

# Nitrogen and phosphorus allocation in leaves, twigs, and fine roots across 49 temperate, subtropical and tropical tree species: a hierarchical pattern

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## Summary

1. Hierarchical branching is a fundamental feature of trees. Understanding how tree architecture is linked to tissue nutrient concentrations, metabolic rates, and life cycles is important for predicting ecosystem processes such as respiration, module turnover, and organic matter decomposition.

2. Here, we examined branch order–nutrient relationships in above- and belowground tree branching systems by analysing nitrogen (N), phosphorus (P), and N : P ratio in leaves, the first three orders of twigs, and the first five orders of roots in 49 tree species, including 21 temperate angiosperm species, six temperate gymnosperm species, and 22 subtropical-tropical angiosperm species.

3. Tissue [N] and [P] declined linearly with increasing twig and root orders across all species. The slope of the linear regression between root order and root [N] was the same between temperate and subtropical-tropical angiosperms, but steeper in angiosperms than gymnosperms. In contrast, root order–[P] relationship differed between biomes but not between phylogenetic groups, probably due to the significantly lower P availability in subtropical-tropical soils. Additionally, the magnitude of change in tissue [N] and [P] between successive shoot and root branch orders was not constant across branching levels. Among all fine root orders, first order root tips had [N], [P], and N : P ratio most similar to those of leaves.

4. These results demonstrate that there is a general inverse order–nutrient relationship in above- and belowground modules, though specific patterns of this relationship differed between N and P, and between species groups. Moreover, among all root orders within the fine root guild, root tips are the best parallels of leaves in nutrient concentrations. The order–nutrient relationships presented here advances our understanding of functional module construction in trees, and provide a basis for modelling tissue chemistry-regulated processes such as respiration and decomposition in forest ecosystems.

**Key-words:** architecture, branch order, carbon and nutrient cycles, fine roots, leaves, nitrogen, phosphorus

## Introduction

Roots generate a network in their development. Large woody roots branch into smaller laterals, which further branch into ephemeral non-woody roots. In mature trees, root branching networks can extend laterally to tens of meters and consist of roots that are very fine (< 0.5 mm) at the distal ends to coarse (> 200 mm) at the root base. This

hierarchical branching allows trees to simultaneously solve multiple challenges such as anchorage, storage, and uptake (Robinson, Hodge & Fitter 2003).

The functional significance of the hierarchical branching in large trees is apparent and well-recognized at the whole root system level, but not so at the fine root level. Fine roots, commonly defined as roots < 2 mm in diameter, are often treated as a homogeneous mass compartment. This approach ignores the distinct branching structure of fine root systems (Pregitzer 2002; Pregitzer *et al.* 2002). By dividing individual root

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segments of fine root systems into different branch orders according to their architectural position, Pregitzer *et al.* (2002) showed in nine temperate trees that root diameter, specific root length, and root nitrogen concentrations ([N]) varied systematically with branch order. Other studies have also shown that branch orders differed in turnover rates, with first order root tips turning over annually (Majdi, Damm & Nylund 2001; King *et al.* 2002; Pregitzer 2002; Wells, Glenn & Eissenstat 2002; Withington *et al.* 2006; Guo *et al.* 2008b) but higher order roots (e.g. fourth and fifth order roots often with a diameter of > 0.5 mm) living for multiple years (Gaudinski *et al.* 2001; Tierney & Fahey 2002; Matamala *et al.* 2003; Guo *et al.* 2008b). Thus, roots of different branch orders play different roles in belowground carbon (C) and nutrient cycling. Additionally, root anatomy varied with branch order such that lower orders (order 1–3) are generally limited to primary development and perform mainly uptake functions, whereas higher orders (fourth or above) often undergo secondary development and perform mainly storage and transport functions (Guo *et al.* 2008c). Clearly, hierarchical branching is a fundamental feature of not only the whole tree root system, but also the fine root branching system.

In this study, we focused on the relationship between branch order and root nutrient concentrations. Because fine root nutrients are tightly related to C and nutrient flux through root respiration (Ryan *et al.* 1996; Pregitzer *et al.* 1998), root mortality (Wells & Eissenstat 2001; Joslin *et al.* 2006; Guo *et al.* 2008b) and decomposition (Silver & Miya 2001), elucidating root order–nutrient relationship may deepen our understanding of how branch order regulates belowground C and nutrient cycling. Whereas it is generally recognized that root branch order is inversely correlated with root [N] (Pregitzer *et al.* 1997, 2002; Guo, Mitchell & Hendricks 2004), many questions regarding order–nutrient relationship are not resolved. In all twelve temperate trees examined thus far, root [N] decreased with branch order (Pregitzer *et al.* 1997, 2002; Guo, Mitchell & Hendricks 2004), yet whether this pattern is ubiquitous across tree species, whether this pattern applies to other elements, e.g. phosphorus (P), and whether there are quantitative relationships between root branch order and nutrient concentrations need to be tested with a wider range of tree species.

Understanding nutrient variations by branch order may also shed light on how the relationship in nutrients between fine roots and leaves should be studied. Although nutrient stoichiometry (e.g. [N], [P], and N : P ratio) above- vs. belowground has often been examined by comparing leaves with fine roots (McGroddy, Daufresne & Hedin 2004; Craine *et al.* 2005; Newman & Hart 2006), recent studies have begun to question the validity of such an approach due to the heterogeneity among fine root segments on the same branching system (Newman & Hart 2006; Withington *et al.* 2006). It has been suggested that in the fine root system, only distal lateral root branches are similar to leaves in terms of resource acquisition and longevity (Wells & Eissenstat 2001; Pregitzer *et al.* 2002; Withington *et al.* 2006). Thus, root tips, instead of the entire fine root system, may have similar nutri-

ent concentrations and metabolic rate (such as respiration rate) to leaves. Understanding whether leaves and root tips, as opposed to leaves and ‘fine roots’, are true parallels in nutrient stoichiometry is important in that it allows us to evaluate the possibility of using leaf nutrients as a proxy of root nutrients at the species and broader level, which may be useful for more accurately parameterizing the belowground component of biogeochemical models.

The objective of this study was to examine the relationship between root branch order and root nutrient (N, P) concentrations in fine root systems and compare this relationship to that in distal shoot systems in forty-nine boreal, temperate, subtropical, and tropical tree species. Specifically, we addressed the following questions: (1) whether declining N and P concentrations from root tips to higher order roots was a general phenomenon and whether parallel patterns could be found in distal shoot systems (leaves and first few orders of twigs); (2) whether the negative correlation between root (twig) order and nutrient concentrations could be described with quantitative relationships, and if so, would this relationship differ systematically between biomes (i.e. temperate vs. subtropical-tropical) and between phylogenetic groups (i.e. angiosperms vs. gymnosperms); and (3) whether root tips represented a better parallel for leaves than fine roots as a whole in N and P concentrations.

## Materials and methods

### SITE DESCRIPTION

This study was conducted at seven forest sites, with six of them located in China and one in Siberia, Russia. These sites covered a latitudinal range of 30° (from 21°N to 51°N), a mean annual temperature range from –2.5 to 21.7 °C, and a mean annual precipitation range from ~400 to 1929 mm. Across these sites, soil nutrients varied markedly: total soil N varied from 0.1 to 0.47%, and total soil P from 0.02 to 0.38%. Of the seven sites, five, i.e. Trans-Baikal region, Bashang, Mt. Baihua, Luliang, and Taihang, were located in the boreal and temperate zone, where we sampled twenty-one angiosperm species and six gymnosperm species. The other two sites, Mt. Dinghu and Xishuangbanna, were located in the subtropical and tropical zone, where we sampled twenty-two angiosperm species. In total, forty-nine species were sampled, of which forty-three were angiosperms and six were gymnosperms. Gymnosperms has a relatively small sample size because (1) fewer gymnosperm tree species occurred in the natural forests we studied compared with angiosperm species; (2) for some gymnosperm species sampled, sparsely branched fine root systems made it difficult to collect sufficient root biomass for chemistry analysis by branch order, particularly for the first two orders, thus these species were excluded from the analysis. The location, climate, forest type, and soil type of each sampling site were listed in Table S1 in Supporting Information. The taxonomic list for all species were presented in Table S2 in Supporting Information.

### PLANT SAMPLING AND DISSECTION

For each species, leaves, intact segments of the distal portion of twig and root branching systems were sampled. Mature leaves and twigs were collected from the top-third of tree canopies, and roots were

excavated from 0–20 cm of soil. Each twig sample included at least three orders and each root branch system included at least five orders. To sample roots, we first identified three trees for each species, and within 2 m distance to the stem of each tree, we used a specially constructed fork (with three teeth, each 20 cm long and 1 cm in diameter and having a pointed tip) to loosen the soil in the sampling area. We then followed root branches to the tree stem to confirm the identity of the species and cut the root branches from the main lateral woody roots. Once collected, root branches were immediately placed on ice in a cooler and transported to the laboratory within a few hours and frozen for later processing. Shoot samples were put into paper envelopes and air-dried. In the laboratory, roots were dissected into different orders as described by Pregitzer *et al.* (2002) with root tips labelled as first order. Twigs were labelled similarly, with the most distal twigs defined as first order. For most species, the first five root orders belonged to fine roots according to traditional definition (<2 mm). The diameters of the fifth order roots fell below 1 mm in twenty-three species, between 1 and 1.5 mm in eighteen species, between 1.5 and 2 mm in three species, and between 2 and 3.5 mm in five species.

#### CHEMICAL ANALYSIS

Samples were oven-dried to a constant weight for 48 h at 68 °C, then grounded to fine powder. Tissue nitrogen was determined with an elemental analyzer (vario EL III Universal CHNOS Elemental Analyzer, Elementar, Hanau, Germany) at the Analytical Instrumentation Center, Peking University. Tissue phosphorus was assessed using a sulfuric acid/perchloric acid digestion and a colorimetric P analysis on a UV-Visible spectrophotometer, with the ammonium

molybdate ascorbic acid method (Kuo 1996). All chemical indices had been expressed on an ash-free, dry-mass basis.

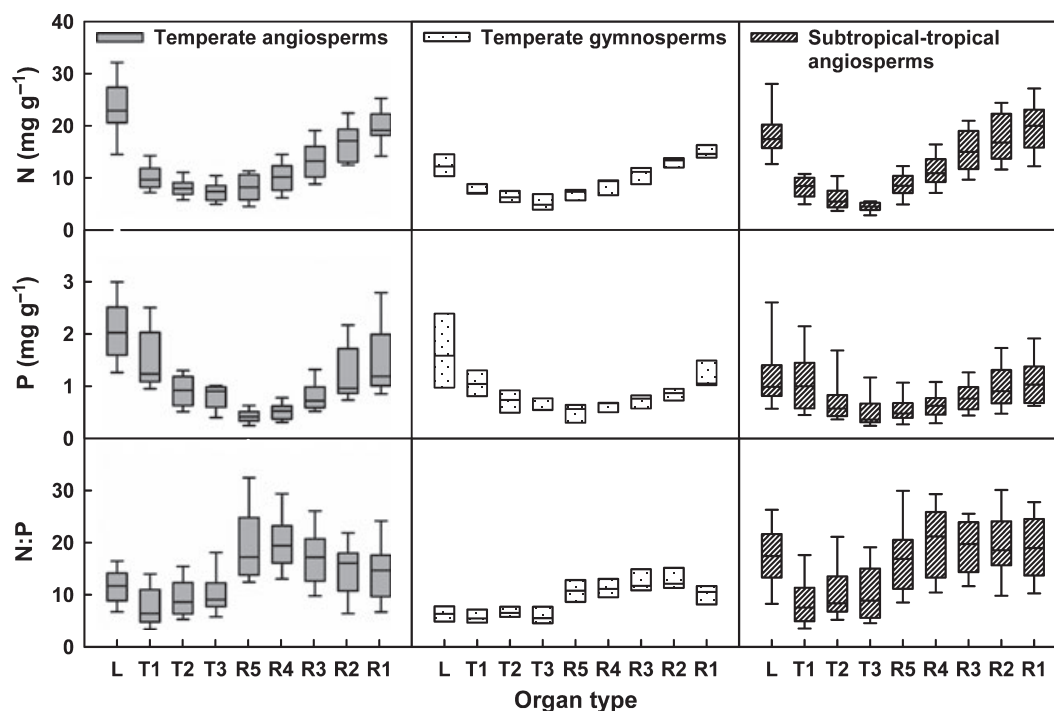
#### DATA ANALYSIS

We classified species into three groups: temperate angiosperms that included twenty-one species, temperate gymnosperms that included six species, and subtropical-tropical angiosperms that included twenty-two species. Since we sampled only two species (both were conifers) from Trans-Baikal region, which is at the southern limit of the boreal zone, we included these two boreal species in the temperate gymnosperm group. We used linear regression analyses to determine the relationship between [N], [P], N : P ratio and branch order in each species, across species of each group, and across all species. Because different branch orders frequently had different variances in [N], [P], and N : P ratio (Fig. 1), weighted least-squares method, a typical method to overcome the problem of heteroskedasticity, was used to adjust the parameters in regression models for each species group and for all species pooled.

We compared order–nutrient relationship between species groups using a multiple regression model that contains a dummy variable to distinguish data from different groups (dummy-variable regression model, Kleinbaum *et al.* 1998). The model used was:

$$Y = \alpha + \beta X + \gamma Z + \delta XZ + \varepsilon, \quad \text{eqn 1}$$

where  $Y$  = nutrient concentration,  $X$  = twig or root order, and  $Z$  was a dummy variable indicating the group to which a species belonged, with 0 representing one group, and 1 representing the other. As the model could be written as:



**Fig. 1.** Box plots showing median and interquartile range for N ( $\text{mg g}^{-1}$ ), P ( $\text{mg g}^{-1}$ ), and N : P ratio of leaf, twig orders, and fine root orders in temperate angiosperms [number of species ( $n$ ) = 21], temperate gymnosperms ( $n$  = 6), and subtropical-tropical angiosperms ( $n$  = 22). Abbreviations: L for leaf; T1, T2, T3 for twig order 1, 2, 3; R5 to R1 for root order 5 to 1.

$$Z = 0 : Y = \alpha + \beta X + \varepsilon \quad \text{eqn 2}$$

$$Z = 1 : Y = (\alpha + \gamma) + (\beta + \delta)X + \varepsilon, \quad \text{eqn 3}$$

the difference between order–nutrient regression equations in comparison can be examined by the significance of  $\delta$  and  $\gamma$ . We used weighted least-squares method to estimate model parameters (Eqn 1) and their significance. When  $\delta$  was not significant, the regression slopes for the two groups were not significantly different (Eqns 2, 3). When both  $\delta$  and  $\gamma$  were not significant, the regression equations for the two groups became statistically identical, meaning that the order–nutrient relationship held consistent between groups.

In addition to linear regression analysis, we calculated the percentage decline of [N] and [P] [N and P decline (%)] from leaves to first order twigs and from distal to successive higher twig and root orders for each species to reveal the detailed patterns of order–nutrient relationship at individual species and species group level. We compared the magnitude of N (P) decline (%) between branching levels in each species group using paired-samples *T* tests, and examined the differences of N (P) decline (%) at the same branching level among different species groups using a one-way ANOVA with Bonferroni *post hoc* tests of significance. Differences between means were considered significant at a *P* value of <0.05.

To explore the relationship between leaf and fine root in nutrient concentrations, we calculated root : leaf ratios in [N] and [P] for all fine root branch orders in each species. To compare the average N : P ratio of leaves with that of the entire fine root guild, we calculated the biomass-weighted average N : P ratio of the first five root orders as an approximate for fine root N : P ratio in each species. All statistical

analyses were performed using spss software (2001, ver. 13.0, SPSS Inc., Chicago, Illinois, USA).

## Results

Tissue [N] and [P] declined from distal to basal locations in above- and belowground branching systems of trees. This general pattern was consistent across biomes, phylogenetic groups, and individual species (Fig. 1; Tables 1, 2).

The general inverse relationship between branch order and tissue [N] and [P] in roots and twigs can be described by linear regressions. For all species pooled, [N] decreased linearly with root order (Fig. 1;  $P < 0.001$ ,  $R^2 = 0.535$ , Table 1). Significant linear relationships were also observed for [P] in roots across all species ( $P < 0.001$ ,  $R^2 = 0.357$ , Table 1). When examined separately for each species group, the linear relationship remained strong between root order and root [N] (all  $R^2 > 0.526$ ), but differences in slopes emerged among the three groups (Table 1). The differences in slopes between species groups were indicated by statistical significance of  $\gamma$  and  $\delta$ , the coefficient of variable *Z* and *XZ*, respectively, in the dummy-variable regressions. For example, when comparing root order–[N] relationships between temperate angiosperms and subtropical-tropical angiosperms,  $\gamma$  and  $\delta$  were not significant ( $P = 0.512$  and  $0.877$ , respectively), but when comparing root order–[N] relationships between each angiosperm group and gymnosperms,  $\gamma$  and  $\delta$  were both significant ( $P = 0.000$  and  $0.019$  in TA vs. TG,  $P = 0.000$  and  $0.038$  in

**Table 1.** Linear regressions between tissue [N], [P], N : P ratio and branch order in roots and twigs for temperate angiosperms (TA,  $n = 21$  species), temperate gymnosperms (TG,  $n = 6$ ), subtropical-tropical angiosperms (SA,  $n = 22$ ), and all species pooled (All,  $n = 49$ ). Std. Errors and *P* values denote the standard error and the significance of regression slopes, respectively

Organ	Nutrient	Group	Intercept	Slope	Std. Error	<i>P</i> value	$R^2$
Fine root	N	TA	2.269 <sup>a</sup>	−0.294 <sup>a</sup>	0.023	< 0.001	0.606
		TG	1.661	−0.195 <sup>b</sup>	0.017	< 0.001	0.798
		SA	2.350 <sup>a</sup>	−0.298 <sup>a</sup>	0.027	< 0.001	0.526
		All	2.220	−0.282	0.016	< 0.001	0.535
	P	TA	1.606 <sup>a</sup>	−0.243 <sup>a</sup>	0.026	< 0.001	0.446
		TG	1.487 <sup>ab</sup>	−0.179 <sup>ab</sup>	0.026	< 0.001	0.598
		SA	1.223 <sup>b</sup>	−0.134 <sup>b</sup>	0.026	< 0.001	0.224
		All	1.465	−0.198	0.017	< 0.001	0.357
	N : P	TA	12.830	1.609 <sup>a</sup>	0.408	< 0.001	0.129
		TG	9.899 <sup>a</sup>	0.702 <sup>b</sup>	0.408	0.095	0.082
		SA	20.045 <sup>b</sup>	−0.328 <sup>b</sup>	0.467	0.485	0.005
		All	15.446	0.662	0.298	0.027	0.020
Twig	N	TA	1.131 <sup>a</sup>	−0.138 <sup>a</sup>	0.035	< 0.001	0.221
		TG	0.870 <sup>a</sup>	−0.117 <sup>a</sup>	0.042	0.014	0.323
		SA	1.037 <sup>a</sup>	−0.201 <sup>a</sup>	0.044	< 0.001	0.314
		All	1.052	−0.156	0.028	< 0.001	0.204
	P	TA	1.644 <sup>a</sup>	−0.305 <sup>a</sup>	0.065	< 0.001	0.281
		TG	1.531 <sup>a</sup>	−0.262 <sup>a</sup>	0.094	0.013	0.327
		SA	1.407 <sup>a</sup>	−0.308 <sup>a</sup>	0.102	0.004	0.171
		All	1.530	−0.294	0.052	< 0.001	0.208
	N : P	TA	7.130 <sup>a</sup>	1.158 <sup>a</sup>	0.661	0.085	0.052
		TG	5.583 <sup>a</sup>	0.552 <sup>a</sup>	0.560	0.339	0.057
		SA	7.534 <sup>a</sup>	1.160 <sup>a</sup>	1.007	0.256	0.029
		All	7.133	1.011	0.527	0.058	0.030

Different superscript letters indicate significant ( $P < 0.05$ ) differences of slopes and intercepts among three species groups (i.e. TA, TG, SA), according to the results of dummy-variable regression analyses.

**Table 2.** Linear regressions between [N], [P] and branch order in roots and twigs at the species level. Statistics of the regression results (i.e. mean value, range, and CV% of slopes, intercepts, and  $R^2$ ) for species within each group, i.e. temperate angiosperms (TA,  $n = 21$ ), temperate gymnosperms (TG,  $n = 6$ ), subtropical-tropical angiosperms (SA,  $n = 22$ ), and for all species pooled (All,  $n = 49$ ) were presented

	Slope			Intercept			$R^2$		
	Mean	Range	CV%	Mean	Range	CV%	Mean	Range	CV%
[N] vs. order									
Root									
TA	-0.30	-0.51–0.17	27	2.28	1.37–3.53	21	0.95	0.84–0.99	4
TG	-0.19	-0.25–0.15	19	1.66	1.34–1.87	11	0.98	0.96–0.99	1
SA	-0.29	-0.46–0.07	37	2.31	1.28–3.28	27	0.92	0.79–0.99	6
All	-0.28	-0.51–0.07	34	2.21	1.28–3.53	25	0.94	0.79–0.99	5
Twig									
TA	-0.19	-0.43–0.01	65	1.29	0.85–1.84	25	0.82	0.04–0.99	34
TG	-0.11	-0.17–0.09	33	0.87	0.71–1.05	16	0.95	0.83–0.99	9
SA	-0.19	-0.29–0.04	45	0.99	0.61–1.43	28	0.79	0.15–0.99	32
All	-0.18	-0.43–0.01	58	1.13	0.61–1.84	29	0.83	0.04–1.00	30
[P] vs. order									
Root									
TA	-0.29	-0.68–0.08	66	1.80	0.90–3.74	50	0.93	0.82–0.99	6
TG	-0.20	-0.36–0.11	52	1.56	1.29–1.93	17	0.90	0.72–0.99	11
SA	-0.14	-0.31–0.01	56	1.23	0.46–2.44	40	0.82	0.00–0.99	32
All	-0.21	-0.68–0.01	74	1.52	0.46–3.74	47	0.88	0.00–0.99	21
Twig									
TA	-0.33	-0.91–0.09	72	1.70	0.73–3.67	45	0.91	0.76–0.99	9
TG	-0.33	-0.64–0.16	67	1.75	1.18–2.71	38	0.98	0.96–0.99	1
SA	-0.31	-1.07–0.09	108	1.39	0.48–4.45	88	0.89	0.72–0.99	10
All	-0.32	-1.07–0.09	81	1.60	0.48–4.45	57	0.91	0.72–0.99	89

SA vs. TG, detailed results not shown). Thus, the slopes for temperate angiosperms (-0.294) and subtropical-tropical angiosperms (-0.298) were similar, but were both steeper than that for temperate gymnosperms (-0.195, Table 1). In comparison, linear relationship between root order and root [P] did not differ between temperate angiosperms and temperate gymnosperms, but temperate angiosperms had steeper slopes than subtropical-tropical angiosperms (Table 1).

Linear relationships between twig order and twig [N], [P] were significant but less strong (Table 1). In addition, both twig order-[N] and twig order-[P] regression equations were similar among the three species groups (Table 1). The regressions between N : P ratio and order was generally not significant, with the only exception of that between N : P ratio and root order in temperate angiosperms (Table 1).

The inverse linear order-nutrient relationships also held at the species level, though with significant species-specific differences (Table 2). The order-nutrient regressions were strong at the species level, as indicated by high  $R^2$  (Table 2), and the regression slopes were negative for all but the twig order-[N] relationship in *Betula platyphylla* (0.01) and the twig order-[P] relationship in *Memecylon nigrescens* (0.09, Table 2), both of which were not significant ( $P = 0.88$  and 0.07, respectively). Among all relationships, root order-[N] relationship showed the smallest variation across species, with lowest CV% for slope (<37%) and intercept (<27%, Table 2).

The magnitude of decline for [N] and [P] between successive shoot and root branch orders varied among branching levels, and species groups. N decline (%) from leaves to first order

twigs averaged 51.9% in all species, that from first to second order roots averaged 10.5% in all species, but that between the other successive twig and root orders was similar and averaged 19.9% (Fig. 2). From leaves to first order twigs, N decline (%) was significantly higher in the two angiosperm groups than in temperate gymnosperms, whereas P decline (%) was higher in the two temperate groups than in subtropical-tropical angiosperms (Fig. 2; detailed results see Table S3 in Supporting Information). From first to second order roots, N decline (%) differed between temperate angiosperms and subtropical-tropical angiosperms (Fig. 2; Table S3). At the other branching levels, the three groups did not differ significantly (Fig. 2; Table S3). P decline (%) showed significant differences among the three groups at many branching levels, with no consistent patterns emerging (Fig. 2; Table S3).

The average root-tip : leaf ratio of [N] for all species pooled was 1.02, with temperate angiosperms (0.88) significantly lower than temperate gymnosperms (1.19) and subtropical-tropical angiosperms (1.12; both  $P < 0.05$ , Fig. 3). The average root-tip : leaf ratio of [P] was 0.84 for all species pooled, with subtropical-tropical angiosperms (1.00) showing statistically similar ratio with temperate gymnosperms (0.80), but significantly greater ratio than temperate angiosperms (0.70;  $P < 0.05$ , Fig. 3). The root-tip : leaf ratios for both [N] and [P] were normally distributed when all species were pooled (Fig. 3;  $P > 0.05$  in Kolmogorov-Smirnov goodness-of-fit test on normal distribution assumption).

N : P ratios of leaves were lower than root tips in two temperate species groups ( $P = 0.008$  for temperate angiosperms, and  $P = 0.021$  for temperate gymnosperms), but were



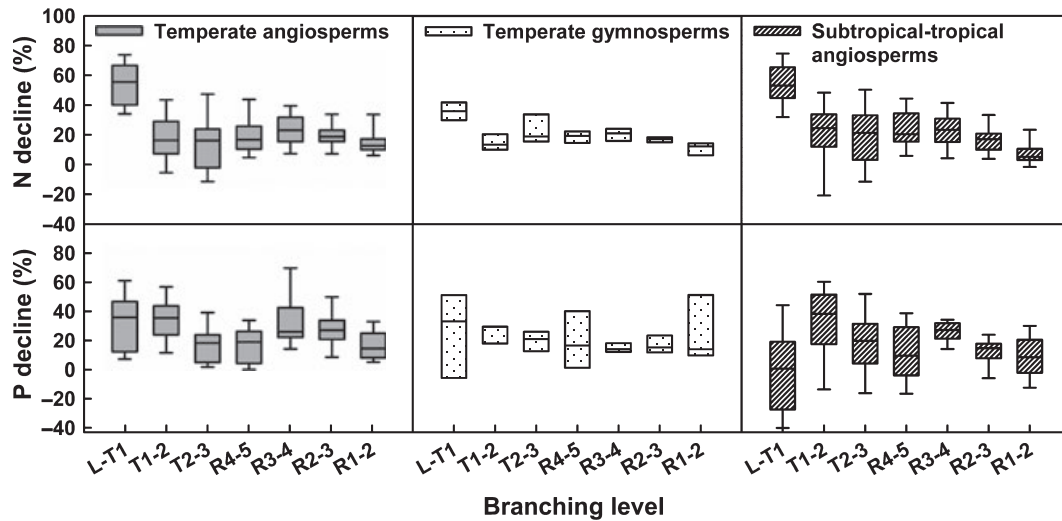


Fig. 2. Box plots showing percentage decline of [N] and [P] between successive above- and belowground branching levels in temperate angiosperms ( $n = 21$ ), temperate gymnosperms ( $n = 6$ ), and subtropical-tropical angiosperms ( $n = 22$ ). Abbreviations: L–T1 for the percentage decline from leaf to twig order 1, T1–2 for that from twig order 1 to 2, T2–3 for that from twig order 2 to 3; R4–5 to R1–2 for that between successive root orders.

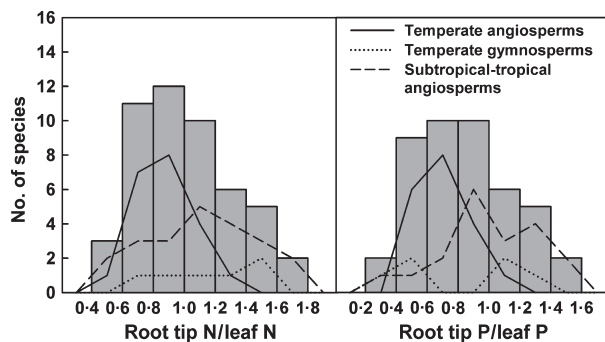


Fig. 3. Histograms of the ratio between root tips and leaves in [N] and [P] of all species (solid bars), and of each species group: temperate angiosperms (solid lines), temperate gymnosperms (dotted lines), and subtropical-tropical angiosperms (dash lines).

similar to root tips in subtropical-tropical angiosperms ( $P = 0.205$ , Fig. 1). In addition, root tips had lower N : P ratio than fine roots as a whole in temperate angiosperms ( $P = 0.013$ ) because root N : P ratios increased with branch order in this group (Table 1).

## Discussion

There was a significant inverse relationship between root branch order and nutrient (N and P) concentrations in temperate and subtropical-tropical tree species (Fig. 1; Table 1, 2). This relationship was consistent across biomes that differed in climate and soil nutrient levels, across phylogenetic groups, and across species that varied dramatically in absolute nutrient concentrations (Fig. 1; Table 1, 2). Similar patterns were also found in the shoot branching system (Fig. 1; Table 1, 2). These results suggest that the negative correlation observed between root branch order and root tissue [N] in the

first three orders of nine temperate trees (Pregitzer *et al.* 2002) also applies to order–[N], and order–[P] relationships in the first five root orders and distal shoot branch orders in forty-nine tree species sampled from diverse forest types. Consequently, a common nutrient ‘hierarchy’ may be identified in trees with highest nutrient concentrations in the distal ends and successively lower concentrations towards more basal tissues in both root and shoot branching systems.

The order–nutrient relationship identified here may be stemmed from basic biophysical and physiological constraints within the tree architecture. For roots, branch order has been shown to correlate systematically with anatomy (Guo *et al.* 2008c). First order roots have mostly primary structure in which cortical cells (which are metabolically active and nutrient-rich) are the dominant cell type. As order increased, the proportion of cortex declined and the total xylem area (which is composed mainly of metabolically inert and nutrient-poor cells) increased. Eventually in higher orders such as the fifth order, the cortex disappeared and secondary tissues such as secondary xylem and cork layers became the major root components. Thus, nutrient concentrations generally decrease with increasing root order. While root anatomy is generally correlated with root nutrient concentrations, anatomy and nutrients differed in the specific patterns of their relationship with branch order: root anatomy seems to shift abruptly from primary to secondary growth at the third or fourth order roots in most trees examined so far (Guo *et al.* 2008c), yet chemistry changed relatively continuously with order (though the first two orders were more similar to each other, see more detailed discussions below). The similar order–nutrient relationship in twigs suggests that anatomical changes in the twig branch system may also explain nutrient variations in twigs. Of course, leaf [N] was much higher than twigs because of its distinct structure

and function, which involves the extra N devoted to photosynthetic enzymes (Reich *et al.* 2008).

The order–nutrient relationships could be quantitatively described by linear regressions (Table 1, 2). The linear regressions for root [N] at the species group level were strong and captured more than 50% of the total variation (Table 1). For individual species in each group, root order–[N] regressions were also strong and showed the least species-specific differences among all regressions (Table 2). When compared between species groups, the linear relationship between branch order and [N] in roots was statistically the same between temperate and subtropical-tropical angiosperms, but differed between the two angiosperm groups and temperate gymnosperms (Table 1), suggesting that order–[N] relationship in roots was highly consistent in angiosperms and differed mainly between the two phylogenetic groups. The consistent linear relationship for each species group, particularly that for angiosperms may be used to predict [N] of different root orders, which, when coupled with the root [N]–respiration relationship reported in Reich *et al.* (2008), can predict respiration of different root orders at a broad scale. Certainly, one needs to be aware of the species-specific differences in order–[N] relationship (Table 2) and in [N]–respiration relationship (Reich *et al.* 2008).

The difference between angiosperms and gymnosperms was mainly due to the markedly lower [N] in distal root orders of temperate gymnosperms than the two angiosperm groups (Fig. 1). In a previous study, we have shown that temperate gymnosperms had higher stele proportion than temperate angiosperms in the first two root orders (Guo *et al.* 2008c), which may in part contribute to the lower [N] in these orders, and thus, more moderate slope of order–[N] regression in gymnosperms in the current study. Moreover, the finding of lower root-tip [N] in gymnosperms corresponds with the well-known fact that gymnosperms have lower leaf (needle) [N] than angiosperms due to their distinct needle structure.

Compared to root order–[N] relationships, root order–[P] relationships were weaker at the species group level (i.e. smaller  $R^2$  compared to [N], Table 1), due to greater species-specific differences within each group (i.e. higher CV% among species for each model parameter, Table 2). Linear relationship between root order and root [P] differed more between biomes than between phylogenetic groups, with the two temperate groups showing similar slopes, which were significantly steeper than the slope of subtropical-tropical angiosperms (Table 1). The more moderate decline in root [P] compared to root [N] as order increased in subtropical-tropical angiosperms may be due to the extremely low P availability in this biome (soil P was  $0.3 \text{ mg g}^{-1}$  in the subtropical-tropical biome, compared to  $1.2 \text{ mg g}^{-1}$  in the temperate biome; also see Han *et al.* 2005). The low soil P availability may constrain [P] in tree tissues, particularly in distal tissues with the highest nutrient concentrations. McGroddy, Daufresne & Hedin (2004) suggested that the P-poor tropical environment exerts a strong selection pressure on plant physiology, leading to lower concentrations but more efficient use of P in leaves in P-poor tropics. Similar selection pressure

may also be at work in roots, and studies have suggested that roots can be more sensitive than leaves in their response to soil fertility (Dighton & Harrison 1983; Adams *et al.* 1987).

Although strong general linear relationship was found between root branch order and root nutrient (particularly N) concentrations, the magnitude of decline in [N] and [P] from distal to successively more basal root branch orders was not constant across branching levels (Fig. 2). N decline (%) between neighbouring root orders was similar from the second to fifth order and averaged at about 20% in all species groups, yet that from the first to second order was much lower and averaged at 15% in temperate angiosperms, 10% in temperate gymnosperms, and 8% in subtropical-tropical angiosperms (Fig. 2; Table S3). The smaller magnitude of decline in [N] in the distal two orders may indicate greater similarity between these two orders as they have similar tissue composition, age, and longevity (Guo *et al.* 2008b, c); and are both preferential sites for mycorrhizal infection (Guo *et al.* 2008c). Similarly, P decline (%) in roots between neighbouring orders also differed between branching levels (Fig. 2; Table S3). These results suggest that branch orders are not equal, and lower root orders appeared to be more similar to each other, and may form a functional module specialized for resource absorption (Guo *et al.* 2008c).

When examining nutrient allocation patterns above- vs. belowground, many studies compared leaves with fine roots as a whole (McGroddy, Daufresne & Hedin 2004; Craine *et al.* 2005; Newman & Hart 2006). However, our results suggest that among all fine root branch orders, root tips had [N] and [P] most similar to those of leaves in all species groups (Fig. 1), though nutrient concentrations were not always identical between root tips and leaves (Fig. 3). This could be explained by the similarity of tissue composition between leaves and root tips, both of which are primarily comprised of nutrient-rich cells. Therefore, within the fine root guild, root tips are the best parallels of leaves in terms of N and P concentrations.

Patterns of N and P stoichiometry also suggest that fine roots as a whole should not be compared to leaves. It has been suggested that average C : N : P ratios for foliage (or foliage and shoots) are similar to those for fine roots (Gordon & Jackson 2000; McGroddy, Daufresne & Hedin 2004). Although in subtropical-tropical angiosperms, N : P ratio for leaves (17.2) was statistically similar to that for root tips (19.4) and fine roots as a whole (18.6), in temperate angiosperms N : P ratio ranked as leaves (11.5) < root tips (14.8) < fine roots (16.5), thus the N : P ratio for leaves was more similar to root tips than fine roots as a whole (Fig. 1).

The order–nutrient relationships found here show promise of being integrated into a set of empirical ‘rules’ for predicting the belowground components of terrestrial C and nutrient cycles. First, nutrient flux via root turnover can be better predicted with the root order–[N] relationships detected in our study, particularly for angiosperms. Second, root order–[N] relationships, in conjunction with [N]–respiration relationships (Reich *et al.* 2008), may be used to predict root respiration of different branch orders. Separating root respiration

by order has value in better understanding the carbon economy of mycorrhizal vs. non-mycorrhizal roots (root tips have higher mycorrhizal colonization than higher orders, and fourth or higher orders generally can not be colonized; Guo *et al.* 2008c), and the respiratory responses of uptake roots (which may be confined to the first two or three orders; Guo *et al.* 2008c) vs. non-uptake roots (fourth or higher orders) as environmental conditions (e.g. temperature, N content) vary. Finally, the comparable nutrient concentrations in leaves vs. root tips suggest that at a large scale, leaf nutrients could be used as a proxy of root-tip nutrients, though direct sampling of roots yield more accurate results.

The patterns and analysis presented here add to a growing recognition that a better understanding of architecture-related functional heterogeneity in fine roots is critical for accurate predictions of belowground ecosystem processes. Root turnover and decomposition were traditionally estimated and studied by treating fine roots as a homogeneous pool, yet functional heterogeneity within the fine root system has now become an undeniable fact (Wells & Eissenstat 2001; Pregitzer *et al.* 2002; Guo, Mitchell & Hendricks 2004; Guo *et al.* 2008b,c). Failure to account for such functional heterogeneity may lead to large errors in estimating root turnover (Guo *et al.* 2008a), nutrient flux (Guo *et al.* 2008b), and decomposition given that different root branch orders of trees have markedly different C fractions (Guo, Mitchell & Hendricks 2004), nutrient levels (Pregitzer *et al.* 1997, 2002; this study), and mycorrhizal colonization patterns (Guo *et al.* 2008c), all of which could have profound influence on root decomposition patterns (Silver & Miya 2001; Langley & Hungate 2003; Langley, Chapman & Hungate 2006). Thus, future studies on root turnover and decomposition, as well as above- and belowground C and nutrient allocations, should give sufficient consideration to the differences in chemistry, physiology and longevity within the root and whole-tree architecture.

## Acknowledgements

We thank Youzhi Han, Jinliang Liu, Oleg Anenkhonov, Nathalia Badmaeva, and Denis Sandanov's assistance in the field and laboratory. We thank Dr Kurt Pregitzer and an anonymous reviewer for insightful comments on a previous version of this manuscript. The funding for this research was provided by Natural Science Foundation of China (NSFC Grants 90511002 and 30130160), and by MaoYugang Undergraduate Research Grant, Peking University.

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Received 2 December 2008; accepted 28 May 2009

Handling Editor: Gareth Phoenix

## Supporting information

Additional supporting information may be found in the online version of this article.

**Table S1.** Sampling site characteristics.

**Table S2.** Taxonomic list of the forty-nine tree species sampled in this study.

**Table S3.** Average percentage decline of tissue [N] and [P] between successive above- and belowground branching orders in the three species groups and all species pooled.

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