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Nitrogen and Potassium Fertilization in a Guava Orchard Evaluated for Five Cycles: Effects on the Plant and on Production

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ABSTRACT: Guava response to fertilization can be monitored through plant tissue analysis. Correct interpretation of these results, based on standard levels, is of great importance for correct nutrient management of the crop. However, standard levels are constantly criticized for not considering interactions among elements. To improve the nutritional diagnosis of 'Paluma' guava (*Psidium guajava* L., Myrtaceae), an experiment was conducted using nitrogen fertilization (0, 0.5, 1.0, and 2.0 kg per plant per cycle of N, with urea as a source with 45 % N), and potassium fertilization (0, 0.55, 1.1, and 2.2 kg per plant per cycle of K_2O, with potassium chloride as a source with 60 % K_2O) in an irrigated commercial area for five consecutive cycles, 2009 through 2012, observing the influence of fertilizers and climate and assessing yield and leaf element content, using the concept of isometric log ratios (ilr) to interpret leaf analysis results (N, P, Ca, Mg, K, and S). This paper showed that nutrient balances and nutrient concentration values can be interpreted coherently using compositional data analysis. Ranges of nutrient balances also were established for "Paluma" guava and validated through ranges grounded in nutrient contents currently used in Brazil. Nitrogen fertilization increased "Paluma" guava yield. The 0.5 kg N application rate per plant and the other studied treatments practically showed the same results, and their values were affected by pruning time as well as the nutrient balances.

Keywords: isometric log ratio (*ilr*), mineral nutrition, nutrient balance, compositional analysis.

INTRODUCTION

Brazil is the world's top producer of red guava, a native South and Central American fruit. The Paluma cultivar of guava (*Psidium guajava* L.) tops Brazilian output, as its fruits are highly marketable for *in natura* consumption and industrial processing. The 2012 crop in the state of São Paulo in southeastern Brazil represented 91 % of all industrialized guava (74,000 Mg of fruits), 70 % coming from the Jaboticabal region alone – an area of approximately 1,500 ha (IEA, 2013).

The Paluma cultivar is highly marketable in Brazil, featuring fine characteristics for both *in natura* consumption and fruit industrialization. Furthermore, Paluma has adapted well to intensive production (which uses pruning, irrigation, and adjusted nutritional management). This allows production cycles of approximately eight months, well-suited for scheduling of fruit output.

Guava is highly responsive to fertilization (Arora and Singh, 1970; Natale et al., 1994, 2002; Anjaneyulu and Raghupathi, 2009). A whole array of tools is used to provide nutritional support, such as tissue and soil analyses (leaf analysis being the most reliable for assessing the nutritional status of perennial plants) grounded on adequate sampling methods and on correct interpretation of analytical data (Bould et al., 1960) These plants access nutrients at deeper depths than it would be possible to determine through standard soil analysis procedures.

In evaluating plant nutritional status, standard nutrient contents are often criticized for not taking into due account interactions among elements (Bates, 1971). In natural systems, a *ceteris paribus* assumption (interactions between nutrients, in which all other factors remain constant) has its constraints, as double or multiple relations have been well-documented in plant nutrition studies. (Fageria, 2001; Malavolta, 2006).

The effects of modifying nutrient proportions due to interactions among the nutrients were first illustrated by Lagatu and Maume (1935). Plant tissue data convey relative information, as they are intrinsically multivariate, i.e., no one component can be interpreted in isolation; it must be related to other components (Tolosana-Delgado and van den Boogart, 2011). Hence, for compositional data (as in plant tissue nutrients), tools should be used that allow analysis of inter-component interactions for the sake of better understanding of plant nutritional status.

Compositional data analysis proposed by Aitchison (1982) has sparked wide-ranging discussion, given the practical importance of this new methodology, albeit some reluctance to its use remains to this day (Pawlowsky-Glahn and Egozcue, 2001). Occasionally, this technique requires interpretation of results in terms of ratios and logarithmic proportions, which are harder to interpret than real vectors in statistical analysis. For the sake of simplifying analysis, components can be ordered so as to cluster them into two or more subsets, which are somehow easier to interpret (Egozcue and Pawlowski-Glahn, 2005).

To avoid numerical bias in compositional analyses, Egozcue and Pawlowski-Glahn (2005) proposed using Isometric Log Ratios (*ilr*) based on the principle of orthogonality (D-1 degrees of freedom) to analyze compositional data. Isometric Log Ratios (*ilr*) coordinates can be projected onto Euclidian space, a geometric structure allowing analyses free from numerical bias (Egozcue and Pawlowski-Glahn, 2011). An *ilr* transformation is a special log-transformation case that preserves the information contained in the new variable, allowing studies of relations among nutrients (Parent et al., 2012).

The *ilr* method is a three-stage method, namely: data represented in *ilr* coordinates; analysis of variance of the coordinates as real random variables; and interpretation of results in terms of balances (Egozcue and Pawlowski-Glahn, 2011); they cannot, however, be transformed back into their initial values.

Unlike conventional methodology, based on the contents of each individual nutrient, this tool is quite promising for the study of plant nutritional status in view of its sturdiness, and as it assesses nutrients taking into accounts the relations among them through nutrient balances. Hence, it is a more adequate instrument for this type of analysis. This concept has been successfully used in plant nutritional studies (Parent, 2011; Hernandes et al., 2012), as well as in soil aggregation (Parent et al., 2012).

Presuming that compositional data analysis is a robust tool to interpretation of leaf analysis because it take in consideration the relationship between nutrients. The aim of this study was improved the nutritional diagnosis of Paluma guava, evaluating rates of nitrogen and potassium fertilization in an irrigated commercial area for five consecutive cycles, with careful observation of the influence of fertilizers and of the climate, using isometric log ratios.

MATERIALS AND METHODS

This experiment was carried out in a seven-year-old Paluma guava orchard irrigated through a micro-sprinkler system. Plant spacing was 7 × 5 m, and production management relied on pruning for five consecutive cycles (2009 through 2012). The experiment was in Vista Alegre do Alto (state of São Paulo, Brazil) belonging to the company *Indústria de Polpas e Conservas VAL Ltda*., a food processing company. Geographic site coordinates are: 21° 08' S, 48° 30' W, and 603 m altitude. The climate is Cwa (subtropical; with a short, moderate, dry winter, and a hot rainy summer) in according to the Köppen (1931) classification system, thus, two distinct yearly seasons. The weather data throughout the whole experimental period is shown in figure 1.

The orchard soil was classified as an *Argissolo Vermelho-Amarelo Distrófico* (Santos et al., 2013), an Ultisol (Soil Survey Staff, 2014). Prior to a three-month treatment, 2 Mg ha^{-1} of limestone were applied (containing 32 % CaO, 18 % MgO, and 90.5 % PRNT) over the entire area on the soil surface without incorporation in order to bring base saturation up to 70 %, as indicated by Natale et al. (1996). At the beginning of the trial, soil samples were collected in the 0.00-0.20 and 0.20-0.40 m layers under the projection of the tree canopies for soil fertility evaluation (Raij et al., 2001) (Table 1).

A randomized block experimental design was used in a 4 × 4 factorial arrangement, with four N application rates (0 ,0.5, 1.0, and 2.0 kg of N per plant per cycle, with urea at 45 % N as the source), and four K rates (0, 0.55, 1.1, and 2.2 kg of K_2O per plant per cycle using potassium chloride at 60 % K_2O), in three replicates. Experimental plots were five
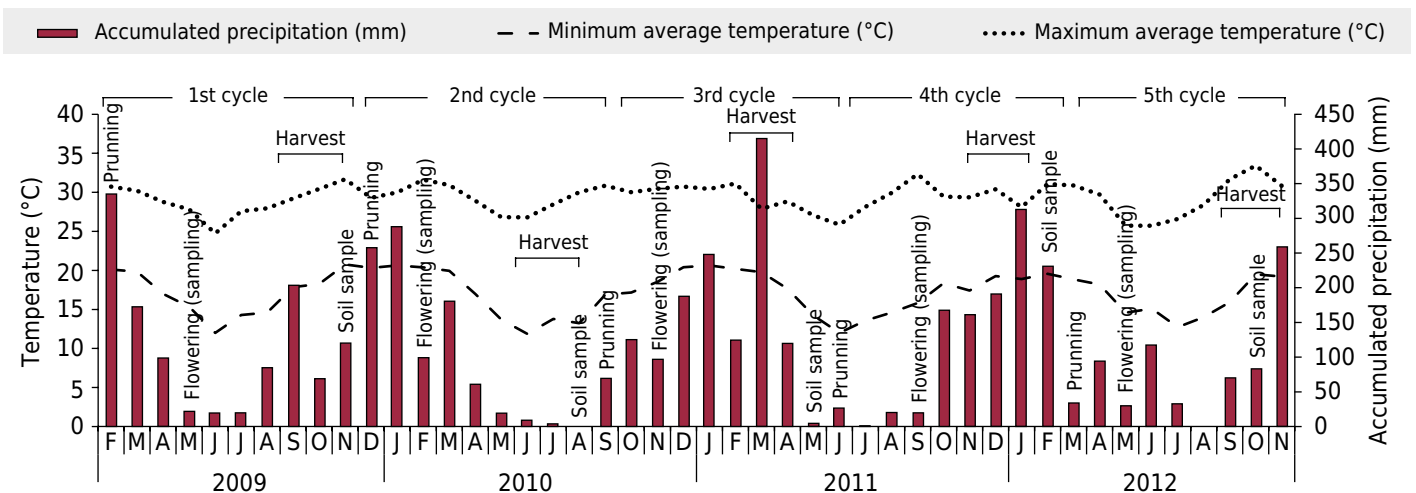

Figure 1. Climate Data at the Citriculture Experimental Station (2009-2012), Bebedouro, SP, Brazil; physiological and research aspects.

plants, and a central area of three guava trees was deemed useful for the evaluations. The experiment proceeded for five consecutive cycles in the same experimental unit.

Fertilizers were equitably distributed on the soil surface around each plant in an area 1.5 to 2.0 m distant from the trunk, parceled out four times at intervals of 25 days, starting at the full bloom plant stage. With the first N-K application, phosphate fertilizer was also applied (70 kg ha^{-1} P$_2$O$_5$), with simple superphosphate as the source, according to the recommendations of Natale et al. (1996).

For plant tissue analysis, the third pair of full bloom mature leaves was collected around the plant at 1.5 m above the soil, for a total of 30 pairs of leaves per plot (Natale et al., 1996). These leaves were washed and dried at 65 °C until reaching constant weight, and their N, P, S, K, Ca, and Mg contents were analyzed according to Bataglia et al. (1983).

To study nutrient balance in guava leaves, a method proposed by Egozcue et al. (2003) was used, and the compositional space was defined as follows:

$S^D = C$ (N, P, S, K, Ca, and Mg)

in which D = six components, and C is the function-closing operator, thus signaling compositional space closure.

Balances were then secured using *ilr* (isometric log ratio) coordinates, and the sequential binary partition (SBP) was arranged following the recommendations of Parent (2011) and Rozane et al. (2012) (Table 2). The SBP can be organized in such a way as to facilitate balance interpretation in relation of the goal of the study. Hence, our study started by contrasting anions (N, S, and P) with cations (K, Ca, and Mg), in order to separate the physiological effects of N and K fertilization. The second and third balances, contrasting N and P with S, and N with P, respectively, also sought to ascertain the effects of N fertilization. The final two balances were organized to evaluate the effect of K fertilization – the former (next to last) contrasting the monovalent cation (K) with the two bivalent cations (Ca and Mg); and, finally, the latter contrasting Ca and Mg.

Isometric Log Ratio (*ilr*) calculations followed the recommendations of Egozcue and Pawlowsky-Glahn (2005), and were expressed by the following equation:

$$ilr_j = \sqrt{\frac{rs}{r+s}} \ln \frac{g(c+)}{g(c-)} \qquad j = [1,2,...,D-1]$$

Table 1. Soil chemical properties in depths of 0.00-0.20 and 0.20-0.40 m, prior to experiment

Depth	OM[1]	pH(CaCl$_2$)	P	K$^+$	Ca^{2+}	Mg^{2+}	H+Al	SB	CEC	V	B	Cu	Fe	Mn	Zn	S
m	g kg^{-1}		mg dm^{-3}			mmol$_c$ dm^{-3}				%			mg dm^{-3}			
0.00-0.20	11	5.9	19	2.2	29	14	12	45.2	57.2	79	0.17	4.5	86.1	42.4	46.2	2
0.20-0.40	9	5.4	7	2.0	22	9	16	33.0	49.0	67	0.16	3.0	71.3	26.5	29.0	3

Methods according to Raij et al. (2001). Extractants: P and K (resin); Ca^{2+}, Mg^{2+}, and Al^{3+} (1 mol L^{-1} KCl); H+Al (calcium acetate at pH 7); S-SO$_4^{2-}$ (calcium phosphate); OM: organic matter (potassium dichromate); B: hot water; Cu, Fe, Mn and Zn (Mehlich-1); SB: sum of bases; V: base saturation.

Table 2. Sequential binary partition of leaf nutrient balance

Ilr	N	P	S	K	Ca	Mg	Nutrient balance	r[1]	s[2]
1	1	1	1	-1	-1	-1	[N, P, S	K, Ca, Mg]	3	3
2	1	1	-1	0	0	0	[N,P	S]	2	1
3	1	-1	0	0	0	0	[N	P]	1	1
4	0	0	0	1	-1	-1	[K	Ca, Mg]	1	2
5	0	0	0	0	1	-1	[Ca	Mg]	1	1

[1] r: number of positive signals; [2] s: number of negative signals.

in which r and s represent the number of positive and negative components, respectively; and $g(c_+)$ is the geometric average of the positive components, and $g(c_-)$ is the geometric average of the negative components. The square root is the balance between the number of positive and negative components.

The *ilr* balances underwent analysis of variance through application of the F test at 5 % probability. Whenever it was significant, polynomial regression analysis was carried out. The statistical procedure used was PROC MIXED (SAS program).

The ranges of critical balance were obtained from the standard contents of nutrients, as indicated by Natale et al. (2002) and Maia et al. (2007). The lower and upper limits were set according to Hernandes et al. (2012), wherein lower limits were set using the lower values of the numerator and the higher values of the denominator, while higher limits were set through the ratio between the highest content values in the numerator and the lowest in the denominator.

The leaves content ranges were suggested by Natale et al. (2002): 20-23 g kg^{-1} N; 1.4-1.8 g kg^{-1} P; 14-17 g kg^{-1} K; 7-11 g kg^{-1} Ca; 3.4-4.0 g kg^{-1} Mg, and 2.5-3.5 g kg^{-1} S for leaves collected during the full bloom stage; Maia et al. (2007) recommended: 20.2-25.3 g kg^{-1} N; 1.4-1.5 g kg^{-1} P; 19-21.7 g kg^{-1} K; 7.7-8.3 g kg^{-1} Ca; 2.7-2.8 g kg^{-1} Mg, and 4-5.1 g kg^{-1} S for leaves collected between budding and fructification.

RESULTS AND DISCUSSION

Guava yield increased significantly (p<0,05) with increasing N application rates, fitting a quadratic adjustment in the five production cycles (Figure 2), as also found in other studies (Natale et al., 1994; Terán et al., 1996; Kumar et al., 2008; Cardoso et al., 2011), confirming a direct N relation to plant yield capacity (Epstein and Bloom, 2006; Malavolta, 2006). Except for the fourth cycle, N application led to yield above 40 Mg ha^{-1} in the fruit per cycle (Figure 2). Low accumulated rainfall and low temperatures in the first half of the fourth cycle (Figure 1) might explain the poor output of this cycle. In contrast, in the other cycles, climate conditions more favorable to plant development resulted in outputs greater than production in the fourth cycle (Figure 2a).

Yield evaluation of irrigated Paluma guava with four different pruning times, carried out in São Francisco do Itabapoana (state of Rio de Janeiro), demonstrated that guava trees pruned in October and December were less productive. Moderate yield occurred in
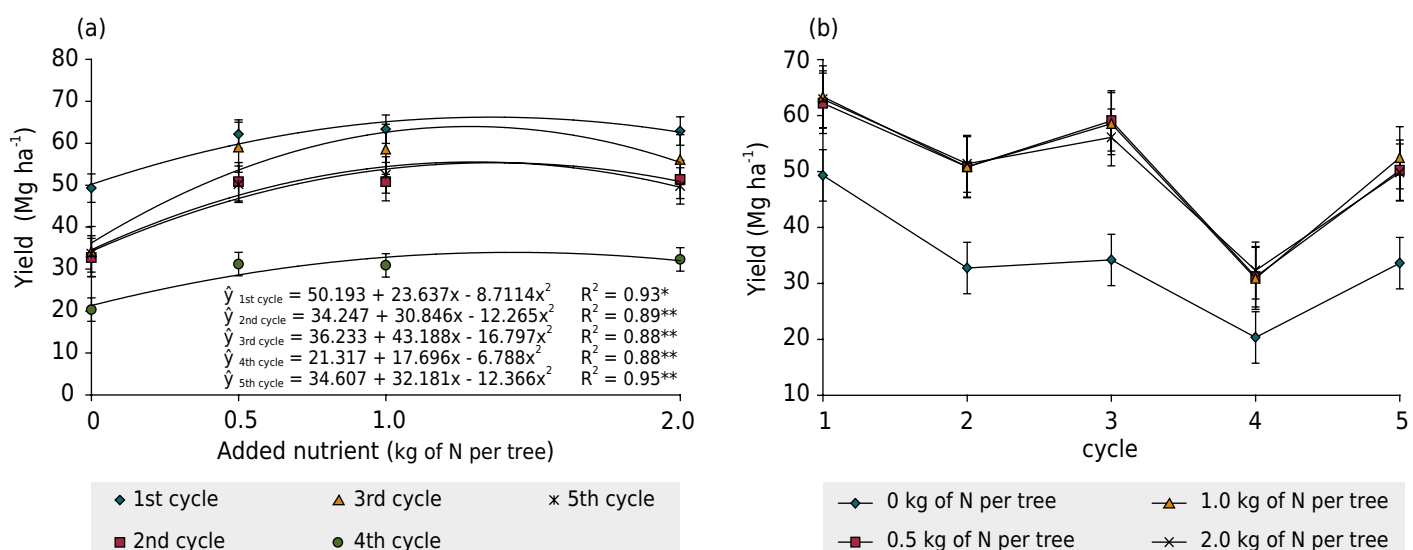

Figure 2. 'Paluma' guava yield in response to nitrogen fertilization (a) and seasonal change of production during the experimental period (b) for five production cycles. Vertical bars at each point represent the standard error of the mean.

August, whereas the highest yield was reached in December pruning due to variations in temperature and rainfall during orchard development (Serrano et al., 2008).

Different times for evaluating fructification pruning (May, June, July, and August) of *Pirassununga Vermelha* and *IAC-4* guava in southeastern Minas Gerais (Manica et al., 2000), and the effect of pruning six times a year (every month from May through October) of 'IAC-4' guava in Novo Hamburgo, Rio Grande do Sul, Brazil (Lopes et al., 1984) showed no effect from different pruning times, either on production or on average fruit weight, possibly due to different climate conditions in the region studied. As noticed in this and in other aforementioned studies, climate variables do influence plant response to pruning, hence directly impacting their yield.

Application rates of K were tested and showed no significant effect ($p > 0.05$) on guava production. In young Paluma guava trees, K supplied through pruning material was 0.232 kg per plant (Maia et al., 2007), which is why studies have shown no influence of K fertilization on guava trees (Terán et al., 1996; Ide and Martelleto, 1997). Soil analysis (Table 1) displays average K contents and a Ca/Mg ratio of 2.07. In this case, it can be inferred that there is competitive inhibition involving K and Ca and Mg. In other words, high K concentration in the rhizosphere has inhibited Ca and Mg uptake (Marschner, 1995). Increased yield in response to K fertilization (0, 90, 180, 360, 540, 720, and 900 g per plant of K_2O) in Paluma guava orchards cultivated with low K content in a *Latossolo Vermelho-Amarelo* with low K content and a 1.75 soil ratio were observed by Hernandes et al. (2012). Such results could be associated with non-cycling of orchard pruning material, as these Guava trees were three years old.

Initial K soil concentration (2.2 $mmol_c$ dm^{-3} in the 0.00-0.20 m layer in the guava rows) and the cycling of pruning material, plus possible characteristics innate to the guava tree itself, may have helped K uptake. The extensive and efficient root system of guava can exploit a huge soil volume, and this could also explain maintenance of plant output.

As for the N and K interaction, no significant effect was observed ($p > 0.05$). Similar results were obtained by Ide and Martelleto (1997), who observed no significant effect of N fertilization on the output of young Paluma guava trees. No influence was verified by either K itself or in its interaction with N.

Paluma guava trees showed seasonal production variation in its output cycles (Figure 2). Albeit orchard management was identical in all five cycles, rainfall and temperature variations (Figure 1) might explain the yield differences. One of pruning's upsides is to avoid the ups and downs of alternating harvests (Pizza Júnior, 1994). Another upside would allow a more homogeneous fruit distribution in the plant itself. However, much yield variation, in view of the fruit development cycle (time), following Paluma guava tree pruning was verified by Rozane et al. (2012).

February pruning established the cycle with greatest fruit production, followed by September, December, and March pruning – all three with similar outputs. June pruning came last, with the smallest production. In São Manuel (another municipality in S.P.), Paluma trees pruned in September and October had a lower output than trees pruned in August (Ramos et al., 2011). In contrast, Serrano et al. (2008), in Pinheiro, state of Espírito Santo, observed greater Paluma fruit output in December-pruned trees (in addition to greater fruit weight), compared to February pruning.

Guava tree pruning is a good management practice to ensure scheduling of production. A caveat, however, to the producer: he/she should be aware that fruit production may vary, depending on pruning time. This could obviously cause an increase or decrease in yield.

Except for the first production cycle, nitrogen fertilization significantly affected ($p < 0.05$) the [N, P, S | K, Ca, Mg] balance (Figure 3). Increasing the N supply encourages growth and, consequently, uptake of other nutrients (Fageria, 2001; Faquin, 2005), thus promoting change in the balance values, as this nutrient (N) is the most interactive with other nutrients in the plant tissue (Faquin, 2005). The effect of N fertilization can translate

into higher or lower nutrient contents, depending on the quantity of nutrients supplied in the root zone (Fageria, 2001). However, no significant effect ($p>0.05$) of K fertilization was observed on this balance.
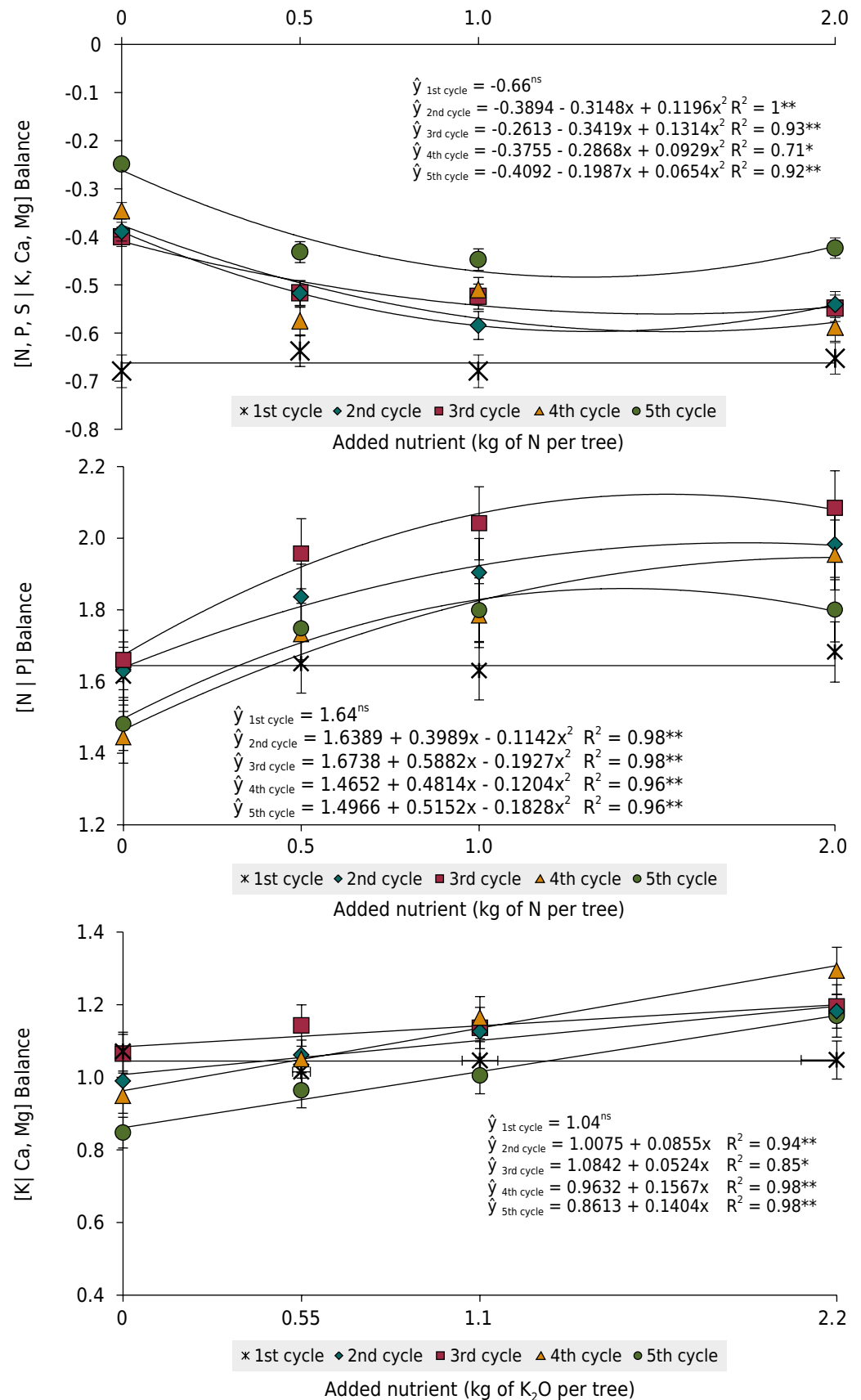

Figure 3. Leaf nutrient balances of 'Paluma' guava. Vertical bars at each point represent the standard error of the mean.

The average [N, P | S] balance was significant in response to nitrogen fertilization, here represented by the equation $\hat{y}_{[N, P \,|\, S] \,average}$ = 0.6669 + 0.0639 x – 0.0139 x^2 (R^2 = 0.99**). The derived balance regression equation revealed a 2.3 kg per plant of N application rate as responsible for the maximum balance value. The increase in average balance value is due to N stimulation of soil nutrient uptake, which triggers an increase in balance value all the way to the maximum application rate verified. Nitrogen nutrition exerts strong a regulatory influence on S assimilation and vice-versa (Duke and Reisenauer, 1986), as N and S plant assimilation are associated with each other (Zhao et al., 1997). Generally, P has a significant positive interaction with N uptake and plant growth (Terman et al., 1977; Summer and Farina, 1986), in synergistic relation with them, as a result of stimulus to growth and appropriate uptake of both elements (Summer and Farina, 1986).

The [N | P] balance varied in response to nitrogen fertilization, fitting a quadratic equation, except for the first cycle only (Figure 3). An increase in the balance in view of N application rates is due to the increase in N concentration in plant tissue and can also relate to greater N uptake in relation to P, on account of its greater availability. Another explanation could be the dilution effect (Andrade et al., 2000), triggered by swift growth of branches in nitrogen-fertilized plants.

The [K | Ca, Mg] balance likewise varied in response to potassium fertilization, showing increases in balance value, except in the first cycle (Figure 3). The [K | Ca, Mg] ratio varied due to increased K concentration in the plant tissue (as the K nutrient is more available). It can also be caused by competitive uptake, as K effectively competes with Ca and Mg, thus reducing uptake of both of these nutrients (Marschner, 1995).

Attention has yet to be given to the antagonistic effect on Ca and Mg uptake whenever K is in higher concentration, which depends on the plant species and on environmental considerations (Malavolta, 2006). Increased K application rates in the corn crop also increased K leaf content but reduced Ca and Mg leaf contents (Vilela and Büll, 1999).

The average [Ca | Mg] ratio showed significant response to potassium fertilization, and is represented by the equation $\hat{y}_{[Ca \,|\, Mg] \,average}$ = 0.8754 + 0.0262 x (R^2 = 0.93*). Potassium fertilization may have interfered more in Mg than in Ca uptake, given the fact that the amount of Ca in the soil was superior to that of Mg (Table 1).

Variations in the environmental conditions of each cycle (Figure 1) influenced average leaf balance [N, P, S | K, Ca, Mg], [N, P | S], [N | P], [K | Ca, Mg] and also average [Ca | Mg], changing balance values in the different cycles evaluated (Figure 4). Although there is no information in the literature on nutrient balance; nevertheless, similar results were verified by Godoy et al. (2012), which indicates that climate conditions, temperature, and rainfall all influenced nutrient leaf contents in banana trees. Lima et al. (2007) also found macronutrient content variability in acerola tree leaves at different sampling times was also found by Lima et al. (2007). In Paluma guava, Rozane et al. (2012) point out that variation in luminosity, rainfall, and temperature are the chief factors of variation in values of nutrient balance.

To assess the nutrient balances in this study, ranges of leaf nutrient balances based on standard element contents indicated by Natale et al. (2002) and by Maia et al. (2007) were drawn up (Table 3). The ranges of leaf balances calculated based on Maia et al. (2007) were outside the scope of leaf balances in higher-yielding guava trees in this study (Figure 4). This particularly applied to leaf balances [N, P | S], [N | P] and [K | Ca, Mg], which had leaf content below Ca and P, and leaf content higher than K and S. Ranges of leaf nutrient balance calculated as based on Natale et al. (2002) seem sufficient for leaf balances [N, P, S | K, Ca, Mg] and [N, P | S], yet they could be adjusted for leaf balances [N | P], [K | Ca, Mg], and [Ca | Mg], as suggested in table 3. The adjustment carried out here sought to consider all the data in high-output guava trees analyzed in this study, taking into due account the management practices chosen and the climate conditions at that time.
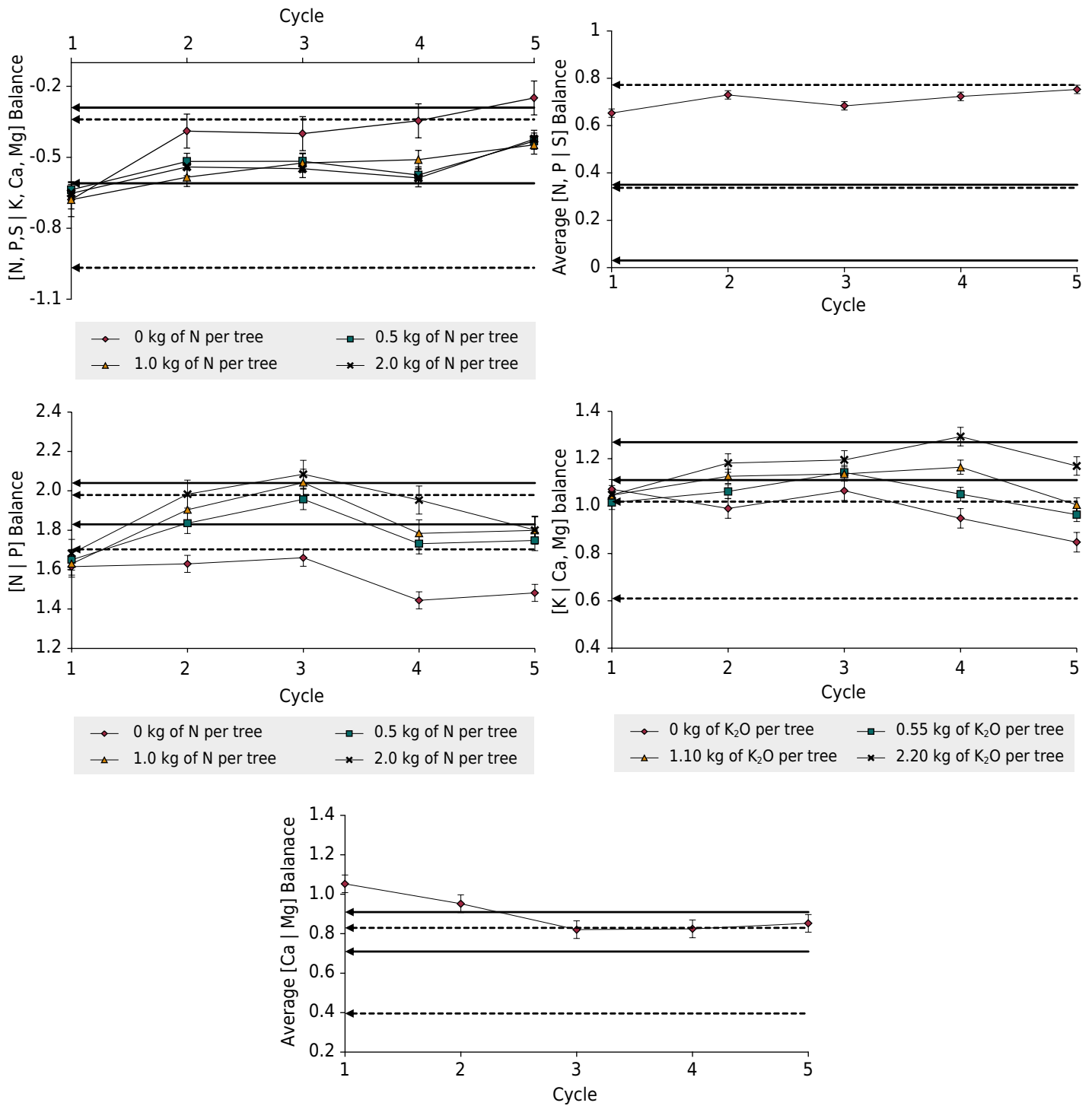
Figure 4. Seasonal variation in nutrient balance of 'Paluma' guava during the trial period. Vertical bars at each point represent the standard error of the mean. Arrows are the ranges of nutrient balances calculated from Natale et al. (2002) (dashed lines) and Maia et al. (2007) (solid lines).

Table 3. Range of nutrient balances obtained by standard nutrient content indicated by literature

Nutrient balance	Range of nutrient balance of leaf					
	Natale et al. (2002)		Maia et al. (2007)		Natale et al. (2002) adjusted	
	LL[1]	UL[2]	LL	UL	LL	UL
[N, P, S | K, Ca, Mg]	-0.97	-0.34	-0.61	-0.29	-0.97	-0.34
[N, P | S]	0.34	0.77	0.03	0.35	0.34	0.77
[N | P]	1.70	1.98	1.83	2.04	1.60	2.20
[K | Ca, Mg]	0.61	1.02	1.11	1.27	0.61	1.30
[Ca | Mg]	0.40	0.83	0.71	0.91	0.40	0.90

[1] LL: lower limit; [2] UL: upper limit.

CONCLUSIONS

The nutrient balances and nutrient concentration values can be interpreted coherently using compositional data analysis.

Ranges of nutrient balances also were established for "Paluma" guava and validated through ranges grounded in nutrient contents currently used in Brazil.

Nitrogen fertilization increased "Paluma" guava yield. The 0.5 kg N application rate per plant and the other studied treatments practically showed the same results, and their values were affected by pruning time as well as the nutrient balances.

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