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Do leaves of plants on phosphorus-impoverished soils contain high concentrations of phenolic defence compounds?

David M. Wright*[,1], Greg J. Jordan[2], William G. Lee[3], Richard P. Duncan[4,5], David M. Forsyth[1,4] and David A. Coomes*[,1,4]

[1]*Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, UK;* [2]*School of Plant Science, University of Tasmania, Private Bag 55, Hobart, Tasmania 7001, Australia;* [3]*Landcare Research, Private Bag 1930, Dunedin, New Zealand;* [4]*Landcare Research, PO Box 40, Lincoln 7640, New Zealand; and* [5]*Bio-Protection Research Centre, PO Box 84, Lincoln University, Lincoln 7647, New Zealand*

Summary

1. Prominent theories of plant defence have predicted that plants growing on nutrient-poor soils produce more phenolic defence compounds than those on richer soils. Only recently has the Protein Competition Model (PCM) of phenolic allocation suggested that N and P limitation could have different effects because the nutrients are involved in different cellular metabolic processes.

2. We extend the prediction of the PCM and hypothesize that N will have a greater influence on the production of phenolic defensive compounds than P availability, because N limitation reduces protein production and thus competition for phenylalanine, a precursor of many phenolic compounds. In contrast, P acts as a recyclable cofactor in these reactions, allowing protein and hence phenolic production to continue under low P conditions.

3. We test this hypothesis by comparing the foliar concentrations of phenolic compounds in (i) phenotypes of 21 species growing on P-rich alluvial terraces and P-depleted marine terraces in southern New Zealand, and (ii) 87 species growing under similar climates on comparatively P-rich soils in New Zealand vs. P-depleted soils in Tasmania.

4. Foliar P concentrations of plants from the marine terraces were about half those of plants from alluvial soils, and much lower in Tasmania than in New Zealand. However, foliar concentrations of N and phenolic compounds were similar across sites in both comparisons, supporting the hypothesis that N availability is a more important determinant of plant investment in phenolic defensive compounds than P availability. We found no indication that reduced soil P levels influenced plant concentrations of phenolic compounds. There was wide variation in the foliar N and P concentrations among species, and those with low foliar nutrient concentrations produced more phenolics (including condensed tannins).

5. Our study is the first trait comparison extending beyond standard leaf economics to include secondary metabolites related to defence in forest plants, and emphasizes that N and P have different influences on the production of phenolic defence compounds.

Key-words: carbon-nutrient balance, herbivory, phenolics, plant–herbivore interactions, protein competition model

Introduction

Herbivores are important drivers of plant community composition and structure in many forests around the world, resulting in strong selective pressure on plants to evolve suitable defence (Martin & Baltzinger 2002; Côté *et al.* 2004; Tremblay, Huot & Potvin 2007). Many plants invest in chemical deterrents that are metabolically costly to both produce and maintain. The resulting channelling of resources away from growth may, in the absence of herbivores, decrease the plant's competitiveness against less defended neighbours (Herms & Mattson 1992; Strauss *et al.* 2002). For instance, the presence of large quantities of monoterpenes and other secondary metabolites in leaves reduces browsing by selective feeders such as Sitka black-tailed deer (*Odocoileus hemionus sitkensis*), thus conferring increased

*Correspondence author. E-mail: dac18@cam.ac.uk

herbivore resistance on plants expressing them, but at the cost of a slower growth rate (Vourc'h *et al*. 2002). Similarly nicotine production in *Nicotiana attenuata* has a fitness cost in terms of seed production in uneaten plants (Baldwin 1998). Yet plants differ greatly in their foliar concentrations of secondary compounds, and the reason for this variation remains poorly understood despite a long history of investigation (Stamp 2003a,b).

The Carbon:Nutrient Balance Hypothesis (CNBH) was an early framework for understanding variation in concentrations of phenolic compounds (Bryant, Chapin & Klein 1983; Koricheva *et al*. 1998). The CNBH predicts that the amount plants invest in secondary compounds depends on the relative supply of carbon and nutrients to the sites of metabolism. For example, the growth of plants on nutrient-poor soils is postulated to be more limited by nutrients than by the carbon supplied by photosynthesis, and as a result carbohydrates accumulate in the leaves, which are then diverted to form constitutive defences including phenolic compounds such as condensed tannins (Bryant, Chapin & Klein 1983). CNBH predicts that if such plants were provided with more nutrients, then they would accelerate biomass growth and decrease allocation to carbon-based secondary metabolites through lack of carbohydrate substrate materials (Koricheva *et al*. 1998). The CNBH has been superseded by the Protein Competition Model (PCM) which is based on a more mechanistic understanding of biochemical pathways (Jones & Hartley 1999). Proteins and phenylpropanoids (phenylalanine-derived phenolic compounds, including lignins and condensed tannins) compete for a common precursor, phenylalanine, in the shikimate biosynthetic pathway (Haukioja *et al*. 1998). The PCM predicts that under low nutrient conditions, demand for growth proteins will decrease and the rate of phenylalanine incorporation into phenolic synthesis will increase, resulting in increased concentrations of phenylpropanoids (Jones & Hartley 1999).

A key issue considered in the PCM but not in the CNBH is whether different nutrients have different influences on the production of phenolic compounds. Here we extend the PCM prediction that deficiency of either nitrogen or phosphorus will lead to increased phenolic concentrations, comparing the effects of these nutrients quantitatively. We argue that N shortage will have a greater influence than P shortage because of the different mechanisms by which N and P influence protein biosynthesis and alter demand for phenylalanine from the shikimate pathway. Specifically, because N comprises 17%, on average, of protein mass (Elser *et al*. 1996), N availability has a direct influence on protein production. Reduced N availability will decrease the quantity of proteins which can be produced in existing cells, leading to reduced demand for phenylalanine and a concomitant increase in production of phenolic compounds. Biosynthesis of phenolics can continue under low N conditions as deamination of phenylalanine is the first committed step, and the resulting amine group can then be recycled to form more phenylalanine (Jones & Hartley 1999). In contrast, most cellular P is contained in ribosomal RNA (Vrede *et al*. 2004), and is an important constituent of enzymes involved in protein biosynthesis. We hypothesize that reduced P availability limits growth primarily through lack of P to form new cells, so the protein production capacity and consequent demand for phenylalanine in existing cells should remain unaffected; overall protein demand will remain approximately constant and according to the PCM, the rate of phenylalanine incorporation into synthesis of phenolic compounds should also remain static.

We assess whether the PCM, that originally focused on intraspecific responses to local-scale environmental variability, can be extended to predict phenolic concentrations among species at much broader spatial scales. We test our hypotheses by comparing concentrations of nutrients and phenolic compounds within two data sets which contrast plants growing on soils supplying different quantities of phosphorus. The first data set is a comparison of 21 species of woody plant sampled from P-rich alluvial terraces and adjoining P-depleted marine terraces in southern New Zealand (Coomes *et al*. 2005; Parfitt *et al*. 2005). The second is a comparison of 87 species growing in forest communities in high-rainfall regions of New Zealand and Tasmania; we anticipated that the highly P-depleted soils of Tasmania (Grant *et al*. 1995) would result in lower foliar P concentrations than found in New Zealand. We compare the foliar traits of plants within New Zealand and Tasmanian forests in order to address the following questions: (i) *Stoichiometry:* Do foliar N : P ratios support the supposition that marine terraces in New Zealand have less available P than alluvial terraces and that Tasmanian forests have less available P than New Zealand forests? (ii) *Nutrients and defence compounds:* How are levels of N and P linked to concentrations of defence-related phenolic compounds? Are concentrations of phenolic compounds similar in both Tasmanian and New Zealand plants as predicted under the PCM? When answering these questions we apply phylogenetically explicit modelling techniques, because interspecific trait comparisons are frequently influenced by the phylogenetic associations of the species sampled (Freckleton, Harvey & Pagel 2002; Duncan, Forsyth & Hone 2007). Finally, we compare our results with pre-existing global comparative analyses of leaf traits (Wright *et al*. 2004), which indicate that the allometric relationships between nutrient content, photosynthetic and respiratory capacity, leaf lifespan and specific leaf area are broadly similar for plants growing in different communities (Díaz *et al*. 2004; Wright *et al*. 2004; Reich, Wright & Lusk 2007). We assess whether similar cross-community trends extend to concentrations of phenolic compounds that have not been included in global analyses.

Materials and methods

STUDY SITES, SPECIES SELECTION AND SAMPLING SCHEME

The first data set was collected at the Waitutu chronosequence situated in the Waitutu Ecological Region of Fiordland National Park, on the south-west coast of the South Island, New Zealand (46·4°S, 167·2°E) (Coomes *et al*. 2005; Parfitt *et al*. 2005). The chronose-

quence consists of a series of elevated marine terraces intersected by watercourses flanked with alluvial deposits (Mark *et al.* 1988). The alluvial areas are regularly rejuvenated with fresh sediment, whereas the marine terraces were uplifted from 80 to 290 ky and are highly depleted in inorganic and organic forms of soil P. Coomes *et al.* (2005) describe the chemistry of these soils in detail: the terrace soils contain much lower concentrations of phosphorus than the alluvial soils (total P in the FH horizon 372 vs. 733 p.p.m., and in the mineral soil 189 vs. 862 p.p.m. respectively). In contrast, the terrace soils contain slightly greater quantities of N than the alluvial soils (total N in organic layers: 1·19% vs. 1·00% and in the mineral soil 0·53% vs. 0·32% respectively).

The second data set contrasts species from Tasmania and New Zealand, remnants of the southern paleo-supercontinent of Gondwana. Wet, cool temperate forests in the west of both landmasses are dominated by characteristic southern hemisphere taxa of *Nothofagus* and *Eucalyptus*. Despite the present geographical separation of 2000 km, both landmasses share many taxa at both the congeneric and conspecific levels, attributable to relatively frequent long-distance dispersal events since separation (Jordan 2001; Knapp *et al.* 2005; Perrie & Brownsey 2007; McDowall 2008). Inherently nutrient-poor rock types, geological inactivity and frequent fires have left Tasmania with typically P-depleted soils compared to New Zealand, particularly in the high rainfall areas with quartzite substrates in the West of the country (Handreck 1997; Read 2001). The types of soils found at our study sites (underlain by Precambrian quartzite or slate, Ordovician conglomerate and sandstone or Permian mudstone) typically contain P concentrations of 38–245 and 14–125 p.p.m. in the organic and mineral layers, respectively (Grant *et al.* 1995), much lower than that found in the Waitutu soils. These soils also contained less N than those in Waitutu forest, with concentrations of 0·10–0·28% in the organic layer and 0·03–0·07% in the mineral layer (Grant *et al.* 1995).

FIELD SAMPLING AND TRAIT MEASUREMENTS

We collected leaf samples from 21 species growing on both soil types in the Waitutu forest, from two to four plants per species on P-depleted terraces and from the alluvial site (Forsyth, Richardson & Menchenton 2005; Parfitt *et al.* 2005). For the interspecific comparison we collected leaf samples from 40 species of Tasmanian and 54 species of New Zealand plants from seven and two different rain forest sites respectively (see Tables S1 and S2, Supporting Information). We selected Tasmanian sites which matched most closely the cool, high rainfall conditions of the Murchison Mountains, where the majority (45) of the New Zealand species were sampled. Species were selected to represent the dominant vegetation in the forest understoreys of each landmass. The majority of samples were taken under closed canopies, and from $< 1{\cdot}5$ m above ground level in order to eliminate the confounding effects of different light environments on concentrations of photoprotective phenolic compounds (Close *et al.* 2003). We sampled up to 20 individuals of each species, collecting 1–10 leaves from each individual (mean = 8 leaves) depending on the size of the plant. One frond was sampled from individual tree ferns.

Leaves were air dried in the field and then oven dried at 45 °C to prevent loss of the more volatile phenolic compounds. Leaves were pooled by species (New Zealand within sites and Tasmania between sites), ground and sent for chemical analysis at an internationally accredited laboratory (Landcare Research Environmental Chemistry Laboratory, Palmerston North, New Zealand). Nitrogen and phosphorus concentrations were measured using colorimetric methods following a Kjeldahl digest. Total fibre (comprising cellulose, hemicellulose and lignin), cellulose and lignin concentrations were determined using acid detergent fibre methods. Concentrations of total phenolics were determined as tannic acid equivalents and the subset of condensed tannins as catechin equivalents. Six species were sampled twice at separate sites in New Zealand, for which we calculated trait means for use in all further comparisons.

STATISTICAL ANALYSIS

Comparisons of bivariate scaling relationships between our data set and the GLOPNET global data base of plant traits (Wright *et al.* 2004) were made using Standardized Major Axis regressions (Warton *et al.* 2006). To account for phylogenetic associations (Rohlf 2001; Freckleton, Harvey & Pagel 2002), we performed phylogenetic generalized least-squares (PGLS) regressions comparing both single trait means and each bivariate trait combination, to determine whether site-level differences were being driven by higher order taxonomic interactions, i.e. whether responses of species are more heavily affected by taxon than the site in which they were sampled (Wright, Westoby & Reich 2002). All traits except lignin and condensed tannins had lognormal species distributions according to the Shapiro–Wilk test and were $\log_{10}$-transformed for further analysis. We generated a phylogeny (Fig. S1, Supporting Information) based on the order and family-level classifications of the Angiosperm Phylogeny Group (APGII, 2003). Genus-level classifications and those for gymnosperms and ferns were collected from recently published cladistic trees and unpublished taxonomies of relevant groups (see Fig. S1). Branch lengths for this composite phylogeny were estimated according to the method of Grafen (1989) using the APE package in R (Paradis, Claude & Strimmer 2004; R Development Core Team, 2008). We set the height of each node equal to the number of daughter taxa minus 1 and calculating branch lengths as the difference in height between upper and lower nodes. Phylogenetic covariance matrices calculated under a Brownian model of evolution were then incorporated into generalized least-squares regressions following Duncan, Forsyth & Hone (2007). We obtained maximum likelihood estimates (MLE) of λ, a measure of phylogenetic correlation associated with a given tree, for each comparison. Traits that are phylogenetically independent have λ values approaching zero whereas those that covary in proportion to their level of relatedness have λ values close to one (Freckleton, Harvey & Pagel 2002). Estimates of λ remained essentially constant for several candidate trees in which polytomies were resolved at random, indicating that λ is robust to taxonomic uncertainties. We fitted linear models at the MLE of λ, which removes the covariance associated with phylogenetic relatedness, leaving only the effects of country in the comparison of mean trait values, in a process analogous to ANCOVA. We also compared pairs of traits by this method. The resulting slope and intercept parameter estimates for each country were compared using *t*-tests, and the strength of bivariate trait relationships within countries was tested using Pearson correlation *r* values. As PGLS parameters differ depending on the direction of the model (regressing *X* on *Y* as opposed to *Y* on *X*), we compared all trait combinations in both directions and, to be conservative, plotted only those lines which were supported under both.

Results

STOICHIOMETRY AND LEAF ECONOMIC SPECTRUM

Mean foliar-N concentrations in plants growing on alluvial soils in Waitutu forest were not significantly different from conspecifics growing on the marine terraces (PGLS single

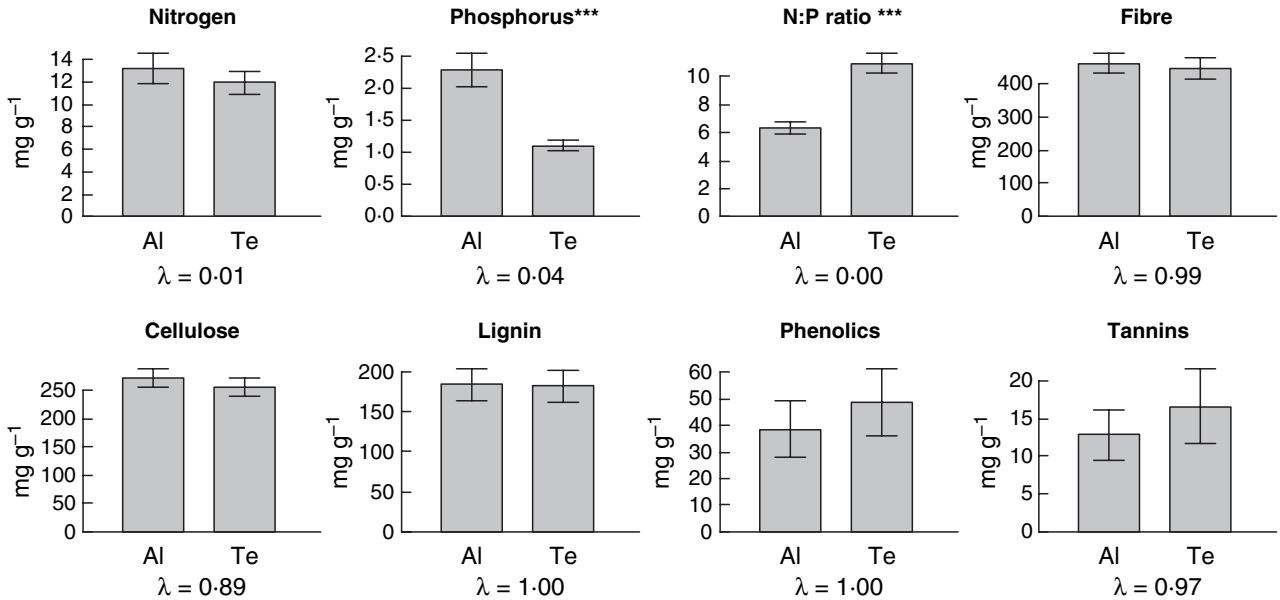

Fig. 1. Mean (±SEM) trait values of 21 species of forest plants sampled on alluvial (Al) and marine terraces (Te) in Waitutu forest, New Zealand. Intraspecific means compared using paired *t*-tests; ***$P < 0{\cdot}001$. Lambda values indicate phylogenetic independence of trait.

trait model: $t = -0{\cdot}76$, $P = 0{\cdot}45$). In contrast, mean foliar-P concentrations were 50% lower on the marine terraces (Fig. 1), contributing towards higher foliar N : P ratios (11·0 ± 0·67) than on the alluvial soils (6·39 ± 0·43, $t = 5{\cdot}76$, $P < 0{\cdot}001$). There was a common positive correlation between N and P across both soil types in Waitutu forest (Table 1, Fig. 2). Interspecific comparisons of Tasmanian and New Zealand species revealed similar patterns; mean foliar-P concentrations were 35% lower in Tasmania than in New Zealand (Fig. 3), with a narrower range of values (Tasmania: 0·35–2·47 mg g^{-1}; New Zealand: 0·40–5·30 mg g^{-1}). In contrast, foliar-N concentrations varied sevenfold among species but the mean concentration (12·7 ± 0·50 mg g^{-1}) did not differ between the landmasses (PGLS single trait model: $t = 0{\cdot}40$, $P = 0{\cdot}69$). Consequently foliar N : P ratios were higher in Tasmania (15·8 ± 0·67) compared to New Zealand (10·7 ± 0·58, $t = 5{\cdot}99$, $P < 0{\cdot}001$). N and P concentrations were positively correlated in both countries, although the slope of the log–log scaling relationship (i.e. the scaling exponent) was greater in New Zealand (1·29, 95% confidence

Table 1. Bivariate trait comparisons of forest plants growing on alluvial and marine terraces in Waitutu forest, New Zealand, by phylogenetic generalized least squares regressions. Measure of phylogenetic correlation, λ. Coefficients and standard errors of $\log_{10}$-transformed traits. Significance of coefficients (*t*-tests) indicated: ***$P < 0{\cdot}001$; **$P < 0{\cdot}01$; *$P < 0{\cdot}05$. Significance of alluvial terrace intercepts and slopes is tested for heterogeneity from zero. Marine terrace intercepts and slopes are tested for heterogeneity from alluvial terrace intercepts and slopes respectively

		Alluvial soils		Terrace soils	
Traits	λ	Intercept (±SE)	Slope (±SE)	Intercept (±SE)	Slope (±SE)
Phosphorus–nitrogen	0·68	−0·86 ± 0·17***	1·07 ± 0·14***	0·15 ± 0·19	−0·37 ± 0·18*
N : P–nitrogen	0·68	0·86 ± 0·17***	−0·07 ± 0·14	−0·15 ± 0·19	0·37 ± 0·18*
Fibre–nitrogen	0·95	2·80 ± 0·13***	−0·11 ± 0·08	−0·00 ± 0·10	−0·02 ± 0·09
Phenolics–nitrogen	1	2·13 ± 0·48***	−0·71 ± 0·21**	−0·32 ± 0·24	0·37 ± 0·23
Tannins–nitrogen	0	2·88 ± 0·83**	−1·99 ± 0·75*	−0·32 ± 1·15	0·32 ± 1·07
N : P–phosphorus	0	0·90 ± 0·04***	−0·39 ± 0·11**	0·13 ± 0·05*	0·21 ± 0·20
Fibre–phosphorus	0·95	2·70 ± 0·10***	−0·08 ± 0·06	−0·04 ± 0·02	−0·03 ± 0·09
Phenolics–phosphorus	1	1·55 ± 0·39***	−0·65 ± 0·15***	−0·08 ± 0·05	−0·09 ± 0·20
Tannins–phosphorus	0·01	1·32 ± 0·21***	−2·00 ± 0·54***	−0·46 ± 0·24	0·01 ± 0·94
Fibre–N : P	0·98	2·70 ± 0·14***	−0·04 ± 0·10	0·07 ± 0·12	−0·08 ± 0·13
Phenolics–N : P	1	1·06 ± 0·51*	0·36 ± 0·29	−0·26 ± 0·34	0·26 ± 0·36
Tannins–N : P	0·98	−0·55 ± 1·07	1·54 ± 0·77	2·38 ± 0·92*	−2·59 ± 0·97*
Phenolics–fibre	1	0·41 ± 1·69	0·35 ± 0·61	1·55 ± 0·84	−0·55 ± 0·32
Tannins–fibre	0·11	−3·79 ± 3·57	1·70 ± 1·34	0·13 ± 4·09	0·00 ± 1·55
Tannins–phenolics	0·54	−1·44 ± 0·48**	1·57 ± 0·29***	0·14 ± 0·50	−0·13 ± 0·34

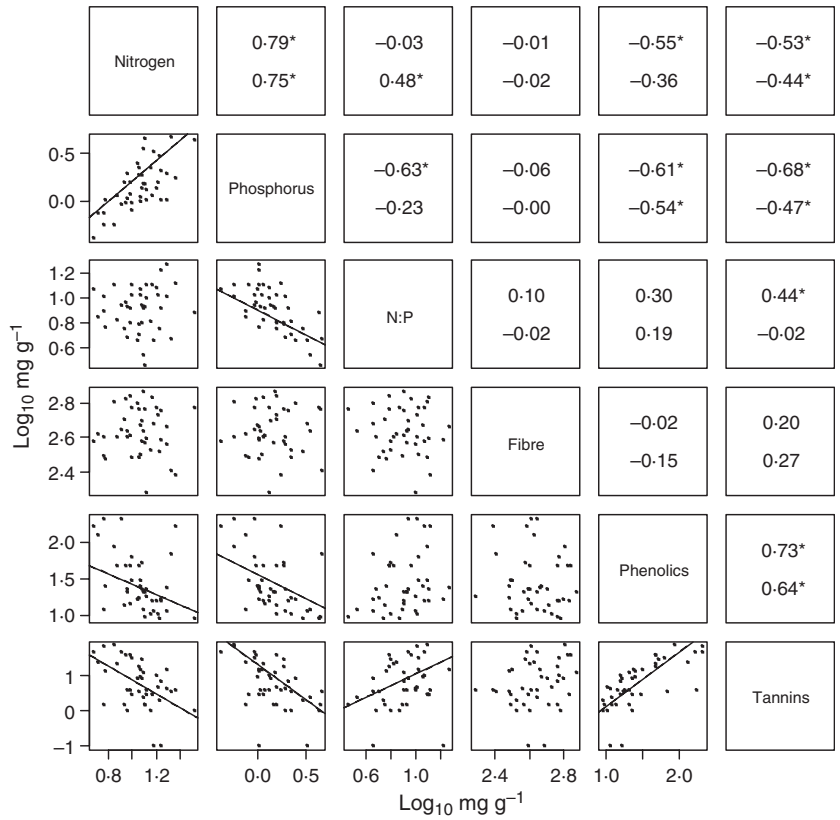

Fig. 2. Bivariate comparisons of traits (plotted on log–log axes) for species sampled on alluvial and marine terraces in Waitutu forest, New Zealand. The upper panels give Pearson correlation coefficients (upper = alluvial, lower = marine, within each box). Asterisks indicate significance of correlation at $P < 0{\cdot}05$. The lower panels show data points and regression lines for relationships that had slopes significantly different from zero (PGLS regression, $P < 0{\cdot}05$).

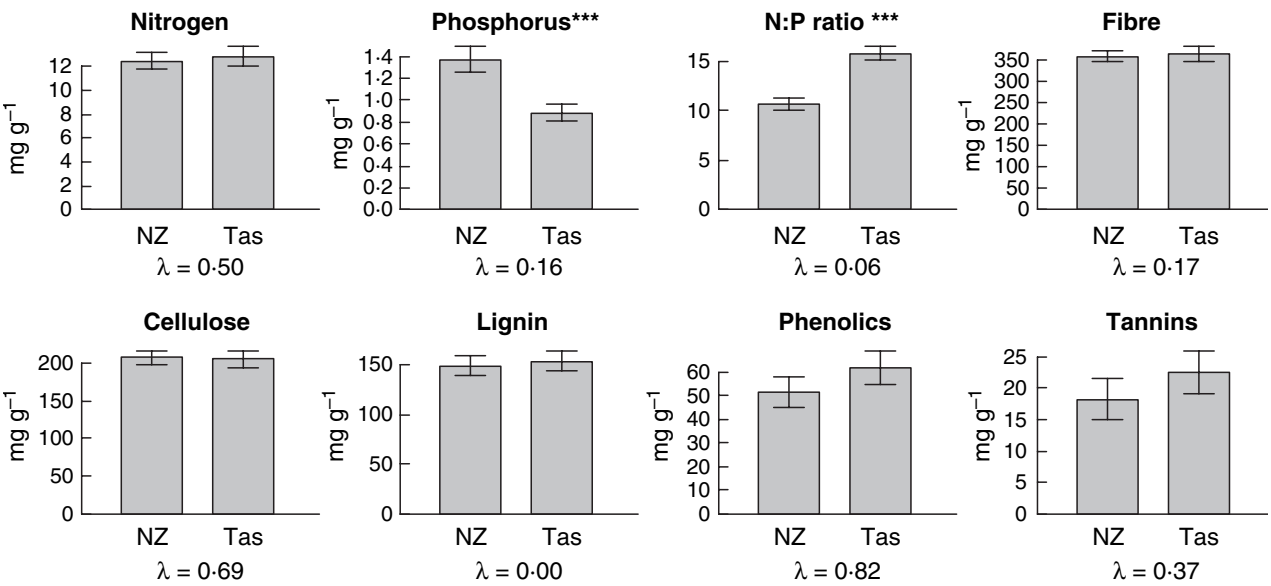

Fig. 3. Mean (±SEM) trait values of forest plants sampled in New Zealand (54 species) and Tasmania (40 species). Means compared using PGLS regression; ***$P < 0{\cdot}001$. Lambda values indicate phylogenetic independence of trait.

interval = 1·01, 1·56) than in Tasmania (0·89 [0·54, 1·24]) (Table 2, Fig. 4). The correlation between these traits was largely independent of phylogeny ($\lambda = 0{\cdot}13$).

NUTRIENTS AND DEFENCE COMPOUNDS

Conspecific comparisons across soil types indicated that the mean concentrations of fibre, cellulose, lignin, total phenolics and condensed tannins were similar on marine and alluvial sites in Waitutu forest despite very large differences in foliar P concentrations (PGLS single trait models: fibre $t = -1{\cdot}46$; cellulose $t = -1{\cdot}76$; lignins $t = -0{\cdot}25$; total phenolics $t = 1{\cdot}71$; condensed tannins $t = 1{\cdot}18$; all tests $P > 0{\cdot}05$, Fig. 1). On both the alluvial and marine terraces N and P were negatively correlated across species with both condensed tannins and total phenolics (Fig. 2). There were no systematic

Table 2. Bivariate trait comparisons of New Zealand and Tasmanian forest plants by phylogenetic generalized least squares regressions. Measure of phylogenetic correlation, λ. Coefficients and standard errors of $\log_{10}$-transformed traits. Significance of coefficients (*t*-tests) indicated: ***$P < 0{\cdot}001$; **$P < 0{\cdot}01$; *$P < 0{\cdot}05$. Significance of New Zealand intercepts and slopes is tested for heterogeneity from zero. Tasmanian intercepts and slopes are tested for heterogeneity from New Zealand intercepts and slopes respectively

		New Zealand		Tasmania	
Traits	λ	Intercept (±SE)	Slope (±SE)	Intercept (±SE)	Slope (±SE)
Phosphorus–nitrogen	0·13	−1·29 ± 0·15***	1·29 ± 0·14***	0·25 ± 0·20	−0·40 ± 0·18*
Fibre–nitrogen	0·22	2·76 ± 0·12***	−0·19 ± 0·11	−0·03 ± 0·16	0·03 ± 0·15
Tannins–nitrogen	0·35	2·39 ± 0·74**	−1·40 ± 0·65*	0·81 ± 0·95	−0·56 ± 0·88
Phenolics–nitrogen	0·77	2·67 ± 0·38***	−1·03 ± 0·28***	−0·12 ± 0·39	0·21 ± 0·36
N : P–nitrogen	0·13	1·29 ± 0·15***	−0·29 ± 0·14*	−0·25 ± 0·20	0·40 ± 0·18*
Fibre–phosphorus	0·26	2·57 ± 0·04***	−0·10 ± 0·07	−0·02 ± 0·03	−0·00 ± 0·11
Tannins–phosphorus	0·39	1·02 ± 0·27***	−1·38 ± 0·38***	−0·09 ± 0·14	−0·30 ± 0·60
Phenolics–phosphorus	0·81	1·66 ± 0·24***	−0·77 ± 0·16***	−0·04 ± 0·06	0·00 ± 0·24
N : P–phosphorus	0	1·04 ± 0·01***	−0·54 ± 0·06***	0·11 ± 0·02***	0·20 ± 0·09*
Tannins–fibre	0·44	−6·54 ± 2·10**	2·92 ± 0·81***	4·36 ± 2·74	−1·63 ± 1·08
Phenolics–fibre	0·93	2·62 ± 0·99**	−0·40 ± 0·35	−0·04 ± 1·06	0·05 ± 0·42
Tannins–phenolics	0·3	−0·79 ± 0·40	1·07 ± 0·22***	−0·06 ± 0·60	0·08 ± 0·36
Fibre–N : P	0·21	2·51 ± 0·11***	0·05 ± 0·10	0·10 ± 0·20	−0·09 ± 0·17
Tannins–N : P	0·49	−0·94 ± 0·67	1·91 ± 0·60**	1·26 ± 1·11	−1·21 ± 0·98
Phenolics–N : P	0·88	0·87 ± 0·40*	0·75 ± 0·25**	0·28 ± 0·45	−0·27 ± 0·40

differences in these trait correlations between soil types (PGLS bivariate trait models, $P < 0{\cdot}05$, Table 1, Fig. 2).

Likewise, mean values for these traits were not significantly different when comparing plants from New Zealand and Tasmania at the interspecific level (PGLS single trait models: fibre $t = 0{\cdot}07$; cellulose $t = -0{\cdot}98$; lignins $t = 0{\cdot}31$; total phenolics $t = 0{\cdot}70$; condensed tannins $t = 0{\cdot}71$; all tests $P > 0{\cdot}05$, Fig. 3). When data were pooled across both countries, N and P were both negatively correlated with both total phenolics and condensed tannins (N–phenolics, $r = -0{\cdot}42$, $t = -4{\cdot}46$, $P < 0{\cdot}001$; P–phenolics, $r = -0{\cdot}49$, $t = -5{\cdot}43$, $P < 0{\cdot}001$; N – tannins, $r = -0{\cdot}33$, $t = -3{\cdot}35$, $P < 0{\cdot}001$; P–tannins, $r = -0{\cdot}39$, $t = -4{\cdot}02$, $P < 0{\cdot}001$). PGLS analysis of these trait correlations did not reveal any systematic differences between countries (Figs 4 and 6). There were no significant correlations between fibre concentration and either N or P.

COMPARISON WITH GLOBAL LEAF TRAIT DATA BASE

The scaling slope of the relationship between N and P for our species (pooling all data) was not significantly different from the slope of the GLOPNET data set [likelihood ratio tests, $P = 0{\cdot}63$; common slope and 95% confidence interval = 1·51 (1·46, 1·57)]. This correspondence with patterns found at the global scale (Fig. 5) indicates that our sample was large enough to reveal general patterns and that the scaling relationships with respect to the leaf economics spectrum are similar (Wright *et al.* 2005). The foliar N concentrations that we observed are mainly within the lower third on the global scale. Our samples had higher P, on average, at a given concentration of N than species recorded in the GLOPNET data set (Wald test: $\chi^2 = 51{\cdot}0$, $P < 0{\cdot}001$, Fig. 5). This may be due to differential leaching rates of N and P under the high rainfall conditions of our study sites, with N being leached

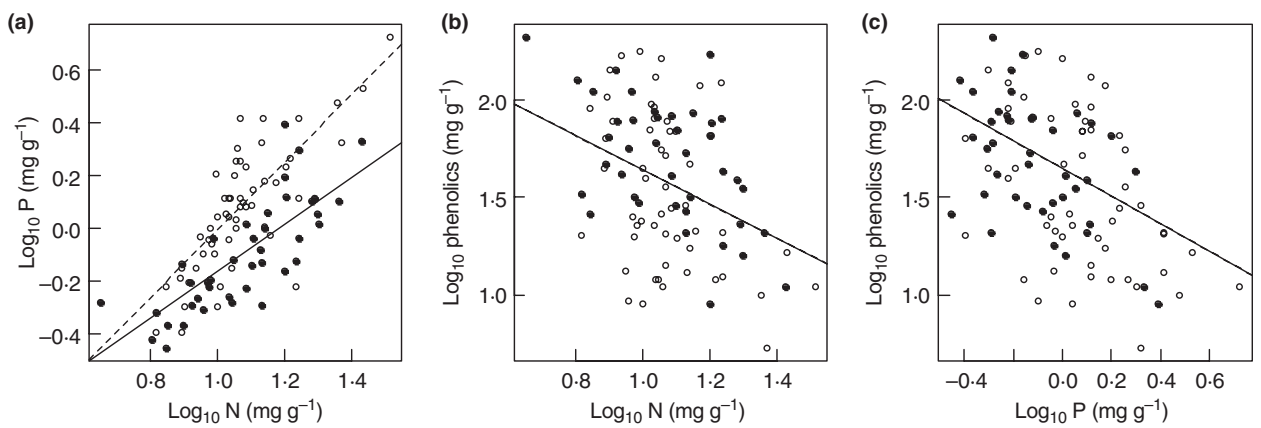

Fig. 4. Bivariate comparisons of foliar concentrations of (a) N and P, (b) N and total phenolic compounds, (c) P and phenolic compounds for all species sampled in New Zealand (open circles) and Tasmania (filled circles). A single PGLS regression line is plotted when relationships were indistinguishable between landmasses, whereas dashed line = New Zealand and solid line = Tasmania when significant differences were observed.

faster than P as leaves age (Chapin 1980), an effect which would be less pronounced in the lower rainfall areas which form the majority of the global survey (Wright *et al.* 2004).

Discussion

INFLUENCES OF FOLIAR NITROGEN AND PHOSPHORUS CONCENTRATIONS ON INVESTMENT IN PHENOLIC COMPOUNDS

Plants growing on relatively P-rich soils had similar concentrations of phenolics and condensed tannins to those growing on P-depleted soils: this was true when comparing alluvial vs. marine terrace sites in southern New Zealand (Fig. 1) and New Zealand vs. Tasmanian sites (Fig. 3). These results support our hypothesis that the availability of phosphorus to plants has little, or no, influence on the production of phenolic compounds. Our premise was that N availability greatly influences protein biosynthesis because N comprises a large proportion of the substrate, whereas the major role of P is to form recyclable cofactors in the intermediate stages. There is strong evidence that plant growth is P rather than N limited on the Waitutu marine terraces with soil P depletion on the marine terraces but similar soil N concentrations across both soil types (Coomes *et al.* 2005; Coomes *et al.* 2009). Foliar N and P concentrations reflected N and P availability; plants growing on the marine terraces had only half the foliar P concentrations of conspecific plants growing on nearby alluvial sites, but there was no significant difference in foliar N concentrations. Although it proved impossible to distinguish the influences of foliar N and P concentrations on phenolic production within sites – because N and P concentrations were closely correlated among species (Figs 2, 4 and 6) – the lack of influence of P in cross-site comparisons suggests that it is N, and not P, availability that is responsible for within-site relationships between phenolics and foliar nutrient concentrations (Figs 2, 4 and 6).

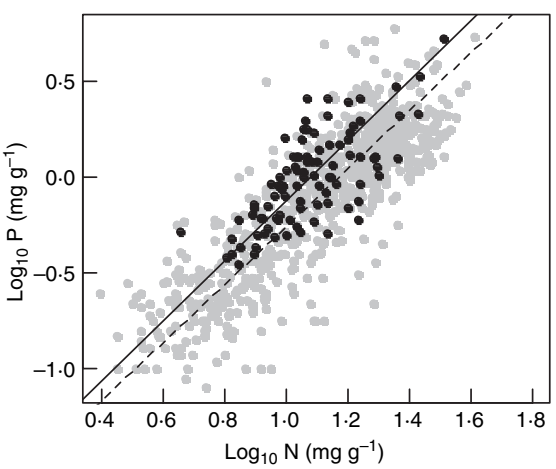

Fig. 5. Scaling relationships between foliar N and P concentrations across New Zealand and Tasmania (black points, solid line) and a global data base of leaf traits [GLOPNET: (Wright *et al.* 2004), grey points, dashed line].

High concentrations of tannins in the leaves of species associated with phosphorus-impoverished soils have strong influences on microbial decomposition of litter and nutrient cycling. There is a general trend towards higher foliar tannin concentrations in forest plants growing on less fertile soils (Kraus, Dahlgren & Zasoski 2003), with trees on tropical white sands leaching large quantities of tannins into blackwater rivers (Janzen 1974). Western Tasmanian waters are known to have similarly high tannin contents (Bowling, Steane & Tyler 1986). The accumulation of foliar tannins in plants growing on less fertile sites can act to regulate nutrient cycling, with reduced rates of decomposition and N mineralization being associated with high tannin concentrations in leaf litter (Kraus, Dahlgren & Zasoski 2003; Schweitzer *et al.* 2008). Subsequent formation of polyphenol–protein complexes in the soil can help to retain inorganic cations by sorption (Hättenschwiler & Vitousek 2000). Increased tannin production may therefore represent a conservative strategy by which plants reduce nutrient losses to the microbial community, although the significance of such processes at the ecosystem level remains to be determined (Hättenschwiler & Vitousek 2000).

The PCM was originally formulated to predict phenotypic variation in phenolic compound concentrations, focusing on the way a plant responds to changes in a single environmental variable, implicitly limiting predictions to relatively homogeneous habitats at local spatial scales. Our results suggest that the PCM provides a useful framework for understanding how phenolic concentrations vary among species at the national scale in response to variation in N availability. In order to predict responses to P limitation, the contrasting roles of N and P in protein synthesis must be considered. Studies of phenotypic variation support our findings: Koricheva *et al.* (1998) conducted a meta-analysis of 147 experimental studies which investigated how N and P fertilization influenced concentrations of various classes of carbon-based secondary compounds, including phenylpropanoids. These experiments indicated that foliar concentrations of phenolic compounds were generally unaffected by P addition, but were strongly affected by N addition (Koricheva *et al.* 1998), although the reasons for this discrepancy were not explored. Low organismal N : P ratios are closely linked to high inherent growth rates (Niklas *et al.* 2005), and manipulation of nutrient availability has been shown to affect community composition and ecosystem processes in a manner predictable using models of the stoichiometry of RNA and protein (Vrede *et al.* 2004).

STOICHIOMETRY AND LEAF ECONOMIC SPECTRUM

The existence of differing growth strategies may contribute towards the high variability which we observed in phenolic and tannin concentrations within sites (eightfold and 30-fold variation in phenolic concentrations in Tasmania and New Zealand respectively). Interspecific variation in foliar P

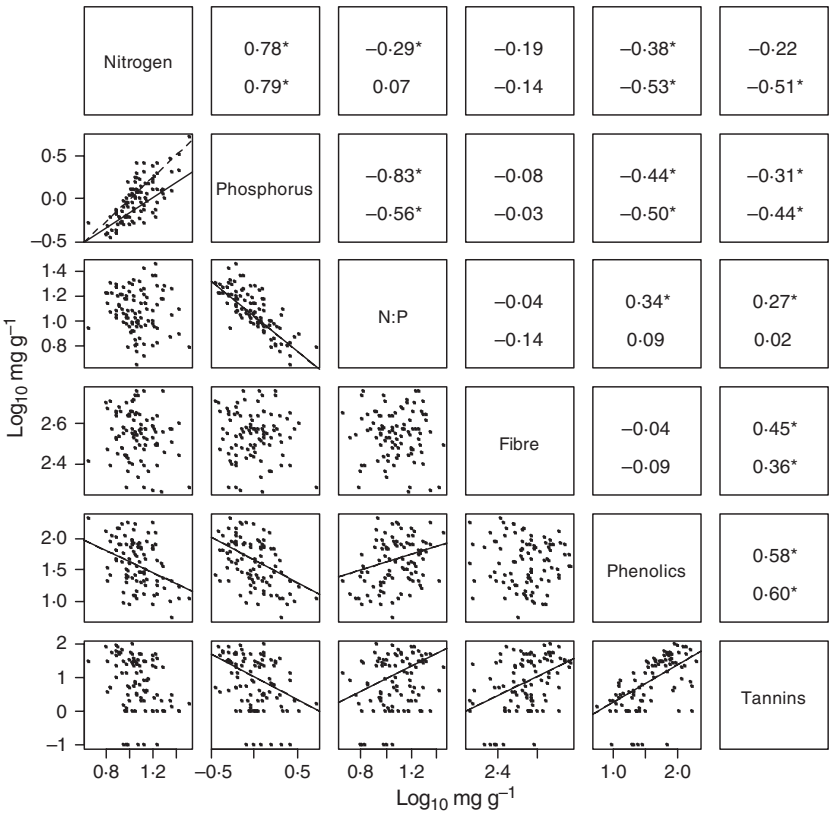

Fig. 6. Bivariate comparisons of traits (plotted on log–log axes) for species sampled in New Zealand and Tasmania. The upper panels give Pearson correlation coefficients (upper = New Zealand, lower = Tasmania, within each box). Asterisks indicate significance of correlation at $P < 0{\cdot}05$. The lower panels show data points and regression lines for relationships that had slopes significantly different from zero (PGLS regression, $P < 0{\cdot}05$); if the slope and/or elevation of the regression line differed between the landmasses then the lines are plotted separately (dashed line = New Zealand, solid line = Tasmania), otherwise the common slope is drawn.

concentrations increased with N concentration (Fig. 4), indicating that species adopt a variety of stoichiometric strategies under similar nutrient regimes. Supporting this conclusion, dominant New Zealand tree species differ in their responses to nutrient supply, with some species maximizing photosynthetic rates under high N situations regardless of P availability, whereas others prefer high N, low P soils (Carswell *et al.* 2005). Likewise, tropical trees exhibit high variation in interspecific N : P ratios within communities, signalling greater diversity of growth strategies in response to edaphic conditions than temperate species (Townsend *et al.* 2007). Increased sclerophylly is one strategy by which plants may sustain high growth rates under low P (Handreck 1997), but we found little indication of increased sclerophylly in Tasmania, as the species that we sampled had similar concentrations of fibre and lignin to those in New Zealand. Plants may form mycorrhizal associations or dense root mats exuding organic acids to extract the maximum amount of P from all soil layers (Lambers *et al.* 2008). Plants may also adopt highly conservative nutrient cycling strategies to retain P, with long leaf lifespan and efficient resorption of P from senescing leaves (Denton *et al.* 2007).

Conclusions

Our comparison of traits extends the standard list of traits used to develop the leaf-economic spectrum theory to include phenolic compounds related to defence in forest plants. Accounting for the effects of phylogenetic association, we show that plants with low foliar nutrient concentrations invest more in total phenolics and the subset of condensed tannins, in agreement with the protein competition model of plant defences. Across very large spatial scales and at both the intra- and inter-specific levels of comparison, N appears to be a more important determinant of plant investment in carbon-based defensive compounds than P.

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References

APGII (2003) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society*, **141**, 399–436.

Baldwin, I.T. (1998) Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 8113–8118.

Bowling, L.C., Steane, M.S. & Tyler, P.A. (1986) The spectral distribution and attenuation of underwater irradiance in Tasmanian inland waters. *Freshwater Biology*, **3**, 313–335.

Bryant, J.P., Chapin, F.S., III & Klein, D.R. (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*, **40**, 357–368.

Carswell, F.E., Whitehead, D., Rogers, G.N.D. & McSeveny, T.M. (2005) Plasticity in photosynthetic response to nutrient supply of seedlings from a mixed conifer-angiosperm forest. *Austral Ecology*, **30**, 426–434.

Chapin, F.S., III (1980) The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics*, **11**, 233–260.

Close, D., McArthur, C., Paterson, S., Fitzgerald, H., Walsh, A. & Kincade, T. (2003) Photoinhibition: a link between effects of the environment on eucalypt leaf chemistry and herbivory. *Ecology*, **84**, 2952–2966.

Coomes, D.A., Allen, R.B., Bentley, W.A., Burrows, L.E., Canham, C.D., Fagan, L., Forsyth, D.M., Gaxiola-Alcantar, A., Parfitt, R.L., Ruscoe, W.A., Wardle, D.A., Wilson, D.J. & Wright, E.F. (2005) The hare, the tortoise and the crocodile: the ecology of angiosperm dominance, conifer persistence and fern filtering. *Journal of Ecology*, **93**, 918–935.

Coomes, D.A., Kunstler, G., Canham, C.D. & Wright, E. (2009) A greater range of shade tolerance strategies among species in relatively nutrient-rich forests: a niche-based explanation for positive richness-productivity relationships? *Journal of Ecology*, in press. DOI: 10.1111/j.1365-2745.2009.01507.x.

Côté, S.D., Rooney, T.P., Tremblay, J.-P., Dussault, C. & Waller, D.M. (2004) Ecological impacts of deer overabundance. *Annual Review of Ecology, Evolution, and Systematics*, **35**, 113–147.

Denton, M.D., Veneklaas, E.J., Freimoser, F.M. & Lambers, H. (2007) Banksia species (Proteaceae) from severely phosphorus-impoverished soils exhibit extreme efficiency in the use and re-mobilization of phosphorus. *Plant, Cell and Environment*, **30**, 1557–1565.

Díaz, S., Hodgson, J.G., Thompson, K., Cabido, M., Cornelissen, J.H.C., Jalili, A., Montserrat-Martí, G., Grime, J.P., Zarrinkamar, F., Asri, Y., Band, S.R., Basconcelo, S., Castro-Díez, P., Funes, G., Hamzehee, B., Khoshnevi, M., Pérez-Harguindeguy, N., Pérez-Rontomé, M.C., Shirvany, F.A., Vendramini, F., Yazdani, S., Abbas-Azimi, R., Bogaard, A., Boustani, S., Charles, M., Dehghan, M., De Torres-Espuny, L., Falczuk, V., Guerrero-Campo, J., Hynd, A., Jones, G., Kowsary, E., Kazemi-Saeed, F., Maestro-Martínez, M., Romo-Díez, A., Shaw, S., Siavash, B., Villar-Salvador, P. & Zak, M.R. (2004) The plant traits that drive ecosystems: evidence from three continents. *Journal of Vegetation Science*, **15**, 295–304.

Duncan, R.P., Forsyth, D.M. & Hone, J. (2007) Testing the metabolic theory of ecology: allometric scaling exponents in mammals. *Ecology*, **88**, 324–333.

Elser, J.J., Dobberfuhl, D.R., Mackay, N.A. & Schampel, J.H. (1996) Organism size, life history, and N:P stoichiometry. *Bioscience*, **46**, 674–684.

Forsyth, D.M., Richardson, S.J. & Menchenton, K. (2005) Foliar fibre predicts diet selection by invasive red deer *Cervus elaphus scoticus* in a temperate New Zealand forest. *Functional Ecology*, **19**, 495–504.

Freckleton, R.P., Harvey, P.H. & Pagel, M. (2002) Phylogenetic analysis and comparative data: a test and review of evidence. *The American Naturalist*, **160**, 712–726.

Grafen, A. (1989) The phylogenetic regression. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences*, **326**, 119–157.

Grant, J.C., Laffan, M.D., Hill, R.B. & Neilson, W.A. (1995) *Forest Soils of Tasmania: A Handbook for Identification and Management*. Forestry Tasmania, Hobart.

Handreck, K.A. (1997) Phosphorus requirements of Australian native plants. *Australian Journal of Soil Research*, **35**, 241–289.

Hättenschwiler, S. & Vitousek, P.M. (2000) The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends in Ecology and Evolution*, **15**, 238–243.

Haukioja, E., Ossipov, V., Koricheva, J., Honkanen, T., Larsson, S. & Lempa, K.S. (1998) Biosynthetic origin of carbon-based secondary compounds: cause of variable responses of woody plants to fertilization? *Chemoecology*, **8**, 133–139.

Herms, D.A. & Mattson, W.J. (1992) The dilemma of plants: to grow or defend. *Quarterly Review of Biology*, **67**, 283–335.

Janzen, D.H. (1974) Tropical blackwater rivers, animals, and mast fruiting by the Dipterocarpaceae. *Biotropica*, **6**, 69–103.

Jones, C.G. & Hartley, S.E. (1999) A protein competition model of phenolic allocation. *Oikos*, **86**, 27–44.

Jordan, G.J. (2001) An investigation of long-distance dispersal based on species native to both Tasmania and New Zealand. *Australian Journal of Botany*, **49**, 333–340.

Knapp, M., Stöckler, K., Havell, D., Delsuc, F.D.R., Sebastiani, F. & Lockhart, P.J. (2005) Relaxed molecular clock provides evidence for long-distance dispersal of *Nothofagus* (southern beech). *PLoS Biology*, **3**, 38–43.

Koricheva, J., Larsson, S., Haukioja, E. & Keinänen, M. (1998) Regulation of woody plant secondary metabolism by resource availability: hypothesis testing by means of a meta-analysis. *Oikos*, **83**, 212–226.

Kraus, T.E.C., Dahlgren, R.A. & Zasoski, R.J. (2003) Tannins in nutrient dynamics of forest ecosystems – a review. *Plant and Soil*, **256**, 41–66.

Lambers, H., Raven, J.A., Shaver, G.R. & Smith, S.E. (2008) Plant nutrient-acquisition strategies change with soil age. *Trends in Ecology and Evolution*, **23**, 95–103.

Mark, A.F., Grealish, G., Ward, C.M. & Wilson, J.B. (1988) Ecological studies of a marine terrace sequence in the Waitutu ecological district of southern New Zealand Part 1: the vegetation and soil patterns. *Journal of the Royal Society of New Zealand*, **18**, 29–58.

Martin, J.-L. & Baltzinger, C. (2002) Interaction among deer browsing, hunting, and tree regeneration. *Canadian Journal of Forest Research*, **32**, 1254–1264.

McDowall, R.M. (2008) Process and pattern in the biogeography of New Zealand – a global microcosm. *Journal of Biogeography*, **35**, 197–212.

Niklas, K.J., Owens, T., Reich, P.B. & Cobb, E.D. (2005) Nitrogen/phosphorus leaf stoichiometry and the scaling of plant growth. *Ecology Letters*, **8**, 636–642.

Paradis, E., Claude, J. & Strimmer, K. (2004) APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, **20**, 289–290.

Parfitt, R.L., Ross, D.J., Coomes, D.A., Richardson, S.J., Smale, M.C. & Dahlgren, R.A. (2005) N and P in New Zealand soil chronosequences and relationships with foliar N and P. *Biogeochemistry*, **75**, 305–328.

Perrie, L. & Brownsey, P. (2007) Molecular evidence for long-distance dispersal in the New Zealand pteridophyte flora. *Journal of Biogeography*, **34**, 2028–2038.

R Development Core Team (2008) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.

Read, J. (2001) Soil and rainforest composition in Tasmania: correlations of soil characteristics with canopy composition and growth rates in *Nothofagus cunninghamii* associations. *Australian Journal of Botany*, **49**, 121–135.

Reich, P.B., Wright, I.J. & Lusk, C.H. (2007) Predicting leaf physiology from simple plant and climate attributes: a global GLOPNET analysis. *Ecological Applications*, **17**, 1982–1988.

Rohlf, F.J. (2001) Comparative methods for the analysis of continuous variables: geometric interpretations. *Evolution*, **55**, 2143–2160.

Schweitzer, J.A., Madritch, M.D., Bailey, J.K., Leroy, C.J., Fischer, D.G., Rehill, B.J., Lindroth, R.L., Hagerman, A.E., Wooley, S.C., Hart, S.C. & Whitham, T.G. (2008) From genes to ecosystems: the genetic basis of condensed tannins and their role in nutrient regulation in a *Populus* model system. *Ecosystems*, **11**, 1005–1020.

Stamp, N. (2003a) Out of the quagmire of plant defense hypotheses. *Quarterly Review of Biology*, **78**, 23–55.

Stamp, N. (2003b) Theory of plant defensive level: example of process and pitfalls in development of ecological theory. *Oikos*, **102**, 672–678.

Strauss, S.Y., Rudgers, J.A., Lau, J.A. & Irwin, R.E. (2002) Direct and ecological costs of resistance to herbivory. *Trends in Ecology and Evolution*, **17**, 278–285.

Townsend, A.R., Cleveland, C.C., Asner, G.P. & Bustamente, M.M.C. (2007) Controls over foliar N:P ratios in tropical rain forests. *Ecology*, **88**, 107–118.

Tremblay, J.-P., Huot, J. & Potvin, F.O. (2007) Density-related effects of deer browsing on the regeneration dynamics of boreal forests. *Journal of Applied Ecology*, **44**, 552–562.

Vourc'h, G., Vila, B., Gillon, D., Escarré, J., Guibal, F., Fritz, H., Clausen, T.P. & Martin, J.L. (2002) Disentangling the causes of damage variation by deer browsing on young *Thuja plicata*. *Oikos*, **98**, 271–283.

Vrede, T., Dobberfuhl, D.R., Kooijman, S.A.L.M. & Elser, J.J. (2004) Fundamental connections among organism C:N:P stoichiometry, macromolecular composition, and growth. *Ecology*, **85**, 1217.

Warton, D.I., Wright, I.J., Falster, D.S. & Westoby, M. (2006) Bivariate line-fitting methods for allometry. *Biological Reviews*, **81**, 259–291.

Wright, I.J., Westoby, M. & Reich, P.B. (2002) Convergence towards higher leaf mass per area in dry and nutrient-poor habitats has different consequences for leaf life span. *Journal of Ecology*, **90**, 534–543.

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.-L., Niinemets, U., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J. & Villar, R. (2004) The worldwide leaf economics spectrum. *Nature*, **428**, 821–827.

Wright, I.J., Reich, P.B., Cornelissen, J.H.C., Falster, D.S., Garnier, E., Hikosaka, K., Lamont, B.B., Lee, W., Oleksyn, J., Osada, N., Poorter, H., Villar,

R., Warton, D.I. & Westoby, M.L.W. (2005) Assessing the generality of global leaf trait relationships. *New Phytologist*, **166**, 485–496.

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Supporting information

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

Table S1. Forest sites in New Zealand and Tasmania at which plants were collected for foliar trait analysis.

Table S2. Forest plant species sampled in New Zealand and Tasmania for foliar trait comparisons.

Figure S1. Phylogeny hypothesis for New Zealand and Tasmanian forest plants used in trait analyses.

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