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The Use of Grape Pomace Residues as a Nutrient Source in Subtropical Viticulture

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Abstract: The global wine industry is shifting towards organic viticulture to reduce dependence on industrial inputs, favoring organic nutrient sources like grape pomace. However, the suitability of grape pomace-based compost and vermicompost in subtropical regions remain unclear. Given the diverse responses of grapevines to fertilization under different climates and soil conditions, field studies are crucial. This research investigated whether these residues, alongside non-industrialized mineral fertilizers, can enhance soil fertility and improve grape yield and quality in subtropical climates. We found that compost and vermicompost exhibit distinct chemical compositions, leading to varied patterns of nutrient release in soils. However, grapevines displayed minimal to negligible responses in terms of leaf tissue levels nutrients. Notably, "Isabella" grapevines displayed a reduction in grape yield with organic fertilizer application and remained unresponsive to mineral fertilization. While there were no substantial alterations in must composition, "Isabella" grapevines demonstrated elevated levels of anthocyanins when cultivated with mineral fertilizer. In contrast, "Chardonnay" grapevines exhibited no changes in both grape yield and quality in response to the various fertilization treatments examined. While organic and mineral fertilizers may have distinct chemical compositions and release patterns, their effectiveness in improving grape yield and quality appears to vary significantly among different grape cultivars.

Keywords: agroecology; grape quality; organic fertilizer; plant nutrition; organic viticulture

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1. Introduction

Most global grape and wine production derives from conventional agroecosystems, which require intense use of industrialized input [1]. There is, however, a desire in developed countries, and more recently in developing ones, to switch from conventional production systems to organic viticulture. In this scenario, organic nutrient sources—such as animal manure or industrial residues, raw or composted—are favored over mineral fertilizers [2], primarily in soils that lack sufficient amounts of nutrients, such as the ones that occur widespread in the subtropical areas in the south of Brazil [3].

The utilization of winery residues, namely grape pomace (skin and seeds), as a source of nutrients, holds considerable promise, and it has already been adopted in organic viticulture [4]. In addition to being low-cost and accessible to farmers, incorporating grape pomace-based compost or vermicompost into soil fertilization can serve as a sustainable waste management solution. This approach aligns with the broader discourse on sustainability in agriculture. The composition of grape pomace residues not only addresses the nutritional requirements of grapevines, but also introduces organic matter that significantly benefits soil health. This includes improvements in soil structure, enhanced water retention, and increased microbial activity, fostering a more ecologically balanced environment [4,5]. Nevertheless, despite their current use, their actual suitability to be a source of nutrients, their ability to secure grapevine nutrition, and their impact on grape production and quality have not been tested in subtropical conditions.

In order to obtain this information and utilize it to refine the technical recommendations related to grape pomace compost and vermicompost in viticulture, long-term field studies are necessary. This is particularly important in perennial crops, which have a long growing cycle and are more susceptible to climate variations among seasons. Grapevines have demonstrated varied responses to fertilization under different climate and soil conditions [6–8]. Besides this, nutritional responses of fruit crops have been proven to be cultivar-specific [9,10], which might be true for European (*Vitis vinifera*) versus hybrid (*V. vinifera* × *V. labrusca*) or red versus green cultivars. This highlights the need to test different nutrient sources on a variety of genotypes, such as grapevine cultivars for red and white wine and grape juice production. With the global shift towards organic viticulture, the challenge lies in enhancing yield while minimizing dependence on industrial inputs in these regions. Notably, the humid subtropical climate fosters faster decomposition of organic materials due to increased soil humidity and higher temperatures, facilitating rapid nutrient release. Consequently, robust experimentation with both conventional and innovative nutrient sources under these conditions becomes imperative.

We hypothesized that the utilization of grape pomace compost and grape pomace vermicompost can enhance soil fertility in vineyards, particularly in terms of nitrogen (N) and potassium (K), considering grape skin composition. We also speculate that the incorporation of non-industrialized mineral fertilizers, such as natural phosphates and potassium sulfate, can contribute to ensuring adequate nutrient availability in vineyard soils. Additionally, the nutritional responses to different fertilizers, together with their influence on grape yield and quality, can be cultivar-specific. In light of these hypotheses, this research aimed to empirically test whether these fertilizers can enhance soil fertility, provide adequate plant nutrition, and improve grape yield and quality, over three growing cycles. The outcomes of this study hold the potential to significantly contribute to the advancement of sustainable viticulture practices, especially in developing subtropical regions worldwide.

2. Materials and Methods

2.1. Experimental Site and Experimental Design

Two experiments were conducted in organic production vineyards located in Veranópolis (28° 47′06″ S; 51° 30′32″ W), Rio Grande do Sul, Brazil, over three growing seasons. The region has a Cfa climate [11] (humid subtropical climate with hot summers) [11]. Spontaneous cover crops (mostly *Lolium multiflorum* and *Raphanus sativus*) were consistently maintained in both rows and inter-rows throughout the year, being mowed during the grapevines’ growing season. One of the vineyards, henceforth called Vineyard 1, featured “Isabella” (*V. labrusca* × *V. vinifera*) grapevines grafted onto Paulsen 1103 rootstocks, employing a pergola system, spaced 2.5 m between rows and 1.5 m between plants (totaling 2666 plants per hectare). The other vineyard, henceforth called Vineyard 2, featured "Chardonnay" (*V. vinifera*) grapevines, grafted onto Paulsen 1103 rootstocks, spaced 2.8 m between rows and 1.5 m between plants (totaling 2380 plants per hectare), also employing a pergola system, under polyethylene translucid coverage. The soil profile in both vineyards was classified as Leptic Cambisols [12]. In Vineyard 1 and in Vineyard 2, grapevines were 10 to 12 and 3 years old (since seedling transplantation), respectively. In both areas, the soil had been previously limed before vineyard establishment.

Three rows were established in Vineyards 1 and 2, and six fertilization treatments were applied in each row, grape pomace compost; grape pomace vermicompost; grape pomace compost plus mineral fertilizer; grape pomace vermicompost plus mineral fertilizer; and only mineral fertilizer, in addition to a negative control (Table 1). The setup comprised a split-plot design, featuring two factors of variation, different organic fertilizers (compost and vermicompost) and the addition of mineral fertilizers (present or absent), with three repetitions (rows) each (Figure S1). The repetitions encompassed five grapevines each.

Table 1. Table of mean nitrogen (N), phosphorus (P), and potassium (K) dry-based content of organic fertilizers applied in the vineyards, and their equivalent mineral doses.

Organic Fertilizers		N	P	K
Grape pomace vermicompost	Content (dry base)	0.568%	0.287%	0.861%
	Equivalent dose	40 kg N ha^{-1}	42.36 kg P_2O_5 ha^{-1}	72.70 kg K_2O ha^{-1}
Grape pomace compost	Content (dry base)	1.024%	0.684%	2.373%
	Equivalent dose	40 kg N ha^{-1}	62.06 kg P_2O_5 ha^{-1}	111.24 kg K_2O ha^{-1}

The compost and vermicompost were supplied in amounts corresponding to 40 kg N ha^{-1}, based on previous research [5] in vineyards in the same region [3]. P_2O_5 and K_2O doses were 160 and 100 kg ha^{-1}, respectively, and were calculated according to the local fertilization manual [13], following soil chemical analysis. The mineral source of phosphorus (P) was natural phosphate which was incorporated into the soil before the first cultivation cycle. The potassium (K) source was potassium sulfate, applied on the surface, and concentrated in rows under the grapevines' canopy projection. As recommended by Brunetto et al. [3], organic fertilizers were applied each year during the growing season at bud burst, in the rows and beneath the grapevines' canopies. Grape pomace compost was obtained in an industrial organic composter, which composted raw winery residues (grape pomace) in open air. Grape pomace vermicompost was made in a closed worm composter at the Federal Institute of Education, Science and Technology of Rio Grande do Sul, campus Restinga, in Porto Alegre (RS), Brazil. Every year, we composted winery residues (grape pomace) for 60 days (thermophilic phase), and then vermicomposted them with *Eisenia* sp. worms for another 120 days (humification phase). During both phases, humidity was maintained between 70% and 80%, with water added as needed. Additionally, the vermicomposting residue was mixed twice a week throughout the process. Both compost and vermicompost were stored at room temperatures after stabilization.

2.2. Mean Temperatures and Accumulated Rainfall

The mean temperatures and accumulated rainfall were summarized from the closest (23.4 km far) meteorological station [14], covering the duration of the experiment (January 2020 to February 2023), and are shown in Figure 1.

Spring temperatures (September, October, and November) were lower in the 2022/23 growing season compared to the other two. However, the summer months (December, January, and February) had temperatures which were slightly higher. The total accumulated rainfall from August to January was 574, 416, and 456 mm in the 2020/21, 2021/22, and 2022/23 growing seasons, with the latter two representing drier conditions for grapevines.

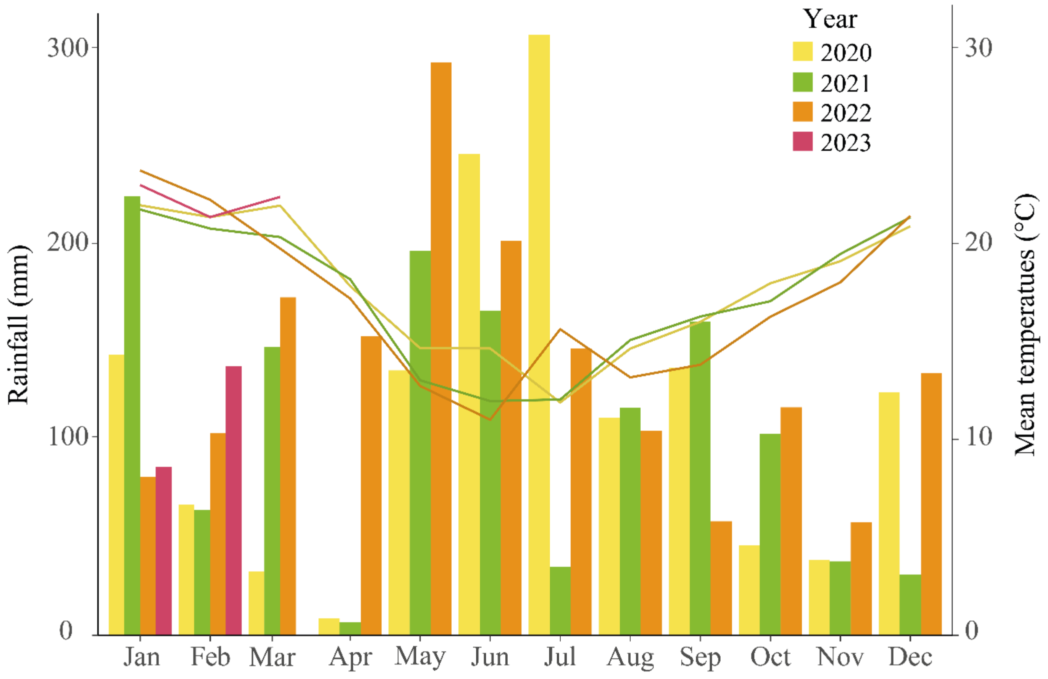

Figure 1. Accumulated monthly rainfall (mm), shown in bars, and monthly mean temperatures (°C), shown in lines, from January 2020 to February 2023, obtained at Bento Gonçalves meteorological station [14].

2.3. Soil Sampling and Analyses

Before the experiment installment in 2020, soil samples, consisting of 15 randomized subsamples, from layers 0–0.10 m and 0–0.20 m were collected from Vineyards 1 and 2. These samples were analyzed for various physicochemical properties, including the following: exchangeable Ca, Mg, and Al (1:20 1 mol L^{-1} KCl), exchangeable K (1:10 Melich-1 extractor), available P (1:10 Melich-1 extractor), EDTA-extracted Cu, Zn, Fe, and Mn (1:40 0.05 mol L^{-1} EDTA), pH (1:10 distilled water), soil organic matter through dry combustion (FlashEA 1112 instrument from Thermo Electron Corp., Milan, Italy), and soil texture [15]. After three growing seasons (in 2023), soil samples (composed of three subsamples) were taken from the row in each experimental unit, and the same variables were reanalyzed, except for soil texture, with the results presented in Table S1 and Table S2.

2.4. Grape Pomace Compost and Vermicompost Composition

Grape pomace compost and vermicompost were analyzed as described in Kokkonen et al. [16], for the same variables. The results are presented in Table S3.

2.5. Leaf Tissue Elementary Analyses and Plant Nutrition

Complete leaves opposed to grape bunches/inflorescences, in the middle section of the branch of the year [13], were collected in the 2020/21, 2021/22, and 2022/23 growing seasons at flowering and veraison. Two leaves were selected from the four quadrants of the three central grapevines in each experimental unit, totaling eight leaves per plant. The leaves were dried in a forced-air circulation oven at around 65 °C until a constant weight was reached, then ground using a Willey-type mill and sifted through a 2 mm mesh. The samples were digested in sulfuric acid and hydrogen peroxide (2:1) [17] and nitric and perchloric acid (3:1) [17]. N content was quantified in the sulfuric–peroxide digestion extract by distillation in a micro-Kjeldahl N distiller (Tecnal, TE-0363, São Paulo, Brazil) and titration with standardized ±0.025 mol L^{-1} of sulfuric acid [17]. The nitric–perchloric extract was diluted and the concentration of phosphorus (P) was quantified using the molybdate-blue technique, in a UV–visible spectrophotometer (Bell Photonics, 1105, Piracicaba–SP, Brazil) at 882 nm [17], which was calibrated at each shift with a calibration curve generated using standard solutions of phosphorus at 0, 0.1, 0.2, 0.4, 0.8, and 1.2 mg L^{-1}, prepared from a potassium dihydrogen phosphate stock solution; potassium (K) content was quantified in flame photometer (910, Analyser, São Paulo–SP, Brazil); and Ca, Mg, Cu, and Zn contents were quantified by atomic absorption spectrophotometry (AAnalyst 200, Perkin-Elmer, Waltham - MA, USA). Also, before collection, we evaluated the SPAD index on the same eight leaves collected for analyses, with a chlorophyllometer (Konica Minolta, SPAD-502, Tokyo, Japan). To minimize photoinhibition effects and ensure stable environmental conditions, all measurements were taken before 9:00 a.m. The SPAD index was measured three times on each leaf: once on each side of the leaf (avoiding major veins) and once near the central-top region. This totaled 24 measurements per plot.

2.6. Grape Yield

All grape bunches from every grapevine were harvested in January (Vineyard 2) and in February (Vineyard 1) in the 2020/21, 2021/22, and 2022/23 growing seasons. All bunches were weighed with a digital scale adjusted to three-digit accuracy (Walmur, Wa4434, Rio Grande do Sul, Brazil) to quantify the grape yield per plant. Next, samples of bunches from each experimental unit were reserved to determine the grape must chemical composition.

2.7. Grape Quality and Must Chemical Composition

A hundred units of the reserved berries were manually peeled, and the pulp and seeds (must) were separated from the grape peels. Subsequently, the chemical composition of the grape must was analyzed by quantifying the total soluble solids (TSS), hydrogen potential (pH), and total titratable acidity (TTA) in the pulp + seeds. The TSS concentration in the samples was quantified in a digital refractometer (Reichert Technologies, Brix/RI-Chek, New York, NY, USA). pH was determined in a benchtop digital pHmeter with automatic temperature control, previously calibrated with pH buffer solution (4.0 and 7.0). TTA was quantified through neutralization titration, utilizing 10 mL of samples, standardized NaOH 0.1 mol L^{-1}, and 1% phenolphthalein as an indicator [18].

Total anthocyanin concentration (TA) and total polyphenol index (TPI) were quantified in red grape (“Isabella”) skin extracts. Fresh grape skins were processed using a blender (Arno, Clic Lav Top, São Paulo–SP, Brazil) with acidified ethanol (0.7% HCl) solution (1:3, m/v) for 40 s at slow speed (1500 RPM), and another 40 s at high speed (3500 RPM) [19]. The resulting solution was transferred to a 250 mL beaker, left to stand in the dark for 30 min at room temperature, and then centrifuged at 3500 RPM for five minutes. TA was quantified by the pH difference method [20] and TPI by the Folin–Ciocalteau method [21] in a UV–visible spectrophotometer (Bell Photonics, 1105, Piracicaba–SP, Brazil) at 515 and 700 nm for TA and 765 nm for TPI.

2.8. Statistical Analyses

All experimental data were subjected to descriptive statistical analyses, and were tested for homogeneity via the Shapiro–Wilk test. The effect of the organic fertilizers (factor 1) and mineral fertilizers (factor 2) upon each response variable was tested with analysis of variance, using the R (version 4.3.0) "ExpDes.pt" package [22]. Whenever the null hypothesis (equal means) was rejected with alpha equal to 0.05, means between different organic fertilizers were compared via the Tukey test, and means between the presence or absence of mineral fertilizers were compared via Fisher's Least Significant Difference (LSD) test. Additionally, principal component analysis (PCA) was performed with the response variables leaf nutrient content, soil nutrient availability, grape yield, and quality parameters in order to identify more complex interactions between them and identify greater differences among treatments. PCA was run using the R "factoextra" package [23]. The R "ggplot2" package [24] was employed for data visualization. All statistical analyses were run on the R software [25].

3. Results

The results of the ANOVA test and the effect of the tested factors (organic fertilizers and mineral fertilizers), and their interaction, on the response variables are shown in Table S4.

3.1. Plant Nutrition and Grape Yield

3.1.1. Leaf Nutrient Content

N and K contents in "Isabella" grapevine leaves collected at flowering and veraison were unaffected by organic or mineral fertilization (Figure 2a,b). The P content in leaves collected at flowering, however, was higher in grapevines fertilized with either CO, VC or +MF (Figure 2c). In "Chardonnay" grapevines, there was not any difference in N, K or P content in leaves among different fertilizer applications (Figure 3).

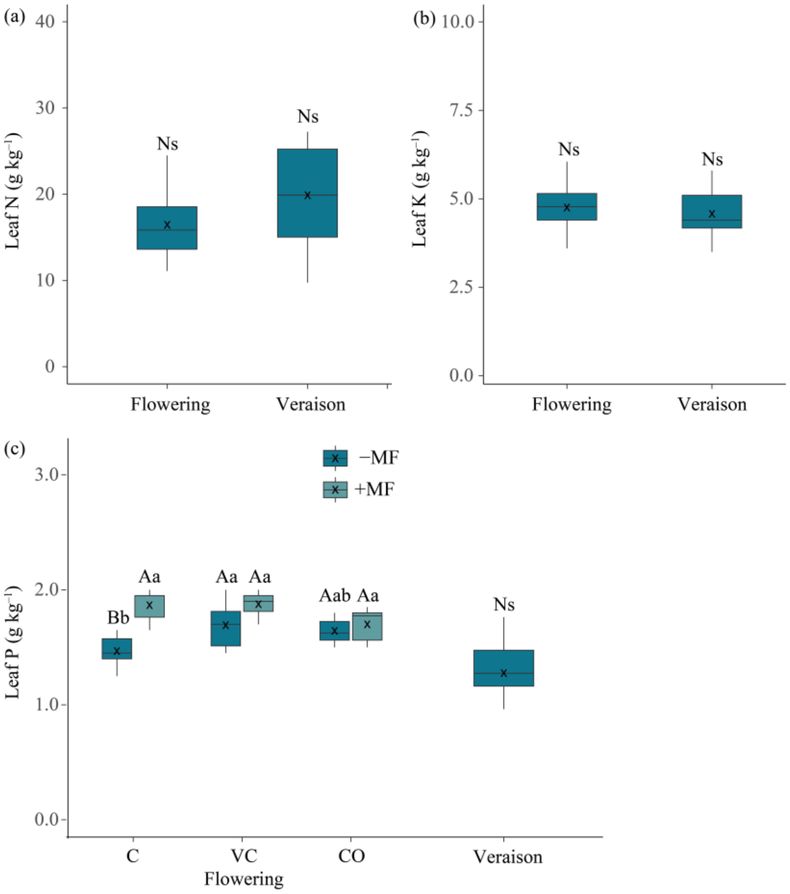

Figure 2. Nitrogen (**a**), potassium (**b**), and phosphorus (**c**) content in "Isabella" grapevine leaves, collected at flowering and veraison, grown with different organic fertilizers: control (C), grape pomace vermicompost (VC), and grape pomace compost (CO), with and without mineral fertilization

(−MF and +MF, respectively, involving natural phosphate and potassium sulfate). Means represent the average values across the three evaluated growing seasons (2020/21, 2021/22, and 2022/23). "Ns" represents no significantly different means. Different uppercase letters indicate different means between −MF and +MF (LSD test, $\alpha = 5\%$); different lowercase letters indicate different means among organic fertilizer applications (Tukey test, $\alpha = 5\%$); "Ns" represents no significantly different means. Dots represent outliers and crosses represent means.

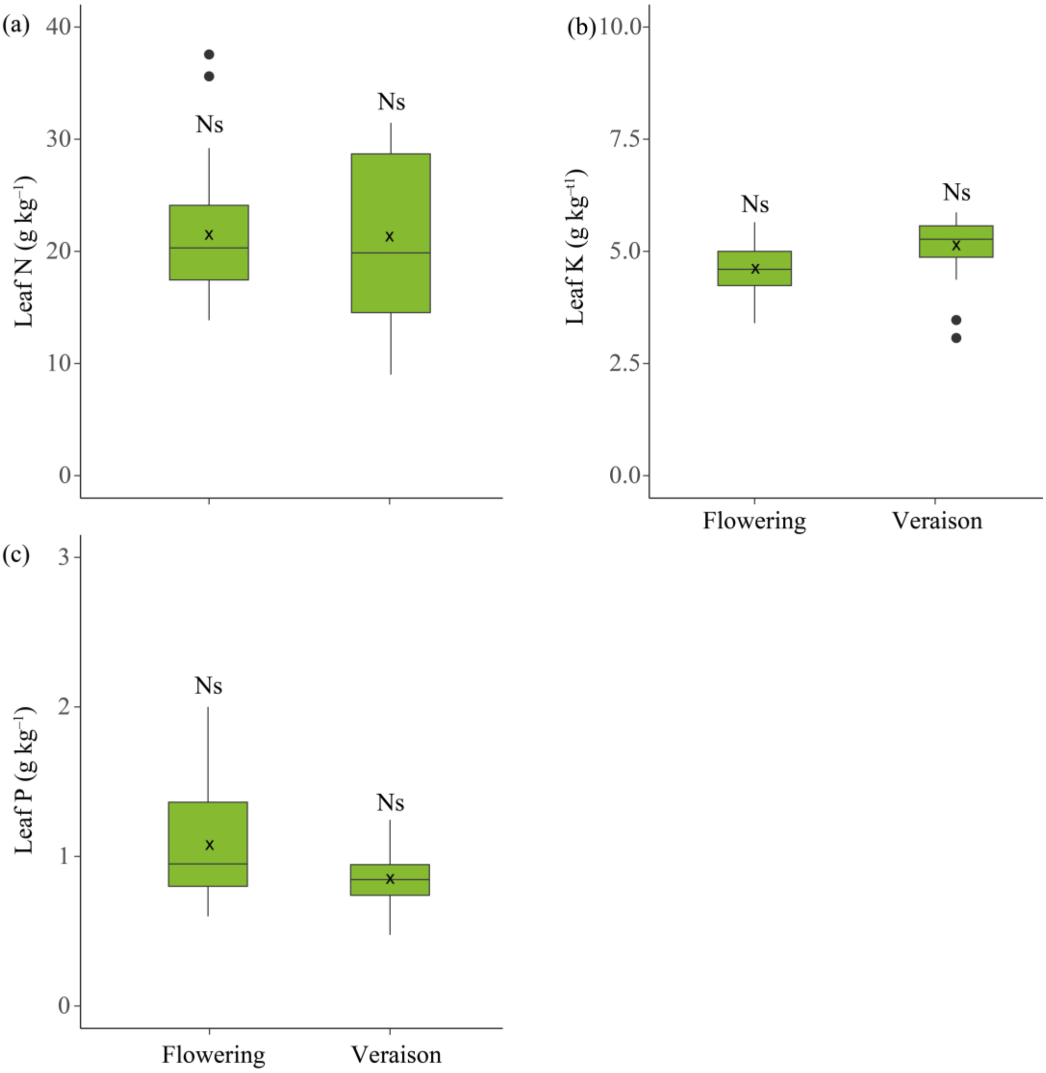

Figure 3. Nitrogen (**a**), potassium (**b**), and phosphorus (**c**) content in "Chardonnay" grapevine leaves, collected at flowering and veraison, grown with different organic fertilizers: control (C), grape pomace vermicompost (VC), and grape pomace compost (CO), with and without mineral fertilization (−MF and +MF, respectively, involving natural phosphate and potassium sulfate). Means represent the average values across the three evaluated growing seasons (2020/21, 2021/22, and 2022/23). "Ns" represents no significantly different means. Dots represent outliers and crosses represent means.

3.1.2. SPAD Index

The SPAD indices of "Isabella" grapevines did not vary among treatments in both flowering and veraison measurements (Figure 4). In "Chardonnay" grapevines, higher indices at flowering were measured in plants which either received no fertilization (C − MF) or were fertilized with grape pomace compost and mineral fertilizers (CO + MF) (Figure 5). This indicates greener leaves. Notably, C − MF leaves had higher SPAD indices than C + MF in "Chardonnay" grapevines at flowering (Figure 5). Also, SPAD indices did not vary among treatments in veraison measurements in "Chardonnay" grapevines.

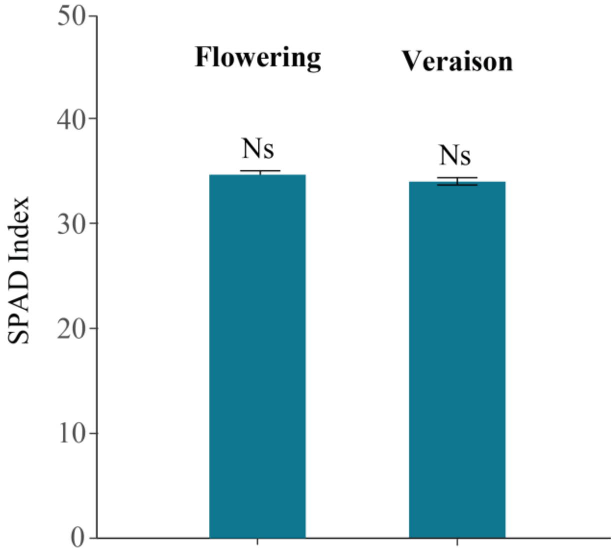

Figure 4. SPAD indices of "Isabella" grapevines, measured at flowering and veraison, grown with different organic fertilizers: control (C), grape pomace vermicompost (VC), and grape pomace compost (CO), with and without mineral fertilization (−MF and +MF, respectively, involving natural phosphate and potassium sulfate). Means represent the average values across the growing seasons 2021/22 and 2022/23. "Ns" represents no significantly different means.

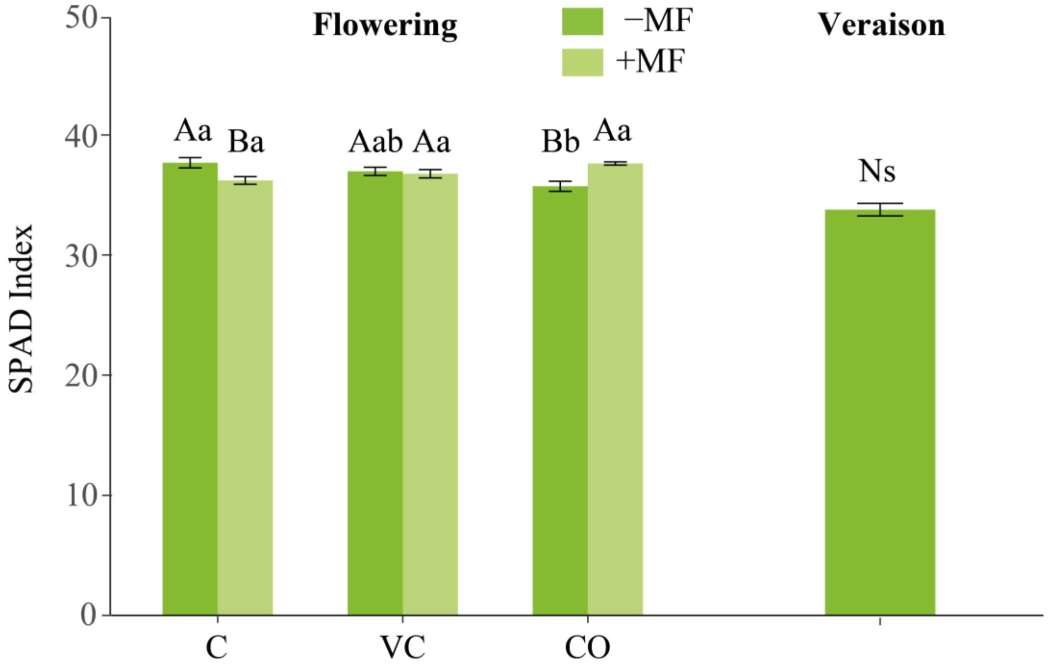

Figure 5. SPAD indices of "Chardonnay" grapevines, measured at flowering and veraison, grown with different organic fertilizers: control (C), grape pomace vermicompost (VC), and grape pomace compost (CO), with and without mineral fertilization (−MF and +MF, respectively, involving natural phosphate and potassium sulfate). Means represent the average values across the growing seasons 2021/22 and 2022/23. Different uppercase letters indicate different means between −MF and +MF (t-lsd test, $\alpha = 5\%$); different lowercase letters represent statistically different means among the organic fertilizer treatments (Tukey test, $\alpha = 5\%$); and "Ns" represents no significantly different means.

3.1.3. Grape Yield

In the three growing seasons evaluated, the different organic fertilizers did not change the mean grape yield of "Isabella" grapevines when mineral fertilizer was not applied (−MF) (Figure 6a). However, in the presence of mineral fertilization (+MF), grapevines fertilized with grape pomace vermicompost (VC) exhibited a lower grape yield compared to those subjected to grape pomace compost (CO) applications and/or control treatment (C) in the 2022/23 growing season, and when the data of all growing seasons were analyzed (Figure 6a). Parallelly, grapevines fertilized with mineral fertilizer (C + MF) showed a higher yield than grapevines without any fertilization (C – MF) in the 2020/21 growing season (Figure 6a). Similarly, grapevines fertilized with CO had a higher yield whenever also fertilized with mineral fertilizers (+MF) across all three seasons, with the exception of season 2021/22 (Figure 6a). The grape yield of "Chardonnay" grapevines did not differ among treatments (Figure 6b).

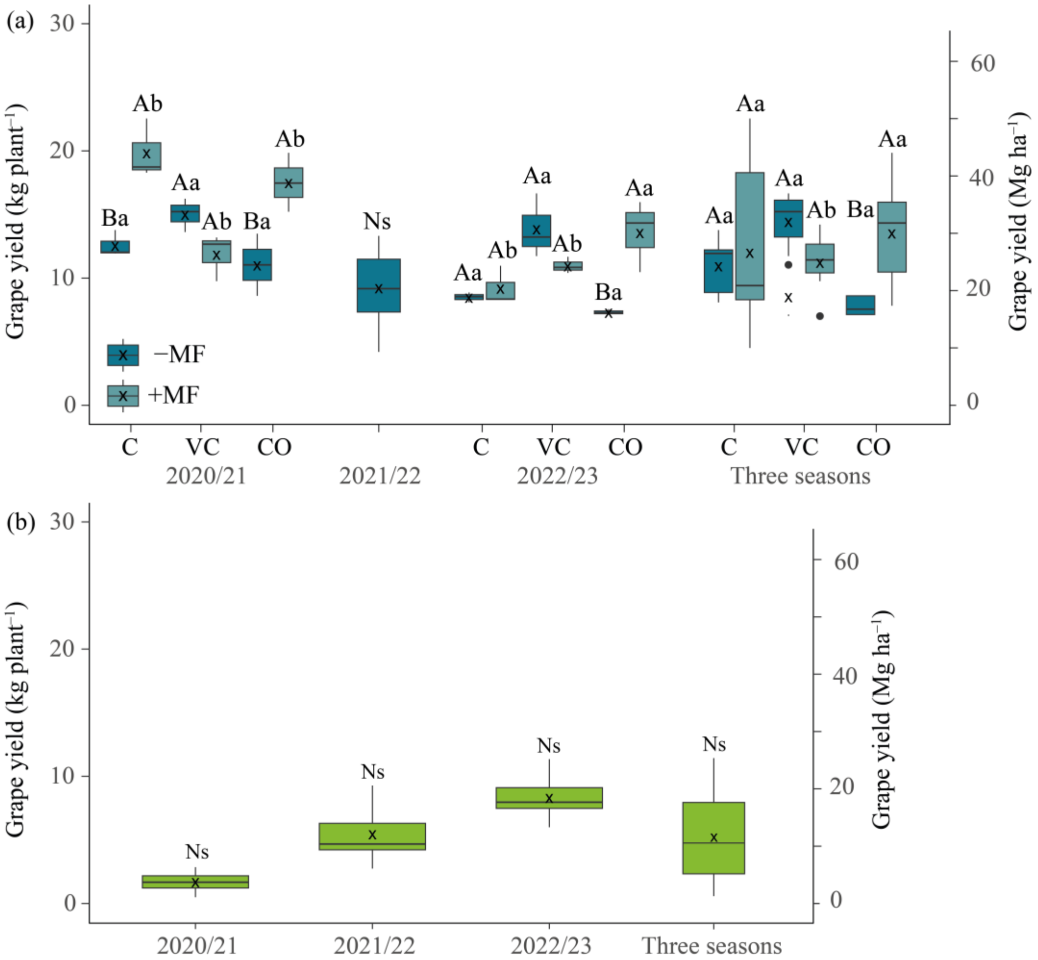

Figure 6. Grape yields of the "Isabella" (**a**) and "Chardonnay" vineyards (**b**) grown with different organic fertilizers, control (C), grape pomace vermicompost (VC), and grape pomace compost (CO), with and without mineral fertilization (−MF and +MF, respectively, involving natural phosphate and potassium sulfate), in three growing seasons (2020/21, 2021/22, and 2022/23). Different uppercase letters indicate different means between −MF and +MF (t-lsd test, α = 5%); different lowercase letters represent statistically different means among the organic fertilizer treatments (Tukey test, α = 5%); and "Ns" represents no significantly different means. Dots represent outliers and crosses represent means.

3.2. Grape Quality and Must Chemical Composition

The quality parameters of must composition total soluble solids (TSS), total titratable acidity (TTA), TSS/TTA ratio, and pH did not differ among treatments in the "Isabella" or "Chardonnay" grapevine berries in the evaluated growing seasons (Figure 7). Higher TSS and TTA levels were found in "Chardonnay" grapevines (Figure 7), which is expected for *V. vinifera* cultivars [7,26]. The application of mineral fertilizers enhanced mean TA concentration in "Isabella" grape skins by 36.4% when data from all three seasons were analyzed, as shown in Figure 8a. However, individual growing season TA means did not differ among treatments (Figure 8a). TPI did not differ among treatments in "Isabella" grapevine berries in the evaluated growing seasons (Figure 8b).

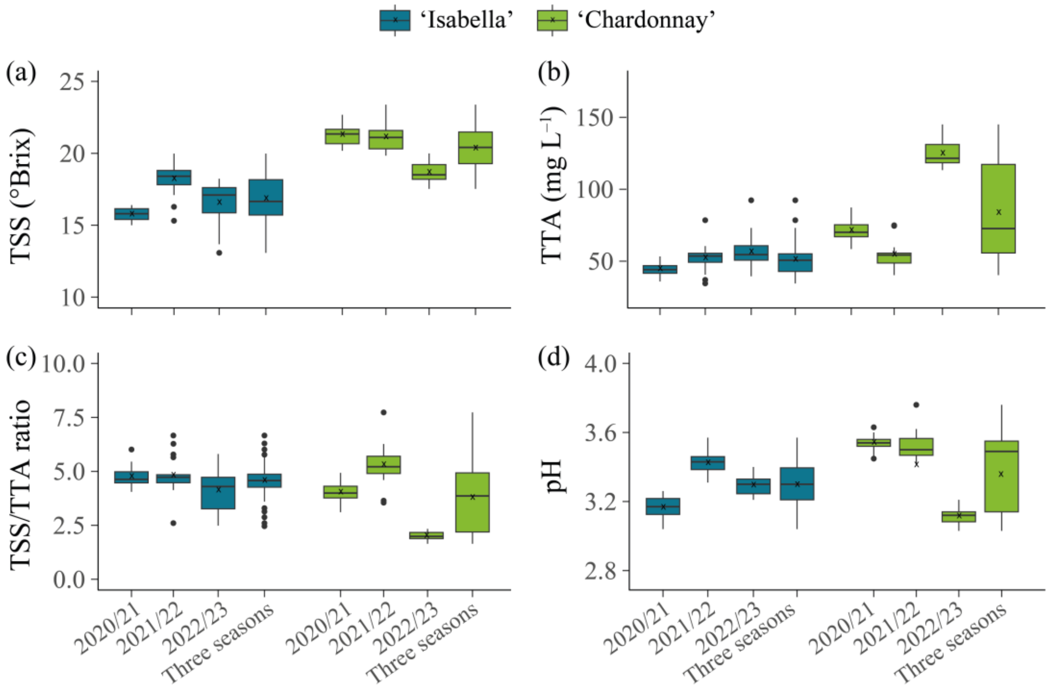

Figure 7. Total soluble solids (TSS) (**a**), total titratable acidity (TTA) (**b**), TSS/TTA ratio (**c**), and pH (**d**) of "Isabella" and "Chardonnay" grapevines must, evaluated in three growing seasons (2020/21, 2021/22, and 2022/23). "Ns" represents no significantly different means. Dots represent outliers and crosses represent means.

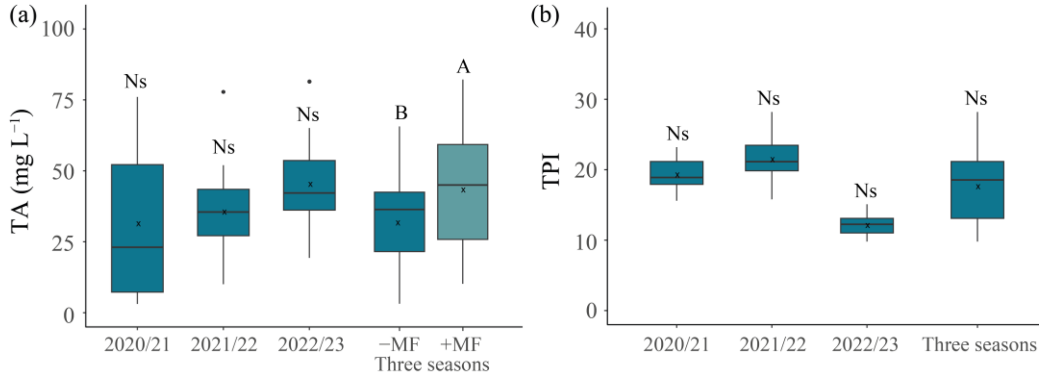

Figure 8. Total anthocyanin concentration (TA) (**a**) and total polyphenol index (TPI) (**b**) in grape skin extracts of "Isabella" grapevines grown with different organic fertilizers, control (C), grape pomace vermicompost (VC), and grape pomace compost (CO), with and without mineral fertilization (−MF and +MF, respectively, involving natural phosphate and potassium sulfate), evaluated in three growing seasons (2020/21, 2021/22, and 2022/23). Different uppercase letters indicate different

means between −MF and +MF (t-lsd test, $\alpha = 5\%$); different lowercase letters represent statistically different means among the organic fertilizer treatments (Tukey test, $\alpha = 5\%$).

3.3. Soil Nutrients

After three years of treatment application in the "Isabella" vineyard, soil exchangeable Ca and Mg in the 0–0.20 m layer were different under different fertilizations (Figure 9a,b). Exchangeable Ca was lower in the soils without any mineral or organic fertilization (C − MF) (Figure 9a). Exchangeable Mg was the highest in soils fertilized with grape pomace compost (CO), which had a mean value 20.4% higher than control and 17.9% higher than soils fertilized with vermicompost (VC) (Figure 9b).

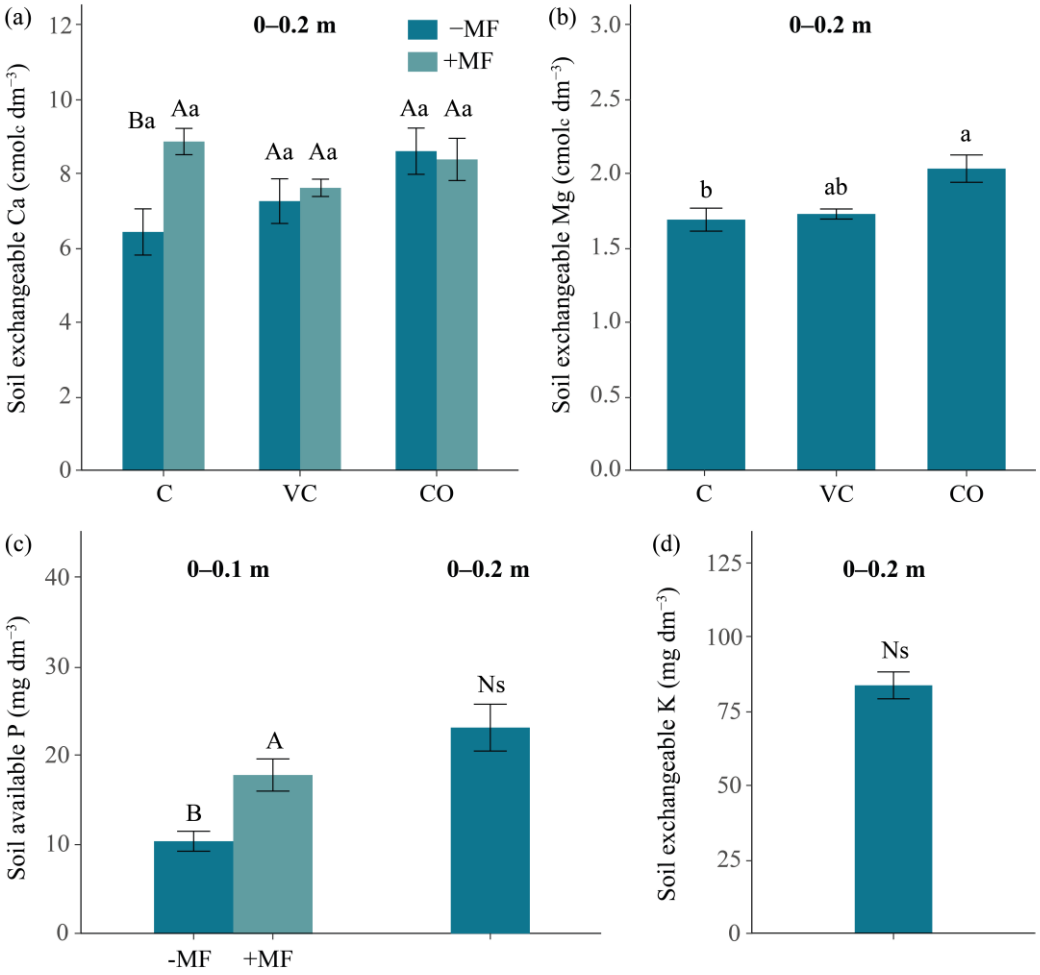

Figure 9. Soil exchangeable Ca (**a**) and Mg (**b**) (cmolc dm^{-3}), soil available P (**c**) (mg dm^{-3}), and soil exchangeable K (**d**) (mg dm^{-3}) in "Isabella" vineyard soil after 3 years (February 2023) of application of the following treatments: control (C), grape pomace vermicompost (VC), and grape pomace compost (CO), with and without mineral fertilization (–MF and +MF, respectively, involving natural phosphate and potassium sulfate). Different uppercase letters indicate different means between –MF and +MF (t–lsd test, $\alpha = 5\%$); different lowercase letters represent statistically different means among the organic fertilizer treatments (Tukey test, $\alpha = 5\%$). "Ns" represents no significantly different means.

The available P content in the 0–0.2 m layer of the "Isabella" vineyard was not affected by organic or mineral fertilizers (Table S1) [13] (Figure 9c). However, when the 0–0.1 m layer was analyzed, the mean values of the soil to which mineral fertilizers were applied (+MF) were 1.7 times higher than those that were not (Figure 9c). Exchangeable K values in the 0–0.2 cm layers of the "Isabella" vineyard did not differ among treatments (Figure 9d). In the "Chardonnay" vineyard, exchangeable Ca, Mg, and K contents in the 0–0.2 m soil layer were not affected by any fertilization (Figure 10a–c). Exchangeable K content in the 0–0.1 m soil layer, however, was significantly higher when mineral fertilizers were applied (+MF) (Figure 10b).

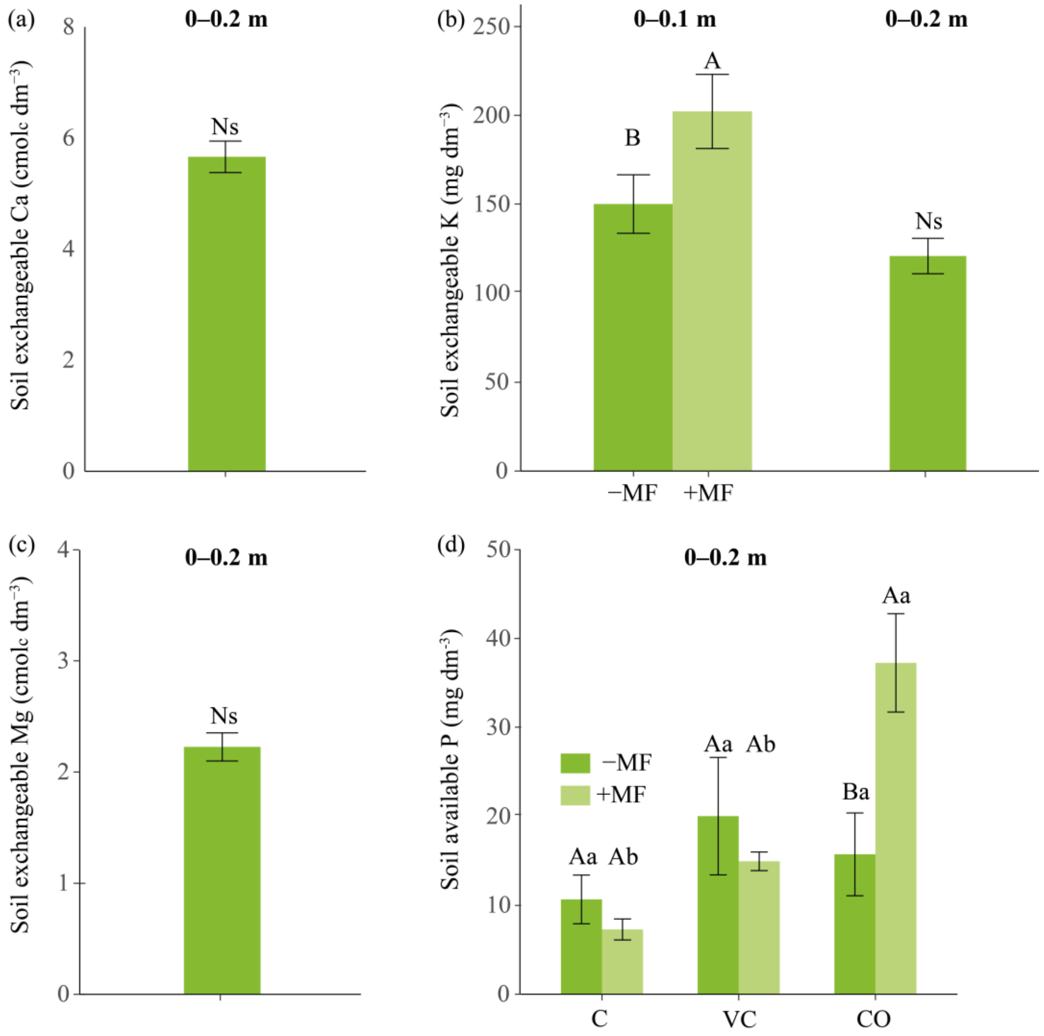

Figure 10. Soil exchangeable Ca (**a**) and Mg (**b**) (cmolc dm^{-3}), soil available P (**c**) (mg dm^{-3}), and soil exchangeable K (**d**) (mg dm^{-3}) in "Chardonnay" vineyard soil after 3 years (February 2023) of application of the following treatments: control (C), grape pomace vermicompost (VC), and grape pomace compost (CO), with and without mineral fertilization (−MF and +MF, respectively, involving natural phosphate and potassium sulfate). Different uppercase letters indicate different means between −MF and +MF (t-lsd test, $\alpha = 5\%$); different lowercase letters represent statistically different means among the organic fertilizer treatments (Tukey test, $\alpha = 5\%$). "Ns" represents no significantly different means.

Although not significantly different, soils fertilized with VC showed available P content near to or slightly above the critical level (Figure 10d). The soil fertilized with both CO and +MF had exceptionally higher content of available P. There was no difference in the concentration of soil EDTA-extracted Cu, Zn, Fe, and Mn concentrations among organic fertilizers (Table S2). However, the addition of mineral fertilizers (+MF), which include phosphate rock, significantly decreased soil Fe concentration in Vineyard 1 and slightly lowered Zn and Mn concentrations (Table S2).

3.4. Principal Component Analysis

The principal component analysis using plant and soil variables was performed, and the first and second principal components (PC1 and PC2) are shown in Figure 11. PC1 explained approximately 22.0% of the variance in the original results and PC2 18.5%, summing up to 40.5%. Therefore, the relation between the PC1 and PC2 efficiently separated for cultivars ("Chardonnay" and "Isabella"), as expected, since the analyzed variables are highly cultivar-specific, as well as for growing seasons. PC1 x PC2 did not, however, discriminate groups according to the factors organic fertilization or mineral fertilization, which suggests that the PCA analysis is not sensitive enough to detect differences in fertilization or plant nutrition, as genetic and climate factors likely overshadow their effects.

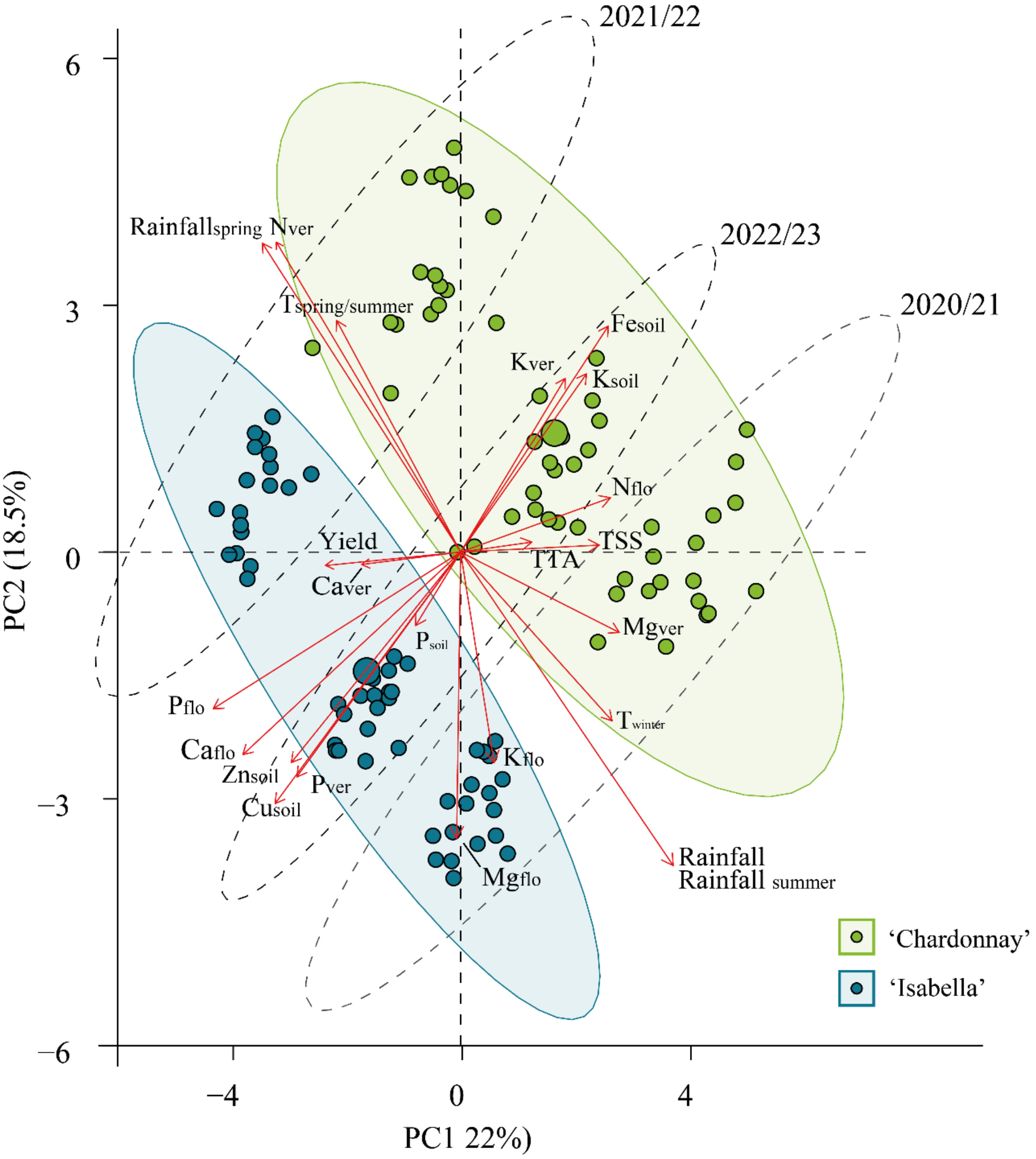

Figure 11. Principal component analysis of grape yield (Yield) and quality (TSS and TTA), plant nutrition (N_{flo}, N_{ver}, K_{flo}, K_{ver}, P_{flo}, P_{ver}, Ca_{flo}, $Ca_{ver,}$ Mg_{flo}, and $Mg_{ver,}$ where $_{flo}$ is nutrient content at flowering and $_{ver}$ at veraison), soil (K_{soil}, P_{soil}, Ca_{soil}, Mg_{soil}, Fe_{soil}, Cu_{soil}, and Zn_{soil}), and climate variables (T = mean temperature; Rainfall = accumulated rainfall).

The grouping of growing seasons is roughly approximately perpendicular to the climatic variables, such as rainfall and mean temperature (Figure 11). The vectors for rainfall (from September to February), summer rainfall (from December to February), and winter temperature (T_{winter}) were positive in the 2020/21 growing season and negative for the 2021/2022 growing season; the opposite was observed for spring rainfall and spring and summer mean temperature ($T_{spring/summer}$). The 2022/23 growing season was grouped in the medial zone between the other two. This indicates different climatic scenarios in each season. Lower summer precipitation and higher winter temperature vectors showed indirect relation to N content in leaves collected at veraison, as well as a direct relation to K (K_{flo}) and Mg (Mg_{flo}) content on leaves collected at flowering (Figure 11).

The grouping of the two cultivars comprised different relations regarding plant (nutrition, grape yield, and quality) and soil variables. The "Chardonnay" vineyard was positively related to exchangeable K content in the soil and also K content in the leaves collected at veraison (K_{ver}) (Figure 11). Also, the "Chardonnay" vineyard presented positive relations to both TSS and TTA (Figure 10). PC1 and PC2 also grouped "Isabella" grapevines with a positive relation to the vectors of soil available P content (P_{soil}), as well as P content in the leaves collected at flowering (P_{flo}) and veraison (P_{ver}) (Figure 11). As for grape yield, its vector was positively related to "Isabella" grapevines (Figure 11).

4. Discussion

The present study sought to evaluate the suitability of grape pomace compost and vermicompost, alongside non-industrialized mineral fertilizers (natural phosphate and potassium sulfate), as sustainable nutrient sources for subtropical vineyards. Notable differences in nitrogen (N), phosphorus (P), potassium (K), and copper (Cu) content between grape pomace compost and vermicompost were observed (Table S3). Usually, the vermicomposting process results in a higher rate of concentrating source material nutrients than composting [27,28], which was observed for P content in grape pomace vermicompost compared to the compost (Table 1). However, N and K content in the vermicompost was lower than in the compost (Table 1). This may have happened due to the necessity of adding water during the vermicomposting process, which could have leached very soluble ions like K^+ and NO_3^- [29]. Similar results were observed when comparing composting to vermicomposting from other sources, in which the additional water leached those ions from the source material [29,30]. The reduced Cu concentration observed in the vermicompost, in comparison to the compost, was expected. Several studies have consistently observed the ability of earthworms to reduce Cu concentration from source material by accumulating the metal within their body tissue [31–34]. The higher N and K content in grape pomace compost suggests its potential to address the nutritional requirements of grapevines, crucial for sustainable viticulture [4,5]. Additionally, the differences in P content underscore the importance of understanding the specific nutrient composition of organic amendments for targeted soil fertility management. Also, grape pomace vermicompost might be more suitable for Cu-contaminated or potentially contaminated areas, due to its lower Cu content. Therefore, based solely on chemical composition, each grape pomace fertilizer (compost or vermicompost) can be chosen accordingly, considering soil analysis and plant requirements.

The results revealed the relations between fertilizer choice, soil properties, and grapevine responses, which did not vary much within each growing season. Due to the higher content in its composition, the application of grape pomace compost (CO) increased soil exchangeable Ca and Mg content in the "Isabella" vineyard (Figure 8a,b), but did not affect grape yield (Figure 5a). Notably, exchangeable Ca and Mg contents in the "Isabella" vineyard soil were already at high levels, which were 4 and 1 $cmol_c\ dm^{-3}$ [13], prior to experiment implementation (Table S1), which explains why it did not lead to any plant response. Interestingly, in the "Chardonnay" vineyard, the fertilization with CO showed a synergistic effect with mineral fertilization, which led to a two-fold increase in soil available P compared to other treatments (Figure 9d). We hypothesize that the microbiota found in grape pomace compost, once added to the soil together with phosphate rock, is able to enhance phosphatase activity and solubilize organic forms of P into inorganic phosphate ions [35], which can be absorbed by plants. Similar results were found by Hu et al. [35] when adding organic fertilizers to soils, also in a Cfa climate zone. These phenomena may also occur due to exudation of organic acids by phophate-solubizing bacteria (PSB), mycorrhiza, and other fungi, which create an acidic microclimate, favorable to phosphate rock solubilization [36–39]. Similarly, the higher P content in VC (Table S3) was able to increase available P content in the 0–0.2 m layer of soil in the "Chardonnay" vineyard near or up to the critical levels, when comparing to C, although no statistical difference was found in the ANOVA test (Figure 9d). However, mean values of P content in grapevines leaves at flowering and at veraison were the same among treatments (Figure 2c). A reasonable explanation would be that the soil had low phosphate content prior to +MF and the addition of organic fertilizers, and thus the absorbed P was used to grow new plant tissue and organs, and/or to restore plant nutrient reserves [40,41], especially in the relatively young "Chardonnay" grapevines.

The use of mineral fertilizers presented both positive and negative consequences regarding soil fertility. The addition of mineral fertilizers (+MF) was able to increase Ca content in the soil when no organic fertilizer was applied (Figure 8a), as well as available P content in the topsoil (0–0.1 m), in the "Isabella" vineyard (Figure 9c). The mean values of P content in grapevine leaves at flowering corroborate that +MF grapevines had significantly higher P content than −MF when no organic fertilizer was used (Figure 2c). Additionally, when no mineral fertilizer was added, VC and CO were also able to increase P content in leaves (Figure 2c), indicating that the P content of these materials was somewhat efficient in mineralizing and providing this nutrient to the grapevines. In parallel, in the "Chardonnay" vineyard, the observed increase in exchangeable K in the 0–0.1 m layer to excessive levels with +MF (Figure 9b) raises concerns about potential over-application in soils already within acceptable K ranges, as in Vineyards 1 and 2 (Table S1). Still, +MF did not have any effect on "Chardonnay" grapevine nutrition (Figure 2b) or grape yield (Figure 6a). Also, CO + MF was able to increase available P content up to the soil excessive threshold in the "Chardonnay" vineyard (Figure 10d). Besides nutrient loss, excessive levels of phosphate on soils raises other concerns. Several studies have previously shown that the constant input of phosphorus to soils results in P adsorption site saturations, which then leads to run-off and P transfer to aquatic environments, ultimately resulting in environmental contamination [42,43]. This emphasizes the importance of careful dose optimization based on soil type and grape variety to avoid unnecessary nutrient accumulation and potential negative impacts on the environment. Notably, the P content in "Isabella" and "Chardonnay" leaves was within the proposed sufficient ranges for this region [13]. Also, the K content in the leaves of both cultivars was considered insufficient, i.e., with values below the lower limit of the proposed sufficient ranges for this region, which are 8 to 16 g kg^{-1} [13].

Notably, neither organic nor mineral fertilizers increased N content in grapevine leaves (Figure 2a, 3a). The mean N content values in both cultivars were within the proposed sufficient ranges for this region [13]. Also, the SPAD index, which is a measure of chlorophyll content and plant nutrition, did not exhibit significant variations among treatments in the "Isabella" grapevines (Figure 4a). In the "Chardonnay" grapevines, however, specific treatments, including no fertilization (C − MF) and grape pomace compost with mineral fertilizers (CO + MF), resulted in higher SPAD indices at flowering, indicative of greener leaves. This, however, might be due to the fact that the leaves were not necessarily more vigorous but rather smaller and more concentrated in chlorophyll [44–46]. In this scenario, grapevines fertilized with C − MF and CO + MF could present undergrowth compared to plants with an adequate nutritional status. This observation aligns with the literature suggesting that nutrient management practices, especially under varying climatic conditions, can influence chlorophyll content and overall plant growth [47–49]. Additionally, grape pomace compost and vermicompost were shown to be ineffective at increasing soil organic matter and nitrogen stock [16]. Therefore, although rapid mineralization of the residues was observed due to the climate conditions [16], they were unable to increase nitrogen nutrition by grapevines, as observed in this study.

Total soluble solids (TSS), total titratable acidity (TTA), TSS/TTA ratio, and pH are common parameters in grape must composition to evaluate grape quality. TSS represents the majority of the berry sugar and is directly related to wine alcoholic content [27]. "Isabella" grapevines in the growing conditions of this study were able to reach the recommended values of TSS for winemaking (14 °Brix) [50] in all evaluated seasons. Similarly, the grape must of "Chardonnay" grapevines presented mean values of 21.3, 21.1, and 18.7 °Brix for TSS in the 2020/21, 2021/22, and 2022/23 growing seasons, which indicate their suitability for winemaking. TSS production and accumulation in grapevines are closely linked to photosynthetic ability and plant growth [47]. The higher the plants' photosynthetic activity, the more primary and secondary sugars are produced [51]. However, increased grape yield might dilute the sugar content in berries, leading to lower TSS values [8,47], which explains why this grape must variable was lower in "Chardonnay" berries in the 2022/23 growing season. The researched organic and mineral fertilizers did not increase or reduce TSS (Figure 6a), which could be mainly because they did not interfere with grapevine photosynthetic activity. Mean values of pH and TTA (Figure 7b,d) of the "Isabella" and "Chardonnay" berries were below 3.5 among all treatments and growing seasons, showing adequate values according to the literature [52,53]. TTA values did not vary much among seasons in the "Isabella" grape must (Figure 7b). However, the same was not true for the "Chardonnay" grape must, which exhibited higher values in the 2022/23 growing season (Figure 7b). Interestingly, +MF increased the "Isabella" grape skin total anthocyanin content when data from all seasons were analyzed (Figure 8a). Potassium plays a key role in determining total anthocyanin content in grape berries, since it is related to enzymatic activation and the synthesis reactions of polyphenols (e.g., anthocyanins) [47]. Therefore, we speculate that the +MF fertilization, which contained K_2SO_4, may have increased K concentration in grape berries, which led to higher anthocyanin content. Also, there was a seasonal variation of TPI in the "Isabella" berries' skin: mean values were lower in the 2022/23 season (Figure 8b), probably due to low precipitation throughout this season (Figure 1), which also means higher global radiation. The synthesis of phenolic compounds is a known plant mechanism to protect against UV radiation damage [51].

Overall, in the "Isabella" grapevines, grape yield did not have much seasonal variation; however, plant responses to treatments were different across the growing seasons (Figure 6a). This highlights that multiple growing seasons should be evaluated in order to minimize season variation and facilitate treatment evaluation in field experiments, especially when aiming to provide fertilization recommendations in viticulture. The "Chardonnay" grapevines grape yield showed the following pattern: 2020/21 << 2021/22 ~ 2022/23 (Figure 8b). The higher yields harvested in the two latter seasons can be explained due to better climatic conditions: fair rainfall during vegetative growth and blossoming (Sep–Oct) and lower rainfall in critical periods such as grape ripening (Jan–Feb), which is desirable (Figure 1).

This study highlights the potential of grape pomace compost and vermicompost, alongside non-industrialized mineral fertilizers, to enhance soil fertility in subtropical vineyards. While these amendments improved soil nutrient availability, particularly P and K, they did not translate into increased grape yield or quality, underscoring the complexity of nutrient management in subtropical climates. Parallelly, grape pomace compost and vermicompost were unable to increase total organic carbon and carbon stocks in vineyards in the short-term period [16]. The unique climatic conditions of these regions, characterized by high temperatures during summer and high rainfall, likely influenced compost and vermicompost mineralization, nutrient dynamics, and plant responses. Given the increasing instability of climate patterns, especially in subtropical climates, due to global warming in recent years, multi-year evaluations are crucial to better understand the long-term impacts of these practices. These findings are particularly relevant for subtropical viticulture, where sustainable nutrient management practices are critical to addressing the challenges of soil fertility, environmental sustainability, and climate resilience in these rapidly developing viticultural regions.

5. Conclusions

The results of this study suggest that organic and mineral fertilizers, despite increasing P and K availability to plants in soils, at the doses applied, may not have direct effects on the nutrient content in grapevine leaves, and, consequently, on grape yield and grape quality. Similarly, the soil fertilization with grape pomace compost or vermicompost did not increase leaf N content. This is likely due to the previous adequate levels of these nutrients in the soil and/or the redistribution of the absorbed nutrients to support the plant growth of perennial organs, such as roots and branches. Also, the observation that certain nutrient values in grapevine leaves fell below critical levels without hindering grape yield suggests that those critical levels might be overestimated for the specific growing conditions found in the "Isabella" and "Chardonnay" vineyards of this study. In addition, the overuse of fertilizers in the soil, e.g., the combination of CO + MF in this study, reiterates the common concern of raising exchangeable nutrient levels in soils and, by consequence, provoking environmental contamination. These findings highlight the potential of organic and non-industrialized mineral fertilizers as sustainable alternatives to industrial inputs in subtropical climates. In these regions, high rainfall increases the risk of nutrient runoff, making fertilization strategies that enhance soil organic matter and nutrient retention particularly valuable. The results suggest that organic and non-industrialized mineral fertilizers can contribute to maintaining soil fertility and improving vineyard resilience under these challenging conditions, offering a viable approach to sustainable nutrient management.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/10.3390/agronomy15051010/s1, Table S1: Soil physical and physicochemical analyses in Vineyard 1 and Vineyard 2 before experiment implementation (August 2020) and after three years of application (April 2023) of the following treatments: control (C), grape pomace vermicompost (VC), and grape pomace compost (CO), with and without mineral fertilization (−MF and +MF, respectively, involving natural phosphate and potassium sulfate).; Table S2: Soil-extracted concentration of micronutrients in Vineyard 1 and Vineyard 2 before experiment implementation (August 2020) and after three years of treatment application (April 2023). The treatments were as follows: control (C), grape pomace vermicompost (VC), and grape pomace compost (CO), with and without mineral fertilization (−MF and +MF, respectively, involving natural phosphate and potassium sulfate). Table S3: Elementary composition and C/N ratio of grape pomace vermicompost and grape pomace compost. Figure S1: Illustrated experimental setup for Vineyards 1 and 2.

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