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Improving the Oenological Potential of Grapes for Prosecco PDO Sparkling Wine Thanks to Nitrogen Fertigation

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Abstract: Several researchers stated that climate change effects are arising quickly in the Mediterranean region. Temperature increasing and droughty summers are two of the most common patterns threatening sparkling wines' grape quality. The present study investigated nitrogen nutrition to enhance acidity and preserve the aromatic compound on *Vitis vinifera* var. Glera for producing white sparkling wine. Half of the one-hectare vineyard placed in northeast Italy was fertigated with nitrogen during summer, while the control half received only mineral fertilization in spring as usual in the area. The trial lasted three years. The grapes' quality was monitored and compared at harvest. The statistical analysis proved an affordable trend among treatments in which the fertigated grapes showed, on average, more free amino acids (+32%), more yeast assimilable nitrogen (+71%), more acidity (+21%), and lower total soluble solids concentration (−3%) than the control grapes. Energy storage, fruit yield, and wood mass were measured too. The study proved the nitrogen supply did not affect either fruit yield or plant vigor. Therefore, nitrogen fertigation has been confirmed to be a reasonable growing practice to preserve wine's aroma and acidity against climate change.

Keywords: nitrogen fertigation; vineyard fertilization; grape acidity; wine aromas; free-amino acids; precision viticulture

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1. Introduction

The grape's quality and yield are the two main factors for assessing the success of a growing season. Concerning wine production, quality consists of the concentration of several chemical compounds, the overall physicochemical traits, and typical aromas and flavors. The sugar-acid ratio and total titratable acidity (TTA) are crucial technological factors for sparkling wines. At the same time, the aromatic ripeness gives the wines all varietal aromas and pleasant odoriferous compounds [1,2]. However, the grape's ripeness balance has been compromised by climate change (CC) in the last decades. CC is also evolving faster in the Mediterranean basin than in the rest of the world. Since the last century, the mean temperatures of Mediterranean countries have risen 0.4 °C more than the global average. The temperature recorded from April to October, also known as grapevine average growing season temperature, had increased by about 2 °C in Spain and the Alsace region [3,4]. Consequently, the rising temperature may jeopardize summer crops such as grapevine. Furthermore, grapevine phenology stages have developed faster over the last two decades [5,6], increasing the sugar content, whereas organic acids concentration decreases [7,8]. Moreover, advancing the grape's maturity in the warmer period led to obtaining pour aromatic wines containing smooth and cooked aromas [4,9,10]. Therefore, grape growers harvest grapes some days before the complete aromatic ripeness, avoiding too much alcohol and preserving the acidity in wines. On the other hand, wines made with early harvested grapes may involve not mature and contain unpleasant veggie-green aromas [11,12].

Total Titratable acidity (TTA) approximates the total acidity and is measured by titration. Tartaric acid (HTH) and malic acid (HMH) are the most abundant organic acids. Leaves produce HTH and HMH from post-anthesis to veraison, while also green berries can produce HMH. Both HTH and HMH are stored in fruit cell vacuoles. Part of HMH is converted into fructose and glucose at the onset of the ripening by pulp cells [2,7]. Acids are essential in molding the wine taste, maintaining good pH values (3–4), and ensuring the stability and longevity of wines during aging [2]. Moreover, high temperatures boost HMH catabolism [2]. Therefore, those wines made in warm climates tend to contain more alcohol and less TTA than wines from cold climates [4,9,10]. Thus, researchers stated that the decoupling between the fruit's ripeness components is the main consequence of climate warming towards grapes quality [10,11].

Among all the agricultural practices available for delaying the grapes ripening and preserving grape acidity, many authors experimented with the management of nitrogen fertilization [13–15]. Vigorous plants have more shoots and leaves, increasing the synthesis of organic acids and reducing light penetration [16,17]. Furthermore, nitrogen fertilization affects the yeast's available nitrogen concentrations (YAN) and free amino acids (FAA) content. Both modulate yeast activity during fermentation and wine's aromas [18]. Many FAA are precursors of aromatic compounds, such as higher alcohols and phenolics. FAA is turned into aromatic compounds during alcoholic fermentation [2,7,19,20]. Both the fertigation and the foliar application of nitrogen supplies were tested for red and white grapes varieties [14,15,21–23]. In every study, authors stated the potential role of nitrogen in-season application for increasing the TTA and FAA concentration.

The present study aims to improve the oenological potential of grapes of *Vitis vinifera* var. Glera for producing Prosecco PDO sparkling wine. Prosecco and other sparkling wines represent a standard viticulture scenario for northern Italy. Because the CC effects were also verified in northeast Italy [24], the temperature warming could jeopardize sparkling wine production. Nitrogen nutrition was chosen as a pattern to enhance both wine acidity and FAA. Acidity, FAA, and aromas are essential for obtaining excellent sparkling wines. Glera is characterized as a high-yield grapevine variety with great vigor. So, the nitrogen was dosed in many events thanks to the fertigation technique, avoiding forcing both the yield and the vigor of plants compared with a standard mineral-fertilized one. The berries' chemicals, the FAA profile, and the energy stocks were monitored in each of the three years trials (2018, 2019, 2020). In addition, wood production, and fruit yield were recorded to monitor the vine's vegetative response against the additional fertigation. The wood production was weighted in 2019 and 2020, while the fruit yield was measured in just the last year of the trial, 2020. In 2019 the grapes were turned into wine separately and a panel taste assessed the wines from the two parcels.

2. Materials and Methods

2.1. Experimental Site and Vine Management

The trials took place in the Veneto region in northeast Italy in the middle of the Prosecco PDO area (45.806259 °N; 12.343800 °E) from 2018 to 2020. It is a floodplain rich in sand and stones. The annual and summer historic mean temperatures record 13.1 °C and 21.9 °C, respectively, while the annual and summer mean rainfall records 1155 mm and 262 mm. The altitude of the location is around 40 m above sea level. The nearest "Regional Agency for Environmental Protection and Prevention of the Veneto" (ARPAV) weather station recorded climate data less than one kilometer far from the experimental site. The one-hectare vineyard was planted in 2010 with white grape Glera (clone ISV19) grafted on Kober 5bb rootstock, with vines and rows spacing of 1.20 m and 2.80 m, respectively. The planting density results in 2976 vines per hectare. The vines are trained vertically in a double Guyot trellis system, and the two main canes are bent toward the ground. This training system for Glera is called "Cappuccina". The irrigation system consists of a drip line 300 mm underground. Budbreak usually happens in April, whereas grapes are harvested from the end of August to the middle of September. Grapes from this area are assigned to the Prosecco PDO sparkling wine production.

2.2. Experimental Design and Fruits and Vines Sampling

The vineyard has been split into two plots. The "control" plot received 35 kg ha^{-1} of nitrogen with mineral fertilizer supplied by about 292 kg ha^{-1} of "Nitrophoska special" (12% of nitrogen) fractioned in two operations from post-harvest to full flowering. The "treated" plot received 15 kg ha^{-1} of nitrogen post-harvest, spreading 125 kg ha^{-1} of "Nitrophoska special". In addition, the treated and control plot was irrigated in three moments, but only the treated plot was fertigated. Each irrigation spread about 250 m^3 ha^{-1} of water. The treated plot received 38 kg ha^{-1} of nitrogen split in three operations between flowering and veraison by dosing 13 g $vine^{-1}$ of nitrogen thanks to the irrigation plant (15 kg + 38 kg per hectare was the total nitrogen supply for the treated plot); the "control" has received, at the same period, only water to avoid any irrigation effects. NIT GG is the fertilizer provided by Haifa Chemicals used for fertigation containing 34.5% of ammonium nitrate. Table 1 shows the fertigation schedule. The timing for the irrigation operations was determined by the leaf's steam water potential (SWP) measured, thanks to a pressure chamber. Each selected leaf was inserted in a humidified aluminum-coated plastic bag while still attached to the shoot for at least one hour before SWP measurement. After this period, the leaves were detached, and SWP was measured [25]. The leaf water potential thresholds were from 0.7 to 0.9 Mpa, chosen to avoid drought stress [26].

Table 1. Dates of the three fertigations that occurred in each year and the dose of nitrogen per vine in the treated parcel, total nitrogen supplied per vine, and total dose of nitrogen per hectare.

Vintage	Date	Date	Date	N Vine (g)	TotN $Vine^{-1}$ (g)	TotN ha^{-1} (kg ha^{-1})
2018	12 June	5 July	17 July	4.33	13.00	38.0
2019	13 June	11 July	31 July	4.33	13.00	38.0
2020	4 June	27 June	31 July	4.33	13.00	38.0

Three consecutive rows from the control parcel next to three consecutive rows from the treated parcel have been considered for the trial. Sampling only the center rows avoided spatial interference among the treatments. Grapes have been sampled from the veraison stage to the harvest date. Each sample counted 300 berries randomly chosen from the whole bunch along three blocks at the same distance in the row, each one consisting of 10 contiguous vines within the selected rows. At the harvest, the sampling was replicated three times. Instead, the samplings between veraison and pre-harvest were not replicated. The chemical parameters of the berries juice were examined. Malic (HMH) and tartaric (HTH) acid content (g L^{-1}) were determined using the RP-HPLC method (Agilent 1220 Infinity LC; Agilent Technologies, Santa Clara, CA, USA). Samples were diluted 50 times and then filtered through 0.2 µm nylon filters. 20 µL of the sample was directly injected into GRACE Alltima HP C18, 5 µm 150 mm × 4.6 mm. Separations were performed under isocratic conditions at 36 °C using a 6×10^{-3} mol L^{-1} H_3PO_4 solution mobile phase at a 0.6 mL min^{-1} flow rate. Organic acids were detected at 210 ηm as described by Kordiš-Krapež [27]. Total titratable acidity (TTA) (expressed as g L^{-1} of tartaric acid equivalents) was determined on 20 mL of clear must by titration with 1N NaOH (ACS reagent Honeywell Fluka 30620) using a Micro TT 2022 automatic titrator (Crison, Barcelona, Spain) equipped with a pH probe Hamilton FlushTrode P/N 238060/08. Soluble solids (SS) were measured by a portable refractometer (Atago PR32) and expressed in grades Brix % refractometers (°BRIX); the instrument requires about one ml of clear must at 20 °C. Yeast-assimilable nitrogen (YAN) was determined using the method described by [28]. In addition, twenty-one amino acids were titrated from three must samples at harvest time, as described by [29]. For the detection and quantification of AAs, the must samples were diluted 50 times in water and injected in the analytical column (Raptor Biphenyl 3 × 150 mm; Restek, Bellefonte, PA, USA) with a mobile phase of 0.1% formic acid (FA; solvent A) and MeOH (solvent B) at a flow rate of 0.5 mL min^{-1}. The chromatographic separation was obtained with a linear ramp: 95% A, 5% B in 1 min; 5% A, 95% B in 15 min and held for 3 min. In 0.5 min, eluents returned to the initial condition and were held for 4 min for column re-equilibration. The injection volume was 10 μL, of which 5 μL was of sample/standard, and 5 μL was O−phthalaldehyde (OPA), a derivatization reagent. In November of every year, samples of thick roots (3–8 mm in diameter) at 250 mm from the row and between 0–350 mm depth and canes (between nodes 4 and 5) were collected. They were then frozen at −20 °C. 150 g of root and cane tissues samples without ross was ground using an "IKA A11 basic" at room temperature, and they were sifted with a 1 mm^2 mesh sieve. The starch and the sugar content were determined following the methods proposed by Hunter J. [30]. An extreme hailstorm close to the harvest compromised the fruit yield in 2018. The short recovery-pruning could affect production in 2019 too, so fruit yield was recorded in 2020, weighing the production of three consecutive vines per three repetitions in each plot. Dormant wood weight was determined every following winter. Usually, the dormant wood was weighed in five vines per repetition, such as in 2020. Because of the 2018 hailstorm, the number and the lengths of shoots were very variable. So, the wood weight determination was avoided in 2018, while in 2019, the sampled vines were increased to 10.

2.3. Winemaking

Grapes from the two treatments have been harvested separately. In 2018 and 2019, 150 kg of grapes were collected from each parcel, crushed, and de-stemmed at harvest. The pulp has been separated from the skins. In steel tanks, musts were inoculated with 20 g hL^{-1} of *Saccharomyces cerevisiae* (Zymaflore FX10, Laffort, Bordeaux, France). Next, sulfur dioxide was added at a 1.5 g hL^{-1} dose as $Na_2S_2O_5$. On the second day, 40 g hL^{-1} of $(NH_4)3PO_4$ was added. Alcoholic fermentation lasted ten days at room temperature (18–20 °C); wine was gravity-settled. Wines were bottled in January and subjected to sensory analysis after 2–3 months. An independent panel taste made by five independent tasters evaluated the two kinds of base wines for producing the "Prosecco PDO", assessing color, flavor, aroma, and overall opinion between control and treated wine. Tasters based their judgment on the card of the "Union Internationale Des Oenologues" [31] but, no statistical analysis was carried out due to a low number of repetitions (just five tasters).

2.4. Statistical Analysis

RStudio (version 1.2.1335 © 2009–2019 RStudio) is the software used for all statistical analysis. A completely randomized design was chosen as the experimental plan. A multifactorial analysis was carried out comparing FAA, YAN, berries chemicals at harvest, and vigor indexes between treatments and vintages. Both treatments and vintages were assessed as fixed effects. A one-way ANOVA was chosen for yield and wood weight due to a lack of repetitions.

3. Results

The main climate records from 2018 to 2020 are presented in Figure 1. In 2018 and 2020, the mean temperatures were comparable with the historical trend of the last 20 years, whereas 2019 was a warmer year since June. All three vintages are warmer in August and September than the historical trend. The summer rainfalls were similar to the historical records, except in May 2019 and June 2020, when it rained double. April 2018, September 2019, April, and May 2020 were the most drought months.

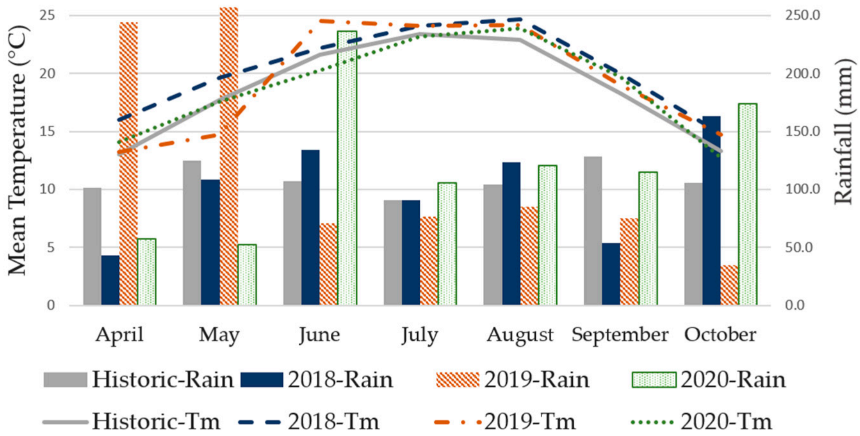

Figure 1. The chart resumes the climate records during the grapevine growing season in the three years compared to the historical trend from 1994 to 2019. The columns represent the total monthly rainfall, while the lines are the daily average temperature.

The grapes production, the pruning wood weight, sugars, and starch contained both in roots and shoots are reassumed in Table 2. Table 2 shows all the available data and the results of the statistical analysis. For each measure, three samples were collected but the number of vines per sample changed in some cases. While the grape yield and the pruning wood weight are expressed in raw production per group of sampled vines, the sugars and starch contents are normalized on the sample dry matter.

Table 2. Mean and standard deviation (SD) of grapevine features. Grape yield and wood weight are expressed in kg per each sample group of vines; starch and sugars concentration in both roots and shoots are expressed in mg per g of dry matter (dm). Data of the same measure paired with different letters "a, b, c" indicate statistical differences among Tukey's multiple comparisons test at $p \leq 0.05$. The last columns reassume the factorial analysis comparing the treatment (T), the vintage (V), and their interaction (T·V) effect. The results mean: "***" p-value < 0.001, "**" p-value < 0.01, "*" p-value < 0.05, while "ns" non-significant.

Measure	Vintage	Vines Sample	Nitrogen		Control		ANOVA	
			Mean	SD	Mean	SD		
		One-way ANOVA						
Grape yield (kg)	2020	3	22.20	3.64	24.12	2.55	T	ns
Wood weight (kg)	2019	10	13.33	1.14	14.80	0.44		
Wood weight (kg)	2020	5	7.82	1.35	7.22	1.35	T	ns
		Two-way ANOVA						
Root's sugars (mg g^{-1} dm^{-1})	2018	5	29.76 b	0.88	35.73 b	3.20	T	ns

Table 2. *Cont.*

Measure	Vintage	Vines Sample	Nitrogen		Control		ANOVA	
			Mean	SD	Mean	SD		
Root's sugars (mg g^{-1} dm^{-1})	2019	5	21.52 b	5.03	39.97 b	23.94	V	***
Root's sugars (mg g^{-1} dm^{-1})	2020	5	16.18 a	2.76	16.17 a	0.20	T·V	*
Root's starch (mg g^{-1} dm^{-1})	2018	5	248.79	10.56	215.70	37.96	T	ns
Root's starch (mg g^{-1} dm^{-1})	2019	5	227.90	7.52	192.84	50.01	V	ns
Root's starch (mg g^{-1} dm^{-1})	2020	5	190.33	19.73	205.01	15.64	T·V	ns
Shoot's sugars (mg g^{-1} dm^{-1})	2018	5	126.28 b	18.21	120.72 b	9.50	T	ns
Shoot's sugars (mg g^{-1} dm^{-1})	2020	5	75.45 a	5.39	72.85 a	11.88	V	**
							T·V	**
Shoot's starch (mg g^{-1} dm^{-1})	2018	5	141.92 b	7.89	130.40 b	15.65	T	ns
Shoot's starch (mg g^{-1} dm^{-1})	2020	5	106.36 a	8.42	120.61 a	7.89	V	**
							T·V	**

The Grapes production has been weighted only in the 2020 vintage, and the yields of plots are completely comparable according to the statistical analysis. According to the ANOVA outputs, fertigation did not affect the sugars and starch in roots or shoots. On the other hand, the sugars and starch stocks show a decreasing trend from 2018 to 2020 vintages. The interaction between the treatment and the vintage effect never emerged as significant regarding the ANOVA on the grapevine features. The chemical parameters analyzed on the berries juice at the harvest time are resumed in Table 3, while the in-season data are listed in Table A1. The tartaric acid concentration (HTH) and the malic acid concentration (HMH) are expressed in grams per liter of must (g L^{-1}) of each compound. The sum of the tartaric and malic acids (HTH+HMH) results from the sum of the two acids contained per liter of must. The sum of all the organic acids (TTA) was converted into grams of tartaric acid per liter of must. As emerged from the multifactorial ANOVA, HMH, HTH+HMH, the soluble solids concentration (SS), the sum of all the organic acids (TTA), and pH significantly differed between treatments. Exception for 2018, HMH and HTH+HMH were higher in the nitrogen treatment than in the no-treated plot, while HTH did not significantly diverge between plots. The SS (correlated to the sugar concentration in the berry's juice) showed a weak divergence between the two treatments, which is relatively higher in the control plot than the treated one in 2020. Finally, in every vintage, TTA was higher in the fertigated plot than the control one, while the pH was lower in the fertigated plot than the no-treated one. HTH, HMH, HTH+HMH, TTA, and SS were susceptible to the vintage effect. 2018 recorded the lowest average values for both HTH and SS. HTH+HMH was smaller in 2018 only if compared with the 2020 average value. The interaction between treatment and the vintage effect was never considered significant regarding the grape quality profile. Yeast assimilable nitrogen (YAN) means the total nitrogen in must be expressed in milligrams of Ammonium nitrate per liter of must. Most organic compounds containing nitrogen are FAA and proteins, whereas mineral compounds are, for example, ions such as ammonium (NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-) [2,7]. YAN concentration is displayed in the last rows of Table 3. More in detail, YAN was higher in the nitrogen plot than the control one in each vintage. Additionally, YAN was lower in 2019 than the other vintages. The seasonal ripening curves for 2018 and 2020 are displayed in Figure A1; the charts reassume

SS and TAA evolution from veraison to the harvest date. The total acidity for the nitrogen treatment was higher than the control one, especially at harvest time.

Table 3. The table shows the mean and the standard deviation (SD) of the main chemical analysis carried out on the berries sampled and analyzed at harvest time. TTA is expressed in grams of tartaric acid per liter of must., while YAN is in mg of Ammonium Nitrate per liter of must. Data of the same measure paired with different letters "a, b, c" indicate statistical differences among Tukey's multiple comparisons test at $p \leq 0.05$. The last columns reassume the factorial analysis comparing the treatment (T), the vintage (V), and their interaction (T·V) effect. The results mean: "***" p-value < 0.001, "**" p-value < 0.01, "*" p-value < 0.05, while "ns" non-significant.

Measure	Vintage	Nitrogen Mean SD		Control Mean SD		Two-Way ANOVA	
HTH (g L^{-1})	2018	5.09 a	1.37	5.56 ab	0.44	T	ns
HTH (g L^{-1})	2019	6.23 ab	0.15	5.64 ab	0.18	V	*
HTH (g L^{-1})	2020	6.70 b	0.10	6.08 ab	0.20	T·V	ns
HMH (g L^{-1})	2018	2.66 ab	0.74	1.84 a	0.27	T	***
HMH (g L^{-1})	2019	3.42 b	0.19	2.20 a	0.11	V	**
HMH (g L^{-1})	2020	3.65 b	0.24	2.41 a	0.21	T·V	ns
HTH+HMH (g L^{-1})	2018	7.75 ab	2.06	7.40 a	0.71	T	**
HTH+HMH (g L^{-1})	2019	9.65 b	0.15	7.84 a	0.08	V	*
HTH+HMH (g L^{-1})	2020	10.34 b	0.22	8.49 a	0.39	T·V	ns
TTA (g L^{-1})	2018	6.76 b	0.47	5.53 a	0.22	T	***
TTA (g L^{-1})	2019	8.60 c	0.79	6.54 ab	0.16	V	***
TTA (g L^{-1})	2020	7.82 c	0.07	6.26 ab	0.30	T·V	ns
SS (°BRIX)	2018	14.47 a	0.31	14.90 a	0.20	T	**
SS (°BRIX)	2019	16.37 b	0.29	16.53 b	0.23	V	***
SS (°BRIX)	2020	15.13 a	0.15	16.07 b	0.31	T·V	ns
pH	2018	3.22 b	0.04	3.30 c	0.01	T	***
pH	2019	3.17 a	0.04	3.31 bc	0.05	V	ns
pH	2020	3.23 ab	0.00	3.33 c	0.04	T·V	ns
YAN (mg L^{-1})	2018	216.5 c	16.91	138.3 b	11.77	T	***
YAN (mg L^{-1})	2019	127.9 b	23.08	60.5 a	12.13	V	***
YAN (mg L^{-1})	2020	200.9 c	2.84	121.6 b	11.38	T·V	ns

However, SS was lower in the nitrogen treatment than the control one since the first sampling in 2018 and 2020. Focusing on the acidity falling during the ripening curve, the HTH and TTA of the treated grapes seem to decrease slower than the control ones, as shown in Figure A2.

Table A2 resumes the most abundant FAA concentration measured in the must at harvest, thanks to the HPLC analysis. The same table reports the results of the multifactorial analysis carried out for each FAA between the two treatments and the three vintages. Arginine, glutamine, aminobutyric acid, alanine, and threonine are the most abundant FAA in the must. Figure 2 illustrates the average FAA concentration among vintages grouped per treatment. All FAA increased thanks to nitrogen fertigation exceptions for aminobutyric acid, ethanolamine, and glycine. Moreover, the vintage effect was significant towards all FAA except for L−histidine and L−threonine. The vintage and the interaction effects were significant for aminobutyric acid, L−aspartic acid, L−arginine, L−citrulline, L−glutamine, and L−lysine. The concentration of the FAA did not increase equally among all. L−Citrulline, L−glutamine, L−arginine, and L−tyrosine rose by more than 25% in concentration in the nitrogen treatment.

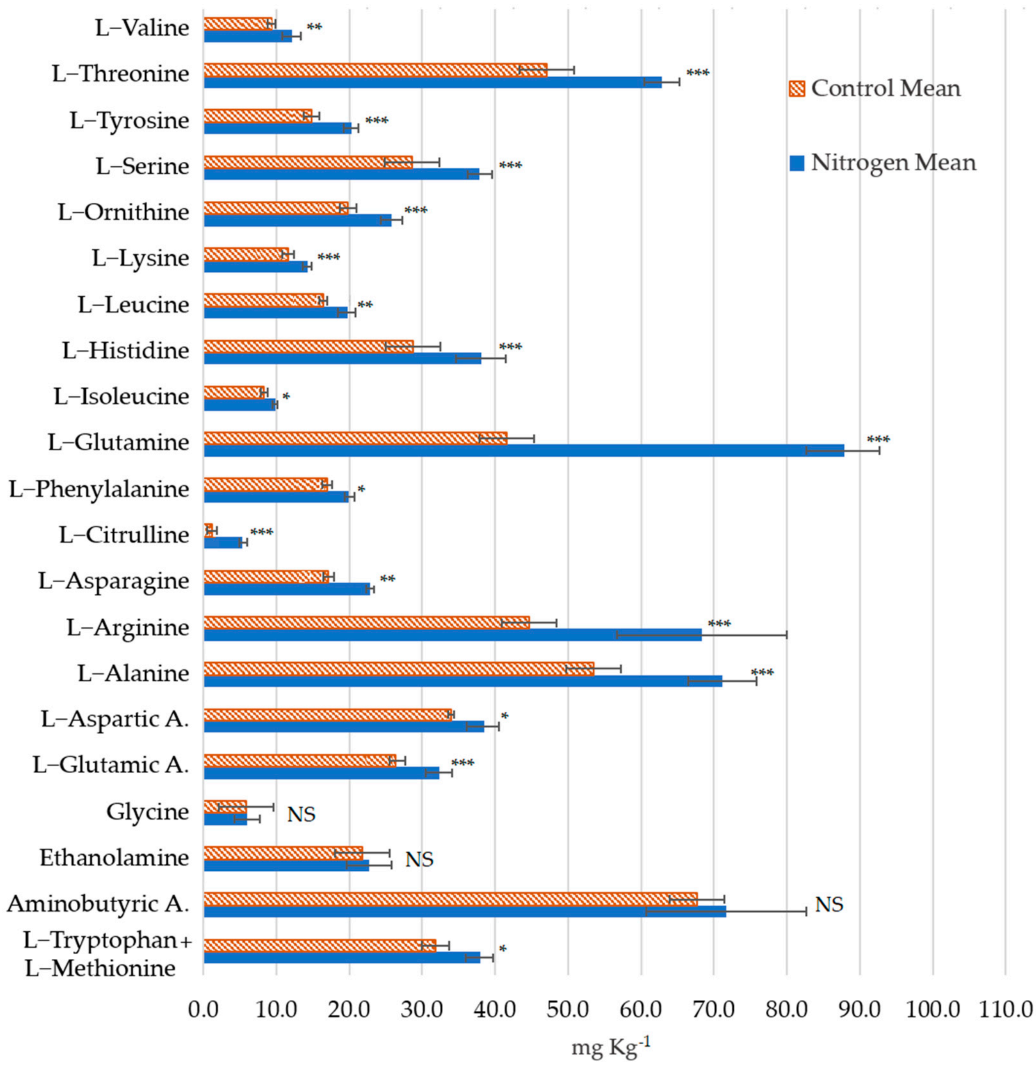

Figure 2. The bar plot resumes the average concentration of the free amino acids (FAA) in must (2018–2019–2020) at the harvest time grouped per treatment. The black bars represent the standard error. L−Arginine concentrations are reduced by ten times. The symbols on the top of the bars are referenced to the treatment effect resulting from the multifactorial ANOVA comparing FAA concentration between vintages and treatments: "***" a p-value< 0.001, "**" a p-value < 0.01, "*" a p-value < 0.05, while "NS" non-significant.

4. Discussion

According to the climate records, the vintage 2019 was the hottest (especially for June and July). Moreover, April and May 2019 were rainier than the historical trend. On the other hand, 2018 and 2020 are comparable with the historical trend, but 2020 was very rainy in June. Therefore, each year's climate condition could boost the vintage effect in the statistical analysis. For example, 2018 recorded the lowest average value for TTA and SS, while 2019 recorded the highest SS average and the lowest YAN average values.

The dormant wood weight and the grape yield were measured to assess the plants' vigor response to nitrogen fertigation. The vigor assessment proved nitrogen fertigation stimulated neither the vines' growth nor the grape production. As a result, the vegetative status of the treatments is comparable, avoiding issues related to dense canopy in grapevines. Dense canopies mean a shoot, leaves, and bunches fastening. Adverse effects on berry juice and the plant's health due to dense canopies are well known [22]. It has also been proved that vigor vines are more susceptible to phytophagous insects and fungal diseases [32]. Starch and sugar content in both roots and shoots indicate the plants' energy stocks. Therefore, the starch and sugars stocks excluded any energy stocks variation related to the treatment effect. On the other hand, the shoots' starch and sugar were affected by the vintage effect. In particular, starch and sugars in shoots were lower in 2020 compared to the other vintages; the hailstorm and short-pruning in 2018 and 2019 could have drained part of the energy stocks to recover the full plant development status. In addition, to support this hypothesis, nitrogen fertigation did not affect yield in 2020, the last year of the experimental trial.

The chemical analysis proved the trend that the berries juice's acidity increased while reducing the sugar concentration thanks to the nitrogen fertigation. In particular, HMH and TTA were higher in the nitrogen treatment, while HTH was similar in both parcels. HMH and TTA showed a higher concentration trend since the first samplings (Figure A2), proving the role of fertilization management in increasing the malic acids synthesis from the early grape ripening stages. Moreover, an early harvest would let harvesting grapes with higher acidity in the fertigated parcel than in the control one. Comparable results were also founded in previous studies [33]. However, a direct effect of nitrogen on must' acidity could not be demonstrated. On the other hand, shaded leaves and bunches can preserve the HMH against a warm climate [34]. So, although no differences were detected in the wood weight between the control and treated, a small number of leaves and leaf area increasing thanks to the nitrogen supply could have reduced the HMH degradation rate. The results above confirmed that the acidity increased thanks to the nitrogen fertigation compared to the standard two steps. The nitrogen supply has also been demonstrated as an affordable agricultural practice for preserving the must and wine acidity against climate warming [35].

YAN depends on many factors, including nitrogen fertilization [36]. YAN is an essential macronutrient for yeast during alcoholic fermentation, and many nitrogen compounds are precursors of phenolics and other aromatics compounds [2,7,34,37]. The YAN of the control plot was every vintage under the optimal threshold of 140 mg L^{-1} to avoid the risk of incomplete alcoholic fermentation [38]. On the contrary, the nitrogen plot recorded an average value of 181.1 mg L^{-1}, considered a low-risk value for vinification issues. FAA is part of the YAN organic fraction. They must contain more than thirty FAA, but only a few represent more than 70% of the overall concentration: L−arginine, L−glutamine, L−alanine, L−glutamic acid, L−serine, and L−threonine [2]. Arginine is the most abundant FAA found in berry juice. Likewise, many other studies proved this. Arginine synthesis is a common way to store nitrogen in berries juice [25,39]. It is also the major nitrogen source for yeasts during alcoholic fermentation [18]. Arginine is the predominant FAA in both treatments, and it also increased by 53% thanks to fertigation in the three vintages mean. As verified by Schreiner, the concentration of 18 out of 21 of the analyzed FAA increased in the must from fertilized grapes [40]. All the monitored FAA, apart from L−histidine and L−threonine, change in concentration across vintages [41]. Indeed, climate conditions, sun exposure, farming practice, and many other unpredictable patterns could have affected the FAA concentration over the years. YAN and FAA are strictly related to wine aroma. Many FAA is turned into long alcohols by yeast during alcoholic fermentation. The reaction between long alcohols and fatty acids produces several esters enriching the wine bouquet of secondary aromas (aromas produced during alcoholic fermentation) [42]. Generally, higher concentrations of FAA and YAN in the berries juice often result in higher concentrations of fruity and floral esters, thiols [7], and terpenes [43]. For example, it has been proven that the crucial role of phenylalanine as a chemical precursor of phenolic compounds, the aromatic molecules responsible for wine aromas [2,7,19,44]. In addition, other studies verify the relation between the concentration of threonine, aspartic acid, and phenylalanine, as the FAA is much related to wine aromas [20,35,44].

In conclusion, the main differences between the wines coming from the two parcels are almost due to the TTA, YAN, and FAA composition, which increased in the treated grapes on average by 21%, 71%, and 32%, respectively. The panel assessed the wine from the nitrogen plot as more pleasant thanks to the higher acidity and more aromatic than the control wine supporting the oenological potential of the summer nitrogen fertigation (Figure A3). The freshness brought by the acidity increases could implement the taste perception of the aroma of the wine from the fertigated parcel. At the same time, the high concentration of FAA could expand the wide flavor profile.

Overall, this study shows that vine growers may adjust the Glera features thanks to nitrogen management in the vineyard. These results are an effort toward preserving the acidity and freshness of Prosecco PDO. Furthermore, the results could contribute to preventing the unwanted effects of the amino acids reduction in the must, due to climate change [45] and, more in general, the qualitative profile of other white grapes cv especially suitable for sparkling white wines.

5. Conclusions

The present research study aimed to improve the oenological potentials of grapes to produce high-quality white sparkling wine. 13 g vine^{-1} of nitrogen has been provided during summer thanks to a soluble mineral fertilizer melted in the irrigation water. Three fertigations supplied 38 kg ha^{-1} of nitrogen from vines flowering to grapes veraison. Dormant wood and nutrients stored in both roots and shoots did not increase in the fertigated plot compared to the control one, although the treated parcel received a higher dose of nitrogen. Therefore, fertigation did not affect the yield. On the other hand, the three fertigation events increase the acidity in the berries juice, the YAN, and FAA concentration. The sugar reduction in the fertigated plot was found relevant just in one vintage. Finally, more acidity and FAA involved more pleasant, aromatic, and fresh wine, respecting the wine obtained from the control parcel. These findings may be relevant for winegrowers demonstrating that well-planned nitrogen management is an affordable alternative to traditional fertilization, which enhances quality factors in wine and hence its commercial value. Finally, the great challenge of the future will be to reduce the dose of nitrogen by optimizing fertilization, towards more sustainability, without changing the quality of musts.

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

Appendix A

The appendix is an optional section that can contain details and data supplemental.

Table A1. Tartaric acid (HTH), malic acid (HMH), the sum of tartaric and malic acids, SS, total titratable acidity (TTA), and pH in berries juice during the grapevines growing season.

Vintage	Date	Treatment	HTH g L^{-1}	HMH g L^{-1}	SS (°BRIX)	HTH+HMH g L^{-1}	TTA g L^{-1}	pH
2018	13-July	Nitrogen	13.14	25.62	4.77	38.76	39.64	2.45
2018	13-July	Control	13.31	26.50	5.30	39.81	41.17	2.47
2018	25-July	Nitrogen	10.49	20.43	7.37	30.92	31.29	2.35
2018	25-July	Control	10.62	18.98	8.20	29.60	30.41	2.36

Table A1. *Cont.*

Vintage	Date	Treatment	HTH g L^{-1}	HMH g L^{-1}	SS (°BRIX)	HTH+HMH g L^{-1}	TTA g L^{-1}	pH
2018	10-August	Nitrogen	6.44	5.88	13.37	12.32	10.16	3.22
2018	10-August	Control	6.61	4.99	13.30	11.60	9.32	3.29
2018	16-August	Nitrogen	5.19	4.27	13.87	9.46	9.06	3.26
2018	16-August	Control	5.67	3.91	13.70	9.58	8.57	3.28
2018	29-August	Nitrogen	5.09	2.66	14.47	7.75	6.76	3.22
2018	29-August	Control	5.56	1.84	14.90	7.40	5.53	3.30
2018	30-August	Nitrogen	7.03	2.49	13.50	9.52	7.23	3.17
2018	30-August	Control	6.38	2.10	14.40	8.48	6.60	3.17
2019	28-August	Nitrogen	8.11	7.51	13.90	15.62	13.51	2.92
2019	28-August	Control	7.67	5.02	13.80	12.69	11.49	2.95
2019	5-September	Nitrogen	7.40	5.29	15.00	12.69	11.48	3.03
2019	5-September	Control	6.63	2.92	14.90	9.55	8.61	3.12
2019	16-September	Nitrogen	6.23	3.42	16.37	9.65	8.60	3.17
2019	16-September	Control	5.64	2.20	16.53	7.84	6.54	3.31
2020	29-July	Nitrogen	12.54	22.21	5.60	34.75	36.10	2.45
2020	29-July	Control	12.73	22.59	5.70	35.32	36.10	2.40
2020	10-August	Nitrogen	10.67	14.34	8.60	25.02	24.10	2.59
2020	10-August	Control	9.69	12.02	9.50	21.71	21.05	2.71
2020	14-August	Nitrogen	8.97	10.46	10.05	19.44	18.39	2.86
2020	14-August	Control	8.77	8.84	11.10	17.61	15.54	2.93
2020	21-August	Nitrogen	7.90	7.53	12.30	15.43	12.50	2.99
2020	21-August	Control	7.49	6.68	12.35	14.18	12.55	3.00
2020	3-September	Nitrogen	7.22	5.13	13.80	12.35	9.91	3.13
2020	3-September	Control	5.66	2.50	15.00	8.16	5.61	3.38
2020	9-September	Nitrogen	6.70	3.65	15.13	10.34	7.82	3.23
2020	9-September	Control	6.08	2.41	16.07	8.49	6.26	3.33
2020	14-September	Nitrogen	6.03	2.63	17.50	8.66	5.92	3.33
2020	14-September	Control	6.25	2.57	17.00	8.82	6.36	3.22

Table A2. FAA mean concentration (three samples) in mg kg^{-1} of must at harvest time. ANOVA represents the multifactorial analysis results from the comparison of treatment "T", vintage "V" and their interaction effect "T·V" on FAA concentration. "***" means a p-value < 0.001, "**" means a p-value < 0.01, "*" means a p-value < 0.05, while "ns" means non-significant.

Vintage	2018				2019				2020				ANOVA		
Treatment	N	SD (n = 3)	C	SD (n = 3)	N	SD (n = 3)	C	SD (n = 3)	N	SD (n = 3)	C	SD (n = 3)	T	V	T·V
L−Tryptophan + L−Methionine	39.70	1.82	33.03	1.01	33.90	2.69	24.70	2.88	40.33	7.46	37.80	10.96	*	*	ns
Aminobutyric A.	75.27	10.12	69.10	8.68	90.83	1.50	73.23	10.37	48.93	2.25	60.73	3.69	ns	***	*
Ethanolamine	16.17	1.01	16.50	0.95	24.03	0.60	20.60	2.79	28.10	0.72	28.27	0.85	ns	***	ns
Glycine	4.23	0.84	3.23	0.45	3.83	0.38	3.33	0.78	10.03	0.25	10.87	1.42	ns	***	ns
L−Glutamic A.	32.50	2.27	25.97	3.40	29.20	2.17	20.70	2.62	35.23	1.45	32.47	3.07	***	***	ns
L−Aspartic A.	31.83	2.00	28.30	3.56	47.43	5.99	36.37	2.51	36.30	1.39	37.27	2.52	*	***	*
L−Alanine	80.27	5.70	66.23	9.65	66.50	6.17	41.77	2.16	66.63	1.39	52.63	1.60	***	***	ns
L−Arginine	488.17	19.64	380.43	18.58	625.00	33.51	392.67	40.53	937.23	31.74	566.50	5.06	***	***	***
L−Asparagine	20.50	3.66	17.13	0.12	31.23	0.70	20.73	2.11	16.70	0.26	13.27	5.95	**	***	ns
L−Citrulline	0.10	0.00	0.10	0.00	0.10	0.00	0.10	0.00	15.83	0.74	4.57	1.59	***	***	***
L−Phenylalanine	21.83	4.92	18.17	1.30	26.07	3.00	19.57	1.19	11.93	0.29	13.00	1.87	*	***	ns
L−Glutamine	62.00	12.28	45.90	5.23	80.23	12.29	41.77	2.02	121.33	2.95	37.27	1.86	***	***	***
L−Isoleucine	10.83	2.44	10.63	0.81	11.77	1.50	8.20	0.66	7.13	0.35	6.13	1.11	*	***	ns

Table A2. *Cont.*

Vintage	2018				2019				2020				ANOVA		
Treatment	N	SD (n = 3)	C	SD (n = 3)	N	SD (n = 3)	C	SD (n = 3)	N	SD (n = 3)	C	SD (n = 3)	T	V	T·V
L−Histidine	33.37	6.77	26.70	4.23	43.23	4.03	26.70	2.33	37.60	1.28	32.70	2.63	***	ns	ns
L−Leucine	16.90	2.34	15.53	1.00	25.13	3.45	17.97	2.46	17.47	0.23	15.73	2.27	**	**	ns
L−Lysine	12.13	0.61	7.60	0.10	12.13	0.50	9.27	0.71	18.70	0.26	17.97	1.22	***	***	**
L−Ornithine	14.77	0.93	8.67	0.55	16.00	0.98	9.57	0.74	46.60	1.05	41.00	0.80	***	***	ns
L−Serine	39.17	1.18	30.93	3.79	34.67	2.91	23.77	1.36	39.80	0.79	31.23	3.20	***	**	ns
L−Tyrosine	28.80	3.82	21.47	3.03	16.97	1.86	11.30	1.48	15.20	0.26	11.87	0.91	***	***	ns
L−Threonine	64.03	2.42	48.53	6.09	60.40	7.01	41.93	4.06	64.13	1.14	50.80	2.60	***	ns	ns
L−Valine	13.57	2.40	10.90	1.73	12.70	1.61	8.27	0.47	10.03	0.35	9.00	1.35	**	*	ns

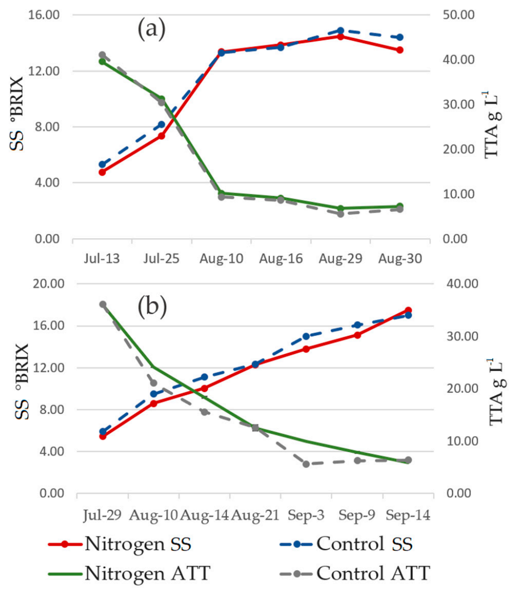

Figure A1. The charts represent the ripening curve of grapes during two vintages: 2018 chart (**a**) and 2020 (**b**). Evolution of SS (left axis) and TTA (right axis) in berries juice from the first available sample to the harvest date.

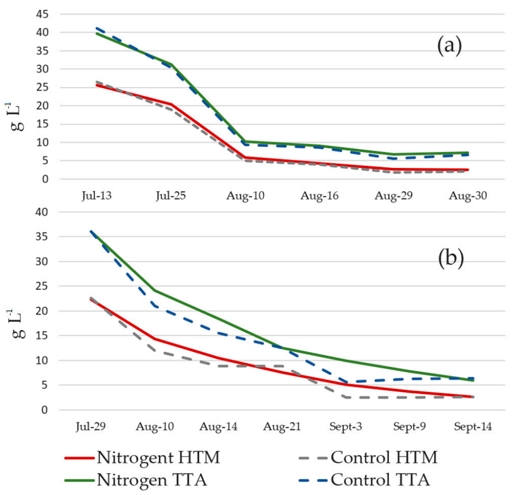

Figure A2. The charts show the acidity falling during the grapevine growing season of each year: 2018 chart (**a**), and 2020 chart (**b**). Malic acid concentration (HMH) and total titratable acidity (TTA) are expressed in g L^{-1}.

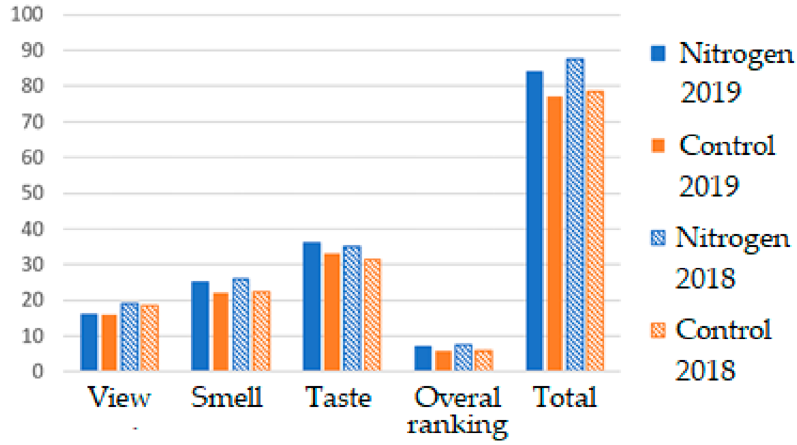

Figure A3. The histogram shows the average scores of each wine made in the 2018 and 2019 vintages. The sensorial analysis carried out by the panel was divided into four judgments (horizontal axis) View, Smell, Taste, and Overall rating. The total ranking is the sum of the four judgments.

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