. 2005) and the removal of leaves to increase vineyard efficiency (Palliotti et al. 2011) using photogrammetry (Torres-Sánchez et al. 2019); others have studied grapevine leaf stripe disease and its characteristics using remote sensing (Rey-Caramés et al. 2015; Di Gennaro et al. 2016; Del Frari et al. 2019) and the stomatal conductance of vine leaves according to their hydraulic properties (Zhang et al. 2012). Many studies have focused on pest and disease prevention via field studies (Smith 1955; Stafford and Jensen 1957; Hibbert and Horne 2001; Daane and Williams 2003; González-Chang et al. 2017; Massa et al. 2020). Few studies have analysed vine leaves and their chemical characteristics using fieldwork.

Some studies have examined the effects of soil and leaf quality on vineyards, focusing on the transpirable soil water content and the leaf water potential (Gaudin et al. 2017), soil composition and leaf water content (Cozzolino et al. 2009), the impact of liming on soil properties and leaf tissue cation composition (Quiroga et al. 2017), the effects of soil liming with dolomitic limestone and leaf nutrient contents (Olego et al. 2016), the effect of green manure on soil fertility and grape leaf nutrient content (Li et al. 2004) and how soil fertility affects the chemical composition of the leaves (Stojanova et al. 2011). The age of

KEYWORDS
Wine quality, soil chemical properties, organic farming, Mediterranean vineyard, wine designation of origin.

PALABRAS CLAVES
Calidad del vino, propiedades químicas del suelo, cultivo orgánico, viñedo mediterráneo, denominación de origen vitivinícola.

PALAVRAS-CHAVE
Qualidade do vinho, propriedades químicas do solo, agricultura biológica, vinha Mediterrânica, denominação de origem vitivinícola.

vineyards and the cultivation of grapevines can affect soil erosion processes (Rodrigo-Comino et al. 2018; Rodrigo-Comino et al. 2017), change the soil nutrient status (Zhao et al. 2019) and soil hydraulic conductivity (Alagna et al. 2018), and affect the nematofauna (Scotto et al. 1988), bacteria and insects (Welch et al. 2015) and wine quality (Kishi and Kanehara 2003). However, no studies have evaluated the variation in soil physicochemical and leaf characteristics in vineyards of different ages, or in organic vineyards. Nowadays, soil and leaf analyses are considered techniques that provide objective information on the condition of soils and plants that can be used to monitor, diagnose and carry out actions aimed at managing the soil–plant system.

Soil and leaf analysis provides useful information for soil and plant nutrition management and is an important topic of study, providing information about soil properties, the state of nutrient reserves, the mineral composition of the plants, and is a tool for nutritional diagnosis. The aim of this study was to determine differences in soil and grapevine quality between different aged vines in organic vineyards. The specific objectives were: a) to analyze the differences in soil physico-chemical properties, b) to evaluate the chemical differences in grapevine leaves and c) to suggest measures to improve the soil and leaf quality without affecting the quality of the wine produced.

2. Material and Methods

2.1. Study area and sampling design

The area of the Rioja Denomination of Origin is located in the north of Spain and is part of the Ebro Depression. It covers an area of approximately 50,000 hectares and produces 250 million liters of wine per year, with more than 15,000 winegrowers and 607 wineries (Ministerio de Agricultura, Alimentación y Medio Ambiente 2011). It covers a total area of 65,326 hectares and three production subzones are distinguished by their orographic and climatic diversity – *Rioja Alta*, *Rioja Oriental* and *Rioja Alavesa*, with. The *Rioja Alavesa*, the site of our study, covers 13,388 hectares (Consejo Regulador DORioja 2009). The most important grape variety is "Tempranillo", producing 87.56% of all wine in this area. According to the Basque Government's Department of Economic Development and Infrastructure, the most characteristic soil type in the *Rioja Alavesa* is highly suitable for quality viticulture, as it has a balanced texture (sand, silt and clay), is slightly alkaline and has moderate water availability during the summer. Clay-limestone soils predominate on terraces and small plots. The whole area benefits from the confluence of two climates, the Atlantic and the Mediterranean, which provide mild temperatures and annual rainfall of just over 400 l/m^2, conditions that are ideal for the development of the vine.

The *Rioja Alavesa* is located in the Tertiary depression of the Ebro Valley. It occupies the sunny part of the Cantabrian mountain range with gentle glacier ramps that connect with the terraces along the Ebro. This depression is filled with synorogenic and postorogenic deposits of detritic origin, such as clays with intercalations of sandstones and conglomerates. The Southern Sierras (Toloño-Cantabria-Joar) slow down the movement of humid Atlantic air on the shady side and cause a foehn effect on the sunny side, thus considerably reducing rainfall. Rainfall barely reaches 600 mm per annum in the foothills of the sierra, and on the banks of the Ebro annual rainfall can be below 400 mm. The pluviometric regime shows a clear Mediterranean component. The equinoctial periods are the rainiest with an absolute maximum in spring. The summer season has at least two dry months, July and August. The area's position far from the sea and at high altitude, about 500 masl on average, results in an appreciable continentalization of temperatures. The winter temperature, at around 5 ºC in the coldest month, is higher than that in Atlantic environments but lower than in the central regions. Summers are hot in the whole province, with average values of 21-22 ºC in the hottest month, and the annual thermal oscillation is around 16 ºC. In the *Rioja Alavesa*, the natural vegetation is made up of *Quercus ilex*, although it is generally very sparse due to the intense agricultural use of the soil. *Quercus faginea* grow in cooler, wetter areas. *Quercus* *coccifera* and *Rosmarinus officinalis*, as well as other aromatic Mediterranean plants, make up the majority of thickets (Meaza 1997).

The physical characteristics and chemical composition of the vineyard soil in Álava are closely linked to the processes that generated it, so it is important to note that the *Rioja Alavesa* vineyard is mainly based on Oligocene sediments deposited in the tectonic depression that in the Tertiary period constituted the inland sea that today is the Ebro Valley. On these Tertiary sediments made up of limestone, marl and sandstone, a type of soil known as "Brown Limestone" is present (Barrios 1994). This soil is classified according to the Soil Taxonomy as a Typic Xerofluvent (Soil Survey Staff 2014) and according to the WRB for Soil Resources it is a Haplic Fluvisol (WRB 2006).

This study focuses on one of the wineries in the *Rioja Alavesa* region, located in the municipality of Lanciego in the province of Álava. The winery is called Lanzaga (http://www.telmorodriguez.com/bodega-lanzaga/). Since 1998, this winery has been acquiring vineyards that are over 80 years old, covering a total of 15 hectares at present. These are traditional vineyards and are cultivated according to organic farming principles (explained below). In this study, we focus on three of the Lanzaga winery's sites: a) "La Estrada" (EST hereafter) at 610 m a.s.l. and covering 0.64 ha, growing mostly Tempranillo and some Graziano grapes. It produces an average of 1,200 bottles per year and the vines were planted in 1940; the vineyard has been farmed organically since 2006; b) "El Velado" (VEL hereafter) at 600 m a.s.l. and covering 1 ha, with a southwest orientation, growing mostly Garnacha and Tempranillo grapes and producing about 500 bottles per year. The vines were planted in 1936, and have been grown organically since 2006; c) "La Encina" (ENC hereafter) is an estate located at 550 m a.s.l. covering 0.30 ha, which was planted in 2013 and grafted onto old vines in 2014, and has been cultivated organically since then. Most of the varieties of La Rioja (tempranillo, garnacha, graciano, granegro, mazuelo, viura, garnacha blanca, alarije and others) are grown in this estate, with the aim of restoring the old abandoned vineyards in the village of Lanciego (**Figure 1**). The soil texture of the studied vineyards are detailed in **Table 1**. Each composite sample was composed of three sub-samples. According to the soil texture triangle, EST and VEL were classified as loam and ENC was classified as sandy loam.

The Lanzaga winery has some well-known vineyards that have received several awards for their wines, the latest being the Atkin 2019 award for the best winery in the Rioja DO. The vines are grown organically from the outset. In the case of new plantations, the ground is prepared with iron fertilizer, generally a legume (vetch) and a cereal, which are incorporated into the soil shortly before flowering. The plantation is cultivated "the old way" with lime and "Herron" marking. The rooted vine is planted and grafted the following spring. Grafting is done in the year N+1, with wood from the mass selection of the old vineyards. The varieties are mixed at the farm in a random way (Field Blend). The fertilizers are made with organic manure and phytosanitary treatments are based on nettle and horsetail herbal teas, sometimes mixed with some copper and sulphur as needed.

In June 2018 the study area was surveyed and three plots that are being organically cultivated and have similar geological, topographical, soil and management characteristics were selected. Soils were sampled every 2 m along a transect of 16 m on each plot. During the sampling campaign, eight composite soil samples were collected at 0.20 cm depth, giving a total of 3 transects and 24 samples for the entire study. Each of the composite samples that were individually derived from a combination of three subsamples were collected using a steel cylinder. The number of samples and soil depth were selected 1) so that the samples were statistically representative and 2) as most of the reactive vine roots in terms of nutrient uptake are found in the uppermost 20 cm of soil (Rodríguez-Salgado et al. 2017; Veiga et al. 2017). Regarding leaf sampling, three samples were analyzed from each site. Each composite sample was individually derived from a combination of three subsamples. One leaf per plant of all varieties was sampled and mixed (Carvalho et al. 2018) with at least 100 other leaves in each subsample (Wells 2011).

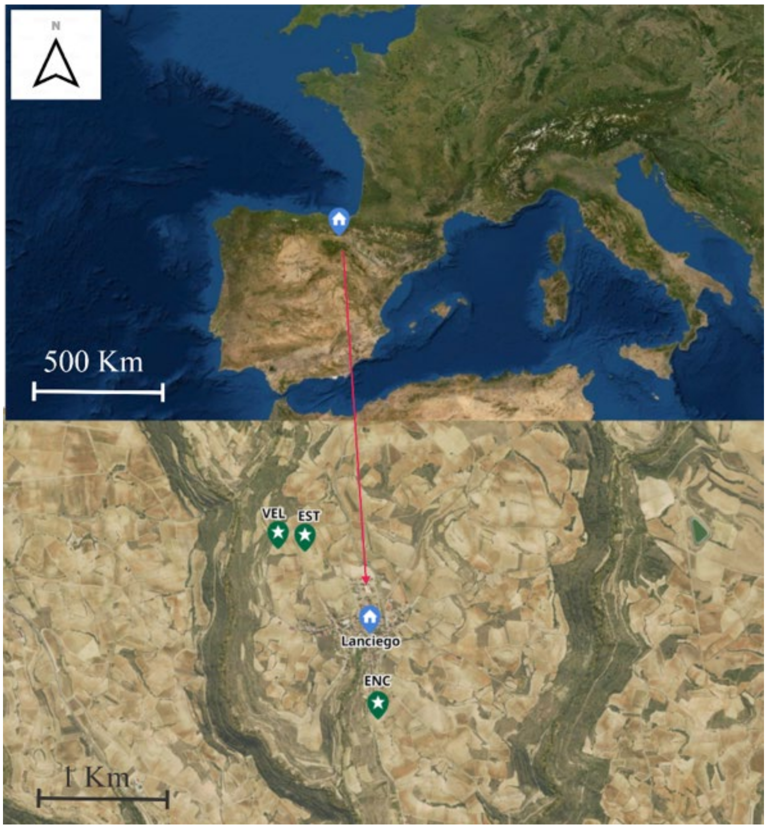

Figure 1. Location of study area and plots.

Table 1. Soil texture characteristics of study sites. N=3

Soil mineralogical characteristics	Study Site	Mean
% Clay	EST	15.1
	VEL	12.1
	ENC	4.2
% Silt	EST	36.3
	VEL	38.3
	ENC	24.3
% Sand	EST	48.6
	VEL	49.6
	ENC	71.5

Sampling sites: La Estrada (EST), El Velado (VEL) and La Encina (ENC).

2.2. Laboratory analysis

Soil samples were dried for 7 days at room temperature (23 ºC) and then sieved at 2 mm to analyze the fine fraction of soil. Total nitrogen (TN) was analyzed using a Flash 112 Series (Thermo-Fisher, Milan) and data calculations were carried out with Eafer 300 software (Pereira et al. 2012). Soil organic matter (SOM) and inorganic carbon (IC) were measured using the loss-on-ignition method (Heiri et al. 2001). The soil C/N ratio was calculated as the proportion of organic carbon to TN. The organic C content in SOM was calculated as follows: Organic C = SOM/1.724 (Al-Gburi et al. 2017). Soil pH [1:2.5] and EC (expressed in µS/cm) [1:2.5] were analyzed with an extraction of deionized water. Extractable major and minor cations were analyzed using an extraction [1:20] of ammonium acetate (Knudsen et al. 1982). Available P was analyzed following the Olsen Gray method (Olsen et al. 1954). Extractable cations and P were expressed in mg/Kg of soil and were analyzed using a PerkinElmer Elan-6000 Spectrometer and a PerkinElmer Optima-3200 RL Spectrometer.

Fresh, green and healthy grapevine leaves, approximately 15-25 cm in length and width, were placed on cellulose filter paper in a well-aerated room and air-dried at room temperature for 30 days. Then 10 g of each sample was macerated with 150 mL of 80% methanol for 2 hours on an orbital shaker. Extractions were carried out as described for the soil samples to determine the chemical composition of the leaves (Šibul et al. 2016). Then, the liquid was dissolved in warm distilled water (40-50 °C, 10 mL per g of dry extract). To remove the nonpolar compounds, the extracts were washed repeatedly with petroleum ether (fraction 40-60 °C). The extracts were dried under vacuum and dissolved in distilled water (w/v) to obtain stock solutions (200 mg/mL) (Pintać et al. 2019).

2.3. Statistical analysis

Data normality and homogeneity of variance were assessed using the Shapiro-Wilk and Levene's tests. In this case all the data were normally distributed, followed a Gaussian distribution and respected the assumption of homogeneity of variance, and thus we conducted a one-way ANOVA with Tukey post-hoc test in order to identify differences between the soils of each sampling site. Significant differences were identified at $p < 0.05$. Statistical analyses were implemented using SPSS 23.0. A redundancy analysis (RDA) was carried out to identify the relations between the variables and the relationship between each site and soil characteristics. Vectors represent soil properties and arrows represent land uses. The longer the vector, the more variance it explains. Vectors that form smaller angles with the axes are explained by this axis and correlated with it. The soil properties used in the RDA were TN, SOM, IC, C/N, pH, EC, extractable Ca, Mg, Na, K, available P, extractable S, Zn, Fe, Si, Cr, Mn, B, Al and Pb. RDA was implemented using version 4.5 of CANOCO software for Windows.

3. Results and Discussion

3.1. Soil

3.1.1. Soil total nitrogen, organic matter, inorganic carbon and C/N ratio

No significant differences were observed in the TN ratio between sites. Soil organic matter, inorganic carbon and the C/N ratio were significantly higher in EST and VEL than in ENC (**Table 2**).

The percentage of nitrogen in the soil of the three plots was above 0.06% and below 0.2%, indicating no excess or deficiency of this element according to INTA (2011) and Villar and Arán (2008). Nitrogen is essential for protein formation and the development of vegetative organs. Excess nitrogen can elongate the shoots and can be harmful since it increases the risk of freezing. The percentage of organic matter was also within the average range, between 2.9 and 0.9%, according to Villar and Arán (2008), and according to Cobertera (1983) the quality of the soil is good. Such levels of organic matter ensure a sufficient supply of nutrients, and the C/N ratio guarantees sufficient mineralization, although according to Cobertera (1983) only the VEL plot provides a balance between humification and mineralization. The fact that in ENC the values of the C/N ratio are lower (7.40) makes than the percentage of organic matter is also lower than in EST and VEL.

Table 2. Descriptive statistics of soil total nitrogen, organic matter, inorganic carbon and C/N ratio. Different letters represent significant differences at p<0.05. N=8

Soil property	Study site	Mean	SD	p value
Total nitrogen (%)	EST	0.09	0.01	n.s.
	VEL	0.10	0.01	
	ENC	0.09	0.01	
Organic matter (%)	EST	2.19a	0.74	***
	VEL	2.19a	0.08	
	ENC	1.16b	00.09	
Inorganic carbon (%)	EST	4.29a	0.75	***
	VEL	3.82a	0.40	
		0.25b	0.24	
C/N ratio	EST	14.54a	6.05	**
	VEL	12.54a	0.72	
	ENC	7.40b	0.83	

Sampling sites: La Estrada (EST), El Velado (VEL) and La Encina (ENC). * p<0.05, ** p<0.01, *** p<0.001, n.s. (no significant differences).

3.1.2. pH, EC, major elements and available phosphorus

Soil pH was significantly higher at EST and VEL than ENC. Electrical conductivity (EC) was significantly higher at VEL than at other sites and significantly lower at ENC than at other sites. Extractable Ca and Mg were significantly higher at EST and VEL than at ENC. Extractable Na was significantly higher at EST than at ENC. Like EC, soil extractable K was significantly higher at VEL than in other areas and significantly lower at ENC than at EST and VEL. No significant differences were observed in the case of available P (**Table 3**).

The soil pH in vineyards is related to inorganic carbon (IC). Stamatiadis et al. (1997) reported a higher pH in areas where IC is higher due to higher amounts of $CaCO_3$, as in the present study. Organic vineyards are characterized by higher amounts of $CaCO_3$, leading to higher EC and more extractable Ca and Mg (Stamatiadis et al. 1997). This can be caused by manure application, changes in earthworm activity and the application of organic fertilizers (Edwards and Lofty 1972). In this study the changes reflect the time since organic procedures were implemented, with the oldest vineyards (EST and VEL) having higher values of pH, EC and extractable Ca and Mg than the newest vineyard (ENC). In comparison with other studies [e.g. Villar and Arán (2008), INTA (2011) and CSR (2019)] the concentrations of Ca and K were at medium-high levels. Calcium is essential for the growth of leaves and roots, as it facilitates the transport of carbohydrates and starch. It also increases sugar production in grapes and their aroma. Available P did not differ between plots due to the lack of use of fertilizers and amendments in the organic vineyards analyzed in this study. Schmitt et al. (2013) and Preston et al. (2017) reported differences in available P due to the application of P to the soil surface at rates that exceeded plant requirements. These studies used the P equilibrium concentration as an environmental indicator of P leaching, and in this sense, as the present study reported similar values for the three sites this indicates that EST, VEL and ENC have similar leaching characteristics.

3.1.3. Minor elements

Soil extractable S was significantly higher at EST and VEL than at ENC. No significant differences were observed in extractable Zn between the studied sites. Soil extractable Fe was significantly higher at EST than at VEL and ENC. Extractable Si and Cr did not differ between the studied

Table 3. Descriptive statistics of soil pH, EC, major elements and available phosphorus. Different letters represent significant differences at $p<0.05$. N=8

Soil property	Study site	Mean	SD	p value
	EST	8.36a	0.05	
pH	VEL	8.38a	0.05	**
	ENC	8.26b	0.08	
	EST	153.29b	32.36	
EC (µS/cm)	VEL	196.63a	15.72	***
	ENC	113.01c	9.50	
	EST	3925.14a	237.41	
Extractable Ca (mg/kg)	VEL	4017.55a	94.43	***
	ENC	1744.52b	494.45	
	EST	373.42a	43.75	
Extractable Mg (mg/kg)	VEL	383.53a	49.20	***
	ENC	169.64b	26.14	
	EST	341.51a	113.57	
Extractable Na (mg/kg)	VEL	290.97ab	97.23	*
	ENC	186.03b	72.54	
	EST	397.04b	30.58	
Extractable K (mg/kg)	VEL	478.14a	71.16	***
	ENC	246.64c	39.80	
	EST	42.60	19.77	
Available P (mg/kg)	VEL	30.78	20.59	n.s.
		26.53	11.56	

Sampling sites: La Estrada (EST), El Velado (VEL) and La Encina (ENC). * $p<0.05$, ** $p<0.01$, *** $p<0.001$, n.s. (no significant differences).

sites. Extractable Mn was significantly higher at ENC than at EST. Significantly higher values of extractable B and Al were observed at EST and VEL in comparison with ENC. No significant differences were observed in extractable P (**Table 4**).

According to INTA (2011), Villar and Arán (2008) and CSR (2019), of all the minor elements, the only concentration that could be considered low was that of Fe. Iron is essential to keep the leaves of the vine green. Iron chlorosis or calcareous chlorosis is a serious problem that can arise if the low iron level is due to the pH level. Sulfate levels were very high in the soil and leaves of the three plots. Sulfate is necessary for the formation of vitamins, helps accumulate heat, and prevents frost. It is used as a remedy against powdery mildew, and increases the production, quality and self-life of wine. The fairly high levels in the three plots are due to the incorporation of S by the farmer. It is the only element allowed by organic agriculture to prevent pests. Regarding heavy metals, these were above those recommended by European Directive 86/278/EEC, but Pb, Mn and Zn would not cause contamination problems as they were well below the maximum limit of 350 mg kg^{-1}. The low levels of contaminants may reflect the organic practices, since such contaminants are associated with the application of plant protection products, as in some of the vines in the study by Marín et al. (2000), who found a high Mn content. Phosphate and nitrogenous fertilizers also contain heavy metals (Alloway 1995) but these are not used on the three organic viticulture plots in this study; in the case of Mn and Zn they were even below the mean for soils considered natural in Spain (Adriano 1997; Marín et al. 2000). A study conducted in

Table 4. Descriptive statistics of minor elements. Different letters represent significant differences at $p<0.05$. N=8

Soil property	Study site	Mean	SD	p value
Extractable S (mg/kg)	EST	105.16a	28.87	***
	VEL	106.99a	12.29	
	ENC	60.34b	17.95	
Extractable Zn (mg/kg)	EST	2.38	0.60	n.s.
	VEL	2.04	0.62	
	ENC	2.09	0.89	
Extractable Fe (mg/kg)	EST	1.45a	0.94	**
	VEL	0.36b	0.22	
	ENC	0.51b	0.16	
Extractable Si (mg/kg)	EST	132.70	90.09	n.s.
	VEL	75.96	52.30	
	ENC	100.01	23.47	
Extractable Cr (mg/kg)	EST	0.19	0.09	n.s.
	VEL	0.11	0.06	
	ENC	0.18	0.08	
Extractable Mn (mg/kg)	EST	43.23b	8.67	**
	VEL	63.49ab	17.86	
		88.41a	33.15	
Extractable B (mg/kg)	EST	3.18a	0.90	***
	VEL	3.16a	0.84	
	ENC	1.55b	0.59	
Extractable Al (mg/kg)	EST	15.44a	1.60	***
	VEL	14.14a	0.74	
	ENC	8.36b	1.98	
Extractable Pb (mg/kg)	EST	9.79	6.77	n.s.
	VEL	12.09	5.63	
		9.78	6.66	

Sampling sites: La Estrada (EST), El Velado (VEL) and La Encina (ENC). * $p<0.05$, ** $p<0.01$, *** $p<0.001$, n.s. (no significant differences).

California by Reeve et al. (2005) compared two vineyard soils, one with organic agriculture and a control, and found that the concentrations of Mn, Fe, Zn and B were lower in organic soils, but these concentrations were still sufficient for good plant growth and quality. The concentration of Fe is our plots was even lower than in the Californian study.

According to Mackenzie and Christy (2005) some elements are more directly involved in grape quality than others, for example Pb and Si. In the three plots under study the quantity of Pb and Si in the soil is sufficient to ensure optimum quality.

3.1.4. Multivariate analysis

The first factor of the RDA explained 49.9% of the variance and the second factor explained 48.2%, which together account for 98.1 of the total variation. Axis 1 separated EST and VEL from ENC. Axis 2 separated VEL and ENC from EST. The variables with the highest explanatory capacity were IC, Ca and Mg, while those with lowest explanatory capacity were Pb, Zn and P. The RDA clearly separates the different sites according to the values obtained in each area, with EST and VEL being more similar. The low angle between the arrows and the vectors shows that these two sites explain the

TN, SOM, IC, C/N, pH, EC, extractable Ca, Mg, Na, K, available P, extractable S, Zn, Fe, Si, Cr, B, Al and Pb; Mn was associated with ENC (**Figure 2**). In this study, EST and VEL had the highest values of most soil properties (and small differences between them) with the exception of Mn, which was higher at ENC. Although EST and VEL are very similar according to our data, EC, extractable K and Fe differed between these sites in the RDA.

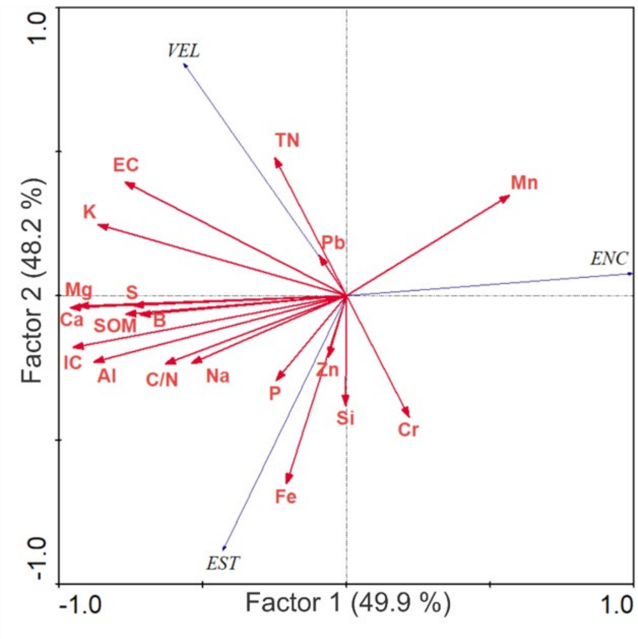

Figure 2. RDA showing the relationships between Factor 1 and 2. Abbreviations: Total nitrogen (TN), soil organic matter (SOM), inorganic carbon (IC), carbon/nitrogen ratio (C/N), pH, electrical conductivity (EC), extractable calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), available phosphorus (P), extractable sulfur (S), zinc (Zn), iron (Fe), silicon (Si), chromium (Cr), manganese (Mn), boron (B), aluminum (Al), and lead (Pb). Sampling sites: La Estrada (EST), El Velado (VEL) and La Encina (ENC).

3.2. Vineyard leaves

Table 5 shows the results of the leaf analysis of the three plots under study (EST, VEL and ENC) compared with two studies that determined the optimal levels (low to high) needed to provide optimal fruit quality and avoid nutritional problems in vineyards (Villar and Arán 2008; García-Escudero and Martín 2019).

Reeve et al. (2005) found that the concentration of N, K, Ca, Mg, B, Zn, and Fe in leaves in California vineyards was lower in leaves from organic plots compared with non-organic vineyards. All the elements in the leaves of our three plots were within the optimal ranges according to the Cascade Analytical Institute and the organic plots in Reeve et al. (2005). Only Fe concentrations were in the low range of optimal concentration, although there was no evidence of a deficiency. Although in general the concentrations of Fe are low in the three plots, no visible symptoms of iron deficiency are seen in the leaves. The fact that it is very calcareous soils and high pH, as we have verified in the results, seems to be a more common consequence of having low iron levels (Díaz et al. 2013). The low amount of iron in the leaves may be due to the fact that it is already rather scarce in the soil, as we have seen in the soil analysis. According to Cibriain and Sagüés (1994) depending on the age of the vineyard, it can influence the lack of iron, it is possible that over the years the iron levels will balance out, due to the fact that the plant acquires more vigor.

According to Villar and Arán (2008) and García-Escudero and Martín (2019) the concentration of K in the vine leaves is high. Potassium stimulates photosynthesis, is responsible for

Table 5. Optimal range of nutrients in leaves for the Tempranillo variety. High and/or very high values in Green; intermediate or normal values in Blue; and low values in Red. N=3.

Grapevine Property	García-Escudero and Martín (2019)	Villar and Arán (2008)	Study Sites		
	Low/Optimal/High	Low/Normal/High/Very high	EST	VEL	ENC
N (%)	<2.08/2.08-2.42/>2.42	<1.8/1.8-2.35/2.35-3/>3	3.21	3.14	3.72
Ca (%)	<2.82/2.82-3.62/>3.62	<1.3/1.3-3/>3/–	9.48	8.27	8.93
Mg (%)	<0.32/0.32-0.56/>0.56	<0.18/0.18-0.5/0.5-0.7/>0.7	1.54	1.39	1.31
Na (mg/kg^{-1})		–/<500/>500/–	1142	1565	1319
K (%)	<0.63/0.63-1.13/>1.13	<0.8/0.8-1.5/1.5-2.1/>2.1	2.08	2.28	2.07
P (%)	<0.13/0.13-0.18/>0.18	<0.11/0.11-0.22/>0.22/–	0.48	0.62	0.64
SO_4 (%)		<0.1/0.1-0.25/0.25-0.4/>0.4	3.76	3.49	3.58
Zn (mg/kg^{-1})	<14/14-23/>23	<15/15-40/40-100/>100	35.74 35.74	38.34 38.34	28.67 28.67
Fe (mg/kg^{-1})	<99/99-205/>205	<40/40-175/>175/–	45.85	35.71	39.83
Mn (mg/kg^{-1})	<77/77-156/>156	<20/20-100/100-200/>200	113 113	58 58	119 119
B (mg/kg^{-1})	<30/30-48/>48	<15/15-75/75-125/>125	32.32	27.10	39.02

Comparison with Villar and Arán (2008) and García-Escudero and Martín (2019). Sampling sites: La Estrada (EST), El Velado (VEL) and La Encina (ENC).

starch formation and is essential for resistance to frost and disease. It improves the quality of grapes and wine, increases their aroma, improves taste, decreases acidity and increases alcoholic strength. A lack of K makes the grapes ripen very slowly and reduces the alcohol content of wine (Gaspar 2010). Magnesium levels in our samples can be classified as medium. Magnesium is important for chlorophyll and is involved in the metabolism of carbohydrates and proteins. Excess Mg damages the assimilation of K while a shortage affects the chlorophyll and leaves tend to dry out and become less green. A large amount of calcium in the soil could impair Mg assimilation (Gaspar 2010), but since there is also a lot of magnesium it seems that it does not affect this nutrient to reach the plant, as can be seen in the results of the foliar analysis. Phosphorus concentrations in our samples were high. Phosphorus is necessary to form vitamins and for the development of roots and stems. There are no known negative effects of excess P (Gras 1995). According to the same authors, based on the conductivity data there is no evidence of salinization problems at our sites and the pH at all sites is moderately basic.

3.3. Overall discussion and implications for vineyard quality

In 2010, an exhaustive study on 123 soils from La Rioja was published, including soils from Rioja Alavesa, which analyzed 12 essential parameters of quality in vineyard soils (Peregrina et al. 2010). In the current study only the percentage of clays in the La Encina plot (4%) was below the average reported in the previous study. Likewise only two parameters (Ca and Na) in the current study were above the maximum reported by Peregrina et al. (2010) three parameters in the El Velado plot (CE, Ca and Na) and one parameter (Na) in the La Encina plot. In any case, these differences are not very important, although the relatively high Ca and Na values in the Lanzaga plots result in a relatively high CE.

We can also compare our results with those of another study from the year 2000 on soils from La Rioja that assessed nine edaphic parameters (Marín et al. 2000). In comparison with that study, in the current study only two parameters (MO and $CaCO_3$) were above the maximum in the VEL plot, one parameter (MO) in the EST plot and one parameter ($CaCO_3$) in the ENC plot. It is noteworthy that the Mn and ZN values were below those reported by Marín et al. (2000) and the Pb values were between the minimum and the mean in the current study.

Ecologically speaking, an interesting topic in any type of agriculture is the carbon footprint of the production system. An analysis of 29 studies by Rugani et al. (2013) found that the total carbon footprint in wine production was 2.17 kg CO_2- eq./per 0.75 L bottle of wine. The planting phase accounts for a total of 0.07 kg CO_2-eq./ per 0.75 L bottle of wine and the growth and production phase of grapes accounts for 0.38 kg CO_2- eq./per 0.75 L bottle of wine. In a separate analysis of 22 studies the same authors found that organic vineyards produce almost 25% less kg CO_2- eq./per 0.75 L bottle of wine than conventional viticulture. The higher carbon footprint of conventional production is believed to be due to the greater use of synthetic substances and other inputs (e.g. diesel, wood) during the agricultural phase (Niccolucci et al. 2008; Point et al. 2012). The authors point out that more studies are needed to reach firm conclusions and other authors stress that many variables must be analyzed in the organic vs. conventional comparison, including the type of soil (Colman and Päster 2009).

Improving the quality of wine has been a subject of interest in recent decades and more specifically in the 21st century (Torresi et al. 2011). It points towards the importance of issues related to the nutrition of cultivated plants. The need to produce more and better food with rising demands and limited resources has led to a renewed interest in production systems that meet the criteria of economic viability and agronomic sustainability (Ramírez 2008; Villar and Aran 2008). Organic viticulture employs a range of techniques for managing the soil, the vineyard and the environment (Pedneault and Provost 2016; Human 2017). Organic viticulture involves not only the substitution of banned synthetic chemicals (pesticides and fertilizers) with others allowed by organic production (Battistelli et al. 2020). The changes in the production system must be more profound, establishing varieties adapted to local agro-climatic conditions, improving the natural fertility of the soil and increasing the biodiversity of the system, so that beneficial natural ecological processes such as those that allow adequate plant nutrition, biotic and environmental regulation are enhanced. For the farmer or producer, organic viticulture means an increase in added value, with greater respect for the environment and a reduction in the use of chemical products (Crescimanno et al. 2002). For the consumer, the benefits include the availability of products produced without pesticides or artificial fertilizers (Remaud et al. 2008; Stolz and Schmid 2008).

4. Conclusions

The soil in the three organic plots showed optimum soil quality parameters for vineyards. However, there were significant differences between the two oldest (EST and VEL) plots and the newer plot (ENC). In comparison with other studies carried out on soils of La Rioja from traditionally cultivated vineyards, similar data are presented, although iron levels were lower in the three study plots compared to those studies and compared with the levels established by technical reports. Organic farming is therefore viable in vineyards, at least in terms of soil and leaf quality, and would avoid the use of pesticides and fertilizers.

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