1 **The root economics spectrum: divergence of absorptive root strategies with root**

2 **diameter**

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Abstract

Plant roots usually vary along a dominant ecological axis, the root economics spectrum (RES),

depicting a tradeoff between resource acquisition and conservation. For absorptive roots,

which are mainly responsible for resource acquisition, we hypothesized that root strategies as

predicted from the RES shift with increasing root diameter. To test this hypothesis, we used

seven contrasting plant species for which we separated absorptive roots into two categories:

thin roots ~~(< 247 μm diameter)~~ and thick roots grouped by a cutoff thickness (247 μm) of root

EC (the epidermis and cortex). For each category, we analyzed a range of root traits closely

related to resource acquisition and conservation, including root tissue density, carbon (C) and

nitrogen (N) fractions as well as root anatomical traits. The results showed that trait

relationships for thin absorptive roots followed the expectations from the RES while no clear

trait relationships were found in support of the RES for thick absorptive roots. Our results

suggest divergence of absorptive root strategies in relation to root diameter, which runs

against a single economics spectrum for absorptive roots.

1 Introduction

Plant traits reflecting a tradeoff between resource acquisition and conservation represents an

essential ecological axis for plant strategies that is important for our understanding of how

plants drive ecosystem processes and ecosystem responses to environmental change

44 (Cornwell et al., 2008; Freschet et al., 2010; Reich, 2014; Westoby et al., 2002). On the one

45 end of this ecological axis, there are species with an acquisitive strategy, i.e., fast acquisition

46 of resources (CO_2 for leaves and nutrients for roots) accompanied with a short lifespan. On

47 the other end of the axis, there are species with a conservative strategy, i.e., slow acquisition

48 accompanied with a long lifespan. Originally, such an ecological axis has been demonstrated

49 for leaves, which is widely known as the leaf economics spectrum (Diaz et al., 2004; Osnas et

50 al., 2013; Wright et al., 2004). More recently, similar trait spectra have been demonstrated

51 across plant organs from leaves to stems and roots, thus forming a whole 'plant economics

52 spectrum' (Freschet et al., 2010; Laughlin et al., 2010; Prieto et al., 2015; Reich, 2014).

53   Resource acquisition in plant roots is performed by absorptive roots, i.e., the first two or

54 three orders of a root branch with primarily-developed tissues which are only a part of the

55 commonly used "fine roots" ($<$ 2mm in diameter) (Guo et al., 2008; Long et al., 2013;

56 Pregitzer et al., 2002). For absorptive roots, the tissue density, i.e., root dry mass per unit root

57 volume, is a key trait of the root economics spectrum (RES) as tissue density is closely linked

58 to the acquisition-conservation tradeoff (Birouste et al., 2014; Craine et al., 2005; Espeleta et

59 al., 2009; Mommer and Weemstra, 2012). In general, absorptive roots with higher tissue

60 density are slower in nutrient acquisition and longer in lifespan whereas absorptive roots with

61 lower tissue density may enable faster acquisition but maintain a shorter lifespan (Ryser, 1996;

62 Wahl and Ryser, 2000; Withington et al., 2006). Recently, tissue density for absorptive roots

63 was found to negatively correlate with root diameter. This could be because root cortex is less

64 dense than root stele and because in thicker roots (except for the monocots with the stele

65 dominating root cross area) a larger proportion of the root cross-sectional area is accounted

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66   for by the cortex (Chen et al., 2013; Kong et al., 2014; Kong and Ma, 2014). On the other

67   hand, compared with thinner absorptive roots, thicker absorptive roots may acquire resources

68   faster because of their greater dependence on mycorrhizal fungi (Kong et al., 2014; Kong and

69   Ma, 2014; St John, 1980), and may also have a longer lifespan due to the larger

70   diameter(Adams et al., 2013; Eissenstat and Yanai, 1997; Wells and Eissenstat, 2001). As

71   such, the trait syndrome for thicker absorptive roots would differ from the predictions of

72   faster acquisition and shorter lifespan. This highlights the importance of discriminating the

73   thicker from the thinner absorptive roots when exploring root strategies. However, we are

74   unaware of any previous studies that have tested for effects root diameter in driving ~~root~~ trait

75   spectra in absorptive roots.

76   In addition to structural traits such as density, the chemical composition of absorptive roots

77   may constitute another important aspect of testing root strategies in relation root diameter

78   (Hidaka and Kitayama, 2011; Meier and Bowman, 2008; Poorter and Bergkotte, 1992;

79   Poorter et al., 2009). For example, carbon (C) and nitrogen (N), the two most abundant

80   elements in plants, are usually bound to organic compounds which may contain labile (e.g.,

81   soluble sugars and proteins in living cells) and recalcitrant fractions (e.g., cellulose and lignin

82   in structural tissues) (Atkinson et al., 2012; Berg and McClaugherty, 2008; Feng et al., 2009;

83   Poorter et al., 2009; Shipley et al., 2006). Generally, absorptive roots with less labile C and

84   more labile N indicate an acquisitive strategy. This is because high root activity may be

85   accompanied by an increased production of metabolism-related proteins with a high labile N

86   content; such roots may be palatable for herbivores and have a relative short lifespan. On the

87   other hand, conservative roots have less labile C and N fractions as more of these chemicals

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88   are used for construction of structural tissues resulting in lower root activity and a longer

89   lifespan. However, compared with thinner absorptive roots, thicker absorptive roots may have

90   higher labile C and N fractions as these labile fractions can be stored in their thick root cortex

91   (Chapin III, 1980; Long et al., 2013; Lux et al., 2004; Withington et al., 2006). As such, the

92   chemical traits of thicker absorptive roots integrate 'opposing' effects of root metabolism and

93   storage suggesting them having neither a true acquisitive nor a true conservative strategy.

94   Therefore, to evaluate the impact of thickness on root strategies it is necessary to examine C

95   and N fractions in relation to root diameter.

96   Here, we selected a variety of plant species common to tropical and subtropical forests in

97   south China with contrasting phylogeny and root structure. The aim of our study was two-fold.

98   First, we examined the influence of root diameter on the root economics spectrum (RES). We

99   hypothesized that root strategies diverge between thinner and thicker absorptive roots, with a

100 trade-off between acquisitive and conservative traits for thinner roots but not for ticker roots.

101 The hypothesis was tested using a series of trait relationships involving both structural and

102 chemical traits. Second, root C and N fraction, have been suggested to vary in predictive ways

103 across branch orders (Fan and Guo, 2010; Goebel et al., 2011). However, we hypothesized

104 that patterns of root C and N fractions across branch orders differ in species varying in

105 absorptive root diameter.

106

107 **2 Materials and methods**

108 **2.1 Plant species and sampling sites**

109 We selected seven plant species with contrasting phylogeny and root structure (Table S1) in

110   tropical and subtropical forests in south China. Three species were sampled at the Heshan

111   Hilly Land Interdisciplinary Experimental Station (22°41′N, 112°54′E), Guangdong province.

112   They were: *Dicranopteris dichotoma* (Gleicheniaceae) (a fern), *Cunninghamia lanceolata*

113   (Taxodiaceae) (a conifer) and *Acacia auriculiformis* (Leguminosae) (a tree). Another tree

114   species, *Paramichelia baillonii* (Magnoliaceae), was sampled in Wutongshan National Forest

115   Park (22°27′-22°52′N, 113°37′-114°37′E) in Shenzhen, Guangdong province. Three other tree

116   species, *Gordonia axillaris* (Theaceae), *Endospermum chinense* (Euphorbiaceae) and

117   *Cryptocarya chinensis* (Lauraceae), were sampled in Jianfengling Nature Reserve

118   (18°23′-18°50′N, 108°36′-109°05′E), Hainan province. More information on sites and species

119   can be found in Long's study (Long et al., 2013) and Table S1.

120

121   **2.2 Root sampling**

122   Roots were collected at a soil depth of 0-10 cm in June and July 2011. For each species, at

123   least three mature trees were selected. We first tracked the main lateral roots by carefully

124   removing surface soil at the base of each plant with a specially manufactured fork. Root

125   branch order was defined according to Pregitzer's study (Pregitzer et al., 2002) with the most

126   terminal branch as the first-order. The intact roots were collected and soil adhering to the

127   roots was carefully removed. We distinguished all four root orders for *D. dichotoma* and the

128   first five orders for the other species. A portion of each root sample was immediately put into

129   Formalin-Aceto-Alcohol (FAA) solution (90 ml 100% ethanol, 10 ml 100% glacial acetic

130   acid) for later anatomical assessment. The remaining unwashed part of each root sample was

131   placed in a plastic bag and transported in a cooler to the laboratory where the. These root

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132 sampless were frozen until measurements of root morphology and chemistry (Pregitzer et al.,

133 2002).

134

2.3 Root tissue density

136 For each species, 50 root segments for the first order, 30 segments for the second order, and

137 20 segments for the third to the fifth order were randomly picked for measuring root diameter

138 and length. The root diameter was measured under a $40\times$ or $20\times$ stereomicroscope (MZ41-2B,

139 MshOt, Guangzhou, China) depending on root size. The length of comparatively short roots

140 was assessed using a stereomicroscope while a measuring tape with the minimum scale of 0.5

141 mm was used for relatively long roots. After root diameter and length were recorded, roots

142 were oven-dried at 65 ℃ for 48 h and weighed. Root tissue density was calculated by dividing

143 root dry mass by root volume assuming roots are cylindrically shape (Kong et al., 2014).

144

2.4 Root anatomy

146 Root segments from the FAA solution were cleaned with deionized water (4 ℃) and then

147 transferred to glass Petri dishes for dissection into different branch orders. Root anatomy was

148 determined following the procedure of Long's study (Long et al., 2013). Briefly, a minimum

149 of 10 root segments were randomly chosen for each root order. All root segments were

150 dehydrated in an ethanol solution series to absolute ethanol, purified in 100% xylene and

151 embedded in paraffin. Root cross-sections were cut into slices of 8μm thick using a

152 microtome (Rotary Microtome KD-2258, Zhejiang, China). After deparaffinage, these root

153 slices were stained first by safranine and then by fast green. Following this staining procedure,

154   the cortex and epidermis was in blue and the stele was in red. The root slices were then

155   photographed by a light microscope (Carl Zeiss Axioscop 20, Jena, Germany). The size of

156   anatomic structures including epidermis, cortex and stele was measured using Image J

157   software (NIH Image, Bethesda, MD, USA). ~~The determination of a~~Absorptive roots in a root

158   branch w~~ereas~~ usually based on root anatomical structures (Guo et al., 2008). Here, root

159   orders were classified as absorptive roots when they had no or little secondary xylem ~~Long's~~

160   ~~study~~ (Long et al., 2013).

161

162   **2.5 Chemical analyses**

163   Before chemical analyses, root samples were put in deionized water to carefully remove soil

164   particles or dead organic matter that adhered to but was not a part of the root (Pregitzer et al.,

165   2002). The root samples were then prepared for chemical measurements after oven-dried

166   (65 ℃ for 24 h) and ground. Root C and N concentrations were determined using an element

167   analyzer (VarioEl, Elementar Analysen-systeme GmbH, Germany). Root C fractions

168   (extractive; acid-soluble fraction; acid-insoluble fraction) were determined by a sulfuric acid

169   digestion method. First, we separated the extractive and labile C fraction from other C

170   fractions. A milled powder sample of c. 100 mg (m_0) was extracted with 15 ml of cetyl

171   trimethylammonium bromide (CTAB) solution for 3 h, filtered, repeatedly washed with

172   de-ionized water until pH was 7.0, and then oven-dried at 60 ℃ to a constant weight, m_1.

173   Second, the filtered residue was digested with 30 ml of sulfuric acid (72 %) at 22 ℃ for 3 h,

174   filtered, repeatedly washed (until pH was 7.0), dried and weighed, m_2. After the

175   acid-digestion step, the ash content, m_3, was determined by combusting 15-30 mg of sample

176 at 550 ℃ for 4 h. Finally, the extractive, acid-soluble fraction, and acid-insoluble fraction

177 were calculated as 100% $\times(m_0-m_1)/(m_0-m_3)$, 100% $\times(m_1-m_2)/(m_0-m_3)$, 100% $\times(m_2-m_3)/$

178 (m_0-m_3), respectively.

179 After acid-digestion, a 5mg subsample of residue after acid-digestion was taken to measure

180 N concentration and N allocation in the acid-insoluble C fraction. The N in the extractive

181 fraction was too low to measure. Thus, estimates of N in the acid-soluble fraction were

182 calculated as the difference between total N and N in the acid-insoluble fraction.

183

184 **2.6 Statistical analyses**

185 Relationships between root tissue density and root N concentration and each of the three C

186 fractions were assessed by linear regressions. Here, we introduced a new term for root tissue,

187 'root EC' which referred to tissues outside the stele including the epidermis plus cortex. Root

188 EC was used for two reasons. First, the thickness of root EC can be a proxy of the size of root

189 diameter (R^2=0.91 and R^2=0.99 for linear regressions in this study and in Kong's study (Kong

190 et al., 2014), respectively). Second, root EC can be an indicator of root chemical compounds

191 as the storage of labile C and most of root N are found in root EC (Chen et al., 2013). The

192 relationships between the thickness of root EC and root tissue density and root chemicals

193 were also investigated with linear regressions.

194 To explore the effect of root diameter on root ecological strategies, the above analyses were

195 repeated for thin and thick absorptive roots, respectively. We used a mean thickness of 247

196 μm for root EC as the cut-off point between thin and thick absorptive roots. The mean

197 thickness of root EC was used because ~~the~~ thickness of root EC for absorptive roots did

198 follow~~ed~~ a normal distribution (Fig. S1a). To avoid the influence of biological N fixation on

199 relationships between root N and root tissue density and root EC, a legume species, *A.*

200 *auriculiformis*, was excluded in these analyses. In addition, for the thin absorptive roots,

201 relationships between the extractive C fraction and root tissue density were further explored

202 by a quadratic polynomial regression using moving average ~~analysis~~data. The moving average

203 data were obtained by the following steps. First, the extractive C fraction was sorted along

204 with the ascending order of root tissue density. Then, the extractive C fraction and root tissue

205 density were averaged by bins (Reich and Oleksyn, 2004), respectively. Here, the bin referred

206 to each of the two neighboring data points. Moving average analyses were used as it improved

207 the goodness of fit. No polynomial regression relationships were found for the other two C

208 fractions.

209   We acknowledge that the seven species we used represent a relative small species pool. To

210 validate the results of our study, we further used the data of 96 woody species from one of our

211 previous studies where only the first-order roots were included (Kong et al., 2014). For the 96

212 species, they followed a skewed normal distribution with abundant species having thinner

213 root EC (Fig. S1b), and hence lower mycorrhizal colonization (Kong et al., 2014). Here, we

214 used a thickness of 182.8 μm for root EC as a cut-off between thin and thick absorptive roots

215 (Kong et al., 2014) which is thinner than in our current study. The thickness of 182.8 μm for

216 root EC corresponded to a transition of mycorrhizal colonization with increasing root

217 diameter (Kong et al., 2014). This may also indicate a divergence of strategy between thin and

218 thick absorptive roots (Baylis, 1975; St John, 1980). In this dataset, relationships between root

219 tissue density and root N concentration and thickness of root EC were examined for both the

220 thin and thick absorptive roots.

221   To test interspecific differences of root chemical fractions among root orders, two-way

222 ANOVAs were used with plant species and root order as fixed factors. Tukey's HSD test was

223 conducted to evaluate differences in chemical fractions among root branch orders within

224 species (Long et al., 2013). All statistical analyses were carried out in SPSS (version 13.0;

225 SPSS Inc. Chicago, USA) with significant level at p=0.05.

226

227 **3 Results**

228 **3.1 Root strategies for thin and thick absorptive roots**

229 Root tissue density was negatively correlated with root N concentration for total and thin but

230 not for thick absorptive roots (Fig. 1). Similarly, using a larger species pool, negative

231 relationships between root tissue density and root N concentration were found for total and

232 thin but not for thick absorptive roots (Fig. S2).

233   For the thin absorptive roots, the extractive C fraction peaked at medium root tissue density

234 (Fig. 2a). Moving average analysis showed a quadratic regression relationship of the

235 extractive C fraction with root tissue density in these thin absorptive roots (Fig. S3), while no

236 relationships were found between acid-soluble and acid-insoluble fractions and root tissue

237 density. It was also noted that in the thin absorptive roots, the acid-soluble and -insoluble

238 fractions were relative higher in the higher and lower range of root tissue density, respectively

239 (Fig. 2b,c). For thick absorptive roots, none of the three C fractions were correlated with root

240 tissue density.

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241   Across total absorptive roots, thickness of root EC was positively correlated with total root

242   N concentration (Fig. 3a) and negatively with root N in the acid-insoluble fraction (Fig. 3b).

243   Thickness of root EC was also positively correlated with the extractive C fraction (Fig. 3c)

244   and negatively with the acid-insoluble fraction (Fig. 3e). However, in each of thin and thick

245   absorptive roots, no relationships were found between thickness of root EC and each of these

246   chemical fractions (all p values>0.05, Fig. 3a-e).

247   Thickness of root EC decreased linearly with root tissue density (Fig. 4), but no

248   relationships were found when separated between thin and thick absorptive roots. Using a

249   large species pool we found a very similar pattern: a significant relationship between

250   thickness of root EC and root tissue density for total absorptive roots, a weaker relationship

251   for thin and no relationship for thick absorptive roots (Fig. S4).

252

253   **3.2 Effects of plant species and root order on root C and N fractions**

254   All chemical fractions except the extractive fraction showed significant differences among

255   species and root orders (p values<0.05, Table 1), and there were significant interactions for all

256   chemical fractions (all p values<0.05) indicating plant species-specific effects of root order on

257   plant chemical traits.

258   The extractive C fraction tended to increase with increasing root order for species with thin

259   absorptive roots such as *D. dichotoma* and *A. auriculiformis,* but decreased for species with

260   thick absorptive roots, except for *C. lanceolata* (Fig. 5a). For both acid-soluble and

261   acid-insoluble fractions, patterns were largely idiosyncratic, including both increases and

262   decreases with root branch orders (Fig. 5b,c). For all species, root N concentration in all

263 species decreased with increasing root order (Fig. 6a), whereas N in the acid-insoluble

264 fraction increased with increasing root order, except for *C. chinensis* (Fig. 6b).

265

266 **4 Discussion**

267 The acquisition-conservation tradeoff in plants has been suggested to be consistent across

268 plant organs (roots, leaves, and stems), as such constituting a key ecological axis, i.e., the

269 'plant economics spectrum' (Freschet et al., 2010; Prieto et al., 2015; Reich, 2014). The

270 negative relationship between root tissue density and root N concentration across total

271 absorptive roots that we found in our study provides support for a root economics spectrum

272 (RES). This is because absorptive roots with higher tissue density usually have longer lifespan

273 (Eissenstat and Yanai, 1997; Ryser, 1996; Withington et al., 2006), while their lower N

274 concentration would be associated with slow resource acquisition (Kong et al., 2010;

275 Mommer and Weemstra, 2012; Reich et al., 2008). However, our results also showed that the

276 negative relationship between root tissue density and root N concentration existed only in thin

277 absorptive roots, but not in thick roots (Fig. 1). Although these results were based on a

278 relative small number of species, reanalysis of data from a previous study using 96 species

279 (Kong et al., 2014) revealed very similar patterns (Fig. S1). This indicates that the

280 conventionally recognized RES may be confined to thin absorptive roots only, as such

281 supporting our first hypothesis.

282 The divergence of absorptive root strategies with root diameter was further supported by

283 the relationships between root tissue density and root C fractions. Theoretically, absorptive

284 roots with lower tissue density would have higher activity, while higher root activity also

285 consumes more labile C thus leaving less labile C and more recalcitrant C fractions in these

286 roots. In contrast, for absorptive roots with higher tissue density, more C is used for structural

287 tissues demanding recalcitrant C fractions (Fan and Guo, 2010). Therefore, we would expect

288 an inverted U-shaped relationship for labile C fractions and a U-shaped relationship for

289 recalcitrant C fractions when correlated with root tissue density. In fact, for thin absorptive

290 roots we found an inverted U-shaped relationship between the labile, extractive C fraction and

291 root tissue density (Fig. 2a, S2). As for recalcitrant C fractions in thin absorptive roots, the

292 acid-insoluble C fraction peaked at lower but not at higher root tissue density which seems to

293 contradict with the expected inverted U-shaped curve. However, the acid-soluble C fraction

294 peaked at higher root tissue density (Fig. 2b). As such, it could be that thin absorptive roots

295 with higher tissue density are constructed with more acid-soluble C compounds, such as

296 cellulose, rather than acid-insoluble C compounds, such as lignin; production of lignin would

297 require more energy than production of cellulose (Novaes et al., 2010). Therefore, with

298 increasing root tissue density, recalcitrant C fractions in thin absorptive roots may follow a

299 pattern opposite to that of labile C fractions. As such, the patterns of labile and recalcitrant C

300 fractions in thin absorptive roots are in support of RES theory. On the other hand, for thick

301 absorptive roots, their lower tissue density was accompanied with higher extractive and lower

302 acid-insoluble C fraction, possibly because of storage of labile C in their thick cortex (Long et

303 al., 2013; Lux et al., 2004). These patterns of root C fractions for thick absorptive roots run

304 against expectations from the RES. Therefore, our study shows that thick absorptive roots

305 may follow a strategy different from that for thin absorptive roots.

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306   Furthermore, observed relationships between thickness of root EC and root C and N

307   fractions provided indirect evidence for our contention of divergence of ecological strategy

308   with root diameter. Across total absorptive roots, thickness of root EC was positively

309   correlated with root N concentration and the extractive C fraction while being negatively

310   correlated with the acid-soluble C fraction and N in the acid-soluble C fraction. This suggest

311   that compared with thin absorptive roots, thick absorptive roots acquire resources at higher

312   rates as indicated by their higher N concentration and lower C and N in recalcitrant fractions.

313   Meanwhile, thick absorptive roots may also have longer lifespan because of their larger root

314   diameter (Adams et al., 2013; Anderson et al., 2003; McCormack et al., 2012; Wells and

315   Eissenstat, 2001). These findings seem to contrast with an acquisition-conservation tradeoff.

316   Further, we showed that relationships between thickness of root EC and root chemical

317   fractions only hold across the full spectrum from thin to thick absorptive roots. Nevertheless,

318   it was also noted that root tissue density showed a greater range of variation for thin than for

319   thick absorptive roots. For thin absorptive roots, variation in root tissue density might arise

320   from secondary thickening of root EC cell walls (Eissenstat and Achor, 1999; Long et al.,

321   2013; Ryser, 2006; Wahl and Ryser, 2000). This could be associated with lower root activity

322   and hence lower root N concentration (Fig. 1, S1), which is consistent with the RES theory.

323   However, for thick absorptive roots, the cell size as well as the cortical cell file number

324   (Chimungu et al., 2014a, b) may be more important than cell wall thickening in determining

325   root activity. If so, root activity may be less affected by thickening of root EC cell walls than

326   by changing the size or number of these cells, and hence no acquisition-conservation trade-off

327   as predicted by the RES for thick absorptive roots. Therefore, relationships between thickness

328   of root EC and root chemical fractions provide further evidence for the idea of divergence of

329   root strategies between the thin and thick absorptive roots which may be underpinned by

330   different mechanisms.

331   Our finding of different ecological strategies in thin and thick absorptive roots has

332   important implications for the emerging debate on the plant economics spectrum. Although

333   the existence of an economics spectrum for roots (RES) has been recognized (Craine et al.,

334   2005; Espeleta et al., 2009; Freschet et al., 2010; Reich, 2014), some recent studies have

335   shown contrasting findings, suggesting no RES (Chen et al., 2013) or positive relationships

336   between root diameter and root N concentration (Kong et al., 2014). Although there may be

337   other mechanisms, one possible explanation for a lack of trade-offs between acquisitive and

338   conservative root traits in these studies is the inclusion of thick absorptive roots which could

339   have altered root trait relationships.

340   Besides the prominent role in influencing root strategy, root thickness may also affect

341   patterns of root chemical traits among root branch orders. The extractive C fraction increased

342   with increasing root order for species with thin absorptive roots, whereas it declined for

343   species with thick absorptive roots. Although both the acid-soluble and acid-insoluble

344   fractions showed no consistent trends across root orders, the total recalcitrant fraction (sum of

345   acid-soluble and acid-insoluble fractions) showed a pattern opposite to that of the extractive

346   fraction. On the other hand, root N concentration and N in recalcitrant C fractions showed

347   relative consistent patterns across root orders. Thus, we only found partial support of our

348   second hypothesis. These patterns of root chemical fractions are important in understanding

349   soil ecosystem processes. For example, it is increasingly recognized that lower-order roots,

350 compared with higher-order woody roots, are faster in root turnover but slower in root

351 decomposition which makes the former a disproportionally greater source for soil organic

352 matter (Clemmensen et al., 2013; Fan and Guo, 2010; Goebel et al., 2011; Xiong et al., 2013).

353 This has been ascribed to higher recalcitrant C fractions in lower-order compared with

354 higher-order woody roots (Goebel et al., 2011). However, our results may challenge the

355 generality of slower decomposition of lower-order relative to higher-order roots as some

356 lower-order roots had less recalcitrant C fractions and hence faster decomposition than

357 higher-order roots.

358 In conclusion, our study revealed for the first time divergence of absorptive root strategies

359 and patterns of root chemical fractions with root diameter and root order. Specifically, the

360 axis of the RES dominated in thin absorptive roots, while thick absorptive roots did not seem

361 to be constrained by an acquisition-conservation tradeoff. The different strategies for the two

362 groups of roots are important in advancing our understanding of root ecology and the links

363 with aboveground plant counterparts. Yet, our knowledge on the functioning of plant roots

364 and their roles in driving soil ecosystem processes is still limited. Future studies should test to

365 what extent our results hold for other (groups of) plant species (i.e. monocots), include more

366 functional traits (including those associated with interactions with rhizosphere biota). One

367 important feature of thick absorptive roots is their mycotrophy, the great dependence on

368 mycorrhizal fungi for nutrient foraging (Baylis, 1975; Eissenstat et al., 2015; Liu et al., 2015).

369 We speculate that the mycotrophy (i.e., species composition of mycorrhizal fungi, their ability

370 in nutrient acquisition and transfer to roots, etc.) may underlie economic strategy in thick

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371 absorptive roots, and needs to be emphasized in future studies, and unravel the mechanisms underlying the economic strategy for thick absorptive roots.

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374 **References**

375 Adams, T. S., McCormack, M. L., and Eissenstat, D. M.: Foraging strategies in trees of different root
376 morphology: the role of root lifespan, Tree Physiology, 33, 940-948, 2013.

377 Anderson, L. J., Comas, L. H., Lakso, A. N., and Eissenstat, D. M.: Multiple risk factors in root
378 survivorship: a four-year study in Concord grape, New Phytologist, 158, 489-501, 2003.

379 Atkinson, R. R. L., Burrell, M. M., Osborne, C. P., Rose, K. E., and Rees, M.: A non-targeted
380 metabolomics approach to quantifying differences in root storage between fast- and slow-growing
381 plants, New Phytologist, 196, 200-211, 2012.

382 Baylis, G.: Magnolioid mycorrhiza and mycotrophy in root systems derived from it. In:
383 Endomycorrhizas, Sanders, F. E., Mosse, B., and Tinker, P. B. (Eds.), Academic Press, 1975.

384 Berg, B. and McClaugherty, C.: Plant litter: decomposition, humus formation, carbon sequestration,
385 Springer, 2008.

386 Birouste, M., Zamora-Ledezma, E., Bossard, C., Pérez-Ramos, I., and Roumet, C.: Measurement of fine
387 root tissue density: a comparison of three methods reveals the potential of root dry matter content,
388 Plant and Soil, 374, 299-313, 2014.

389 Chapin III, F. S.: The mineral nutrition of wild plants, Annu. Rev. Ecol. Evol. Sys., 11, 233-260, 1980.

390 Chen, W., Zeng, H., Eissenstat, D. M., and Guo, D.: Variation of first-order root traits across climatic
391 gradients and evolutionary trends in geological time, Global Ecology and Biogeography, 22, 846-856,
392 2013.

393 Chimungu, J. G., Brown, K. M., and Lynch, J. P.: Large root cortical cell size improves drought tolerance
394 in maize, Plant Physiology, 166, 2166-2178, 2014a.

395 Chimungu, J. G., Brown, K. M., and Lynch, J. P.: Reduced root cortical cell file number improves
396 drought tolerance in maize, Plant Physiology, 166, 1943-1955, 2014b.

397 Clemmensen, K. E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., Finlay,
398 R. D., Wardle, D. A., and Lindahl, B. D.: Roots and associated fungi drive long-term carbon
399 sequestration in boreal forest, Science, 339, 1615-1618, 2013.

400 Cornwell, W. K., Cornelissen, J. H. C., Amatangelo, K., Dorrepaal, E., Eviner, V. T., Godoy, O., Hobbie, S.
401 E., Hoorens, B., Kurokawa, H., Pérez-Harguindeguy, N., Quested, H. M., Santiago, L. S., Wardle, D. A.,
402 Wright, I. J., Aerts, R., Allison, S. D., Van Bodegom, P., Brovkin, V., Chatain, A., Callaghan, T. V., Díaz, S.,
403 Garnier, E., Gurvich, D. E., Kazakou, E., Klein, J. A., Read, J., Reich, P. B., Soudzilovskaia, N. A., Vaieretti,
404 M. V., and Westoby, M.: Plant species traits are the predominant control on litter decomposition rates
405 within biomes worldwide, Ecology Letters, 11, 1065-1071, 2008.

406 Craine, J. M., Lee, W. G., Bond, W. J., Williams, R. J., and Johnson, L. C.: Environmental constraints on
407 a global relationship among leaf and root traits of grasses, Ecology, 86, 12-19, 2005.

408 Diaz, S., Hodgson, J. G., Thompson, K., Cabido, M., Cornelissen, J. H. C., Jalili, A., Montserrat-Martí, G.,
409 Grime, J. P., Zarrinkamar, F., Asri, Y., Band, S. R., Basconcelo, S., Castro-Díez, P., Funes, G., Hamzehee,

410 B., Khoshnevi, M., Pérez-Harguindeguy, N., Pérez-Rontomé, M. C., Shirvany, F. A., Vendramini, F.,
411 Yazdani, S., Abbas-Azimi, R., Bogaard, A., Boustani, S., Charles, M., Dehghan, M., de Torres-Espuny, L.,
412 Falczuk, V., Guerrero-Campo, J., Hynd, A., Jones, G., Kowsary, E., Kazemi-Saeed, F., Maestro-Martínez,
413 M., Romo-Díez, A., Shaw, S., Siavash, B., Villar-Salvador, P., and Zak, M. R.: The plant traits that drive
414 ecosystems: Evidence from three continents, Journal of Vegetation Science, 15, 295-304, 2004.
415 Eissenstat, D. M. and Achor, D. S.: Anatomical characteristics of roots of citrus rootstocks that vary in
416 specific root length, New Phytologist, 141, 309-321, 1999.
417 Eissenstat, D. M., Kucharski, J. M., Zadworny, M., Adams, T. S., and Koide, R. T.: Linking root traits to
418 nutrient foraging in arbuscular mycorrhizal trees in a temperate forest, New Phytologist, 208, 114-124,
419 2015.
420 Eissenstat, D. M. and Yanai, R. D.: The ecology of root lifespan, Advances in Ecological Research 27,
421 1-60, 1997.
422 Espeleta, J. F., West, J. B., and Donovan, L. A.: Tree species fine-root demography parallels habitat
423 specialization across a sandhill soil resource gradient, Ecology, 90, 1773-1787, 2009.
424 Fan, P. and Guo, D.: Slow decomposition of lower order roots: a key mechanism of root carbon and
425 nutrient retention in the soil. , Oecologia, 163, 509-515, 2010.
426 Feng, Y. L., Lei, Y. B., Wan, R. F., Callaway, R. M., Valiente-Banuet, I., Li, Y. P., and Zheng, Y. L.:
427 Evolutionary tradeoffs for nitrogen allocation to photosynthesis versus cell walls in an invasive plant, P.
428 Natl. Acad. Sci. USA 106, 1853-1856, 2009.
429 Freschet, G. T., Cornelissen, J. H., Van Logtestijn, R. S., and Aerts, R.: Evidence of the 'plant economics
430 spectrum'in a subarctic flora, J. Ecol., 98, 275-301, 2010.
431 Goebel, M., Hobbie, S. E., Bulaj, B., Zadworny, M., Archibald, D. D., Oleksyn, J., Reich, P. B., and
432 Eissenstat, D. M.: Decomposition of the finest root branching orders: linking belowground dynamics
433 to fine-root function and structure, Ecol. Monogr. , 81, 89-102, 2011.
434 Guo, D., Xia, M., Wei, X., Chang, W., Liu, Y., and Wang, Z.: Anatomical traits associated with absorption
435 and mycorrhizal colonization are linked to root branch order in twenty-three Chinese temperate tree
436 species, New Phytologist, 180, 673-683, 2008.
437 Hidaka, A. and Kitayama, K.: Allocation of foliar phosphorus fractions and leaf traits of tropical tree
438 species in response to decreased soil phosphorus availability on Mount Kinabalu, Borneo, Journal of
439 Ecology, 99, 849-857, 2011.
440 Kong, D., Ma, C., Zhang, Q., Li, L., Chen, X., Zeng, H., and Guo, D.: Leading dimensions in absorptive
441 root trait variation across 96 subtropical forest species, New Phytologist, 203, 863-872, 2014.
442 Kong, D., Wu, H., Wang, M., Simmons, M., Lü, X., Yu, Q., and Han, X.: Structural and chemical
443 differences between shoot- and root-derived roots of three perennial grasses in a typical steppe in
444 Inner Mongolia China, Plant and Soil, 336, 209-217, 2010.
445 Kong, D. L. and Ma, C. E.: Acquisition of ephemeral module in roots: a new view and test, Sci. Rep., 4,
446 5078, 2014.
447 Laughlin, D. C., Leppert, J. J., Moore, M. M., and Sieg, C. H.: A multi-trait test of the leaf-height-seed
448 plant strategy scheme with 133 species from a pine forest flora, Functional Ecology, 24, 493-501,
449 2010.
450 Liu, B., Li, H., Zhu, B., Koide, R. T., Eissenstat, D. M., and Guo, D.: Complementarity in nutrient foraging
451 strategies of absorptive fine roots and arbuscular mycorrhizal fungi across 14 coexisting subtropical
452 tree species, New Phytologist, 208, 125-136, 2015.

453 Long, Y., Kong, D., Chen, Z., and Zeng, H.: Variation of the linkage of root function with root branch
454 order, PLoS ONE, 8, e57153, 2013.

455 Lux, A., Luxova, M., Abe, J., and Morita, S.: Root cortex: structural and functional variability and
456 responses to environmental stress, Root Research, 13, 117-131, 2004.

457 McCormack, M. L., Adams, T. S., Smithwick, E. A., and Eissenstat, D. M.: Predicting fine root lifespan
458 from plant functional traits in temperate trees, New Phytologist, 195, 823-831, 2012.

459 Meier, C. L. and Bowman, W. D.: Links between plant litter chemistry, species diversity, and
460 below-ground ecosystem function, P. Natl. Acad. Sci. USA 105, 19780-19785 2008.

461 Mommer, L. and Weemstra, M.: The role of roots in the resource economics spectrum, New
462 Phytologist, 195, 725-727, 2012.

463 Novaes, E., Kirst, M., Chiang, V., Winter-Sederoff, H., and Sederoff, R.: Lignin and biomass: a negative
464 correlation for wood formation and lignin content in trees., Plant Physiology, 154, 555-561, 2010.

465 Osnas, J. L., Lichstein, J. W., Reich, P. B., and Pacala, S. W.: Global leaf trait relationships- mass, area,
466 and the leaf economics spectrum, Science, 340, 741-744, 2013.

467 Poorter, H. and Bergkotte, M.: Chemical composition of 24 wild species differing in relative growth
468 rate, Plant Cell Environ, 15, 221-229, 1992.

469 Poorter, H., Niinemets, Ü., Poorter, L., Wright, I. J., and Villar, R.: Causes and consequences of
470 variation in leaf mass per area (LMA): a meta-analysis, New Phytologist, 182, 565-588, 2009.

471 Pregitzer, K. S., DeForest, J. L., Burton, A. J., Allen, M. F., Ruess, R. W., and Hendrick, R. L.: Fine root
472 architecture of nine North American trees, Ecological Monographs, 72, 293-309, 2002.

473 Prieto, I., Roumet, C., Cardinael, R., Dupraz, C., Jourdan, C., Kim, J. H., Maeght, J. L., Mao, Z., Pierret,
474 A., Portillo, N., Roupsard, O., Thammahacksa, C., and Stokes, A.: Root functional parameters along a
475 land-use gradient: evidence of a community-level economics spectrum, Journal of Ecology, 103,
476 361-373, 2015.

477 Reich, P. B.: The world-wide 'fast–slow' plant economics spectrum: a traits manifesto, J. Ecol., 102,
478 275-301, 2014.

479 Reich, P. B. and Oleksyn, J.: Global patterns of plant leaf N and P in relation to temperature and latitud,
480 P. Natl. Acad. Sci. USA, 101, 11001-11006, 2004.

481 Reich, P. B., Tjoelker, M. G., Pregitzer, K. S., Wright, I. J., Oleksyn, J., and Machado, J. L.: Scaling of
482 respiration to nitrogen in leaves, stems and roots of higher land plants, Ecol. Lett., 11, 793-801 2008.

483 Ryser, P.: The Importance of Tissue Density for Growth and Life Span of Leaves and Roots: A
484 Comparison of Five Ecologically Contrasting Grasses, Functional Ecology, 10, 717-723, 1996.

485 Ryser, P.: The mysterious root length, Plant and Soil, 286, 1-6, 2006.

486 Shipley, B., Lechowicz, M. J., Wright, I., and Reich, P. B.: Fundamental trade-offs generating the
487 worldwide leaf economics spectrum, Ecology, 87, 535-541, 2006.

488 St John, T. V.: Root size, root hairs and mycorrhizal infection: a re- examination of Baylis's hypothesis
489 with tropical trees, New Phytologist, 84, 483-487, 1980.

490 Wahl, S. and Ryser, P.: Root tissue structure is linked to ecological strategies of grasses, New
491 Phytologist, 148, 459-471, 2000.

492 Wells, C. E. and Eissenstat, D. M.: Marked Differences in Survivorship among Apple Roots of Different
493 Diameters, Ecology, 82, 882-892, 2001.

494 Westoby, M., Falster, D. S., Moles, A. T., Vesk, P. A., and Wright, I. J.: Plant ecological strategies- some
495 leading dimensions of variation among species, Annu. Rev. Ecol. Evol. Sys., 33, 125-159, 2002.

496 Withington, J. M., Reich, P. B., Oleksyn, J., and Eissenstat, D. M.: Comparisons of structure and life
497 span in roots and leaves among temperate trees, Ecological Monographs, 76, 381-397, 2006.
498 Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin,
499 T., Cornelissen, J. H. C., Diemer, M., Flexas, J., Garnier, E., Groom, P. K., Gulias, J., Hikosaka, K., Lamont,
500 B. B., Lee, T., Lee, W., Lusk, C., Midgley, J. J., Navas, M.-L., Niinemets, U., Oleksyn, J., Osada, N., Poorter,
501 H., Poot, P., Prior, L., Pyankov, V. I., Roumet, C., Thomas, S. C., Tjoelker, M. G., Veneklaas, E. J., and
502 Villar, R.: The worldwide leaf economics spectrum, Nature, 428, 821-827, 2004.
503 Xiong, Y. M., Fan, P. P., Fu, S. L., Zeng, H., and Guo, D. L.: Slow decomposition and limited nitrogen
504 release by lower order roots in eight Chinese temperate and subtropical trees., Plant and Soil, 363,
505 19-31, 2013.

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514

515   **Table 1.** *F* values of two-way ANOVAs testing effects of plants species and root branch order

516   on the extractive C fraction, acid-soluble C fraction, acid-insoluble C fraction, N

517   concentration, and N in acid-insoluble C fraction. *, **, *** were significant level at 0.05,

518   0.01, 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***

519

Figure legends

Fig. 1 Relationships between root tissue density and root N concentration over the total, thin
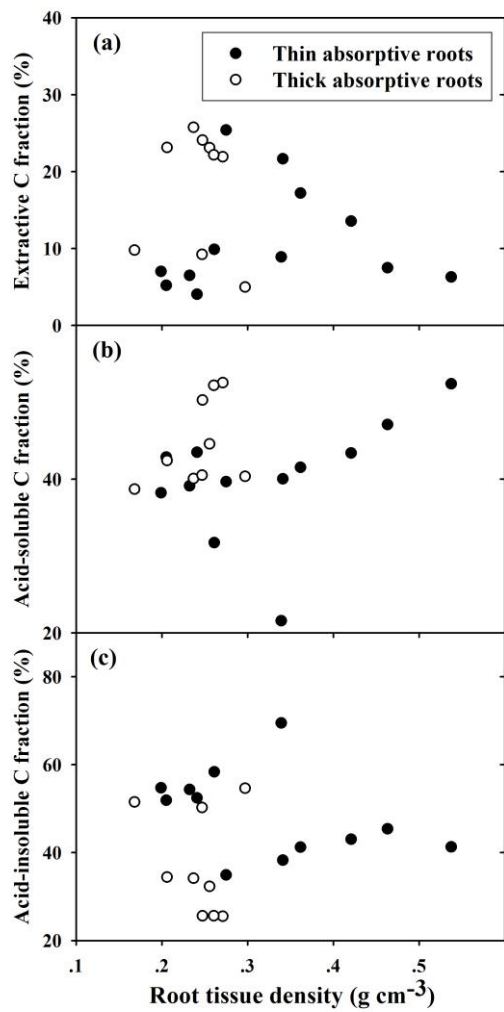
(solid circles) and thick (open circles) absorptive roots.
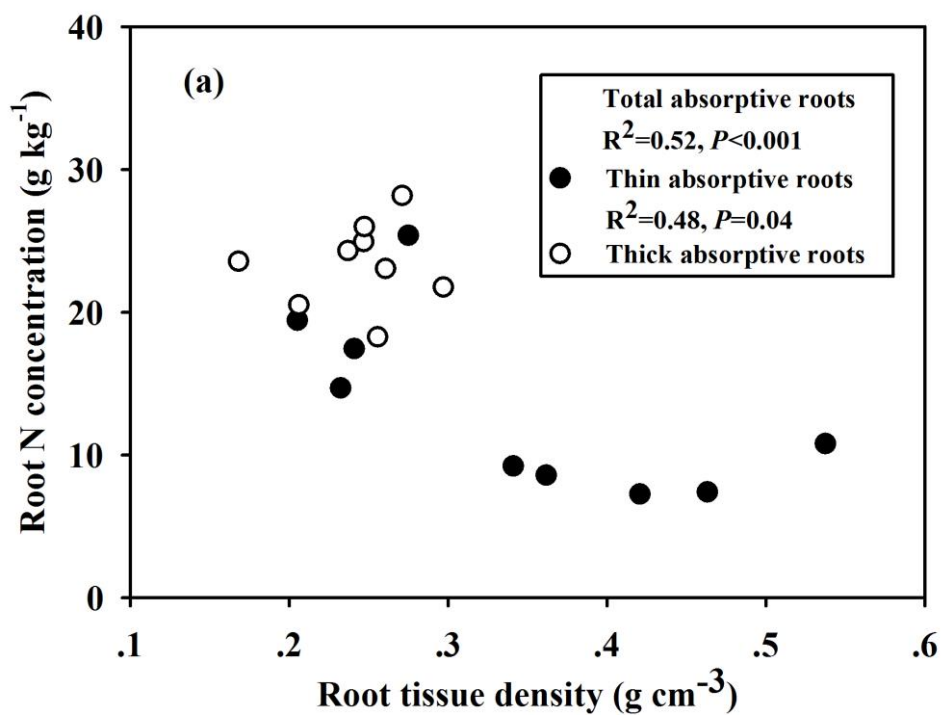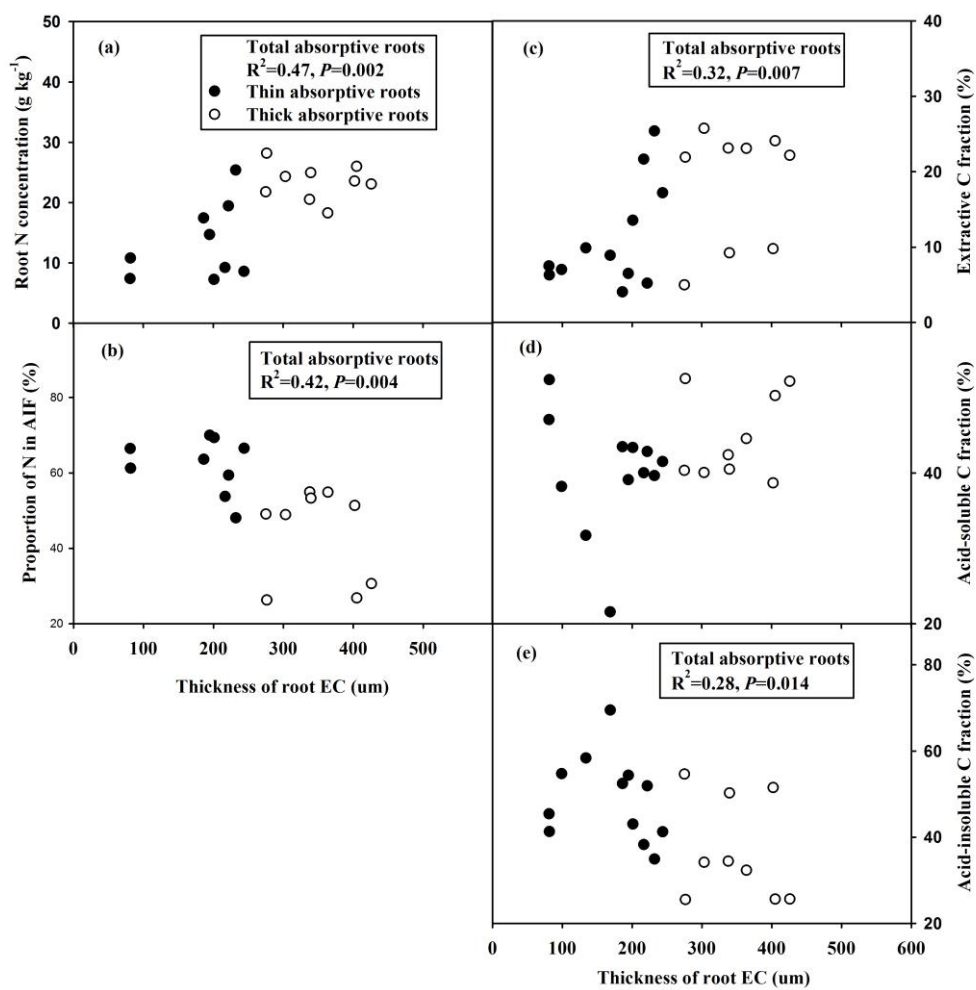
525　　Fig. 2 Relationships between root tissue density and three C fractions, extractive C fraction

526　　(a), acid-soluble C fraction (b) and acid-insoluble C fraction (c), for the thin (solid circles)

527　　and thick (open circles) absorptive roots.

528

529

530   Fig. 3 Relationships between thickness of root EC and root N concentration (a), N in

531   acid-insoluble C fraction (b), extractive C fraction (c), acid-soluble C fraction (d) and

532   acid-insoluble C fraction (e) for the thin (solid circles) and thick (open circles) absorptive

533   roots.

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535

536   Fig. 4 Relationships between root tissue density and thickness of root EC over the total, thin

537   (solid circles) and thick (open circles) absorptive roots.
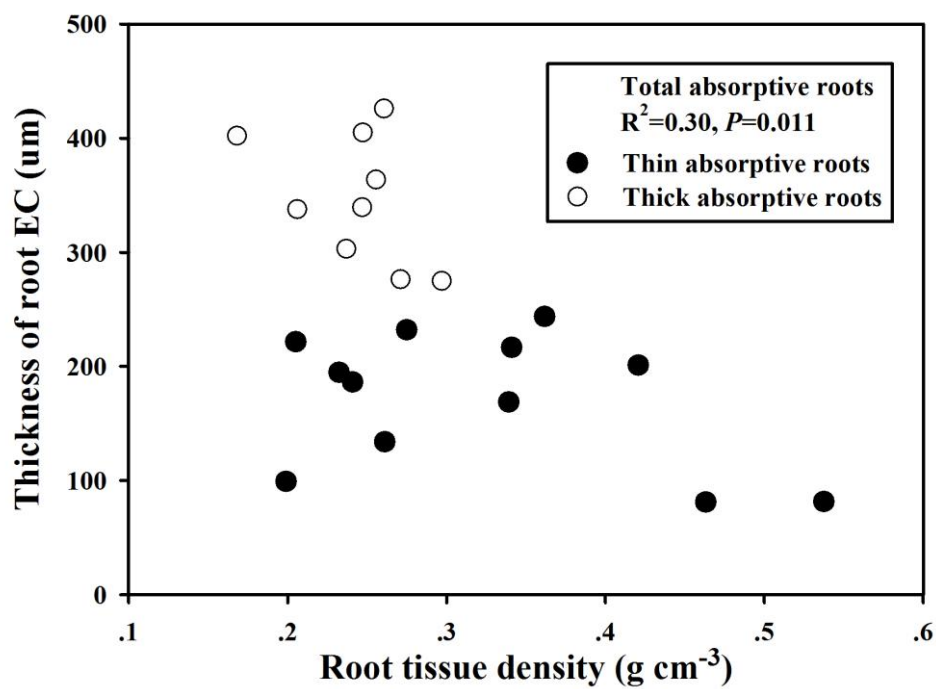
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539

540   Fig. 5 Three C fractions, extractive C fraction (a), acid-soluble C fraction (b) and

541   acid-insoluble C fraction (c), among different root orders in seven species. R1-R5 were the

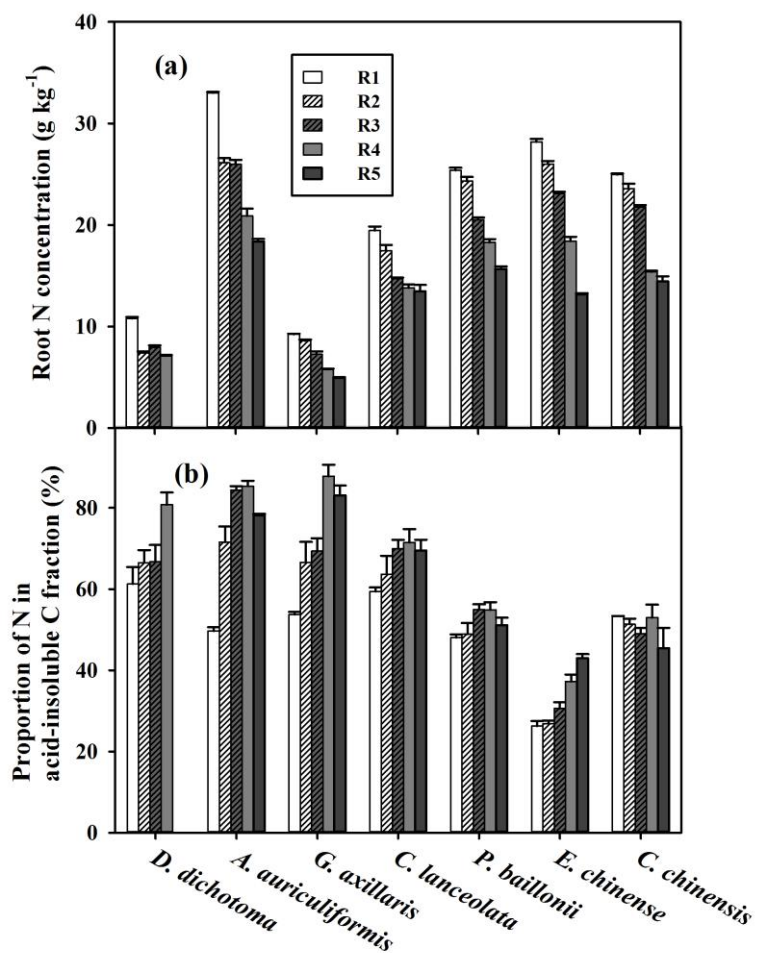542   first to the fifth root branch order.
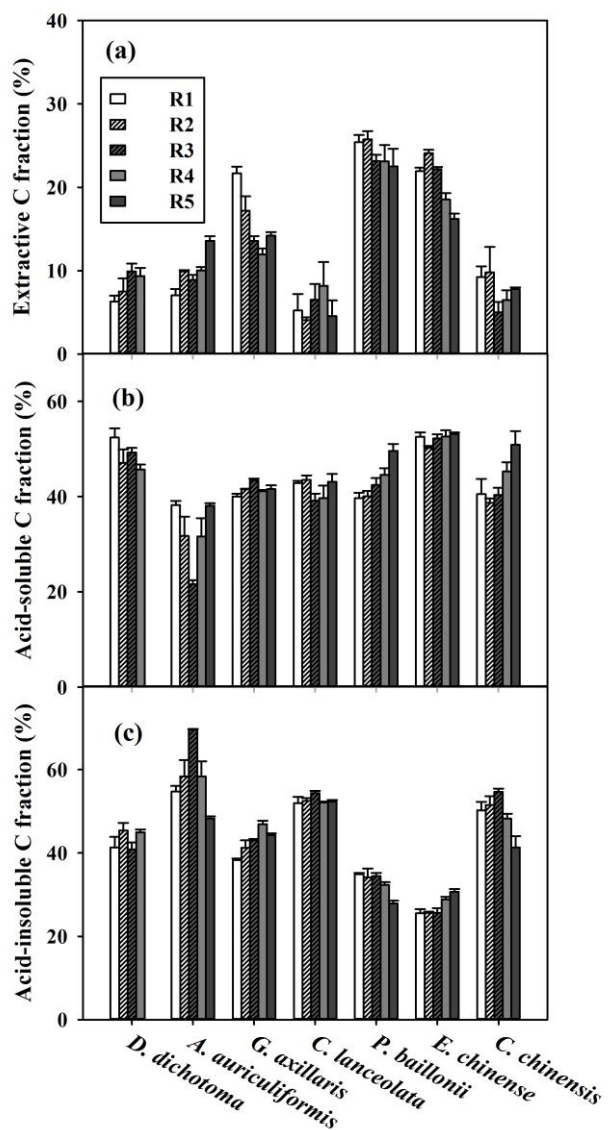
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544

545   Fig. 6 Root N concentration (a) and N in acid-insoluble C fraction (b) among different orders

546   for seven species. R1-R5 were the first to the fifth root branch order.