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Article

MECHANICAL PRUNING AND SOIL ORGANIC AMENDMENTS IN VINEYARDS OF 'SYRAH': EFFECTS ON WINE MINERAL COMPOSITION

PODA MECÂNICA E FERTILIZAÇÃO ORGÂNICA DO SOLO EM VINHAS DE 'SYRAH': EFEITOS NA COMPOSIÇÃO MINERAL DO VINHO

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SUMMARY

The interaction of mechanized pruning systems and soil organic amendment can affect vine vegetative and reproductive growth. However, since organic amendments supply several mineral elements, namely heavy metals, this study aimed to understand the effects of the interaction between these two practices on the mineral composition of wine. Two field trials were implemented in 'Syrah' vineyards in two Portuguese wine regions (Lisboa and Tejo). Mechanical hedge pruning was compared with hand spur pruning and four different organic amendments were tested: biochar, municipal solid waste compost, cattle manure and sewage sludge. The nitrogen (N), phosphorus (P) and potassium (K) wine contents were significantly reduced by mechanical pruning while calcium (Ca) and magnesium (Mg) contents were tendentially higher in this pruning system. Mechanical pruning also reduced the content of some minor elements, such as arsenic (As), molybdenum (Mo) and nickel (Ni). In 2014, the year with the higher reproductive growth, some other elements also decreased as a consequence of the mechanical pruning (gallium - Ga; lithium – Li; rubidium - Rb, thallium – Tl; yttrium - Y). Concerning the organic amendments, sewage sludge was associated with the wines with the lowest P and iron (Fe) content. Ca content was tendentially higher in municipal solid waste compost and sewage sludge treatments. Mechanical pruning and organic amendments had different effects on the mineral composition of wine, according to each specific element. However, the legal limits, recommended by OIV and established by European Union, as well as the technical limits, adopted by winemakers, were never exceeded and the interaction of both practices does not seem to be a problem in what concerns to the mineral composition of the produced wines.

RESUMO

A interação entre a poda mecânica e a aplicação de corretivos orgânicos ao solo pode afetar o crescimento vegetativo e reprodutivo da videira. No entanto, uma vez que os corretivos orgânicos fornecem vários elementos minerais, nomeadamente metais pesados, este estudo teve como objetivo compreender os efeitos da interação entre estas duas práticas na composição mineral do vinho. Foram implementados dois ensaios em vinhas de 'Syrah', em duas regiões vitivinícolas Portuguesas (Lisboa e Tejo). A poda mecânica em sebe foi comparada com a poda manual e quatro diferentes corretivos orgânicos foram testados: biochar, resíduos sólidos urbanos compostados, estrume de bovino e lamas de uma estação de tratamento de águas residuais. Os teores de azoto (N), fósforo (P) e potássio (K) no vinho foram significativamente reduzidos pela poda mecânica, enquanto os teores de cálcio (Ca) e magnésio (Mg) foram tendencialmente maiores neste sistema de poda. A poda mecânica também reduziu o teor de alguns elementos minoritários, como arsénio (As), molibdénio (Mo) e níquel (Ni). Em 2014, o ano de maior crescimento vegetativo e reprodutivo, alguns outros elementos também foram reduzidos pela poda mecânica (gálio - Ga; lítio – Li; rubídio – Rb; tálio – Tl; ítrio Y). No que diz respeito aos corretivos orgânicos, as lamas de depuração produziram os vinhos com os menores teores de P e ferro (Fe). O teor de Ca foi tendencialmente mais elevado na modalidade com resíduos sólidos urbanos compostados e lamas de estação de tratamento de águas residuais. A poda mecânica e os corretivos orgânicos tiveram efeitos diferentes na composição mineral do vinho, de acordo com cada elemento específico. No entanto, os limites legais, recomendados pela OIV e estabelecidos pela legislação da União Europeia, e os limites técnicos adotados pelos enólogos nunca foram ultrapassados, pelo que a interação de ambas as práticas não parece ser um problema no que diz respeito à composição mineral dos vinhos produzidos.

Keywords: mechanical pruning, organic amendments, wine mineral composition, ICP-OES, ICP-MS.
Palavras-chave: poda mecânica, corretivos orgânicos, composição mineral do vinho, ICP-OES, ICP-MS.

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INTRODUCTION

The mineral content of wines depends on several aspects, including environmental conditions, soil, grape variety, and viticultural and enological practices. Some elements must be determined due to their toxicological and physiological properties and some others can lead to wine spoilage (Catarino *et al.*, 2006, 2008a). The levels of some elements, such as arsenic (As), cadmium (Cd), cupper (Cu), lead (Pb) and zinc (Zn), at different phases of the winemaking process are also a concerning because of legal requirements.

Vineyard soil is an important source of the minerals that are present in wine. The minerals absorption is influenced, among other factors, by soil geochemistry and vine rootstock (Catarino *et al.*, 2018). There are also other factors that can affect wine mineral content, namely soil amendments and fertilizers, irrigation water, atmospheric pollution, pesticides, contact with materials during transport, vinification and ageing processes, and enological processing aids and additives (Catarino *et al.*, 2008b; Volpe *et al.*, 2009). On the other hand, the decrease of some elements occurs over time, particularly during alcoholic fermentation. The precipitation of K and Ca as tartrate salts starts during alcoholic fermentation and remains during the ageing period. Heavy metals precipitate as insoluble salts, namely as sulfides, a phenomenon that is favored by the addition of sulfur dioxide during winemaking (Ribéreau-Gayon *et al.*, 2000).

The major concern about the application of organic amendments, particularly municipal solid waste compost (MSWC) and sewage sludge (Sludge), is their heavy metal content that may contaminate the soil and enter the food chain. The environmental risks are linked to the mobility of metals and to their concentration in soil solution rather than to the total soil concentration (Diacono and Montemurro, 2010). This highlights the importance of long-term experimentation when studying the environmental fate of metals in compost-amended soils on a large scale.

Regarding MSWC, over a 10-year application, Businelli *et al.* (2009) found that the most abundant heavy metals in topsoil were Cu, Zn and Pb. Bartl *et al.* (2002), in a 5-year study, observed significant differences only in Zn and Pb. Pinamonti *et al.* (1999), using municipal solid waste with high amount of metals for six years, observed a significant accumulation of Ni, Pb, Cd and Cr in soil, vegetation and grape juice. On the other hand, Erhart *et al.* (2008), after ten years of application of high quality biowaste compost, found no variation in either total heavy metal concentration or available fractions.

In what concerns to Sludge, Smith (2009) refers that Zn is the element with higher potential to impact soil microbial activity and fertility. Fliessbach *et al.* (1994) observed that high heavy metals contamination of soil resulted in a decrease of soil microbial biomass carbon. Pinamonti *et al.* (1999) and Korboulewski *et al.* (2002) reported that after two and one application of Sludge compost, respectively, neither the total nor the available heavy metals concentration in soil, plant and grape juice were increased.

As far as we know, previous studies focused on the effects of MSWC and Sludge in grapes and grape juice and no studies analyzed the effects of these two amendments in wine composition.

Manure can also contribute to the increase of some heavy metals in soil, namely Zn and Cu (Nicholson *et al.*, 2003). However, to the best of our knowledge, no studies were performed analyzing the effect of Manure in wine mineral content.

Biochar is known to increase nutrients retention in soil (Lehmann *et al.*, 2003), reduce the bioavailability and phytotoxicity of heavy metals (Park *et al.*, 2011), improve plant water availability (Baronti *et al.*, 2014), improve soil structure (Case *et al.*, 2012) and stimulate soil microbial activity (Sánchez-Monedero *et al.*, 2019). The effects of biochar application in vineyard soil on grape and wine quality has not been yet fairly studied. However, the existing works point to a lack of effects on grape and wine composition (Sánchez-Monedero *et al.*, 2019).

The influence of pruning on the nutritional status of grapevines has already been studied by several authors, such as Balasubrahmanyam and Diofasi (1978), Wample (1989), Rühl and Clingeleffer (1993), Bovio and Lisa (1996) and Pérez-Bermúdez *et al.* (2015). However, to the best of our knowledge, the influence of pruning system on the mineral composition of wines, considering major, minor and trace elements of wines, has not yet been studied.

Concerning the effects of pruning in nutrient concentration in vegetative organs, Balasubrahmanyam and Diofasi (1978) observed a decrease of mineral elements in canes with increase of bud load, while Rühl and Clingeleffer (1993) noticed a decrease of nitrogen in perennial organs with minimal pruning. On the other hand, Bovio and Lisa (1996) observed that the concentration of macronutrients, in petioles and blades, was not affected by mechanical pruning. Wample (1989) found relatively small and, most of the time, inconsistent differences between pruning systems based on data from petioles and leaf blades collected in six trial fields over nine years.

However, many factors may affect the net accumulation of mineral nutrients in the berry through their effects on root cation uptake, translocation from root to shoot, re-translocation of cations from shoot back to root, the mineral nutrient reserve, and the number of berries and berry growth rates in relation to vine vigour, complicating any simple explanation of the regulation of cation accumulation in grape berries (Etchebarne *et al.*, 2009).

The present work is part of a broader study, from which the main outcomes about vine vegetative and reproductive growth have already been published in Botelho *et al.* (2020) and about grape composition in Botelho *et al.* (2021).

From the reviewed literature, organic amending of vineyard soil increases productivity and tackle the problems associated with the predicted climatic changes, while mechanical pruning significantly increases bud load, affecting both vegetative and reproductive growth (Botelho *et al.* 2020), and seems to be a suitable strategy to reduce production costs and increase productivity. However, since both practices can have impact in the mineral content of wines, it is necessary to understand the effects of the interaction between them on wine mineral content. To the best of our knowledge this is the first experimental work involving the interaction between the two factors (mechanical pruning and soil organic amendments).

MATERIALS AND METHODS

Site description and experimental design

The trial, carried out over four years (2012 to 2015), was installed in two vineyards of *Vitis vinifera* L. cv. ‘Syrah’. Quinta do Côro (QC) is located in Tejo wine region and Quinta do Gradil (QG) in Lisboa wine region. The vineyards and the experimental layout are described in Botelho *et al.* (2020).

The studied factors were pruning system and organic amendments, which were compared in a strip-plot design, with three blocks. Each block held eight adjacent rows where pruning treatment was randomly assigned, creating two groups of four adjacent lines each with a different pruning treatment. The 60 m rows were divided into five parts of twelve meters each, in which organic amendments were randomly assigned. Each one of the 30 plots consisted of 48 vines.

Regarding pruning, two treatments were imposed: MAN - manual spur pruning, retaining six to seven 2-bud spurs per vine; MEC - mechanical pruning, simulating the pruning effect of four cutting bars (2 parallel to the ground and 2 perpendicular to the ground) working at a distance of 15 cm from the cordon.

Five treatments of organic amendments were imposed: Ctrl – no application of organic amendment neither fertilizer; Bioc – application of 8500 kg/ha/year of char dust resulting from the pyrolysis of wood; MSWC – application of 16100 kg/ha/year of municipal solid waste compost; Manure – application of 24000 kg/ha/yea of cattle manure; Sludge - application of 34000 kg/ha/year of sewage sludge. The referred quantity of each organic amendment is expressed in fresh weight and its definition was based on the application of 5000 kg of dry organic matter per hectare and per year. The composition of each organic amendment is shown in Table I.

Winemaking

In both field trials, grapes from the three replicates per treatment were pooled respectively for winemaking; 16 kg of grapes were harvested per plot and pooled, thus 48 kg of grapes were used for each treatment.

Before the harvest, the grapes from the vineyards involved in this project were controlled, in order to access their quality and maturation stage. The parameters controlled in this phase were weight of a hundred berries (g), °Brix, potential alcohol content (%), pH and total acidity (g of tartaric acid/L). These results are presented in Botelho *et al.* (2021).

When the grapes were at the ideal stage of maturation the manual harvest was performed and the grapes were transported to the experimental winery of Instituto Superior de Agronomia (Lisboa), where the vinification took place. The grapes were de-stemmed, crushed and sulfur dioxide was added (50 mg/L). The crushed grapes were placed into 60 L stainless steel tanks and inoculated with the yeast Zymasil® Bayanus. After these operations, a sample of must from each vineyard and treatment was taken to analyze potential alcoholic content, pH and total acidity, using the methods recommended by OIV (OIV, 2019).

The alcoholic fermentation lasted between seven and nine days at the average temperature of 24 °C, and the maceration time was extended to 15 days. During this period the cap was punched down three times a day. After maceration the skins were separated from the juice using a vertical press, and the pressed juice was added to the free-run juice. After fermentation was completed, the free sulfur dioxide content was adjusted to 30 mg/L. When alcoholic fermentation ended, wines were analyzed to determine alcoholic content, pH, total acidity and volatile acidity.

The malolactic fermentation occurred after the alcoholic fermentation, spontaneously, and its progression was controlled using paper chromatography (Ribéreau-Gayon *et al.*, 1982). In February, this process was ended for all the wines. The wines were racked to remove the lees that settled, and then a new analysis took place to control total and free sulfur dioxide, volatile acidity and pH. Free sulfur dioxide content was then adjusted to 30 mg/L, and the wines were stored in 750 mL bottles.

Table I

Average composition of the organic amendments along the four years (Botelho *et al.* 2020)

	Bioc	MSWC	Manure	Sludge
Fresh matter basis				
pH	8.99	7.71	7.00	9.64
Electrical conductivity (mS/m)	69.1	413.7	522.0	263.3
Moisture (%)	23.8	44.2	63.0	78.0
Dry matter basis				
Organic Matter (%)	72.3 ± 12.32	46.5 ± 10.03	67.5 ± 9.48	67.8 ± 6.48
Total N (%)	1.0 ± 0.44	2.1 ± 0.16	2.4 ± 0.72	6.8 ± 0.26
Total P (g/kg)	0.8 ± 0.45	6.9 ± 0.46	4.2 ± 1.22	13.5 ± 1.94
Total K (g/kg)	5.2 ± 1.44	7.8 ± 0.25	18 ± 1.36	3.2 ± 0.77
Total Ca (g/kg)	36.3 ± 6.94	72.7 ± 18.00	16.4 ± 0.55	66.5 ± 16.49
Total Mg (g/kg)	2.2 ± 0.49	14.9 ± 3.04	4.8 ± 0.44	4.6 ± 0.63
Total S (g/kg)	1.4 ± 0.23	2.9 ± 0.16	3.3 ± 2.59	7.6 ± 0.18
Total Na (g/kg)	0.5 ± 3.02	6 ± 2.65	6.6 ± 2.68	0.8 ± 5.50
Total Fe (g/kg)	5.2 ± 0.07	8 ± 0.08	3.1 ± 0.07	9.3 ± 0.06
Total Mn (mg/kg)	144.9 ± <0.01	249.9 ± 0.02	223.3 ± 0.04	105.4 ± 0.02
Total Cu (mg/kg)	10.8 ± 0.00	132.2 ± 0.08	45.2 ± 0.05	137.6 ± 0.18
Total Zn (mg/kg)	18.1 ± 0.01	360.8 ± 0.02	134.1 ± 0.01	831.4 ± <0.01
Total B (mg/kg)	18.7 ± 1.49	26.1 ± 0.93	22 ± 0.50	28.2 ± 3.19
Total Ni (mg/kg)	2.9 ± <0.01	10 ± 0.01	5.2 ± 0.01	6 ± 0.01
Total Cd (mg/kg)	0.03 ± <0.01	0.03 ± <0.01	0.07 ± <0.01	0.05 ± <0.01
Total Pb (mg/kg)	14.8 ± <0.01	79.6 ± 0.07	3.4 ± <0.01	24.9 ± 0.02
Total Cr (mg/kg)	8.6 ± 0.01	27.8 ± 0.03	4.6 ± <0.01	20.3 ± 0.02
Total Hg (mg/kg)	0.01 ± 0.01	0.41 ± 0.29	0.02 ± 0.01	0.51 ± 0.41

Results are the four years average ± standard deviation. For standard deviation lower than 0.01, the value was replaced by <0.01. Biochar (Bioc), municipal solid waste compost (MSWC), cattle manure (Manure) and sewage sludge compost (Sludge).

After the bottling process, mineral elements contents were assessed.

Mineral elements analysis

The determination of total N was performed according to the method recommended by OIV (OIV, 2019).

For the quantification of As, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mo, Na, Ni, P, Pb, S and Zn, inductively coupled plasma optical emission spectrometry (model iCAP 7000 Series - Thermo Fisher Scientific) was used. A Sigma Aldrich (USA) stock solution containing P, K, S, Ca, Mg, Na, Cu, Fe, Zn, B, Mo, Cr, Ni, Cd, Pb and As (1000 mg/L for each element) was used to prepare calibration standards. The calibration range for P, K, and S was 1-500 mg/L, for Ca, Mg and Na was 0.2-100 mg/L, for Cu, Mn, Fe, Zn, B and Mo was 0.02-10 mg/L and for Cr, Ni, Cd, Pb and As was 0.002-1 mg/L. The suprapure 65 % HNO_3 (m/m) from Merck (USA) and double distilled water were used for sample dilution. The samples were previously diluted 10 times, without any prior preparation as described by Zioła-Frankowska and Frankowski (2017).

In order to quantify other mineral elements in the wines, namely minor and other trace and sub-trace elements (Li, Be, Al, V, Co, Ga, Rb, Sr, Y, Cs, Pr, Eu, Dy, Ho, Lu, Tl, Zr, Nb, Ce, Tb, Tm and Se), inductively coupled plasma mass spectrometry (Perkin- Elmer SCIEX Elan 9000 ICP-MS - Perkin-Elmer SCIEX) was applied and a semi-quantitative method was used, as described by Catarino *et al.* (2006), at the Laboratory of Mineral Analysis of Instituto Nacional de Investigação Agrária e Veterinária - Dois Portos. A PerkinElmer SCIEX Elan 9000 was utilized with a Gilson pump and a Scott-type spray chamber, a crossflow nebulizer, and nickel cones. To optimize operational conditions, monoelement standard solutions of Be, Co, and In in 1000 mg/L (Merck) and a multielement solution with Mg, Cu, Rh, Cd, In, Ba, Ce, Pb, and U 10 μg/L (PerkinElmer) were used. Wash, blank, and standard solutions were prepared with ultrapure concentrated HNO_3 Ultrex II 70% (v/v) (J. T. Baker). Analytical calibration was established using a standard solution with 30 elements (PerkinElmer, 10 mg/L), reproducing the wine mineral composition, in a final concentration of 10 μg/L. To avoid contamination, all polyethylene material (volumetric flasks, micropipette tips, and autosampler vessels) was immersed at least for 24 h in 20% (v/v) HNO_3, and rinsed thoroughly with purified water before use. For decontamination solution preparation, reagent grade HNO_3 was double-distilled using an infra-red subboiling distillatory system (model BSB-939-IR, Berghof, Germany). Purified water (conductivity < 0.1 μS/cm) was produced using a Seralpur Pro 90CN apparatus (Seral, Ransbach-Baumbach, Germany). Due to analytical constraints it was only possible to analyze by ICP-MS the wines from 2013 and 2014.

Statistical Analysis

All data were tested to verify if the assumptions of analysis of variance (ANOVA) using Shapiro-Wilk's test , and then subjected to three-way (pruning x organic amendment x site) ANOVA, using the general linear procedure for strip-split–plot design and F-test. The significance level was set at p=0.05 and means were separated using Tukey's honestly significant difference test. The statistical analysis was performed using Statistix software package (version 9.0; Analytical Software, Tallahassee, FL).

In the following Tables the values presented for the pruning system and for the site are an average of 10 wines, while for the organic amendment they are an average of four wines.

RESULTS AND DISCUSSION

The results presented in this work correspond only to the last three years (harvests) of the research project, since, in 2012, no significant effects were observed in wine mineral composition. The main outcomes, concerning grape and wine composition, from the first experimental year (2012) were reported by Correia (2014).

Since some viticultural data are important to understand the effects of the studied factors on mineral composition of wines, some of the data already reported in Botelho *et al.* (2020), are presented: Mechanical pruning induced a significant increase in bud load (MAN – 13.9 buds/vine; MEC – 54.9 buds/vine) and consequently in the shoot number per vine (MAN – 20.4 shoots/vine; MEC – 32.6 shoots/vine) and in yield (MAN – 4.74/kg/vine; MEC – 6.23/kg/vine). Berry weight was lower in mechanical pruning (MAN – 1.69 g; MEC – 1.42 g). Globally, there was a tendency for lower pruning weights per vine in mechanical pruning (MAN – 0.931 kg/vine; MEC – 0.727/kg/vine).

Organic amendments did not affect bud load (Botelho *et al.*, 2020). However, the shoot number per vine increased from 2014 on, and yield was significantly increased from 2013 on (Ctrl – 4.68 kg/vine; Bioc - 5.04 kg/vine; MSWC – 5.92 kg/vine; Manure – 5.88/kg/vine; Sludge – 6.68 kg/vine) (Botelho *et al.*, 2020). Berry weight was higher with the organic amendments (Ctrl – 1.51 g; Bioc – 1.53 g; MSWC – 1.56 g; Manure – 1.58 g; Sludge – 1.60 g) (Botelho *et al.*, 2020). The pruning weight per vine increased through the organic amendments (Ctrl – 0.754 kg/vine; Bioc – 0.808 kg/vine; MSWC – 0.878 kg/vine; Manure – 0.795 kg/vine; Sludge – 0.915 kg/vine) (Botelho *et al.*, 2020).

Since leaf area was not affected by the studied factors, the leaf area to fruit ratio was lower in the treatments with higher yield (Botelho *et al.*, 2020). The interaction of pruning system and organic amendments effect on yield was significant with the differences between organic amendments being significant only in MEC treatment (Botelho *et al.*, 2020).

Some oenological data are also important to understand the following results: Mechanical pruning produced wines with lower alcoholic strength (MAN – 14.0% vol.; MEC – 12.9% vol) and lower pH (MAN – 3.62; MEC – 3.46).

Major elements

The concentration of the major elements (N, P and K) in wine decreased significantly by the mechanical pruning, while the concentration of Ca, Mg and S was less affected by pruning system and was tendentially equal or higher in mechanical pruning (Table II).

The decrease of the concentration of N, P and K in MEC wines was probably related to the increase in yield that takes to a growth-induced nutrient dilution in berries. The decrease of N and K concentration in grapes, due to the mechanical pruning, has already been observed by Pérez-Bermúdez *et al.* (2015). The N concentration in berries tends to be higher at technological and phenolic maturity (Garde-Cerdán *et al.*, 2009), so the already referred ripening delay in MEC, due to the lower leaf to fruit ratio, was likely the cause of the observed differences.

Concerning K accumulation in the berries, according to Etchebarne *et al.* (2009), it is directly related to the plant water status, so the reduction of K concentration observed in MEC was likely related to the lower water availability in this treatment (Botelho, 2021). Moreover, the wine K content also reflects the precipitation that naturally occurs with tartaric acid (Boulton *et al.*, 1995), which was tendentially higher in MEC (Botelho *et al.*, 2021) and, probably, led to a higher precipitation of K, reducing its level in wine.

Table II

Effect of the pruning system, the organic amendment and the site on the content of major elements in wine (mg/L)

	N	Na	Mg	P	S	K	Ca
Year				2013			
MAN	201.30 a	7.36	106.70 b	170.87 a	150.54	854.74 a	41.95
MEC	176.28 b	6.73	118.09 a	157.27 b	174.97	750.36 b	42.88
Prun. effect	**	n.s.	*	**	n.s.	***	n.s.
Ctrl	198.10	8.19	124.18 a	182.14 a	153.16	787.86 ab	42.54
Bioc	180.03	6.29	112.68 ab	160.97 ab	141.46	837.95 a	42.57
MSWC	185.59	7.52	113.02 ab	172.22 ab	156.18	816.65 a	43.40
Manure	180.73	6.97	108.35 ab	159.54 b	200.36	811.29 a	41.89
Sludge	199.49	6.25	103.74 b	145.46 b	162.59	758.98 b	41.69
Amend. effect	n.s.	n.s.	*	**	n.s.	*	n.s.
QC	234.11 a	4.29 b	112.96	222.26 a	155.88	740.65 b	41.21
QG	143.47 b	9.80 a	111.83	105.87 b	169.62	864.45 a	43.62
Site effect	**	***	n.s.	***	n.s.	***	n.s.
Prun * Amend	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Prun * Site	*	n.s.	n.s.	**	n.s.	**	n.s.
Amend * Site	n.s.	n.s.	n.s.	*	n.s.	*	n.s.
Year				2014			
MAN	280.03 a	7.96 a	104.00	299.51 a	169.58 b	877.88 a	42.82 b
MEC	162.70 b	6.91 b	103.61	273.54 b	174.28 a	786.01 b	47.25 a
Prun. effect	**	*	n.s.	**	n.s.	**	**
Ctrl	181.37 c	8.11	108.12 a	310.93 a	172.14	836.90	43.08 b
Bioc	176.62 c	7.42	104.88 ab	294.06 a	164.83	841.96	44.82 b
MSWC	244.81 ab	7.57	102.61 bc	298.59 a	173.09	815.61	45.71 ab
Manure	222.69 b	7.53	103.45 bc	283.76 a	175.45	852.59	41.65 b
Sludge	281.33 a	6.55	99.95 c	245.27 b	174.14	812.66	49.91 a
Amend. effect	*	n.s.	**	**	n.s.	n.s.	**
QC	190.26 b	3.51 b	88.45 b	267.62 b	176.15 a	734.79 b	49.77 a
QG	252.46 a	11.37 a	119.15 a	305.43 a	167.71 b	929.10 a	40.30 b
Site effect	*	***	***	***	*	***	***
Prun * Amend	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
Prun * Site	n.s.	n.s.	**	*	n.s.	n.s.	*
Amend * Site	n.s.	n.s.	*	*	*	n.s.	*
Year				2015			
MAN	220.32 a	6.75	111.59 b	289.50 a	201.85	707.23	50.50
MEC	168.23 b	6.56	127.93 a	258.69 b	198.60	652.75	54.29
Prun. effect	**	n.s.	*	**	n.s.	n.s.	n.s.
Ctrl	194.64	7.14 a	128.72	292.95 a	198.89	717.11	51.53
Bioc	183.83	6.64 a	121.33	306.90 a	205.54	698.76	50.28
MSWC	195.03	6.77 a	116.68	287.14 a	197.68	694.08	54.82
Manure	197.05	6.70 a	121.31	275.91 a	203.05	661.51	49.70
Sludge	200.82	6.02 b	110.75	207.58 b	195.97	628.49	55.63
Amend. effect	n.s.	**	n.s.	**	n.s.	n.s.	n.s.
QC	231.06 a	3.36 b	102.87 b	290.87 a	205.76	632.32 b	56.15
QG	157.49 b	9.95 a	136.64 a	257.32 b	194.69	727.66 a	48.63
Site effect	**	***	**	**	n.s.	*	n.s.
Prun * Amend	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.
Prun * Site	*	**	n.s.	**	n.s.	n.s.	n.s.
Amend * Site	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.

Statistical significance of the effects of pruning system, organic amendment, experimental site and their interactions: n.s. not significant 5% level by F test; *, **, *** significant at $p<0.05$, $p<0.01$ and $p<0.001$, respectively. Within each column and for each factor, mean values followed by a different letter are significantly different at $p<0.05$ by Tukey's test. Pruning system: hand pruning (MAN) and mechanical pruning (MEC). Organic amendments: control (Ctrl), biochar (Bioc), municipal solid waste compost (MSWC), cattle manure (Manure) and sewage sludge compost (Sludge). Site: Quinta do Côro (QC), Quinta do Gradil (QG).

In the case of Ca and Mg a tendency for higher values in MEC was observed. This behavior was probably related to the higher transpiration observed in MEC (Botelho, 2021). Indeed, according to Lazaroff and Pitman (1966), the uptake of Ca and Mg is largely influenced by transpiration. Concerning Ca, the higher values in MEC may also be due to the lower pH and alcoholic strength which lead to less precipitation of Ca as calcium bitartrate (Ribéreau-Gayon *et al.*, 2000). However, the Ca content was always lower than 60 mg/L, indicating a low probability of Ca precipitation problems (Ribéreau-Gayon *et al.*, 2000).

The S uptake is not simply related to transpiration (Hawkesford and Kok, 2006) since S is usually applied in vineyard to control powdery mildew, it is not a limiting nutrient so the plants absorbed according to their needs and no differences between pruning systems were observed.

The application of organic amendments to soil affected the mineral composition of wine. Concerning the principal major elements, P was the most affected by organic amendments, being Sludge the treatment that produced wines with the lowest P levels. The reduction of P content in wines was probably related to the higher N supplied by sewage sludge. Hilbert *et al.* (2003) also report a negative correlation between N and P petiole content in grapevine

Although the organic amendments increased assimilable N in musts (data not shown), the effect on the N content of wines was not significant, and was probably related to the fact that N compounds are used by yeasts during fermentation and that proteins and peptides precipitate with tannins during fermentation (Boulton *et al.*, 1995). Only in 2014, when water availability was higher, the treatments with none or low supply of N, Ctrl and Bioc respectively, produced wines with lower N content. In 2014 the soil had a higher moisture what, probably, led to a faster diffusion of NO_3^- in soil and favored the N uptake that the organic amendments supplied (Vuuren *et al.*, 1997), namely MSWC, Manure and Sludge.

In the case of Mg and Na, there was a tendency for treatments with higher yield (MSWC, Manure and Sludge) to produce wines with a slightly lower content of these minerals, which was probably related to the aforementioned growth-induced dilution that was not offset by a higher absorption. Na is an element limited to 80 mg/L (Na in excess) according to OIV maximum acceptable limits (OIV 2019), however this threshold was never overwhelmed.

Ca content was tendentially higher in MSWC and Sludge because these organic amendments supplied a higher amount of this nutrient than Bioc and Manure.

The site effect was significant in some elements content in wine. However, the one with the most expressive differences was K, which was higher in QG. These differences were due to the soil composition, which was significantly richer in K in QG (Botelho, 2021).

Minor elements

Mechanical pruning significantly decreased the content of some minor elements in wine (Table III). The elements which content was reduced were Ni, in 2013, As (which legal limit is 200 µg/L), Mo and Ni, in 2014, and As, in 2015. This reduction is probably related to the growth induced dilution, that the increase in yield, observed in this treatment, origins. Moreover, 2014, which was the year with the highest yield differences between pruning systems (Botelho *et al.*, 2020), was the year with differences in more elements. Pruning system had no significant effects on Cr, Cu and Pb.

Organic amendments did not affect wine Cr, Ni, Cu, Zn, As, Mo, Cd and Pb content.

The effect of pruning on Fe content in wine was low or null (Table III). Since the demand of this nutrient by the plant is low, the increase in productivity and the consequent dilution effect was, most of the times, compensated by a higher uptake from soil. When differences in yield between pruning systems were higher (2014), the higher demand for Fe was not completely offset and wines from MEC had significantly lower concentrations of Fe. It is generally accepted that total Fe content in wines should be below 8 to 10 mg/L in order to avoid any haze issues. Furthermore, MEC wines are less susceptible to Fe precipitations as ferric phosphate and ferric hydroxide since the pH is lower (Ribéreau-Gayon *et al.*, 2000).

The B content in wines from MEC was always significantly lower than in those from MAN (Table III). Since B uptake occurs as boric acid transported by the transpiration flow (Brown and Shelp, 1997), it could be expected that the higher transpiration in MEC (Poni *et al.*, 2011) would compensate the higher demand due to the larger crop. However, B availability in soil was low (0.34 mg/kg) and B has low mobility within grapevine (Brown and Shelp, 1997). Consequently, the increase in productivity in MEC led to a decrease of B concentration in berries.

Table III

Effect of the pruning system, the organic amendment and the site on the content of minor (B, Fe, Cu, Zn and Mo), trace (Cr, Ni, As and Pb) and sub-trace (Cd) elements in wine (B, Fe and Zn – mg L^{-1}; Cr, Ni, Cu, As, Mo, Cd and Pb - µg L^{-1})

	B	Cr	Fe	Ni	Cu	Zn	As	Mo	Cd	Pb
Year					**2013**					
MAN	2.93 a	3.83	1.53	11.30 a	27.31	0.55 b	54.03	15.10	UDL	4.71
MEC	2.48 b	6.40	1.51	9.30 b	25.49	0.84 a	43.93	13.02	UDL	7.41
Pruning	**	n.s.	n.s.	*	n.s.	*	n.s.	n.s.	-	n.s.
Ctrl	3.12 a	6.23	1.54	9.44	34.79	0.91	59.02	16.59	UDL	6.31
Bioc	2.92 ab	2.06	1.52	11.96	14.85	0.70	47.36	13.82	UDL	3.85
MSWC	2.63 ab	5.41	1.51	10.63	25.86	0.65	48.39	14.55	UDL	7.12
Manure	2.48 b	7.24	1.44	8.11	29.27	0.54	52.05	12.88	UDL	6.88
Sludge	2.36 b	4.65	1.59	11.34	27.20	0.66	38.10	12.47	UDL	6.15
Amend.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-	n.s.
QC	1.60 b	4.15	1.62	0.51 b	35.08	0.63	76.38 a	16.49 a	UDL	3.26
QG	3.81 a	6.08	1.43	20.08 a	17.71	0.75	21.58 b	11.63 b	UDL	8.86
Site effect	***	n.s.	n.s.	***	n.s.	n.s.	***	**	-	n.s.
Prun *	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-	n.s.
Prun * Site	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-	n.s.
Amend *	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	-	n.s.
Year					**2014**					
MAN	3.24 a	6.02	1.54 a	8.49 a	27.31	0.78 a	55.09 a	16.47 a	UDL	9.81
MEC	2.55 b	6.49	1.28 b	7.05 b	25.49	0.45 b	34.49 b	12.28 b	UDL	7.24
Pruning	***	n.s.	*	**	n.s.	**	**	*	-	n.s.
Ctrl	3.20 a	5.92	1.29	6.56	57.52	0.69	48.20	17.24	UDL	6.08
Bioc	2.96 b	6.85	1.43	9.10	77.23	0.55	49.34	14.86	UDL	7.88
MSWC	2.84 bc	6.16	1.40	7.16	94.96	0.59	48.61	14.45	UDL	9.79
Manure	2.79 bc	5.82	1.48	7.19	113.52	0.58	44.47	13.44	UDL	10.58
Sludge	2.68 c	6.54	1.45	8.83	61.31	0.67	33.33	11.88	UDL	8.31
Amend.	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-	n.s.
QC	1.49 b	5.73	1.41	2.91 a	72.35	0.44 b	49.92	16.51 a	UDL	1.26 a
QG	4.31 a	6.78	1.41	12.63 b	89.46	0.79 a	39.66	12.24 b	UDL	15.80 b
Site effect	***	n.s.	n.s.	***	n.s.	**	n.s.	*	-	***
Prun *	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-	n.s.
Prun * Site	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-	n.s.
Amend *	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-	n.s.
Year					**2015**					
MAN	2.52 a	4.90	1.15	10.54	4.28	0.68	62.18 a	13.86	UDL	4.76
MEC	2.24 b	4.92	1.09	8.96	5.09	0.63	46.25 b	10.75	UDL	2.95
Pruning	**	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	-	n.s.
Ctrl	2.69 a	5.44	1.23 a	9.61	10.72	0.69	57.75	15.26	UDL	5.97
Bioc	2.36	5.82	1.16 ab	10.26	7.79	0.61	66.63	13.35	UDL	4.35
MSWC	2.51 ab	5.48	1.17 a	10.33	4.76	0.69	55.57	11.65	UDL	2.20
Manure	2.26 bc	2.76	1.14 ab	8.21	0.26	0.66	54.74	11.28	UDL	0.64
Sludge	2.08 c	5.06	0.91 b	10.35	0.12	0.64	36.37	9.99	UDL	6.12
Amend.	*	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	-	n.s.
QC	1.39 b	5.72	1.30 a	3.11 b	6.53	0.54 b	62.37 a	14.63	UDL	4.47 a
QG	3.37 a	4.10	0.94 b	16.39 a	2.84	0.78 a	46.05 b	9.98	UDL	3.24 b
Site effect	***	n.s.	***	**	n.s.	**	*	n.s.	-	*
Prun *	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-	n.s.
Prun * Site	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-	n.s.
Amend *	*	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	-	n.s.

Statistical significance of the effects of pruning system. organic amendment. experimental site and their interactions: n.s. not significant 5% level by F test; *. **. *** significant at $p<0.05$, $p<0.01$ and $p<0.001$. respectively. Within each column and for each factor. mean values followed by a different letter are significantly different at $p<0.05$ by Tukey's test. Pruning system: hand pruning (MAN) and mechanical pruning (MEC). Organic amendments: control (Ctrl). biochar (Bioc). municipal solid waste compost (MSWC). cattle manure (Manure) and sewage sludge compost (Sludge). Site: Quinta do Côro (QC). Quinta do Gradil (QG). UDL – value lower than the detection limit of the method, which, in the case of Cd, is 0.01mg L^{-1}.

In 2015, the significantly lower Fe content in wines associated with Sludge might be related only to the growth induced dilution, since the Fe content of the raw material was the highest of the four organic amendments. The obtained wines are far from the common technical security limit of 8-10 mg/L generally accepted by the oenologists to predict Fe hazes in wine. Concerning B content, the levels were lower in Manure and Sludge and intermediate in Bioc and MSWC. Since the B concentration in the amendments was low, the growth induced dilution was probably the reason for the observed differences in Manure, Sludge and MSWC. Concerning the effect of Bioc, the high binding capacity (Lehmann *et al.*, 2003) may have reduced the bioavailability of B and, consequently, reduced its content in wine.

The Zn content is limited according to OIV indications (OIV, 2019). However, the values observed in this work were quite below the referred limits (5 mg/L). The variation in Zn content due to pruning, although significant, was variable among years and no clear trend was observed.

As, Cd, Cu and Pb content in organic amendments is limited due to the national law (DL103/2015). In this study, the amendments used did not exceeded the legal limits. In addition, the total plant uptake of heavy metals applied with the organic amendments is generally low, being the most concerning elements, for human health, Pb, supplied especially by MSWC (Smith, 2009), and Cd, supplied especially by sewage sludge (Dean and Suess, 1985). However, these elements were present in a low concentration, in the used organic amendments. Thus, the low concentration in the raw material and the high affinity for binding heavy metals that composted residuals have (Smith, 2009) limited the absorbance of these elements by the plants and, consequently, reduced the differences of these elements content in the wine. It is relevant to note that there are legal limits for the contents of As (200 µg/L), Cd (10 µg/L), Cu (1000 µg/L) and Pb (150 µg/L), which are toxic elements, but the levels of those elements in the wines produced in this study were all significantly below them.

Comparing the two sites, it is noteworthy that the levels of Ni are higher in QG, which was probably related to the soil composition.

Other elements

Mechanical pruning significantly reduced the content of some other elements in wine: Ga and Tm, in 2013, and Rb, Li, Ga, Y, Tl, Be and Se, in 2014 (Table IV). This reduction was likely due to the growth induced dilution. In the case of Sr, globally, the pruning system had no effect on its concentration in wine, although in 2013 it was slightly higher in MEC.

Regarding the organic amendments, the reduction in Sr and Eu, in 2013, as well as in Li and Ga, in 2014, was probably due to the growth induced dilution. In the case of Cs, in 2013, and Be, in 2014, which were significantly affected by organic amendments, the growth induced dilution effect was also visible, although a higher value was observed in Sludge. Although the analysis of Cs and Be content in Sludge has not been performed, these higher values were possibly related to a higher content of these elements in the raw material.

Generally, the observed concentrations were in accordance with the literature (Eschnauer, 1982; Nicolini *et al.*, 2004; Thiel *et al.*, 2004; Greenough *et al.*, 2005; Catarino *et al.*, 2006; Catarino *et al.*, 2008a; Moreno *et al.*, 2008). However, the concentration of some elements was below the values referred in the reviewed bibliography, like Ce, Al, Tb, Dy and V. On the other hand, Rb and Tl were above those levels. In the present work, the highest observed Rb content was 9,2 mg/L, while Nicolini *et al.* (2004) assessed 2,16 mg/L of this element in an Italian wine. In the case of Tl, the highest value observed in the present work was 5,5 µg/L, which is higher than the 4,2 µg/L obtained by Thiel *et al.* (2004).

CONCLUSIONS

Mechanical pruning and soil organic amending had significant effects on wine mineral composition. The wine analysis revealed lower N, P and K contents in wines from treatments with mechanical pruning, while Ca and Mg were tendentially higher in this pruning system. The contents of some minor elements, such as As, Mo and Ni, also decreased by mechanical pruning. In 2014, the year with the higher reproductive growth, some other elements also decreased by mechanical pruning (Ga, Li, Rb, Tl and Y). Concerning the organic amendments, sewage sludge produced the wines with the lowest P and Fe content. Ca was tendentially higher in municipal solid waste compost and sewage sludge treatments.

According to each mineral element, mechanical pruning and soil organic amendments had different effects. It should be highlighted that the legal limits, recommended by OIV and established by the European Union, as well as technical limits adopted by winemakers were never exceeded. Therefore, the interaction of both practices does not seem to be a problem in what concerns to the mineral composition of the produced wines

Table IV

Effect of the pruning system, the organic amendment and the site on minor (Al, Rb and Sr), trace (Li, Co, Ga, Zr and Cs) and sub-trace (Be, Y, V, Nb, Tl, Ce, Pr, Tb, Eu, Dy, Ho and Lu) elements content of wine (Rb and Sr – mg/L; Al, Ce, Co, Cs, Eu, Ga, Li, Y, Tl, Be, Se and V – µg/L; Dy, Lu, Ho, Nb, Pr, Tb, Tm and Zr – ng/L)

Year	**2013**																		
	Li	**Al**	**Co**	**Ga**	**Rb**	**Sr**	**Y**	**Zr**	**Nb**	**Cs**	**Tl**	**Ce**	**Pr**	**Eu**	**Tb**	**Dy**	**Ho**	**Tm**	**Lu**
MAN	2.81	69.04	1.55	2.60 a	3.78	0.35 b	0.32	4.27	0.08	37.77	1.89	0.18	27.13	0.16	7.12	29.73	12.56	12.53 a	10.16
MEC	2.82	68.53	1.31	2.15 b	3.71	0.38 a	0.26	7.97	0.08	31.64	1.46	0.21	25.79	0.17	4.84	22.97	8.94	9.52 b	7.44
Pruning	n.s.	n.s.	n.s.	*	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.
Ctrl	3.08	70.52	1.46	2.62	5.27	0.41 a	0.35	5.34	0.10	36.86 ab	1.64	0.26	31.73	0.17	8.42	32.12	10.97	13.39	10.72
Bioc	2.74	88.94	1.48	2.39	3.42	0.37 a	0.29	8.91	0.07	27.54 b	1.72	0.17	24.03	0.18	6.08	23.89	9.49	12.48	7.33
MSWC	2.83	59.43	1.40	2.45	3.45	0.36 ab	0.29	5.65	0.07	28.17 b	1.60	0.21	25.55	0.16	4.49	24.59	9.50	8.25	8.66
Manure	2.78	69.99	1.22	2.29	3.19	0.36 ab	0.26	5.47	0.07	23.82 b	1.55	0.17	22.56	0.19 a	4.51	18.79	13.15	12.57	9.44
Sludge	2.63	55.04	1.59	2.13	3.40	0.32 b	0.26	5.21	0.09	57.15 a	1.85	0.19	28.44	0.14 b	6.41	32.35	10.65	8.46	7.85
Amend.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.
QC	2.36 b	62.92	1.41	3.11 a	5.17	0.38 a	0.41 a	5.63	0.08	67.16 a	3.24 a	0.22 a	28.62	0.16	7.90	36.25	14.00	12.96 a	10.06
QG	3.26 a	74.65	1.45	1.64 b	2.33	0.35 b	0.17 b	6.61	0.08	2.26 b	0.11 b	0.18 b	24.31	0.17	4.07	16.45	7.50	9.10 b	7.54
Site effect	**	n.s.	n.s.	***	n.s.	*	**	n.s.	n.s.	***	***	*	n.s.	n.s.	n.s.	*	n.s.	**	n.s.
Prun *	*	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Prun * Site	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Amend *	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Year	**2014**													
	Li	**Be**	**Al**	**V**	**Co**	**Ga**	**Se**	**Rb**	**Sr**	**Y**	**Cs**	**Tl**	**Ce**	**Eu**
MAN	5.34 a	0.37 a	73.47	0.23	3.58	7.19 a	0.67 a	4.05 a	0.53	0.02 a	39.66	2.62 a	0.03	0.02
MEC	4.97 b	0.29 b	73.35	0.34	3.17	6.12 b	0.52 b	3.23 b	0.56	0.01 b	65.08	2.25 b	0.03	0.02
Pruning	*	**	n.s.	n.s.	n.s.	***	*	***	n.s.	**	n.s.	*	n.s.	n.s.
Ctrl	5.57 a	0.35 ab	65.64	0.26	3.25	7.29 a	0.71	3.71	0.56	0.01	34.11	2.42	0.03	0.02
Bioc	5.37 ab	0.38 a	69.92	0.24	3.49	7.01 ab	0.56	3.63	0.56	0.01	71.81	2.45	0.02	0.02
MSWC	4.83 b	0.25 b	99.39	0.23	3.39	6.94 ab	0.51	3.73	0.52	0.01	71.64	2.24	0.02	0.02
Manure	5.10 ab	0.28 ab	67.84	0.32	3.39	6.46 b	0.64	3.53	0.57	0.02	26.67	2.36	0.03	0.02
Sludge	4.92 ab	0.38 a	64.26	0.36	3.35	5.57 c	0.54	3.60	0.52	0.01	57.61	2.70	0.03	0.02
Amend.	*	*	n.s.	n.s.	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
QC	3.08 b	0.06 b	65.49 b	0.31	2.47 b	5.79 b	0.35 b	5.46 a	0.54	0.02	83.87	4.65 a	0.03	0.02 a
QG	7.23 a	0.60 a	81.32 a	0.25	4.28 a	7.51 a	0.83 a	1.82 b	0.54	0.01	20.87	0.23 b	0.02	0.01 b
Site effect	***	***	*	n.s.	***	***	***	***	n.s.	n.s.	n.s.	***	n.s.	***
Prun *	***	**	n.s.	n.s.	n.s.	**	*	**	n.s.	n.s.	n.s.	*	n.s.	n.s.
Prun * Site	*	*	n.s.	n.s.	n.s.	**	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Amend *	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Statistical significance of the effects of pruning system, organic amendment, experimental site and their interactions: n.s. not significant 5% level by F test; *, **, *** significant at $p<0.05$, $p<0.01$ and $p<0.001$, respectively. Within each column and for each factor, mean values followed by a different letter are significantly different at $p<0.05$ by Tukey's test. Pruning system: hand pruning (MAN) and mechanical pruning (MEC). Organic amendments: control (Ctrl), biochar (Bioc), municipal solid waste compost (MSWC), cattle manure (Manure) and sewage sludge compost (Sludge). Site: Quinta do Côro (QC), Quinta do Gradil (QG).

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