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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 km². 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 ¹⁵N uptake was dependent on the species. *X. aromatica* trees
26 took up ¹⁵N from a maximum depth of 9 m and exploited a much larger soil volume
27 than the two other species, with the uptake of ¹⁵N 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 ¹⁵N only in the 0-1.5 m soil layer,
30 within 2 m horizontally from the injection site. The depth of ¹⁵N 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:** ¹⁵N, 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 km² 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 CO₂ 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 ± 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}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}N determinations within leaves after
80 injection of ^{15}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}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}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}N) is injected into deep soil layers then the leaves of only
96 some species will be enriched in ^{15}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) ≥ 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}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}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}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 ¹⁵N natural abundance.
208 Leaves were sampled about 5 months after tracer injection in both seasons (Fig. 1).

209

210 2.5. Isotopic analyses of ¹⁵N

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 ¹⁵N 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 ¹⁵N 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 ¹⁵N.

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_α 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}N by X. aromatica, C. hydrangeifolia and M. albicans*

251

252 The foliar enrichment in ^{15}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 $^{15}\text{N}\text{-NO}_3$ 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 $^{15}\text{N}\text{-}$
258 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 $^{15}\text{N}\text{-NO}_3$ was injected at a depth of 0.1 m, a high foliar enrichment in
261 ^{15}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 $^{15}\text{N}\text{-NO}_3$ 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}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 $^{15}\text{N}\text{-NO}_3$ 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}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}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}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}N when $^{15}\text{N}\text{-NO}_3$ was injected at depths of 9 and 12 m.

291

292 3.2. ^{15}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}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}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}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}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}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}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}N within 5 m horizontally of
319 the injection site, the absorption of ^{15}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}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}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}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}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 ^{15}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 ^{15}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 ^{15}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 ^{15}N at the depths of 9 and 12 m during the dry season, we
371 did not detect a clear enrichment in foliar ^{15}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}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 ^2H and ^{18}O measurements to detect small amounts of water taken up by
390 plants. We considered that leaves can only be significantly enriched in ^{15}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}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}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}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}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}N . Studies based on the natural abundance of deuterium or ^{18}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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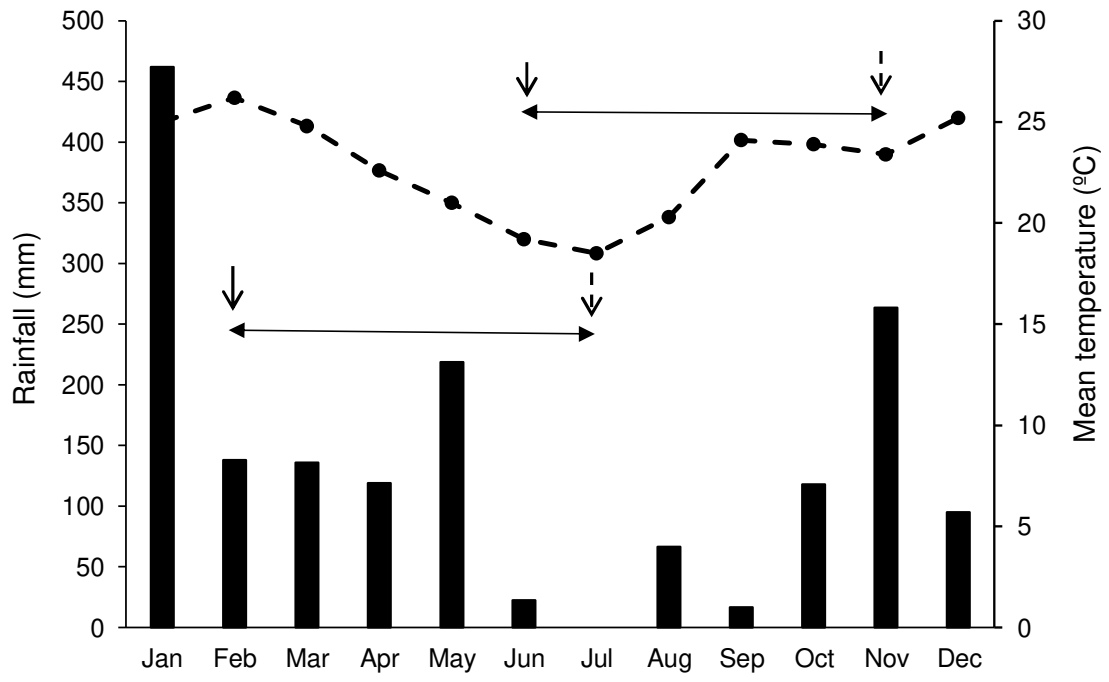


Fig. 1.

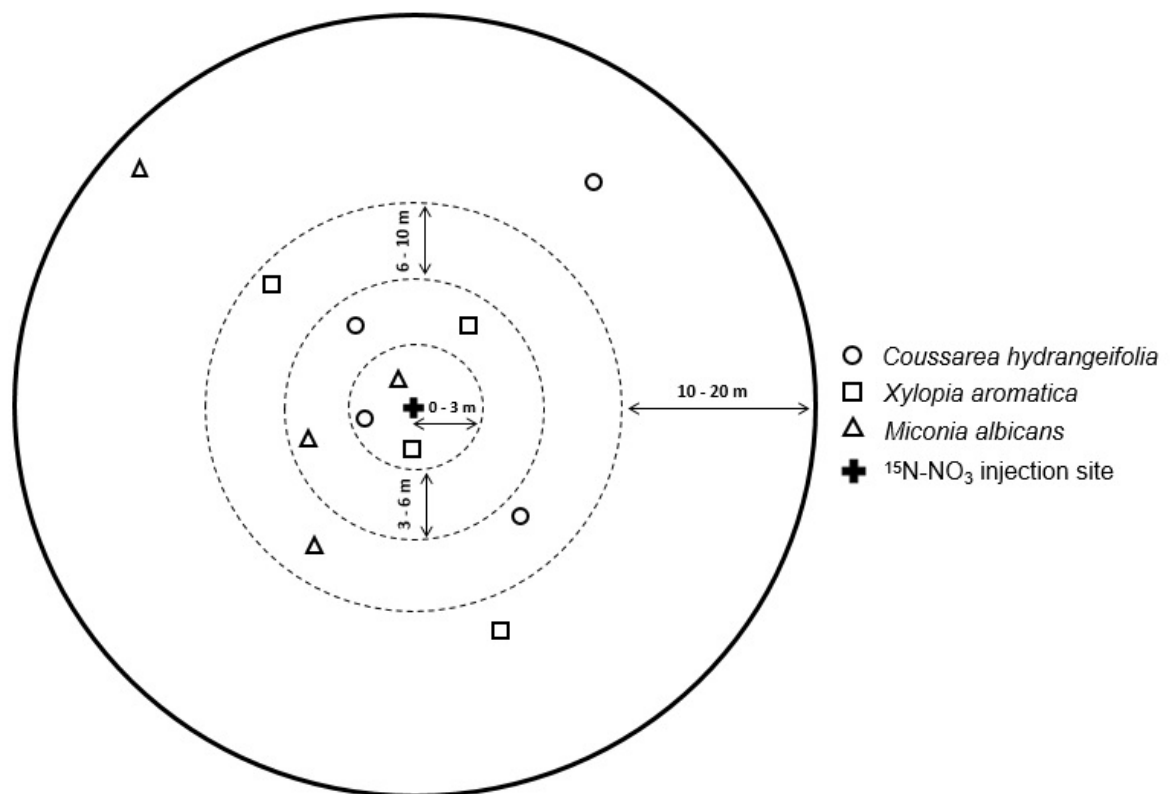


Fig. 2.

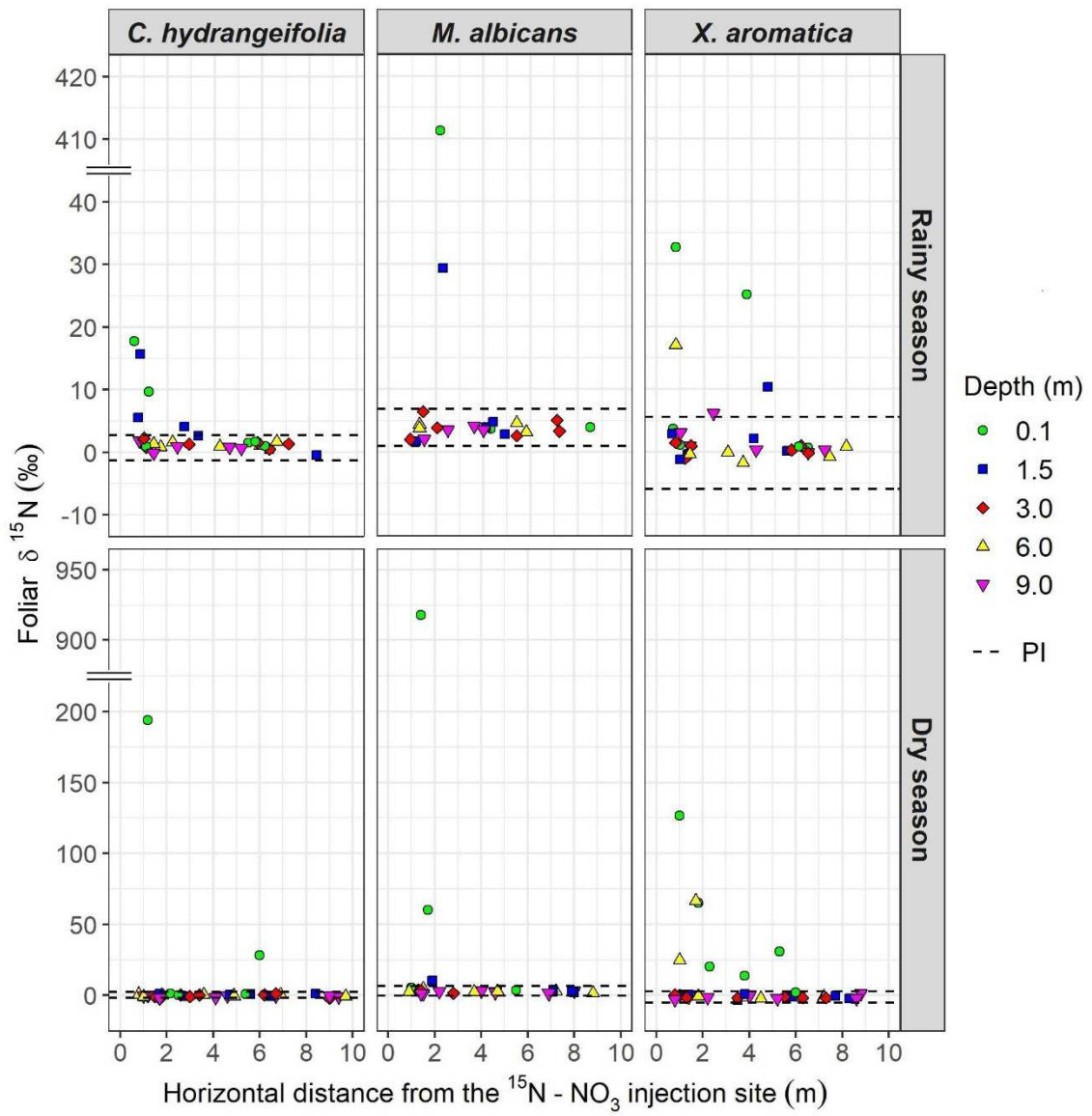


Fig. 3.

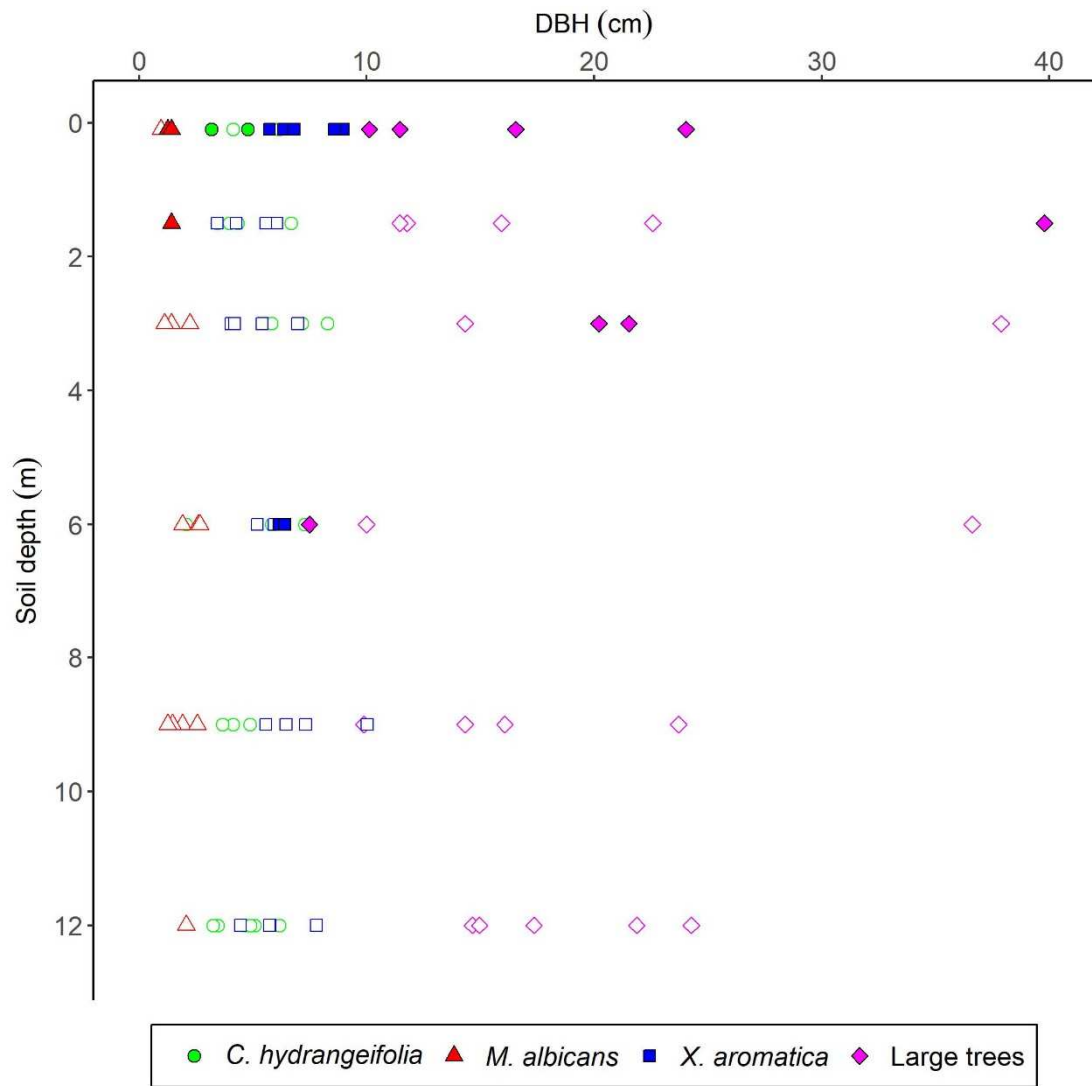


Fig. 4.

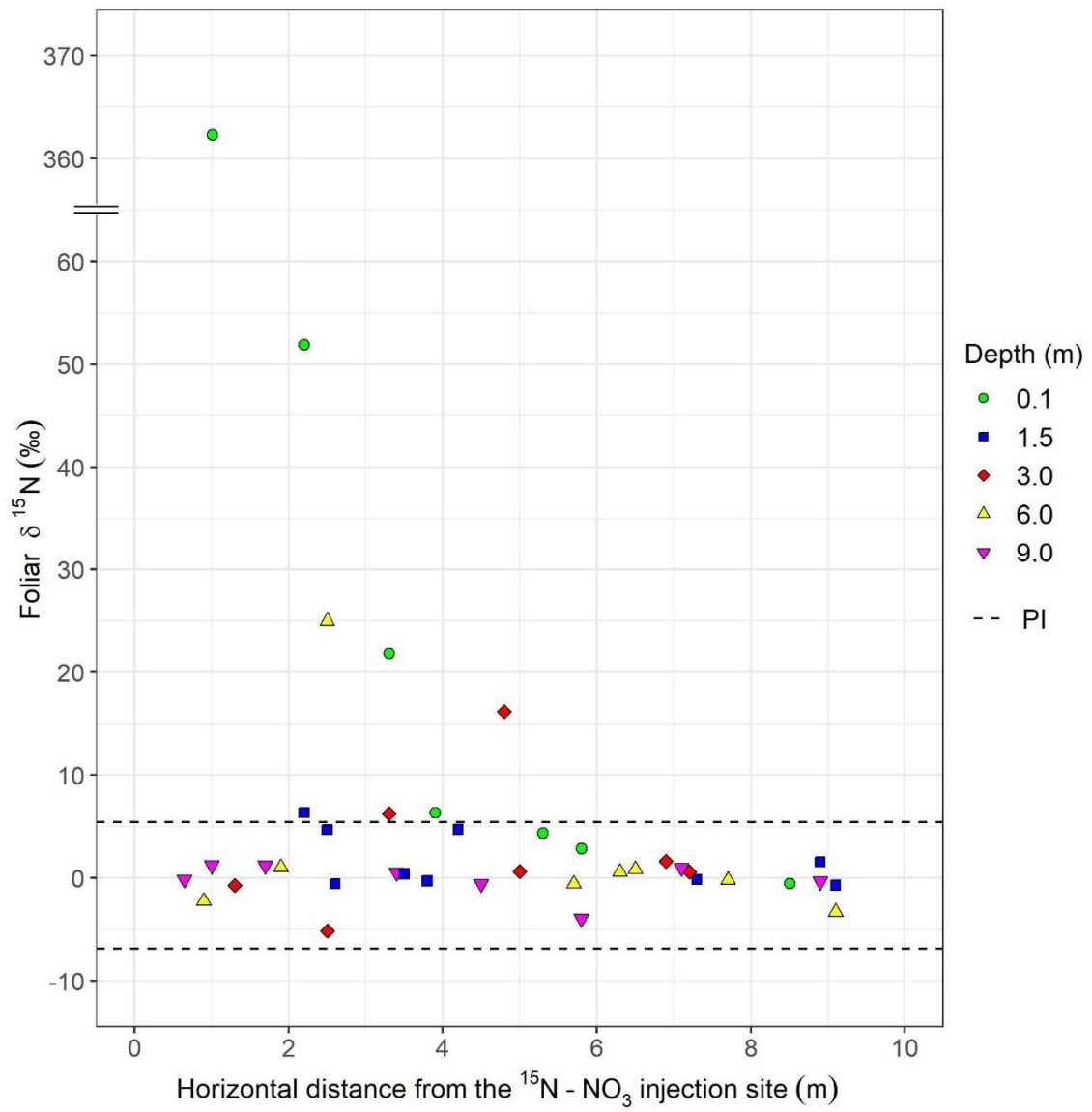


Fig. 5.

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 ⁻¹)	Clay	pH	O.M.	P _{resin}	H+Al	K	Ca	Mg	SB	CEC
				CaCl ₂	(g kg ⁻¹)	(mg kg ⁻¹)	(mmol _c kg ⁻¹)					
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.

Species	Height (m)	DBH (cm)	Basal area (m² ha⁻¹)	Density (# plants ha⁻¹)
<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)