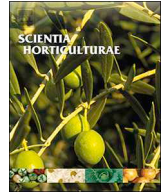




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## Nitrogen fertilization affects yield and fruit quality in pear

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### ABSTRACT

Mineralized nitrogen (N) from organic matter and decomposing residues in most soils are not always taken into account. Little is known about the correct N application dose for pear trees to promote yield and fruit quality, without increase the risk of N loss into the environment. The study aimed to evaluate the effect of N doses application on N soil content, tree yield and fruit quality. The treatments consisted of the application of 0, 40, 80, 120 and 160 kg N ha<sup>-1</sup> year<sup>-1</sup>, whereas urea was applied on the soil surface in September and February for 5 seasons (2011/12 to 2015/16). Leaves were collected, prepared and submitted to nutrient analysis. Fruit weight, number and yield were evaluated. Soil samples were collected at 0-0.20 m for 3 seasons (2013/2014 to 2015/2016), prepared and submitted to NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N analysis, and then mineral N content was calculated. Peel color, ethylene production, and respiration rate were analyzed in the 2015/2016 crop season after 90 days inside the controlled atmosphere storage chamber. The fruits were kept at ambient conditions for 7 days and evaluated again for peel color, ethylene production and respiration rate, as well as destructive parameters such as titratable acidity, soluble solids and pulp firmness. The doses of N application affect the fruit number and yield, but did not affect leaf nutrient concentration. The highest N levels in the soil were observed in the 2014/2015 and 2015/2016 crops. The most economical doses were 122.0, 66.4, 22.5 and 96.0 kg N ha<sup>-1</sup> in the crops of 2011/2012, 2012/2013, 2013/2014 and 2015/2016 respectively.

### 1. Introduction

Brazil imports approximately 150 thousand tons of pear per year, which is equivalent to 86% of the total fruit consumed in the country (FAO, 2019). This is related to the fact that only 1.305 ha are cultivated with pear in Brazil, which generates a total production of 22,108 thousand Mg ha<sup>-1</sup> year<sup>-1</sup> (IBGE, 2019).

The fruit import can represent a high cost for the importing countries, reducing the generation of jobs linked to the productive chain and, consequently, diminishing the profitability of the producers. For this reason, it is desirable to prompt cultivation and production of fruits in rural areas of the consuming country, especially in regions with favorable soil and climatic conditions for fruit trees like the pear tree (Fachinello et al., 2011; Pasa et al., 2015, 2011). The average yield of the pear tree in Brazil is 12.9 Mg ha<sup>-1</sup> year<sup>-1</sup>, which is lower than what

is achieved in traditional producing countries, such as Argentina, which obtain yields of 36.2 Mg ha<sup>-1</sup> year<sup>-1</sup> (FAO, 2019).

This low pear yield may be related to the difficulty of cultivars, rootstocks and cultivar-rootstock combinations in adapting to climatic conditions, as well as a lack of knowledge on the best management practices to reduce the incidence of pest and disease, but also because of a lack of definition of the correct nitrogen (N) application rate. This is because N uptake and storage by the pear trees can impact fruit growth, yield, and quality (Botelho et al., 2010; Ikinici et al., 2014).

The state of Santa Catarina (SC), which is located in the southern region of Brazil, is the first largest pear producer in the country (IBGE, 2019) with 408 hectares (IBGE, 2019). Some areas of Santa Catarina have adequate climatic conditions for pear cultivation (Fachinello et al., 2011) and also the industrial infrastructure already in place for apple production, that can be extended and used for pear production (Mello,

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2013). In general, pear orchards are established in flat or undulating relief, in Typic Haplumbrept or Lithic Udorthents soils with medium to high organic matter content (Soil Survey Staff, 2014). Cover crop plants of the legume family, that cohabit within these orchards, may promote biological fixation of atmospheric N. However, grass family plants may absorb nutrients, such as N from deeper soil layers (Gómez-Muñoz et al., 2014). Cover crops residues can be deposited and decomposed on the soil surface to mineralize N (Montanaro et al., 2017). As a result, the soils hypothetically provides satisfactory N demand to pear trees (Neto et al., 2009). However, the orchards are located at altitudes of approximately 1300 m, with an average temperature of 13 °C, which reduces the mineralization of organic matter residues in the soil, decreasing N availability to the plants (Dar et al., 2013; Mota et al., 2011; Rodrigues et al., 2013). Furthermore, soils in this region are typically shallow, which reduces the volume explored by the roots, decreasing the uptake of water and nutrients, such as N (Ernani et al., 2008). Therefore, there is a real for N application in the soil that can be found in sources such as urea, which is a nitrogenous fertilizer with a low cost that can be easily found and purchase.

The pear tree N requirement and doses can be established based on soil organic matter (OM) contained on the soil, leaf N concentration and vegetative growth (CQFS-RS/SC, 2016). However, soil OM offers an idea of the potential N available on the soil over medium to long-term periods but does not predict values in short-term periods, such as in an agricultural crop season (Ernani et al., 2008; Ismaili, 2015). Furthermore, leaf N concentration is not always sensitive to diagnose N availability for plants and often has no relation with yield or fruit quality parameters (Dar et al., 2013). Therefore, performing calibration experiments in the long run, to compare different N application, can provide results and information needed to enhance the crops (Stüpp et al., 2015). Thus, an increase in fruit yield, quality, peel color, pulp firmness, TA, TSS, in both pre and post-harvest (Martin et al., 2015) are expected along with farm income (Souza et al., 2013).

Pear trees grown in soils with low N availability may exhibit a leaf area reduction, which decreases the photosynthetic rate, reflecting in a smaller fruit size and lower yield. On the other hand, plants submitted to high N application doses can increase their concentration inside the plant, stimulating the leaf area and, consequently, the incidence of foliar and fruit diseases. However, N surplus can increase fruit diameter, which may dilute TSS and increase respiration. Adequate N application doses pear orchard soils may increase soil mineral N content (mainly  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N) and supply plant nutrient demand (Brunetto et al., 2015; Gómez-Muñoz et al., 2014). Yet, in periods of rainfall (typical in subtropical conditions), most N forms (mainly N- $\text{NO}_3^-$  that is not absorbed by pear trees) may be lost by leaching or surface runoff, especially in soils located in sloping relief. The study aimed to evaluating the effect of N application doses on soil N concentration, tree yield, and fruit quality in a commercial pear orchard in South Brazil.

## 2. Material and methods

### 2.1. Location and treatments

The experiment was conducted in a pear orchard of cv. Rocha grafted on the quince (*Cydonia oblonga*) BA29 rootstock, at a density of 2500 plants  $\text{ha}^{-1}$  (4 m between rows x 1 m between plants). Trees were planted in 2006 in São Joaquim, located in the state of Santa Catarina, southern Brazil (28° 17'38" S, 49° 55' 54" W and an average altitude of 1.353 m). The climate is humid mesothermal (Cfb) according to Köppen classification (Peel et al., 2007). Average annual rainfall ranges from 1360 to 1600 mm, and there are 20 to 29 frosts throughout the year. The average temperature (°C) and rainfall (mm) during the whole experiment are shown in Fig. 1.

The soil was a Typic Humudept (Soil Survey Staff, 2014) having the following characteristics at 0-0.20 m: 450 g  $\text{kg}^{-1}$  of clay (Pipette method); 43 g  $\text{kg}^{-1}$  of organic matter (Walkley-Black method); 6.6 pH in

water (1:1 ratio); 4.6 mg  $\text{kg}^{-1}$  of available P and 65 mg  $\text{kg}^{-1}$  of available K (both extracted by Mehlich-1); 0.0, 9.5 and 4.0  $\text{cmol}_c \text{kg}^{-1}$  of exchangeable Al, Ca and Mg, respectively (extracted by KCl 1 mol  $\text{L}^{-1}$ ). Prior to the transplanting of the seedlings, 100 kg  $\text{P}_2\text{O}_5 \text{ha}^{-1}$  (CQFS-RS/SC, 2004) and 25 kg  $\text{K}_2\text{O ha}^{-1}$  (CQFS-RS/SC, 2004) were applied to the soil surface and incorporated up to the 0-20 cm layer with plowing, followed by harrowing.

Later approximately 10% of the trees were grafted with Asian pear (*P. serotina*) cv. Housui, to aid the orchard pollination as well as the installation of beehives (*Apis mellifera*), which were added between the rows every year at the flowering period. The pear trees were handled in a central leading system, abiding 2 m spacing of between plants and 4 m between rows (density of 1250 plants per hectare). Every 2 months, the cover crops located at the rows were desiccated with 2.0 L  $\text{ha}^{-1}$  of non-residual herbicide (N-(phosphonomethyl) glycine, glyphosate).

The cover crops contained in the orchard consisted of the following species: *Trifolium repens*, *Trifolium pratense*, *Trifolium vesiculosum*, *Plantago major*, *Paspalum sp*, *Pimpinella anisum* and *Graminea sp*. The vegetation between the rows was mowed approximately every two months and kept at a height of 10 cm. The residues were deposited on the soil surface between the planting rows. The cover plants management was carried out with periodic mowing along the vegetative and productive cycle of the pear trees, leaving the residues over the soil surface. The experimental design chosen was randomized blocks implemented in October 2010, which consisted of five plants sequentially arranged in a row where the three central plants were evaluated. The treatments consisted of annual applications of 0, 40, 80, 120 and 160 kg N  $\text{ha}^{-1}$  by the use of urea (44% total N). Urea was applied twice a year on the soil surface (September and February), without incorporation and in the treetop projection area. The application of fungicides and insecticides if required were carried out following technical recommendations for pear tree crop.

### 2.2. Leaf collection and nutrient analysis

From January 15 to February 15 of each crop season, 20 leaves were picked up per plant in the middle third of the year branches, on opposite sides of the plant following CQFS-RS/SC (2016) references. Subsequently, the leaves were dried in a forced air circulation at 65 °C, to be grounded in a Willey mill with a 2 mm sieve and directed to sulfur digestion (Tedesco et al., 1995). An amount of 0.200 g of dry matter was placed it in a digestion tube (25 x 250 mm) to be then added 1 mL of  $\text{H}_2\text{O}_2$ , 2 mL  $\text{H}_2\text{SO}_4$  and 0.7 g of the digestion mixture (90.9%  $\text{Na}_2\text{SO}_4$  and 9.1%  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ). The tubes were then placed and heated in a digester block at 150 °C, in which the temperature was raised gradually, 50 °C every 30 min up to 350 °C. After complete digestion of the leaves, until a predominance of a yellow-greenish color, the tubes remained in the digester block for another 60 min at 350 °C. Sequentially, the total N was determined in a semi-micro Kjeldahl steam distillation unit (Tecnal TE-0364, Brazil). The leaves collected in 2014 and 2015, P and K concentrations were also analyzed. The P concentrations were determined by spectrophotometer (Pro analyse UV 51-00, Brazil), with 882 nm absorbance, according to Murphy and Riley's method (1962). The K concentration was determined by a flame photometer (Digimed DL-62, Brazil) (Tedesco et al., 1995).

### 2.3. Yield and chemical analysis of fruits after harvesting and storing

At full physiological maturation of pears in all crops happened in February, in which every fruit per plant was counted, collected and weighed using a scale Shimadzu (AUY 220, Japan), in order to determine yield per plant and per hectare. In the 2015/2016 crop season, 20 fruits were randomly collected per treatment, discarding the damaged fruits, to be later stored in a refrigerated atmosphere for 90 days at a temperature of  $-0.5 \pm 0.1$  °C and  $95 \pm 2\%$  RH. Afterwards, the fruits were withdrawn from the cold chamber and submitted to peel

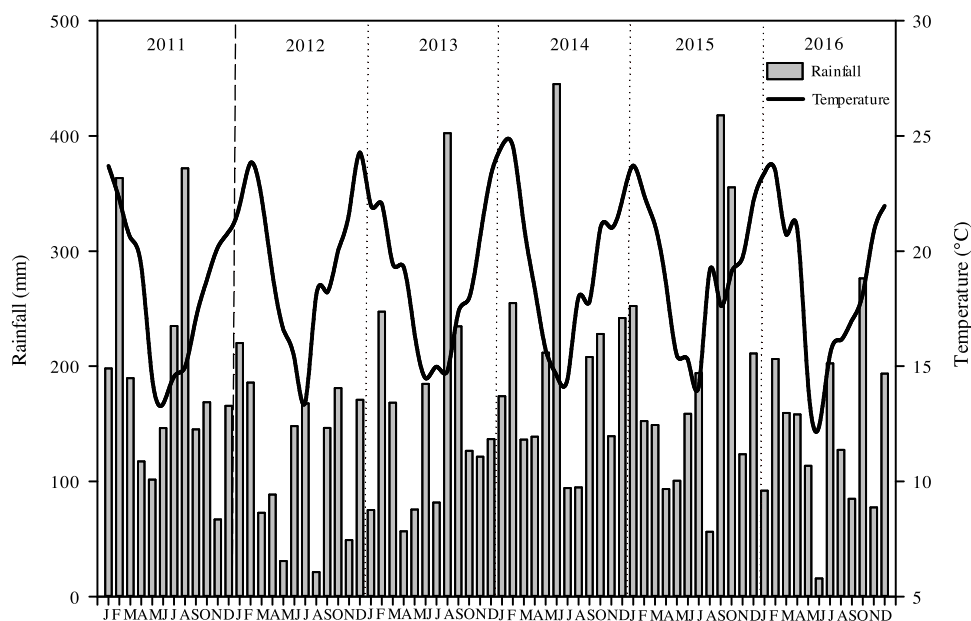


Fig. 1. Average monthly rainfall (mm) and air temperature (°C) in 2011, 2012, 2013, 2014, 2015 and 2016.

color, ethylene production, and respiratory rate analysis (Brackmann et al., 2013). These same parameters in the fruits were assessed after 7 days under ambient conditions ( $20 \pm 2^\circ\text{C}$  and  $80 \pm 5\%$  RH), and sequentially determined pulp firmness, total titratable acidity (TFA) and soluble solids (SS) (Adolfo Lutz Institute, 2008).

The peel color readings were determined using a colorimeter (Konica Minolta CR 400, Japan), from the average of three readings performed in the equatorial region of the fruits, which had their color expressed in the following variables: L, corresponding to the luminosity (brightness, clarity or reflectance; 0 = dark/opaque and 100 = white); C, chroma (saturation or color intensity, 0 = impure color and 60 = pure color); H, Hue angle (color angle,  $0^\circ$  = red,  $180^\circ$  = green,  $270^\circ$  and  $360^\circ$  = black). The ethylene production of the fruits was determined by gas chromatography, where the fruits were individually allocated in a hermetically sealed 5 L container for approximately one hour. Then, two 1.0 mL gas samples were subsequently injected into a chromatograph (Varian® model Star CX 3400, Palo Alto, CA, USA), with a flame detector (FID) and a Porapak column N80/100 with controlled temperature (90, 140 and  $240^\circ\text{C}$ ). The ethylene production was expressed in nmol of ethylene  $\text{kg}^{-1} \text{s}^{-1}$ . The respiratory rate was measured by the amount of  $\text{CO}_2$  produced by the fruits in an electronic gas analyzer (Isolcell, Italy), expressed in  $\text{mL CO}_2 \text{kg}^{-1} \text{h}^{-1}$ .

The TTA was performed by processing in a centrifuge (Philips Walita, Brazil) for manual titration. Ten milliliters of the fruit juice extract obtained was pipetted, and then 100 mL of distilled  $\text{H}_2\text{O}$  was added to the titrated with  $\text{NaOH}$   $0.1 \text{ mol L}^{-1}$  until pH 8.1. The SS contents were obtained using a manual refractometer (Atago Master  $\alpha$ , Japan), and expressed in  $^\circ\text{Brix}$  units. Pulp firmness was measured with an automatic penetrometer (Effegi Systems, Milan, Italy), and expressed in Newton (N). The penetrometer readings were performed at two opposed locations in the equatorial region of the fruits after removal of a peel portion with an 8.0 mm diameter tip.

#### 2.4. Soil collection and analysis of N forms

On 14 March, April, June and July 2014; and on 15 September, October, November, and December 2015 and January 2016, three samples were collected at 0.0-0.20 m using an auger in the treetop projection area of the three central plants of each treatment. The sample was placed into plastic bags and stored in a Styrofoam cooler with ice, where 5 g of moist soil was selected in the lab and placed into

a 90 mL snap cap flask. Then, 50 mL of a  $1 \text{ mol L}^{-1}$  KCl solution was added and mixed for 30 min to be decanted for 30 min. Soon after, 20 mL of the supernatant was added in digestion tubes with 0.7 g of MgO and distilled in a semi-micro Kjeldahl steam distillation unit. After distillation, the extract ( $\pm 35 \text{ mL}$ ) was collected in an Erlenmeyer flask containing 5 mL of indicator-boric acid mixture, and titrated with  $\text{H}_2\text{SO}_4$  ( $0.0025 \text{ mol L}^{-1}$ ), allowing us to determine  $\text{NH}_4^+\text{-N}$  content. In the same sample, 0.7 g of Devarda's was added and subjected again to distillation, following the same procedure to determine  $\text{NO}_3\text{-N}$  content. With  $\text{N-NH}_4^+$  e  $\text{N-NO}_3^-$  data, it was possible to calculate N mineral content (Tedesco et al., 1995).

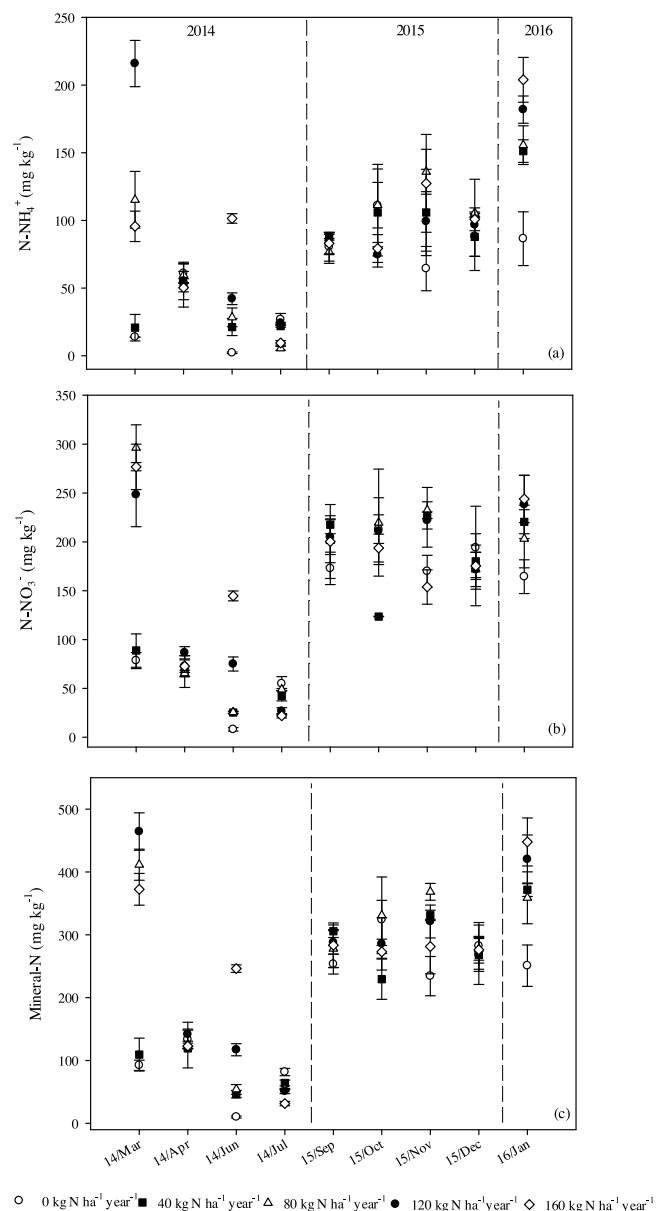
#### 2.5. Statistical analyses and calculations

Polynomial regression equations were adjusted for leaf N, P and K content, production parameters (number, fruit mass and yield) and quality parameters (peel color, ATT, SS, pulp firmness, ethylene production and respiration rate), when there was a significant effect over the variance analysis. Due to the lack of "independence" among the observations and N content results in the soil throughout the evaluation period, made a polynomial regression analysis impossible. Moreover, the impossibility to control some environmental variables experimentally implied in faulty assumptions for the variance analysis. As a result, the averages of the results were presented with their respective standard deviations.

N doses with superior economic viability in the pear orchard were calculated for each crop according to (Natale et al., 1996). It was considered the average price of a fresh pear kilogram sold US \$ 1.44, and urea US \$ 0.42 with regards to the average price of the last six months of 2018 (Infoagro, 2019). In order to set values according to the fruits exchange rate fluctuation, a relation of exchange was chosen instead of the current currency to have a more stable data. In this way the "currency" used in the calculations was the "pear" itself, considering the following equivalence relationship: kg KCL applied per kg of pear paid, which is equal to  $\text{US } 0.42/1.44 = 0.29$ . The most economical dose was calculated based on the derivative from the regression equation between the applied N doses and the fruit yield, making it equal to the exchange ratio by Eq. 1:

$$dy/dx = a1 + 2ax = \text{exchange ratio} \quad (1)$$

Where: most economical dose ( $x'$ ) is calculated by the Eq. 2:



**Fig. 2.** N-NH<sub>4</sub><sup>+</sup> (a), N-NO<sub>3</sub><sup>-</sup> (b) and mineral N (c) concentration at 0.0-0.20 m in a pear orchard submitted to nitrogen doses. Vertical bars indicate the standard error of the mean.

$$x' = a1 - \text{relação de troca} / 2. (-a2) \quad (2)$$

The cumulative production of the four crops was used to calculate the most economical doses of N fertilizer.

### 3. Results

#### 3.1. N in soil

On March 14 (2014), the highest NH<sub>4</sub><sup>+</sup>-N concentration collected at layer 0-0.20 m in the soil presented a value higher than 200 mg kg<sup>-1</sup>, being induced by the application of doses of 120 kg N ha<sup>-1</sup> followed by 160 and 80 kg N ha<sup>-1</sup>. The lowest NH<sub>4</sub><sup>+</sup>-N concentration in soil was identified at doses of 0 and 40 kg N ha<sup>-1</sup> (Fig. 2a). On April 14 and July (2014), NH<sub>4</sub><sup>+</sup>-N soil concentration was not affected by N rates, but on June 14th, (2014), N dose application of 160 kg ha<sup>-1</sup> promoted the highest NH<sub>4</sub><sup>+</sup>-N soil concentration. On September, October and December (2015), the soil content NH<sub>4</sub><sup>+</sup>-N was not affected by N doses

(Fig. 2a).

On March 14th (2014), the highest content of NO<sub>3</sub><sup>-</sup>-N in the soil was induced by the application of N doses of 80, 120 and 160 kg N ha<sup>-1</sup> (Fig. 2b). On April 14 and July 14th (2014), N-NO<sub>3</sub><sup>-</sup> soil concentration was not affected by N application rates. On June 14th (2014), the highest NO<sub>3</sub><sup>-</sup>-N concentration was found at 160 kg N ha<sup>-1</sup> dose application, which matches with the same NH<sub>4</sub><sup>+</sup>-N concentration in the soil at the same evaluation period (Fig. 1a). On September and December 15th (2015), soil NO<sub>3</sub><sup>-</sup>-N concentration was not affected by N rate. On October 15th (2015), the lowest NO<sub>3</sub><sup>-</sup>-N was found at doses application of 40 kg N ha<sup>-1</sup> and on 15th (2015), at 40, 80 and 120 kg N ha<sup>-1</sup>. On January 16th (2016), the highest soil NO<sub>3</sub><sup>-</sup>-N was prompted by doses application of 160 kg N ha<sup>-1</sup> (Fig. 2b).

On March 14th (2014), the highest soil mineral N concentration was found at the application doses of 80, 120 and 160 kg N ha<sup>-1</sup> (Fig. 1c). On April and July 14th (2014), the soil mineral N content did not diverge statistically among N rates. In June 2014, the highest mineral N concentration was identified at an application dose of 160 kg N ha<sup>-1</sup>. On September, October and December 15th (2015), the soil mineral N concentration was not affected by N application rates, but on November 15th (2015), the highest mineral N soil concentration was identified in response to the application doses of 80 kg N ha<sup>-1</sup>. On January 16th (2016), the application dose of 160 kg N ha<sup>-1</sup> promoted the highest N mineral concentration in soil (Fig. 2c).

#### 3.2. Leaf nutrient concentration tree yield and fruit quality

The N doses applied in the soil over five years affected the number of fruits and yield at the pear tree orchard for four seasons (Table 1). N doses applied in the soil did not affect the concentration of N, P, and K in the leaves, neither weight or pear trees (Table 1). The N application did not also affect, peel color, ethylene production and respiration rate of the pear fruits after 90 days in refrigerated storage (Table 2). Likewise, after 7 days under ambient conditions at 20 °C, peel color, titratable acidity and ethylene production in fruit were not affected. However, the respiration rate increased in a quadratic way, along with increasing N application dose (Table 2).

#### 3.3. Most economical doses

The most economical dose was calculated for from five crops from 2011/12 to 2015/16. In the four crops, the response curve to the N fertilizer was quadratic (Fig. 3) and, by using regression equations shows in Natale et al., 1996, the highest fertilizer dose of 2011/12 crop was:  $x' (49.1 - 0.29) / (2 \times 0.2) = 122.0 \text{ kg N ha}^{-1}$  (or divided by 2500 plants/ha = 48.8 g N/plant). The estimated income due to N fertilization can be calculated by yield increase (8314.0 kg fruits/ha<sup>-1</sup>), fertilizer cost (35 kg fruits/ha<sup>-1</sup>) and by the revenue obtained (8314.6 kg fruits/ha<sup>-1</sup>). The same system was used for 2012/2013, 2013/2014 and 2015/2016 season crops, where the most economical dose was 66.4, 22.5 and 96.0 kg N ha<sup>-1</sup>, and the revenue obtained was 7430.7, 10,701.6 and 6762 kg pear ha<sup>-1</sup>, respectively.

In the 2014/2015 crop, the relationship between the increase of the N doses and the fruit yield was linear, not allowing the calculation of the most economical dose.

### 4. Discussion

The N mineral data collected during the experiment revealed an N soil content variation following seasonal tree demand for nutrients. In winter period (i.e. June-August), there was a decrease of N availability on the soil during tree dormancy cycle, and in spring and summer (i.e., September – February), there was an increase of N availability on soil due to higher pear tree demand for nutrients and warmer temperatures. In this study, the average temperature was 20 °C in the summer, which is considered suitable for C and N mineralization in soil temperatures.

**Table 1**  
Leaf nutrient concentration, fruit number and weight and yield in pear trees submitted to nitrogen application.

Variable	N dose (kg N ha <sup>-1</sup> year <sup>-1</sup> )					Equation	R <sup>2</sup>	CV(%)
	0	40	80	120	160			
2011/2012 crop								
Leaf N (g kg <sup>-1</sup> )	15.8	14.7	14.9	14.6	16.3	ns	–	15.1
Number of fruits per plant	33	33	31	28	33	y = 35.03-0.14x + 0.0007x <sup>2</sup>	0.25	15.5
Fruit weight (g)	164.5	163.1	167.0	164.7	156.5	ns	–	9.3
Yield (kg plant)	5.3	5.4	5.3	4.4	5.3	y = 5.67-0.01x + 0.00008x <sup>2</sup>	0.25	16.5
Yield (Mg ha <sup>-1</sup> )	13.4	13.5	13.2	11.0	13.2	y = 14.18-0.04x + 0.0002x <sup>2</sup>	0.25	16.5
2012/2013 crop								
Leaf N (g kg <sup>-1</sup> )	12.7	15.5	14.8	14.4	16.7	ns	–	11.2
Number of fruits per plant	39	26	28	39	37	y = 36.56-0.20x + 0.001x <sup>2</sup>	0.46	16.7
Fruit weight (g)	122.0	115.9	122.1	128.0	122.1	ns	–	11.3
Yield (kg plant)	4.8	3.2	3.4	4.7	4.4	y = 4.34-0.02x + 0.0001x <sup>2</sup>	0.33	21.0
Yield (Mg ha <sup>-1</sup> )	11.9	7.5	8.5	12.7	11.1	y = 10.86-0.05x + 0.003x <sup>2</sup>	0.34	21.1
2013/2014 crop								
Leaf N (g kg <sup>-1</sup> )	13.9	14.9	14.9	14.4	14.3	ns	–	13.2
Number of fruits per plant	47	33	37	49	46	y = 43.82-0.18x + 0.001x <sup>2</sup>	0.43	17.0
Fruit weight (g)	106.0	131.0	139.0	119.0	135.0	ns	–	14.3
Yield (kg plant)	4.9	4.3	5.1	5.8	6.2	y = 4.73-0.003x + 0.00008x <sup>2</sup>	0.83	15.3
Yield (Mg ha <sup>-1</sup> )	12.4	10.1	12.8	14.6	15.5	y = 11.84-0.009x + 0.0002x <sup>2</sup>	0.84	15.3
2014/2015 crop								
Leaf N (g kg <sup>-1</sup> )	15.2	16.0	17.7	19.3	21.4	y = 15.15 + 0.02x-0.0001x <sup>2</sup>	0.99	9.4
Leaf P (g kg <sup>-1</sup> )	1.4	1.2	1.3	1.4	1.7	ns	–	21.7
Leaf K (g kg <sup>-1</sup> )	2.8	3.2	3.1	3.0	3.3	ns	–	23.1
Number of fruits per plant	39	36	41	29	35	y = 39.65-0.04x + 0.00003x <sup>2</sup>	29.70	15.5
Fruit weight (g)	122.5	120.5	134.0	124.7	132.5	ns	–	12.5
Yield (kg plant)	5.1	3.9	4.8	4.3	4.4	ns	–	26.7
Yield (Mg ha <sup>-1</sup> )	12.7	9.8	12.0	10.7	10.9	ns	–	26.7
2015/2016 crop								
Leaf N (g kg <sup>-1</sup> )	15.2	19.3	16.0	16.8	18.5	ns	–	18.3
Leaf P (g kg <sup>-1</sup> )	2.9	3.3	3.1	2.8	3.2	ns	–	10.7
Leaf K (g kg <sup>-1</sup> )	2.9	2.2	3.5	3.3	3.2	ns	–	28.5
Number of fruits per plant	66	28	28	49	37	y = 59.46-0.61x + 0.003x <sup>2</sup>	0.48	10.7
Fruit weight (g)	98.5	99.6	123.2	111.7	106.1	y = 95.26 + 0.41x-0.002x <sup>2</sup>	0.60	6.67
Yield (kg plant)	6.5	2.7	3.5	5.5	3.9	y = 5.72-0.04x + 0.0002x <sup>2</sup>	0.29	13.0
Yield (Mg ha <sup>-1</sup> )	16.3	6.8	8.8	13.8	9.8	y = 14.31-0.11x + 0.0006x <sup>2</sup>	0.29	13.0

Ns = not significant.

Also, soil N mineral concentration was considered high throughout the experiment along with NH<sub>4</sub><sup>+</sup> N concentration, which remained above 200 mg kg<sup>-1</sup> and having season peaks, in March 2014 and January 2015. The reason of this high concentration of ammonium-N is linked probably to the low pH and the low concentration of O<sub>2</sub> in the soil, as a result of the frequent rainfall and the heavy (clay of 45%) texture of the soil. Ammonium-N only represents a fraction of N uptake by the roots, which may cause a toxic effect in cell cytosol if accumulated in excess. To avoid it, ammonium-N is assimilated by plant roots and transported

quickly to other organs into amino acids form (Marschner, 2012). However, the assimilation produces the excretion of one proton per ammonium-N molecule, which decreases the rhizosphere pH. At low external pH, like in the experiment conditions, proton excretion is impaired and cytosol pH drops, having a negative effect on plant growth.

All these results portraits a paradox identified during the experiment, where the high N content available in the soil was not followed by an N uptake increase of pear tree, nor a rise in leaf N concentration. In a matter fact, regardless which treatment was applied, N

**Table 2**

Quality parameters of pear (cv. Rocha) submitted to N doses after 90 days of refrigerated storage and after 7 days at ambient conditions in the 2015/2016 crop season.

N dose (Kg N ha <sup>-1</sup> year <sup>-1</sup> )	Peel color (°h)	Pulp firmness (N)	Titratable acidity (%)	Soluble solids (°Brix)	Ethylene production (µmol C <sub>2</sub> H <sub>4</sub> kg <sup>-1</sup> s <sup>-1</sup> )	Respiration rate (µmol CO <sub>2</sub> kg <sup>-1</sup> s <sup>-1</sup> )
After 90 days of refrigerated storage						
0	74.8 <sup>ns</sup>	na	na	na	19.3 <sup>ns</sup>	9.7 <sup>ns</sup>
40	74.1	na	na	na	22.5	10.9
80	77.0	na	na	na	18.8	9.3
120	74.8	na	na	na	21.2	10.3
160	75.6	na	na	na	19.6	10.0
CV(%)	3.05	–	–	–	14.8	7.3
After 7 days at ambient conditions at 20 °C						
0	77.1 <sup>ns</sup>	70.4 <sup>(1)</sup>	1.6 <sup>ns</sup>	14.3 <sup>ns</sup>	8.2 <sup>ns</sup>	13.0 <sup>(2)</sup>
40	77.1	69.6	1.6	14.6	9.6	13.8
80	77.4	69.0	1.5	14.4	11.6	15.4
120	78.8	60.6	1.6	14.3	10.3	17.9
160	79.7	60.2	1.7	14.0	10.2	19.1
CV (%)	2.62	6.21	14.9	3.44	9.6	5.3

<sup>(1)</sup>y = 70.83-0.02x + 0.003x<sup>2</sup>, (R<sup>2</sup> = 0.86\*); <sup>(2)</sup>y = 12.18 + 0.11x-0.005x<sup>2</sup> (R<sup>2</sup> = 0.75\*); ns = not significant; \* = Significant at 5% probability; Na = not analyzed.

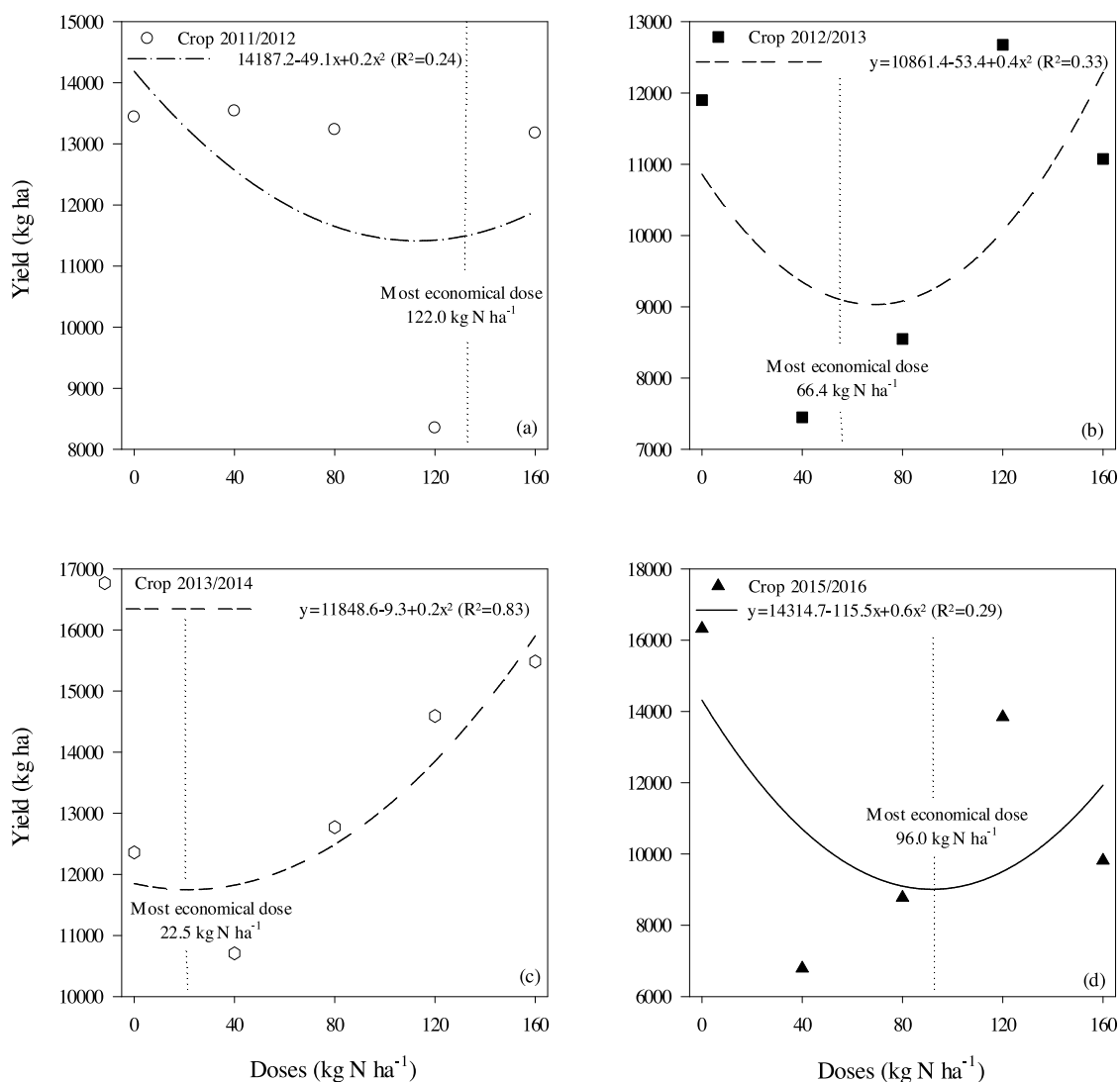


Fig. 3. Relationship between doses of urea (N) and yield in the period from (a) crop 2011/2012; (b) crop 2012/2013; (c) crop 2013/2014 and (d) 2015/2016, to establish the most economical dose.

concentration in the leaves remained always lower than 20 g kg<sup>-1</sup>, with only one exception (trees fertilized with 160 kg N ha<sup>-1</sup>, in 2015), whereas optimal N leaf concentration for cultivar Rocha is higher than 20 g kg<sup>-1</sup> (Neto et al., 2011). One possible solution for this problem can be than reduction of N mineral availability in the soil, by introducing high N demanding intercrop with high C: N ration, that can promote microbial biomass development (Wei et al., 2017). However, in the winter, there was a reduction of soil mineral N (Oliveira et al., 2016), since there was intense precipitation during the period (average rainfall of 445 mm).

Unexpectedly, N application exhibited a positive effect on fruit respiration and a negative effect on pulp firmness, after 90 days of refrigeration storage and 7 days under ambiente conditions at 20 °C. Typically, high N application delays fruit maturation lowers fruit respiration rate and ripeness.

The fertilizer cost represents less than 0.5% of the crop yield in 2011/2012; 0.3% in the 2012/2013 crop, 0.1% in the 2013/2014 and 0.5% in the 2015/2016 crop, which clearly compensates the use of N fertilizer in the orchard.

## 5. Conclusions

The application of high N doses in orchards of cultivar Rocha

affected the number of fruits and yield parameters. However, it did not increase N, K, and P concentration in the leaves. The highest N levels in the soil were observed in the 2014/2015, and 2015/2016 crops with the highest N doses applied of 120 and 160 kg N ha<sup>-1</sup>. The most economical N doses applied in the soil was 122.0 at the 2011/2012 crop, 66.4 at the 2012/2013 crop, 22.5 at the 2013/2014 and 96.0 at the 2015/2016 crop.

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