








Article

Grass Cover in Vineyards as a Multifunctional Solution for Sustainable Grape Growing: A Case Study of Cabernet Sauvignon Cultivation in Serbia

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Abstract: Faced with the challenges posed by climate change, Serbian viticulture is looking for sustainable solutions for adaptable production. This study shows that grass is a multifunctional tool for overcoming the challenges of intensive viticulture while maintaining the quality of the grapes. In a three-year research experiment (2020–2022), the maintenance of an inter-row sward in a vineyard with four certified high-quality French Cabernet Sauvignon clones was investigated, and its effects on the ampelographic composition of the grapes and the quality of the grape juice (must) were studied as a function of wine quality. A grass sward was established between the rows as a biological soil management system and as a climate change adaptation measure in a high-intensity viticultural system. A grass–legume mixture was used as an inter-row cover crop, with nitrogen applied in two doses (50 and 100 kg ha^{−1}) in spring. The growth of the grasses responded to the nitrogen fertilisation, which was reflected in the biomass production, surface cover and nitrogen content in the biomass. At the end of the study, the biomass of the grass increased threefold when a high dose of nitrogen was applied compared to the non-fertilised grass. In contrast to the effects of nitrogen on the sward, N has no effect on the quantitative or qualitative parameters of the grapes. Clone 169 was separated for most grape mechanical parameters such as the bunch mass, all berries and the bunch stem; clone 15 showed the best grape juice quality parameters such as the sugar content and glycoacidometric index. The results show an option for climate change adaptation in viticulture that can mitigate the effects of rising temperatures, contribute to soil conservation and carbon storage in biomass and enable timely interventions in vineyards after heavy rainfall by creating accessible paths within the vineyards. The three-year effect of the different nutrient management of the sward in the inter-rows of Cabernet Sauvignon showed that the interaction between the two systems, sward and vine, is low and has no negative impact on the ampelographic and qualitative grape parameters.

Keywords: Cabernet Sauvignon; grass cover; N application; grape quality; climate change; adaptation



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1. Introduction

According to the latest census of vineyards in the Republic of Serbia, $19,973 \pm 136$ ha (2020–2022) are planted with vines, of which 15,773 ha are planted with wine varieties and 4200 ha are planted with table varieties. In the total agricultural production, viticulture has a share of 0.6%, with an average yield of 8 ± 0.23 t ha⁻¹. In the overall structure of fruit production, grapevine is the most important fruit species in the Republic of Serbia, along with apple, plum and raspberry [1,2].

According to the reports of the International Organisation of Vine and Wine (OIV) [3–7] for the period from 2010 to 2019, the Republic of Serbia ranks 17th to 19th in the world in terms of annual wine production (2227 ± 68 million hectolitres). The average annual wine consumption was 2.0 ± 0.49 litres per capita. Cabernet Sauvignon is the most important international grape variety for the production of premium red wines [8,9] and also the grape variety with the largest share (20%) of the total wine assortment in the Republic of Serbia [2,10]. It is cultivated in all wine-growing regions of Serbia, with a focus on the cultivation of clones imported from Italy and France [11].

Traditionally, the vineyards were cultivated in a system of continuous mechanical tillage. The modern approach includes a methodology that increasingly relies on so-called biological soil management systems that utilise cover crops [12] as soil management functions and with the principles of sustainable agriculture [13–15].

Grass covering as a biological system and as a method of soil conservation [16] is used in different regions with different amounts of precipitation. These regions have higher rainfall or are located in semi-arid [17–19] and arid geographical areas where the vineyards are irrigated or the soils are prone to waterlogging [20,21]. In the context of climate change in Serbian agriculture [22–25], protecting the grape yield and quality through grass cover could be a solution for reducing the risk of climate hazards such as extreme rainfall, heat waves and soil degradation. Experience around the world shows that various measures are being taken to adapt viticultural production to climate change and make it sustainable [26–28].

The establishment of a grass cover could be a motivation for producers due to its multifunctionality and multiple benefits compared to other adaptation measures. For example, effects on soil have been found through the reduction in erosion [29,30], the reduced use of machinery and the risk of soil compaction [31], an increase in surface organic matter content and an improvement in soil biodiversity status [32,33]. Better rainfall management is reflected in the reduction in surface water runoff and the reduction in temperature extremes [34,35], the cover raises the possibility of increasing the nitrogen content in the soil [36] and the use of herbicides is reduced as weeds are successfully suppressed [37,38]. The grass cover facilitates passage after extreme rainfall and enables rapid intervention to combat diseases. On the other hand, the possible negative aspects of the cover have been pointed out, such as the unfavourable effects on yield quality [35,39] and competition for soil moisture during drought [40,41].

Grass covering can be accomplished with only one plant species, but it is considered better to sow a mixture of grasses and legumes, which complement each other and reduce the risk of the rapid deterioration of the sward due to unfavourable weather conditions, pest infestation, diseases and weeds [42]. The varieties for the grass mixtures are selected so that they are as long-lived as possible but have acceptable water, nutrients, weaker growth and tillering requirements so that they do not compete with the vines. Low-growing varieties are more suitable because they do not hinder work in the vineyard. The root system should be well connected to the soil in order to be resistant to trampling and tolerate frequent mowing [43]. If you choose such species for grass mixtures, nitrogen fertilisation is necessary, especially in the uppermost soil layers [44], so that a dense

sward can develop and form. There is a risk that fertilisation in combination with other agrotechnical measures can lead to a reduction in the vigour of the varieties (especially in the case of very vigorous varieties such as Cabernet Sauvignon) and to changes in the yield components. The risk of the influence of fertilisation on the qualitative parameters of the grapes is lower and is generally influenced by the interaction of meteorological seasonal phenomena and ampelotechnical measures on the variety [35,36,45,46]. The nitrogen requirement of grapevines is relatively low and is generally stated to be less than 100 kg ha⁻¹ during the growing season [47]. The vine root system spreads deeper in the soil to utilise the water resources at a greater depth, leaving the mineralisation zone with the highest inorganic nitrogen content. Nitrogen can easily be leached into the bare soil and reach greater depths through the soil profiles. This can be prevented by a temporary or permanent plant cover. Due to the shorter uptake time and the time needed for the annual cover crop to develop its root system, nitrogen is less taken up by a temporary sward than by a permanent sward [47,48]. For this reason, a long-term cover is the better option, as the risk of leaching is lower and less nitrogen can reach the roots of the vines. The aim of the work was to evaluate the effects of nitrogen fertilisation for grass cover maintenance in a vineyard planted with Caberne Sauvignon clones. The study utilised a sown sward with high nitrogen requirements to maintain persistence and cover, which is in conflict with the limited requirements of the productive Cabernet Sauvignon clones. In this conflict of interest between two systems, the benefits and risks of each system and the possible interaction of parameters on one side and the other are analysed in the context of climate change.

2. Materials and Methods

2.1. Experimental Site and Design

The trial was conducted in central Serbia in the Šumadija wine-growing region and the Krnjevo subregion (GPS N 44° 25' 47" E 21° 02' 14", 220 m altitude) over three growing seasons (2020, 2021 and 2022). The vineyard was designed with an inter-row spacing of 2.4 m and 0.9 m between vines in a row. The training system is characterised by a trunk height of 90 cm, to which the Guyot pruning method is applied. All experimental vines were pruned uniquely, leaving one spur with two buds and one arch with eight buds per trunk. The trials were carried out with four certified, high-quality French Cabernet Sauvignon clones 15, 169, 191 and 412. During the growing season, the usual agrotechnical (tillage in the row, removal of grass, protection against diseases and pests, etc.) and ampelotechnical (removal of excess shoots, pulling the shoots into the trellis, shortening the shoots, etc.) measures were carried out in the rows of the vineyard. A sward was established between the rows as a biological soil management system and as a measure to adapt to climate change in a high-intensity viticulture system. For the grass–legume mixture, on the recommendation of the Institute for Forage Crops, Kruševac, Serbia, the following species were used: 60% *Festuca rubra*, 30% *Lolium perenne* and 10% *Trifolium repens* with a seed rate of 40 kg ha⁻¹. Although the mixture contained white clover (legume), which was supposed to improve the nitrogen supply of the sward, it practically disappeared due to the dry conditions in spring and the relatively high acidity of the soil. To ensure good grass cover, nitrogen was applied each spring in two doses of 50 and 100 kg ha⁻¹ N, but only to the sown sward, not to the vines. The trial was set up in randomised plots of 10 m² in three replicates for all clones and treatments (control, 50 and 100 kg ha⁻¹ N). The plot size for each treatment was 1.45 m wide (width of the sward per row, between two consecutive rows of vines) along the slope and 21 m long, divided into three subplots (7 × 1.45 = 10 m²). Under the vines, there is a substrate without plant cover. This cultivation method is common for this type of vineyard in Serbia. The slope of the terrain was gentle, characteristic of

the vineyards of Šumadija, Serbia, and no leaching or soil erosion was expected. Each subplot was mowed to a height of 3 cm each year. Grass samples were collected annually by cuttings and measurements were taken, i.e., the fresh and dry biomass was measured and presented cumulatively for the growing season. The total fresh biomass (TFB) was determined by weighing the freshly cut grass on a scale directly in the vineyard after mowing, and the dry matter (TDM) was measured by weighing it on a scale after drying.

The quality of the sward was assessed on a scale of 0 to 9 in three test years in spring before mowing. The visual quality of the sward was rated as the Cover Quality Index (CQI) on a scale of 1 to 9, where 1 = worst quality, 6 = lowest acceptable quality for a sward and 9 = optimum colour, density and uniformity [49]. The assessment of sward quality was primarily based on uniformity, density, texture and freedom from weeds and diseases [50]. The Cover Quality Index and N content were determined for each cutting and the average for the growing season was presented (Figure 1).



Figure 1. Experimental vineyard.

2.2. Climate Factors

The meteorological data were obtained from the nearest observation station (Smederevska Palanka) of the Republic Hydrometeorological Service of Serbia (RHMSS) with high-quality long-term data, located at a similar altitude as the experimental site and 10 km away from it. The use of long-term station data enabled a better understanding of the weather conditions during the duration of the experiment, by comparing them with the climate values of selected indices. Daily data series of temperature and precipitation can be obtained from the RHMSS reports for the selected station [51,52] and from the Digital Climate Atlas of Serbia until 2020 [53].

The standard (mean annual, growing season and monthly temperatures in °C and accumulated precipitation in mm) and specific climate indices defined by the International Organisation of Vine and Wine (OIV) in Resolution OIV-Viti 423-2012 Rev1, Appendix 2 [54] were calculated for the period 1991–2020 (period for which the official climate normals, used for climate monitoring in the RHMSS, are determined) and separately for the years of the experiment (2020, 2021, 2022). The indices recommended by the OIV and used in this paper refer to the analysis of the accumulated heat during the growing season (Winkler and Huglin indices), the general humidity conditions (Dryness index), the heat conditions during ripening (Cool night index) and the risks of low and high temperatures (number of days with temperatures below 0 °C during the growing season, days with temperatures below 15 °C and above 35 °C). In addition, the hydrothermal index (K) of

water conservation was included in the analysis, which is important for grass growth and was calculated according to the Selianinov index [55]. The following formula was used to calculate the value of the hydrothermal index (K):

$$K = (Mo \times 10) / (Dt \times \text{days})$$

K is the hydrothermal coefficient for a single month during the growing season, Mo is the total monthly precipitation and Dt is the mean daily temperature in a given month.

2.3. Soil and Plant Sampling and Chemical Characterisation

For a bulk sample, three individual samples were taken from each clone at three depths in 2020. As the soil among the clones was fairly uniform, a single index was given by depth. No soil analyses were carried out in subsequent springs as the aim was to apply the same amounts of nitrogen in order to determine the possible effects on the vine over several years. The soil samples were taken with a pedological probe at 3 depths: 0–30 cm (surface mineral layer of soil), 30–60 cm (subsurface soil layer) and 80–100 cm (deep soil layer). The soil samples were dried, crushed and sieved through sieves with an opening of <2 mm.

The pH of the soil was determined at a solid/liquid ratio of 1:2.5 (*w/v*) (method ISO 10390:1994—Determination of pH in soil) using a pH metre (Iskra, Ljubljana, Yugoslavia (Slovenia), model MA 5730). To determine the active acidity ($\text{pH}_{\text{H}_2\text{O}}$), 10 g of dry soil was weighed and 25 mL of distilled water was added and stirred. The exchangeable acidity (pH_{KCl}) was determined in a 1 M KCl solution.

The total organic C content of the soil was determined by sulfochromic oxidation (SRPS ISO 14235:2005). The total N content of the soil samples was determined using the semi-micro Kjeldahl method (SRPS ISO 11261:2005).

Available phosphorus (P_2O_5) and potassium (K_2O) in the soil were extracted by the Egner–Riehm method using 2 M AL solution (mixture of 0.1 M ammonium lactate and 0.4 M acetic acid). The P_2O_5 concentration was determined by the molybdenum blue method using a spectrophotometer (580 nm, Shimadzu UV-1900i) and the K_2O concentration was determined by the Shimadzu AA-7000 flame emission spectrophotometer using a calibration curve prepared after measuring a standard of a known concentration.

For chemical characterisation, samples of the grass cover were collected each year after mowing, dried and ground by crushing. The N concentration in the plant material was determined using the semi-micro Kjeldahl method [56]. The sample of plant material was decomposed by heating with concentrated sulphuric acid in the presence of a catalyst (30% H_2O_2). The resulting solution was then quantitatively transferred to distillation flasks, treated with 40% NaOH solution and subjected to vapour distillation. The N concentration was calculated after the titration of the distillate.

2.4. Grape Samples

To analyse the mechanical composition of the grapes and berries as well as the quality of the grape juice (must), the grapes were harvested when fully mature. The mechanical analysis of the grapes and berries was carried out according to the methodology described in [57,58]. The results for the bunch length, bunch width, bunch stem mass and berry mass of 10 randomly selected bunches per treatment were presented in a paper. A sample of 10 representative bunches was collected randomly along the length of the trellis, ensuring that the sample consisted of bunches that were both exposed to the sun and in the shade. The length and width of bunches were measured with a ruler before the bunches were placed on a surface of graph paper. To determine the mass of the berries, the pedicel of each berry was carefully cut off with scissors so that as little mesocarp (brush) as possible

remained on the grape stem; after this procedure, the mass of berries per bunch and the mass of the bunch stems were measured on an analytical balance.

The quality parameters of the grape juice (must) are represented by the content of accumulated sugar (%), the total acidity expressed as tartaric acid (g/L) and the pH value. The sugar content was determined by physico-chemical methods using the Oeschle hydrometer and the values were calculated using the Dujardin–Salleron tables. The total acidity was determined by a titration method using 0.1 M NaOH and the pH was determined using a pH metre. The glycoacidometric index-GAI (ripeness index) was calculated from the ratio of sugar and acid content.

2.5. Statistical Analysis

The results obtained were subjected to an analysis of variance (two-way factorial ANOVA). The differences between the mean values were tested with a *t*-test at the 0.05 and 0.01 probability levels. In addition, the determined parameters of the sward and the qualitative and quantitative characteristics of the grapes were correlated separately for each year in order to determine a possible correlation (probability level 0.05) and the effects of the sward on the grapes.

3. Results

All years in which the experiment was conducted were warmer than the climate values for the period 1991–2020 (Table 1). The warmest year in the period in which the 2020–2022 experiment was conducted was 2022, with a mean temperature anomaly (TAN) of 0.9 °C and a growing season temperature anomaly (TVEG) of 0.6 °C, compared to 1991–2020. The Winkler index (WIN) and the Huglin index (HI), which indicate heat accumulation during the growing season, were also highest in this year, as was the Cool night index (CI). In 2021, the highest number of days with temperatures above 35 °C (NT35) was observed, more than double the normal for 1991–2020. However, 2021 was the coldest year on average, with an anomaly in the average annual temperature of 0.4 °C and a negative anomaly in the average temperature during the growing season (−0.2 °C) compared to 1991–2020. While WIN was slightly below average, HI was above average for 1991–2020, which was the result of a very hot period in July (Figure 2). CI was below average (−0.3 °C). There was no risk of high temperatures in 2020 (NT35 is zero). In terms of frost risk, the number of days with frost (NT0) was higher in all years than the 1991–2020 average.

Table 1. Values of selected indices: average annual temperature (TAN), average temperature during the growing season (TVEG), accumulated annual precipitation (PAN), accumulated precipitation during the growing season (PVEG), Winkler Index (WIN), Huglin Heliothermal Index (HI), Cool Night Index (CI), Dryness Index (DI), number of days with a minimum daily temperature below 0 °C during the growing season, from 1 April to 31 October (NT0), number of days with temperatures below 15 °C (NT15) and number of days with temperatures above 35 °C (NT35). The values for the climate period were calculated as an average for 1991–2020.

	TAN	TVEG	PAN	PVEG	WIN	HI	CI	DI	NT0	NT35	NT15
1991–2020	12.3	18.0	669	439	1755	2336	11.2	170	4.6	8.7	2.4
2020	13.0	18.1	724	550	1765	2367	11.7	216	8.0	0.0	0.0
2021	12.7	17.8	796	443	1739	2404	9.9	156	9.0	20.0	1.0
2022	13.2	18.6	809	556	1876	2475	12.1	259	6.0	11.0	1.0

The accumulated precipitation in all three years was above the normal values for 1991–2020, both for the year and for the growing season. The largest annual and seasonal precipitation amounts were observed for 2022 (PAN 21% above normal and PVEG 27% above normal), with the Dryness index (DI) well above the 1991–2020 average. Similar

precipitation in the growing season was observed in 2020, and in 2021, the PVEG was over 100 mm lower than in the other two years, but close to the 1991–2020 climate average. The lowest DI was in 2021, lower than the climate average.

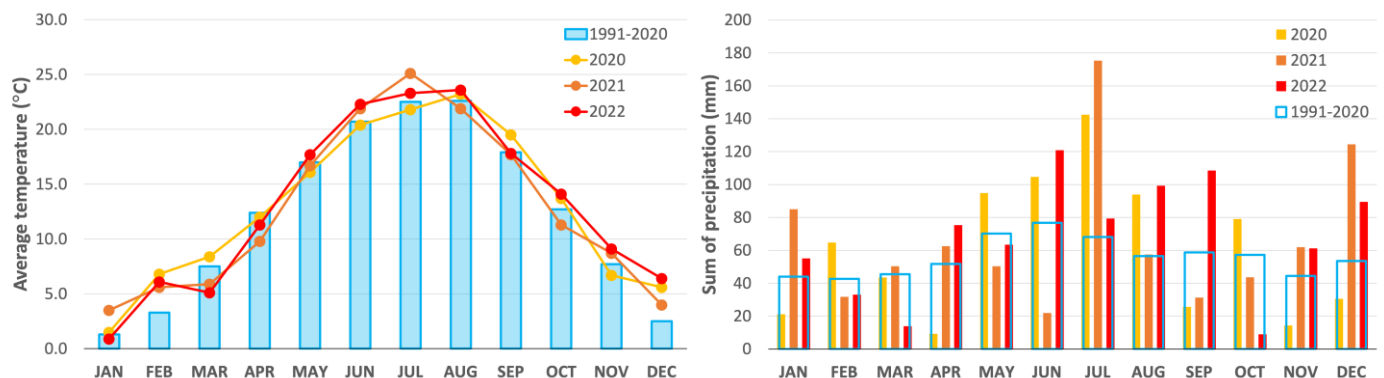


Figure 2. Monthly values of temperature and accumulated precipitation; average for 1991–2020 and values for the experimental years 2020, 2021, 2022.

The year-to-year variability of monthly average temperatures and cumulative precipitation for 2020, 2021 and 2022 and the average for 1991–2020 are shown in Figure 2. There was high year-to-year variability in precipitation, particularly high in 2021, when the highest precipitation amounts were recorded in July, reaching 175 mm, which is 157% compared to the average for July over the 1991–2020 period. Note that this month was also categorised as the warmest during the experimental period. On the other hand, June 2021 was one of the driest months.

In addition to the climate analysis presented above, which is standard practice according to the OIV recommendation, selected values of the hydrothermal Selianinov index are presented in Table 2 to further investigate the potential impact of the combined temperature and precipitation conditions and their annual variability. Lower values of this index were recorded in April and September 2020 and in June 2021. Apart from the fact that July had the highest monthly precipitation, four of the six months (April–September) had drier conditions, which was also reflected in the lowest DI (Table 1).

Table 2. Hydrothermal Selianinov index (K) in the growing season (April to September).

	April	May	June	July	August	September
1991–2020	1.45	1.22	1.43	0.95	0.80	1.04
2020	0.26	1.90	1.71	2.11	1.31	0.44
2021	2.13	0.97	0.33	2.25	0.85	0.59
2022	2.22	1.16	1.81	1.10	1.36	2.03

Selianinov Index (K): <0.5—drought, 0.5–1.0—semi-drought, 1.0–1.5—border of optimal moisture, >1.5—excessive moisture.

The most important soil properties are listed in Table 3. The surface layer of the soil is characterised by an acid reaction that becomes neutral with increasing depth. The vineyard is located on loose humus soil with a low total nitrogen content, which provides a basis for nitrogen fertilisation. Measured by the content of available P_2O_5 and K_2O , the analysed soil is poor. The C/N ratio is within the usual range of values for vineyard soils.

In all three years, the effect of N fertilisation on the concentration in the plant tissue was observed, which increased steadily with the amount applied (Table 4). At the same time, the difference in nitrogen concentration in sward biomass after the treatments is the lowest in the first year of the research (up to 11% in 2020), while it is the highest in the third year (up to 38% in 2022). Although the nitrogen content of the soil is low, the concentration of nitrogen in the sward during the experimental period is relatively high in the treatment

without nitrogen fertilisation ($\geq 1.67\%$). Nitrogen fertilisation also had an effect on the increase in biomass and the quality index of the sward.

Table 3. Basic soil fertility planted by Cabernet Sauvignon clones.

Depth	pH _{H2O}	pH _{KCl}	N _{tot} (%)	Available (mg 100 ⁻¹ g of Soil)		Total Organic C (%)	C/N
				P ₂ O ₅	K ₂ O		
0–30 cm	6.81	4.84	0.08	2.42	0.32	0.82	9.9
30–60 cm	6.56	5.06	0.07	2.79	0.30	0.66	8.9
80–100 cm	7.86	6.75	0.04	4.57	0.49	0.39	10.5

Table 4. Ground cover characteristics during the period 2020–2022.

	Total Fresh Biomass (g)	Total Dry Biomass (g)	Cover Quality Index	N Content (%)
Fertiliser (A)		2020		
0	4299 b	1478 b	5.52 c	1.71 c
50	4598 b	1554 b	6.19 b	1.83 b
100	4949 a	1860 a	6.83 a	1.90 a
Clone (B)				
15	2903 C	955 C	5.81 B	1.50 C
169	1614 D	694 D	4.22 C	1.67 B
191	7368 A	2337 B	7.28 A	2.08 A
412	6575 B	2537 A	7.42 A	2.01 A
ANOVA				
A	322 **	145 **	0.26 **	0.11 **
B	372 **	167 **	0.30 **	0.14 **
Fertiliser (A)		2021		
0	950 c	375 c	4.8 c	1.67 c
50	1948 b	724 b	5.8 b	1.95 b
100	2983 a	1015 a	7.0 a	2.18 a
Clone (B)				
15	1466 B	548 B	5.6 C	1.85 AB
169	1463 B	401 B	5.2 D	2.08
191	2213 A	852 A	6.0 B	1.80 B
412	2699 A	1019 A	6.6 A	2.01 AB
ANOVA				
A	481 **	188 **	0.17 **	0.21 **
B	555 **	217 **	0.19 **	0.24 **
Fertiliser (A)		2022		
0	1533 c	549 c	7.36 b	1.68 c
50	3506 b	1205 b	7.48 b	1.87 b
100	5287 a	1604 a	7.81 a	2.32 a
Clone (B)				
15	4651 A	1594 A	7.92 A	1.82 B
169	2669 C	869 C	7.13 C	2.08 A
191	2695 C	859 C	7.56 B	1.85 B
412	3788 B	1154 B	7.61 AB	2.06 A
ANOVA				
A	611 **	220 **	0.28 **	0.20 **
B	706 **	254 **	0.33 **	0.23 **

Significant at $p < 0.01$ (**). The numbers in the columns followed by the same letter are not statistically different according to the t -test ($p < 0.05$); lowercase letters stand for differences caused by fertilisation and uppercase letters stand for differences between the clones.

Comparing all three years with the weather conditions during the growing season based on the Selianinov index (Tables 2 and 4), it can be seen that in the first year of the study, the total fresh biomass and dry biomass were high due to two harvests (one in May and one in August). Dry conditions in April were followed by extremely wet conditions from May to July, resulting in high biomass in August. The final yield balance showed equal yields in the control treatment and the lower fertiliser treatments, with biomass increasing significantly with higher nitrogen application and yields differing between clones. In the second year, spring conditions followed a different trend, changing from wet conditions in April to increasingly dry months until July (harvests took place in May and July). With almost half the yield of fresh and dry biomass compared to the first year, there were clear differences between the nitrogen fertiliser treatments, while the yields of the different clones became more uniform. In the third year of the study, conditions were optimal for grass growth in spring, resulting in different grass yields due to a significant interaction between weather conditions and the nitrogen fertiliser applied. Yields increased significantly from the control variant to the higher nitrogen dose, exceeding the yield of the control variant by more than three times.

When analysing the plant cover indicators over three years, we observe significant differences ($p < 0.01$) between the treatments with nitrogen application and between the clones. In 2020, the fresh and dry grass biomass yield and cover quality index were highest when $100 \text{ kg ha}^{-1} \text{ N}$ was applied. In addition, N application at the highest fertiliser dose had an influence on the nitrogen content in the grass biomass. Grass growth was more productive under clones 412 and 191.

In 2021, all parameters increased continuously with the dose of nitrogen applied. Grass cover continued to be better under clones 191 and 412, while the differences in the nitrogen content of biomass between the different clones were not as consistent. In 2022, nitrogen application again affected the increase in fresh and dry matter as well as sward quality (cover and nitrogen content in biomass). The morphological parameters of the sward were most pronounced in clone 15.

From the results shown in Table 5, it can be concluded that in the first year (2020) of applying different fertiliser doses to the sward, no influence on the mechanical parameters of the bunches was observed. Clone 169 stands out in all parameters compared to the other clones analysed. In 2021, there were differences between the fertiliser treatments with the same tendency for clone 169. In 2022, the two clones 15 and 169 were separated based on the determined mass of bunches, all berries and bunch stems.

The application of different doses of nitrogen fertiliser in the first year had no effect on the values of the qualitative parameters of the grape juice-must (Table 5). On the other hand, differences were found in the values between the clones tested. In the second year of the trial (2021), the same trend of variation depending on the fertiliser treatment was observed. Clone 169 stood out in terms of sugar content and clone 191 stood out in terms of total acids expressed as tartaric acid. In 2022, an effect of nitrogen fertilisation was observed on total acidity (dose 100 kg ha^{-1}) and on the glycoacidometric index (50 kg ha^{-1}). Clone 15 stood out in terms of the sugar content and glycoacidometric index, while clone 412 stood out in terms of total acidity. The effect of nitrogen fertilisation was more pronounced in the third year of the study, which is due to the cumulative effect of applying the same fertiliser concentrations. These variations could therefore be a consequence of the different nitrogen uptake from the soil substrate in the different years, making the cumulative effect even more pronounced.

The factor of vineyard management must be completely disregarded, considering that it is a very intensive plantation where the same agro- and ampelotechnical measures were applied every year. The timely application of the aforementioned measures is essential

in this type of production, especially considering that the grapes from the vineyard are destined for the production of premium wine. The differentiation of clone 15 by most of its characteristics can be attributed to its genetics, considering that it is an isolated superior clone and not a varietal population of Cabernet Sauvignon.

Table 5. Grape characteristics (bunch and berry) and grape quality parameters.

	Bunch Characteristics (cm and g)				Berry Mass (g)	Grape Quality Parameters			
	Length	Width	Mass	Stem Mass		% Sugar	TAC (g L ⁻¹)	GAI	pH
2020, Fertiliser (A)									
0	13	8.23	162	6.98	154	20.8	7.21	3.08	2.95
50	13.3	8.81	173	7.03	165	21.6	7.16	3.10	2.95
100	13.5	8.15	165	7.63	157	21.0	7.33	3.00	2.91
Clone (B)									
15	14.2 B	8.90 AB	184 B	7.03	177 B	18.6 C	5.97 C	3.17 A	3.16 A
169	15.4 A	9.48 A	211 A	7.87	202 A	24.3 A	7.60 AB	3.21 A	2.59 C
191	11.7 C	8.10 BC	140 C	7.27	130 C	20.5 BC	8.13 A	2.80 B	2.98 B
412	11.8 C	7.10 C	132 C	6.67	125 C	21.2 B	7.23 B	3.04 AB	3.03 B
ANOVA									
A	1.02 NS	0.91 NS	21 NS	1.32 NS	20 NS	1.86 NS	0.69 NS	0.27 NS	0.06 NS
B	0.89 **	1.05 **	24 **	1.52 NS	23 **	2.15 **	0.80 **	0.31 *	0.07 **
2021, Fertiliser (A)									
0	11.5	7.2 a	119 ab	4.45 ab	114 ab	24.0	7.12	3.40	3.10
50	11.1	6.5 ab	111 b	4.00 b	105 b	24.5	6.67	3.73	3.10
100	11.7	6.2 b	127 a	4.49 a	122 a	24.0	6.77	3.58	3.12
Clone (B)									
15	11.6 AB	6.5 AB	122 AB	4.75 A	114 B	25.5 A	6.54 B	3.95 A	3.10 B
169	12.1 A	7.2 A	131 A	4.47 AB	127 A	24.9 A	6.71 B	3.73 AB	3.13 A
191	11.1 B	6.9 AB	117 BC	4.19 BC	112 BC	23.1 B	7.37 A	3.15 C	3.10 B
412	11.0	6.0 B	107 C	3.84 C	102 C	23.2 B	6.79 B	3.45 BC	3.08 B
ANOVA									
A	0.56 NS	0.96 *	11 **	0.47 *	10 **	1.4 NS	0.48 NS	0.34 NS	0.02 NS
B	0.64 **	1.11 *	12 **	0.55 **	11 **	1.7 **	0.56 **	0.40 **	0.03 **
2022, Fertiliser (A)									
0	14.3 a	8.23	166	7.18 a	159	21.2	7.11 ab	3.01 ab	3.17
50	13.4 b	7.97	152	5.91 b	145	21.5	6.46 b	3.38 a	3.19
100	13.9 ab	8.20	153	6.23 ab	147	21.2	7.25 a	2.84 b	3.20
Clone (B)									
15	15.1 A	8.16 A	176 A	6.77 AB	169 A	22.8 A	6.33 B	3.72 A	3.26 A
169	14.5 AB	8.57 A	186 A	7.31 A	177 A	21.6 B	7.08 AB	2.82 B	3.19 B
191	12.1 C	7.55 B	125 B	5.70 B	120 B	19.9 C	7.15 AB	2.82 B	3.19 B
412	13.7 B	8.26 A	141 B	5.97 B	135 B	20.9 B	7.20 A	2.93 B	3.11 C
ANOVA									
A	0.86 *	0.52 NS	16 NS	1.10 **	14.6 NS	0.86 NS	0.76 *	0.41 **	0.05 NS
B	0.99 **	0.60 **	18 **	1.27 **	16.8 **	0.99	0.88 *	0.48 **	0.06 **

TAC—total acid content; GAI—glycoacidometric index. Significant at $p < 0.05$ (*) and $p < 0.01$ (**); not significant (NS). The numbers in the columns followed by the same letter are not statistically different according to the t -test ($p < 0.05$); lowercase letters stand for differences caused by fertilisation and uppercase letters stand for differences between the clones.

The highest correlation dependence in all three years (Figure 3) was in three clusters where sward indicators, bunch indicators and grape quality were demonstrably linked (positive and negative), while the correlation between clusters was only significant in 2020. The first year of the study is also characterised by the favourable weather conditions for all parameters, so a mutual correlation also occurred, while in the following years, the correlations were statistically significant in some clusters, but the pale colour indicates a low intensity.

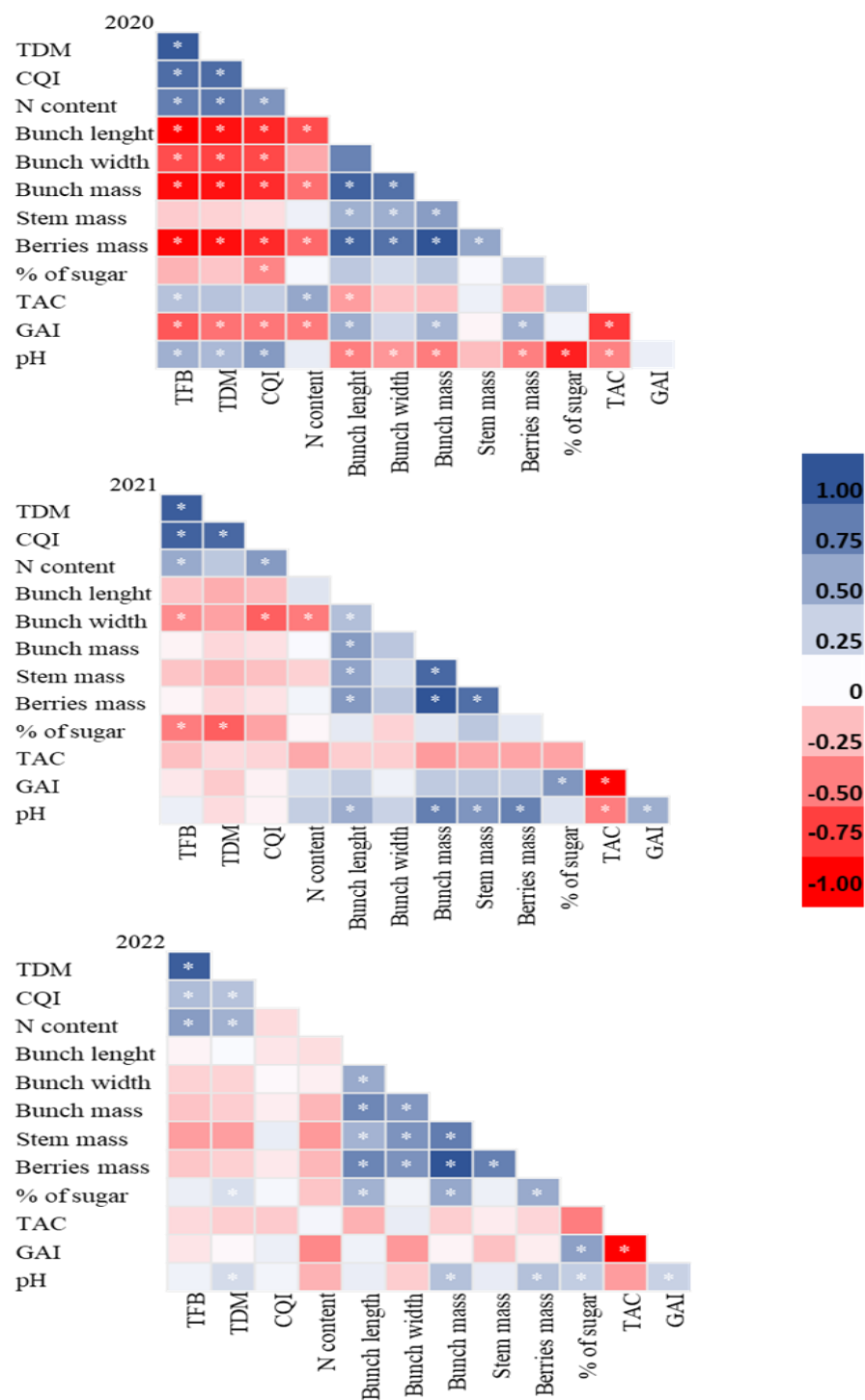


Figure 3. Correlation of grape parameters and sward indicators. TFB—total fresh biomass; TDM—total dry matter; CQI—cover quality index; N content—N content in sward; TAC—total acid content; GAI—glycoacidometric index; significance level * $p < 0.05$.

4. Discussion

Climate change in Serbia is affecting grape production due to the rapid increase in average temperature and the frequency and severity of heat waves, droughts and extreme rainfall [22–24]. The increase in drought and the intensification of precipitation increase the risk of soil degradation [59]. Thermal conditions have changed so that at lower altitudes they correspond to the climatic optimum for the production of quality wine, which is assessed according to the Winkler index as defined in the Multicriteria Classification System [23,60,61] and accepted by the International Organisation of Vine and Wine [54] as one of the indices for the zoning of vine growing areas. It is predicted that these conditions will continue in the coming decades [25]. This is by far the only positive effect of climate change. In this study, the resilience of viticulture under changing climatic conditions is analysed with regard to adaptation to climate change. It takes into account the plant cover between rows, which helps to mitigate soil degradation, while at the same time considering the negative effects of climate change on water availability during the growing season. The years in which the experiment presented here was conducted (2020, 2021, 2022) were all above normal compared to the 1991–2020 average. This baseline period is about 1 °C warmer than 1961–1990 [51,53], meaning that the experiment was conducted under warmer conditions caused by climate change. As shown in the results, extreme conditions occurred in 2020, 2021 and 2022: extreme heat periods, extreme precipitation periods, dry periods, etc. This exposed the vineyard to increasing climate variability and extreme conditions, which are expected to increase in the future [25]. To mitigate these effects, a grass cover was introduced.

Grass covering in vineyards prevents erosion [62,63], reduces soil compaction, increases the organic matter content in the surface layer of the soil through the decomposition and mineralisation of the mown above-ground mass and improves the rhizogenic properties [32]. The chemical properties of the soil in this study were poor, especially the N content, indicating poor water retention and low potential for sward maintenance. Many authors point out that organic matter in the vineyard soil and nitrogen are crucial as indicators of the water capacity of the substrate as well as the competitive relationship between the sward and the vine. Most studies have focused on comparing clean tillage and vineyard cover cropping treatments on the competition of water and nutrients in the soil. For example, several studies found that cover crops decreased the yield and pruning weight in Spain [64] by increasing water competition with vines, decreased the berry size in Portugal [65], increased the total soluble solids in grapes [66] in France, affected most physical and chemical indices in grapes and increased total soluble solids, but they had no significant effects on titrated acid and pH [67]. In studies by Peng et al. [45], the cover crop had some effect on the physical and chemical indices of grape berries, such as soluble solids, titrated acidity, pH and 100 berry weight. Some trials were conducted with inter-row Cabernet Sauvignon and found that the effects of cover cropping on the chemical composition of the grapes were minimal and more influenced by seasonal variations. Cover cropping did not reduce the yield and berry weight was slightly reduced by seasonal variations [36,68]. The overall results showed no effect on yield, while changes in must composition were observed after 2–3 years [69,70].

Nitrogen application has not been sufficiently tested in the sward under vines in Serbia, as the spacing is traditionally left without a sward or with spontaneous vegetation. When sampling the initial condition, it was assumed that the nitrogen mobility in the soil is high and that fertilisation is necessary every spring for the vitality of the sward. In our study, the application of nitrogen had a clearly positive effect on the growth and development of the biomass as well as on the quality of the sward (density of the grass cover), which is also a result of the successful assimilation of nitrogen by the grass, which

is expressed in the nitrogen content in the biomass. In addition, the nitrogen treatments achieved higher yields in each season than the control without N application, confirming the observations of Morlat and Jacquet [32] in this regard. They point out that one of the problems lies in the way mineral fertilisers, especially nitrogen, are applied to the vineyard cover crop. While the sward increases the organic nitrogen content of the topsoil in the inter-rows, the nitrogen uptake of the vines is reduced due to fewer roots and less available root moisture. In addition, organic matter content, nitrogen, exchangeable K_2O , pH and soil moisture increased under the permanent grass cover up to field capacity. Our results show that sward management by nitrogen application strongly influences sward properties, but without significant effects on grapevine production. This confirms the observations of Pornaro et al. [71] in the Cabernet Sauvignon vineyard that the parameters of the vines were not influenced by the type of vegetation in the inter-rows. In contrast to them, other authors found that cover crops increased the soluble solids content in Cabernet Sauvignon berries but decreased the titrated acidity. They hypothesised that this result was due to reduced leaf area, increased canopy spacing, increased light transmittance, and improved illumination conditions as a result of inter-row competition for nutrients [72]. Regarding below-ground competition between grasses and vines, the availability of water in the soil is probably the most important factor for source competition, especially under conditions of increased nitrogen supply to the grasses, which favours greater root development. Clone 15 showed the best quality performance, and clones 15 and 169 stood out for morphological traits over three years.

Our results show that a negative correlation between grass cover and grape characteristics could only be calculated for the year 2020, indicating a negative influence of grass development on grapes, which does not necessarily have a negative impact on wine production. The correlation between the cluster of monitored cover indicators and the vines in 2020 shows that under certain conditions we can expect the impact of the cover system on the supersystem. For a regularity or trend, a longer observation period is needed to accurately determine whether grass production, cover or nitrogen uptake increases or decreases the main grape parameters.

To bring out the above-mentioned multifunctionality of the sward, it is necessary to maintain it through nitrogen fertilisation, which is particularly important considering that the biomass formed during the growing season and mowing removes large amounts of nutrients. In addition, N fertilisation improved the composition of the sward by reducing empty patches and the presence of weeds.

5. Conclusions

The study investigated the role of grass cover in the row spacing of the Cabernet Sauvignon vineyard and the potential impact of maintaining and improving cover on grape production in a climate condition of Šumadija, the most important wine region in Serbia. In the context of climate change and increasing temperatures with decreasing precipitation during the growing season, the question of how the combined system of vine and grass can be maintained over a longer period of time was investigated. Under the conditions of our experiment, treating the sward with different amounts of nitrogen improved the performance of the grasses (yield, nitrogen content in the sward and cover quality). The conclusion is that the sward developed successfully with nitrogen fertilisation and that it improved over the years with increasing application rates. The present study has shown that the management of the sward with different N rates had no effect on the ampelometric and quality–chemical indices of the grapes.

There was no consistent correlation between grass cover or nitrogen dose and the parameters of grape quality and yield. Fluctuations in grass cover and grape parameters

were observed over the growing seasons, which can be attributed to fluctuations in rainfall over the years and increasing temperatures in recent decades. The use of grass in the inter-rows of the vineyard, grown with varying amounts of nitrogen in the soil, is an excellent practice that does not affect the yield and quality of the Cabernet Sauvignon clones. During the three-year study, the qualitative parameters of the grape juice (must), indicated by the sugar content (%) and the total acidity, expressed as tartaric acid, varied between a minimum of 18.6% and 25.5% and between 5.97 and 8.13 g/L. Based on the results obtained, the qualitative parameters of the clones studied, with the fluctuations observed, were within the range of grape quality suitable for the production of premium wine.

In the face of climate change, which is undoubtedly making viticultural production more risky every year, producers are forced to find and implement quick and appropriate solutions. Multifunctionality and the use of grass cover in viticulture are a suitable and very reliable response to climate change. In order to demonstrate the multifunctionality of grass cover, the climatic characteristics of the site and the agrochemical composition of the soil, as the most rapidly changing variables of a given site, must be taken into account in addition to the biological characteristics of the variety. Taking the above factors into account, a recommendation is given for the selection of a suitable grass mixture that is expected to best demonstrate its multifunctionality without negatively affecting the qualitative parameters of the grapes produced. Moreover, the research and the results obtained are of great practical importance, considering the rezoning of Serbian wine-growing areas and recommendation on the selection of varieties and clones for all rezoned wine-growing areas where optimal climatic and pedological conditions for growing vines prevail. The contribution of the work from the practical side is demonstrated by the fact that there are very few such studies in the wine-growing regions of Serbia on which producers can rely. In further research and in view of the increasing impact of climate change, it is essential to conduct as many trials as possible in different locations, with different microclimatic conditions, varieties and clones, with the aim of adapting to climate change.

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