



Reference Nutrient Levels in Soil and Leaves for Vines Cultivated in a Subtropical Climate Using Boundary Line

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Abstract

The study aimed to establish nutritional reference values in the soil and leaves of *Vitis* spp. vines, cultivated in a subtropical climate to obtain high yield, using the boundary line (BL) method. The research was conducted in the 2021/22 and 2022/23 harvests, in the Serra Gaúcha region, Southern Brazil, one of the main winemaking regions in South America. Six cultivars were evaluated: four *Vitis labrusca* (‘Isabel’, ‘Bordô’, ‘BRS Magna’, and ‘BRS Lorena’) and two *Vitis vinifera* (‘Pinot Noir’ and ‘Chardonnay’). A total of 207 vineyards were evaluated, and seven plants were selected and marked in each vineyard. Complete leaves (blades + petiole) opposite to the bunch were collected to evaluate total nutrient contents during flowering. At harvest, soil samples (0.00–0.20 m layer) were collected for soil fertility evaluation, and the number of bunches was counted to estimate yield. The relative fruit yield (RFY) was related to soil chemical attributes and foliar nutrient contents through scatter plots, using the BL method to obtain soil fertility classes and nutrient sufficiency ranges. The sufficiency ranges considered adequate in the soil for pH(H₂O), soil organic matter, P, K, Ca, Mg, Cu, Zn, Mn, CEC_{pH7.0}, and Ca-Mg-K saturation were proposed. Also, the nutrient sufficiency ranges in the leaves for N, P, K, Ca, Mg, S, Cu, Zn, Fe, Mn, and B were defined. These results will contribute to greater accuracy in fertilization, maintenance of high grape yields, reduction of environmental impacts from excess fertilizers, and reduction of viticulture production costs.

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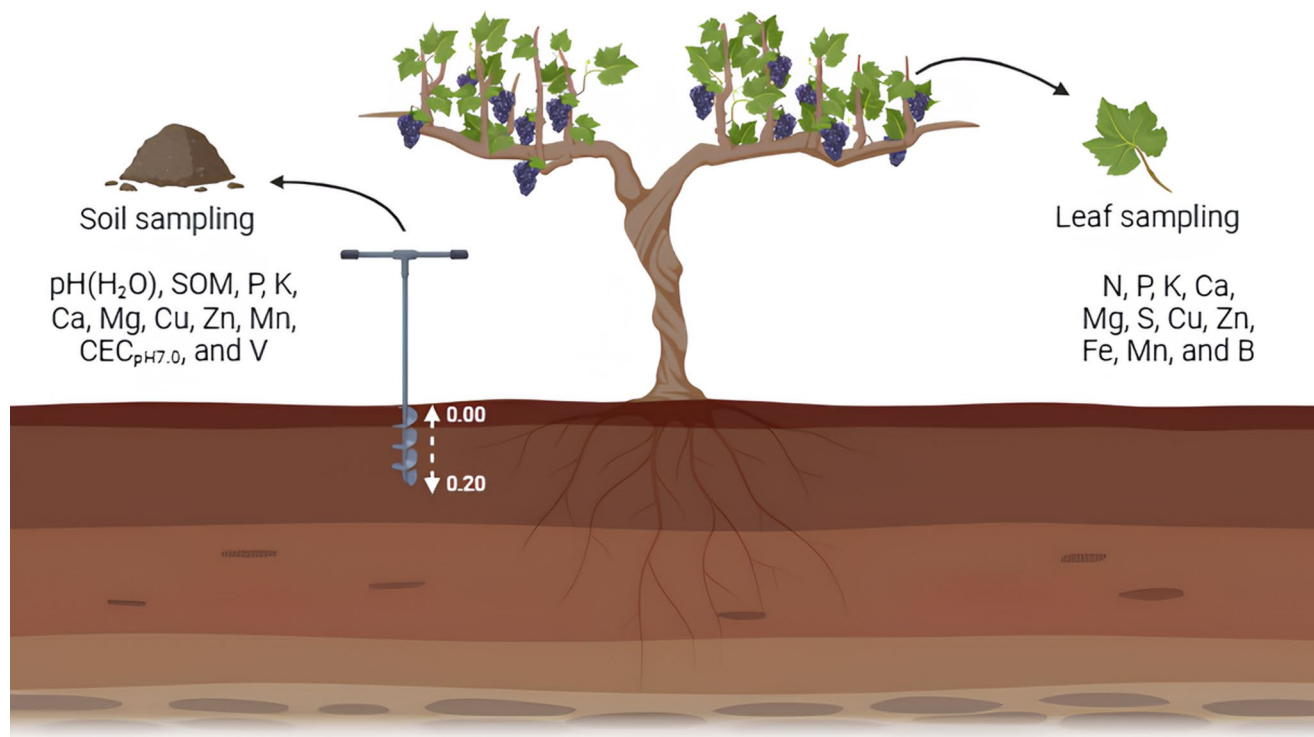
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Graphical Abstract

Soil and leaf nutritional reference values
to improve grape yield

Highlights

- *Vitis vinifera* and *Vitis labrusca* vineyards were evaluated.
- Nutrient contents were correlated with relative fruit yield by the boundary line method.
- Soil and leaf nutrient reference values for subtropical vines were established.
- Nutrient sufficiency range can be used to monitor the nutrient status.

Keywords *Vitis* spp. · vineyards · crop fertilization · database · sustainable agriculture

1 Introduction

Viticulture is a significant worldwide economic agricultural industry, including nearly 7.2 million ha in vineyards, 48% of total production used in winemaking, representing 34.1 Mt of wine grapes (OIV 2024). Spain, France, China, Italy, Turkey, and the United States account for 56% of global grape production (OIV 2023). Brazil represents 1.1% of global winegrape production with approximately 76.7 thousand ha producing 1.7 Mt of grapes (IBGE 2024). The state of Rio Grande do Sul represents 62% of Brazilian winegrape area, 53% of the total grape production, and 90% of wine production, wherein

the Serra Gaúcha region represents the largest wine grape area in Brazil (SEAPDR 2023, 2022), and one of the largest wine regions in South America.

Tropical and subtropical soils where vineyards are cultivated in Brazil do not supply naturally sufficient nutrient quantities to meet the high yields. Therefore, in producing vineyards, it is necessary to carry out fertilization, whose nutrient rates can be established based on the nutrient contents in the soil and leaves of the vines (Ciotta et al. 2020; Mostashari et al. 2018) and also based on the yield expectation, which is related to the amount of nutrients exported by the bunches (Pradubsuk and Davenport 2010; Tong et al.

2010; Verdenal et al. 2021). However, the establishment of the current critical levels (CL) or sufficiency ranges (SR) of nutrients in the soil or leaves, used to define fertilizer rates, is established for vines in a generalized way, not considering soil types, climate, and cultivars, such as *Vitis labrusca* and *Vitis vinifera*. This fact often results in the absence of an increased yield of vines with fertilization.

Critical levels for vines can be obtained in correlation experiments under field conditions, where fertilizer rates are added to soils to increase nutrient concentrations in the soil and plant tissues, and are diagnosed through chemical analyses (Baby et al. 2021; Havlin et al. 2022). Thus, the available nutrient levels in the soil and leaves evaluated over several years of experimentation are related to yield and/or must quality parameters (Koureh et al. 2019; Schmitt et al. 2020; Schreiner and Osborne 2018). However, correlation/calibration experiments are long-term, have high maintenance costs (Kleinman et al. 2018), and are quite restricted, being able to represent the reality of only one cultivar, soil, climate, and management characteristics (Ciotta et al. 2020; Pushpavathi et al. 2021).

Alternatively, soil and leaf samples can be collected from commercial vineyards, located in different sites (Beauchet et al. 2020; Likar et al. 2015), and used to compose robust databases, which enable the use of statistical calculations. Thus, the data can be explored by methods such as the boundary line (BL) so that critical nutrient values in the soil and plant tissues can be established for diagnosing the plant's nutritional status (Ali 2023; Lima Neto et al. 2024; Smith et al. 2024; Walworth et al. 1986). The use of robust databases allows the generation of regionalized results, considering environmental and cultivation management conditions (Krug et al. 2023; Stefanello et al. 2023), which is still a national and international challenge for fruit growing (Betemps et al. 2020). From this, it will be possible to explore the maximum potential of vines in each insertion environment.

This study hypothesizes that regional nutrient reference values can be defined using the boundary line method, which allows more efficient fertilization and, consequently, greater grapevine productivity. Therefore, the present study aimed to establish nutritional reference values for soil and leaves of *Vitis labrusca* and *Vitis vinifera* vines cultivated in a subtropical climate to achieve high yields, using the boundary line (BL) method.

2 Materials and Methods

2.1 Vineyards

The study area is in an altitude range varying from 150 to 750 m above sea level. The region presents a complex relief and high variability of soil types (Sarmiento et al. 2006,

2012), with a predominance of Inceptisols and Entisols (Soil Survey Staff, 2014), which corresponds to Cambissolos and Neossolos (Santos et al. 2018). The region's climate is classified, according to Köppen-Geiger, as Cfa, with hot summers, and Cfb, with temperate summers (Alvares et al. 2013). Climatic data were obtained from a meteorological station located in the Bento Gonçalves municipality (INMET 2024), and are presented in Fig. 1.

A total of 207 vineyards were evaluated in the 2021/22 and 2022/23 harvests, in Southern Brazil, located in the municipalities of Bento Gonçalves, Veranópolis, Pinto Bandeira, Monte Belo do Sul, and Farroupilha, in the Serra Gaúcha region, Rio Grande do Sul state (Fig. 2). The vineyards were selected through a survey of the main cultivars planted in the region and with the greatest distribution in the highlighted municipalities, whose purpose of grape cultivation (red and white) is for the production of juices, wines, and sparkling wines. Six cultivars were evaluated, four *Vitis labrusca*: 'Isabel', 'Bordô', 'BRS Magna', and 'BRS Lorena', destined for juice production, and two cultivars *Vitis vinifera*: 'Pinot Noir' and 'Chardonnay'. In all the evaluated areas, the plants are grown in a pergola system.

2.2 Leaves and Soil Sampling

In each plot, seven vines with homogeneous characteristics were selected and marked sequentially in the same planting row. Four complete leaves (blades+petiole) per plant were collected in the middle third of the productive shoot, opposite the bunch, at full flowering (September/October), as recommended by Brunetto et al. (2020). The complete leaves were washed with distilled water, dried in a forced-air oven at 60 °C until they reached a constant mass, ground in a Wiley mill, passed through a 1.0 mm mesh sieve, and reserved for analysis.

Soil samples were collected at the grape harvest time (January to March), representing the 0.00–0.20 m layer, covering the row and inter-row of planting (Fig. 2). The collected soil was manually crumbled, added to a plastic container, identified, dried in a forced-air oven at 60 °C, passed through a 2.0 mm mesh sieve, and reserved for analysis.

2.3 Nutrient Analysis of Leaves and Soil

Plant tissue samples were digested with sulfuric acid, according to Tedesco et al. (1995), for the total N content determination through distillation in a micro-Kjeldahl steam distillation apparatus (Tecnal, TE-0363, Brazil). The total contents of P, K, Ca, Mg, Cu, Zn, Fe, and Mn were determined by nitroperchloric digestion (3:1) (Embrapa 2009). Quantification of Ca, Mg, Cu, Zn, Fe, and Mn was made in an atomic absorption spectrophotometer (Perkin Elmer,

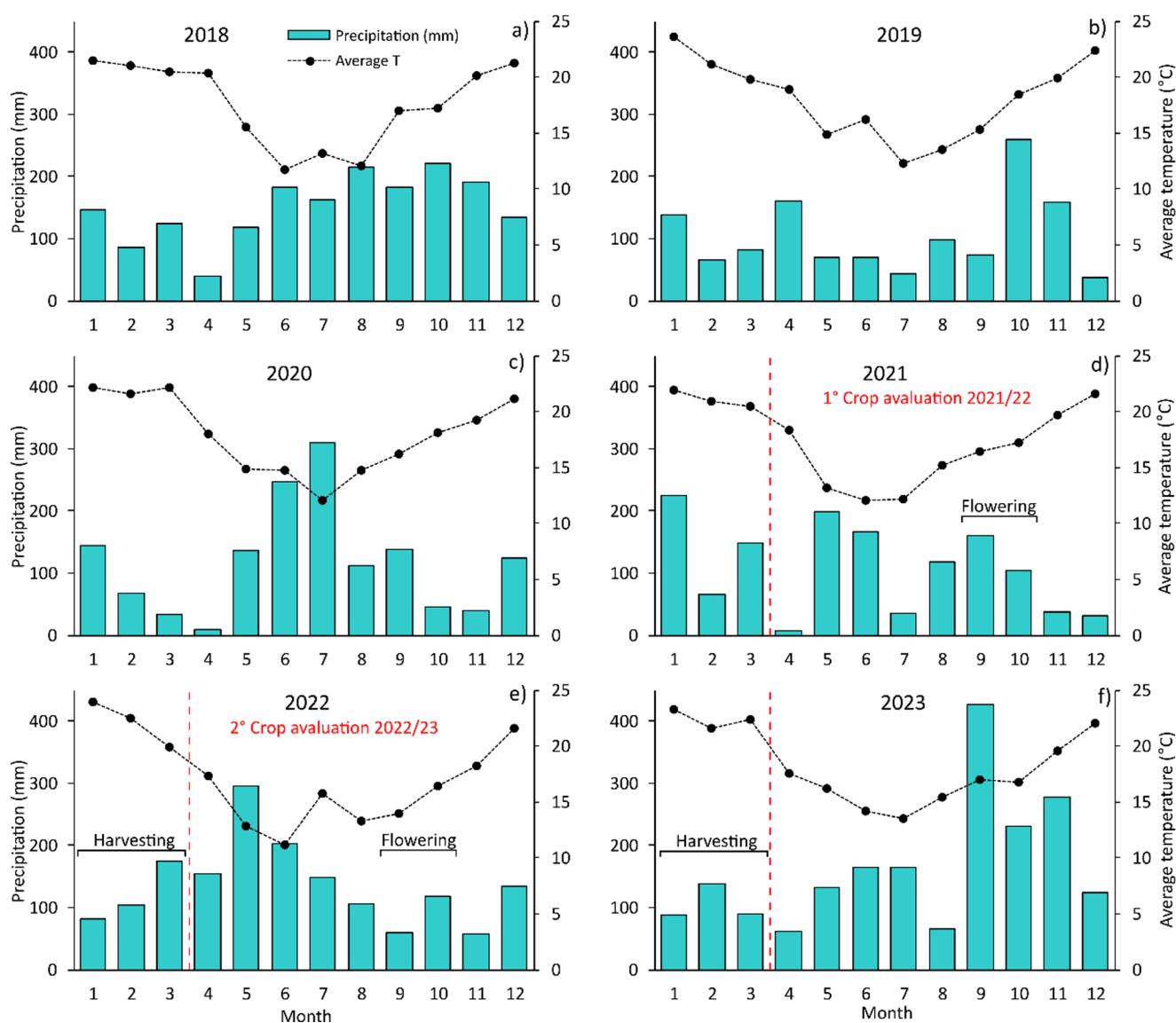


Fig. 1 Monthly precipitation and average temperature observed before and during crop seasons 2021/22 and 2022/23 (vertical red dotted line) in Bento Gonçalves municipality, in Rio Grande do Sul state, Southern Brazil. Annually accumulated precipitation was 1,806, 1,256, 1,407, 1,298, 1,634, and 1,963 mm, respectively, for the six years presented. Phenological stages: dormancy (May–July), sprouting

(August–September), flowering (September–October), development of fruits (October–November), ripening of berries (December–February), harvest (January–March), senescence (March–May) (Lorenz et al. 1995). Meteorological station location (29°09′52.5″S, 51°32′03.1″W, altitude: 623 m)

AAAnalyst 200, USA); P contents were determined by the method described by Murphy and Riley (1962), with readings taken on a molecular absorption spectrophotometer at 882 nm wavelength (Model V-5000, Metash, China); and K contents were determined by flame photometry (DM-62, Digimed, Brazil). The descriptive statistics of foliar nutrient contents are presented in Table S1.

Soil samples were subjected to physical analysis for clay content determination by the pipette method (Teixeira et al. 2017). The pH(H₂O) was determined through the soil:

solution ratio (1:1), and the pH-TSM was determined following Toledo et al. (2012). P, K, Cu, Zn, and Mn were extracted with Mehlich-1 solution (0.0125 mol L⁻¹ H₂SO₄ and 0.050 mol L⁻¹ HCl). In the extract, P was determined by the method described by Murphy and Riley (1962), using a molecular absorption spectrophotometer at 882 nm wavelength (Model V-5000, Metash, China). Potassium was determined by flame photometry (DM-62, Digimed, Brazil). The total organic carbon (TOC) content was determined by the modified Mebius method, after wet oxidation

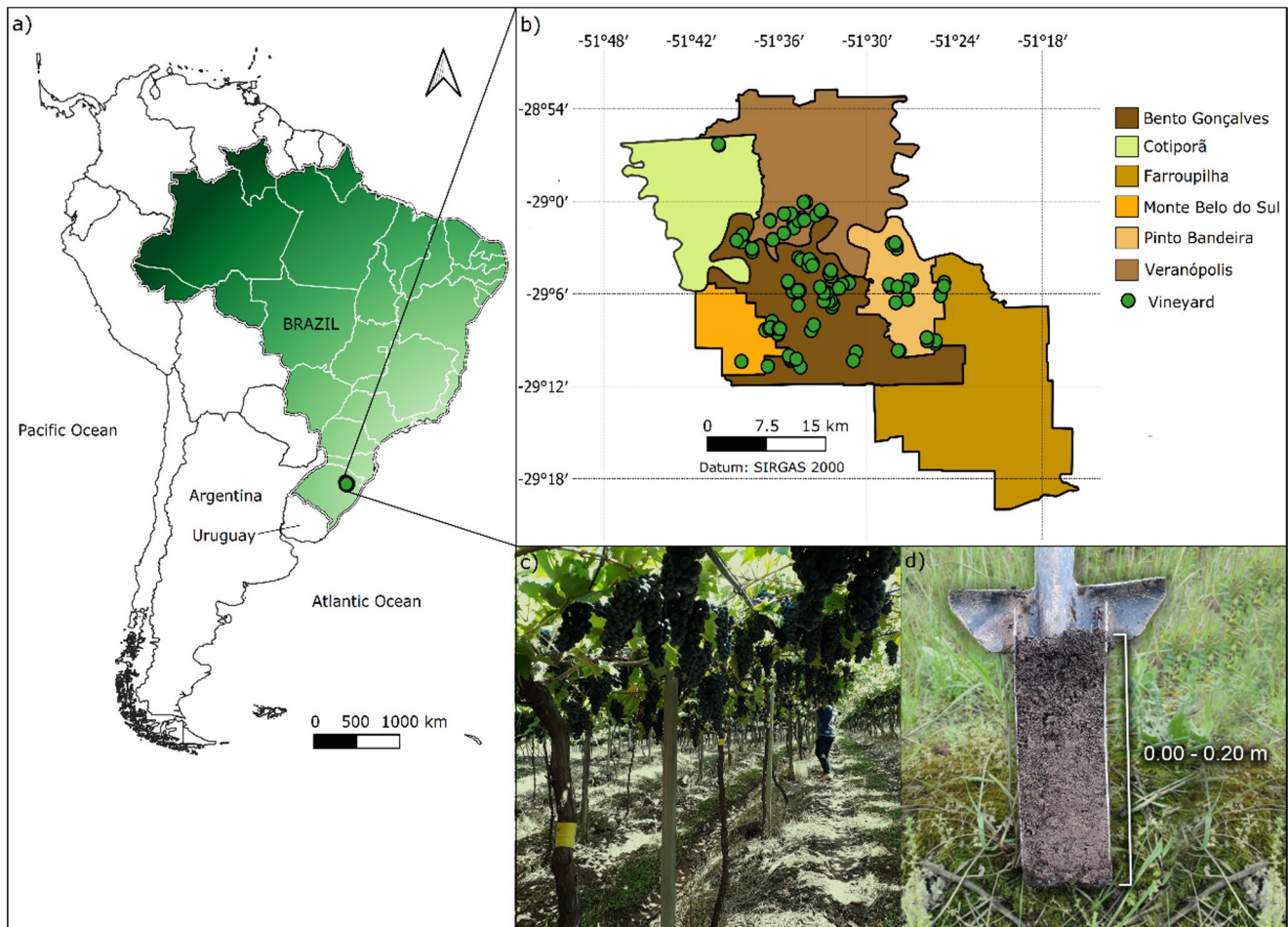


Fig. 2 Location of the evaluated vineyard areas within South America (a), during the 2021/22 and 2022/23 harvests in the Serra Gaúcha region (b), Southern Brazil, and vineyard view where leaf sampling,

yield sampling (c), and soil sampling in the 0.00–0.20 m diagnostic layer (d) were performed

in sulfochromic solution - $K_2Cr_2O_7 + H_2SO_4$ (Yeomans and Bremner 1988), and the values were multiplied by 1.724 to convert to soil organic matter (SOM). Aluminum, Ca, and Mg were extracted with 1.0 mol L^{-1} KCl (Tedesco et al. 1995). Quantification of Ca, Mg, Cu, Zn, and Mn was made in an atomic absorption spectrophotometer (Perkin Elmer, AAnalyst 200, USA). Aluminum was determined by titration using $0.0125 \text{ mol L}^{-1}$ NaOH (Tedesco et al. 1995). Potential acidity ($H+Al$), $CEC_{pH7.0}$ obtained by the sum of exchangeable elements Ca, Mg, K, and $H+Al$, and saturation by Ca-Mg-K (V) were calculated using equations described by CQFS-RS/SC (2016). The soil descriptive statistics are presented in Table S1.

2.4 Grape Yield and Yield Components

During the grape harvest in the 2021/22 and 2022/23 seasons, seven plants per vineyard were evaluated, the total

number of bunches per plant was counted, and then harvested and weighed to calculate production (kg), using a digital scale with three-digit precision (Walmur, 50 kg, Brazil). According to the spacing between plants and fruit production, yield per hectare was determined. The relative fruit yield (RFY) was calculated by assigning the value of 100 to the vineyard with the maximum yield (Eq. 1). The grape yield characterization is presented in Table S1.

$$Relative\ fruit\ yield\ (RFY) = \frac{yield\ in\ each\ vineyard}{highest\ yield\ among\ vineyards} \times 100 \quad (1)$$

2.5 Statistical Analysis

The database was subjected to descriptive statistical analysis for exploration purposes. The response variable, yield, was subjected to analysis of variance (ANOVA), considering the factors ‘cultivar’ and ‘crop season’, as well as the

interaction between these factors (fixed effect). The normality of the residuals was tested using the Shapiro-Wilk test. Whenever the null hypothesis (equal means) was rejected with alpha equal to 0.05, the means were compared by Tukey's test ($p < 0.05$). ANOVA was performed using the 'ExpDes.pt' package in the R Studio version 4.2.2 statistical program (R Core Team 2022).

To explore the results, all data (yield, nutrient concentrations in the soil, and nutrient contents in the leaves) were subjected to principal component analysis (PCA), allowing the identification of more complex interactions between variables, as well as verifying the similarity/dissimilarity between cultivars. PCA was performed using the 'FactoMineR' and 'factoextra' packages in the R Studio version 4.2.2 statistical program (R Core Team 2022).

The critical levels (CL) and sufficiency ranges (SR) of nutrients in the soil ($\text{pH}(\text{H}_2\text{O})$, SOM, P, K, Ca, Mg, Cu, Zn, Mn, $\text{CEC}_{\text{pH}7.0}$, and V) and in the leaves (N, P, K, Ca, Mg, S, Cu, Zn, Fe, Mn, and B) were estimated in relation to yield, using the total number of observations in the database ($n=207$). To establish soil fertility classes and foliar nutrient sufficiency ranges, the boundary line (BL) approach was used (Walworth et al. 1986). Soil chemical attributes or foliar nutrient contents as the independent variable (x-axis) were related to the relative fruit yield (RFY) as the dependent variable (y-axis), disregarding discrepant data to better adjust the determination coefficient (R^2). The values at the upper limit of the point cloud were selected and used to generate regression equations (Lima Neto et al. 2020, 2024), using the Curve Expert Basic version 2.2.3 software (Hyams 2020), selecting the best-fit model, based on significance and the coefficient of determination (R^2). Finally, the BL equations were used to establish soil fertility classes and foliar sufficiency ranges: low ($\text{RFY} < 70\%$), medium ($70\% \leq \text{RFY} < 90\%$), high ($90\% \leq \text{RFY} < 100\%$), and excessive ($\text{RFY} \geq 100\%$), according to Lima Neto et al. (2024).

3 Results

3.1 Grape Yield

The average fruit yield differed among cultivars, with lower yield observed in *Vitis vinifera* species ('Pinot Noir' and 'Chardonnay') compared to *Vitis labrusca* (Fig. 3). The red cultivar 'Isabel' showed a yield 141% higher than the cultivar 'Pinot Noir'. The white cultivar 'BRS Lorena' showed 143% higher yield than the cultivar 'Chardonnay'. The average yield was $31.3 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ for the six cultivars evaluated, and no difference was observed between the 2021/22 and 2022/23 seasons.

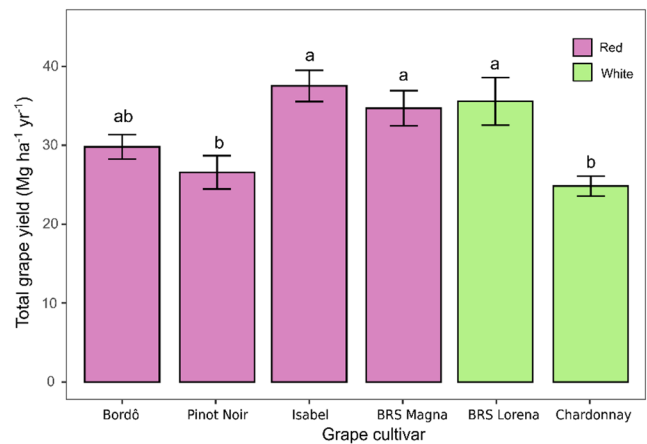


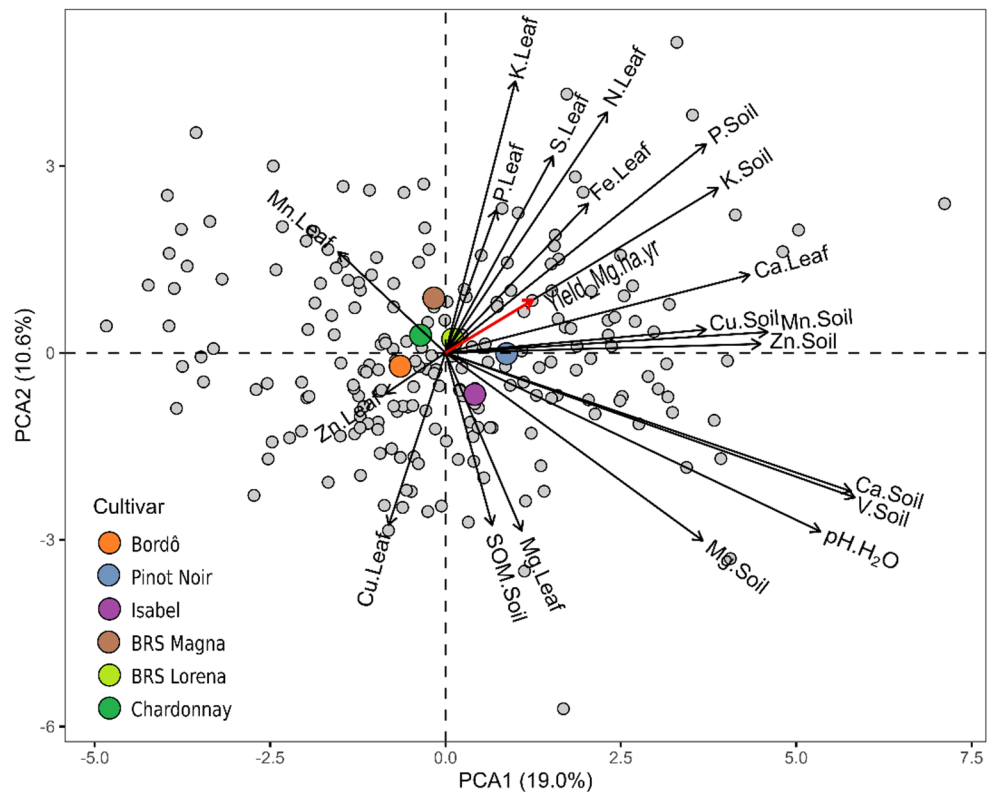
Fig. 3 Average yield of 'Bordô', 'Pinot Noir', 'Isabel', 'BRS Magna', 'BRS Lorena', and 'Chardonnay' grapes, evaluated in the 2021/22 and 2022/23 crop seasons in the Serra Gaúcha region, Southern Brazil. Equal letters do not differ from each other using the Tukey test ($p < 0.05$). Vertical lines represent the standard error of the mean

Cultivars did not show distinction among themselves regarding the parameters evaluated in the principal component analysis (Fig. 4). Yield showed a positive correlation with the K concentration in the soil. The concentrations of micronutrients Cu, Zn, and Mn in the soil showed a positive correlation with each other. In addition, negative correlations were observed between the P concentration in the soil and the Zn content in the leaf, between the Mg concentration in the soil and the Mn content in the leaf, and between the Cu and P contents in the leaves of the vines.

3.2 Critical Nutrient Levels and Soil Fertility Classes

Reference values for the soil were established based on the affinity between the relative fruit yield (RFY) and soil chemical variables, using the BL method (Fig. 5). The fertility class considered adequate (high class) for $\text{pH}(\text{H}_2\text{O})$ was 5.3–5.9, and SOM was 2.8–4.5% (Table 1). The adequate ranges for P and K in the soil were 62–108 and 120–227 mg dm^{-3} , respectively. The adequate Ca and Mg ranges were 6.6–8.3 and 2.1–2.9 $\text{cmol}_c \text{ dm}^{-3}$, maintaining the Ca: Mg ratio of 3:1. Regarding the micronutrients Cu, Zn, and Mn, approximately 22, 30, and 46% of the vineyards reached the excessive class, with values above 210; 36 and 210 mg dm^{-3} , respectively (Fig. 5g, h, i). The values of $\text{CEC}_{\text{pH}7.0}$ were 14.4–18.7 $\text{cmol}_c \text{ dm}^{-3}$ and V of 67.5–77.6% (Fig. 5j, k). We observed that the vineyards had high nutrient concentrations in the soil (Fig. 5). In the case of P, about 33% of the soils presented concentrations in the excessive class ($> 108 \text{ mg dm}^{-3}$) (Table 1). Regarding K, 36% of the vineyards have concentrations above the high class ($> 227 \text{ mg dm}^{-3}$) in the soil (Fig. 5b).

Fig. 4 Principal component analysis (PCA) according to grape yield and soil and leaf variables, evaluated in the 2021/22 and 2022/23 crop seasons, in Southern Brazil. The colored dots represent the average for each grape cultivar. The red arrow represents the grape fruit yield. Leaf: represents the nutrients evaluated in plant tissue. Soil: represents the soil nutrients evaluated in the 0.00–0.20 m layer. V.Soil: Ca–Mg–K saturation; SOM.Soil: soil organic matter; pH.H₂O: water pH in the soil solution, soil: water ratio (1:1)



3.3 Leaf Critical Nutrient Levels

The relationship between RFY and nutrient contents in grapevine leaves is presented in scatter plots (Fig. 6). The equations obtained by the BL method indicate that RFY increased up to a maximum limit, with a subsequent decrease as foliar contents became elevated. The ranges of foliar contents considered adequate for each nutrient were established, comprising the RFY between 90 and 100% (Table 2). The adequate ranges of macronutrients N, P, K, Ca, Mg, and S for ‘Bordô’, ‘Pinot Noir’, ‘Isabel’, ‘BRS Magna’, ‘BRS Lorena’, and ‘Chardonnay’ grapevines were, respectively, 26.6–30.8, 3.4–4.2, 8.4–10.1, 9.0–12.4, 2.1–2.6, and 1.7–2.1 (g kg⁻¹) (Fig. 6). For the micronutrients Cu, Zn, Fe, Mn, and B, the adequate contents were 8.3–11.1, 109–136, 68.1–84.9, 368–450, and 37.2–49.6 (mg kg⁻¹), respectively.

4 Discussion

The evaluated vineyards showed a wide variation in soil chemical attributes and clay content (Table S1). The soils in the study were characterized by medium clay contents, medium to high SOM contents, and high CEC_{pH7.0} values, according to CQFS-RS/SC (2016), which favors adsorption and, consequently, reduces nutrient losses from the soil.

The six cultivars showed differences in yield, with the red cultivars ‘Isabel’ and ‘BRS Magna’ and the white cultivar ‘BRS Lorena’ being the most productive (Fig. 3). These differences are related to the genetic characteristics of the cultivars themselves, with the *V. vinifera* cultivars ‘Pinot Noir’ and ‘Chardonnay’ being the least productive. The red cultivar ‘Isabel’ has the largest cultivated area in the Serra Gaúcha region, being the most traditional, with centenary vineyards and reaching high yields (>60 Mg ha⁻¹) compared to the national average yield of 22.8 Mg ha⁻¹ (IBGE 2024). However, principal component analysis was not able to identify differences between cultivars when plotting yield values in relation to soil and leaf variables (Fig. 4). Therefore, the reference values for the interpretation of soil and leaf analyses were established jointly, covering all cultivars (Figs. 5 and 6).

The Serra Gaúcha climate favors a low rate of organic matter mineralization, which contributes to soil carbon accumulation, as evidenced by the high organic matter content found in the vineyards (>5.0%), as well as higher nutrient concentrations compared to other regions of Brazil (Bortoluzzi et al. 2012; Schmitt et al. 2014). In addition, the long history of vineyard cultivation, with fertilizer applications above nutrient export by harvests, provides a soil nutrient accumulation, as observed by the wide range of P values, which vary from 8 to 607 mg dm⁻³, as well as K, which varied from 49 to 610 mg dm⁻³ (Table S1). The soil

Fig. 5 Scatter plots and boundary lines of the relation between soil fertility attributes (0.00–0.20 m) and the relative yield of ‘Bordô’, ‘Pinot Noir’, ‘Isabel’, ‘BRS Magna’, ‘BRS Lorena’, and ‘Chardonnay’ grapes, evaluated in the 2021/22 and 2022/23 crop seasons in Serra Gaúcha region, Southern Brazil. The soil chemical evaluation was performed at harvesting time. SOM: Soil organic matter; $\text{CEC}_{\text{pH}7.0}$: cation exchange capacity at pH 7.0; V: Ca-Mg-K saturation. Brown dots: boundary line, points used to create the mathematical equations. Gray dots: points lower than the boundary line

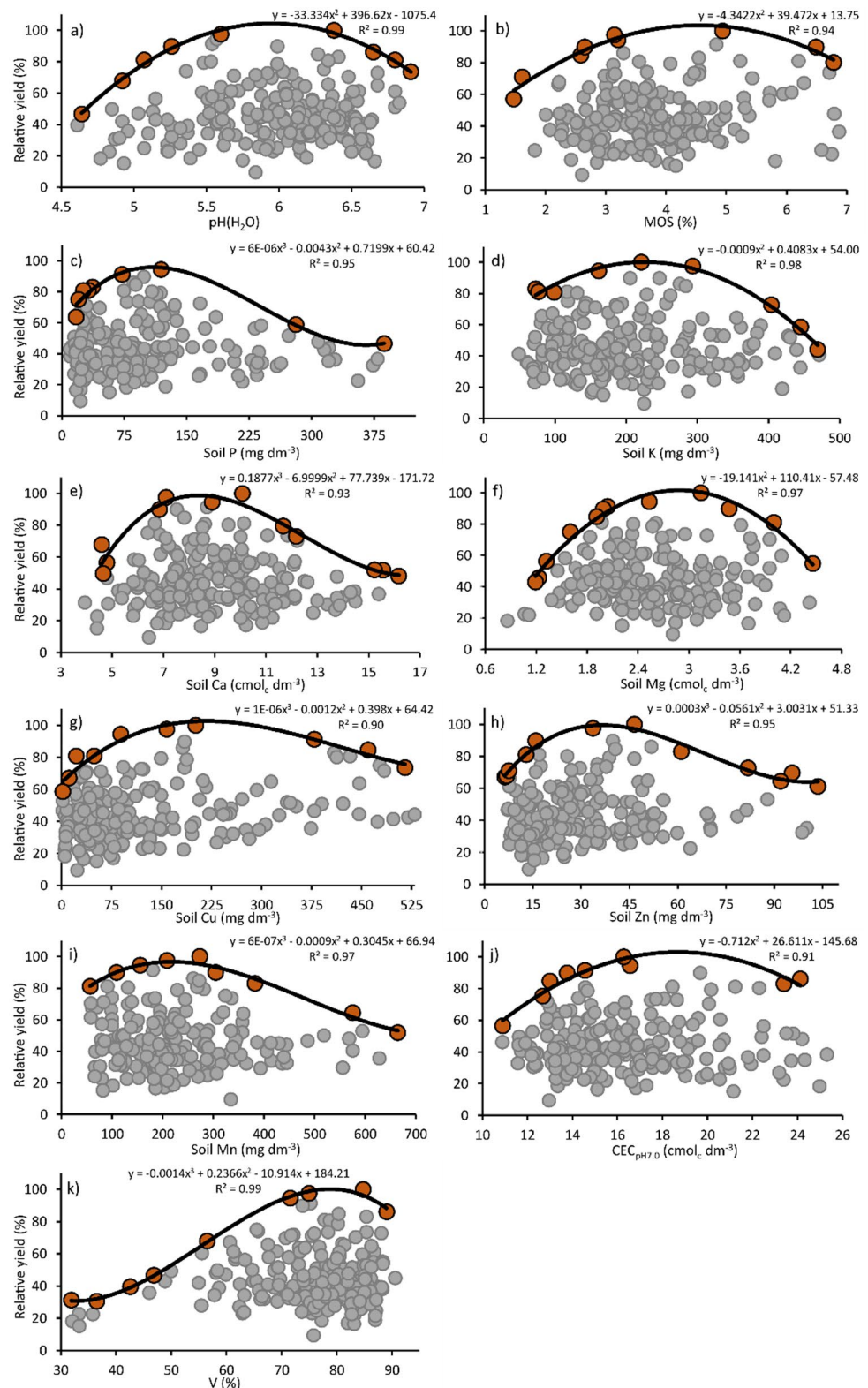


Table 1 Soil sufficiency ranges of nutrients in ‘Bordô’, ‘Pinot Noir’, ‘Isabel’, ‘BRS Magna’, ‘BRS Lorena’, and ‘Chardonnay’ grapes, cultivated in Southern Brazil, established by the boundary line (BL) method

Relative fruit yield	pH(H ₂ O)	SOM	P	K	Cu	Fertility Classes	
%		%	mg dm ⁻³				
<70	<4.9	<1.8	<14.6	<43.3	<14.7		Low
70–90	4.9–5.3	1.8–2.8	14.6–62.2	43.3–119.8	14.7–84.1		Medium
90–100	5.3–5.9	2.8–4.5	62.2–107.5	119.8–226.8	84.1–210.2		High
>100	>5.9	>4.5	>107.5	>226.8	>210.2		Excessive
	Zn	Mn	Ca	Mg	CEC _{pH7.0}	V	
	mg dm ⁻³			cmol _c dm ⁻³		%	
<70	<7.1	<10.3	<5.2	<1.6	<11.9	<57.5	Low
70–90	7.1–18.8	10.3–107.4	5.2–6.6	1.6–2.1	11.9–14.4	57.5–67.5	Medium
90–100	18.8–36.2	107.4–210.3	6.6–8.3	2.1–2.9	14.4–18.7	67.5–77.6	High
>100	>36.2	>210.3	>8.3	>2.9	>18.7	>77.6	Excessive

P, K, Cu, Zn, and Mn extracted by Mehlich-1 solution; Ca and Mg extracted by KCl 1.0 mol L⁻¹ solution; pH(H₂O) determined through soil: solution relation (1:1); SOM: soil organic matter determined by sulfochromic solution (K₂Cr₂O₇+H₂SO₄); CEC_{pH7.0}: cation exchange capacity at pH 7.0 and V: Ca-Mg-K saturation obtained by CQFS-RS/SC (2016) equations

chemical characteristics, such as CEC_{pH7.0} and parent material, justify the high natural fertility observed in forest soils from the Serra Gaúcha region (Grando et al. 2025a).

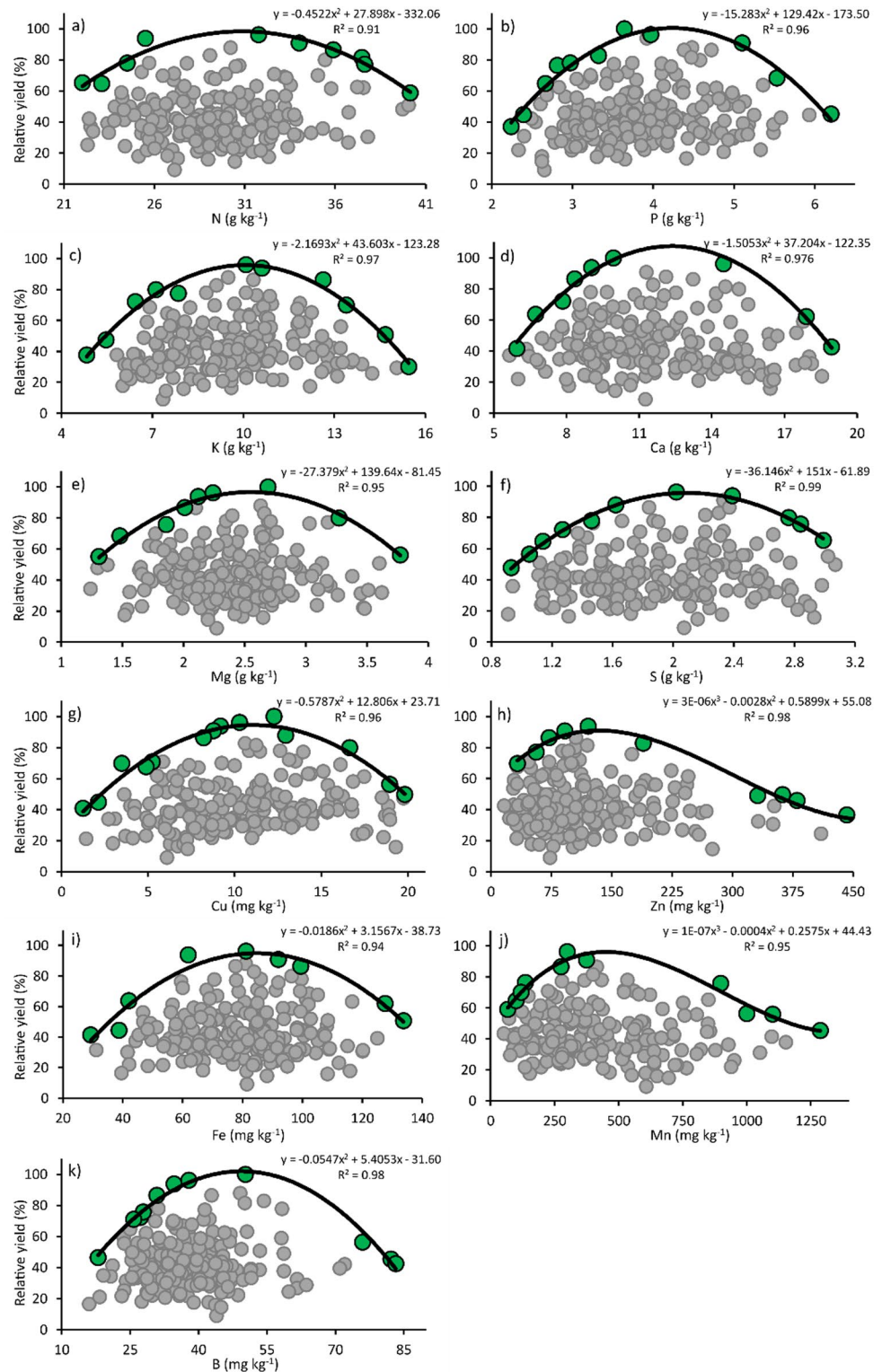
The P reference values in the soil (Table 1), obtained by the BL method (62–108 mg dm⁻³), were about three times higher than the values presented in the current regional fertilization recommendation system (18–36 mg dm⁻³) for the 21–40% clay class (CQFS-RS/SC 2016), evidencing the excess of nutrients in the soil. Only 2% of the evaluated vineyards presented P concentrations in the low class (<14.6 mg dm⁻³), which makes it difficult to determine reference values for this fertility class. It should also be noted that about 33% of the soils in the evaluated areas presented excessive P concentrations, above 108 mg dm⁻³ (Fig. 5a), which may overestimate the critical P concentration. Thus, it is recommended to maintain the P concentration within the high availability class proposed, since if the excessive class is reached, in addition to representing an additional and unnecessary cost with fertilization, the increase in P in the soil above the adequate value can intensify the process of transfer to aquatic environments, causing environmental contamination (Gatiboni et al. 2020; Grando et al. 2021; Schmitt et al. 2014). The adequate K concentration range in the soil (120–227 mg dm⁻³) showed lower limits compared to the current fertilization recommendation system (181–360 mg dm⁻³) for the CEC_{pH7.0} class, ranging between 15 and 30 cmol_c dm⁻³ (CQFS-RS/SC 2016). On the other hand, the concentrations of Ca (6.6–8.3 cmol_c dm⁻³) and Mg (2.1–2.9 cmol_c dm⁻³) in the soil were higher than the regional reference values of Ca: 4.0 cmol_c dm⁻³ and Mg: 1.0 cmol_c dm⁻³. These Ca and Mg values are unprecedented for vines cultivated in the study region, also contributing to the definition of acidity correction applications. The average values of saturation by Ca, Mg, and K in the CEC_{pH7.0} of the vineyard soils were 55.0, 16.2, and 3.3%, similar to forest soils in the region (Grando et al. 2025b). The balance

of exchangeable cations, based on the increase in soil CEC, can improve nutrient availability and reduce the effects of competition for charges between Ca, Mg, and K in the soil (Wacal et al. 2019; Yang et al. 2024).

We highlight that the BL method is affected by the auto-correlation, and the cause-and-effect relationship can be misleading. For example, when the soil pH is very high (>6.5), it is common to have Mn, Zn, and Cu deficiency, but at the same time, there is greater Ca availability. So, when a graph shows Ca reducing yield in the “very high” range, it is likely not because Ca is excessively high, but because Mn, Cu, and Zn are deficient (this is a misleading cause-and-effect). Based on this, we recommend using these values generated by the BL method, maintaining the technical criteria, and visualizing nutrient interactions to avoid interpretation mistakes. Based on the results obtained, concentrations of Cu, Zn, and Mn in the soil, higher than 210, 36, and 210 mg dm⁻³ are considered excessive (Table 1), and may negatively affect the growth and development of the vine (Mir et al. 2021; Morsch et al. 2022). These values are much higher than 0.4, 0.5, and 5.0 mg dm⁻³, from which the concentrations of Cu, Zn, and Mn, respectively, are interpreted as high (adequate levels) by the current regional recommendation (CQFS-RS/SC 2016). It is also important to highlight that the values presented in this study are indicated for producing vineyards, with already established plants; this is because some research indicates greater susceptibility of vine seedlings to the effects of Cu toxicity (De Conti et al. 2019; Giroto et al. 2016; Morsch et al. 2022; Trentin et al. 2022). However, these studies were conducted in nutrient solutions or in soils with low clay and SOM contents, conditions that differ from those of the Serra Gaúcha region, that have high buffer capacity (Grando et al. 2025a).

Values of Cu, Zn, and Mn based on the diethylene-triaminepentaacetic acid (DTPA) method differ from those based on the Mehlich-1 extractor. However, there

Fig. 6 Scatter plots and boundary lines of the relation between the relative yield and the leaf contents of macro and micronutrients in ‘Bordô’, ‘Pinot Noir’, ‘Isabel’, ‘BRS Magna’, ‘BRS Lorena’, and ‘Chardonnay’ grapes, cultivated in Southern Brazil, in the 2021/22 and 2022/23 crop seasons. Total nutrients in leaves opposite the bunch were determined at flowering time. Green dots: boundary line, points used to create the mathematical equations. Gray dots: points lower than the boundary line



is a significant correlation between these methods, with Mehlich-1 values generally being higher than DTPA values (Sobral et al. 2013). Generally, available Cu and Zn (DTPA) contents greater than 25 and 15 mg kg⁻¹ in sandy soils, respectively, are considered phytotoxic for vines

(Kabata-Pendias 2010). In the present study, the high SOM content is the main contributor to the adsorption of these cations (Fontes et al. 2021; Grando et al. 2025c), which can reduce plant toxicity and increase excessive levels (Trentin et al. 2022; Oliveira et al. 2025). Additionally, Zn, P, and

Table 2 Leaf sufficiency ranges of nutrients at flowering time in ‘Bordô’, ‘Pinot Noir’, ‘Isabel’, ‘BRS Magna’, ‘BRS Lorena’, and ‘Chardonnay’ grapes, cultivated in Southern Brazil, established by the boundary line (BL) method

Relative fruit yield	N	P	K	Ca	Mg	S	Sufficiency range
%	g kg ⁻¹						
< 70	22.9	2.8	6.6	7.4	1.6	1.2	Low
70–90	22.9–26.6	2.8–3.4	6.6–8.4	7.4–9.0	1.6–2.1	1.2–1.7	Medium
90–100	26.6–30.8	3.4–4.2	8.4–10.1	9.0–12.4	2.1–2.6	1.7–2.1	High
> 100	30.8	4.2	10.1	12.4	2.6	2.1	Excessive
	Cu*	Zn*	Fe	Mn*	B		
	mg kg ⁻¹						
< 70	4.6	29.2	48.1	121.6	26.0		Low
70–90	4.6–8.3	29.2–109.0	48.1–68.1	121.6–368.2	26.0–37.2		Medium
90–100	8.3–11.1	109.0–135.9	68.1–84.9	368.2–450.0	37.2–49.6		High
> 100	11.1	135.9	84.9	450.0	49.4		Excessive

* Ranges with a physiological meaning cannot be precisely determined due to fungicide residues, which are inherent to the cultivation of vines in a high-yielding system

Fe deficiencies, and combinations thereof, can inhibit plant growth and photosynthetic parameters (Gao et al. 2023), resulting in plant imbalances. Also, Mn is bound by soil clay minerals and carbonates, but not by organic matter (Ochmian and Malinowski 2024). Manganese bioavailability is related to pH changes, with higher contents in acid soils (pH < 5.0) and increases by a factor of 100 when pH decreases by one unit (Kabata-Pendias and Pendias 2001). High Mn concentrations affect plant energy metabolism, decrease photosynthetic parameters (Nable et al. 1988), and cause oxidative stress (Fecht-christoffers et al. 2003).

The cultivation history of the evaluated vineyards, characterized by the constant application of fertilizers and fungicides, is the primary reason for the high nutrient concentrations in the soil, which often occurs without technical monitoring (Santos et al. 2019). This can be observed in the high values of Cu and Zn in the vineyard soils, with concentrations 455 and 179% higher in the ‘Isabel’ cultivar, compared to ‘Bordô’ (Fig. S1). The average age of the ‘Isabel’ vineyards is 37 years, while for the other cultivars, this average dropped to 10 years. In addition, many of the areas are undergoing renovation, that is, after a long period under cultivation, the plants are uprooted, and a new vineyard is implanted, often using different cultivars. For this reason, due to soil turnover and the reduction of SOM levels, symptoms of Cu, Zn, and Mn toxicity are often observed in vine seedlings (Brunetto et al. 2018; Mou et al. 2011; Sonoda et al. 2019).

The pH(H₂O) value of 6.0 is considered ideal for fruit trees in general, including vines (CQFS-RS/SC 2016), corroborating the pH values obtained in this study (Table 1). In addition, it was observed that the adequate values of K-Ca-Mg saturation (V) are between 68 and 78%. The wide range of SOM contents (1.5–8.1%) and, consequently, CEC_{pH7.0} (10.9–30.1 cmol_c dm⁻³) provides high adsorption capacities. Therefore, the maintenance and even the

increase of SOM levels in vineyards are desirable (Visconti et al. 2025). As observed in ‘Isabel’ vineyards, with a long cultivation history, there is an increase in SOM due to the permanence of cover crops and the deposition of vineyard crop residues, in addition to the soil non-tillage (Fig. S1h). Thus, subtropical climate viticulture is a strategy to increase carbon storage in the soil, contributing to climate change mitigation (Don et al. 2024; Nazir et al. 2024).

The foliar contents of N, P, K, Ca, Mg, and S, associated with the maximum RFY were 30.8, 4.2, 10.1, 12.4, 2.6, and 2.1 g kg⁻¹, respectively (Table 2). Observing the quadratic behavior of the equations that relate fruit yield to nutrient contents in the foliar tissue of grapevines (Fig. 6), it is possible to observe that RFY decreased when the values were excessive. This effect is caused by nutritional imbalance, which tends to toxicity, and is accompanied by a reduction in grape yield (Ndabamenye et al. 2013; Sharma et al. 2005). However, the leaf nutrients did not correlate with soil nutrients (Fig. 4). This can be related to the root system exploration of grapes, which grows in a deeper soil layer, allowing nutrient uptake below 0.00–0.20 m (diagnostic layer), which reduces correlation effects from soil to leaf variables. Also, grapes can make natural associations with arbuscular mycorrhiza fungi, increasing plant growth and nutrition by better access to soil nutrients (Trouvelot et al. 2015).

The micronutrient contents in the foliar tissue, associated with the maximum RFY, for Cu, Zn, Fe, Mn, and B were 11, 136, 85, 450, and 50 mg kg⁻¹, respectively (Table 2). Most of these values differ from those observed by Melo et al. (2018) for *V. vinifera* with 12, 203, 115, 484, and 32 mg kg⁻¹. The leaf Mn contents were about 64% lower in ‘Bordô’, compared to the other cultivars (Fig. S2j), which can be justified by the non-application of fungicides containing Mn in its composition in this cultivar (Table S3). The high Mn critical level was also observed in subtropical vineyards, 380 mg kg⁻¹ (Oliveira

et al. 2025). These high values can be explained because grape species are tolerant to Mn, using detoxification mechanisms such as accumulation in the cell wall or cell vacuole to avoid Mn-toxicity (Yao et al. 2012). In addition, constant applications of Bordeaux mixture (CuSO_4) provide a thick protective layer on the leaf blade. Thus, spraying phytosanitary products stimulates deposition and/or absorption processes on the leaf surface, increasing the nutrient content and preventing references with real physiological significance, making a safe diagnosis impossible (García-Escudero et al. 2013). Thus, although presented in this study, the contents of Cu, Zn, and Mn need to be critically evaluated, mainly in *V. vinifera* cultivars that receive frequent pesticide applications (Table S3).

The foliar nutrient contents proposed for the Serra Gaúcha were compared with those in the literature, obtained in other Brazilian regions (Table S2). Similar findings were recorded for other fruit plants like citrus (Krug et al. 2023), banana (Lima Neto et al. 2022), and peach (Betemps et al. 2020). It is possible to observe that the sufficiency ranges varied between regions, as well as the values proposed for the Serra Gaúcha have a smaller range in relation to the CQFS-RS/SC (2016) indications, in addition to including the reference values for S and Cu in the leaves (Table S2). Also, the reference values observed by Rozane et al. (2025) for the ‘Bordô’ cultivar in the same region were similar to those proposed in our study. The narrower amplitude observed in the present values is considered positive due to the greater accuracy in understanding the results of the foliar contents, minimizing the possibility of obtaining low yields, but with the requisite plant nutrient levels (Melo et al. 2018).

Due to the lack of research in viticulture, the reproduction of reference values are still observed between some regions and even from other countries. This is a result of the difficulty of experimenting with perennial crops, which require many years of field trials to consolidate results, demanding human resources and long-term funding. Therefore, the importance of developing research to improve recommendation systems is highlighted, and it is necessary to constantly update nutritional reference values. This is because there is a change over the years in yield levels affected by soil conditions, climate, and climate change, management of the varieties used, and improvements in production systems (Andrade et al. 2023; Fraga et al. 2016; Lima Neto et al. 2024).

5 5. Conclusion

This study established regionalized nutritional reference values for *Vitis vinifera* and *Vitis labrusca* cultivars, using the Boundary Line (BL) method. These values demonstrate a marked discrepancy in relation to current regional

recommendations, highlighting the crucial importance of specific data for effective and sustainable management in the context of a subtropical climate and soil with high nutrient retention capacity. The high natural fertility is overshadowed by the chronic and massive accumulation of nutrients. It is noteworthy that the new P reference values are approximately three times higher than those currently recommended, confirming a widespread excess in the soil. Equally concerning is the accumulation of Cu, Zn, and Mn, which reach potentially toxic concentrations, resulting from decades of applying fertilizers and fungicides above the actual harvest export needs. The lack of correlation observed between nutrient levels in the traditional diagnostic soil layer (0.00–0.20 m) and leaf levels suggests that deep root exploration by the vine minimizes the plant’s response to surface variations, challenging conventional sampling methods. Thus, the adoption of these new reference values allows for the monitoring of vine nutrition, enabling the correction of deficiencies in situ and, most crucially, avoiding excess nutrition that imposes unnecessary costs on producers and increases the risk of environmental contamination through nutrient transfer to aquatic ecosystems.

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Data Availability All data were generated or analyzed in this study.

Declarations

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