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Understanding seedling growth relationships through specific leaf area and leaf nitrogen concentration: generalisations across growth forms and growth irradiance

Received: 17 January 2000 / Accepted: 1 October 2000 / Published online: 7 November 2000
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Abstract Seedling relative growth rate (RGR) achieved under favourable growth conditions can be thought of as a useful bioassay of the potential ability of species to take advantage of favourable growth opportunities; that is, of a species' growth strategy. The consistency of relationships between RGR and its component attributes leaf nitrogen productivity (LNP), leaf N per area (LNCA), specific leaf area (SLA) and leaf mass ratio (LMR) was assessed across 12 datasets comprising three growth forms (grasses, herbaceous dicots and woody plants; 250 species in total). These relationships were characterised in terms of scaling slopes (regressions on log-log axes, the slopes giving the proportional relationship between the variables). Mathematically, the expected scaling slope between RGR and each component is 1.0, giving an appropriate null hypothesis to test against (whereas the widely used null hypothesis of zero correlation is in fact inappropriate for this situation). Deviations below 1:1 scaling slopes indicate negative covariance between the components. Consequently, the correlation structure between the components of RGR should also be investigated. Biologically, RGR should scale 1:1 with SLA at a given LNCA and somewhat more weakly with LNCA at a given SLA. SLA and LNCA should themselves scale with a slope of between 0 and –1, with the actual slope indicating the extent to which between-species variation in SLA dilutes leaf N on an area basis versus the ability of species to maintain LNCA at a given growth irradiance. On average, across the 12 datasets RGR scaled close-to-proportionally with SLA, and 1:1 with LNCA at a given SLA. RGR scaled with LNCA with null or negative slopes, since SLA and LNCA scaled negatively (with slopes generally shallower than –1); however, RGR scaled positively (but less than proportionally) with LNCA at a given SLA. For these key relationships there were no qualitatively different conclusions with respect

to the growth form under consideration or the growth irradiance at which the seedlings were grown. RGR also scaled close-to-proportionally with LNP, while LNP and LNCA were negatively associated. These relationships involving LNP are difficult to interpret since it can be shown that they are, at least potentially, the result of the interactions between RGR, SLA and LNCA, as well as reflecting intrinsic differences in the efficiency of nitrogen use in the growth process.

Keywords Nitrogen productivity · Ecological strategies · Relative growth rate

Introduction

Broadly speaking, species characteristic of low-resource habitats are not capable of high seedling relative growth rate (RGR) even when grown under favourable conditions (Grime and Hunt 1975; Chapin 1980; Poorter and Remkes 1990; Wright and Westoby 1999). This appears to be the result of selection in resource-poor environments favouring attributes such as those that enhance leaf longevity and, hence, conservation of nutrients, rather than selection acting on RGR itself (Aerts and van der Peijl 1993; Reich 1993; van der Werf et al. 1993). Thus, seedling RGR achieved under favourable growth conditions can be thought of as a useful bioassay of the potential ability of species to take advantage of favourable growth opportunities.

Seedling RGR (mass increase per time per mass) can be factored into the mass increase per time and unit leaf nitrogen (leaf nitrogen productivity, LNP), leaf nitrogen concentration per unit area (LNCA), specific leaf area (leaf area per unit leaf mass, SLA), and leaf mass ratio (ratio of leaf to total mass, LMR). That is:

$$\text{RGR} = \text{LNP} \times \text{LNCA} \times \text{SLA} \times \text{LMR},$$

or

$$\left[\frac{dM_T}{M_T dt} \right] = \left[\frac{dM_T}{N_L dt} \right] \left[\frac{N_L}{A_L} \right] \left[\frac{A_L}{M_L} \right] \left[\frac{M_L}{M_T} \right] \quad (1)$$

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where M_T is plant dry mass, M_L leaf dry mass, N_L the nitrogen content of leaves, A_L leaf area and t time. This factorisation recognises that nitrogen-rich proteins are largely responsible for carbon gain and that the apparent “efficiency” with which nitrogen is used in this process may vary; that light capture is an area-based phenomenon, and that the leaf area deployed per unit leaf mass, and the amount of nitrogen displayed per unit leaf area may vary; that the proportion of biomass invested in leaves may vary; and, finally, that these attributes may vary independently from one another, at least in principle.

A more common decomposition of RGR involves only three terms, NAR (net assimilation rate, the mass increase per time and unit leaf area), SLA and LMR (i.e. $NAR=LNP \times LNCa$). In this three-component factorisation, SLA has frequently been the strongest correlate of between-species variation in RGR in laboratory or glass-house trials spanning a wide range of growth forms (e.g. Poorter and Remkes 1990; Garnier 1992; Cornelissen et al. 1996; Reich et al. 1998b; Wright and Westoby 1999). By contrast, little generality has emerged for the relationships between RGR and LMR or between RGR and NAR. For NAR, there have been suggestions that the strength of the relationship may vary between growth forms or with the light intensity at which seedlings are grown (van der Werf et al. 1998; Veneklaas and Poorter 1998; but see Poorter and van der Werf 1998), but no consensus has been reached on the matter.

Routine significance testing of correlations between RGR and its component attributes suffers from a drawback. It adopts a null hypothesis that RGR and its component attributes are not correlated. This is inappropriate because, in reality, variation in any one attribute *must* produce variation in RGR unless there is a counteracting trend involving another component, in which case the two components in question will covary negatively. A complementary approach is to investigate the “scaling relationships” between RGR and its components, and between the components themselves. Scaling relationships are log-log regressions, the slopes of which give the proportional relationship between variables. For the decomposition of RGR this produces an additive equation (Eq. 2), which also meets the assumptions underlying regression analysis better than a multiplicative equation.

$$\log RGR = \log SLA + \log LNP + \log LNCa + \log LMR. \quad (2)$$

More importantly though, this approach allows for specific tests of the relationship between variables, with the test not being of a particular strength of correlation, but of slope (proportionality). Specifically, from Eq. 2 it can be seen that the expected slope for a regression of $\log RGR$ on any one $\log(\text{component})$ is 1.0, with a flatter slope indicative of negative covariance between any two (or more) components, while a steeper slope would indicate positive covariance. Thus, investigating this covariance structure along with the RGR-component relationships may help in understanding between-species variation in RGR.

From a biological perspective, directly proportional cross-species scaling relationships can be predicted for some relationships, while for others the expected outcome is less clear. For example, all else being equal, a doubling in leaf area per unit mass (SLA) should result in double the amount of light captured and a doubling in RGR (providing self-shading is minimal). By contrast, a doubling of LNCa (all else held equal) may not result in a doubling of RGR if the growth irradiance is insufficient to saturate the photosynthetic systems of higher LNCa species. That is, the slope may be 1.0 or somewhat less, depending on the extent to which other factors become limiting at increasingly higher leaf N per area.

The relationship between leaf area per mass (SLA) and leaf N per area (LNCa) is of particular interest. SLA is a key component in, and index of, a species' growth strategy (Westoby 1998). It describes the light capture area deployed per unit leaf mass and it tends to scale negatively with leaf lifespan (Reich et al. 1997). Since leaf area is the numerator in SLA and the denominator in LNCa, the natural tendency of an increase in SLA is towards a concomitant decrease in LNCa. On the other hand, LNCa tends to be adjusted within plants according to the light level (Werger and Hirose 1991; Terashima and Hikosaka 1995; Kull and Niinemets 1998). Thus, if there was a single optimal LNCa for carbon gain at a given growth irradiance, convergence towards this concentration would be expected across the species in an experiment. If LNCa was invariant in a set of species (because of this convergence, or because it simply did not vary between species in general or within any given habitat), the scaling slope of LNCa on SLA would be 0. For this to be the case, a doubling in SLA would have to correspond with a doubling in leaf N per mass (LNCm); i.e. the slope of LNCm on SLA would be 1.0. On the other hand, if LNCm was invariant between species, then SLA would scale on LNCa with a slope of -1 , and LNCm would scale on SLA with slope 0. Thus, for tests of the scaling of LNCa on SLA we can identify at least two alternative hypotheses, with slopes of 0 and -1 . Each has a biological interpretation, consequently neither should be considered a null hypothesis.

Wright and Westoby (2000) grew seedlings of 28 species of woody dicots under favourable conditions, finding that, in the four-component factorisation of RGR (Eq. 1) SLA was the strongest determinant of RGR. However, both LNP and leaf N per area explained approximately 20% of variation in RGR, with RGR and LNP being positively associated, LNCa negatively associated with RGR, and LNCm uncorrelated with RGR. On the face of it, this suggested that RGR was influenced more by the efficiency with which leaf nitrogen was used than by the concentration of nitrogen in the leaves, with this efficiency decreasing with increased LNCa, and perhaps being modulated via variation in SLA. The aim of the analysis reported here was to examine the generality of those results by comparing results from a number of cross-species studies. The emphasis was on understanding seedling growth relationships

through the scaling of RGR, SLA and LNCA. Specific questions addressed were:

1. What are the observed scaling slopes of RGR on each of its component attributes (Eq. 2)? In particular, does RGR scale 1:1 with SLA, and 1:1 with LNCA at a given SLA?
2. What is the covariance structure underlying RGR – component scaling relationships which are less than 1:1? In particular, how does LNCA scale with SLA (0, -1, or somewhere in between)?
3. To what extent can the relationships between LNP and other attributes be understood via those between RGR, SLA and LNCA?
4. Do the relationships between these traits differ between seedlings of grasses, herbaceous dicots and woody plants, and do they vary predictably with growth irradiance?

Methods

A literature search was undertaken to identify cross-species datasets about early growth of seedlings, for which RGR could be decomposed according to Eq. 2 (i.e. requiring RGR, SLA, LMR, leaf N concentration, LNP or NAR). Initially the minimum sample size was set at ten species, but this was relaxed to six since few suitable datasets were found. Ten datasets were located, covering a range of growth forms, floras and growth conditions. Two contained both grasses and herbaceous dicots. These were each split into two prior to analysis and the subsets treated as separate datasets since a primary aim was to identify the extent to which trait relationships differed between grasses, herbs and woody species. These groupings were used in the loose sense: a small number of Cyperaceae and Juncaceae were included in one grass dataset. Datasets containing woody species were not further divided into shrubs versus trees nor deciduous versus evergreen, and in some cases included both gymnosperms and angiosperms. Herbs and grasses were not further subdivided into annuals versus perennials. The resulting 12 datasets covered 271 species-dataset combinations (250 species in all; Table 1). Datasets are hereafter described by combination of growth form and first author (apologies to all

secondary authors). Five contained grasses (average sample size 13.2 species), two contained herbaceous dicots (“herbs”; average 22.5 species) and five comprised woody plants only (“woodies”; average 32 species). There was large variation in taxonomic spread as well as species number, with datasets composed of 6–79 species, in 1–56 genera. Altogether 15 species (12 grasses, 3 herbs) occurred in more than one dataset but only 4 of the 15 occurred in more than two. Within-species comparisons were not made.

Seven seedling attributes were considered in each dataset: RGR, NAR, LNP, LNCA, LNCm, SLA and LMR. LNP was inferred from NAR and LNCA for datasets where LNP was not calculated directly, or inferred from whole-plant nitrogen productivity where this had been reported instead (from which LNP can be calculated if both leaf and whole-plant nitrogen concentrations are known). No distinction was made between studies which reported total N versus organic N. Justification for inferring LNP from NAR and LNCA was given by Wright and Westoby (2000), who found a tight relationship between LNP inferred in this manner and directly calculated LNP (22 species, $r^2=0.98$, $\beta=1.02$, $SE=0.04$). A similar comparison was made in a subset of datasets presented here; again the two indices were tightly related (data not shown). A point of concern was whether NAR itself had been calculated directly (e.g. Roumet et al. 1996; Wright and Westoby 1999) or inferred from RGR, SLA and LMR (e.g. Atkin et al. 1998). Comparison of the two possible variants of NAR revealed a generally very tight relationship, providing justification for inferring NAR for the data of Cornelissen et al. (1996), and for not distinguishing datasets by the method of calculation of NAR (comparison of the two NAR variants across datasets from Garnier, Poorter, Reich, Roumet and van der Werf: r^2 values ranged from 0.86 to 0.99, slopes from 0.93 to 1.33, with none significantly different from 1). Still, it should be noted that RGR will not necessarily exactly equal the product of its components when some are calculated over an interval (LNP, NAR) and some from specific points in time (SLA, LMR, LNCA).

Various methods can be used in synthesising data from disparate sources. In general a fairly descriptive approach was adopted here, first treating each dataset as a replicate with equal status, irrespective of the number of species within it. Bivariate trait relationships were characterised predominantly in terms of log-log regression (“scaling”) slopes, while the covariance structure between RGR components was assessed with Pearson correlation r values. All variables were log-transformed prior to analyses. Growth response coefficients (GRCs; Poorter and van der Werf 1998) were reported to enable comparison with other recent studies. The GRC for RGR and a component attribute is simply the

Table 1 Description of the datasets used in analyses and distinction of the five growth form-light (GFL) classes ($n1$ number of species, $n2$ number of genera; light conditions: L , M , H denote (relatively) low-, medium- and high-light conditions, respectively; Gh glasshouse, PAR photosynthetically active radiation). Data from Poorter and Remkes (1990); Poorter et al. (1990); Garnier

(1992); Garnier and Vancaeyzeele (1994); Garnier et al. (1995); Huante et al. (1995); Atkin et al. (1996a, 1996b, 1998); Cornelissen et al. (1996, 1997); Roumet et al. (1996); Reich et al. (1998b, 1998c); van der Werf et al. (1998); Wright and Westoby (2000); and unpublished data from the first-named authors of several of these studies

GFL class	Dataset	$n1, n2$	Light ($\mu\text{mol m}^{-2} \text{s}^{-1} \text{ PAR} \times \text{photoperiod}$)
Grass, medium	Poorter grasses	11, 11	315×14 h
	van der Werf grasses	26, 20	300×14 h
Grass, high	Atkin grasses	6, 1	520×14 h
	Garnier grasses	12, 5	550×16 h
	Roumet grasses	11, 2	500×13 h
Herbs, medium	Poorter herbs	13, 13	315×14 h
	van der Werf herbs	32, 25	300×14 h
Woodies, low	Cornelissen woodies	79, 56	135×14 h
	Wright woodies	28, 13	160×16 h
Woodies, medium	Atkin woodies	10, 1	Gh. 590 at midday ^a
	Huante woodies	34, 29	Gh: unspecified
	Reich woodies	9, 6	Gh: 25% of outside PAR

^a Photoperiod extended to 16 h with 5×75 W incandescent bulbs

scaling slope of the component (dependent) on RGR (i.e. the inverse regression to the regular scaling slope). Since the x -axis data ($\log RGR$) are the same for each regression in a dataset, a GRC slope can be thought of as indicating the relative importance of that attribute in explaining variation in RGR (with no error, the GRCs would sum to 1).

Studies were also ranked as being undertaken at low, medium or high light (for these particular studies, this ranking applied equally well to instantaneous light levels or to daily integrated PAR), and common regression slopes (ANCOVA) calculated across the resulting growth form-light classes (GFLs: grasses, medium light; grasses, high light; herbs, medium light; woodies, low light; woodies, medium light). There was substantial confounding with categorisation by growth form, since the three high-light studies all used grasses and the two low-light studies both involved woody plants. However, comparisons between GFL common scaling slopes could be made between light classes within growth form (medium versus high light within grasses; low versus medium light within woodies), and between the three growth forms (medium light only). Differences between common GFL slopes were assessed using the conservative method of checking for overlap of the 95% confidence intervals.

Results

Across all species, relative growth rate (RGR) ranged approximately 26-fold (mean variation within datasets 3.9-fold, Fig. 1), leaf area per mass (SLA) 6.5-fold (mean 2.9), leaf nitrogen productivity (LNP) 24-fold (mean 4.5) and leaf mass ratio (LMR) varied 5-fold (mean 1.9). Leaf nitrogen concentration ranged between 0.3 and 4.9 $g\ m^{-2}$ (14-fold) on an area basis (LNCA; mean 2.9) and between 1.0 and 6.7% on a mass basis (LNCm; mean 2.3). In general, there was a greater range of variation in the (larger) woody datasets, particularly in LNP, LNCA and LMR. Inspection of Fig. 1 indicates that, in general, RGR was lower for woody species than for grasses and herbs, with the lower RGRs associated with generally lower mean SLA, LNP and LNCm (but not LNCA). No clear differences emerged with differences in growth irradiance, although there was a tendency for grasses grown at high light to have lower SLA and lower LNCA than those grown at medium light.

Bivariate relationships between RGR and components

Specific leaf area was strongly associated with RGR in most datasets, whether considered via coefficients for scaling slopes, correlations or growth response coefficients (GRCs; Fig. 2). Scaling slopes varied between 0.34 and 1.5 (average 0.85), being significantly lower than 1.0 in 5 of 12 individual datasets. Common scaling slopes within growth form-light (GFL) classes ranged from 0.48 to 0.81 (Table 2), with overlapping 95% confidence intervals suggesting no differences between growth forms or with growth irradiance within growth forms (comparisons made for grasses and woody species only). Heterogeneity between datasets within high light grasses (slope flatter for Roumet grasses than for Atkin or Garnier grasses) rendered this test only approximate.

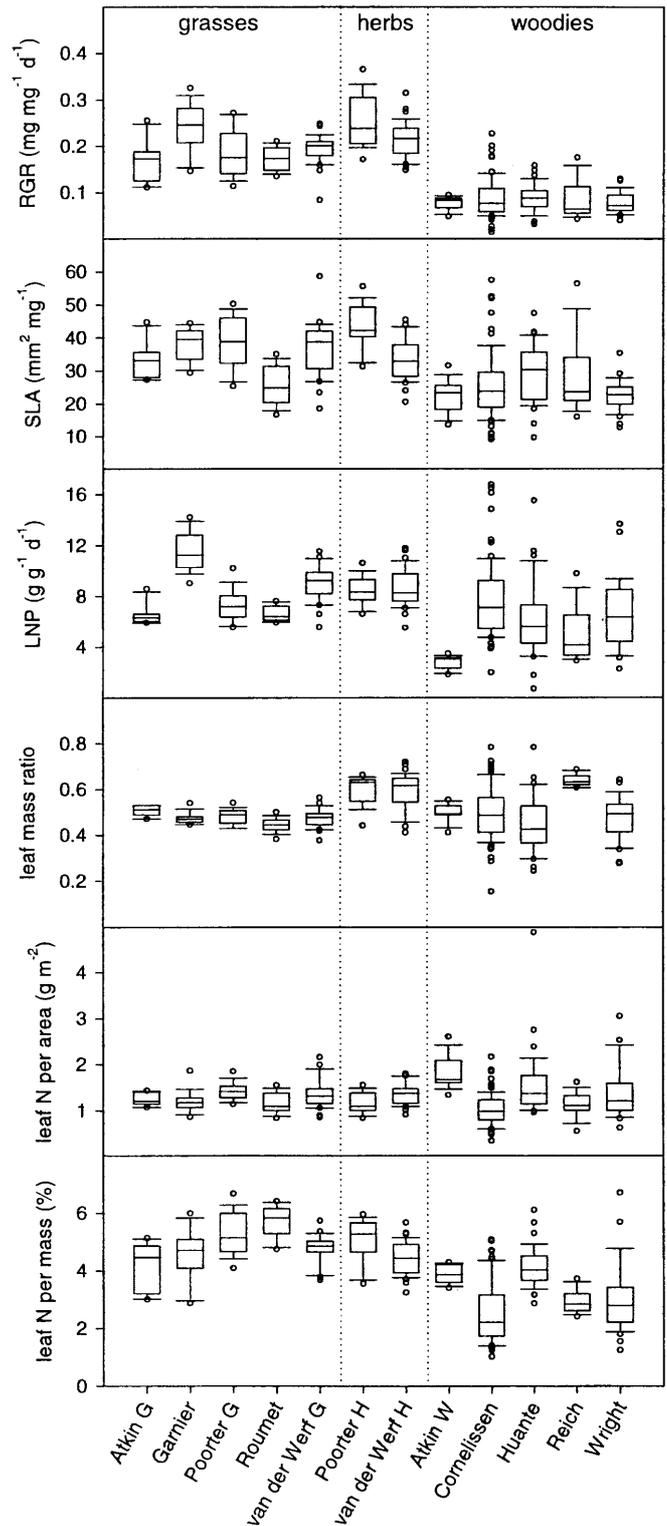


Fig. 1 Boxplots of the 12 datasets, showing means, 10th, 25th, 75th and 90th percentiles, and outliers for seedling relative growth rate (RGR), specific leaf area (SLA), leaf nitrogen productivity (LNP), leaf mass ratio (LMR), leaf N per area (LNCA) and leaf N per mass (LNCm). Dataset definitions given in Table 1

Table 2 Common slopes from ANCOVAs run within the five growth form-light (GFL) classes (95% confidence intervals given in parentheses). Dependent variables are listed first. Dataset was

treated as a random factor (RGR seedling relative growth rate, SLA specific leaf area, LNP leaf nitrogen productivity, LNCa leaf N concentration per area, LMR leaf mass ratio)

GFL class	RGR, SLA	RGR, LNP	RGR, LNCa	RGR, LMR	LNCa, SLA	LNP, LNCa
Grass, medium	0.81 (0.61, 1.02)	1.19 (0.93, 1.44)	-0.65 (-1.0, -0.30)	0.33 (-0.68, 1.33)	-0.72 (-0.87, -0.57) *	-0.55 (-0.78, -0.31)
Grass, high	0.78 (0.40, 1.16) *	1.07 (0.42, 1.72)	0.17 (-0.28, 0.63)	-0.11 (-1.17, 1.48) *	-0.60 (-0.96, -0.23) *	-0.11 (-0.35, 0.13)
Herb, medium	0.48 (0.19, 0.78)	0.79 (0.50, 1.07)	-0.23 (-0.56, 0.11)	0.42 (-0.004, 0.84)	-0.67 (-0.88, -0.45)	-0.35 (-0.61, -0.09)
Woody, low	0.70 (0.51, 0.90)	0.39 (0.18, 0.59)	-0.23 (-0.47, 0.01) *	1.14 (0.87, 1.41) *	-0.37 (-0.54, -0.19)	-0.65 (-0.83, -0.47)
Woody, medium	0.58 (0.30, 0.86)	0.51 (0.35, 0.68) *	-0.54 (-0.85, -0.23)	-0.10 (-0.58, 0.38)	-0.84 (-0.96, -0.72)	-0.77 (-1.2, -0.38)

* Significant interaction term ($P < 0.05$), i.e. heterogeneity of slopes between datasets within a GFL class

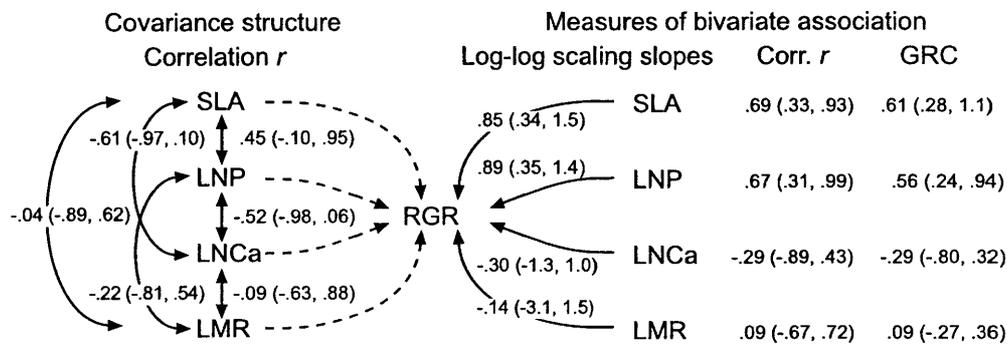


Fig. 2 Comparison of results from various methods used to assess strength of relationships between RGR and components. Numbers given are averages from across the 12 individual datasets, with minimum and maximum values given in parentheses. Covariance structure: Pearson correlations between RGR components indicated with double-headed arrows. Bivariate measures of association: log-log scaling slopes (RGR as dependent variable; coefficients give proportionality of bivariate relationships, with expected slopes of 1 – see Introduction), Corr. r Pearson correlation coefficients, GRC growth response coefficients (log-log slopes with RGR as independent variable, see Methods)

Leaf nitrogen productivity (LNP) was also strongly associated with RGR in most datasets, indeed to a similar extent as was SLA. Scaling slopes for individual datasets varied between 0.35 and 1.4 (average 0.89, significantly less than 1.0 in 3 of 12 cases). Common GFL slopes varied between 0.39 and 1.19, with only those for woody species significantly less than 1.0. While there were no differences associated with irradiance within either grasses or woodies, the common slope was significantly flatter for woody species than for grasses at medium light (although, note the slope heterogeneity within medium light woodies, with that for the Huante dataset flatter than that for the Atkin and Reich datasets).

On average, leaf nitrogen per area (LNCa) was negatively associated with RGR (considered as scaling slopes, correlations or GRCs), with scaling slopes for individual datasets ranging between -1.25 and 1.02 (average -0.29, significantly less than 1.0 in 10 of 12 cases

and significantly less than 0 in 4 of 12, but not different from 0 in the other 8). Common GFL slopes were significantly less than 1 in all cases and significantly less than 0 for both grasses and woodies at medium light (not different from 0 in the remaining 3). No differences were apparent between growth forms (overlapping CIs); however, the slope was more steeply negative at higher light within grasses. When an outlier in the Huante dataset with high LNCa and RGR was removed (*Mimosa tenuiflora*), the slope for medium light woodies dropped from -0.54 to -0.95 (CIs -1.26 to -0.64), which was significantly steeper than that for low-light woodies, and the opposite of the trend with light for grasses.

On average, leaf mass ratio (LMR) showed little relationship with RGR, although scaling slopes and correlations for individual datasets varied between being significantly positive and significantly negative. Common GFL slopes tended to have wide confidence intervals and suggested no difference between growth forms, but a steeper (approximately proportional) slope was suggested at lower light for woody species (although individual slopes were heterogeneous within the low-light woody GFL class).

Relationships between RGR components

Deviations from the expected 1:1 bivariate scaling relationship between RGR and each of its components are

reflected in the covariance (correlation) structure between the components. Mean correlation coefficients calculated across the 12 individual datasets indicated that in almost all cases negative covariance between LNCa and each of SLA and LNP contributed to the observed null or negative scaling slopes between RGR and LNCa (Fig. 2). These negative correlations would also have the effect of flattening the RGR-SLA and RGR-LNP relationships; however, positive covariance between SLA and LNP in most cases (in addition to positive covariance with LMR in others) must have contributed to their generally steeper slopes scaling slopes with RGR. The remaining covariance relationships were on average relatively weak, but varied greatly between individual datasets.

Returning to bivariate relationships, LNP scaled significantly negatively with LNCa in 7 of 12 datasets (average -0.56 , range -1.1 to 0.04). In four of these the slope was not different from -1 ; in two datasets (both grasses) the slope was not different from either 0 or -1 . Common GFL slopes ranged from -0.77 to -0.11 (Table 2), with no difference between growth forms at medium light, but with a less negative slope (not significantly different from 0) at higher light within the grasses. Thus, in general, species within higher LNCa appeared to have lower leaf nitrogen productivity.

LNCa scaled on SLA with a significantly negative slope in 9 of 12 datasets (ranging from 0.06 to -0.97 , with an average slope of -0.57 ; data not shown). Of these nine, six were significantly shallower than -1 but less than 0 (i.e. the two alternative “null” hypotheses). Two of the three slopes that were not significantly negative were also not significantly different from -1 , due to their very wide confidence intervals. Within GFL classes, common LNCa-SLA slopes ranged between -0.37 and -0.84 (Table 2), with all slopes significantly negative but not as negative as -1 . Thus, there was strong evidence that the hypothesis of zero slope could be generally rejected (high-SLA species do in fact have less N per leaf area than low-SLA species), but the alternative hypothesis of a slope of -1 was also generally inconsistent with the results: a doubling in SLA (across species) generally went along with a less-than-halving in LNCa. No difference was evident between growth forms in their LNCa-SLA relationship, while a difference with growth irradiance was found for woody species only (more negative at higher light).

Finally, multiple regressions of $\log RGR$ on $\log SLA$ and $\log LNCa$ were run in order to further explore the relationship between these traits. In each of the 12 datasets the RGR response to SLA at a given LNCa was greater than that to increased LNCa at a given SLA. On average, RGR scaled more or less proportionally with SLA at a given LNCa (1.06 , range 0.26 to 1.8 ; data not shown), yet increased at around half that rate with increased LNCa at a given SLA (average 0.43 , range -0.24 to 1.27). This trend was less pronounced (but not qualitatively different) in the grass datasets than for herbs or woody species, whether considered across all datasets or for

those grown at medium light only (SLA and LNCa partial regression coefficients for grasses: $c. 1.4$ vs. 0.8 ; herbs $c. 0.8$ vs. 0.2 ; woody species $c. 0.9$ vs. 0.2), and did not differ systematically with growth irradiance in grasses and woody species.

Discussion

The key results presented here are that, on average, (1) RGR scaled close-to-proportionally with SLA, and 1:1 with SLA at a given leaf N per area; (2) RGR scaled with LNCa with null or negative slopes, since SLA and LNCa scaled negatively, as did LNCa with LNP (in both cases with slopes generally shallower than -1); however, (3) RGR scaled positively (but less than proportionally) with LNCa at a given SLA; (4) RGR scaled close-to-proportionally with LNP. For these key relationships there were no qualitatively different conclusions with respect to the growth form under consideration. Perhaps this is not surprising: there is no reason to believe that there are intrinsic differences in the way that RGR is determined in different growth forms. Still, grasses, herbs and woody species differed somewhat in terms of mean trait values (RGR, SLA, LNP and LNCm were generally lower in woody species). In some cases scaling slopes varied with growth irradiance (especially those involving LNCa), but never systematically across both grasses and woody species (no comparisons with growth irradiance were possible for herbs).

The role of SLA and leaf N in explaining variation in seedling RGR

Mathematically, RGR and SLA are expected to scale proportionally across species. Where this did not occur, our analyses suggested that this was mostly due to negative covariance between SLA and LNCa, or in some cases due to negative covariance between SLA and LMR. Across species, proportional scaling between RGR and SLA at a given LNCa makes straightforward physiological sense, providing self-shading is minimal and sufficient water and nutrients can be supplied by the root system. Mathematically, a 1:1 response in RGR to leaf N is also expected (considered on their own or at a given SLA), yet the observed slopes were generally flatter than this. Three potential explanations for this result stand out immediately, but are not necessarily mutually exclusive. All potentially contribute to the observed negative relationship between LNP and LNCa.

First, for a proportional RGR-N relationship to hold across a group of species, the net carbon return on each unit of extra nitrogen (LNP) would have to be maintained across the range of LNCa values. However, if higher-LNCa species were more light-limited at a given growth irradiance, the scaling between RGR and leaf N (at a given SLA) would be less than proportional, and higher LNCa species would (by definition) have lower

LNP which, indeed, was found. Secondly, the relationship between N and carbon gain could become partially uncoupled if many species exhibited “luxury consumption” of nitrogen (Chapin 1980), such that increased N led to only a modest increase in growth. Again, in this scenario the apparent efficiency of N use (i.e. LNP) might decrease with increasing nitrogen concentration since the maintenance costs (protein turnover) would increase more or less proportionally with increasing N (Penning de Vries 1975; Ryan 1995) but the gross photosynthetic rate would not. Thirdly, the RGR-N relationship would be weakened if LNCa did not reliably reflect the amount of photosynthetic machinery in leaves, such that higher LNCa species allocated proportionally less leaf N to photosynthetic functions (e.g. Poorter and Evans 1998 reported lower proportional N allocation to photosynthetic function in low SLA species, which are likely to have high LNCa). Again, even if all photosynthetic N was being used optimally, the apparent efficiency of N use would be lower in the high LNCa species. On the other hand, it has also been argued that high RGR (and thus probably high SLA, and perhaps low LNCa) species should have a greater proportion of nitrogen-based defences than low RGR species (Coley et al. 1985), and thus possibly a lower proportion of photosynthetic N in their leaves, by implication.

Leaf nitrogen productivity and RGR

Leaf nitrogen productivity has been described as a complex function of photosynthesis, respiration, nitrogen allocation within leaves, and biomass allocation between leaves and other plant parts (Lambers et al. 1989; Pons et al. 1994; Garnier et al. 1995). LNP has been found to be tightly correlated with the rate of photosynthesis per unit leaf N, at least in herbs and grasses (Garnier et al. 1995), and may reflect variation in N allocation between electron transport versus light harvesting components and perhaps variation in specific Rubisco activity, as well as to allocation of nitrogen to photosynthetic versus non-photosynthetic functions (Poorter and Evans 1998). Because of these many potential contributing factors, variation in LNP is rather difficult to interpret. Indeed, a puzzling aspect of our results is the extent to which cross-species variation in LNP was due to inherent differences in the way species allocate and use nitrogen in the growth process, and to what extent it was an artefact of the relationships between RGR, SLA and leaf nitrogen concentration. Below, we outline arguments which demonstrate that both interpretations are plausible, although both are likely true to some extent.

The strong positive relationship between RGR and LNP suggests that LNP is a key trait which should be considered along with SLA and leaf N as a fundamental component of a species' growth strategy. On the other hand, it is possible that this apparent relationship is also largely artefactual. For example, compare these three

scenarios contrasting two hypothetical species in terms of Eq. 2:

1. With LNCa and LMR held constant, if SLA was doubled so too would RGR and also total leaf N. Hence, LNP would not differ between the species and RGR and LNP would show no relationship.
2. If LMR and total leaf N were held constant but SLA doubled, LNCa would be halved (since the same total N would be spread over twice the leaf area). If RGR then scaled 1:1 with SLA, LNP would double also (it would scale 1:1 with RGR), and LNCa and LNP would scale with a slope of -1 .
3. Given the same conditions as in (2), if RGR less-than-doubled with the doubling in SLA and halving in LNCa, LNP would less-than-double to the same extent as did RGR. Again, RGR and LNP would scale proportionally, while LNCa and LNP would scale negatively but not as steeply as -1 .

Thus, a substantial contribution to the strength of the RGR-LNP relationship in a dataset could come via the RGR-SLA-LNCa relationships, particularly from that between SLA and LNCa.

Still, SLA and LNP were, on average, positively correlated across the 12 individual datasets. To some extent this might have been mediated through their negative relationships with LNCa; however, lower SLA may also be associated with slower intercellular diffusion of CO_2 (Parkhurst 1994) or with greater internal shading of chloroplasts (Terashima and Hikosaka 1995), either of which could decrease the carbon gain per unit leaf N. Similarly, woody species tended to have flatter RGR-LNP relationships, which could possibly be related to their generally lower SLAs through the same mechanisms.

Negative relationship between SLA and leaf N per area

Since light capture, CO_2 uptake and transpiration are inherently area-based phenomena, quantifying leaf nitrogen in terms of concentration per area is perhaps more appropriate than concentration per mass. On the other hand, since leaves vary widely in thickness and density, variation in SLA should perhaps be considered simultaneously (Reich et al. 1998a; Peterson et al. 1999). Leaf nitrogen per area is equivalent to leaf N per mass (LNCm) divided by leaf area per mass (SLA); alternatively, LNCm is the product of leaf N per area and SLA. Scaling slopes between these attributes are easily inter-converted since the slope of LNCm on SLA is simply $1 + \text{slope of LNCa on SLA}$. The fact that SLA and LNCa are negatively correlated might not be surprising; after all, leaf area appears as the numerator in the first attribute and the denominator in the second. However, comparing across species it is not clear why this should result in any specific relationship. In fact, it is the size of variation in SLA compared to LNCm which determines the degree to which SLA and LNCa are negatively correlated in a dataset.

Since SLA is a function of leaf thickness and density (Witkowski and Lamont 1991; Wilson et al. 1999), the nitrogen concentrations of tissue types contributing to variation in thickness and density could affect the relationship between SLA and LNCA (Garnier et al. 1997). For example, if SLA variation was due to variation in leaf thickness, and increased thickness due to thicker N-rich mesophyll, then lower SLA would be associated with higher LNCA. On the other hand, if variation in SLA was due mostly to leaf tissue density, and higher density resulted from a higher proportion of relatively N-poor cell wall or sclerified tissue per unit volume, then the opposite result might be found.

Interaction with growth irradiance

The voluminous literature dealing with optimal nitrogen distribution throughout canopies details examples of within-species adjustment of LNCA according to light conditions (e.g. Werger and Hirose 1991; Kull and Niinemets 1998; Schieving and Poorter 1999 and references therein). Self-shading is probably minimal in most seedling growth studies since plants are generally harvested while exponential growth is still occurring and before a large total leaf area has developed. Still, it is likely that such seedlings can adjust their leaf N content to an appropriate LNCA for a given growth irradiance. If LNCA was optimal for carbon gain in all species in a growth experiment (and their inherent LNP did not vary), LNCA would be maintained at a similar level across species despite between-species variation in SLA, and the two attributes would scale with a slope not different from 0. In fact, they were generally negatively associated; furthermore, there was variation in LNCA within any dataset (2.9-fold, on average), suggesting that either LNCA was not closely adjusted to growth irradiance, or the inherent LNP did differ between species.

For woody species but not grasses, the scaling slope of LNCA on SLA was less negative at lower light. This indicates that LNCA responded less per unit change in SLA; i.e. that LNCA was maintained in the face of SLA variation more at low light than at high light. If this was also the case in field situations, one interpretation might be that LNCA was being adjusted to light conditions, while variation in SLA may be more closely related to variation in tissue turnover (lower tissue turnover being more advantageous at low light, for example). In our minds, these issues emerge as interesting questions for the future: whether differences between growth relationships at low light and higher light levels (e.g. Veneklaas and Poorter 1998; Walters and Reich 1999) can be understood in terms of the scaling of SLA and leaf nitrogen. Further, we believe that the use of scaling relationships in conjunction with the identification of the covariance structure in a dataset will help interpret seedling growth relationships in a more sophisticated way than is possible when bivariate RGR-component correlations are viewed on their own.

Acknowledgements Many thanks to Owen Atkin, Eric Garnier, Peter Reich, Catherine Roumet, Hendrik Poorter, and Adrie van der Werf for cheerfully supplying data for analyses (in some cases, unpublished data), and especially to the latter two for also reading an early draft of this manuscript.

References

- Aerts R, Peijl MJ van der (1993) A simple model to explain the dominance of low-productive perennials in nutrient-poor habitats. *Oikos* 66:144–147
- Atkin OK, Botman B, Lambers H (1996a) The causes of inherently slow growth in alpine plants – an analysis based on the underlying carbon economies of alpine and lowland *Poa* species. *Funct Ecol* 10:698–707
- Atkin OK, Botman B, Lambers H (1996b) The relationship between the relative growth rate and nitrogen economy of alpine and lowland *Poa* species. *Plant Cell Environ* 19:1324–1330
- Atkin OK, Schortemeyer M, McFarlane N, Evans JR (1998) Variation in the components of relative growth rate in ten *Acacia* species from contrasting environments. *Plant Cell Environ* 21:1007–1017
- Chapin FS (1980) The mineral nutrition of wild plants. *Annu Rev Ecol Syst* 11:233–260
- Coley P, Bryant J, Chapin F (1985) Resource availability and plant antiherbivore defense. *Science* 230:895–899
- Cornelissen JHC, Castro Diez P, Hunt R (1996) Seedling growth, allocation and leaf attributes in a wide range of woody plant species and types. *J Ecol* 84:755–765
- Cornelissen JHC, Werger MJA, Castro-Diez P, Rheejan JWA van, Rowland AP (1997) Foliar nutrients in relation to growth, allocation and leaf traits in seedlings of a wide range of woody plant species and types. *Oecologia* 111:460–469
- Garnier E (1992) Growth analysis of congeneric annual and perennial grass species. *J Ecol* 80:665–675
- Garnier E, Vancaeyzeele S (1994) Carbon and nitrogen content of congeneric annual and perennial grass species: relationships with growth. *Plant Cell Environ* 17:399–407
- Garnier E, Gobin O, Poorter H (1995) Nitrogen productivity depends on photosynthetic nitrogen use efficiency and on nitrogen allocation within the plant. *Ann Bot* 76:667–672
- Garnier E, Cordonnier P, Guillermin J-L, Soni L (1997) Specific leaf area and leaf nitrogen concentration in annual and perennial grass species growing in Mediterranean old-fields. *Oecologia* 111:490–498
- Grime JP, Hunt R (1975) Relative growth-rate: its range and adaptive significance in a local flora. *J Ecol* 63:393–422
- Huante P, Rincón E, Acosta I (1995) Nutrient availability and growth rate of 34 woody species from a tropical deciduous forest in Mexico. *Funct Ecol* 9:849–858
- Kull O, Niinemets Ü (1998) Distribution of leaf photosynthetic properties in tree canopies – comparison of species with different shade tolerance. *Funct Ecol* 12:472–479
- Lambers H, Freijns N, Poorter H, Hirose T, Werf A van der (1989) Analyses of growth based on net assimilation rate and nitrogen productivity. Their physiological background. In: Lambers H, Cambridge ML, Konings H, Pons TL (eds) Causes and consequences of variation in growth rate and productivity of higher plants. SPB Academic, The Hague, pp 1–17
- Parkhurst DF (1994) Diffusion of CO₂ and other gases inside leaves. *New Phytol* 126:449–479
- Penning de Vries FWT (1975) The cost of maintenance processes in plant cells. *Ann Bot* 39:77–92
- Peterson AG, participants C (1999) Reconciling the apparent difference between mass- and area-based expressions of the photosynthesis-nitrogen relationship. *Oecologia* 118:114–150
- Pons TL, Werf A van der, Lambers H (1994) Photosynthetic nitrogen use efficiency of inherently slow- and fast-growing species: possible explanations for observed differences. In:

- Roy E, Garnier E (eds) A whole plant perspective on carbon-nitrogen interactions. SPB Academic, The Hague, pp 61–77
- Poorter H, Evans JR (1998) Photosynthetic nitrogen-use efficiency of species that differ inherently in specific leaf area. *Oecologia* 116:26–37
- Poorter H, Remkes C (1990) Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth. *Oecologia* 53:553–559
- Poorter H, Werf A van der (1998) Is inherent variation in RGR determined by LAR at low irradiance and by NAR at high irradiance? A review of herbaceous species. In: Lambers H, Poorter H, Van Vuuren MMI (eds) Inherent variation in plant growth. Physiological mechanisms and ecological consequences. Backhuys, Leiden, pp 309–336
- Poorter H, Remkes C, Lambers H (1990) Carbon and nitrogen economy of 24 species differing in relative growth rates. *Plant Physiol* 94:621–627
- Reich PB (1993) Reconciling apparent discrepancies among studies relating life span, structure and function of leaves in contrasting plant life forms and climates: “the blind men and the elephant” retold. *Funct Ecol* 7:721–725
- Reich PB, Walters MB, Ellsworth DS (1997) From tropics to tundra: global convergence in plant functioning. *Proc Natl Acad Sci USA* 94:13730–13734
- Reich PB, Ellsworth DS, Walters MB (1998a) Leaf structure (specific leaf area) modulates photosynthesis-nitrogen relations: evidence from within and across species and functional groups. *Funct Ecol* 12:948–958
- Reich PB, Tjoelker MG, Walters MB, Vanderklein DW, Bushena C (1998b) Close association of RGR, leaf and root morphology, seed mass and shade tolerance in seedlings of nine boreal tree species grown in high and low light. *Funct Ecol* 12:327–338
- Reich PB, Walters MB, Tjoelker MG, Vanderklein D, Buschena C (1998c) Photosynthesis and respiration rates depend on leaf and root morphology and nitrogen concentration in nine boreal tree species differing in relative growth rate. *Funct Ecol* 12:395–405
- Roumet C, Bel MP, Sonie L, Jardon F, Roy J (1996) Growth response of grasses to elevated CO₂: a physiological plurispecific analysis. *New Phytol* 133:595–603
- Ryan MG (1995) Foliar maintenance respiration of subalpine and boreal trees and shrubs in relation to nitrogen content. *Plant Cell Environ* 18:765–772
- Schieving F, Poorter H (1999) Carbon gain in a multispecies canopy: the role of specific leaf area and photosynthetic nitrogen-use efficiency in the tragedy of the commons. *New Phytol* 143:201–211
- Terashima I, Hikosaka K (1995) Comparative ecophysiology of leaf and canopy photosynthesis. *Plant Cell Environ* 18:1111–1128
- Veneklaas E, Poorter L (1998) Growth and carbon partitioning of tropical tree seedlings in contrasting light environments. In: Lambers H, Poorter H, Van Vuuren MMI (eds) Inherent variation in plant growth. Physiological mechanisms and ecological consequences. Backhuys, Leiden, pp 337–361
- Walters MB, Reich PB (1999) Low-light carbon balance and shade tolerance in the seedlings of woody plants: do winter deciduous and broad-leaved evergreen species differ? *New Phytol* 143:143–154
- Werf A van der, Nuenen M van, Visser AJ, Lambers H (1993) Contribution of physiological and morphological plant traits to a species’ competitive ability at high and low nitrogen supply. *Oecologia* 94:434–440
- Werf A van der, Geerts RHEM, Jacobs FHH, Korevaar H, Oomes MJM, Visser W de (1998) The importance of relative growth rate and associated traits for competition between species during vegetation succession. In: Lambers H, Poorter H, Van Vuuren MMI (eds) Inherent variation in plant growth. Physiological mechanisms and ecological consequences. Backhuys, Leiden, pp 489–502
- Wenger MJA, Hirose T (1991) Leaf nitrogen distribution and whole canopy photosynthetic carbon gain in herbaceous stands. *Vegetatio* 97:11–20
- Westoby M (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant Soil* 199:213–227
- Wilson PJ, Thompson K, Hodgson JG (1999) Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. *New Phytol* 143:155–162
- Witkowski ETF, Lamont BB (1991) Leaf specific mass confounds leaf density and thickness. *Oecologia* 88:486–493
- Wright IJ, Westoby M (1999) Differences in seedling growth behaviour among species: trait correlations across species, and trait shifts along nutrient compared to rainfall gradients. *J Ecol* 87:85–97
- Wright IJ, Westoby M (2000) Cross-species relationships between seedling relative growth rate, nitrogen productivity and root versus leaf function in 28 Australian woody species. *Funct Ecol* 14:97–107