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Do macro-nutrient balanced compositions in a subspace of the ionome of *Opuntia ficus-indica* (L.) Miller maximize fruiting?

Fidel Blanco-Macías[1], Jesús Salvador Rodríguez-Barrientos[2], Miguel Márquez-Madrid[1], Alfredo Lara-Herrera[2], José Hernández-Martínez[2], Ricardo David Valdez-Cepeda[1,3*]

[1]Universidad Autónoma Chapingo, Centro Regional Universitario Centro-Norte, Cruz del Sur No. 100, Col. Constelación. Apdo. Postal 196, El Orito, CP 98085 Zacatecas, Zac., Mexico.
[2]Universidad Autónoma de Zacatecas, Unidad Académica de Agronomía, Km 15.5 Carretera Zacatecas-Guadalajara, CP 98183, Zacatecas, Zac., Mexico
[3]Universidad Autónoma de Zacatecas, Unidad Académica de Matemáticas, Paseo Solidaridad s/n, CP 98064 Zacatecas, Zac., Mexico

*Corresponding Author: vacrida@hotmail.com

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Abstract. The current state of knowledge on *Opuntia ficus-indica* nutrient requirements has important limitations. Macro-nutrient mean concentrations proposed as standards do not consider balances between (or among) elements, and the available compositional nutrient diagnosis norms are not supported by orthogonal references easy to estimate. We hypothesize that *O. ficus-indica* plants pose macro-nutrient requirements in terms of balanced compositions for producing fruits. The isometric log-ratios allowed us to test that *O. ficus-indica* variety 'Rojo Pelón' plants maximize fruit yield under the basis of true-negative (T-N) or macro-nutrient-balanced compositions in their 1-year-old cladodes. These T-N compositions can be estimated in a straightforward manner using two optimal delimiters, that is, the Mahalanobis distance across the involved isometric log-ratios linked with the highest value of Class Sum of Squares, and a target yield previously defined. When used as a target, these balanced compositions allowed us to identify that true-positive compositions were characterized by P deficiencies and N luxurious consumptions. Novel techniques to perform suitable recommendations based on correct diagnosis interpretation should be developed to address objectively the needed change to enhance the nutrient status of true-positive imbalanced compositions.

Keywords: *Calcium, Magnesium, Nitrogen, Phosphorus, Potassium, Nutrient interactions*

Introduction

Opuntia ficus-indica (L.) Miller species is cultivated because its tender shoots are widely used for human consumption as vegetables mainly in Mexico, its mature cladodes are commonly used for animal feed, and its fruits are considered of high value (Russell and Felker, 1987; Blanco-Macías *et al.,* 2010). Today, it is a very important crop in at least 25 countries around the world (Valdez-Cepeda *et al.*, 2013). Its whole fruit, pulp, flowers, seeds, and peel contain various groups of bioactive compounds. Among these compounds are phenolic acids, flavonoids, anthocyanins, carotenoids, betalains, sterols, lignans, saponins, vitamin E, and vitamin C (Tahir *et al*., 2019). The identified bioactive compounds are demonstrated to be endowed with biologically relative activity like antioxidant, antimicrobial, anticancer, anti-diabetes Mellitus, hypertension, hypercholesterolemia, rheumatic pain, antiulcerogenic activity, gastric mucosa diseases, and asthma (Tahir et al., 2019). As an example, its fruit extract has pH-sensitive, antioxidant, and antimicrobial abilities (Yao *et al*., 2020).

Hitherto, although *O. ficus-indica* species is becoming a very important crop in various countries around the world, studies on its nutrient requirements are outdated and fragmented (Mayer and Cushman, 2019). An occasional number of studies have documented the macro-, and micro-nutrient concentrations in cladodes. Some of them are focused on the mineral contents of cladodes to demonstrate their forage potential. Other studies involved mineral contents in tender pads and fruits to see their nutritional contribution to the human diet.

Other research works are focused on the effect of soil fertilization on fruit production. For instance, nitrogen fertilization (0, 60, 120 kg ha^{-1}) did not affect flower bud formation in *O. ficus-indica* (Nerd and Mizrahi, 1994). No *O. ficus-indica* fruit response was obtained within the case of fertilizer application even comparing different rates of N, P, K, and Mg to the control that had never been fertilized (Karim *et al*., 1997; Galizzi *et al*., 2004). Three treatments of nitrogen and phosphorus fertilization (N-P_2O_5) had nil effect on *O. ficus-indica* fruit yielding within the first year; however, the doses of 60 kg ha^{-1} N or 80 kg ha^{-1} N-P_2O_5 alone increased the fruit yield by +3 and + 6.1 kg plant^{-1}, respectively, compared with the control (Arba *et al*., 2017). Those results indicate that fertilizer response is difficult thanks to the cladodes' high moisture content and also the large mass of *Opuntia*'s buffers' nutrient changes (Felker and Bunch, 2009). Notably, all these prior research works did not relate cladode nutrient concentrations or their interactions or balances with yield or quality of yield in terms of fruit (prickly pear or cactus pear) through statistical trends or functions.

A few research works have involved 1-year-old cladode mineral contents and biomass or fruit production relationships. Recently, macro-nutrient norms for *O. ficus-indica* (L.) Miller variety 'Rojo Pelón' fruiting were estimated employing the Boundary-Line Approach (B-LA) by Hernández-Vidal *et al.* (2021a); the estimated optimum concentrations were N = 10.2 g kg^{-1}, P = 3.04 g kg^{-1}, K = 35.18 g kg^{-1}, Ca = 36.65 g kg^{-1}, and Mg = 13.83 g kg^{-1} as linked to maximum fruit yield that varies between 1901.13 and 1984.41 g cladode^{-1}. Moreover, *O. ficus-indica* (L.) Miller variety 'Rojo Pelón' fruiting Compositional Nutrient Diagnosis (CND) norms were proposed by Hernández-Vidal *et al*. (2021b); these CND standards are associated with the following 1-year-old fruiting cladode mean concentrations: N = 9.58 g kg^{-1}, P = 3.18 g kg^{-1}, K = 35.07 g kg^{-1}, Ca = 42.28 g kg^{-1}, and Mg = 14.48 g kg^{-1}.

These last findings mean that both nutrient storage (at the plant and cladode levels), and best-fit plant nutrient requirements for *O. ficus-indica* fruiting had been remaining unknown practically (Inglese *et al*., 1995) until a very short time ago. However, the current state of knowledge on *O. ficus-indica* nutrient requirements has important limitations. The B-LA does not consider interactions or balances between (or among) nutrients (Hernández-Vidal *et al*., 2021a). In addition, the CND norms are supported by the centered log-ratio (*clr*) transformation to properly handle compositional data. However, the *clr* transformation linear restriction in the components allows a D–1 dimensional representation, and then orthogonal references in that subspace are not obtained in a straightforward manner (Egozcue *et al*., 2003). Therefore, the isometric log-ratio (*ilr*) technique was developed to structure D components into D–1 orthogonal parts or balances amenable to multivariate analysis (Egozcue *et al*., 2003). These balances can be developed as orthogonal contrasts considering the knowledge of nutrient interactions in plants (Parent *et al*., 2013a), and soil (Parent *et al*., 2013b).

In this context, nutrient-balanced compositions (i.e. orthonormal balances, *irl*'s) in 1-year-old cladodes as compositional spaces –i.e. complex interaction systems or concentration vectors representative of *O. ficus-indica* plant ionome– for fruiting remain unknown yet (Hernández-Vidal *et al*., 2021a). It deserves to be noted that, in compositional spaces, a component is inherently

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related to each other because changing a proportion inherently affects at least another proportion (Aitchison, 1994; Parent *et al*. 2013a). Focusing on plant nutrition, nutrient balances may be considered as components or parts of the plant (i.e. tissue of reference) as compositional data or ionome data (see Parent *et al*., 2013ab). So, we hypothesize that *O. ficus-indica* plants pose macro-nutrient requirements in terms of balanced compositions for producing fruits. Therefore, this research work aimed i) to develop macro-nutrient orthonormal hierarchical balances in a subspace of the ionome of *Opuntia ficus-indica* (L.) Miller variety 'Rojo Pelón' for fruit production, and ii) to spot macro-nutrient balanced compositions and their corresponding optimum concentrations related to high-yield (g Cladode^{-1}).

Material and Methods

Study site

An orchard was established in 2006 at the experimental field of the "Centro Regional Norte Universitario Centro Norte" of the "Universidad Autónoma Chapingo" at 22° 44′ 49.6″ north latitude, 102° 46′ 28.2″ west longitude, and 2296 masl, near the city of Zacatecas, Mexico. The regional climate is classified as BS1kw (w), i.e., temperate semiarid climate, with an average annual temperature that varies between 12 and 18 °C, an average annual rainfall of 472 mm, and most of the precipitation (> 65%) occurs from June to August. The soil at the site had a clay loam texture, a very slightly alkaline pH (7.5), and high content matter (3.23%). Extractable nutrient levels were as follows: Availability for inorganic N was low (15 mg kg^{-1}), very high for P (40.5 mg kg^{-1}), medium for K (230 mg kg^{-1}), high for Ca (4371 mg kg^{-1}), moderately high for Mg (569 mg kg^{-1}), moderately low for iron (Fe, 7.85 mg kg^{-1}), very high for copper (Cu, 7.47 mg kg^{-1}), excessive for zinc (Zn, 14.6 mg kg^{-1}), moderately for manganese (Mn, 6.13 mg kg^{-1}), and medium for boron (B, 1.59 mg kg^{-1}). The high content of organic matter can be due to the plot has been used as a fruit orchard for the previous 50 years, involving regular organic soil amendment with cow manure and incorporation of tree foliage on the ground (Valdez-Cepeda *et al*., 2013). The Ca high content could be associated with the calcareous origin of the soil. The orchard was established using 20 mother cladodes. Plant density was 625 plants ha^{-1} (4 m between rows and 4 m between trees). Then, 20 trees with a natural vessel-shaped structure were growing in the field. The management of the orchard consisted of removing weeds each year at the end of spring and summer through minimum tillage. Fertilization, irrigation, and other agronomic practices were not performed.

Sample collection and analysis

Two hundred twenty-eight fruiting cladodes and 1744 fruits of *O. ficus-indica* variety "Rojo Pelón" were considered in this research. Fruiting cladodes and their fruits were taken for four consecutive years (2012–2015). The collection was carried out as follows: 2012, 60 cladodes and 480 fruits; 2013, 52 cladodes and 364 fruits; 2014, 56 cladodes and 420 fruits; and 2015, 60 cladodes and 480 fruits. All cladodes were selected from the uppermost part of the trees to ensure they were 1-year-old. Cladodes having from 1 to 15 fruits were chosen; four cladodes having each of these numbers of fruits were selected from different plant orientations (north, south, east, and west). None of these cladodes had young shoots. All fruits were harvested when most of the fruits showed peel coloration change indicating the beginning of fruit ripeness. All 1-year-old cladodes and their fruits were identified. Each fruit weight was registered. Besides, all 228 detached fruiting cladodes were cleaned with distilled water and immediately weighed. Afterward, the cladodes were cut into slices and dehydrated to constant weight in an oven at 75 °C for 36 h, and then their dry weights were registered. Dry tissue of cladode samples was milled and then digested with a mixture of hydrochloric and nitric acids ($HCl:HNO_3$, 3:1). Afterward, these samples were used to determine macro-nutrient concentrations. The N concentration was determined by the Kjeldahl method, whereas the P content was estimated by reduction with the molybdo-vanadate technique using an optical photo spectrometer (Thermo Spectronic, Helios Epsilon model, USA®). The K, Ca, and Mg concentrations were determined with an atomic absorption spectrophotometer (UNICAM Solar model 9626).

Macro-nutrient orthogonal hierarchical balances

A database of N, P, K, Ca, and Mg concentrations (g kg^{-1}) in all 228 fruiting cladodes and their fruit weights (g) was used to develop macro-nutrient orthogonal hierarchical balances in a subspace of *O. ficus-indica* variety "Rojo Pelón". Such a technique was described by Parent *et al*. (2013ab), Lima de Deus *et al*. (2018), Leitzke Betemps *et al*. (2020), and Vahl de Paula *et al*. (2020). This approach obeys the principle that points out nutrient concentrations (as compositional data) are parts of a whole (Parent *et al*., 2013ab) –really a complex system–, between zero and the unit of measurement (for instance, 1 if measurements are in parts per 1 or 100 if they are in percentages); then, not transforming such a kind of data will probably yield biased results. Consequently, it is a need to estimate an additional nutrient (named filling value, F_v) to form a d-dimensional nutrient arrangement, i.e., a simplex (S^d) made of d+1 nutrient proportions defined as (Parent and Dafir, 1992):

$$S^d = [(\mathrm{N, P, K}, \ldots, F_v): \mathrm{N}>0, \mathrm{P}>0, \mathrm{K}>0, \ldots, F_v>0, \mathrm{N+P+K+K}+\ldots+ F_v = 100], \quad (1)$$

where 100 is the dry matter concentration (%); N, P, K, …, and F_v are nutrient proportions computed (as proposed by Aitchison, 1982) by

$$F_v = 100 - (\mathrm{N + P + K} + \ldots). \quad (2)$$

Of course, the main components of the F_v value are C, O, and H, as found in products of photosynthesis.

Another form of obtaining the simplex S^d, as row vectors, is dividing each component by the sum of all the components and then multiplying by the unit of measurement or closure operator (it can be 1, 100, or 1000, among others depending on the measurement unit) in each composition, as pointed out by Aitchison (2002). Whatever the way to obtain the unit simplex S^d, it provides scale invariance to compositions and any meaningful function of it must be expressible in terms of ratios of components, and the obvious fact ratios are unaltered in the process of forming sub-compositions (Aitchison, 1994).

This kind of compositional data can be analyzed using known Aitchison geometry. There is a function that transforms compositions from simplex (S) to the real ($\mathbb{R}$) space, that is (Egozcue *et al*., 2003):

$$ilr : \mathrm{S}^{\mathrm{D}} \rightarrow \mathbb{R}^{\mathrm{D}-1}, \quad (3)$$

where *irl* = isometric log-ratio, and D = components (macro-nutrients in the present work). In this context, rows (row vectors or compositions) and columns (macro-nutrients or components) may be arranged as a matrix of proportions. Thus, we must have in mind the idea that D–part compositions can be compressed into D–1 isometric log-ratios (ilr_i's) or orthonormal hierarchical balances (Egozcue *et al*., 2003), the exact number of degrees of freedom available in compositions (Aitchison, 1994; Leitzke Betemps *et al*., 2020). Therefore, the orthonormal balances between selected subsets of components at the numerator and denominator can be estimated by

$$ilr_i = \sqrt{\frac{rs}{r+s}} ln\left(\frac{G_{Num}}{G_{Den}}\right), \quad (4)$$

where r and s are numbers of components at the numerator and denominator, respectively; and G_{Num} and G_{Den} are geometric means of components at the numerator and denominator, respectively.

In the present study, components (*i.e.* macro-nutrients) were arranged as meaningful orthogonal and orthonormal balances in a sequential binary partition (SBP) as appreciated in Table 1 under the consideration that the compositional vector mapped in the space is $\mathbb{R}^{D-1}$, and $\mathbb{R}^2$ neatly contains the compositions in their corresponding distribution in an elliptical spatial region (Aitchison, 1982). In the present case, D–1 = 4 orthonormal ilr_i's or 5 orthonormal ilr_i's when F_v is involved. Having in mind such a goal, this work involved N, P, K, Ca, Mg, and F_v components from their 0 to 100% constrained space compositional data to estimate the ilr_i's.

As a result, the SBP describes the D–1 orthonormal balance between parts and groups of parts. It is a (D–1) x D matrix (Table 1), in which parts labeled '+1' (group numerator) are balanced with parts labeled '–1' (group denominator)'. A part labeled '0' is excluded from the balance between parts. In this way, the geometric subspace of the ionome of *Opuntia ficus-indica* (L.) Miller was partitioned sequentially into contrasts at every hierarchically ordered row until each of the (+1) and (–1) groups contain a single part. As a result, there is a sound SBP for this subspace under the basis of widely known macro-nutrient interactions. N and P are anions, whereas K, Ca, and Mg are cations. N, P, K, and Mg are phloem-mobile, while Ca does not (Tagliavini *et al*., 2000). N contributes to protein synthesis, and P does to yield energy. Macro-nutrients K, Ca, and Mg may be competing (Marschner, 2012). Ca and Mg may reflect the geographical position and soil mineralogy (Walworth and Sumner, 1987).

Afterward, the ilr_i's were used to compute the Mahalanobis distance (M) for each composition through

$$M=\sqrt{(ilr_i - Median\ ilr_i)^T\ COV^{-1}(ilr_i - Median\ ilr_i)}\ , \quad (5)$$

where *Median* ilr_i represents the barycenter of the sample (n = 228 compositions in this study) and COV its covariance matrix. The Mahalanobis distance across each ilr_i is a measure of the multivariate distance between each composition and the corresponding median reference balance. *COV* is the covariance matrix, and T indicates that the ilr_i vector is transposed. It deserves to be noted that M^2 is distributed like a χ^2 variable. As a consequence, and by its definition, M can account for the usual inclined hyper ellipsoidal shape of plant ionome scatters (Parent *et al*., 2012).

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Table 1. Sequential binary partition (SBP) designed to compute nutritional orthonormal balances as isometric log-ratios (*ilr*$_i$'s) in a subspace of the ionome of *Opuntia ficus-indica* (L.) Miller.

ilr$_i$	SBP Orthogonal Contrasts						Balance designation	*r* (Counts of +1)	*S* (Counts of –1)	*ilr* estimation
	N	P	K	Ca	Mg	F_v				
Ilr$_1$	+1	+1	+1	–1	–1	0	[N, P, K | Ca, Mg]	3	2	$\sqrt{\frac{3x2}{3+2}}ln\left(\frac{G(C_N, C_P, C_K)}{G(C_{Ca}, C_{Mg})}\right)$
Ilr$_2$	+1	+1	–1	0	0	0	[N, P | K]	2	1	$\sqrt{\frac{2x1}{2+1}}ln\left(\frac{G(C_N, C_P)}{G(C_K)}\right)$
Ilr$_3$	+1	–1	0	0	0	0	[N | P]	1	1	$\sqrt{\frac{1x1}{1+1}}ln\left(\frac{G(C_N)}{G(C_P)}\right)$
Ilr$_4$	0	0	0	+1	–1	0	[Ca | Mg]	1	1	$\sqrt{\frac{1x1}{1+1}}ln\left(\frac{G(C_{Ca})}{G(C_{Mg})}\right)$
Ilr$_5$ (Optional)	+1	+1	+1	+1	+1	–1	[N, P, K, Ca, Mg | Fv]	5	1	$\sqrt{\frac{5x1}{5+1}}ln\left(\frac{G(C_N, C_P, C_K, C_{Ca}, C_{Mg})}{G(C_{F_v})}\right)$

N is nitrogen; P is phosphorus: K is potassium; Ca is calcium; Mg is magnesium; F_v is a filling value that represents plant nutrients not involved in the analysis [(F_v = 100 (i.e. Total composition) – (N + P + Ca + Mg))]; *G* is the geometric mean, and *C* is the concentration of the corresponding macro-nutrient

Binary Classification Procedure

We used a Binary Classification Procedure (BCP) as proposed by Berrar (2019) to define an optimal decision boundary when dividing the sample or S^d into two classes. *M* was used as a predictor delimiter to separate balanced from imbalanced compositions. To define an optimal predictor delimiter, i.e. an *M* value as nutrient balance index, we used the maximum value of Class Sum of Squares (CSS) as proposed by Nelson and Anderson (1977). So, we were able to identify two groups of compositions (balanced, and imbalanced) separated by an *M* value as the optimal predictor delimiter. Later, the sample was divided into high- and low-yielding groups considering the target yield (1166.67 g Cladode^{-1}) as calculated through the Compositional Nutrient Diagnosis (CND) approach; it was proposed by Vidal-Hernández *et al*. (2021b).

Both, the *M* optimal predictor value and the mentioned target yield were useful to identify 4 groups of compositions as classification results represented in a 2 X 2 table or confusion matrix like that proposed by Berrar (2019). A group of cases belongs to high-yield (>1166.67 g Cladode^{-1}) and an adequate nutrient balance; this group is integrated by True-Negative (T-N) compositions; it can be used as a reference group to perform diagnosis and correct defective compositions at the specified combination of the involved macro-nutrients. An additional group of cases is integrated by low yielders (<1166.67 g Cladode^{-1}) despite adequate nutritional balance (some other factor limiting yield); it involves False-Negative (F-N) compositions. The third group of cases is integrated by high yielders (>1166.67 g Cladode^{-1}) despite nutrient imbalance (excess or luxury consumption of some nutrients); it is integrated by False-Positive (F-P) compositions. The fourth group of cases belongs to low-yield (<1166.67 g Cladode^{-1}) and nutritional imbalance (these cases could positively respond to improvements in nutrient availability); this group is integrated by True-Positive (T-P) compositions.

This BCP allows the estimation of its different performance measurements (see Berrar, 2019), for instance, the following. The probability that a balance's diagnosis returns high performance can be estimated using the negative predictive value (NPV = TN/(TN + FN). On the contrary, the probability that a balance's diagnosis returns low performance can be estimated using the positive predictive value (PPV = TP/(TP + FP). In addition, the probability that a composition is correctly identified as balanced, or imbalances is estimated in terms of accuracy (Accuracy = (TN + TP)/(TN + FN + TP + FP). The probability that a high yield composition is balanced is computed as Specificity = TN/(TN + FP). The probability that a low yield composition is imbalanced is computed as Sensitivity = TP/(TP + FN).

Original data was captured and organized in an Excel spreadsheet (Microsoft Inc., 2016). Most of the statistical work was also carried out with Excel. A balance-dendrogram was carried out, once original compositions were closed to 100%, using the CoDaPack v2.03.01 program considering four identified groups of compositions (Comas-Cufí and Thió-Henestrosa, 2011). Besides, nutrient concentrations and orthonormal balanced comparisons between T-N and T-F groups were carried out using the Tukey test and discriminant analysis through the corresponding procedures in Minitab 16 (Minitab, LLC, 2016).

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Results

Basic statistics of yield and nutrient concentrations in 1-year-old fruiting cladodes can be appreciated in Table 2. Results suggest that yield shows high variability (CV = 59.08%); P, Mg, and Ca concentrations have moderate variability (CV = 19.38%, CV = 20.88%, and CV = 24.62%, respectively); and K and N show high variability (CV = 36.48% and CV = 30.69%, respectively). Variability is an important issue in getting the planned aims.

Table 2. Basic statistics of *Opuntia ficus-indica* variety 'Rojo Pelón' fruit yield per cladode and N, P, K, Ca, and Mg concentrations in 1-year-old fruiting cladodes (n = 228).

Statistic	Yield (g cladode^{-1})	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})
Mean	795.16	11.67	2.86	32.06	38.45	13.65
Standard Deviation	469.79	3.58	0.55	11.69	9.47	2.85
Coefficient of Variation	59.08	30.69	19.38	36.48	24.62	20.88
Minimum	60.00	6.00	1.56	13.12	13.00	5.94
Maximum	2186.00	21.70	4.20	65.36	63.50	21.50

Binary Classification Procedure

The CSS approach allowed the estimation of a Mahalanobis distance = 1.557 linked with the highest value of CSS (6,095,603.04). This *M* value and the target yield = 1166.67 g Cladode^{-1} were used to carry out the BCP. Both optimal delimiters classified 30, 21, 46, and 131 compositions in the T-N, F-P, F-N, and T-P quadrants, respectively (Figure 1, Table 3). Both optimal delimiters have a compromise defined by Specificity = 0.5882 and Sensitivity = 0.7401. Most of the compositions were correctly diagnosed by the *M* predictor (Accuracy = 0.7061) (Table 3). Most of the imbalanced cases (F-P + T-P) yielded less than 1166.67 g Cladode^{-1} (PPV=0.8618). Almost two-fifths (NPV = 0.3947) of balanced compositions yielded more than the cut-off yield.

Notably, the mean yield of the T-P group of compositions (572.9 g Cladode^{-1}) is near two-fifths lower than that of the T-N group of cases (1503.4 g Cladode^{-1}) (Table 3); this suggests that yields of T-P compositions could be enhanced through improvements of nutrients availability when the correct diagnoses are performed in an opportune way; in other words, pertinent identification of their limiting factors is a needed issue to improve yield suitably.

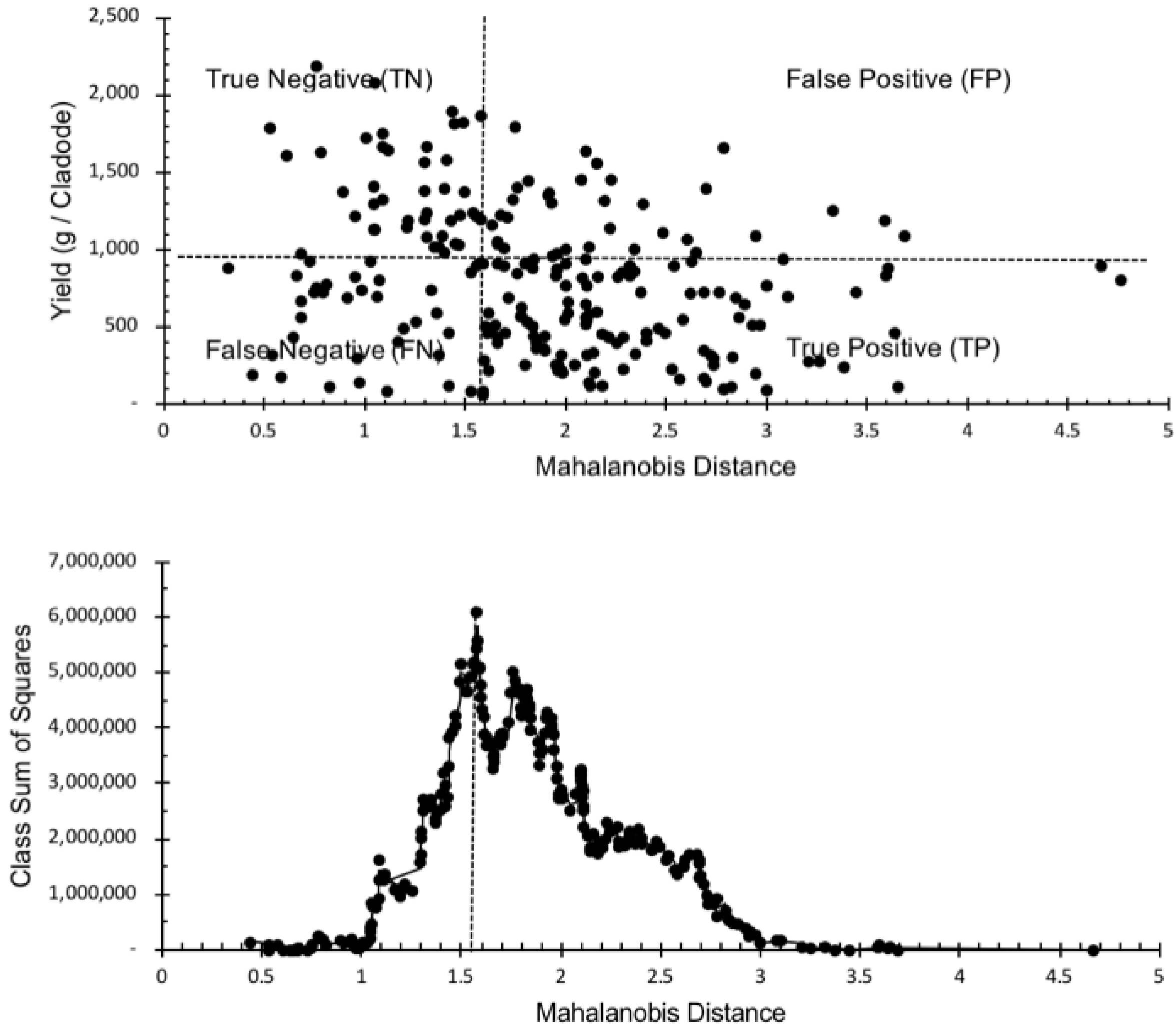

Figure 1. Binary classification of data with indexes of performance (yield) at the top, and Class Sum of Squares at the bottom against the Mahalanobis Distance across the geometric coordinates or isometric log-ratios: *ilr* [N, P, K | Ca, Mg], *ilr* [N | P], *ilr* [N, P | K], and *ilr* [Ca | Mg] in a subspace of the ionome of *Opuntia ficus-indica* (L.) Miller variety 'Rojo Pelón' (*n* = 228 compositions).

Table 3. Definitions in the binary classification of data with the index of performance (yield) against the Mahalanobis Distance across the geometric coordinates or isometric log-ratios: *ilr* [N, P, K | Ca, Mg], *ilr* [N | P], *ilr* [N, P | K], and *ilr* [Ca |Mg] in a subspace of the ionome of *Opuntia ficus-indica* (L.) Miller variety 'Rojo Pelón (*n* = 228 compositions).

Yield (g Cladode^{-1})	Negative predictive value = 0.3947	Positive predictive value = 0.8618	Accuracy = 0.7061
1166.67	**True Negative** n=30 Mahalanobis Distance / Yield (g Cladode^{-1}) Mean 1.1957978 / 1503.415385 Standard Deviation 0.2879463 / 284.112588	**False Positive** n=21 Mahalanobis Distance / Yield (g Cladode^{-1}) Mean 2.1401835 / 1430.117216 Standard Deviation 0.5457494 / 191.359418	Specifity = 0.5882
0	**False Negative** n=46 Mahalanobis Distance / Yield (g Cladode^{-1}) Mean 1.0559805 / 676.217391 Standard Deviation 0.3359397 / 326.523704	**True Positive** n=131 Mahalanobis Distance / Yield (g Cladode^{-1}) Mean 2.30920225 / 572.938931 Standard Deviation 0.60243195 / 298.639859	Sensitivity = 0.7401
	0	1.575	

Mahalanobis Distance

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On the other hand, the mean yield of the F-N compositions (676.2 g $Cladode^{-1}$) is less than half as it should be considering the mean yield of the T-N group of cases. In addition, the mean yield of the F-P group of compositions is 95% of that of the T-N group of cases.

Yield and *M* basic statistics (means and standard deviations) are appreciated for each group of compositions in Table 4. In addition, means, standard error of the mean, standard deviation, coefficient of variation, minimum, median, and maximum of the 4 *irl*'s, and the linked 5 macro-nutrients for the T-N compositions (i.e. the target group) can be appreciated in Table 5.

Macro-nutrient orthonormal balances, concentrations, and comparisons between T-N and T-F groups

As expected, the designed SBP allowed the formalization of a balance dendrogram (Figure 2). This balance dendrogram is a graphical representation of the SBP with statistical summaries of balances. The dendrogram shows clearly that the *ilr* [N, P, K | Ca, Mg] functions as an initiator. Such an orthonormal hierarchical balance reflects sequentially the relationships [N | P], [N, P | K], and [Ca | Mg]. Strongly, these associations form hierarchical clusters of orthonormal balances (*irl*'s).

In the balance-dendrogram, each colored vertical line has a length determined by the proportion of variance explained by the corresponding *irl*. Notably, the *irl* [N, P | K] and the *irl* [N | P] explained much more variance than the remaining *irl*'s. Markedly, the higher explained variance belongs to the T-P group as linked to green vertical lines. On the other hand, the mean yield of the F-N compositions (676.2 g $Cladode^{-1}$) is less than half as it should be considering the mean yield of the T-N group of cases. In addition, the mean yield of the F-P group of compositions is 95% of that of the T-N group of cases.

Yield and *M* basic statistics (means and standard deviations) are appreciated for each group of compositions in Table 4. In addition, means, standard error of the mean, standard deviation, coefficient of variation, minimum, median, and maximum of the 4 *irl*'s, and the linked 5 macro-nutrients for the T-N compositions (i.e. the target group) can be appreciated in Table 5.

The mean belonging to each balance is the point where the vertical line ends on the corresponding-colored box plot. Each box plot includes the percentiles 5, 25, 50 (median), 75, and 95 on its horizontal bar. Then, the greater box plot corresponds to the *irl* [N, P | K] balance of the T-P group suggesting it explained the most variance of the total. The means of the T-N and T-P groups of the *irl* [N, P | K], and the *irl* [N | P] are well separated and thus quite different, and the variances could also be different. In other words, both *irl*'s are good candidates for discrimination.

In this context, Tukey tests allowed the detection in which nutrient and orthonormal balance important differences occurred between T-N and T-P groups of compositions (Table 5). The order of nutrient significant differences is N = P = Mg > Ca > K. Strongly, the N mean of the T-N group is lower than that of the T-P group. On the other hand, P, Mg, Ca, and K, the means of the T-N group are higher than those of the T-P group. In addition, the most significantly different orthonormal balances are *ilr* [N | P], and *ilr* [N, P, K | Ca, Mg] which confirms the interpretation of the balance-dendrogram. The lower positive mean of *ilr* [N | P] corresponds to the T-N group; it suggests that T-N compositions tended to load more P (the minus, –, part or denominator) than N (the plus, +, part or numerator) compared to the T-P cluster; this result can be appreciated by considering the corresponding macro-nutrient concentrations in both groups. Besides, the greater negative mean of the *ilr* [N, P, K | Ca, Mg] belongs to the T-N group; this indicates that T-N compositions tended to be characterized by a greater load in the minus (–) parts or group denominator than in the plus (+) parts or group numerator compared to T-P. This means that Ca and Mg loaded more than N, P, and K in the T-N compositions; this is specific for the macro-nutrient Ca. Moreover, the greater negative mean of the *ilr* [N, P | K] corresponds to the T-N cluster; then, T-N compositions tended to load more the minus part (K) than the plus part (N, P) compared to the T-P group.

Under the basis of the *irl*'s, the performed discriminant analysis correctly identified 120 of 161 compositions. The probability of correctly classifying a T-P composition was lower (94/131 or 71.8%) than the probability of correctly classifying a T-N case (26/30 or 86.7%). Moreover, the coefficients of the estimated linear discriminant functions for the four groups are shown in Table 6. The *ilr* [N | P] result is the most discriminant balance between T-P and T-N compositions as demonstrated by the greater balance coefficient difference.

Theoretically, to classify newly-compositions, anybody could compute the linear discriminant functions associated with the four groups. Thus, it is possible to classify each new composition as being of a particular group depending upon which discriminant function value is higher, however, this process requires estimation of the *ilr*'s. Thenceforth, new compositions of interest are those lying in the T-P group due they could be enhanced through the improvement of the nutrient(s) availability condition. The magnitude of the needed change can depend on the *M* between the position of the new composition and the position of the centroid of the reference (1.196 as the mean of *M* corresponding to the T-N group, Table 3) within the subspace of the ionome of *O. ficus-indica*; testing this procedure was out of the scope of our study. Otherwise, T-N's mean or median of the orthonormal balances may be considered as norms and they could be also used as a reference to diagnose the nutrient status of any new composition though with caution as explained in the next section; and of course, these norms are supported by the linked macro-nutrient concentrations (Table 4).

Discussion

Macro-nutrient orthonormal nutrient balances as norms and related concentrations

Prior works have documented macro-nutrient requirements of *O. ficus-indica* as linked to yield through the B-LA and the CND technique. However, those standards have serious limitations. The B-LA does not consider interactions or balances between and/or among nutrients. Besides, the CND approach sacrifices variate and orthogonal references are not obtained straightforwardly. On the other hand, we tested the extent to which a compositional data analysis under the basis of orthonormal hierarchical balances (i.e. orthogonally arranged balances) reflected macro-nutrient interactions and adequate macro-nutrient status in a subspace of the ionome of *O. ficus-indica* variety 'Rojo Pelón' for fruiting.

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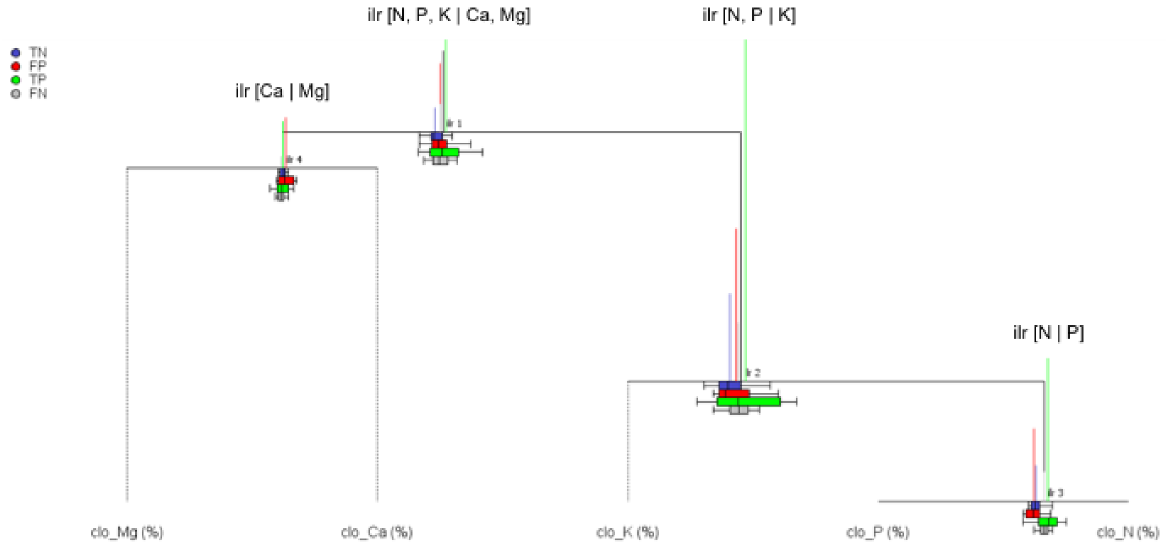
Table 4. Basic statistics of isometric log-ratios: *ilr* [N, P, K | Ca, Mg], *ilr* [N | P], *ilr* [N, P | K], and *ilr* [Ca | Mg] for True-Negative compositions (*n* = 30) in a subspace of the ionome of *Opuntia ficus-indica* (L.) Miller variety 'Rojo Pelón, and their associated macro-nutrient concentrations.

Variable	Mean	Standard Error of the Mean	Standard Deviation	Coefficient of Variation	Minimum	Median	Maximum
ilr [N, P, K | Ca, Mg]	-1.0048	0.0235	0.1288	-12.81	-1.2929	-1.0185	-0.7277
ilr [N, P | K]	-1.5218	0.0453	0.2482	-16.31	-1.9175	-1.5572	-0.8610
ilr [N | P]	0.7933	0.0292	0.1598	20.14	0.6138	0.7476	1.1840
ilr [Ca | Mg]	0.7367	0.0154	0.0842	11.42	0.5119	0.7411	0.8951
N (g kg^{-1})	9.6930	0.3440	1.8860	19.46	7.4000	9.4000	14.3000
P (g kg^{-1})	3.1227	0.0626	0.3430	10.98	2.4000	3.1000	3.6000
K (g kg^{-1})	36.1500	1.5500	8.4700	23.43	17.4100	36.2500	55.0000
Ca (g kg^{-1})	43.0400	1.0300	5.6300	13.08	26.4000	43.1000	5.2000
Mg (g kg^{-1})	15.1810	0.3870	2.1200	13.97	11.8000	15.0150	20.6000

Table 5. Macro-nutrient mean concentrations and isometric log-ratios (*ilr*'s) comparisons between True-Negative, T-N (*n* = 30) and True-Positive, T-P (*n* = 131) groups of compositions in a subspace of the ionome of *Opuntia ficus-indica* (L.) Miller variety 'Rojo Pelón' through the Tukey test. Different letters indicate significant differences between groups.

Group	Variable									
	N (g kg^{-1}) $p<0.0001$		P (g kg^{-1}) $p<0.0001$		K (g kg^{-1}) $p=0.038$		Ca (g kg^{-1}) $p=0.001$		Mg (g kg^{-1}) $p<0.0001$	
T-P	12.256	a	2.720	b	30.74	b	36.18	b	12.96	b
T-N	9.690	b	3.120	a	36.15	a	43.04	a	15.18	a
	ilr [N, P, K | Ca, Mg] $p=0.008$		*ilr* [N, P | K] $p=0.019$		*ilr* [N | P] $p<0.0001$		*ilr* [Ca | Mg] $p=0.666$			
T-P	–0.867	a	–1.303	a	1.059	a	0.722	a		
T-N	–1.005	b	–1.522	b	0.793	b	0.737	a		



	Mg (g kg^{-1})	Ca (g kg^{-1})	K (g kg^{-1})	P (g kg^{-1})	N (g kg^{-1})
TN	15.58	43.04	36.15	3.12	9.690
FP	13.49	41.21	33.54	3.26	9.424
TP	12.96	36.68	30.74	2.72	12.256
FN	14.69	39.24	32.46	2.87	11.463

Figure 2. Balance-dendrogram showing 4 orthonormal hierarchical balances (variables as isometric log-ratios –*ilr*'s–) that represent a subspace of macro-nutrients (N, Nitrogen; P, Phosphorus; K, Potassium; Ca, Calcium; and Mg, Magnesium) in the ionome of *Opuntia ficus-indica* (L.) Miller variety 'Rojo Pelón'. Data in the bucket are mean concentrations belonging to four groups of compositions: TN, true-negative; FP, false-positive; TP, true-positive; and FN, false-negative.

Table 6. Discrimination functions for True-Positive (T-P) and True-Negative (T-N), False-Positive (F-P) and False-Negative (F-N) compositions, and the differences between the isometric log-ratios (*irl*'s) of T-P and T-N groups in *Opuntia ficus-indica* (L.) Miller variety 'Rojo Pelón'.

	T-P	T-N	Difference	F-P	F-N
Constant	-54.234	-52.621		-60.583	-67.113
ilr [N, P, K | Ca, Mg]	-20.514	-22.872	-2.358	-26.343	-28.250
ilr [N, P | K]	-22.168	-22.450	-0.282	-26.647	-28.884
ilr [N | P]	38.778	35.798	2.980	43.053	48.182
ilr [Ca | Mg]	28.736	26.737	1.999	32.988	29.195

The present finding of orthonormal balances (*ilr*'s) allowed us to identify a group of compositions (T-N) linked to higher yields (g Cladode^{-1}) in *O. ficus-indica* variety 'Rojo Pelón). For that, such a nutrient-balanced group (T-N) is characterized by an adequate macro-nutrient status which markedly differs from the imbalanced group (T-P). The T-P cluster is defined by an N trend to load more than P, and by an N, P, and K trend to load more than Ca and Mg, especially a high level of N linked possibly with a P deficiency. Theoretically, any composition of the T-P group or a new case diagnosed that lies in this category could improve its nutrient status. However, moving from an imbalanced situation to a balanced one like the target (T-N) composition is a difficult subject.

Macro-nutrient concentrations and orthonormal balances use and implications

Performing a correct diagnosis is not any trouble easy to solve although an ideal composition is known as it may be as the orthonormal hierarchical balances statistical estimators belonging to the present T-N group for *O. ficus-indica* variety 'Rojo Pelón' fruiting. For instance, the macro-nutrient mean concentrations of the T-P group could be considered as a new composition; then, there remains unanswered the question: How to move its macro-nutrient imbalanced situation toward one balanced?

Someone could perform a comparison between a composition diagnosed as T-P and a target composition (e.g. T-N macro-nutrient mean or median concentrations). Considering the T-P's mean concentrations as a new composition, P of the new imbalanced composition is in shortage by 87%, K, Ca and Mg are in shortage by 85% as they should be, all at the expense of N. Do this imply P, K, Ca, and Mg deficiencies and N excess? Of course not! In this context, Aitchison (2015) pointed out that "...compositions provide information only about the relative magnitudes of the compositional components and so interpretations involving absolute values as in the above example cannot be justified."

To define how to move an imbalanced situation toward one balanced, readers must not forget that in compositional spaces, or complex interaction systems, or concentration vectors, a component is inherently related to each other because changing a proportion inevitably affects at least another. For instance, an increment of the concentration of a nutrient will inevitably decrease the concentration of at least another. For that reason, there is important that interpretation be carried out through a multivariate and compositional data focus. To perform a correct diagnosis, the balance-dendrogram was proposed by Thió-Henestrosa *et al*. (2008); it has been used widely (e.g. Pawlowsky-Glahn and Egozcue, 2011), and a slightly different balance-dendrogram was used by Parent *et al*. (2013b). To reinforce the diagnosis, Lima de Deus *et al*. (2018) proposed employing the centered log-ratio (*clr*) transformation to diagnose plant ionome; that procedure implies the estimation of ratios between the *clr*'s of the new composition and that of the target one just like the exercise performed *ut supra* using macro-nutrient means. However, there remains no identified approach to perform a correct recommendation procedure considering the diagnosis carried out as demonstrated in the Parent *et al*. (2013) and the present case. This could be due to visualizing a compositional space in a multidimensional mobile of parts in equilibrium remains also to be a hard matter. Even so, though the diagnosis is correct concerning an important difference between both nutrient balances through this procedure, the only fact consists of the new composition needs a change toward enhancing its orthonormal balances in its compositional space, not how to achieve such a change.

Achieving the mentioned change could be performed by identifying what happened in T-N compositions having orthonormal balanced status. Knowing what happened implies that specialized agronomists and growers must consider experimental results as well as knowledge of nutrient interactions in plants and soil, among other issues to adapt new compositions to local environmental conditions. For instance, there is widely known K and Ca are connected in the plant; thus K content may decrease according to Ca increase, particularly when the plant is growing in a calcareous soil that is rich in available Ca (*e.g.* the present case); however, this interaction did not occur importantly in the present case because both nutrients were not identified as limiting factors although the concentration of both nutrients in the T-P group were lower than those in the T-N group. What happened is that the *irl* [N | P] was detected as the most sensitive orthonormal balance probably linked to a P deficiency and luxury consumption of N situation (it deserves to be noted that someone could be diagnosing an N excess instead of a luxury consumption of N when nutrient

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concentrations between the new case and the reference are used for comparison); so, an improvement should be performed to enhance this balance in compositions diagnosed as T-P without modifying another orthonormal balance in a significant way which affects yield. There is known that a P deficiency in plants can be alleviated by an improvement of P availability. This improvement can be performed through soil or foliar fertilization practice which requires field trials to generate a suitable recommendation or through the promotion of plant's roots release chemical compounds (*e.g.* citric acid through over-secretion) which react in the soil to increase the dissolution of insoluble phosphate thus improving phosphate uptake and plant performance (López-Bucio *et al*., 2000).

In summary, the nutrient orthonormal balances technique involving a compositional subspace of the *O. ficus-indica* variety 'Rojo Pelón' has been demonstrated to be a meaningful measure of macro-nutrients signature. Most notably, this is the first study to our knowledge to investigate the ionome of *O. ficus-indica* as a complex system or macro-nutrient concentration vector through the nutrient orthonormal balances approach. Our findings extend those of Hernández-Vidal *et al*. (2021 a, b) because there appears that the B-LA sub-estimated the Ca and Mg standards, and the CND technique sub-estimated the Mg standard, that is, they might be in shortage by 85.15%, 91.1%, and 95.38%, respectively, as they should be considering the mean concentrations linked to orthonormal balances as a reference (Table 7).

Table 7. Macro-nutrient optimum concentrations in 1-year-old fruiting cladodes for *Opuntia ficus-indica* (L.) Miller variety 'Rojo Pelón' as proposed by several authors and ratios between the Boundary-Line Approach (B-LA) or the Compositional Nutrient Diagnosis technique (CND)' standards and the mean concentrations linked with the True-Negative (T-N) compositions estimated in this work.

Source	Target yield (g Cladode^{-1})	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})
Hernández-Vidal *et al*., B-LA (2021a)	>1901.13	10.20	3.04	35.18	36.65	13.83
Ratio B-LA/ T-N compositions		1.0523	0.9735	0.9732	0.8515	0.9110
Hernández-Vidal *et al*., CND (2021b)	>1166.67	9.58	3.18	35.07	42.28	14.48
Ratio CND/T-N compositions		0.9883	1.0183	0.9701	0.9823	0.9539
This work (T-NC)	1503.415±28	9.693	3.1228	36.15	43.04	15.18

Besides, our results on orthonormal balances provide compelling evidence of macro-nutrient disorders in terms of P deficiency and luxury consumption of N as estimated by contrasting ideal (balanced) compositions (T-N group) with those imbalanced (T-P group). However, some limitations are worth noting. First, the used sample size is small (n = 228), therefore, it must be increased to evaluate whether the orthonormal balances statistics of the T-N group remain stable once the sample is greater. Second, the sample corresponds to a unique variety, thus, this kind of research should involve more *O. ficus-indica* varieties and *Opuntia* species with diverse purposes of production. Third, the original sample is representative at the local level, thus, more and great size samples must be taken in other regions to evaluate their representativeness. Fourth, our study only involved five nutrients but objectively this kind of work should ideally involve all those defined as essential for plants. Fifth, this kind of result should be accomplished by an approach that allows simplifying suitable recommendations under the basis of correct diagnosis through a technique focused on visualizing a compositional space in a multidimensional mobile of parts in equilibrium and not. Sixth, fieldwork such as fertilization trials should be carried out because fertilizer recommendations must involve information on the availability of nutrients in the soil. This last point of view is also based on the fact that a large proportion of the plant biomass lies in the roots, which adjust their growth according to the proximity of neighboring specimens (González and Manavella, 2021) and soil nature. Therefore, future research works should be focused on at least one of these limitations.

Conclusions

This study is, to our knowledge, the first addressed to know the ionome of *Opuntia ficus-indica* under the basis of macro-nutrient requirements in terms of orthonormal balances (sets of true-negative compositions) for producing fruits. Regarding the title of the paper, our results indicate that macro-nutrient balanced compositions in a subspace of the ionome of *O. ficus-indica* (L.) Miller maximizes fruiting (i.e. g Cladode^{-1}). These balanced compositions as requirements or norms (Table 4) can be used as a reference to promote improvements in *O. ficus-indica* variety 'Rojo Pelón' nutrient-imbalanced compositions characterized as true-positive compositions at the local scale. These norms allowed us to identify a near-ideal 1-year-old fructification cladode composition in terms of macro-nutrient concentrations. Besides, it was possible to define that most of the true-positive macro-nutrient compositions were mainly characterized by a P deficiency and luxury consumption of N. However, the true-negative orthonormal balances (norms) should be evaluated in future research works by increasing the database size, involving more varieties and species, and using novel techniques to perform suitable recommendations under the basis of correct diagnosis interpretation by visualizing compositional spaces in a multidimensional mobile of parts in equilibrium and not. Of course, these actions should be accomplished with prior knowledge of nutrient interactions and balances in plants and soil, and additional issues to address objectively the needed change to enhance the nutrient status of true-positive imbalanced compositions.

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Author contributions

R.D. Valdez-Cepeda, F. Blanco-Macías, and M. Márquez-Madrid contributed to the study's conception and design. R.D. Valdez-Cepeda, F. Blanco-Macías, M. Márquez-Madrid, and A. Lara-Herrera performed material preparation and data collection. R.D. Valdez-Cepeda, and J.S. Rodríguez-Barrientos carried out statistical analyses. R.D. Valdez-Cepeda and J.S. Rodríguez-Barrientos wrote the first draft of the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

Data availability

Data will be available through reasonable request.

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