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

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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.

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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 form of the response curve. 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 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 CO₂, such an approach has been fruitfully used to show that plants grown in small pots were restricted in their response to CO₂ (Arp, 1991) and that C₄ species, although responding less strongly than C₃ species, nonetheless increase biomass at elevated CO₂ 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
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 (m² 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...
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 transform (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 xth 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
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

\[ y = a + bx + cx^2 \]  

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 can now 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 (Diaz 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>
<thead>
<tr>
<th>Variable</th>
<th>Environmental factor</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>df</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLA</td>
<td>Irradiance</td>
<td>1.20</td>
<td>-0.642</td>
<td>0.324</td>
<td>1050</td>
<td>0.75***</td>
</tr>
<tr>
<td></td>
<td>R:FR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>70</td>
<td>0.00***</td>
</tr>
<tr>
<td></td>
<td>UV-B</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>70</td>
<td>0.00***</td>
</tr>
<tr>
<td></td>
<td>CO( \text{}_2 )</td>
<td>1.86</td>
<td>-0.811</td>
<td>0.139</td>
<td>670</td>
<td>0.27***</td>
</tr>
<tr>
<td></td>
<td>O( \text{}_3 )</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>140</td>
<td>0.00***</td>
</tr>
<tr>
<td></td>
<td>Nutrients</td>
<td>-0.194</td>
<td>0.184</td>
<td>-</td>
<td>720</td>
<td>0.06***</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>-0.344</td>
<td>0.334</td>
<td>-</td>
<td>330</td>
<td>0.20***</td>
</tr>
<tr>
<td></td>
<td>Waterlogging</td>
<td>0</td>
<td>-0.174</td>
<td>-</td>
<td>90</td>
<td>0.19***</td>
</tr>
<tr>
<td></td>
<td>Submergence</td>
<td>0</td>
<td>-0.904</td>
<td>-</td>
<td>70</td>
<td>0.40***</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>-2.56</td>
<td>1.21</td>
<td>0.249</td>
<td>390</td>
<td>0.44***</td>
</tr>
<tr>
<td></td>
<td>Salinity</td>
<td>0.0351</td>
<td>-0.304</td>
<td>-</td>
<td>190</td>
<td>0.24***</td>
</tr>
<tr>
<td></td>
<td>Compaction</td>
<td>0.216</td>
<td>-0.162</td>
<td>-</td>
<td>70</td>
<td>0.06***</td>
</tr>
<tr>
<td>LMF</td>
<td>Irradiance</td>
<td>0.961</td>
<td>-0.794</td>
<td>0.0920</td>
<td>420</td>
<td>0.16***</td>
</tr>
<tr>
<td>SMF</td>
<td>Irradiance</td>
<td>0.064</td>
<td>-0.0074</td>
<td>-</td>
<td>410</td>
<td>0.10***</td>
</tr>
<tr>
<td>RMF</td>
<td>Irradiance</td>
<td>-0.889</td>
<td>0.508</td>
<td>0.261</td>
<td>420</td>
<td>0.48***</td>
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<td>Leaf DMC</td>
<td>Irradiance</td>
<td>-0.841</td>
<td>0.486</td>
<td>0.275</td>
<td>150</td>
<td>0.84***</td>
</tr>
<tr>
<td>Stem DMC</td>
<td>Irradiance</td>
<td>-0.511</td>
<td>0.156</td>
<td>0.547</td>
<td>80</td>
<td>0.78***</td>
</tr>
<tr>
<td>Root DMC</td>
<td>Irradiance</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>80</td>
<td>0.00***</td>
</tr>
</tbody>
</table>

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

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

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.

<table>
<thead>
<tr>
<th>Environmental factor</th>
<th>Range considered</th>
<th>Reference Unit</th>
<th>Plasticity index SLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiance</td>
<td>1–50</td>
<td>mol m$^{-2}$ day$^{-1}$</td>
<td>3.12</td>
</tr>
<tr>
<td>R:FR</td>
<td>0.2–1.2</td>
<td>mol$^{-1}$</td>
<td>1.00</td>
</tr>
<tr>
<td>UV-B</td>
<td>1–20</td>
<td>kJ m$^{-2}$ d$^{-1}$</td>
<td>1.00</td>
</tr>
<tr>
<td>CO( \text{}_2 )</td>
<td>200–1200</td>
<td>( \mu )mol$^{-1}$</td>
<td>1.39</td>
</tr>
<tr>
<td>O( \text{}_3 )</td>
<td>5–100</td>
<td>nmol$^{-1}$</td>
<td>1.00</td>
</tr>
<tr>
<td>Nutrients</td>
<td>0.02–1</td>
<td>Relative units</td>
<td>1.13</td>
</tr>
<tr>
<td>Water</td>
<td>0.05–1</td>
<td>Relative units</td>
<td>1.25</td>
</tr>
<tr>
<td>Waterlogging</td>
<td>Absent/present</td>
<td></td>
<td>1.06</td>
</tr>
<tr>
<td>Submergence</td>
<td>Absent/present</td>
<td></td>
<td>1.81</td>
</tr>
<tr>
<td>Temperature</td>
<td>5–35</td>
<td>°C</td>
<td>2.19</td>
</tr>
<tr>
<td>Salinity</td>
<td>0–1</td>
<td>Fraction of seawater</td>
<td>1.23</td>
</tr>
<tr>
<td>Compaction</td>
<td>1.0–1.6</td>
<td>g cm$^{-3}$</td>
<td>1.07</td>
</tr>
</tbody>
</table>
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 Portsmouth 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
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, 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.

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 most land plants can endure for only

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.
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 submersion 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 the least information on R:FR, UV-B, and soil compaction. 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...
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
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

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**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.
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 CO2 (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


