

1 **Economic strategies of plant absorptive roots vary with root diameter**

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21

22 **Abstract**

23 Plant roots typically vary along a dominant ecological axis, the root economics spectrum,  
24 depicting a tradeoff between resource acquisition and conservation. For absorptive roots,  
25 which are mainly responsible for resource acquisition, we hypothesized that root economic  
26 strategies differ with increasing root diameter. To test this hypothesis, we used seven plant  
27 species (a fern, a conifer, and five angiosperms from south China) for which we separated  
28 absorptive roots into two categories: thin roots (thickness of root cortex plus epidermis < 247  
29  $\mu\text{m}$ ) and thick roots. For each category, we analyzed a range of root traits related to resource  
30 acquisition and conservation, including root tissue density, different carbon (C) and nitrogen  
31 (N) fractions (i.e., extractive, acid-soluble and acid-insoluble fractions) as well as root  
32 anatomical traits. The results showed significant relationships among root traits indicating an  
33 acquisition-conservation tradeoff for thin absorptive roots while no such trait relationships  
34 were found for thick absorptive roots. Similar results were found when reanalyzing data of a  
35 previous study including 96 plant species. The contrasting economic strategies between thin  
36 and thick absorptive roots, as revealed here, may provide a new perspective on our  
37 understanding of the root economics spectrum.

38

39 **Key-words:** chemical fractions, plant functional traits, root diameter, root economics  
40 spectrum, root tissue density

41

42 **1 Introduction**

43 Plant traits reflecting a tradeoff between resource acquisition and conservation represent an  
44 essential ecological axis for plant strategies that is important for our understanding of how  
45 plants drive ecosystem processes and responses to environmental change (Cornwell et al.,  
46 2008; Freschet et al., 2010; Reich, 2014; Westoby et al., 2002). On the one end of this axis,  
47 there are species with acquisitive strategies, i.e., fast acquisition of resources (e.g., CO<sub>2</sub> for  
48 leaves and nutrients for roots) accompanied with a short lifespan. On the other end of the axis,  
49 there are species with conservative strategies, i.e., slow resource acquisition accompanied  
50 with a long lifespan. Originally, such an ecological axis has been demonstrated for leaves,  
51 which is widely known as the leaf economics spectrum (Diaz et al., 2004; Osnas et al., 2013;  
52 Wright et al., 2004). More recently, similar trait spectra have been demonstrated across plant  
53 organs from leaves to stems and roots, thus forming a whole ‘plant economics spectrum’  
54 (Freschet et al., 2010; Laughlin et al., 2010; Prieto et al., 2015; Reich, 2014).

55 Resource acquisition in plant roots is performed by absorptive roots, i.e., the first two or  
56 three orders of a root branch with primarily-developed tissues which are part of the commonly  
57 used category of ‘fine roots’ (< 2mm in diameter) (Guo et al., 2008; Long et al., 2013;  
58 Pregitzer et al., 2002). For absorptive roots, tissue density, i.e., root dry mass per unit root  
59 volume, is a key trait of the root economics spectrum as tissue density is closely linked to the  
60 acquisition-conservation tradeoff (Bardgett et al., 2014; Birouste et al., 2014; Craine et al.,  
61 2005; Espeleta et al., 2009; Mommer and Weemstra, 2012; Roumet et al., 2006). In general,  
62 absorptive roots with higher tissue density are slower in nutrient acquisition and longer in  
63 lifespan whereas absorptive roots with lower tissue density may enable faster acquisition but  
64 maintain a shorter lifespan (Ryser, 1996; Wahl and Ryser, 2000; Withington et al., 2006).

65 Recently, tissue density for absorptive roots was found to negatively correlate with root  
66 diameter. This could be because root cortex is less dense than root stele and because in thicker  
67 roots a larger proportion of the root cross-sectional area is accounted for by the cortex (Chen  
68 et al., 2013; Kong et al., 2014; Kong and Ma, 2014). On the other hand, compared with  
69 thinner absorptive roots, thicker absorptive roots may acquire resources faster because of their  
70 greater dependence on mycorrhizal fungi (Eissenstat et al., 2015; Kong et al., 2014; Kong and  
71 Ma, 2014; St John, 1980), and may also have a longer lifespan due to the larger diameter  
72 (Adams et al., 2013; Eissenstat and Yanai, 1997; Wells and Eissenstat, 2001). As such, the  
73 trait syndrome for thicker absorptive roots would differ from the predictions of faster  
74 acquisition and shorter lifespan. This highlights the importance of discriminating thicker and  
75 thinner absorptive roots when exploring root strategies. However, few studies have tested for  
76 effects of root diameter in driving trait economics spectra in absorptive roots.

77 In addition to structural traits such as density, the chemical composition of absorptive roots  
78 may constitute another important aspect of testing root strategies in relation to root diameter  
79 (Hidaka and Kitayama, 2011; Meier and Bowman, 2008; Poorter and Bergkotte, 1992;  
80 Poorter et al., 2009). For example, carbon (C) and nitrogen (N), the two most abundant  
81 elements in plant tissues, are usually bound to organic compounds which may contain labile  
82 fractions (e.g., soluble sugars and proteins in living cells) and recalcitrant fractions (e.g.,  
83 cellulose and lignin in structural tissues) (Atkinson et al., 2012; Berg and McClaugherty, 2008;  
84 Feng et al., 2009; Poorter et al., 2009; Shipley et al., 2006). From the perspective of C and N  
85 fractions, absorptive roots with less labile C and more labile N may indicate an acquisitive  
86 strategy. This is because high root activity may be accompanied by an increased production of

87 metabolism-related proteins with a high labile N content; such roots may be palatable for  
88 herbivores and have a relative short lifespan. On the other hand, conservative roots contain  
89 less labile C and N fractions as more of these compounds are used for construction of  
90 structural tissues resulting in lower root activity and a longer lifespan. However, compared  
91 with thinner absorptive roots, thicker absorptive roots may have higher labile C and N  
92 fractions as these labile fractions can be stored in their thick root cortex (Chapin III, 1980;  
93 Long et al., 2013; Lux et al., 2004; Withington et al., 2006). As such, the chemical traits of  
94 thicker absorptive roots integrate ‘opposing’ effects of root metabolism and storage,  
95 suggesting them having neither a true acquisitive nor a true conservative strategy. Therefore,  
96 in evaluating the impact of thickness on root economic strategies it is necessary to examine C  
97 and N fractions in relation to root diameter.

98 Here, we selected a variety of plant species common to tropical and subtropical forests in  
99 south China with contrasting phylogeny and root structure. The aim of our study was two-fold.  
100 First, we examined the influence of root diameter on the root economic strategies in  
101 absorptive roots. We hypothesized that the root economic strategies differ between thinner  
102 and thicker absorptive roots, with trait relationships indicating acquisitive-conservative  
103 trade-off for thinner roots but not for thicker roots. The hypothesis was tested using a series of  
104 trait relationships involving both structural and chemical traits. Second, root C and N  
105 fractions have been suggested to vary in predictive ways across branch orders (Fan and Guo,  
106 2010; Goebel et al., 2011). However, we hypothesized that patterns of root C and N fractions  
107 across branch orders differ in species varying in absorptive root diameter.

108

## 109 **2 Materials and methods**

### 110 **2.1 Plant species and sampling sites**

111 We selected seven plant species with contrasting phylogeny and root structure (Table S1) in  
112 tropical and subtropical forests in south China. Three species were sampled at the Heshan  
113 Hilly Land Interdisciplinary Experimental Station (22°41'N, 112°54'E), Guangdong province.  
114 The species were: *Dicranopteris dichotoma* (Gleicheniaceae) (a fern), *Cunninghamia*  
115 *lanceolata* (Taxodiaceae) (a conifer) and *Acacia auriculiformis* (Leguminosae) (a tree).  
116 Another tree species, *Paramichelia baillonii* (Magnoliaceae), was sampled in Wutongshan  
117 National Forest Park (22°27'-22°52'N, 113°37'-114°37'E) in Shenzhen, Guangdong province.  
118 Three other tree species, *Gordonia axillaris* (Theaceae), *Endospermum chinense*  
119 (Euphorbiaceae) and *Cryptocarya chinensis* (Lauraceae), were sampled in Jianfengling  
120 Nature Reserve (18°23'-18°50'N, 108°36'-109°05'E), Hainan province. Roots of these species  
121 are mycorrhized. Including plant species from the fern, conifer to angiosperms could  
122 extrapolate to some extent our idea of root economic strategies across different groups of  
123 plant species. In addition, anatomical structures of some species have been explored in one of  
124 our previous studies (see Long et al., 2013) and their results may be instructive for our current  
125 study. More information on sites and species can be found in Table S1 and in Long et al.  
126 (2013).

127

### 128 **2.2 Root sampling**

129 Roots were collected at a soil depth of 0-10 cm in June and July 2011. For each species, at  
130 least three mature trees were selected. We first tracked the main lateral roots by carefully

131 removing surface soil at the base of each plant with a specially manufactured fork. Root  
132 branch order was defined according to Pregitzer's study with the most terminal branch as the  
133 first-order (Pregitzer et al., 2002). The intact roots were collected and soil adhering to the  
134 roots was carefully removed. We distinguished all four root orders for *D. dichotoma* and the  
135 first five orders for the other species. A portion of each root sample was immediately put into  
136 Formalin-Aceto-Alcohol (FAA) solution (90 ml 100% ethanol, 10 ml 100% glacial acetic  
137 acid) for later anatomical assessments. The remaining unwashed part of each root sample was  
138 placed in plastic bags and transported in a cooler to the laboratory. These root samples were  
139 then frozen until measurements of root morphology and chemistry (Pregitzer et al., 2002).

140

### 141 **2.3 Root tissue density**

142 For each species, 50 root segments for the first order, 30 segments for the second order, and  
143 20 segments for the third to the fifth order were randomly picked for measuring root diameter  
144 and length. Depending on root size, the root diameter was measured under a 40× or 20×  
145 stereomicroscope (MZ41-2B, MshOt, Guangzhou, China). The length of comparatively short  
146 roots was assessed using a stereomicroscope with an ocular micrometer ( $\pm 0.025$  mm) while a  
147 measuring tape with the minimum scale of 0.5 mm was used for relatively long roots (Guo et  
148 al., 2008). After root diameter and length were recorded, roots were oven-dried at 65 °C for  
149 48 h and weighed. Root tissue density was calculated by dividing root dry mass by root  
150 volume assuming roots are cylindrically shaped (Kong et al., 2014). In addition, specific root  
151 length (SRL) was calculated as the root length divided by its dry mass.

152

## 153 **2.4 Root anatomy**

154 Root segments from the FAA solution were cleaned with deionized water (4 °C) and then  
155 transferred to glass Petri dishes for dissection into different branch orders. Root anatomy was  
156 determined according to Long et al. (2013). Briefly, a minimum of 10 root segments were  
157 randomly chosen for each root order. All root segments were dehydrated in an ethanol  
158 solution series to absolute ethanol, purified in 100% xylene and embedded in paraffin. Root  
159 cross-sections were then cut into slices of 8 µm thick using a microtome (Rotary Microtome  
160 KD-2258, Zhejiang, China). After deparaffinage, the root slices were stained first by safranin  
161 and then by fast green. Following this staining procedure, the cortex and epidermis was in  
162 blue and the stele was in red. The root slices were then photographed by a light microscope  
163 (Carl Zeiss Axioscop 20, Jena, Germany). The size of anatomical structures including  
164 epidermis, cortex and stele was measured using Image J software (NIH Image, Bethesda, MD,  
165 USA). Absorptive roots in a root branch were defined based on root anatomy (Guo et al.,  
166 2008). Here, root orders were classified as absorptive roots when they had no or little  
167 secondary xylem (Long et al., 2013). Specifically, absorptive roots referred to the first two  
168 orders for *D. dichotoma*, the first three orders for *A. auriculiformis*, *G. axillaris*, *C. lanceolata*,  
169 *E. chinense* and *C. chinensis*, and the first four orders for *P. baillonii*, respectively (Fig. S1).

170

## 171 **2.5 Chemical analyses**

172 The frozen root samples were put into deionized water to carefully remove any soil particles  
173 or dead organic matter that adhered to but was not part of the root (Pregitzer et al., 2002). The  
174 samples of each root branch order were then oven-dried (65 °C for 24 h), milled (ZM200,



175 Retsch, Germany), and mixed homogeneously for chemical analyses. Root C and N  
176 concentrations were determined using an element analyzer (VarioEl, Elementar  
177 Analysen-systeme GmbH, Germany). Root C fractions (extractive, acid-soluble fraction,  
178 acid-insoluble fraction) were determined by a sulfuric acid digestion method. First, we  
179 separated the extractive and labile C fraction from other C fractions. A subsample of c. 100  
180 mg ( $m_0$ ) was extracted with 15 ml of cetyl trimethylammonium bromide (CTAB) solution for  
181 3 h, filtered, repeatedly washed with de-ionized water until pH was 7.0, and then oven-dried  
182 at 60 °C to a constant weight ( $m_1$ ). Second, the filtered residue was digested with 30 ml  
183 sulfuric acid (72 %) at 22 °C for 3 h, filtered, repeatedly washed (until pH was 7.0), dried and  
184 weighed ( $m_2$ ). After the acid-digestion step, the ash content ( $m_3$ ), was determined by  
185 combusting 15-30 mg of sample at 550 °C for 4 h. Finally, the extractive, acid-soluble and  
186 acid-insoluble fractions were calculated as  $100\% \times (m_0 - m_1) / (m_0 - m_3)$ ,  $100\% \times$   
187  $(m_1 - m_2) / (m_0 - m_3)$ , and  $100\% \times (m_2 - m_3) / (m_0 - m_3)$ , respectively. Here, the extractive fraction  
188 was considered as the labile C fraction while acid-soluble and acid-insoluble fractions were  
189 considered as the recalcitrant C fraction.

190 An about 5mg subsample of residue left after the above acid-digestion procedure was used  
191 to measure N concentration and N allocation in the acid-insoluble C fraction. The N in the  
192 extractive fraction was too low to measure. Thus, estimates of N in the acid-soluble fraction  
193 were calculated as the difference between total N and N in the acid-insoluble fraction.

194

## 195 **2.6 Data analyses**

196 Relationships between root tissue density and root N concentration and each of the three C  
197 fractions were assessed by linear regressions. Here, we introduced a new term, 'root EC'  
198 referring to tissues outside the stele including the epidermis and cortex. Root EC was used for  
199 two reasons. First, the thickness of root EC can be a proxy of the size of root diameter  
200 ( $R^2=0.91$  and  $R^2=0.99$  for linear regressions in this study and in Kong et al. (2014),  
201 respectively). Second, root EC can be used as an indicator of root chemical composition as  
202 the storage of root labile C and most of root N is found in root EC (Chen et al., 2013). The  
203 relationships between the thickness of root EC and root tissue density and root chemical  
204 fractions were also investigated with linear regressions. In addition, the relationship between  
205 SRL and thickness of root EC was fitted by exponential regression.

206 To explore the effect of root diameter on root ecological strategies, the above analyses were  
207 repeated for thin and thick absorptive roots, respectively. A mean thickness of 247  $\mu\text{m}$  was  
208 used for root EC as the cut-off point between thin and thick absorptive roots. The mean  
209 thickness of root EC was used because the thickness of root EC for absorptive roots followed  
210 a normal distribution ( $p>0.05$ , indicating that thickness was statistically no different from a  
211 normal distribution; Fig. S2a). To avoid the influence of biological N fixation on relationships  
212 between root N and root tissue density and root EC, a legume species, *A. auriculiformis*, was  
213 excluded in these analyses. In addition, the relationship between the extractive C fraction and  
214 root tissue density was further explored by a quadratic polynomial regression using moving  
215 average data (Fig. S4). Polynomial regressions were run both for the thin and thick absorptive  
216 roots. The moving average data were obtained as follows. First, the extractive C fraction was  
217 sorted along with the ascending order of root tissue density. Then, the extractive C fraction

218 and root tissue density were averaged by bins (Reich and Oleksyn, 2004), with bins referring  
219 to each of the two neighboring data of extractive C fraction or root tissue density, respectively.  
220 Moving average analyses were used as it improved the goodness of fit. No polynomial  
221 regression relationships were found for the other two C fractions.

222 We acknowledge that the seven species we used represent a relative small species pool. To  
223 validate the results of our study, another dataset of 96 woody species from one of our  
224 previous studies was used where only the first-order roots were measured (Kong et al., 2014).  
225 For these 96 species, we did not use the average root EC thickness as the cut-off between thin  
226 and thick absorptive roots. This was because root EC of these species followed a skewed  
227 normal distribution with abundant species having thinner root EC ( $p < 0.05$ , indicating that  
228 thickness was statistically different from a normal distribution; Fig. S2b). In the case of a  
229 skewed normal distribution, the cut-off point based on mean root EC might cause bias in  
230 separating thin and thick absorptive roots. Here, a thickness of 182.8  $\mu\text{m}$  for root EC was used  
231 as a cut-off between thin and thick absorptive roots for these species (Kong et al., 2014). The  
232 thickness of 182.8  $\mu\text{m}$  for root EC corresponded to a transition from lower to higher  
233 mycorrhizal colonization with increasing root diameter (Kong et al., 2014). This transition  
234 may also indicate a divergence of strategy between thin absorptive roots (depending mainly  
235 on roots themselves for resource acquisition) and thick absorptive roots (depending mainly on  
236 mycorrhizal fungi for resource acquisition, or the mycotrophy) (Baylis, 1975; Eissenstat et al.,  
237 2015; Liu et al., 2015; St John, 1980). In this dataset, relationships between root tissue density  
238 and root N concentration and thickness of root EC were examined for both the thin and thick  
239 absorptive roots.

240 To test interspecific differences of root chemical fractions among root orders, two-way  
241 ANOVAs were used with plant species and root order as fixed factors. Tukey's HSD test was  
242 conducted to evaluate differences in chemical fractions among root branch orders within  
243 species (Long et al., 2013). All statistical analyses were carried out in SPSS (version 13.0;  
244 SPSS Inc. Chicago, USA) with significant level at  $p < 0.05$ .

245

### 246 **3 Results**

#### 247 **3.1 Root trait relationships for thin and thick absorptive roots**

248 Root tissue density was negatively correlated with root N concentration for total and thin but  
249 not for thick absorptive roots (Fig. 1). Similarly, using a larger species pool, negative  
250 relationships between root tissue density and root N concentration were found for total and  
251 thin but not for thick absorptive roots (Fig. S3).

252 For thin absorptive roots, the extractive C fraction peaked at medium root tissue density  
253 (Fig. 2a). Moving average analysis revealed a quadratic relationship between the extractive C  
254 fraction and root tissue density in thin absorptive roots (Fig. S4a), while no relationships were  
255 found between acid-soluble and acid-insoluble fractions and root tissue density. The  
256 recalcitrant C fraction (acid-soluble C + acid insoluble C) in thin absorptive roots showed a  
257 quadratic relationship with root tissue density (Fig. S4b). It was also noted that in the thin  
258 absorptive roots, the acid-soluble and acid-insoluble fractions were relatively higher in the  
259 higher and lower range of root tissue density, respectively (Fig. 2b,c). For thick absorptive  
260 roots, none of the three C fractions were correlated with root tissue density (Fig. 2, Fig. S4).

261 Across total absorptive roots, thickness of root EC was positively correlated with total root  
262 N concentration (Fig. 3a) and negatively with root N in the acid-insoluble fraction (Fig. 3b).  
263 Thickness of root EC was also positively correlated with the extractive C fraction (Fig. 3c)  
264 and negatively with the acid-insoluble fraction (Fig. 3e). However, in each of thin and thick  
265 absorptive roots, no relationships were found between thickness of root EC and either of these  
266 chemical fractions (all  $p$  values  $>0.05$ , Fig. 3a-e).

267 Thickness of root EC decreased linearly with root tissue density (Fig. 4), but no  
268 relationships were found when separated between thin and thick absorptive roots. Using a  
269 large species pool we found a very similar pattern: a significant relationship between  
270 thickness of root EC and root tissue density for total absorptive roots, a weaker relationship  
271 for thin absorptive roots and no relationship for thick absorptive roots (Fig. S5). In addition,  
272 we found exponential relationships between SRL and thickness of root EC for the species in  
273 our current study as well as for the larger species pool from a previous study (Fig. S6).

274

### 275 **3.2 Effects of plant species and root order on root C and N fractions**

276 All chemical fractions except the extractive fraction showed significant differences among  
277 species and root orders ( $p$  values  $<0.05$ , Table 1), and there were significant interactions for all  
278 chemical fractions (all  $p$  values  $<0.05$ ) indicating plant species-specific effects of root order on  
279 plant chemical traits.

280 The extractive C fraction tended to increase with increasing root order for species with thin  
281 absorptive roots such as *D. dichotoma* and *A. auriculiformis*, but decreased for species with  
282 thick absorptive roots, except for *C. lanceolata* (Fig. 5a). For both acid-soluble and

283 acid-insoluble fractions, patterns were largely idiosyncratic, including both increases and  
284 decreases with increasing root branch order (Fig. 5b,c). For all species, root N concentration  
285 decreased with increasing root branch order (Fig. 6a), whereas N in the acid-insoluble fraction  
286 increased with increasing root branch order, except for *C. chinensis* (Fig. 6b).

287

#### 288 **4 Discussion**

289 The negative relationship between root tissue density and root N concentration supports the  
290 acquisition-conservation tradeoff, and hence, the existence of economic strategies in  
291 absorptive roots because absorptive roots with higher tissue density usually have longer  
292 lifespan (Eissenstat and Yanai, 1997; Ryser, 1996; Withington et al., 2006) while their lower  
293 N concentration indicates slow resource acquisition (Kong et al., 2010; Mommer and  
294 Weemstra, 2012; Reich et al., 2008). However, our results further showed that the negative  
295 relationship between root tissue density and root N concentration held for thin but not for  
296 thick absorptive roots (Fig. 1). Although these results were based on a relatively small number  
297 of species, reanalysis of data from a previous study including 96 species (Kong et al., 2014)  
298 revealed very similar patterns (Fig. S1). As such, trait relationship between root N  
299 concentration and root tissue density supports our first hypothesis of different economic  
300 strategies for thin and thick absorptive roots.

301 The trait relationships between root tissue density and root C fractions provide further  
302 support for our hypothesis. Theoretically, absorptive roots with lower tissue density would  
303 have higher activity, while higher root activity also consumes more labile C thus leaving less  
304 labile and more recalcitrant C fractions in these roots. In contrast, in absorptive roots with

305 higher tissue density, more C is used for structural tissues demanding recalcitrant C fractions  
306 (Fan and Guo, 2010). Therefore, we would expect an inverted U-shaped relationship for labile  
307 C fractions and a U-shaped relationship for recalcitrant C fractions when these C fractions  
308 would be correlated with root tissue density. As expected, for thin absorptive roots we found  
309 an inverted U-shaped relationship between the labile C fraction and root tissue density (Fig.  
310 S4a) and a U-shaped relationship between recalcitrant C fractions (acid-soluble C + acid  
311 insoluble C) and root tissue density (Fig. S4b). The higher acid-soluble C fraction with  
312 increasing root tissue density (Fig. 2b) suggest that thin absorptive roots with higher tissue  
313 density are constructed with more acid-soluble C compounds, such as cellulose, rather than  
314 acid-insoluble C compounds, such as lignin, possibly because of higher energy demands for  
315 the production of lignin than for the production of cellulose (Novaes et al., 2010).. However,  
316 different from thin absorptive roots, there were no relationships between root C fractions and  
317 root tissue density for thick absorptive roots (Fig. 2, Fig. S4). Therefore, trait relationships  
318 between root C fractions and root tissue density provide further evidence for an  
319 acquisition-conservation tradeoff for thin absorptive roots, but not for thick absorptive roots.

320 Furthermore, observed relationships between thickness of root EC and root C and N  
321 fractions provide the third piece of support for our hypothesis of contrasting economic  
322 strategies with root diameter. Across total absorptive roots, thickness of root EC was  
323 positively correlated with root N concentration and the extractive C fraction while negatively  
324 correlated with the acid-soluble C fraction and N in the acid-soluble C fraction. This suggests  
325 that compared with thin absorptive roots, thick absorptive roots acquire resources at higher  
326 rates as indicated by their higher N concentration and lower C and N in recalcitrant fractions.

327 Meanwhile, thick absorptive roots may also have longer lifespan because of their larger root  
328 diameter (Adams et al., 2013; Anderson et al., 2003; McCormack et al., 2012; Wells and  
329 Eissenstat, 2001). These findings seem to contrast with an acquisition-conservation tradeoff.  
330 Further, we showed that relationships between thickness of root EC and root chemical  
331 fractions only held across the full spectrum from thin to thick absorptive roots. Nevertheless,  
332 it was also noted that root tissue density showed a greater range of variation for thin than for  
333 thick absorptive roots. For thin absorptive roots, variation in root tissue density might arise  
334 from secondary thickening of root EC cell walls (Eissenstat and Achor, 1999; Long et al.,  
335 2013; Ryser, 2006; Wahl and Ryser, 2000). This could be associated with lower root activity  
336 and hence lower root N concentration (Fig. 1, Fig. S3). As such, an acquisition-conservation  
337 tradeoff in thin absorptive roots would be expected. However, for thick absorptive roots, the  
338 cell size, as well as the cortical cell file number (Chimungu et al., 2014a, b), may be more  
339 important than cell wall thickening in determining root activity. If so, root activity may be  
340 less affected by thickening of root EC cell walls than by changing the size or number of these  
341 cells. As such, there would be no obvious economic strategies for thick absorptive roots.

342 Recent studies have revealed different nutrient foraging strategies for thin and thick  
343 absorptive roots with the former depending on roots themselves and the latter depending more  
344 on mycorrhizal fungi (Baylis, 1975; Eissenstat et al., 2015; Liu et al., 2015). These  
345 observations are supported by the SRL-thickness relationship we found in our study where  
346 thin roots had larger SRL than thick roots (Fig. S6). Here, our results further indicate that thin  
347 and thick absorptive roots may follow different economic strategies when foraging for  
348 nutrients. These findings may have important implications for the emerging debate on the root



349 economics spectrum. For example, the existence of an economic strategy for plant roots has  
350 been commonly accepted (Craine et al., 2005; Espeleta et al., 2009; Freschet et al., 2010;  
351 Reich, 2014). However, some recent studies have challenged the ubiquity of root economics  
352 spectra by showing no (Chen et al., 2013) or positive (Kong et al., 2014) relationships  
353 between root diameter and root N concentration. One possible explanation for these  
354 contrasting findings is the inclusion of many species with thick absorptive roots. Including  
355 these species may potentially obscure trait relationships indicating acquisition-conservation  
356 tradeoffs. On the other hand, the lack of evidence of an acquisition-conservation tradeoff may  
357 have resulted from the larger proportion of root cross-section area accounted for by root EC  
358 compared to the stele (Table S2; Kong et al., 2014). Notably, for species like monocots, the  
359 area of root stele is much larger than the area of root EC. We did not include monocots in our  
360 study, but it would be interesting to test whether the contrasting economic strategies for thin  
361 and thick absorptive roots, as presented here, can be applied across mono-dicots. Furthermore,  
362 our findings of different economic strategies for thin and thick absorptive roots are important  
363 for understanding plant impacts on soil processes. Acquisitive species are usually associated  
364 with bacterial-dominated soil microbial communities, faster carbon and nutrient cycling, and  
365 stronger plant-soil feedbacks, while conservative species are usually associated with  
366 fungal-dominated soil microbial communities, slower carbon and nutrient cycling, and weaker  
367 plant-soil feedbacks (Bardgett et al., 2014; Kardol et al., 2015; Wardle et al., 2004). This  
368 suggests that the impacts of absorptive roots on soil processes would depend on root diameter.

369 Besides the prominent role in influencing root strategy, root thickness may also affect  
370 patterns of root chemical traits among root branch orders. The extractive C fraction increased

371 with increasing root order for species with thin absorptive roots, whereas it declined for  
372 species with thick absorptive roots. Although both the acid-soluble and acid-insoluble  
373 fractions showed no consistent trends across root branch orders, the total recalcitrant fraction  
374 (sum of acid-soluble and acid-insoluble fractions) showed a pattern opposite to that of the  
375 extractive fraction. On the other hand, root N concentration and N in recalcitrant C fractions  
376 showed relative consistent patterns across root orders. As such, our findings provided only  
377 partial support of our second hypothesis. These patterns of root chemical fractions, however,  
378 are important in understanding soil ecosystem processes. For example, it is increasingly  
379 recognized that lower-order roots, compared with higher-order woody roots, are faster in root  
380 turnover but slower in root decomposition which makes the former a disproportionately greater  
381 source of soil organic matter (Clemmensen et al., 2013; Fan and Guo, 2010; Goebel et al.,  
382 2011; Xiong et al., 2013). This has been ascribed to higher recalcitrant C fractions in  
383 lower-order compared with higher-order woody roots (Goebel et al., 2011). However, our  
384 results may challenge the generality of slower decomposition of lower-order relative to  
385 higher-order roots as some lower-order roots had less recalcitrant C fractions and hence faster  
386 decomposition than higher-order roots.

387 In conclusion, the results of our study suggest an acquisition-conservation tradeoff for thin  
388 absorptive roots but not for thick absorptive roots. In addition, we found different patterns of  
389 root chemical fractions with root diameter and root order. The contrasting economic strategies  
390 between thin and thick absorptive roots are important in advancing our understanding of root  
391 ecology and the links with aboveground plant counterparts. Yet, our knowledge on the  
392 functioning of plant roots and their roles in driving soil ecosystem processes is still limited.

393 We hope that our study presents an instructive perspective on the root economics spectrum  
394 that will stimulate further research in this field. Future studies may test to what extent our  
395 results hold for other (groups of) plant species (e.g., monocots, ferns, or conifers), including a  
396 larger spectrum of functional traits (including those associated with interactions with  
397 rhizosphere biota), and unravel the mechanisms underlying the ‘non-economics strategy’ for  
398 thick absorptive roots. Further, we anticipate that the mycotrophy (i.e., plant species  
399 association with mycorrhizal fungi for resource acquisition) may underlie economic strategies  
400 in thick absorptive roots; however, empirical studies are needed to confirm this.

401

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### 551 **Acknowledgements**

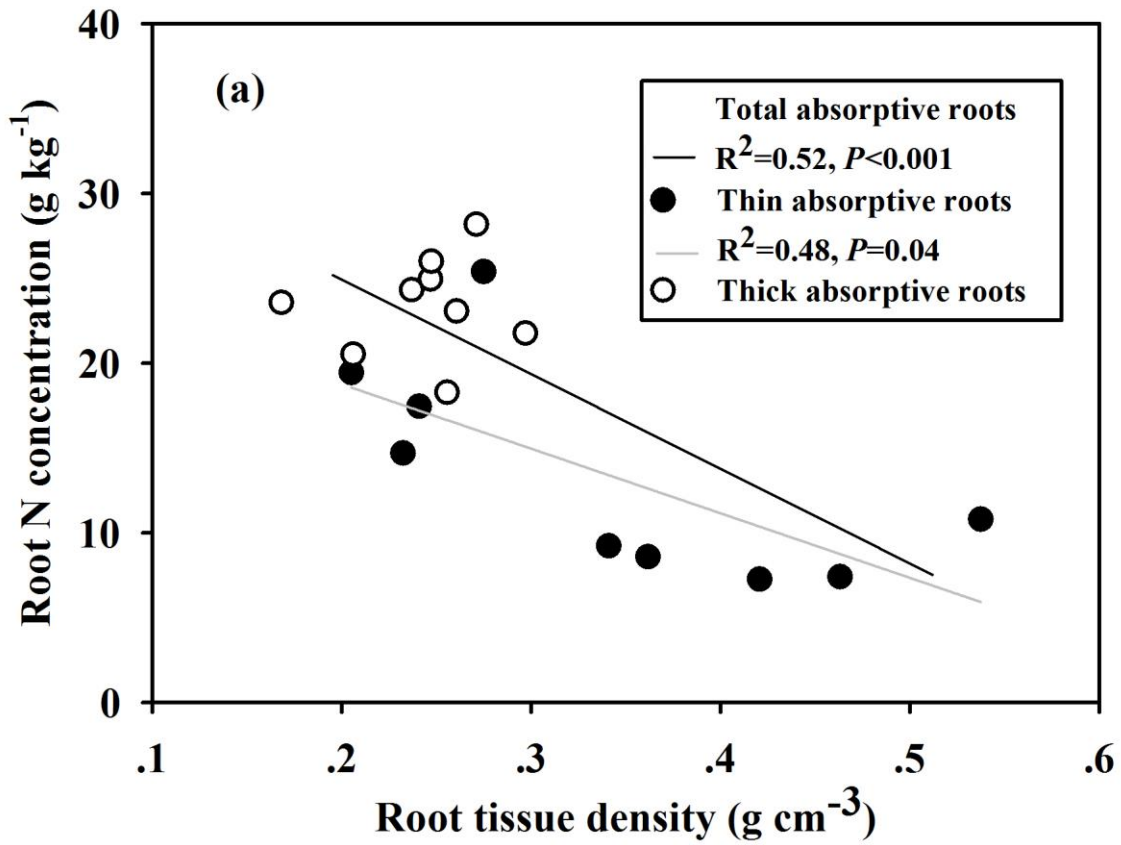
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559 **Table 1.** *F* values of two-way ANOVAs testing effects of plants species and root branch  
560 order on the extractive C fraction, acid-soluble C fraction, acid-insoluble C fraction, N  
561 concentration, and N in acid-insoluble C fraction. \*, \*\*, \*\*\* are significant level at 0.05, 0.01,  
562 0.001, respectively.

	Extractive C fraction	Acid-soluble C fraction	Acid-insoluble C fraction	N concentration	N in acid-insoluble C fraction
Species	132.97***	51.57***	188.51***	1578.85***	142.40***
Root order	1.63	11.76***	17.78***	521.22***	19.61***
Species × Root order	4.46***	2.59**	3.53***	29.33***	3.83***

563 **Figures**

564 Fig. 1 Relationships between root tissue density and root N concentration for total (black line),  
565 thin (solid circles, grey line) and thick (open circles) absorptive roots.

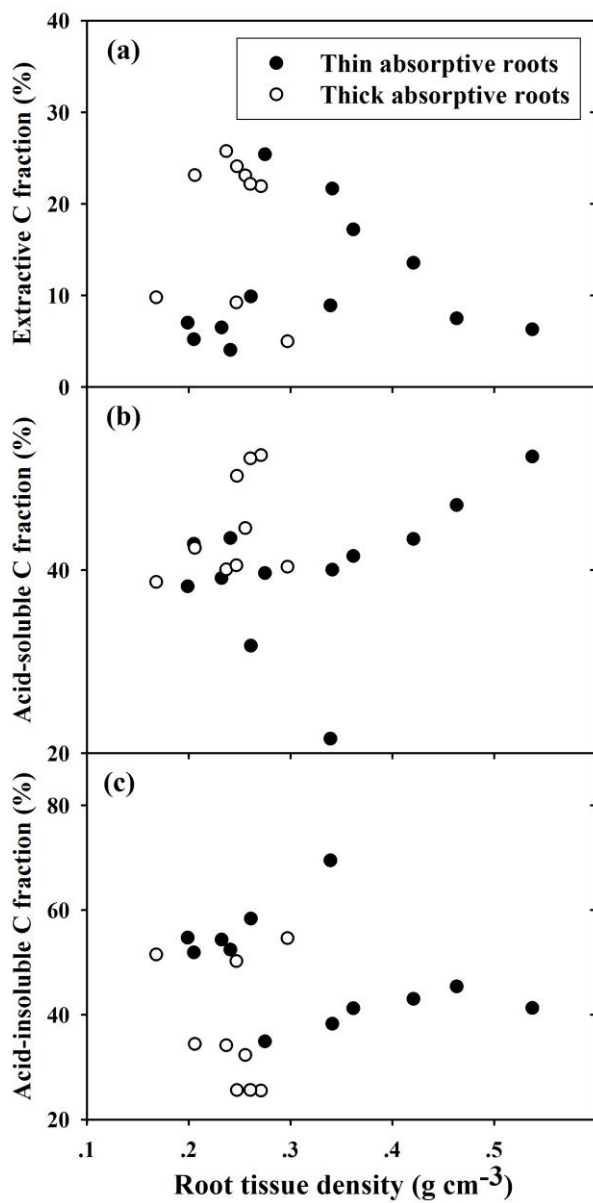


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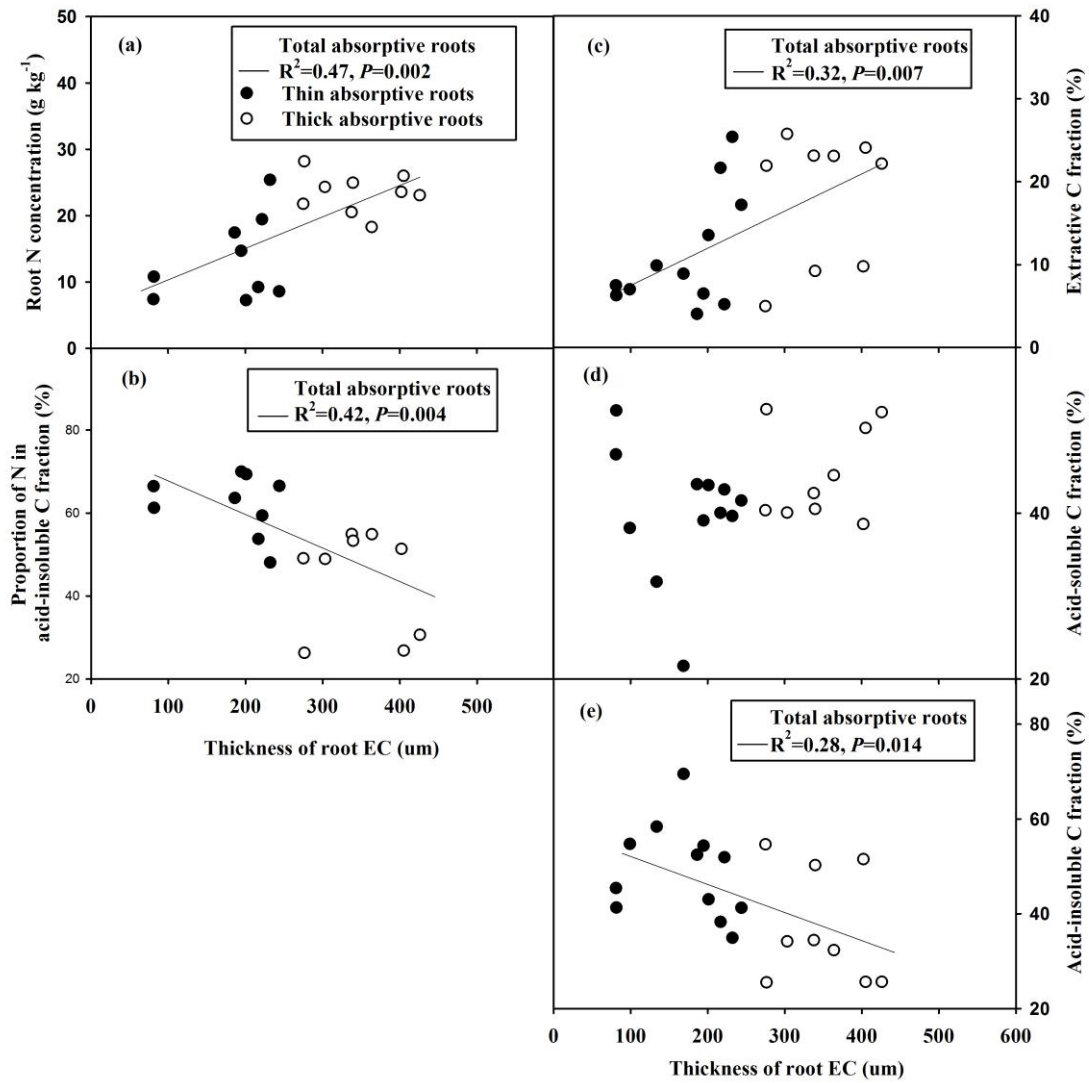
569 Fig. 2 Relationships between root tissue density and extractive C fraction (a), acid-soluble C  
570 fraction (b) and acid-insoluble C fraction (c), for thin (solid circles) and thick (open circles)  
571 absorptive roots.



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573

574 Fig. 3 Relationships between thickness of root EC and root N concentration (a), N in  
 575 acid-insoluble C fraction (b), extractive C fraction (c), acid-soluble C fraction (d) and  
 576 acid-insoluble C fraction (e) for total (black line), thin (solid circles) and thick (open circles)  
 577 absorptive roots.

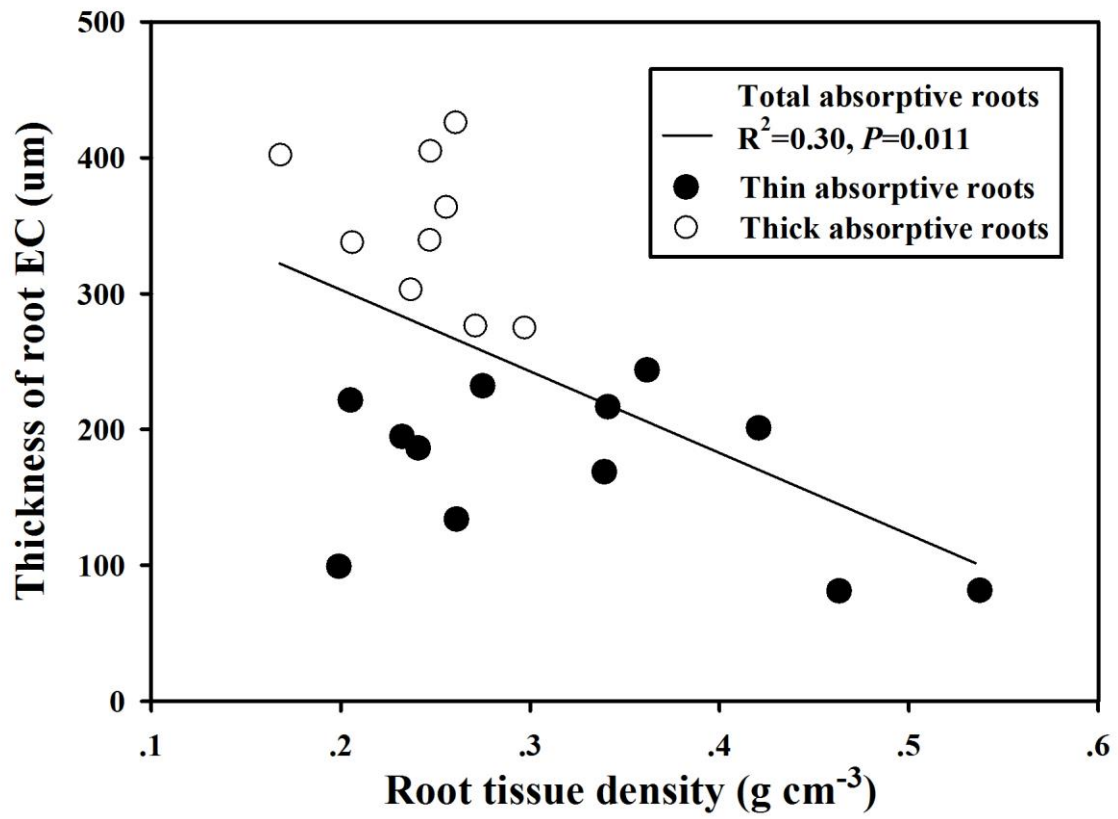


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581 Fig. 4 Relationships between root tissue density and thickness of root EC for total, thin (solid  
582 circles, black line) and thick (open circles) absorptive roots.

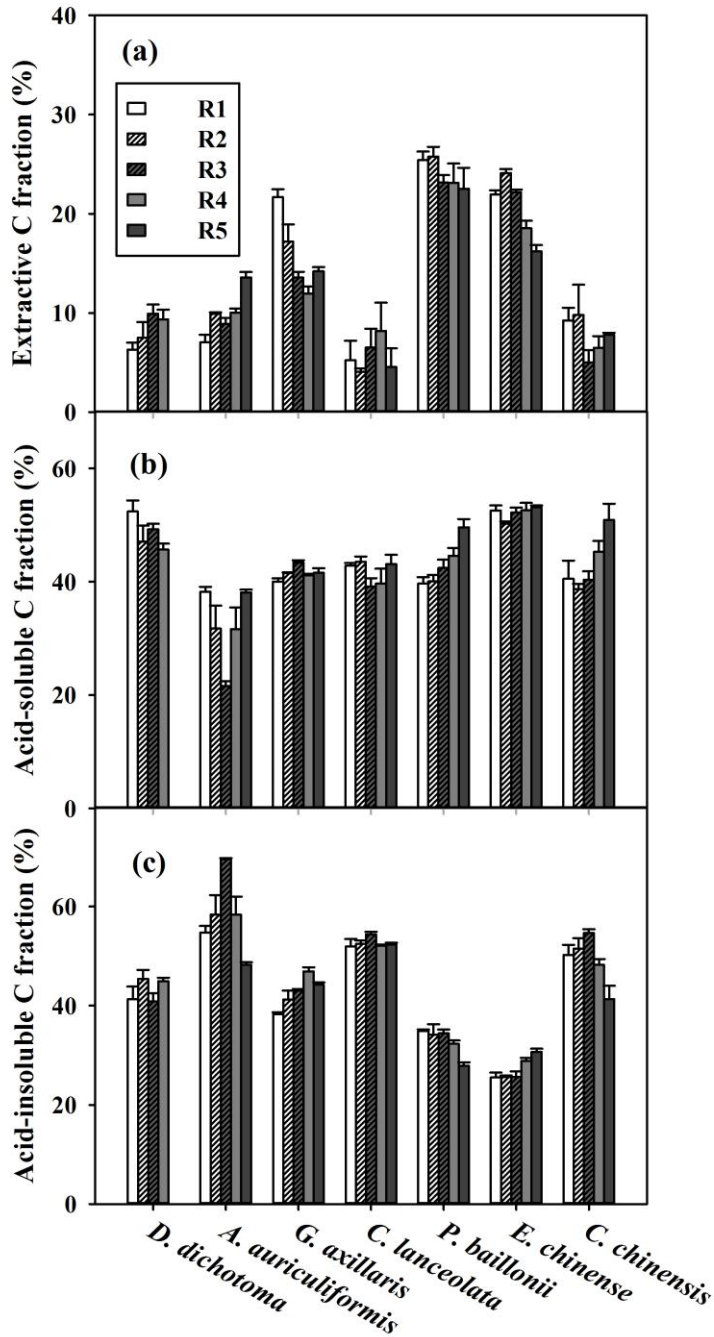


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586 Fig. 5 The extractive C fraction (a), acid-soluble C fraction (b) and acid-insoluble C fraction  
 587 (c) for the first five root orders for each of seven plant species. R1-R5 refer to the first to fifth  
 588 order.

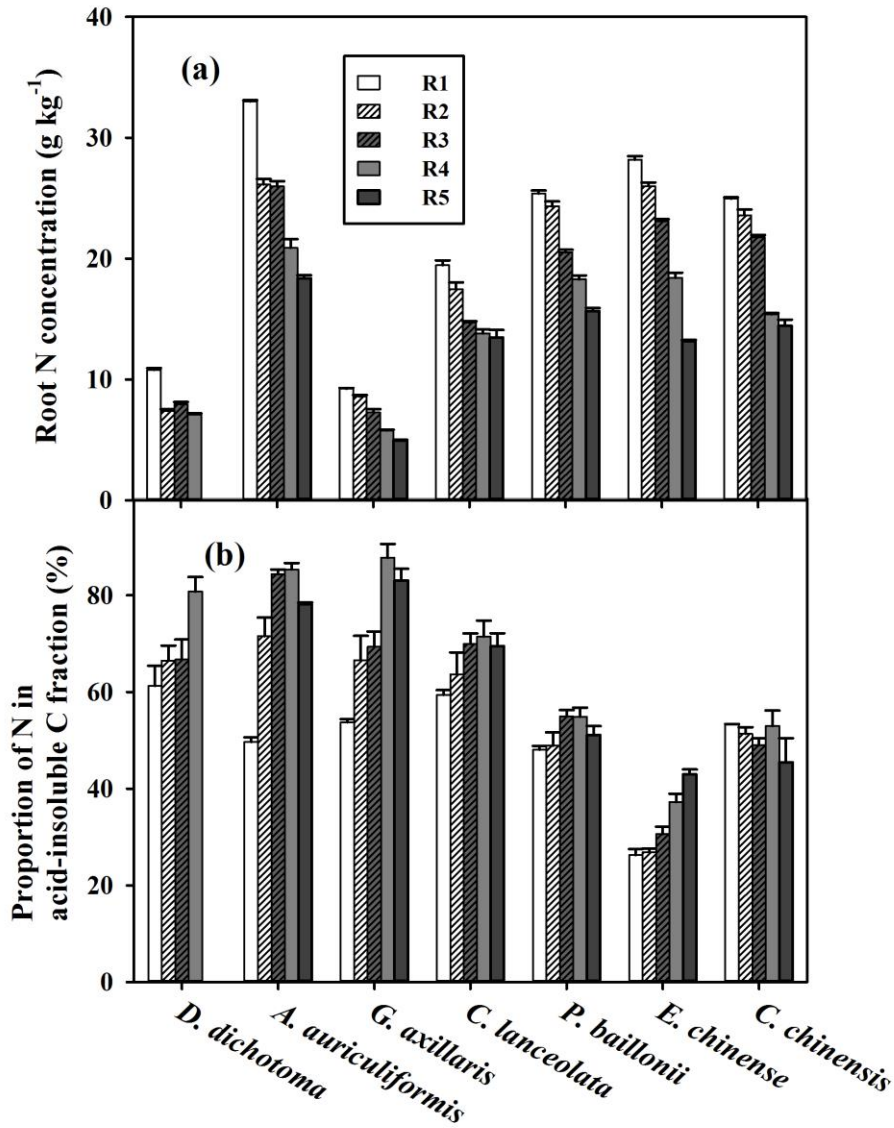


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591 Fig. 6 Root N concentration (a) and N in acid-insoluble C fraction (b) for the first five root  
 592 branch orders for each of seven plant species. R1-R5 refer to the first to fifth order.



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