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**Research Article**

# Mechanical Pruning and Soil Organic Amending in Two Terroirs. Effects on Wine Chemical Composition and Sensory Profile

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**Abstract:** The knowledge about the interaction between mechanical pruning and soil organic amending is still scarce. This study aimed to examine the effects of the interaction between these two practices on wine quality. Syrah grapes from two trial fields in Portugal subjected to two different pruning systems (mechanical pruning – MEC; hand spur pruning – MAN) and five different organic amendments treatments (control – Ctrl; biochar - Bioc; municipal solid waste compost - MSWC; cattle manure - Manure; sewage sludge - Sludge) were harvested and vinified

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for four years. Mechanical pruning significantly reduced wine alcoholic strength, pH and total anthocyanins. Mechanical pruning and organic amendments, tendentially reduced wine total phenols and tannin power, known as an estimation of the astringency potential of the wines. Tasters found low but significant differences in global appreciation with pruning system. Sludge tended to reduce wines global appreciation more than MSWC and Manure, while Bioc had no effect on tasters' preference, when compared to Ctrl. There was strong relation between yield and tasters' preference only above 6 and 8 kg/vine depending on the terroir. Mechanical pruning tendentially has significant effects on wine quality when yield raises above a certain level. Thus, with this pruning system, the choice of the organic amendment and its amount, must be done considering the destiny of the produced grapes. Impacts of the interaction of mechanical pruning with soil organic amending on wine quality are a novelty, to the best of our knowledge.

**Key words:** fertilization, pruning, sensory discrimination, Syrah, wine composition

## Introduction

The increase in yield generally originated by mechanized pruning systems (Sims et al. 1990, Keller and Mills 2007) is not normally connected with the loss of grape and wine quality (Sims et al. 1990), except when the canopy efficiency does not compensate the rise of production (Poni et al. 2004). In a wine market that requires higher efficiency and competitiveness, a pruning system that produces grapes with overall unaffected yield and composition is a reliable tool for improving vine growers economic performance.

The application of organic amendments affects the chemical properties of soils, increasing the availability of nutrients (Fangueiro et al. 2012, Illera-Vives et al. 2015), changing the nutrient status of the vine and affecting wine composition (Morlat and Symoneaux 2008).

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The effects of nitrogen (N), that can be supplied by the mineralization of organic matter, have already been extensively studied. In terms of grape and wine composition, high levels of N delay grape maturation (Hilbert et al. 2003, Morlat and Symoneaux 2008), that can be related to higher yields (Spayd et al. 1994) and/or to the increase in plant vigor, that affects carbon partitioning favoring vegetative growth in detriment of reproductive growth (Delgado et al. 2004, Bell and Henschke 2005). Excessive N supply tend to decrease total soluble solids, increase or maintain pH, maintain titratable acidity and decrease polyphenols content (Spayd et al. 1994, Delgado et al. 2004, Morlat and Symoneaux 2008) even in low vigor vineyards (Gatti et al. 2020).

Organic amendments also supply phosphorus (P), particularly sewage sludge (Sludge), which effects on grapevine are not widely studied, since the grapevine requires only small quantities of this nutrient. The P application to soil is regulated in Portugal as well as in many other areas. Conradie and Saayman (1989) found no differences in grape composition with P fertilization. However, Kakegawa et al. (1995), observed that, when in excess, P may inhibit the induction of phenylalanine ammonia-lyase and chalcone synthase activity leading to a reduction of anthocyanin content of berries.

Potassium (K) is another of the macronutrients that is usually supplied by organic amendments. Mpelasoka et al. (2003) refer that, although a relationship between total soluble solids (TSS) and berry K content can be established, it is not clear that high levels of K in berries have a positive correlation with sugar accumulation. Most of the already performed works refers no effects of K supply on grape sugar content (Conradie and Saayman 1989, Delgado et al. 2004). High levels of K in berries increase must and wine pH (Conradie and Saayman 1989, Mpelasoka et al. 2003). However, the influence in total acidity is not consensual since some authors found a

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reduction of this variable (Mpelasoka et al. 2003, Delgado et al. 2004) while others observed an increase (Conradie and Saayman 1989) or even no influence (Freeman and Kliewer 1983).

Organic amendments also have a role on micronutrients availability in soil. With a long-term application of biosolids, Richards et al. (2011) observed a significant increase of soil extractable Cu, Fe, Mo and Zn while with the application of beef manure an increase in the levels of B, Cu, Fe, Mn, Mo, and Zn was observed. Zhang et al. (2015) refer that manure plus inorganic fertilizers application to soil significantly augmented soil-available Fe, Mn, Cu and Zn contents relative to controls.

Not many studies have been performed regarding the effects on grape and wine quality of the application of municipal solid waste compost (MSWC) and Sludge. However, Messiga et al. (2016) observed no differences in grape sugar content and phenolics with the application of 13.4 Mg/ha of municipal solid food waste. Pinamonti (1998) observed no differences in grape quality of Merlot, with the application of MSWC and Sludge.

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 and stimulate soil microbial activity (Sánchez-Monedero et al. 2019), in general leading to low but significant increases in crop productivity (approximately 10%) across different crops, soils, biochar types and application rates (Jeffery et al., 2011). The effects on grape and wine quality, of biochar application in vineyard soil, has not been yet fairly studied. However, the existing works point to a lack of effects on grape and wine quality parameters (Sánchez-Monedero et al. 2019).

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According to the reviewed literature, mechanical pruning seems to be an appropriate strategy to face the increasingly scarcity of skilled hand-labour, to decrease production costs and increase productivity while organic amendments increase productivity, tackle the problems associated with predicted climatic changes and, when obtained from human residues, are a tool to implement circular economy. However, the interaction between mechanical pruning and soil organic amending have significant effects in vegetative and reproductive growth (Botelho et al., 2020) and in grape composition (Botelho et al., 2021). Consequently, it is likely that the interaction between these two practices can affect wine quality and the present work aims to evaluate it.

## Materials and Methods

**Site description, experimental design, and yield assessment.** The trial, run 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, in Portugal. The vineyards and the cultural practices adopted are described in Botelho et al. (2020).

The soil in QC was a Hypereutric Regosol (USS Working Group WRB, 2015), with a sandy-loam texture, a pHH2O of 6.4, a low organic matter content (1.54%), an extractable K and P contents of 70.7 mg K/kg and 59.8 mg P/kg (ammonium lactate extraction – Egnér et al., 1960), respectively. In QG soil was also a Hypereutric Regosol (USS Working Group WRB, 2015), with a sandy-loam textures, a pHH2O of 5.9, a low organic matter content (1.07%), an extractable K and P contents of 167.0 mg K/kg and 61.2 mg P/kg (ammonium lactate extraction – Egnér et al. 1960), respectively. The climate in QC is a Csa and in QG is a Csb, according to the Köppen-Geiger climate classification (IPMA, 2020). Monthly total rainfall and mean air temperature data, during the course of the study, are presented in Botelho et al. (2020).

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The studied factors were pruning system and organic amendments that were compared in a strip-plot design, with three blocks (Figure 1). 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 in five parts of twelve meters each, where organic amendments were randomly distributed. Each one of the 30 plots consisted of 48 vines.

In what concerns to pruning, two treatments were imposed during all the experiment: 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 and 2 perpendicular to the ground) working at a distance of 15 cm from the cordon.

In relation to organic amendments, five treatments were imposed all the years of the experiment: 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/year 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 presented in Botelho et al. (2020).

To estimate yield, six vines per experimental unit were harvested and the production weight per plant was assessed.

**Winemaking.** In both trial fields grapes from the three replicates per treatment were pooled respectively for wine making. Sixteen kilograms of grapes were harvested per plot and pooled,

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thus 48 kg of grape were used for each treatment. Twenty vinifications were performed each year, to obtain ten wines from each experimental site.

Before the harvest, the grapes from the vineyards involved in this project were monitored, in order to access their quality and maturation stage. The parameters controlled in this phase were weight of one hundred berries (g), °Brix, potential alcohol content (%), pH and total acidity (g tartaric acid/L). Total anthocyanins and total phenols in grapes were assessed also at harvest. The results of grape analysis before fermentation are presented in Botelho et al. (2021).

When the grapes were at the ideal stage of maturation the manual harvest was performed, in the same day for all treatments, and the grapes were transported to the experimental winery of Instituto Superior de Agronomia (Lisbon), where the vinification took place. In the same day of harvest, grapes were de-stemmed, crushed and sulphur dioxide was added (50 mg/L-1). The crushed grapes were placed into 60 L stainless-steel tanks and inoculated with the yeast Zymasil® Bayanus (AEB®). After these operations, a sample of must from each vineyard and treatment was taken to analyze potential alcoholic content, pH and total acidity.

The alcoholic fermentation lasted between 7 and 9 days at the average temperature of 24 °C and the maceration time was extended to 15 days in all treatments. During this period the cap was punched down three times a day. After the maceration the skins were separated from the juice using a vertical press and pressed juice added to the free-run juice. When alcoholic fermentation ended, wines were analysed to determine all classical parameters like alcoholic content, pH, total acidity (TA), volatile acidity and free and total $SO_2$.

The malolactic fermentation developed after the alcoholic fermentation, spontaneously, and its progression was followed using paper chromatography (Ribéreau-Gayon et al. 1982) to

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monitor the presence of malic and lactic acids in the wines. In February, this process was ended for all the wines. In order to remove the lees that settled, the wines were racked and then a new analysis took place to control total and free sulphur dioxide, volatile acidity and pH. Free SO2 content was then adjusted to 30 mg/L and the wines were stored in 750 mL bottles.

After the bottling process, the wine's chromatic characteristics, phenolic composition and sensory analysis were performed.

**Classical chemical parameters.** The wine analysis was performed in the Enology Laboratory of Instituto Superior de Agronomia. Alcoholic content (distillation and densimetry), total acidity (titration with sodium hydroxide with bromothymol blue as indicator), fixed acidity (FA), volatile acidity (steam distillation followed by acid-base titrimetry), total and free sulphur dioxide (by titration with iodine) and reducing substances (clarification with neutral lead acetate, reaction with alkaline copper salt solution and iodometry) in wines were analyzed according to OIV described methods (OIV 2021).

**Color parameters and phenolic compounds evaluation**. Color intensity (sum of absorbencies at 420nm, 520nm and 620nm wavelengths) and hue (ratio of absorbencies at 420nm and 520nm wavelengths) were analyzed according to OIV described methods (OIV 2021).

Total and ionized anthocyanins, total and polymeric pigments, total phenols and tannin power were analyzed according to the following procedures:

. total and ionized anthocyanins (mg/L) and total and polymeric pigments (a.u.) determinations were performed by spectrophotometry according to the methodology developed by Somers and Evans (1977), using sodium metabisulphite solution (20%), in the first part, and HCl (1M), in the second part;

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. total phenols index (a.u.) were analyzed according to the methodology proposed by Somers and Evans (1977), that consists in the measurement of the absorbance at wavelength 280 nm (A280) of the diluted wine sample;

. tannin power (NTU/mL), which is actually a way to estimate the potential astringency of a wine, was determined by the method developed by De Freitas and Mateus (2001), which measures the turbidity caused by the aggregates of tannins and proteins by nephelometry (nephelometer Hach 2100N), after adding BSA (bovine serum albumin) to cause the precipitation.

For the quantification of K inductively coupled plasma optical emission spectrometry (model iCAP 7000 Series - Thermo Fisher Scientific) was used. The samples were previously diluted 1:10 as described by Zioła-Frankowska and Frankowski (2017).

**Descriptive sensory analysis**. Each wine sample was stored for 24 hours at room temperature before sensory analysis, which was performed at 20-22 °C in a sensory analysis room with individual booths for each expert, fluorescent light and tables with white surfaces (ISO 8589:2007). All evaluations were conducted in the morning from 10:00 to 12:00 h. Twelve expert judges with wine tasting experience, most of them winemakers, evaluated the wine samples during a single sensory evaluation session 9 months after the harvest of each year. In each session, wines from the two sites were divided in two flights that were tasted with a 20 min interval. Each wine was served in tasting glasses (ISO 3591:1977) coded with a random three-digit code and filled with 25mL of wine at a temperature of 18º ± 2ºC. Wines were presented to the tasters in a randomized order. Water and crackers were used as palate cleaners.

All expert judges were previously selected and trained during 6 months. During this training period several sessions were carried out in order to get a judges training about the meaning

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of each attribute and achieving intensity rating in a reliable way. The procedures for monitoring the performance of the panel are described in ISO 11132:2012 and the practices explained in general guidelines for the selection, training and monitoring of selected assessors and expert sensory assessor in ISO 8586:2012.

The sensory attributes used were the following: color (“red” and “violet”), aroma (“fruit”, “floral, “vegetal”, “jam”, "intensity" and “balance”), taste (“body”, “bitterness”, “astringency”, “acidity”, "persistency" and “balance”), and “global appreciation”.

The experts scored each sensory attribute on the following 5-point scales:

. nonexistent (0), not very intense (1), moderately intense (2), intense (3) and very intense (4);

. mediocre (0), satisfactory (1), good (2), very good (3), excellent (4), being this scale used only for “balance”, of aroma and taste, and “global appreciation”.

***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 $\alpha = 0.05$ 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). Regression analysis was used to study relationships between continuous variables and the curves were fitted using the least squares method.

In the tables presented in the Results section the values presented for the pruning system are an average of 10 wines (two sites × five organic amendments), while for the organic

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amendment are an average of 4 (two sites × two pruning systems) and for site are an average of 10 (two pruning systems × five organic amendments).

## Results

The results presented in this paper correspond only to the last three years (harvests) of the research project, since in 2012 no significant effects were observed in grape and wine composition. The main outcomes, concerning grape and wine composition, from the first experimental year (2012) were reported by Correia (2014). The grape composition is presented in Botelho et al. (2021b).

**Classical chemical parameters.** The interaction between the pruning system and organic amendments was not significant in any of the evaluated physical-chemical characteristics.

The alcoholic strength (Table 1 and Supplemental Table 1) shows a significant decrease in MEC when compared to MAN, although in 2015 the differences were significant only in QC site (Supplemental Table 2). Regarding to organic amendments effect, significant differences were observed only in 2015 when Sludge had lower alcoholic strength (11.9 % vol.), MSWC and Manure had an intermediate behavior (13.2 and 13.0 %vol respectively) Ctrl and Bioc presented the highest values (14.0 and 13.8 % vol. respectively).

The pH (Table 1 and Supplemental Table 1) was significantly reduced by MEC every year, but in 2013 the differences were significant only in QG and in 2015 only in QC (Supplemental Table 2).

In what concerns to TA and FA (Table 1 and Supplemental Table 1), there is a significant increase of these variables in MEC treatments, with the exception of FA in 2013. The analysis of the interaction between pruning system and site, which, excepting for TA in 2014, is always

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significant, shows that in 2013 the difference of TA is significant only in QG, in 2014 and 2015 only in QC (Supplemental Table 2).

Volatile acidity (Table 1 and Supplemental Table 1) was affected by pruning only in 2015 and MAN wines presented higher values (15 % more).

Mechanical pruning originated a significant reduction of K content of wines (Table 1 and Supplemental Table 1) in 2013 and 2014, while the organic amendment had a significant effect only in 2013. Concerning the organic amendments, a reduction of wine K content in Sludge was observed. Regarding the differences between sites, there is a significantly higher K content in QG in all the studied years, with differences that are between 13.1 % (2015) and 20.9 % (2014).

In Figure 2 is presented the relation between yield and wines alcoholic strength. Globally, there is a tendency for a decrease in wines alcoholic strength with the increase of yield. There is a difference between QC and QG which is constant and the covariance analysis shows that the lines of the two sites are parallel, with QG wines having less alcohol when compared to QC. The relationship between the two variables is relatively high, especially in QC. pH has a negative relation with yield in both sites (Figure 3), although in QG that relation is more negative than in QC. Figure 4 illustrates the regression of FA on yield and the results show a weak correlation between both variables.

**Color parameters and phenolic compounds.** The imposed treatments had few effects on color intensity and color hue of wines (Table 2 and Supplemental Table 3). However, in 2014 color hue was significantly lower in MEC (11 % less). The significant differences found in color hue in 2014, due to the pruning system, occurred only in QC experimental site (Supplemental Table 4). Concerning organic amendments effect, significant differences were observed in color intensity

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only in 2015 when Sludge had lower color intensity (6.4 a.u.), MSWC, Manure and Bioc had an intermediate behavior and Ctrl presented the highest values of this variable (11.8 a.u.). Color hue was not significantly affected by organic amendments and the interaction between pruning and organic amendments was never significant.

The effect of pruning system on total phenols is significant only in 2014, with lower values in MEC (11 % less). In the other two years there was also a tendency for inferior values in MEC. As far as tannin power is concerned, MEC treatments led to a decrease in this variable in 2014 and 2015 (32 % and 15 % less, respectively), although in 2015 the difference was not significant in QG experimental site (Supplemental Table 4).

The organic amendments affected more the total phenols concentration than the tannin power since, in the first case, the differences were significant in 2014 and 2015 and the results trend was similar every year with higher values in Ctrl and Bioc, followed by MSWC and Manure and with Sludge presenting the lowest total phenols values., In tannin power the results were significant only in 2015 with a reduction of this variable with soil organic amending, especially with Sludge.

The interaction of pruning system with organic amendments was not significant in any of the variables related to phenolic composition.

Total anthocyanins (Table 3 and Supplemental Table 5) were significantly higher in MAN wines in 2014 and 2015, even if in 2015 the differences were significant only in QC (Supplemental Table 4). In what regards to wine pigments, polymerized pigments and polymerization index (Table 3 and Supplemental Table 5), in a global approach, none of these variables were significantly affected by pruning.

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The organic amendments influenced total anthocyanins in 2013 and 2015. Wines from Sludge treatments had the lowest values of total anthocyanins (339 and 225 mg/L) and those from Ctrl had the highest (481 and 402 mg/L) and intermediate levels were observed in Bioc, MSW and Manure.

Total and polymerized pigments were significantly influenced by the different amendments only in 2015. Regarding to polymerization index, significant differences occurred only in 2014, with higher values of this variable in Bioc (10.2 %).

The interaction between pruning system and organic amendments was never significant.

Both total anthocyanins and pigments had a negative relationship with yield (Figure 5 and Supplemental Figure 1). However, the relationship tended to be more negative in QG, when compared to QC.

Total phenols had a negative relation with yield (Supplemental Figure 2). Tannin power also had a negative relation with yield in QC (Figure 6). However, in QG a weak relation was observed, with no differences in tannin power through a noteworthy range of yields.

**Descriptive sensory analysis.** The main sensory attributes of the wines (fruity, floral aromas and aroma balance, body astringency and global appreciation) from the different treatments are presented in Table 4 (other parameters are presented in Supplemental Tables 6 to 11).

Wines from MEC were, tendentially, less red and, in the last year, more violet than those from MAN. Concerning the descriptors used by the tasters to characterize the aroma of wines from different pruning system, there was no differences between pruning systems, except for jam aroma, in 2014 (Supplemental Table 7), that was higher in MAN. On the other hand, there were no

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differences on the aroma intensity, while the aroma balance tended to be lower in MEC. In 2015, no differences were found between tastes of the pruning systems, while in the first two years wines from MEC were less bodied and balanced. In 2013 MEC wines were also less astringent and persistent. Finally, in 2013 and in 2014, wines from MEC had lower global appreciation (8 and 10 % respectively).

When comparing organic amendments, the differences in red color were not substantial, with Manure and Sludge being significantly lower than the other treatments in 2013 and 2014, respectively. However, in what concerns to violet color differences were higher, with Sludge wines being, consistently, the less violet, followed by Manure and MSWC. Excepting 2013, Bioc did not significantly differed from Ctrl, which had the highest values of violet color.

Concerning the aroma, with exception of MSWC wines, in 2015, that were less balanced than those from Ctrl, the only treatment that differed significantly from Ctrl was Sludge. When comparing to Ctrl, in 2013 Sludge wines were less intense and with lower levels of jam aroma, while in 2014 were less balanced. In 2015, wines from Sludge were less intense and balanced, with lower levels of fruit, floral and jam aromas.

The wines from Sludge were the less bodied of all organic amendment treatments, differing significantly from Ctrl in all the evaluated vintages. The effect of this factor on bitterness and astringency of wines was not always significant, but wines from Sludge showed to be the less astringent wines followed by MSWC and Manure. Tasters, found no significant differences in the acidity of wines from different organic amendments, while persistency and balance were always affected. In the two first years, Sludge was the less persistent and balanced wines and the only that differed significantly from Ctrl, while in 2015 MSWC had a similar behavior. The interaction

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between pruning system and organic amendments effects on body (Supplemental Table 12) shows that there were no significant differences between MAN/Bioc, MAN/Ctrl, MEC/Bioc and MEC/Ctrl treatments and that wines from MEC/Sludge were the less bodied.

Tasters, consistently, classified Sludge wines with the lowest global appreciation score (with an average of 2.68). MSWC and Manure had intermediate scores (with averages of 3.01 and 2.97 respectively) and did not significantly differ from each other in any of the analyzed years. Ctrl and Bioc had the highest scores in global appreciation and a similar performance (with averages of 3.30 and 3.17 respectively). The interaction between pruning system and organic amendments effects on global appreciation (Supplemental Table 12) shows that there were no significant differences between MAN/Bioc, MAN/Ctrl, MEC/Bioc and MEC/Ctrl treatments and that wines from MAN/Sludge and MEC/Sludge had the lowest global appreciation.

The relation between yield and global appreciation of wines is presented on Figure 7. Until 6 kg/vine, in QG, and 8 kg/vine, in QC, the relationship between these variables is weak and there is no decrease in quality, with the increase in yield. When yield exceeds the referred thresholds, there is a tendency for lower wines global appreciation.

## Discussion

**Classical chemical parameters.** Some physical-chemical characteristics of wines (Table 1) were significantly affected by the two factors in study. In an overall point of view, the alcoholic strength of wine was negatively affected by MEC in all the three years, as observed by Reynolds (1988) and Pérez-Bermúdez et al. (2015). However, as shown in Figure 2, there is a high association between the TSS decrease and the yield increase that MEC induces, particularly with the organic amendments that provide more principal macronutrients and increase the total dry mass

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production, namely Manure, MSWC and Sludge (Botelho et al., 2020). When the yields are similar there are no considerable TSS differences between MEC and MAN treatments (Botelho et al. 2021).

According to Clingeleffer (1988), Spayd et al. (1994) and Wessner and Kurtural (2013), higher yields and the associated lower leaf area to fruit ratios delay ripening. In the case of QC, some delay of the harvest is not problematic, because September is, usually, a dry month in Tejo wine region (IPMA 2020). However, in QG that can be a problem since the harvest will be postponed to October when rainfall usually occurs in Lisboa wine region (IPMA 2020) and Botrytis cinerea infections are highly probable (Elmer and Michailides 2004). When comparing the relationship between wine alcohol content and yield in both sites, it is noteworthy that the regression lines have similar slopes, but for the same level of production, QG wines had lower alcohol content. The higher alcoholic content in QC is associated with the higher average temperatures observed in this site during all the three years (Botelho et al. 2020) These results are in accordance to those referred by other authors (Jackson and Lombard 1993).

In addition to the reduction of alcoholic strength caused by the increase of productivity, there is a significant tendency for wines of Sludge treatment to have lower alcohol content, respectively, when compared to the other organic amendments, even with similar yields. Hilbert et al. (2003) and Delgado et al. (2004) also observed a delay in ripening due to high nitrogen supply, even without an increase in productivity neither a decrease in leaf to fruit ratio. Delgado et al. (2004) and Korboulewsky et al. (2004), attribute the decrease in wine alcohol content to the increase of vine vigor, caused by high N supply, which changes the balance in carbon partitioning, favoring vegetative growth in detriment of reproductive growth. In this study, vine vigor (Botelho

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et al., 2020) tended to be higher in Sludge, when comparing to Ctrl and Bioc, and is likely to be the cause of the inferior alcoholic strength value observed in the corresponding wines.

The wine pH showed a clear tendency for decrease with MEC, what was also observed by Morris and Cawthon (1981) and Holt et al. (2008). The reduction of pH in MEC is, probably related to the referred delay in ripening and/or to a lower K content, which was associated to a growth induced dilution. A negative relationship between yield and wine pH was observed, with QG having a stronger decline when compared to QC. According to Jackson and Lombard (1993) there is a negative relation between pH and crop load, which was always higher in MEC and increased with yield growth (Botelho et al. 2020). Surprisingly, the wine pH was lower in QC, when compared to QG, what was not expected since the average temperatures in this site were higher and a higher malic acid degradation would be expected (Keller 2010). However, the wine K content was significantly higher in QG (Table 1), what led to a higher precipitation of tartaric acid as potassium hydrogen tartrate (Ribéreau-Gayon et al. 2000) and, consequently, to a higher pH (Conradie and Sayman 1989).

Usually, grape total acidity is not affected by mechanical pruning (Clingeleffer 1988, Holt et al. 2008), as occurred in the present work (Botelho et al. 2021). However, although TA of must was not significantly different due to pruning system, in wine, total and fixed acidity were both lower in MAN. These differences are, probably, related with the differences found in K concentration between treatments. As already referred, the lower concentration of K in MEC wines led to less precipitation of tartaric acid as potassium hydrogen tartrate (Ribéreau-Gayon et al. 2000), and, consequently, higher concentrations of this acid remained in the wines from these treatments.

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Although slight significant differences were observed in 2014 in must pH, among different organic amendments, they are not relevant in a practical point of view. Thus, globally, organic amendments affected neither pH nor TA of must and wine. Identical results were obtained by Morlat and Symoneaux (2008).

Wine fixed acidity had a weak correlation with yield in both sites, as observed by other authors (González-Flor et al. 2014). The present results show that fixed acidity is more related to the site, with QG having less acidity than QC due to the K content, than to yield.

**Color parameters and phenolic compounds.** In what concerns to color (Table 3), globally, the pruning system had low influence on color intensity and color hue of wines, as observed by Keller and Mills (2007). Although pruning did not affect wines color intensity, the total anthocyanins content (Table 3) was significantly lower in MEC, contrarily to what was reported by Holt et al. (2008) and Wessner and Kurtural (2013), but in accordance to what Poni et al. (2004) and Main and Morris (2008) observed with minimal pruning. The lower anthocyanins content, in MEC, may be related to the delay in sugar accumulation, essential in the regulation of color development (Castellarin et al., 2011), which, in this case, overlapped the effect of the higher skin-to-flesh ratio of MEC berries (Botelho et al., 2020). However, since pH in MEC wines was lower than in MAN, the anthocyanins ionization index was higher and more anthocyanin molecules were in the red colored form of flavylium cation (Somers and Evans 1977, Ribéreau-Gayon et al. 2000), compensating their inferior content and maintaining wines color intensity.

Total pigments were significantly lower in MEC in 2014 and 2015, which is in accordance to the difference observed in total anthocyanins content and is probably a consequence of the yield increase promoted by MEC that reduced the leaf area to production ratio (Botelho et al. 2020).

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Striegler and Lake (2002) also found a significant decrease in total pigments with machine pruning, while Main and Morris (2008) found no differences. Since in 2014 the polymerization index was not different between pruning treatments, MAN had more polymerized pigments than MEC. In 2015, although total pigments were higher in MAN, the polymerization index was higher in MEC and there were no significant differences in polymerized pigments content. So, although there is a tendency for lower total pigments content in MEC, in some years, when the polymerization index is higher, in this pruning system, the result is an identical level of polymerized pigments, which, comparing to anthocyanins, are much less sensitive to changes in pH and are quite resistant to decolorization by sulphur dioxide (Somers, 1971).

In what concerns to the effect of organic amendments on color, though the differences in color intensity, between treatments, are significant only in 2015, there is always a tendency for higher color intensity in Ctrl and lower in MSWC, Manure and Sludge. This trend is corroborated by the reduction observed in total anthocyanins content, as well as in total pigments. The higher yield observed in these treatments and the consequent delay in ripening, may be related with the decrease in anthocyanins content and color intensity, as already referred. However, according to Hilbert et al. (2003), a high nitrogen supply interferes with the metabolic pathway of anthocyanins, delaying quantitative and qualitative biosynthesis, and enhances their degradation in the final steps of berry maturation. So, both these two facts may concur to the lower color intensity anthocyanin content and total pigments in MSWC, Manure and, specially, in Sludge. In Sludge treatment, the high phosphorus supply can also be in the origin of the lower total anthocyanins content, since, when in excess, P may inhibit the induction of phenylalanine ammonia-lyase and chalcone synthase activity leading to a reduction of anthocyanin content of berries (Kakegawa et al. 1995).

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A negative relationship between wine total anthocyanins and yield was observed (Figure 5). A similar trend was observed for total pigments (Supplemental Figure 1). This decrease in total anthocyanins is probably related with grape sugar content, that also decreased with yield. The relation between sugar and anthocyanin accumulation was demonstrated by Pirie and Mullins (1976) and Yokotsuka et al. (1999) also found a positive correlation between grape sugar content and total anthocyanins and pigments.

Wine total phenols content (Table 4) was slightly reduced by MEC only in one of the four years in study (2014), while in the other years no significant differences were observed between pruning systems. Pérez-Bermúdez et al. (2015) also found a reduction in total phenols content, in mechanical pruning, only in one of three years of trial, while Wessner and Kurtural (2013) found no differences between pruning systems and Holt et al. (2008) observed higher total phenols levels in machine pruned treatments.

The organic amendments significantly affected total phenols in 2014 and in 2015. In both years Ctrl and Bioc were the treatments with the highest value of this variable and Sludge was the one with the lowest, while MSWC and Manure presented intermediate values. Delgado et al. (2004) report a reduction in total phenols content with the application of nitrogen to soil, so the observed results are probably related to the nitrogen supplied by Sludge, MSWC and Manure. Delgado et al. (2004) also refer that total phenols content decrease with the application of potassium. MSWC and Manure provide less nitrogen and more potassium than Sludge and, perhaps because of this, total phenols content is higher than in Sludge wines.

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Total phenols were negatively correlated with yield (Supplemental Figure 2). This reduction in total phenols may be related to a growth induced dilution phenomenon, due to the yield increase, and/or to a decrease in grape sugar content, as observed by Yokotsuka et al. (1999).

Tannin power, which is defined as the tannin specific activity of the wine (De Freitas and Mateus, 2001), is used to characterize the reactivity of polyphenols towards proteins (De Freitas and Mateus, 2001) and has a positive correlation with the wine astringency (Mateus at al., 2004). The observed lower tannin power in MEC wines is indicative of a lower astringency perception in our mouth and is coherent with the results obtained concerning the wine total phenols. The same trend is observed in what concerns to organic amendments, since significant differences in tannin power were observed only in 2015, which is also the year when wine total phenols had more differences among organic amendments.

Concerning the relation between tannin power and yield (Figure 6), a negative correlation was found in QC in line with the total phenols behavior. However, in QG tannin power had a weak correlation with yield, although total phenols were negatively correlated with yield. For some reason, that deserves further studies, the tannin power levels are more resistant to yield fluctuations.

**Descriptive sensory analysis.** The sensory analysis (Table 4) shows that the pruning system tends to induce significant differences in less wine quality parameters than organic amendments.

MEC wines color (Supplemental Tables 3, 4 and 5) have a tendency for being less red and, in some years, more violet, corroborating the results of color hue and showing a younger color. Globally, no differences were found in the descriptors used to characterize the wine aroma neither

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in the aroma intensity (Supplemental Tables 3, 4 and 5), between pruning systems. However, the aroma balance was tendentially reduced by mechanical pruning. Sims et al. (1990) observed lower aroma intensity in wines obtained from mechanically pruned Muscadine grapes, while Reynolds (1988) found no differences in wine aroma with mechanical box pruning.

Wine taste was also influenced by pruning system, with MEC tendentially reducing body and balance (Supplemental Tables 3, 4 and 5) and, in a lesser extent, astringency and persistence (Supplemental Tables 3, 4 and 5). Morris and Cawthon (1981) found a decrease in the score given by tasters to the taste of wines from mechanically pruned vines, even with lower yields, when comparing to the spur pruned ones. On the other hand, Reynolds (1988) found no differences in taste between pruning systems with significantly higher yields in mechanical pruning. It is interesting to note that the astringency results, given by tasters, are in accordance to those obtained for tannin power (Table 4), this one being a chemical approach to what can be the astringency perception of a wine by a taster.

In what concerns to global appreciation, tasters found significant differences between pruning systems, with MEC wines presenting lower values in two of the three years. However, even when significant, the differences were low (0.25 in 2013 and 0.32 in 2014) and when comparing the pruning system in the same organic amendment, year and trial field, several times the quality of MEC wines was superior, especially in Ctrl where 67% of the times MEC wines had higher global appreciation than MAN (data not shown). Holt et al. (2008) also found small but significant differences in the quality score of Cabernet Sauvignon wines in two of three years of comparison between hand and mechanical pruning (an average difference of 0.30 points in a 20-

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point scale). Morris and Cawthon (1981) found larger and more significant differences in the overall quality of wines from mechanical and hand pruning.

In a global perspective, the organic amendments decreased wine color, especially Sludge. In terms of aroma, Sludge was the only treatment that presented significant differences when compared to Ctrl. Wines from Sludge treatment had less intense and balanced aroma with lower levels of fruit, flowers and jam. In palate, wines form Sludge also obtained the lowest scores with less body, astringency, persistence and balance. The high levels of nitrogen supplied by Sludge may be in the origin of this result, as referred by Treeby et al. (2000) who, working with Syrah, observed a decrease in wine color, palate intensity and final wine score with the application of nitrogen to the vineyard soil. Korboulewsky et al. (2004) also report that wines from high rates of sewage sludge compost have low olfactory quality and less overall wine quality. A likely explanation for these observations is that nitrogen application in the vineyard increases the assimilable amino nitrogen concentration of musts, which shortens fermentation and may reduce the wine contact with skins (Bell and Henscke, 2005). However, in the present study, though the juice nitrogen content was higher in Sludge, the maceration time and temperature were equal between all the treatments and the same for all years and locals. Thus, the reduced quality in Sludge wines is probably related to the several effects that nitrogen triggers in the vine that result in grapes and wines with different sensory profiles.

In what is related to Bioc, MSWC and Manure effects on sensory attributes of wines, these treatments tend to have an intermediate behavior between Ctrl and Sludge and, globally, do not differ significantly from Ctrl. However, in what concerns to global appreciation, MSWC is significantly lower from Ctrl in one year, while Manure is in two of the three years. In a global

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analysis, MSWC is not significantly different from Ctrl, while Manure is. Although the yields of these two treatments are similar, Manure has more available nitrogen than MSWC and, as discussed previously, this may be the reason for the differences observed.

As it has already been referred, yield had a negative relation with grapes TSS. However, the relation between yield and global appreciation is weak, especially when productivity is below 6 kg/vine in QG and 8 kg/vine in QC (Figure 7). Above these thresholds, a tendency for lower quality levels was observed, but below them there was no relation between yield and global appreciation and MAN and MEC wines had similar global appreciation.

It is also evident that the lower global appreciation observed in MEC is associated to the high yields that this pruning system achieve, when it was combined with MSWC, Manure and Sludge. However, even when combined with the referred organic amendments, if yield did not exceed the already referred thresholds, tasters did not penalize these wines (Figure 7).

The relationship between wine global appreciation and yield is more negatively correlated in QG than in QC. The threshold above which the tasters penalized wine quality was lower in QG when compared to QC. To the best of our knowledge, there are no studies comparing the relation between wine quality and yield in different climates. However, the higher radiation and temperature availability in QC probably led to higher photosynthetic and metabolic activities (Jackson and Lombard, 1993) allowing a higher amount of fruit to be correctly ripen.

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## Conclusions

Mechanical pruning associated with soil organic amending significantly reduced wines alcoholic strength. This reduction is related to a delay in grape sugar accumulation, due to an increase in productivity. In warm regions, this fact is not a problem since harvest can be delayed with no problems of bunch rot. However, in cooler areas, it must be considered that the application of organic amendments in high rates may increase productivity, but may also delay harvest to periods when autumn precipitation can trigger Botrytis cinerea Pers. infections.

Mechanical pruning tended to reduce pH and increase total and fixed acidity, while the organic amendments had no effects on these parameters. Mechanical pruning affected wines color components but not color intensity, had few effects on wine total phenols and reduced tannin power (astringency potential). On the other hand, organic amendments induced a significant reduction in color components as well in color intensity, in total phenols and in tannin power.

Municipal solid waste compost had similar effects, when compared to cattle manure. Thus, it seems an interesting alternative to cattle manure and a good destination for these residues from human settlements. Sewage sludge originated wines with inferior quality, but, due to the high productivity that induces, it can be an interesting alternative for the production of cheaper wines, that can be considered entry level wines in the portfolio of a wine company.

Yield had no relationship with fixed acidity, but a negative one with several other assessed variables such as pH, total anthocyanins, total pigments, total phenols and tannin power. Mechanical pruning significantly reduced wine balance, body and global appreciation. However, when yield was below 6 kg/vine in QG (cooler climate) and 8 kg/vine in QC (warmer climate) mechanical pruning had few effects on wine sensory analysis. Above that threshold, which was

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exceeded only in some years and by treatments with mechanical pruning associated to soil organic amending, there was a tendency for the production of wines with lower global appreciation. Thereby, the results of this study allow to conclude that mechanical pruning associated with the organic amending of soil is a powerful tool to regulate vine yield and to produce a range of wines with different quality.

The valorization of human residues is a key challenge in today's economy. This work shows that the use of non-conventional organic amendments is a powerful tool to increase vineyards profitability and a step more towards circular economy.

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**Table 1** - Effect of the pruning system, the organic amendment and the site on the physical-chemical characteristics (classical parameters) of wine.

| Treatment | Alcoholic strength (% vol.) | | | pH | | | Total acidity (g/L)[1] | | | Volatile acidity (g/L)[2] | | | Fixed acidity (g/L)[1] | | | K content (mg/L) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **2013** | **2014** | **2015** | **2013** | **2014** | **2015** | **2013** | **2014** | **2015** | **2013** | **2014** | **2015** | **2013** | **2014** | **2015** | **2013** | **2014** | **2015** |
| MAN | 14.4 a | 13.7 a | 13.8 a | 3.67 a | 3.69 a | 3.51 a | 5.20 b | 6.24 b | 6.17 b | 0.38 | 0.40 | 0.42 a | 4.72 | 5.74 b | 5.64 b | 854.74 a | 877.88 a | 707.23 |
| MEC | 13.3 b | 12.7 b | 12.6 b | 3.53 b | 3.49 b | 3.35 b | 5.51 a | 6.59 a | 6.65 a | 0.36 | 0.36 | 0.36 b | 5.06 | 6.14 a | 6.20 a | 750.36 b | 786.01 b | 652.75 |
| *Pruning effect* | * | * | ** | ** | *** | * | * | *** | * | *n.s.* | *n.s.* | ** | *n.s.* | * | * | *** | ** | *n.s.* |
| Ctrl | 14.6 | 13.8 | 14.0 a | 3.55 | 3.62 | 3.47 | 5.49 | 6.30 | 6.38 | 0.37 | 0.40 | 0.37 | 4.86 | 5.79 | 5.91 | 787.86 ab | 836.90 | 717.11 |
| Bioc | 14.0 | 13.8 | 13.8 a | 3.61 | 3.56 | 3.46 | 5.35 | 6.56 | 6.49 | 0.39 | 0.38 | 0.40 | 4.60 | 6.08 | 5.99 | 837.95 a | 841.96 | 698.76 |
| MSWC | 13.5 | 13.2 | 13.2 ab | 3.62 | 3.60 | 3.45 | 5.35 | 6.45 | 6.19 | 0.36 | 0.40 | 0.41 | 5.07 | 5.95 | 5.68 | 816.65 a | 815.61 | 694.08 |
| Manure | 13.8 | 12.9 | 13.0 ab | 3.68 | 3.60 | 3.42 | 5.10 | 6.38 | 6.41 | 0.40 | 0.35 | 0.39 | 4.90 | 5.94 | 5.93 | 811.29 a | 852.59 | 661.51 |
| Sludge | 13.4 | 12.2 | 11.9 b | 3.54 | 3.57 | 3.36 | 5.50 | 6.38 | 6.56 | 0.34 | 0.37 | 0.39 | 5.02 | 5.92 | 6.08 | 758.98 b | 812.66 | 628.49 |
| *Amendment effect* | *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 | 16.5 a | 13.7 a | 13.9 a | 3.57 b | 3.38 b | 3.33 b | 5.92 a | 6.74 a | 6.51 a | 0.41 | 0.30 b | 0.34 b | 5.40 a | 6.36 a | 6.09 | 740.65 b | 734.79 b | 632.32 b |
| QG | 11.2 b | 12.6 b | 12.4 b | 3.63 a | 3.8 a | 3.53 a | 4.79 b | 6.09 b | 6.30 b | 0.33 | 0.45 a | 0.45 a | 4.38 b | 5.52 b | 5.74 | 864.45 a | 929.10 a | 727.66 a |
| *Site effect* | *** | * | ** | * | *** | * | *** | *** | * | *n.s.* | ** | *** | ** | *** | *n.s.* | *** | *** | * |
| *Interactions* | | | | | | | | | | | | | | | | | | |
| *Prun x 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.* |
| *Prun x Site* | *n.s.* | *n.s.* | ** | * | * | * | * | * | * | *n.s.* | *n.s.* | *n.s.* | *n.s.* | * | ** | ** | *n.s.* | *n.s.* |
| *Amend x 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.* |

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 (Sludge). Site: Quinta do Côro (QC), Quinta do Gradil (QG).

[1] expressed in tartaric acid.

[2] expressed in acetic acid.

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**Table 2** - Effect of the pruning system, the organic amendment and the site on chromatic characteristics and phenolic composition of wine.

| Treatment | Color intensity (a.u.[1]) | | | Color hue | | | Total Phenols (a.u.[1]) | | | Tannin power (NTU[2]/mL) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2013 | 2014 | 2015 | 2013 | 2014 | 2015 | 2013 | 2014 | 2015 | 2013 | 2014 | 2015 |
| MAN | 12.6 | 10.0 | 9.0 | 0.639 | 0.626 a | 0.595 | 53.0 | 50.5 a | 45.0 | 208 | 175 a | 172 a |
| MEC | 11.1 | 10.2 | 9.9 | 0.609 | 0.558 b | 0.582 | 52.3 | 45.0 b | 43.6 | 199 | 120 b | 147 b |
| *Pruning effect* | *n.s.* | *n.s.* | *n.s.* | *n.s.* | ** | *n.s.* | *n.s.* | * | *n.s.* | *n.s.* | ** | ** |
| Ctrl | 15.4 | 11.3 | 11.8 a | 0.578 | 0.588 | 0.590 | 59.7 | 50.9 ab | 52.1 a | 211 | 159 | 205 a |
| Bioc | 13.5 | 12.0 | 10.3 ab | 0.597 | 0.577 | 0.543 | 53.1 | 51.6 a | 47.7 ab | 201 | 139 | 186 ab |
| MSWC | 11.2 | 9.5 | 8.9 ab | 0.634 | 0.593 | 0.611 | 52.8 | 48.0 ab | 43.9 b | 204 | 155 | 155 c |
| Manure | 8.7 | 9.3 | 9.9 ab | 0.673 | 0.606 | 0.601 | 50.3 | 47.6 ab | 44.5 b | 188 | 142 | 156 bc |
| Sludge | 10.2 | 8.5 | 6.4 b | 0.636 | 0.597 | 0.597 | 47.5 | 40.8 b | 33.2 c | 215 | 141 | 96 d |
| *Amend. effect* | *n.s.* | *n.s.* | * | *n.s.* | *n.s.* | *n.s.* | *n.s.* | * | ** | *n.s.* | *n.s.* | *** |
| QC | 17.7 a | 12.9 a | 9.7 | 0.607 | 0.528 b | 0.582 | 66.0 a | 50.1 a | 42.5 b | 264 a | 166 a | 153 b |
| QG | 6.0 b | 7.3 b | 9.3 | 0.640 | 0.657 a | 0.594 | 39.3 b | 45.4 b | 46.1 a | 143 b | 129 b | 166 a |
| *Site effect* | ** | ** | *n.s.* | *n.s.* | *** | *n.s.* | ** | * | * | *** | * | * |
| | | | | | | *Interactions* | | | | | | |
| *Prun x 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.* |
| *Prun x Site* | *n.s.* | *n.s.* | *n.s.* | * | * | *n.s.* | *n.s.* | *n.s.* | ** | *n.s.* | *n.s.* | * |
| *Amend x 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.* |

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 (Sludge). Site: Quinta do Côro (QC), Quinta do Gradil (QG).

[1] a.u. – absorbance units

[2] NTU – nephelometric turbidity units.

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**Table 3** - Effect of the pruning system, the organic amendment and the site on the pigments of wine.

| **Treatment** | **Total anthocyanins (mg/L)[1]** | | | **Ionized anthocyanins (mg/L)[1]** | | | **Ionization index (%)** | | | **Total pigments (a.u.)[2]** | | | **Polymerized pigments (a.u.)[2]** | | | **Polymerization index (%)** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **2013** | **2014** | **2015** | **2013** | **2014** | **2015** | **2013** | **2014** | **2015** | **2013** | **2014** | **2015** | **2013** | **2014** | **2015** | **2013** | **2014** | **2015** |
| MAN | 432 | 538 a | 347 a | 67 | 56 b | 47 | 14.6 | 11.2 b | 13.9 | 27.30 | 31.12 a | 21.66 a | 3.40 | 2.64 a | 2.59 | 11.8 | 8.8 | 12.0 b |
| MEC | 407 | 455 b | 307 b | 60 | 76 a | 59 | 13.5 | 17.7 a | 18.8 | 25.45 | 26.36 b | 19.73 b | 3.04 | 2.17 b | 2.64 | 11.3 | 8.2 | 13.6 a |
| *Pruning effect* | *n.s.* | * | * | *n.s.* | * | *n.s.* | *n.s.* | ** | *n.s.* | *n.s.* | * | * | *n.s.* | * | *n.s.* | *n.s.* | *n.s.* | * |
| Ctrl | 481 a | 551 | 402 a | 98 | 76 | 69 | 20.1 | 14.3 | 17.6 | 30.32 | 31.87 | 25.28 a | 3.77 | 2.59 | 3.11 a | 11.8 | 8.2 ab | 12.4 |
| Bioc | 444 ab | 505 | 358 ab | 83 | 79 | 58 | 18.3 | 16.7 | 16.7 | 27.75 | 29.83 | 22.81 ab | 3.32 | 2.94 | 2.95 a | 11.2 | 10.2 a | 12.9 |
| MSWC | 443 ab | 498 | 317 b | 61 | 59 | 49 | 13.1 | 12.6 | 15.5 | 27.08 | 28.78 | 19.86 b | 2.95 | 2.31 | 2.41 ab | 10.2 | 8.2 b | 12.3 |
| Manure | 391 ab | 503 | 332 ab | 28 | 58 | 54 | 7.2 | 12.7 | 16.2 | 24.68 | 28.94 | 21.19 ab | 3.07 | 2.28 | 2.76 ab | 11.9 | 8.1 b | 13.1 |
| Sludge | 339 b | 422 | 225 c | 47 | 59 | 35 | 11.5 | 16.1 | 15.7 | 22.04 | 24.27 | 14.34 c | 3.07 | 1.90 | 1.85 b | 12.7 | 7.9 b | 13.2 |
| *Amendment effect* | * | *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 | 449 a | 436 b | 307 b | 94 a | 91 a | 51 | 20.1 a | 21.4 a | 17.1 | 30.66 a | 26.71 | 20.13 | 4.93 a | 3.02 a | 2.88 a | 15.9 a | 11.3 a | 14.6 a |
| QG | 390 b | 557 a | 347 a | 32 b | 42 b | 55 | 8.0 b | 7.5 b | 15.6 | 22.08 b | 30.77 | 21.26 | 1.54 b | 1.79 b | 2.35 b | 7.2 b | 5.8 b | 11.0 b |
| *Site effect* | * | ** | * | ** | *** | *n.s.* | ** | *** | *ns* | ** | *n.s.* | *n.s.* | *** | ** | * | *** | *** | ** |
| | | | | | | | | | *Interactions* | | | | | | | | | |
| *Prun x Amend* | *ns* | *ns* | *ns* | *n.s.* | *n.s.* | *n.s.* | *ns* | *ns* | *ns* | *n.s.* | *n.s.* | *n.s.* | *n.s.* | *n.s.* | *n.s.* | *n.s.* | *n.s.* | *n.s.* |
| *Prun x Site* | *ns* | *ns* | ** | *n.s.* | ** | *n.s.* | *ns* | * | *ns* | *n.s.* | *n.s.* | ** | *n.s.* | *n.s.* | *n.s.* | *n.s.* | *n.s.* | *n.s.* |
| *Amend x Site* | *ns* | *ns* | *ns* | *n.s.* | *n.s.* | *n.s.* | *ns* | *ns* | *ns* | *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 (Sludge). Site: Quinta do Côro (QC), Quinta do Gradil (QG).

[1] expressed in Malvidin-3-O-glucoside.

[2] a.u. – absorbance units.

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**Table 4** - Effect of the pruning system, the organic amendment and the site on the wine sensory attributes.

| **Treatment** | **Fruity (Aroma)** | | | **Floral (Aroma)** | | | **Balance (Aroma)** | | | **Body (Taste)** | | | **Astringency (Taste)** | | | **Global appreciation** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **2013** | **2014** | **2015** | **2013** | **2014** | **2015** | **2013** | **2014** | **2015** | **2013** | **2014** | **2015** | **2013** | **2014** | **2015** | **2013** | **2014** | **2015** |
| MAN | 3.17 | 3.28 | 3.09 | 2.00 | 2.19 | 2.23 | 3.17a | 3.23a | 3.00 | 3.26a | 3.44a | 3.14 | 2.97a | 2.74 | 2.88 | 3.14a | 3.23a | 3.05 |
| MEC | 3.04 | 3.14 | 3.14 | 1.92 | 2.04 | 2.08 | 2.96b | 2.98b | 2.95 | 3.05b | 3.03b | 3.03 | 2.78b | 2.63 | 2.65 | 2.89b | 2.91b | 2.94 |
| *Pruning effect* | *n.s.* | *n.s.* | *n.s.* | *n.s.* | *n.s.* | *n.s.* | * | ** | *n.s.* | * | ** | *n.s.* | * | *n.s.* | *n.s.* | *** | ** | *n.s.* |
| Ctrl | 3.21 | 3.26 | 3.38a | 1.95 | 2.18 | 2.29a | 3.17 | 3.35a | 3.28a | 3.45a | 3.51a | 3.41a | 2.98a | 2.80 | 2.91ab | 3.25a | 3.36a | 3.29a |
| Bioc | 3.14 | 3.21 | 3.14ab | 1.94 | 2.11 | 2.22ab | 3.09 | 3.05ab | 3.08ab | 3.13b | 3.35a | 3.30ab | 2.97ab | 2.74 | 3.02a | 3.06abc | 3.13ab | 3.31a |
| MSWC | 3.21 | 3.23 | 3.09ab | 1.98 | 2.03 | 2.30a | 3.13 | 3.14ab | 2.86b | 3.16ab | 3.22ab | 3.02b | 2.93ab | 2.73 | 2.71abc | 3.10ab | 3.09ab | 2.83bc |
| Manure | 3.00 | 3.29 | 3.14ab | 1.94 | 2.11 | 2.10ab | 3.01 | 3.05ab | 2.96ab | 3.10b | 3.20ab | 3.19ab | 2.85ab | 2.65 | 2.66bc | 2.91bc | 2.98b | 3.03ab |
| Sludge | 2.96 | 3.05 | 2.82b | 2.00 | 2.15 | 1.85b | 2.92 | 2.93b | 2.70b | 2.94b | 2.89b | 2.51c | 2.65b | 2.50 | 2.54c | 2.75c | 2.79b | 2.49c |
| *Amendment effect* | *n.s.* | *n.s.* | * | *n.s.* | *n.s.* | * | *n.s.* | * | ** | *** | *** | *** | * | *n.s.* | *** | *** | *** | *** |
| QC | 3.18 | 3.30 | 3.17 | 1.96 | 2.25 | 2.18 | 3.12 | 3.37 | 3.09a | 3.48a | 3.48a | 3.11 | 3.08a | 2.82a | 2.69 | 3.21a | 3.17 | 3.04 |
| QG | 3.03 | 3.12 | 3.06 | 1.97 | 1.99 | 2.13 | 3.01 | 3.15 | 2.87b | 2.83b | 2.99b | 3.06 | 2.68b | 2.55b | 2.85 | 2.82b | 2.97 | 2.94 |
| *Site effect* | *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 x 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.* | ** |
| *Prun x 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.* | * |
| *Amend x 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.* |

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 (Sludge). Site: Quinta do Côro (QC), Quinta do Gradil (QG).

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| | | | | | |
|---|---|---|---|---|---|
| **MEC** | Ctrl | MSWC | Bioc | Manure | Sludge |
| **MAN** | Ctrl | MSWC | Bioc | Manure | Sludge |
| **MAN** | Bioc | Sludge | Ctrl | MSWC | Manure |
| **MEC** | Bioc | Sludge | Ctrl | MSWC | Manure |
| **MEC** | Sludge | MSWC | Manure | Ctrl | Bioc |
| **MAN** | Sludge | MSWC | Manure | Ctrl | Bioc |

**Figure 1** – Experimental design of the trials installed in each of the two sites (Quinta do Côro and Quinta do Gradil). 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 (Sludge).

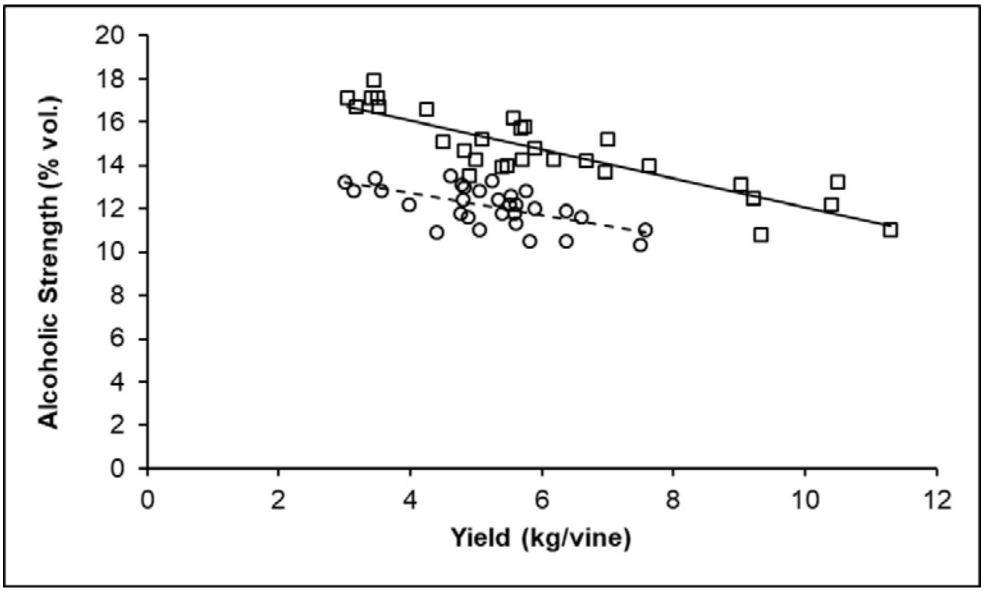


**Figure 2** – Influence of the site on the relation between yield and alcoholic strength of Syrah wines. Data represent single treatment (pruning system × organic amendment × site) averages and data were pooled over repetitions: □ - Quinta do Côro (QC); ○ - Quinta do Gradil (QG). Regression equations:
$y = -0.67x + 18.79$, $r^2 = 0.76$, p-value $< 0.0001$ (QC);
$y = -0.51x + 14.75$, $r^2 = 0.38$, p-value $= 0.0003$ (QG).

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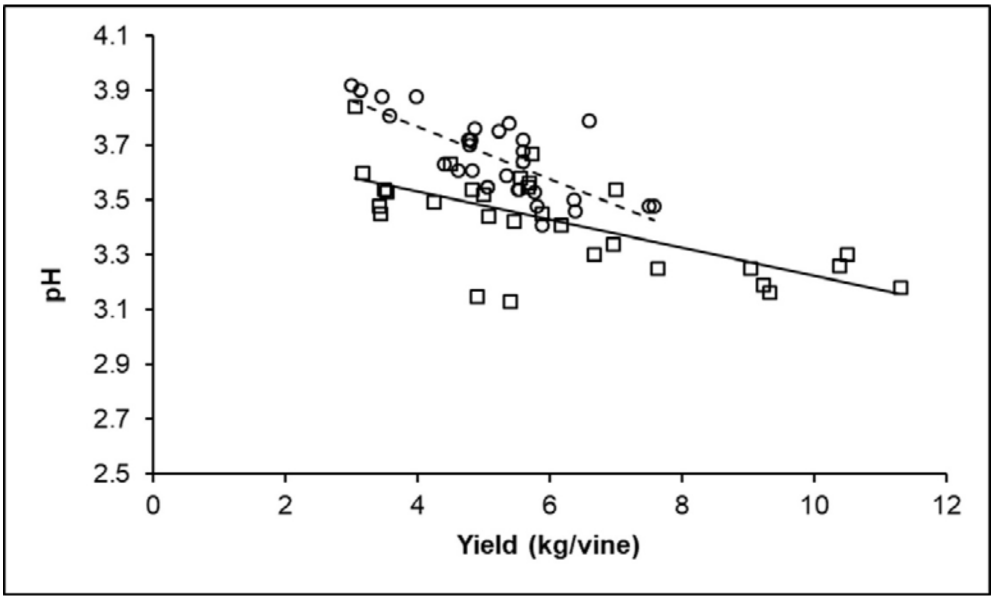


**Figure 3** – Influence of the site on the relation between yield and pH of Syrah wines. Data represent single treatment (pruning system × organic amendment × site) averages and data were pooled over repetitions: □ - Quinta do Côro (QC); ○ - Quinta do Gradil (QG). Regression equations:
$y = -0.051x + 3.73$, $r^2 = 0.45$, p-value = 0.0001 (QC);
$y = -0.097x + 4.16$, $r^2 = 0.54$, p-value < 0.0001 (QG).

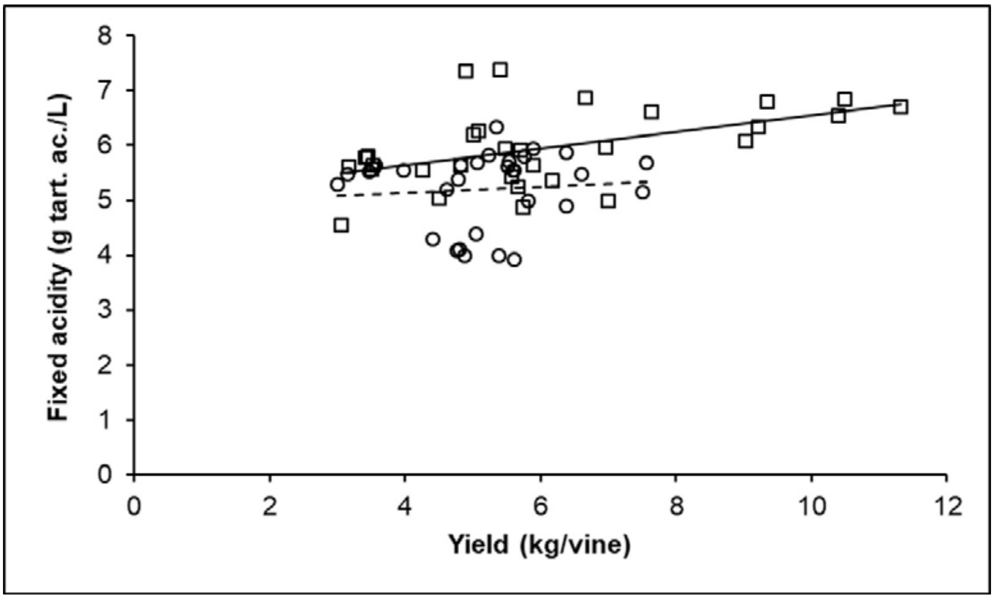


**Figure 4** – Influence of the site on the relation between yield and fixed acidity of Syrah wines. Data represent single treatment (pruning system × organic amendment × site) averages and data were pooled over repetitions: □ - Quinta do Côro (QC); ○ - Quinta do Gradil (QG). Regression equations:
$y = -0.152x + 3.73$, $r^2 = 0.241$, p-value = 0.0059 (QC);
$y = -0.056x + 4.92$, $r^2 = 0.008$, p-value = 0.6342 (QG).

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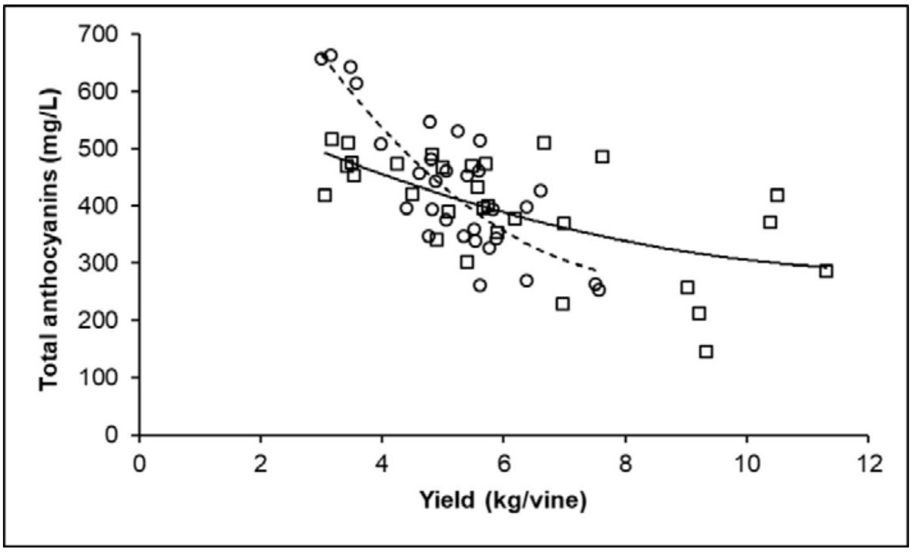


**Figure 5** – Influence of the site on the relation between yield and total anthocyanins of Syrah wines. Data represent single treatment (pruning system × organic amendment × site) averages and data were pooled over repetitions: □ - Quinta do Côro (QC); ○ - Quinta do Gradil (QG). Regression equations:
$y = 2.09x^2 - 54.24x + 638.9$, $r^2 = 0.39$, p-value = 0.0004 (QC);
$y = 12.75x^2 – 217.32x + 1201.6$, $r^2 = 0.68$, p-value < 0.0001 (QG).

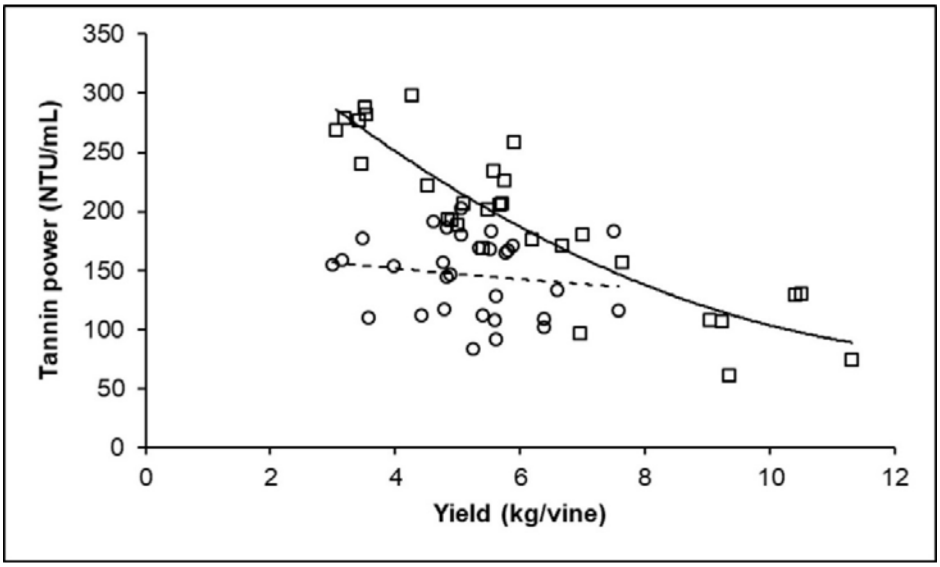


**Figure 6** – Influence of the site on the relation between yield and tannin power of Syrah wines. Data represent single treatment (pruning system × organic amendment × site) averages and data were pooled over repetitions: □ - Quinta do Côro (QC); ○ - Quinta do Gradil (QG). Regression equations:
$y = 1.86x^2 – 50.63x + 423.53$, $r^2 = 0.39$, p-value < 0.0001 (QC);
$y = 0.14x^2 – 5.91x + 173.10$, $r^2 = 0.02$, p-value = 0.4286 (QG).

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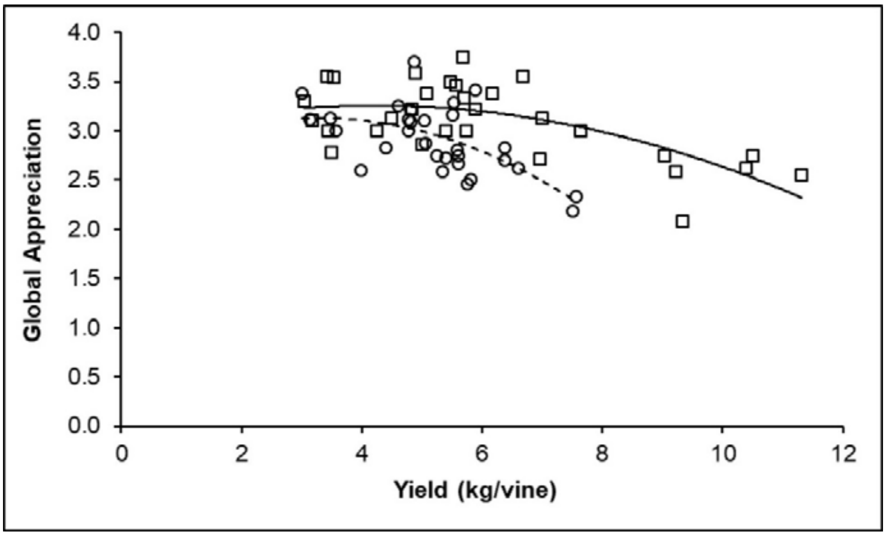


**Figure 7** – Influence of the site on the relation between yield and global appreciation of Syrah wines. Data represent single treatment (pruning system × organic amendment × site) averages and data were pooled over repetitions: □ - Quinta do Côro (QC); ○ - Quinta do Gradil (QG). Regression equations:
$y = -0.019x^2 + 0.156x + 2.926$, $r^2 = 0.44$, p-value = 0.0004 (QC);
$y = -0.047x^2 + 0.313x + 2.609$, $r^2 = 0.38$, p-value = 0.0010 (QG).

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**Supplemental Table 1** - Effect of the pruning system, the organic amendment and the site on the physical-chemical characteristics (classical parameters) of wine.

| Treatment | Alcoholic strength (% vol) | | | pH | | | Total acidity (g/L)[1] | | | Volatile acidity (g/L)[2] | | | Fixed acidity (g/L)[1] | | | K content (mg/L) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2013 | 2014 | 2015 | 2013 | 2014 | 2015 | 2013 | 2014 | 2015 | 2013 | 2014 | 2015 | 2013 | 2014 | 2015 | 2013 | 2014 | 2015 |
| QC/MAN/Ctrl | 17.1 | 14.7 | 16.2 | 3.48 | 3.54 | 3.58 | 6.30 | 6.15 | 5.85 | 0.41 | 0.41 | 0.34 | 0.41 | 5.64 | 5.43 | 809.4 | 971.6 | 933.6 |
| QC/MAN/Bioc | 16.7 | 15.2 | 15.7 | 3.53 | 3.44 | 3.55 | 6.23 | 6.75 | 5.70 | 0.46 | 0.39 | 0.36 | 0.46 | 6.26 | 5.25 | 895.2 | 973.8 | 944.7 |
| QC/MAN/MSWC | 16.7 | 14.3 | 15.2 | 3.60 | 3.56 | 3.54 | 6.15 | 6.30 | 5.40 | 0.43 | 0.31 | 0.34 | 0.43 | 5.91 | 4.98 | 941.0 | 1064.6 | 977.7 |
| QC/MAN/Manure | 17.1 | 14.3 | 14.3 | 3.84 | 3.52 | 3.41 | 5.10 | 6.60 | 5.85 | 0.45 | 0.32 | 0.39 | 0.45 | 6.20 | 5.36 | 1054.3 | 1047.7 | 793.9 |
| QC/MAN/Sludge | 17.1 | 14.0 | 13.7 | 3.54 | 3.42 | 3.34 | 6.00 | 6.30 | 6.45 | 0.34 | 0.29 | 0.39 | 0.34 | 5.94 | 5.96 | 804.4 | 909.8 | 716.9 |
| QC/MEC/Ctrl | 17.9 | 14.2 | 13.5 | 3.45 | 3.30 | 3.15 | 6.30 | 7.20 | 7.65 | 0.40 | 0.26 | 0.23 | 0.40 | 6.88 | 7.36 | 726.5 | 850.0 | 687.6 |
| QC/MEC/Bioc | 16.6 | 14.0 | 13.9 | 3.49 | 3.25 | 3.13 | 6.08 | 6.90 | 7.80 | 0.43 | 0.23 | 0.34 | 0.43 | 6.61 | 7.38 | 791.0 | 863.0 | 680.8 |
| QC/MEC/MSWC | 15.1 | 13.2 | 12.5 | 3.63 | 3.30 | 3.19 | 5.55 | 7.20 | 6.75 | 0.41 | 0.28 | 0.34 | 0.41 | 6.85 | 6.33 | 827.4 | 773.3 | 665.8 |
| QC/MEC/Manure | 15.8 | 12.2 | 13.1 | 3.67 | 3.26 | 3.25 | 5.40 | 6.90 | 6.45 | 0.42 | 0.28 | 0.29 | 0.42 | 6.55 | 6.09 | 882.0 | 852.5 | 678.4 |
| QC/MEC/Sludge | 14.8 | 11.0 | 10.8 | 3.45 | 3.18 | 3.16 | 6.08 | 7.05 | 7.20 | 0.36 | 0.27 | 0.33 | 0.36 | 6.71 | 6.79 | 769.3 | 717.3 | 629.9 |
| QG/MAN/Ctrl | 12.4 | 13.2 | 12.8 | 3.72 | 3.92 | 3.55 | 4.50 | 5.85 | 6.30 | 0.31 | 0.46 | 0.50 | 0.31 | 5.28 | 5.68 | 1096.0 | 1283.4 | 932.5 |
| QG/MAN/Bioc | 11.8 | 12.8 | 12.6 | 3.78 | 3.81 | 3.54 | 4.35 | 6.15 | 6.30 | 0.30 | 0.42 | 0.47 | 0.30 | 5.63 | 5.71 | 1157.7 | 1245.8 | 921.9 |
| QG/MAN/MSWC | 11.6 | 13.4 | 12.8 | 3.76 | 3.88 | 3.53 | 4.39 | 6.15 | 6.45 | 0.33 | 0.51 | 0.52 | 0.33 | 5.52 | 5.80 | 1083.9 | 1264.8 | 865.4 |
| QG/MAN/Manure | 11.8 | 12.8 | 12.4 | 3.72 | 3.90 | 3.59 | 4.50 | 6.00 | 6.90 | 0.33 | 0.42 | 0.46 | 0.33 | 5.48 | 6.33 | 1116.1 | 1281.2 | 982.5 |
| QG/MAN/Sludge | 11.3 | 12.2 | 11.9 | 3.68 | 3.88 | 3.46 | 4.47 | 6.15 | 6.45 | 0.45 | 0.48 | 0.46 | 0.45 | 5.54 | 5.88 | 986.8 | 1292.2 | 818.8 |
| QG/MEC/Ctrl | 11.0 | 13.1 | 13.5 | 3.55 | 3.70 | 3.61 | 4.84 | 6.00 | 5.70 | 0.37 | 0.49 | 0.41 | 0.37 | 5.39 | 5.19 | 1009.1 | 1092.4 | 997.6 |
| QG/MEC/Bioc | 10.9 | 13.3 | 13.0 | 3.63 | 3.75 | 3.61 | 4.73 | 6.45 | 6.15 | 0.35 | 0.50 | 0.42 | 0.35 | 5.83 | 5.63 | 1151.2 | 1290.7 | 1007.7 |
| QG/MEC/MSWC | 10.5 | 11.8 | 12.2 | 3.48 | 3.64 | 3.54 | 5.32 | 6.15 | 6.15 | 0.27 | 0.49 | 0.43 | 0.27 | 5.54 | 5.61 | 1109.2 | 1044.9 | 938.6 |
| QG/MEC/Manure | 10.5 | 12.2 | 12.0 | 3.50 | 3.72 | 3.41 | 5.38 | 6.00 | 6.45 | 0.39 | 0.36 | 0.41 | 0.39 | 5.55 | 5.94 | 1027.6 | 1148.5 | 882.6 |
| QG/MEC/Sludge | 10.3 | 11.6 | 11.0 | 3.48 | 3.79 | 3.48 | 5.44 | 6.00 | 6.15 | 0.23 | 0.42 | 0.37 | 0.23 | 5.48 | 5.69 | 943.8 | 1186.3 | 897.3 |

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 (Sludge). Site: Quinta do Côro (QC), Quinta do Gradil (QG).

[1] expressed in tartaric acid.

[2] expressed in acetic acid.

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**Supplemental Table 2** - Interaction between pruning system and site effect in wine classical parameters.

| | **Alcoholic strength (% vol.)** | **pH** | | **Total acidity (g / L)[1]** | | **Fixed acidity (g / L)[1]** | | **K content (mg/L)** |
|---|---|---|---|---|---|---|---|---|
| | **2015** | **2013** | **2015** | **2013** | **2015** | **2014** | **2015** | **2013** |
| QC/MAN | 15.0 a | 3.60 b | 3.48 a | 5.96 a | 5.85 b | 5.99 b | 5.40 b | 901 b |
| QC/MEC | 12.8 b | 3.54 b | 3.18 b | 5.88 a | 7.17 a | 6.72 a | 6.79 a | 799 c |
| QG/MAN | 12.5 b | 3.73 a | 3.53 a | 4.44 c | 6.48 ab | 5.49 b | 5.88 b | 1088 a |
| QG/MEC | 12.3 b | 3.53 b | 3.53 a | 5.14 b | 6.12 b | 5.56 b | 5.61 b | 1048 a |
| *Sig.*[1] | ** | * | * | * | ** | * | ** | ** |

1 Sig. – Significance level: n.s. – non-significant at $p < 0.05$ level by F test; significant at $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) by F test. In each column values followed by the same letter do not significantly differ by Tukey HSD test at $\alpha = 0.05$.

Pruning system: hand pruning (MAN) and mechanical pruning (MEC). Site: Quinta do Côro (QC) and Quinta do Gradil (QG).

[1] expressed in tartaric acid.

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**Supplemental Table 3** - Effect of pruning system, organic amendment and site on chromatic characteristics and phenolic composition of wine.

| Treatment | Color intensity (a.u.[1]) | | | Color hue | | | Total Phenols (a.u.[1]) | | | Tannin power (NTU[2]/mL) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2013 | 2014 | 2015 | 2013 | 2014 | 2015 | 2013 | 2014 | 2015 | 2013 | 2014 | 2015 |
| QC/MAN/Ctrl | 22.3 | 11.2 | 10.9 | 0.560 | 0.583 | 0.633 | 69.6 | 51.3 | 52.9 | 277 | 193 | 234 |
| QC/MAN/Bioc | 18.4 | 13.7 | 10.6 | 0.570 | 0.554 | 0.630 | 59.0 | 55.0 | 50.7 | 282 | 206 | 206 |
| QC/MAN/MSWC | 22.1 | 11.4 | 9.5 | 0.580 | 0.611 | 0.636 | 72.0 | 55.9 | 47.3 | 279 | 207 | 180 |
| QC/MAN/Manure | 11.9 | 11.9 | 11.1 | 0.720 | 0.591 | 0.575 | 63.4 | 52.4 | 46.4 | 269 | 189 | 176 |
| QC/MAN/Sludge | 22.4 | 11.9 | 8.6 | 0.580 | 0.546 | 0.572 | 72.0 | 49.8 | 34.4 | 288 | 201 | 96 |
| QC/MEC/Ctrl | 24.3 | 17.4 | 13.8 | 0.540 | 0.475 | 0.503 | 80.0 | 55.9 | 49.2 | 240 | 171 | 192 |
| QC/MEC/Bioc | 23.2 | 18.0 | 12.8 | 0.550 | 0.496 | 0.501 | 76.0 | 56.2 | 44.6 | 298 | 157 | 169 |
| QC/MEC/MSWC | 10.9 | 11.8 | 6.9 | 0.670 | 0.493 | 0.573 | 58.0 | 46.4 | 34.6 | 222 | 131 | 107 |
| QC/MEC/Manure | 11.3 | 10.9 | 7.7 | 0.650 | 0.492 | 0.601 | 58.0 | 43.7 | 37.0 | 226 | 129 | 108 |
| QC/MEC/Sludge | 9.8 | 10.8 | 4.9 | 0.650 | 0.437 | 0.599 | 52.0 | 34.8 | 27.4 | 258 | 75 | 61 |
| QG/MAN/Ctrl | 6.5 | 8.8 | 9.2 | 0.656 | 0.672 | 0.609 | 42.8 | 50.3 | 48.3 | 145 | 155 | 202 |
| QG/MAN/Bioc | 5.7 | 8.4 | 7.4 | 0.662 | 0.655 | 0.409 | 38.9 | 48.7 | 44.8 | 112 | 110 | 184 |
| QG/MAN/MSWC | 6.4 | 8.0 | 7.7 | 0.659 | 0.657 | 0.632 | 42.4 | 49.4 | 43.5 | 146 | 177 | 165 |
| QG/MAN/Manure | 6.1 | 8.3 | 8.3 | 0.700 | 0.680 | 0.653 | 40.3 | 50.1 | 45.2 | 156 | 159 | 169 |
| QG/MAN/Sludge | 3.7 | 6.4 | 6.7 | 0.700 | 0.710 | 0.598 | 29.5 | 42.4 | 36.0 | 129 | 154 | 109 |
| QG/MEC/Ctrl | 8.7 | 7.9 | 13.2 | 0.557 | 0.623 | 0.615 | 46.4 | 45.9 | 58.0 | 180 | 117 | 192 |
| QG/MEC/Bioc | 6.6 | 8.0 | 10.4 | 0.605 | 0.604 | 0.631 | 38.4 | 46.5 | 50.6 | 113 | 84 | 186 |
| QG/MEC/MSWC | 5.4 | 6.7 | 11.4 | 0.628 | 0.612 | 0.604 | 38.7 | 40.2 | 50.2 | 167 | 108 | 168 |
| QG/MEC/Manure | 5.4 | 6.1 | 12.4 | 0.624 | 0.661 | 0.573 | 39.4 | 44.2 | 49.5 | 102 | 92 | 171 |
| QG/MEC/Sludge | 5.0 | 4.7 | 5.7 | 0.615 | 0.693 | 0.620 | 36.6 | 36.0 | 34.9 | 183 | 133 | 117 |

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 (Sludge). Site: Quinta do Côro (QC), Quinta do Gradil (QG).

[1] a.u. – absorbance units.

[2] NTU – nephelometric turbidity units.

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**Supplemental Table 4** - Interaction between pruning system and site effect in wine classical parameters.

| | Color hue | Total anthocyanins (mg / l)[1] | Total pigments (a.u.)[2] | Tannin power (NTU[3]/ml) |
|---|---|---|---|---|
| | 2014 | 2015 | 2015 | 2015 |
| QC/MAN | 0.577 b | 362 a | 23.14 a | 178 a |
| QC/MEC | 0.478 c | 252 b | 17.13 b | 128 b |
| QG/MAN | 0.675 a | 332 a | 20.19 ab | 166 a |
| QG/MEC | 0.638 a | 362 a | 22.34 a | 167 a |
| *Sig.*[1] | * | ** | ** | * |

1 Sig. – Significance level: n.s. – non-significant at $p < 0.05$ level by F test; significant at $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) by F test. In each column values followed by the same letter do not significantly differ by Tukey HSD test at $\alpha = 0.05$.
Pruning system: hand pruning (MAN) and mechanical pruning (MEC). Site: Quinta do Côro (QC) and Quinta do Gradil (QG).
[1] expressed in Malvidin-3-O-glucoside.
[2] a.u. – absorbance units.
[3] NTU – nephelometric turbidity units.

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**Supplemental Table 5** - Effect of the pruning system, the organic amendment and the site on the pigments of wine.

| Treatment | Total anthocyanins (mg/L)[1] | | | Ionized anthocyanins (mg/L)[1] | | | Ionization index (%) | | | Total pigments (a.u.)[2] | | | Polymerized pigments (a.u.)[2] | | | Polymerization index (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2013 | 2014 | 2015 | 2013 | 2014 | 2015 | 2013 | 2014 | 2015 | 2013 | 2014 | 2015 | 2013 | 2014 | 2015 | 2013 | 2014 | 2015 |
| QC/MAN/Ctrl | 470 | 490 | 434 | 137 | 63 | 50 | 29.1 | 12.8 | 11.6 | 32.79 | 29.52 | 27.27 | 5.62 | 3.05 | 3.35 | 17.1 | 10.3 | 12.3 |
| QC/MAN/Bioc | 452 | 391 | 397 | 109 | 86 | 46 | 24.2 | 22.0 | 11.7 | 30.53 | 24.69 | 25.48 | 4.76 | 3.80 | 3.39 | 15.6 | 15.4 | 13.3 |
| QC/MAN/MSWC | 516 | 473 | 371 | 135 | 57 | 45 | 26.2 | 11.9 | 12.3 | 34.70 | 29.21 | 23.15 | 5.35 | 3.34 | 2.80 | 15.4 | 11.4 | 12.1 |
| QC/MAN/Manure | 420 | 467 | 378 | 18 | 64 | 62 | 4.3 | 13.6 | 16.4 | 29.43 | 28.88 | 24.06 | 5.08 | 3.33 | 3.09 | 17.3 | 11.5 | 12.9 |
| QC/MAN/Sludge | 475 | 469 | 230 | 129 | 78 | 46 | 27.1 | 16.7 | 20.0 | 33.43 | 28.31 | 15.72 | 5.81 | 2.95 | 2.54 | 17.4 | 10.4 | 16.1 |
| QC/MEC/Ctrl | 511 | 511 | 341 | 152 | 144 | 91 | 29.9 | 28.1 | 26.8 | 35.73 | 31.09 | 23.09 | 6.11 | 3.33 | 3.63 | 17.1 | 10.7 | 15.7 |
| QC/MEC/Bioc | 473 | 485 | 303 | 146 | 133 | 75 | 30.9 | 27.4 | 24.9 | 33.16 | 31.03 | 21.51 | 5.69 | 4.06 | 3.83 | 17.2 | 13.1 | 17.8 |
| QC/MEC/MSWC | 420 | 418 | 212 | 41 | 92 | 36 | 9.7 | 22.1 | 16.8 | 27.00 | 25.05 | 14.21 | 3.58 | 2.48 | 2.16 | 13.3 | 9.9 | 15.2 |
| QC/MEC/Manure | 401 | 371 | 258 | 45 | 89 | 38 | 11.1 | 23.9 | 14.9 | 26.19 | 22.11 | 16.85 | 3.68 | 2.14 | 2.38 | 14.1 | 9.7 | 14.1 |
| QC/MEC/Sludge | 354 | 286 | 145 | 32 | 102 | 22 | 9.0 | 35.8 | 15.5 | 23.68 | 17.20 | 9.98 | 3.58 | 1.73 | 1.64 | 15.1 | 10.1 | 16.4 |
| QG/MAN/Ctrl | 482 | 657 | 376 | 38 | 49 | 55 | 7.9 | 7.5 | 14.6 | 26.62 | 36.47 | 22.59 | 1.56 | 2.14 | 2.31 | 5.9 | 5.9 | 10.2 |
| QG/MAN/Bioc | 454 | 614 | 339 | 34 | 46 | 48 | 7.5 | 7.5 | 14.0 | 25.00 | 34.12 | 20.57 | 1.35 | 2.14 | 2.17 | 5.4 | 6.3 | 10.5 |
| QG/MAN/MSWC | 442 | 643 | 327 | 38 | 47 | 42 | 8.5 | 7.2 | 12.8 | 24.58 | 35.28 | 19.80 | 1.48 | 1.87 | 2.06 | 6.0 | 5.3 | 10.4 |
| QG/MAN/Manure | 347 | 662 | 348 | 20 | 44 | 40 | 5.8 | 6.7 | 11.6 | 20.77 | 36.43 | 21.38 | 2.08 | 2.10 | 2.38 | 10.0 | 5.8 | 11.1 |
| QG/MAN/Sludge | 261 | 509 | 271 | 14 | 32 | 37 | 5.3 | 6.3 | 13.8 | 15.10 | 28.26 | 16.60 | 1.24 | 1.67 | 1.83 | 8.2 | 5.9 | 11.0 |
| QG/MEC/Ctrl | 461 | 548 | 458 | 63 | 48 | 80 | 13.7 | 8.8 | 17.4 | 26.12 | 30.41 | 28.17 | 1.80 | 1.83 | 3.16 | 6.9 | 6.0 | 11.2 |
| QG/MEC/Bioc | 397 | 531 | 394 | 43 | 53 | 64 | 10.8 | 9.9 | 16.3 | 22.32 | 29.49 | 23.70 | 1.46 | 1.77 | 2.39 | 6.5 | 6.0 | 10.1 |
| QG/MEC/MSWC | 394 | 460 | 359 | 31 | 42 | 73 | 8.0 | 9.1 | 20.3 | 22.04 | 25.60 | 22.28 | 1.38 | 1.56 | 2.60 | 6.3 | 6.1 | 11.7 |
| QG/MEC/Manure | 399 | 515 | 344 | 31 | 33 | 76 | 7.7 | 6.5 | 22.0 | 22.31 | 28.35 | 22.48 | 1.43 | 1.54 | 3.18 | 6.4 | 5.4 | 14.1 |
| QG/MEC/Sludge | 264 | 427 | 254 | 12 | 24 | 34 | 4.4 | 5.6 | 13.5 | 15.95 | 23.33 | 15.05 | 1.64 | 1.23 | 1.41 | 10.3 | 5.3 | 9.4 |

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 (Sludge). Site: Quinta do Côro (QC), Quinta do Gradil (QG).
[1] expressed in Malvidin-3-O-glucoside.
[2] a.u. – absorbance units.

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**Supplemental Table 6** - Effect of the pruning system, organic amendment and site on the sensory analysis of the wines produced in 2013.

| | Colour | | Aroma | | | | | | Taste | | | | | | Global Apprec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Red** | **Violet** | **Fruit** | **Floral** | **Veget.** | **Jam** | **Intens.** | **Balan.** | **Body** | **Bitter.** | **Astring.** | **Acid.** | **Persist.** | **Balan.** | |
| MAN | 3.21 a | 2.76 | 3.17 | 2.00 | 1.85 | 2.34 | 3.26 | 3.17 a | 3.26 a | 2.07 | 2.97 a | 3.01 | 3.17 a | 3.10 a | 3.14 a |
| MEC | 2.99 b | 2.73 | 3.04 | 1.92 | 1.80 | 2.29 | 3.24 | 2.96 b | 3.05 b | 2.06 | 2.78 b | 2.97 | 3.01 b | 2.87 b | 2.89 b |
| *Pruning effect* | * | *n.s.* | *n.s.* | *n.s.* | *n.s.* | *n.s.* | *n.s.* | * | * | *n.s.* | * | *n.s.* | * | ** | *** |
| Ctrl | 3.26 a | 3.07 a | 3.21 | 1.95 | 1.75 | 2.46 a | 3.35 a | 3.17 | 3.45 a | 1.96 ab | 2.98 a | 2.93 | 3.19 a | 3.21 a | 3.25 a |
| Bioc | 3.22 a | 2.78 b | 3.14 | 1.94 | 1.83 | 2.45 a | 3.23 ab | 3.09 | 3.13 b | 2.28 a | 2.97 ab | 3.01 | 3.22 a | 2.97 ab | 3.06 abc |
| MSWC | 3.13 ab | 2.67 bc | 3.21 | 1.98 | 1.72 | 2.50 a | 3.47 a | 3.13 | 3.16 ab | 1.94 b | 2.93 ab | 3.06 | 3.16 ab | 3.03 ab | 3.10 ab |
| Manure | 2.89 b | 2.76 b | 3.00 | 1.94 | 2.00 | 2.17 ab | 3.16 ab | 3.01 | 3.10 b | 2.08 ab | 2.85 ab | 2.99 | 3.03 ab | 2.97 ab | 2.91 bc |
| Sludge | 3.02 ab | 2.42 c | 2.96 | 2.00 | 1.81 | 1.99 b | 3.04 b | 2.92 | 2.94 b | 2.06 ab | 2.65 b | 2.96 | 2.87 b | 2.76 b | 2.75 c |
| *Amendment effect* | ** | *** | *n.s.* | *n.s.* | *n.s.* | *** | ** | *n.s.* | *** | * | * | *n.s.* | * | ** | *** |
| QC | 3.57 a | 2.91 | 3.18 | 1.96 | 1.77 | 2.50 | 3.42 a | 3.12 | 3.48 a | 2.04 | 3.08 a | 2.76 b | 3.33 a | 3.23 a | 3.21 a |
| QG | 2.64 b | 2.57 | 3.03 | 1.97 | 1.87 | 2.13 | 3.08 b | 3.01 | 2.83 b | 2.09 | 2.68 b | 3.22 a | 2.85 b | 2.74 b | 2.82 b |
| *Local effect* | ** | *n.s.* | *n.s.* | *n.s.* | *n.s.* | *n.s.* | ** | *n.s.* | *** | *n.s.* | * | ** | * | * | * |
| *Prun x 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.* |
| *Prun x Local* | *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 x Local* | *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.* |

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 (Sludge). Site: Quinta do Côro (QC), Quinta do Gradil (QG).

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**Supplemental Table 7** - Effect of the pruning system, organic amendment and site on the sensory analysis of the wines produced in 2014.

| | Colour | | Aroma | | | | | | Taste | | | | | | Global Apprec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Red | Violet | Fruit | Floral | Veget. | Jam | Intens. | Balan. | Body | Bitter. | Astring. | Acid. | Persist. | Balan. | |
| MAN | 3.67 | 2.65 | 3.28 | 2.19 | 2.05 | 2.42 a | 3.34 | 3.23 a | 3.44 a | 1.95 | 2.74 | 2.68 | 3.27 | 3.17 a | 3.23 a |
| MEC | 3.40 | 2.50 | 3.14 | 2.04 | 2.00 | 2.08 b | 3.18 | 2.98 b | 3.03 b | 1.86 | 2.63 | 2.70 | 3.11 | 2.83 b | 2.91 b |
| *Pruning effect* | *n.s.* | *n.s.* | *n.s.* | *n.s.* | *n.s.* | ** | *n.s.* | ** | ** | *n.s.* | *n.s.* | *n.s.* | *n.s.* | ** | ** |
| Ctrl | 3.60 a | 2.89 a | 3.26 | 2.18 | 1.94 | 2.48 | 3.43 | 3.35 a | 3.51 a | 1.80 | 2.80 | 2.64 | 3.41 a | 3.28 a | 3.36 a |
| Bioc | 3.74 a | 2.83 a | 3.21 | 2.11 | 1.93 | 2.31 | 3.26 | 3.05 ab | 3.35 a | 1.94 | 2.74 | 2.65 | 3.29 a | 3.09 a | 3.13 ab |
| MSWC | 3.76 a | 2.44 b | 3.23 | 2.03 | 2.14 | 2.10 | 3.23 | 3.14 ab | 3.22 ab | 1.88 | 2.73 | 2.63 | 3.12 ab | 3.00 ab | 3.09 ab |
| Manure | 3.46 ab | 2.49 b | 3.29 | 2.11 | 1.98 | 2.21 | 3.28 | 3.05 ab | 3.20 ab | 1.94 | 2.65 | 2.66 | 3.24 a | 2.98 ab | 2.98 b |
| Sludge | 3.11 b | 2.24 b | 3.05 | 2.15 | 2.15 | 2.15 | 3.10 | 2.93 b | 2.89 b | 1.96 | 2.50 | 2.86 | 2.88 b | 2.65 b | 2.79 b |
| *Amendment effect* | *** | *** | *n.s.* | *n.s.* | *n.s.* | *n.s.* | *n.s.* | * | *** | *n.s.* | *n.s.* | *n.s.* | *** | *** | *** |
| QC | 3.69 | 2.68 | 3.30 | 2.25 | 2.18 | 2.34 | 3.26 | 3.37 | 3.48 a | 1.87 | 2.82 a | 2.61 | 3.33 | 3.25 a | 3.17 |
| QG | 3.38 | 2.47 | 3.12 | 1.99 | 1.88 | 2.17 | 2.95 | 3.15 | 2.99 b | 1.94 | 2.55 b | 2.77 | 3.05 | 2.75 b | 2.97 |
| *Local effect* | *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 x Amend* | *n.s.* | *** | *n.s.* | *n.s.* | *n.s.* | * | *n.s.* | *n.s.* | *** | *n.s.* | *n.s.* | *n.s.* | * | *n.s.* | *n.s.* |
| *Prun x Local* | *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 x Local* | *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.* |

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 (Sludge). Site: Quinta do Côro (QC), Quinta do Gradil (QG).

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**Supplemental Table 8** - Effect of the pruning system, organic amendment and site on the sensory analysis of the wines produced in 2015.

| | Colour | | Aroma | | | | | | Taste | | | | | | Global Apprec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Red** | **Violet** | **Fruit** | **Floral** | **Veget.** | **Jam** | **Intens.** | **Balan.** | **Body** | **Bitter.** | **Astring.** | **Acid.** | **Persist.** | **Balan.** | |
| MAN | 3.53 a | 2.13 b | 3.09 | 2.23 | 2.00 | 2.21 | 3.16 | 3.00 | 3.14 | 2.22 | 2.88 | 3.31 | 3.24 | 3.03 | 3.05 |
| MEC | 3.12 b | 2.64 a | 3.14 | 2.08 | 1.92 | 2.23 | 3.16 | 2.95 | 3.03 | 2.03 | 2.65 | 3.41 | 3.19 | 2.94 | 2.94 |
| *Pruning effect* | * | ** | *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 | 3.16 | 2.99 a | 3.38 a | 2.29 a | 1.92 | 2.39 a | 3.43 a | 3.28 a | 3.41 a | 2.23 | 2.91 ab | 3.38 | 3.28 ab | 3.25 a | 3.29 a |
| Bioc | 3.38 | 2.72 ab | 3.14 ab | 2.22 ab | 1.88 | 2.36 a | 3.22 a | 3.08 ab | 3.30 ab | 2.08 | 3.02 a | 3.35 | 3.45 a | 3.23 a | 3.31 a |
| MSWC | 3.43 | 2.28 b | 3.09 ab | 2.30 a | 2.04 | 2.22 ab | 3.24 a | 2.86 b | 3.02 b | 2.10 | 2.71 abc | 3.25 | 3.05 b | 2.79 bc | 2.83 bc |
| Manure | 3.47 | 2.30 b | 3.14 ab | 2.10 ab | 1.94 | 2.22 ab | 3.11 ab | 2.96 ab | 3.19 ab | 2.13 | 2.66 bc | 3.33 | 3.28 ab | 3.11 ab | 3.03 ab |
| Sludge | 3.19 | 1.63 c | 2.82 b | 1.85 b | 2.02 | 1.91 b | 2.80 b | 2.70 b | 2.51 c | 2.08 | 2.54 c | 3.49 | 3.00 b | 2.55 c | 2.49 c |
| *Amendment effect* | *n.s.* | *** | * | * | *n.s.* | ** | *** | ** | *** | *n.s.* | *** | *n.s.* | * | *** | *** |
| QC | 3.19 b | 2.34 | 3.17 | 2.18 | 1.89 | 2.40 a | 3.12 | 3.09 a | 3.11 | 2.15 | 2.69 | 3.36 | 3.19 | 2.99 | 3.04 |
| QG | 3.45 a | 2.43 | 3.06 | 2.13 | 2.03 | 2.04 b | 3.20 | 2.87 b | 3.06 | 2.10 | 2.85 | 3.36 | 3.24 | 2.99 | 2.94 |
| *Local effect* | * | *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 x Amend* | *n.s.* | ** | *n.s.* | *n.s.* | *n.s.* | *n.s.* | * | *n.s.* | ** | *n.s.* | *n.s.* | *n.s.* | *n.s.* | *n.s.* | ** |
| *Prun x Local* | *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 x Local* | *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.* |

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 (Sludge). Site: Quinta do Côro (QC), Quinta do Gradil (QG).

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**Supplemental Table 9** - Effect of the pruning system, organic amendment and site on the sensory analysis of the wines produced in 2013.

| | Colour | | Aroma | | | | | | Taste | | | | | | Global Apprec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Red | Violet | Fruit | Floral | Veget. | Jam | Intens. | Balan. | Body | Bitter. | Astring. | Acid. | Persist. | Balan. | |
| QC/MAN/Ctrl | 3.73 | 3.17 | 3.00 | 2.00 | 1.85 | 2.64 | 3.33 | 3.10 | 3.82 | 1.91 | 3.18 | 2.75 | 3.64 | 3.60 | 3.56 |
| QC/MAN/Bioc | 3.55 | 2.83 | 3.42 | 2.00 | 1.92 | 2.69 | 3.55 | 3.55 | 3.64 | 2.23 | 3.23 | 3.00 | 3.75 | 3.46 | 3.55 |
| QC/MAN/MSWC | 3.64 | 2.75 | 3.08 | 2.08 | 1.92 | 2.91 | 3.58 | 3.00 | 3.40 | 1.75 | 3.36 | 2.91 | 3.27 | 3.17 | 3.10 |
| QC/MAN/Manure | 3.33 | 2.73 | 3.09 | 1.92 | 2.00 | 2.31 | 3.33 | 3.17 | 3.50 | 2.00 | 3.00 | 2.67 | 3.09 | 3.40 | 3.30 |
| QC/MAN/Sludge | 3.50 | 3.10 | 3.09 | 2.00 | 1.42 | 2.18 | 3.00 | 3.00 | 3.40 | 2.00 | 2.80 | 2.73 | 2.89 | 2.91 | 2.78 |
| QC/MEC/Ctrl | 3.64 | 2.70 | 3.25 | 2.10 | 1.64 | 2.50 | 3.50 | 3.09 | 3.73 | 2.09 | 2.90 | 2.82 | 3.27 | 3.33 | 3.00 |
| QC/MEC/Bioc | 3.55 | 3.09 | 3.00 | 1.82 | 1.85 | 2.75 | 3.36 | 2.85 | 3.00 | 1.92 | 3.00 | 2.67 | 3.20 | 2.91 | 3.00 |
| QC/MEC/MSWC | 2.90 | 2.67 | 3.46 | 1.83 | 1.54 | 2.82 | 3.64 | 3.10 | 3.36 | 1.92 | 2.82 | 2.75 | 3.27 | 3.33 | 3.13 |
| QC/MEC/Manure | 3.27 | 2.67 | 3.00 | 2.00 | 1.77 | 2.33 | 3.33 | 2.90 | 3.18 | 1.82 | 2.83 | 2.62 | 3.17 | 3.00 | 3.00 |
| QC/MEC/Sludge | 3.33 | 2.70 | 3.18 | 2.18 | 1.58 | 2.31 | 3.42 | 2.85 | 3.25 | 2.17 | 2.82 | 2.64 | 3.27 | 3.20 | 3.22 |
| QG/MAN/Ctrl | 3.08 | 2.73 | 3.00 | 2.00 | 1.92 | 2.18 | 3.10 | 2.92 | 3.00 | 1.75 | 2.70 | 3.08 | 2.80 | 2.91 | 3.09 |
| QG/MAN/Bioc | 2.73 | 2.33 | 3.17 | 2.00 | 1.92 | 2.17 | 3.00 | 3.15 | 2.92 | 2.33 | 2.92 | 3.17 | 2.64 | 2.75 | 2.73 |
| QG/MAN/MSWC | 2.92 | 2.64 | 3.42 | 2.08 | 1.67 | 2.67 | 3.58 | 3.36 | 3.17 | 1.92 | 3.08 | 3.09 | 3.45 | 3.45 | 3.70 |
| QG/MAN/Manure | 2.50 | 3.20 | 3.09 | 2.00 | 1.92 | 2.08 | 2.90 | 3.15 | 3.00 | 2.00 | 2.67 | 3.00 | 3.00 | 3.00 | 3.00 |
| QG/MAN/Sludge | 2.36 | 1.91 | 3.00 | 2.15 | 1.73 | 1.62 | 2.85 | 2.92 | 2.64 | 2.00 | 2.42 | 3.23 | 2.75 | 2.62 | 2.67 |
| QG/MEC/Ctrl | 2.18 | 3.55 | 3.42 | 1.85 | 1.54 | 2.33 | 3.38 | 3.25 | 3.00 | 1.73 | 2.83 | 3.00 | 3.00 | 3.09 | 3.10 |
| QG/MEC/Bioc | 2.80 | 2.83 | 2.91 | 2.08 | 1.62 | 2.25 | 3.00 | 2.83 | 2.67 | 2.33 | 2.54 | 2.90 | 2.92 | 2.69 | 2.83 |
| QG/MEC/MSWC | 2.64 | 2.50 | 2.91 | 2.00 | 1.69 | 1.92 | 3.10 | 2.83 | 2.58 | 1.83 | 2.31 | 3.10 | 2.42 | 2.45 | 2.50 |
| QG/MEC/Manure | 2.25 | 2.17 | 2.69 | 2.00 | 2.31 | 1.92 | 3.00 | 2.75 | 2.64 | 2.31 | 2.77 | 3.50 | 2.77 | 2.67 | 2.70 |
| QG/MEC/Sludge | 2.58 | 1.83 | 2.64 | 1.75 | 2.31 | 1.83 | 2.73 | 2.83 | 2.38 | 2.00 | 2.38 | 3.18 | 2.17 | 2.31 | 2.18 |

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 (Sludge). Site: Quinta do Côro (QC), Quinta do Gradil (QG).

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**Supplemental Table 10** - Effect of the pruning system, organic amendment and site on the sensory analysis of the wines produced in 2014.

| | Colour | | Aroma | | | | | | Taste | | | | | | Global Apprec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Red | Violet | Fruit | Floral | Veget. | Jam | Intens. | Balan. | Body | Bitter. | Astring. | Acid. | Persist. | Balan. | |
| QC/MAN/Ctrl | 3.56 | 2.80 | 3.50 | 2.20 | 1.75 | 2.60 | 3.56 | 3.60 | 3.70 | 2.00 | 2.90 | 2.78 | 3.44 | 3.44 | 3.22 |
| QC/MAN/Bioc | 3.78 | 2.89 | 3.40 | 2.30 | 2.30 | 2.50 | 3.44 | 3.30 | 3.50 | 1.89 | 2.78 | 2.56 | 3.44 | 3.30 | 3.38 |
| QC/MAN/MSWC | 4.13 | 2.44 | 3.44 | 2.44 | 2.56 | 2.13 | 3.67 | 3.78 | 4.11 | 1.75 | 3.13 | 2.38 | 3.43 | 3.86 | 3.33 |
| QC/MAN/Manure | 3.75 | 2.56 | 3.00 | 2.20 | 2.00 | 2.30 | 3.22 | 3.00 | 3.88 | 1.67 | 2.90 | 2.33 | 3.38 | 3.38 | 2.86 |
| QC/MAN/Sludge | 3.38 | 2.33 | 3.30 | 2.50 | 2.22 | 2.70 | 3.44 | 3.22 | 3.67 | 1.70 | 2.56 | 2.50 | 3.38 | 3.38 | 3.50 |
| QC/MEC/Ctrl | 3.67 | 3.11 | 3.30 | 2.40 | 1.67 | 2.56 | 3.44 | 3.40 | 3.60 | 1.56 | 3.00 | 2.44 | 3.56 | 3.60 | 3.56 |
| QC/MEC/Bioc | 3.78 | 3.00 | 3.40 | 2.10 | 1.78 | 2.33 | 3.30 | 3.22 | 3.70 | 1.56 | 2.88 | 2.40 | 3.38 | 3.44 | 3.00 |
| QC/MEC/MSWC | 3.67 | 2.40 | 3.11 | 1.70 | 2.33 | 1.67 | 2.88 | 2.56 | 2.80 | 1.78 | 2.67 | 2.50 | 2.89 | 2.63 | 2.75 |
| QC/MEC/Manure | 3.33 | 2.40 | 3.25 | 2.22 | 2.00 | 1.89 | 3.29 | 3.00 | 3.11 | 2.11 | 2.89 | 2.63 | 3.33 | 2.78 | 2.63 |
| QC/MEC/Sludge | 3.22 | 2.00 | 3.20 | 2.30 | 1.78 | 1.90 | 3.22 | 3.20 | 2.80 | 1.75 | 2.63 | 3.00 | 2.70 | 2.56 | 2.56 |
| QG/MAN/Ctrl | 3.67 | 2.70 | 3.00 | 2.20 | 1.90 | 2.11 | 3.40 | 3.30 | 3.44 | 1.44 | 2.44 | 2.56 | 3.11 | 3.00 | 3.38 |
| QG/MAN/Bioc | 3.80 | 2.30 | 3.10 | 2.10 | 1.50 | 2.20 | 3.10 | 2.80 | 3.00 | 2.20 | 2.60 | 3.00 | 3.10 | 2.90 | 3.00 |
| QG/MAN/MSWC | 3.56 | 2.70 | 3.40 | 1.90 | 1.70 | 2.40 | 3.10 | 3.30 | 3.33 | 1.90 | 2.70 | 2.60 | 3.20 | 2.90 | 3.13 |
| QG/MAN/Manure | 3.50 | 2.80 | 3.56 | 2.10 | 1.70 | 2.60 | 3.30 | 3.00 | 3.00 | 1.67 | 2.56 | 2.70 | 3.00 | 2.89 | 3.11 |
| QG/MAN/Sludge | 3.10 | 2.40 | 3.00 | 2.00 | 2.30 | 2.30 | 3.10 | 2.89 | 2.70 | 2.44 | 2.67 | 3.00 | 2.80 | 2.56 | 2.60 |
| QG/MEC/Ctrl | 3.11 | 2.80 | 3.10 | 1.90 | 1.90 | 2.40 | 3.40 | 3.10 | 3.30 | 1.70 | 2.70 | 2.60 | 3.40 | 2.90 | 3.11 |
| QG/MEC/Bioc | 3.33 | 2.80 | 3.00 | 1.89 | 1.78 | 2.10 | 3.20 | 2.89 | 3.11 | 1.78 | 2.60 | 2.67 | 3.20 | 2.70 | 2.75 |
| QG/MEC/MSWC | 3.50 | 2.20 | 2.89 | 2.10 | 1.90 | 1.80 | 3.20 | 2.90 | 2.70 | 1.78 | 2.56 | 2.80 | 2.80 | 2.60 | 2.80 |
| QG/MEC/Manure | 3.20 | 2.00 | 3.30 | 1.80 | 1.90 | 1.90 | 3.10 | 3.00 | 2.70 | 2.00 | 2.20 | 2.80 | 3.10 | 2.70 | 2.75 |
| QG/MEC/Sludge | 2.60 | 2.00 | 2.78 | 1.80 | 2.00 | 1.70 | 2.60 | 2.40 | 2.40 | 1.70 | 2.20 | 2.80 | 2.60 | 2.10 | 2.63 |

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 (Sludge). Site: Quinta do Côro (QC), Quinta do Gradil (QG).

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**Supplemental Table 11** - Effect of the pruning system, organic amendment and site on the sensory analysis of the wines produced in 2015.

| | Colour | | Aroma | | | | | | Taste | | | | | | Global Apprec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Red | Violet | Fruit | Floral | Veget. | Jam | Intens. | Balan. | Body | Bitter. | Astring. | Acid. | Persist. | Balan. | |
| QC/MAN/Ctrl | 3.21 | 2.58 | 3.25 | 2.17 | 2.00 | 2.63 | 3.29 | 3.21 | 3.67 | 2.08 | 2.92 | 3.21 | 3.33 | 3.38 | 3.46 |
| QC/MAN/Bioc | 3.29 | 2.29 | 2.96 | 2.17 | 2.08 | 2.33 | 3.29 | 3.25 | 3.75 | 2.33 | 3.33 | 3.38 | 3.88 | 3.50 | 3.75 |
| QC/MAN/MSWC | 3.54 | 2.25 | 3.13 | 2.33 | 2.08 | 2.46 | 3.38 | 3.00 | 3.25 | 2.17 | 2.88 | 3.13 | 3.13 | 3.00 | 3.13 |
| QC/MAN/Manure | 3.50 | 2.58 | 3.38 | 2.33 | 1.83 | 2.50 | 3.33 | 3.42 | 3.67 | 2.25 | 2.79 | 3.25 | 3.29 | 3.21 | 3.38 |
| QC/MAN/Sludge | 3.46 | 1.83 | 3.13 | 2.08 | 1.75 | 2.46 | 3.00 | 3.04 | 2.63 | 2.42 | 2.46 | 3.42 | 2.92 | 2.71 | 2.71 |
| QC/MEC/Ctrl | 2.92 | 3.38 | 3.67 | 2.50 | 1.67 | 2.75 | 3.58 | 3.67 | 3.54 | 2.00 | 2.50 | 3.42 | 3.17 | 3.42 | 3.58 |
| QC/MEC/Bioc | 2.83 | 3.46 | 3.46 | 2.13 | 1.75 | 2.96 | 3.33 | 3.25 | 3.13 | 2.08 | 2.88 | 3.33 | 3.29 | 2.96 | 3.00 |
| QC/MEC/MSWC | 3.25 | 1.67 | 3.04 | 2.21 | 1.67 | 2.13 | 2.88 | 2.88 | 2.54 | 2.08 | 2.42 | 3.50 | 3.00 | 2.58 | 2.58 |
| QC/MEC/Manure | 3.13 | 1.83 | 2.83 | 1.83 | 2.08 | 1.92 | 2.58 | 2.50 | 2.83 | 2.08 | 2.29 | 3.46 | 3.29 | 3.04 | 2.75 |
| QC/MEC/Sludge | 2.79 | 1.50 | 2.83 | 2.00 | 2.00 | 1.83 | 2.50 | 2.67 | 2.08 | 2.00 | 2.42 | 3.50 | 2.58 | 2.08 | 2.08 |
| QG/MAN/Ctrl | 3.33 | 2.42 | 3.17 | 2.50 | 2.17 | 1.92 | 3.17 | 2.92 | 2.75 | 2.58 | 3.17 | 3.46 | 3.21 | 2.92 | 2.88 |
| QG/MAN/Bioc | 3.79 | 2.08 | 3.08 | 2.33 | 1.75 | 2.33 | 3.00 | 3.00 | 3.00 | 2.08 | 3.00 | 3.25 | 3.33 | 3.25 | 3.29 |
| QG/MAN/MSWC | 3.58 | 1.83 | 3.13 | 2.50 | 2.17 | 1.79 | 3.13 | 2.67 | 2.88 | 2.25 | 2.71 | 3.38 | 2.92 | 2.54 | 2.46 |
| QG/MAN/Manure | 3.92 | 1.83 | 3.08 | 2.17 | 1.83 | 2.00 | 3.08 | 2.83 | 2.83 | 2.00 | 2.67 | 3.08 | 3.04 | 3.00 | 2.58 |
| QG/MAN/Sludge | 3.63 | 1.58 | 2.58 | 1.75 | 2.33 | 1.67 | 2.96 | 2.67 | 3.00 | 2.00 | 2.88 | 3.54 | 3.33 | 2.83 | 2.83 |
| QG/MEC/Ctrl | 3.17 | 3.58 | 3.42 | 2.00 | 1.83 | 2.25 | 3.67 | 3.33 | 3.67 | 2.25 | 3.04 | 3.42 | 3.42 | 3.29 | 3.25 |
| QG/MEC/Bioc | 3.58 | 3.04 | 3.04 | 2.25 | 1.92 | 1.83 | 3.25 | 2.83 | 3.33 | 1.83 | 2.88 | 3.46 | 3.29 | 3.21 | 3.21 |
| QG/MEC/MSWC | 3.33 | 3.38 | 3.08 | 2.17 | 2.25 | 2.50 | 3.58 | 2.92 | 3.42 | 1.92 | 2.83 | 3.00 | 3.17 | 3.04 | 3.17 |
| QG/MEC/Manure | 3.33 | 2.96 | 3.25 | 2.08 | 2.00 | 2.46 | 3.46 | 3.08 | 3.42 | 2.17 | 2.88 | 3.54 | 3.50 | 3.21 | 3.42 |
| QG/MEC/Sludge | 2.88 | 1.58 | 2.75 | 1.58 | 2.00 | 1.67 | 2.75 | 2.42 | 2.33 | 1.92 | 2.42 | 3.50 | 3.17 | 2.58 | 2.33 |

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 (Sludge). Site: Quinta do Côro (QC), Quinta do Gradil (QG).

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**Supplemental Table 12** - Interaction between pruning system and organic amendments effect in wine sensory analysis.

| | **Body (Taste)** | | **Global appreciation** |
|---|---|---|---|
| | **2014** | **2015** | **2015** |
| MAN/Ctrl | 3.58 a | 3.21 abc | 3.13 abc |
| MAN/Bioc | 3.28 abc | 3.38 ab | 3.50 a |
| MAN/MSWC | 3.69 a | 3.00 abc | 2.75 cd |
| MAN/Manure | 3.48 ab | 3.25 abc | 2.96 abc |
| MAN/Sludge | 3.18 abcd | 2.79 cd | 2.75 cd |
| MEC/Ctrl | 3.45 ab | 3.58 a | 3.42 ab |
| MEC/Bioc | 3.43 ab | 3.21 abc | 3.04 abc |
| MEC/MSWC | 2.75 cd | 2.96 bc | 2.83 bcd |
| MEC/Manure | 2.93 bcd | 3.13 abc | 3.08 abc |
| MEC/Sludge | 2.60 d | 2.21 d | 2.21 d |
| *Sig.*[1] | *** | ** | ** |

1 Sig. – Significance level: n.s. – non-significant at $p < 0.05$ level by F test; significant at $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) by F test. In each column values followed by the same letter do not significantly differ by Tukey HSD test at $\alpha = 0.05$. 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 (Sludge).

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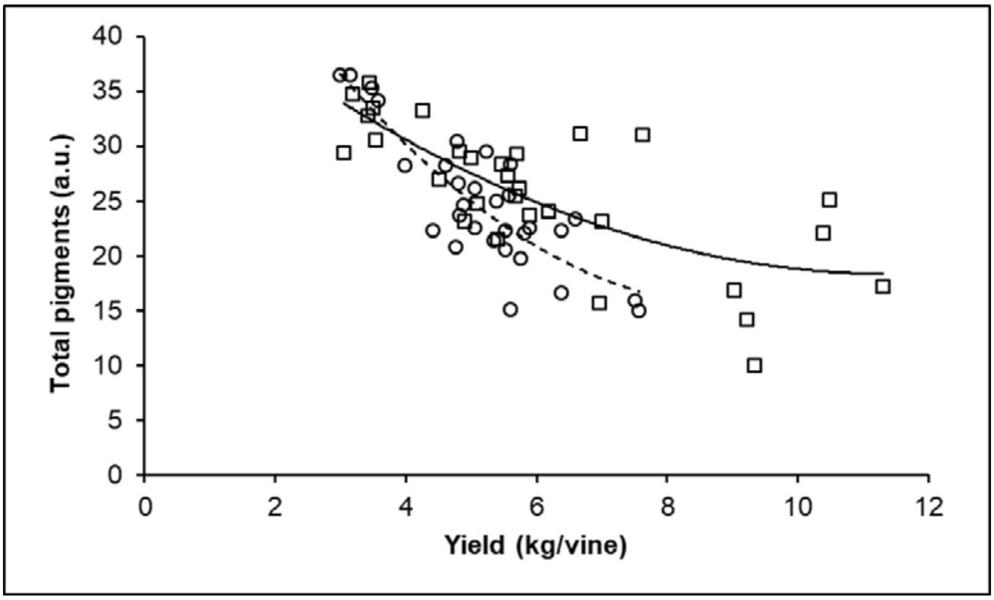
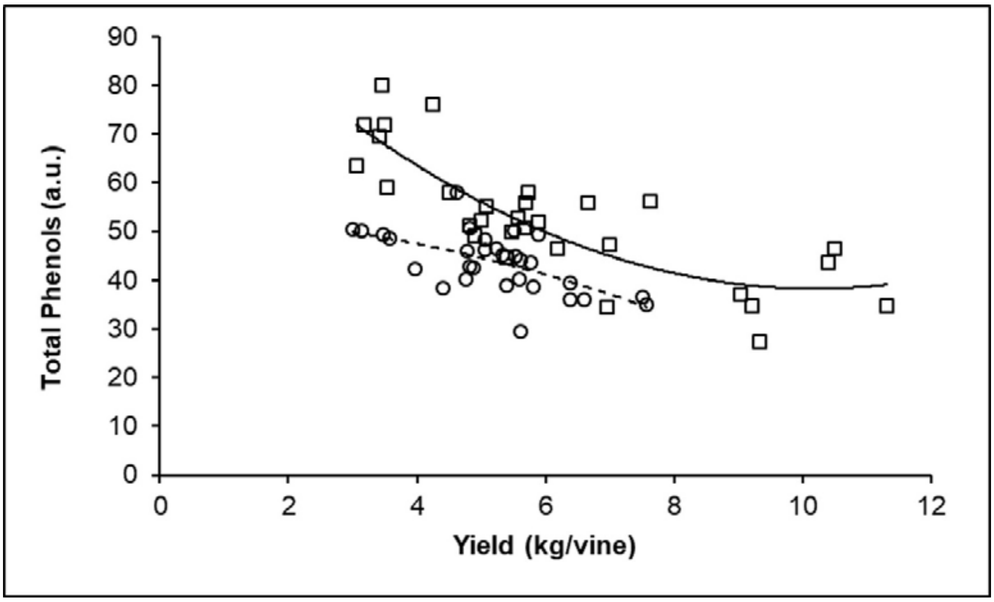



**Supplemental Figure 1** – Influence of the site on the relation between yield and total pigments of Syrah wines. Data represent single treatment (pruning system × organic amendment × site) averages and data were pooled over repetitions: □ - Quinta do Côro (QC); ○ - Quinta do Gradil (QG). Regression equations:
$y = 0.22x^2 - 5.06x + 47.28$, $r^2 = 0.57$, p-value $< 0.0001$ (QC);
$y = 0.57x^2 – 10.34x + 62.43$, $r^2 = 0.71$, p-value $< 0.0001$ (QG).




**Supplemental Figure 2** – Influence of the site on the relation between yield and total phenols of Syrah wines. Data represent single treatment (pruning system × organic amendment × site) averages and data were pooled over repetitions: □ - Quinta do Côro (QC); ○ - Quinta do Gradil (QG). Regression equations:
$y = 0.66x^2 – 13.47x + 106.88$, $r^2 = 0.69$, p-value $< 0.0001$ (QC);
$y = -0.29x^2 – 0.30x + 53.29$, $r^2 = 0.68$, p-value $= 0.0005$ (QG).