Journal of Experimental Botany, Vol. 61, No. 8, pp. 2043–2055, 2010
doi:10.1093/jxb/erp358 Advance Access publication 4 January, 2010

Journal of Experimental Botany
www.jxb.oxfordjournals.org

REVIEW PAPER

A method to construct dose–response curves for a wide range of environmental factors and plant traits by means of a meta-analysis of phenotypic data

Hendrik Poorter[1,2,*], Ülo Niinemets[3], Achim Walter[1], Fabio Fiorani[4] and Uli Schurr[1]

[1] ICG-3 (Phytosphäre), Forschungszentrum Jülich, D-52425 Jülich, Germany
[2] Ecophysiology of Plants, Institute of Environmental Biology, PO Box 800.84, 3508 TB Utrecht, The Netherlands
[3] Department of Plant Physiology, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, Tartu 51014, Estonia
[4] CropDesign NV, Technologiepark 3, B-9052 Zwijnaarde, Belgium

* To whom correspondence should be addressed. E-mail: H.Poorter@fz-juelich.de

Received 27 July 2009; Revised 27 October 2009; Accepted 2 November 2009

Abstract

In the past, biologists have characterized the responses of a wide range of plant species to their environment. As a result, phenotypic data from hundreds of experiments are publicly available now. Unfortunately, this information is not structured in a way that enables quantitative and comparative analyses. We aim to fill this gap by building a large database which currently contains data on 1000 experiments and 800 species. This paper presents methodology to generalize across different experiments and species, taking the response of specific leaf area (SLA; leaf area:leaf mass ratio) to irradiance as an example. We show how to construct and quantify a normalized mean light–response curve, and subsequently test whether there are systematic differences in the form of the curve between contrasting subgroups of species. This meta-analysis is then extended to a range of other environmental factors important for plant growth as well as other phenotypic traits, using >5300 mean values. The present approach, which we refer to as 'meta-phenomics', represents a valuable tool in understanding the integrated response of plants to their environment and could serve as a benchmark for future phenotyping efforts as well as for modelling global change effects on both wild species and crops.

Key words: Biomass allocation, dry matter percentage, environment, meta-phenomics, plasticity, response curve, specific leaf area.

Introduction

The last 100 years have seen a substantial increase in efforts invested in plant biology research, and an even greater rise in the number of scientific publications documenting the outcome of these efforts. While the first investigations of botanists focused on the analysis of plants growing in an agricultural setting or in their natural habitat (Kreusler, 1879; Hanson, 1917), gradually the research focus has shifted to include pot-grown plants raised in experimental gardens or glasshouses. Although this allowed for a more standardized supply of nutrients and water, such plants still experience strong variation of light and temperature over the day, from day to day, and across seasons, complicating comparisons across experiments. The use of plant growth chambers enabled an even better control of the environmental conditions in which the plants were grown and allowed them to be challenged with a reproducible environment (Went, 1957). In this way, the effect of a range of environmental factors on the growth and development of plants could be studied, by comparing two or more test groups exposed to the same target set of environmental

Abbreviations: DMC, dry matter content; DPI, daily photon irradiance; LMF, leaf mass fraction; SLA, specific leaf area; SMF, stem mass fraction; RMF, root mass fraction.

© The Author [2010]. Published by Oxford University Press [on behalf of the Society for Experimental Biology]. All rights reserved.
For Permissions, please e-mail: journals.permissions@oxfordjournals.org

JXB
Journal of Experimental Botany
www.jxb.oxfordjournals.org
Downloaded from jxb.oxfordjournals.org at Universite Laval on September 9, 2010

conditions except for the factor(s) of interest (Evans *et al.*, 1985).

Thousands of experiments have been carried out in these different settings, and an amazing variety of plant species have been tested for their response to a suite of environmental conditions. With the increasing number of published data sets, the necessity arose to summarize results and generalize from case studies to broad-scale patterns. The first attempts to produce a synthesis of the published research consisted of an expert-based description. Scientists working in a certain area outlined general trends by combining information from a range of publications and presenting these in a generally narrative way. This approach, which has proven valuable up to today, allows for a flexible form of reviewing and emphasizes paradigms and patterns considered of major significance by leading experts. However, narrative reviews could potentially suffer from drawbacks. They focus mainly on qualitative (i.e. directionality of response) rather than on quantitative differences and they may contain varying levels of subjective judgement, as it is almost impossible for the authors not to express their personal view. Although conceivable, it is hard to devise standardized procedures for this traditional form of review.

The last 30 years have seen the development of a more quantitative approach to reviewing a certain research area, in the form of so-called 'meta-analyses' (Hedges and Olkin, 1985). This type of analysis aims at generalizing in a formal way across a number of independent experiments. To this end, a suitable effect–size metric is defined, for example the value of the variable of interest measured in environment A divided by its value in environment B (Osenberg *et al.*, 1997; Hedges *et al.*, 1999). Following such an approach, the response of various plant traits to specific environmental factors has been analysed [e.g. Searles *et al.* (2001) for UV-B; Morgan *et al.* (2003) for ozone; Poorter and Navas (2003) for CO_2]. Meta-analyses, carefully restricted to discrete treatment levels, such as the effect of elevated CO_2 in the 600–800 μmol mol^{-1} range compared with a baseline level in the 300–400 μmol mol^{-1} range, may give a synthetic overview of the response of plants to the expected doubling of the CO_2 concentration. As such, they can provide a focused answer to practical questions, such as the effect of global change on agricultural productivity in 50 years time, taking the current situation as a baseline. However, nearly all environmental factors that affect plant growth are intrinsically continuous variables, and we would gain much better insight into a plant's physiology if we account for this by generating overall response curves. The most basic questions would then be whether the response is positive or negative, and whether there are any non-linearities (De Groot *et al.*, 2002). From the archetypical response curves as described by Mitscherlich (1909) for nutrients, we know that such non-linearities may well occur and, in some cases, their modelling has advanced our understanding significantly (Farquhar *et al.*, 2001). Unfortunately, for many plant traits we have as yet no proper insight into the exact form of the response curve.

A second goal of a systematic analysis of the literature that could significantly advance our understanding of plant responses to their environment is the quantitative parameterization of such response curves. Quantifying general relationships for a given plant trait—or a combination of traits—across the full range of environmental conditions in which plants generally occur could not only improve ecological models of global change effects, but could also be valuable for breeders to predict the phenotypic performance in environments of varying complexity. A good example is the analysis of Wright *et al.* (2004), who described quantitative relationships between leaf morphology, photosynthetic capacity and leaf nitrogen across a wide range of wild species growing in their natural habitat. These quantitative estimates have been subsequently used to develop further theory on the relationship between growth and nitrogen uptake as observed in the laboratory (Hikosaka and Osone, 2009). An additional asset to the quantification of response curves across a wide variety of experiments with an array of plant species is that it would allow the establishment of 'normal limits'. The concept of normal limits is used advantageously in the medical field as a guide to doctors with respect to the normally expected biological variation for a trait across healthy persons (Bezemer *et al.*, 1982). Observed values beyond these limits do not necessarily indicate serious illness, but are a reason for increased awareness. We believe that the establishment of normal limits could be similarly helpful in plant biology. It could serve as an early warning for scientists that plants in their experiments are 'off' because of unwanted effects, for example a failure in the temperature regulation of the growth room, that have escaped their attention. Having excluded such a possibility, the definition of normal limits could also serve as a quantitative handle to show that a given plant species shows specialized adaptations to its environment, different from most other plant species.

A third goal of such an analysis is to analyse retrospectively whether variation in the response to the environment can be ascribed to differences in the experimental design, or to differences between functional groups of species. In the case of elevated CO_2, such an approach has been fruitfully used to show that plants grown in small pots were restricted in their response to CO_2 (Arp, 1991) and that C_4 species, although responding less strongly than C_3 species, nonetheless increase biomass at elevated CO_2 concentrations (Poorter, 1993). Highly relevant questions that have received little attention so far, for example, are: (i) to what extent is the form of the response curve determined by the cultivation system (outdoor, glasshouses, or in growth chambers); (ii) to what extent is the form of the response curve phylogenetically constrained and; (iii) do ecologically different groups of species respond differently? Although the literature is rich in data documenting a wide variety of stress experiments, plant species, and traits, specific groups of species and phenotypic traits have received more attention than others. An additional result of this type of analysis can be directed awareness about potential gaps in

Downloaded from jxb.oxfordjournals.org at Universite Laval on September 9, 2010

our knowledge that could result in concerted efforts in prioritizing certain types of experiments.

Taking into account the above considerations, we set out to devise an approach that could generalize data across a range of experiments by constructing dose–response curves in a quantitative manner, enabling comparisons across different environmental factors as well as a range of phenotypic traits. After an introduction to this procedure in the next section, we exemplify the method by comparing the response of an important growth-related trait, SLA (specific leaf area), across 12 different environmental factors. Finally, by comparing light–response curves of SLA, biomass allocation, and the dry mass:fresh mass ratio we show how this approach can be successfully scaled up to encompass a wide range of plant traits. We propose to name the methodology where this specific method of meta-analysis is used to describe the phenome of the plant as 'meta-phenomics'.

A method to calculate generalized response curves

To infer proper response curves for a specific organism preferably requires five or more different levels of a given environmental factor over the range that plants are likely to encounter. Although such experiments occasionally have been carried out (MacDowall, 1972; Van de Vijver *et al.*, 1993; Juurola, 2003), the large majority of papers we reviewed (>85%) focused on two or three levels at most. How then can we generalize across such data?

A way to achieve this goal is to construct response curves by combining information from various experiments. To this end, we set out to produce a large compilation of literature data on experiments with individually grown plants, subjected to the experimental manipulation of one or more environmental factors. In the case of multiple factors, experiments were only considered when treatments were applied fully factorially. This compilation currently consists of 1000 experiments, with observations on >800 different species.

To construct response curves from such a database is not straightforward, because experiments have been conducted under widely different conditions, using many different species. A proper analysis requires an appropriate scaling of both the environmental factor (on the x-axis) and the plant response variable (on the y-axis). We achieve this goal using a three-step procedure, which we illustrate by constructing the response curve of SLA (m^2 leaf kg^{-1} leaf dry matter) as dependent on the available light during plant growth. The SLA determines how much light-intercepting leaf area is made given a certain plant biomass investment in leaves, and is as such one of the key factors for the growth of plants (Lambers and Poorter, 1992). It is a widely used plant trait in a range of fields, from plant physiology to ecology (Roumet *et al.*, 1996; Wright *et al.*, 2004). To exemplify the procedure, we start with literature data for four species from four different experiments (Fig. 1A).

(i) Various light levels are generally obtained by applying different neutral density filters, a different number of lamps or by various layers of netting. In the database we collated, about half of the experiments have been carried out in growth rooms in which the light intensity is mostly applied as a square wave, whereas the other half took place in

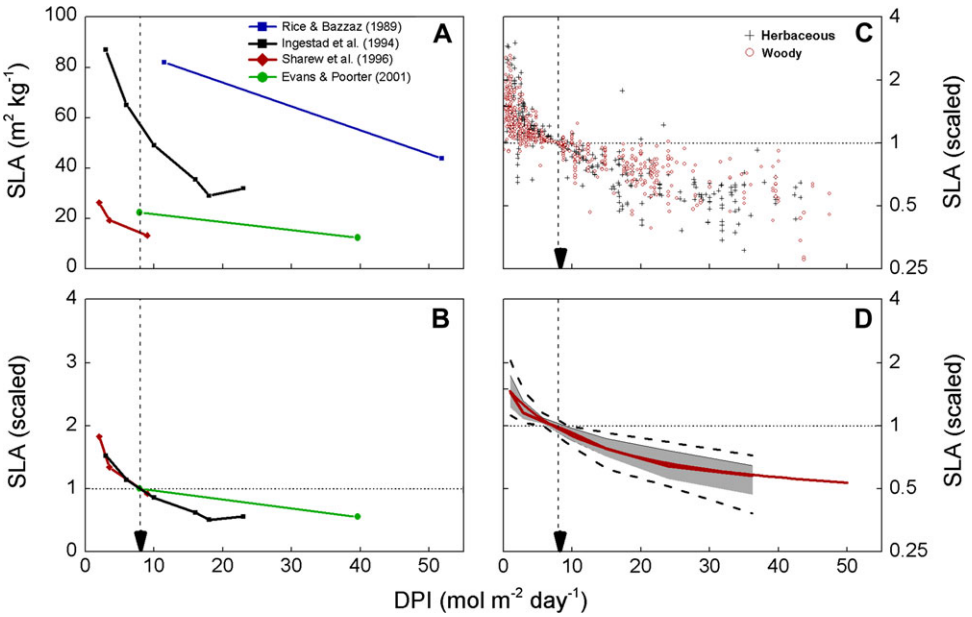

Fig. 1. (A) SLA as dependent on daily photon irradiance (DPI) for four different species measured in different experiments. (B) The same data after scaling (interpolated) SLA to 1 at a DPI of 8 mol m^{-2} d^{-1}. (C) Literature data for ~1050 SLA observations from 150 experiments on a total of 330 species. (D) Response curve constructed from various segments of the data presented in C, indicated by the median value (bold line), the interquartile range (the shaded area indicating the range between the 25th and the 75th percentile), and the 10th and 90th percentile (broken lines). Note that the y-axis of A and B is on a linear scale, whereas those of C and D are logarithmically scaled. The bold line in red in panel D indicates the fitted equation. The reference value of DPI is indicated by the black triangle.

Downloaded from jxb.oxfordjournals.org at Universite Laval on September 9, 2010

glasshouses or outdoor in which light varies greatly over the day. Is there a way to scale light availability in a manner that enables the combination of data from both types of growth environments? A suitable solution is to consider the daily amount of photosynthetic irradiance (DPI), which is the integrated value of light intensity over the day, because it correlates much better with, for example, leaf morphology (Chabot *et al.*, 1979) and plant growth rate (Poorter and Van der Werf, 1998) than light intensity or photoperiod alone.

(ii) Having chosen the appropriate variable on the *x*-axis, the next challenge is to choose the units on the *y*-axis. Plants differ inherently in SLA (Poorter *et al.*, 2009), and experiments vary in the DPI applied, which precludes an analysis of absolute values. We therefore chose to extract relative values, by defining for each experiment the SLA found at a DPI of 8.0 mol m^{-2} d^{-1} as the reference value to which all measurements of an experiment were normalized (Fig. 1B). This value was chosen because most collated experiments cover a range of DPIs that includes the value of 8.0. It is not very common, though, that one of the light treatments is exactly 8.0 mol m^{-2} d^{-1}. In this case we linearly interpolated a reference value for SLA from the two adjacent light levels. If a DPI of 8.0 was outside the range considered for a given experiment, we excluded those data from further analysis, with the exception of experiments where the highest or lowest light levels differed by <10% from the reference value. Thus, of the four studies shown in Fig. 1A, the data of Rice and Bazzaz (1989) were excluded from further analysis. For each of the other experiments, observed SLA values at each DPI are divided by their calculated reference value. Although the three remaining experiments were characterized by very different DPIs and species with inherently different SLAs, they converge well after this scaling procedure (Fig. 1B). Note that this analysis allows an evaluation of the form of the response curve, but does not differentiate between species with inherently different maximum values. A similar normalization procedure is used by Tardieu and Parent (2010), with interesting insights into the short-term effect of temperature on leaf elongation rate.

(iii) The third step is to calculate all SLA values for each experiment and light level relative to the SLA observed or calculated at a DPI of 8.0 (Fig. 1C). The scaling procedure yields normalized values (ratios) rather than absolute values. By their nature, ratios do not show a normal distribution in a statistical sense, as values <1 can range from 0 to 1, but values >1 may approach infinity. As it is of interest to know whether a given plant trait varies linearly or non-linearly with an environmental factor, we $\log_2$-transformed the ratios of plant traits before any statistical analysis, as well as the relevant axes of graphs that we will use further on.

The light–response curve of SLA as an example

The full database we collated for SLA as dependent on irradiance currently consists of 160 experiments, with a total of >300 species and 1200 average values. Approximately 15% of those data are from experiments that do not include the reference value of 8.0 mol m^{-2} d^{-1}. They are therefore excluded from the analysis, although most of them confirmed the trends described here (data not shown). The remaining data set is remarkably diverse: *Helianthus annuus* is the most frequently measured species, yet it represents only 2% of all observations. The single largest experiment is that of Poorter (1999) on 15 species and six light intensities, which represents 7% of the observations. In the subsequent analyses, we no longer consider the separate experiments, but rather analyse all data points observed across all experiments concurrently. The resulting data set reveals a strong decrease in SLA with increasing DPI (Fig. 1C). However, there is also considerable variation present in the response. This variation may be caused by: (i) different species responding distinctly; (ii) different levels of environmental factors other than light for different experiments; (iii) the plant's ontogenetic stage at the time of harvest; (iv) possible errors during data collection and/or calculation by the original authors; and/or (v) errors or inaccuracies occurring during our analysis of the literature. In so far as errors in the SLA measurement involve a linear transformation (e.g. a wrong calibration factor or unit of expression) they do not affect the current analysis because all values are expressed in a relative way. A more serious problem arises if, for example, data are labelled in the paper as SLA values (leaf area:leaf mass), whereas in reality calculations pertain to LMA values (leaf mass:leaf area). This yields data characterized by a similar numerical range, but by an inverse relationship. In case of doubt, we contacted authors to double check. However, especially for older literature, this is not always possible. As the overall trend is at first more interesting than possible outliers, we decided to show the trend of the median and the interquartile range. This was done by categorizing the data points in seven DPI ranges (0–2, 2–4, 4–8, 8–12, 12–20, 20–30, and >30 mol m^{-2} d^{-1}) and calculating the median response, as well as the 25th and 75th percentile for each DPI range. The *x*th percentile is that value in a group of observations at which *x*% of the total observations show a smaller number and 100–*x*% a higher value. The advantage of percentiles is that they do not require any assumptions about the distribution of the underlying data and that they are relatively strongly buffered against occasional outliers. The median and the interquartile range are plotted in Fig. 1D as a bold line and grey area, respectively, and show the 'main trend' across all data. Furthermore, we used this approach to set 'normal limits', by calculating the 10th and the 90th percentile. They are indicated in Fig. 1D as broken lines. As mentioned before, observations outside this range are not necessarily abnormal, but rather should be considered by researchers with greater awareness of possible unintended effects.

An explicit aim of our approach is not only to provide an overall summary of a wide range of experiments, but also to make the approach quantitative. This may serve as a benchmark for future experiments as it can be analysed whether

Downloaded from jxb.oxfordjournals.org at Universite Laval on September 9, 2010

a given species responds more or less strongly compared with the 'average' species. To this end, we carried out a stepwise regression through all data, starting with a quadratic polynomial equation. In cases where the second-order equation was significant, we fitted the data with the formula

$$y = a + b \cdot x^c \quad (1)$$

where y is the $\log_2$-transformed scaled dependent variable (in this case SLA), and x is the environmental factor of interest. Conversely, in cases where the quadratic term or the whole equation was non-significant, we tested a linear equation. In the case of light, the relationship for SLA was clearly negative and non-linear (P <0.001 for the quadratic term), with an overall r^2 of 0.75 (Table 1). The resulting trend line is shown in Fig. 1D in red and can be used as an average approximation of the SLA response of plants to light intensity. We set the likely range of DPI that plants experience as lying between 1 mol m^{-2} d^{-1} and 50 mol m^{-2} d^{-1}, extreme specialists not included. From the fitted curve we now can calculate a plasticity index, which we define as the highest SLA value fitted in this range divided by the lowest value. In the present example, the plasticity index is 3.1 (Table 2), implying a 3-fold change in SLA over a 50-fold range in light. This index captures in a nutshell the sensitivity of a given trait within the entire testing range for a given environmental factor.

The overall trend is calculated across all data, with a minimum of assumptions. Thus, we did not weight experiments depending on the variability of the data or number of independent experimental units underlying the mean observations in each experiment (Hedges *et al.*, 1999). However, randomly categorizing data in two separate groups yielded very similar results (data not shown). A potential biological problem is that the data underlying the calculated response curve may over-represent a set of species selected for their relevance in agriculture, their presumed importance for the functioning of ecosystems, or because of other considerations of researchers. With >250 000 known higher plant species with very different ecological niches, it can be expected that not all species will have exactly the same form of the light–response. Although there is generally too little information for each species, sharper insights can be sought by categorization of species into a limited number of 'functional' groups, which have certain characteristics in common (Díaz and Cabido, 1997). Functional groups can share a certain ancestry (monocots), anatomy (woody species), or physiology (type of photosynthesis, nitrogen fixation). Alternatively, changes can be evaluated that form a continuous scale, such as species with an inherently low or high SLA. To test differences in response between groups of species experimentally is logistically challenging if one does not know *a priori* which groups to compare. With various groups to consider, even large-scale experiments with >30 species, such as carried out by Reich *et al.* (2003), result in a relatively small number of species per functional group (<6), making a comprehensive evaluation difficult. The present approach is a more 'soft' one, in the sense that not all experiments were carried out under exactly the same levels of light. Moreover, other conditions also varied. We

Table 1. General response curves of scaled SLA values as affected by 12 environmental factors

As ratios do have a logarithmic distribution by nature, we $\log_2$-transformed all scaled SLA values prior to the statistical analysis. For each factor we tested whether the relationship was linear (only linear component significant), non-linear (also the quadratic component significant), or no relationship at all. Coefficients of the linear equation (constant *a* and slope *b*) or the non-linear equation (*a*, *b*, and *c* in equation 1) are given. For each relationship the degrees of freedom (df) and the r^2 are indicated.

Variable	Environmental factor	*a*	*b*	*c*	df	r^2
SLA	Irradiance	1.20	–0.642	0.324	1050	0.75***
	R:FR	–	–	–	70	0.00^{ns}
	UV-B	–	–	–	70	0.00^{ns}
	CO_2	1.86	–0.811	0.139	670	0.27***
	O_3	–	–	–	140	0.00^{ns}
	Nutrients	–0.194	0.184	–	720	0.06***
	Water	–0.344	0.334	–	330	0.20***
	Waterlogging	0	–0.174	–	90	0.19***
	Submergence	0	–0.904	–	70	0.40***
	Temperature	–2.56	1.21	0.249	390	0.44***
	Salinity	0.0351	–0.304	–	190	0.24***
	Compaction	0.216	–0.162	–	70	0.06*
LMF	Irradiance	0.961	–0.794	0.0920	420	0.16***
SMF	Irradiance	0.064	–0.0074	–	410	0.10***
RMF	Irradiance	–0.889	0.508	0.261	420	0.48***
Leaf DMC	Irradiance	–0.841	0.486	0.275	150	0.84***
Stem DMC	Irradiance	–0.511	0.156	0.547	80	0.78***
Root DMC	Irradiance	–	–	–	80	0.00^{ns}

ns, non-significant; *P <0.05; **P <0.01; ***P <0.001.

Table 2. The plasticity index of SLA for 12 environmental factors, over the range considered to be ecologically relevant for physiologically active (non-dormant) plants. The plasticity index is defined as the highest value of the response curve over the range considered divided by the lowest value

Plasticity indices >1.5 are given in bold.

Environmental factor	Range considered	Reference value	Unit	Plasticity index SLA
Irradiance	1–50	8	mol m^{-2} day^{-1}	**3.12**
R:FR	0.2–1.2	0.9	mol mol^{-1}	1.00
UV-B	1–20	7	kJ m^{-2} d^{-1}	1.00
CO_2	200–1200	400	µmol mol^{-1}	1.39
O_3	5–100	20	nmol mol^{-1}	1.00
Nutrients	0.02–1	1	Relative units	1.13
Water	0.05–1	1	Relative units	1.25
Waterlogging	Absent/present	Absent	–	1.06
Submergence	Absent/present	Absent	–	**1.81**
Temperature	5–35	20	°C	**2.19**
Salinity	0–1	0.1	Fraction of seawater	1.23
Compaction	1.0–1.6	1.2	g cm^{-3}	1.07

Downloaded from jxb.oxfordjournals.org at Universite Laval on September 9, 2010

therefore cannot exclude the possibility that in some experiments another factor, for example a suboptimal nutrient supply, interacted with the response of plants to light. In the case of factorial combinations of treatments, we therefore focused on that level of the environmental factors outside our direct interest that yielded plants with the highest biomass. In the case of unifactorial experiments, we simply have to rely on the expertise of the researchers in choosing the appropriate growth conditions. Variability in the environmental factors that are not directly of interest is not only a nuisance: the wide variety of experiments compiled here also offers an advantage, as it implies that the observed trends are probably more generally valid than the results of one large experiment which was carried out under one specific combination of environmental factors. However, to exclude fully the possibility of confounding effects, any result from this analysis should be independently tested in a directed experiment.

Analysing response curves for contrasting subgroups

As an extension to the above analysis we classified species in a number of categories, as listed in the 'species trait' box of Fig. 2. Furthermore, we characterized general experimental conditions, as listed in the conditions box of the same figure. The third classification was the most challenging, as we categorized species in accordance with their ecological niche. For each environmental factor considered, species were classified on a three-point scale, discriminating between species generally found in shaded conditions, a group of species found mainly in light-exposed habitats, and an intermediate group. Separate response curves were constructed for each subgroup of species, and the plasticity index calculated as the most concise index of variation in the response curve.

Particular cultivation conditions seem to matter, as the plasticity index is higher for plants grown in growth cabinets than for those growing outdoors, and plants grown in hydroponics respond more strongly than those in a solid rooting medium (Table 3). At the same time, woody species from both the Gymnosperm and Angiosperm clades responded less strongly than herbaceous monocots and dicots. In our data set, these factors are strongly confounded: 90% of the data for woody species are from open shade houses constructed in experimental gardens, whereas 85% of the data from growth chambers are for herbaceous plants, often (>50%) grown in hydroponics. It is therefore as yet impossible to separate accurately the importance of life form and growth environment. However, documentation of this strong confounding between life form and growth conditions may help in data interpretation as well as decisions concerning future experimentation.

Three other biological classifications that we made yielded differences in plasticity, with little confounding of growth environment. Deciduous woody species had a somewhat greater plasticity than evergreen woody species, although the difference was relatively small and not significant (Table 3). A second classification pertains to the debate as to whether species with their ecological niche in shady habitats show less plasticity for SLA than those characteristic of sun-exposed environments (see Portsmuth and Niinemets, 2007 for an extended discussion). Taken over all experiments and plant species, we found this to be statistically true, as there was a significant interaction between light class and tolerance group. However, the

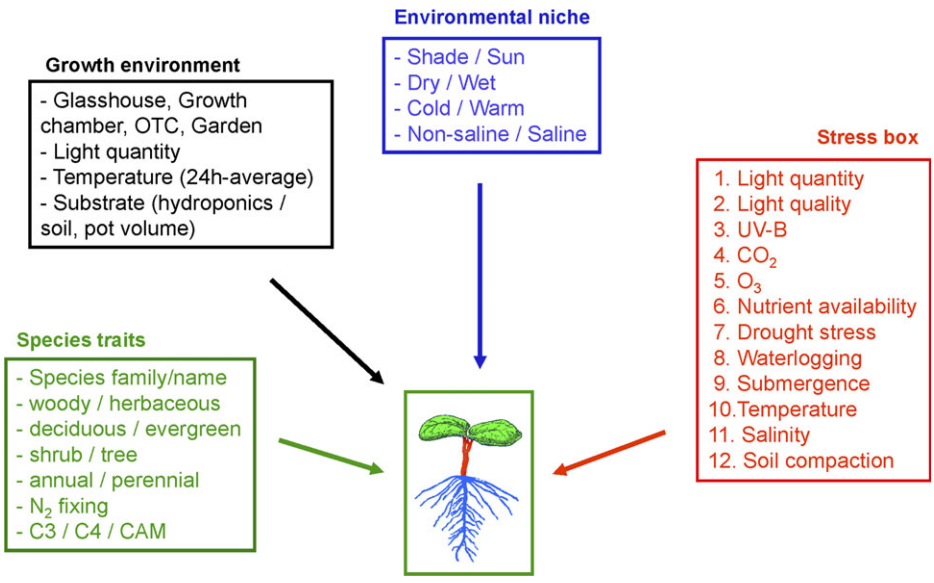

Fig. 2. The characterization of experiments, as carried out in the current meta-phenomics approach. Plant species are classified according to the species box by a number of general characteristics. General experimental conditions are given in the experimental box. The ecological niche of species is estimated by a three-stage scale for the relevant environmental factors. The last box shows the 12 environmental factors considered in this paper. In the case of nutrients, experiments are considered that apply limitations by nitrogen (N), phosphorus (P), or nutrients in general (G).

Downloaded from jxb.oxfordjournals.org at Universite Laval on September 9, 2010

Table 3. Plasticity indices for specific leaf area (SLA) of different subgroups over the daily photon irradiance (DPI) range of 1–50 mol m^{-2} d^{-1}

A non-linear equation was fitted to various subgroups of observations. For some groups this included some extrapolation of the data, but ranking of differences remained the same when a smaller trajectory was considered. The plasticity index is defined as the highest value of the response curve over the range considered divided by the lowest value. The last column indicates the significance of differences in plasticity between subgroups. To this end, we tested with orthogonal polynomials for a significant subgroup×light class interaction, considering only the linear component. This indicates whether there are differences in plasticity between subgroups, which linearly increase or decrease over the whole light range considered, neglecting higher order fluctuations. In the case of the ecological classifications this implies an increasing or decreasing plasticity across the species groups

Subgroup		Plasticity index SLA	df	*P*, between subgroups
Growth environment	Cabinets	3.52	290	***
	Glasshouses	3.07	210	
	Experimental garden	2.73	330	
Root substrate	Hydroponics	4.09	140	***
	Soil	2.90	660	
Phylogenetic/life form	Herbaceous dicots	3.90	280	***
	Herbaceous monocots	3.67	80	
	Woody dicots	2.87	630	
	Woody gymnosperms	2.74	30	
Leaf habit	Woody dicots deciduous	2.90	260	ns
	Woody dicots evergreen	2.59	400	
Shade tolerance	Low	3.45	480	***
	Intermediate	2.76	370	
	High	2.71	150	

ns, non-significant; ***P <0.001.

differences seem not to be very large (Fig. 3A). The difference became stronger when experiments carried out in growth chambers were excluded (56% difference; data not shown). A last comparison we made relates to a continuous trait rather than a categorical one. During the normalization procedure all observed SLA values were scaled to the reference value calculated at a light intensity of 8 mol m^{-2} d^{-1}. Although we statistically corrected for what is most probably innate variation between species to compare curves in a standardized way, we can still use the information to discriminate between inherently low SLA and high SLA species. Given that there is such a large difference in SLA between herbaceous and woody species, an unconditional comparison would yield a result discussed above already. Therefore, we contrasted the plants with the 35% lowest and 35% highest SLA values within each life form. Differences were more pronounced in this case, with high SLA species from both life forms responding more strongly than low SLA species (Fig. 3B; P <0.001 in both cases).

The response of SLA to 12 environmental factors

The above analysis provides a condensed summary of the response of SLA to light over a wide range of experiments.

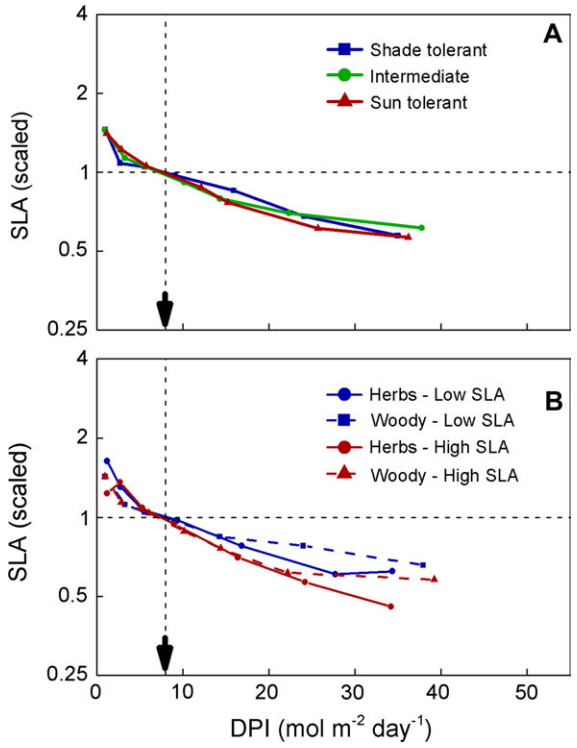

Fig. 3. SLA response to daily photon irradiance as dependent on various subgroups. (A) The median response of plant species characteristic of shaded habitats (blue line), sun-exposed habitats (red line), or from intermediate environments (green line). (B) The median response of species with a relatively high (red line) or low (blue line) SLA at the reference irradiance of 8 mol m^{-2} d^{-1}, analysed separately for woody (broken line) and herbaceous species (continuous line). The reference value for the different environmental factors is indicated by black arrows.

All data were systematically expressed in the same units of irradiance as well as the same units describing leaf morphology. Although highly informative in itself, light forms only one axis in a multidimensional space of environmental factors. Far more insight could be achieved if we were able to have similar information for the other environmental dimensions as well. In a recent review, Poorter *et al.* (2009) considered the response of LMA to a wide range of environmental factors. Among these are the 'general' factors that received the greatest attention in the scientific field up to now: light, CO_2, nutrients, temperature, and water limitation. However, also more specific stresses, such as UV-B, ozone, waterlogging, submergence, salinity, and soil compaction can be highly relevant for plant functioning and are included in the analysis. We did not include abiotic stresses such as trampling, wind, SO_2, and NO_x, not because they are irrelevant, but simply because too little information is available to allow for a proper generalization. As for light, we focus on plants that are generally grown for the longest period of their active growing time under contrasting environmental conditions, without experimentally designed switches between environments. The only exception is complete submergence, which is a stress factor that most land plants can endure for only

Downloaded from jxb.oxfordjournals.org at Universite Laval on September 9, 2010

a limited amount of time. A detailed description of the restrictions used for this review is given in Appendix 1.

Here we extend the analysis of Poorter *et al.* (2009), with ~20% more experiments, and present all leaf area:biomass ratios as SLAs. Although SLA and LMA carry the same information, they are inversely related, which can make analysis of linear and non-linear responses difficult. Moreover, for a large group in the scientific community, SLA is a more appropriate parameter to use, as it scales in principle linearly with the relative growth rate of plants (Evans, 1972). In total, we considered SLA responses to 12 environmental factors. For 10 factors, an objectively measurable reference value could be chosen. A critical criterion for the choice of the reference value is that it falls in the range of values usually measured. In principle, the actual level of choice does not affect the final result. Reference values are listed in Table 2 and indicated by black triangles in Fig. 4. The main problem we faced was choosing a reference level for nutrient and drought stress. There are many ways in which nutrient stresses can be applied (Ingestad *et al.*, 1982; Van de Vijver *et al.*, 1993), with a wide range of results possible, which depend on the details of the experimental design on the one hand and the size as well as the growth rate of the plants—and therefore the demand for nutrients—on the other. In the case of drought stress, the experimental designs vary as greatly as for nutrients (Fernández and Reynolds, 2000; Granier *et al.*, 2006). The only possible way to scale the severity of these stresses is by expressing them relative to the total biomass gained by control plants. This is not ideal, as the control plants may have suffered from stress in some experiments and not in others, but it is possibly as close as one can get in generalizing the severity of a stress over such a variety of experiments. For the factors waterlogging and complete submergence, we only considered two levels: either fully waterlogged or submerged, or well-watered controls, neglecting a more fuzzy intermediate level such as '70% submerged'. A level of 100% waterlogging or submergence is still objectively definable.

The results of the analysis are shown in Fig. 4A–L. The number of data we have been able to find and that underlie these response curves varies greatly between factors, with the least information on R:FR, UV-B, and soil compaction. These are at the same time the factors that turn out to have little impact on SLA, which may actually be the prime reason why they are not reported as often. Another environmental factor, for which little information is present, is complete submergence. In this case, the increase in SLA is large, and has been thought to be one of the important traits determining the ability to survive in such an environment (Mommer *et al.*, 2006). For a more quantitative analysis, we fitted for each factor general response curves over all data. Taking then into account the biologically relevant range for each environmental factor (Table 3), plasticity indices were calculated over these ranges. Plasticity for SLA varied widely, being highest for light, submergence, and temperature, and only modest for CO_2, nutrients, drought, and salinity. An extended discussion of the underlying mechanisms is outside the scope of this paper, and can be found in Poorter *et al.* (2009).

Variability, as judged from the interquartile range, is also an important issue in judging these response curves. As a consequence of the normalization procedure, variability is smallest close to the reference values, indicated by the black triangles. The factors with the strongest variability are those for which the response is generally strong anyway, which may be caused by species specialization. We included two clear examples of this phenomenon in Fig. 4. The median response to CO_2 is stronger than average for C_3 species (Fig. 4D). The response of C_4 species is in stark contrast; it does not respond to elevated CO_2 up to a range of 800 μmol mol^{-1} and—surprisingly—even increases at higher concentrations. Although the number of data on C_4 plants at high CO_2 levels is low, the difference is significantly different from unity. The other example is that of temperature (Fig. 4J), highlighting that tropical species show a much higher plasticity in SLA for this factor than plants from temperate climates.

The response curves of different traits can be compared

The above analysis shows how the response of one trait can be analysed over a range of environmental factors. However, the approach can be fruitfully extended to other variables. As an example, we show here the response of biomass allocation and the dry matter content (DMC) with respect to growth irradiance. The allocation of biomass over the various plant organs has received attention for a long time, in both a physiological and ecological context. Brouwer (1962) coined the appealing term 'functional equilibrium' for the way biomass was allocated to shoots and roots under various environmental conditions, and Tilman (1988) used it as cornerstone for his theory on the ecological success of species. Following the same approach as for SLA, we compiled ~440 observations on the fractions of biomass invested in leaves, stems, and roots (termed LMF, SMF, and RMF, respectively). Response curves are shown in Fig. 5, at the same scale as was used for the SLA data. As can be seen from this graph, the changes are very modest. There is some shift towards a decreased allocation to roots and an increased allocation to leaves at lower light levels, but only when the light level is very low (<3 mol m^{-2} d^{-1}) does the shift in LMF become more apparent. Although this trend can be considered to agree well with a 'functional equilibrium' paradigm, the changes are overall marginal, with a plasticity index for LMF of 1.26, which is small compared with the 3-fold change in SLA. We therefore conclude that the differences in SLA are more important than the variation in LMF in understanding the variation in relative growth rate with light. A second point that is nicely illustrated by these data is the care that has to be taken in their interpretation. Presented on the same relative scale, LMF seems to be less plastic than RMF (plasticity index = 1.91). However, compared with leaves, roots generally

Downloaded from jxb.oxfordjournals.org at Universite Laval on September 9, 2010

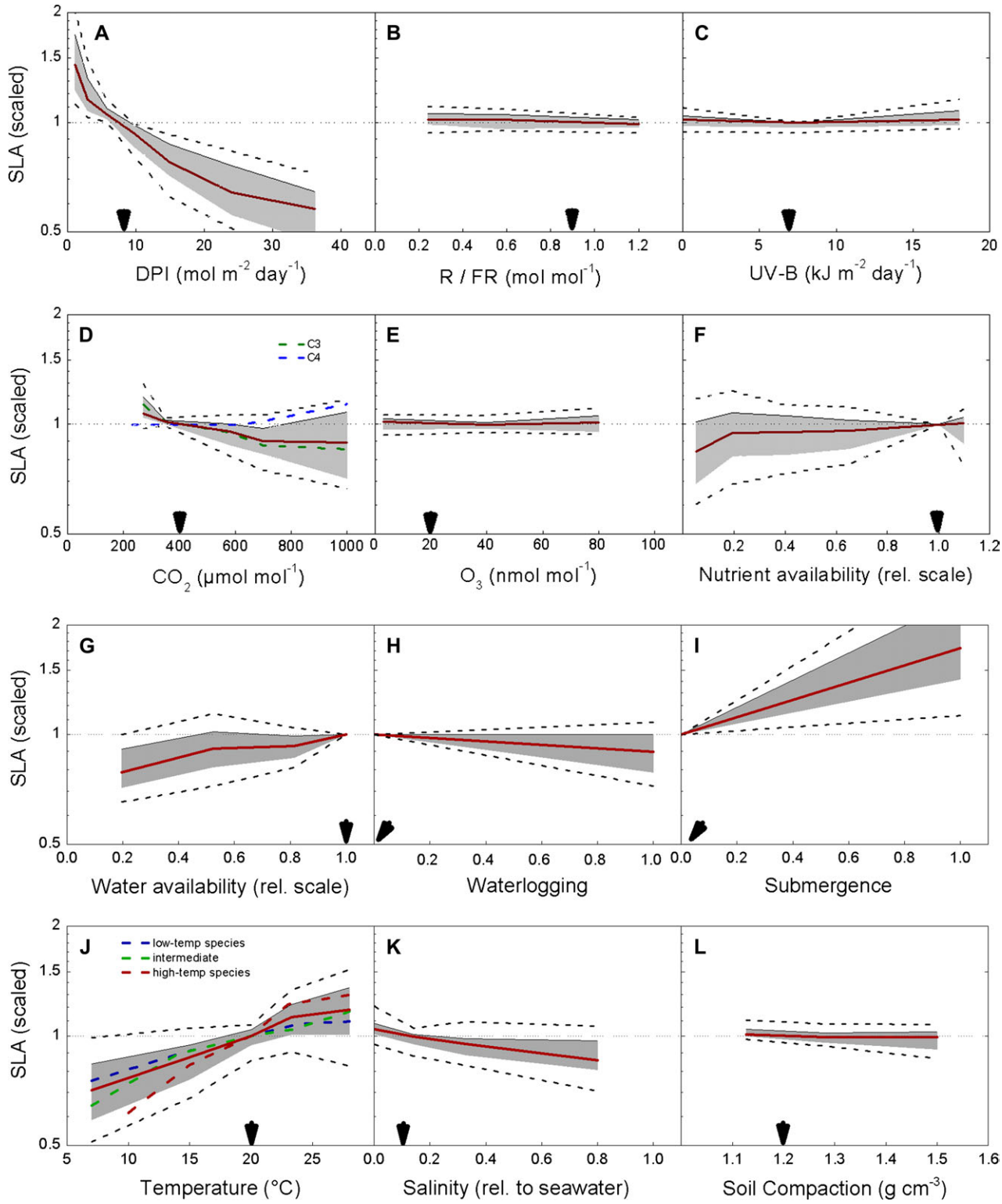

Fig. 4. (A–L) The response of specific leaf area (SLA) to 12 environmental factors. The bold line indicates the median value, and the shaded area the interquartile range. The number of observations on which this graph is based is listed in Table 1. The reference value for the various environmental factors is indicated by black arrows. D also shows the median response of C_3 and C_4 species separately and J shows the median response of species characteristic of environments with different temperatures during the growing period.

comprise a smaller fraction of biomass of young trees and herbaceous plants. Thus, if a plant allocates 1 g less to leaves but rather invests this in roots, the relative decrease in LMF will be smaller than the relative increase in RMF.

Another trait that has received little attention so far is the DMC (dry mass:fresh mass) of various organs. There are strong and inherent differences in DMC for ecologically different species. In fact, in the ecological literature it has

Downloaded from jxb.oxfordjournals.org at Universite Laval on September 9, 2010

been suggested that leaf DMC would be a better parameter to determine a plant's ecological niche than SLA (Wilson *et al.*, 1999). However, also for physiological research, the DMC of the various organs is an important variable to consider, not least because various publications use various ways to scale rates of physiological processes or chemical amounts across treatments or species. Thus, some scientists prefer to express rates of processes or amounts of compounds per unit area, especially in photosynthesis-related research (Hurry *et al.*, 1995; Pons and De Jong, 2004), others generally use dry masses (De Groot *et al.*, 2003), and still others report their results routinely on a fresh mass basis (Smith and Stitt, 2007; Usadel *et al.*, 2008). It follows that if we do not know the relationships between these three parameters, it is hard to make a useful integration across experiments. Therefore, we looked at how irradiance affects the DMC of the various organs.

In strong contrast to SLA and biomass allocation, there are very few reports on the DMC of organs as dependent on the environment, notwithstanding the fact that fresh and dry masses are routinely measured in many laboratories. Therefore, the majority of the data on which Fig. 6 are based are not from the literature, but are unpublished data kindly shared by colleagues mentioned in the Acknowledgements section. Leaf DMC turned out to be surprisingly strongly affected by light, with an almost linear increase in DMC when light increases. The plasticity index for this trait

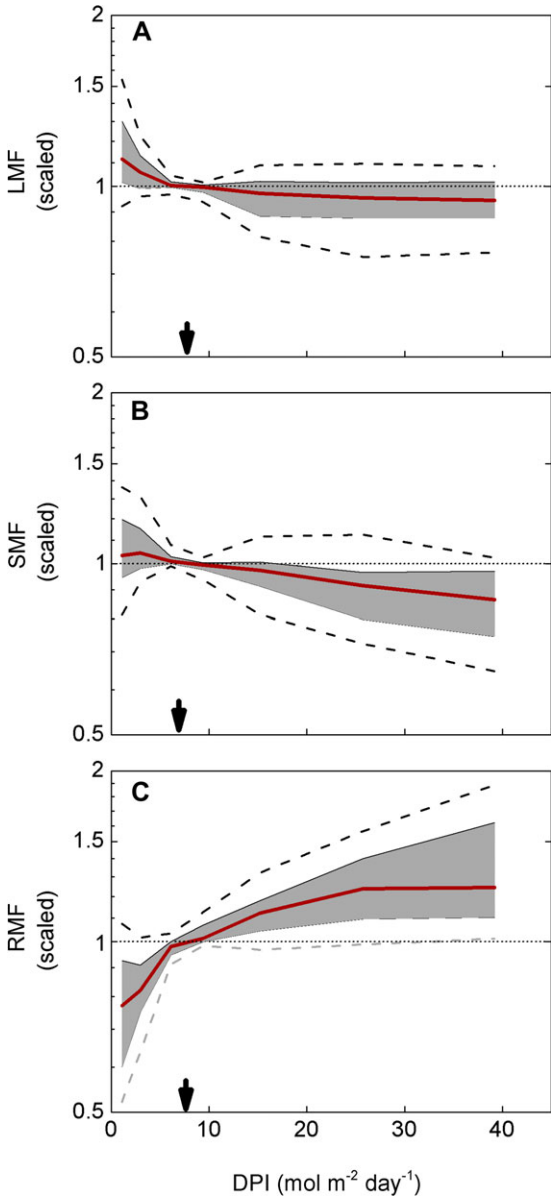

Fig. 5. The response of the allocation of biomass to (A) leaves (LMF), (B) stems (SMF), and (C) roots (RMF) to irradiance. The bold line indicates the median value and the shaded area the interquartile range. The number of observations on which this graph is based is listed in Table 1. The reference value of the DPI is indicated by black arrows.

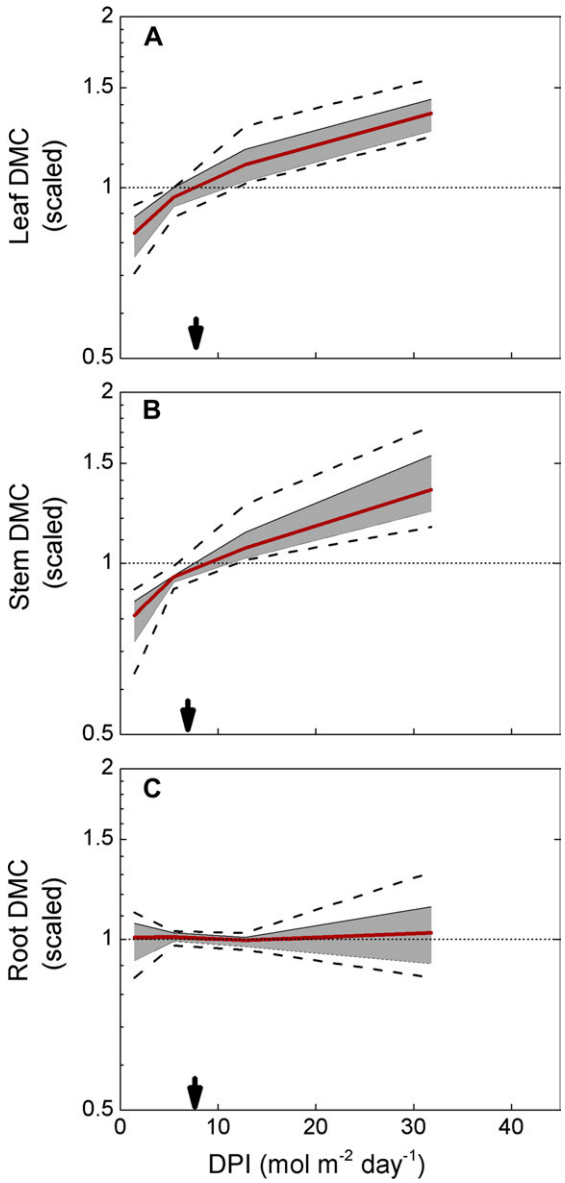

Fig. 6. Response curves of the dry matter content (DMC) of (A) leaves, (B) stems, and (C) roots to irradiance. The number of observations on which this graph is based is listed in Table 1. The reference value of the DPI is indicated by black arrows.

Downloaded from jxb.oxfordjournals.org at Universite Laval on September 9, 2010

is 1.92, which is less than that for SLA, but still considerable. The same holds for the DMC of the stems (2.25), but, very surprisingly, the DMC of the roots is hardly affected by the light environment. In general, the underlying basis for differences in DMC can be 3-fold. First, there can be a difference in the concentration of cell wall compounds, because of higher allocation to cell walls, a shift from water-rich epidermal tissue to other tissues, such as sclerenchymatic cells, or because of changes between leaf veins and interveinal areas (cf. Van Arendonk and Poorter, 1997; Niinemets, 1999; Walter and Schurr, 1999; Niinemets and Sack, 2006). Secondly, the cell size can be affected, with smaller cells and (much) smaller vacuoles, which will also increase the relative fraction of dry matter in cell walls (Niinemets and Sack, 2006). Thirdly, the content can be affected by accumulation of large quantities of, for example, starch. Although starch concentrations are higher at high light, these differences are modest compared with those of plants grown at elevated CO_2 (Roumet *et al.*, 1999) or in cold conditions (Venema *et al.*, 1999). Currently, we do not have a satisfactory understanding of the quantitative importance of each of these factors.

Conclusions and outlook

The procedures presented here build on a large database of phenotypic observations and provide a quantitative method to construct response curves. Using SLA as an example of an important phenotypic trait, we were able to show that the use of this methodology enabled: (i) the construction of quantitative relationships with 12 environmental factors; (ii) the estimation of variability around median trends; (iii) the characteristic response of certain pre-defined experimental subgroups; and (iv) the definition of a plasticity index over the full range of an environmental factor. The quantitative relationships found can form a reference for results of future experiments, and provide the framework of prior knowledge as required, for example, in Bayesian statistics (McCarthy, 2007).

We have shown that this meta-analytical approach can be fruitfully extended to other phenotypic data. We will target a larger number of physiological, morphological, chemical, and anatomical plant traits, such as photosynthetic capacity, biomass allocation, and nitrogen content. In future analysis, another focal point will be the interaction between different variables. We refer to this approach as 'meta-phenomics', which provides us with a more systematic and formal way to structure information on the response of plants to their environment. This will be advantageous, in understanding both the constraints to plant productivity by limiting factors and the response of plants to global change.

Supplementary data

Supplementary data are available at *JXB* online.

Supplementary appendix 1. List of papers used for the analysis of the effect of 12 environmental factors on SLA.

Supplementary appendix 2. List of papers used for the analysis of the effect of irradiance on allocation and dry matter content.

Acknowledgements

We thank Ismael Aranda, Owen Atkin, Corine de Groot, Yulong Feng, Keith Funnell, Yaskara Hayashida, Vaughan Hurry, Maarit Mäenpää, Kerstin Nagel, Leo Marcelis, Thijs Pons, Peter Reich, Dina Rhonzina, Francesco Ripullone, Catherine Roumet, Peter Ryser, Dylan Schwilk, Susanne Tittman, Jan Henk Venema, and Rafael Villar, for generously providing (partly) unpublished results for this analysis. Lea Hallik, Harry Olde Venterink, and Hans Schepers as well as the reviewers provided insightful comments on a previous version of the manuscript. HP acknowledges support from the Estonian University of Life Sciences and the Estonian Ministry of Education and Science (grant SF1090065s07) for his stay during the writing of this manuscript.

Appendix 1. Inclusion criteria for the meta-analyses

We believe that this approach could be used to generalize across a wide variety of experiments and conditions. Accordingly, we adopted by default an inclusive approach in considering previously published work. However, we wish to list explicitly the few decisional criteria that we applied consistently to define a final selection of data.

(i) We only considered plants that were subjected to some form of controlled experimental treatment involving the direct manipulation of environmental variables, and thus excluded plants growing in the field for which correlations were made with measured environmental variables *a posteriori*. However, these observations can form interesting comparisons with our results (Ogaya and Penuelas, 2007).

(ii) We only considered plants grown in pots, hydroponics, or other types of containers, in the absence of competition with neighbouring plants. Thus we excluded plants growing in intra- or interspecific competition for light (such as in artificial vegetations), or nutrients (such as in experimental gardens in naturally occurring soil).

(iii) We considered three plant organs: leaves, stems, and roots. In cases where concentrations or biomass allocation were presented on a shoot basis, these observations were disregarded. An exception was made for rosette plants, where the caudex would form a small proportion of the shoot anyway.

(iv) Plants in the generative phase may show a different response to those in the vegetative phase, especially at the whole plant level, and for this review we focus on the vegetative phase only.

(v) In the case of an experiment with a factorial combination of environmental factors, we choose the response of plants to the factor of interest at the level of the other environmental conditions that were least limiting.

References

Arp WJ. 1991. Effects of source–sink relations on photosynthetic acclimation to elevated CO_2. *Plant, Cell and Environment* **14,** 869–875.

Downloaded from jxb.oxfordjournals.org at Universite Laval on September 9, 2010

Bezemer PD, Netelenbos JC, Mulder C, Theune JA, Stamhuis IH, Straus JP. 1982. Determining reference (normal) limits in medicine: an application. *Statistics in Medicine* **2,** 191–198.

Brouwer R. 1962. Distribution of dry matter in the plant. *Netherlands Journal of Agricultural Sciences* **10,** 399–408.

Chabot BF, Jurik TW, Chabot JF. 1979. Influence of instantaneous and integrated light-flux density on leaf anatomy and photosynthesis. *American Journal of Botany* **66,** 940–945.

De Groot CC, Marcelis LFM, Van den Boogaard R, Kaiser WM, Lambers H. 2003. Interaction of nitrogen and phosphorus nutrition in determining growth. *Plant and Soil* **248,** 257–268.

De Groot CC, Marcelis LFM, Van den Boogaard R, Lambers H. 2002. Interactive effects of nitrogen and irradiance on growth and partitioning of dry mass and nitrogen in young tomato plants. *Functional Plant Biology* **29,** 1319–1328.

Díaz S, Cabido M. 1997. Plant functional types and ecosystem function in relation to global change. *Journal of Vegetation Science* **8,** 463–474.

Evans GC. 1972. *The quantitative analysis of plant growth*. Oxford: Blackwell.

Evans LT, Wardlaw IF, King RW. 1985. Plants and environment: two decades of research at the Canberra phytotron. *Botanical Review* **51,** 203–272.

Farquhar GD, Von Caemmerer S, Berry JA. 2001. Models of photosynthesis. *Plant Physiology* **125,** 42–45.

Fernandez RJ, Reynolds JF. 2000. Potential growth and drought tolerance of eight desert grasses: lack of a trade-off? *Oecologia* **123,** 90–98.

Granier C, Aguirrezabal L, Chenu K, *et al*. 2006. PHENOPSIS, an automated platform for reproducible phenotyping of plant responses to soil water deficit in Arabidopsis thaliana permitted the identification of an accession with low sensitivity to soil water deficit. *New Phytologist* **169,** 623–635.

Hanson HC. 1917. Leaf-structure as related to environment. *American Journal of Botany* **4,** 533–560.

Hedges LV, Gurevitch J, Curtis PS. 1999. The meta-analysis of response ratios in experimental ecology. *Ecology* **80,** 1150–1156.

Hedges LV, Olkin I. 1985. *Statistical methods for meta-analysis*. Orlando: Academic Press.

Hikosaka K, Osone Y. 2009. A paradox of leaf-trait convergence: why is leaf nitrogen concentration higher in species with higher photosynthetic capacity? *Journal of Plant Research* **122,** 245–251.

Hurry VM, Strand A, Tobiaeson M, Gardestrom P, Oquist G. 1995. Cold hardening of spring and winter wheat and rape results in differential effects on growth, carbon metabolism, and carbohydrate content. *Plant Physiology* **109,** 697–706.

Ingestad T. 1982. Relative addition rate and external concentration; driving variables used in plant nutrition research. *Plant, Cell and Environment* **5,** 443–453.

Juurola E. 2003. Biochemical acclimation patterns of Betula pendula and Pinus sylvestris seedlings to elevated carbon dioxide concentrations. *Tree Physiology* **23,** 85–95.

Kreusler U, Prehn A, Hornberger R. 1879. Beobachtungen über das Wachstum der Maispflänze (Bericht über die Versuche vom Jahre 1878). *Landwirtschaftliche Jahrbücher* **8,** 617–622.

Lambers H, Poorter H. 1992. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. *Advances in Ecological Research* **23,** 187–261.

Macdowall FDH. 1972. Growth kinetics of Marquis wheat. I. Light dependence. *Canadian Journal of Botany* **50,** 89–99.

McCarthy MA. 2007. *Bayesian statistics for ecology*. Cambridge: Cambridge University Press.

Mitscherlich E. 1909. Das Gesetz des Minimum, das Gesetz des Abnahmenden Bodenertrages. *Landwirtschaftliches Jahrbuch* **38,** 537–552.

Mommer L, Lenssen JPM, Huber H, Visser EJW, de Kroon H. 2006. Ecophysiological determinants of plant performance under flooding: a comparative study among seven plant families. *Journal of Ecology* **94,** 1117–1129.

Morgan PB, Ainsworth EA, Long SP. 2003. How does elevated ozone impact soybean? A meta-analysis of photosynthesis, growth and yield. *Plant, Cell and Environment* **26,** 1317–1328.

Niinemets Ü. 1999. Research review. Components of leaf dry mass per area—thickness and density—alter leaf photosynthetic capacity in reverse directions in woody plants. *New Phytologist* **144,** 35–47.

Niinemets Ü Sack L. 2006. Structural determinants of leaf light-harvesting capacity and photosynthetic potentials. In: Esser K, Lüttge UE, Beyschlag W, Murata J, eds. *Progress in botany*, vol. 67. Berlin: Springer Verlag, 385–419.

Ogaya R, Peñuelas J. 2007. Leaf mass per area ratio in Quercus ilex leaves under a wide range of climatic conditions. The importance of low temperatures. *Acta Oecologia* **31,** 168–173.

Osenberg CW, Sarnelle O, Cooper SD. 1997. Effect size in ecological experiments: the application of biological models in meta-analysis. *American Naturalist* **150,** 798–812.

Pons TL, De Jong-van Berkel YEM. 2004. Species-specific variation in the importance of the spectral quality gradient in canopies as a signal for photosynthetic resource partitioning. *Annals of Botany* **94,** 725–732.

Portsmuth A, Niinemets Ü. 2007. Structural and physiological plasticity in response to light and nutrients in five temperate deciduous woody species of contrasting shade tolerance. *Functional Ecology* **21,** 61–77.

Poorter H. 1993. Interspecific variation in the growth response of plants to an elevated ambient CO_2 concentration. *Vegetatio* **104/105,** 77–97.

Poorter H, Navas ML. 2003. Plant growth and competition at elevated CO_2: on winners, losers and functional groups. *New Phytologist* **157,** 175–198.

Poorter H, Niinemets Ü, Poorter L, Wright IJ, Villar R. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist* **182,** 565–588.

Poorter H, van der Werf A. 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*. Leiden, The Netherlands: Backhuys Publishers, 309–336.

Downloaded from jxb.oxfordjournals.org at Universite Laval on September 9, 2010

Poorter L. 1999. Growth responses of 15 rain-forest tree species to a light gradient: the relative importance of morphological and physiological traits. *Functional Ecology* **13,** 396–410.

Reich PB, Buschena C, Tjoelker MG, Wrage K, Knops J, Tilman D, Machado JL. 2003. Variation in growth rate and ecophysiology among 34 grassland and savanna species under contrasting N supply: a test of functional group differences. *New Phytologist* **157,** 617–631.

Rice SA, Bazzaz FA. 1989. Quantification of plasticity of plant traits in response to light intensity: comparing phenotypes at a common weight. *Oecologia* **78,** 502–507.

Roumet C, Bel MP, Sonie L, Jardon F, Roy J. 1996. Growth response of grasses to elevated CO_2: a physiological plurispecific analysis. *New Phytologist* **133,** 595–603.

Roumet C, Laurent G, Roy J. 1999. Leaf structure and chemical composition as affected by elevated CO2: genotypic responses of two perennial grasses. *New Phytologist* **143,** 73–81.

Smith A, Stitt M. 2007. Coordination of carbon supply and plant growth. *Plant, Cell and Environment* **30,** 1126–1149.

Tardieu F, Parent B. 2010. Modelling the effects of genes and QTLs: on the plant sensitivity to environmental conditions. *Journal of Experimental Botany,* in press.

Tilman D. 1988. *Plant strategies and the dynamics and structure of plant communities*. Princeton, NJ: Princeton University Press.

Usadel B, Gibon Y, Bläsing OE, Poree F, Höhne M, Günter M, Trethewey R, Kamlage B, Poorter H, Stitt M. 2008. Multilevel genomics analysis of the response of transcripts, enzyme activities and metabolites in Arabidopsis rosettes to a progressive decrease of the temperature in the non-freezing range. *Plant, Cell and Environment* **31,** 518–547.

Van Arendonk JJCM, Poorter H. 1994. The chemical composition and anatomical structure of leaves of grass species differing in relative growth rate. *Plant, Cell and Environment* **17,** 963–970.

Van de Vijver CADM, Boot RGA, Poorter H, Lambers H. 1993. Phenotypic plasticity in response to nitrate supply of an inherently fast-growing species from a fertile habitat and an inherently slow-growing species from an infertile habitat. *Oecologia* **96,** 548–554.

Venema JH, Posthumus F, De Vries M, Van Hasselt PR. 1999. Differential response of domestic and wild Lycopersicon species to chilling under low light: growth, carbohydrate content, photosynthesis and the xanthophyll cycle. *Physiologia Plantarum* **105,** 81–88.

Walter A, Schurr U. 1999. The modular character of growth in Nicotiana tabacum plants under steady state nutrition. *Journal of Experimental Botany* **50,** 1169–1177.

Went FW. 1957. *The experimental control of plant growth*. New York: The Ronald Press Company.

Wilson PJ, Thompson K, Hodgson JG. 1999. Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. *New Phytologist* **143,** 155–162.

Wright IJ, Reich PB, Westoby M, *et al*. 2004. The leaf economics spectrum worldwide. *Nature* **428,** 821–827.

Downloaded from jxb.oxfordjournals.org at Universite Laval on September 9, 2010