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1 **Roots take up labeled nitrogen from a depth of 9 m in a wooded savanna in**  
2 **Brazil**

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13 **ABSTRACT**

14 The savannas (called Cerrado) are the second-largest vegetation formation in Brazil  
15 after the Amazon rainforest, with about 2 million km<sup>2</sup>. Roots have been found in very  
16 deep soil layers in Cerrado ecosystems, which suggests a crucial role of deep rooting  
17 in the supply of water and nutrients over dry periods. The aim of our study was to  
18 gain insight into the complementarity of common Cerrado woody species in taking up  
19 mobile nutrients throughout deep soil profiles. In a closed-canopy savanna with  
20 dense woody understory, labeled nitrate was injected into the soil at six soil depths  
21 (0.1, 1.5, 3.0, 6.0, 9.0, or 12.0 m) with three plots per depth, at two dates (in rainy  
22 and dry seasons). Five months after labeled nitrate injection, young leaves were  
23 sampled and foliar  $\delta^{15}\text{N}$  was determined in each plot in the three most common  
24 woody species (*Coussarea hydrangeifolia*, *Miconia albicans* and *Xylopia aromatica*).  
25 The maximum depth of <sup>15</sup>N uptake was dependent on the species. *X. aromatica* trees  
26 took up <sup>15</sup>N from a maximum depth of 9 m and exploited a much larger soil volume  
27 than the two other species, with the uptake of <sup>15</sup>N at a horizontal distance of up to 5  
28 m between the trunk and the injection site. The behavior of *M. albicans* and *C.*  
29 *hydrangeifolia* was similar, with a strong uptake of <sup>15</sup>N only in the 0-1.5 m soil layer,  
30 within 2 m horizontally from the injection site. The depth of <sup>15</sup>N uptake over the dry  
31 season was not related to the diameter at breast height of the woody plants sampled.  
32 We show that roots can take up labeled nitrogen from a depth of 9 m in tropical  
33 wooded savannas, which suggests an important role of deep-rooted species in  
34 closing biogeochemical cycles on highly weathered tropical soils.

35 **Keywords:** <sup>15</sup>N, Cerrado, deep roots, nutrient, tropical savanna, subsoil.

## 36 **1. Introduction**

37

38 Prolonged droughts will be more frequent in many tropical regions due to climate  
39 change, and access to water stored in the subsoil of certain species is likely to  
40 influence the composition of tropical savannas. The Cerrado biome in Brazil is the  
41 world's richest savanna and one of the global 35 biodiversity hotspots (Myers et al.,  
42 2000). This biome is the second most deforested area in Brazil, with a loss of  
43 152,706 km<sup>2</sup> between 2000 and 2018 (IBGE, 2020). While deep rooting can help  
44 plants survive over extended drought periods by shifting the uptake of water to  
45 deeper soil layers (Sharp and Davies, 1985; Hoekstra et al., 2014; Christina et al.,  
46 2017), a recent study suggests that severe drought episodes in successive years  
47 preventing subsoil water recharge could, on the contrary, increase mortality rates of  
48 deep-rooted species (Chitra-Tarak et al., 2017). Deep rooting is common in  
49 seasonally dry environments such as neotropical savannas, and provides access to  
50 water stored in deep soil layers, which makes it possible to maintain plant  
51 transpiration and CO<sub>2</sub> assimilation over dry periods (Oliveira et al., 2005). Canadell et  
52 al. (1996) pointed out that tropical savannas are the biome with the highest average  
53 rooting depth ( $15 \pm 5.4$  m), and with the deepest rooting ever recorded (68 m). The  
54 deep rooting habit of some Cerrado plants has been recognized for decades  
55 (Rawitscher, 1948; Jackson et al., 1999; Franco, 2002).

56 The contribution of deep rooting to water and nutrient acquisition has been little  
57 explored in Cerrado ecosystems (Oliveira et al., 2005). Palhares et al. (2010)  
58 indicated in a review paper that the roots of adult trees can reach depths greater than  
59 8 m (shown by Rawitscher, 1948) and that water can be taken up horizontally from  
60 12 m away (shown using deuterium injection by Sternberg et al., 2005). Some

61 studies suggest that evergreen tree species take up water preferably at a depth of 1-  
62 2 m, whereas deciduous trees take up water preferably at 3-4 m, but this trend is  
63 debated (Palhares et al., 2010). While niche partitioning of soil resources is  
64 considered to fulfil a crucial role in the coexistence of plants in native ecosystems,  
65 methodological difficulties have considerably limited measurements of the timing and  
66 location of nutrient uptake in deep soil layers (Kulmatiski and Beard, 2013; Maeght et  
67 al., 2013). Hydrological niche segregation was recently demonstrated in a seasonal  
68 Amazon forest, with differences in depth of water uptake strongly related to the  
69 diameter at breast height of the trees and with a trade-off between access to deep  
70 water and tolerance of very low water potentials (Brum et al., 2018). Isotopic  
71 approaches are commonly used to trace water and nutrient dynamics in forest soils  
72 (Poszwa et al., 2002). The depth of water uptake can be estimated using the natural  
73 abundance of stable isotopes (Bertrand et al., 2014). However, the natural isotopic  
74 abundance of deuterium and  $^{18}\text{O}$  can only be used when there is a clear gradient  
75 with soil depth. Moreover, the climatic conditions of the days before sampling can  
76 strongly influence the results (Berry et al., 2017). Modeling approaches based on an  
77 inversion of water availability at different soil depths can be useful to estimate the  
78 depth of water uptake in forests (Chitra-Tarak et al., 2017; Christina et al., 2017).  
79 Studies in eucalypt plantations suggest that  $^{15}\text{N}$  determinations within leaves after  
80 injection of  $^{15}\text{N}$ -labeled nitrates in a specific soil area could also be useful to gain  
81 insight into the localization of water uptake by tree roots. An enrichment in  $^{15}\text{N}$  was  
82 only detected in young leaves of 6-year-old *Eucalyptus* trees when the gravitational  
83 soil solutions reached the depth of 3 m where the labeled nitrate had been injected  
84 (da Silva et al., 2011), which suggested that  $^{15}\text{N}\text{-NO}_3$  uptake could be a tracer of  
85 water uptake in forests. The largest trees (with the largest diameter at breast height)

86 in monoclonal eucalypt plantations took up the labeled nitrate more deeply than the  
87 small trees in the first years after planting (Pinheiro et al., 2019). However, several  
88 assumptions are implicit in the use of  $^{15}\text{N}$  tracers and the limitations resulting from  
89 the methodology used need to be discussed in detail. Basic information as the  
90 rooting depth and the architecture of root systems are lacking for Cerrado tree  
91 species, which is a limitation for the interpretation of results of studies using tracers to  
92 better understand the partition of soil resources between species.

93 Our study aimed to gain insight into the complementarity of common Cerrado  
94 woody species in taking up mobile nutrients in different soil layers. We hypothesized  
95 that i) if labeled nitrogen ( $^{15}\text{N}$ ) is injected into deep soil layers then the leaves of only  
96 some species will be enriched in  $^{15}\text{N}$  because nutrient absorption niches are different  
97 among Cerrado species, ii) if the water content over the entire soil profile drops  
98 during the dry season compared to the rainy season, then the deep-rooted species  
99 will take up labeled N more deeply because the strong competition in the topsoil will  
100 force them to take up water and mobile nutrients in deep soil layers, and iii) if woody  
101 plants of very different sizes coexist in the Cerrado ecosystem, then large trees will  
102 absorb the labeled nitrate at a greater depth than small trees because, as shown  
103 recently in an Amazonian forest, dominant trees have access to water in deeper soil  
104 layers than dominated trees.

105

## 106 **2. Materials and Methods**

107

### 108 *2.1. Study site*

109

110 Our study was carried out at the Jardim Botânico Municipal de Bauru in São  
111 Paulo state, southeast Brazil (22°20'30" S, 40°00'30" W; average altitude of 530 m  
112 above sea level). This botanic garden preserves one of the biggest Cerrado  
113 remnants of the São Paulo state, with an area of 277 ha composed of woodland  
114 savanna (the *cerradão* physiognomy) (Weiser, 2007). We chose the *cerradão*  
115 physiognomy in this study because it is the dominant Cerrado type in São Paulo state  
116 nowadays.

117 The regional climate of Bauru is humid subtropical (*Cwa*, Köppen's  
118 classification), with dry winters and hot summers. Over the study period in 2017,  
119 annual precipitation was 1,655 mm and the average temperature was 22.9 °C (Fig.  
120 1). The average annual precipitation has been 1,394 mm over the last 10 years and  
121 the average temperature 22.7 °C. The wettest month in 2017 was January (462 mm)  
122 while no rainfall was recorded in July. The hottest month was February (31.8 °C)  
123 while the minimum temperature was in July (12.6 °C). The soil was sandy  
124 (Arenosols, FAO classification) with high acidity ( $\text{pH} \leq 4.3$ ) and low nutrient contents  
125 (Table 1). The water table was deep (not found in our soil sampling to a depth of 12  
126 m).

127

## 128 2.2. *Experimental design*

129

130 Based on a preliminary botanical survey at the same site (Weiser, 2007), we  
131 selected the three most common species for our study: i) *Xylopia aromatica* (Lam.)  
132 Mart. (Annonaceae); ii) *Coussarea hydrangeifolia* (Benth.) Müll. Arg. (Rubiaceae);  
133 and iii) *Miconia albicans* (Sw.) Steud. (Melastomataceae). While *X. aromatica* and *C.*

134 *hydrangeifolia* are tree species, *M. albicans* is a treelet (Fig. S1). *X. aromatica* is an  
135 evergreen species with a widespread but irregular and discontinuous distribution,  
136 occurring typically at low frequency (Lorenzi, 2008). *C. hydrangeifolia* is another  
137 evergreen pioneer species common in Cerrado formations and preferentially found  
138 on sandy soils (Lorenzi, 2008). *M. albicans* is a semi-deciduous treelet species  
139 (Santos et al., 2012) typical of Cerrado formations (Martins et al., 1996), which loses  
140 some of its leaves in the dry season.

141 Thirty-six circular plots (20 m in radius) were randomly delimited in the studied  
142 Cerrado. Labeled nitrate ( $^{15}\text{N-NO}_3$ ) was injected at a single depth in each plot (0.1,  
143 1.5, 3.0, 6.0, 9.0, or 12.0 m) in two seasons (rainy and dry), with three plots for each  
144 depth. The centers of the plots were at least 50 m apart (Fig. 2). An inventory of  
145 plants with a diameter at breast height (DBH)  $\geq 1.5$  cm was made in a radius of 10 m  
146 from the  $^{15}\text{N-NO}_3$  injection site in each of the 36 plots, which showed that the basal  
147 area of *M. albicans* was much lower than those of *X. aromatica* and *C. hydrangeifolia*  
148 (Fig. S2). The basal areas of the three selected species were low compared to the  
149 total basal area of the Cerrado. However, these three species were the only ones  
150 found in all the plots (Table S1). Among the three studied species, *C. hydrangeifolia*  
151 accounted for the largest basal area in the stand and the highest density, with on  
152 average 1,103 trees per ha (Table 2). The height of *C. hydrangeifolia* trees ranged  
153 from 2.8 to 8.1 m with a DBH ranging from 3.0 to 10.4 cm. The mean density of *X.*  
154 *aromatica* trees was 756 trees per ha, with heights ranging from 4.3 to 10.5 m and  
155 DBHs from 2.5 to 10.8 cm. *M. albicans* treelets had a lower density than the two  
156 other species, with 337 trees per ha on average, with heights ranging from 1.2 to 6.4  
157 m and DBHs from 0.1 to 7.0 cm.

158



### 159 2.3. $^{15}\text{N}\text{-NO}_3$ injections

160

161 A labeled solution was prepared with 16.6 g of  $\text{NH}_4^{15}\text{NO}_3$  commercial compound  
162 (10 atom %  $^{15}\text{N}$ , Sigma-Aldrich Corporation) dissolved in 20 mL of distilled water, and  
163 was injected at a single depth at the center of each plot (indicated as 'injection site'  
164 hereafter). Holes were drilled to the target application depth and soil water contents  
165 (SWCs) were measured every meter in all the samples collected during the drilling  
166 (Fig. S3). Soil blocks from the inner part of the auger were collected for the deepest  
167 meter in each hole to assess fine root density (< 2 mm in diameter) close to the area  
168 of  $\text{NO}_3\text{-}^{15}\text{N}$  injection (Pineiro et al., 2016). Gravitational SWCs from the soil surface  
169 to a depth of 11 m ranged from 17 to 25% in February 2017 (middle of the rainy  
170 season), and from 5 to 10% in June 2017, at the onset of the dry season (Fig. S3).  
171 SWCs sharply increased below a depth of 10 m, which might reflect a decrease in  
172 water withdrawal by tree roots relative to upper soil layers since the clay and silt  
173 contents and the soil water retention capacity did not increase below 10 m (Table 1).

174 A PVC tube (2.5 cm in diameter) was inserted into each hole to avoid any  
175 contamination of the soil in  $^{15}\text{N}$  during the  $^{15}\text{N}\text{-NO}_3$  injection. A 0.4-cm polyethylene  
176 tube, attached to an iron rod, was inserted into the PVC tube, with a length  
177 depending on the application depth. A plastic sheet was placed around each hole to  
178 avoid any contamination with  $^{15}\text{N}$  at the soil surface, and 20 mL of the labeled  
179 solution was injected at the selected depth using a syringe. Thereafter, 280 mL of  
180 distilled water was injected to rinse the polyethylene tube and to increase soil  
181 moisture in the area where  $^{15}\text{N}\text{-NO}_3$  was applied, so as to promote nitrate uptake.  
182 Lastly, the polyethylene and PVC tubes were carefully withdrawn from the holes,  
183 which were filled with the soil removed during drilling, respecting the original order of

184 the soil layers. Similar methodologies were used by Bordron et al. (2019) and  
185 Pinheiro et al. (2019).

186

#### 187 2.4. Leaf sampling

188

189 Young leaves were sampled in the upper part of the crown of one individual of  
190 each selected species, for each interval of distance from the injection site at each  
191 plot. Leaves were sampled in July 2017 in the plots where the labeled nitrate was  
192 injected in February to study N uptake over the rainy season, and in November 2017  
193 in the plots where the labeled nitrate was injected in June to study N uptake over the  
194 dry season (Fig. 1).

195 In addition to the sampling of the three species studied, another leaf sampling  
196 was carried out in November 2017 to assess whether the largest trees (selected  
197 solely based on their DBH, regardless of species) could take up the tracer at a larger  
198 distance from the injection site than the three studied species (*M. albicans*, *X.*  
199 *aromatica* and *C. hydrangeifolia*). Young leaves were then sampled from the upper  
200 part of the crown of the tree with the largest DBH (whatever the species) at the first  
201 three predefined intervals of distance from the  $^{15}\text{N}\text{-NO}_3$  injection site (as shown in  
202 Fig. 2) in each plot (1 tree x 3 distance intervals from the center of the plot x 18 plots  
203 in the dry season = 54 trees).

204 Young leaves were sampled in 8 (rainy season) and 12 (dry season) control  
205 trees of each species, far from the injection sites (> 10 m), to determine foliar  $\delta^{15}\text{N}$   
206 values in natural abundance. Control trees were sampled far from each other

207 throughout the study area to cover the spatial variability of <sup>15</sup>N natural abundance.  
208 Leaves were sampled about 5 months after tracer injection in both seasons (Fig. 1).

209

## 210 2.5. Isotopic analyses of <sup>15</sup>N

211

212 The sampled leaves were washed in deionized water and oven-dried at 65 °C  
213 for 72 hours. The dried samples were ground (< 60 μm) in a cryogenic mill (2010  
214 Geno Grinder, SPEX Sample Prep, Metuchen, USA) at -196 °C for homogenization  
215 and stored until isotopic analysis. An aliquot of 4.5-5.0 mg of each dry and milled  
216 sample was weighed into a cylindrical tin capsule (D1106 - Elemental Microanalysis,  
217 Okehampton, UK) with a 1 μg resolution scale (XP6 - Mettler Toledo, Greifensee,  
218 Switzerland). The <sup>15</sup>N analyses were performed using a continuous-flow isotope ratio  
219 mass spectrometer system (Flash 2000 / ConFlo IV / Delta V Advantage - Thermo  
220 Scientific, Bremen, Germany) that determines the isotope ratio of sample  
221  $R(^{15}\text{N}/^{14}\text{N})_{\text{sample}}$ .

222 The <sup>15</sup>N values in relative difference of isotope-amount ratios ( $\delta^{15}\text{N}$ , expressed  
223 in ‰) were calculated from Coplen (2011):

$$224 \quad \delta^{15}\text{N} = R(^{15}\text{N}/^{14}\text{N})_{\text{sample}} / R(^{15}\text{N}/^{14}\text{N})_{\text{std}} - 1 \quad (1)$$

225 where  $R(^{15}\text{N}/^{14}\text{N})_{\text{std}}$  is the isotopic ratio of N atmospheric air (0.0036765) as an  
226 international standard. The standard uncertainty in  $\delta^{15}\text{N}$  was  $\pm 0.3\text{‰}$  for samples  
227 slightly enriched in <sup>15</sup>N.

228

## 229 2.6. Data analyses

230

231 For the three selected species, a prediction interval (PI) of  $\delta^{15}\text{N}$  in natural  
232 abundance (with a threshold of 99%) was calculated from the  $\delta^{15}\text{N}$  values of control  
233 trees using equation (2):

$$234 \quad \text{PI} = \bar{X}_n \pm T_\alpha s_n \sqrt{1 + 1/n} \quad (2)$$

235 where  $\bar{X}_n$  and  $s_n$  were the average and the standard deviation of  $\delta^{15}\text{N}$  values in  
236 control trees, respectively,  $n$  was the number of observations, and  $T_\alpha$  the 99.5  
237 percentile of a Student's t-distribution with  $(n - 1)$  degrees of freedom (Geisser,  
238 1993). The statistical software R was used.

239 Foliar  $\delta^{15}\text{N}$  values higher than the upper boundary of the prediction interval were  
240 considered significantly different from the control population ( $P < 0.01$ ), which  
241 indicated that the sampled trees took up  $^{15}\text{N}\text{-NO}_3$  injected in the same plot. A general  
242 linear model procedure was used in a three-way analysis of variance to test for  
243 differences due to species, season, injection depth, and the interactions between  
244 these variables on foliar  $\delta^{15}\text{N}$  values for the three studied species (*C. hydrangeifolia*,  
245 *M. albicans* and *X. aromatica*). The homogeneity of variances was tested using  
246 Levene's test.

247

### 248 **3. Results**

249

#### 250 *3.1. Uptake of $^{15}\text{N}$ by X. aromatica, C. hydrangeifolia and M. albicans*

251

252 The foliar enrichment in  $^{15}\text{N}$  of the plants sampled near the injection sites was  
253 significantly influenced by the injection depth of  $^{15}\text{N}\text{-NO}_3$  ( $P < 0.01$ ). While the effect

254 of the species on the foliar values of  $\delta^{15}\text{N}$  was only marginally significant ( $P < 0.10$ ),  
255 the significant interaction between  $^{15}\text{N-NO}_3$  injection depth and plant species showed  
256 that N uptake patterns along the soil profile were different for the species studied  
257 (Table S2). Foliar  $\delta^{15}\text{N}$  values were not significantly influenced by the season of  $^{15}\text{N-}$   
258  $\text{NO}_3$  injection and the species x season interaction was not significant, which showed  
259 a similar behavior of the three species in rainy and dry seasons.

260 When the  $^{15}\text{N-NO}_3$  was injected at a depth of 0.1 m, a high foliar enrichment in  
261  $^{15}\text{N}$  was observed for some individuals of the three species (Fig. 3). At a horizontal  
262 distance from the trunk of less than 3 m, the highest foliar  $\delta^{15}\text{N}$  value in the rainy  
263 season was measured in one *M. albicans* individual (411‰). At distances between 3  
264 and 6 m from the injection site, the highest foliar  $\delta^{15}\text{N}$  value was 25.2‰ for one *X.*  
265 *aromatica* tree. Over the dry season, the three species studied took up large amounts  
266 of  $^{15}\text{N-NO}_3$  applied in the topsoil within 3 m from the injection site, with a peak of  
267 foliar  $\delta^{15}\text{N}$  at 917‰ for one individual of *M. albicans*. One tree of the species *C.*  
268 *hydrangeifolia* exhibited a foliar  $\delta^{15}\text{N}$  value of 194‰ at a distance of 1.2 m from the  
269 injection site. Between 3 and 6 m from the injection site, the highest foliar value of  
270  $\delta^{15}\text{N}$  was found in one individual *X. aromatica* (30.9‰). Large amounts of  $^{15}\text{N}$  were  
271 also taken up by another *X. aromatica* tree (13.9‰) and one individual *C.*  
272 *hydrangeifolia* (28.4‰) at 3.8 and 6 m from the injection site, respectively.

273 When the  $^{15}\text{N-NO}_3$  was injected into deep soil layers, the uptake of labeled N led  
274 to much lower foliar  $\delta^{15}\text{N}$  values than when it was injected into the topsoil (Fig. 3). At  
275 the injection depth of 1.5 m, the highest  $\delta^{15}\text{N}$  value was observed in one individual *M.*  
276 *albicans* (29.4‰) in the rainy season. Once again, at more than 3 m from the  
277 injection site, the highest foliar  $\delta^{15}\text{N}$  value (10.3‰) was measured in one individual *X.*

278 *aromatica*. At an injection depth of 3 m, all the foliar  $\delta^{15}\text{N}$  values were within the  
279 prediction interval of natural abundance, whatever the species and the season. In  
280 contrast, large amounts of  $^{15}\text{N}$  were taken up by some plants when the labeled nitrate  
281 was injected at a depth of 6 m during both seasons. In the rainy season, a high foliar  
282  $\delta^{15}\text{N}$  value (17.1‰) was measured in one individual *X. aromatica*. During the dry  
283 season, two *X. aromatica* individuals located within 2 m from the injection site took up  
284 large amounts of  $^{15}\text{N}$ , with a peak of foliar  $\delta^{15}\text{N}$  of 66.7‰. The foliar  $\delta^{15}\text{N}$  value  
285 (6.2‰) in one individual *X. aromatica* sampled in the rainy season was 0.7‰ above  
286 the upper boundary of the prediction interval for a tracer injection depth of 9 m. A  
287 small amount of labeled nitrogen was therefore taken up, consistently with the strong  
288 foliar enrichment in  $^{15}\text{N}$  for the same species when the tracer was injected at a depth  
289 of 6 m. Regardless of species and seasons, the sampled leaves of all the other trees  
290 were not enriched in  $^{15}\text{N}$  when  $^{15}\text{N}\text{-NO}_3$  was injected at depths of 9 and 12 m.

291

### 292 3.2. $^{15}\text{N}$ uptake of large trees during the dry season

293

294 Whatever the depth of injection of  $^{15}\text{N}\text{-NO}_3$  and whatever the species, we did not  
295 observe a clear relationship between the size of the trees and the uptake of  $^{15}\text{N}$  at  
296 the end of the dry season (Fig. 4). When  $^{15}\text{N}\text{-NO}_3$  was injected at a depth of 0.1 m,  
297 the peak of foliar  $\delta^{15}\text{N}$  was 362‰ in one large tree located at 1 m from the injection  
298 site (Fig. 5). The largest trees in each plot sampled at more than 3.5 m from the  
299 injection site did not exhibit high foliar  $\delta^{15}\text{N}$  values (maximum of 6.4‰ for one  
300 individual at 4 m from the injection site). When the tracer was injected at a depth of  
301 1.5 m, the only foliar  $\delta^{15}\text{N}$  value above the upper boundary of the prediction interval

302 (6.4‰) was found in one large tree located at 2.2 m from the injection site. At a tracer  
303 injection depth of 3 m, the leaves of two large trees were significantly enriched in  $^{15}\text{N}$ .  
304 When the tracer was injected at a depth of 6 m, the leaves of only one large tree  
305 were significantly enriched in  $^{15}\text{N}$ . At the tracer injection depths of 9 and 12 m, all the  
306 foliar  $\delta^{15}\text{N}$  values were within the prediction interval of natural abundance.

307

## 308 **4. Discussion**

309

### 310 *4.1. Complementarity between species in taking up soil resources*

311

312 In agreement with our first hypothesis, when the labeled nitrate was injected at  
313 the depths of 3 m, 6 m and 9 m, a foliar enrichment in  $^{15}\text{N}$  was consistently observed  
314 for some individuals of the *X. aromatica* species, but not for the other studied  
315 species. A marked absorption of  $^{15}\text{N}$  was observed for *X. aromatica* trees in both  
316 seasons when the labeled nitrate was injected at the depths of 0.1 and 6 m, while a  
317 clear uptake of  $^{15}\text{N}$  was only detected to a depth of 1.5 m for the other two studied  
318 species. In addition, while *X. aromatica* trees absorbed  $^{15}\text{N}$  within 5 m horizontally of  
319 the injection site, the absorption of  $^{15}\text{N}$  was only detected within 2 m for the other two  
320 species. In a review of hydrological niches in plant communities ranging from dry to  
321 wet environments, Silvertown et al. (2015) show that niche segregation is  
322 widespread, occurring in 43 of the 48 studies reviewed. Kulmatiski and Beard (2013)  
323 reported that niche partitioning increased when the availability of resources  
324 decreased and highlighted that niche partitioning can occur on short time and spatial

325 scales. Other studies in forest ecosystems have shown that the depth of water and/or  
326 nutrient uptake can vary between tree species (Jackson et al., 1999; Oliveira et al.,  
327 2005; Silvertown et al., 2015). The horizontal distance of  $^{15}\text{N}$  uptake in our Cerrado  
328 ecosystem is consistent with studies in other environments. Göttlicher et al. (2008)  
329 showed that the lateral root spread of trees in monospecific boreal forests was 4–5 m  
330 on average. As in our study, only a small proportion of the trees close to the injection  
331 site were enriched in  $^{15}\text{N}$ , which suggests a high asymmetry of the root system. In an  
332 Amazonian forest, while high deuterium concentrations in the leaves of some species  
333 were measured about 10 m horizontally from the injection site, most of the small  
334 trees sampled (DBH from 2.5 to 22.0 cm) took up the deuterium tracer close to the  
335 trunk (Sternberg et al., 2002).

336         Consequently, we assume that nutrient uptake niches among Cerrado species  
337 allow for the uptake of nutrients in gravitational soil solutions by different plant  
338 species depending on the depth of absorption in very deep soils. However, we  
339 acknowledge that there are many implicit assumptions when we use  $^{15}\text{N}$  as a tracer.  
340 Comparisons between species could in particular be influenced by the preferential  
341 form of N absorbed for each species and by a contrasting water demand during the  
342 dry season for evergreen and deciduous species. However, the methodology used  
343 here made it possible to minimize the potential drawbacks. Although the preferential  
344 form of N absorbed has never been investigated for the species studied, a clear foliar  
345 enrichment in  $^{15}\text{N}$  when the labeled nitrate was applied in the topsoil shows that the  
346 three studied species absorb our tracer. Most tree species take up both nitrate and  
347 ammonium (Wang and Macko, 2011). Applying small amounts of water and nitrate at  
348 each injection site avoided creating soil patches with high water and N availability,  
349 which made it possible to detect the actual uptake of labeled N by trees in a little



350 disturbed environment. Fine roots already established close to the injection area  
351 were little affected by the injection of labeled nitrate. The effect of different foraging  
352 effects between species, with more competitive root systems likely to target the  
353 nitrate-rich patches, was therefore minimized by the low amounts of water and nitrate  
354 applied at each site. Although plants in Cerrado ecosystems are known to explore  
355 very deep soil layers, fine root densities drop below a depth of 1 m (Abdala et al.,  
356 1998; Castro and Kauffman, 1998; Lilienfein et al., 2001; Oliveira et al., 2005). In  
357 further studies, we would recommend several injection points close to the center of  
358 each plot to increase the probability that the roots of neighboring trees explore the  
359 <sup>15</sup>N-rich areas. This method would be more effective in assessing whether trees of a  
360 specific species take up N from deep soil layers without major disturbance to soil  
361 functioning resulting from the injection of large amounts of water and N.

362

#### 363 4.2. *Seasonality of <sup>15</sup>N uptake throughout deep soil profiles*

364

365 Our results do not support the second hypothesis that if the water content over  
366 the entire soil profile drops during the dry season compared to the rainy season, then  
367 the deep-rooted species will take up labeled N more deeply. Indeed, the foliar  
368 enrichment in <sup>15</sup>N of the three species sampled close to the tracer injection sites was  
369 not significantly different between the rainy and dry seasons. While we expected the  
370 uptake of large amounts of <sup>15</sup>N at the depths of 9 and 12 m during the dry season, we  
371 did not detect a clear enrichment in foliar <sup>15</sup>N in the three studied species, or in large  
372 trees sampled close to the injection zones. A comprehensive study combining eddy  
373 covariance measurements and ecophysiological modeling in a neighboring area  
374 shows that eucalypt roots established at a depth of more than 10 m take up water

375 only when the soil is dry in the upper layers (Christina et al., 2017). The lack of  
376 detection of  $^{15}\text{N}$  uptake at the depth of 12 m during the dry season in our study could  
377 be the result of many factors, such as absorbed amounts of labeled N too low to be  
378 detected in the foliage, and/or a period not dry enough to require the absorption of  
379 very deep water by plant roots. Another explanation might be that low soil water  
380 contents in deep soil layers, when the tracer was injected at the beginning of the dry  
381 season, limited the diffusion of nitrate ions to the roots. Large changes in soil water  
382 contents between the two dates of tracer injection suggest that tree roots had already  
383 withdrawn large amounts of water between the depths of 4 m and 10 m when the  
384 tracer was injected in June 2017 (Fig. S3).

385         Consequently, we assume that the uptake of water in the Brazilian Cerrado  
386 could be deeper over dry periods than the depth of 9 m shown in our study. We  
387 acknowledge that the simple method in our study using the injection of nitrates in the  
388 soil as a proxy of water uptake is probably less sensitive than isotopic methods  
389 based on  $^2\text{H}$  and  $^{18}\text{O}$  measurements to detect small amounts of water taken up by  
390 plants. We considered that leaves can only be significantly enriched in  $^{15}\text{N}$  if large  
391 amounts of water are withdrawn since mass flow is necessary for nitrate ions to  
392 reach the few roots present in deep soil layers (Oyewole et al., 2014; McMurtrie and  
393 Näsholm, 2018). The period of 5 months between tracer injection in the soil and leaf  
394 sampling in our study was a compromise between a short period, with an attendant  
395 risk that different N statuses between species and/or contrasting water uptake  
396 between evergreen and deciduous species will modify the comparisons between  
397 species, and a long period, with an accompanying risk of leaching of the labeled  
398 nitrate and leaf shedding after enrichment in  $^{15}\text{N}$ . We would recommend a  
399 characterization of the hydrodynamic properties of the soil to estimate the migration

400 of the tracer over the period between injection and leaf sampling. The simultaneous  
401 injection of deuterium and  $^{18}\text{O}$ -labeled water with labeled nitrate during a particularly  
402 dry period would be useful to verify the interest of using labeled nitrate as a proxy of  
403 water uptake in deep soil layers.

404

#### 405 4.3. *Relationship between plant DBH and the depth of nitrogen uptake*

406

407 Our third hypothesis that if woody plants of very different sizes coexist in the  
408 Cerrado ecosystem, then large trees will absorb the injected tracer at a greater depth  
409 than small trees is not accepted because we did not observe a clear relationship  
410 between the DBH of the trees and the depth of  $^{15}\text{N}$  uptake. Nevertheless, only trees  
411 with a DBH greater than 5 cm were able to take up the tracer at more than 3 m from  
412 the bottom of the trunk, horizontally and vertically (except for a single *C.*  
413 *hydrangeifolia* individual when  $^{15}\text{N}\text{-NO}_3$  was injected in the topsoil). The largest trees  
414 sampled in the dry season (irrespective of the species) did not take up detectable  
415 amounts of  $^{15}\text{N}$  at the depths of 9 and 12 m, although some fine roots were sampled  
416 (Fig. S4). In a seasonal Amazon forest, Brum et al. (2018) showed that the  
417 differences in rooting depth of 12 dominant species were strongly related to tree size.  
418 Although larger trees have been reported to preferentially tap deeper water than  
419 smaller trees, the reverse has also been shown (Meinzer et al., 2001). In another  
420 Cerrado in Brazil, Jackson et al. (1999) pointed out a specific division of water  
421 resources among 10 woody species at different depths to 4 m during the dry season,  
422 with deciduous species preferentially tapping deeper sources of soil water than  
423 evergreen species. However, the size of the plant did not influence the partitioning of  
424 water in the soil among the woody species in this Cerrado. Furthermore, using a

425 dual-isotope labeling approach in an Amazonian forest, Stahl et al. (2013) did not find  
426 a strong relationship between tree dimensions (diameter or height) and depth of  
427 water uptake. Similarly, Chitra-Tarak et al. (2017) did not find a clear relationship  
428 between the depth of water uptake and stem size in a dry tropical forest in India.

429         Consequently, we assume that the relationship between the DBH of the trees  
430 and the depth of N and water uptake is weak in the Brazilian Cerrado, in agreement  
431 with previous studies (Jackson et al., 1999; Oliveira et al., 2005). However, we  
432 acknowledge that our methodology was probably not the most appropriate to study  
433 the relationship between the depth of N uptake and tree DBH, because the  
434 probability of sampling leaves of trees with roots exploring exactly the area of tracer  
435 injection was low, which probably explains why many plants did not show a marked  
436 uptake of  $^{15}\text{N}$ . Studies based on the natural abundance of deuterium or  $^{18}\text{O}$  would be  
437 better suited to test this hypothesis.

438         Studies using tracers are scarce in tropical ecosystems and, as far as we are  
439 aware, stable isotopes have never been injected in tropical savannas at depths of  
440 more than 5 m. We show that very low densities of roots in deep soil layers can take  
441 up detectable amounts of labeled nitrogen, which suggests that deep roots of some  
442 tree species could help reduce nutrient losses through deep leaching. Further studies  
443 dealing with nutrient and water uptake in very deep soil layers are needed to improve  
444 the forecasting of tropical savanna response to climate change.

445

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447

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455

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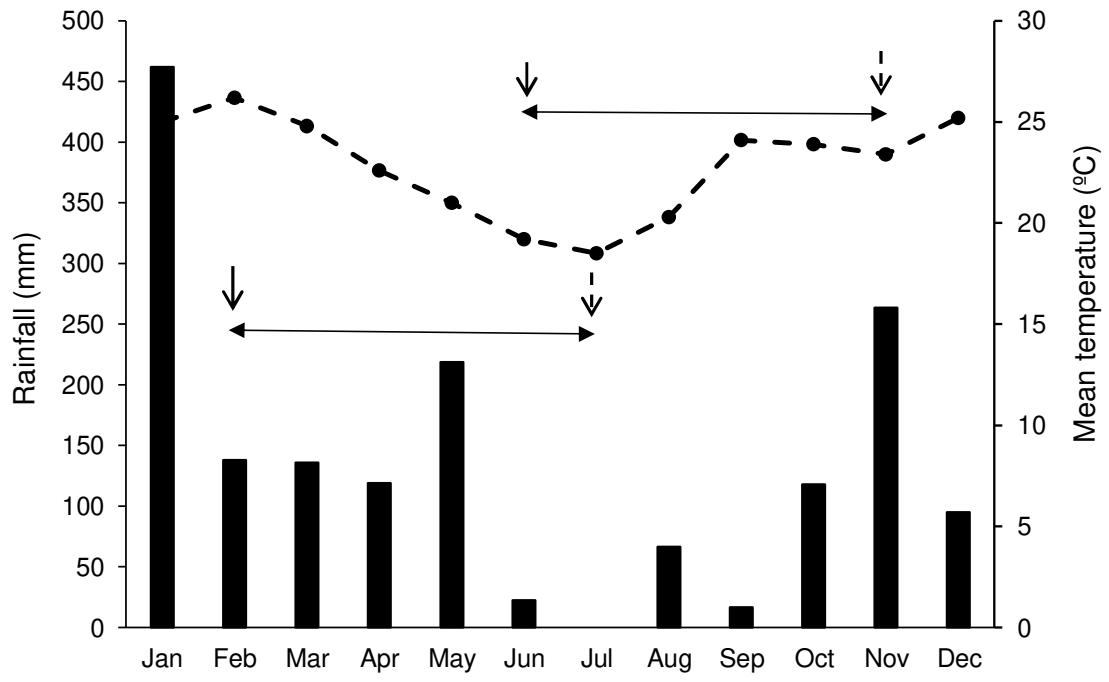


Fig. 1.

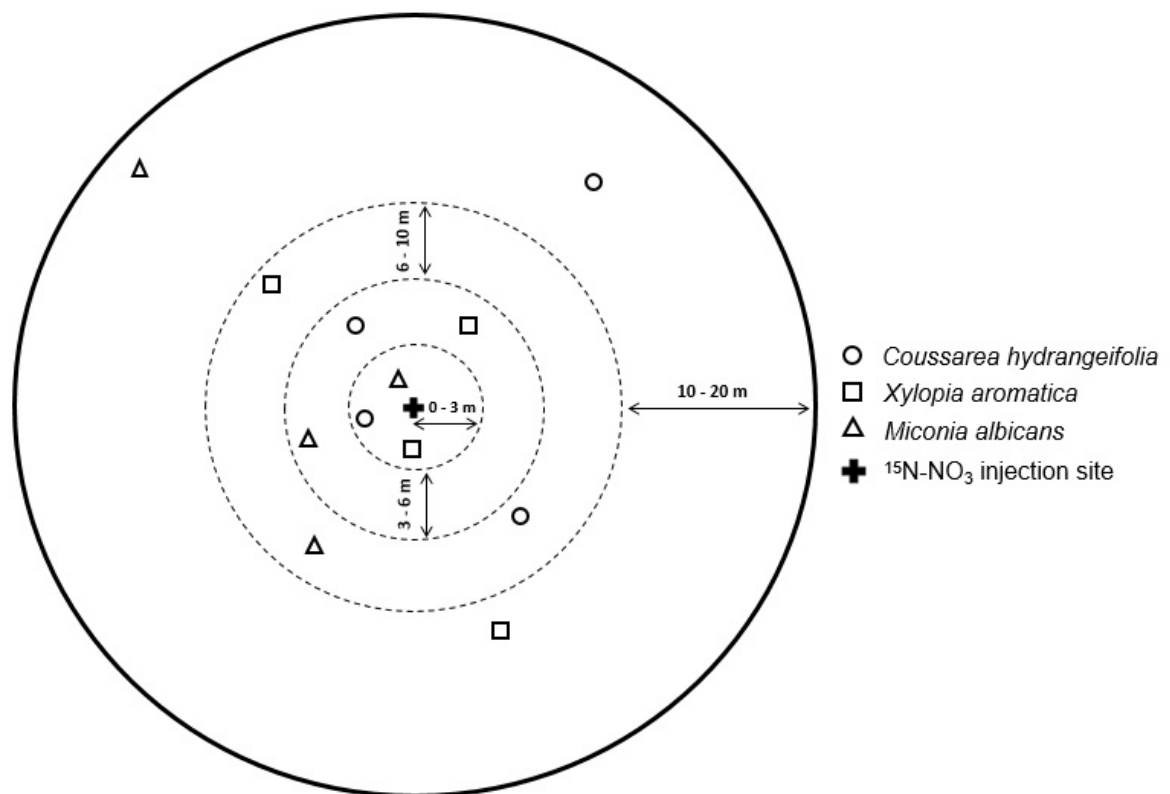


Fig. 2.

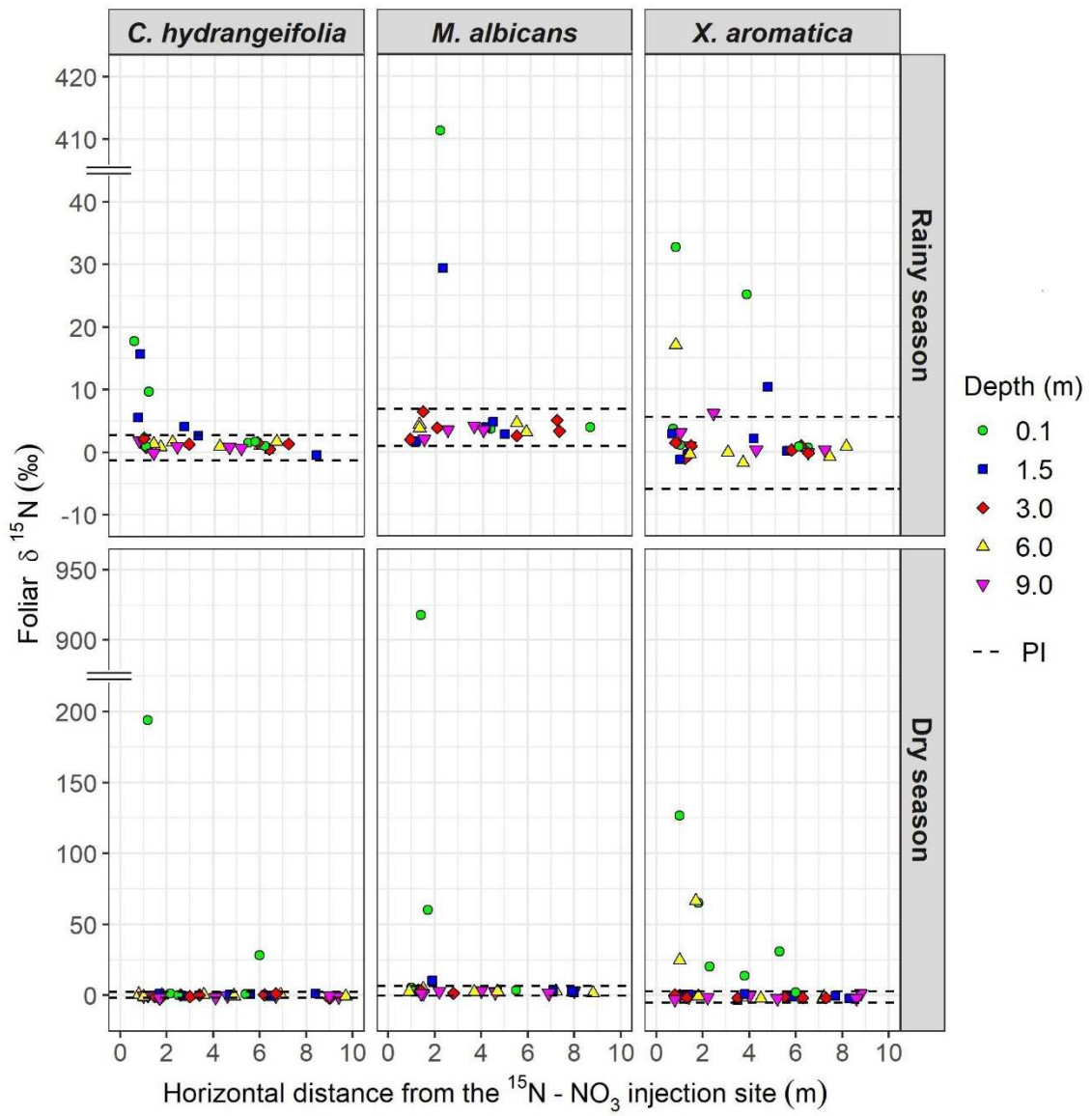


Fig. 3.



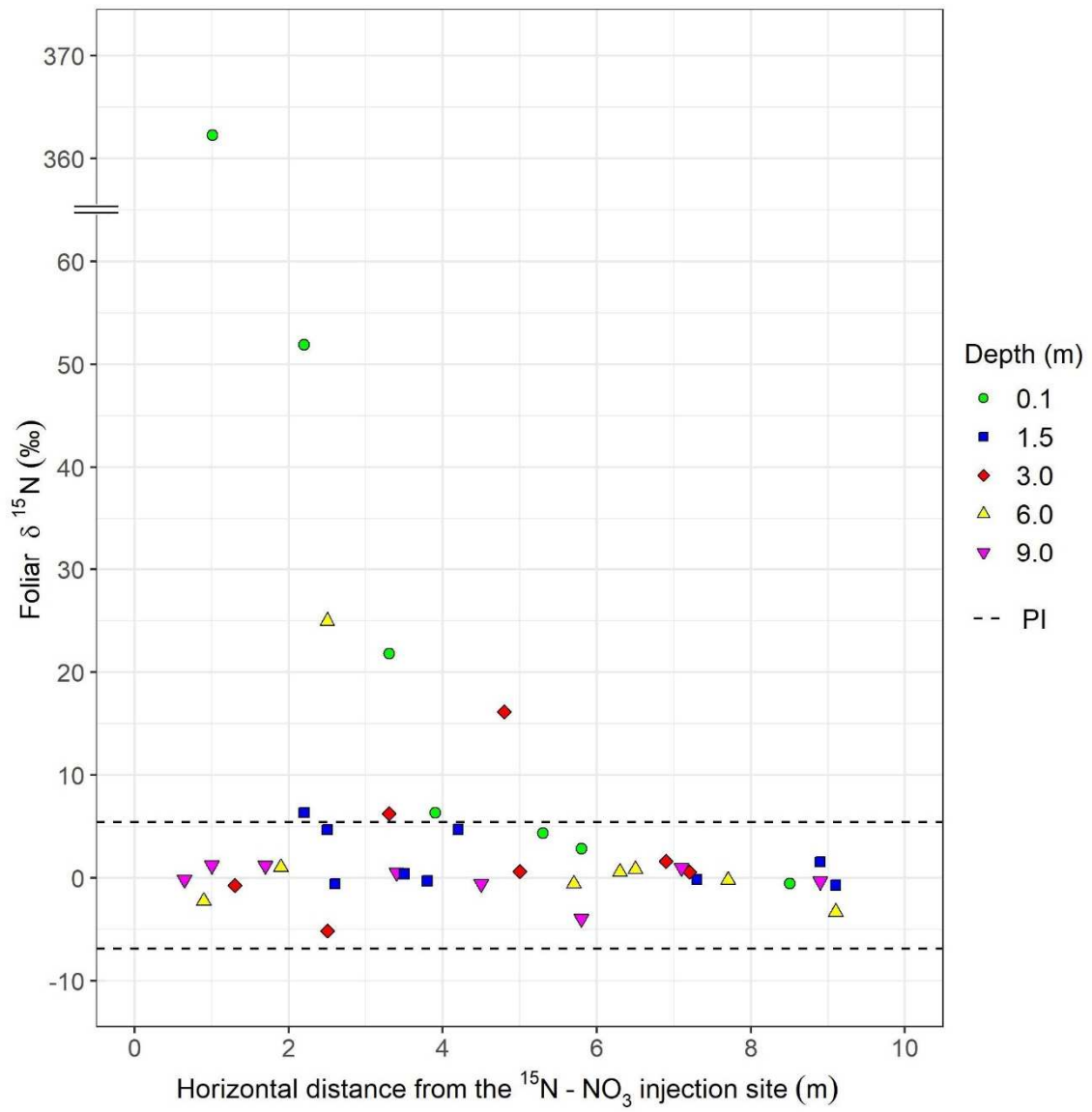


Fig. 5.



**Table 1**

Main physical and chemical soil properties of the Cerrado area at Bauru, São Paulo state, southeast Brazil. Mean values are indicated ( $n = 3$ ).

Soil layer (m)	Sand	Silt (g kg <sup>-1</sup> )	Clay	pH	O.M.	P <sub>resin</sub>	H+Al	K	Ca	Mg	SB	CEC
				CaCl <sub>2</sub>	(g kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(mmol <sub>c</sub> kg <sup>-1</sup> )					
0 - 0.25	896	21.7	82.3	3.9	14	3.6	34	0.6	2.0	1.4	4.0	38
0.25 - 0.5	876	24.3	99.7	3.8	10	2.3	32	0.5	1.7	1.0	3.1	35
0.5 - 1	857	29.3	114	3.7	5.3	1.8	27	0.2	1.1	0.5	1.8	29
1 - 2	836	33.0	131	3.9	4.3	1.8	20	0.3	1.2	0.5	1.9	22
2 - 4	832	28.7	139	4.1	2.7	1.8	16	0.2	1.5	0.5	2.1	18
4 - 6	807	34.3	159	4.0	2.2	2.1	16	0.1	1.5	0.5	2.2	19
6 - 8	801	35.3	163	4.0	2.1	1.9	18	0.2	1.2	0.5	1.9	20
8 - 10	802	42.7	156	4.0	3.4	1.8	21	0.3	1.2	0.6	2.1	23
10 - 12	787	61.3	152	3.9	3.4	2.2	26	0.7	1.2	2.5	4.5	30

Organic matter (O.M.) determined using sodium dichromate. Resin extraction of phosphorus and exchangeable elements. SB, sum of bases. CEC, cation exchange capacity.

**Table 2**

Mean height, diameter at breast height (DBH), basal area and density of the three species studied in the Cerrado area at Bauru, São Paulo state, Brazil. DBH (> 1.5 cm) was measured for all plants within a radius of 10 m around the tracer injection point in 36 plots. Heights were measured only for plants whose leaves were sampled for isotopic analysis. Standard deviations are indicated.

<b>Species</b>	<b>Height (m)</b>	<b>DBH (cm)</b>	<b>Basal area (m<sup>2</sup> ha<sup>-1</sup>)</b>	<b>Density (# plants ha<sup>-1</sup>)</b>
<i>Coussarea hydrangeifolia</i>	5.3 (1.1)	4.3 (2.0)	2.3 (0.8)	1103 (560)
<i>Miconia albicans</i>	3.1 (1.0)	2.1 (1.2)	0.2 (0.2)	337 (175)
<i>Xylopia aromatica</i>	7.3 (1.0)	4.5 (2.1)	1.8 (0.9)	756 (335)
Large trees sampled	-	23 (9.5)	5.2 (2.2)	106 (15)
Other shrubs and trees	-	6.4 (6.0)	21 (7.3)	3185 (1100)