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Effect of Nitrogen Fertilization on Savvatiano (*Vitis vinifera* L.) Grape and Wine Composition

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Abstract: Nitrogen nutrition is one of the most effective cultural practices in vineyards. The vine nitrogen status influences the berries' quality characteristics and the produced wines. The current study investigated the effect of traditional nitrogen fertilization in the form of ammonium sulfate compared to nitrogen fertilization coupled with the nitrification inhibitor 3,4-Dimethylpyrazole phosphate (DMPP) on the agronomic characteristics of grapes and the produced wines of the white variety Savvatiano from a productive vineyard in the Attiki region. Must and wine quality was evaluated by a chemical analysis and sensorial evaluation by trained panelists. The different forms of nitrogen fertilizers did not significantly affect the aroma and sensory profile in contrast to unfertilized grapevines. In addition, the applied fertilization increased some important aroma compounds in the wine, compared to no fertilization. The significance of this work is to add information about the effect of nitrogen fertilization on the wine volatile composition of the Greek white grapevine Savvatiano.

Keywords: *Vitis vinifera*; Savvatiano; nitrogen nutrition; nitrification inhibitor; DMPP; aroma compounds; wine quality

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1. Introduction

Nitrogen (N) is one of the most important macronutrients in terms of crop productivity and more specifically, nitrification is the geochemical process which ensures the conversion of ammonia to nitrate, which is the preferred nitrogen source for plants, hence promoting soil fertility [1].

It is well known that soil type affects the grapevine behavior through its contents of nutrients, such as nitrogen (N), and is a key factor for the vine's vigor. Vineyard N fertilization has a significant effect on the successful completion of the fermentation process, due to the N effects on berry composition [2]. Yeast assimilable nitrogen (YAN) content is a key factor for the fermentation process. A low content of YAN in must could negatively influence the alcoholic fermentation, mainly by slowing the procedure and resulting in an undesired presence of thiols and higher alcohols in the wine [2,3]. Moreover, a low N content reduces the ability of the must to ferment and might lead to sluggish or stuck fermentations.

The effect of N application on grape composition is highly dependent on the form of N used, the time of application, and the variety. While primary metabolites, except amino acids, are rarely directly affected, YAN and secondary metabolites, such as phenolics

and aromas, are usually improved [4–6]. The total N content and YAN in grape must were found to be correlated and highly responsive to fertilization practices and could provide a good overview of the fertilization strategies. Must N composition not only affect alcoholic fermentation (AF) kinetics, but also the formation of aromatic compounds [7]. Furthermore, deficiencies in N uptake from red varieties, such as Cabernet Sauvignon and Merlot, may induce phenolic biosynthesis [8,9]. Unlike in red grapes, phenolic compounds do not play a positive role in white grape quality. A high phenolic content in white grape juice is responsible for unstable color and bitterness. Conversely, what concerns the white wines as quality factors are the potential aroma and the phenolic content. The phenolic content in grapes contributes to the color, flavor, and texture of wine and to its antioxidant properties [10]. Among other viticultural practices, fertilization is well known to affect the proportion and the amount of phenolic compounds in berries [11].

Except from phenolic compounds, N affects the content of thiols in wines, such as 4-methyl-4-sulfanylpentan-2-one (4MSP) and 3-sulfanylhexan-1-ol (3SH) in wine and its precursors in grapes [12]. However, 4MSP and 3SH are not present in berries and musts, but they are generated during the alcoholic fermentation from non-volatile and odorless berry precursors. Wines produced by Sauvignon blanc grapes are significantly affected by the soil nitrogen supplies [3,4]. Besides Sauvignon Blanc, only a few other research works have demonstrated the influence of N fertilization on the wine's aromatic profile [13–16].

N application in vineyards and in other crops can be expensive, with important economic and environmentally negative consequences [17]. At the same time, up to 60% of the applied-N through fertilizers is lost to the environment, resulting in atmospheric and groundwater pollution by nitrous oxide emissions and nitrate production, respectively [18,19]. Therefore, modern technologies in fertilizers couple mineral ammonium with nitrification inhibitors to achieve a slow release of nitrates and to improve nitrogen use efficiency or reduce N losses from agricultural ecosystems [18]. While the pollution linked to N fertilization in different cropping systems around the world represents a main environmental concern, eco-friendly fertilization strategies are the main goal of scientists as well as in agricultural governmental policies [20,21].

The aim of this one-year experiment was to analyze the effect of typical mineral N fertilizer, with or without the addition of the nitrification inhibitor DMPP (3,4-dimethylpyrazole phosphate), on the Savvatiano (*Vitis vinifera* L.) grape and wine composition. The cultivar Savvatiano was selected as the most cultivated Greek white cultivar, mainly at the Attica region.

2. Materials and Methods

2.1. Vineyard Description

The study was conducted during the 2020 vegetative period at the productive vineyard of the Agricultural University of Athens located in Spata, Attiki (37.98637, 23.90722).

2.2. Plant Material Vineyard Management and Fertilization Treatments

The vineyard is on a lean clay soil with the following properties: pH 7.63, Electrical Conductivity (EC) (μS/cm) 379.4, Organic Matter 2.33%, Total N 0.07%, Olsen-P (mg/kg) 5.3, Mg (mg/kg) 741.06, Ca (mg/kg) 9269.4, K (mg/kg) 742.2, and Na (mg/kg) 86. The cultivar used in the current study was the Savvatiano variety, a Greek widespread white grape variety, mainly in the viticulture area of Attika. The vineyard was planted during 1995 onto a 110 Richter rootstock (planting density of 2 × 1.6 m) and trained on a vertical shoot positioning trellis system into unilateral cordons 50 cm above-ground. The pruning system followed was 4–6 spurs in each cordon. All vines were thinned to the same number of fruit clusters per shoot (2 clusters/ shoot). The vineyard was arranged in a randomized complete block design, with three replications within each fertilization application plus the control. Each block contained three rows of vines with four vines per row. Row orientation was north–south with 1.2 m between rows and 0.8 m between vines. Standard commercial and cultural practices for the Attika vineyards were implemented. During February of

each year, vines were pruned. The different N fertilizers were applied to the vineyard in the phenological stage just before flowering, so as to meet the increased N uptake rates of grapevine [22]. More specifically, 50 g of N per vine was applied, either in the form of ammonium sulfate ($(NH_4)_2SO_4$—N treatment) or coupled with the nitrification inhibitor DMPP (formulation: 27% DMPP/tn ($(NH_4)_2SO_4$)—N + DMPP treatment). Vines without N fertilization (Control treatment) and only with DMPP (DMPP treatment) served as controls. Three spatial replications of each of the four treatments were applied at the field (plots), and each plot contained 10–12 vines.

2.3. Grape Sampling

At veraison (07/08/2020), 100 berries were randomly sampled from each block (replicate) to monitor the fruit ripening of each treatment. Sampling was conducted weekly early in the morning until the harvest. Berry samples were crushed into juice by hand and analyzed for Total Soluble Solid (ºBrix), Titratable Acidity (TA) (expressed in g/L tartaric acid equivalents), and pH, according to the analytical methods recommended by the OIV [23].

2.4. Amonia and Primary Amino Nitrogen

Yeast Assimilable Nitrogen was determined and calculated as the sum of primary amino nitrogen (PAN) and NH_4^+ nitrogen. They were determined using a Y15 enzymatic autoanalyzer (Biosystems, Barcelona, Spain) and appropriate kits supplied by the manufacturer [24].

2.5. Vinification Process

Grapes from each replicate (20 kg) were manually harvested with care to ensure consistency in the method of harvesting. The harvest was conducted early in the morning, and all the grapes were transported to the experimental winery of the Laboratory of Enology and Alcoholic Beverages of the Agricultural University of Athens into grape harvest containers to start the vinification process immediately. Grapes were destemmed, crushed, and softly pressed with a hydraulic grape press (0.5–0.7 bars). The produced grape juice was sulfited with 15 mg/L of sulfur dioxide (SO_2), which was added during the crushing. The pressed juice was placed in 10 L plastic fermenters, and thereafter 3 mg/L of enzymes were added (AB Enzymes, Augustdorf, Germany) to facilitate sedimentation. The fermenters' headspace was purged with N_2 and, afterwards, were sealed and left overnight at 4 °C for sedimentation. Clear juice, coming from each replicate, was racked off the sediment and sulfited with 30 mg/L of sulfur dioxide (SO_2). A conventional analysis (pH, ºBrix and Titrabable Acidity) was conducted on the clear must. Clear must was placed into a 10 L plastic tank and inoculated with yeasts (Vivace, VIC-23, Renaissance Yeast Inc., Zug, Switzerland), prepared in accordance with the manufacturer's instructions. Fermentations were conducted in a temperature-controlled room at 18–20 °C.

Alcoholic fermentation (AF) showed a regular trend and was considered finished when the reducing sugar concentration was lower than 2 g/L. At the end of fermentation (approximately after 10 days), wines were racked and sulfited (30 mg/L SO_2), and containers' headspaces were purged with N_2 before sealing. Finally, they were stored for the stabilization process at a controlled temperature (4 °C).

2.6. Determination of Organic Acids, Sugars, and Alcohols

The analysis was performed using a Shimadzu HPLC system, model LC-20 (Shimadzu Scientific Instruments Inc., Columbia, MD, USA), equipped with a quaternary solvent pump (LC 20AT model), degasser (DGU 20A model), thermostatted column compartment (CTO 20AC model), and autosampler (SIL 20 AC model) coupled to a diode array detector (DAD) (SPD-M20A model) and a refractive index detector (RID) (Shimadzu RID-10A). Data were obtained and processed using Lab Solutions Multi LC Software (Shimadzu).

The analytical procedure was carried out using the chromatographic conditions previously described by Coelho et al. [25]. Detection of the compounds was performed on an ion exchange resin column Agilent Hi-Plex (H+ model, L = 300 mm, internal = 7.7 mm, and 8 µm particle size) (Agilent Technologies, CA, USA) fitted with a pre-column Hi-Plex, 5 mm × 3 mm (Agilent Technologies). Samples of wine were centrifuged at 6000 rpm for 10 min and filtered through a syringe filter of 0.2 µm, and a volume of 10 µL of the filtered samples was injected. The temperature of the column compartment was maintained at 70 °C, and the RID flow cell was kept at 50 °C. The flowrate applied was 0.5 mL/min, with a run time of 20 min. The phase was 4.0 mM of H_2SO_4 in ultrapure water.

For the determination of organic acids, detection was conducted in the DAD at 210 nm. For sugars and alcohols, detection was carried out by RID. Compounds in the samples were identified based on the retention time of the standards, and the quantification of each compound was accomplished using calibration curves based on the peak areas of the standards.

2.7. Quantitative Determination of Volatile Compounds

The analytical procedure for the determination of volatile compounds was adapted based on the method previously described by Ivanova-Petropulos et al. [26], optimized for the white wine matrix. For the isolation of the volatile compounds from the wine samples, a liquid–liquid extraction was performed. Thus, 40 mL of wine was spiked with the 3 internal standards (3-octanol, ethyl heptanoate, and heptanoic acid) so that their final concentration was 10 mg/L for each of them, and the sample was placed in a glass-capped Erlenmeyer flask. To the spiked wine sample, a volume of 5 mL of dichloromethane was added, followed by continuous stirring for 15 min on a magnetic stirrer. The mixture was then centrifuged at 4000 rpm for 10 min at a temperature of 4 °C. Once the phases separated, the dichloromethane layer was collected, and the extraction process was repeated. Afterwards, the vial containing the total organic phase was evaporated under a nitrogen stream to a volume of approximately 500 µL of extract, and then, a volume of 1 µL was injected into the GC-MS system. All extractions were performed in triplicate.

An analysis of volatile compounds in the wine samples was performed using a Perkin Elmer Clarus SQ8S mass spectrometer coupled to a Perkin Elmer Clarus 590 gas chromatograph (Perkin Elmer, Waltham, MA, USA). The polar capillary column used for the separation of the compounds was a DB-WAX type from Agilent (ID: 0.20 mm, film thickness: 0.20 µm, and length: 50 m). The working parameters were as following: injector temperature of 250 °C, MS source of 250 °C, and impact energy of 70 eV operating in EI mode. The initial temperature was 40 °C for 2 min and was then increased to 240 °C at a rate of 5 °C/min. The carrier gas was He at a flow rate of 1.0 mL/min. Samples were injected in split/splitless mode. A mass range of 40–400 m/z was acquired at one scan per second. A quantitative analysis and identification using commercial standards and external calibration curves was performed.

2.8. Sensorial Analysis

The sensory assessment was carried out by a group of 12 trained and experienced judges. The panelists attended two training sessions. In the first session, the panelists were trained using appropriate standard solutions and then were served samples for assessment. They were asked to identify the sensations and aromas perceived using a predetermined descriptor list. The selected attributes were grouped into three categories: visual descriptors (color intensity), olfactory descriptors (aroma intensity, white flowers, citrus fruit, tropical fruit, vegetal aroma, green apple, and banana), and gustative descriptors (acidity and after-taste). The evaluation of the wine samples (4 wines in two replicates, i.e., 8 samples in total) was divided into two sessions over a period of one week. Between samples, a minute break was enforced. The tests were conducted in individual booths, and each sample was served in random order. The panelists were provided with 30 mL of wine in ISO wine glasses at room temperature (18–20 °C). Samples were presented with 3-digit blinding

codes in a monadic sequence, according to a Latin Square Design. Data were collected using Compusense Cloud, Academic Consortium software (Compusense, Guelph, ON, Canada). The intensity of the sensory attributes examined was evaluated using a 10-point scale (1: null; 10: very strong).

2.9. Statistical Analysis

All values are presented as the mean and standard deviation. Statistical analyses were performed using Statgraphics Centurion application (version 1.0.1.C). The significance of the results was determined with an unpaired t-test or one-way ANOVA with Tukey's test. A multivariate statistical data analysis (MVA) of the samples was performed with SIMCA P+ version 15 (Umetrics AB, Umeå, Sweden) and with XLstat (XLSTAT 2017: Data Analysis and Statistical Solution for Microsoft Excel; Addinsoft, Paris, France, 2017). The sensory test results were analyzed by a non-parametric Kruskal–Wallis one-way analysis of variance using Statgraphics Centrurion. When the *p*-values were <0.05, a Post-Hoc Mann–Whitney–Wilcoxon Test was applied to compare, one by one, the wines for each variable The odor activity values (OAVs) were calculated as the ratio of a single compound's concentration to its odor threshold [27].

3. Results and Discussion

3.1. Grape Berries Maturity and Must Composition

The effect of different N fertilizations on the Savvatiano grape berry and must composition was investigated to determine if the rate of maturity of berries was affected by the fertilization. Grapes were harvested at their optimum technological maturity to produce dry white wine, determined by measuring the grape berry total soluble solids (TSS) and total acidity. In the present study, treatments affected berry weight. Especially, the N and N + DMPP recorded the highest levels, following the lower value of DMPP-treatment and the unfertilized (control) (Table 1). Grape seeds' weight followed similar trend. The N- and N + DMPP-treated vines provided larger grape seeds than the DMPP-treatment and the control one (Table 1). The presented data of this research showed that nitrogen fertilization significantly increased the berry's and seeds' weight. The additional N may have improved the fruit set and thereby increased the berries per cluster. The influence of nitrogen fertilization of vineyards on bunch size and weight has been reported in previous studies but with conflicting results [28].

An evaluation of the grape must composition (TSS, total acidity, and pH) is presented in Table 1. For all treatments, little or no significant differences were observed. Different treatments led to a similar reduction in the sugar content of grape berries, which was similar to the study of Perez-Alvarez et al. [29]. They concluded that leaf nitrogen application had no significant effect on some physicochemical parameters of the grapes. Similar results were noted in Sauvignon Blanc berries at the areas of Sancerre and Bordeaux after the application of different N treatments [30].

Tartaric and citric acid demonstrated similar concertation among the treatments. On the other hand, malic acid presented the highest concertation upon N treatment, followed by N + DMPP treatment, control, and DNPP treatment (Table 1). Similar results were observed in Sauvignon Blanc grape berries in the area of Bordeaux [31].

The concentration of glucose and fructose did not differ among the treatments. Therefore, in Total Sugars, no difference was observed (Table 1).

Table 1. Physicochemical parameters of the four experimental musts in response to three different treatments/ fertilization and a control during the experimental year 2020.

Treatment/ Fertilization	Control	DMPP	N	N + DMPP
Weight/ Berry (g)	2.39 ± 0.17 c	2.49 ± 0.09 bc	2.65 ± 0.05 a	2.52 ± 0.08 b
TSS (°Brix)	19.78 ± 0.83	20.38 ± 0.95	20.43 ± 0.54	19.81 ± 0.46
T.A. (g/L of Tart. Acid)	5.31 ± 0.31	5.41 ± 0.49	5.23 ± 0.66	5.83 ± 0.35
pH	3.38 ± 0.05	3.35 ± 0.04	3.42 ± 0.10	3.35 ± 0.08
Weight/Seed (mg)	29.79 ± 1.12 c	30.23 ± 0.85 c	35.59 ± 1.71 a	32.37 ± 2.14 b
Organic Acids				
Tartaric Acid (g/L)	5.24 ± 0.28	5.32 ± 0.21	5.27 ± 0.29	5.37 ± 0.08
Malic Acid (g/L)	0.84 ± 0.14	0.77 ± 0.05	0.93 ± 0.17	0.86 ± 0.10
Citric Acid (g/L)	0.19 ± 0.03	0.20 ± 0.03	0.22 ± 0.02	0.21 ± 0.01
Sugars				
Glucose (g/L)	97.65 ± 1.51	96.79 ± 5.45	99.26 ± 3.21	94.97 ± 2.12
Fructose (g/L)	109.88 ± 4.58	111.58 ± 6.61	113.04 ± 4.1	109.32 ± 2.39
Total Sugars (g/L)	207.53 ± 8.24	208.37 ± 12.03	212.31 ± 7.31	204.29 ± 4.49

Values followed by different letters in each row indicate significant differences ($p < 0.05$) among different samples.

3.2. Berry Nitrogen Status

The grape berry's N status was assessed in order to check if this nutrient was correctly assimilated in the fertilized vines with the different treatments. The N status (determined by Y15 enzymatic auto analyzer) and the NH_4^+, PAN, and YAN levels of N and N + DMPP treatments were higher for berries compared to the control and DMPP treatment during the harvest. Differences were statistically significant for all measurements (Table 2). Differences among treatments were confirmed by YAN measurements. This parameter is considered as one of the most reliable indicators for the vine nitrogen status [32]. These findings concur with the work of Bell and Henschke [2], in which they concluded that the effect of vineyard N application on grape berry composition is an increase in nitrogenous compounds and is expressed as an increase in YAN. Grape juice concentrations of YAN were more sensitive to N fertilizations. This is not particularly surprising, given that the effects of N fertilizations on fruit composition are often mediated by changes in canopy growth [33,34]. This implies that the time of N application is an important consideration for increasing nitrogen composition of the grape berries. A similar conclusion, that N fertilizer applied at the fruit set was more effective in increasing the must N of Riesling, was reached from a long-term N-timing study [35].

Table 2. Total nitrogen and NH_4^+ in grape juice at harvest.

Treatment/ Fertilization	Control	DMPP	N	N + DMPP
NH_4^+ (mg/L)	11.1 ± 2.9 b	10.6 ± 6.1 b	25.2 ± 2.7 a	30.1 ± 7.2 a
PAN (mg/L)	58.7 ± 4.8 b	52.9 ± 14.1 b	81.1 ± 4.0 a	80.0 ± 2.8 a
YAN (mg/L)	69.8 ± 2.3 b	63.5 ± 19.0 b	106.3 ± 3.1 a	110.2 ± 7.7 a

Values followed by different letters in each row indicate significant differences ($p < 0.05$) among different samples.

3.3. Alcoholic Fermentation Kinetics

The progress of the fermentations was monitored by measuring the Baume degree of the fermenting must. A comparison of fermentation kinetics in the control and the three fertilization treatments is shown in Figure 1. The kinetics of alcoholic fermentations depended on YAN concentrations. According to Figure 1, the duration of the AF for musts produced by Nitrogen and N + DMPP treatments in vines lasted 13 days. In contrast, the control and DMPP treatment musts were characterized by a 48-h delay. In all the cases, the rate of fermentation was quite similar. Most of the sugar was consumed within 11 days

from the beginning of the fermentation for all the alcoholic fermentations. However, the control and DMPP treatment showed a slower consumption rate and lasted two days more than the N- and N-DMPP treatment musts. In the case of musts containing more than 100 mg N/L, the AF speed was higher. Especially, in the three to four first days, the evolution of alcoholic fermentation was slow, while from the fifth until the 10th day, the consumption rate of sugars was higher, with the must of N treatment going faster. Finally, the ethanol content of the four wines produced did not show any statistically important differences. No significant differences were observed regarding the conversion yield of ethanol between ferments of the fertilizations provided with a different initial YAN. This outcome could indicate that the relatively low fermentation temperature and the yeast strain pose a more significant role compared to YAN.

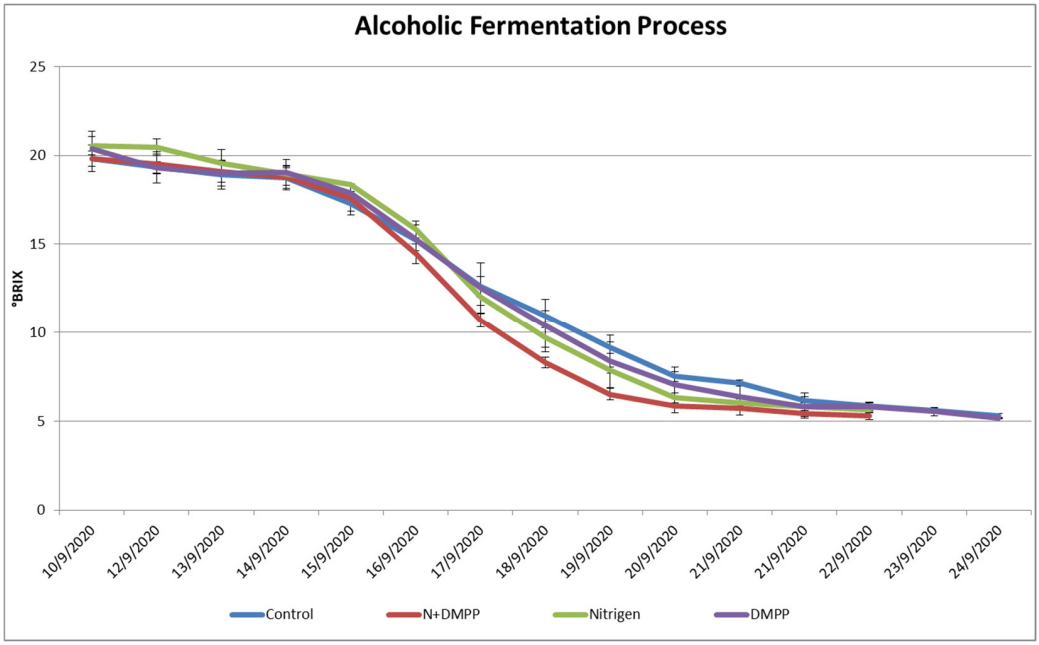


Figure 1. Alcoholic fermentation procedure of Savvatiano must at a temperature of 18–20 °C.

3.4. Physicochemical Analysis—Organic Acids

Table 3 summarizes the chemical composition of the wines obtained. The residual sugar content showed that treatments fermented the wines to dryness and that the degree of alcohol ranged from 12.1 to 12.5% vol., which agreed with the amount of sugars in the must. The total acidity ranged from 4.22 to 4.65 g/L, expressed as tartaric acid. The pH ranged from 2.95 to 3.11. The observed high TA (and low pH) was within the range commonly measured in wines produced from Savvatiano grapes. Thus, although slight differences were detected in some parameters according to the vineyard fertilization treatments, no statistical differences among the physicochemical properties were found.

At the end of AF, an average reduction of tartaric acid was observed. Tartaric acid is the most abundant acid in grapes and wines. Significant differences were observed between produced wines by musts containing different amounts of YAN. A higher value was detected in the wines of the N + DMPP-treated vines. The immediately lower value was recorded by the control and DMPP treatments, and the lowest was recorded in the wine produced by the vines treated with N. There were no differences in the citric acid concentrations. However, differences were observed in the concentration of malic, succinic, and lactic acid, with the higher values observed in the N and N + DMPP treatments and the lowest in the control and the DMPP-treated vines (Table 3). This negative correlation between the malic acid concentration in the wine and the N content of the must is assumed to be a result of slow fermentation due to lower nitrogen content (Table 3). Succinic acid is the main carboxylic acid produced by yeast during alcoholic fermentation. This

acid is formed in the citric acid cycle. Various components (yeast strains, fermentation conditions, temperature, aeration and must composition, and nitrogen availability) can influence the succinic acid concentration during fermentation [36]. These compounds influence the quality and character of the wine, since they are key components, and they reflect the process of wine production. Further research is necessary to verify whether these fertilization treatments, due to different oenological practices, mask the modulation of nitrogen status on the vineyard.

The presence of important differences between the AF of the same strain with different YAN contents constitutes an indication of expression of the GLN3 and URE2 genes associated with alternative nitrogen assimilatory pathways connected with the TCA cycle [37]. A moderated decrease of acetic acid was achieved in wines produced by the N+ DMPP and a statistically significant decrease in wines produced with N treatment, in comparison with control and DMPP treatments (Table 3). The influence of the initial assimilable nitrogen concentration in must on the acetic acid production by the yeasts was reported by other research groups [38,39]. The increase in nitrogen concertation is reversably correlated with acetic acid production, probably since nitrogen is a key factor for yeast growth with an influence on the yeast growth rate, final cell population, and, consequently, restricts on volatile acidity production [38]. However, all the wines had low concentrations of acetic acid, which is an indication of no-fault wines.

Glycerol is a significant by-product of alcoholic fermentation, as it contributes to the organoleptic properties of wine by increasing viscosity and sweetness [40]. A slightly higher increase of glycerol was recorded in fermentation, with a higher concentration of YAN (N treatment) compared to the other fermentation, although these differences were not statistically significant (Table 3). Bely et al. (2003) [38] observed that the initial nitrogen content of the fermented must did not affect the final glycerol content, while controversial results were observed by other research groups [41,42], suggesting that nitrogen limitation increases glycerol production. In the present study, the increase of the YAN concentration after the application of the nitrogen fertilization was still at lower levels than that recommended to avoid sluggish or stuck fermentations, and for that reason, probably, any clear trend was not observed.

The ethanol content of the four wines produced did not show any statistically important differences. No significant differences were observed regarding the conversion yield of ethanol between ferments of different N-treatment fermentations provided with a different initial YAN. This phenomenon could be correlated with the relatively low fermentation temperature and the selected yeast strain, which gives rise to a more significant role compared to YAN [43].

3.5. Formation of Volatile Compounds in Response to Different N Fertilizations in the Vineyard

Besides the known effect on fermentation rate, nitrogen had an impact on the concentration of volatile fermentation products. Individual classes of volatile compounds are discussed below (Table 4).

The final concentrations of fermentation-derived volatile compounds (four higher alcohols, six ethyl esters, four volatile acids, three acetate esters, and two monoterpenes) are shown in Table 4. Alcohols, esters, and acids were detected in the produced wines, originating mainly from the alcoholic fermentation, whereas terpenes could have originated mainly from the grapes, contributing directly to the varietal aroma of wine [44].

Higher alcohols are qualitatively the most important fractions and might be responsible for the fermentative aroma of wines depending on their levels. In the studied wines, this group was mainly composed of methyl propanol, 3 methyl thio propanol, isoamyl alcohol, and 2 phenyl alcohol. The sum of total alcohols was different among the wines, with the wines produced by the vines treated with DMPP fertilization providing significantly higher values. Similar results were recorded by Maigre [45] and Spring [46]. They observed that N application in the vineyard increased must nitrogen concentration, reduced fermenta-

tion time, and decreased the concentration of phenyl 2 ethanol in Gamay and Chasselas wines, respectively.

Table 3. Oenological parameters, color, and phenolic parameters of the four experimental wines in response to three different treatments/ fertilization and a control during the experimental year, 2020.

Treatment/ Fertilization	Control	DMPP	N	N + DMPP
Alcoholic Volume (*v/v* %)	12.2 ± 0.2	12.1 ± 0.4	12.5 ± 0.2	12.1 ± 0.2
T.A. (g/L of Tart. Acid)	4.22 ± 0.24	4.65 ± 0.34	4.32 ± 0.22	4.57 ± 0.30
pH	3.11 ± 0.23	2.95 ± 0.09	3.05 ± 0.11	2.96 ± 0.04
Residual Sugar (g/L)	0.09 ± 0.008 a	0.09 ± 0.02 a	0.03 ± 0.008 b	0.03 ± 0.01 b
Glycerol	9.44 ± 1.10	9.60 ± 1.34	10.73 ± 1.34	10.36 ± 0.20
		Organic Acids		
Tartaric Acid (g/L)	1.65 ± 0.40 ab	1.65 ± 0.34 ab	1.38 ± 0.1 b	1.77 ± 0.20 a
Malic Acid (g/L)	0.85 ± 0.12 b	0.84 ± 0.84 b	1.09 ± 0.14 a	0.97 ± 0.09 ab
Citric Acid (g/L)	0.72 ± 0.24	0.93 ± 0.06	0.84 ± 0.05	0.77 ± 0.09
Succinic Acid (g/L)	0.80 ± 0.09 c	1.10 ± 0.28 ab	1.29 ± 0.16 a	1.10 ± 0.30 ab
Lactic Acid (g/L)	0.11 ± 0.01 b	0.14 ± 0.00 ab	0.15 ± 0.01 a	0.16 ± 0.01 a
Acetic Acid(g/L)	0.29 ± 0.03 a	0.29 ± 0.06 a	0.19 ± 0.02 b	0.25 ± 0.05 ab
		Color and Phenolic parameters		
420 nm	0.12 ± 0.03 a	0.10 ± 0.01 a	0.12 ± 0.01 a	0.10 ± 0.01 a
Total Phenolic Index	12.03 ± 0.90 a	11.53 ± 1.52 ab	11.5 ± 0.74 ab	10.44 ± 1.14 b
Folin (Gal. Ac. Mg/L)	13.17 ± 0.13 a	12.87 ± 0.17 ab	12.71 ± 0.07 b	12.68 ± 0.03 b
K factor	0.0077 ± 0.0002 b	0.0075 ± 0.0001 b	0.0080 ± 0.0005 ab	0.0084 ± 0.0001 a

Values followed by different letters in each row indicate significant differences ($p < 0.05$) among different samples.

Among the ethyl esters, ethyl octanoate and ethyl hexanoate also recorded high concentrations. Differences were observed in some of the esters among the different wines; however, the sum of the esters was similar. More so, wines produced by the vines treated with the N fertilization recorded higher values of ethyl hexanoate, ethyl decanoate, and ethyl 3 methyl butyrate among all treatments, while for the other ethylic esters, there was not a specific trend recorded.

Vines treated with N fertilization showed an increased final concentration of hexanoic, isobutyric, and isovaleric acid, recording high levels in wines of the N-treated vines, while for the concentration of butyric acid, no significant differences were observed among the different treatments and the control wines.

Acetates are aroma-active esters, resulting from the esterification of a higher alcohols with acetic acid, which are formed intracellularly by yeast cells diffusing through the cellular membrane into the fermenting medium [47], comprising the most abundant group of esters in industrial fermentations, with a major impact on the fruity flavor of wine [48]. An increase in specific acetates mediated by treatment could potentially benefit wine aroma. In general, the sum of acetates was different among the treatments, with the wines produced by the N treatment providing the highest levels of total acetates. In addition to that, isoamyl acetate and 2 phenyl ethyl acetate (both play a major role in wine aroma) recorded higher values in wines produced by the vines treated with the N fertilization. Inversely, hexyl acetate demonstrated a. higher value in the control and DMPP-treated vines.

Terpenes are considered to be important volatiles for the expression of varietal aroma characteristics in wine. Terpenes have a low olfactory threshold and are generally associated with floral and citrus aromas. In the studied wines, two terpenes were detected, linalool and geraniol. The increased levels of these two terpenes in the control wines could favor wine aroma, as these terpenes have low perception thresholds, contributing a pleasant lemon and sweet rose odor [49]. The different fertilizations led to a lower concentration of terpenes in the wines made from these grapes, decreasing their total concentrations compared with the levels found in the control wines.

These data demonstrate that vineyard N fertilizations had a slight effect on the terpenes. Other viticultural techniques, like canopy manipulation and sunlight exposure, were found to alter the concentrations of terpenes in Gewurztraminer berries [50], and Riesling berries and juices [51,52] observed dense canopies for N-fertilized vines and possible N deficiencies for unfertilized vines. It is possible that these two factors may have a limited influence in terpene synthesis in all treatments.

Table 4. Mean values and standard deviation of volatile compounds (mg/L) measured in wines produced from control vines and vines treated with different Nitrogen Fertilizations.

Treatment/ Fertilization	Control	DMPP	N	N + DMPP
		ALCOHOLS		
2 methyl 1 propanol	32.51 ± 6.43 b	45.60 ± 10.43 a	36.21 ± 7.38 b	32.54 ± 6.16
isoamyl alcohol	237.49 ± 29.80 b	311.41 ± 32.27 a	246.74 ± 59.81 b	236.51 ± 42.77 b
methionol	0.26 ± 0.06 b	0.30 ± 0.08 ab	0.20 ± 0.05 b	0.37 ± 0.11 a
2 phenylethanol	67.71 ± 6.51 ab	78.99 ± 19.44 a	61.68 ± 15.13 b	60.35 ± 12.11 b
Total Alcohols	337.49 ± 10.05 b	436.30 ± 20.01 a	344.44 ± 18.65 b	329.77 ± 7.97 b
		ETHYL ESTERS		
ethyl octanoate	3.64 ± 0.56	3.29 ± 0.60	3.43 ± 0.89	3.13 ± 0.76
ethyl hexanoate	1.11 ± 0.14 a	0.75 ± 0.18 b	1.12 ± 0.24 a	0.82 ± 0.14 b
ethyl decanoate	0.44 ± 0.07 b	0.34 ± 0.05 bc	0.67 ± 0.16 a	0.21 ± 0.05 c
ethyl butyrate	0.22 ± 0.06 bc	0.18 ± 0.06 c	0.40 ± 0.07 a	0.32 ± 0.05 ab
ethyl isobutyrate	0.024 ± 0.004	0.028 ± 0.006	0.023 ± 0.008	0.022 ± 0.002
ethyl isovalerate	0.046 ± 0.002 b	0.058 ± 0.011 a	0.053 ± 0.006 a	0.045 ± 0.009 b
Total Ethyl Esters	5.48 ± 0.24 a	4.64 ± 0.17 b	5.69 ± 0.22 a	4.54 ± 0.20 b
		ACIDS		
hexanoic acid	2.14 ± 0.29 a	0.55 ± 0.18 b	1.72 ± 0.44 a	1.72 ± 0.42 a
isobutyric acid	0.09 ± 0.009	0.10 ± 0.002	0.11 ± 0.02	0.11 ± 0.01
butyric acid	0.58 ± 0.12 b	0.61 ± 0.08 b	0.91 ± 0.29 a	0.63 ± 0.10 b
isovaleric acid	0.58 ± 0.13 b	0.44 ± 0.11 b	1.29 ± 0.42 a	0.59 ± 0.11 b
Total Acids	3.39 ± 0.19 a	1.70 ± 0.05 b	4.03 ± 0.19 a	3.05 ± 0.20 a
		ACETATES		
isoamyl acetate	4.58 ± 0.95 b	3.64 ± 1.04 b	7.10 ± 1.60 a	4.77 ± 1.30 b
2 phenyl ethyl acetate	2.40 ± 0.60 a	1.54 ± 0.33 c	2.17 ± 0.32 a	1.67 ± 0.41 bc
hexyl acetate	0.20 ± 0.05 ab	0.25 ± 0.03 a	0.18 ± 0.04 b	0.17 ± 0.04 b
Total Acetates	7.18 ± 0.51 b	5.43 ± 0.89 c	9.45 ± 0.30 a	6.61 ± 0.56 bc
		TERPENES		
Linalool	0.012 ± 0.001 a	0.012 ± 0.001 ab	0.010 ± 0.001 c	0.010 ± 0.001 bc
Geraniol	0.152 ± 0.024 a	0.121 ± 0.027 b	0.125 ± 0.023 ab	0.104 ± 0.008 b
Total Terpenes	0.164 ± 0.012 a	0.133 ± 0.014 b	0.135 ± 0.011 b	0.114 ± 0.004 b

Values followed by different letters in each row indicate significant differences ($p < 0.05$) among different samples.

3.6. Odor Activity Values

From the compounds analyzed, the ones displaying OAVs > 1 were considered to contribute to wine aroma, although they are studies that even use an OAV > 0.5, in synthetic model wines, to build sensory prediction models [27]. OAVs are useful to determine the possible relevance and contribution of each compound to the wine aroma. However, it should also be considered that compounds even under their detection threshold could potentially contribute to the wine aroma due to additive-synergistic effects or emergent properties, and in the opposite way, compounds above their detection threshold could decrease their aroma intensity due to masking effects [53].

The contribution of each volatile compound with OAVs > 1 to the aroma of each wine could be evaluated qualitatively by means of its associate descriptor and quantitatively by means of its OAVs. Table 5 lists the OAVs for the 19 odor-active compounds for all of the wines. The Control, N, and N + DMPP wines had the same odorants, with OAVs above 1 (14), while DMPP wine recorded 15. Moreover, the OAVs for ethyl decanoate, ethyl butyrate, and isoamly acetate in wines produced by the N-treated vines were almost two-times higher than the other treatments.

Based on the high OAVs values of aroma compounds, those ones are able to provide a significant influence on wine aroma. Due to the fact that they are significant aroma compounds in terms of sensory evaluation, sensory assays are needful to confirm the effect of the odor-active compounds already identified.

Table 5. Odor activity values (OAVs) for the aroma compounds in wines produced from control vines and vines treated with different Nitrogen Fertilizations.

Treatment/ Fertilization	Sensory Descriptor	Reference	Odor Threshold (mg/L)h	OAV [a]			
				Control	DMPP	N	N + DMPP
ALCOHOLS							
2 methyl 1 propanol	wine, solvent, bitter	[54]	40	0.8	1.1	0.9	0.8
isoamyl alcohol	whiskey, malt, burnt	[55]	1	7.9	10.4	8.2	7.9
methionol	sweet, potato	[54]	1	0.3	0.3	0.2	0.4
2 phenylethanol	honey, spice, rose, lilac	[54]	14	4.8	4.8	4.4	4.3
ETHYL ESTERS							
ethyl octanoate	fruit, fat	[54]	0.005	728	658	686	626
ethyl hexanoate	apple peel, fruit	[54]	0.014	79.3	53.6	80.0	58.6
ethyl decanoate	grape	[54]	0.200	2.2	1.7	3.4	1.1
ethyl butyrate	apple	[54]	0.02	11.0	9.0	20.0	16.0
ethyl isobutyrate	apple	[54]	0.018	1.3	1.6	1.3	1.2
ethyl isovalerate	fruit	[54]	0.03	1.5	1.9	1.8	1.5
ACIDS							
hexanoic acid	sweat	[54]	0.42	5.1	1.3	4.1	4.1
isobutyric acid	rancid, butter, cheese	[54]	8.1	0.011	0.012	0.014	0.014
butyric acid	rancid, cheese, sweat	[54]	0.173	3.4	3.4	5.3	3.4
isovaleric acid	sweat, acid, rancid	[54]	0.033	17.6	1.3	39.1	17.9
ACETATES							
isoamyl acetate	Banana	[54]	0.03	152.7	121.3	236.7	159.0
2 phenyl ethyl acetate	rose, honey, tobacco	[55]	0.25	9.6	6.2	8.7	6.7
hexyl acetate	fruit, herb	[56]	1.5	0.1	0.2	0.1	0.1
TERPENES							
Linalool	flower, lavender	[54]	0.025	0.5	0.5	0.4	0.4
Geraniol	rose, geranium	[57]	0.036	4.2	3.4	3.5	2.9

[a] OAVs are expressed as the mean concentration of an aroma compound divided by its odor threshold value.

3.7. Sensory Profile

In order to enhance the differences observed in volatile compound levels of the wines produced by the different nitrogen treatments described above, all Savvatiano wines were evaluated by aa sensory expert panel. The use of a sensory panel accustomed to the descriptive analysis of Savvatiano allowed some rudimentary relationships to be made between aroma compounds detected and sensory descriptors. Panelists were asked to evaluate descriptors related to color, aroma, and mouthfeel sensations. Figure 2 shows the mean scores of 10 sensory characteristics, with a maximum score of 10, of the wines produced (Control, N, DMPP, and N + DMPP). In order to identify in which attributes and wines significant differences were observed, a Kruskal–Wallis test was performed, accompanied by a Mann–Whitney–Wilcoxon Test (Table 6). Despite their superficial appearance, sensorial data are not truly numeric variables, because intervals between consecutive categories cannot be assumed to be equal. Moreover, the assumptions of parametric tests were not met on the sample data. Therefore, parametric tests may not be the most appropriate method of analysis, because, in such cases, the statistics may not be a good estimate of the parameter. It does not seem appropriate to ignore these restrictions and to go ahead with the analysis.

The aroma profile of the various wines (Figure 2) showed a significant change among the treatments and the control wines. Concerning color parameters, the statistical analysis did not show any significant differences, indicating that the panelists could not perceive any differences in the color intensity of the wines (Table 6). These findings correlated

well with the results obtained from the absorption at 420 nm. Regarding the aroma of the wines, the panelists judged the wines produced by the Nitrogen treatment with the highest value for aroma intensity (Table 6). Moreover, the same trend was observed regarding the white flowers, tropical fruits, green apple, and banana attributes. In particular, Nitrogen-treatment wines evaluated by the panelists had a significantly higher value ($p < 0.05$) (Table 6). In relation to the vegetal character of wines, the ones produced with DMPP treatment were found to have the highest vegetal aroma, while in the control wines, the vegetal notes were minimized ($p < 0.05$) (Figure 2 and Table 6). Sensory results were consistent with the concentration of volatile compounds in wines.

Regarding the higher aroma intensity of white flowers, citrus, and tropical fruits described by the panelists for the control and Nitrogen-treated wines, this could be justified due to the fact that esters and acetates tend to present fruity aromas and may play a major sensory role, especially in neutral grape varieties containing negligible amounts of terpenes [58].

The high values of total terpenes ($p < 0.05$) in the control wines had no impact on the attributes of white flowers and citrus fruit. This could be explained due to the fact that wine is a complex matrix, and there could be a possibility of mixture suppression effects. Consequently, as the perceived intensity of one compound decreases, another compound could be more perceived as the inhibition effect diminishes [59].

According to the statistical analysis, differences were found among the wines produced with different nitrogen-fertilization levels. Similar results presented from previous studies [2] showed that the aroma profile of the wines produced with a higher YAN concentration exhibited significant changes in comparison with wines produced from musts with a lower YAN concentration.

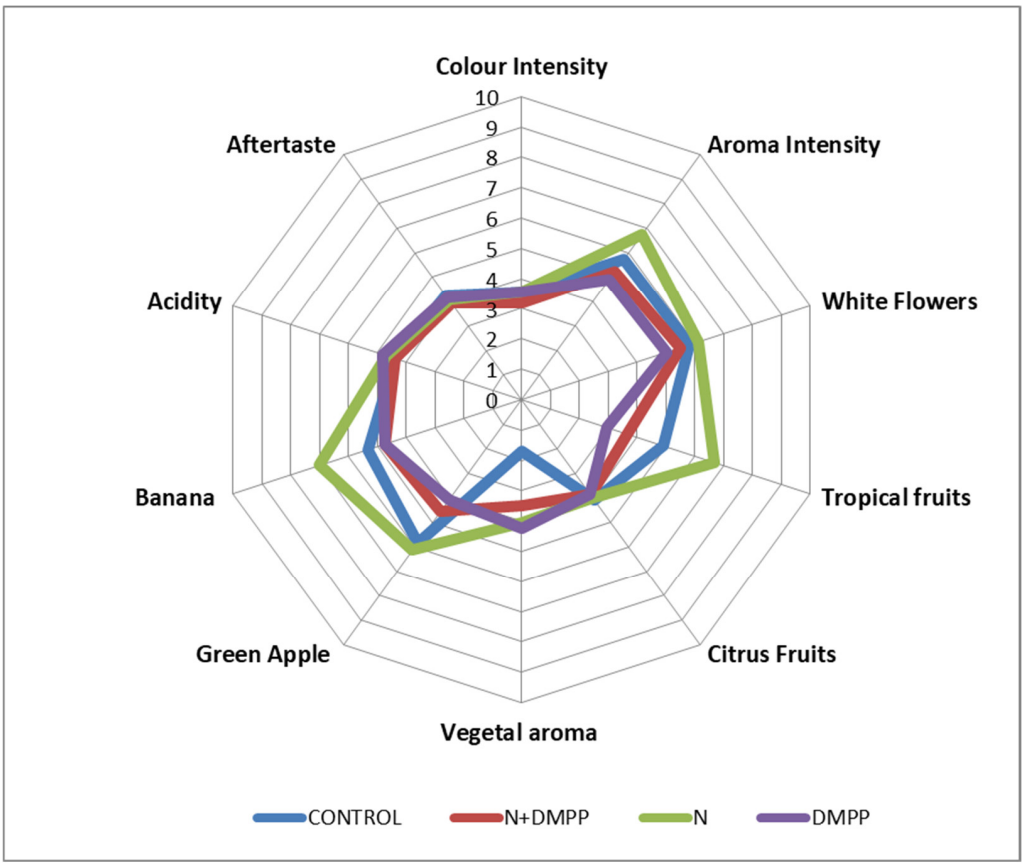


Figure 2. Spider plots of the sensory profile of the experimental wines from *Vitis vinifera* L. cv. Savvatiano in response to three different fertilization treatments and a control. Wines were judged using predefined quality attributes on a scale from 1 (absent) to 10 (high).

Table 6. The Kruskal–Wallis test and, when significant, ($p < 0.05$) the Mann–Whitney–Wilcoxon Test were applied for multiple comparisons to the results of the sensory scores for the wines produced by the different fertilization treatments.

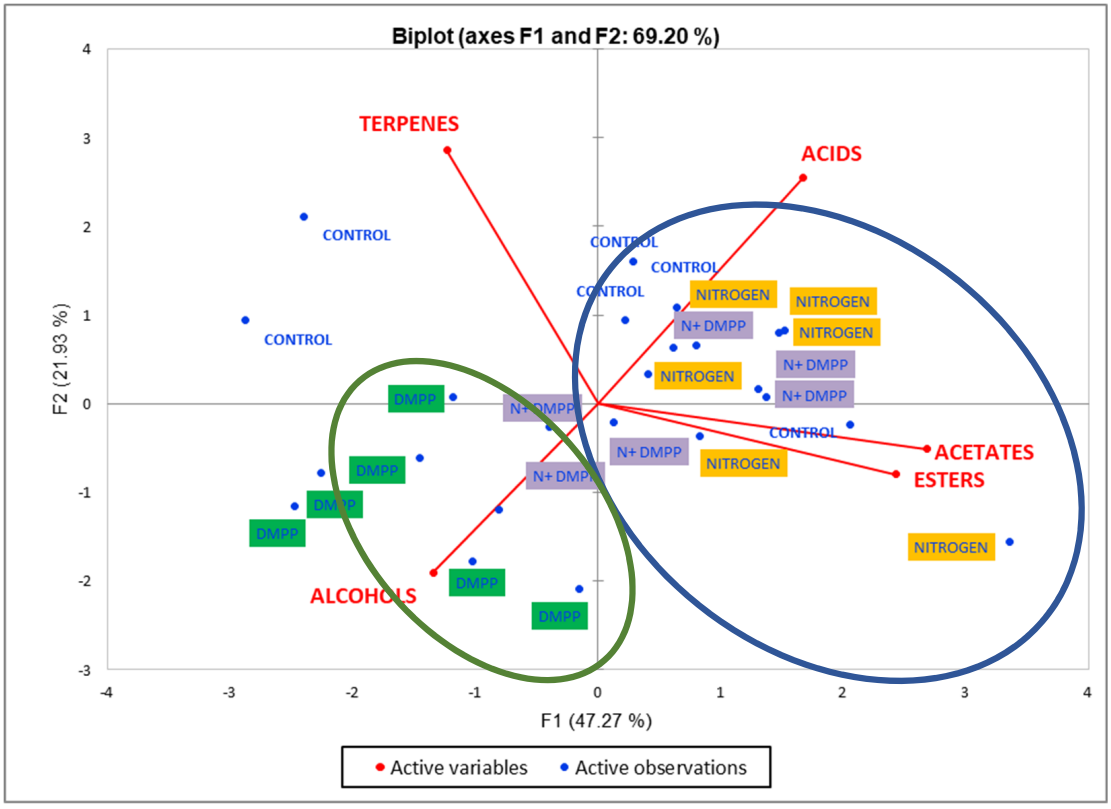
	Kruskal–Wallis Test *p*-Value	**Post-Hoc Mann–Whitney–Wilcoxon Test**			
		Control	**DMPP**	**N**	**N + DMPP**
Colour Intensity	0.496				
Aroma Intensity	0.0057	ab	a	b	a
White Flowers	0.043	ab	a	b	ab
Tropical Fruits	1.005×10^{-9}	a	b	c	b
Citrus Fruits	0.889				
Vegetal aroma	1.45×10^{-9}	a	b	b	b
Green Apple	0.00015	b	a	b	a
Banana	0.00015	a	a	b	a
Acidity	0.619				
Aftertaste	0.944				

Test statistics; the Kruskal–Wallis test was statistically significant when $p < 0.05$. Different letters in each row indicate significant differences ($p < 0.05$) among different samples

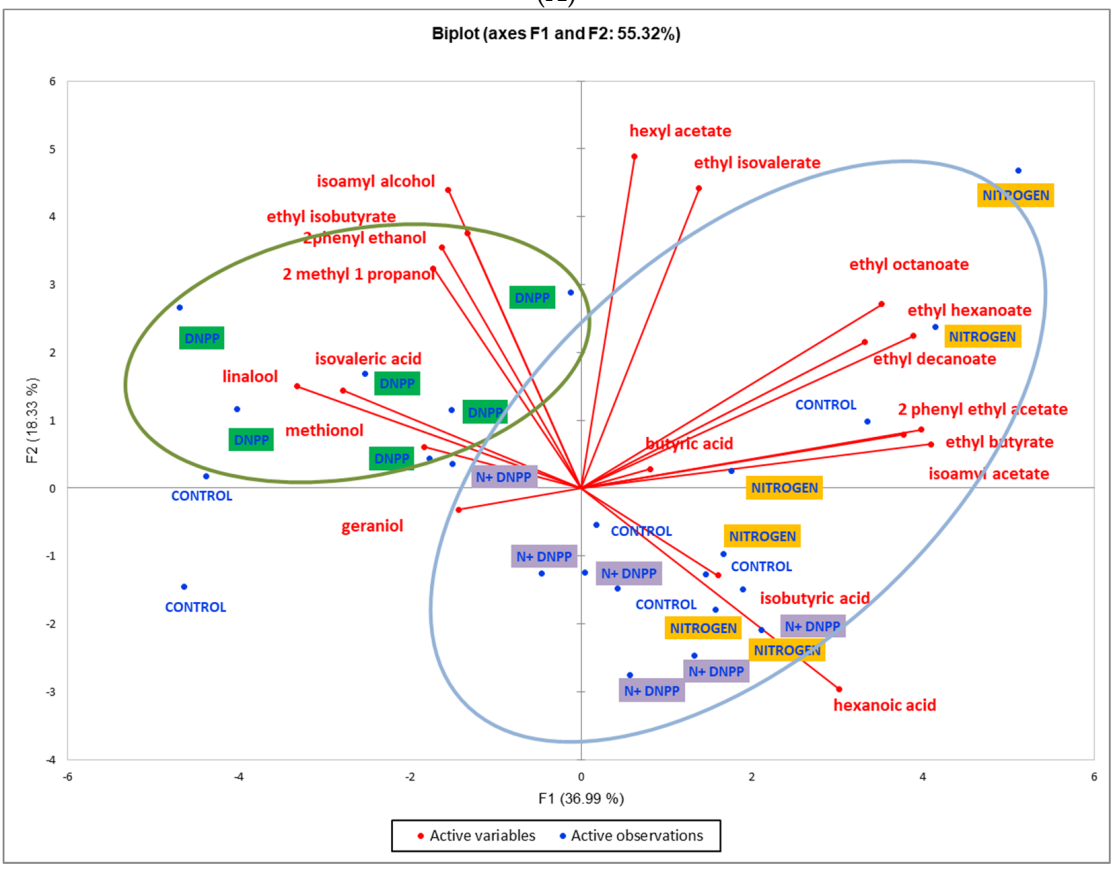
3.8. PCA

Supervised OPLS-DA score plots showed a separation of the wines by treatment/fertilization regarding volatile compounds of the aroma (alcohols, acetates, acids, esters, and terpenes) (Figure 3a) and the specific aroma compounds (Figure 3b). PC1 and PC2 explained, respectively, 69.2% (Figure 3a) and 55.32% (Figure 3b) of the variation of the data. Variables in the score plots were colored according to the fertilization treatment. Wines of the unfertilized vines were spread on the plot not showing any specific grouping, while different N fertilizers, as well as the DMPP treatments, were differentiated and grouped accordingly. That means that all the treatments, including the DMPP, achieved homogenization of the sample set in comparison with the untreated control samples. More so, there was a transaction from DMPP treatment to N + DMPP and finally to N treatment, implying the availability of both the mineral form of available N ammonium from DMPP and N + DMPP and nitrate from N treatment. However, the amount of it (DMPP treatment has much lesser ammonium than N + DMPP) that determined the wine characteristics showed that nitrogen fertilization was the main factor of discrimination with a separation along the PC1 axis in both PCA plots. A form of grouping of the wines could be determined: the wines of the nitrogen and the DMPP treatment were co-located in different parts of the biplot (Figure 3a,b). The principal component analysis showed that acetates and esters were associated with nitrogen treatments, whereas alcohols were associated with the DMPP treatment.

The corresponding aroma compounds responsible for the separation between wine samples treated with different fertilizations and untreated/control are shown in Figure 3c. For Savvatiano wines (Figure 3c), the major aroma compound classes involved in sample segregation were alcohols, terpenes, and acetates. Therefore, in this experiment, the loading plot also showed that some aroma compounds were clustered in relation to their structural class.

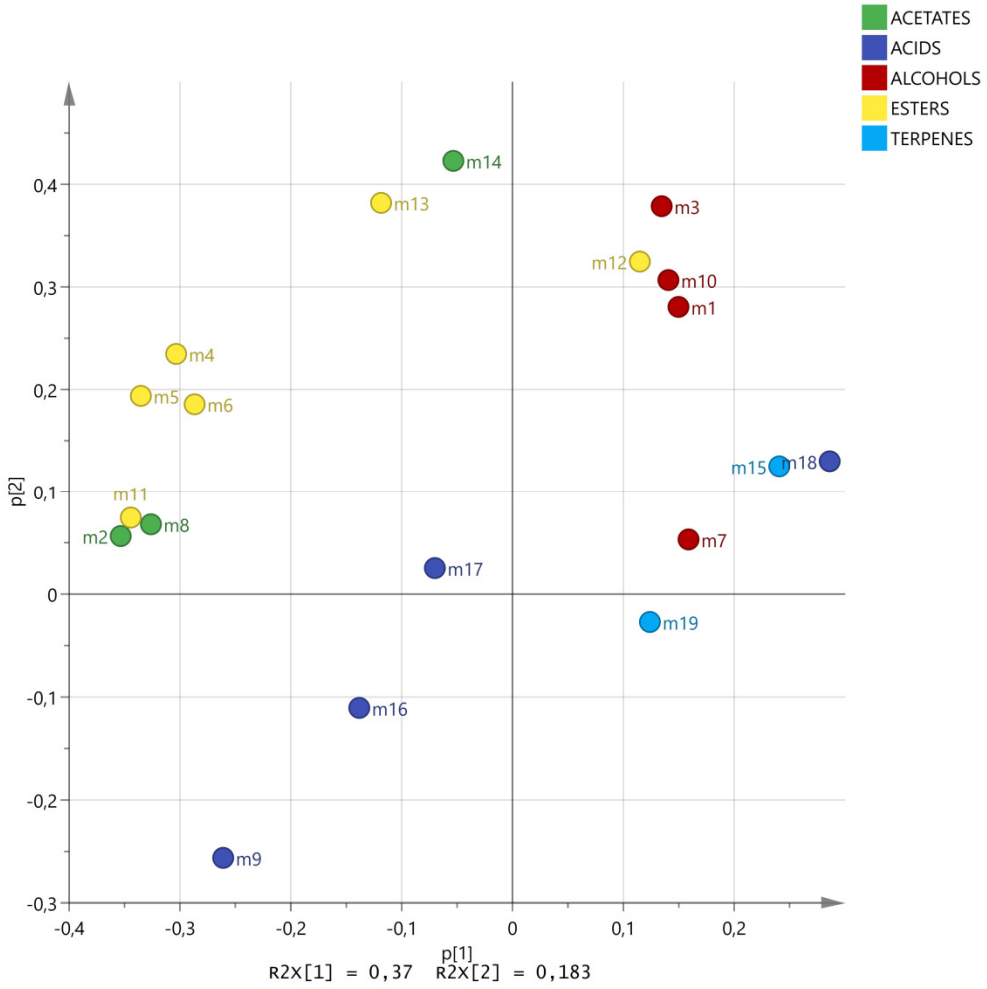


(**A**)



(**B**)

Figure 3. *Cont.*



(**C**)

Figure 3. Unsupervised classification using a principal component analysis on metabolomic data (aroma compounds) from wines of tge cultivar Savvatiano, depending on different fertilizations (Nitrogen, Nitrogen + DMPP, DMPP, and untreated−control). Samples in the score plots (**A**,**B**) were colored according to the treatments, and variables in loading plots (**C**) were colored according to the metabolic class. Numbers indicate the ID of aroma compounds, as follows: 2 methyl 1 propanol (m1), isoamyl alcohol (m2), methionol (m3), 2 phenyl ethanol (m4), isoamly acetate (m5), 2 phenyl ethyl acetate (m6), hexyl acetate (m7), ethyl octanoate (m8), ethyl hexanoate (m9), ethyl decanoate (m10), ethyl butyrate (m11), ethyl isobutyrate (m12), ethyl isovalerate (m13), hexanoic acid (m14), isobutyric acid (m15), butyric acid (m16), isovaleric acid (m17), geraniol (m18), and linalool (m19).

4. Conclusions

N fertilization is an agricultural/ viticultural technique with a great effect on the grapevine yield and the quality of wine. However, interactions between nutrients should be considered in a complete nutrient management program in vineyards. A mineral fertilization plan should take into account the related changes in nutrient solubility, nutrient resupply from the solid phase, and the resulting bioavailability to plants.

Balanced soil fertilization with N can increase the vine's nitrogen status, yield, and vigor and the susceptibility of grapes to Botrytis [60]. An N deficiency should be avoided, as well as excessively high vine N levels. The same recommendations can be given for a number of other white grape varieties of *Vitis vinifera* L. (Gewurztraminer, Petit Manseng, Gros Manseng, and Semillon), as volatile thiols also make up part of their aroma [61].

N increases vegetative growth, which, in turn, has an influence on the ripening of the grapes. Hence, there could be an effect on their quality. Delayed maturity affects the biochemical composition of the berry [62] and, thus, affects the formation of aroma compounds and tastes. This impact is based on changes in flavonoid metabolism, attributable to a competition for sugar between the leaves and grape berry growth [63]. A higher supply of N might favor plant biomass and, therefore, less compounds biosynthesis in grape berries. As a result, less aroma precursors accumulate in the berries, and a decreased aromatic intensity of the wines results. This could explain the results of the present study, in which the produced wines from the control vines showed significantly smaller berries than the wines produced by the treated ones.

Must composition and fermentation conditions are two major factors determining the volatile profile of wines [64]. Hence, it can be expected that these applications speed up the fermentation and might avoid sluggish fermentations. Moreover, nitrogen addition to the must can be limited or avoided. A quick and clean fermentation is a quality factor in wine making. However, it has been reported that the nitrogen content of grape juice can vary over a very wide range (60–2400 mg N/L) [65]. Few studies have investigated the impact of "severe" nitrogen limitation on yeast metabolism. This study confirmed that low nitrogen ferments are generally characterized by continuously slow, essentially linear, rates of sugar consumption (Figure 1), which may or may not lead to a stuck (incomplete) fermentation [66]. This low rate of fermentation has been attributed to a low biomass [67].

Total acidity and pH are two of the most important quality factors in grapes and wine. In addition to wine stability and microbiological control, both parameters have an influence on sensorial parameters [68]. Lower values within this range in musts are preferred, because pH increases during or after fermentation [53]. For all treatments, no significant differences were observed (Table 1). It could be assumed that the chemical analysis of must can provide a broad indication of the final produced wine quality, while a high/low quality cannot be predicted on all occasions. In several fertilization strategies, "wine quality" is still defined using analytical data. It is crucial for all fertilization treatments to perform a sensorial evaluation of the produced wines.

Leaving aside the effects of nitrogen application in the vineyard on grapevine productivity, the main objective of winemakers is to achieve an adequate concentration of YAN in grape juice that will foster a healthy fermentation, as far as this objective is in parallel with other fruit requirements. Harvest time is usually dictated by factors other than YAN concentration. These constituents, like TSS, total acidity, pH, or phenolic ripeness, which could not be easily altered by winemaking procedures, have been taken into consideration in the last decades.

At the harvest point, although the grape berry composition could influence wine composition, the winemakers have many options available to transform the wine style. Additionally, nitrogen, being a growth-limiting nutrient in grape, must affect yeast growth and the capacity to ferment sugars. Consequently, yeast activity will affect the production of many of the volatile products which are sensorially important for the wine quality. No significant differences were observed regarding the conversion yield of ethanol between ferments of the different fertilization treatments provided with a different initial YAN into the musts. This outcome could indicate that the relatively low fermentation temperature and the yeast strain perform a more significant role compared to YAN [43].

The little information that is available concerning the impact of different N forms in the vineyard system and their effect on the grapevine metabolome and wine is conflicting. Furthermore, data on phenolic content and their effect on aroma and flavor composition are rare.

The results of this research study suggest that the initial concentration of grape must YAN does not significantly improve the sensory profile of the produced wines, as previously thought. A trend was observed of increasing aroma intensity and white flowers and a significant increase in tropical fruits and a banana aroma, which is in accordance with other research studies [69]. On the other hand, control wines recorded a lower k factor and high

phenolic content (Table 4). Practically, these wines would develop a brown color later than the others, taking into consideration previous reports [70,71]. Phenolic compounds could affect flavor, appearance, taste, and color. In addition to their organoleptic properties, they are the main substrates for must and wine oxidation [72,73]. Similar results were showed by the research groups of Nikolantonaki et al. [12] and by Romanet et al. [74].

Deciphering the link between N fertilization and volatile compound biosynthesis will provide useful information to monitor the fermentative aroma profile of wine. Future studies could lead to a better comprehension of N concentrations interconnected with other pathways, like amino acids, which will assist to describe the production of volatile compounds responsible for the pleasant aroma and the control of AF by avoiding sluggish fermentation, providing innovative techniques to winemakers.

Herein, we conclude that nitrogen fertilization and the use of the nitrification inhibitor DMPP increased the productivity and the yield, as the weight/berry was increased significantly, and at the same time, YAN increased over 50%. Other basic grape and wine characteristics were not affected. Moreover, the quality of the produced wines was similar and even slightly better, with tendency of a higher aroma intensity, white flowers aroma, and tropical fruits and banana aroma attributes. This indicates that productivity can be increased with the adequate cultivar treatments without affecting the aroma profile, at least for neutral varieties, such as Savvatiano. The other important output is that nitrogen fertilization and the use of the nitrification inhibitor DMPP achieved a homogenization of the sample set in comparison with the untreated control samples, as the PCA analysis showed. This could be potentially important to decrease the heterogeneity that appears in the grape berry chemical composition during the harvest time. More studies need to be done to improve this supposition. Finally, the emerged technique to use nitrification inhibitors with the aim to decrease N losses and improve N-fertilization efficiency and N availability to vine, which can improve vine productivity without negatively affecting the wine quality, is one of the future challengers for the viticulture and wine sectors.

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Informed Consent Statement: Panelists gave informed consent before participating in this study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author (pending privacy and ethical considerations).

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