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Exploring the Interplay of Bud Load and Pruning Type in Shaping 'Xinomavro' (*V. vinifera* L.) Vine Growth, Yield, and Berry Composition

Serafeim Theocharis [1], Theodoros Gkrimpizis [1], Christina Karadimou [1], Kleopatra-Eleni Nikolaou [1], Stefanos Koundouras [1] and Dimitrios Taskos [2,*]

[1] Laboratory of Viticulture, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; sertheo@agro.auth.gr (S.T.); gkrimpiz@agro.auth.gr (T.G.); cckaradi@agro.auth.gr (C.K.); skoundou@agro.auth.gr (S.K.)
[2] Institute of Olive Trees, Subtropical Crops and Viticulture, Hellenic Agricultural Organization-Demeter, 1 S. Venizelou Str., Lykovryssi, 14123 Athens, Greece
* Correspondence: taskos@elgo.gr

Abstract: 'Xinomavro' (*V. vinifera* L.) is an important native red wine grape variety in Northern Greece, particularly in PDO regions. Despite its significance, there is limited research on the effects of pruning type and severity on 'Xinomavro' vine physiology, yield, and berry quality across diverse environmental conditions. This study aims to address this knowledge gap and provide growers with crucial information for optimizing vineyard management practices. The study was conducted over two consecutive years (2016 and 2017) in a vineyard in Thessaloniki, Northern Greece. Four treatments (B12: 12 buds on 6 spurs; B24: 24 buds on 12 spurs; M12: 12 buds on 2 canes; M24: 24 buds on 4 canes) combining two bud load levels (12 or 24 count nodes) and two pruning types (short spurs or long canes) were applied to 'Xinomavro' vines in complete block randomized design. Measurements included vine water status, gas exchange, canopy characteristics, yield components, and berry composition. Bud load and pruning type significantly influenced vine canopy development, microclimate, and yield components. Short pruning with high bud load (B24) resulted in denser canopies and higher yields, while cane pruning (M12 and M24) led to more open canopies and improved berry quality indicators. Treatment effects on berry composition were inconsistent across years but showed a tendency for higher anthocyanin and total phenol content in cane-pruned vines. The study demonstrates that pruning type (short or long fruiting units) may have a greater impact on vine growth, yield, and berry composition than bud load alone in 'Xinomavro' vines. Cane pruning appears to be a more effective strategy for achieving vine balance and potentially improving grape quality under the given experimental conditions.

Keywords: xinomavro; bud load; pruning type; vine balance; canopy management; berry composition; grape yield; viticulture practices; winegrape quality

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1. Introduction

Modern viticulture faces the challenge of achieving a complex set of management objectives to ensure both financial viability and sustainability. These objectives include consistently meeting the stringent grape quality standards of the targeted market while simultaneously achieving economically sustainable grapevine growth and yields. Achieving and maintaining an optimal equilibrium between vegetative and reproductive growth while achieving high quality grapes defines the concept of 'vine balance' [1]. While widely discussed in recent decades, the idea of 'vine balance', as discussed by [2], originates from the earlier work of [3]. Despite these pioneering and sound earlier works, traditional winemaking often assumes an inverse relationship between vine yield and grape quality, but modern research suggests an optimum curve to describing this relationship: quality initially rises with yield, plateaus, then declines as yield increases further [4]. Thus, achieving "vine balance" allows for a range of crop levels to produce suitable grape quality, demonstrating that optimal yield and quality are achievable within a balanced vineyard [4]. It follows that achieving and maintaining an appropriate vine balance is important to effective vineyard management in the context of modern viticulture.

Adjusting the bud load, or the number of nodes, retained after winter pruning, offers a practical method for regulating, though only in part, crop levels in grapevines [5]. Previous research [5–8] suggests a positive relationship between pruning severity and yield: as pruning severity decreases (more buds retained), yield generally increases. However, this relationship isn't always linear due to compensatory changes in other yield components [5,7,8]. A meta-analysis using mixed models by [9] revealed that the increases in vine yield due to reduced pruning severity is attributed to a higher number of clusters, even though individual cluster weight decreases. Individual studies report varying responses of cluster weight and other yield components to bud load, ranging from no observed effect [5] to inconsistent across years to inconsistent effects across years [7].

The microclimate within a grapevine canopy is primarily governed by the amount and spatial distribution of leaf area, which interacts with prevailing atmospheric conditions [10]. Canopy density, a key factor influencing this microclimate, directly affects the light environment within the canopy [11]. This light exposure, modulated by canopy density, subsequently influences berry temperature [12,13]. Adjusting the number and spatial arrangement of retained latent buds, in conjunction with vine yield, influences canopy wall characteristics, including size and specific properties [14]. These modifications to vine microclimate and to canopy structure subsequently impact grapevine growth, productivity [15,16] and berry composition [17]. While previous studies have investigated the impact of pruning severity on berry composition [5,6,18,19], among others, their findings were not always consistent across different experiments. In summary, while increasing bud numbers through less severe pruning generally leads to higher yields, this relationship is complex and moderated by the vine's compensatory mechanisms, long-term adjustments, and interactions with environmental and management factors. Therefore, given the context-dependent nature of bud load effects on grapevine growth, yield, and berry composition, conducting region-specific research for diverse grape-growing scenarios is crucial. Pruning to a specific number of buds per unit of dormant pruning weight was suggested as a method for achieving optimal vine balance [2,20].

Beyond bud load, pruning types also vary based on the fruiting units that retain buds after winter pruning. "Long" pruning, with canes of usually 6-12 buds as fruiting units, reduces vine vigor by limiting resource reserves, making it suitable for vigorous varieties or those with low bud fertility, especially in cool climates. It promotes bud fruitfulness, creates less dense canopies with better air circulation, and reduces disease incidence. However, it can lead to uneven bud burst and variable shoot growth. "Short" pruning, which retains short spurs (2-3 buds) on permanent cordons, typically enhances shoot vigor, bud burst uniformity, and grape maturation due to greater resource reserves in the permanent wood. However, it can increase disease incidence due to more pruning cuts and necessitate more shoot thinning.

Several studies have shown that pruning type may affect grapevine growth, yield and berry composition [16,21–23]. For example, [22] found that long pruning resulted in decreased berry and cluster weight but higher vine yield. Responses of berry juice composition were inconsistent, while no changes were observed in berry skin phenolics. Similarly, [23] did not observe significant differences in juice composition, anthocyanin content, or total phenol content between short and long pruning. However, this study applied both pruning types simultaneously on the same vines during a single growing season. Not all of these studies maintained an exact common bud load across pruning type treatments [16,22].

'Xinomavro', a distinguished native red winegrape of Northern Greece, holds a prominent position within the modern Greek vineyard landscape, particularly in the PDO regions of Naoussa, Amyndeon, Rapsani, and Goumenissa. Its late-maturing characteristic has garnered increasing attention within the Greek wine sector, especially in light of climate change projections. Despite its

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cultivation across diverse environmental conditions, research on the impacts of pruning type and severity on 'Xinomavro' vine physiology, yield, and berry quality remains limited. This lack of region-specific knowledge deprives growers of crucial information needed to optimize vineyard management practices and maximize grape production within their unique environmental contexts. These objectives can encompass both quality and yield considerations. In some cases, the focus might be on enhancing grape quality while adhering to yield restrictions for PDO wine production. In other cases, objectives can target maximum vine yield.

Research on the effects of pruning severity and type on the performance of 'Xinomavro' grapevines is scarce. [24] investigated the effect of three different training systems on 'Xinomavro' vines (Lyre, Guyot, bilateral Royat) in a one year study. Short (Royat) and long (Guyot) pruning were compared on a common load of 10 buds. While no significant effects were observed on main growth, yield, ad berry composition traits, grapes from the long pruned vines had higher concentrations of tannin monomers. In contrast, the short pruned vines yielded grapes richer in skin tannins, potentially resulting in a less astringent character. Despite being subtle, these effects may dictate that grapes from short pruned vines are more suitable for longer maceration during vinification.

This study focuses on 'Xinomavro', an important native red wine grape variety in Northern Greece, particularly in PDO regions like Naoussa, Amyndeon, Rapsani, and Goumenissa. Despite its significance, there is a notable gap in research regarding the impact of pruning type and severity on 'Xinomavro' vine physiology, yield, and berry quality across diverse environmental conditions. This lack of region-specific knowledge poses a challenge for growers seeking to optimize vineyard management practices. This study directly addresses this knowledge gap by investigating the effects of bud load (pruning severity) and pruning type on 'Xinomavro' grapevine performance. The ultimate goal is to provide growers with the critical information needed to tailor their vineyard management strategies and maximize grape production within their unique environmental contexts. This study completes a trilogy of research dedicated to optimizing important aspects of 'Xinomavro' cultivation [25,26].

2. Materials and Methods

2.1. Experimental Vineyard and Trial Design

This study spanned two consecutive years (2016 and 2017) and was conducted in a 0.6-hectare, 10-year-old vineyard situated in Thessaloniki, Northern Greece (37° 79′ N, 22° 61′ E) at an altitude of 60 meters above mean sea level. The vineyard soil, classified as loamy-clay (30% sand, 25% silt, and 45% clay), was managed using a clean surface cultivation system. The vineyard featured 'Xinomavro' (*Vitis vinifera* L.) grafted onto 1103P rootstock (*V. rupestris* × *V. berlandieri*) at a density of 4,000 vines per hectare. Vines were planted in a 1.0 m × 2.5 m within and between row layout, with rows oriented east-northeast to west-southwest (246° heading). A bilateral Royat training system with a vertical trellis system (three fixed pairs of foliage wires) was employed.

Drip irrigation delivered consistent water amounts across the two experimental years (91 mm in 2016 and 84 mm in 2017). Irrigation scheduling involved applying half the water approximately 15 days before veraison, with the remainder split into two doses applied 10 days after veraison and 15 days before harvest. An on-site automatic weather station (iMETOS, Pessl Instruments GmbH, Weiz, Austria) recorded weather data. Crop evapotranspiration was estimated based on potential evapotranspiration calculated using the Penman-Monteith method. Standard local viticultural practices were followed for pest and canopy management, as well as fertilization.

Within the vineyard, three vine rows were selected for the study, separated by two buffer rows. Each row contained four plots, with each plot comprising ten consecutive vines. Two bud load treatments were applied: 12 or 24 count nodes retained per vine after winter pruning. These bud loads were further divided into two pruning type treatments: either retaining the nodes on 2-bud short spurs or on 6-bud canes. The combination of the two bud load treatments (12 or 24 count nodes) and the two pruning type treatments (2-bud short spurs or 6-bud canes) resulted in four treatment combinations applied to individual vines (B12: 12 buds on 6 spurs; B24: 24 buds on 12 spurs; M12: 12

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buds on 2 canes; M24: 24 buds on 4 canes). These combinations were randomly assigned to the four plots within each row, following a randomized complete block design with three replications (rows).

2.2. Vine Water Potential and Leaf Gas Exchange

Measurements of stem water potential (Ψstem) were taken from the end of veraison to maturity on selected dates during two growing seasons: 2016 (days of the year—DOYs: 218, 226, 235, 244, 253 and 260) and 2017 (DOYs: 216, 223, 234, 249, 260, and 271). These measurements utilized a pressure chamber, as outlined in [27]. On each measurement date, three mature leaves from the central vines of each plot were sampled. Ψstem was taken at solar noon (12:30–14:30 p.m. local time) from leaves positioned between the 7th and 9th nodes of the primary shoots. To facilitate equilibrium between the leaf and stem water statuses for Ψstem measurements, leaves were bagged for 1 h in light-excluding, black plastic bags with an aluminum foil cover for thermal insulation [27]. The average values of three leaves for each type of water potential were used for statistical analysis.

Concurrently, the net assimilation rate (A, µmol CO_2 m^{-2} S^{-1}), stomatal conductance (gs, mol H_2O m^{-2} S^{-1}), transpiration (E, mmol H_2O m^{-2} S^{-1}), and leaf intrinsic water-use efficiency (WUEi, calculated as A/gs, µmol CO_2 mol^{-1} H_2O) were measured using an LCi portable gas exchange system, ADC BioScientific Ltd., Hoddesdon, UK. Data were collected from three fully expanded, recently matured, sunlit leaves in each plot that received a photosynthetic photon flux density exceeding 1200 µmol m^{-2} s^{-1}, in proximity to the leaves chosen for water potential measurements.

2.3. Leaf Area, Shoot Growth Production, and Grape Yield

Vine yield and its components were assessed at commercial harvest maturity, reached on September 29, 2016, and September 25, 2017. During harvest, clusters were collected from the four central vines within each plot. All clusters were counted and weighed to determine vine yield (kg $vine^{-1}$) and average cluster weight (g). For each central vine, a representative sample of 10 clusters was randomly selected from the total yield. These clusters were transported to the lab in insulated coolers for further analysis. In the lab, cluster weight, length (cm), and width (cm) were measured. Berries per cluster were counted to determine cluster compactness, calculated as the ratio of berry count to peduncle length (cm).

At full ripening, the total leaf area of four vines from each treatment plot was estimated using the non-destructive method outlined in [28]. Exposed surface area (m^2 $vine^{-1}$) was calculated according [29] for vertical shoot positioned vines. Canopy density (%) was determined as the percentage of actual total leaf area per vine relative to the exposed surface area. In cases where the actual total leaf area exceeded the exposed surface area, a canopy density of 100% was assigned. Canopy density measurements exceeding 100% indicate shaded leaf area, calculated as the surplus leaf area relative to total leaf area. Conversely, canopy densities below 100% indicated the presence of canopy gaps, expressed as the percentage of unfilled surface area. During dormancy, these same four vines were assessed for pruning wood weight and cane count. Total pruning wood weight (kg) was recorded, and the mean weight per cane was calculated by dividing the total wood weight by the number of canes for each vine.

2.4. Berry Sampling and Must Analysis

Berry chemical composition was analyzed across six sampling points, from veraison to harvest, during 2016 (days of the year [DOYs] 218, 226, 235, 244, 253 and 260) and 2017 (DOYs 216, 223, 234, 249, 260, and 271). At each sampling point, 200 berries were randomly selected from clusters on the four central vines within each plot and transported to the laboratory in portable coolers. In the lab, the 200-berry samples were weighed to determine average berry weight. Each sample was then divided into four 50-berry subsamples. One subsample was manually pressed to extract juice for analysis of total soluble solids (°Brix) using a digital refractometer (HI96841, HANNA Instruments, Woonsocket, RI, USA), pH using a laboratory pH meter (HI2020-02, HANNA Instruments, Woonsocket, RI, USA), and titratable acidity (g/L tartaric acid equivalent) via titration against 0.1 N sodium hydroxide. The remaining 150 berries (three 50-berry subsamples) were stored at -30°C for later phenolic compound analysis.

2.5. Phenolic Content and Anthocyanins

Berry phenolic content was determined using whole berries following established protocol in [30]. For each plot, 50 berries were homogenized in a 125 mL plastic beaker using a Polytron at 25,000 rpm for 30 seconds. A 1 g aliquot of the homogenate was transferred to a centrifuge tube (in triplicate), combined with 10 mL of 50% (v/v) aqueous ethanol (pH 2), and mixed for 1 hour. Following centrifugation at 3,500 rpm for 10 minutes, 0.5 mL of the supernatant was added to 10 mL of 1 M HCl and mixed thoroughly. After a 3-hour incubation, absorbance was measured at 520 nm and 280 nm using a 10 mm cell. Anthocyanin content (mg $berry^{-1}$) was calculated from the absorbance at 520 nm, while total phenolics (absorbance units $berry^{-1}$) were calculated from the absorbance at 280 nm.

2.6. Statistical Analysis

Data were averaged per plot, and these mean values were used for statistical analysis. Results are presented as the means of three replicates ($n = 3$). An analysis of variance was conducted with rootstock variety as the main factor. Duncan's multiple range test was used to identify significant differences among the main effect means at $p < 0.05$. Principal component analysis was performed using nine variables measured at the 2016 and 2017 harvests, and a biplot was generated. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 22.0.

3. Results

3.1. Stem Water Potential and Gas Exchange

Treatment effects on stem water potential and gas exchange during berry maturation were generally insignificant, except for stem water potential in 2017, where short pruned vines with a bud load of 24 (B24) exhibited higher values (Table S1). However, a significant growth season effect was observed, resulting in higher overall mean of Ψstem, stomatal conductance (gs), and net CO_2 assimilation (A) values in the first year of the experiment (Table S1). No significant interactions were found between treatments or between treatments and growth season (Table S1).

3.2. Vine Canopy, Shoot Growth, and Microclimate

3.2.1. Leaf Area and Shoot Growth

Significant variation in total leaf area and main leaf area was observed across bud load and pruning type treatments (Figure 1A, 1B). Total main leaf area ranged from 1.5 to 3.4 m^2 $vine^{-1}$ in 2016 and from 1.2 to 3.5 m^2 $vine^{-1}$ in 2017 (Figure 1B). Regardless of pruning type, vines with higher bud loads consistently exhibited significantly greater total leaf area at harvest in both years (Figure 1A). This effect was more pronounced in the short pruning treatment (Figure 1A). Consequently, B24 vines (short pruned with 24 buds) developed the greatest total leaf area among all treatments, reaching 6.2 m^2 $vine^{-1}$ in 2016 and 5.9 m^2 $vine^{-1}$ in 2017 (Figure 1A). Conversely, when the higher bud load was maintained on canes (M24), the increase in total leaf area between M12 and M24 treatments was less pronounced (Figure 1A). M24 vines exhibited similar total leaf area values to B12 vines (Figure 1A).

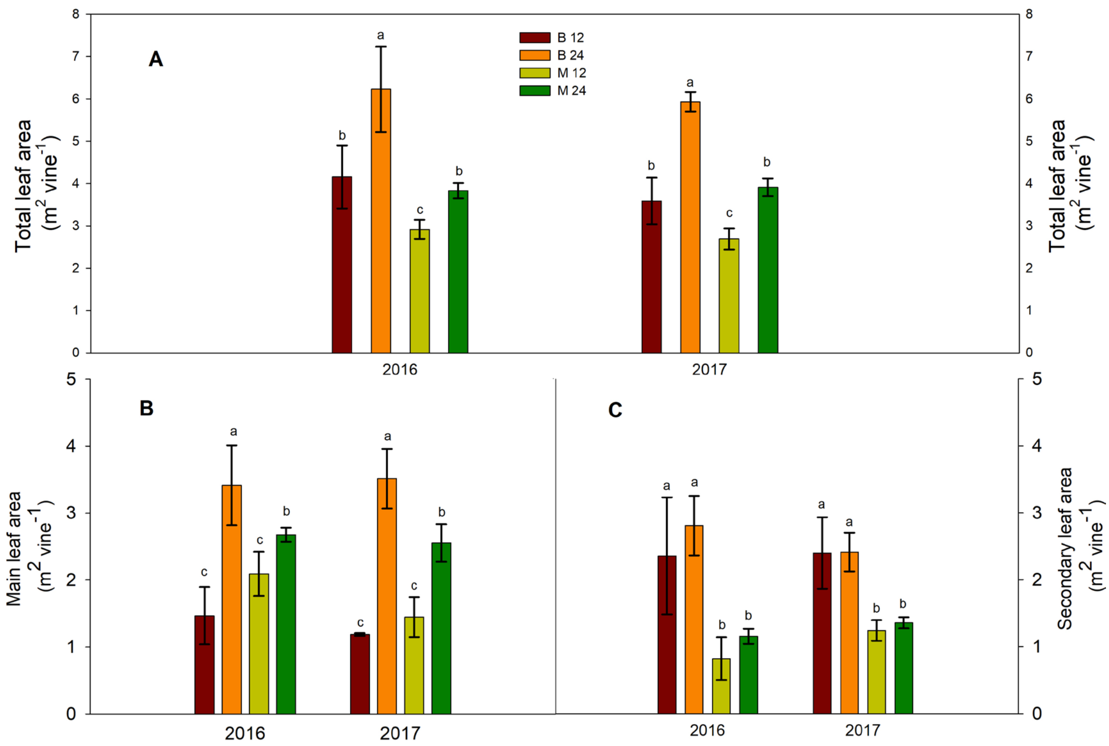

Figure 1. The effect of bud load and pruning type on leaf area characteristics: (A) Total, (B) Main and (C) Secondary area. Significant differences ($p<0.05$) among treatments are indicated by different letters.

Mirroring the trend observed in total leaf area, the total area of main leaves responded similarly to both bud load and fruiting unit type treatments (Figure 1B). However, vine secondary leaf area differed only between short and long pruning treatments, with no significant bud load effects observed (Figure 1C). Short pruned vines consistently displayed greater secondary leaf area compared to their long pruned counterparts (Figure 1C).

Vines pruned to the higher bud load developed more shoots compared to the lower bud load treated vines (Figure 2A). The mean shoot number per vine was consistently higher on the B24 treatment compared to M24 (Figure 2A). This influence of fruiting unit type on mean shoot number was not consistent across the two years of the experiment for the lower bud load treatment (Figure 2A). Mean shoot per bud followed a reverse trend compared to mean shoot per vine: vines with the high bud load had lower ratio of shoots for each bud retained at pruning (Figure 2B).

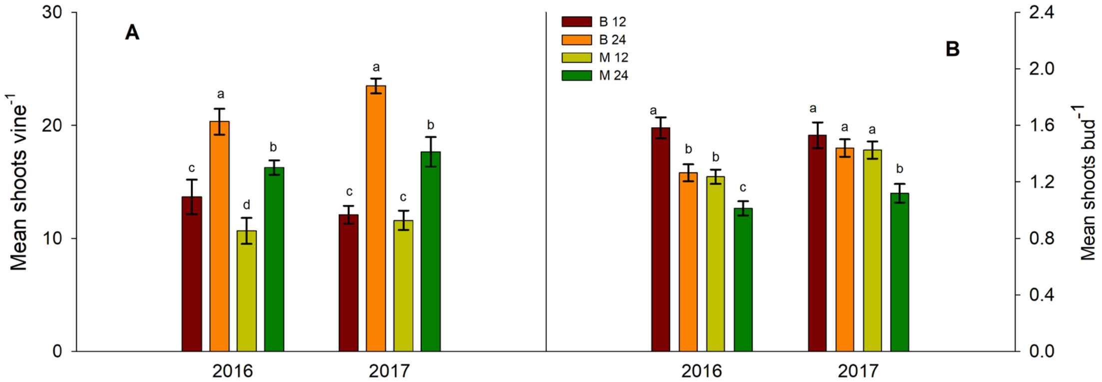

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Figure 2. The effect of bud load and pruning type on (A) shoots per vine and (B) shoots per bud. Significant differences (p<0.05) among treatments are indicated by different letters.

Consistent with the observed trends in total leaf area (Figure 1A), the total weight of pruning wood in the following winter season was significantly influenced by both bud load and fruiting unit type treatments (Figure 3A). Pruning wood weight was notably higher in the B24 and M24 treatments compared to the B12 and M12 treatments, respectively (Figure 3A). Furthermore, within a given bud load, short pruned vines (B12 and B24) consistently exhibited greater pruning wood weight than their long pruned counterparts (M12 and M24) (Figure 3A). In contrast to the observed variations in mean shoot number per vine, no significant differences in mean cane weight were detected between treatments in either experimental year (Figure 3B).

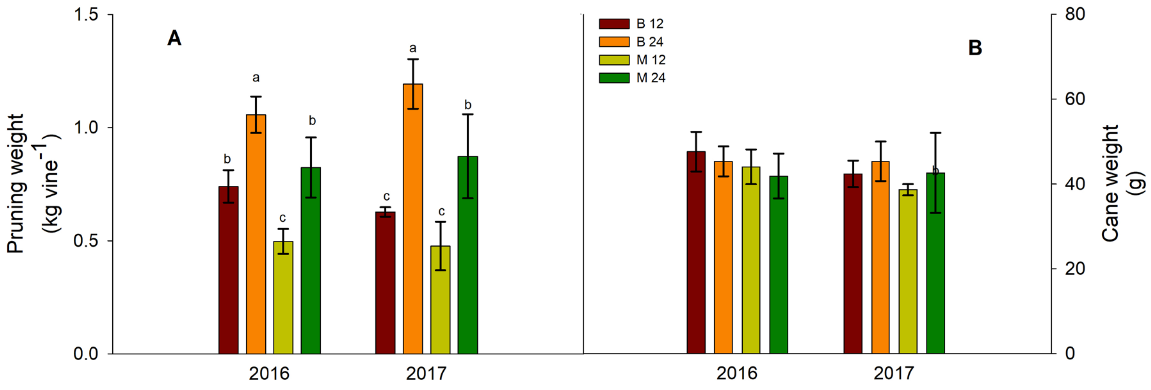

Figure 3. The effect of bud load and pruning type on (A) pruning weight and (B) cane weight. Significant differences (p<0.05) among treatments are indicated by different letters.

3.2.2. Canopy Density and Cluster Temperature

The actual total leaf area expressed as percentage of the exposes leaf area ranged at 100% for the B24 treatment for both years of experimentation, between 70-80% for B12 and M24 vines, while the lowest percentage of exposed leaf area was for the M12 vines (61% and 57% for the two years respectively) (Figure 4A). Conversely, M12 vines had the lowest canopy density and the highest number of gaps while, in contrast, B24 vines had a high percentage of overlapping leaves at 37% (2016) and 19% (2017) (Figure 4B). Vines from the B12 and M24 treatments showed intermediate values of canopy density, with no difference between them (Figure 4B).

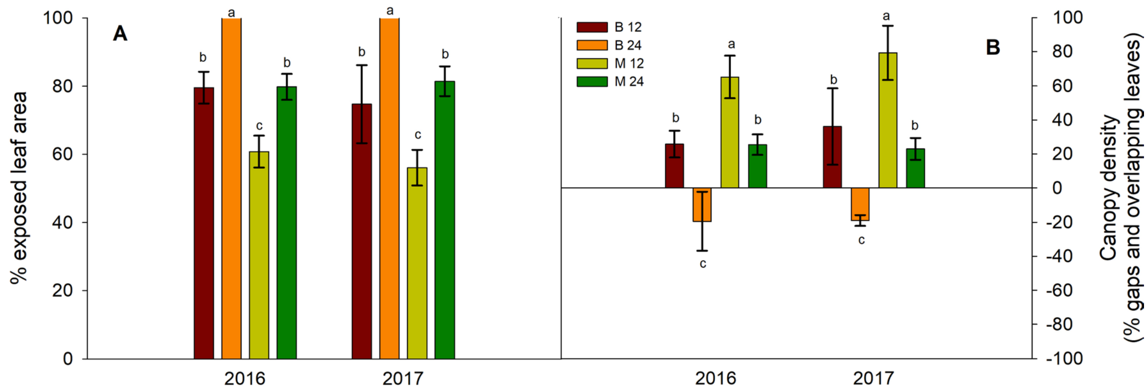

Figure 4. The effect of bud load and pruning type on (A) % of exposed leaf area and (B) canopy density. Significant differences (p<0.05) among treatments are indicated by different letters.

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Berry temperature was significantly increased in treatments receiving long pruning (M12 and M24) in particular compared to B24, whose grapes recorded the lowest temperature in both years of experimentation, apparently as a result of denser foliage (Figure 5).

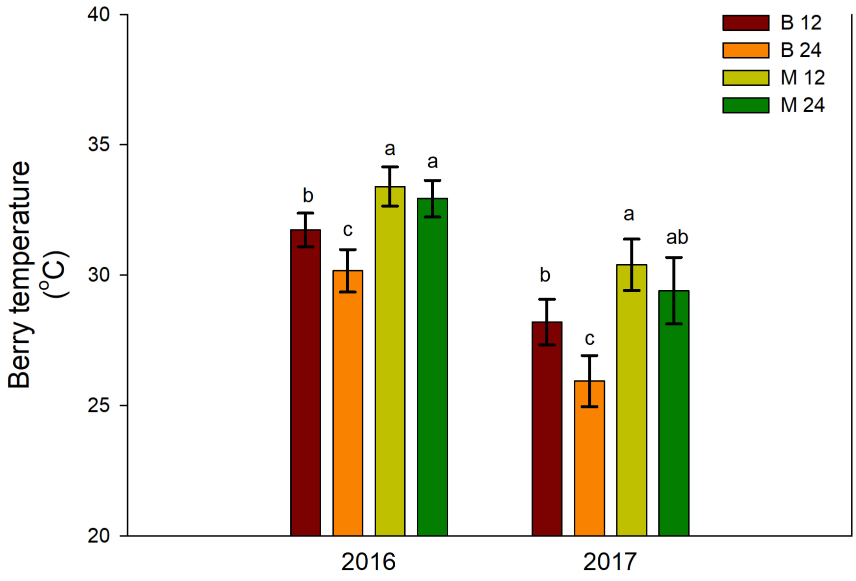

Figure 5. The effect of bud load and pruning type on berry temperature. Significant differences ($p<0.05$) among treatments are indicated by different letters.

3.3. Grape Production, Cluster Properties, and Vine Balance

Short-pruned vines consistently yielded a higher grape yield under higher bud load treatment. Conversely, long-pruned vines under the M24 treatment exhibited higher yields only in 2017 (Figure 6A).

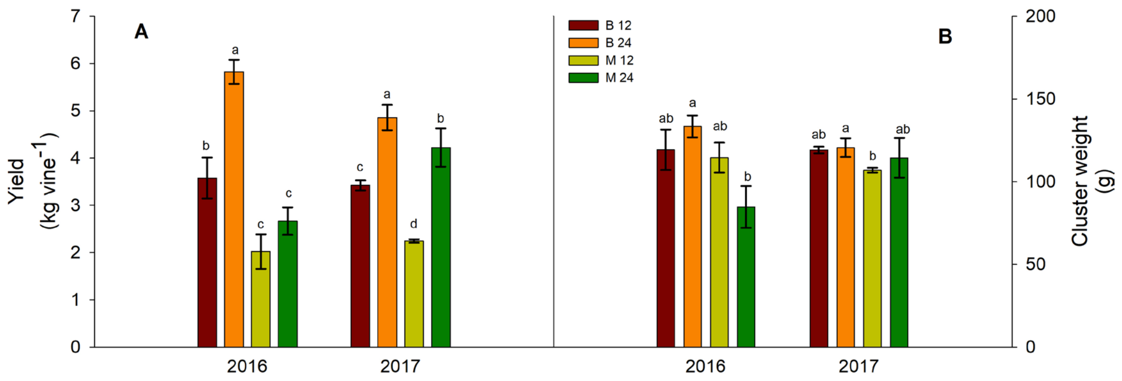

Figure 6. The effect of bud load and pruning type on (A) yield and (B) cluster weight. Significant differences ($p<0.05$) among treatments are indicated by different letters.

While no consistent responses in mean cluster weight were observed across bud load and fruiting unit type treatments (Figure 6B), increased bud load resulted in a higher mean number of clusters per vine, irrespective of pruning type (Figure 7A). In contrast, lower bud load in short-pruned vines resulted in a greater number of clusters per shoot across both years of the experiment (Figure 7B). Across both years of the experiment, the M24 treatment consistently resulted in lower berry weight throughout the ripening process (Figure S1). Despite the differences in grape yield, we did not observe consistent and significant differences in vine balance indices (ratios of total leaf area to grape yield and total pruning weight to grape yield; Figure S4).

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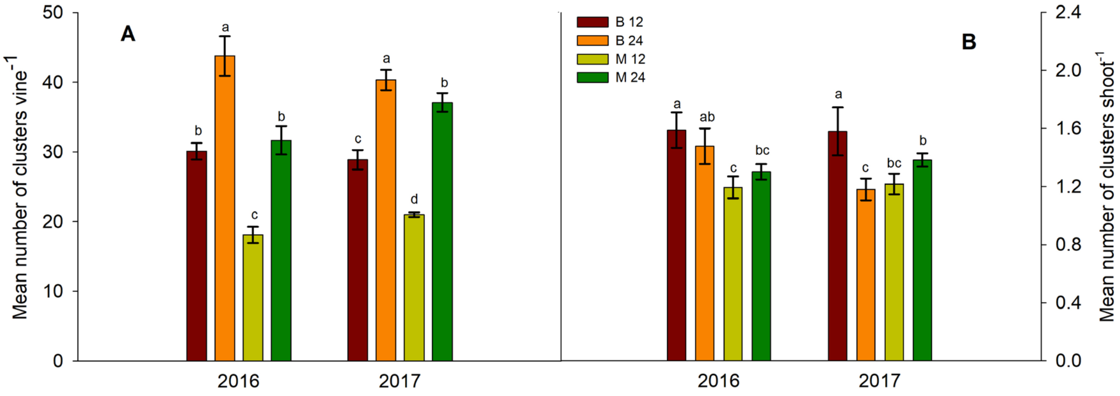

Figure 7. The effect of bud load and pruning type on (A) clusters per vine and (B) clusters per shoot. Significant differences (p<0.05) among treatments are indicated by different letters.

Treatment effects were significant on several attributes of cluster architecture and berry components (Table 1). Cane-pruned vines generally exhibited less compact clusters with fewer berries per cluster (Table 1). However, this trend was consistent only within the M24 treatment (Table 1). In addition, cane pruning had a significant effect on berry anatomy of 'Xinomavro' vines with a consistent trend in both years, as the berries of treatments M12 and M24 tended to have an increased contribution of skin and seeds in the total weight of their berries compared to treatments B12 and B24 (Table 1).

Table 1. The effect bud load (L) [count (C): 12 or 24 nodes per vine and type (T): 2-bud short spurs or on 6-bud canes] and year on cluster architecture (length, width, berries per cluster, compactness) and berry components (% skin and seed weight). Within each column and parameter, means followed by a different letter are significantly different at P < 0.05 based on Duncan test. *. **: interaction between bud load (L) and year (L x year) at P < 0.05 and P < 0.01. ns: absence of interaction.

Year	Treatments	Cluster Length (cm)	Cluster Width (cm)	Berries per cluster	Cluster compactnes s	(% skin weight)	(% seed weight)
2016	B 12	17,4	11,0	143 a	8,3 a	8,58 b	2,18 b
	B 24	16,4	9,6	133 ab	8,1 a	8,97 b	1,39 c
	M 12	15,6	9,5	109 bc	6,9 ab	12,08 a	3,63 a
	M 24	15,7	9,9	98 c	6,2 b	10,27 ab	3,55 a
C x T		*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
2017	B 12	16,7 b	10,8 b	169 b	10,2 a	7,91 b	2,97 ab
	B 24	17,6 a	13,0 a	198 a	11,3 a	8,40 b	2,34 b
	M 12	15,3 c	9,7 c	121 c	7,9 b	12,67 a	3,62 a
	M 24	15,5 c	9,2 c	124 c	8,0 b	12,70 a	3,21 ab
C x T		*ns*	**	*	*ns*	*ns*	*ns*
*L * year*		*ns*	*	*	*ns*	*ns*	*ns*

3.4. Berry Composition

A significant year effect was observed across several berry composition variables (Table 2). In the second year of the experiment, berry juice exhibited significantly higher titratable acidity, anthocyanin, and total phenol concentrations, but lower pH values (Table 2). While significant main effects on berry composition were evident within each year, these effects were not consistent across treatments and years (Table 2). Notably, significant interactions between bud load and pruning type were observed on berry composition (Table 2). The seasonal evolution of berry juice composition (Figure S2) and phenolic compounds (Figure S3) aligned with these responses to treatment and year

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effects. Despite the lack of consistency of main effects on berry composition, the cane pruned vines tended to have higher anthocyanin and total phenol content (Table 2; Figure S3).

Table 2. The effect bud load (L) [count (C): 12 or 24 nodes per vine and type (T): 2-bud short spurs or on 6-bud canes] and year on berry chemical attributes at harvest. Within each column and parameter, means followed by a different letter are significantly different at P < 0.05 based on Duncan test. *. **. ***: interaction between bud load (L) and year (L x year) at P < 0.05, P < 0.01 and P<0.001. ns: absence of interaction.

Year	Treatments	Total soluble solids (°Brix)	Titratable acidity (g L^{-1})	pH	Total anthocyanins (mg $berry^{-1}$)	Total phenols (au $berry^{-1}$)	Total anthocyanins (mg g $berry^{-1}$)	Total phenols (au g $berry^{-1}$)
2016	B 12	22,7 a	6,9 b	3,1 b	0,77 b	2,18 c	0,47 b	1,32 b
	B 24	20,5 b	7,7 a	3,2 a	0,62 b	2,40 b	0,37 b	1,45 b
	M 12	21,6 ab	7,7 a	3,3 a	1,09 a	2,32 b	0,69 a	1,48 b
	M 24	21,1 b	8,3 a	3,3 a	1,07 a	3,00 a	0,74 a	2,10 a
C x T		*	ns	ns	ns	ns	ns	***
2017	B 12	22,2 a	8,6	3,2 a	0,78 bc	2,30 b	0,66 c	1,93 b
	B 24	19,6 b	8,8	3,0 b	0,71 c	2,37 b	0,88 bc	2,88 a
	M 12	22,8 a	8,6	3,1 ab	1,02 a	2,72 a	1,19 a	3,25 a
	M 24	22,7 a	9,2	3,1 ab	0,87 b	2,33 b	1,03 ab	2,77 a
C x T		**	*ns*	*	*ns*	*ns*	***	***
*L * year*		*	*ns*	*	*	*	*	*

3.5. Multivariate Analysis and Data Overview

A principal component analysis was conducted using nine parameters related to vine growth, microclimate, yield, and berry composition. Applying Kaiser's criterion (eigenvalue > 1), two principal components were identified, explaining 76.28% of the total variation (Figure 8). PC1 accounted for 55.49% of the variance, while PC2 explained 20.79% (Figure 8).

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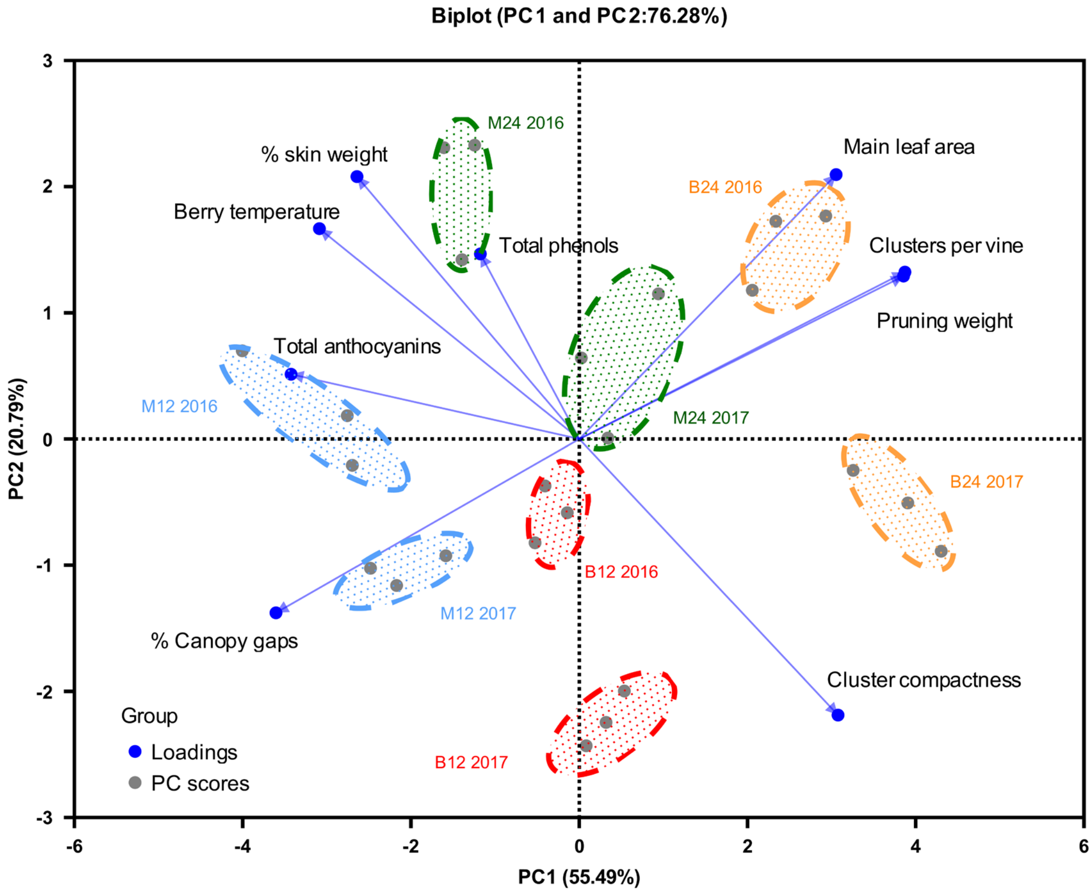

Figure 8. Biplot of the Principal component analysis (PCA) of cv. Xinomavro vine growth, microclimate, yield, and berry composition.

Five parameters - percentage of canopy gaps, cluster temperature, clusters per vine, pruning wood weight, and total anthocyanins - exhibited high loadings (>0.500) only in PC1 (Figure 8). Total phenolics loaded highly only in PC2, while main leaf area, % skin weight, and grape density showed high loadings in both PCs. Figure 8 can be summarized as follows:

Treatment B24 (high bud load on short fruiting units) correlated positively with higher grape yield, vegetative growth (vigor (main leaf area and pruning wood weight). Conversely, treatment B24 showed a negative correlation with % canopy gaps and cluster temperature. Treatment M12 (cane pruning with lower bud load) displayed the opposite trend in both years, exhibiting less crowded canopy and lower yield. Treatment M24 (cane pruning with higher bud load) followed a similar pattern, though less pronounced and only observed in 2016. These findings suggest that increasing bud load on short pruning systems may not effectively control vine vegetative and vine balance. Cane pruning appears to be a more effective strategy for achieving this balance.

Interestingly, the B12 treatment (low bud load on short fruiting units), particularly in 2016, did not show strong correlations with most variables except for grape density, which was notably higher in both short pruning treatments (B12 and B24). Cane pruning was positively associated with increased skin weight and phenolic content, especially in 2016.

These observations suggest that load distribution (short or long fruiting units) may have a greater impact on vine growth, yield, and berry composition than bud load alone. Specifically, B12 and B24 treatments resulted in higher shoot vegetative growth, grape yield, and cluster density. In contrast, M12 and M24 treatments exhibited improved quality indicators, potentially linked to the observed increase in skin weight percentage. This increase in skin weight is likely due to lower cluster density and reduced canopy density, leading to greater berry exposure to light. This hypothesis is supported by the higher berry temperatures observed in M12 and M24 treatments (Figure 5).

4. Discussion

4.1. Vine Water Status and Gas Exchange

Stem water potential is widely recognized as a robust measure of a grapevine's water status [27]. According to [31], the recorded mean stem water potential values, as presented in Table S1, generally suggest that vines across all treatments experienced moderate water stress during berry maturation. The absence of significant and consistent treatment effects on stem water potential (Table S1) indicates that variations in bud load and pruning type did not significantly alter the water status of 'Xinomavro' vines. Water availability is a critical factor influencing grapevine physiology, profoundly affecting growth, yield, and berry composition [27,31]. Therefore, given the observed stability of vine water status across treatments in 'Xinomavro' vines (Table S1), it is plausible that any responses in productivity and berry composition are likely independent of alterations in vine water status.

In grapevines subjected to mild to moderate water deficits, stomatal conductance serves as a sensitive indicator of water stress intensity [32]. Specifically, gs rates within the range of 0.15 to 0.05 mol H2O m^{-2} s^{-1} are indicative of a moderate level of water deficit [33]. Thus, the gs values in Table S1, are consistent with the Ψstem responses to bud load and pruning type treatments.

The higher mean values for Ψstem, gs, and A observed in 2016 (Table S1) can be attributed to the contrasting climate profiles of the two growing seasons, as previously reported in [26]. Specifically, the final month of berry maturation in 2017 experienced drier and warmer conditions compared to 2016. However, the lack of significant interaction between treatments and growth season (Table S1) suggests that these climatic differences, while influencing overall vine physiology, did not substantially alter the main effects of bud load and pruning type on vine water status.

4.2. Vine Canopy and Microclimate

4.2.1. Leaf Area

The significant variation of total leaf area and its components – the main and the secondary leaf area – in response to main effects (Figure 1A,B,C) indicates that bud load and pruning type treatments modified the vegetative growth of 'Xinomavro' vines. This variation in total leaf area was primarily driven by treatment-induced effects on two key factors: the number of shoots per vine and the constituent components of total leaf area. The higher number of shoots observed in the high bud load vines, as depicted in Figure 2A, support this conclusion.

Since the total area of main leaves responded to both bud load and pruning type (Figure 1B) but the secondary leaf area was affected by pruning type only, being higher in short pruned vines (Figure 1C), it appears that leaf development was in part driven by the treatment effects on the secondary leaf area. Thus, the less pronounced effect of higher bud load in the long pruned vines that eventually resulted to similar total leaf area for B12 and M24 vines (Figure 1A) can be explained by the differential increase in primary and secondary leaf area in response to pruning type effects (Figure 1C). Because of this differential increase, the B12 vines had the highest secondary leaf area (Figure 1C) from fast-growing lateral shoots that developed below the height of shoot trimming due to increased vigor of the main shoots.

In contrast, in the long pruning treatments (M12 and M24), the lower growth vigor of main shoots was accompanied by the lower percentage contribution of secondary leaf area to the total leaf area of the vine (28.2% for M12 and 30.1% for M24 in 2016 and 38.5% and 34.8% respectively for 2017 compared to 56.6% for B12 and 45.2% for B24 in 2016 and 66.4% and 46.8% respectively for 2017). The lowest leaf area was recorded in the case of M12 treatment (simple load on cane), i.e., 2.7 m^2 $vine^{-1}$ (2016) and 2.6 m^2 $vine^{-1}$ (2017), which showed the lowest values for both primary and secondary leaf area.

The observed differences in shoot number per vine across treatments (Figure 2A) may have led to the differential expression of vine growth capacity, significantly impacting the allocation of resources between short and long fruiting units. Specifically, vines pruned to short heads (B12 and B24) exhibited a more pronounced increase in secondary leaf area surface, typically associated with fast-growing shoots, compared to their long-pruned counterparts (M12 and M24). This difference could be attributed to the potentially greater availability of stored carbohydrates within the vine arms of short-pruned vines, as suggested by [34].

4.2.2. Shoot Growth

Variations in shoot number per vine between short and long pruning under equivalent bud loads can be attributed to differences in shoot number per bud (Figure 2B). Short pruning treatments (B12, B24) exhibited a higher average number of shoots per bud compared to cane treatments (M12, M24) (Figure 2B). Notably, the B12 treatment (simple load on short heads) demonstrated an average exceeding 1.5 shoots per bud (Figure 2B), indicating that excess vigor of B12 vines was manifested as non-count shoot development [8]. This phenomenon was less pronounced in B24 and M12 vines and absent in M24 vines (double load in canes), where the value approached 1(Figure 2B). Given that excessive development of non-count shoots often signifies high shoot vigor in grapevines, the M24 treatment, combining the higher bud load with cane pruning, appears more favorable for 'Xinomavro' shoot growth in terms of shoot vigor under the given experimental conditions.

High bud loads can potentially reduce bud burst rates in grapevines. Research by [6] and evidence from viticultural practice have shown that bud burst rates can fall below 100% (<1 shoot per bud left after pruning) under high load conditions. This reduction is exacerbated in warm climates, with bud burst rates potentially dropping to 50% due to incomplete winter bud differentiation [35]. Additionally, combining high bud loads with cane pruning can lead to poor bud burst in the middle of the canes [36]. However, bud burst response to load manipulation is not consistent and depends on factors like varietal vigor and vineyard conditions. For instance, [8] observed a bud burst rate close to 120% in 'Chambourcin' (*V. vinifera* × *V. rupestris*) vines, even with a load of 40 buds per vine. Similarly, in the present study the higher bud load (24 buds per vine) distributed across canes of 6-7 buds each did not negatively impact bud burst rate (Figure 2B).

The patterns observed for dormant pruning weight in response to bud load and pruning type treatments were similar to those observed for total leaf area (Figure 1A) and total area of main leaves (Figure 1B). These responses are supported by a strong, significant linear relationship observed between total leaf area and pruning wood weight across both years of the experiment ($R^2 = 0.698$, $p = 0.05$ for 2016 and $R^2 = 0.843$, $p = 0.05$ for 2017). This strong relationship suggests that pruning weight was a reliable indicator of vine vegetative growth during the previous season [2].

4.2.3. Canopy Density, Cluster Shading and Microclimate

Canopy density is primarily determined by both the number of shoots per unit length of row and the vigor of individual shoots [37]. In our study, both shoot number and shoot vigor were found to vary in response to treatment effects. The higher ratio of actual leaf area to the exposed vine surface in B24 vines (Figure 4A), coupled with a lower percentage of canopy gaps and more shadowed leaves (Figure 4B), indicates increased canopy density in this treatment. Because each vine has specified space defined by the within-row vine distance in a given vineyard, the increased shading in the canopy of B24 vines was a direct result of their increased total leaf area (Figure 1A). The responses of canopy density to bud load and pruning type effects in the rest of treatments can be explained in an analogous manner.

The microclimate within a grapevine canopy is largely determined by the amount and spatial distribution of leaf area, as well as its interaction with the surrounding atmospheric conditions [10]. Canopy density affects the light environment within the grapevine canopy [11]. In turn, the degree of light exposure in the canopy, which is affected by canopy density, influences berry temperature [12,13]. Clusters and berries in dense, shaded canopies tend to have lower temperatures compared to those in more exposed, less dense canopies [12]. Consistent with these previous findings, our study observed lower berry temperatures in the denser canopies of B24 vines compared to other treatments, particularly those subjected to long pruning (Figure 5). Overall, our data show that bud load and pruning type had a significant influence on canopy microclimate in 'Xinomavro' vines.

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4.3. Yield Components

Previous research [5–8] indicates that as pruning severity decreases (i.e., more buds are retained), yield generally increases. For example, [7] found that increasing retained nodes from 24 to 72 per vine led to a linear yield increase from 4.8 to 12.7 kg vine^{-1} in the first year of their study in a 'Sauvignon blanc' vineyards. Similarly, [38] found that retaining over five-fold more buds significantly increased yield compared to retaining twice or fewer buds. The higher yields observed in high bud load treatments (Figure 6A), except for M24 vines in 2016, support these findings. However, this relationship is not always linear [5,7] due to compensation that occurs through changes in other yield components. This highlights the need for longer-term studies (4-7 years) to assess the sustainability of pruning regimes and their impact on vine yield and growth [7].

Vine production is determined by both the number of clusters per vine and the average cluster weight [7]. In the present study, a significant positive linear relationship was observed between grape number and bud number per vine across both years ($R^2 = 0.55$, $p < 0.01$ for 2016 and $R^2 = 0.83$, $p < 0.001$ for 2017). This relationship explains the lower number of clusters per vine in the lower bud treatments observed within each pruning type treatment (Figure 7A). The absence of any significant and consistent influence of bud load and pruning type on mean cluster weight (Figure 6B) suggests that grape yield per vine was primarily determined by cluster number. This hypothesis is supported by the results of [9] which performed data meta-analysis using mixed models to estimate the response ratio of vine yield components to several management practices: vine yield increases when pruning severity decreases due to increased cluster number despite reduced cluster weights.

Given that the number of clusters per shoot was consistently higher only in the B12 vines (Figure 7B), it appears that the number of clusters per vine in the other treatments was primarily determined by bud load rather than shoot fertility. Adequate bud fruitfulness in grapevines depends on sufficient light exposure of buds during flower development [10]. The negative relationship between bud load and bud fertility has been explained in terms of increased shading within the vine canopy [6]. While significant differences in canopy density and shading were observed between treatments (Figure 4A,B) the lack of a corresponding consistent effect on shoot fertility suggests that these variations in canopy microclimate did not significantly impact this yield component.

When studying pruning system effects on inflorescence primordia initiation and inflorescence architecture in 'Sauvignon Blanc' vines, [21] reported that pruning type had no influence on flower number per inflorescence and the extent of primary branch development. Our study revealed that pruning type influenced cluster architecture, as evidenced by changes in cluster dimensions, berry number, and density, but these changes were consistent only in the M24 vines (Table 1). Berry dimensions and weight, as factors influencing vine yield, tend to exhibit less variability compared to the number of clusters per vine or berries per cluster [7]. In our study, M24 vines consistently produced the smallest berries, while B12 vines yielded the largest (Figure S1, A, B). This aligns with previous research indicating that cane pruning generally results in smaller berries compared to spur pruning [22], and that berry weight tends to decrease with increasing pruning severity [6,8].

Smaller berries in red varieties are often preferred due to their supposedly higher skin-to-pulp ratio, which can lead to more concentrated wines [39]. However, this isn't always true, as berry components can decrease proportionally with size [40]. Despite smaller berries in M24 vines (Figure S1, A, B), both skin and seed contributions to overall berry mass increased (Table 1). This is likely due to a combined effect of reduced berry size and increased sunlight exposure in M24 vines [41], attributed to their more open canopies compared to B24 vines (Figure 4).

4.4. Berry Composition

Early studies report significant changes in berry juice composition in response to pruning treatments. For example, [6] found that sugar and acid concentrations decreased as the bud load increased while pH remained unchanged. The authors partially attributed these results to a concurrent reduction in leaf surface area available to ripen the fruit. Apart from berry juice constituents, color density of berry skins has been reported to vary inversely to bud load [18]. However, this was not consistent across years, probably because of influence of environmental factors

[18]. Inconsistent responses of berry composition to pruning type have been reported also by [22,42,43] in winegrapes. In table grapes, [44] reported that the influence of bud load on berry composition depended on the variety.

The inconsistent treatment effects and the strong interaction between bud load and pruning type on berry composition (Table 2) highlight the complex interplay of factors influencing berry composition. However, analysis of the interaction between bud load and pruning type on total anthocyanin ($F=24.23$, $p=0.0004$) and total phenol ($F=425.79$, $p<0.0001$) concentrations provides insights into their combined effects. While bud load had variable effects on anthocyanin concentration, within each pruning type cane pruned vines consistently yielded grapes with higher anthocyanin concentrations compared to spur pruning across bud loads and years. This suggests pruning type has a more consistent effect on anthocyanin concentration than bud load. For total phenols, the interaction between bud load and pruning type was even more pronounced as indicated by the high F value. At lower bud loads, cane pruning resulted in higher total phenol concentrations compared to spur pruning. However, this effect was reversed at higher bud loads. Given the unclear effects of pruning severity and type on total anthocyanin and phenol *content* (Table 2), it's possible that observed differences in their *concentrations* were partly driven by the generally lower berry weights observed in cane-pruned treatments at harvest (Figure S1A,B).

Variations in berry composition are frequently linked to corresponding shifts in vine balance, which can be influenced by both management practices and environmental conditions [4]. To estimate vine balance, various indices are employed, including the ratio of total leaf area to grape yield and the dimensionless Ravaz index, which represents the ratio of total pruning weight to grape yield [45]. For instance, a Ravaz index value between 5 and 10 is generally considered indicative of balanced vines [46]. However, this range may change because of varietal differences in growth characteristics [14,45]. Based on the typical Ravaz index range (5-10), the vines in this study can generally be classified as balanced, as evidenced by Figure S4B. The lack of significant treatment effects on the Ravaz index (Figure S4B) and the inconsistent effects observed in the ratio of foliar area to grape weight (Figure S4A) suggest that any observed impacts of bud load and pruning type on berry composition were not linked to vine balance. Given that the cane pruned vines had more open canopies, particularly in the lower bud treatment (Figure 4B), any berry composition responses may have been driven by differences in grape exposure to light and cluster temperature (Figure 5).

5. Conclusions

This study provides valuable insights into the effects of bud load and pruning type on 'Xinomavro' grapevine performance. Our findings demonstrate that:

Bud load and pruning type significantly influence vine vegetative growth, canopy structure, and microclimate. Short pruning with high bud load (B24) resulted in denser canopies and lower cluster temperatures, while cane pruning (M12 and M24) led to more open canopies and higher cluster temperatures.

Yield components were affected by treatments, with higher bud loads generally increasing yield, primarily through increased cluster numbers. Cane pruning, particularly M24, consistently produced smaller berries with a higher proportion of skin and seeds.

Berry composition showed complex responses to treatments, with significant interactions between bud load and pruning type. Cane-pruned vines tended to produce grapes with higher anthocyanin and total phenol content, likely due to increased light exposure and smaller berry size.

The effects of bud load and pruning type on vine balance indices were not consistent, suggesting that observed differences in berry composition were more likely due to changes in canopy microclimate than alterations in source-sink relationships.

Multivariate analysis revealed that load distribution (short vs. long fruiting units) may have a greater impact on vine growth, yield, and berry composition than bud load alone.

These findings highlight the importance of pruning practices in managing 'Xinomavro' vineyards. Cane pruning, especially with higher bud loads, appears to offer a good balance between yield and quality parameters. However, the choice between short and long pruning should consider specific vineyard conditions and production goals. Future research should focus on longer-term studies to assess the sustainability of these pruning regimes over multiple seasons. Additionally, investigating the interaction of these pruning practices with other management techniques, such as irrigation strategies or canopy management, could provide a more comprehensive understanding of optimizing 'Xinomavro' production in various environmental contexts.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Table S1. The effects of bud load and pruning type on physiology parameters; Figure S1. The effect of bud load and pruning type on berry weight. Figure S2. The effect of bud load and pruning type on on the total soluble solids, titratable acidity, and pH; Figure S3. The effects of bud load and pruning type on the total anthocyanins and total phenolics per berry; Figure S4. The effect of bud load and pruning type on the ratio of total leaf area to grape yield and the ratio of yield to total pruning weight.

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