

## Research Article

# Effects of the Annual Nitrogen Fertilization Rate on Vine Performance and Grape Quality for Winemaking: Insights from a Meta-Analysis

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Sustainability in grapevine cultivation requires the precise use of water and fertilizers, particularly nitrogen (N), to produce grapes of the highest quality for winemaking, while simultaneously avoiding harm to the surrounding waters and atmosphere by reducing  $\text{NO}_3^-$  losses and  $\text{N}_2\text{O}$  and  $\text{NH}_3$  emissions from the vineyards. To address the challenge of optimizing N use in viticulture, many N fertilization trials have been carried out over the last decades, and a compilation and analysis of worldwide trials was therefore needed. The present study tackled this challenge through a meta-analysis of published research, which included 374 fertilization trials. From the compiled data, six vine production parameters and eight grape quality traits were extracted and normalized to enable comparisons between experiments. The Mitscherlich law of diminishing returns was able to satisfactorily describe the set of vine production parameters against nitrogen application rate, and the same occurred with the yeast assimilable nitrogen (YAN). In vines, both reproductive and vegetative growth similarly responded to the N application rate. In general, the nitrogen requirements for 95% of the maximum grape yield amounted to rates between 30 and 40  $\text{kg}\cdot\text{N}\cdot\text{ha}^{-1}$ , which increased nitrogen use efficiency (NUE) to values between 0.27 and 0.36  $\text{t}\cdot\text{kg}\cdot\text{N}^{-1}$ . Although several grape quality traits could not be described against the N rate in terms of any mathematical relationship, an N rate between 20 and 25  $\text{kg}\cdot\text{N}\cdot\text{ha}^{-1}$  could be considered as maximizing grape quality for winemaking. Such N fertilization range increases NUE up to values between 0.41 and 0.47  $\text{t}\cdot\text{kg}\cdot\text{N}^{-1}$ , thus almost doubling the known NUE standards when grape quality is targeted instead of yield, although soil fertility could be exhausted in the mid-to-long term. Whatever the case, anthocyanins and polyphenols are well preserved in red grapes at such low N rates, although YAN is not. The results of this work will be useful for guiding new vine N nutrition research and N nutrition management in vineyards, thus increasing wine growing sustainability.

## 1. Introduction

Nitrogen represents 1.5% of vines on a dry-weight basis [1]. Most of the nitrogen absorbed by vines is assimilated to synthesize amino acids, peptides, proteins, nucleotides, nucleic acids, chlorophylls, and other secondary metabolites of great importance, such as adenosine triphosphate (ATP).

Therefore, N plays a key role in plant metabolism, both structurally and energetically, strongly controlling the synthesis of carbohydrates, their buildup, and use in vine organs [2]. Since grapevine N requirements are rather low when compared to nonperennial crops [3–5], the vine's N uptake capacity may be easily exceeded with inadequate fertilization practices. In such circumstances, the N surplus

may be lost to water as  $\text{NO}_3^-$ , and to the atmosphere as  $\text{N}_2\text{O}$  and  $\text{NH}_3$ , which cause harmful effects to these parts of the environment [6–10].

Nitrogen uptake is mainly controlled by vine demand and thus varies according to growth requirements [4, 11]. In this sense, limiting the nitrogen supply decreases shoot growth to a rate consistent with this supply [12]. However, both N uptake and shoot growth in grapevines have saturation-type responses to increasing soil N [13, 14], thus likely obeying the diminishing-returns law, i.e., the Mitscherlich law [15]. Moreover, the heterogeneous distribution of N in the soil, together with potential water deficits, hinders the uptake of N by the vines [16]. In this context, fertilization is a powerful tool for improving vine performance and modulating grape composition [11]. However, nitrogen application to grapevines must be performed with caution, given its conflicting effects on vegetative growth, yield, and grape composition [17, 18].

For the sustainable production of  $12 \text{ t}\cdot\text{ha}^{-1}$  of grapes, the N requirements of vines have been estimated to be  $50 \text{ kg}\cdot\text{N}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$  [3], which results in a nitrogen use efficiency (NUE) of  $0.24 \text{ t}\cdot\text{kg}\cdot\text{N}^{-1}$ , considering that NUE stands for the weight of harvest produced per unit weight of applied nitrogen [19]. Nevertheless, these requirements may vary depending on the grapevine cultivar, soil type, and management history; for instance, the N requirements of cv. Viosinho Blanc in the North of Portugal were quantified as  $34.2 \text{ kg}\cdot\text{N}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$  [20]. A deficit in N leads to lower vegetative development and yield, in addition to slower ripening with small rates of sugar buildup [5, 21]. In contrast, an excess of N can foster an unrestricted and lengthy vegetative development of the canopy [22]. Such excessive development withdraws resources, specifically carbohydrates, from grape formation [1] and increases bunch shading, which may decrease the concentration of phenolic compounds in the berries [23], thus resulting in detrimental effects to grapes and wine quality [1, 5]. Therefore, these findings suggest that grape and wine quality traits do not strictly follow the Mitscherlich law and that further integrative research is required for quantitatively establishing fertilization requirements to maximize grape and wine quality.

Consequently, it is expected that both yield and sugars will increase in the berries as vine N absorption increases. However, as vine N absorption continues, yield is expected to asymptotically approach its maximum and stabilize, whereas, in contrast, sugars are expected to reach their maximum and then decrease. Regarding other grape quality traits, such as phenolic compounds, they may even monotonically decrease as vine N uptake increases, although the response of these traits to the N dose differs between studies [24, 25]. In addition, as the N excess makes the canopy grow comparatively more than the roots, a surplus of N makes the vine more vulnerable to water stress [11] and *Botrytis* infestations [26]. Because of all these effects, an excess of N decreases NUE, which results in higher N losses to the environment, thereby diminishing the sustainability of grapevine cultivation. Therefore, optimal grapevine NUE requires not only balancing N status between vegetative

growth and yield but also promoting the accumulation of amino acids in the grapes, as well as other related metabolites, to enhance wine quality [5, 27]. This optimal NUE can contribute to a reduction in N inputs, and a decrease in the potential risks of N losses to the environment.

An optimal management of N fertilization can improve other berry quality traits, such as yeast assimilable nitrogen (YAN), relevant for efficient must fermentation [17]. This trait seems to have a linear response to the increases in N doses, as reported in several studies [28, 29]. Therefore, increases in grape must YAN can be modulated by smart decisions regarding N fertilization, including the application rate, through both amount and timing [30].

In the last decades, many fertilization trials in vineyards have been carried out to estimate the N rates that maximize NUE for yield and grape quality. These works have been reviewed by, among others, Bell and Henschke [17], Keller [11], and Verdenal et al. [1]. As an alternative approach to the casual and somewhat subjective extraction and synthesis of results that characterizes traditional literature reviews, we find meta-analyses. These analyses use statistical techniques that jointly analyse the large collection of data obtained in all of the independent studies that aim to answer a common question [31–33]. In viticulture, meta-analysis techniques have been applied to understand the effects of various vine management practices on ripening delay [34], on *Botrytis* bunch rot [35], on vineyard ecology and yield [36, 37], and on soil organic carbon build-up [38]. However, a meta-analysis about the effects of the annual N fertilization rate on vine production and grape quality has not yet been carried out. The results of such meta-analysis will help in quantifying grapevine N needs for both enhancing yield and promoting grape composition improvements, accounting for different soil types, grapevine varieties, and viticultural regions. In addition, the Mitscherlich law has never been applied before to understand the effects of N fertilization on vines, even though such equation seems to have the potential for at least modelling vine production, and may therefore serve as a basis for making N rate estimations that maximize vine NUE.

Therefore, the aim of the present work was to carry out a meta-analysis on the effects of the annual nitrogen rate on the main vine production parameters and grape quality traits for winemaking, whose results could be useful for consolidating knowledge on general rates of nitrogen fertilization of vines, particularly for increasing NUE.

## 2. Materials and Methods

**2.1. Variable Selection.** Before carrying out the data search, the variables that were *a priori* considered key for characterizing vine production, as well as grape quality for winemaking, were selected as target variables for the meta-analysis. In the case of vine production, the following parameters were selected: grape yield, pruning weight, bunch number, bunch weight, berry number, and berry weight. Regarding grape quality, the following traits, which had been determined on grape must and expressed on a fresh volume basis where appropriate, were selected: total soluble solids

(TSS), titratable acidity (TA), pH, malic acid concentration, tartaric acid concentration, anthocyanin concentration, total polyphenol index, and yeast assimilable nitrogen (YAN).

**2.2. Data Collection.** In April 2022, the term “grapevine nitrogen fertilisation” was searched in the abstract databases of Clarivate Web of Science™ (WOS) and Elsevier Scopus® in the search fields of “Topic” and “Title-Abstract-Keywords,” respectively. Although more words with the root “fertil” could have been used, the aim was to obtain a representative sample of the studies conducted on the subject, and not the complete set of studies on the N fertilization of grapevines. Then, following this procedure, 242 records were retrieved from WOS, and 129 from Scopus. After removing duplicates, 283 articles were collected. After reading the abstracts, the ones that (i) did not deal with grapevine for winemaking, e.g., table grapes, (ii) were literature reviews, (iii) implied the botanical meaning of fertilisation, or (iv) did not report any of the target variables were rejected. Therefore, 122 articles were kept, from which the complete text of 117 could be obtained. These were read in-depth, and the values of the target variables were extracted. In the process, some articles were combined, as they reported data from the same study. In contrast, some other articles that were not found in the previous literature search, but that reported data from the same study, were added to the selection. Finally, the variables from 95 studies were obtained. Next, since nitrogen fertilization was tested in each study, along with other biological, environmental, and cultural factors, the data in each study was split into several experiments so that each one featured the same irrigation treatment, soil type, vine variety, rootstock, cultural practices, site, and year. Therefore, nitrogen was left in each experiment as the only factor that determined vine yield and must quality, as explained below. As a result, the total number of trials included in the present study’s database was 374.

**2.3. Cell Means Estimation.** As previously mentioned, in many N fertilization studies, the nitrogen fertilization factor was tested along with other factors that may determine vine yield and must quality. In such studies, the outputs of the multifactor experimental designs were analysed through multiway ANOVAs, and a summary of marginal means was often shown to the reader. However, the presentation of marginal means leads to information losses. To take care of this inconvenience, in the current meta-analysis, the means of each combination of factor levels, i.e., the cell means, were estimated from the marginal means in the original studies. For example, the mean of the  $ijk \dots$  cell ( $\bar{x}_{ijk\dots}$ ) in an  $m$ -factor experimental design was estimated using the following equation:

$$\bar{x}_{ijk\dots} = \bar{x}_{i\dots} \frac{\bar{x}_{\cdot j\dots}}{1/n_2 \sum_{j=1}^{n_2} \bar{x}_{\cdot j\dots}} \frac{\bar{x}_{\cdot\cdot k\dots}}{1/n_3 \sum_{k=1}^{n_3} \bar{x}_{\cdot\cdot k\dots}} \dots, \quad (1)$$

where  $\bar{x}_{i\dots}$ ,  $\bar{x}_{\cdot j\dots}$ , and  $\bar{x}_{\cdot\cdot k\dots}$  are the marginal means of the  $i$ ,  $j$ , and  $k$  levels of, respectively, the first, second, and third factors and  $n_2$  and  $n_3$  are the total number of levels for, respectively, the second and third factor. This approach assumes that the interactions are negligible as compared to the main effects, which may impose limitations when this premise is not fulfilled. However, it is a reasonable assumption, providing the authors do not indicate otherwise, as was the case in most of the articles.

**2.4. Homogenization of Units.** The same variable was often reported in different units, depending on the study considered. Therefore, when needed, adequate transformations were performed to ease comparisons as indicated in the following lines.

**2.5. Vine Fresh Production.** To have grape yield and pruning wood always expressed in  $\text{kg}\cdot\text{ha}^{-1}$  of fresh matter, equation (2) was used, where  $w_w$  is the water mass fraction in the material of interest. If  $w_w$  was not reported in the same article, and neither the TSS, which is a percentage of the dry matter in must [39] and thus  $w_w \approx (100 - \text{TSS})/100$ , a generic  $0.795 \text{ g}\cdot\text{g}^{-1}$  value was used according to Doymaz [40], whereas a generic  $0.5 \text{ g}\cdot\text{g}^{-1}$  value was used for pruning wood according to Porceddu et al. [41].

$$Y_f = \frac{Y_d}{(1-w_w)}. \quad (2)$$

Additionally, to transform fresh yield of must volume ( $Y_{vf}$ ), expressed as  $\text{L}\cdot\text{ha}^{-1}$ , into fresh yield of grapes ( $Y_{mf}$ ), expressed as  $\text{kg}\cdot\text{ha}^{-1}$ , equation (3) was used, where  $E_{eff}$  is the must extraction efficiency for which a  $0.6 \text{ L}\cdot\text{kg}^{-1}$  value was used as an estimate.

$$Y_f = \frac{Y_{vf}}{E_{eff}}. \quad (3)$$

**2.6. Must Total Soluble Solids.** To always express the total soluble solids (TSS) in °Brix, a simple linear transformation was applied to the alternative units ( $a.u.$ ) using equation (4) and the adequate coefficients reported in Table 1 [39, 42].

$$^\circ\text{Brix} = a + b \text{ a.u.} \quad (4)$$

**2.7. Must Titratable Acidity.** To always express must titratable acidity (TA) as the equivalent mass of tartaric acid per unit volume of must ( $\rho_{TA}$ ) in  $\text{g}\cdot\text{L}^{-1}$ , the equivalents of tartaric acid per unit volume ( $N_{TA}$ ) in  $\text{meq}\cdot\text{L}^{-1}$  were multiplied by the tartaric acid equivalent weight ( $\text{eq.w.}_{TA}$ ), i.e.,  $75.0435 \text{ g}\cdot\text{eq}^{-1}$ , and by  $10^{-3}$ , which is needed as a transformation factor as follows:

$$\rho_{TA} = 10^{-3} \text{ eq.w.}_{TA} N_{TA}. \quad (5)$$

TABLE 1: Coefficients to obtain °Brix from several alternative units using equation (4).

Alternative units	$a$	$b$
Total sugars (g·L <sup>-1</sup> )	3.5	0.0855
Reducing sugars (g·L <sup>-1</sup> )*	3.5	0.0900
Probable alcohol (% vol.)	3.5	1.44
*Baumé	-0.365	1.8391

\*Reducing sugars are considered 95% of total sugars.

Then, the remaining issue of the different titration endpoints, usually 7.0 in Europe and often 8.2 elsewhere, was addressed by normalizing the TA data within each experiment.

**2.8. Must pH.** In this meta-analysis, the hydrogen ion activity ( $a_{H^+}$ ) in mol·L<sup>-1</sup> was used instead of directly using the pH values of the must. Accordingly,  $a_{H^+}$  was calculated based on the following relationship:

$$a_{H^+} = 10^{-pH}. \quad (6)$$

**2.9. Must Analyte Concentrations.** To express the concentrations of the analytes determined in the must ( $A$ ), e.g., YAN, always in mass per unit volume ( $\rho_A$ ) in mg·L<sup>-1</sup>, the mass fractions in must ( $w_A$ ) in g·g<sup>-1</sup> were transformed. Specifically, equations (7) and (8) were used, depending on whether  $w_A$  had been expressed on a dry ( $w_{A,dw}$ ) or a fresh ( $w_{A,fw}$ ) weight basis, respectively.

$$\rho_A = 10^6 w_{A,dw} \rho (1 - w_w), \quad (7)$$

$$\rho_A = 10^6 w_{A,fw} \rho. \quad (8)$$

In these equations,  $10^6$  is a transformation factor,  $\rho$  is the must density in g·cm<sup>-3</sup>, and  $w_w$  the must water mass fraction in g·g<sup>-1</sup>. If  $\rho$  or  $w_w$ , i.e., TSS, were not reported in the same article, generic  $\rho$  and  $w_w$  values of 1.1 g·cm<sup>-3</sup> [43, 44] and 0.795 g·g<sup>-1</sup> [40] were used, respectively.

**2.10. Data Normalization.** Before data analysis, both vine production and grape quality data in each of the 374 experimental trials included in the database were normalized. The calculation of normalized values enables the comparison between trials, by avoiding the effects of several biological, environmental, and management factors, which, in addition to the nitrogen rate, determine vine production and grape quality. In this regard, normalization is commonly used in meta-analyses, and whenever it is necessary to neutralize the sources of variation that add up to the one of interest, e.g., irrigation rate in crop water production functions [45], or salinity in crop salinity tolerance functions [46]. In addition, to help neutralize the effects of factors that include, among others, irrigation, cultural practices, rootstock, vine variety, soil features, plant age, vintage, and incidences of pests and diseases, the normalization helps in neutralizing the effects due to the different methodological approaches between studies. In the current investigation, these methodological

differences included the different titration endpoints for TA determination, and the different determination methods and units used for the contents of anthocyanins and polyphenols. Although there are several normalization methods available, in the present study, the mean vine production or must quality trait in each different N treatment within an experimental trial ( $y_i$ ) was divided by the trial maximum value ( $y_{\max}$ ) as follows:

$$y_{n,i} = 100 \frac{y_i}{y_{\max}}. \quad (9)$$

In this regard,  $y_{\max}$  is not the potential vine production or must quality trait, but a convenient benchmark for normalization. Interestingly, the alternative use of a closer-to-potential value, such as the maximum value of the experimental trials throughout all years, soil types, and cultural practices, would allow these factors to contribute to variation, in addition to the annual nitrogen rate and randomness.

**2.11. Vine Production Response to Nitrogen.** The Mitscherlich law in the form described by Baule, as pointed out by Dhanoa et al. [47], was fitted to the point clouds of the trial-normalized vine production parameters against the annual nitrogen rate as follows:

$$y_n = 100 \left( 1 - e^{-a(Na+N)} \right). \quad (10)$$

In the Mitscherlich law, as represented by equation (10) and shown in Figure 1(a),  $y_n$  is the normalized vine production parameter of interest, i.e., grape yield, pruning weight, bunch number, bunch weight, berry number, or berry weight. Since the vine production parameters were expressed on a normalized scale featuring a maximum given by 100% (equation (9)), a 100 factor was included in equation (10). Then,  $N$  is the annual rate of nitrogen from fertilizer, and  $N_a$  is the annual rate of N from sources alternative to the intentional fertilizer application. These nondeliberate alternative N contributions may include the N stored in the preceding years in the vine's permanent organs, the N mineralized from the soil organic matter, the N dissolved and applied with the irrigation water, or that contributed by several environmental N fixation processes unfolding in the vineyard agroecosystem. Since achieving 95% of the maximum grape yield can be considered as a reasonable target for maximizing NUE, the corresponding vine N uptake could be obtained by adding  $N_a$  to  $N_{95}$ , thus giving  $N_{\rightarrow,95}$ , as represented in Figure 1. Finally, in equation (10),  $a$  is an efficiency factor of vine nitrogen uptake, which stands for the curve steepness change as N increases (Figure 1(a)).

**2.12. Grape Quality Response to Nitrogen.** The data from the trial-normalized grape quality traits against annual nitrogen rate were fitted to the Mitscherlich law depicted by equation (10). However, if equation (10) could not fit the data satisfactorily, a logistic curve featuring a sigmoid shape (Figure 1(b)), and represented by the following equation, was tried instead as follows:

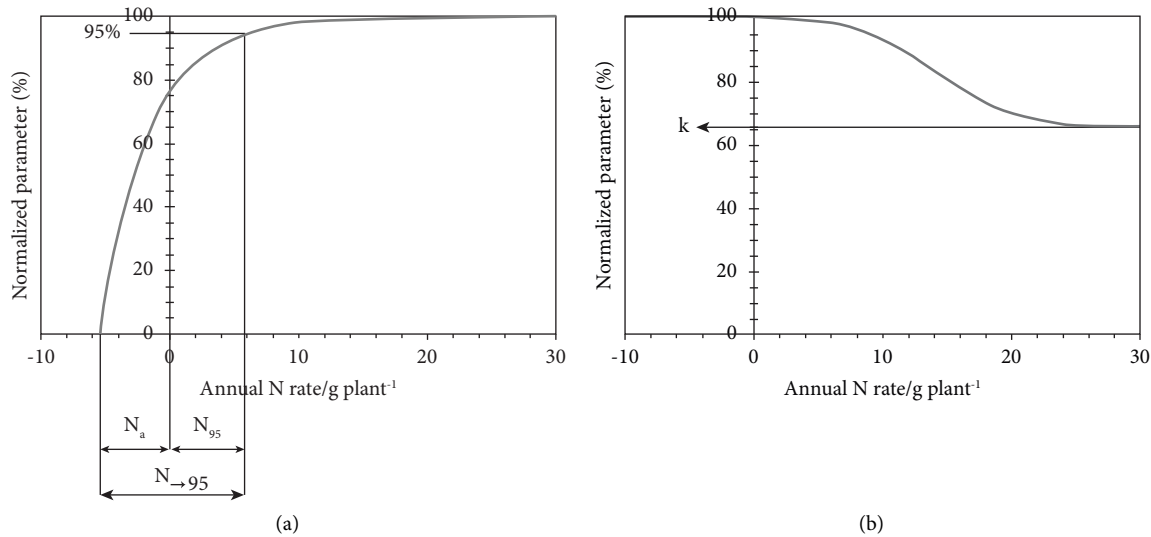


FIGURE 1: Hypothetical functional dependence of vine production or grape quality traits on the annual N rate. (a) Mitscherlich law. (b) Logistic curve.

$$y_n = 100 + \frac{k - 100}{1 + qe^{-b(N_a + N)}} \quad (11)$$

In equation (11),  $y_n$  is the normalized grape quality trait of interest. Then, similar to the Mitscherlich law (equation (10)) as the quality traits are also expressed on a normalized scale having a maximum of 100% (equation (9)), the value of 100 was included as both addend and subtrahend in equation (11). Finally,  $k$  is the value that  $y_n$  asymptotically approaches to as the annual nitrogen rate increases, and  $q$  and  $b$  jointly account for the speed at which the normalized quality trait approaches its right asymptote as the annual nitrogen rate increases (Figure 1(b)).

**2.13. Curve Fitting.** Fitting the Mitscherlich law and logistic curve to the data point clouds was directly performed by minimizing the summation of the squared differences between predictions and observations of the production parameter or quality trait of interest. In this regard, the generalized reduced gradient (GRG) nonlinear optimization algorithm [48] was used. However, the direct fitting may not provide reasonable results in the case that the point cloud lacks sufficient shape. To solve this issue, we propose taking advantage of the point density. Specifically, a density surface may be estimated through Kernel smoothing by means of the KernSmooth package [49] in *R* [50]. Then, only the points defining the ridgeline of the density surface may be used for fitting. If, additionally, the point density, i.e., the surface height, is used as the weight for each of the ridgeline points, the corresponding weighted GRG nonlinear optimization may further improve fitting.

**2.14. Fitting Errors.** Kernel smoothing is taken from geography, where coordinates boast negligible errors. However, in the present application of the technique, the ordinate would be a normalized vine production or grape quality

trait, and the abscissa is the annual nitrogen rate. Therefore, the coordinates, specifically the annual N rate, are defined as the variables subjected to estimation, and the errors associated to the curve parameters should be estimated by means of a model-independent method, such as bootstrapping. Therefore, bootstrapping may be applied by taking 1000 random samples with replacement from the pool of ridgeline points obtained from each production parameter and quality trait. Then, the fitting may be recalculated, and the 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles of the fitting coefficients may be assessed to estimate the 95% confidence interval for their means. In this work, comparisons between pairs of means were made at the 95% confidence level using the Student's *t*-test.

### 3. Results

**3.1. General Characteristics of the Database.** Most of the vine N fertilization studies were carried out in the northern hemisphere (73%), predominantly in Europe (63%), followed by the Americas (28%), and in outdoor conditions, either directly on soil (81%) or in pots (14%), and without irrigation (48%). Soil organic carbon was only reported in 47% of the analysed studies, where it ranged from 0.06 to 3.6% with an average of 1.0% for those studies performed in outdoors conditions on soil.

Planting density ranged between 1250 and 10000 vines·ha<sup>-1</sup>, with an average of 3800 ± 400 vines·ha<sup>-1</sup>. Vine age ranged between 1 and 32 years with an average of 11 ± 2 years, and the studies were both annual (47%) and multiannual (53%). The most-utilized rootstocks were SO4 (23%) and Paulsen 1103 (16%), followed by own-rooted *Vitis vinifera* (9%). *Vitis vinifera* varieties were used in 96% of the trials, whilst the rootstock alone in 4%. Specifically, up to 36 vine varieties were used, mainly red grapes (66%) ones, over white grapes (34%), and studies with Cabernet Sauvignon (23%) and Syrah (11%) particularly prevailed in the dataset.

Most of the experimental trials aimed at testing different rates of mineral N fertilizer (69%), supplied mainly as urea and ammonium nitrate. The rest aimed at comparing different types of mineral N fertilizers (10%), and at testing different organic N rates (9%). In the mineral N rate tests, the nutrient was conventionally applied through soil in 64% of the cases, or through fertigation in 26% of the cases, while the remaining 10% of studies dealt with foliar applications of N fertilizers. The fresh grape yields were  $11.5 \pm 0.9$ ,  $9.0 \pm 1.6$  and  $8.0 \pm 1.3$  t·ha<sup>-1</sup>, for soil, fertigation, and foliar applications of mineral N fertilizers, respectively. Therefore, since the world grape yield average is 10.2 t·ha<sup>-1</sup> [51], then for N application through soil, yields were significantly above average ( $p = 0.008$ ); for N application through fertigation, these were not significantly different from the average ( $p = 0.17$ ); and for N application through leaves, these were significantly below average ( $p = 0.0009$ ). The mean TSS values at harvest for each kind of N application were, respectively,  $20.3 \pm 0.4$ ,  $21.2 \pm 0.6$ , and  $21.8 \pm 0.8$ °Brix, i.e., within the target range of 20 to 23°Brix for achieving standard wine alcohol concentrations, which range between 11.5 and 13.5% vol. Therefore, based on the similarity observed for grape yield and quality standards for winemaking, the results that are presented in the following sections are constrained to the tests of mineral N fertilizer rate applied through both soil and fertigation.

The N rates throughout the different trials included in the database were remarkably variable, as shown by the minimum and maximum N values in each trial. The lowest N rate was, in general, 0 g·N·vine<sup>-1</sup>·yr<sup>-1</sup>, but the highest one reached an outstanding 400 g·N·vine<sup>-1</sup>·yr<sup>-1</sup>, both through soil and fertigation applications. Nevertheless, on average, the maximum N rate was 50 g·N·vine<sup>-1</sup>·yr<sup>-1</sup> for both types of applications. Interestingly, vine age at the beginning of the trial seemed not to have been a criterion for selecting the maximum N rate in the trials.

**3.2. Vine Production Response to Nitrogen.** Grape yield was reported in most of the studies included in the database, followed by berry weight and bunch number, then bunch weight, pruning weight, and, finally, berry number per bunch (Table 2). The mean grape yield of  $3.7 \pm 0.3$  kg·vine<sup>-1</sup> and the mean pruning weight of  $0.58 \pm 0.04$  kg·vine<sup>-1</sup> of the dataset provided a mean Ravaz index of  $6.4 \pm 0.7$ , which is well within the optimum range of 5–10 for most cultivars and soil and climate conditions [52–54]. The number of collected records per variable for the ensuing analysis ranged between 146 and 541 (Table 2).

The scatter plots of the vine production parameters against the annual nitrogen rate in the many mineral N fertilizer trials with either soil or fertigation application are shown in Figure 2. In general, the variability of the normalized production parameters ( $y_n$ ) decreased as the annual N rate increased. The high variability at low annual N rates can be interpreted as a consequence of a steep increase in  $y_n$  around zero N, which gradually flattens by approaching the asymptote at 100%. Therefore, the sets of points present

some resemblance with the Mitscherlich law depicted in Figure 1 and described by equation (10)

Despite the similarity with the Mitscherlich law, fitting equation (10) to the points by direct minimization of the sum of square deviations between  $y_n$  predictions and observations led to unsatisfactory results, which were reflected by unreal estimates of equation (10) coefficients  $a$  and  $N_a$ . In fact, to at least obtain a meaningful estimate of the Mitscherlich  $a$  coefficient, the line had to be forced to pass through, e.g.,  $y_n = 50\%$  at  $N = 0$ , but with the unwanted consequence that  $N_a$  was fixed in the process.

The most shapeless parts of vine production against N rate scatter plots precluded the satisfactory direct fitting of Mitscherlich law (Figure 2). However, these shapeless parts could be advantageously exploited, by first calculating the corresponding density surfaces (Figure 3), and then by fitting equation (10) to the ridgeline points that consequently appeared using the point density, i.e., the surface height, in the ridgeline points as weights.

Importantly, in Kernel smoothing, density is estimated at equally-spaced grid nodes, whose number along the abscissa and ordinate axes is specified by the user as an input. Since the density estimation changes with the number of grid nodes along either axis, this parameter was established as the one up from which the N rate of the maximum density does not appreciably change. In this regard, a value of 101 fulfilled this requirement for most of the vine production parameters, and additionally, grape quality traits. See Figures S1 and S2 in the supplementary material, which show the graphs of the N rate of the maximum density, as a function of the number of grid nodes along each axis, for each variable included in the meta-analysis.

The fitting of the Mitscherlich law to the ridgeline points for every vine production parameter is shown on the density contour plots in Figure 4, whereas the estimates of equation (10)  $a$  and  $N_a$  coefficients are displayed in Table 3. Note that for berry weight, no reasonable estimates were obtained for the coefficients of the Mitscherlich law, and berry weight was therefore not reported in Figure 4 and Table 3.

As shown in Table 3, the value of the Mitscherlich  $a$  coefficient did not significantly change between the vine production parameters, giving rise to just one homogeneous  $a$  group. On the contrary, the  $N_a$  coefficient significantly changed between the vine production parameters, giving rise to two different, although overlapping,  $N_a$  groups. One of the groups included grape yield and pruning weight, featuring a lower  $N_a$ . Another group included bunch weight, featuring around four times higher  $N_a$  than the previous group. Because of the values the Mitscherlich  $a$ , and especially the  $N_a$  coefficients presented, the N for 95% ( $N_{\rightarrow,95}$ ) also split into two different groups. The low  $N_{\rightarrow,95}$  group featured grape yield, pruning weight, and bunch number, and the high  $N_{\rightarrow,95}$  group featured bunch weight and berry number.

**3.3. Grape Quality Response to Nitrogen.** The TSS value was the grape quality trait most reported in the mineral N fertilizer research included in the database, followed by TA and pH, then YAN, tartaric, and malic acids, and finally, the

TABLE 2: Statistical summary of the vine production parameters and grape quality traits in the mineral N trials included in the database.

Data	Counts			Units	Mean $\pm$ 95% CI	Maximum	Minimum
	Articles	Trials	<i>n</i>				
Grape yield	43	160	541	kg.vine <sup>-1</sup>	3.7 $\pm$ 0.3	15.8	0.0
Pruning weight	22	119	346	kg.vine <sup>-1</sup>	0.58 $\pm$ 0.04	2.00	0.01
Bunch number	26	66	253	—	29 $\pm$ 3	100	4
Bunch weight	24	62	232	g.bunch <sup>-1</sup>	140 $\pm$ 10	380	20
Berry number	11	29	146	—	104 $\pm$ 7	213	34
Berry weight	31	80	302	g.berry <sup>-1</sup>	1.45 $\pm$ 0.06	3.04	0.55
TSS	42	100	377	°Brix	20.6 $\pm$ 0.4	28.3	11.6
Titrateable acidity	35	81	296	g.L <sup>-1</sup>	7.3 $\pm$ 0.4	23.5	2.0
pH	29	77	281	—	3.45 $\pm$ 0.03	4.00	2.80
Malic acid	14	30	110	g.L <sup>-1</sup>	3.5 $\pm$ 0.4	11.2	0.9
Tartaric acid	16	35	137	g.L <sup>-1</sup>	4.3 $\pm$ 0.5	9.4	0.1
Anthocyanins	13	32	117	mg.L <sup>-1</sup>	1600 $\pm$ 300	5200	30
Polyphenols	11	27	94	mg.L <sup>-1</sup>	800 $\pm$ 300	4200	10
YAN	20	53	165	mg.L <sup>-1</sup>	120 $\pm$ 10	600	20

*n* = number of collected records per variable; CI = confidence interval; TSS = total soluble solids; YAN = yeast assimilable nitrogen.

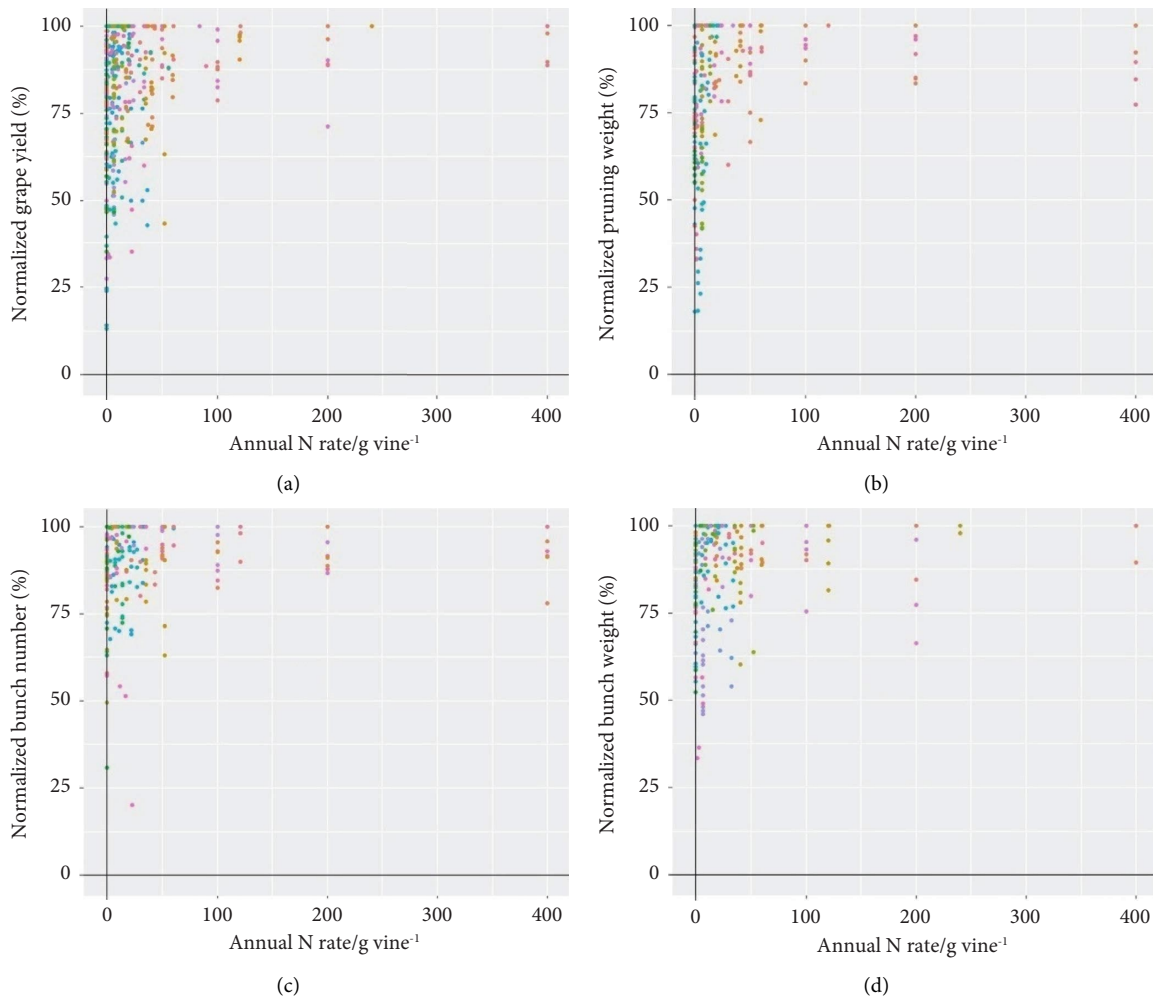


FIGURE 2: Continued.

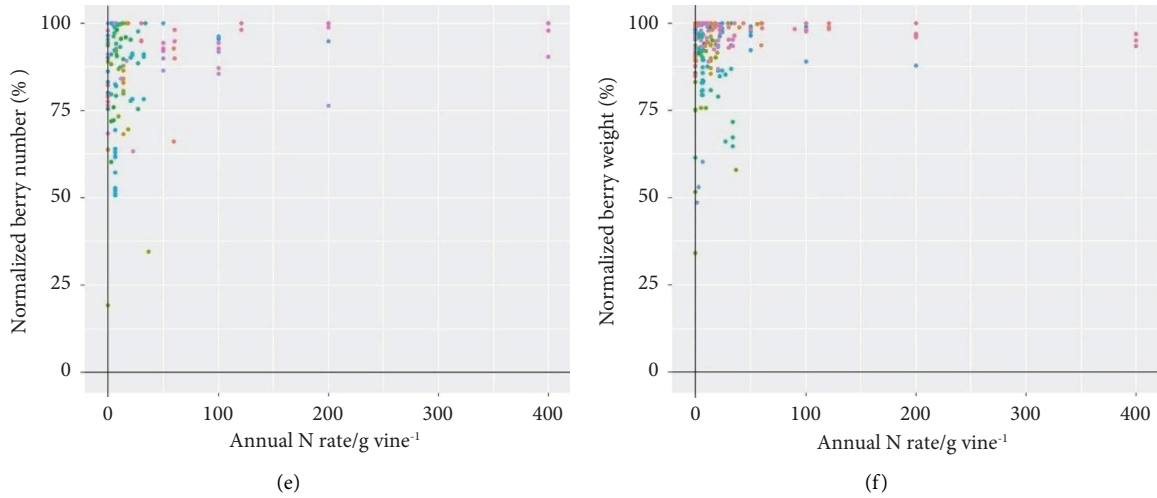


FIGURE 2: Trial-normalized vine production parameters against the annual nitrogen rate. Within the same scatter plot each colour stands for a single trial. (a) Grape yield. (b) Pruning weight. (c) Bunch number. (d) Bunch weight. (e) Berry number. (f) Berry weight.

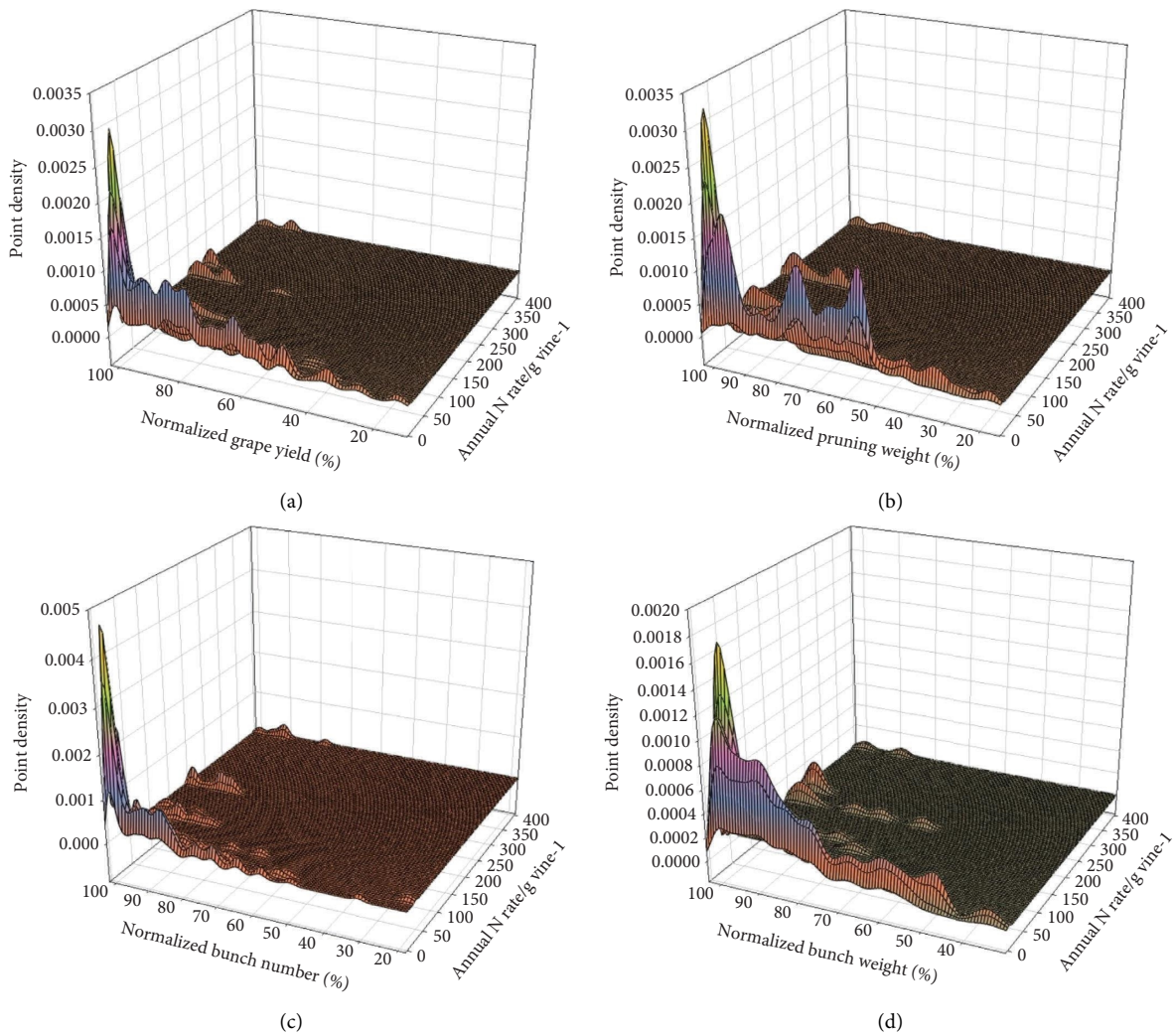


FIGURE 3: Continued.



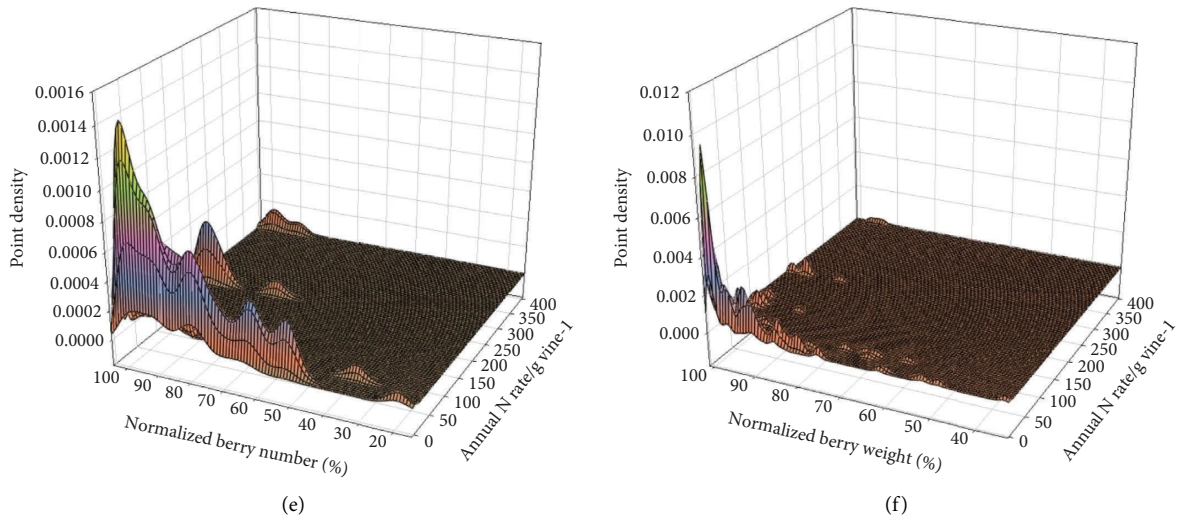


FIGURE 3: Density surfaces of the scatter plots in Figure 2 for the trial-normalized vine production parameters against the annual nitrogen rate. (a) Grape yield. (b) Pruning weight. (c) Bunch number. (d) Bunch weight. (e) Berry number. (f) Berry weight.

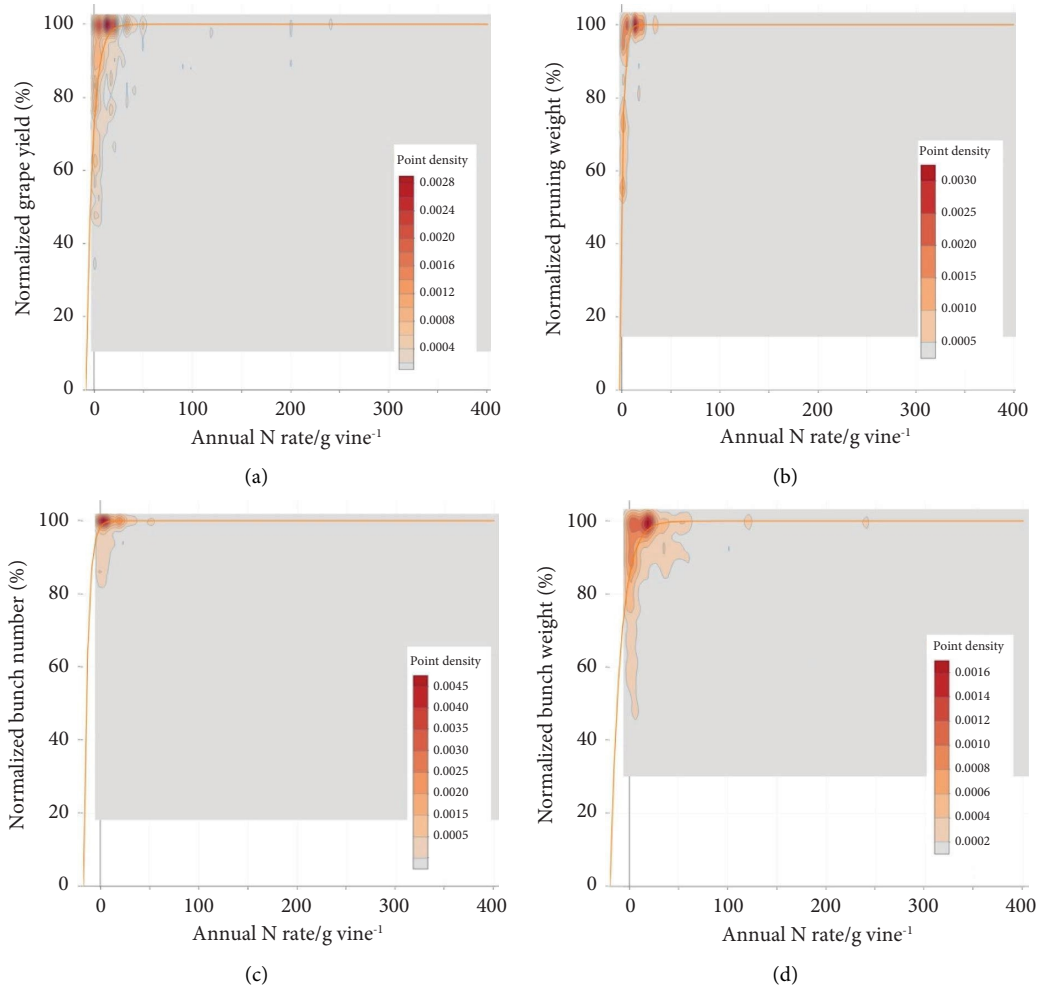


FIGURE 4: Continued.

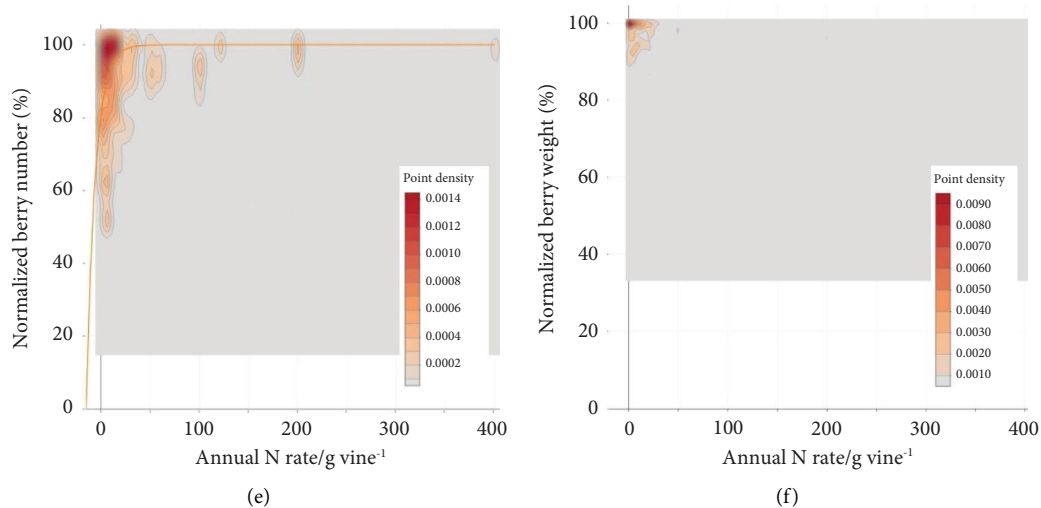


FIGURE 4: Contour plots of the density surfaces in Figure 3 and the corresponding Mitscherlich law fitted to the ridgeline points where fitting leads to reasonable results. The fitting was obtained by density-weighted generalized reduced gradient (GRG) nonlinear optimization of the sum of squared deviations between calculations and observations of the trial-normalized vine production parameters. (a) Grape yield. (b) Pruning weight. (c) Bunch number. (d) Bunch weight. (e) Berry number. (f) Berry weight.

TABLE 3: Mean plus 95% confidence interval for the coefficients of the Mitscherlich law fitted to the vine production parameters and the yeast assimilable nitrogen, along with the N for 95% of each parameter ( $N_{\rightarrow,95}$ ).

Normalized parameter	$a$	$N_a$ (g.vine <sup>-1</sup> )	$N_{\rightarrow,95}$ (g.vine <sup>-1</sup> )
Grape yield	$0.26 \pm 0.15^a$	$5.5 \pm 4.1^a$	$12 \pm 7^a$
Pruning weight	$0.29 \pm 0.24^{ab}$	$3.9 \pm 3.5^a$	$12 \pm 7^a$
Bunch number	$0.42 \pm 0.61^{ab}$	$12 \pm 16^{ab}$	$12 \pm 16^{ac}$
Bunch weight	$0.11 \pm 0.05^{ab}$	$17 \pm 9^b$	$29 \pm 12^{bcd}$
Berry number	$0.12 \pm 0.10^{ab}$	$18 \pm 42^{ab}$	$31 \pm 53^{ade}$
Berry weight	—	—	—
Yeast assimilable nitrogen	$0.07 \pm 0.02^b$	$25 \pm 9^b$	$42 \pm 14^{bc}$

For each curve coefficient, the means sharing at least one letter are non-significantly different at the 95% confidence level.

concentration of anthocyanins and the total polyphenol index (Table 2). A mean TSS value of  $20.6 \pm 0.4$ °Brix and TA of  $7.3 \pm 0.4$  g·L<sup>-1</sup> provided a mean TSS/TA ratio of  $2.8 \pm 0.2$ . The number of collected records per variable for the ensuing analysis ranged from 94 to 377, depending on the grape quality trait considered, i.e., between 30 and 35% less data than for the vine production parameters (Table 2).

The scatter plots of the normalized grape quality traits against the annual nitrogen rate in the several mineral N fertilizer trials with either soil or fertigation application are shown in Figure 5. For TSS, TA,  $a_{H^+}$ , malic and tartaric acid concentrations, and YAN, the  $y_n$  variability decreased as the annual N rate increased, i.e., similar to what was observed in the production parameters. Accordingly, it can be interpreted that  $y_n$  steeply increases around zero N, and then gradually flattens as it approaches the asymptote at 100%, for TA,  $a_{H^+}$ , tartaric acid concentration, and YAN. For TSS, the variability was too small at low annual N rates to observe any clear trend. For the malic acid concentration, the variability

was higher, but no clear trends could be discerned either. For the concentration of anthocyanins and the total polyphenol index, variability did not seem to depend on the annual N rate, and a different progression that did not match the Mitscherlich law can be suggested (Figure 5). Specifically, for both anthocyanins and polyphenols, their values were close to 100% around zero N, then gradually decreased with the annual N rate, to finally stabilise.

Given the visual similarity with the Mitscherlich law of the data of titratable acidity,  $a_{H^+}$ , tartaric acid concentration, and YAN, fitting equation (10) seemed adequate. In addition, the fitting of equation (10) was also tried for TSS and malic acid concentration, although the resemblance with the Mitscherlich law had been barely anticipated for them. However, direct fitting to the data points was avoided because of the previous unsatisfactory results with the vine production data. Instead, the density surfaces were calculated (Figure 6), after which, a density-weighted GRG nonlinear optimization was performed to the ridgeline points in the corresponding graphs. In spite of this, no reasonable estimates for the Mitscherlich  $a$  and  $N_a$  coefficients were obtained, with the sole exception of YAN.

The fitting of the Mitscherlich law to the ridgeline points of the YAN contour plot is shown in Figure 7(c), whereas the estimates of the equation coefficients are displayed in Table 3, bottom row. Interestingly, YAN featured an  $a$  coefficient that was significantly lower than that of grape yield, although it was not significantly different from those of the other vine production parameters. In addition, the  $N_a$  of the YAN was not significantly different from the higher  $N_a$  among the vine production parameters, i.e., bunch weight and berry number.

The general evolution of the normalized concentration of anthocyanins and the total polyphenol index with the annual N rate did not follow the Mitscherlich law, but instead followed another relationship, which may be a line or

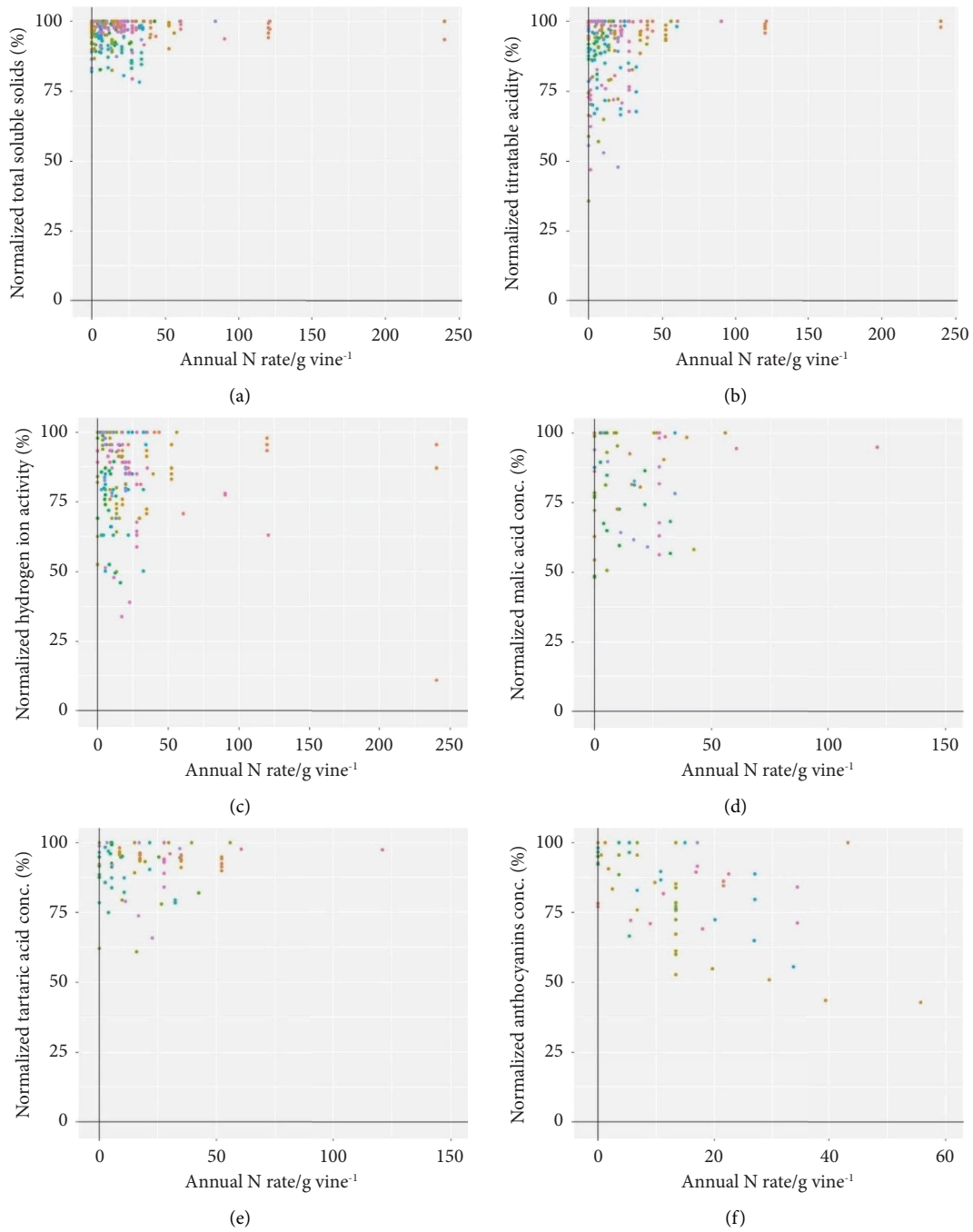


FIGURE 5: Continued.

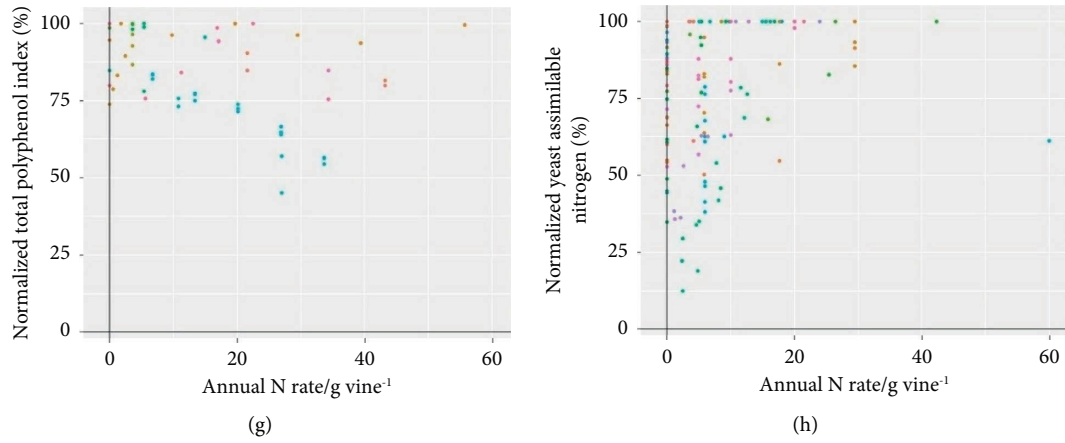


FIGURE 5: Trial-normalized grape quality traits against the annual nitrogen rate. Within the same scatter plot each colour stands for a single trial. (a) Total soluble solids. (b) Titratable acidity. (c) Hydrogen ion activity. (d) Malic acid concentration. (e) Tartaric acid concentration. (f) Anthocyanin concentration. (g) Total polyphenol index. (h) Yeast assimilable nitrogen.

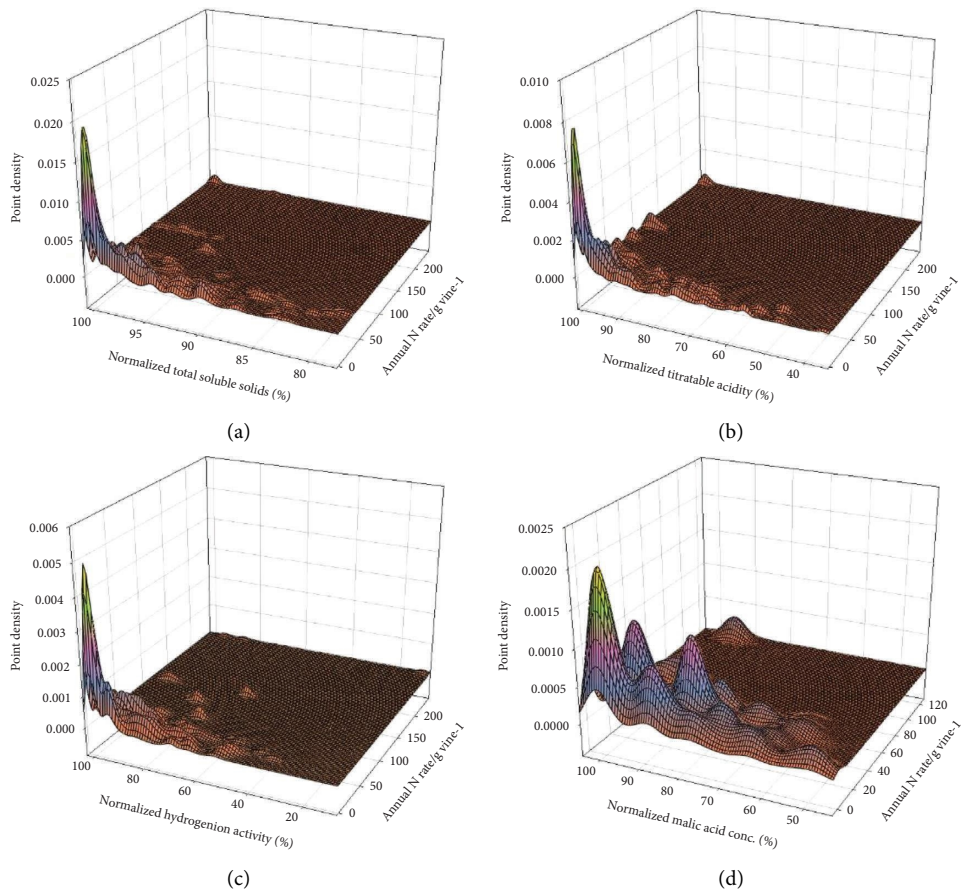


FIGURE 6: Continued.

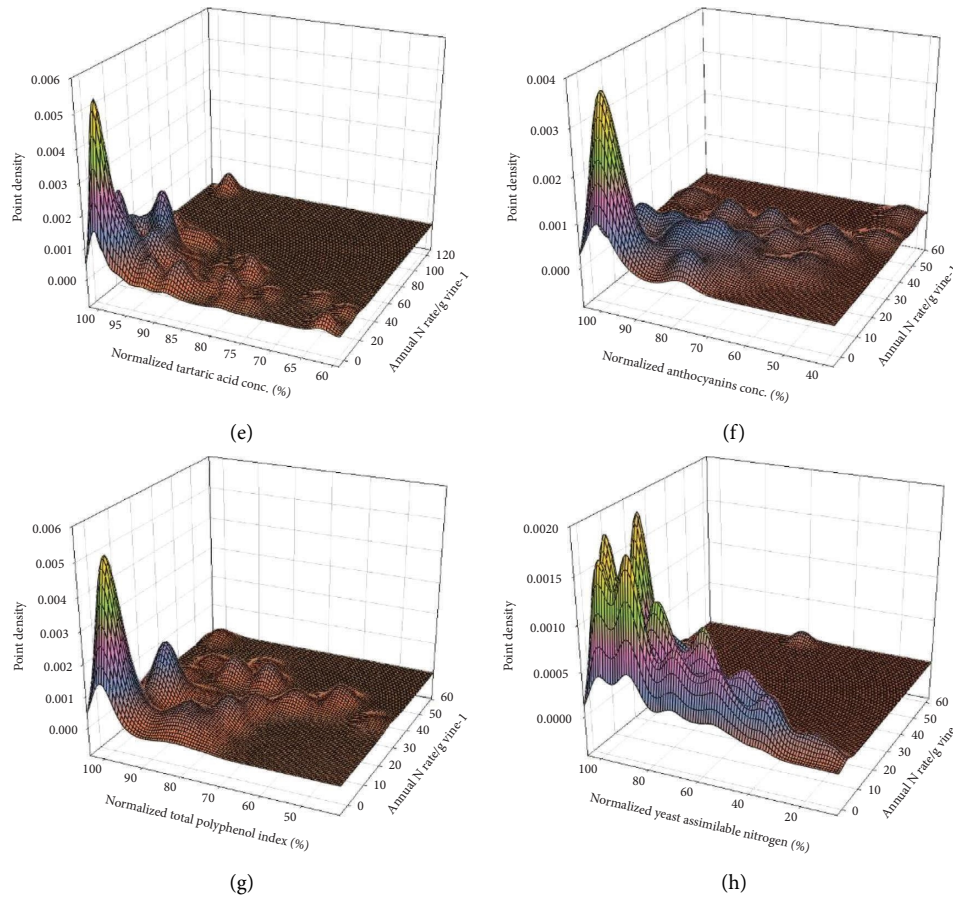


FIGURE 6: Density surfaces of the scatter plots in Figure 5 for the trial-normalized grape quality traits against the annual nitrogen rate. (a) Total soluble solids. (b) Titratable acidity. (c) Hydrogen ion activity. (d) Malic acid concentration. (e) Tartaric acid concentration. (f) Anthocyanin concentration. (g) Total polyphenol index. (h) Yeast assimilable nitrogen.

a sigmoid logistic curve; therefore, fitting of equation (11) was tried in both cases. The fitting of the sigmoid logistic curve to the ridgeline points for both traits is shown on the density contour plots in Figures 7(a) and 7(b), whereas the estimates of the  $N_a$ ,  $k$ ,  $b$ , and  $q$  factors are displayed in Table 4. The values of the coefficients of the logistic curves did not differ between these normalized quality traits.

For the grape quality traits for which no reasonable estimates for the Mitscherlich law or logistic curve coefficients were obtained, i.e., TSS, TA,  $a_{H^+}$ , malic acid, and tartaric acid, the annual N rate needed to attain their respective maxima ( $N_{max}$ ) was assessed. Therefore, at least a rough estimate of the annual N rate effect on these traits could be obtained (Table 5).

The annual N rates for achieving the maximum ( $N_{max}$ ) TSS, titratable acidity, and malic acid concentration were very similar and ranged from 1.5 to 3.1 g·plant<sup>-1</sup>, whereas for hydrogen ion activity,  $N_{max}$  was significantly different, i.e., 3 to 4 times higher (Table 4). The fact that the N rate for maximum hydrogen ion activity was remarkably above the N rate for maximum titratable acidity is surprising, as the former is directly proportional to the latter. For the tartaric acid concentration,  $N_{max}$  presented negative nonreasonable values (see Figure S2(c)) and it is not reported in Table 5.

#### 4. Discussion

The Mitscherlich law of diminishing-returns is often applied to annual field and horticultural crops [55, 56]. However, to the authors' best knowledge, it has not been used for multiyear woody crops, although it has been applied to study the N, P, K, and Ca nutrition of forest trees [57, 58]. Whatever the case, the lack of use of the Mitscherlich law in viticulture contrasts with the diminishing increases of the vine production parameters as a function of the annual N rate, as revealed in this meta-analysis. Indeed, the results from the current meta-analysis suggest that the Mitscherlich law can be regarded as adequately representing the vine production response to the annual N rate.

Grapevines for wine production are highly managed to optimise grape composition at the expense of yield. Therefore, it could be argued that the effects of increasing annual N application on yield would have been obtained under conditions mostly below the vine yield potential due to limiting factors other than N, such as crop level regulation, among others. However, the Mitscherlich law approach in this work is based on the normalization of vine production and quality traits, and even though the agronomic practices can change the absolute maximum yield, it

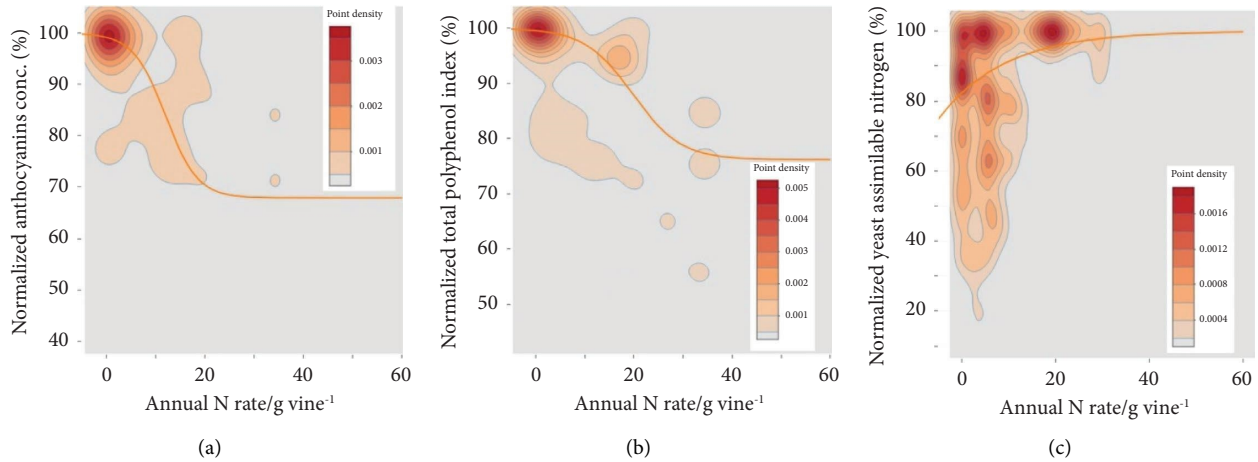


FIGURE 7: Contour plots of the density surfaces for which either the Mitscherlich law or a logistic curve could be reasonably fitted to the ridgeline points. The fitting was obtained by density-weighted generalized reduced gradient (GRG) nonlinear optimization of the sum of squared deviations between calculations and observations of the trial-normalized grape quality parameters. (a) Anthocyanin concentration. (b) Total polyphenol index. (c) Yeast assimilable nitrogen.

TABLE 4: Mean plus 95% confidence interval for the coefficients of the logistic curves fitted to the concentration of anthocyanins and the total polyphenol index.

Normalized parameter	$N_a$ (g·plant <sup>-1</sup> )	$k$	$b$	$q$
Anthocyanins concentration	$(0 \pm 5) 10^{-4a}$	$66 \pm 10^a$	$0.32 \pm 0.26^a$	$90 \pm 280^a$
Total polyphenol index	$(0 \pm 4) 10^{-9a}$	$76 \pm 6^a$	$0.22 \pm 0.12^a$	$110 \pm 300^a$

For each curve coefficient, the means sharing at least one letter are nonsignificantly different at the 95% confidence level.

TABLE 5: Nitrogen rate interval featuring the maximum in the normalized parameter density surface as represented in Figure 6 for the grape quality traits for which the Mitscherlich law or logistic curve could not be reasonably fitted.

Normalized parameter	$N_{max}$ (g·plant <sup>-1</sup> )
Total soluble solids	1.5–3.0
Titrate acidity	2.3–3.1
Hydrogen ion activity	7.0–8.8
Malic acid concentration	2.0–2.1
Tartaric acid concentration	—

has been previously shown that the amount of one specific nutrient needed to achieve such maximum yield or a fraction of it thereof is not affected [59–61]. In fact, in the present work, the data normalization left the annual N rate as the only controlled source of variation affecting the dependent variables (yield, TSS, etc.), in each trial. Consequently, the data normalization provided variables that could be used to compare the effects of the annual N rate on the dependent variables throughout trials differing in many other factors, including management practices, variety, rootstock, weather conditions, and soil.

Upon accepting the adequacy of the Mitscherlich law for grapevine, the values of Mitscherlich  $a$  and  $N_a$  coefficients obtained through curve fitting bear meaningful information about vine N nutrition characteristics. The Mitscherlich  $a$  coefficient is considered as a nutrient efficiency factor [47]. Furthermore, this efficiency can be interpreted as expressing the sensitivity of each vine production parameter, or when

appropriate, the quality traits, to the N rate. In the literature, the N nutrition effects on the build-up of reproductive and vegetative biomass in vines have been somewhat inconsistent [5, 20, 21, 28, 30]. These contrasting outcomes could be related to differences in soil and climate conditions, as well as diverse plant material and management practices. However, based on the nonsignificant differences in the  $a$  coefficient between grape yield and pruning weight found in this meta-analysis, it can be suggested that both reproductive and vegetative biomasses respond similarly to the annual N rate, and that the observed differences are likely caused by variations in cultivar, rootstock, soil and climate conditions, and/or management practices. Moreover, as the  $a$  coefficients for all the vine production parameters were found to be nonsignificantly different between themselves, all the grape yield components can be regarded as almost equally sensitive to the annual N rate.

Despite the absence of significant differences in the Mitscherlich  $a$  coefficient among the vine production parameters considered in the current study, some trends can still be discerned that are worth highlighting. In general, bunch number tended to be slightly more sensitive to the annual N rate than either bunch weight or berry weight. Therefore, the annual N rate seems to increase grape yield, to a greater degree by increasing the number of bunches, than the bunch or berry weight, or the berry number per bunch [62, 63]. Note that the  $a$  coefficient could not be computed for this last component of grape yield. Nevertheless, the berry number per bunch ( $n_{berry}$ ) is related to the bunch weight ( $w_{bunch}$ ) and berry weight ( $w_{berry}$ ) through the

following equation, and can thus be inferred that its sensitivity to the annual N rate could be similar.

$$n_{\text{berry}} = \frac{w_{\text{bunch}}}{w_{\text{berry}}}. \quad (12)$$

The likely reason why the Mitscherlich law had not previously used for multiyear perennial crops such as vines, is that, unlike most annual crops, perennials have permanent organs. These are the roots, trunk, and branches, which may store and carry over nutrients from one season to the next, thus buffering ongoing fertilization effects. As commonly used for annual field crops and vegetables, the Mitscherlich  $N_a$  coefficient is considered as the N rate that the plant absorbs from other sources different from the applied mineral fertilizer [47]. Therefore,  $N_a$  bears some information that is unconnected to the plant's characteristics, but connected to environmental features such as soil properties and others. In the case of multiyear woody crops,  $N_a$  can be considered to bear, in addition to the information on N uptake from the orchard environment, some information about the N storage in the plants' permanent organs. According to this point of view,  $N_a$  would equal the addition of the plants' internal and external N sources that are different from the deliberate N fertilization. Therefore, the Mitscherlich law would be an adequate choice for understanding the N fertilization effects on woody crops including vines.

Taking the Mitscherlich law as valid for vines, the annual N rate for 95% ( $N_{\_,95}$ ) expresses whole vine nutrition needs. Then, according to the  $N_{\_,95}$  estimates for grape yield, bunch number, and pruning weight (Table 3), both the reproductive and vegetative developments present the same N requirements. Specifically, as the annual N rate increases, vine N uptake is higher, and hence grape N uptake as well. However, berry weight also increases with the annual N rate, mainly because of water accumulation [64, 65], thus diluting the grape N content. This latter process somewhat counteracts the former, resulting in that significantly more N is needed to achieve 95% of maximum YAN than to achieve a grape yield of 95%.

According to the database built for this meta-analysis, the grape yield's  $N_{\_,95}$  value expressed on a per hectare basis was  $36 \pm 19 \text{ kg}\cdot\text{N}\cdot\text{ha}^{-1}$ , considering that in the mineral N trials fertilized through soil or fertigation, vine spacing was on average  $3.45 \pm 0.11 \text{ m}^2\cdot\text{vine}^{-1}$ . This  $N_{\_,95}$  is not significantly different ( $p = 0.16$ ) from the standard  $50 \text{ kg}\cdot\text{N}\cdot\text{ha}^{-1}$  [3] and other specific annual N rates for a given grapevine cultivar, such as  $34 \text{ kg}\cdot\text{N}\cdot\text{ha}^{-1}$  for "Viosinho" cultivar [20]. In the trials examined in this meta-analysis, soil organic matter and nitrogen contents were not often reported. However, the fitting of the Mitscherlich law has allowed unveiling this vineyard environmental N source, which is included within  $N_a$ , along with other vine internal and external N sources. Whatever the case, the  $N_a$  magnitude reveals the importance of these nonintentional N sources on vine nutrition. Specifically, these may contribute over 40% of  $N_{\_,95}$  to vines, thereby showing that  $50 \text{ kg}\cdot\text{N}\cdot\text{ha}^{-1}$  is rather an upper safe fertilization limit than anything else. Therefore, to fulfill the grape yield target,

while preserving the soil organic N, the average annual N rate found in this meta-analysis, which was between 30 and  $40 \text{ kg}\cdot\text{N}\cdot\text{ha}^{-1}$ , may suffice for fertilizing most vineyards, thus achieving a NUE ranging from 0.27 to  $0.36 \text{ t}\cdot\text{kg}\cdot\text{N}^{-1}$ , which is higher than the standard  $0.24 \text{ t}\cdot\text{kg}\cdot\text{N}^{-1}$  [3]. Note that to assess this NUE, a joint soil fertilization and fertigation grape yield of  $10.7 \pm 0.8 \text{ t}\cdot\text{ha}^{-1}$  was considered.

The  $N_{95}$  for grape yield was  $7 \pm 3 \text{ g}\cdot\text{N}\cdot\text{plant}^{-1}$ , i.e., significantly higher than the  $N_{\text{max}}$  for several quality traits, including total soluble solids, titratable acidity, and malic acid concentrations, which, in general, had  $N_{95}$  values ranging from 1.5 to  $3.1 \text{ g}\cdot\text{N}\cdot\text{plant}^{-1}$ . In this regard, note that  $N_{95} = N_{\_,95} - N_a$  (Figure 1) and  $N_{95}$  is conceptually the value to which  $N_{\text{max}}$  should be compared. Therefore, to fertilize for 95% of the maximum grape yield means reducing total soluble solids and acidity from their maxima in musts. Conversely, to obtain maximum total soluble solids and acidity in grapes means fertilizing with N up to a target between 84% and 89% of grape yield, i.e., between 6 and 11% less than 95%, which is not a significant loss and could be assumed by grapevine growers due to the enhancement in quality. Then, to maximize TSS and TA, between  $7.0$  and  $8.6 \text{ g}\cdot\text{N}\cdot\text{plant}^{-1}$  ( $N_{\text{max}} + N_a$ ), i.e., between  $20$  and  $25 \text{ kg}\cdot\text{N}\cdot\text{ha}^{-1}$ , should be applied. Accordingly, a NUE between  $0.41$  and  $0.47 \text{ t}\cdot\text{kg}\cdot\text{N}^{-1}$  would be achieved, which is between 69 and 96% greater than the standard of  $0.24 \text{ t}\cdot\text{kg}\cdot\text{N}^{-1}$ . Based on NUE, these lower N fertilization rates for optimizing grape quality can be interpreted as an important sustainability improvement of vineyards. Apart from this enhancement in NUE, these low N fertilization rates for optimizing grape quality can also decrease most of the variability in the vine production parameters, as shown in Figure 2, thus further improving grapevine management. However, these low annual N rates for optimizing grape quality might not be enough to replenish the soil organic N, and hence to keep the soil organic matter stock, whose exhaustion would threaten vineyard soil health and sustainability in the mid-to long-term.

Aside from the mid-to long-term sustainability issue, fertilizing to maximize TSS and TA by adding N at rates ranging from  $7.0$  to  $8.6 \text{ g}\cdot\text{N}\cdot\text{plant}^{-1}$  keeps the concentration of anthocyanins and the total polyphenol index over 98% of their respective maxima, according to Table 4 and Figure 7. Therefore, the annual N rates that maximize TSS and TA can also maximize their phenolic contents and decrease their variability, along with the vine production variability, as previously indicated. In contrast to overall quality, with such annual N rates, the yeast assimilable nitrogen concentration would be, on average, 90% of its maximum value. Consequently, the maximum YAN concentration achievable is not very far, and this does not pose an issue, as the objective for winemaking purposes, is to attain a minimum YAN concentration (usually,  $150 \text{ mg}\cdot\text{L}^{-1}$ ) that allows successful fermentations rather than a maximum YAN concentration. In fact, being under maximum achievable YAN values is adequate, because high nitrogen contents could be detrimental to the must quality for winemaking [17], and if, whatever the case, YAN is found to be too low, musts may be readily fortified with N in the winery [66].

Finally, vine N requirements change throughout the growing season. However, this subject was not an objective of the current meta-analysis. Therefore, since a higher efficiency is attained by applying the nutrient plants need in the precise moment that it is required, the annual N fertilization rates here obtained must be split accordingly. In this regard, the known evolution of the N uptake throughout the vine growing cycle [3] should be taken advantage of, and whatever the case, further investigated to quantify the exact N requirements of each vine developmental stage. Overall, the annual N rates obtained in this meta-analysis provide recommendations for average vineyard conditions, and further investigations should consider, in addition to the changes in vine N requirements throughout the growing season, the role played by vineyard variability in N fertilization scheduling. Particularly, the variability in terms of soil properties, including the important factor of vine water-availability, should be tackled to delineate zones with different management approaches under a precision viticulture context [67]. In this regard, in semiarid terroirs, the irrigation regime and application method, if any, should be considered to better adapt the N rates.

## 5. Conclusions

The current meta-analysis produced annual rates of nitrogen fertilization for vines that can be considered general for maximizing nitrogen use efficiency depending on production objectives, either yield or quality-oriented. With the aim of maximizing yield, 30 to 40 kg·N·ha<sup>-1</sup> would suffice, whereas when aiming at optimizing grape quality for winemaking, 20 to 25 kg·N·ha<sup>-1</sup> would be enough. Importantly, the former annual N rates may replenish the soil organic N pool and hence maintain the organic matter stock, whereas the latter may not. From a methodological perspective, this work has shown the utility of the Mitscherlich law for explaining vine response to annual N fertilization rates. Specifically, the hypothesis that the required amount of one specific nutrient does not depend on the absolute maximum yield was used as the basis, and furthermore, this hypothesis was expanded to other vine production parameters and quality traits besides yield. Therefore, the Mitscherlich law coefficients obtained in this work may allow practitioners and viticulturists to forecast relative grape yields, or conversely, to assess annual N fertilization rates in vineyards. Moreover, the use of this equation may improve soil N uptake modelling for vine growing. However, since the N fertilization guidelines produced are for general application, they are not completely definite, and further tuning might be required to account for vine age, cultivar-rootstock combination, and irrigation regime and method, among other distinctive vineyard features. In this regard, this work also provides the basis for planning future vine N fertilization trials.

## Data Availability

The data associated to this work are available upon reasonable request to the authors.

## Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the research and publication of this paper.

## Acknowledgments

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## Supplementary Materials

Figures S1 and S2 are provided as Supplementary Materials. In Figure S1, the N rate featuring the maximum point density for the vine production parameters is plotted against the number of grid nodes on each axis in the Kernel Smoothing estimation. (a) Grape yield. (b) Pruning weight. (c) Bunch number. (d) Bunch weight. (e) Berry number. (f) Berry weight. In Figure S2, the N rate featuring the maximum point density for the grape quality traits is plotted against the number of grid nodes on each axis in the Kernel Smoothing estimation. (a) Total Soluble Solids. (b) Titratable acidity. (c) Hydrogen ion activity. (d) Malic acid concentration. (e) Tartaric acid concentration. (f) Anthocyanins concentration. (g) Total polyphenol index. (h) Yeast Assimilable Nitrogen. (*Supplementary Materials*)

## References

- [1] T. Verdenal, Á. Dienes-Nagy, J. E. Spangenberg et al., "Understanding and managing nitrogen nutrition in grapevine: a review," *Oeno One*, vol. 55, no. 1, pp. 1–43, 2021.
- [2] L. Cheng, G. Xia, and T. Bates, "Growth and fruiting of young 'Concord' grapevines in relation to reserve nitrogen and carbohydrates," *Journal of the American Society for Horticultural Science*, vol. 129, no. 5, pp. 660–666, 2004.
- [3] O. Löhnertz, "Studies on the time course of nutrient uptake in *Vitis vinifera* (cv. Riesling)," in *German: Untersuchungen zum zeitlichen Verlauf der Nährstoffaufnahme bei Vitis vinifera* (cv. Riesling), p. 228, Forschungsanst, Geisenheim, Geisenheim, Germany, 1988.
- [4] A. Metay, J. Magnier, N. Guilpart, and A. Christophe, "Nitrogen supply controls vegetative growth, biomass and nitrogen allocation for grapevine (cv. Shiraz) grown in pots," *Functional Plant Biology*, vol. 42, no. 1, pp. 105–114, 2015.
- [5] R. P. Schreiner, J. Osborne, and P. A. Skinkis, "Nitrogen requirements of Pinot noir based on growth parameters, must composition and fermentation behavior," *American Journal of Enology and Viticulture*, vol. 69, no. 1, pp. 45–58, 2018.
- [6] Y. Guo, Y. Ji, J. Zhang, Q. Liu, J. Han, and L. Zhang, "Effects of water and nitrogen management on N<sub>2</sub>O emissions and NH<sub>3</sub> volatilization from a vineyard in North China," *Agricultural Water Management*, vol. 266, Article ID 107601, 2022.
- [7] M. L. Longbottom and P. R. Petrie, "Role of vineyard practices in generating and mitigating greenhouse gas emissions," *Australian Journal of Grape and Wine Research*, vol. 21, no. S1, pp. 522–536, 2015.
- [8] E. Nistor, A. G. Dobrei, A. Dobrei, D. Camen, F. Sala, and H. Prundeanu, "N<sub>2</sub>O, CO<sub>2</sub>, production, and C sequestration



- in vineyards: a review,” *Water, Air, and Soil Pollution*, vol. 229, no. 9, 2018.
- [9] D. J. Silva, L. H. Bassoi, M. G. D. Rocha, A. O. D. Silva, and M. D. I. Deon, “Organic and nitrogen fertilization of soil under ‘Syrah’ grapevine: effects on soil chemical properties and nitrate concentration,” *Revista Brasileira de Ciência do Solo*, vol. 40, 2016.
- [10] M. Sprenger, M. Erhardt, M. Riedel, and M. Weiler, “Historical tracking of nitrate in contrasting vineyards using water isotopes and nitrate depth profiles,” *Agriculture, Ecosystems & Environment*, vol. 222, pp. 185–192, 2016.
- [11] M. Keller, “From the ASEV soil environment and vine mineral nutrition symposium: deficit irrigation and vine mineral nutrition,” *American Journal of Enology and Viticulture*, vol. 56, no. 3, pp. 267–283, 2005.
- [12] F. Gastal and G. Lemaire, “N uptake and distribution in crops: an agronomical and ecophysiological perspective,” *Journal of Experimental Botany*, vol. 53, no. 370, pp. 789–799, 2002.
- [13] S. E. Spayd, R. L. Wample, R. G. Stevens, R. G. Evans, and A. K. Kawakami, “Nitrogen fertilization of White Riesling in Washington. Effects on petiole nutrient concentration, yield, yield components and vegetative growth,” *American Journal of Enology and Viticulture*, vol. 44, no. 4, pp. 378–386, 1993.
- [14] M. Keller, B. Hess, and H. Schwager, “Carbon and nitrogen partitioning in *Vitis vinifera* L.: responses to nitrogen supply and limiting irradiance,” *Vitis*, vol. 34, no. 1, pp. 19–26, 1995.
- [15] E. A. Mitscherlich, “The law of minimums and the law of decreasing soil yield (in German: das gesetz der minimums und das gesetz des abnehmenden bodenertrags),” *Landwirtsch. Jahrb.*, vol. 38, pp. 537–552, 1909.
- [16] W. J. Conrady, “Partitioning of mineral nutrients and timing of fertilizer applications for optimum efficiency,” in *Proceedings of the Soil Environment and Vine Mineral Nutrition Symposium*, P. Christensen and D. R. Smart, Eds., pp. 69–81, American Society for Enology and Viticulture, Davis, CA, USA, 2005.
- [17] S.-J. Bell and P. A. Henschke, “Implications of nitrogen nutrition for grapes, fermentation and wine,” *Australian Journal of Grape and Wine Research*, vol. 11, no. 3, pp. 242–295, 2005.
- [18] G. Brunetto, G. W. B. D. Melo, M. Toselli, M. Quartieri, and M. Tagliavini, “The role of mineral nutrition on yields and fruit quality in grapevine, pear and apple,” *Revista Brasileira de Fruticultura*, vol. 37, no. 4, pp. 1089–1104, 2015.
- [19] G. Xu, X. Fan, and A. J. Miller, “Plant nitrogen assimilation and use efficiency,” *Annual Review of Plant Biology*, vol. 63, no. 1, pp. 153–182, 2012.
- [20] M. Arrobas, I. Q. Ferreira, S. Freitas, J. Verdial, and M. Â. Rodrigues, “Guidelines for fertilizer use in vineyards based on nutrient content of grapevine parts,” *Scientia Horticulturae*, vol. 172, pp. 191–198, 2014.
- [21] N. Guilpart, A. Metay, and C. Gary, “Grapevine bud fertility and number of berries per bunch are determined by water and nitrogen stress around flowering in the previous year,” *European Journal of Agronomy*, vol. 54, pp. 9–20, 2014.
- [22] T. Thomidis, E. Zioziou, S. Koundouras, C. Karagiannidis, I. Navrozidis, and N. Nikolaou, “Effects of nitrogen and irrigation on the quality of grapes and the susceptibility to Botrytis bunch rot,” *Scientia Horticulturae*, vol. 212, pp. 60–68, 2016.
- [23] M. Oliveira, J. Teles, P. Barbosa, F. Olazabal, and J. Queiroz, “Shading of the fruit zone to reduce grape yield and quality losses caused by sunburn,” *OENO One*, vol. 48, no. 3, pp. 179–187, 2014.
- [24] A. Habran, M. Commisso, P. Helwi et al., “Rootstocks/scion/nitrogen interactions affect secondary metabolism in the grape berry,” *Frontiers in Plant Science*, vol. 7, 2016.
- [25] J. Portu, P. Santamaría, R. López, and T. Garde-Cerdán, “Phenolic composition of Tempranillo grapes following foliar applications of phenylalanine and urea: a two-year study,” *Scientia Horticulturae*, vol. 219, pp. 191–199, 2017.
- [26] D. C. Mundy, “A review of the direct and indirect effects of nitrogen on botrytis bunch rot in wine grapes,” *New Zealand Plant Protection*, vol. 61, pp. 306–310, 2008.
- [27] T. Garde-Cerdán, G. Gutiérrez-Gamboa, J. Fernández-Novales, E. Pérez-Álvarez, and M. Diago, “Towards the definition of optimal grape harvest time in Grenache grapevines: nitrogenous maturity,” *Scientia Horticulturae*, vol. 239, pp. 9–16, 2018.
- [28] K. D. Hannam, G. H. Neilsen, T. Forge, and D. Neilsen, “The concentration of yeast assimilable nitrogen in Merlot grape juice is increased by N fertilization and reduced irrigation,” *Canadian Journal of Plant Science*, vol. 93, no. 1, pp. 37–45, 2013.
- [29] A. W. Linsenmeier, U. Loos, and O. Löhnertz, “Must composition and nitrogen uptake in a long-term trial as affected by timing of nitrogen fertilization in a cool-climate Riesling vineyard,” *American Journal of Enology and Viticulture*, vol. 59, no. 3, pp. 255–264, 2008.
- [30] T. Tian, M. Ruppel, J. Osborne, E. Tomasino, and R. P. Schreiner, “Fertilize or supplement: the impact of nitrogen on vine productivity and wine sensory properties in Chardonnay,” *American Journal of Enology and Viticulture*, vol. 73, no. 3, pp. 156–169, 2022.
- [31] G. V. Glass, “Primary, secondary, and meta-analysis of research,” *Educational Researcher*, vol. 5, no. 10, pp. 3–8, 1976.
- [32] M. Borenstein, L. V. Hedges, J. P. T. Higgins, and H. R. Rothstein, *Introduction to Meta-Analysis*, John Wiley & Sons, Ltd, Chichester, UK, 2009.
- [33] D. Mitchell-McCallister, A. Cano, and C. West, “Meta-analysis of crop water use efficiency by irrigation system in the Texas High Plains,” *Irrigation Science*, vol. 38, no. 5–6, pp. 535–546, 2020.
- [34] P. Previtali, F. Giorgini, R. S. Mullen, N. K. Dookozlian, K. L. Wilkinson, and C. M. Ford, “A systematic review and meta-analysis of vineyard techniques used to delay ripening,” *Horticulture Research*, vol. 9, 2022.
- [35] E. González-Domínguez, G. Fedele, T. Caffi et al., “A network meta-analysis provides new insight into fungicide scheduling for the control of Botrytis cinerea in vineyards,” *Pest Management Science*, vol. 75, no. 2, pp. 324–332, 2019.
- [36] S. Winter, T. Bauer, P. Strauss et al., “Effects of vegetation management intensity on biodiversity and ecosystem services in vineyards: a meta-analysis,” *Journal of Applied Ecology*, vol. 55, no. 5, pp. 2484–2495, 2018.
- [37] N. Katayama, I. Bouam, C. Koshida, and Y. G. Baba, “Biodiversity and yield under different land-use types in orchard/vineyard landscapes: a meta-analysis,” *Biological Conservation*, vol. 229, pp. 125–133, 2019.
- [38] F. T. Payen, A. Sykes, M. Aitkenhead, P. Alexander, D. Moran, and M. MacLeod, “Soil organic carbon sequestration rates in vineyard agroecosystems under different soil management practices: a meta-analysis,” *Journal of Cleaner Production*, vol. 290, Article ID 125736, 2021.
- [39] P. Ribéreau-Gayon, D. Dubourdieu, B. Donèche, and A. Lonvaud, *Handbook of Enology*, vol. 1, John Wiley & Sons, Chichester, UK, 2006.

- [40] I. Doymaz, "Sun drying of seedless and seeded grapes," *Journal of Food Science and Technology*, vol. 49, no. 2, pp. 214–220, 2012.
- [41] P. R. Porceddu, L. Rosati, and M. Dionigi, "Evaluation of temporal variations in moisture and calorific value of vine and olive pruning," *Journal of Agricultural Engineering*, vol. 41, no. 4, pp. 9–13, 2012.
- [42] OIV, *Compendium of International Methods of Wine and Must Analysis*, International Organisation of Vine and Wine, Dijon, France, 2022.
- [43] C. A. Zuritz, E. M. Puentes, H. H. Mathey et al., "Density, viscosity and coefficient of thermal expansion of clear grape juice at different soluble solid concentrations and temperatures," *Journal of Food Engineering*, vol. 71, no. 2, pp. 143–149, 2005.
- [44] R. Morlat and R. Symoneaux, "Long-term additions of organic amendments in a Loire Valley vineyard on a calcareous sandy soil. III. Effects on fruit composition and chemical and sensory characteristics of cabernet franc wine," *American Journal of Enology and Viticulture*, vol. 59, no. 4, pp. 375–386, 2008.
- [45] S. A. Saseendran, L. R. Ahuja, L. W. Ma et al., "Developing and normalizing average corn crop water production functions across years and locations using a system model," *Agricultural Water Management*, vol. 157, pp. 65–77, 2015.
- [46] E. V. Maas and G. J. Hoffman, "Crop salt tolerance-current assessment," *Journal of the Irrigation and Drainage Division*, vol. 103, no. 2, pp. 115–134, 1977.
- [47] M. S. Dhanoa, R. Sanderson, and L. M. Cardenas, "Overview and application of the Mitscherlich equation and its extensions to estimate the soil nitrogen pool fraction associated with crop yield and nitrous oxide emission," *Advances in Agronomy*, vol. 174, pp. 269–295, 2022.
- [48] L. S. Lasdon, A. D. Waren, A. Jain, and M. Ratner, "Design and testing of a generalized reduced gradient code for non-linear programming," *ACM Transactions on Mathematical Software*, vol. 4, no. 1, pp. 34–50, 1978.
- [49] M. P. Wand and M. C. Jones, *Kernel Smoothing*, Chapman & Hall/CRC Monographs on Statistics and Applied Probability, Boca Raton, FL, USA, 1995.
- [50] R-project, "The R foundation for statistical computing 2022 R," 2022, <https://www.r-project.org/>.
- [51] OIV, *Annual Assessment of the World Vine and Wine Sector in 2021*, International Organisation of Vine and Wine, Dijon, France, 2022.
- [52] F. Champagnol, "Elements of vine physiology and general viticulture," in *French: Eléments de Physiologie de la Vigne et de Viticulture Générale*, p. 351, Dehan. Montpellier, France, 1984.
- [53] R. E. Smart, "Principles of grapevine canopy microclimate manipulation with implications for yield and quality. A review," *American Journal of Enology and Viticulture*, vol. 36, no. 3, pp. 230–239, 1985.
- [54] B. Bravdo, Y. Hepner, C. Loinger, S. Cohen, and H. Tabacman, "Effect of crop level and crop load on growth, yield, must and wine composition, and quality of Cabernet Sauvignon," *American Journal of Enology and Viticulture*, vol. 36, no. 2, pp. 125–131, 1985.
- [55] P. E. McNall, "The law of diminishing returns in agriculture," *Journal of Agricultural Research*, vol. 47, no. 3, pp. 167–178, 1993.
- [56] I. E. P. Ferreira, S. S. Zocchi, and D. Baron, "Reconciling the Mitscherlich's law of diminishing returns with Liebig's law of the minimum. Some results on crop modeling," *Mathematical Biosciences*, vol. 293, pp. 29–37, 2017.
- [57] G. G. Wang and K. Klinka, "White spruce foliar nutrient concentrations in relation to tree growth and soil nutrient amounts," *Forest Ecology and Management*, vol. 98, no. 1, pp. 89–99, 1997.
- [58] E. G. Mason, M. F. Skinner, N. J. Barrow, A. Lowe, and H. E. Bown, "Predicting phosphorus requirements of young *Pinus radiata* using sequential Bray soil extraction," *Plant and Soil*, vol. 339, no. 1-2, pp. 425–434, 2011.
- [59] E. A. Mitscherlich, "Determination of the soil fertilizer requirements," in *German: Die bestimmung des düngerbedürfnisses der bodens*, Verlagsbuchhandlung Paul Parey, p. 119, Berlin, Germany, 1930.
- [60] C. T. de Wit, "Resource use efficiency in agriculture," *Agricultural Systems*, vol. 40, no. 1–3, pp. 125–151, 1992.
- [61] H. J. M. van Grinsven, P. Ebanyat, M. Glendining et al., "Establishing long-term nitrogen response of global cereals to assess sustainable fertilizer rates," *Nature Food*, vol. 3, no. 2, pp. 122–132, 2022.
- [62] M. Botelho, A. Cruz, J. Ricardo-da-Silva, R. de Castro, and H. Ribeiro, "Mechanical pruning and soil fertilization with distinct organic amendments in vineyards of Syrah: effects on vegetative and reproductive growth," *Agronomy*, vol. 10, no. 8, 2020.
- [63] L. O. Stefanello, R. Schwalbert, R. A. Schwalbert et al., "Nitrogen supply method affects growth, yield and must composition of young grape vines (*Vitis vinifera* L. cv Alicante Bouschet) in southern Brazil," *Scientia Horticulturae*, vol. 261, Article ID 108910, 2020.
- [64] Z. W. Dai, P. Vivin, F. Barrieu, N. Ollat, and S. Delrot, "Physiological and modelling approaches to understand water and carbon fluxes during grape berry growth and quality development: a review," *Australian Journal of Grape and Wine Research*, vol. 16, no. S1, pp. 70–85, 2010.
- [65] J. Zhu, M. Génard, S. Poni et al., "Modelling grape growth in relation to whole-plant carbon and water fluxes," *Journal of Experimental Botany*, vol. 70, no. 9, pp. 2505–2521, 2019.
- [66] P. Santamaría, L. González-Arenzana, P. Garijo, A. R. Gutiérrez, and R. López, "Nitrogen sources added to must: effect on the fermentations and on the Tempranillo red wine quality," *Fermentation*, vol. 6, no. 3, p. 79, 2020.
- [67] L. G. Santesteban, "Precision viticulture and advanced analytics. A short review," *Food Chemistry*, vol. 279, pp. 58–62, 2019.