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Mechanical Pruning and Soil Fertilization with Distinct Organic Amendments in Vineyards of Syrah: Effects on Vegetative and Reproductive Growth

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Received: 18 June 2020; Accepted: 22 July 2020; Published: 28 July 2020

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Abstract: The effects of fully mechanized pruning systems on physiology, vigor and yield of grapevines have been studied for more than forty years and are an interesting way to reduce production costs. Organic amending of vineyard soil is a way to increase vine productivity. Thus, this study aims to understand the effects of the interaction between these two practices, on vine growth and productivity. Two trial fields were implemented on Shiraz vineyards in 2 different wine regions. Mechanical hedge pruning was compared with hand spur pruning and four different organic amendments were tested: biochar; municipal solid waste compost; cattle manure; sewage sludge. Mechanical pruning and organic amendments, by themselves, did not significantly increase yield. However, the interaction between both factors reduced the impact of self-regulation mechanisms (budburst, bud fruitfulness and cluster weight) in mechanical pruning and successfully increased yield, without decreasing vegetative growth. The effects of mechanical pruning with the application of organic amendments to soil on yield is significant and, thereby, the choice of the organic amendment and its amount, must be done considering the destiny of produced grapes.

Keywords: cattle manure; dry mass production; leaf area; municipal solid waste compost; sewage sludge; water availability; yield

1. Introduction

The effects of fully mechanized pruning systems on physiology and yield of grapevines and on grapes and wines quality have been studied since the early 1970's [1]. However, the interaction between the mechanical pruning and plant nutrition has not yet been evaluated, despite the fact that the interactions of irrigation with mechanical pruning [2] as well as with pruning level have given significant results [3]. It is known that mechanical pruning strongly increases the bud load per vine [4,5] and, due to that, the plant autoregulation capacity, which is influenced among other factors by soil fertility, becomes the major regulator of yield and, consequently, grape and wine quality.

Fully mechanized pruning systems induce a significant increase in bud load, which leads to compensation mechanisms that downregulate bud break [6], shoot fruitfulness [7] and, in a lesser extent, cluster weight [6,8]. However, the higher number of clusters per vine [4,8], usually increases yield [4], although it does not always occur [8,9] due to the referred autoregulation mechanisms.

Mechanically pruned grapevines develop a higher leaf area in the beginning of the season [9], nevertheless the differences are not always maintained until the end of the season [10]. The higher the bud load difference is, the later the leaf area become equal between pruning systems [11] and, at veraison, there are no differences [2,10]. Besides, photosynthetic rates are higher in free growing canopies [12], which are more suitable for mechanical pruning, than in those forced between catch wires.

Mechanical pruning leads to an increase in the shoot number per plant and to a decrease of the individual shoot weight [8,13]. The total pruning weight per vine is usually lower in mechanically pruned grapevines [10,14], reflecting the higher competition of reproductive growth for available assimilates.

One of the limiting factors of vineyard productivity in Portugal is the lack of fertility of the soils, which is strictly correlated with the low organic matter content [15]. In a climate change scenario, Mediterranean viticulture is facing serious risks and adaptation measures are needed, in order to face this problem. Among those measures, organic fertilization of soil is highlighted [16] because organic matter plays an important role in long-term soil conservation and/or restoration by sustaining its fertility, due to the improvement of physical, chemical and biological properties of soils [15].

In Europe, vineyards are one of the agricultural ecosystems with the lowest carbon content in the soil [17] and the one with the highest risk of erosion [18]. The decrease of carbon content in vineyard soils is intensified in Mediterranean regions, due to climatic conditions, namely high temperatures associated with large periods of drought, during late spring and summer, which are expected to worsen with the expected climate changes [16].

No tillage, no removal of pruning wood from the vineyard soil or the usage of crop rotations, are some of the strategies that may be used to raise the soil organic matter content [19]. However, soil fertility can also be improved with organic waste application which generally presents high contents of organic matter, substantial quantities of nutrients and their use in agriculture can contribute to close the natural ecological cycles [19,20].

The reduction of the availability of animal manures, which led to a decrease in the practice of replacing soil organic matter, is compensated by the increasing availability of "unconventional" organic fertilizers, obtained from alternative residues [21]. There is a particular interest in composted municipal solid waste (MSWC) and sewage sludge (Sludge), due to their availability, and in coal resulting from the pyrolysis of biomass (biochar), due to its potential of carbon fixation in soil.

The chemical modifications of soil characteristics due to the application of organic amendments can play a significant role in the nutrient status of the vine and, consequently, affect yield [22].

The effects of nitrogen (N) on the grapevine have already been extensively studied. Nitrogen fertilization tends to induce an increase in yield [23] up to a certain level, that is around 40 and 56 kg N ha^{-1} [24,25]. In agreement with these results, Morris et al. [26], with 152 kg N ha^{-1} and 228 kg N ha^{-1}, did not notice any increase in yield. However, the response to N is not always the same, as reported by Wolf and Pool [27] who observed a tendential decrease in yield with the increase of N application rate from 39 to 84 kg N ha^{-1}. Usually the organic amendment that provides the larger amount of this nutrient is sewage sludge (Sludge), while municipal solid waste compost (MSWC) and cattle manure also provide it but in a lower extent [21,28,29].

Organic amendments supply also phosphorus (P), particularly Sludge, which effects on grapevine are not widely studied, since the grapevine requires only small amounts of this nutrient. According to Schreiner et al. [23], P fertilization did not affect growth and yield, while Conradie and Saayman [24] observed an increase in yield and growth with only 9 kg P ha^{-1} $year^{-1}$, in a soil that was originally deficient in this nutrient.

Potassium (K) is another of the macronutrients that is supplied by organic amendments. However, the results of its application are not consistent with some authors finding slight increases in yield [24], while others found no differences [30] or even small reductions [31].

The effects of Sludge and MSW amending on yield, vegetative growth and grape composition have been poorly studied, but an increase of yield was observed with the application of a sewage sludge compost in a Cabernet Sauvignon vineyard [32], while, in a Merlot experiment, no differences were found in yield, but only a decrease in pruning weight [33]. Concerning MSW application, no differences were found in vegetative and reproductive growth [33,34]. About soil amending with cattle manure the results are not always consistent, while in one trial significant increases in yield were observed since the second year of application [35], in another study, no significant differences were found with a 28-year application [22].

Biochar is a co-product of a thermochemical conversion of biomass that is recognized to be a beneficial soil amendment which when incorporated into the soil increases the retention and availability of water [36] and nutrients [37]. Few works have been made regarding biochar application in vineyards and its influence on yield and quality of grape and wine. Nevertheless, with the application of 22 Mg ha^{-1} year^{-1} of biochar, an increase in productivity was observed, mainly due to water availability, without loss of quality [38]. However, in another work, biochar and biochar-compost treatments induced only small, economically irrelevant and mostly non-significant effects on yield and quality over the three years of the experiment [39].

Mechanical pruning appears to be an interesting way to reduce annual production costs. On the other hand, organic amendments improve soil fertility and tends to increase productivity. However, the interaction between mechanical pruning and the organic amending of vineyard soil was not previously studied, to the best of our knowledge. The objective of this work is to study if the increase of soil fertility by the organic amending will reduce the impact of self-regulation mechanisms in mechanically pruned vines maintaining or increasing vegetative and reproductive growth.

2. Materials and Methods

2.1. Vineyard Sites and Experimental Layout

The trial, run over four years (2012 to 2015), was installed in two vineyards of *Vitis vinifera* L. cv. Shiraz. Quinta do Côro (QC) experimental site is located in Tejo wine region and the vines had been grafted on 99R in 1999 and spaced 1.0 m × 2.5 m, for a density of 4000 plants ha^{-1}. In Quinta do Gradil (QG) experimental site, which was located in Lisboa wine region, the vines, grafted on 1103P, were planted in 2005 and spaced 1.0 m × 2.6 m, for a density of 3846 plants ha^{-1}. Row orientation is N-S in both cases.

The soil in QC was a Hypereutric Regosol [40], with a sandy-loam texture, a pH_{H2O} of 6.4, a low organic matter content (1.54%), an extractable K and P contents of 70.7 mg K kg^{-1} and 59.8 mg P kg^{-1} (ammonium lactate extraction—[41]), respectively. In QG soil was also a Hypereutric Regosol [40], with a sandy-loam textures, a pH_{H2O} of 5.9, a low organic matter content (1.07%), an extractable K and P contents of 167.0 mg K kg^{-1} and 61.2 mg P kg^{-1} (ammonium lactate extraction—[41]), respectively.

The climate in QC is a Csa and in QG is a Csb, according to the Köppen-Geiger climate classification [42]. Total rainfall and monthly mean air temperature data (Figures 1 and 2), were collected in the IPMA's weather station of Alvega (12 km from QC trial field) and in a weather station located in QG (200 m from the trial field), show differences between the two trial fields and among four years of the work. The mean air temperature amplitude was higher in QC, with cooler winters but warmer summers. Among the four years, the average of the highest mean air temperatures was 23.6 °C in QC while in QG was 20.3 °C. The warmer summer in QC led to a earlier grape maturation when compared to QG.

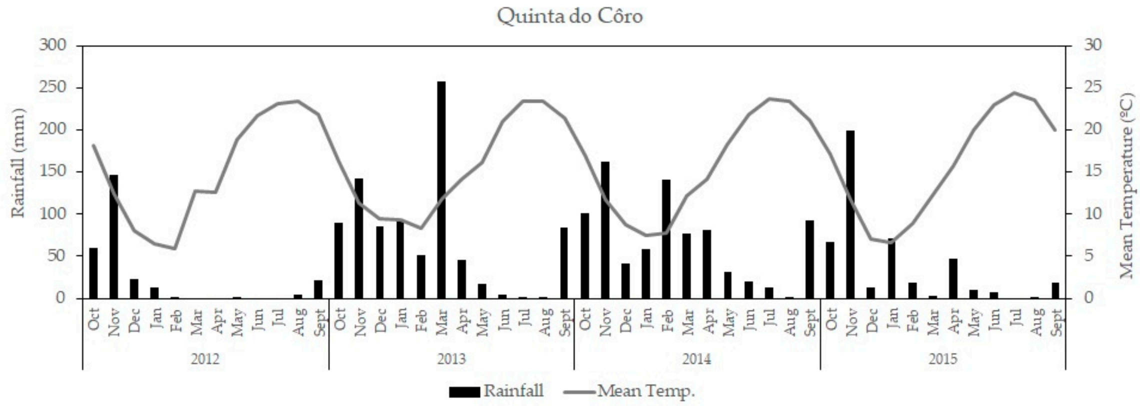

Figure 1. Monthly total rainfall and mean air temperature in Quinta do Côro.

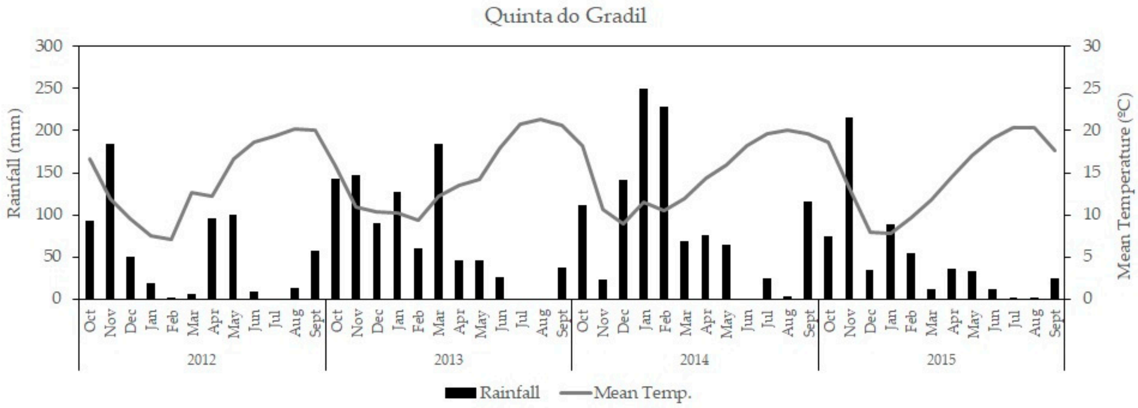

Figure 2. Monthly total rainfall and mean air temperature in Quinta do Gradil.

In what concerns to precipitation, the rainier experimental site was QG (Table 1) with an average annual rainfall of 812 mm comparing to 603 mm in QC. The precipitation was the highest in both experimental sites in 2014, with 819 mm in QC and 1112 in QG, while 2012 was the driest year only with 269 mm in QC and 635 mm in QG.

The studied factors were the pruning system and organic amendment that were compared in a strip-plot design, with three replications, installed in both trial fields. Each block held eight adjacent rows where pruning treatment was randomly assigned, creating two groups of four adjacent rows 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. The organic amendments were spread all over the seven interrows, creating a stripe perpendicular to the row orientation. Each one of the 30 experimental units consisted of 48 vines.

In what concerns to pruning, two treatments were imposed: MAN—manual spur pruning, retaining six to seven 2-bud spurs per vine; MEC - mechanical pruning, simulating the pruning effect of four cutting bars (2 parallel and 2 perpendicular to the ground) working at a distance of 15 cm from the cordon. All wood with ventral insertion was removed. In MAN treatment, the training system was a spur pruned Royat cordon, established at 70 cm above soil surface, with vertical shoot positioning. Mobile wires were moved once in the season (right before bloom) in order to position shoots which were later trimmed in order to create a parallelepipedic canopy. In MEC treatment, the cordon was also established at 70 cm above soil surface and the wires stayed in the same position (40 cm and 80 cm above the cordon) the whole year, and shoots were not positioned. Thus, some shoots grabbed their tendrils to the wires and maintained a vertical position, while the others grew freely in oblique or horizontal positions, creating a larger and sparser canopy. Canopy management, in MEC, was limited to light and wide mechanical shoot trimming performed to promote upright growth and optimal machine operating conditions for the harvest and the next winter pruning. Shoots were tipped before bloom and veraison to retain approximately 9 to 10 leaves on the main shoots.

Table 1. Average composition of the organic amendments along the 4 years.

	Bioc	**MSWC**	**Manure**	**Sludge**
fresh matter basis				
pH	8.99	7.71	7.00	9.64
Electrical conductivity (mS/m)	69.1	413.7	522.0	263.3
Moisture (%)	23.8	44.2	63.0	78.0
dry matter basis				
Organic Matter(%)	72.3 ± 12.32	46.5 ± 10.03	67.5 ± 9.48	67.8 ± 6.48
Total N (%)	1.0 ± 0.44	2.1 ± 0.16	2.4 ± 0.72	6.8 ± 0.26
Total P (g kg^{-1})	0.8 ± 0.45	6.9 ± 0.46	4.2 ± 1.22	13.5 ± 1.94
Total K (g kg^{-1})	5.2 ± 1.44	7.8 ± 0.25	18 ± 1.36	3.2 ± 0.77
Total Ca (g kg^{-1})	36.3 ± 6.94	72.7 ± 18.00	16.4 ± 0.55	66.5 ± 16.49
Total Mg (g kg^{-1})	2.2 ± 0.49	14.9 ± 3.04	4.8 ± 0.44	4.6 ± 0.63
Total S (g kg^{-1})	1.4 ± 0.23	2.9 ± 0.16	3.3 ± 2.59	7.6 ± 0.18
Total Na (g kg^{-1})	0.5 ± 3.02	6 ± 2.65	6.6 ± 2.68	0.8 ± 5.50
Total Fe (g kg^{-1})	5.2 ± 0.07	8 ± 0.08	3.1 ± 0.07	9.3 ± 0.06
Total Mn (mg kg^{-1})	144.9 ± <0.01	249.9 ± 0.02	223.3 ± 0.04	105.4 ± 0.02
Total Cu (mg kg^{-1})	10.8 ± 0.00	132.2 ± 0.08	45.2 ± 0.05	137.6 ± 0.18
Total Zn (mg kg^{-1})	18.1 ± 0.01	360.8 ± 0.02	134.1 ± 0.01	831.4 ± <0.01
Total B (mg kg^{-1})	18.7 ± 1.49	26.1 ± 0.93	22 ± 0.50	28.2 ± 3.19
Total Ni (mg kg^{-1})	2.9 ± <0.01	10 ± 0.01	5.2 ± 0.01	6 ± 0.01
Total Cd (mg kg^{-1})	0.03 ± <0.01	0.03 ± <0.01	0.07 ± <0.01	0.05 ± <0.01
Total Pb (mg kg^{-1})	14.8 ± <0.01	79.6 ± 0.07	3.4 ± <0.01	24.9 ± 0.02
Total Cr (mg kg^{-1})	8.6 ± 0.01	27.8 ± 0.03	4.6 ± <0.01	20.3 ± 0.02
Total Hg (mg kg^{-1})	0.01 ± 0.01	0.41 ± 0.29	0.02 ± 0.01	0.51 ± 0.41

In each cell is presented the four years average ± standard deviation. When the standard deviation is lower than 0.01, the value is replaced by <0.01. Biochar (Bioc), municipal solid waste compost (MSWC), cattle manure (Manure) and sewage sludge compost (Sludge).

In relation to organic amendments, five treatments were imposed: Ctrl—no application of organic amendment neither fertilizer; Bioc—application of 8500 kg ha^{-1} year^{-1} of char dust; MSWC—application of 16,100 kg ha^{-1} year^{-1} of municipal solid waste compost; Manure—application of 24,000 kg ha^{-1} year^{-1} of cattle manure; Sludge—application of 34000 kg ha^{-1} year^{-1} 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 the organic amendments is presented in Table 1.

- The organic amendments were spread over the soil and incorporated with a light disk harrow, before bud burst. The nutrients supplied by each organic amendment are the following:
- Bioc—72.0 kg ha^{-1} year^{-1} of total N, 7.2 kg ha^{-1} year^{-1} of total P and 43.8 kg ha^{-1} year^{-1} of total K;
- MSWC—226.0 kg ha^{-1} year^{-1} of total N, 110.4 kg ha^{-1} year^{-1} of total P and 124.9 kg ha^{-1} year^{-1} of total K;
- Manure—179.4 kg ha^{-1} year^{-1} of total N, 101.9 kg ha^{-1} year^{-1} of total P and 432.2 kg ha^{-1} year^{-1} of total K;
- Sludge—503.7 kg ha^{-1} year^{-1} of total N, 460.5 kg ha^{-1} year^{-1} of total P and 108.6 kg ha^{-1} year^{-1} of total K.

2.2. Reproductive and Vegetative Growth

In order to determine yield components, the number of clusters per vine and their weight were assessed at harvest in six previously selected vines in each replication. Berry weight was assessed in 100 berries samples, randomly collected in each experimental unit, and the number of berries per cluster was estimated by the ratio between cluster and berry weight. During dormant pruning, the number of canes per vine, including water sprouts, and their weight was measured, in order to evaluate the effect of the different treatments on vegetative growth. The dry matter production was calculated as proposed by Carbonneau and Cargnello [43]: DMP = 0.2 × yield + 0.5 × pruning weight. The Ravaz index was calculated by dividing the yield per vine by the dormant pruning weight of the same vine in the following winter. Right after pruning, the bud load was assessed in the same six vines. The vines where the pruning data was collected were the same that were evaluated at harvest.

2.3. Statistical Analysis

Data were tested to verify if the assumptions of analysis of variance (ANOVA) were met using Shapiro-Wilk's test and then subjected to three-way ANOVA (site × pruning × organic amendment), 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, USA).

In the following tables the presented values are averages of both trial fields.

3. Results

3.1. Shoot and Cluster Number

On Table 2 are presented the results of bud load as well as shoot, water sprout and cluster number per vine. The bud load as well as the number of shoots per vine was significantly higher in MEC. The budburst percentage was lower in MEC (60%) when compared to MAN (100%). The differences between pruning systems in what concerns to bud load were higher than to shoots number per vine (74% and 37% average, respectively), due to the higher water sprout number in MAN. While the number of water sprouts in MEC was residual (between 1 and 3%), in MAN it accounted for almost 41% of the total number of shoots per vine. In result of the higher number of shoots, the number of clusters per vine in MEC was also significantly higher than in MAN (52%). Even though the interaction between pruning and experimental site was significant in three years in each site the cluster number per vine was always significantly higher in MEC.

Organic amendments had no significant effects, neither on bud load nor on water sprout number per vine. However, the shoot number was affected in the last two years of the trial, being higher in MSWC, Manure and Sludge. In consequence of the higher shoots number per vine, these treatments also had more clusters per vine.

In shoot number per vine, the interaction between the two studied factors was significant in the last two years of the trial, because shoot number was significantly affected by organic amendments only in MEC. The interaction between pruning and organic amendment was significant for cluster number per vine in 2015, also because it was significantly affected by organic amendment only in mechanically pruned treatments.

Table 2. Pruning system and organic amendment effect in bud load, shoot, water sprouts and cluster number per vine.

	Bud Load				**Shoot Number Per Vine**				**Water Sprout Number per Vine**				**Cluster Number Per Vine**			
	2012	**2013**	**2014**	**2015**	**2012**	**2013**	**2014**	**2015**	**2012**	**2013**	**2014**	**2015**	**2012**	**2013**	**2014**	**2015**
MAN	15.0	12.1	14.0	14.3	19.2	20.3	20.4	21.6	8.63	8.19	8.16	8.23	31.6	30.9	27.8	43.5
MEC	43.3	49.3	64.2	62.8	26.5	33.2	36.4	34.1	0.83	0.66	0.54	0.75	57.1	68.1	72.6	81.2
Sig. [1]	**	***	***	***	**	**	***	***	**	***	**	**	**	**	**	***
Ctrl	29.3	30.5	39.6	38.4	23.1	26.2	26.9 c	26.7 ab	5.25	4.34	4.08	4.12	42.5	50.1	45.2 b	54.5 b
Bioc	29.4	29.0	37.1	35.3	22.3	25.9	27.4 bc	25.4 b	4.30	4.92	4.38	4.60	44.4	45.2	49.2 ab	52.7 b
MSWC	29.5	29.9	39.7	38.5	23.3	26.9	29.3 ab	29.0 a	4.58	4.29	4.69	4.72	46.0	47.9	53.1 a	65.8 a
Manure	29.0	32.1	38.6	38.3	22.7	27.0	28.6 abc	28.7 a	5.11	4.38	3.94	4.32	45.8	51.2	50.8 ab	65.7 a
Sludge	28.5	30.4	40.5	40.0	22.9	27.2	29.9 a	28.7 a	4.41	4.56	4.66	5.01	43.1	51.4	52.7 ab	70.6 a
Sig. [1]	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	33.6	34.1	42.7	41.3	26.6	30.1	31.9	29.0	4.75	5.96	5.81	6.11	52.5	52.7	60.8	66.8
QG	24.7	26.5	35.5	35.0	19.1	23.1	24.9	26.5	4.71	2.97	2.89	3.05	36.2	45.6	39.6	57.4
Site	*	n.s.	n.s.	n.s.	**	**	*	n.s.	n.s.	**	**	*	**	n.s.	*	n.s.
Pruning × O.A.	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.	***
Site × Pruning	*	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	**	*	*	*	n.s.	**	**
Site × O.A.	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.	**
Site × Pruning × O.A.	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.

[1] Sig.—Significance level: n.a.—not applicable; 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 compost (Sludge). Site: Quinta do Côro (QC) and Quinta do Gradil (QG).

3.2. Cluster and Berry Weight

Table 3 presents the results of berry and cluster weight as well as the berry number per cluster. Cluster weight was, consistently, significantly lower in MEC, with a 39% difference between treatments. In 2014 the cluster weight was the highest of the four years of trial in both treatments and, among years, cluster weight was more stable in MAN than in MEC.

Table 3. Pruning system and organic amendment effect in berry and cluster weight and berry number per cluster.

	Berry Weight (g)			Berry Number Per Cluster			Cluster Weight (g)			
	2012	**2013**	**2014**	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**	**2015**
MAN	1.55	1.46	2.05	91.3	92.9	78.2	141	139	155	140
MEC	1.30	1.24	1.73	72.6	62.0	58.8	95	77	100	82
Sig.[1]	*	**	*	*	**	*	**	**	**	***
Ctrl	1.40	1.31	1.82 bc	82.1	69.4 b	64.4	117	95 b	117 b	101
Bioc	1.45	1.34	1.79 c	80.8	80.8 ab	68.0	119	112 ab	121 b	113
MSWC	1.42	1.34	1.92 ab	81.7	77.5 ab	69.6	118	108 ab	132 ab	114
Manure	1.46	1.34	1.94 ab	83.9	77.1 ab	68.3	124	106 ab	130 ab	111
Sludge	1.40	1.43	1.97 a	81.2	82.9 a	72.4	114	122 a	138 a	118
Sig.[1]	n.s.	n.s.	***	n.s.	**	n.s.	n.s.	*	**	n.s.
QC	1.40	1.21	1.65	74.5	65.5	80.7	106	80	134	121
QG	1.45	1.49	2.13	89.4	90.3	56.3	130	138	120	102
Site	n.s.	*	**	n.s.	*	*	n.s.	**	n.s.	n.s.
Pruning × O.A.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Site × Pruning	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	*	n.s.	n.s.
Site × O.A.	n.s.	n.s.	**	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.
Site × Pruning × O.A.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[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 compost (Sludge). Site: Quinta do Côro (QC) and Quinta do Gradil (QG).

Regarding the effect of the organic amendments on cluster weight, significant differences were observed only in 2013 and 2014. In 2013, the difference was significant only between Sludge and Ctrl (22%). In 2014, Sludge was significantly higher than Bioc and Ctrl, being the differences of 12% and 15% respectively. In 2012 and 2015 there were no significant differences in cluster weight between organic amendments.

The berry weight and its number per cluster were assessed in 2012, 2013 and in 2014 and the results show that the decrease in cluster weight in MEC is due to a significant decrease in berry weight as well as in berry number per cluster in the three years. The effect of the organic amendments is not so consistent since in 2013 the berry weight was not affected by this factor while the berry number was significantly increased by Sludge. On the other hand, in 2014 was the berry weight that was increased by Sludge, only in QG, while the berry weight was not significantly affected in any of the trial fields. In this year, in QG, the lowest berry weight was obtained in Ctrl and in Bioc (2.00 g in both treatments) and it was significantly lower than Sludge (2.31 g).

3.3. Yield

In Table 4 is presented the effect of the studied factors in yield per plant. Yield was significantly increased by MEC only in 2014. In the other three years a not significant tendency for higher yield in MEC was observed. Globally, MEC produced 24% more than MAN. The organic amendments had a significant effect on yield since the second year of the trial. Sludge was the organic amendment with a faster effect on yield, while MSWC and Manure took more time to take effect. In 2013, Sludge produced more than Ctrl, Bioc and MSWC. In 2014, Sludge had a significantly higher yield when compared to Ctrl and Bioc. In 2015, Sludge, manure and MSWC produced more than Ctrl and Bioc.

Table 4. Pruning system and organic amendment effect in yield.

	Yield (kg vine^{-1})			
	2012	**2013**	**2014**	**2015**
MAN	4.47	4.21	4.33	5.95
MEC	5.50	5.33	7.43	6.67
Sig.[1]	n.s.	n.s.	**	n.s.
Ctrl	4.70	4.18 b	4.82 c	5.03 b
Bioc	5.05	4.40 b	5.38 bc	5.35 b
MSWC	5.15	4.59 b	6.31 ab	6.87 a
Manure	5.34	4.98 ab	6.04 ab	6.61 a
Sludge	4.71	5.63 a	6.84 a	7.56 a
Sig.[1]	n.s.	***	***	***
QC	5.44	4.05	7.26	6.98
QG	4.53	5.50	4.50	5.65
Site	n.s.	n.s.	**	**
Pruning × O.A.	n.s.	**	**	**
Site × Pruning	n.s.	n.s.	*	n.s.
Site × O.A.	n.s.	n.s.	n.s.	*
Site × Pruning × O.A.	n.s.	n.s.	n.s.	n.s.

[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 compost (Sludge). Site: Quinta do Côro (QC) and Quinta do Gradil (QG).

One interesting fact is that in the three years when the organic amendments originated significant effects on yield, there was a significant interaction between this factor and the pruning system. The analysis of the interaction between both factors (Figure 3) reveals that the effect of the organic amendments was significant only in MEC, while in MAN the organic amendments had no significant effect on yield.

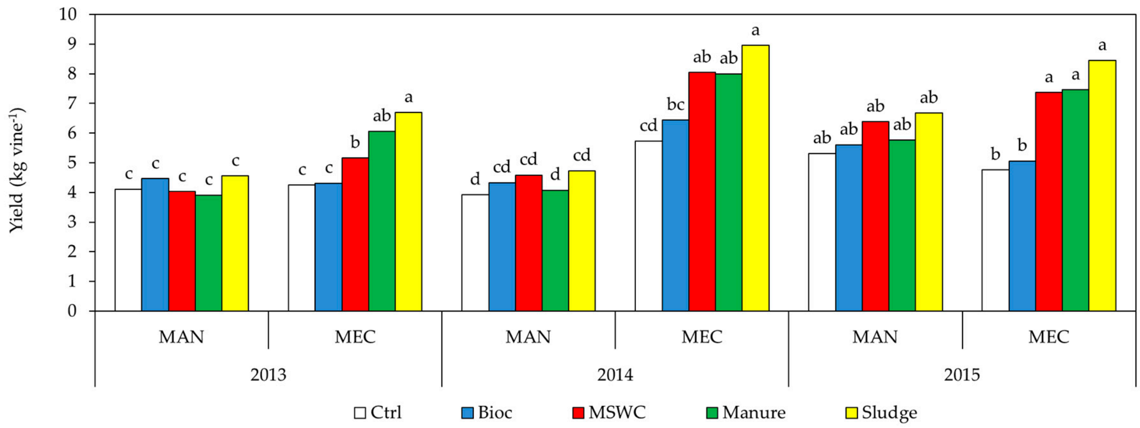

Figure 3. Interaction between pruning system and organic amendment effect on yield. In each year columns with by the same letter do not significantly differ by Tukey HSD test at $\alpha = 0.05$. Pruning system: manual pruning (MAN), mechanical pruning (MEC); Organic Amendment: control (Ctrl), biochar (Bioc), municipal solid waste compost (MSWC), cattle manure (Manure), sewage sludge (Sludge).

In MEC, the higher yield was obtained in Sludge, followed by MSWC and Manure, while Bioc did not significantly differ from Ctrl in any year. MEC*Ctrl and MEC*Bioc were not significantly different from MAN treatments in any year.

3.4. Vegetative Growth

Table 5 presents the results of shoot weight and of the pruning weight per vine. Mechanical pruning significantly reduced the shoot individual weight. In the first year, the reduction in shoot weight was lower (37%) than in the next three years (between 51% and 61%).

Table 5. Pruning system and organic amendment effect on shoot weight and pruning weight per vine.

	Shoot Weight (g)				Pruning Weight (kg vine^{-1})			
	2012	**2013**	**2014**	**2015**	**2012**	**2013**	**2014**	**2015**
MAN	41.8	43.5	55.0	43.9	0.802	0.888	1.087	0.945
MEC	26.3	21.3	24.1	17.2	0.699	0.736	0.874	0.600
Sig.[1]	*	*	**	**	n.s.	n.s.	n.s.	**
Ctrl	32.8	30.2	36.5 b	27.5 b	0.733	0.735	0.867 b	0.680 b
Bioc	34.7	33.4	39.4 ab	31.4 ab	0.749	0.814	0.942 b	0.727 b
MSWC	33.5	33.9	40.3 ab	33.6 a	0.761	0.841	1.028 ab	0.881 a
Manure	36.0	30.6	36.8 b	27.0 b	0.773	0.795	0.909 b	0.703 b
Sludge	33.2	35.0	44.6 a	34.5 a	0.737	0.882	1.158 a	0.881 a
Sig.[1]	n.s.	n.s.	**	***	n.s.	n.s.	***	***
QC	31.4	32.7	33.5	36.3	0.833	0.947	0.954	0.926
QG	36.7	32.4	45.6	25.5	0.668	0.675	1.008	0.629
Site	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	*
Pruning × O.A.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Site × Pruning	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	*
Site × O.A.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	**
Site × Pruning × O.A.	n.s.	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.

[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 compost (Sludge). Site: Quinta do Côro (QC) and Quinta do Gradil (QG).

Pruning weight per vine was not significantly different between pruning systems in the first three years of trial. In the last year, pruning weight was significantly lower in MEC. Although the differences were not significant, in all the three first years there was a tendency for lower pruning weight in mechanically pruned vines. The difference between pruning systems oscillated between 103 g (13%), in the first year, and 345 g per plant (36%), in the last one.

The organic amendments started to have effect in vegetative growth only from 2014 on. Sludge and MSWC treatments were those where shoot and pruning weight were higher, while Ctrl and Manure were the lowest. Bioc had an intermediate behavior and did not significantly differ from all the other treatments, in terms of shoot weight, and, like Ctrl and Manure, had a significantly lower pruning weight when compared to Sludge and MSWC.

3.5. Dry Matter Production and Ravaz Index

Dry matter production and Ravaz Index results are presented in Table 6. Mechanical pruning induced a significant increase of 26% in dry matter production, in 2014. Dry matter production tended to be higher also in 2012 and in 2013, nevertheless, in 2015, both treatments had equal values of this variable. On the other hand, Ravaz Index was consistently increased by mechanical pruning, due to the previously referred rise in yield and reduction of the pruning weight. The difference among pruning systems was higher in 2014 (55%) and the higher values of this index were obtained in 2015, in both pruning systems.

Table 6. Pruning system and organic amendment effect on dry matter production per vine and Ravaz Index.

	Dry Matter Production (kg vine^{-1})				Ravaz Index			
	2012	**2013**	**2014**	**2015**	**2012**	**2013**	**2014**	**2015**
MAN	1.30	1.29	1.41	1.66	5.98	5.25	4.39	7.01
MEC	1.45	1.43	1.92	1.63	7.97	8.34	9.68	11.59
Sig. [1]	n.s.	n.s.	**	n.s.	**	*	**	**
Ctrl	1.31	1.20b	1.40c	1.35c	6.76	6.81	6.45	8.75
Bioc	1.38	1.29b	1.55bc	1.43bc	7.23	6.17	6.72	8.32
MSWC	1.41	1.34ab	1.78ab	1.82a	7.06	6.43	7.75	8.90
Manure	1.45	1.39ab	1.66abc	1.67ab	7.20	7.04	7.45	10.16
Sludge	1.31	1.57a	1.95a	1.95a	6.62	7.33	6.78	10.09
Sig. [1]	n.s.	**	***	***	n.s.	n.s.	n.s.	n.s.
QC	1.51	1.28	1.93	1.86	6.72	4.54	9.00	8.77
QG	1.24	1.44	1.40	1.44	7.22	9.07	5.06	9.73
Site	n.s.	n.s.	*	**	n.s.	**	*	n.s.
Pruning × O.A.	n.s.	**	*	*	n.s.	n.s.	*	n.s.
Site × Pruning	n.s.	n.s.	n.s.	n.s.*	n.s.	n.s.	n.s.	n.s.
Site × O.A.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.
Site × Pruning × O.A.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[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 compost (Sludge). Site: Quinta do Côro (QC) and Quinta do Gradil (QG).

Organic amendments started to have effect on dry matter production from the second year on. Sludge was significantly higher than Ctrl and Bioc since the second year of the trial. MSWC significantly increased dry matter production, when compared to Ctrl, from 2013 on and Manure only induced significant effects in the last year of the trial. Bioc was never significantly different from Ctrl. Organic amendments had no significant effects on Ravaz Index along the trial.

The interaction between the pruning system and organic amendment effects in dry matter production is presented in Figure 4. The differences among organic amendments, in MAN, are not significant with the exception of Sludge that, in 2013, had more dry matter production than Manure and, in 2015, than Manure, Bioc and Ctrl. However, even though the differences were statistically significant, the maximum dry matter production difference among MAN treatments was 20% (between Sludge and Ctrl in 2015). On the other hand, in MEC, the dry matter differences, between organic amendments, was higher. The lower difference between Sludge and Ctrl was 34% and the higher was 42%. MSWC and Manure were significantly different from Ctrl every year and 2015 was the only season in which these two treatments were significantly different from Sludge. Bioc was never different from Ctrl.

The only year in which MEC*Ctrl and MEC*Bioc were lower than MAN*Ctrl and MAN*Bioc was 2015, with a difference of 21% and 19% respectively.

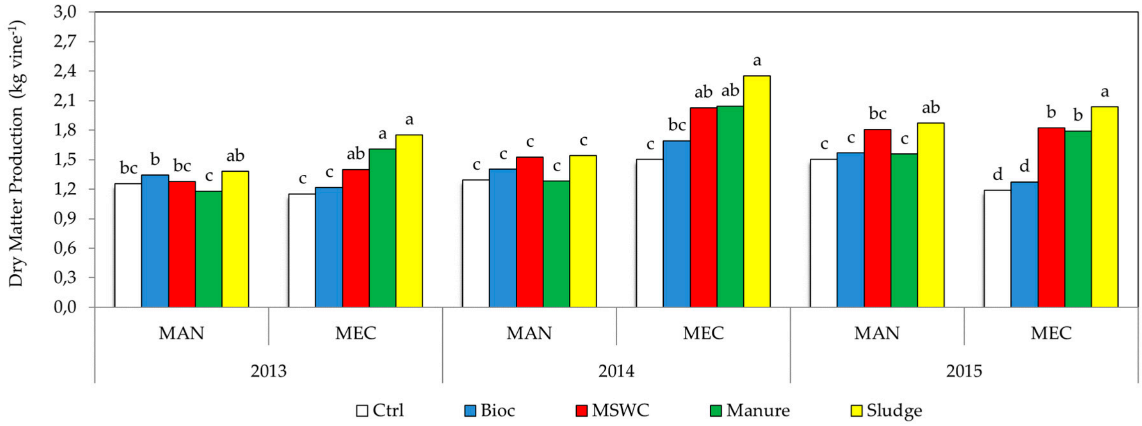

Figure 4. Interaction between pruning system and organic amendment effect on dry matter production. In each year columns with by the same letter do not significantly differ by Tukey HSD test at $\alpha = 0.05$. Pruning system: manual pruning (MAN), mechanical pruning (MEC); Organic Amendment: control (Ctrl), biochar (Bioc), municipal solid waste compost (MSWC), cattle manure (Manure), sewage sludge (Sludge).

4. Discussion

4.1. Shoot and Cluster Number

The bud load was significantly affected by the pruning system and, as observed in previous trials, the budburst percentage was lower in MEC due to self-regulation [6,44]. In consequence of the higher bud load, even with a lower budburst percentage, shoot number per vine was significantly higher in MEC, as reported in other works [8,13]. In MAN, a high proportion of water sprouts grew to compensate the lower bud load [6], but it was not enough to reach the shoot number per vine of MEC. In MEC, the water sprouts growth was residual due to the high bud load [45].

Shoot number per vine was affected by organic amendments only in the last two years of the trial, probably due to the significant residual effect of the cumulative applications, which effects on soil organic matter content and nutrients availability becomes visible only after some years [28].

One interesting fact is that shoot number per vine responded to organic amendments only in MEC, while in MAN no significant differences were recorded. Probably due to the higher bud load per vine, in MEC the higher vegetative growth, promoted by MSWC, Manure and Sludge, was directed preferably to the increase in budburst instead of individual shoot growth. Other authors, when comparing different nitrogen supplies using hand pruned vines, also found significant increases in shoot weight but not in shoot number per vine [46,47].

In what concerns to the number of clusters per vine, there was a significant difference between pruning systems. In MEC, the higher bud load and consequent higher shoot number per vine led to a significant increase in cluster number, as reported by other authors [5,8,48,49]. In addition to the lower shoot number, due to the lower bud load, in MAN, 30 to 50% of the shoots were water sprouts, that have lower fertility [45] and whose clusters are smaller [50].

Organic amendments, namely MSWC, Manure and Sludge, increased the cluster number per vine only in MEC and in the last two years. In MEC, the increase of cluster number observed in MSWC, Manure and Sludge is related to the higher budburst in these treatments. Working with Cabernet Sauvignon, Gaiotti et al. [35] also observed an increase in this variable with the application of 4 Mg ha^{-1} $year^{-1}$, of cattle manure, during 5 years. In that work, the definition of the bud load was based on the pruning weight of the previous year, so the amended treatments, which had higher vegetative growth, were pruned with a higher bud load and, consequently, had more clusters per vine. In MAN, where the bud load was similar among treatments, there were no differences in cluster number between organic amendments. With equal bud load per vine between treatments, no increases in the number of clusters per vine were recorded with 10 and 20 Mg ha^{-1} year^{-1} of cattle manure, in a Cabernet Franc vineyard, along 28 years [22].

4.2. Cluster Weight

The cluster weight was significantly and consistently reduced by mechanical pruning. Similar result was obtained in other studies [6,8,48,51]. The reduction in cluster weight occurred due to a decrease in berry weight, which is related to its size, and to a decrease in berry number per cluster, although in each year only one of the results was observed. Other studies reported a decrease in cluster weight due to a reduction in both variables [51,52] or only in berry weight [6].

Concerning to the effect of the organic amendments on cluster weight, Sludge was the only treatment that originated significant differences, when compared to Ctrl, as already observed in another work [32]. The higher cluster weight observed in Sludge was related both to the higher berry number per cluster and berry weight.

The organic amendments effects are probably related to the nutrients supplied to the plants, what is particularly relevant in sewage sludge due to the high amounts of N that this amendment provide to the soil. An increase in cluster weight with higher N availability has already been observed, related to the higher berry number per cluster [25,47] and to the berry weight [53].

4.3. Yield

In an overall analysis, the yield was significantly increased by mechanical pruning. However, although there was a tendency for higher yield in MEC in all the four years, the differences were significant only in 2014. Increases in yield with mechanical pruning were already reported [4,7,54]. However, in several works, the increase in yield was not significant in all the seasons, although, globally, mechanically pruned grapevines produced more than the manually pruned ones [8,55,56]. There are also some studies where mechanical pruning did not increase yield, when compared to manual spur pruning [9,57].

The higher bud load is the main reason for the increase in yield in fully mechanized pruning systems. However, although the higher bud load usually leads to significantly higher yields [58], this does not always occur [59]. According to the literature, the increase in yield due to the bud load occurs up to a certain level [58,60], which is different depending on the variety [58] and if that level is exceeded there may even occur decreases in production [60]. The increase in yield is not proportional to the increase of the bud load because, due to the self-regulation, there is a reduction in budburst [61], in bud fertility [62] and in cluster weight [63].

In the present work, the differences in yield due to pruning were significant only in 2014, corresponding to the year with the lower water deficit [64].Freeman et al. [3] observed a yield increase with bud load only in irrigated vines and no yield variation in non-irrigated vines in years when water was a limiting factor.

Concerning to the effect of organic amendments on yield, Sludge significantly increased yield in three of the four years of the trial. The increase of reproductive growth with the application of a sewage sludge compost was already observed in Cabernet Sauvignon [32], while no differences were found in Merlot [33]. In both referred works, the control treatment was supplied with a NPK fertilizer, while in the present study there was no fertilization in Ctrl and, thus, the differences between treatments were maximized.

Municipal solid waste compost and cattle manure only originated significant yield increase from the third year on. The faster response of yield in Sludge application when compared to MSWC and Manure is probably related to the higher N content of the first amendment, when compared to the others. An increase in yield since the second year of cattle manure application has already been reported [35]. However, Pinamonti [33] found significant differences in the first year of a MSWC application, but not in the next three years.

Biochar did not increase yield in any year. It is reported that biochar increases nutrient availability for plants as the result of both the direct nutrient additions and the greater nutrient retention [65]. However, the biochar used in this study supplied low amounts of nutrients to the soil (Table 1) and, since there was no addition of other fertilizers, the role in nutrient retention is negligible. Schmidt et al. [39] did not observe significant differences in reproductive growth applying 8 Mg ha^{-1}, an amount close to what was used in the present study. On the other hand, Genesio et al. [38] found a 66% increase in yield, but with an application of 22 Mg ha^{-1}.

The interaction between pruning system and organic amendment is significant from the second year on. The results show that the application of organic amendments with a low bud load, in MAN, did not produce significant increases in yield. On the other hand, the increase in bud load, due to mechanical pruning, without the enhancement of soil fertility promoted by MSWC, Manure and Sludge, did not also produce any significant increment in yield. Thus, was the interaction between high bud load and enhanced soil fertility that led to a significant increase in yield.

The interaction between pruning level and vine nutrition has not been yet studied, to the best of our knowledge. However, some internal self-regulation mechanisms can be appointed to be the reason for the observed results. The compensation of the high bud load imposed by mechanical pruning can occur through reductions in budburst, bud fruitfulness and/or cluster weight. The present results show that the enhancement of soil fertility can attenuate these self-regulation mechanisms.

The budburst usually decreases in response to the high bud load that mechanical pruning imposes [7,46]. The improvement of plant nutrition in mechanically pruned vines seems to reduce this self-regulation mechanism intensity and lead to an increase in shoot number per vine.

Bud fruitfulness generally decreases when canopy or shoot physiology imposes some limitation on the induction and differentiation processes. Since buds only import carbon directed basipetally from leaves on the same side of the shoot [66], the lower leaf area per shoot in mechanical pruning [5,49] exerts a source limitation on bud differentiation and lower bud fertility is usually observed in mechanically pruned vines. However, the improvement in vine nutrition promoted by the application of organic amendments, can mitigate this effect since an adequate nutrients supply is needed on flower formation. Bud fertility responds positively to N fertilization, if not excessive, [46] as well as to P [67] and K fertilization [68]. In the present work, in 2015, an increase of bud fruitfulness was observed with the organic amendments in MEC, although the differences were significant only between Sludge and Control (2.7 and 2.1 clusters per shoot, respectively).

Finally, the cluster weight was influenced by organic amendments both in MAN and in MEC, so it does not justify the different response of the pruning systems to organic amendments.

4.4. Vegetative Growth

Vegetative growth was significantly affected by mechanical pruning. Shoot individual vigor was significantly decreased by MEC, as reported in other studies [8,13,69,70]. This result is related to the higher shoot number per vine that obliges to a partitioning of the available carbohydrates to more sinks, leading to a lower growth of each one.

Vegetative growth, assessed by pruning weight per vine, was tendentially lower in MEC, although in a global analysis differences were not significant. Significant reductions in pruning weight in mechanically pruned vines were already reported [10,13,14], nevertheless, in other studies, no significant differences were found [8,57]. The increase in cluster number per vine leads to a higher competition for carbohydrates between vegetative and reproductive growth. Since grapes are a stronger sink than apical meristem [71], assimilates are preferentially directed to reproductive growth.

Along the four years of the trial, vegetative growth of MEC vines did not decrease, as reported by other authors [8], showing no evidences of overcropping, such as vine decline and alternate or biennial bearing [6].

Organic amendments had significant effects on vegetative growth since the third year of the experiment. Sludge and MSWC increased both, shoot individual vigor and total pruning weight per vine, although the MSWC effect only became significant in the fourth year. Liu et al. [32] observed an increase on vegetative growth in response to sewage sludge compost, while Pinamonti [33] found a decrease in the first four years of the vine and no significant differences after that period. In the same work, a decrease in vegetative growth with the application of MSWC was observed only in the plantation year and no significant differences in the other five years of trial [33].

Even though Manure significantly increased yield, it had no significant effect both on shoot and pruning weight. No differences in pruning weight with the application of cattle manure in a rate of 10 Mg ha^{-1} $year^{-1}$ were already reported [22]. On the contrary, in other studies, with lower amounts of manure (1.6 and 4 Mg ha^{-1} $year^{-1}$), an increase in vegetative growth was observed [33,72].

Biochar had no significant effects on vegetative growth, when compared to control as already reported [39].

4.5. Dry Matter Production and Partitioning

Dry matter production allocated in grapes and canes was not affected by the pruning system except in 2014, when the water deficit was lower [64] allowing mechanically pruned vines to have a higher dry matter production. An increase in dry matter production with mechanical pruning was also observed in Grenache under drought conditions [8].

As well as in yield, dry matter production was significantly affected by the organic amendments from the second year on. This response is, probably related to the N supply, since the dry matter production has a positive relation to N supply in non-restrictive light conditions [46]. As already discussed, the differences in the time of response to each treatment are, probably, related with the amendments nutrient composition, as well as with their rate of release.

The interaction between the two factors was significant also from the second year on. Like in yield, the dry matter production was increased by the conjugation of the soil organic amending and the bud load increase. The dry matter production is the result of the vegetative potential subtracted from the losses due to the lack of bud load and those resultant from damages of the vessels [73]. In MEC, the losses due to the lack of bud load are reduced or even eliminated, since the bud load is high, contrarily to MAN. On the other hand, the organic amendments, by improving soil fertility, increase the vegetative potential.

The Ravaz Index was consistently increased by mechanical pruning as already reported [56]. Although there were no significant differences in yield in most of the years, as well as in pruning weight, the tendency of higher yield and lower pruning weight in MEC, led to a significant increase in the Ravaz Index in all years.

Organic amendments had no effect on Ravaz Index, since the increase in growth promoted by soil fertilization was both reproductive and vegetative as observed by other authors [22].

5. Conclusions

Mechanical pruning and the organic amendments had a positive effect in shoot and cluster number per vine. However, while mechanical pruning significantly reduced shoot and cluster weight, the organic amendments had an opposite effect

Although the present work was performed in non-irrigated vineyards and the results showed a good performance, even under drought conditions, mechanically pruned vines tend to consume more water. Therefore, the use of irrigation in this system should be considered.

The obtained results show that the interaction between mechanical pruning and soil organic amending successfully increases yield, without decreasing vegetative growth. Both tools, when combined, create a high yielding production system with low hand-labor input, increasing the activity profitability.

The growth in waste generation all over the world has quickly become one of the major challenges for today's economy and is a key issue for circular economy. This work shows that the use of organic amendments together with mechanical pruning are an effective way to valorize human residues, to reduce the dependence of winegrowers on the increasingly scarce hand labor and increase vineyards profitability.

Author Contributions: Conceptualization, M.B., A.C., R.d.C., H.R.; methodology, M.B., A.C.; investigation, M.B., A.C.; writing—original draft preparation, M.B.; writing—review and editing, A.C., J.R.-d.-S., R.d.C., H.R.; supervision, J.R.-d.-S., R.d.C., H.R.; project administration, H.R.; funding acquisition, M.B., A.C., H.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by ProDeR (Measure 4.1 "Cooperation for Innovation", PA 24071, Partnership 397, FERTILPODA Project), by Caixa Geral de Depósitos and ISA (doctoral grant to Manuel Botelho) and by the research center LEAF (UID/AGR/04129).

Conflicts of Interest: The authors declare no conflict of interest.

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