Economic strategies for plant absorptive roots vary with root diameter

Deliang Kong[1,2*], Junjian Wang[3], Paul Kardol[4], Huifang Wu[5], Hui Zeng[6], Xiaobao Deng[1], Yun Deng[1]

[1]Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, Yunnan, China

[2] College of Biosciences and Biotechnology, Shenyang Agricultural University, Shenyang, China

[3] Belle W. Baruch Institute of Coastal Ecology & Forest Science, Clemson University, Georgetown, South Carolina, USA

[4] Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, Umeå, Sweden

[5] School of Life Sciences, Henan University, Kaifeng, China

[6] Key Laboratory for Urban Habitat Environmental Science and Technology, Peking University Shenzhen Graduate School, Shenzhen, China

Author for correspondence:

Tel: +86-024-88487163, Fax: +86-024-88492799

E-mail: deliangkong1999@126.com

Abstract

Plant roots typically vary along a dominant ecological axis, the root economics spectrum, depicting a tradeoff between resource acquisition and conservation. For absorptive roots, which are mainly responsible for resource acquisition, we hypothesized that root economic strategies differ with increasing root diameter. To test this hypothesis, we used seven plant species (a fern, a conifer, and five angiosperms from south China) for which we separated absorptive roots into two categories: thin roots (thickness of root cortex plus epidermis < 247 μm) and thick roots. For each category, we analyzed a range of root traits 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 significant relationships among root traits indicating an acquisition-conservation tradeoff for thin absorptive roots while no such trait relationships were found for thick absorptive roots. Similar results were found when reanalyzing data of a previous study including more species. The contrasting economic strategies between thin and thick absorptive roots, as revealed here, may provide a new perspective on our understanding of the root economics spectrum.

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

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 (Cornwell et al., 2008; Freschet et al., 2010; Reich, 2014; Westoby et al., 2002). On the one end of this ecological axis, there are species with acquisitive strategies, i.e., fast acquisition of resources (e.g., CO_2 for leaves and nutrients for roots) accompanied with a short lifespan. On the other end of the axis, there are species with conservative strategies, i.e., slow resource acquisition accompanied with a long lifespan. Originally, such an ecological axis has been demonstrated for leaves, which is widely known as the leaf economics spectrum (Diaz et al., 2004; Osnas et al., 2013; Wright et al., 2004). More recently, similar trait spectra have been demonstrated across plant organs from leaves to stems and roots, thus forming a whole ‘plant economics spectrum’ (Freschet et al., 2010; Laughlin et al., 2010; Prieto et al., 2015; Reich, 2014).

Resource acquisition in plant roots is performed by absorptive roots, i.e., the first two or three orders of a root branch with primarily-developed tissues which are part of the commonly used category of ‘fine roots’ (< 2mm in diameter) (Guo et al., 2008; Long et al., 2013; Pregitzer et al., 2002). For absorptive roots, the tissue density, i.e., root dry mass per unit root volume, is a key trait of the root economics spectrum as tissue density is closely linked to the acquisition-conservation tradeoff (Bardgett et al., 2014; Birouste et al., 2014; Craine et al., 2005; Espeleta et al., 2009; Mommer and Weemstra, 2012; Roumet et al., 2006). In general, absorptive roots with higher tissue density are slower in nutrient acquisition and longer in lifespan whereas absorptive roots with lower tissue density may enable faster acquisition but maintain a shorter lifespan (Ryser, 1996; Wahl and Ryser, 2000; Withington et al., 2006). Recently, tissue density for absorptive roots was found to negatively correlate with root diameter. This could be because root cortex is less dense than root stele and because in thicker roots a larger proportion of the root cross-sectional area is accounted for by the cortex (Chen et al., 2013; Kong et al., 2014; Kong and Ma, 2014). On the other hand, compared with thinner absorptive roots, thicker absorptive roots may acquire resources faster because of their greater dependence on mycorrhizal fungi (Eissenstat et al., 2015; Kong et al., 2014; Kong and Ma, 2014; St John, 1980), and may also have a longer lifespan due to the larger diameter(Adams et al., 2013; Eissenstat and Yanai, 1997; Wells and Eissenstat, 2001). As such, the trait syndrome for thicker absorptive roots would differ from the predictions of faster acquisition and shorter lifespan. This highlights the importance of discriminating thicker and thinner absorptive roots when exploring root strategies. However, we are aware of few studies that have tested for effects root diameter in driving trait economics spectra in absorptive roots.

In addition to structural traits such as density, the chemical composition of absorptive roots may constitute another important aspect of testing root strategies in relation root diameter (Hidaka and Kitayama, 2011; Meier and Bowman, 2008; Poorter and Bergkotte, 1992; Poorter et al., 2009). For example, carbon (C) and nitrogen (N), the two most abundant elements in plant tissues, are usually bound to organic compounds which may contain labile fractions (e.g., soluble sugars and proteins in living cells) and recalcitrant fractions (e.g., cellulose and lignin in structural tissues) (Atkinson et al., 2012; Berg and McClaugherty, 2008; Feng et al., 2009; Poorter et al., 2009; Shipley et al., 2006). Generally, absorptive roots with less labile C and more labile N indicate an acquisitive strategy. This is because high root activity may be accompanied by an increased production of metabolism-related proteins with a high labile N content; such roots may be palatable for herbivores and have a relative short lifespan. On the other hand, conservative roots contain less labile C and N fractions as more of these chemicals are used for construction of structural tissues resulting in lower root activity and a longer lifespan. However, compared with thinner absorptive roots, thicker absorptive roots may have higher labile C and N fractions as these labile fractions can be stored in their thick root cortex (Chapin III, 1980; Long et al., 2013; Lux et al., 2004; Withington et al., 2006). As such, the chemical traits of thicker absorptive roots integrate ‘opposing’ effects of root metabolism and storage suggesting them having neither a true acquisitive nor a true conservative strategy. Therefore, to evaluate the impact of thickness on root economic strategies it is necessary to examine C and N fractions in relation to root diameter.

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

2 Materials and methods

2.1 Plant species and sampling sites

We selected seven plant species with contrasting phylogeny and root structure (Table S1) in tropical and subtropical forests in south China. Three species were sampled at the Heshan Hilly Land Interdisciplinary Experimental Station (22°41′N, 112°54′E), Guangdong province. The species were: *Dicranopteris dichotoma* (Gleicheniaceae) (a fern), *Cunninghamia lanceolata* (Taxodiaceae) (a conifer) and *Acacia auriculiformis* (Leguminosae) (a tree). Another tree species, *Paramichelia baillonii* (Magnoliaceae), was sampled in Wutongshan National Forest Park (22°27′-22°52′N, 113°37′-114°37′E) in Shenzhen, Guangdong province. Three other tree species, *Gordonia axillaris* (Theaceae), *Endospermum chinense* (Euphorbiaceae) and *Cryptocarya chinensis* (Lauraceae), were sampled in Jianfengling Nature Reserve (18°23′-18^{o}50′N, 108°36′-109°05′E), Hainan province. Roots of these species are mycorrhizas. More information on sites and species can be found in Table S1 and Long et al. (2013).

2.2 Root sampling

Roots were collected at a soil depth of 0-10 cm in June and July 2011. For each species, at least three mature trees were selected. We first tracked the main lateral roots by carefully removing surface soil at the base of each plant with a specially manufactured fork. Root branch order was defined according to Pregitzer's study with the most terminal branch as the first-order (Pregitzer et al., 2002). The intact roots were collected and soil adhering to the roots was carefully removed. We distinguished all four root orders for *D. dichotoma* and the first five orders for the other species. A portion of each root sample was immediately put into Formalin-Aceto-Alcohol (FAA) solution (90 ml 100% ethanol, 10 ml 100% glacial acetic acid) for later anatomical assessment. The remaining unwashed part of each root sample was placed in plastic bags and transported in a cooler to the laboratory. These root samples were then frozen until measurements of root morphology and chemistry (Pregitzer et al., 2002).

2.3 Root tissue density

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

2.4 Root anatomy

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

2.5 Chemical analyses

The frozen root samples were put into deionized water to carefully remove any soil particles or dead organic matter that adhered to but was not a part of the root (Pregitzer et al., 2002). The samples of each root branch order were then oven-dried (65 ℃ for 24 h), milled (ZM200, Retsch, Germany), and mixed homogeneously for chemical analyses. Root C and N concentrations were determined using an element analyzer (VarioEl, Elementar Analysen-systeme GmbH, Germany). Root C fractions (extractive, acid-soluble fraction, acid-insoluble fraction) were determined by a sulfuric acid digestion method. First, we separated the extractive and labile C fraction from other C fractions. A subsample of c. 100 mg (m_0) was extracted with 15 ml of cetyl trimethylammonium bromide (CTAB) solution for 3 h, filtered, repeatedly washed with de-ionized water until pH was 7.0, and then oven-dried at 60 ℃ to a constant weight (m_1). Second, the filtered residue was digested with 30 ml sulfuric acid (72 %) at 22 ℃ for 3 h, filtered, repeatedly washed (until pH was 7.0), dried and weighed (m_2). After the acid-digestion step, the ash content (m_3), was determined by combusting 15-30 mg of sample at 550 ℃ for 4 h. Finally, the extractive fraction, acid-soluble fraction, and acid-insoluble fraction were calculated as $100\% \times (m_0-m_1)/(m_0-m_3)$, $100\% \times (m_1-m_2)/(m_0-m_3)$, and $100\% \times (m_2-m_3)/(m_0-m_3)$, respectively. Here, the extractive fraction was considered as the labile C fraction while acid-soluble and acid-insoluble fractions were considered as the recalcitrant C fraction.

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

2.6 Statistical analyses

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

To explore the effect of root diameter on root ecological strategies, the above analyses were repeated for thin and thick absorptive roots, respectively. A mean thickness of 247 μm was used for root EC as the cut-off point between thin and thick absorptive roots. The mean thickness of root EC was used because the thickness of root EC for absorptive roots followed a normal distribution ($p>0.05$, indicating that thickness was statistically no different from a normal distribution; Fig. S2a). To avoid the influence of biological N fixation on relationships between root N and root tissue density and root EC, a legume species, *A. auriculiformis*, was excluded in these analyses. In addition, the relationship between the extractive C fraction and root tissue density was further explored by a quadratic polynomial regression using moving average data (Fig. S4). Polynomial regressions were run both for the thin and thick absorptive roots. The moving average data were obtained as follows. First, the extractive C fraction was sorted along with the ascending order of root tissue density. Then, the extractive C fraction and root tissue density were averaged by bins (Reich and Oleksyn, 2004), with bins referring to each of the two neighboring data of extractive C fraction or root tissue density, respectively. Moving average analyses were used as it improved the goodness of fit. No polynomial regression relationships were found for the other two C fractions.

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

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

3 Results

3.1 Root trait relationships for thin and thick absorptive roots

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

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

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

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

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

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

The extractive C fraction tended to increase with increasing root order for species with thin absorptive roots such as *D. dichotoma* and *A. auriculiformis,* but decreased for species with thick absorptive roots, except for *C. lanceolata* (Fig. 5a). For both acid-soluble and acid-insoluble fractions, patterns were largely idiosyncratic, including both increases and decreases with increasing root branch order (Fig. 5b,c). For all species, root N concentration decreased with increasing root branch order (Fig. 6a), whereas N in the acid-insoluble fraction increased with increasing root branch order, except for *C. chinensis* (Fig. 6b).

4 Discussion

The acquisition-conservation tradeoff in plants has been suggested to be consistent across plant organs (roots, leaves, and stems), as such constituting a key ecological axis, i.e., the ‘plant economics spectrum’ (Freschet et al., 2010; Prieto et al., 2015; Reich, 2014). The negative relationship between root tissue density and root N concentration across total absorptive roots supports the existence of economic strategies in absorptive roots. This is because absorptive roots with higher tissue density usually have longer lifespan (Eissenstat and Yanai, 1997; Ryser, 1996; Withington et al., 2006), while their lower N concentration would be associated with slow resource acquisition (Kong et al., 2010; Mommer and Weemstra, 2012; Reich et al., 2008). However, our results further showed that the negative relationship between root tissue density and root N concentration held for thin but not for thick absorptive roots (Fig. 1). Although these results were based on a relative small number of species, reanalysis of data from a previous study including 96 species (Kong et al., 2014) revealed very similar patterns (Fig. S1). As such, trait relationship between root N concentration and root tissue density supports our first hypothesis of different economics strategies for the thin and thick absorptive roots.

The trait relationships between root tissue density and root C fractions provide further support for the hypothesis. Theoretically, absorptive roots with lower tissue density would have higher activity, while higher root activity also consumes more labile C thus leaving less labile and more recalcitrant C fractions in these roots. In contrast, in absorptive roots with higher tissue density, more C is used for structural tissues demanding recalcitrant C fractions

(Fan and Guo, 2010). Therefore, we would expect an inverted U-shaped relationship for labile C fractions and a U-shaped relationship for recalcitrant C fractions when these C fractions would be correlated with root tissue density. As expected, for thin absorptive roots we found an inverted U-shaped relationship between the labile C fraction and root tissue density (Fig. S4a) and a U-shaped relationship between recalcitrant C fractions (acid-soluble C + acid insoluble C) and root tissue density (Fig. S4b). The higher acid-soluble C fraction with increasing root tissue density (Fig. 2b) suggest that thin absorptive roots with higher tissue density are constructed with more acid-soluble C compounds, such as cellulose, rather than acid-insoluble C compounds, such as lignin, possibly because of higher energy demands for the production of lignin than for the production of cellulose (Novaes et al., 2010).. However, different from thin absorptive roots, there were no relationships between root C fractions and root tissue density for thick absorptive roots (Fig. 2, Fig. S4). Therefore, trait relationships between root C fractions and root tissue density provides further evidence for an acquisition-conservation tradeoff economics strategy in thin absorptive roots, but not for thick absorptive roots.

Furthermore, observed relationships between thickness of root EC and root C and N fractions provides the third piece of support for our hypothesis of different economic strategies with root diameter. Across total absorptive roots, thickness of root EC was positively correlated with root N concentration and the extractive C fraction while being negatively correlated with the acid-soluble C fraction and N in the acid-soluble C fraction. This suggest that compared with thin absorptive roots, thick absorptive roots acquire resources at higher rates as indicated by their higher N concentration and lower C and N in recalcitrant fractions. Meanwhile, thick absorptive roots may also have longer lifespan because of their larger root diameter (Adams et al., 2013; Anderson et al., 2003; McCormack et al., 2012; Wells and Eissenstat, 2001). These findings seem to contrast with an acquisition-conservation tradeoff. Further, we showed that relationships between thickness of root EC and root chemical fractions only hold across the full spectrum from thin to thick absorptive roots. Nevertheless, it was also noted that root tissue density showed a greater range of variation for thin than for thick absorptive roots. For thin absorptive roots, variation in root tissue density might arise from secondary thickening of root EC cell walls (Eissenstat and Achor, 1999; Long et al., 2013; Ryser, 2006; Wahl and Ryser, 2000). This could be associated with lower root activity and hence lower root N concentration (Fig. 1, Fig. S3), and an acquisition-conservation tradeoff in thin absorptive roots could be expected. However, for thick absorptive roots, the cell size as well as the cortical cell file number (Chimungu et al., 2014a, b) may be more important than cell wall thickening in determining root activity. If so, root activity may be less affected by thickening of root EC cell walls than by changing the size or number of these cells, and there would thus be no clear economic strategies for thick absorptive roots.

Recent studies have revealed different nutrient foraging strategies for thin and thick absorptive roots with the former depending on roots themselves and the latter depending more on mycorrhizal fungi (Baylis, 1975; Eissenstat et al., 2015; Liu et al., 2015). These observations are supported by the SRL-thickness relationship we found in our study where thin roots had larger SRL and SRL of thick roots was constantly smaller (Fig. S6). Here, our results further indicate that thin and thick absorptive roots may follow different economic strategies when foraging for nutrients. These findings may have important implications for the emerging debate on the root economics spectrum. For example, the existence of an economic strategies for plant roots has been commonly accepted (Craine et al., 2005; Espeleta et al., 2009; Freschet et al., 2010; Reich, 2014). However, some recent studies have challenged the ubiquity of root economics spectra by showing no (Chen et al., 2013) or positive (Kong et al., 2014) relationships between root diameter and root N concentration. One possible explanation for the conflicting findings of these studies is the inclusion of many species with thick absorptive roots. Including these species may potentially obscure trait relationships indicating acquisition-conservation tradeoffs. On the other hand, the lack of evidence of an acquisition-conservation tradeoff may have resulted from the larger proportion of root cross-section area accounted for by root EC compared to the stele (Table S2; Kong et al., 2014). Notable, for species like monocots, the area of root stele is much larger than the area of root EC. We did not included monocots in our study, but it would be interesting to test whether the contrasting economic strategies for thin and thick absorptive roots, as presented here, can be applied across mono-dicots.

Besides the prominent role in influencing root strategy, root thickness may also affect patterns of root chemical traits among root branch orders. The extractive C fraction increased with increasing root order for species with thin absorptive roots, whereas it declined for species with thick absorptive roots. Although both the acid-soluble and acid-insoluble fractions showed no consistent trends across root branch orders, the total recalcitrant fraction (sum of acid-soluble and acid-insoluble fractions) showed a pattern opposite to that of the extractive fraction. On the other hand, root N concentration and N in recalcitrant C fractions showed relative consistent patterns across root orders. Thus, the findings provided only partial support of our second hypothesis. These patterns of root chemical fractions, however, are important in understanding soil ecosystem processes. For example, it is increasingly recognized that lower-order roots, compared with higher-order woody roots, are faster in root turnover but slower in root decomposition which makes the former a disproportionally greater source of soil organic matter (Clemmensen et al., 2013; Fan and Guo, 2010; Goebel et al., 2011; Xiong et al., 2013). This has been ascribed to higher recalcitrant C fractions in lower-order compared with higher-order woody roots (Goebel et al., 2011). However, our results may challenge the generality of slower decomposition of lower-order relative to higher-order roots as some lower-order roots had less recalcitrant C fractions and hence faster decomposition than higher-order roots.

In conclusion, the results of our study suggest an acquisition-conservation tradeoff for thin absorptive roots but not for thick absorptive roots. In addition, we found different patterns of root chemical fractions with root diameter and root order. The contrasting economic strategies between thin and thick absorptive roots are important in advancing our understanding of root ecology and the links with aboveground plant counterparts. Yet, our knowledge on the functioning of plant roots and their roles in driving soil ecosystem processes is still limited. We hope that our study presents an instructive perspective on the root economics spectrum that will stimulate further research in this field. Future studies may test to what extent our results hold for other (groups of) plant species (i.e. monocots), include a larger spectrum of functional traits (including those associated with interactions with rhizosphere biota), and unravel the mechanisms underlying the ‘non-economics strategy’ for thick absorptive roots.

Furthermore, we speculate that the mycotrophy (i.e., species composition of mycorrhizal fungi, their ability in nutrient acquisition and transfer to roots, etc.) may underlie economics strategy in thick absorptive roots, and needs to be emphasized in future studies.

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Table 1. *F* values of two-way ANOVAs testing effects of plants species and root branch order on the extractive C fraction, acid-soluble C fraction, acid-insoluble C fraction, N concentration, and N in acid-insoluble C fraction. *, **, *** were significant level at 0.05, 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***

Figures

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

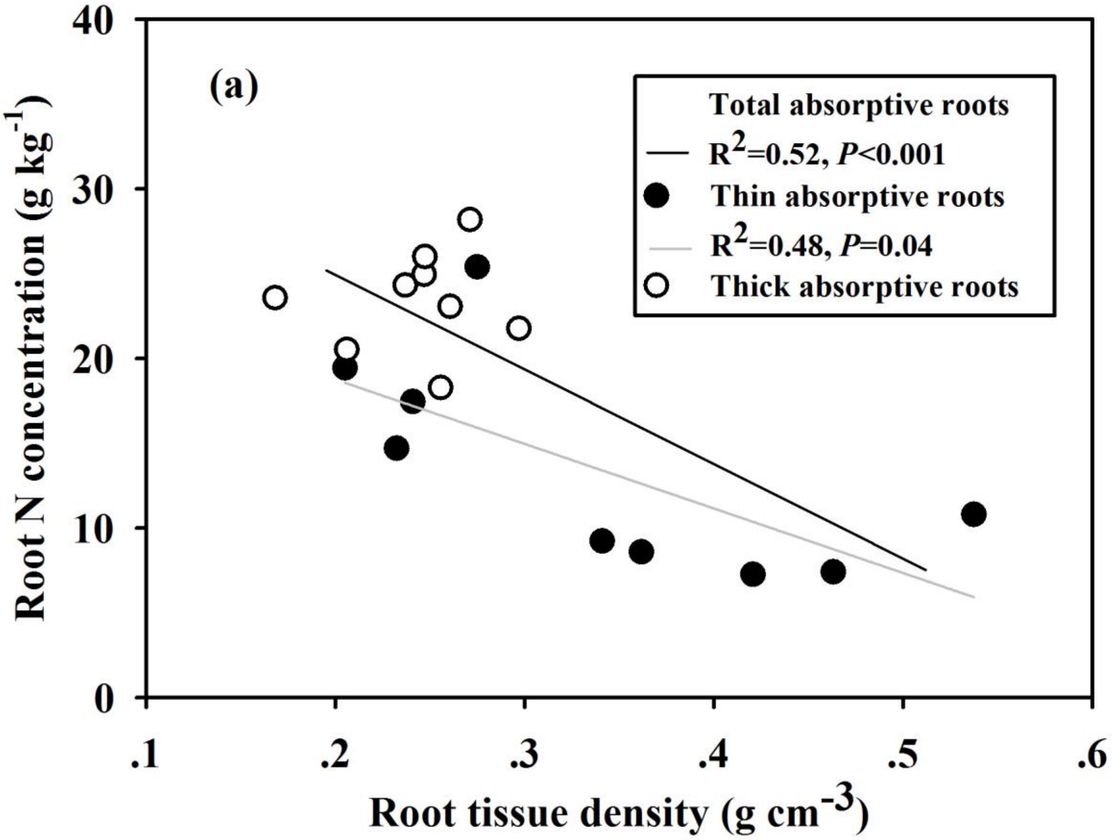


Fig. 2 Relationships between root tissue density and extractive C fraction (a), acid-soluble C fraction (b) and acid-insoluble C fraction (c), for thin (solid circles) and thick (open circles) absorptive roots.

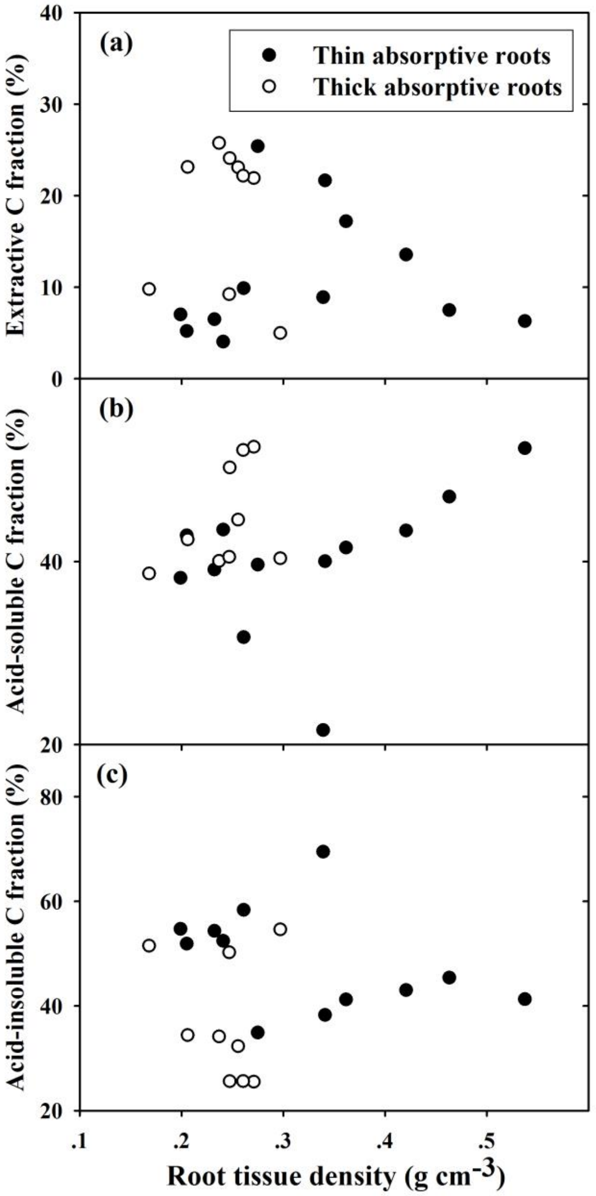


Fig. 3 Relationships between thickness of root EC and root N concentration (a), N in acid-insoluble C fraction (b), extractive C fraction (c), acid-soluble C fraction (d) and acid-insoluble C fraction (e) for total (black line), thin (solid circles) and thick (open circles) absorptive roots.

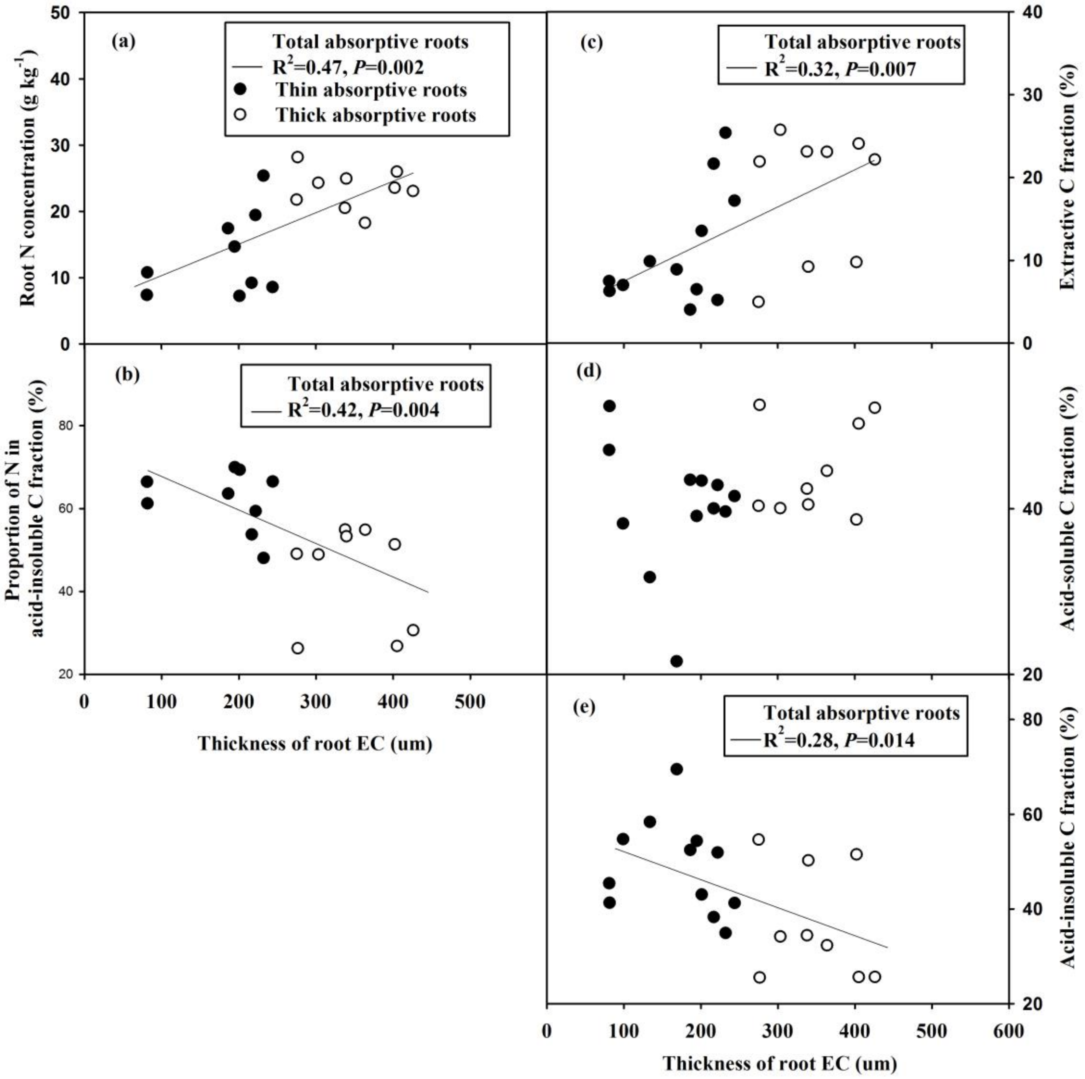


Fig. 4 Relationships between root tissue density and thickness of root EC for total, thin (solid circles, black line) and thick (open circles) absorptive roots.

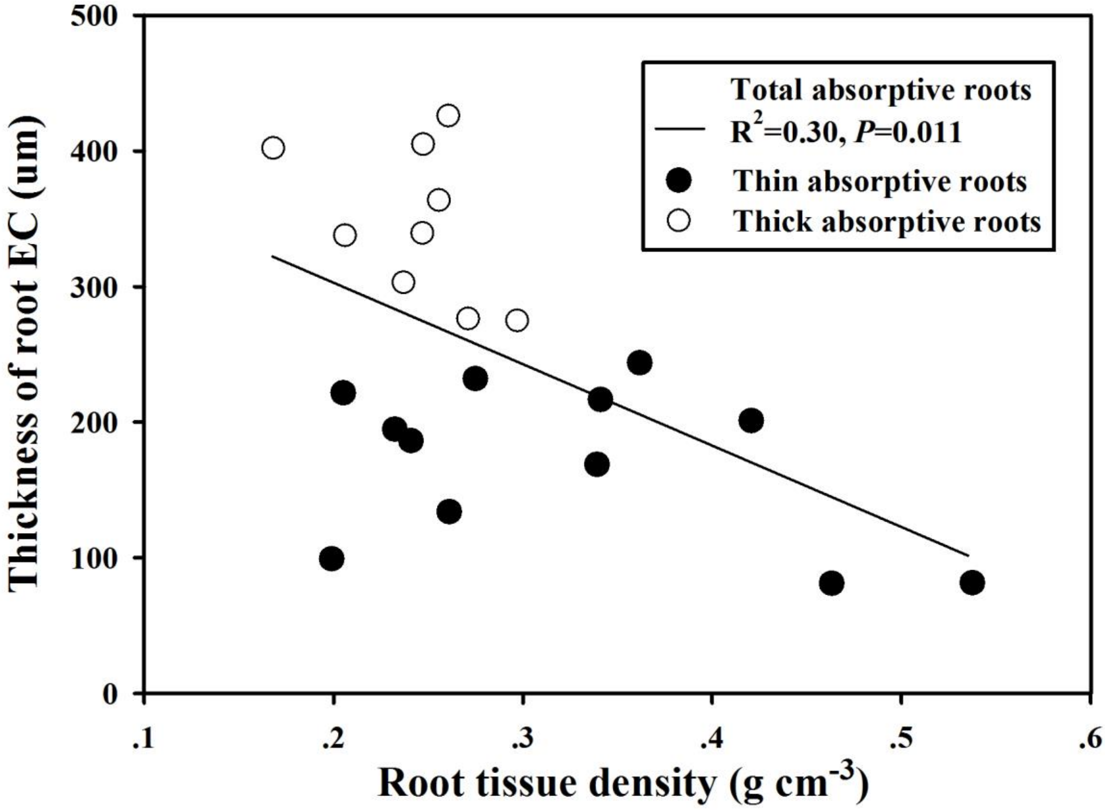
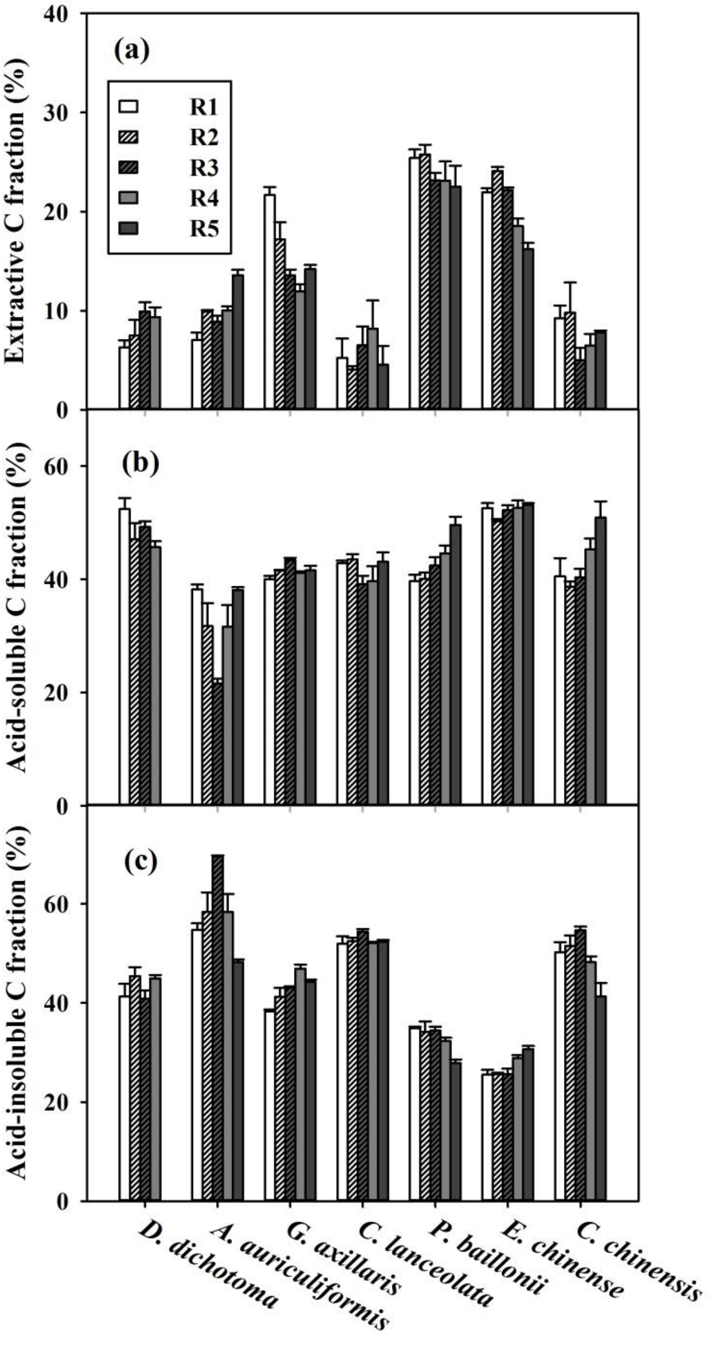
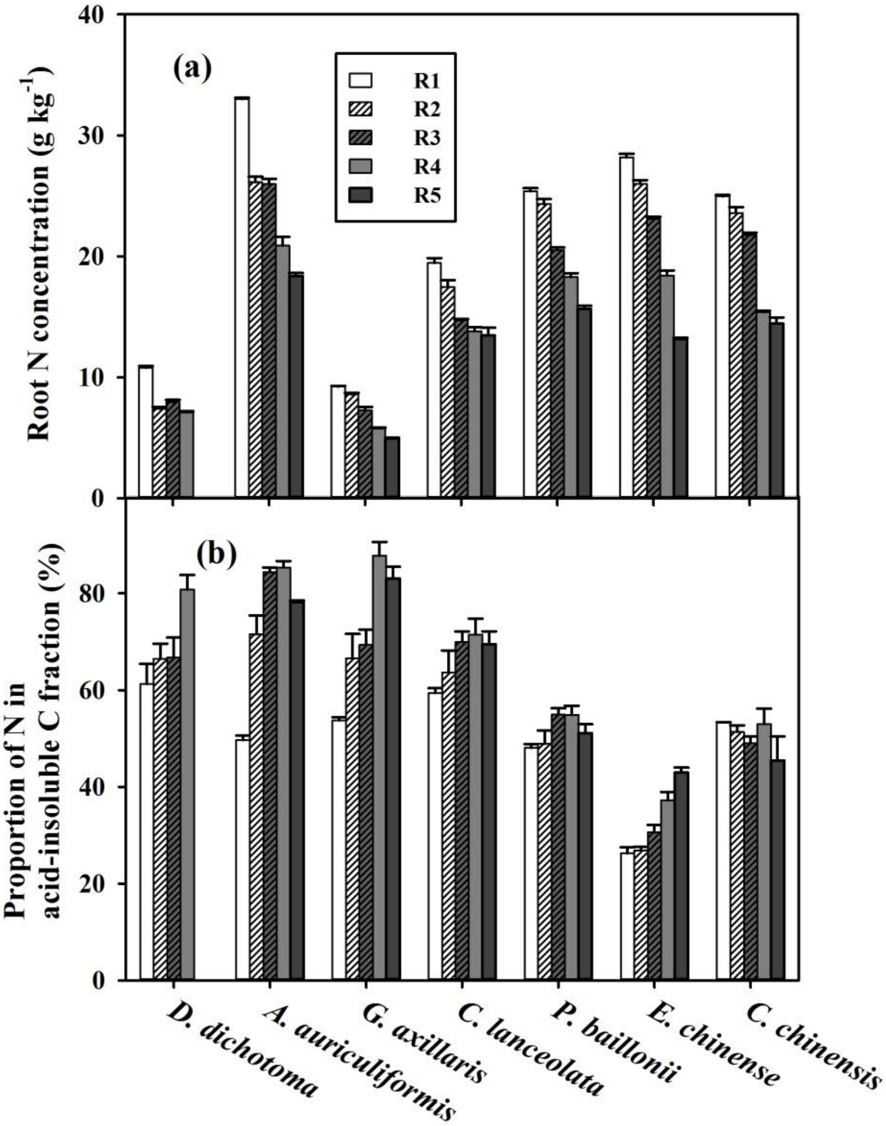


Fig. 5 The extractive C fraction (a), acid-soluble C fraction (b) and acid-insoluble C fraction (c) for the first five root orders for each of seven plant species. R1-R5 refer to the first to fifth order.



593 Fig. 6 Root N concentration (a) and N in acid-insoluble C fraction (b) for the first five root

594 branch orders for each of seven plant species. R1-R5 refer to the first to fifth order.



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