The authors appreciate the valuable comments from the reviewers and the editor on our manuscript. Our point by point response to these comments is listed below.

Reviewer #1

General comments *The aims of the paper were to evaluate (1) the influence of root diameter on the root economics spectrum (RES) and (2) that the root chemical traits (C, N) vary across branch orders. Recently it has been argued that roots should be categorized based on their function or order with the architecture more than that based on a diameter cutoff, typically 2 mm (see McCormack et al 2015). The distal roots, called absorptive, could be considered as a main group because of their position in the root system. The authors would like to demonstrate this is not the case and that absorptive roots could follow different patterns. The authors consider that a RES exists in plants in general, but it has not been yet demonstrated at large scales (see debates given by Mommer & Weenstra 2012, Reich 2014 or Bardgett et al 2015). Defining a RES needs to observe similar traits syndromes related to resource acquisition and conservation in a large number of species. In the present study only a limited number of traits (mainly chemical and anatomy) for 7 species were measured. For these reasons the title gives a false message of the paper and RES should be removed from the title. Additional traits related to resource acquisition (SRL, SRA) in order to confirm the separation between thin and thick roots are expected. In addition the size of cortex (root EC) seems to be a promising trait more than diameter itself, as it drives values of root tissue density (RTD), C and N. But this trait has not been enough underlined in the hypotheses. Similarly for mycorrhiza colonization as it seems to contrast thin and thick absorptive roots.*

I consider this paper addresses relevant scientific questions within the scope of BG and presents novel data on absorptive roots by considering separation of thin and thick based on diameter. However the attractive title does not reflect the data shown. The conclusions should take into account this point of view.

Response: We appreciate the constructive comments on our manuscript. In this manuscript, we aim to provide a new perspective on the commonly accepted root economics spectrum. We hope our findings of different economics strategies for thin and thick absorptive roots may be instructive for our understanding the emerging debate on the existence of 'root economics spectrum'.

We also acknowledge that there is a big problem of this study, testing a big topic of root economics spectrum using only a few species. This is the main reason why we used the data of 96 species in another study for validation of our perspective. Interestingly, the results of reanalyzing data of 96 species are consistent with our primarily results from a few species. Although the results of both datasets support our perspective on economics strategies in absorptive roots, we argue in the revised version that it is only a first-step in further elucidating root economics spectrum (Line 389-390 **in the revised version**). Findings of this study may arouse many interests of ecologists in this field. Additionally, we have changed the title with a new one, '*Economic strategies for plant absorptive roots vary with root diameter*'. The new title may be more suitable for this study.

As the reviewer concerns, the number of root traits measured in our study is relative small. However, traits used in this study represent key aspects of root morphology (i.e., diameter, root tissue density), chemicals (i.e., C and N fractions) and anatomy (i.e., EC) that are closely related to resource acquisition in absorptive roots. Further, our perspective is supported by the results of three suits of root trait relationships (root N-root tissue density, root tissue density-root C fractions, root EC-root C and N fractions). As such, we feel that our perspective is reasonable and ecologically meaningful.

In the revised version, we haven't covered root EC in the 'introduction' section. The reason for this is that the main focus of the introduction is on effects of root diameter on root economics strategies. Root EC emerges as a proxy for the size of root diameter. Therefore, we feel that it may be proper to introduce the term 'root EC' in the 'material and method' section, and there we also explain why we introduced this new term (Line 196-199). On the other hand, we feel that the reviewer's suggestion of giving the term of root EC in the introduction section may lie in the fact that there are other types of plant species, i.e., monocots, for which the area of root stele is much larger than the area of root EC (see the reviewer's comment below). Unfortunately, our study did we haven't included such species in our study. However, the reviewer's suggestion of these species is important for extrapolation of our results. Therefore, we have added some new text on this topic in the Discussion (Line 358-364). Further, in the summary section we explicitly suggest that future studies should stress on species such as monocots (Line 391).

In this study, we haven’t included mycorrhizal colonization rate. Despite mycorrhizal colonization rate can be quantified to some extent (i.e. the percentage of root length or total number of roots infected with mycorrhizal fungi), it is very difficult to accurately determine resource acquisition rate through mycorrhizal fungi. Additionally, there are usually many different mycorrhizal fungi species even in a single absorptive root which also adds to hardness of precisely determining resource acquisition through this pathway. Therefore, mycorrhizal colonization rate may not be a very meaningful trait in our studies on root economics strategies.

We haven’t included SRL in our analysis. This is because diameter-related root traits including SRL and root anatomical structures have been found to be closely correlated and constitute a key ecological dimension for absorptive roots (see Kong et al. 2014). However, in responses to your comment,, we have now included a figure showing the relationship between SRL and thickness of root EC for absorptive roots for the current study and 96 species for our previous study (see Fig. S6 of the revised manuscript). The relationships are both strong and consistent for the two dataset. In the relationships, SRL co-varied with root EC in thin roots whereas SRL remained constantly small for thick roots. SRL is also a trait related to resource absorption in roots. Therefore, the small SRL of thick roots as well as limited root branching (Baylis 1975; Fitter 2004 in New Phytologist) may suggest these roots may depend less on roots for resource absorption and nutrient foraging but instead depend more on symbioses with, for example,, i.e., mycorrhizal fungi, for nutrient foraging. This idea has been supported by some early and recent studies (St John 1980; Eissenstat et al. 2015; Liu et al. 2015). The different foraging strategies for thin and thick absorptive roots may suggest them potentially having different economics strategies when foraging nutrient.

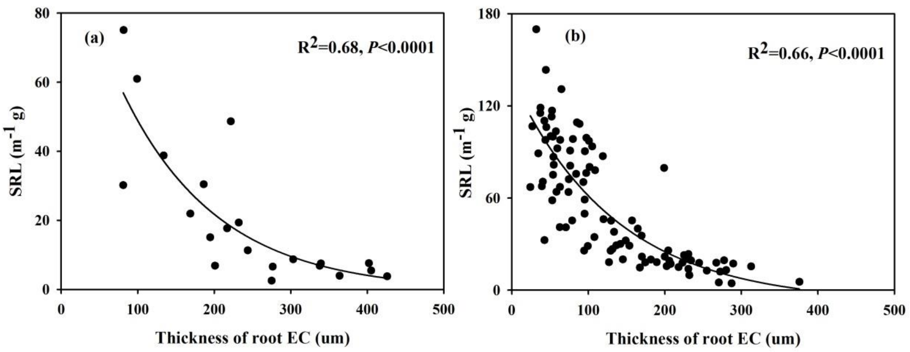

Fig. S6 Relationship between specific root length (SRL) and thickness of root EC for data of absorptive roots in the current study (a) and our previous study (Kong et al. 2014) (b). The relationship is fitted by exponential regression for each dataset.

Finally, we highly appreciate the valuable comments from the reviewer. Considering the reviewer’s concerns, in the summary section we suggest that more traits and trait relationships as well as more species should be investigated in future studies to further the ideas proposed in our study.

Specific comments *Choice of the measured root traits. It is surprising that for absorptive roots (distal part of root system including apices) the authors did not measure specific root length or root surface area, nor mycorrhiza colonization, traits considered to be linked with resource acquisition whereas the chosen traits (anatomy, chemical) are more related to transport or construction cost. How can you estimate acquisition strategy with such traits?*

Root tissue density is more related to construction cost of tissue (mainly stele, see Wahl & Ryser 2000) and not to resource acquisition.

Response: The anatomical and chemical traits apart from SRL and mycorrhizal colonization are also key traits closely related to resource acquisition in plant roots. These traits have been shown to form a trait syndrome, the diameter-related trait dimension or ecological axis (Kong et al. 2014). Therefore, the traits we examined in this study, although not including all root traits, may represent key aspects of resource acquisition and preservation in absorptive roots.

We admit that root tissue density is a trait directly reflecting construction cost of root tissues. However, the construction costs may be associated with root lifespan (see Eissenstat et al. 2000. 'Building roots in a changing environment: implications for root longevity'). For example, following cost-benefit theory, it is not economic for roots with higher construction cost to live shorter. Therefore, roots with higher construction cost may have longer lifespan while roots with lower construction cost may have shorter lifespan. On the other hand, root activity may also be affected by root tissue density (i.e., Picon-Cochard et al 2012. Plant and Soil, 353:47-57). For example, higher root tissue density may result from thickened cell walls or more secondary tissues which can cause reduced root activity. Therefore, we argue that root tissue density can influence resource acquisition and preservation and hence is a key trait of root economics strategies.

Regarding, SRL and mycorrhizal colonizaition, see our response to the general comments of the reviewer.

Root diameter in driving root trait spectra. Comments on two sentences given page 13044, line 21-22: "Traits syndrome for thicker absorptive roots would differ from the predictions of faster acquisition and shorter lifespan"; and page 13044, line 23-24: "This highlights the importance of discriminating the thicker for the thinner absorptive roots when exploring root strategies". I agree but this is because in case of your species thick roots have higher proportion of cortex than thin roots while for other species including monocots this is the opposite. What is then important is the proportion of cortex in the surface area, more than the diameter per se. Thus the link between diameter and lifespan is not applicable.

Response: Our study unfortunately did not included species like monocots with stele size larger than cortex size. The size of root diameter has been found to be contributed more by the size of cortex than stele in many species (see Gu et al. 2014 in Tree Physiology, Kong et al. 2014 in New Phytologist, and the current study). However, in species like monocots, the size of stele rather than cortex dominates the size of root diameter or root cross section area. Even though there is no much known about root lifespan in monocots, we speculate that thick roots in monocots may also have longer lifespan than thin roots because the construction of thick roots are usually more costly than the construction of thin roots. Therefore, it is possible that monocot roots also show a positive diameter-lifespan relationship but with the slope or R square different than the included in our study.

For species like monocots, the stele rather than the cortex dominates the size of root diameter or root cross-section area. In the steles of monocots, besides the vascular conduits there are many parenchyma cells that may serve the storage function and potentially alter root trait relationships. However, as there is few data in this regard, we canno't make further inference. Anyway, we appreciate these valuable comments. In the revised manuscript, we have taken these species into account in the discussion section (Line 358-364).

Furthermore, the presence of mycorrhiza in thick roots also changes the capacity of the roots to uptake nutrients, independently of their morphology. Thus defining a RES with/without mycorrhiza should be explored.

Response: It has been acknowledged that thick roots depend mainly on mycorrhizal fungi for resource acquisition (see Baylis 1975, Kong et al. 2014, Eissenstat et al. 2015 in New Phytologist). The great dependence on mycorrhizal fungi may be one of the reasons of no acquisition-conservation tradeoff in thick roots. However, we know little about how mycorrhizal fungi alter the trait relationships in these thick roots. Unfortunately, assessment of resource acquisition through mycorrhizal fungi is beyond the scope of our current study. See also our response to the general reviewer comment. In the revised version, we also advocate that mycorrhizal fungi in thick roots should be emphasized in future studies for better understanding the nature the ‘non-economic’ strategy in thick absorptive roots (Line 394-396).

Page 13044, line 24-25: Contrary to the sentence, the effect of root diameter in driving root traits spectra has been tested in monocots (see Drouet et al 2005. European Journal of Agronomy, 22:185–193 ; Picon-Cochard et al 2012. Plant and Soil, 353:47–57; and see Zobel. 2003. New Phytologist, 160:276–279).

Response: We thank the review for providing these important references. We note these papers have explored the effects of root diameter on root trait spectra. However, roots used in these study do not all belong to absorptive roots, and hence comparisons would be not be ecologically meaningful. For example, thick roots in Picon-Cochard et al. (2012) referred to shoot-born roots which may be similar to the higher-order roots in this study.

As in our previous studies, there is significant heterogeneity between these shoot-born roots and root-derived roots (roots produced from shoot-born roots), and the shoot-born roots are less active than root-born roots, see Kong et al. (2010 Plant and Soil). We speculate that the shoot-born roots in Picon-Cochard et al. (2012) may not be the dominant parts of absorptive roots or be weakly absorptive relative to the abundant and active root-derived roots. Therefore, although root diameter effects have been explored in monocots and other species (i.e., Prieto et al., 2015), roots in those studies may not be the real absorptive roots or the dominant part of absorptive roots. Anyway, we appreciate reminding of studies in monocots.

In the revised version, we have edited the sentence and use “aware of few previous” instead of “unaware of any previous” in the corresponding sentence (Line 75-76).

Methods. Page 13046, line 6-12: precise if all species hold mycorrhiza

Response: Correct. Yes,the species examined in this study hold mycorrhiza and we have added this information in this line (Line 121-122).

Page 13047, line 1-2: Precise if the roots collected in plastic bags were washed or not before or after freezing. This is important for chemical analyses.

Response: Root samples for chemical analyses haven’t been washed when they were put in plastic bags and transported in a cooler. Before chemical measurements, root samples were washed in deionized water. The procedure of root sampling and collection followed previous studies, i.e., Pregitzer et al. (2002 Ecological Monographs) and Guo et al. (2008 New Phytologist). In the revised manuscript, we have added more detailed information on how we processed the root samples (Line 134-136).

Page 13047, line 7: The type and company of the stereomicroscope should be given

Response: we have added information of the stereomicroscope in the revised manuscript.

Page 13048, line 1-2: determination of absorptive roots should be developed a bit even always described earlier.

Response: We have added detailed information for determination of the absorptive roots in these seven species (Line 163-166).

Page 13048, line 25: "root EC": why there is no link with hypotheses?

Response: In light of the first hypothesis, we test different economic strategies with root diameter. Here, we use root EC to indicate the size of root diameter. We then separate the thin and thick absorptive roots according to the thickness of root EC. In the sentences following line 25, we have given reasons for using of root EC in this study. In this way, the 'root EC' could be indirectly related to the hypothesis in this study.

Page 13049, line 9: 247_m for root EC: have you tested the normal distribution of fig S1a, because it seems there are 2 groups, 250-300_m being in the middle.

Response: Yes, we do test the normal distribution of the data in Fig. S1a. The statistical test shows that the frequency distribution in Fig. S1a has no difference from normal distribution (P=0.995). In other words, they follow exactly the normal distribution. We have also supplied this information in the revised manuscript (Line 207-208; Line 224-225).

Page 13049, line 16: Moving average analyses should be more described as there are different methods

Response: We have added detailed information about procedures used for moving average analysis in the revised manuscript (Line 213-216).

Page 13049, line 17: a point is missing between fit and No.

Response: We appreciate this careful comment on this error. We have added a point between fit and No.

Results. thin vs thick absorptive roots: thick roots do not follow the same pattern as thin one: in conclusion can you consider that thick roots are still absorptive roots? The use of RES is not correct in your work (see comments above).

Response: Our results show that thick absorptive roots may not follow similar patterns as the thin absorptive roots. However, these thick roots are still thick absorptive roots as indicated by their anatomical structures. For these thick roots, they have been reported to have a different nutrient foraging strategy, i.e., depending mainly on mycorrhizal fungi rather than roots themselves.

Fig S3: different symbols between thin and thick should be shown

Response: In the revised manuscript, Fig. S3 in previously submitted version has been changed to Fig. S4. In this figure, we have provided relationships between extractive C fraction and root tissue density for both thin and thick absorptive roots, using different symbols for thin and thick roots. Furthermore, we have also added a figure for the relationship between the recalcitrant C fraction (sum of acid-soluble and acid-insoluble fractions) and root tissue density. The new figure (Fig. S4) more clearly shows the difference between thin and thick roots in these trait relationships.

Discussion. Page 13052, line 8-10: fig S1 shows distribution of root EC thickness for your species and previous work, but the two distributions seem to be different not similar. The comparison of your dataset with previous studies (supplementary material) raises more questions than answers. For example, fig S1: the two distributions seem different.

Response: We respectfully disagree with the reviewer that comparison of our dataset with previous studies raises more questions than answers. Yes, the distributions of root EC are different for species of the current study and the previous study. Presentation of the two different distributions mainly aims to explain why cutoff points between thin and thick absorptive roots are different for the two datasets. Root EC of our current study follow a normal distribution, and then we use the average root diameter (root EC=247 μm) as the cutoff point. While for the data of 96 species from our previous study, they follow a skewed normal distribution with a bias towards thin absorptive roots. In the case of skewed normal distribution, the cutoff point based on average of root EC may cause bias on separating thin and thick absorptive roots. Therefore, for this dataset, we use a cutoff point (root EC=182.8 μm) corresponding to the transition of mycorrhizal colonization. As such, the frequency distributions are used to justify selection of different cutoff points for the two datasets.

Although we provide limited approaches to separate thin and thick absorptive roots, a range of difference for the two root groups has increasingly been revealed (i.e., Baylis 1975; St John 1980; Eissenstat et al. 2015; Liu et al. 2015). The difference of economic strategies between thin and thick absorptive roots may add further evidence for the claim of different entity for the two root groups. On the other hand, we acknowledge that there is no commonly accepted cutoff point to separate thin and thick absorptive roots. We hope new ways to discriminate the two root groups may be developed in future studies.

Reviewer #2

General comments

This is an interesting study on the relationships between root diameter and root strategies for resource acquisition. This study is based on seven contrasting tree species from tropical and subtropical forest, and a range of root traits to test (1) the influence of root diameter on the root economic spectrum and (2) the influence of root branch order on root C and N fractions. The gradient of plant trait variation, called economic spectrum, has been found world-wide describing the existence of a fundamental tradeoff between acquisition and conservation of resources in plant species. However, our knowledge of below-ground trait variation and their economics remains limited and inconsistent (Chen et al., 2013; Bardgett et al., 2014; Poorter et al., 2014; Reich, 2014). Consequently, the aim of this study is very relevant. But the authors only used 7 seven three species from tropical and subtropical forests, which is inadequate and quite ambitious to extent this study to the root economic spectrum as indicated in the title. The choice of plant species and root traits are justified but this study will gain in interest with more vegetation types to test the root economic spectrum as announced by the title. More chemical traits implied in root absorption would have been appreciated to test the hypothesis *and to gain more insight of root absorption strategies for nutrient capture as expected. The authors wanted to demonstrate the importance of the cortex and epidermis thickness in the root absorption strategy, which seem to be an important root trait for future research in root ecophysiology. Although this study is interesting, it does not correspond to the title. This manuscript is well written but some more proofreading would have been appreciated to avoid few mistakes. Consequently, some parts should be rewrite and correct to improve the quality of the manuscript.*

Response: We appreciate these pertinent comments on our manuscript. As the reviewer concerns, it is a bit ambitious to test the idea of root economics spectrum using only a few plant species and root traits. This weakness of a few species included has been appreciated in our study. To overcome the weakness, we reanalyze a dataset of 96 species from one of our previous studies. Results of this reanalysis are largely consistent with our current study. Therefore, results of the two datasets both support our hypothesis of different economic strategies for thin and thick absorptive roots. See also our responses to Reviewer #1.

After carefully consideration of the reviewer's concerns, we feel that the conclusion of our previous version is too strong. In this revised version, we have toned down our statements (see, for example, Lines 33-36, 389-390) and we have adopted reviewer's suggestion for a more appropriate new title: '*Economic strategies for plant absorptive roots vary with root diameter*'. This new title better reflects the scope of our study. Furthermore, in the current version, we argue that results of our study present an instructive perspective for understanding economics strategies in absorptive roots rather than a final conclusion on the existence of the root economics spectrum. We hope the findings of this study are interesting to stimulate more future research in this field by including more species and root traits.

We apologize for errors in grammar, phrasing and citations in previously submitted version. We have carefully checked in the revised version.

Specific comments

Page 13043, line 6: It would have been appreciated to read more details on the studied vegetation in the abstract. Could the authors specify which kind of plant species are considered in this study and where they come from ?

Response: We have added the information in the revised version (Line 27).

Introduction is clear but few references are missing in the 'Reference' section, while more references would have been appreciated to justify the choice of root traits.

Response: We have supplied some important and recent reference on root traits, i.e. Roumet et al. (2006), Bardgett et al. (2014), Eissenstat et al. (2015).

Material and Methods are too concise and sometimes informal. Some parts of the 'Material and Methods' section should be rewrite to improve the clarity of the work realized.

Response: We note that some parts of the "Material and Methods" are too few. We have supplied more detailed information in this section (see, for example, Lines 163-166, 169-172, 184-186, 213-216). We are grateful for reminding of the missing information. Further, we have revised much of text of the Materials and Methods in order to improve clarity. A revised version with tracked change has been provided to make clear the changes we have made.

Methods use to separate thin and thick roots should be better explained and easy to reproduce to gain in interest and to ensure the repeatability of this work among studies.

Response: We have thoroughly revised the Methods section, better explaining how we separated between thin and thick roots (see, for example, Line 223-233).. We also give a detailed response to a similar question following this one (see the latter part of this response letter).

In addition, some important details are missing to gain in clarity on the representativeness of the root subsamples used for root trait measurements.

Response: We feel that the reviewer may concern about procedure for root chemical measurements. In our study, root samples were ground not by hand but by an automatic mill (ZM200, Retsch, Germany) and well mixed for homogeneity before chemical measurements. We have added this information in revised manuscript (Line 169-172).

In addition, I suggest to use the passive form and remove few parts of the 'Statistical analysis' paragraph to the Results section to improve the quality of the text.

Response: We follow this suggestion and use the passive form in the section of Statistical analysis. However, respectfully, we decided to not move 'the few parts in this section' to the 'Results' section. We feel that these parts present details of the methods and our arguments for employing these statistical analyses. These are not the real *results* after the data analyses. Anyway, we appreciate the reviewer for this comment.

Page 13046, line 22: Could the author specify the root mass or fraction of subsample collected to gain more insight of the subsample representativeness.

Response: We appreciate reminding of details for preparing root samples before chemical measurements. We did not measure the exact weight of the subsamples, but we are confident that the subsamples were representative. In our study, root samples have been ground not by hand but by an automatic mill (ZM200, Retsch, Germany) and well mixed for homogeneity for chemical measurements. We have added the information in the revised version (Line 171-172).

Page 13047, line 8: It is very surprising to measure the root length with a tape whereas high efficient image software would have been more precise to analyses the root length and the root diameter. Could the authors justify this choice?

Response: The authors thank for the suggestion of "high efficient image software" method for root length measurements. In this study, we only used a measuring tape for measuring length of relatively long roots.. For relative short roots, we used a stereomicroscope with an ocular micrometer (±0.025 mm). We have added this information in this corresponding sentence (Line 142-143). The combination of using a stereomicroscope and measuring tape has been commonly used in previous studies (e.g., Guo et al. (2008_New Phytologist).

Page 13047, line 24 : This work is very long and impressive, I suggest to insert root slices pictures of the seven species in Supplement.

Response: Great suggestion! We have supplied some pictures to show the absorptive and non-absorptive roots. See the new Fig. S1. Here, we have provided pictures for *E. chinense* and *C. chinensis*, and for pictures of the other species we refer to Long et al. (2013).

Page 13048, line 1 : As the study deals on root order and thin vs. thick roots, it would have been appreciated to briefly describe the determination of absorptive roots.

Response: We have revised this sentence and given t more detailed information on the classification of absorptive roots (Line 163-166).

Page 13048, lines 4 - 21 : Only two fractions are defined in the Introduction (labile vs. recalcitrant fractions). Could the author unify the terms used in the introduction with the following parts to gain in clarity?

Response: Thanks for the suggestion. As pointed out by the reviewer, only two fractions of root carbon are referred to in the introduction while three root carbon fractions are measured in the chemical analyses section. To improve clarity, we have classified extractive C as the labile C fraction and the other two C fractions, the acid-soluble and acid-insoluble C fractions, as the recalcitrant fraction. We have added a sentence to explain this in the Methods section (Lines 184-186).

Page 13048, lines 10 - 13 : Parenthesis are missing.

Response: Corrected.

Page 13048, line 17 : Please, could the authors correct the sentence.

Response: We have corrected the sentences.

Page 13048, line 25 and Page 13049, line 21 : It is also very surprising to introduce a new root trait and new set of plant species at the end of this Material & Method section. It would have been appreciated to present the additional plant species in the 'Plant species and sampling site' section.

Response: In this study, we have introduced a new root trait, root EC referring to the tissues outside the stele including root cortex and epidermis. We have also given explanations for the using of this trait in our study (see the text for details).

Regarding the ‘additional plant species’: We feel it is not appropriate to introduced this set of plant species in the “Plant species and sampling site” section. This is because these additional species were sampled as part of our previous study (Kong et al. 2014). In the current study, we *reanalyzed* them from this previous study to validate our results.

Page 13049, lines 9 and 23 : The cutting point between the thick and thin absorptive roots should be introduced earlier in the text. This study will gain in clarity by better explaining how thin and thick absorptive root are determined, and by using a common cutting point between the studied plant species and the additional set of 96 plant species. Could the authors explain why the cutting point was not similar between the two set of plant species ?

Response: The reason for the different cut-off points is the different frequency distribution of the two datasets. For species of the current study, root EC follows a normal distribution, while for the 96 species of the previous study, data of root EC follows a skewed normal distribution with a bias towards thin root species. In the case of skewed normal distribution, using the average of root EC as the cutoff point may cause bias on separating thin and thick roots. Therefore, we used 182.8 µm root EC as a cutoff to separating thin and thick roots for the 96 species. This cutoff point also corresponds to the functional transition from lower to higher mycorrhizal colonization when increasing root diameter (see Kong et al. 2014; Eissenstat et al. 2015; Liu et al. 2015). See also our response to the comments of reviewer #1.

In addition, we note that there has been no commonly accepted cutoff point to separate thin from the thick absorptive roots. In this study, separation of thin and thick roots is based on frequency distribution as well as root mycotrophy. The methods used here represent one kind of strategies to discriminate the two root groups. We also hope that more convenient and precise ways will be developed in future studies.

Results section are too concise and would have been easier to understand by presenting first the effects of plant species on the measured root traits before presenting the root strategies and root trait relationships. In addition, it would have been appreciated to see the regression lines on the Figures presenting root traits relationships, and a multivariate analysis to better synthesize the results and to clearly understand the trade-offs between root strategies presented in this study.

Response: We appreciate these comments by the reviewer. However, the results actually track the main findings of this study, first describing the results related to Hypothesis 1 and then the results related to Hypothesis 2. As such, we are somewhat hesitant changing the order. In the revised version, we have added regression lines in the figures. We appreciate the suggestion of multivariate analyses. However, multivariate analyses are often based on linear relationships. This may not be suitable for traits of chemical fractions that are usually in non-linearly relationships with root tissue density. On the other hand, although multivariate analyses could somehow synthesize the findings of our study, we feel that adding more results would make the manuscript too long. In our study, the bivariate trait relationships are arranged as three pieces of support for our perspective of different economic strategies for thin and thick absorptive roots. Anyways, we appreciate these valuable suggestions.

Page 13050, lines 14 and 19 : What does 'medium', 'higher' and 'lower' mean ? Please, could the authors specify the thresholds used ?

Response: The "lower", "medium" and "higher" refer to root tissue density. They are used to indicate relative size of root tissue density. Here, we do not aim to clearly present definition of "lower", "medium" and "higher" root tissue density. These terms are used for comparisons only in discussing relationships of root C fractions with root tissue density (see, for example, Line 302-306 in the discussion of the revised version).

Supplement, line 20 : It is very surprising to modify the dataset. Please, could the author explain why they removed some points to arrange the results ?

Response: Two outliers are removed in analysis of thick absorptive roots for the dataset of 96 species. This is because the relationship between root tissue density-root N concentration in these thick roots is greatly influenced by the two outliers. For example, the relationship is significant (R^2=0.24, P=0.01) when including these two data points, but not significant (R^2=0.025, P=0.45) when excluding them.

These values may represent rare cases and can lead to inflated error rates and distoration of statistic estimates and as such inappropriately affect the overall results. We have added these justifitions to the supplemenatry information.

Discussion : Conclusions of this study seem to be highly influenced by the methods used to separate thin and thick roots, and the definition of C and N fractions as well, which imply to better define these traits in the 'Introduction' and 'Material and Methods' sections.

Response: As in the revised "statistical analyses" section, we clearly justify the separation of thin and thick absorptive roots. Although there are no commonly accepted criteria for classifying thin and thick absorptive roots, we feel that our method to separate these roots may not greatly influence conclusion of this study. This is because reanalysis of the previous 96 species also demonstrate different trait relationships between the two root groups. Therefore, our perspective, despite based on results of a relative few species, may not be a biased but rather a common rule. Moreover, it has been revealed recently that thin and thick absorptive roots do follow different nutrient foraging strategies: the thin ones depend mainly on roots themselves and the thick ones depend on mycorrhizal fungi (see Eissenstat et al. (2015_New Phytologist), and Liu et al. (2015_New Phytologist)). The different foraging strategies for the thin and thick absorptive roots suggest that they potentially have different economics strategies when foraging for nutrient.

Our definition and measurements of these C and N fractions has been used in previous studies, i.e., Fan and Guo (2010) and Xiong et al. (2013). These chemical fractions are physiologically and ecologically important (see the introduction section). For example, the recalcitrant fractions are energy costly in chemical synthesis and are usually used for structural tissues such as cell walls, vascular conduit and fibers. For plant organs like leaves and roots, greater investments in recalcitrant fractions can result in them less active (see Feng et al. (2009 PNAS), Eissenstat and Achor (1999)). In this study, our discussion on these chemical fractions and hence the perspective on root economic strategies are based on these commonsense of chemical fractions. Therefore, the definition of C and N fraction may not greatly affect our conclusion.

Page 13050, lines 20 - 27 : Discussion of the root traits relationships should be better supported by showing the regression lines, which are not obvious to see on the presented figures.

Response: We have added the regression lines for these figures.

As there are many questions from the two reviewers (see the above response letter), we have not prepared a list of all the change we made in the revised manuscript. All the relative change in the revised manuscript can be found in our point by point response to the reviewers. The lines where we have made a change are also indicated in the response letter. Additionally, the change for this revised version can be found in the following marked-up manuscript.

Economic strategies for plant absorptive roots vary with root diameter~~The root economics spectrum: divergence of absorptive root strategies with root diameter~~

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Abstract

Plant roots ~~usually~~ typically 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 economic strategies ~~as predicted from the RES shift~~ differ with increasing root diameter. To test this hypothesis, we used seven ~~contrasting~~ 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 ~~diameter~~) and thick roots. 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 significant relationships among root traits indicating an acquisition-conservation tradeoff~~that trait relationships~~ for thin absorptive roots ~~followed the expectations from the RES~~ while no ~~clear~~ such trait relationships were found – ~~in support of the RES~~ for thick absorptive roots. Similar results were found when reanalyzing data of a previous study including more species. ~~Our results suggest~~ 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. ~~divergence of absorptive root strategies in relation to root diameter, which runs against a single economics spectrum for absorptive roots.~~

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 ~~an~~ acquisitive strategies~~y~~, 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 ~~a~~ conservative strategies~~y~~, 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 ~~only a~~ 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 ~~(RES)~~ 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

~~Catherine 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 ~~the~~ thicker ~~from the~~and thinner absorptive roots when exploring root strategies. However, we are ~~un~~aware of ~~any previous~~few studies that have tested for effects root diameter in driving ~~root~~ 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.,

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cellulose and lignin in structural tissues) (Atkinson et al., 2012; Berg and McClaugherty, 2008;

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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 have 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;

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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 economics strategiesspectrum (RES) in absorptive roots. We hypothesized that the root economic strategies diverge differ between thinner and thicker absorptive roots, with trait relationships indicating a trade-off between acquisitive and conservative trade-off traits for thinner roots but not 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~~y~~ 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°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 ~~Long's study (Long et al., 2013) and~~ 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

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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)~~ with the most terminal branch as the first-order~~. 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 ~~a~~ 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, ~~T~~the root diameter was measured under a 40× or 20× stereomicroscope (MZ41-2B, MshOt, Guangzhou, China)~~depending on root size~~. 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.,

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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)~~following the procedure of Long's study (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~~se~~ 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). ~~The determination of a~~Absorptive roots in a root branch were~~as~~ 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's study~~ (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).

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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 sub~~milled powder~~ sample 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 ~~of~~ 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% × $(m_0-m_1)/(m_0-m_3)$, 100% × $(m_1-m_2)/(m_0-m_3)$, and 100% × $(m_2-m_3)/(m_0-m_3)$, 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.

~~After acid-digestion, a~~An about 5mg subsample of residue left after the above acid-digestion procedure was ~~taken~~ 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,

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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, for root tissue, 'root EC' which referred 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)'s study (Kong et al., 2014), respectively). Second, root EC can be used as an indicator of root chemical compositionounds as the storage of root labile C and most of root N are 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. We used Aa 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. S21a). To avoid the influence of biological N fixation on relationships between root N and root tissue density and root EC, a legume species, *A.*

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auriculiformis, was excluded in these analyses. In addition, ~~for the thin absorptive roots,~~ the relationship~~s~~ between the extractive C fraction and root tissue density ~~were~~ was further explored by a quadratic polynomial regression using moving average data ~~analysis~~(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, ~~we further used the~~ 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 ~~they~~ 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. S2~~1~~b)~~, and hence lower mycorrhizal colonization~~ ~~(Kong et al., 2014)~~. 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, ~~we used~~ 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) ~~which is thinner than in our current study~~. The thickness of 182.8 μm for root EC corresponded to a

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transition from lower to higher ~~of~~ 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 ~~strategies~~ 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~~2~~).

For ~~the~~ thin absorptive roots, the extractive C fraction peaked at medium root tissue density (Fig. 2a). Moving average analysis ~~showed~~ revealed a quadratic ~~regression~~ relationship ~~of~~ between the extractive C fraction ~~with~~ and root tissue density in ~~these~~ thin absorptive roots

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(Fig. S~~3~~4a), 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 ~~each~~ 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~~4~~). 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~~s~~ (Fig. 5b,c). For all species, root N concentration ~~in all species~~ 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 ~~that we found in our study provides~~ supports ~~for~~ the existence of~~a~~ ~~root~~ economic~~s~~ ~~spectrum (RES)~~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

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resource acquisition (Kong et al., 2010; Mommer and Weemstra, 2012; Reich et al., 2008). However, our results ~~also~~ further showed that the negative relationship between root tissue density and root N concentration ~~existed only~~held for ~~in~~ thin ~~absorptive roots,~~ but not ~~in~~ 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 ~~using~~ 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 ~~This indicates that the conventionally recognized RES~~economics strategies for the thin and thick absorptive roots.~~may be confined to thin absorptive roots only, as such supporting our first hypothesis.~~ ~~The divergence of absorptive root strategies with root diameter was further supported by the~~ 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 ~~C~~ and more recalcitrant C fractions in these roots. In contrast, ~~for~~ 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~~In fact~~, for thin absorptive roots we found ~~ound~~ an inverted U-shaped relationship between the labile~~, extractive~~ C fraction and root tissue density (~~Fig. 2a, S2~~Fig. S4a) and a U-shaped relationship between ~~.~~ ~~As for~~ recalcitrant C fractions (acid-soluble C + acid insoluble C) and root tissue density (Fig. S4b). ~~in thin absorptive roots,~~ The higher acid-soluble C fraction~~the acid-insoluble C fraction~~

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~~peaked at lower but not at higher root tissue density which seems to contradict with the expected inverted U-shaped curve. However, the acid-soluble C fraction peaked~~ ~~at~~ with increasing ~~higher~~ root tissue density (Fig. 2b) suggest ~~. As such, it could be~~ 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 ~~would require more energy~~ than for the production of cellulose (Novaes et al., 2010). ~~Therefore, with increasing root tissue density, recalcitrant C fractions in thin absorptive roots may follow a pattern opposite to that of labile C fractions. As such, the patterns of labile and recalcitrant C fractions in thin absorptive roots are in support of RES theory~~. 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. ~~On the other hand, for thick absorptive roots, their lower tissue density was accompanied with higher extractive and lower acid-insoluble C fraction, possibly because of storage of labile C in their thick cortex (Long et al., 2013; Lux et al., 2004). These patterns of root C fractions for thick absorptive roots run against expectations from the RES. Therefore, our study shows that thick absorptive roots may follow a strategy different from that for thin absorptive roots.~~

Furthermore, observed relationships between thickness of root EC and root C and N fractions provide~~s~~~~d~~ ~~indirect~~ the third piece of ~~evidence~~ support for our ~~contention~~ hypothesis of different economic strategies ~~divergence of ecological strategy~~ with root diameter. Across

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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~~1~~), ~~which is consistent with~~and ~~the RES theory~~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 ~~hence~~ no clear economic strategies~~acquisition-conservation trade-off as predicted by the RES~~ for thick absorptive roots.

~~Therefore, relationships between thickness of root EC and root chemical fractions provide~~

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further evidence for the idea of divergence of root strategies between the thin and thick absorptive roots which may be underpinned by different mechanisms.

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. -in thin and thick absorptive rootsThese findings- may havehas important implications for the emerging debate on the plant root economics spectrum. For example, Although the existence of an economics spectrum strategies for plant roots (RES) has been commonly recognized 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 shown contrasting findings, suggestingby showing no RES (Chen et al., 2013) or positive (Kong et al., 2014) relationships between root diameter and root N concentration (Kong et al., 2014). Although there may be other mechanisms, oOne possible explanation for the conflicting findingsa lack 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.trade-offs between acquisitive and conservative root traits in these studies is the inclusion of thick absorptive roots which could have altered root trait relationships. On the other hand, the lack of evidence of an acquisition-conservation tradeoff may have resulted from the larger proportion of root

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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 ~~we~~ provided only ~~found~~ 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 ~~for~~ 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

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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 ~~revealed for the first time divergence of absorptive root strategies and~~ different patterns of root chemical fractions with root diameter and root order. ~~Specifically, the axis of the RES dominated in thin absorptive roots, while thick absorptive roots did not seem to be constrained by an acquisition-conservation tradeoff.~~ The ~~different~~ contrasting economic strategies between thin and thick absorptive roots ~~for the two groups of 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 ~~should~~ may test to what extent our results hold for other (groups of) plant species (i.e. monocots), include a larger spectrum of ~~more~~ 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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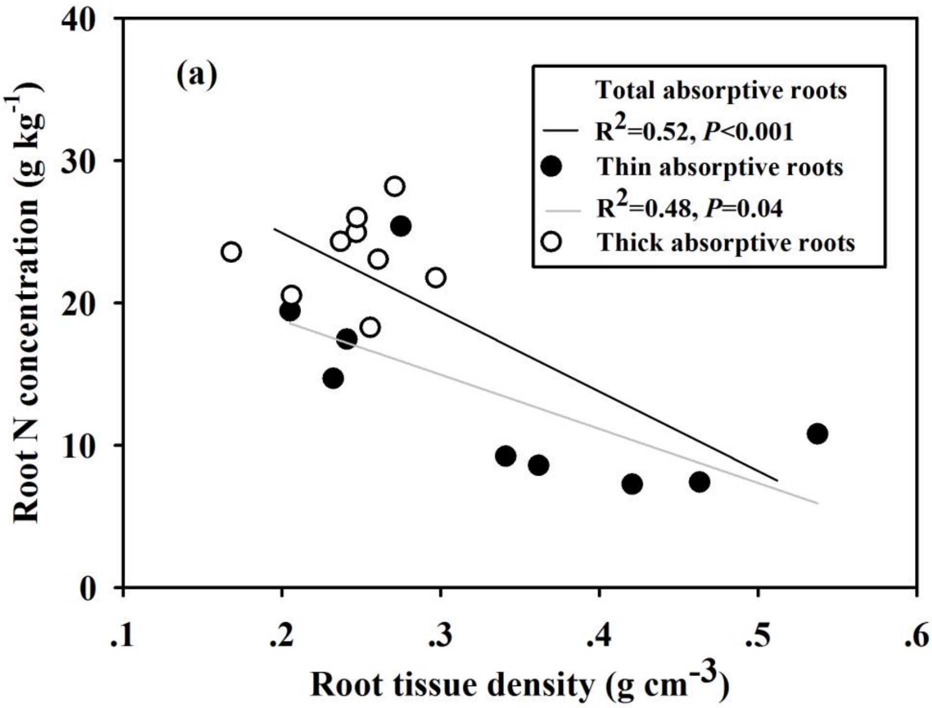
Table 1. *F* values of two-way ANOVAs testing effects of plants species and root branch order on the extractive C fraction, acid-soluble C fraction, acid-insoluble C fraction, N concentration, and N in acid-insoluble C fraction. *, **, *** were significant level at 0.05, 0.01, 0.001, respectively.

	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***

Figure legends

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



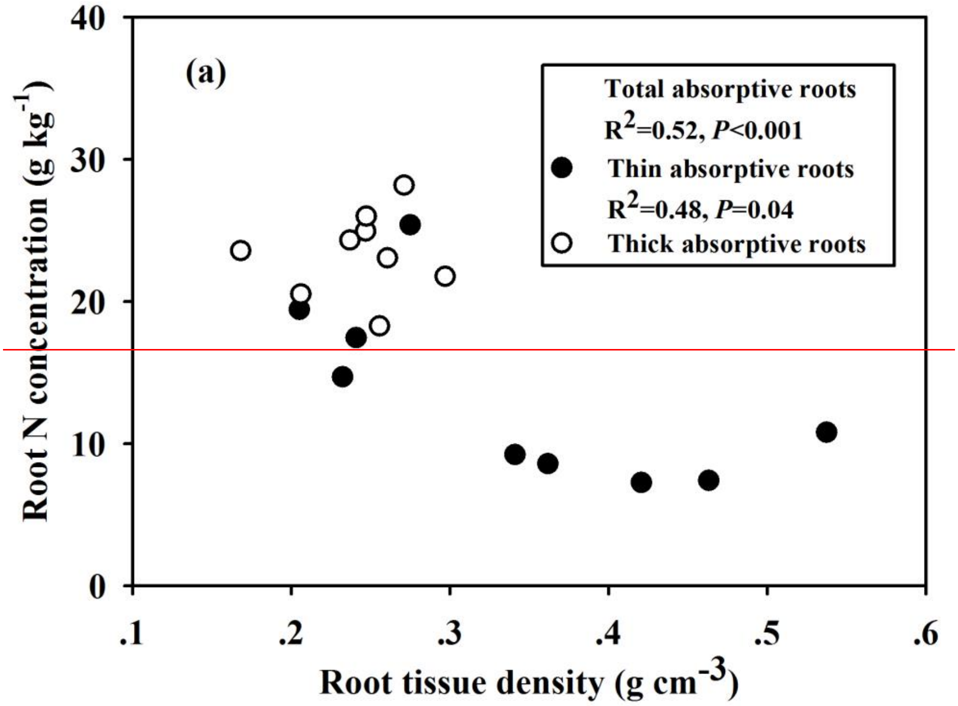

(a)
Total absorptive roots
R2=0.52, P<0.001
Thin absorptive roots
R2=0.48, P=0.04
Thick absorptive roots
Root N concentration (g kg-1)
Root tissue density (g cm-3)
0
10
20
30
40
.1
.2
.3
.4
.5
.6

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

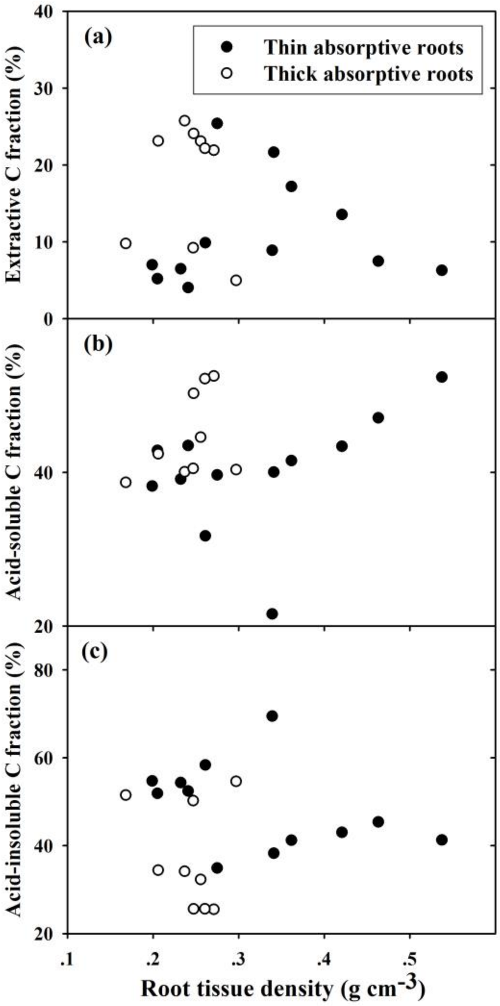
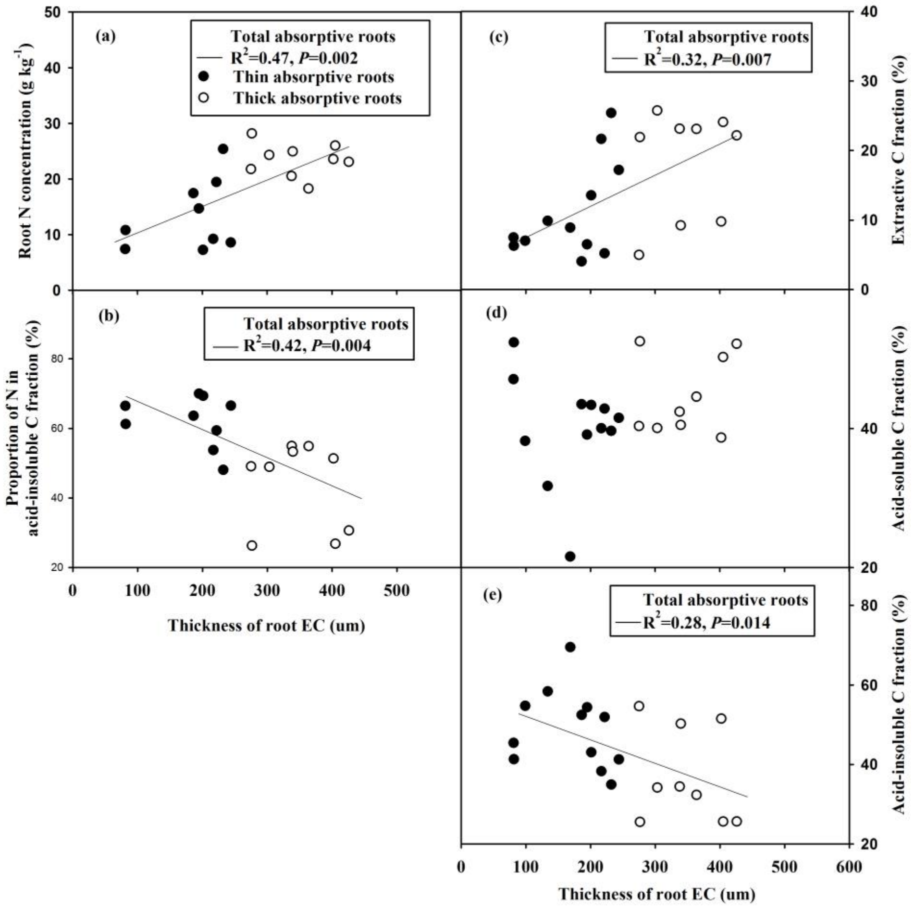
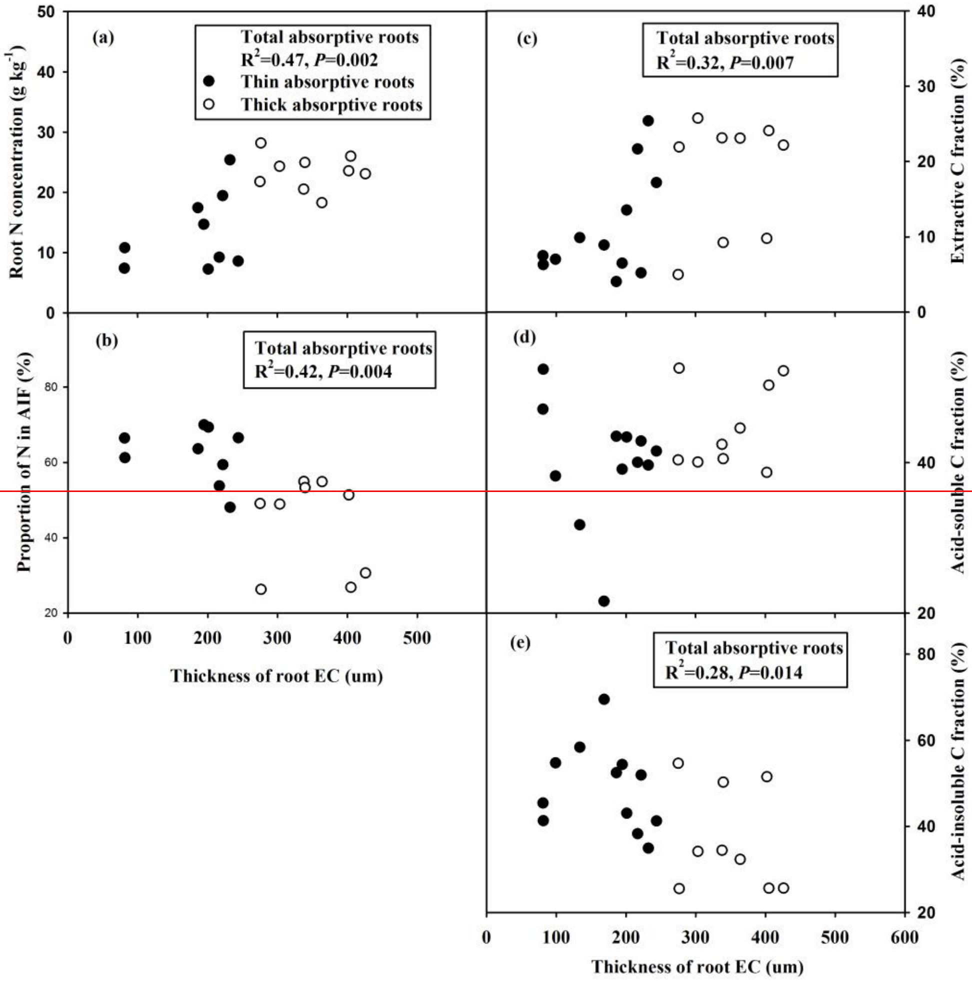


Fig. 3 Relationships between thickness of root EC and root N concentration (a), N in acid-insoluble C fraction (b), extractive C fraction (c), acid-soluble C fraction (d) and acid-insoluble C fraction (e) for total (black line), ~~the~~ thin (solid circles) and thick (open circles) absorptive roots.

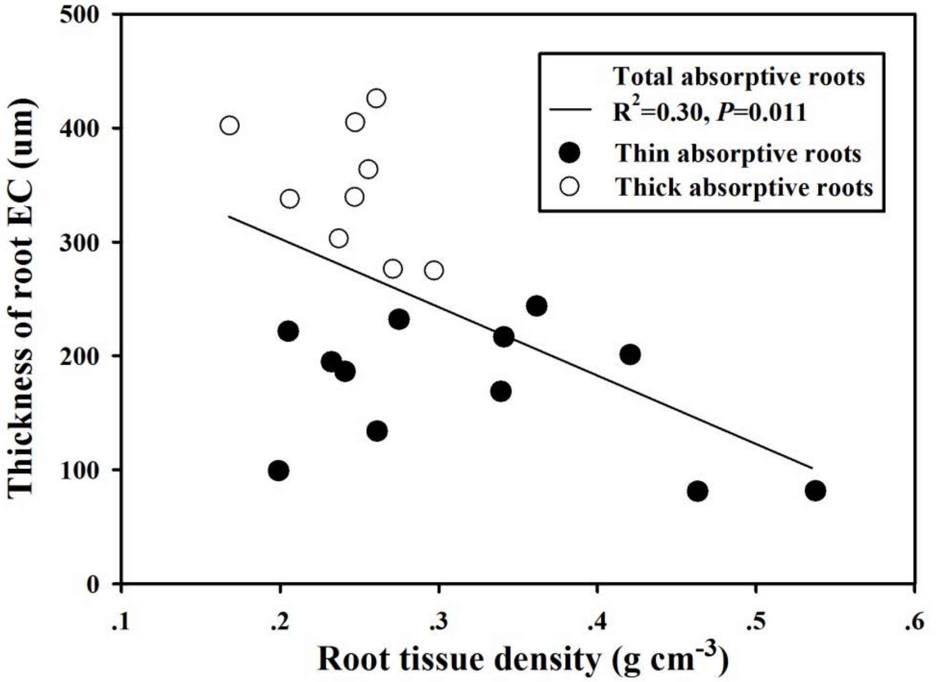


1160 

(a)
Total absorptive roots
R²=0.47, P=0.002
Thin absorptive roots
Thick absorptive roots
Root N concentration (g kg⁻¹)
(b)
Total absorptive roots
R²=0.42, P=0.004
Proportion of N in AIF (%)
Thickness of root EC (um)
(c)
Total absorptive roots
R²=0.32, P=0.007
Extractive C fraction (%)
(d)
Acid-soluble C fraction (%)
(e)
Total absorptive roots
R²=0.28, P=0.014
Acid-insoluble C fraction (%)
Thickness of root EC (um)

1161

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



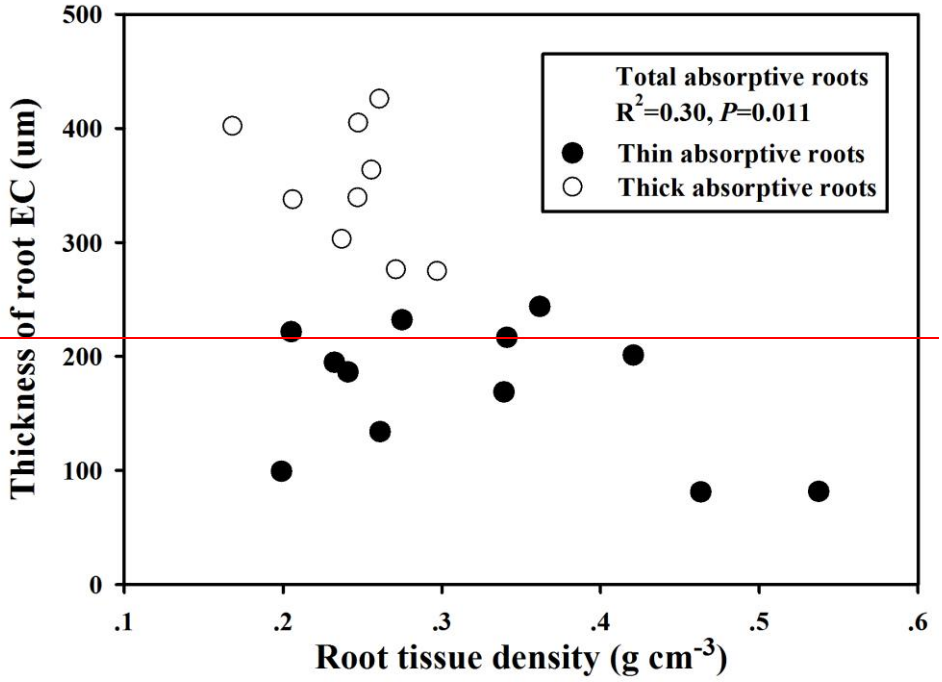

Total absorptive roots
R2=0.30, P=0.011
Thin absorptive roots
Thick absorptive roots
Thickness of root EC (um)
Root tissue density (g cm-3)
500
400
300
200
100
0
.1
.2
.3
.4
.5
.6

Fig. 5 The~~ree C fractions,~~ extractive C fraction (a), acid-soluble C fraction (b) and acid-insoluble C fraction (c) for the first five ~~, among different~~ root orders ~~in~~ for each of seven plant species. R1-R5 ~~were~~ refer to the first to ~~the~~ fifth ~~root branch~~ order.~~–~~

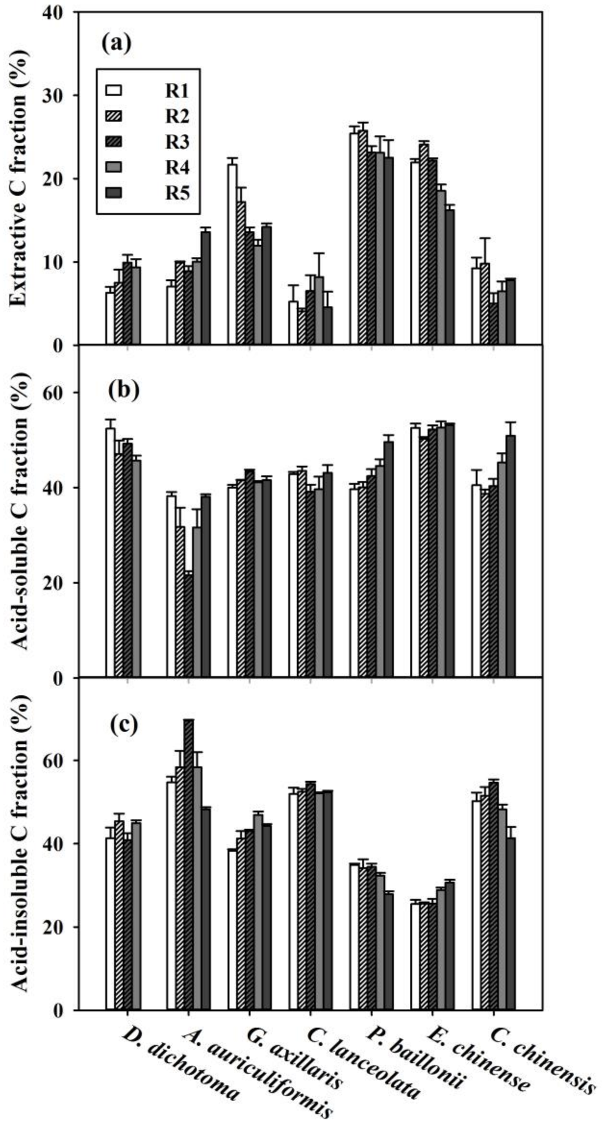
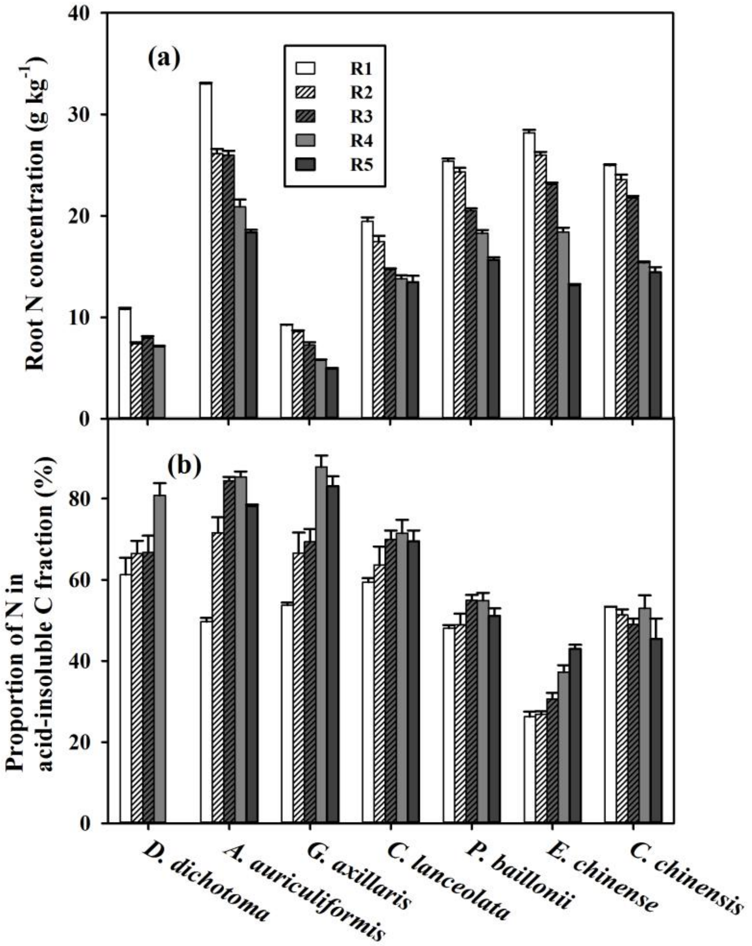


Fig. 6 Root N concentration (a) and N in acid-insoluble C fraction (b) for the first five root branch ~~among different~~ orders for each of seven plant species. R1-R5 ~~were~~ refer to the first to ~~the~~ fifth ~~root branch~~ order.



Supporting information

Fig. S1 Root cross-sections for *Cryptocarya chinensis* (Lauraceae) (a, order 1; b, order 3; c, order 5) and *Endospermum chinense* (Euphorbiaceae) (d, order 1; e, order 3; f, order 5). The cortex and stele for root order 1 and order 3 and the secondary xylem for root order 5 are indicated by arrows.

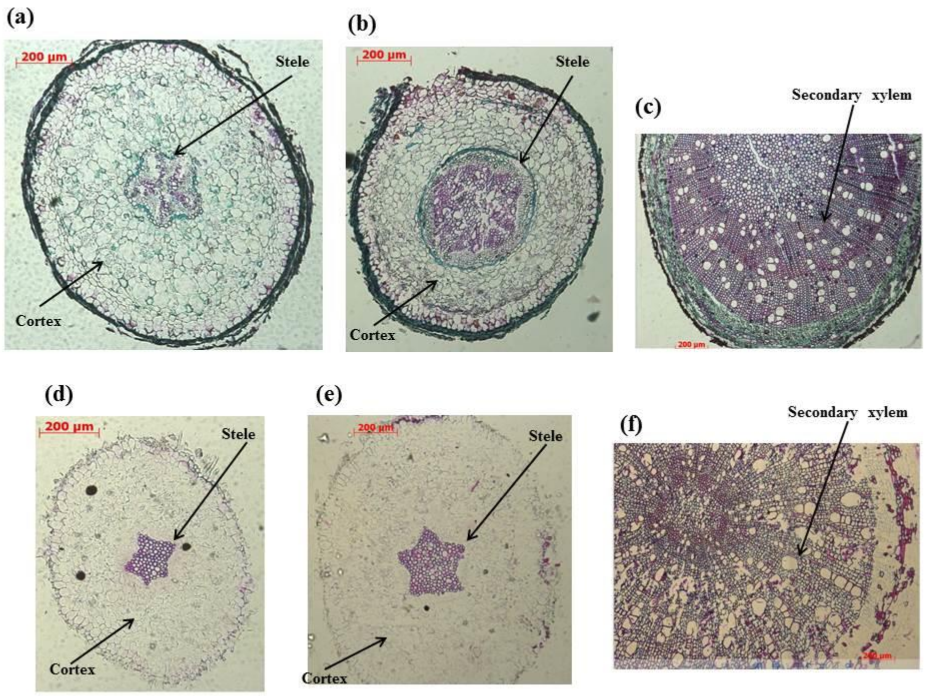


Fig. S2 Frequency distribution of thickness of root EC for absorptive roots in the current study (a) and a previous study (Kong et al. 2014) (b). Root EC is defined as the tissue outside the stele including the epidermis and the cortex.

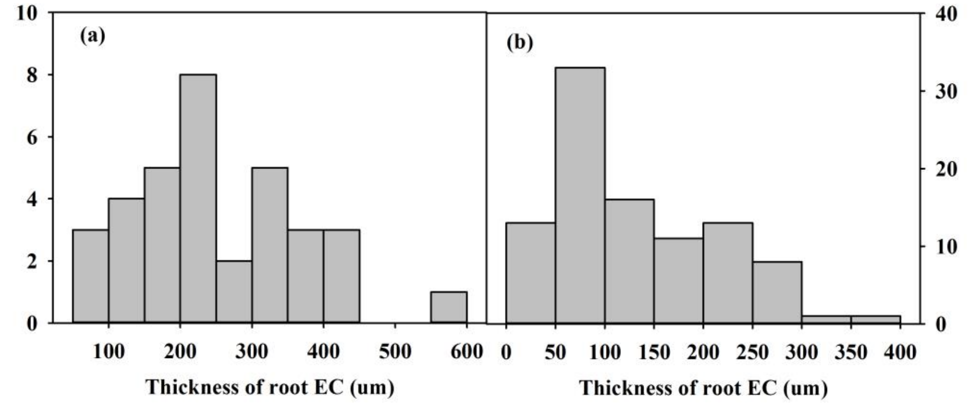


Fig. S3 Relationships between root tissue density and root N concentration for total (black line), thin (solid circles, grey line) and thick (open circles) absorptive roots. Data are from 96 species recalculated from Kong et al. (2014). For the thick absorptive roots, two outlying values are identified because such relationship is significant when they included (R^2=0.24, p=0.01) and nonsignificant when they excluded (R^2=0.025, p=0.45). These two values may represent rare cases and can lead to inflated error rates and distoration of statistic estimates and as such inappropriately affect the overall results. Therefore, the two outlying values are excluded for regression analysis of the thick absorptive roots.

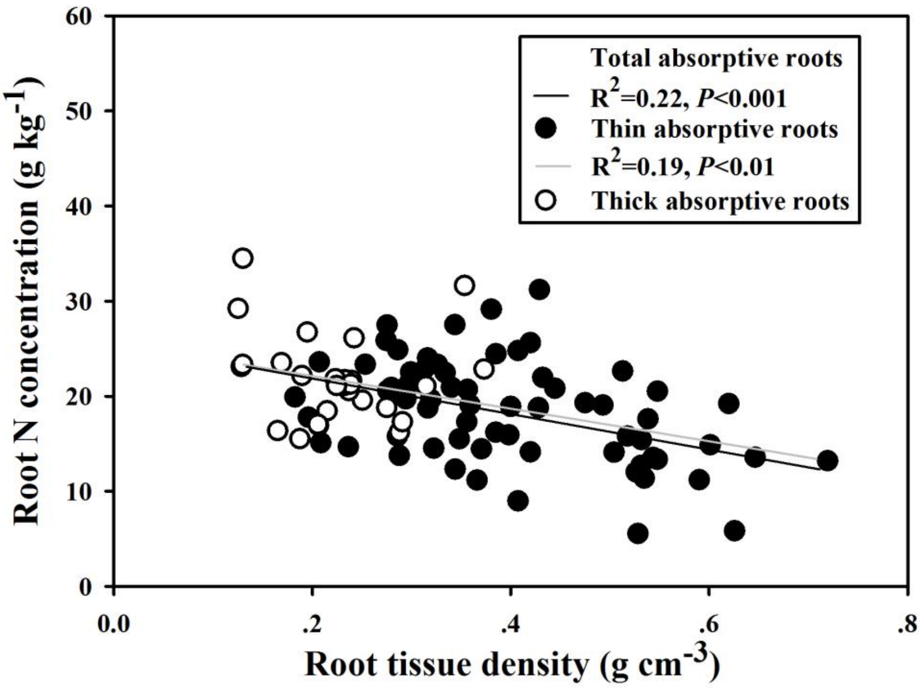


Fig. S4 Quadratic relationships between the extractive C fraction and root tissue density (a) and between the recalcitrant C fraction and root tissue density (b) for thin (solid circles, black line) and thick (open circles, grey line) absorptive roots using moving average data. The recalcitrant C fraction is the sum of the acid-soluble C fraction and the acid insoluble C fraction.

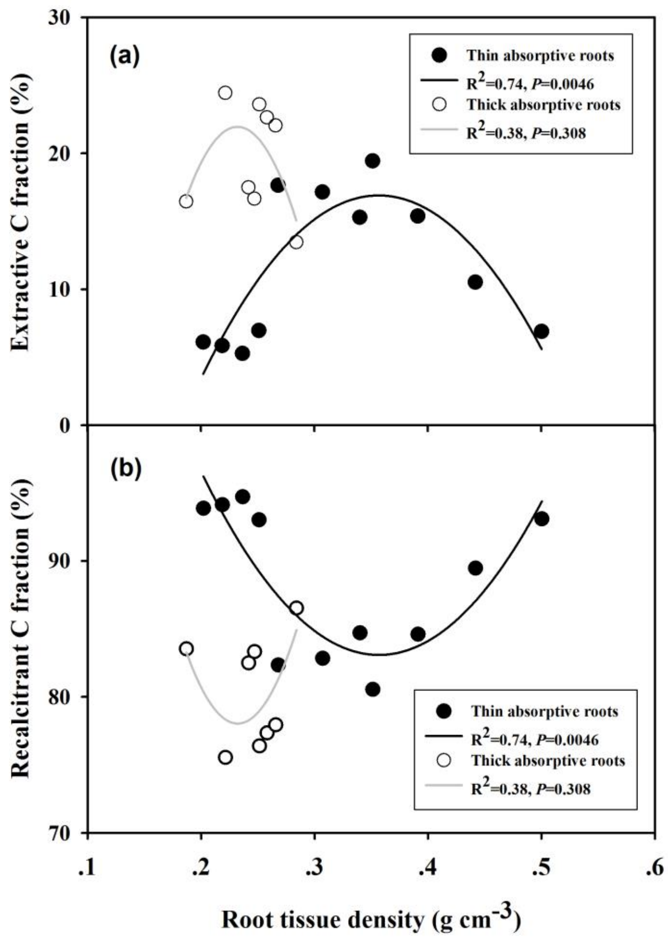


Fig. S5 Relationships between root tissue density and thickness of root EC over the total (black line), thin (solid circles, grey line) and thick (open circles) absorptive roots. Data are from 96 species recalculated from a previous study Kong et al. (2014). Root EC is defined as the tissue outside the stele including the epidermis and the cortex.

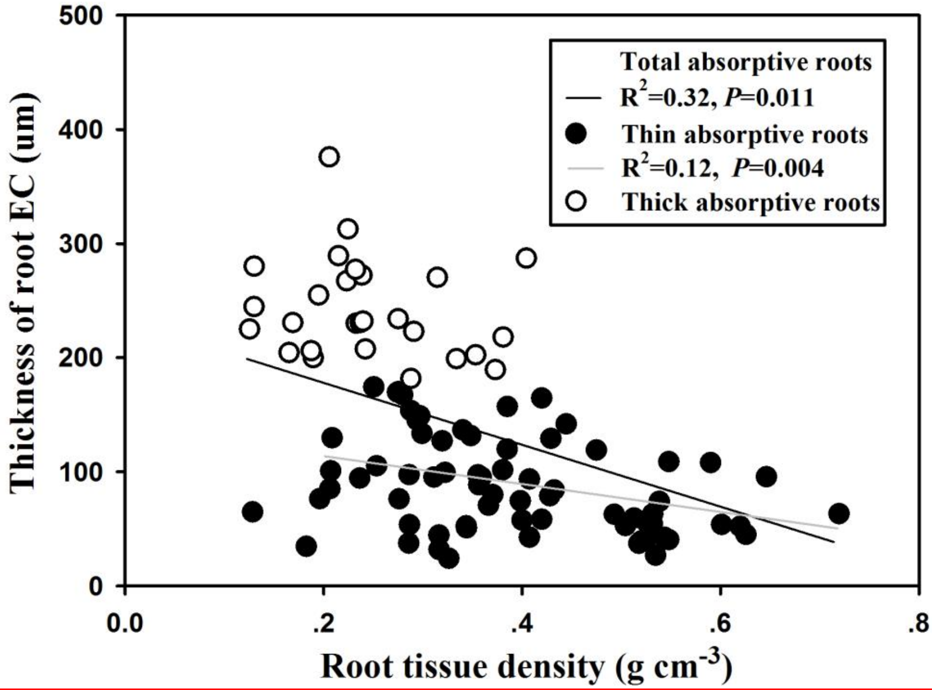


Fig. S6 Relationship between specific root length (SRL) and thickness of root EC for data of absorptive roots in the current study (a) and a previous study (Kong et al. 2014) (b). The relationships are fitted by exponential regressions.

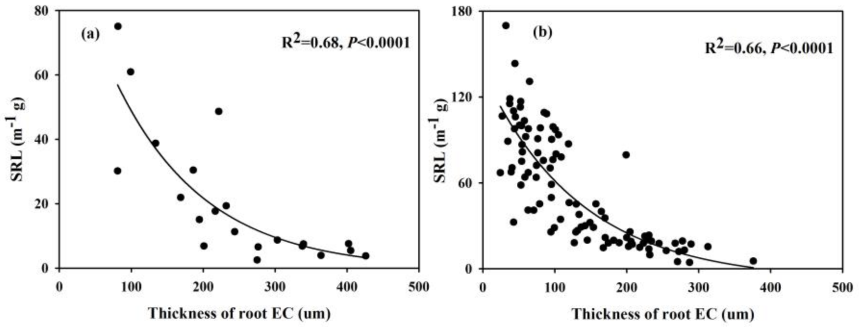


Table S1 Summary of root morphology and anatomical traits for each of the seven plant species used in our study. Data are presented as mean value with standard error in parentheses. Root EC refers to the tissue outside the stele including the epidermis and the cortex in absorptive roots. SRL = specific root length.

Plant species	Root order	Root diameter (μm)	Root tissue density (g cm^{-3})	Thickness of root EC (μm)	SRL (m g^{-1})
D. dichotoma	1	196.80(9.01)	0.60(0.05)	70.5(3.10)	75.1(3.94)
	2	255.59(20.94)	0.47(0.06)	81.06(5.16)	30.18(4.47)
	3	412.34(27.99)	0.50(0.08)	119.45(7.25)	13.31(2.38)
	4	623.32(128.96)	0.50(0.04)	169.86(19.09)	6.78(0.68)
A. auriculiformis	1	286.47(12.46)	0.22(0.02)	98.81(4.85)	60.96(5.4)
	2	362.03(18.26)	0.27(0.03)	134.19(9.51)	38.78(2.62)
	3	509.85(34.16)	0.34(0.06)	168.68(16.51)	21.93(1.98)
	4	552.44(22.39)	0.33(0.03)	160.63(13.40)	6.36(0.82)
	5	852.78(29.42)	0.35(0.03)	146.21(0)	2.47(0.31)
G. axillaris	1	539.9(15.82)	0.36(0.02)	216.76(5.43)	17.68(1.66)
	2	630.63(20.14)	0.37(0.02)	242.84(9.46)	11.31(0.99)
	3	659.87(32.32)	0.43(0.03)	150.6(19.45)	6.86(0.65)
	4	687.50(19.21)	0.60(0.04)	201.07(23.67)	3.70(0.34)
	5	1289.20(75.31)	0.57(0.04)	161.12(22.05)	1.17(0.12)
C. lanceolata	1	558.09(18.42)	0.21(0.02)	221.51(8.28)	48.68(4.25)
	2	488.53(12.37)	0.25(0.02)	186.1(6.53)	30.43(2.85)
	3	532.01(21.27)	0.24(0.02)	194.69(9.81)	15.08(1.57)
	4	773.20(48.83)	0.31(0.03)	235.91(34.07)	7.24(0.51)
	5	1071.33(42.59)	0.26(0.02)	236.28(18.40)	2.98(0.23)
P. baillonii	1	574.50(14.78)	0.28(0.03)	232.07(6.18)	19.33(1.15)
	2	745.19(31.45)	0.24(0.02)	301.8(11.55)	8.71(0.39)
	3	866.27(40.11)	0.21(0.02)	337.76(15.79)	6.83(0.38)
	4	1021.15(79.76)	0.26(0.04)	363.79(23.80)	3.94(0.33)
	5	1672.37(236.49)	0.24(0.02)	550.6(34.15)	2.3(0.24)
E. chinense	1	748.89(39.21)	0.28(0.02)	266.12(16.59)	6.57(0.31)
	2	1133.34(57.74)	0.25(0.02)	405.84(26.84)	5.45(0.41)
	3	1240.00(46.05)	0.27(0.02)	426(22.00)	3.77(0.2)
	4	2065.00(107.3)	0.31(0.02)	341.5(25.01)	2.74(0.2)
	5	2460.00(229.35)	0.29(0.02)	364(12.89)	0.56(0.15)
C. chinensis	1	982.23(27.63)	0.20(0.03)	339.17(11.75)	7.51(1.15)
	2	1133.75(89.98)	0.25(0.03)	275(16.47)	7.57(0.4)
	3	1170.00(67.21)	0.49(0.02)	393.19(24.46)	2.51(0.48)
	4	1815.72(179.61)	0.36(0.02)	347.15(73.75)	1.61(0.33)

	5	2766.67(120.19)	0.33(0.03)		353.34(20.47)	0.70(0.27)