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**Annual and residual urea nitrogen contribution to the nutrition of peach trees (*Prunus persica* L.) grown under subtropical climate**

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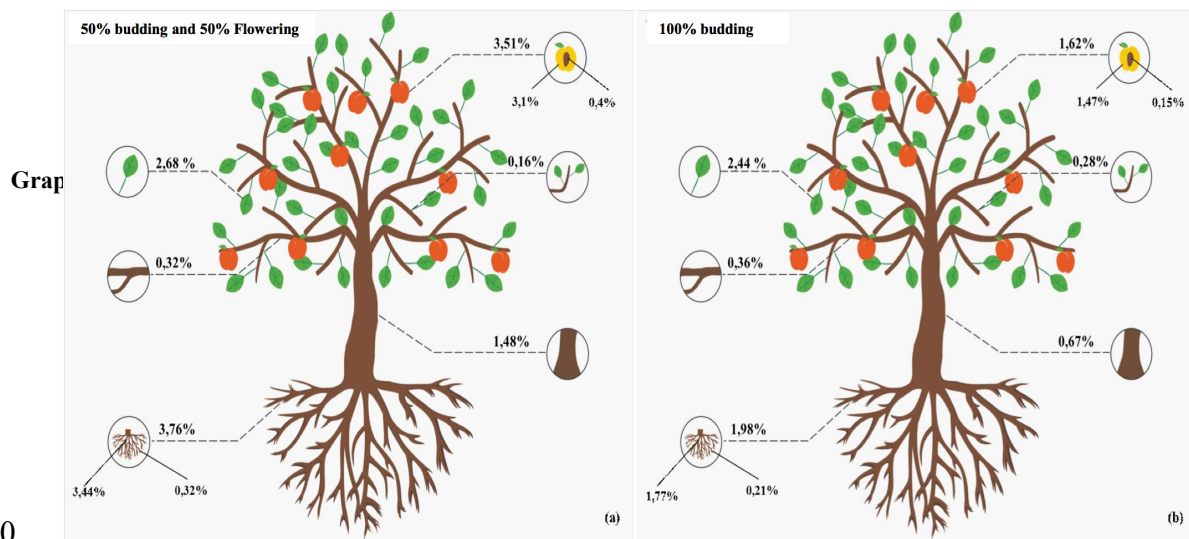
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**Highlights**

- Residual urea nitrogen was found in the soil after two years.
- Higher N deriving from fertilizer in leaves and fruits when the dose is split.
- Most N found in trees is from sources other than nitrogen fertilizer.



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Recovery of N deriving from fertilizer (%) in peach organs subjected to the application of 40 kg N ha<sup>-1</sup> in its urea form at budding (100% of the dose at budding - 100B) and split into two doses (50% of the dose at budding and 50% at flowering - 50B + 50F).

#### Abstract

Nitrogen (N) fertilizers such as urea are applied to peach orchards worldwide whenever the soil cannot meet plants' N demand. However, the actual nutritional contribution of different N supply modes applied to peach crops, or the contribution of residual N in the subsequent cycle, is yet to be fully known. The current study aims to assess the annual and residual urea N contribution to the nutrition of peach trees grown under subtropical climate. Forty kilograms of N per hectare supplied as enriched urea (3.0 at. % <sup>15</sup>N), were applied to peach in full production at a single rate (100% at budding) or split in two rates (50% at budding and 50% at flowering). Total <sup>15</sup>N and N concentrations in the application year and in the year after treatments were assessed in peach leaves throughout the cycle, in fruits (pulp and stone) at harvest and in stratified soil samples. Total <sup>15</sup>N, N concentrations and total dry mass were measured in annual and perennial tree organs in the year after treatment application. Peach trees evidenced higher N deriving from fertilizer (NDFP) in leaves and fruits (pulp and stone) in the year N was applied to the soil, as well as in the following year when N application was split into two rates. The highest NDFP amounts recorded in the year following N application were observed in leaves and fruits (annual organs) and thick roots (perennial organ), mainly when N was splitted. However, mainly in the year following its application, due to the small residual N, the N found in trees derived from sources other than N fertilizer; this justifies annual

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59<sup>N</sup> applications, whenever necessary. The cultivation of cover crops and the preservation  
60 of organic matter could help N peach nutrition that **seems to take advantage more of re-  
61sidual N** in soil than on fertilizers.

61**Keywords:** Fruit farming, <sup>15</sup>N isotope, N distribution, N fertilization, orchard.

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### 63 1. Introduction

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64 Nitrogen (N) deriving from the mineralization of organic matter (OM) and de-  
65 composition of plant residues is not always able to **satisfy the N** demand of peach trees  
66 (*Prunus persica* L.). To avoid N deficiency, **which can cause a decrease in crop** yield and  
67 negatively affect fruit quality parameters (Damour et al., 2014; Jannoyer et al., 2011), N  
68 fertilizers are often applied **to the soil** surface. To establish the correct N rates to supply,  
69 soil OM concentration and plant nutritional status, as well as growth and yield parameters,  
70 should be evaluated (Brunetto et al., 2016b).

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71 Urea is the N fertilizer most used in orchards thanks to its high N concentration  
72 and low cost per nutrient unit (Brunetto et al., 2016b). However, urea **in the soil** is rapidly  
73 hydrolyzed by extracellular ureases enzymes produced by microorganisms such as bac-  
74 teria and fungi, and produce ammonium carbonate ( $(\text{NH}_4^+)_2\text{CO}_3$ ), which is not stable **in the  
75 soil**. Urea decomposes into bicarbonate ion ( $\text{HCO}_3^-$ ), hydroxide ion ( $\text{OH}^-$ ) and ammonium  
76 ion ( $\text{NH}_4^+$ ) when it gets in contact with water.  $\text{NH}_4^+$  can react to  $\text{OH}^-$  and stimulate am-  
77 monia ( $\text{NH}_3$ ) loss due to volatilization; however, part of  $\text{NH}_4^+$  in the soil can be trans-  
78 formed into nitrite ( $\text{NO}_2^-$ ) due to biological oxidation and, later, into nitrate ( $\text{NO}_3^-$ ) (Bru-  
79 netto et al., 2016a), which is often the prevalent form of N found in drained soils. How-  
80 ever,  $\text{NO}_3^-$  in the soil is extremely mobile; therefore, it can be easily leached and contam-  
81 inate subsurface water in orchards (Baram et al., 2016; Nevison et al., 2016). Therefore,  
82 it is recommended to split N supply during the vegetative season, according **to the phe-  
83 nological** stage and plant needs focusing on the period of maximum requirement. Nitro-  
84 gen supply at budding and spring can be able to increase root emission and longevity in  
85 the soil since this highly active organ accounts for the absorption of larger volumes of  
86 water and nutrients such as N (Jordan, 2015). In addition, intense cell division and elon-  
87 gation in shoot organs can be mainly observed just after flowering, a fact that leads to dry  
88 matter increase and increases plant demand for N (Ventura et al., 2010). However, peach  
89 trees planted in soil presenting clayey texture and average organic matter contents, which  
90 was subjected to the application of single or split low N doses such as 40 kg N ha<sup>-1</sup>  
91 (CQFS-RS/SC, 2016), may absorb similar N amounts from the fertilizer. This outcome

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93 would be the desirable one since it would decrease costs with split nitrogen fertilizer ap-  
94 plications, as well as potential water-contamination rates and increase the amount of N  
(from the fertilizer) absorbed by peach trees.

95 Part of the N absorbed by roots is preferably transported to growing organs such  
96 as leaves, annual shoots and fruits (El-Jendoubi et al., 2013; Jordan, 2015). Moreover,  
97 part of the N accumulated in annual organs is redistributed, after harvesting, to storage  
organs such as stem, shoots older than one year and, mainly to the roots (Rivera et al.,  
98 2016; Zhang et al., 2012). If large amounts of N from the fertilizer accumulate inside  
99 peach trees in the fertilizer application year, it may not be necessary applying large N  
100 rates to the soil in the next crop to reduce soil dependence on the fertilizer (Jordan et al.,  
2012) or even on N, since this element often derives from organic matter mineralization  
101 and the decomposition of plant residues (Sabahi et al., 2016; TerAvest et al., 2010).

102 It is not clear whether the peach tree absorbs a greater amount of N, when the  
103 fertilizer rate is applied in a fractional way and if the highest concentration of N present  
in the plant is from the origin of the fertilizer or other sources. However, the N amount  
104 accumulated in plant organs in the fertilizer application year, and in the subsequent year  
105 when N different application modes (single or split N rates) are adopted are yet to be fully  
106 known and this information could be more reliable when  $^{15}\text{N}$  is used as a tracer (Brunetto  
107 et al., 2014). The hypothesis of the study is that peach trees absorb and accumulate greater  
amounts of N of the fertilizer when the application is split and that the greater amount of  
108 N absorbed is derived from other N sources than not of the fertilizer.

109 The current study aimed to evaluate the annual and residual contribution of N  
110 derived from the urea at a single rate or split for the nutritional status of peach trees.

## 111 2. Materials and methods

### 112 2.1. Experimental Site and Treatments

113 The experiment was conducted in July 2016, in Bento Gonçalves County, Rio  
Grande do Sul State, Southern Brazil (latitude 29°9'54.50"S; longitude 51°32'3.87"W)  
114 on a peach orchard of the cultivar 'Chimarrita' grafted on 'Capdeboscq' rootstock. Trees,  
115 trained as in "Epsilons" system were planted in 2009 at density of 1,666 plants ha<sup>-1</sup> (1.5  
116 m between plants and 4 m between rows). The orchard was planted in Cambisol Humic  
(Sibcs, 2013) and Typic Hapludalf soil (Soil Survey Staff, 2014) and presented, before  
117 planting, in the 0-0.2 m layer, the following characteristics: clay (310 g kg<sup>-1</sup>), silt (468 g  
118 kg<sup>-1</sup>) and sand (280 g kg<sup>-1</sup>); OM (26.5 g kg<sup>-1</sup>); pH in water 5.7 (1:1 ratio); exchangeable

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127 Al ( $0.0 \text{ cmol}_c \text{ dm}^{-3}$ ), Ca ( $7.4 \text{ cmol}_c \text{ dm}^{-3}$ ) and Mg ( $2.3 \text{ cmol}_c \text{ dm}^{-3}$ ) extracted through  $1 \text{ mol}$   
128  $\text{KCl L}^{-1}$ ; available P ( $8.6 \text{ mg dm}^{-3}$ ) and K ( $207 \text{ mg dm}^{-3}$ ) - both extracted through Mehlich-  
129 annual rainfall is  $1,736 \text{ mm}$  (Table S1).

130 The following N application strategies were compared as in completely random-  
131 ized block design with three replicates: 100% N rate supplied at budding (100B) and N  
132 splitted as 50% of total rate and at budding + 50% of N at the end of flowering (50B +  
133 50F). Each replicate comprised five plants; the three central plants were subjected to N  
134 application and evaluated. Nitrogen was applied at a rate of  $40 \text{ kg N ha}^{-1}$ , which is equiv-  
135 alent to  $54.5 \text{ g N plant}^{-1}$ ; this N rate is the quantity recommended for soils presenting  
136 2.6% to 5.0% OM (CQFS-RS/SC, 2016) and was supplied as enriched urea (3.0 at. %  
137  $^{15}\text{N}$ ) in 2016. The fertilizer was applied on the soil surface of a  $1\text{-m}^2$  area considering the  
138 tree stem in the center of the area. Ground cover plants found in the urea application area  
139 were manually removed at treatment application time. Cover plants found in the treatment  
140 application region were desiccated with non-residual herbicide (glyphosate was the active  
141 ingredient) every 30 days, throughout the peach tree cycle.

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## 140 2.2. Assessment and analyses

141 Ten full expanded leaves per plant were collected at budding, flowering, fruit  
142 growth, harvest and senescence in 2016 and 2017. Leaves were then washed, dried,  
143 weighted, milled and analyzed for N and  $^{15}\text{N}$  concentration determined with mass spec-  
144 trometry (Finnigan MAT mass spectrometer, Delta Plus model), according to Brunetto  
(2014).

145 At harvest of both years, the yield was recorded and fruits were counted; in addi-  
146 tion, a sample of 10 fruits was collected, fruit pulp was manually separated from the stone;  
147 organs were dried, weighted and analyzed as described for leaves. In 2017, plants were  
148 uprooted with the aid of a tractor and separated into leaves, annual shoots, branches older  
149 than one year and stem. Roots were manually separated from the soil and divided into  
150 thin (diameter  $\leq 2 \text{ mm}$ ) and thick (diameter  $> 2 \text{ mm}$ ) roots (Hendrick and Pregitzer, 1992).  
151 They were washed with running water and, subsequently, with distilled water. All organs  
152 were weighted to determine fresh weight; a subsample of each organ was then collected  
153 and fresh and dry weight was determined. All organs were then ground and analyzed as  
154 described before. Soon after fruit harvest in 2016 and 2017, soil samples were collected  
155 at the depth of 0.0-0.025, 0.026-0.05, 0.051-0.10 and 0.11-0.20 m in the crown projection

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161 area where treatments were applied. The soil was **air-dried**, subjected to 2-mm-mesh sieve  
162 and stored for total N and <sup>15</sup>N analysis.

### 163 **2.3. Calculations and statistical analysis**

164 Excess <sup>15</sup>N atoms was calculated according to the following equation

165 (Equation 1):

$$166 \text{ Excess } ^{15}\text{N atoms in the sample (\%)} = \% ^{15}\text{N atoms in the sample} - 0.3663\% \quad \text{Equation 1}$$

167 The percentage of excess <sup>15</sup>N atoms in the sample, total N amount and the  
168 percentage of <sup>15</sup>N in the fertilizer applied to the soil were used to calculate N deriving  
169 from the fertilizer (NDFE) (Equations 2 and 3):

$$170 \text{ NDFE (\%)} = (\% \text{ excess } ^{15}\text{N atoms in the sample} / \% \text{ excess } ^{15}\text{N atoms in the fertilizer}) \times 100 \quad \text{Equation 2}$$

$$171 \text{ NDFE (g)} = \text{Total N in the sample (g)} \times (\% \text{ excess } ^{15}\text{N atoms in the sample} / \% \text{ excess } ^{15}\text{N atoms in the fertilizer}) \quad \text{Equation 3}$$

172 Afterwards, results of the aforementioned equations were used to calculate  
173 N deriving from soil (NDFS) (Equation 4):

$$174 \text{ NDFS (\%)} = 100 - \text{NDFE (\%)} \quad \text{Equation 4}$$

175 Recovery of N deriving from the fertilizer by plants (R) was calculated  
176 according equation 5:

$$177 \text{ R (\%)} = \text{NDFE} / \text{Amount N fertilizer applied to the soil (mg)} \times 100 \quad \text{Equation 5}$$

178 Total N, NDFE and NDFS content in each organ for calculated by multiplying N  
179 concentration for organ dry weight.

180 Results were subjected to the **D'Agostino-Pearson** normality test. Data of total N,  
181 excess <sup>15</sup>N and NDFE in 2016 and 2017 in leaves, were analyzed as in a factorial experi-  
182 mental design with application mode (2 levels: 100B and 50B+50F) and sampling time  
183 (4 levels: budding, end of flowering, fruit growth, fruit harvest) as main factors. Data of  
184 total N, excess <sup>15</sup>N NDFE in 2016 in fruits, were analyzed as in a factorial experimental  
185 design with application mode (2 levels: 100B and 50B+50F) and organ (2 levels: pulp  
186 and stone) as main factors. Total N and <sup>15</sup>N in soil were analyzed as in a factorial experi-  
187 mental design with application mode (2 levels: 100B and 50B+50F) and sampling depth  
188 (4 levels: 0-0.025, 0.026-0.05, 0.051-0.01, 0.011-0.02) as main factors. When analysis of  
189 variance showed a statistical effect of treatments ( $P \leq 0.05$ ), means were separated by  
190 Student Newman Keuls test. **When the interaction** between factors was significant, 2  
191 times standard error of means (2SEM) was used as the minimum difference between two  
means statistically different for  $P \leq 0.05$ .

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### 196 **3. Results**

197 Interaction between treatment and sampling time was not significant for total N,  
198 excess  $^{15}\text{N}$  and NDFP in leaves in 2016 and 2017, consequently **Tables 1 and 2** report the  
199 effects of main factors. Total N was higher in 100B than in 50B+50F in 2016 (Table 1)  
200 and 2017 (Table 2); excess  $^{15}\text{N}$  and NDFP were higher in 50B+50F than in 100B both in  
201 2016 (Table 1) and 2017 (Table 2). The highest total N concentrations were observed in  
202 leaves collected at budding and end of flowering, followed by those sampled at fruit har-  
203 vest, senescence and fruit growth (Table 1). Excess  $^{15}\text{N}$  atoms and NDFP rates were  
204 higher in leaves collected at end of flowering and fruit harvest than those sampled at fruit  
205 growth, senescence and budding (Table 1). Leaves sampled at fruit growth showed higher  
206  $^{15}\text{N}$  excess and NDFP than those at budding and senescence that showed similar values  
207 (Table 1).

208 In 2017, total N concentration, as well as excess  $^{15}\text{N}$  atoms and NDFP, were higher  
209 in leaves collected at the fruit growth than all other sampling data (Table 2). Excess  $^{15}\text{N}$   
210 and NDFP showed similar values between all other sampling data. Leaves total N was  
211 similar at budding and end of flowering and higher than the values measured at fruit har-  
212 vest and senescence (Table 2).

213 Interaction between treatment and sampling time was not significant for total N,  
214 excess  $^{15}\text{N}$  and NDFP in fruits in 2016, consequently table 3 only reports the effects of  
215 main factors. Total N concentration, as well as excess  $^{15}\text{N}$  atoms and **NDFP, have ob-**  
216 **served** fruits of trees subjected to 50B + 50F than those supplied with a single treatment  
217 (Table 3). All values were higher in pulp than in stone (Table 3).

218 Fruits (pulp and stone), leaves, shoot and stem dry weight was higher in  
219 50B+50F than in 100B; the opposite was observed for thick roots; no **significant** differ-  
220 ences were observed **for the 1-year** shoot and fine roots (Table 4).

221 Total N content in leaves, fruit pulp and stone and thick roots was higher in trees  
222 subjected to 50B+50F applications than 100B; the opposite was observed **for the shoot**  
223 (Table 5). No **significant** difference was observed for other organs (Table 5).

224 N deriving from fertilizer in fruit stone and pulp and thick roots was higher in trees  
225 subjected to 50B+50F applications in comparison to 100B; no **significant** differences  
226 were observed between treatments for other organs (Table 6).

227 N deriving from soil in fruits (pulp and stone), leaves, shoot and thick roots was  
228 higher in 50B+50F than 100B; no differences were observed for other treatments (Table  
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230 Total N content in the soil profile between 0 and 0.10 m was higher when N was  
231 split in comparison to the entire rate in 2016; while at 0.011-0.02 m of depth the values  
232 of total N were higher for 100B than for 50B+50F (Table 8). In 2017 total N was higher  
233 in soil 50B+50F than in 100B (Table 8). Total N decreased with depth both in 2016 and  
234 2017 (Table 8).

234 The excess  $^{15}\text{N}$  atoms were higher, in 2016 and 2017, in all layers of soil subjected  
235 to 100B applications (Table 9) and, for both treatments, the values decreased with depth  
236 (Table 9).

236 Soil NDFFF was higher in 50B+50F than 100B along with the entire soil profile  
237 both in 2016 and 2017; moreover, the values decreased with depth for all treatments and  
238 in all years (Table 10).

#### 239 4. Discussion

240 The split of N rates in two different phenological phase enhanced leaves  $^{15}\text{N}$  atoms  
241 excess and NDFFF in both years probably because this application method improved the  
242 synchronism between the availability of mineral N in the soil and N uptake by the root  
243 system (Radicetti et al., 2017; Sabahi et al., 2016). According to some authors (Brunetto  
244 et al., 2016a; Neto et al., 2008; Rocuzzo et al., 2012) at flowering peach trees start pro-  
245 ducing new roots that enlarged the surface area and increased the volume of soil explored  
246 by the root system, enhancing, consequently, the uptake of water and nutrients. In the  
247 period immediately after flowering, there is also intense vegetative activity due to the  
248 formation of new shoots that become a sink for nutrients, mainly N (Brunetto et al.,  
249 2016a; Carranca et al., 2018).

248 In 2017, the year after the application of enriched urea, leaves showed lower  $^{15}\text{N}$   
249 atoms and NDFFF in both N application techniques (100B and 50B + 50F) probably be-  
250 cause plants allocated part of the  $^{15}\text{N}$  assimilated to fruits as also evidenced previously  
251 (Muhammad et al., 2015). According to our results, we evidenced that N was mainly  
252 allocated to the pulp and, to a lesser extent, to stones with more evident results in 2016  
253 than in 2017. Fruits from plants fertilized with a split mode (50B+50F) evidenced, in both  
254 years, higher  $^{15}\text{N}$  values, reinforcing the hypothesis of a greater synchronism between N  
255 applications and absorption. Moreover, peach pulp recorded higher excess  $^{15}\text{N}$  atoms and  
256 NDFFF than stone as also demonstrated previously (Kuo et al., 2016; Pescie et al., 2018).  
257 However, excess N allocation to the pulp can lead to worse fruit quality and an increase  
258 of fungal diseases in the field or during storage (Brunetto et al., 2015; Bush et al., 2018).

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264 Peach trees subjected to split N applications (50B + 50F) accumulated **more N**  
265 **than those subjected to a single application** mainly in leaves and pulp, which are annual  
266 organs that present intense growth and increased dry mass throughout the phenological  
267 stages (Pescie et al., 2018; Roccuzzo et al., 2017) being the main N sinks during the veg-  
268 etative season.

268 The N plant content in the second **year was derived** mainly from sources other  
269 than the fertilizer, probably from organic matter mineralization and from waste decom-  
270 position, since the NDFP rate in most organs did not exceed 2.5%, except for the thick  
271 roots, which recorded values close to 3.5%.

271 Decreased total N, excess <sup>15</sup>N atoms and NDFP values in leaves at senescence, or  
272 even at fruit growth, are probably due to protein degradation and remobilization of N  
273 forms from leaves to reserve organs such as stems, branches older than one year, or roots  
274 (Brunetto et al., 2016a; Roccuzzo et al., 2017). Part of the N accumulated in storage or-  
275 gans may be redistributed to annual organs growing in the subsequent cycle, a fact that  
276 can decrease fruit tree dependence on N applied in the year (Carranca et al., 2018;  
277 Roccuzzo et al., 2017).

276 It is well known that in spring, peach trees use N stored in perennial organs and it  
277 was estimated that the majority of N remobilization occurs before root uptake starts  
278 (Rufat and DeJong 2001. The remobilization process is little affected by the amount of  
279 soil N (Tagliavini and Millard, 2005) but the duration depends on the amount of stored  
280 N, being longer in trees with large storage pools (Grassi et al., 2003). Once remobilization  
281 finishes, root uptake provides the remainder of the N used for growth; consequently, from  
282 this stage until the end of the season it is important to maintain adequate N **in the soil**.

282 As a consequence of soil OM mineralization in the soil there is an increase of N  
283 availability that not derives from enriched urea and **determines** an increase of the amount  
284 of N deriving from the soil **in the plant** (García-Orenes et al., 2016). This partly justifies  
285 the low leaf NDFP rates in the N application year (2016) and in the following year (2017)  
286 which did not exceed 17% and 3% respectively, throughout leaf collection times in both  
287 N supply techniques. Moreover, it is also evidenced by the higher NDFS content that  
288 NDFP showed in plants at the end of the experiment.

287 The higher excess <sup>15</sup>N atoms and NDFP values observed in topsoil layers, mainly  
288 in soil subjected to split N application (50B+50F), can be attributed to the complexa-  
289 tion/adsorption of part of the N applied to organic compounds of organic matter on the  
290 soil (Zhang et al., 2015), a fact that was observed in orchards subjected to different N

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298 rates and application times (Brunetto et al., 2016a; Rocuzzo et al., 2017). Thus, the split  
299 mode should induce greater soil mineral N availability in the most superficial layers, those  
300 more explored by roots, explaining the greater incidence of applied N in split mode  
(50B+50F) on leaves and fruits N concentration.

301 The lower values measured in 2017 could be due to loss of the N supplied in 2016  
302 due to volatilization (Carranca et al., 2018; Dominghetti et al., 2016; Pescie et al., 2018),  
denitrification (Nevison et al., 2016), leaching (Sparks, 2018; Lynch and Wojciechowski,  
303 2015) and runoff (Baram et al., 2016; Dominghetti et al., 2016; Pescie et al., 2018).

304 Data from this experiment evidence that N should be applied yearly and possibly  
305 in split mode to reduce as much as possible loss of N in the environment and at the same  
time, meet plant needs. Moreover, the maintenance of high soil OM levels with different  
306 techniques such as minimum soil tillage, organic fertilization, ground cover plant culti-  
307 vation and maintenance of plant residues on the soil (Baldi et al., 2016; Brunetto et al.,  
308 2014; Radicetti et al., 2017) could help to reduce N loss in the environment and improve  
309 plant nutritional status.

## 310 5. Conclusion

311 Peach trees allocated more N derived from urea in leaves and fruits than in other  
312 organs, to a higher extent in the year of fertilizer application more than in the following  
313 year, probably due to N loss in the environment or absorbed by plants. The best results in  
314 terms of N concentration were observed as a consequence of the split mode showing that  
with this technique it is easier to meet plants' need.

315 For the purpose of recommendation, it is important to make it clear that although  
316 the plants show better results with the fractionation of N rates; however, at the time of  
317 application it should be checked whether the costs for fertilizer supply (which includes  
318 double use of machinery and labor) offset the productivity gain and guarantee profits for  
the producer.

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### 325 **Author Contributions**

326 Betania Vahl de Paula carried out the assembly and experimental collection, analysis and  
interpretation of data and co-wrote the paper. Beatriz Baticini Vitto, Paula Beatriz Sete,

327 Talita Trapp and Jovani Zalameña collected the data, laboratory analysis and statistical  
328 analysis. Gustavo Brunetto and Elena Baldi analyzed the data and co-wrote the paper.

329 George Wellington Bastos de Melo, Moreno Toselli, Danilo Eduardo Rozane revised the  
paper.

330

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### **Conflicts of Interest**

337 The authors have declared that no competing interests exist.

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Table 1. Effect of urea application and sampling time on total N, excess <sup>15</sup>N atoms and N deriving from fertilizers (NDFP) on peach leaves in 2016.

<b>Treatment</b>	<b>Total N (% DW)</b>	<b>Atom <sup>15</sup>N excess (% DW)</b>	<b>NDFP (% DW)</b>
50B+50F	2.92	0.326	10.9
100B	3.08	0.242	8.08
<i>Significance</i>	*	***	***
<b>Sampling Time</b>			
Budding	3.55 a	0.120 c	4.00 c
End of flowering	3.51 a	0.403 a	13.4 a
Fruit growth	2.20 d	0.313 b	10.4 b
Fruit harvest	3.32 b	0.401 a	13.4 a
Senescence	2.42 c	0.184 c	6.14 c
<i>Significance</i>	***	***	***
<i>Treatment × Time</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>

Values followed by the same letter are not statistically different according to Student Neuman Keul test ( $P \leq 0.05$ ). n.s., \*, \*\*\*: effect not significant, significant at  $P \leq 0.05$  and  $P \leq 0.001$ , respectively.

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Table 2. Effect of urea application and sampling time on total N, excess  $^{15}\text{N}$  atoms and N deriving from fertilizers (NDFF) on peach leaves in 2017.

<b>Treatment</b>	<b>Total N (% DW)</b>	<b>Atom <math>^{15}\text{N}</math> excess (% DW)</b>	<b>NDFF (% DW)</b>
50B+50F	3.15	0.048	1.61
100B	3.30	0.039	1.30
<i>Significance</i>	***	***	***
<b>Sampling Time</b>			
Budding	3.42 b	0.0388 b	1.29 b
End of flowering	3.45 b	0.0345 b	1.15 b
Fruit growth	3.74 a	0.0745 a	2.49 a
Fruit harvest	2.76 c	0.0341b	1.14 b
Senescence	2.76 c	0.0357 b	1.19 b
<i>Significance</i>	***	***	***
<i>Treatment</i> × <i>Time</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>

Values followed by the same letter are not statistically different according to Student Neuman Keul test ( $P \leq 0.05$ ). n.s., \*, \*\*\*: effect not significant, significant at  $P \leq 0.001$ , respectively.

Table 3. Effect of urea application and organ on total N, excess  $^{15}\text{N}$  atoms and N deriving from fertilizers (NDFF) on peach fruit in 2016.

<b>Treatment</b>	<b>Total N (% DW)</b>	<b>Atom <math>^{15}\text{N}</math> excess (% DW)</b>	<b>NDFF (% DW)</b>
50B+50F	0.738	0.234	7.88
100B	0.580	0.182	5.68
<i>Significance</i>	**	*	***
<b>Organ</b>			
Pulp	0.893	0.242	8.17
Stone	0.424	0.174	5.40
<i>Significance</i>	***	*	***
<i>Treatment</i> × <i>Organ</i>	<i>n.s.</i>	<i>n.s.</i>	**

n.s., \*, \*\*, \*\*\*: effect not significant, significant at  $P \leq 0.05$ ,  $P \leq 0.01$  and  $P \leq 0.001$ , respectively.

Table 4. Effect of urea application on organ biomass ( $\text{g pt}^{-1}$ ) at the end of the experiment.

<b>Treatment</b>	<b>Stone</b>	<b>Pulp</b>	<b>Leaves</b>	<b>Branches</b>	<b>Shoot</b>	<b>Stem</b>	<b>Fine roots</b>	<b>Thick roots</b>
50B+50F	929	6447	3142	698	1335	8966	305	4480
100B	712	4699	2554	518	1230	5836	349	4961
<i>Significance</i>	*	*	*	*	<i>n.s.</i>	*	<i>n.s.</i>	*

*n.s.*, \*: effect not significant and significant at  $P \leq 0.05$ , respectively.

Table 5. Effect of urea application on organ total N content (g pt<sup>-1</sup>) at the end of the experiment.

<b>Treatment</b>	<b>Stone</b>	<b>Pulp</b>	<b>Leaves</b>	<b>Shoot</b>	<b>Shoot (1 year old)</b>	<b>Stem</b>	<b>Fine roots</b>	<b>Thick roots</b>
50B+50F	9.52	62.8	94.4	5.60	7.84	26.2	3.21	51.9
100B	5.11	39.0	64.4	8.53	6.14	15.9	3.21	40.5
<i>Significance</i>	*	*	*	*	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	*

*n.s.*, \*: effect not significant and significant at  $P \leq 0.05$ , respectively.

Table 6. Effect of urea application on organ N deriving from fertilizer ( $\text{g pt}^{-1}$ ) at the end of the experiment.

<b>Treatment</b>	<b>Stone</b>	<b>Pulp</b>	<b>Leaves</b>	<b>Shoot</b>	<b>Shoot (1 year old)</b>	<b>Stem</b>	<b>Fine roots</b>	<b>Thick roots</b>
50B+50F	0.140	1.12	0.964	0.056	0.129	0.397	0.114	1.24
100B	0.053	0.528	0.877	0.100	0.115	0.240	0.074	0.638
<i>Significance</i>	*	*	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	**

*n.s.*, \*, \*\*: effect not significant, significant at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively.

Table 7. Effect of urea application on organ N deriving from soil ( $\text{g pt}^{-1}$ ) at the end of the experiment.

<b>Treatment</b>	<b>Stone</b>	<b>Pulp</b>	<b>Leaves</b>	<b>Shoot</b>	<b>Shoot (1 year old)</b>	<b>Stem</b>	<b>Fine roots</b>	<b>Thick roots</b>
50B+50F	9.38	61.6	93.5	5.55	7.72	25.8	3.10	50.6
100B	5.05	38.4	63.5	7.84	6.01	15.6	3.14	39.9
<i>Significance</i>	*	*	*	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	*

*n.s.*, \*: effect not significant and significant at  $P \leq 0.05$ , respectively.

Table 8. Effect of urea application and depth (m) on soil total N content ( $\text{g kg}^{-1}$ ) in 2016 and 2017.

<b>Treatment</b>	<b>0-0.025</b>	<b>0.026-0.05</b>	<b>0.051-0.01</b>	<b>0.011-0.02</b>
<b>2016</b>				
50B+50F	20.5	19.5	14.6	12.7
100B	19.0	15.6	13.4	13.4
<i>Significance</i>	<i>2SEM=0.412</i>			
<b>2017</b>				
50B+50F	20.7	18.3	16.8	15.5
100B	18.7	17.0	15.7	15.3
<i>Significance</i>	<i>2SEM=0.146</i>			

Values differing by 2 standard error of means (SEM) are statistically different. Interaction treatment\*depth significant at  $P < 0.05$  in 2016 and  $P < 0.01$  in 2017.

Table 9. Effect of urea application and depth (m) on soil  $^{15}\text{N}$  atom excess (%) in 2016 and 2017.

<b>Treatment</b>	<b>0-0.025</b>	<b>0.026-0.05</b>	<b>0.051-0.01</b>	<b>0.011-0.02</b>
<b>2016</b>				
50B+50F	0.032	0.018	0.006	0.003
100B	0.043	0.032	0.024	0.010
<i>Significance</i>	<i>2SEM=0.0011</i>			
<b>2017</b>				
50B+50F	0.019	0.009	0.007	0.005
100B	0.029	0.021	0.011	0.009
<i>Significance</i>	<i>2SEM=0.0010</i>			

Values differing by 2 standard error of means (SEM) are statistically different. Interaction treatment\*depth significant at  $P < 0.05$  for both 2016 and 2017.



Table 10. Effect of urea application and depth (m) on soil N deriving from fertilizer (%) in 2016 and 2017.

<b>Treatment</b>	<b>0-0.025</b>	<b>0.026-0.05</b>	<b>0.051-0.01</b>	<b>0.011-0.02</b>
<b>2016</b>				
50B+50F	1.44	1.05	0.806	0.333
100B	1.08	0.602	0.211	0.103
<i>Significance</i>	<i>2SEM=0.038</i>			
<b>2017</b>				
50B+50F	0.965	0.715	0.353	0.291
100B	0.634	0.295	0.231	0.181
<i>Significance</i>	<i>2SEM=0.035</i>			

Values differing by 2 standard error of means (SEM) are statistically different. Interaction treatment\*depth significant at P <0.05 for both 2016 and 2017.

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**Supplementary**

Table S1. Mean monthly rainfall, air temperature and relative humidity during the experimental months in 2016 and 2017.

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Month	Phenological stage	Average air temperature (°C)		Average rainfall (mm)		Relative air humidity (%)	
		2016	2017	2016	2017	2016	2017
February	Start of senescence	22.6	22.2	146	147	79.2	79.7
June	Budding	10.0	13.8	7	160	78.2	81.5
July	Flowering	12.8	14.5	192	29	77.5	69.5
September	Fruit Growing	14.2	19.1	84	107	74.1	73.9
November	Start of fruiting	18.6	18.2	104	1620	70.9	70.4

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