

Coordinating leaf functional traits with branch hydraulic conductivity:
resource substitution and implications for carbon gain

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Summary

We examined relationships amongst branch hydraulic conductivity, xylem embolism, mean stomatal conductance, foliar N concentration and specific leaf area of seven tree species growing at four temperate woodland sites spanning a 464 - 1350 mm rainfall gradient. In particular we addressed the question: are mean stomatal conductance and foliar N concentration coordinated with branch hydraulic conductivity and, if so, what are the implications for C assimilation? Mean rate of assimilation expressed on an area basis (A_{area}) was uniquely and positively correlated with stomatal conductance (g_s) and foliar N concentration. Multiple regression analyses showed that when variability in SLA was controlled for, the (positive) partial slope for each predictor remained significant. In contrast there was a negative correlation between g_s and N_{mass} such that for any given A_{area} leaves with a large g_s allocated less N to foliage than leaves with a small g_s . Foliar N concentration was negatively correlated with branch hydraulic conductivity and g_s was positively correlated with branch hydraulic conductivity. These relationships were also significant when variability in leaf area to sapwood area ratio, g_s and SLA were controlled for in a multiple regression, suggesting that the relationship was unique and independent of other confounding factors. Trees with low water transport capacity were able to support a high A_{area} by increasing the investment in foliar N. Resource substitution occurred such that there was a trade-off between g_s and foliar N in relation of branch hydraulic conductivity. A large A_{area} could be sustained either through having a large branch hydraulic conductivity and hence g_s and a low allocation of foliar N, or the impact of a small branch hydraulic conductivity and hence g_s could be offset by allocating a large foliar N. These results are discussed in relation to strategies for minimizing the negative effects of limited water availability on C gain.

Key words: leaf traits, resource substitution

Introduction

The pathway of water movement from soil to atmosphere comprises a series of liquid and gas phase conductances (Meinzer 2002). The largest resistance to water loss must be stomatal conductance (Parkhurst 1994), for if hydraulic conductance of roots, stems or branches were the chief limitation, water supply to leaves would be insufficient to prevent desiccation (Meinzer 2002). Consistent with these assumptions, positive correlations between hydraulic conductance and stomatal conductance have been observed (Hubbard et al. 2001, Rust and Roloff 2002). Although reduction of stomatal conductance with decreasing hydraulic conductance prevents excessive water loss and limits the potential for leaf damage caused by low leaf water potentials, it simultaneously restricts carbon dioxide uptake and hence limits tree and stand productivity (Gower et al. 1996, Ryan and Yoder 1997, Ryan et al. 1997, Hubbard et al 2001, Santiago et al. 2004). Nevertheless, in contrast to a number of other studies, Macinnis-Ng et al. (2004) reported a negative relationship between mass-based photosynthetic rate and sapwood specific hydraulic conductivity. They proposed that the negative slope arose because of the need for leaves on branches with low hydraulic conductivity and large Huber value to fix sufficient C to maintain the large H_v (Macinnis-Ng-Ng et al. 2004). The present paper explores this further.

Branch hydraulic conductance is strongly influenced by embolism of xylem vessels. High evaporative demand and transpiration rate increase xylem sap tension, which increases the risk of xylem cavitation (Hacke and Sperry 2001; Macinnis-Ng-Ng et al. 2004). Increased levels of embolism decreases xylem hydraulic conductance of branches or whole trees (Tyree and Ewers 1991; Jones and Sutherland 1991; Prior and Eamus 2000; Cruiziat et al 2001; Hacke and Sperry 2001; Martínez-Vilalta 2002). Given that g_s must be the largest resistance to water loss if leaf desiccation is to be avoided, we may postulate that the degree of xylem embolism will be related, either directly or indirectly, to vapour phase stomatal conductance.

If stomatal conductance coordinates with hydraulic conductance (Meinzer 2002), and xylem embolism reduces both conductances (Sperry 2000), one should expect indirect coordination of photosynthetic and hydraulic capacity. However, stomatal conductance is not the only link between hydraulic conductance and C gain. Foliar rates of photosynthesis are strongly correlated with the amount of foliar nitrogen (N) as well as specific leaf area (SLA) (Evans 1989; Wright et al. 2001; Prior et al. 2003). Thus, it is possible that photosynthesis–hydraulic architecture relations can be mediated via coordinating water transport capacity with leaf traits that control or limit photosynthesis (e.g. foliar N or SLA). The results of modeling (Farquhar et al. 2002, Buckley et al. 2002, Buckley and Roberts 2006a) and experiment (Buckley and Roberts 2006b, Santiago et al. 2004) support this hypothesis. The study described in the present paper examines whether resource substitution, that is, a trade-off between stomatal conductance (and hence water use) and foliar N, has implications for photosynthetic rate when branch hydraulic conductance varies.

The purpose of this work was to examine relationships amongst branch hydraulic conductivity, xylem embolism, leaf attributes (stomatal conductance (g_s), foliar N and SLA) and leaf-scale photosynthesis of several Australian tree species. Correlations amongst these variables will provide insight into how trees allocate resources in order to balance water loss, water transport capacity and C gain. We address the following questions:

- 1) How do g_s , foliar nitrogen and SLA influence photosynthetic rate?
- 2) Do trees with higher g_s allocate less nitrogen to foliage?
- 3) How are mean stomatal conductance and foliar nitrogen coordinated with branch hydraulic conductivity, and what are the implications of this for C assimilation?
- 4) Can resource substitution allow C assimilation rates to be maintained across a gradient of water availability?

We hypothesize that g_s , foliar nitrogen and SLA will exert independent effects on photosynthetic rate, and that for a given rate of light saturated photosynthesis, trees with higher g_s will have lower foliar N concentrations. We also hypothesize that foliar N concentration will be positively correlated with hydraulic conductivity. It may be assumed that trees with intrinsically lower capacity to supply their leaves with water will necessarily have a reduced stomatal conductance. However, by increasing foliar N concentration, trees with lower branch hydraulic conductivity will fix more C for a given stomatal conductance (resource substitution *sensu* Buckley and Roberts 2006a, b). Consequently, we predict that rates of light saturated C assimilation will remain relatively constant across widely varying water transport capacities.

Materials and methods

Site and species descriptions

We measured A_{area} , g_s , leaf N and SLA, hydraulic architecture and branch structural traits at four woodland sites differing in mean annual rainfall. The sites were the Woggoon Nature Reserve (32°48'45.52"S, 146°56'00.54"E; mean annual rainfall (MAR) \approx 464 mm; elevation = 212 m); a stand of remnant woodland at Windsor NSW within the bounds of land managed by Waste Services NSW (33°39'41.54"S, 150°46'58.27"E; MAR \approx 800 mm; elevation = 34 m); the Royal National Park (RNP; 34°05'53.37"S, 151°03'33.85"E; MAR \approx 1200 mm); and the Jiliby State Conservation area (33°11'27"S, 151°18'59"E; MAR \approx 1350 mm). The Woggoon Nature reserve is dominated by *Eucalyptus populnea* (F. Muell.) and *Callitris glaucophylla* (J. Thompson. & L. Johnson); the Windsor site by *Angophora bakeri* (E.C. Hall) and *Eucalyptus sclerophylla* ((Blakely) L. Johnson & Blaxell); the RNP by *Eucalyptus haemastoma* (Smith) and *Angophora hispida* (Smith); and Jiliby SCA by *E. haemastoma* (Smith) and *Angophora costata* (Smith).

At each site, we established a single 50×50 m plot within which 10 randomly selected trees were chosen for sampling. Individual trees were selected by dividing the quadrat into approximately 2×2 m sub-quadrats, selecting 10 sub-quadrats using a table of random numbers and choosing a single tree within each sub-quadrat. All sampling was conducted in summer, between December 2005 and February 2006.

Measuring A_{area} , g_{sv} , foliar N and SLA

A single, sun-exposed branch was cut from each tree of each sub-quadrat and the cut end immediately placed in a bottle of distilled water. Using a Walz HCM-1000 portable infra-red gas analyzer (Walz, Effeltrich, Germany), light saturated photosynthetic rate (A_{area}) and stomatal conductance (g_s) were measured on two young, fully expanded leaves per branch, within 5 minutes of having their basal end immersed in water. This method allowed us to measure A_{area} on upper crown branches that were clearly light saturated, since most branches in lower parts of the canopies were relatively shaded for most of the day. All measurements were taken between 09:00 and 11:00 h (Australian Eastern Standard Time) at ambient CO_2 concentrations, temperatures and at solar irradiances exceeding $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density. We validated that the measurements taken on excised branches did not differ from that of attached branches by randomly selecting three trees of each species, choosing a single, accessible (from the lower parts of each tree), sun-exposed branch from each tree and measuring A_{area} and g_s . We then excised each branch, placed it in distilled water and measured A_{area} and g_s at 5 minute intervals for 15 minutes. The results of these tests showed that there was no difference between A_{area} and g_s of attached and detached branches over this time period, and that neither variable declined significantly over the 15 min period (repeated measures ANOVA; insignificant time main effect; $P > 0.5$ for all species; data not shown). All branches and leaves sampled were free of disease and insect damage. Our measurements of A_{area} and g_s do not represent maximum rates of CO_2 assimilation and g_s , which are usually measured under conditions of non-limiting light, temperature and water availability. While our measurements of A_{area} and g_s are not temperature or light limited, soil moisture may have been limiting at many of the sites as the region where they are located experienced drought during the measurement period. Thus, our measurements of A_{area} and g_s represent maximum observed rates of CO_2 assimilation and g_s at the time and place of sampling, not a maximum rate for each species/tree examined. Atmospheric water content and leaf temperature were not controlled in any measurements, but leaves were sampled only under saturating light conditions.

In some cases, individual leaves were too small to encompass the entire area of the measuring cuvette. In these cases, we excised the area of the leaf within the cuvette and returned it to the laboratory for leaf area measurement with a LI-COR 3000A leaf area meter. Photosynthetic rates were then recalculated to $A_{\text{area}} = (A_{\text{measured}} \times \text{cuvette area})/\text{projected leaf area}$. Stomatal conductance was similarly re-calculated. For needle-leaved *Callitris* trees, gas exchange rates were expressed on a needle surface area rather than projected area

basis. We assumed that *Callitris* needles were cylindrical and A_{area} was subsequently modified to $A_{\text{area}} = (A_{\text{measured}} \times \text{cuvette area})/0.5(\text{projected leaf area} \times \pi)$.

At the cessation of gas exchange measurements, branches were bagged, placed in darkness and returned to the laboratory for analyses of leaf N, SLA and hydraulic architecture traits. Leaves were dried to a constant mass at 70 °C then ground to a powder with a ball-mill grinder. Approximately 0.2 g of ground material was analyzed for leaf nitrogen concentration using a Leco total C and N analysis system (Leco Corporation, St. Joseph, MI).

Branch hydraulic conductivity

Branch hydraulic conductivity was measured according to the method of Macinnis-Ng et al. (2004). The terminal segment of branches of 3 – 6 mm diameter was selected from the sunlit portion of the canopy. Current year's growth only was sampled and therefore leaves on the branch were those that had formed in the current growing season. Excision of the branch and all leaves from each branch (at the petiole-branch junction) occurred under distilled water to prevent embolism. The basal end of each branch was also re-cut underwater approximately 5 mm from the point of excision. Branches were then inserted into the lid of a custom built vacuum chamber. The basal end of each branch was quickly connected to a 1cm³ graduated pipette via flexible tubing filled with distilled, filtered (0.22 µm) and acidified (pH 2.0 with a 10% hydrochloric acid solution; Sperry et al. 1994) water. Hydraulic conductivity was measured by increasing pressure in the vacuum chamber in 5 steps ($\Delta P \approx -20, -30, -40, -50$ and -60 kPa). At each pressure, sap flow (ml s⁻¹) was measured by recording the rate of change in volume of water in the pipette. Branches were allowed to equilibrate at each pressure for 10 minutes before measurement began. Hydraulic conductivity (ml cm s⁻¹ MPa⁻¹) was calculated as the ordinary least-squares regression slope of the relationship between sap flux and $\Delta P/l$, where l is the length of the branch segment (Prior and Eamus 2000). Previous work has established that the absence of salts from the perfusion solution does not affect the value of conductivity obtained (Prior and Eamus 2000; Macinnis-Ng et al. 2004). There was no evidence that flow rates declined with time in the vacuum chamber.

After these measurements of hydraulic conductivity, xylem emboli were removed by forcing acidified, filtered and degassed solution through each branch at a positive pressure of 175 kPa for 30 to 50 minutes. Branch hydraulic conductivity was then re-measured. The percentage loss of conductivity due to embolism (PLC) was then calculated from the difference in the slopes of the relationship between initial and maximum conductivity when emboli were removed (Prior and Eamus 2000) as:

$$\text{PLC} = \left(\frac{k_{\text{max}} - k}{k_{\text{max}}} \right) \times 100$$

Where k is branch hydraulic conductivity and k_{max} is maximum hydraulic conductivity after emboli have been removed.

To confirm that branch lengths were greater than mean xylem vessel length, the mean vessel length of each branch was measured by forcing nitrogen gas through stem segments at a positive pressure of 0.5 MPa, placing the distal end of the branch under water and gradually shortening it by progressively cutting 0.5 cm lengths back from the distal end and until bubbles began to emerge (Drake and Franks 2004). Branches on which conductivity measurements were taken were at least 20 % longer than the longest vessel lengths.

Once these measurements were complete, sapwood and leaf areas of each branch were measured. Sapwood area was determined by dipping thin cross-sections of branch in methyl orange and measuring the diameter of the stained (heartwood) and unstained (sapwood) sections with a calibrated eyepiece micrometer. Leaf area distal to the basal end of each branch was measured with a leaf area meter (LI-COR 3000A). Sapwood specific hydraulic conductivity (k_s) was calculated by dividing branch conductivity by its sapwood area.

Statistical analyses

Univariate variance components

We sampled four sites, two dominant species within each site and 4-6 replicate trees within each species. We built a series of general linear models, with site and species (specified as nested within sites) treated as random factors, to break down univariate variance components (adjusted type III sums of squares) between site, species and tree (residual) scales. These analyses allow us to answer questions such as: does most of the variability in hydraulic conductivity occur from site to site or from species to species? All response variables, except k_s and PLC, were $\ln x + 1$ transformed before analyses to approach error normality and variance homogeneity.

Relationships amongst leaf traits and hydraulic architecture

Bivariate relationships between leaf traits were modeled using simple linear regressions or Pearson's correlations. We also used multiple regressions to explore the unique contribution of each predictor in explaining the variance of the dependant variable. The unique relationship of each predictor is assessed in terms of a partial slope and "partial r^2 " value (Quinn and Keogh 2003) which is the amount of variance explained by a predictor after controlling for all the other predictors in the model. The use of multiple regression allowed us to look at the unique relationship between two variables while holding potentially confounding effects of other variables constant (Tabachnick and Fidell 2001; Hair et al. 2006). For example, we looked at the amount of variance in A_{area} explained by nitrogen concentration after controlling for SLA, which is known to co-vary highly with

nitrogen concentration. Multiple regression relationships were visualized using 3-dimensional plots (two predictors) or partial regression plots (which allow visualization of the unique relationship between two variables in a multi-dimensional model; Norousis 2003). These analyses were performed with SPSS version 12.0.1 (SPSS Inc). Individual trees were treated as independent experimental units for all statistical analyses.

Results

Variation in leaf and hydraulic architecture traits

There was significant variability in leaf nitrogen concentration (% dry mass) between sites and significant variability in specific leaf area between species nested within sites (Table 1). Sapwood-specific hydraulic conductivity varied significantly among species nested within sites and PLC did not vary significantly between species or sites. Moreover, with the exception of N_{mass} , 'site' explained less than 21% of the variance of any trait, meaning that all of the measured traits were more variable within sites than they were between sites (Table 1). Averages of each variable for each species are given in Table 2.

Mean rates of A_{area} ranged from 1.77 to 7.6 $\mu\text{mol m}^{-2} \text{s}^{-1}$ while mean g_s varied between 27 and 1024 $\text{mmol m}^{-2} \text{s}^{-1}$. Mass based foliar nitrogen concentration (N_{mass}) ranged from 0.9 to 1.35 % and SLA from 33.55 to 60.0 $\text{cm}^2 \text{g}^{-1}$. Sapwood-specific conductivity (k_s) ranged between 1.17 and 28.3 $\text{ml cm s}^{-1} \text{MPa}^{-1} \text{cm}^{-2}$ and percentage loss of conductivity (PLC) due to embolism ranged between 7.5 and 57.3%.

Relationships among assimilation rate, g_s , N concentration and sapwood specific hydraulic conductivity

CO_2 assimilation was uniquely and positively correlated with both mean stomatal conductance and mass based leaf nitrogen concentration, suggesting that each have an independent effect on photosynthesis (Figure 1; Table 3). Moreover, the partial slope of each predictor was significant when variability in SLA was also controlled for, showing that the relationship between N_{mass} and A_{area} is not an artifact of the cross correlation of N_{mass} and A_{area} with SLA (Table 3). Thus, as g_s or N_{mass} increased, A_{area} increased. In contrast, the unique relationship between mean g_s and N_{mass} for any given A_{area} revealed a negative correlation, and this relationship also held when SLA was controlled for (Figure 2; Table 3). Thus, for any given A_{area} , leaves with higher mean g_s allocate less nitrogen to foliage and this relationship is independent of SLA.

CO_2 assimilation rate was not correlated with k_s (Figure 3a), but there was a positive relationship between g_s and k_s (Table 4; Figure 3b). Mass based N concentration was negatively correlated with k_s (Table 4; Figure 3c). Moreover, the negative relationship between N_{mass} and k_s was also significant when SLA was controlled (Table

5) meaning that the relationship between N_{mass} and k_s is not a result of changes in SLA. The results were qualitatively similar whether N concentration was expressed on a mass or area basis (Table 5).

Instantaneous water-use-efficiency (A_{area} /transpiration rate) was negatively correlated with k_s ($r = 0.41$; $P = 0.034$; Figure 4a) and positively correlated with PLC ($r = 0.53$; $P = 0.028$; Figure 4b). Thus, although trees with lower k_s , had lower than average g_s and were more sensitive to embolism, they fixed more CO_2 for a given unit of water loss.

Both N_{mass} and g_s were unique predictors of C_i/C_a (Figure 5a-b). Trees with higher than average N_{mass} had lower than average C_i/C_a (partial slope = -0.154; partial $r^2 = 17.1\%$; $P = 0.029$; Figure 5), while trees with higher than average g_s had higher than average C_i/C_a (partial slope = 0.013; partial $r^2 = 24.0\%$; $P = 0.008$; Figure 5). Consequently leaves investing more in foliar N operated at a lower C_i and this pattern was independent of SLA.

Discussion

Variation in leaf traits and water transport capacity and PLC

Rates of light saturated photosynthesis in the present study (1.5 to $7.6 \mu\text{mol m}^{-2} \text{s}^{-1}$) were at the low end of the range observed globally (0.8 to $42 \mu\text{mol m}^{-2} \text{s}^{-1}$; Wright et al. 2004), whereas the ranges of g_s , hydraulic conductivity, leaf specific conductivity, percentage loss of conductivity due to embolism and SLA all lie within published ranges (Prior et al. 1997, Prior and Eamus 2000, Eamus and Prior 2001, Macinnis-Ng et al. 2004, Wright et al. 2004). The low values of photosynthesis and to some extent g_s , reflect the impact of the drought that has prevailed over much of the eastern Australia from 2002 to 2006. However, the presence of resource substitution in drought conditions is perhaps where the benefits are largest.

Most of the variability in A_{area} , g_s and SLA was due to differences between species nested within sites, consistent with Wright et al. (2004, 2005). In contrast, the source of most of the variability in hydraulic conductivity and PLC occurred at the scale of individual trees and there was no evidence that hydraulic conductivity or PLC varied between species or sites. The fact that most traits were more variable within a single site suggests that phylogenetic, genetic and within-site environmental heterogeneity are larger sources of variation than between site factors such as climate (Wright and Westoby 2003).

Stomatal conductance and leaf nitrogen concentration independently control A_{area}

Both g_s and N_{mass} exerted independent and positive effects on A_{area} . Both g_s and N have a large influence on photosynthetic rate (Ryan and Yoder 1997; Hubbard et al. 1999; 2001), the former through diffusional

limitations, the latter through the N-cost associated with photosynthetic enzymes and light-harvesting pigments (Evans 1989). Independence of stomatal and N control of A_{area} can also be observed in the data of Wright et al. (2004). Although Wright et al. (2004) did not look for an effect of g_s as a predictor of A_{area} , our reanalysis of the species GLOPNET data shows that both g_s and N_{mass} independently affect photosynthesis (g_s partial slope = 0.39; $P < 0.0001$ and N_{mass} partial slope = 0.22; $P < 0.0001$). The independent effects of g_s and N_{mass} are not mediated through variation in SLA, because when SLA is controlled for, the relationships between g_s and N_{mass} remain significant in both GLOPNET and our data.

That g_s and foliar N exert independent effects on photosynthetic rate means that C gain can be coordinated with other plant traits (such as branch water transport capacity) either via changing g_s or via changes in N allocation, or both. Although SLA was not uniquely correlated with A_{area} in our data, for the global GLOPNET data, photosynthesis was uniquely correlated with SLA (Wright et al. 2004). Therefore it is possible for trees to coordinate water transport capacity with C gain through coordination of the area available for light capture relative to dry mass.

Trade-off between g_s and N_{mass}

For a given A_{area} , there was a strong negative relationship between foliar N and g_s and the relationship remained significant when SLA was held constant. Therefore, to achieve a given rate of C assimilation, leaves with higher mean g_s tend to contain less N than leaves with lower mean g_s and this rate of C assimilation is maintained with a smaller investment in foliar N by maintaining a high intercellular CO_2 concentration (C_i), as was observed in the current study. A similar pattern was also found by Meziane and Shipley (2001), who analyzed cross-species patterns among SLA, N_{mass} , A_{max} and g_s across contrasting irradiance and nutrient regimes. The best model describing their patterns of correlations amongst traits assumes that N_{mass} and g_s exert independent effects on photosynthesis (Meziane and Shipley 2001). However, much of the lack of fit of their model to several datasets was due to the assumption that g_s is independent of N_{mass} for a given photosynthetic rate. In fact, they observed that for species with the same average A_{max} , those with above-average g_s have below average N_{mass} , a pattern paralleled by our data. Furthermore, when the best-fitting model was modified to allow for a direct effect of N_{mass} on g_s independent of A_{max} , the resultant model provided a good fit to the results of Meziane and Shipley (2001), Reich et al. (1999) and Shipley and Lechowicz (2000). Thus the negative relationship between g_s and foliar N, while rarely investigated, is widely apparent across species and also within individual trees.

What is the adaptive significance of a trade-off? This strategy optimizes photosynthetic rate relative to the cost of both water loss and N investment, rather than maximizing C gain. If the evolutionary benefit of a trade-off between N and g_s is to maximize photosynthetic rate, then N and g_s should be positively correlated, which implies a large cost in water use and N investment. This may be adaptive when water and N resources are

abundant, or when C gain is limited to a short period of each year (in deciduous trees; Eamus et al. 1999). However, where water and N availability are limiting, an optimum relationship between N and g_s is one that maximises C assimilation relative to the cost incurred for both water and N (Farquhar et al. 2002). This results in a negative correlation of g_s and N_{mass} for a given A_{area} and supports the prediction that water-use-efficiency and nitrogen-use-efficiency should be inversely related (Buckley et al. 2002).

Co-ordination of g_{max} , nitrogen and water transport capacity: implications for A_{max}

A strong positive relationship between branch hydraulic conductivity and mean g_s was observed, similar to that of others (Comstock 2000; Schäfer et al. 2000; Hubbard et al. 2001). Such correlations ensure a balance between water loss and water supply to leaves (Tyree and Ewers 1991; Cruiziat et al. 2002), thereby ensuring the maintenance of a favourable leaf water status (Tyree and Ewers 1991; Sperry 2000).

Besides the positive relationship between g_s and k_s , we observed a strong negative correlation between k_s and foliar N concentration and this relationship was independent of changes in SLA. On average, trees with low branch hydraulic conductivities achieved similar rates of photosynthesis to trees with high conductivities. The positive and negative correlations of g_s and N (respectively) with k_s results from a trade-off (or substitution) of g_s and investment in foliar N in relation to the water transport capacity of the branch. Thus, trees with high branch conductivities achieved a given rate of C assimilation by having higher g_s and investing less in foliar N, while trees with lower branch conductivities achieve a given rate of photosynthesis by investing more N, but having lower g_s . Trees with lower branch conductivities reduce their rate of water use by maintaining a lower g_s because of the requirement to balance water loss with the capacity to supply water (Tyree and Ewers 1991). However, by allocating more N to foliage, trees with lower branch hydraulic conductivities and g_s , can fix more C for a given unit of water loss (so have higher water-use-efficiency). Given this trade-off, C assimilation rate appears to be independent of hydraulic conductivity. This contradicts a number of previous studies showing that trees with lower branch conductivities have lower photosynthetic rates (Brodribb & Field 2000; Hubbard et al. 2001; Rust & Roloff 2002). However, our results are consistent with Macinnis-Ng et al. (2004) who observed that sapwood specific hydraulic conductivity was independent of assimilation rate during summer. We observed that g_s and N_{mass} were differentially correlated with hydraulic conductivity and as a consequence an approximately constant photosynthetic rate was maintained when water transport capacity decreased. We conclude that leaves offset stomatal limitations on photosynthesis resulting from reduced hydraulic conductivity by allocating more N to foliage, as observed by Macinnis-Ng et al. (2004).

Species invest more N in foliage as rainfall declines (Wright et al. 2005). Similarly, Farquhar et al. (2002) and Buckley et al. (2002) predict that as water availability declines, foliar N concentration should increase while g_s should decrease. The inverse relationship of g_s and N with increasing rainfall in the GLOPNET data acts to

minimizes changes in photosynthetic rate across large rainfall gradients (Wright et al. 2005). Data presented in the present study support the view that changes in foliar N and g_s work in concert to allow trees to minimize the decline in photosynthetic rate as branch water transport capacity (rather than rainfall) declines.

Although resource substitution as a function of height growth has been examined (Buckley and Roberts (2006a, 2006b Ryan et al. 2006), few have observed a trade-off between g_s and foliar N in relation to hydraulic conductivity. Schulze et al. (1998) observed that plants with high foliar N concentration conserve water, in agreement with the results presented here. In contrast, Santiago et al. (2004) found no correlation between foliar N concentration and leaf specific branch hydraulic conductivity. There are a number of possible reasons for these contrasting results. First, Santiago et al. (2004) looked only at bivariate relationships amongst traits and it is likely that the cross-correlation of leaf N with other variables may have obscured any relationship between N and hydraulic conductivity. Thus, when SLA was held constant, we observed a significant relationship between N_{mass} and hydraulic conductivity. Second, trees occupying mesic sites (e.g Santiago et al. 2004, MAR = 3100 mm) are likely to adopt a strategy that maximizes C gain (given the abundance of water resources) rather than optimizes resource use. Rates of C assimilation measured by Santiago et al. (2004) were between 10-20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ which is much higher than the rates we recorded and generally higher than those observed in water limited environments (Wright et al. 2005). One would expect selection pressures to result in optimization of resource use to ensure that C gain is maximized relative to water transport capacity. We might expect a similar situation in deciduous tree species or for species occupying highly seasonal environments. Deciduous trees fix C during periods of abundant water supply (Eamus et al. 1999; Eamus and Prior 2001) and therefore are subject to selective pressures to maximize C gain, rather than optimizing the use of water and N resources. The same might also apply to evergreen trees that occupy highly seasonal environments. Substituting g_s for N_{mass} is a strategy that optimizes the use of water and N resources.

Conclusions

This study examined the relationship between C gain and branch water transport capacity. We conclude that 1) mean g_s and foliar N concentration can exert independent effects on instantaneous rates of C uptake in water and N-limited environments; 2) where C assimilation is simultaneously limited by water and N, water may be substituted for N, or N for water, to achieve the same assimilation rate; (3) trees with low branch water transport capacities can achieve a high rate of C gain for a given g_s by investing a lot of N in leaves whilst trees with high water transport capacities can reduce their allocation of N to photosynthesis but maintain a high g_s and hence a high rate of C fixation. Consequently the reduction in C fixation capacity that may be expected to occur in water limited environments is much smaller than would occur in the absence of this strategy of resource substitution.

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Table 1. Results of several fully nested ANOVA models for leaf and hydraulic architecture traits.

Variable [‡]	Scale	<i>df</i>	MS	<i>F</i>	<i>P</i> -value	VC [†] (%)
A_{area} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Site	3	0.2695	0.55	0.67260	< 0.01
	Species(Site)	4	0.5049	4.53	0.00658	46.61
	Trees	26	0.1116			53.39
g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)	Site	3	6.2453	1.18	0.42336	5.36
	Species(Site)	4	5.3395	5.86	0.00147	49.63
	Trees	28	0.9107			45.01
N_{mass} (%)	Site	3	0.0648	11.70	0.01862	66.10
	Species(Site)	4	0.0056	1.85	0.14617	6.50
	Trees	29	0.0030			29.70
SLA (cm g^{-1})	Site	3	0.2678	1.51	0.33996	15.88
	Species(Site)	4	0.1780	10.47	0.00002	57.19
	Trees	29	0.0170			26.93
k_s ($\text{ml cm s}^{-1} \text{MPa}^{-1} \text{cm}^{-2}$)	Site	3	147.35	1.74	0.273	20.95
	Species(Site)	4	86.53	5.43	0.002	43.55
	Trees	27	15.94			35.50
PLC (%)	Site	3	68.6	0.40	0.760	< 0.01
	Species(Site)	4	165.7	0.64	0.644	< 0.01
	Trees	15	260.2			> 99.99

[‡]Units prior to $\ln x + 1$ transformation are shown; k_s and PLC were not \ln -transformed

[†]VC; variance component expressed as a percentage of total variation

Table 2. Mean values of leaf traits and branch hydraulic architecture traits for each species.

Site	Species	A_{area}	A_{mass}	g_s	E^\dagger	N_{mass}	SLA	k_s	PLC
Wogoon NR	<i>Callitris glaucophylla</i>	7.6	10.9	53.1	3.2	1.35	33.6	15.7	21.2
	<i>Eucalyptus populnea</i>	1.9	7.7	92.0	3.2	1.32	39.7	3.1	49.5
Windsor	<i>Angophora bakeri</i>	1.5	9.0	27.0	1.0	1.19	60.0	10.5	29.2
	<i>Eucalyptus sclerophylla</i>	2.9	15.0	135.3	4.2	1.28	51.1	11.4	33.1
Royal NP	<i>Eucalyptus haemastoma</i>	3.4	15.3	1024.5	7.2	1.05	44.2	21.9	30.2
	<i>Angophora hispida</i>	2.2	11.5	164.2	2.3	0.90	55.6	15.09	35.2
Jilliby SCA	<i>Angophora costata</i>	1.8	9.4	101.7	2.2	1.00	53.3	13.6	22.2
	<i>Eucalyptus haemastoma</i>	2.4	7.9	248.9	4.3	0.93	33.5	14.6	31.04

[†]Transpiration rate

Note: units for A_{area} are $\mu\text{mol m}^{-2} \text{s}^{-1}$, A_{mass} are $\text{nmol s}^{-1} \text{g}^{-1}$, g_s are $\text{mmol m}^{-2} \text{s}^{-1}$, E are $\text{mmol m}^{-2} \text{s}^{-1}$, N_{mass} are %, SLA are $\text{cm}^2 \text{g}^{-1}$, k_s are $\text{cm}^3 \text{cm s}^{-1} \text{MPa}^{-1} \text{cm}^{-2}$ and for PLC are %.

Table 3. Results of multiple regression analyses of the relationship between A_{area} , g_s and N_{mass} , and the relationship between g_s and N_{mass} .

Dependent	Predictor	Partial slope	<i>P</i> -value	Variance explained (%)
A_{area}	g_s	0.18	0.0001	38.68
	N_{mass}	1.75	0.0165	17.70
A_{area}	g_s	0.15	0.0016	29.47
	N_{mass}	1.81	0.0122	19.76
	SLA	-0.40	0.1549	6.85
g_s	A_{area}	2.15	0.0001	38.68
	N_{mass}	-6.96	0.0049	23.48
g_s	A_{area}	1.91	0.0016	29.47
	N_{mass}	-6.29	0.0135	19.27
	SLA	-0.98	0.3290	3.29

Note; the results of these regression models are qualitatively similar whether nitrogen is expressed on a mass or area basis. We used mass based nitrogen because it is independent of SLA. *P*-values of relationships referred to in the text are in bold.

Table 4. Pearson's correlation coefficient (*r*) matrix of water transport capacity and leaf trait variables.

Variable		A_{area}	g_s	N_{mass}	k_s	PLC
g_s	<i>r</i>	0.527				
	<i>P</i> -value	0.002				
	<i>N</i>	33				
N_{mass}	<i>r</i>	0.171	-0.291			
	<i>P</i> -value	0.332	0.086			
	<i>N</i>	34	36			
k_s	<i>r</i>	0.289	0.584	-0.328		
	<i>P</i> -value	0.109	≤ 0.0001	0.018		
	<i>N</i>	32	34	35		
PLC	<i>r</i>	0.275	-0.215	0.190	-0.554	
	<i>P</i> -value	0.215	0.336	0.384	0.006	
	<i>N</i>	22	22	23	23	
SLA	<i>r</i>	-0.388	-0.387	0.027	0.091	-0.012
	<i>P</i> -value	0.023	0.020	0.872	0.604	0.958
	<i>N</i>	34	36	37	35	23

Note; the results are qualitatively similar whether mass or area based expressions of leaf nitrogen are used. Significant relationships are in bold with one *P*-value italicized indicating a marginally significant ($P < 0.1$) result.

Table 5. Results of multiple regression analyses of the relationship between foliar nitrogen concentration (N_{mass} and N_{area}) and branch hydraulic conductivity. Relevant relationships referred to in the text are in bold.

Dependent	Independent	Slope	<i>P</i> -value	Variance explained (%)
N_{mass}	k_s	-0.005	0.018	15.8
N_{mass}	k_s	-0.006	0.015	17.0
	SLA	0.055	0.363	2.6
N_{area}	k_s	-0.01	0.054	10.8
N_{area}	k_s	-0.008	0.012	18.0
	SLA	-0.643	≤ 0.0001	66.2

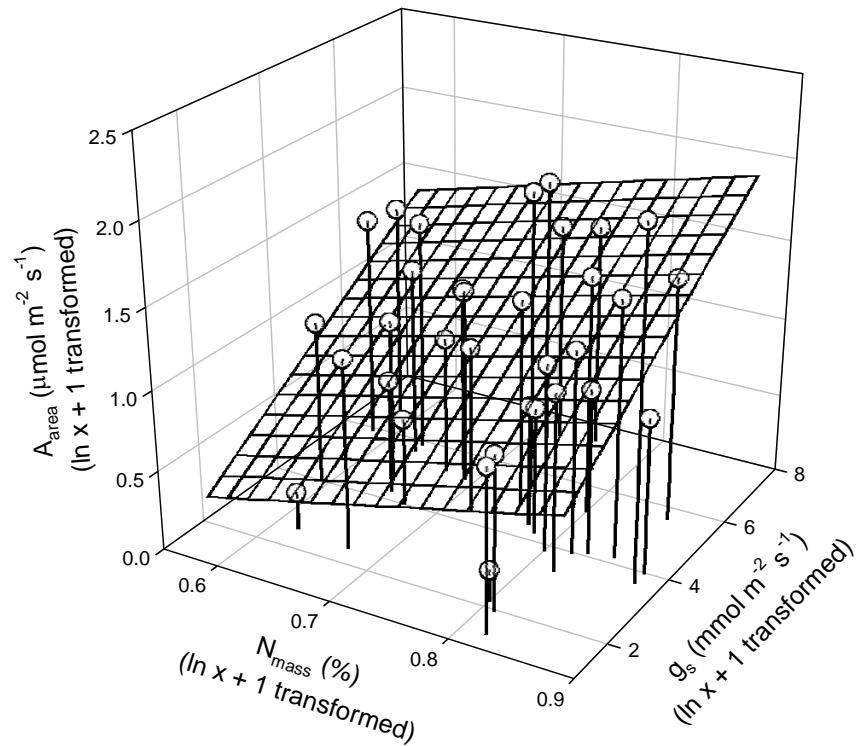


Figure 1. The relationship between photosynthetic rate, mass based foliar nitrogen (N_{mass}) and mean stomatal conductance (g_s). The partial regression coefficients for both g_s and N_{mass} were significant ($P = 0.001$ and 0.016 respectively). The regression equation describing the plane is: $A_{\text{area}} = (g_s \times 0.18) + (N_{\text{mass}} \times 1.75) - 0.935$. The values on each axis are $\ln x + 1$ transformed values.

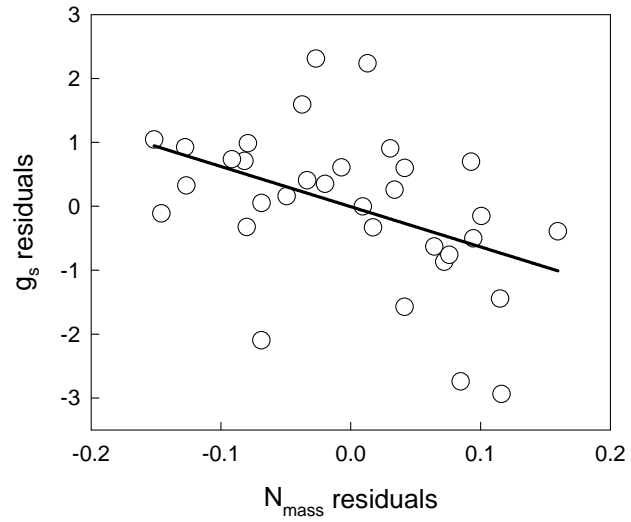


Figure 2. Partial regression plot of g_s against N_{mass} . The plot shows the unique relationship between g_s and N_{mass} for any given A_{area} and SLA. The interpretation of this plot is outlined in the text.

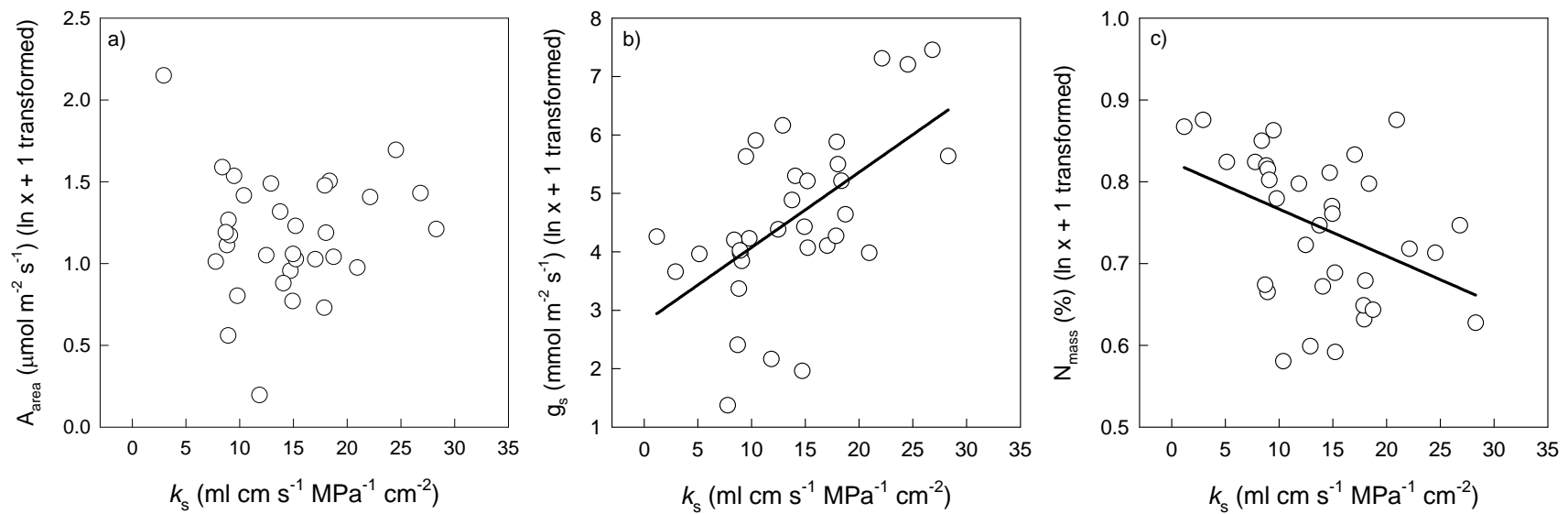


Figure 3. The relationship between sapwood-specific branch hydraulic conductivity (k_s) and (a) area based photosynthetic rate, (b) mean stomatal conductance and (c) mass based foliar nitrogen. Regressions shown in (b) and (c) are significant ($P < 0.05$). The values on each axis are $\ln x + 1$ transformed.

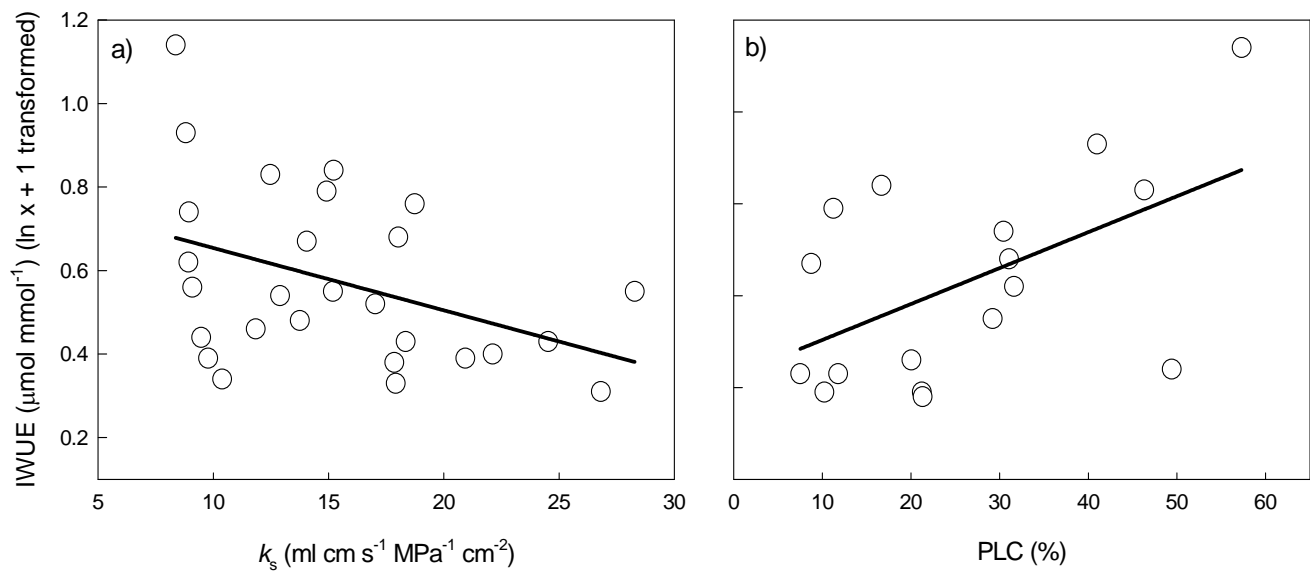


Figure 4a-b. The relationship between instantaneous water use efficiency (IWUE) and sapwood-specific branch hydraulic conductivity (k_s) (Panel a) and IWUE and percentage loss of conductivity due to embolism (PLC) (Panel b). The linear regressions shown are significant ($P < 0.05$). The values IWUE values are ln x + 1 transformed.

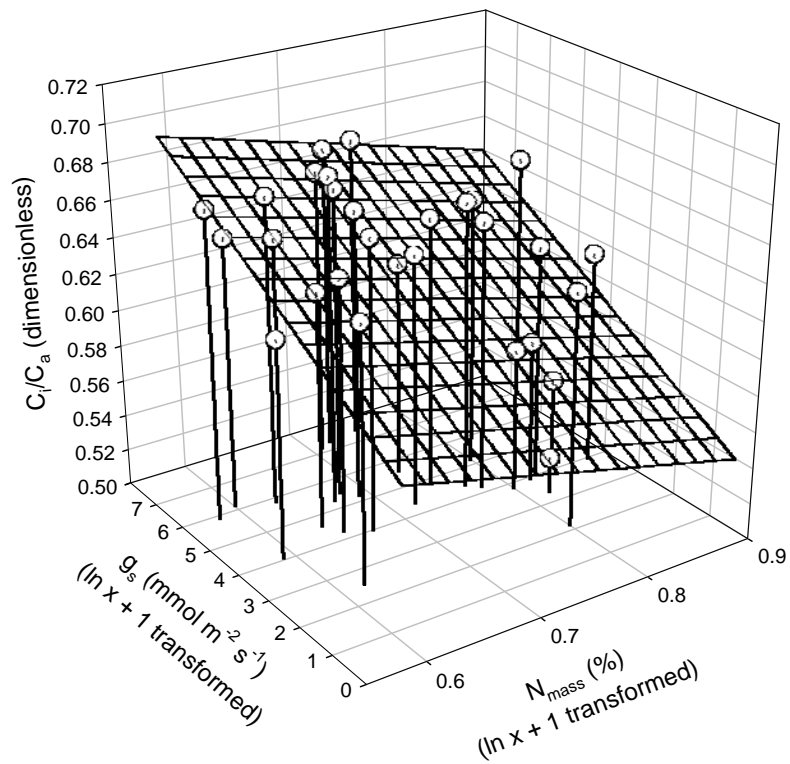


Figure 5. 3-dimensional plot showing the relationship between C_i/C_a vs. g_s and N_{mass} . The partial regression slopes of C_i/C_a vs. g_s and C_i/C_a vs. N_{mass} are each significant. C_i/C_a is negatively related to N_{mass} and positively to g_s .